A PROTEOMICS-BASED APPROACH TO STUDYING THE IMPACT OF TRANSGENIC MAIZE (MON810) IN RATS AS A MODEL

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Declaration

I declare that this work has not been accepted for any degree or qualification before and is not currently being submitted in candidature for any degree other than the degree of Doctor of Philosophy of the Newcastle University.

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Abstract

Transgenic maize MON810 is a maize variety that has been genetically modified to express Cry1Ab isolated from the soil bacterium *Bacillus thuringiensis* (*Bt*) to produce a natural insecticide (*Bt* toxin) which kills larvae of the European corn borer (ECB), a major pest of maize. The mode of action of *Bt* toxin in ECB is through binding to specific receptors on the epithelial cells of the highly alkaline midgut of the insect, resulting in pore-formation, osmotic imbalance, cell lysis and subsequent death of the insect. In contrast, this *Bt* toxin is considered to be harmless or nontoxic to mammals due to acidified gut pepsinolysis and the lack of Cry protein binding-sites on the mammalian gut epithelial cells. However, to date, no studies have investigated the cellular effects of these Cry proteins at the proteome level. The aim of this study was therefor to investigate the *in vivo* and *in vitro* effects of MON810 maize expressing the δ-insecticidal protein Cry1Ab from *Bacillus thuringiensis*, on differential gene expression at the proteome level in the epithelial cells of the small intestine of the rat as a model for mammals. Proteomic profiling techniques were included for the *in vivo* and *in vitro* studies to obtain a better understanding of the underlying molecular responses in rat to MON810. Transgenic *Bt* maize (MON810), the corresponding parental non-transgenic maize (MON CONV CORN), and 3 other maize varieties, MON Garst 8450, MON Gold HVST H8920 and MCert Rod commercial control (used as internal controls), were provided by Monsanto, USA. All diets were formulated by TestDiet and contained approximately 33% (w/w) corn grain; other diet components were adjusted to provide approximately equal levels of protein, calories and nutrients. Different reference varieties were used in this study to determine whether the changes that may occur with the consumption of MON810 maize lay within the expected range for several different unmodified reference varieties. Two rats feeding trials (7-day and 28-day) were conducted to assess the safety of MON810 maize using forty immature male Wistar rats (rats were between 6 to 7 weeks of age at the beginning of the study). Rats were assigned to the above 5 experimental groups based on body weight means. No adverse behavioural effects on rats were observed and there were no significant differences in absolute body weights, body weight gains, food consumption and feed conversion efficiency between rats fed MON810 in the diet when compared to rats fed diets containing grain from the other maize varieties. Thus the transgenic variety MON810 had no adverse effects on these parameters. Following these feeding studies, rats were sacrificed and the total proteins extracted from the small intestinal epithelial cells were separated by 2D gel electrophoresis. Differentially expressed proteins were identified using SameSpot Progenesis software followed by liquid chromatography–mass spectrometry (LC-MS/MS); the mass spectrometry data were analysed by Global Proteome Machine (GPM) search engine for protein identification. The same proteomic profiling techniques were used for the *in vitro* approaches for MON810 maize safety evaluation where primary intestinal epithelial cells and HCT116 cell line were used. *In vivo* effects of these different maize varieties on the proteome of the epithelial cells of the small intestine when all five groups were compared showed that there were 5, 4, 3, 0, 0, differentially expresed protein spots for Mcert, Mon Conv Corn, MON810, Mon Garst and MON Gold, respectively for the 7-day trial. Two stress-related proteins (LDLR chaperone MESD precursor and peroxiredoxin-6) were up-regulated (2.4 fold) in the MON810 group and 1 stress-related protein (thioredoxin-dependent peroxide reductase) was up-regulated (2.6 fold) in the Mcert group. For the 28-day trial, only 2 proteins spots (representing 6 proteins) were up-regulated in the small intestinal epithelial cells of rats fed MON810, one of which was a stress-related protein (stress-induced phosphoprotein 1; 3.1 fold). *In vitro* effects of MON810 and its near isogenic line on the proteome of the epithelial cell lines were negligible, with only 4 protein spots (5 stress-related protein) being up-regulated in the small intestinal primary epithelial cells (IE cells) when exposed to *Bt* maize extracts and 2 protein spots (1 stress-related protein) being up-regulated when exposed to non-*Bt* maize extracts. There were no differentially expressed spots between the HCT116 cell lines. The findings from the *in vivo* and *in vitro* studies both suggest that MON810 has negligible effects on rats at the cellular level. They also confirm the lack of mammalian toxicity when using rats as a model system.

List of Abbreviations

AChE	Acetylcholine esterase.
AGBIOS	Agriculture & Biotechnology Strategies (Canada) Inc. Is a Canadian company dedicated to providing public policy, regulatory, and risk assessment expertise for products of biotechnology.
ALP	Alkaline phosphatase.
ALT	Alanine aminotransferase.
APS	Ammonium persulfate.
AST	Aspartate transaminase, also known as glutamic oxalacetictransaminase.
ATP	Adenosine triphosphate.
BHA	Butylated hydroxyanisole.
Biotech	Biotechnology.
BSA	Bovine serum albumin**.**
Bt	*Bacillus thuringiensis*
CA	Carrier ampholytes.
CAT	Catalase.
cDNA	Complementary deoxyribonucleic acid.
CHAPS	3-[(3-Chloamidopropyl) dimethylammonio]-1-propane-sulfonate.
cRAP	Common Repository of Adventitious Proteins.
Cry	Crystal protein
DMSO	Dimethyl sulfoxide.

DNA	Deoxyribonucleic acid.
DTT	Dithiothreitol.
ECB	European corn borer
ECL	Electrochemiluminescence.
EDTA	Ethylenediaminetetraacetic acid.
EFSA	European food safety authority.
EGF	Epidermal growth factor.
ELISA	enzyme-linked immunosorbent assay.
EPA	Environmental Protection Agency.
EST	Expressed sequence tag.
EU	European union.
FBS	Fetal bovine serum.
FCE	Feed conversion efficiency.
FDR	False discovery rate.
GI	Gastrointestinal impairment.
GIT	Gastrointestinal tract.
GM	Genetically modified.
GMOs	Genetically modified organisms.
GPM	Global proteome machine (GPM) search engine for protein identification.
GSH	Glutathione.
Gy	Gray is a unit of ionizing radiation dose in the International System of Units (SI).
H_2O_2	Hydrogen peroxide.

HCT-116	Colorectal carcinoma cell line.
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid**.**
HIFCS	Heat-inactivated fetal calf serum**.**
HnRNP	heterogeneous nuclear ribonucleoprotein.
HOP	heat shock protein-organizing protein.
HPLC	High-performance liquid chromatography.
HRP	Horseradish peroxidase.
HSPs	Heat shock proteins
IECs	Intestinal primary epithelial cells.
IEF	Isoelectric focusing.
IgE	Immunoglobulin E.
ILSI	The international life sciences institute.
IPG	Immobilized pH-gradient.
IRES	Institute for research on environment and sustainability.
kDa	Kilodalton
LDH	Lactate dehydrogenase.
LDLR chaperone MESD precursor	low-density lipoprotein receptor chaperone mesoderm development protein precursor.
MALDI-TOF-MS	matrix assisted laser desorption/ionization time of flight mass spectrometry.

MCert Rod	Commercial purina rodent chow.
ME	Mercaptoethanol.
MEM	Minimum essential medium.
MON CONV CORN	The corresponding parental non-transgenic maize of MON810.
MON Garst	Non-genetically modified maize variety developed by Monsanto.
MON Gold HVST	Golden Harvest is a non-genetically modified maize variety developed by Monsanto.
MON810 maize	Transgenic maize expressing Cry1Ab protein.
mRNA	messenger ribonucleic acid
MS	Mass spectrometry.
MS/MS	Tandem mass spectrometry.
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
MW	Molecular weight.
OECD	Organization for economic cooperation and development.
PBS	Phosphate-buffered saline
pH	Power of hydrogen. Is a measure of the acidity or basicity of an aqueous solution.
pI	Isoelectric point.
PMI	Purina mills international.
PMSF	Phenylmethanesulphonyl fluoride.

Prdx6	Peroxiredoxin 6.
qPCR	quantitative real-time polymerase chain reaction.
RNA	Ribonucleic acid.
RTCA	Real-time cell analysis.
RT-PCR	Real-time polymerase chain reaction.
s.i.	Small intestine
SD	Standard deviation.
SDS	Sodium dodecyl sulphate**.**
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis.
SE	Standard error.
SOD	Superoxide dismutase.
STIP1	Stress-induced phosphoprotein 1
STIP1	Stress-induced phosphoprotein 1.
TBS	Tris-buffered saline.
TC	Tissue culture.
TEER	Transepithelial electrical resistance.
TEMED	N,N,N,N-Tetramethylethylendiamine.
2-D	Two-dimensional.
UK	United Kingdom.
UniProt	Universal protein resource.
V	Volts.
VIB	Vlaams Instituut voor Biotechnologie (English: Flanders Institute for

	Biotechnology), is a research institute located in Flanders, Belgium.
WHO	World health organization.
WST-1	Water soluble tetrazolium salts.
ICP	Insecticidal crystalline protein.
BBMV	Brush border membrane vesicles.
APN	Aminopeptidase N.
ALP	Alkaline phosphatase.

Chapter 1

1 General Introduction

Global interest surrounding the use of genetically modified (GM or transgenic) crops is continuously increasing because of the possibility of the food produced being more nutritious and agronomic productivity being higher, both being affected without the use of pesticides (Shintani and DellaPenna, 1998; Ye *et al.*, 2000). However, concerns relating to their safety for human consumption are widespread and need to be fully addressed before their acceptance into the market. Although several studies have been conducted to evaluate the safety of GM crops, there is still a debate on the potential risk of GM derived food products and there exists widespread and vocal demand for additional evidence of GM food safety (Haslberger, 2006; Hug, 2007).

1.1 Genetically modified crops

According to the World Health Organization (WHO), Genetically Modified Organisms (GMOs) are those organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally (Domingo, 2007).

Crops developed through biotechnology have been marketed and used by farmers since the mid-1990s. Biotechnology was used in the first generation of so-called GM crops to provide growers with complementary and sometimes alternative crop management solutions to the use of pesticides. Genes identified from other plants or non-plant sources or selected host genes are modified and transferred to a crop plant. The altered or new protein expression resulting from these modifications confer on the plant a desired physiological trait, such as resistance to particularinsect pests or herbicides. Second generation modifications provide traits such as enhanced nutritional or health-promoting characteristics that are of (claimed) benefit to consumers (Malarkey, 2003).

The technology used allows selected individual genes to be transferred from an organism into another, and also between non-related species. Such methods are used to create GM plants, which are then used to grow GM food crops. The GM crops currently on the market are mainly aimed at achieving an increased level of crop protection through the introduction of resistance against plant pest diseases and through

increased tolerance towards herbicides. Taking into account that different GMOs include different genes inserted in different ways, the WHO indicates that individual foods and their safety should be assessed on a case-by-case basis, and that it is not possible to make general statements on the safety of all GM foods. In general, the safety assessmentof GM foods should investigate: a) toxicity, b) allergenicity, c) specific components thought to have nutritional or toxic properties, d) stability of the inserted gene, e) nutritional effects associated with genetic modification, and f) any unintended effects which could result from the gene insertion (Cellini *et al.*, 2004; Domingo, 2007).

A record 175.2 million hectares of biotech crops were grown globally in 2013, at an annual growth rate of 3%, up 5 million from 170 million hectares in 2012 (Figures 1.1, 1.2 and 1.3). The year 2013, was the 18th year of commercialization, of the period 1996-2013, when the overall growth volume continued to be seen after a remarkable previous 17 consecutive years of increases; notably 12 of the 17 years witnessed double-digit growth rates. The global hectarage of biotech crops has increased more than 100-fold from 1.7 million hectares in 1996 to over 175 million hectares in 2013 – this makes biotech crops the fastest adopted crop technology in recent history. This adoption rate speaks for itself in terms of its benefits in its delivery to farmers and consumers alike (James, 2013).

1.1.1 Types of GM crops

There are many different types of GM crops that are currently being developed that are able to enhance the quality of life in agricultural communities (Cohen, 2005).

GM crops can reduce the use of conventional pesticides, which has quantifiable environmental and human health benefits, as well as reducing application costs per acre. Of the transformation events reported in many studies, 35 confer insect-resistant traits to crops, reflecting the perceived importance of action on the effect of pests on crops and thus on regional economies (Table 1.1). In addition, GM crops can reduce the use of other agrochemicals which are widely used to fight virus, fungus or other diseases. Eighty-four transformation events target this area, which if brought to the market successfully, can target reduced costs and increased production. Also, genetic modification can improve abiotic stress crop tolerance, such as drought and salinity that place limitations on poor farmers located in less favoured regions. Of the 201 events, 11 are being developed in this area. The GM crops can produce better product quality,

such as prolonged shelf life or enhanced product characteristics (foods delivering alternative carbohydrate or fat composition) that would improve transportation and consumer appeal. Of 15 transformation events being developed for product qualities, 5 are in the area of nutritional enhancement and 6 are to prolong shelf life. The other 4 are for product characteristics, such as increased sucrose. There are also major public initiatives, such as HarvestPlus, that seeks to reduce micronutrient malnutrition to breed nutrient-dense staple foods. Furthermore, GM crops can produce alternative and more efficient provision of essential vitamins and vaccines. Nine transformation events are being developed for plant-based vaccine deployment as seen in Table 1.1 (Cohen, 2005).

1.1.2 Commercially growing GM crops

In 2013, 27 countries planted biotech crops. Of the 27 countries which planted these in 2013 (Table 1.2 and Figure 1.4), 19 were developing and 8 were industrial countries. Each of the top 10 countries, of which 8 were developing, grew more than 1 million hectares providing a broad-based worldwide foundation for continued and diversified growth in the future. More than half the world's population, 60% or ~4 billion people, live in the 27countries planting biotech crops (James, 2013).

In 2013, five EU countries planted a record 148,013 hectares of *Bt* maize, up 18,942 hectares or 15% from 2012. Spain led the EU with a record 136,962 hectares of *Bt* maize, up 18%. Portugal was lower by approximately 1,000 hectares, caused by a seed shortage, and Romania's figure was the same as in 2012. The other countries, Slovakia and Czechia, planted lower and small hectarages attributed to onerous and over-demanding EU reporting procedures for farmers (James, 2013).

1.1.3 Advantages of GM-derived foods

Pest resistance

The loss of crops from insect pests can be staggering, resulting in devastating financial loss for farmers and starvation in developing countries. Farmers typically use many tons of pesticides annually. However, consumers would prefer not to consume foods that have been treated with pesticides because of the potential health hazards, and run-off of agricultural wastes from excessive use of pesticides and fertilizers which contaminate the water supply and adversely affect the environment. Therefore, it is widely held in sections of the scientific and lay community that growing GM foods such

as *Bt* maize can help eliminate the application of chemical pesticides and reduce the cost of bringing a crop to market (Baum *et al.*, 2001; Moellenbeck *et al.*, 2001).

Insects not only cause direct yield losses by damaging and consuming plants, but also act as vectors for many viral diseases and the damage they inflict often facilitates secondary microbial infections (Ferry and Gatehouse, 2009).

Herbicide tolerance

Weeds compete with crops for resources, in some cases by parasitism. The cost of weeds, measured in terms of reduced yields, the application of herbicides and the mechanical and manual labour required to remove them, is probably the largest single input into agriculture (Ferry and Gatehouse, 2009).

Removing weeds from crop plants by physical means such as tilling is not cost-effective, so farmers often spray large quantities of different herbicides (weed-killer) as a means of weed control. This is an expensive and a time-consuming process which requires care to ensure that the herbicide does not harm the crop plant or the environment. The genetically-engineered crop plants which are resistant to herbicides can help prevent environmental damage by reducing the amount of herbicides needed (Whitman, 2000).

For this reason, weed control has been identified as the primary target for first-generation genetically modified technology and most of the transgenic plants grown in the world today have been modified for herbicide tolerance, allowing the use of a safe, broad spectrum of herbicides such as glyphosate. Glyphosate, for example, is active against a wide range of plants but has a very low toxicity to wildlife, farm animals and humans; in soil, it is rapidly bound to soil particles and inactivated by bacteria (Ferry and Gatehouse, 2009).

Furthermore, Monsanto which is an agriculture biotechnology company based in USA has created a strain of soybeans genetically modified so as not to be affected by their herbicide product under the trade name Roundup. Growing these soybeans requires only one application of weed-killer instead of multiple applications and this will reduce the production cost and limiting the dangers of agricultural waste run-off (Whitman, 2000).

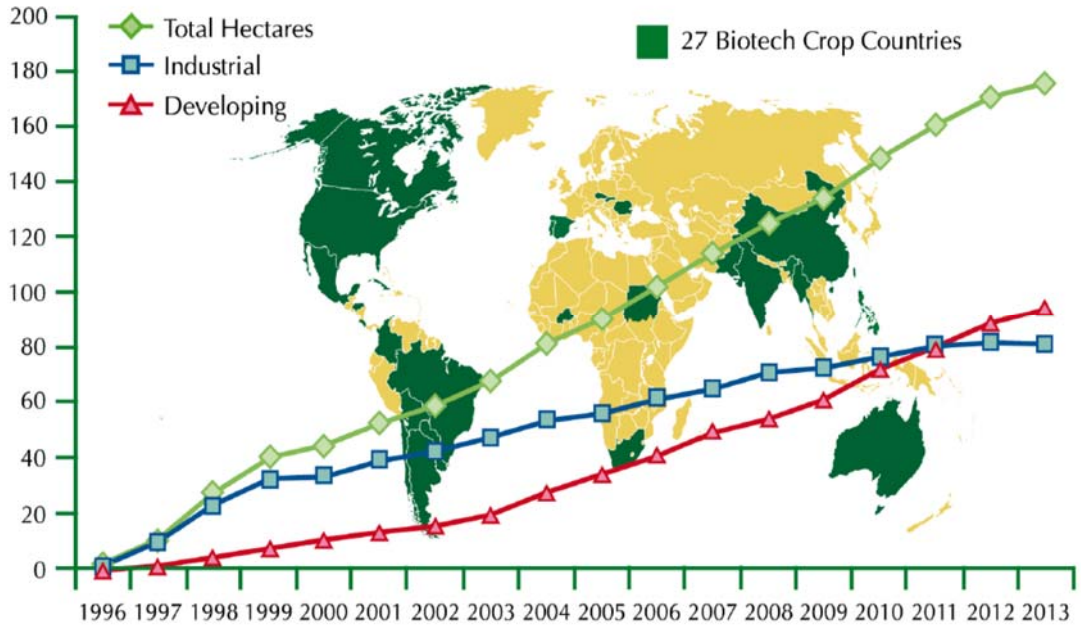


Figure 1.1: Global area of biotech crops.
A record 18 million farmers, in 27 countries, planted 175.2 million hectares (433 million acres) in 2013, a sustained increase of 3% or 5 million hectares (12 million acres) over 2012.
Source: James, 2013.

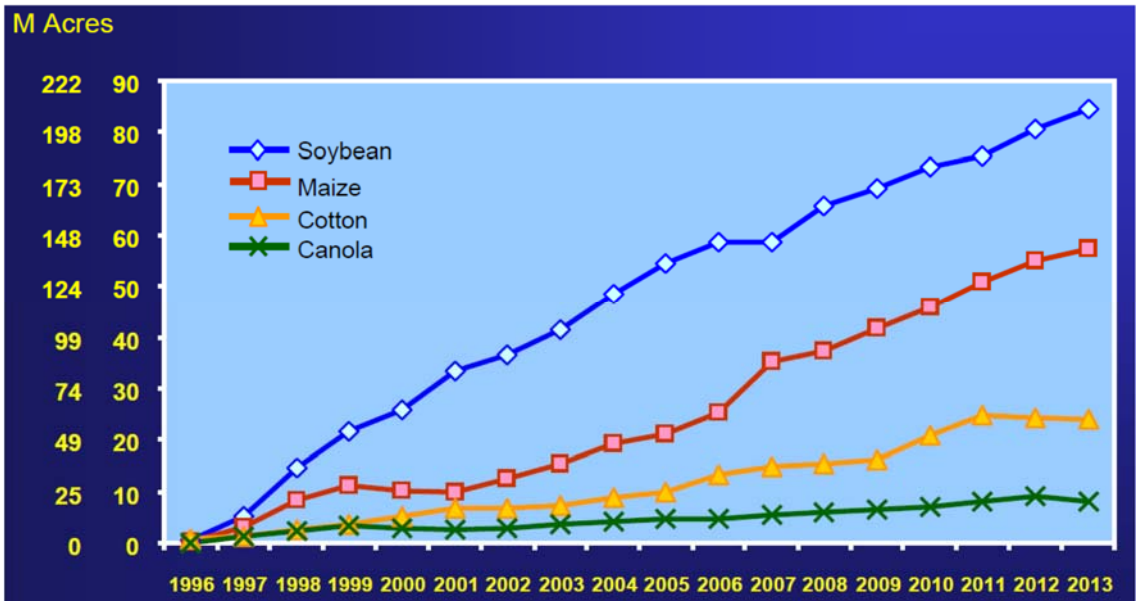


Figure 1.2: Global area of biotech crops, 1996 to 2013: by crop (million hectares, million acres).
Source: James, 2013.

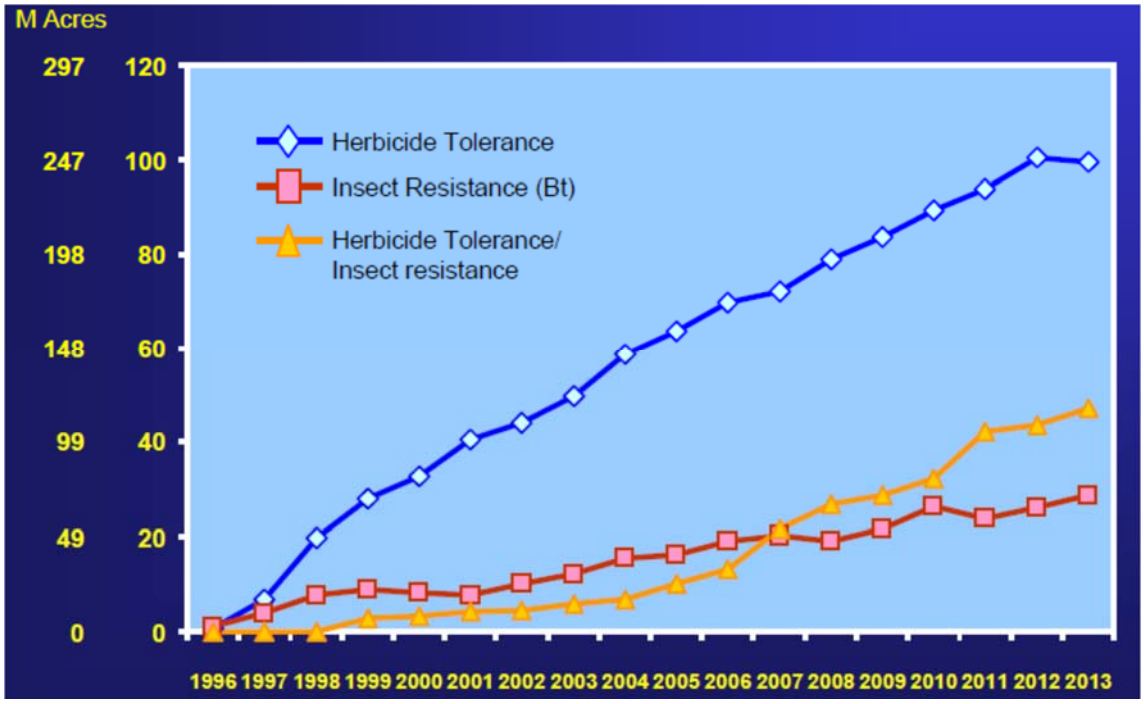


Figure 1.3: Global area of biotech crops, 1996 to 2013: by trait (million hectares, million acres).
Source: James, 2013.

Table 1.1: Transformation events created at public research institutions related to quality-of-life categories.
Source: Cohen, 2005.

Trait	No. of events
Insect resistance	35
Lepidoptera	35
Disease resistance	82
Bacteria	8
Fungi	21
Viruses	53
Abiotic stress tolerance	11
Drought	7
Salinity	4
Quality improvement	15
Nutritional and other	9
Enhancing shelf-life	6
Other	9
Vaccines	9
Total in this table	152
Total number of reported events	201
Percentage of all events related to quality-of-life traits	75.6%

Table 1.2: Global area of biotech crops in 2013: by country (million hectares).
Source: James, 2013.

Rank	Country	Area (million hectares)	Biotech Crops
1	USA*	70.1	Maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash
2	Brazil*	40.3	Soybean, maize, cotton
3	Argentina*	24.4	Soybean, maize, cotton
4	India*	11.0	Cotton
5	Canada*	10.8	Canola, maize, soybean, sugar beet
6	China*	4.2	Cotton, papaya, poplar, tomato, sweet pepper
7	Paraguay*	3.6	Soybean, maize, cotton
8	South Africa*	2.9	Maize, soybean, cotton
9	Pakistan*	2.8	Cotton
10	Uruguay*	1.5	Soybean, maize
11	Bolivia*	1.0	Soybean
12	Philippines*	0.8	Maize
13	Australia*	0.6	Cotton, canola
14	Burkina Faso*	0.5	Cotton
15	Myanmar*	0.3	Cotton
16	Spain*	0.1	Maize
17	Mexico*	0.1	Cotton, soybean
18	Colombia*	0.1	Cotton, maize
19	Sudan*	0.1	Cotton
20	Chile	<0.1	Maize, soybean, canola
21	Honduras	<0.1	Maize
22	Portugal	<0.1	Maize
23	Cuba	<0.1	Maize
24	Czech Republic	<0.1	Maize
25	Costa Rica	<0.1	Cotton, soybean
26	Romania	<0.1	Maize
27	Slovakia	<0.1	Maize
	Total	**175.2**	

* 19 biotech mega-countries growing 50,000 hectares, or more, of biotech crops

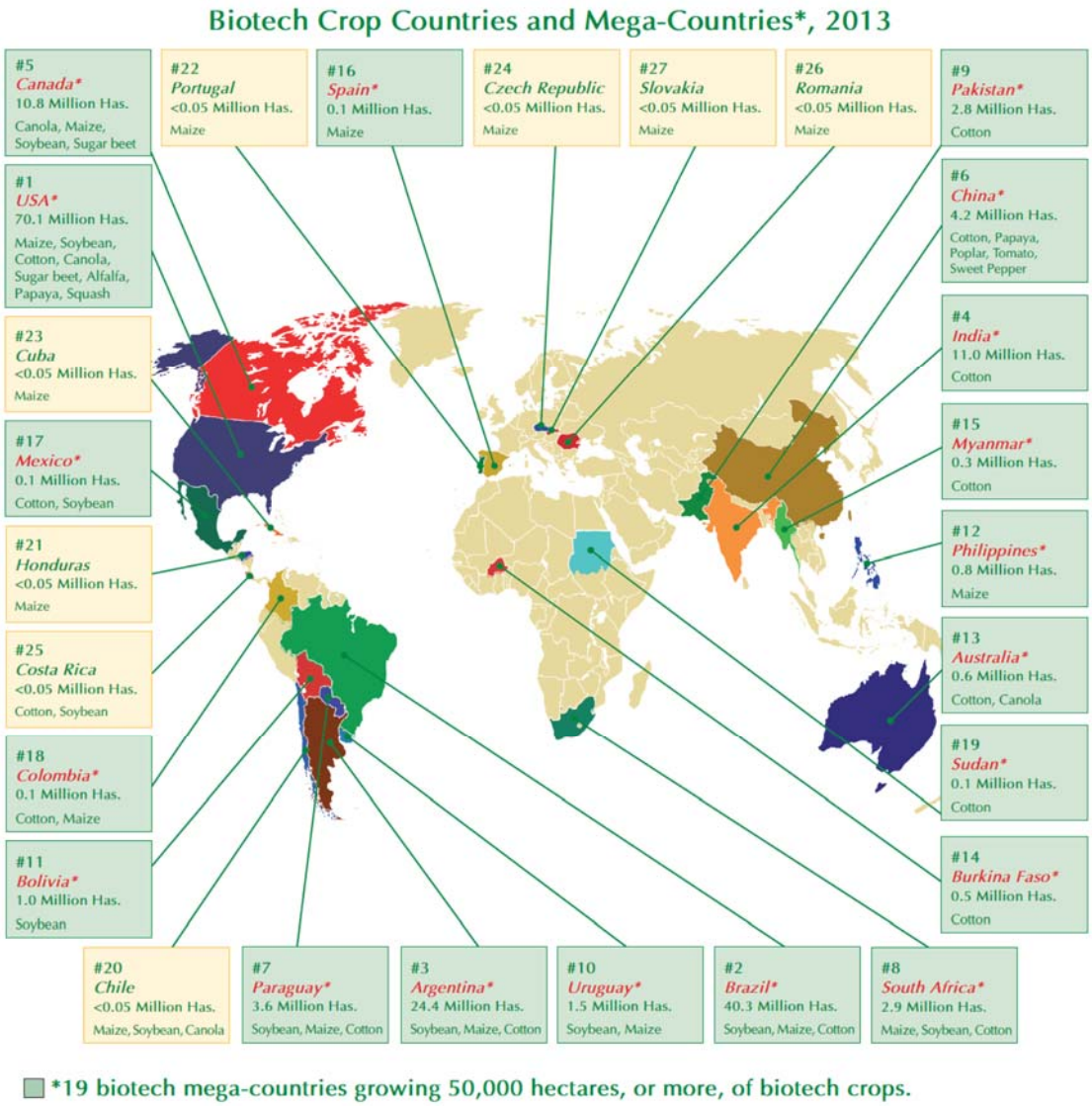


Figure 1.4: Global map of biotech crop countries and mega-countries in 2013.
Source: James, 2013.

Disease resistance

There are many nematodes, fungi, bacteria and viruses that can cause disease to plants. Therefore, plant biologists are working to create plants with genetically-engineered resistance to such diseases (Dahleen *et al.*, 2001; Scorza *et al.*, 2001).

In Africa, the major cause of disease in lowland rice ecosystems is rice yellow mottle virus (RYMV). The only naturally occurring resistance genes to RYMV are found in African landraces, which are difficult to cross with the cultivated varieties. Therefore, transgenes were constructed from the RNA polymerase gene of RYMV, which encodes a highly conserved component of the virus replicative machinery. The gene was transferred to three West African cultivated rice varieties that are grown in regions with the worst records of viral disease and where the yield gaps are the highest. All three varieties were shown to be resistant to RYMV, and one of the varieties was resistant to isolates of the virus from several different locations in Africa. In the best performing lines, viral replication was completely blocked over several generations (Ferry and Gatehouse, 2009).

Cold tolerance

Cold stress is one of the major environmental factors limiting the agricultural productivity of crop plants. Plants vary greatly in their abilities to survive freezing temperatures (Zhang *et al.*, 2011). Frost, for example, can destroy sensitive seedlings. Therefore, an antifreeze gene from cold water fish has been introduced into plants such as tobacco and potato. With this antifreeze gene, these plants are able to tolerate cold temperatures that may normally kill unmodified seedlings (Kenward *et al.*, 1999).

Drought tolerance/salinity tolerance

As the world population grows and more land is utilized for housing instead of food production, farmers are increasingly needing need to grow crop plants in places unsuited for plant cultivation. Therefore, creating plants that can withstand high salt content in soil and groundwater or long periods of drought is necessitated to enable the cultivation of crops in formerly inhospitable places (Tang, 2000; Zhang and Blumwald, 2001).

Conventional breeding has helped to create varieties with increased tolerance to drought, but progress in introducing new genes through genetic manipulation has been slow. Despite decades of research, only one drought-tolerant genetically modified crop

has been approved in the United States: Monsanto's DroughtGard maize, which expresses a stress-response gene from bacteria (Jones, 2013).

Nutrition

Malnutrition is a common problem in the third world countries where impoverished peoples rely on a single crop such as rice for the main staple of their diet. In this case and other instances of reliance on single crops, sufficient amounts of all necessary nutrients are absent and thus malnutrition often results. If rice, as but one example, could be genetically engineered to contain additional vitamins and minerals, nutrient deficiencies could be alleviated. For example, blindness due to vitamin A deficiency is a common problem in third world countries. Researchers at the Swiss Federal Institute of Technology Institute for Plant Sciences have created a variety of golden rice containing an unusually high content of beta-carotene (vitamin A). Since this rice was funded by the Rockefeller Foundation, a non-profit organization, the Institute hopes to offer the golden rice seed free to any third world country that requests it. Plans were also underway to develop golden rice that also has increased iron content. However, the grant that funded the creation of these two rice strains was not renewed, perhaps because of the vigorous anti-GM food protest movement in Europe, and so this nutritionally-enhanced rice may not come to market (Whitman, 2000).

The creation of 'Golden Rice' with enhanced vitamin A content is an example of the metabolic engineering approach to nutritional improvement. Vitamin A deficiency is prevalent in the developing world, and is probably responsible for the death of 2 million children every year. Humans can synthesize vitamin A if provided with the precursor molecule β-carotene (also known as pro-vitamin A), a pigment found in many plants but not cereal grains. Therefore, a strategy was devised to introduce the correct metabolic steps into rice endosperm to facilitate β-carotene synthesis (Ferry and Gatehouse, 2009). Although developed primarily for animal feed, maize has been genetically engineered to express enhanced levels of β-carotene, ascorbate and folate (Naqvi *et al.*, 2009). Mice fed on a diet enriched with this genetically engineered multivitamin corn showed no sub-acute effects and no sub-chronic toxicity (Arjó *et al.*, 2012).

There are also many examples of transgenic technology being employed to increase the nutritional value of plants, either through direct interference with nutrient accumulation or through the modification of primary or secondary metabolism. An example of the former strategy is the expression of seed storage proteins or

developmental regulators to increase the protein content of food. Specific reports include the expression of the *AmA1* seed albumin gene from *Amaranthus hypochondriacus* in potato, which has been shown to double protein content and increase the content of essential amino acids, and the expression of the *Gpc-B1* gene in wheat, which improved grain protein, zinc and iron content (Ferry and Gatehouse, 2009).

Pharmaceuticals

Vaccines and medicines are often costly to produce and may require special storage conditions not readily available in third world countries. Researchers are working to develop edible vaccines in potatoes and tomatoes and these vaccines will be much easier to store, ship and administer than traditional injectable vaccines (Daniell *et al.*, 2001; Kong *et al.*, 2001).

Clinical trials showed that an oral vaccine expressed in plants gives protection against a virulent viral pathogen in livestock. One of these trials was conducted on swine using an edible form of avaccine for transmissible gastroenteritis virus (Ferry and Gatehouse, 2009).

There have been several human clinical trials involving plant-derived, oral vaccines. Yusibov *et al.* (2002) conducted a trial involving 14 volunteers given spinach infected with alfalfa mosaic virus vectors expressing the rabies virus glycoprotein and nucleoprotein. Five of these individuals had previously received a conventional rabies vaccine. Three of the five and all nine of the initially naive subjects produced antibodies against the rabies virus while no such response was seen in those given normal spinach.

Phytoremediation

Not all GM plants are grown as crops. Soil and ground water pollution continues to be a problem in all parts of the world. Plants such as poplar trees have been genetically engineered to clean up heavy metal pollution from contaminated soil (Bizily *et al.*, 2000).

Hyper-accumulators are model plants for phytoremediation as they are tolerant to heavy metals. The hyper-accumulation of heavy metals in some plants has been recorded by many researchers in the last few decades emphasising the importance of further advanced research into the molecular basis of phytoremediation technology. In

metal biology, it is experimentally proved that even some metals that are essential for normal plant growth (such as iron and copper) may become toxic, depending on the dose and mode of exposure. Therefore, remediation of metal-contaminated soils has become a goal for many research laboratories in the world (Sarma, 2011).

1.1.4 Concerns regarding GM foods for human use

Allergenicity

Many children in the Europe and US have developed allergies to certain foods. An allergic reaction to nuts is an example of an ordinary familiar food that has become allergenic to some people which can be serious and even fatal. There is a possibility that introducing a gene into a plant may create a new allergen or cause an allergic reaction in susceptible individuals (Nordlee *et al.*, 1996).

The insertion of novel genes into crops, such as a gene from the Brazil nut to enhance the nutritional content of the soybean, can create health problems in people who are allergic to various plant proteins, including those of nuts. Several investigators have documented how, when genetically engineered soybeans were fed to mice and rabbits, their cells and enzymatic activities were altered. These studies emphasize the need for careful consideration of what traits are to be expressed and what the consequences may be, if any, for human health (Ferry and Gatehouse, 2009).

Unknown effects on human health

There is a concern that introducing foreign genes into food plants may have an unexpected and negative impacts on human health. On the whole, with the exception of possible allergenicity, scientists believe that GM foods do not present a risk to human health (Whitman, 2000).

Genetic engineers have experimentally produced drugs and other chemicals by manipulating the genetic make-up of various plants. There areseveral risks associated with this technology including contaminating the food supply. The best approach would be to produce these possibly hazardous materialsin secured greenhouses where insects and pollen cannot invade or escape (Ferry and Gatehouse, 2009).

1.1.5 Environmental concerns

Pesticide resistant crops could be toxic to non-target organisms

Transgenic crops that express insecticidal transgenes to control agricultural pests may also affect non-target organisms. The ecological risks of releasing transgenic *Bt* plants could be toxic effects on organisms that are not pests of the crop itself but are predators and parasites of the pests and, therefore, of benefit to agriculture. However, these problems may be directly related to the application of *Bt* toxin and not due to cultivation of crops carrying the *Bt* gene, as is evident from the studies on the effects of spraying of *Bt*-toxin on non-target organisms or pests (Singh *et al.*, 2006).

In addition, the *Bt* toxin could be harmful to insects related to the European corn borer, including moths and butterflies. In 1999, a group of Cornell scientists expressed concern specifically regarding the monarch butterfly. In 2001, the Environmental Protection Agency (EPA) announced its findings: that *Bt* does not harm monarch butterflies. Furthermore, the roots of *Bt* plants have been found to release the toxin into the soil and following harvest, decaying plants still contain trace amounts (Hanten, 2002).

Reduced effectiveness of pesticides

The release and wide spread cultivation of GM crops with pest or disease resistance has raised concerns that this may impose intense selection pressure on pest and pathogen populations to adopt to the resistance mechanism (Singh *et al.*, 2006).

Bt crops expose insects to the toxins whether or not there is a major infestation. Many people are concerned that insects may become resistant to *Bt* or other crops that have been genetically-modified to produce their own pesticides (Whitman, 2000). Thus, to delay the onset of resistance in pest population it is mandatory in many countries growing *Bt* crops, including USA, that at least 20% of the crops is non-transgenic, thus providing a refugia.

All of these environmental hazards are compounded by genetic pollution

Many crop plants disperse pollen which may be carried by wind or insect pollinators so that genetically engineered plants may cross pollinate non-engineered plants, introducing new genes into wild plant populations and the ecosystem, thus affecting the

whole food chain. This may result in some organic farmers, whose fields are near GM crops, not being able to certify their crops as organic (Hanten, 2002).

Another concern is that crop plants engineered for herbicide tolerance and weeds will cross-breed, resulting in the transfer of the herbicide resistance genes from the crops into the weeds and these super-weeds would then be herbicide tolerant as well (Whitman, 2000).

1.1.6 Genetically modified maize/corn

The use of biotech crops has been rising since their commercialization in 1996. Maize is the world's third leading cereal crop, following wheat and rice. It is grown as a commercial crop in over 25 countries worldwide. In 1995–1997, 66% of all the maize produced worldwide was used for animal feed and 17% for human consumption. In developing countries, 30% of the maize produced was used for human consumption and 57% for animal feed, whereas in Western Europe, North America and other high income countries, 4% was used for human consumption and 76% for animal feed during the same period. GM maize is one of the most extensively cultivated genetically modified crops, with traits introduced in these lines being, basically, resistance to herbicides and increased tolerance to insects and pests. Genetically modified maize expressing gene encoding *Bacillus thuringiensis* (*Bt*) occupying 37.3 million hectares, was the second most frequently cultivated GM plant after the GM soybean (Liu *et al.*, 2012).

The first-commercial-scale plantings of insect-protected field corn hybrids, commonly referred to as *Bt* corn, occurred in 1996, following regulatory approval by USA and Canadian authorities. These first field corn hybrids derived from a genetic modification designated Event 176, express a gene that enables the plants to produce an insecticidal protein, Cry1Ab, similar to that produced in nature by certain subspecies of the common soil bacterium *Bacillus thuringiensis* (Domingo, 2007).

Definition of MON 810

MON810 is a variety of maize that has been genetically modified in order to produce a natural insecticide (*Bt* toxin) which kills the larvae of the European corn borer (*Ostrinia nubilalis).* Scientists have developed *Bt* corn by transferring the genetic information of the *Bacillus thuringiensis* bacterium, responsible for the production of a toxin, to corn (VIB, 2010).

MON 810 maize contains a truncated cry1Ab transgene originated from *Bacillus thuringiensis* (*Bt*) and inherited in its several subspecies. The full length coding sequence of the *Bacillus thuringiensis ssp. kurstaki* cry1Ab gene produces a 131 kDa Cry1 protoxin, while MON 810 cry1Ab transgene expresses a 91 kDa N-terminal fragment of it—a so called pre-activated toxin form, as documented in the official registration and reassessment documents of *Bt* plants of genetic event MON 810, listed in the database of AGBIOS, which is a Canadian company dedicated to providing public policy, regulatory, and risk assessment expertise for products of biotechnology. Cry pro-toxins of *Bacillus thuringiensis* act as trypsin-activated toxins in sensitive insect larval groups. However, to become active and exert their insecticidal activity, pro-toxin molecules have to be processed in the insect midgut by trypsin-like and other proteases. Proteolytic activation usually leads to rapid removal of the highly conserved C-terminal pro-toxin tail. Various cysteine residues in this part are involved in intramolecular disulfide bridges that cross-link the different pro-toxin molecules in the unsolubilized crystal. At the N-terminal end, a few residues are cleaved off more slowly resulting in a final toxic fragment of molecular size between 60 and 70 kDa. Smaller pro-toxins lacking the conserved C-terminal region are minimally trimmed at both ends upon proteolytic activation. Although MON 810 cultivars (Agrigold, Asgrow, DeKalb, Garst, Golden Harvest, Monsanto, Pioneer) were registered in the USA and Europe as early as 1996 and 1998, respectively, Cry1A toxin production of the cultivars has to date not been fully explored with extremely variable levels being reported. For example, in Monsanto's documents registered in the databases of AGBIOS, the U.S. Environmental Protection Agency and the U.S. Department of Agriculture, mostly the same, non-published measurements are cited, referring to Cry1Ab toxin content of 7.93 to 10.61, 0.09, and 0.19 to 0.91 mg Cry1Ab/g fresh weight in leaf, pollen, and grain, respectively, in the U.S. documentation, and 7.59 to 15.06 and 0.35 to 0.69 mg Cry1Ab/g fresh weight in leaf and grain, respectively, in European Union documentation. Furthermore, these studies also mention levels of Cry1Ab toxin expressed in the first-, second-, and third-leaf levels in pooled over season samples from four-leaf to pollination stage, that is, 9.78, 8.43, and 4.91 mg Cry1Ab/g fresh weight, respectively. These reports thus suggest that expression levels are spatially and temporarily variable, and dependent upon the regions in which they are grown (Székács *et al.*, 2010).

Protein expression levels – whether transgenic or endogenous – can be quite variable *in planta* and can be affected by a number of factors. In fact, Monsanto regularly acknowledges year of cultivation, geographical location, and environmental conditions as potential sources of this variability in the expression levels of Cry1Ab protein in MON810 (Michael Koch, 2014, pers.comm.). The expression levels of Cry1Ab protein were derived from grain produced from field trials conducted according to international guidance. These studies are very well controlled and take extensive steps to account for numerous sources of variability that can skew results and are considered the most reliable expression values for the Cry1Ab protein in MON810. The expression values which are of specific interest to the present study are those in grain; the fraction consumed by humans. The range of mean expression levels for Cry1Ab in MON 810 grain from four separate field trials was 0.31-0.57 µg/g (Safety Assessment of YieldGard®Insect-Protected Corn Event MON 810, 2002). Of note, this range of mean values of the expression level of Cry1Ab protein is consistent with the information in the technical assessment from Brazil (National Technical Biosafety Commission-CTNBio, 2006), which indicates an expression level in the grain of 0.31µg/g. Therefore, the more conservative (i.e., high) estimate of intake for Cry1Ab in MON810 grain is 0.57 µg/g.

History of MON 810

Maize is one of the most widely grown crops for both feed and fodder, producing high-fructose corn syrup, dextrose, glycose, starch, oil, flour and meal. By means of transferring an insecticidal trait to maize (*Bt* corn), it is possible to control damaging pests that cause 7% loss of maize products (Kılıç and Akay, 2008).

In 1988, MON810 maize was one of the first genetically modified plants to be allowed for cultivation in the European Community, where it currently takes place mainly in Spain. This corn variety, developed by Monsanto, produces its own insecticide, which is specifically directed against the European corn borer, a lepidopteran pest that can seriously damage the corn harvest. The most important advantage of MON810 is the increased harvest certainty for farmers. They pay a slightly higher premium for the seed, but in exchange they have a higher certainty that the harvest will be successful. Moreover, farmers need to use fewer insecticides keeping costs down and further lowering the environmental impact of the crop. Millions of tons of MON810 are harvested and consumed worldwide every year. Scientific research

shows that MON810 does not have any harmful consequences on human health or on the environment. However, various European Union (EU) member states have blocked its cultivation in their countries. Public opinion in some member states is still actively opposed to the introduction of genetically modified crops (VIB, 2010).

MON810 is a group of tens of varieties

Monsanto developed the MON810 corn variety in the 1990s. Once such a variety is created, an intensive refinement program starts with the goal of deriving as many different varieties as possible from it. Each region needs its own variety adapted to the local soil and climatic circumstances. Thus, there are MON810 varieties that grow well close to the Canadian border and others that flourish in warmer climates. Therefore, MON810 is not a single corn variety: it is the name for tens of corn varieties, derived from the MON810 line. In Spain today, there are more than 100 MON810 corn varieties on the market. The majority of these are included not only in the Spanish but also in the European list of crop varieties (European Commission, 2009).

Monsanto was not alone in bringing MON810 varieties onto the market: around ten other seed companies also adopted Monsanto's technology under license (VIB, 2010).

The European stalk borer

The European corn borer (*Ostrinia nubilalis*) is a lepidopteran insect whose larvae eat the corn stems and cobs. The European corn borer appears mainly in Southern and Eastern Europe and in large parts of the USA and Canada. In the years that the moth appears in large quantities, it can damage up to 30% of the corn harvest (Bohn *et al.*, 1999).

In the United States, Germany, Canada, France, Italy and many other countries, the corn borer constitutes an important pest for the cultivators of corn. Consequently, despite its name, the European corn borer was originally a problem in America with regard to corn cultivation. Since the larvae of the moth live inside the stem, they are hard to reach with insecticides that are sprayed on the crop. The development of corn/maize varieties with resistance to this insect pest, such as *Bt* expressing maize, thus provides a viable means of controlling this pest (VIB, 2010).

Plants affected by the corn borer lose their vigour and as a consequence are easily levelled during exposure to gusting winds. In addition, the tissues where the corn has been eaten by the larvae are an ideal point of growth for moulds, in particular Fusarium

species. These moulds produce mycotoxins. In affected plants, a much higher concentration of these mycotoxins is found, some of which are carcinogenic to mammals (Magg *et al.*, 2002).

Mode of action of Bt Cry toxins

The most successful insect pathogen used for insect control is the bacterium *Bacillus thuringiensis* (*Bt*), which presently occupies ~2% of the total insecticidal market. *Bt* is almost exclusively active against larval stages of specific insects and kills the insect by disruption of the mid-gut tissue followed by septicemia (Bravo *et al.*, 2011).

Bacillus thuringiensis toxin is a collective term for a family of toxins produced in nature by the soil bacterium *Bacillus thuringiensis* (the full version of the abbreviation *Bt*). This bacterium is present throughout the world in various strains, each of which produces another form of *Bt* toxin. Each of these forms has a specific effect on certain types of insects. The Cry1 form is toxic for the larvae of specific lepidoptera and Cry3 for certain types of coleopteran (beetles). In contrast to many chemical pesticides, *Bt* is not toxic to humans and other mammals, since the activated toxin requires the presence of specific receptors that are unique for these insects and with which the toxin can bind, since binding is a prerequisite for toxicity (VIB, 2010).

Cry proteins show highly species-specific toxicity against certain insects and only a few insect species are affected by each of the Cry proteins. The mode of action in the insect is through specific receptors in the gut, which is highly alkaline, with binding of the toxin resulting in pore-formation, osmotic imbalance, cell lysis and subsequent death of the insect as illustrated in Figure 1.5 (Schrøder *et al.*, 2007).

The transformation of Cry proteins from a relatively inert crystalline protoxin to a cytotoxic form is a multistep process. Following ingestion of inclusion body proteins by a susceptible larva, the Cry1 proteins (lepidopteran specific) are solubilised in the insect mid-gut to release 130 kDa pro-toxins, which are then activated by proteases in the insect mid-gut to form the active truncated 65 kDa toxin molecules that subsequently bind to specific receptors on the mid-gut epithelium. Several mechanisms of toxicity have been proposed. Toxin sub units oligomerize to form pore structures capable of insertion into the membrane. These pores allow ions and water to pass freely into the cells, resulting in swelling, lysis, and the eventual death of the host. More recently, an alternative hypothesis has been proposed that suggests Cry toxicity is

independent of toxin oligomerization and that pore formation may be a secondary effect of the toxic events triggered by toxin binding to the receptor. Cry toxin binding to insect mid gut epithelial receptors is an important determinant of specificity. The correlation between binding and toxicity was first demonstrated using brush border membrane vesicles (BBMV) prepared from microvilli by a technique developed by Wolfersberger (1984). In lepidopterans, the optimum characterized receptors are the aminopeptidase N (APN) receptors and the cadherin-like receptors.

Recent work by Arenas *et al.* (2010) suggests that the GPI-anchored proteins, aminopeptidase (APN) and alkaline phosphatase (ALP) are important for *Bt* toxicity, first as initial receptors promoting the localization of toxin monomers in the midgut microvilli before interaction with cadherin, and then as secondary receptors mediating oligomer insertion into the membrane. Work with the non-toxic Domain III Cry1Ab toxin (L511A mutant) suggests that ALP (which is preferentially present in neonate larvae) may have a predominant role in toxin action, as this mutant did not bind ALP but retained APN binding suggesting that the initial interaction with ALP is critical for toxicity. Thus ALP may have a predominant role in toxin action since Cry toxins are highly effective against neonate larvae that are the target for pest control programmes (Xu *et al.*, 2013).

The Cry proteins are regarded as harmless or nontoxic to mammals, including humans, probably due to acidified gut pepsinolysis and the lack of Cry protein binding-sites on mammalian gut epithelial cells. Numerous data from toxicity studies show no significant adverse effects of the Cry proteins on body weight gain or clinical observations. Furthermore, no signs of pathogenicity to mammals, including humans, have been reported (Schrøder *et al.*, 2007).

Yield advantages for the cultivation of the MON810 crop

Insect pests, weeds or weather circumstances can ruin farmer's harvests. Therefore, farmers look for methods of increasing harvest certainty and for this reason most would be willing to pay an additional fee for *Bt* seeds as some form of assurance (VIB, 2010).

In places where the European corn borer is a pest for corn growers (such as the USA, Canada, Spain, Southern Germany or Italy), farmers can ensure a good yield by replacing conventional corn varieties with MON810 plants. This is the most important motive for farmers to sow it, as by doing so, they improve their chances of having a

good harvest. In the European Union, Spain is the country with the longest experience in cultivating *Bt* corn. Scientific research shows that, under normal circumstances, farmer's income increases by 15% with *Bt* corn. This is mainly due to the fact that farmers use half of the amount of pesticides with *Bt* corn as with conventional corn because the plant produces its own pesticide and farmers can save on spraying (Gomez-Barbero *et al.*, 2008).

Furthermore, the quality of MON810 corn can offer advantages. Since the plants are less damaged by the corn borers, there is a reduced likelihood of moulds infecting corn plants. On average, *Bt* plants contain lower levels of mycotoxins, leading to forage that contains fewer harmful substances. In addition, when using corn for conversion into biofuel, *Bt* corn is better than normal corn because of the low quantities of mycotoxins, since those molecules are also harmful for the microorganisms that must convert the starch in the corn into ethanol (VIB, 2010).

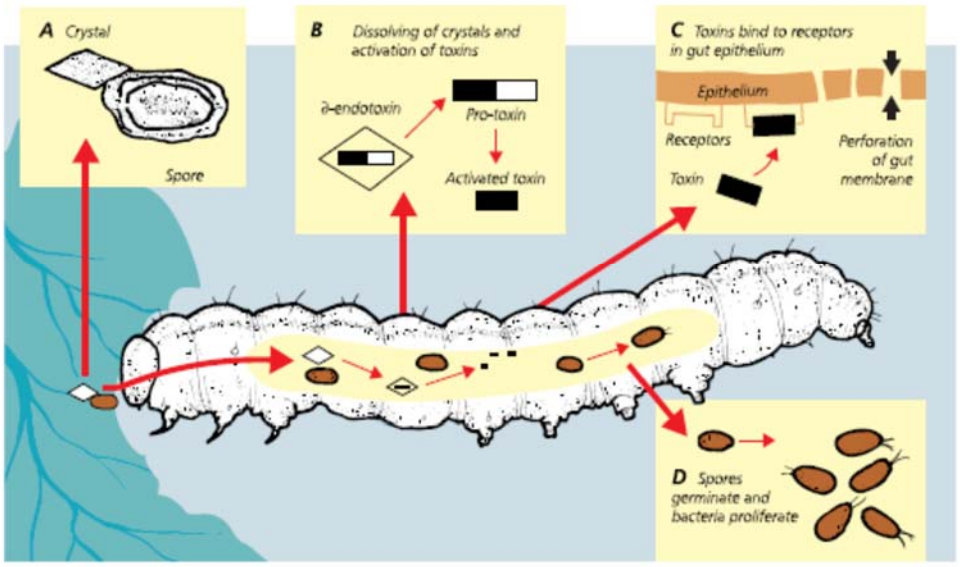


Figure 1.5: Mechanism of toxicity of *Bt*.
The mode of action of *Bt* can be summarised in the following stages: 1) ingestion of sporulated *Bt* and specific insecticidal crystalline protein (ICP) by an insect larva; 2) solubilization of the crystalline ICP in the midgut; 3) activation of the ICP by proteases; 4) binding of the activated ICP to specific receptors in the midgut cell membrane; 5) insertion of the toxin in the cell membrane and formation of pores and channels in the gut cell membrane, followed by destruction of the epithelial cells and 6) subsequent *Bt* spore germination and septicaemia may enhance mortality.
Source: World Health Organization, 1999.

1.2 Omic based technologies in food safety

Omic technologies can, in principle, allow visualization of all of the changes that take place when the genetics, nutrition or environment of an organism is altered. Targeted compositional analysis is today a key component of the food safety assessment paradigm in which known nutrients, anti-nutrients, toxicants, allergens, and other molecules of potential biological importance to humans or animals are quantitatively analysed. In recent years new transgenic crop varieties possessing a variety of useful agronomic traits have been developed using modern biotechnology. An important characteristic of transgenic plants is that well-defined genes that encode specific desirable traits are inserted into crop plants *in vitro* to produce new varieties. Plant varieties developed using conventional methods of plant breeding are typically not subject to pre-market regulatory review; however, transgenic crops are required to pass a pre-market review before they can be widely cultivated. One component of safety assessment is the evaluation of safety for transgenic crops as food or feed. The safety review seeks to establish that foods and feeds produced using modern molecular methods of *in vitro* gene insertion are as safe as any other in the market; in particular, that a new variety is as safe as a conventional counterpart, often called a comparator. Since safety is a relative concept, the degree of safety sought must be defined. A cornerstone of the food safety assessment is the Substantial Equivalence paradigm that asserts that differences in safety between a new variety and its conventional comparator can arise only from differences observed between them. The comparator selected is usually a variety that is very similar to the parental strain from which the transgenic was developed, although in the case of crop varieties in which the composition has been intentionally altered, it is conceivable that alternative comparators could be selected. A primary focus of comparative assessment has been a thorough evaluation of the composition of a given crop. Typically, 50–100 or more individual components are determined for each crop variety; the selection of analytes follows recommendations of the OECD Consensus Documents that describe key nutrients, anti-nutrients, and other compounds of biological interest for many crops. This evaluation of crop composition is sometimes referred to as targeted analysis; it should be noted that the target of the analysis typically comprises greater than 95% of the crop composition. The emergence of omic technologies over the last two decades has contributed powerful discovery tools for medical, pharmaceutical and biological research. Transciptomic, proteomic and metabolomics analysis of crops plants have been described. To date,

omic technologies have not been formally applied to the food safety assessment of transgenic crops (Chassy, 2010).

1.2.1 Assessing the safety of whole foods

No food or feed poses zero risk or is absolutely safe. It is also difficult to evaluate the safety of a plant-derived food since the food can be a mixture of 100s or even 1000s of compounds. The mycoprotein food Quorn is a good example of the difficulty of assessing the safety of a wholly novel food. Quorn is a single-cell protein product that can be formed into cheese, meat or poultry-like foods. Researchers performed extensive compositional studies, fed the material to animals, rubbed it on their skin, injected it under their skin, and fed it to human volunteers without ill effect being observed. The Food and Drug Administration (FDA) approved the product even though there was no specific test that definitively demonstrated the product was safe to consume; what could be demonstrated was that its composition was similar to other high quality protein foods and that it was apparently innocuous when consumed by living subjects. The FDA did not object to the developers claim that Quorn should be considered generally as safe (GRAS) in spite of the fact that about one in every 100,000 people that consume it experience an adverse reaction (Miller and Dwyer, 2001).

Although transgenic crops are categorized as novel foods, the safety assessment process applied to transgenic crops, foods and feeds should in principle be much more straightforward than that described for Quorn since only one, or at most a few genes, are inserted and the changes that are introduced are small, well-defined and usually predictable. Transgenic crops are not wholly novel foods as was the case with Quorn. In the production of a transgenic crop, the crop composition usually remains largely unchanged, except for the presence of a few well-defined additions. When a gene is inserted into a plant, three questions are appropriate about safety for consumption: Is the inserted DNA safe to consume? Is the protein product(s) of the gene, if applicable, safe to consume? Are the intended, and any unintended changes, safe to consume? (Chassy, 2010).

Since DNA, including antibiotic resistance makers used in the development of the transgenic plant, is considered safe to consume, the safety assessment focuses primarily on the safety of any newly synthesized proteins, any newly introduced compounds, and any changes in composition that may have occurred during plant breeding. It is noteworthy in this regard that conventional plant breeding produces genetic and

compositional changes of a similar or greater magnitude than transgenic breeding but resulting cultivars are not required to undergo pre-market safety assessment (Cellini *et al.*, 2004; Parrott, 2005; Herman *et al.*, 2009; Chassy, 2010).

Prior to selection of a gene to insert into a plant, a bioinformatics analysis is performed to ensure that the gene product bears no resemblance (similarity) to potentially toxic or allergenic proteins (Delaney *et al.*, 2008; Goodman *et al.*, 2008).

The safety of inserted proteins is also assessed through evaluation of the digestibility of the protein, and by toxicological evaluation of the protein in laboratory animals exposed by an oral route of administration. Since the great majority of proteins are safe for humans to consume, digestible proteins that do not have a structural resemblance to known allergens or toxins, and which produce no adverse reactions in animal tests, are regarded as safe to consume (Chassy, 2010).

As noted previously, extensive targeted compositional analysis is a key element of the safety assessment paradigm. Compositional analysis provides direct evidence that intended changes have occurred as well as revealing if any unintended changes in composition have occurred. The safety and nutritional value of a food or feed can be deduced from the composition. Moreover, the absence of large unexpected changes and the presence of intended changes in composition are also interpreted as an indicator that no adverse unintended effects have occurred in the breeding process (Chassy, 2010).

Although animal studies are exceedingly difficult to perform and may bear only limited influence when completed (ILSI, 2003; Chassy *et al.*, 2004; Hartnell *et al.*, 2007; ILSI, 2008), over two-hundred animal studies on whole transgenic foods and feeds have also been conducted in order to provide an additional level of assurance that a new crop variety is safe (EFSA, 2008). While a number of reports, beginning with Ewen and Pusztai (1999) flawed experiments using transgenic potato, have claimed adverse effects resulting from feeding transgenic crops to laboratory animals, none of these studies has been proven to have revealed any negative effect(s) that was the result of the transgenic breeding process nor have these studies been repeatable. Studies that make claims of adverse effects of consumption of GM crops are often poorly designed, inadequately documented and conducted, suffer from experimenter bias, and make conclusions that are not rooted in any meaningful biological differences (Chassy *et al.*, 2004; EFSA, 2008).

The safety of the inserted gene and its products, taken along with the compositional analysis are the keystones of an effective safety evaluation. It should also be noted that the rigorous screening for absence of changes in a long list of key phenotypes that is performed throughout the development process, as well as the extensive back-crossing into elite varieties that is required to produce a commercial cultivar, doubtless serve as an effective screen against unintended and undetected changes being present in a new transgenic variety. More importantly, change in and of itself does not increase risk since changes can be beneficial, neutral or deleterious. This is evidenced by fact in that the crop varieties in use today differ significantly in composition from historical stock (Chassy, 2010).

1.2.2 Can–omics of novel food/feed inform a safety assessment?

As discussed previously, the Omic technologies are powerful analytical tools for discovery research. The potential applicability of Omics to safety assessment remains under review. Furthermore, it must be remembered that they only represent a 'snap shot' in time.

Transcriptomics

The current status of transcriptomic analysis of food crops has been reviewed. Transcriptome profiling tools allows the identification of differences in the transcription of genes representing a significant portion of the genome between two or more samples of a plant to be made. The research reported to date demonstrates that reproducibility is a major challenge for transcriptomic analyses. Duplicate analysis of a sample can produce different differential gene expression patterns, and it is clear that variables such as time of sampling, method of mRNA isolation, and differences in soil and weather during cultivation, can profoundly impact expression patterns observed. Perhaps more important is that different varieties of the same crop can display very different gene expression patterns when cultured side-by-side in the same field. It is not immediately apparent how differences in the expression of genes observed in two varieties of the same food or feed crop can be interpreted from a safety perspective. For a variety of reasons, differences in transcription do not necessarily translate into differences in the proteome or metabolome. The transcriptome is therefore not necessarily predictive of the composition of the final food or feed that will be consumed. Catalogues of gene expression patterns that correlate with ‘‘safe” and ‘unsafe” samples of a crop plant do not exist since: (1) to date, virtually all examples of commonly eaten crops are equally

safe when consumed as intended, and (2) no extensive databases of ‘‘normal transcriptome” patterns have been compiled as has been done for compositional analysis of crops. If a plant is known to produce an anti-nutrient or an allergen, using expression analysis to evaluate expression of the genes associated with biosynthesis of these components would not be predictive for the quantitative measure of anti-nutrient content. Transcription analysis targeted in this way would also be simply an alternative, and likely an inferior alternative, to a direct analysis of a constituent, such as a protein, that is currently analysed through composition studies. An underlying motivation for the suggestion that transcriptomics could be useful in safety assessment arises from concerns that unintended changes may have occurred as a result of transgene insertion (Chassy, 2010).

The hypothesis to be evaluated here is that transcriptomics could be a tool for revealing unintended effects. The comparatively few studies reported to date indicate that transgene insertion is less likely to elicit major changes in the transcriptome than other modalities of crop breeding as, for example, radiation mutagenesis (Baudo *et al.*, 2006; Batista *et al.*, 2008).

Proteomics

Proteomics is the large-scale study of proteins, particularly their structures and functions (Anderson and Anderson, 1998; Blackstock and Weir, 1999). Proteins are vital parts of living organisms, as they are the main components of the physiological metabolic pathways of cells. The term "proteomics" was first coined in 1997 to make an analogy with genomics, the study of the genes (James, 1997). The word "proteome" is a blend of "protein" and "genome", and was coined by Marc Wilkins in 1994 while working on the concept as a PhD student. The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system (Wilkins *et al.*, 1996).

The proteome has also been defined as the protein complements expressed by the genome of an organism or cell type. The most common implementation of proteome analysis is the separation of proteins extracted from the cell or tissue by high-resolution two-dimensional (2-D) gel electrophoresis, and identification of separated proteins by sensitive mass spectrometric techniques (Anderson and Anderson, 1998).

Proteome analysis is technically complex compared to genomic and transcriptomic profiling techniques. It is however essential for the comprehensive analysis of biological systems, as protein expression levels are not predictable from the messenger RNA (mRNA) expression levels, proteins are dynamically modified and processed in ways, which are not necessarily apparent from the gene sequence. In their biologically active form, many proteins are post-translationally modified by glycosylation, phosphorylation, acylation or other modifications. Many proteins are only functional if associated with other molecules, including DNA, RNA, proteins, and organic and inorganic cofactors. These modifications are dynamic and reversible and may alter the three dimensional structure and the state of protein activity. Collectively, the type of protein modification and the sites modified cannot be determined from the gene sequence alone. Therefore, it is expected that proteomics will be able to provide the most precise and detailed molecular description of the state of a cell or tissue (Haynes *et al.*, 1998).

There are a number of difficulties in protein analysis, which complicate the task. Proteins cannot be amplified (like DNA/RNA) from a template found in the sample. They cannot even be expressed in large amounts in the form in which they finally function in the biological system, due to post-translational modifications. In addition, proteins vary dramatically with respect to their solubility in commonly used solvents. There are no solvents in which all proteins are soluble and which would also be compatible with protein analysis. Detergents, in particular sodium dodecyl sulphate (SDS), are frequently added to aqueous solvents to maintain protein solubility. The compatibility of SDS is a big advantage of SDS polyacrylamide gel electrophoresis (SDS-PAGE) and 2-D gel electrophoresis, which are the most preferred methods for the separation of small amounts of proteins. However, the use of salts and detergents is limited by their incompatibility with mass spectrometric analyses (Haynes *et al.*, 1998).

The most commonly used technique in proteome analysis is based upon the separation of proteins by 2-D gel electrophoresis (2DE) and their subsequent identification by mass spectrometry (MS) or tandem mass spectrometry (MS/MS). In 2-D gel electrophoresis, proteins are first separated by isoelectric focusing (IEF) and then by SDS-PAGE in the second perpendicular dimension. Separated proteins are visualized with high sensitivity by staining or autoradiography, producing 2-D arrays that contain thousands of proteins. The technique is widely used due to its compatibility with high concentrations of detergents, protein denaturants and other additives

promoting protein solubility. However, other separation techniques, such as liquid chromatography (LC), are replacing 2DE (Haynes and Yates, 2000).

Ideally, all expressed proteins should be present in the 2-D gel, but in practice at least 10-15% of proteins cannot be included in the gel. Very basic proteins (pI>11) are not easy to analyse with 2-D gel electrophoresis due to the lack of suitable conditions for IEF in this pH range. Also some hydrophobic proteins (example transmembrane proteins) and high molecular weight proteins (>200 KDa) resist entry into 2-D gel. Many regulatory proteins are expressed in low copy numbers and hence cannot be visualised by the applied detection methods (Westermeier, 2006).

The identification of individual proteins from polyacrylamide gels has traditionally been carried out using N-terminal sequencing, internal peptide sequencing, immunoblotting or comigration with known proteins. The dramatic growth of large-scale proteomic, genomic and expressed sequence tag (EST) sequence databases have resulted in a fundamental change in the way proteins are identified. The current method relies on excising spots from gels, proteolytically digesting them and then extracting the peptides. The final stage is the analysis of these peptides by MS or tandem mass spectrometry MS/MS and the correlation of the mass spectral data derived from the peptides with information contained in databases (Haynes and Yates, 2000).

Proteomic applications in the safety assessment of transgenic crops have been recently reviewed. Research has focused primarily on the use of modern protein analytical techniques—the techniques used in proteomics—for the analysis of changes in the abundance of a few well characterized proteins of interest in a particular crop (e.g. soybean allergens). However, it should be noted here that the application of modern protein chemistry techniques to measure the concentration of multiple allergens is not proteomic profiling, rather it is a very sophisticated and useful form of targeted analysis. As it is generally understood, proteomic profiling is an attempt to provide a rough estimate of the concentration of all of the proteins that are expressed in an organism. Although the relative concentration of dozens of major proteins can be estimated using 2D-gel electrophoresis, and the presence or absence of hundreds more can be detected, proteomic technology is only able to detect about 1000 of potentially 10,000–30,000 proteins that could be encoded in the plant genome. Proteomic analysis is also not typically quantitative, is time consuming and expensive, and requires specialized equipment and software. The lack of laboratory-to-laboratory

reproducibility and lack of standards for methodology, data handling and analysis all present challenges to an industry-standard approach (Chassy, 2010).

From a food safety assessment perspective, the proteome is not likely to be a good predictor of the metabolome and if it is, it is as yet unknown how the prediction made by differences observed between two proteomic displays may be deciphered. An additional complication is that repeat analyses are highly variable, difficult to reproduce, and there is a total absence of accumulated knowledge about what would be a safe display and what should raise a red flag of caution (noting of course that food is inherently safe). Although proteomic analysis can reveal that differences exist between two or more samples, it is not yet clear that differences in the protein context are relevant to food safety risk since it is not likely that the proteome composition rigidly controls the metabolome. From a food safety perspective, it is the composition of a crop that determines the safety and nutritional value. Metabolic and cellular regulatory circuits, differences in substrate availability and product utilization, as well as differential rates of protein turnover and differences in the external environment, all work to disconnect the proteome from having a predictive value for the composition of the metabolome. Proteomic analysis does not appear to be useful for the detection of unintended effects either since conventional varieties with long histories of safe consumption appear to have larger proteomic differences compared with varieties developed through transgene insertion. There is much ongoing research directed at developing a system with which the concentration of allergens that are present in specific samples of a food crop can be rapidly measured. This targeted application of proteomic techniques is likely to be successful in the near future in defining a system for profiling and estimation of allergenic protein content. There is no published evidence that differences between varieties in allergen concentration materially change the degree of clinically relevant allergenicity of any crop. There is little quantitative information and even less agreement among allergists on the amount of any specific protein allergen that is required for sensitization and subsequent elicitation. Since food allergens are generally abundant proteins, it may well be that they are always present in ample amounts to participate in the allergy process. Food allergy is an inappropriate response by an individual to a protein, or proteins present in a food, that are innocuous to more than 99% of human subjects under similar conditions of exposure. The adverse reaction appears to be caused by (1) host genetics, (2) the food matrix and environment of the exposure, (3) the physiological and nutritional state of the host, and (4) repeated

exposure to an allergen. A key observation is that patients who are allergic to various foods can have IgE molecules in their serum that react with different, and sometimes totally unrelated, proteins and combinations of proteins in the food. This formation of IgE antibodies points to a failure of the immune system to ignore or become tolerant to a protein more than it does to the allergic potential of specific proteins. At present no regulatory decision about safety could be justified on the basis of proteomic displays of foods as a way to categorize foods to which people already are or may become allergic (Chassy, 2010).

Metabolomics

The potential for application of metabolomic analysis to food and agricultural research have been reviewed. It has been suggested that the primary value of metabolomic analysis is in support of plant breeding and selection. However, it has also been suggested that metabolomic analysis can be used in support of the food safety assessment of transgenic crops (Chassy *et al.*, 2004; Dixon *et al.*, 2006).

Although an extensive compositional data set is required, information derived directly from metabolomic analysis is not one of the tests currently required by food safety assessment or in support of applications for the approval of new transgenic crops. Comparative compositional analysis provides a direct insight into any compositional changes that have occurred in the plant breeding process. The metabolomic analysis offers the possibility of analyzing a significantly greater number of metabolites in a short period of time than is possible with conventional analysis. Metabolomic analysis also offers the prospect of reducing the cost of analysis for each analyte. It has also been suggested that analysis targeted at specific compounds might overlook an unintended and unexpected change in an unanalysed component of the crop that could be of significance to health or nutrition. Unintended changes can and do occur in all forms of plant breeding and it is for this reason that changes in nutrients and potentially deleterious compounds are evaluated in the safety assessment. More importantly, since intracellular pathways and metabolic pools are tightly interconnected, targeted analysis of 50–100 or more analytes that are major components of the food (which can represent more than 95% of the composition) provides significant additional information about the metabolome. One potential outcome of research using metabolomic techniques, however, may be the identification of a small set of key indicator metabolites whose concentration can be used to predict the plant metabolome. Metabolite profiling

techniques have been applied to potato, wheat, rice, tomato, arabidopsis, and gerbera. These studies have demonstrated that profiling can provide a quick snapshot that is useful in comparing varieties and evaluating changes in metabolic pools. There exist some significant hurdles to be crossed before metabolomics profiling can be applied to food safety assessment. The cost of analytical equipment is high and few laboratories are prepared to perform the analysis. Owing to methodological differences, as well as differences in data analysis, statistical analysis and reporting, results obtained in different laboratories are difficult to compare thus limiting the ability to compare data over many years across the food industry. The data that are generated are often expressed as relative concentrations. Most metabolomic profiles include a significant number of unidentified metabolites. The absolute concentrations of most metabolites are reported with poor run-to-run and sample-to-sample reproducibility, and unacceptably poor accuracy and precision. Databases with which to compare results with normal concentrations do not exist. The findings that transgenic plants composition closely resembles that of parental varieties, and that the range of concentrations observed in transgenic varieties falls within the normal ranges for those constituents found in conventional varieties, parallels the observations made using targeted analysis (Chassy, 2010).

It is concluded that while metabolomic profiling is a powerful research tool that can be used effectively by plant breeders, it does not at present offer advantages for food safety assessment. It is not clear that metabolome profiling will ever meet currently applicable standards for compositional analysis. Safety assessment requires internationally validated and standardized measurement techniques and robust databases that establish norms and safety intervals (Chassy, 2010).

1.2.3 Use of proteomic based technologies to study toxicology and animal response to stress

Toxicology is likely to prove one of the most important applications of proteomics. 2DE is a highly sensitive means of screening for toxicity and probing toxic mechanisms. By comparing proteins expressed following treatment with a given drug with those present under untreated conditions, it is possible to identify changes in biochemical pathways via observed alterations in sets of proteins that may be related to the drugs efficacy or toxicity. When a large enough library of proteomic signatures has been compiled for compounds of known toxicity, it will be possible to use it to assess the toxicity of novel compounds. Many large-scale proteomics studies in toxicology

have entered the public domain. A group at Imperial College London/SmithKline Beecham reported a 2DE- and NMR-based study of glomerular nephrotoxicity in an experimental rat following exposure to puramycin aminonucleoside. By monitoring the proteins in urine, the study has permitted a more detailed understanding of the nature and progression of the proteinuria associated with glomerular nephrotoxicity than has previously been possible. In a second example, a study of lead toxicity in a rabbit model identified a number of proteins that change expression following increased lead exposure, of which several molecules, provisionally identified as glutathione-S transferase variants, may be developed into valuable markers of lead toxicity in humans. Other notable reports to emerge recently in proteomic toxicology include an analysis of cyclosporin A toxicity, which revealed a novel toxic mechanism involving the calcium binding protein calbindin-D, and the ongoing studies of liver protein changes following exposure of rodents to peroxisome proliferators being conducted by Argonne National Laboratories and Large Scale Proteomics Corporation. However, although numerous other toxicological studies have used proteomics tools such as 2DE and MS, the potential of proteomics in routine toxicology has yet to be realized (Anderson *et al.*, 2000).

Carboni *et al.* (2006) identified the molecular changes in protein levels induced in rat hippocampus by repeated exposure to psychosocial stress with a proteomic technique. In the social defeat model, the experimental animal was defeated by a dominant male eight times. Additional groups of rats were submitted to a single defeat or placed in an empty cage (controls). The open field test was carried out on parallel animal groups. The day after the last exposure, levels of hippocampal proteins were compared between groups after separation by 2-D gel electrophoresis and image analysis. Spots showing significantly altered levels were submitted to peptide fingerprinting mass spectrometry for protein identification. The pattern of changes induced by repeated stress was quantitatively and qualitatively different from that observed after a single exposure. From their study, they found that several changed proteins have already been associated with stress-related responses; some of them are described for the first time in relation to stress.

In addition, Mairesse *et al.* (2012) investigated the exposure of rats to early life stress. Rats exposed to early life stress are considered to be a valuable model for the study of epigenetic programming leading to mood disorders and anxiety in the adult life. They found that rats submitted to prenatal restraint stress (PRS) are characterized

by an anxious/depressive phenotype associated with neuro-adaptive changes in the hippocampus. They used this PRS model to identify proteins that are specifically affected by early life stress. Therefore, they performed a proteomic analysis in the hippocampus of adult male PRS rats. Pregnant females were randomly assigned to stressed or control groups (n= 12 per group). From day 11 of pregnancy until delivery, pregnant female rats were subjected to three stress sessions daily (45 min each), during which they were placed in transparent plastic cylinders and exposed to bright light. Mairesse and his colleagues found that PRS induced changes in the expression profile of a number of proteins, involved in the regulation of signal transduction, synaptic vesicles, protein synthesis, cytoskeleton dynamics, and energetic metabolism. Immunoblot analysis showed significant changes in the expression of proteins, such as LASP-1, fascin, and prohibitin, which may lie at the core of the developmental programming triggered by early life stress (Mairesse *et al.*, 2012).

The epithelial cells of the small intestine were selected as the target tissue for studying changes in the proteome profile because the small intestine is the main organ for the absorption of nutrients and water in mammals. Further more, early studies showed weak or nonspecific binding of Cry1Ab to the small intestinal epithelial cells of mammalian cells, specifically bovine and porcine cells (Shimada *et al.*, 2006).

1.3 Aims of the project

The overall project aim is to:

- Study the effects of a commercial GM crop, insect resistant maize expressing *Bt* Cry 1Ab, on both feed conversion efficiency and differential gene expression in the epithelial cells of the small intestine of the rat as a non-target model system.

This will be achieved through the following specific objectives:

- To study the *In vivo* effects of transgenic maize (MON810) expressing the δ-insecticidal protein Cry 1Ab from *Bacillus thuringiensis*, on weight gain and food consumption in rat as a non-target species. Rat will serve as a model system for both humans and livestock (Chapter 3).
- To study the *In vivo* effects of transgenic maize (MON810) expressing the δ-insecticidal protein Cry 1Ab on differential gene expression at the proteome level in the epithelial cells of the small intestine of the rat (Chapter 4).

- To conduct comparative *in vitro* studies of Cry 1Ab on mammalian epithelial cells as a possible alternative to animal studies. In these studies both rat small intestinal primary epithelial cells and one human epithelial cell line will be investigated (Chapter 5).

Chapter 2

2 Materials and Methods

2.1 Materials

The following reagents were purchased from Sigma-Aldrich, USA: Dulbecco's modified eagle media high glucose with 4500 mg/L glucose, L-glutamine, and sodium pyruvate, without sodium bicarbonate, powder, suitable for cell culture (Catalogue # D7777); Hydrochloric acid, Molecular biology grade (Catalogue # H1758); Dimethyl sulfoxide (DMSO) for molecular biology 99.9% (Catalogue # D8418); Urea (Ultra pure) for electrophoresis (Catalogue # U6504); Tris (Ultra Pure)or (Tris(hydroxymethyl)aminomethane) (Catalogue # 93362); Thiazolyl Blue Tetrazolium Bromide Powder (MTT) (Catalogue # M5655); Glacial acetic acid ≥99.99% (Catalogue # 338826); Trypan Blue powder, cell culture tested (Catalogue # T6146); Methanol, BioReagent, suitable for protein sequencing (Catalogue # M1770), Mineral oil, light (Catalogue # 330779); Bromophenol blue for electrophoresis (Catalogue # B5525); Coomassie Brilliant Blue G250 (Catalogue # 27815), Acrylamide, for electrophoresis ≥99% (Catalogue # A8887); Agarose, Ultra pure, for electrophoresis (Catalogue # 05070); Concentrated phosphoric acid approx. 85% H_3PO_4 (aqueous) (Catalogue # 438081); Sodium Hydroxide, reagent grade, ≥98% (Catalogue # S5881); Ammonium persulfate (APS), ACS reagent grade (Catalogue # 248614), N,N-Methylene-Bisacrylamide (Catalogue # M7256); 2-Mercaptoethanol (99%) for electrophoresis (Catalogue # M7154); Sodium pyruvate (Catalogue # 15990); Fetal Bovine Serum (FBS) for cell culture (Catalogue # 12106C); L-Glutamine, cell culture tested, from non-animal source (Catalogue # G8540); Penicillin-Streptomycin solution stabilized, with 10,000 units penicillin and 10 mg streptomycin/mL, sterile-filtered, BioReagent, suitable for cell culture (Catalogue #P4333); Ethylenediaminetetraacetic acid (EDTA) (Catalogue # E6758); Ammonium sulfate for molecular biology (Catalogue # A4418); Ammonium bicarbonate (Catalogue # 09830);MEM Vitamin Solution (100×) (Catalogue # M6895); Sodium bicarbonate, cell culture tested (Catalogue#S5761), Thiourea, very pure (Catalogue # T7875), Phenylmethanesulphonyl fluoride (PMSF) (Catalogue # P7626); Sodium carbonate (Catalogue # S7795); Sodium azide (Catalogue # S2002); Trypsin from porcine pancreas (Catalogue # T4799); Phenol Red, powder,

BioReagent, cell culture tested (Catalogue # P3532); *n*-Butanol for molecular biology, ≥99% (Catalogue # B7906); Diethyl ether (Catalogue # 309966); and HEPES buffer solution, 1M in H_2O (Catalogue # 83264).

Glycine for molecular biology (Catalogue # 17-1323-01); Gycerol, 87% for molecular biology (Catalogue # 17-1325-01); immobilized pH-gradient (IPG) Strips with pH 3-10L, 18 cm long (Catalogue # 17-1234-01);Sodium Dodecyl Sulfate (SDS) for electrophoresis (Catalogue # 17-1313-01);N,N,N,N-Tetramethylethylendiamine (TEMED) (Catalogue # 17-1312-01);IPG Buffer pH 3-10 (Catalogue # 17-6000-87);3-[(3-Chloamidopropyl)dimethylammonio]-1-propane-sulfonate (CHAPS) (Catalogue # 17-1314-01); 2-D Quant Kit, 500 assay (Catalogue # 80-6483-56);2-D Clean up kit (Catalogue # 80-6484-51); Dithiothreitol (DTT) (Catalogue # 17-1318-01);Iodoacetamide, proteomic-grade (Catalogue # RPN6302); andProtease Inhibitor Mix (Catalogue # 80-6501-23) were all purchased from GE healthcare, Sweden.

SDS-PAGE Molecular weight standard, broad range (Catalogue # 161-0317) was purchased from Bio-Rad, USA.

Sequencing Grade Modified Trypsin (Catalogue # V5111) was purchased from Promega, UK.

Petri dishes and sterile 96/24 well clear flat bottom polysterene Tissue Culture (TC.) treated micro plates with lids were obtained from BD Falcon, as were 25 cm^2 and 75 cm^2 cell culture flasks. Sterile pipette tips were purchased from Eppendoeff, USA.

Cryogenic tubes with cap for Storage of Biological Material in low temperature liquid nitrogen were purchased from Corning, USA.

Syringe filter units, Diameter 25mm, pore size 0.2µl were obtained from Nalgene, USA.

2.2 Methods

2.2.1 Effects of different maize lines on weight gain and food consumption of male Wistar rats

Test materials

Transgenic *Bt* maize MON 810, its corresponding parental non-transgenic (near isogenic) maize (MON Conv Corn), and two different maize varieties, MON Garst 8450

as reference 1, and MON Gold HVST H8920 as reference 2 were kindly provided by Monsanto, St Louis, USA. In addition, commercial Purina rodent chow (MCert Rod control), was provided by Purina Mills. All diets were shipped to the Institute for Research on Environment and Sustainability (IRES), School of Biology, University of Newcastle, Newcastle upon Tyne, UK before it was shipped to King Abdulaziz University, King Fahad Research Centre, Jeddah, Saudi Arabia. All shipping and handling was conducted so as to protect the quality and freshness of the maize grains.

Diet formulation for rat feeding trials

All control, test, reference diets and the commercial rodent chow were formulated by Purina Mills (St. Louis, Missouri) using corn grains supplied by Monsanto Company (St. Louis, Missouri). Approximately 6.0 kg of each rodent diet were prepared. The diets were formulated according to the specifications of Purina Mills International, LLC (PMI) Certified Rodent LabDiet 5002. Approximately 33% (w/w) corn grains were used for all diets, adjusting other components of the diet to provide approximately equal levels of protein, calories and nutrients in all diets as detailed in Table 3.1 (Chapter 3). The basal diet used for all treatments is a proprietary formula developed by Purina. Further details of diet components, other than as shown in Table 3.1 are therefore not available as it is marketed under and protected by a registered trade name. For the four different experimental diets, the standard corn variety used by Purina was substituted with either MON810, its near isogenic line, MON Garst 8450 or MON Gold HVST H8920. All diets were shipped under ambient conditions by all laboratories and were storied at ambient temperature upon receipt. Corn-based diets prepared by Purina Mills are known to be stable for at least 6 months under these conditions.

Compositional analyses of rat diets

Rats in Group I were fed only with diet containing approximately 33% corn from *Bt* parental non-transgenic variety, those in Group II with test diet containing 33% transgenic *Bt* corn grain and those in Groups III and IV with diet containing 33% reference corn varieties (as described above). Rats in Group V were fed commercial Purina rodent chow containing approximately 33% standard corn. These percentages were chosen in order to maintain a balanced rodent diet for both the 7-day and 28-day feeding study. The mixing ratios of experimental diets are provided in Table 2.1. All diets were mixed by Purina to ensure homogeneity.

To quantify levels of *Bt* Cry1Ab in the MON810 diet (fed to rats in Group II) a 50g sample was collected by Purina TestDiet (Richmond, IN) on the day of diet preparation and sent frozen to Monsanto Company where Cry1Ab protein analysis was carried out; the diet containing the parental non-transgenic maize (fed to rats in Group I) was used as the control for these analyses. The Cry1Ab protein served as marker to confirm that the appropriate grains were used to prepare the test and control diets.

Analysis for nutritional composition and environmental contaminants

Approximately 100 gram samples of each formulated diet were shipped from Purina TestDiet to Covance Laboratories (Madison, WI) for analysis of nutritional components and environmental contaminants.

Animals and housing

Forty locally bred immature male Wistar rats, obtained from the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia were between 6 to 7 weeks of age at the beginning of the study. The study conforms to the National Research Council guidelines for animal experimentation (National Research Council, 1996). The Wistar rats were allowed to acclimate to the housing conditions for 1 week during which they kept on basal diet. Rats had free access to food and water and all rats were provided with tap water *ad libitum* through the study and were housed singly in polycarbonate cages with a stainless steel cover. The Laboratory conditions were maintained 12 h light/dark cycle, at temperature of 23 ± 3 ^{0}C and a relative humidity of 47 ± 5. Animals were observed two times daily for general wellbeing and care.

Experimental design: 7-day and 28-day Rat Feeding Trials

Two animal feeding studies were conducted in one laboratory at King Fahad Research Center, King Abdulaziz University, Jeddah, Saudi Arabia under conditions described above. The study design is an adaptation of the Organization for Economic Cooperation and Development (OECD) guideline No. 407 (OECD, 2008) for a repeated dose 28-day oral toxicity study in rodents. Rats were randomly assigned to five experimental groups based on body weight means. However, the different groups were not randomly assigned within the cage rack for logistical reasons. The rats were fed with the different experimental diets for either 7 or 28 days. Four rats per group were used for both 7 and 28 days feeding trials (Table 2.2). In line with requirements governing the use of animals for experimentation (The three Rs), four rats per group is

considered to be the minimum, but acceptable, number used by the industry in such studies (Cockburn, pers. comm.). The animals were observed twice daily; body weight and food consumption were measured once daily. All animals were fed at 08:00 am daily and water was freely available throughout the study. The cumulative food consumption for each cage was recorded daily and casual observations were made of animal development and behavior.

Before the above studies were undertaken a preliminary study using commercial rodent diet was carried out following the conditions stated above. This provided a growth curve for the basal diet under the proposed experimental conditions, and importantly highlighted any potential problems that needed to be addressed before the actual experiments were carried out.

2.2.2 Isolation of small intestine epithelial cells

Intestinal epithelial cells (IECs) were isolated from the rat small intestine according to the method described by Bjerknes and Cheng (Bjerknes and Cheng, 1981) with minor modifications. At the end of each trial the animals were anesthetized with diethyl ether for 3 minutes and killed by cervical dislocation. The abdomen of each rat was opened and the small intestine was removed. The lumen of the small intestine was perfused with cold phosphate-buffered saline (PBS), pH 7.4 and gently everted using a 4-mm-diameter glass rod and flushed with PBS with its two ends enveloped. The swollen intestine was transferred to a clean flask, and immersed into 20 ml of warm PBSPE solution (PBS containing 1mM PMSF and 1 mM EDTA), and shaken at 150 cycles per min for 5 minutes. Shaking procedures was carried out on an orbital shaking platform. After shaking, the intestine was transferred to another clean flask containing 20 ml of warm PBSPE solution, and vibrated for another 5 min. The vibrating procedure was carried out on a vortexer. The shed intestinal epithelial cells were centrifuged at 500×g for 10 minutes. The cell pellets harvested as described above were then washed in ice-cold PBS three times. Cell counts of the isolated viable epithelial cells were performed by using viable cells analyzer (BECKMAN Coulter Counter, U.S.A) and ViCellXR 2.03 software. The isolated cells were stored at -80^{0}C until further use.

Table2.1: Ratios of experimental diets (%).
Approximately 33% (w/w) corn grains were used for all diets

Groups	Diets (%)				
	Control Maize (MON Conv Corn)	Transgenic Maize (MON810)	Reference Maize 1	Reference Maize 2	Commercial Purina rodent chow
Group I	33	0	0	0	0
Group II	0	33	0	0	0
Group III	0	0	33	0	0
Group IV	0	0	0	33	0
Group V	0	0	0	0	33

2.2.3 Protein separation and identification

All solutions were prepared using double de-ionised water (Ultra Pure Water) with resistance of higher than 18 Ohm (18.2 Ω), prepared on a MilliQ system from Millipore (Direct-Q® 3 UV Water Purification system, U.S.A).

Protein extraction from isolated rat intestinal epithelial cells

Sample preparation is of the utmost importance and very critical to acquisition of highquality data from proteome analysis based on 2-DE. The solubilisation process includes denaturation of the protein to break non-covalent bindings within and among the proteins, while maintaining the native charge and molecular weight of soluble proteins (Rabilloud, 1999). Addition of protease inhibitor cocktails have extensively been used in order to prevent degradation of proteins that otherwise would result in artefactual spots on the 2-D gel and loss of high molecular weighted proteins (Kim and Kim, 2007). In addition, it has been shown that urea, thiourea and CHAPS in combination are very effective inhibitors of proteolytic activity (Castellanos-Serra and Paz-Lago, 2002); furthermore, detergents such as CHAPS are commonly included in the solubilisation mixture due to its efficiency for solubilising hydrophobic proteins (Görg *et al.*, 2004).

Total proteins were extracted from the isolated epithelial cells with an appropriate volume (200 µl) of lysis buffer (sample buffer) containing 7 M urea, 2 M thiourea, 65 mM DTT, 2% CHAPS, 2 mM PMSF, 0.5 % IPG buffer, and protease inhibitor mixture (GE healthcare). The extraction mixture was sonicated using Sonic Dismembrator model 100 (Fisher Scientific), and then centrifuged at 12000×g at 4^0C for 20 minutes, for removal of particulate materials. After the protein solution was collected and transferred to a clean tube, the supernatant was then directly applied to 2D gel electrophoresis or stored at -20 ^{0}C as aliquots until use.

Determination of protein concentration

Total soluble protein concentrations were determined using the 2-D Quant kit (GE healthcare, Sweden) to ensure that appropriate and accurate amounts of protein were loaded, following the Manufacture's instructions. Samples prepared for electrophoresis (including SDS-PAGE and IEF) are often difficult to quantify due to the presence of detergent and reductant. 2-D electrophoresis samples represent a particularly difficult quantification challenge due to the possible presence of interfering carrier ampholyte

and thiourea in addition to the detergents and reductants typically used in sample preparation for electrophoresis. The 2-D Quant Kit circumvents these limitations and can be used to accurately quantify protein samples prepared for 2-D electrophoresis. A calibration curve was prepared according to Table 2.3 using a stock solution of 2 mg/ml bovine serum albumin (BSA) provided with the kit, over the range 0 – 50µg. Following the manufacture's instructions, precipitant (500 µl) were added to 5µl of each sample to be analysed, including to the BSA samples required to construct the calibration curve. The tubes were vortexed briefly and incubated for 2 to 3 minutes at room temperature. Co-precipitant (500 µl) were then added to each sample, mixed briefly by vortexing or inversion and centrifuged at a minimum of 10 000xg for 5 min to pellet the proteins. The supernatants were decanted and the tubes were re-centrifuged to remove the remaining supernatant. Copper solution (100 µl) and distilled or de-ionized water (400 µl) were added to each tube. The tubes were vortexed briefly to dissolve the precipitated protein. Working color reagent (1ml) was added to each tube (color reagent was prepared by mixing 100 parts of color reagent A with 1 part color reagent B to prepare the color reagent used to measure unbound copper ion as described in the manufacture's instructions). The tubes were mixed by inversion and incubated at room temperature for 15-20 minutes. The absorbance of each sample and standard was read at 480 nm and water was used as a reference. The absorbance was read within 40 minutes of the addition of the working color reagent. Unlike most protein assays, the absorbance of the assay solution decreases with increasing protein concentration. Therefore, the blank reading was not subtracted from the sample reading or used the assay blank as the reference. A calibration curve was plotted using the absorbance readings of the standards against the quantity of the protein. Unknown protein concentrations of the samples were then determined from the standard curve.

Table 2.2: Organisation of the study groups.

Group Number	Treatment	Group diet Identification	MON 810 (% in Diet)	Control or reference (% in Diet)	7 day Feeding Males	28 day Feeding Males
1	Conventional	Control	0	33	4	4
2	MON 810	Test	33	0	4	4
3	Garst 8450	Reference 1	0	33	4	4
4	Golden Harvest H8920	Reference 2	0	33	4	4
5	PMI Commercial diet	Commercial	0	33	4	4

Table 2.3: Construction of standard calibration curve for protein concentration using bovine serum albumin (BSA) as a standard protein.

Tube number	1	2	3	4	5	6
Volume of 2mg/ml BSA standard solution	0µl	5µl	10µl	15µl	20 µl	25 µl
Protein quantity	0 µg	10µg	20µg	30µg	40µg	50µg

Cleaning up and preparing the samples for 2-D electrophoresis

The protein samples were cleaned using 2-D Clean-Up kit (GE Healthcare, Sweden). The ability to analyze a sample effectively by 2-D electrophoresis is often limited by the presence of non-protein impurities in the sample. For example excess salts and buffers from sample preparation can render the solution too conductive for effective first dimension isoelectric focusing (IEF). Charged detergents, lipids, phenolics and nucleic acids can also interfere both with first dimension IEF separation and visualization of the 2-D result. Protein precipitation is therefore often employed to selectively separate proteins in the sample from contaminating substances as well as for concentrating samples. Recovery is generally above 90%. The procedure does not result in spot gain or loss, or changes in spot position relative to untreated samples. The precipitated proteins are easily resuspended in 2-D sample solution. Protein samples (1300 to 1500 µg of total protein) were cleaned using the 2-D Clean up kit. The protein samples were transferred into microcentrifuge tubes and centrifuged at 8000g for 10 mins. For each volume of sample, 3 volumes of precipitant were added and mixed well by vortexing and incubated on ice (4-5 ^{0}C) for 15 minutes. This process was repeated, the supernatant discarded and the pellet retained. Co-precipitant was added 3-4x times the size of the pellet. Samples were again centrifuged at 8000 xg for 5 minutes. The wash was discarded. De-ionised distilled water was pipetted on top of the pellet to cover the pellet and the tube was vortexed for several seconds to disperse the pellet. Wash buffer (1 ml), pre-chilled for at least 1 h at -20°C was added to the protein sample. Wash additive (5 µl) were added to the protein sample and vortexed until the pellet was fully dispersed. Samples were incubated at -20^{o}C for at least 30 minutes, being vortexed for 20-30 seconds once every 10 minutes, followed by centrifugation at 8000 xg for 10 minutes. Then, the supernatant was carefully discarded and the pellet was allowed to air dry briefly (no more than 5 min) because if it becomes too dry, it will be difficult to resuspend in rehydration solution for first dimension IEF. Pellets were resuspend in 350 µl of the rehydration solution (8M urea, 2% CHAPS, 0.5% IPG buffer, 0.002% bromophenol blue, 0.3% DTT) and vortexed for 30 seconds to fully dissolve the pellets. Finally, samples were centrifuged at 8000 xg for 10 minutes to remove any insoluble material and to reduce any foam. The supernatant may be loaded directly onto first dimension IEF or transferred to another tube and stored at -80°C for later analysis.

Two-dimensional gel electrophoresis (2-DE)

Two-dimensional gel electrophoresis was performed according to Görg *et al.* (1988), with modifications introduced by Bjellqvist *et al.* (1993).

First dimensional electrophoresis (Isoelectric Focusing (IEF))

The first dimension of 2-DE consists of isoelectric focusing, where proteins are separated on basis of their charge. The isoelectric point (pI) of a protein is the pH value of the proteins surroundings at which the protein has a zero net charge. In order to overcome the limitations of the presence of Carrier ampholytes (CA), the use of immobilised pH gradients (IPG) was developed by Bjellqvist *et al.* (1982) and further improved by Gorg and colleagues (Görg *et al.*, 1988) and now represents the method of choice (Görg *et al.*, 2009). The IPG can be cast in different ranges between pH 2.5-pH 12 in both narrow or wide ranges (Görg *et al.*, 2004) and the choice of range is dependent on sample complexity and scientific purpose. Temperature during IEF has a significant effect on the 2-DE pattern, since the position of protein spots on the gel vary along the pH gradient, when different temperatures are applied. Thus, to achieve high levels of reproducibility of 2-DE gels, it is very important that IEF is conducted under a defined temperature, where 20^0C has been shown to provide optimal conditions (Görg *et al.*, 1991).

Isoelectric focusing dry polyacrylamide gel strips with immobilised pH-gradients (IPG dry strips; GE healthcare) were applied for IEF. Wide range immobiline dry strips with pH 3-10, 18 cm long (IPG pH 3-10, 18 cm, linear) were used. The IPG strips were rehydrated overnight in 350 µl of urea rehydration buffer (8M urea, 2% CHAPS and 0.3% DTT) supplemented with 0.5% IPG buffer (GE healthcare) and 0.002% bromophenol blue. The recommended rehydration time is 12 hours. Preparative sample loads was used. The protein samples were loaded in the rehydration step (rehydration loading). Proteins (1300µg to 1500µg) were treated with the 2-D Clean Up Kit (GE Healthcare, Sweden) as previously described above and loaded per strip; focusing was performed under light mineral oil (paraffin oil) to avoid drying of the strips during the focusing process. Active rehydration was applied using 50V to the IPG strips during rehydration to facilitate uptake of the sample into the IPG strip. The current limit was set to 50 µA per strip to prevent overheating or burning of the IPG strips. The rehydrated IPG strips were focused on an Ettan IPGphor 3 isoelectric focusing unit (GE Healthcare, Sweden). The running conditions for the samples were

optimised and determined according to the standard protocol of the 2-D Electrophoresis Handbook (GE Healthcare, Sweden) with the following modifications: Initially, the samples were run in a step mode for 2h at 250 volts (V) followed by one step in gradient mode for 3h at 10000 V. Finally, the samples were left in step mode at 10000 V for 4h (Table 2.4) or until the steady state was reached; this was achieved using Ettan IPGphor 3 Control Software, which allows easy programming and viewing of run data on a PC (Figure 2.1). The current for each strip remained stable at 50 μA and isoelectric focusing was run at 20^0C. During the run, the damp electrode wicks were changed 2 times to remove high amounts of salts and nucleic acids. After IEF separation, the IPG strips were rinsed with double deionised water, to remove the excess mineral oil, and either frozen or directly transferred into 10 ml of freshly prepared first equilibration buffer (reducing buffer; 50 mM Tris-HCl, pH8.8, 6 M urea, 30 % glycerol, 2 % SDS, and 0.002% of bromophenol blue) for 15 min. The DTT (1%) was added in the first equilibration buffer. This solution was replaced after 15 min by 10 ml of freshly prepared second equilibration buffer (alkylation buffer; 50 mM Tris-HCl, pH8.8, 6 M urea, 30 % glycerol, 2 % SDS, and 0.002% of bromophenol blue) for another 15 min at room temperature. Iodoacetamide (2.5%) was added in the second equilibration buffer. The equilibration processes were carried on a rocker at room temperature before the strips were rinsed again with SDS-electrophoresis buffer (25mM Tris-HCL, pH 8.3, 192mM glycine, 0.1% SDS in 5L sterile double deionised water) and placed on top of SDS polyacrylamide gels for the second stage of electrophoresis.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Following the separation of proteins by first dimensional IEF, the second dimension was carried out by SDS-PAGE in an electric field, where proteins are separated according to their molecular weights.

Gels were cast using PROTEAN® II xi gel casting stands recommended by the manufacturer (BIO-RAD, U.S.A). To achieve a smooth surface water saturated with n-butanol was added to the top of the gels and then the gels were left for about an hour to set and used immediately. The gels were prepared according to the standard protocol of the 2-D Electrophoresis Handbook (GE healthcare, Sweden) (Table 2.5).

IEF Strips were loaded on 12.5% SDS-polyacrylamide gels (20 cm x 20 cm, 0.1 cm thick) and run over night at 1W at 15 °C until the bromophenol blue dye had reached

the bottom of the gel using PROTEAN® II xi Cell, PROTEAN® II xi Cell IPG conversion kit (BIO-RAD, U.S.A) with a chiller circulator (JULABO, U.S.A). A broad range molecular weight standard was run on the same gel to determine the relative molecular masses of the proteins. Powder-free nitrile gloves were worn when handling the gels to avoid contamination with keratin. Following electrophoresis gels were then stained to enable visualisation of protein spots.

SDS-PAGE molecular weight standards, broad range

Broad range molecular weight standards, as shown in Figure 2.2 (BIO-RAD, U.S.A), were applied to the second dimension; prior to use they were diluted 1:20 in SDS reducing sample bufferas detailed in the manufacturer's guidelines (BIO-RAD, U.S.A). These were heated for five minutes at 95^0C, then cooled and 10 µl/well loaded for full length (16-20 cm) gels (Table 2.6 and 2.7).

Table 2.4: Running conditions of the isoelectric focusing for the protein samples.

Step	Voltage (V)	Step mode	Time (h)	Voltage hour (Vh)
1	250	Step	2	500
2	10000	Gradient	3	15375
3	10000	Step	4	40000
Total			9	55875

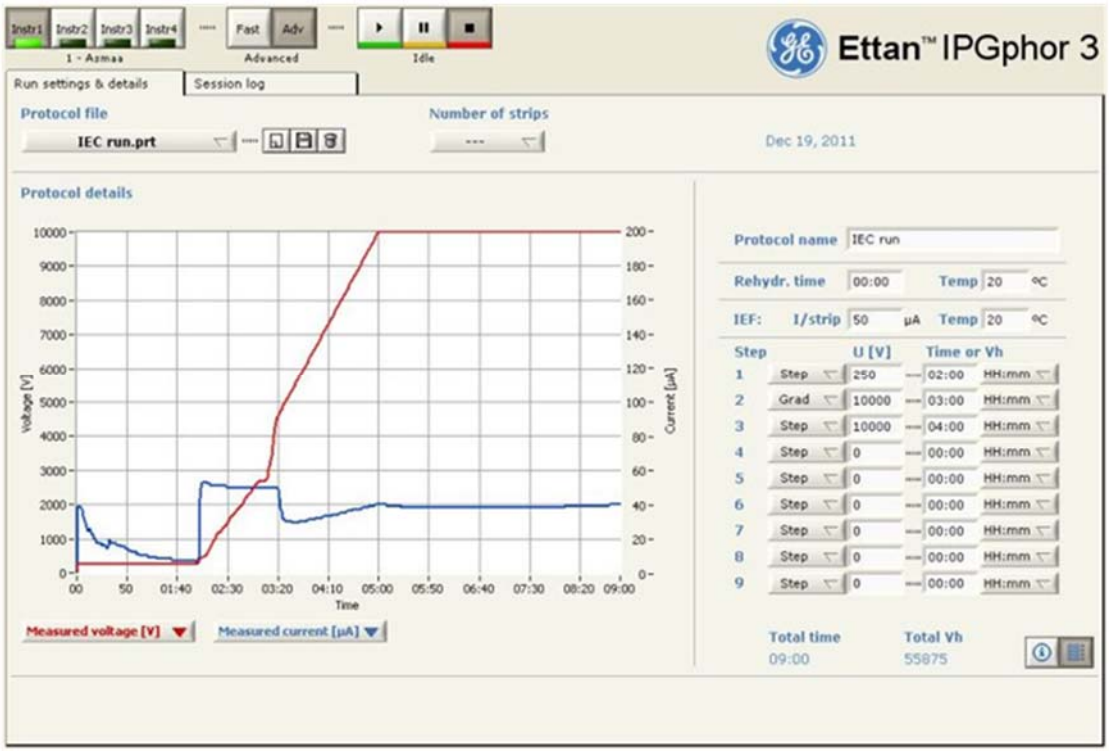


Figure 2.1: Ettan IPGphor 3 control software which allows easy programming and viewing of run data on a computer.

Table 2.5: Gel Composition**.**

30%/0.8% Acrylamide/Bisacrylamide	20.85ml
1.5M Tris-HCl, pH 8.8	12.5 ml
10% SDS	500 µl
Double distilled water	15.9 ml
*10% Ammonium persulfate (APS)	500 µl
*TEMED	20 µl

*APS and TEMED were added immediately after degasing before casting.

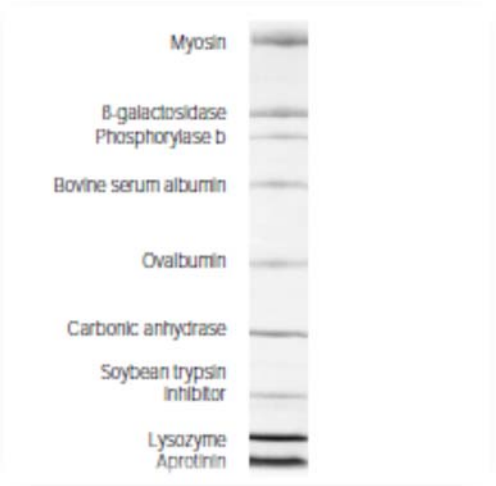


Figure 2.2: Broad molecular weight standards run on 12.5% gel, as supplied by BIO-RAD.

Table 2.6: Stock sample buffer (stored at room temperature).

	Amount
Distilled water	**4.8 ml**
0.5M tris-HCl pH 6.8	**1.2 ml**
Glycerol	**1 ml**
10% (w/v) SDS	**2 ml**
0.1% (w/v) Brompphenol blue	**0.5 ml**
Total volume	**9.5 ml**

Table 2.7: SDS reducing sample buffer
(Prepared immediately before use).

	Amount
2-Mercaptoethanol or ß-Mercaptoethanol	**25 µl**
Stock Sample buffer	**475 µl**
Total volume	**500 µl**

Visualisation of proteins

Following 2-DE, the separated proteins were visualised with Colloidal Coomassie Brilliant Blue G-250 stain (stock solution: 10% ammonium sulfate, 1% (w/v) phosphoric acid, 0.1% Coomassie Blue G-250 in 500 ml sterile deionized water). Staining with Colloidal Coomassie Brilliant Blue G-250 is about ten times more sensitive (about 10-40 ng of protein) than conventional staining with Coomassie R-250 and fully compatible with mass spectrometry. The staining method used in this study has been modified from Neuhoff *et al.* (1988). Briefly, the peptides in the gels were fixed for at least 30 min in fixing solution composed of 10% (v/v) acetic acid and 40% (v/v) ethanol. After that, the fixing solution was decanted and the gels were washed with deionized water then stained overnight with Colloidal Coomassie Brilliant Blue G-250 (working concentration: 8% (w/v) ammonium sulfate, 0.8% (w/v) phosphoric acid, 0.08% (w/v) coomassie blue G-250, 20% (v/v) methanol in 500 ml of sterile deionized water). To remove the residual stain, gels were rinsed repeatedly with sterile deionized water (Neuhoff *et al.*, 1988).

Image acquisition and analysis

After protein staining, it is of vital importance that proteins spots are aligned accurately to ensure high quality data from proteome experiments. PDQuest version 8.0.1 was used for image acquisition and Progenesis SameSpot software version 4.1 was used for image analysis. Since slight variations in protein load per gel and staining efficiency can have a considerable impact on the spot volumes, spot volume normalisation, a necessary step in 2-DE data acquisition to minimize the analytical variation caused by gel-to-gel variations, is very important for quantification of spot volumes.

The polyacrylamide gels were scanned in reflective mode (600 dots per inch (dpi)) using a GS-800™ Calibrated Imaging Densitometer (BIO-RAD, U.S.A), saved as a gray-scale TIFF file, and analysed by the image analysis software Progenesis SameSpot. Parameters of intensity and contrast were chosen so that the protein spots were calculated. All other parameters were set as proposed by the manufacturer.

Mass spectrometry based identification of proteins of interest

The classical 2-DE based approach coupled with identification by mass spectrometry has become the most widely used approach in proteomics to effectively identifying the proteins of tissues, cells and bio-fluids (Fuchs *et al.*, 2005).

Tryptic in gel digestion for liquid chromatography-mass spectrometry (LC-MS/MS)

Trypsin (Sequencing Grade Modified Trypsin, Promega) stock solution was prepared according to the manufacturer protocol. All washing and digestion steps were performed in a laminar flow hood. Protein spots were excised from the gel manually using specialised spot pickers (Harris UNI-CORE^{TM}, Sigma- Aldrich) and transferred into 1.5 ml microcentrifuge tubes (Eppendorf). Gel pieces were washed with 500µl of 100mM ammonium bicarbonate for 30 mins on a shaker. The gels were subsequently washed twice with 500µl of 50% acetonitrile/100mM ammonium bicarbonate for 30 mins at 37^{0}C on a shaker, and the liquids were discarded. After discarding the wash, 50µl of acetonitrile was added to shrink the gel pieces. After 10 minutes, the solvent was removed and the gel pieces were completely dried under vacuum (Concentrator 5301, Eppendorf). Then, 20µl of 12.5 ng µl^{-1} of trypsin was added to each sample followed by addition of sufficient 25mM ammonium bicarbonate to just cover the gel piece. Protein samples were allowed to digest overnight at 37˚C. After digestion, the tubes were briefly centrifuged to pellet the gel pieces. All liquids were transferred to fresh labelled 0.5 ml Eppendorf tubes. Then 20µl 5% formic acid was added to each sample and the tubes were incubated for 20mins. Subsequently, 40µl acetonitrile was added to each tube containing the protein sample and the tubes were incubated for 20 mins at room temperature. The tubes were briefly centrifuged to pellet any remaining gel pieces. All liquids were transferred to the same labelled tubes. The volume of the pooled samples were reduced to about 2-3 µl in a Speed vac, then 10µl of 0.1% formic acid was added to each sample prior to analysis by mass spectrometer.

Nanoflow HPLC electrospray tandem mass spectrometry (nLC-ESI-MS/MS)

An ion-trap mass spectrometer (amaZon ETD, Bruker Daltonics, Bremen, Germany) equipped with an on-line nanospray source was used for mass spectrometry data acquisition. Briefly, solubilised peptides were fractionated on a nanoflow HPLC system (Thermo RSLCnano) before online analysis by electrospray ionisation (ESI) mass spectrometry on an amaZon speed ion ETD trap (Bruker Daltonics). Peptide separation

was performed on a Pepmap C18 reversed phase column (LC Packings), using a 5 - 85% v/v acetonitrile gradient (in 0.5% v/v formic acid) run over 45 min. at a flow rate of 0.2 µl/min. Mass spectrometric (MS) analysis was performed using a continuous duty cycle of survey. The mobile phase consisted of 0.1% v/v formic acid/H_2O and the peptides were eluted at 200 nl/min. A survey scan was conducted in standard enhanced mode from *m/z* 400 to *m/z* 1600MS scan, followed by up to ten MS/MS analyses of the most abundant peptides, choosing the most intense multiply charged ions with dynamic exclusion for 120 s.

LC-MS/MS is a powerful analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with those of mass spectrometry. It therefore enhances sensitivity and facilitates precise detection of biomolecules. The spectra that were obtained through the Bruker DataAnalysis 3.4 software from the MS/MS experiments were subsequently used for the confident identification of the proteins present in the mixture.

Database search for protein identification

For the analysis of mass spectrometry data, different search programs are available on the Internet. Protein identifications were assigned using the Global Proteome Machine (GPM) search engine (www.thegpm.org) to interrogate protein sequences in the ENSEMBL *Rattus Norvegicus* database. The following settings were applied: method, ion trap (4 Da); cleavage site, trypsin [RK]|{P}; may missed cleavage, 1; complete modifications, carbamidomethyl (CU); variable modification, oxidation (M); fragment mass error, 0.4 Da; parent mass error, ±0.3 Da; fragment type, monoisotopic; maximum parent charge, 4. Only identification with log(e) values ≤ -3 were regarded as significant hits regardless of the number of peptides.

2.2.4 In vitro effects of transgenic maize (*Bt* maize MON810) expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) on mammalian epithelial cells

Test material

The transgenic Bt maize (*Product safety summary for MON 810 corn*) and the corresponding parental non-transgenic maize were kindly provided by Monsanto, St Louis, USA and shipped to the Institute for Research on Environment and Sustainability (IRES), School of Biology, University of Newcastle, Newcastle upon Tyne, UK. All

shipping and handling was conducted to protect the quality and freshness of the maize grains.

Protein extraction of maize (MEB307Bt) Cry1Ab MON810

The seed coat was removed and seeds ground to a fine powder and extracted at 100mg/ml over night at 4°C using 1X TBS (0.1M Tris, 150 mM NaCl) and 1% SDS extraction buffer. Extracts were centrifuged at 13000 xg, and the supernatant dialyzed against dH_2O at 4°C. Buffer was changed 4X in 24 hours. Finally, the dialysed extracts were flash frozen and freeze dried.

Immunoassy by Western blotting of test material

The presence of the Cry1Ab protein in seeds of the transgenic maize line MON810 and its absence in the parental control maize was verified by immunological assay (Western blotting after analysis of total protein by SDS-PAGE). Ten mg of lyophilized protein was dissolved in 1 ml of 1xTBS (0.1 M tris, 150 mM NaCl) + 0.1% SDS buffer and 20 µl from this solution was then mixed with 5 µl 6x SDS + ME (mercaptoethanol) loading dye. The mixture was boiled for 10 minutes and then loaded onto 12.5% polyacrylamide gel (200 µg of proteins were loaded onto the gel and electrophoresed). The marker used was Mark 12 Unstained Standard (Invitrogen) and the gel was run at 80 V for first 30 minutes and then at 150 V for 1 h 45 min. The proteins were then transferred onto a nitrocellulose membrane in a transfer unit running at 0.2 A for 2 hrs. The membrane was incubated on a shaker for 1 h in blocking solution (20% w/v dried milk powder, 0.15 tween20 in 1x PBS) followed by washes in 30 ml double distilled water. The washed membranes were incubated on a shaker for 2 h with Cry1Ab antibody (primary antibody) followed by incubation with goat anti-rabbit-HRP conjugate (secondary antibody) for 1 h. Both the antibodies were diluted 1:5000 in PBS-T before use. Membranes were then washed thrice with PBS-T, 5 min each wash, and finally subjected to ECL (chemiluminescence) detection (Amersham) and quantified by densitometric scanning using Bio-Rad Molecular Analyst software.

Cell culture methods

Phosphate Buffered Saline (PBS) was used to wash the cells. It was prepared by dissolving 9 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.2 g KH_2PO_4 and 1 g $NaHCO_3$ in 800 ml double de-ionised water (d.d. H_2O) with a resistance of higher than 18 Ohm, prepared on a MiliQ system from Millipore. The PBS pH was adjusted to 7.4 and the

volume was completed to 1000 ml with d.d. H_2O. Trypsin/EDTA solution was used to detach the cells from the tissue culture flasks and plates (Table 2.8). All solutions were sterile filtered using 0.2 μm vacuum filters (Millipore) and were of cell culture grade. All media preparation and other cell culture work were performed in a safety cabinet. All surfaces were surface sterilised with 70% ethanol before beginning the work. Sterile pipettes and disposable test tubes were used for cell culture. Pipette tips were autoclaved prior to use. All cell culture vessels, test tubes, centrifuge tubes and pipette tips boxes were opened in the laminar flow hood to avoid contamination.

All cell lines were incubated in DMEM medium in a humidified cell culture incubator (SANYO) at 36^0C, 5% CO_2. All cell lines were cultured in optimised medium at pH 7.4. All procedures were performed under the restricted sterile conditions in a laminar flow hood. If medium contamination was suspected, the medium was discarded and fresh medium prepared.

Isolation of epithelial cells from rat small intestine

The isolation of small intestinal epithelial cells was carried out as described in section 2.2.2 above.

Establishment in culture of epithelial cells isolated from rat small intestine and the commercial human epithelial cell line HCT116

Intestinal epithelial cells which were isolated from rat small intestine, as described above, and human colon cancer cell line HCT-116 which, was obtained from National Cancer Institute, Cairo, Egypt, as frozen ampoules were cultured in Dulbecco's Modified Eagle's Medium-high glucose (with 4500 mg/L glucose, 0.584 g/L L-glutamine, and 110 mg/L sodium pyruvate (Sigma) supplemented with 10% Heat-Inactivated Fetal Calf Serum (HIFCS) (Sigma), 3.7 g sodium bicarbonate (Sigma), 2 mM glutamine (Sigma), 26.18 mM NaHCO3 (Sigma), 10 ml of 1M HEPES (Sigma), 10 ml of Minimum Essential Medium vitamin solution (MEM vitamin solution 100x) (Sigma) and 1 % Penicillin/Streptomycin solution with 10,000 units penicillin and 10 mg streptomycin/mL (Sigma)) at 37^oC in a humidified atmosphere with 5% CO_2 until monolayer (complete sheet) was observed.

Cell count and viability count

Determination of viable cell numbers for appropriate cell seeding was performed by using a viable cells analyzer (BECKMAN Coulter Counter, U.S.A) together with ViCellXR 2.03 software. The tissue culture flask was transfered from the cell culture incubator to a laminar flow hood. Medium was removed from the tissue culture flask. Trypsin/EDTA solution (2 ml) was added and removed directly after 5 seconds and another 2ml of Trypsin/EDTA solution was added and incubated for 2 minutes at 36^0C. The tissue culture flask was jarred against the palm of the hand to complete cell detachment and fresh medium was added to inactivate trypsin. To 25 cm^2 tissue culture flask, cells were resuspended in 10 ml medium and to 75 cm^2 tissue culture flask, cells were resuspended in 25 ml medium. Cell number and viability were assessed as proposed by the BECKMAN Coulter Counter manufacture.

A cryogenic vial (approximately 1x 10^6 cells per vial) was removed from gas phase liquid nitrogen storage and allowed to stand at room temperature for 1 minute, after which the vial was then transferred to a 37^0C water bath and left for 10 minutes until thawed. The vial was first cleaned using a cloth impregnated with 70% ethanol and the content was transferred to a 25 cm^2 tissue culture flask with a vented cap containing 9 ml of pre-warmed medium at 37^0C. The tissue culture flask neck was sterilized with 70% ethanol and transferred to the cell culture incubator at 36^0C containing 5% CO_2. All procedures were performed under sterile conditions in a laminar flow hood. Cells were incubated overnight and inspected 24 hours later. Medium was removed and new medium (10 ml) was added. Cells were allowed to proliferate and subcultured again when confluent.

Cells were inspected every two days, followed by replacement of old medium with fresh medium. Cells were examined under an inverted microscope for signs of metabolic stress, which was typically characterized by blabbing of cells membranes and rounding-up of cells followed by detachment from the tissue culture flask surface. When detachments become excessive, the tissue culture flask and its contents were discarded. If fungal or yeast infection was evident or suspected, the tissue culture flask content and stock medium were discarded.

Subculturing of cells

Medium was removed from the tissue culture flask. Trypsin/EDTA solution (2 ml) was added and removed directly after 5 seconds and another 2ml of Trypsin/EDTA solution was added and incubated for 2 minutes at 36^0C. The tissue culture flask was jarred against the palm of the hand to complete cells detachment and fresh medium was added to inactivate trypsin. To 25 cm^2 tissue culture flask, cells were resuspended in 10 ml medium and to 75 cm^2 tissue culture flask, cells were resuspended in 25 ml medium. Cells number and viability were also assessed. Medium was changed 24 hours later and subsequently every two days until confluent.

Cell staining

Cells were washed three times with 1x PBS. After that, the cells were fixed in 4% formaldehyde for 5 minutes at room temperature. Then, the cells were stained with Coomassie Brilliant Blue for 3 minutes followed by repeated washing with distilled water. The stained cells were allowed to dry at room temperature for one day before visualization under an inverted microscope (OLYMPUS 1x71) using the software OLYMPUSDP2-BSW, 2006.

Table 2.8: Recipe of trypsin/EDTA solution

	Amount
Phosphate Buffer Saline	350 ml
Trypsin	1.25 g
Ethylenedinitrilotetraacetic acid, EDTA (F.W 292.24)	0.1 g
Sodium Bicarbonate ($NaHCO_3$) (F.W 84.01)	0.75 g
Phenol red (0.5%) (F.W 354.38)	0.5 ml
Phosphate Buffer Saline	To 500 ml

Cryopreservation

For long-term storage, the medium was removed from a confluent tissue culture flask. Cells were trypsinised and centrifuged and the pellets were resuspended in ice cold frozen medium containing 10% DMSO in 30% FBS and 60% medium followed by quick and gentle mixing. Aliquots (1.5 ml) were transferred into cryovials which were labeled with cell name, passage number and date and frozen at -80^{0}C for one day before being transferred to liquid nitrogen at -192^{0}C for long-term storage. For re-cultivation, cells were thawed in 37^{0}C water bath and resuspended in the Dulbecco's Modified Eagle's Medium-high glucose (with 4500 mg/L glucose, 0.584 g/L L-glutamine, and 110 mg/L sodium pyruvate (Sigma) supplemented with 10% Heat-Inactivated Fetal Calf Serum (HIFCS) (Sigma), 3.7 g sodium bicarbonate (Sigma), 2 mM glutamine (Sigma), 26.18 mM NaHCO3 (Sigma), 10 ml of 1M HEPES (Sigma), 10 ml of Minimum Essential Medium vitamin solution (MEM vitamin solution 100x) (Sigma) and 1 % Penicillin/Streptomycin solution with 10,000 units penicillin and 10 mg streptomycin/mL (Sigma)) then incubated at 37^{o}C in a humidified atmosphere with 5% CO_2 until monolayer was observed.

Cell viability assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay)

To determine the effects of the transgenic *Bt* maize (MON 810) and the corresponding parental non-transgenic maize extracts on both IECs and HCT 116 cell line, a [3-(4,5-dimethyl thiazole-2-yl)]-2,5-diphenyl tetrazolium bromide (MTT) assay was performed. For cell viability assay, 1×10^5 cells (HCT 116 and IECs)/well were plated in 100 µl of DMEM media into a 96-well plate. The cells were allowed to attach and grow for 24 h at 37°C in humidified atmosphere of 5% CO_2. After that the old medium was replaced with fresh medium containing the maize extracts at various concentrations for determining the effect on cells growth. The transgenic *Bt* maize (MON 810) and the corresponding parental non-transgenic maize extracts were added at various concentrations ranging from 0, 6.25, 12.5, 25, 50, 100 and 200µg/ml after 24 h incubation. After 48 h incubation in a humidified CO_2 incubator at 37˚C, 20 µl of MTT solution (2 mg/ml) was added to all wells of the plate at the final concentration of 0.5 mg/ml. Then, 200 µl of isopropanol was added under dark to each well. The resulting MTT-products were determined by measuring the absorbance at 550 nm using an

ELISA plate reader (Molecular Devices, U.S.A). The optical density obtained is directly related to the viability of cells.

$$\%\ viability = \frac{Optical\ density\ of\ sample}{Optical\ density\ of\ control} \times 100$$

Calibration of growth curve through the Growth Cell Cycle

Cells were trypsinised and the cells number and viability were determined. After appropriate dilution they were seeded at 5000 cells/well in 24 well plate (BD Falcon) containing 1.5 ml growth medium with 10% FBS and incubated at 36^0C, 5% CO_2. Quadruplicate wells were taken at daily intervals for determining cell numbers per well as described in section above.

Determination of exposure time of Bt and non-Bt maize extracts for both IE cells and HCT116 cell line

To determine the optimal exposure time of IE cells and HCT116 cell line to maize extracts, growth curves were constructed using the control medium (without any maize extracts) and medium with non-cytotoxic concentrations of both *Bt* and non-*B t*maize extract. For the both the control plate and the experimental plate, 5000 cells/well were seeded at day 0 in 24-well plate (BD Falcon) containing medium supplemented with 10% FBS and incubated at 36^0C, 5% CO_2 for 24 hours. After this duration, for the controls the medium was removed and new medium was added to each well, whereas for the experimental plates the medium was removed and new medium containing non-cytotoxic concentrations of either *Bt* maize extract or non-*Bt* maize extract (based on results from the MTT assay) was added to each well. All plates were incubated at 36^0C, 5% CO_2 for another 24 hours. At day 1, the cells of 5 wells were trypsinised and the cells number and viability were determined. This was repeated for days 2, 3 and 4. All experiments were done triplicate for each treatment.

Protein separation and identification

All solutions were prepared using double de-ionised water (Ultra Pure Water) with resistance of higher than 18 Ohm (18.2 Ω), prepared on a MilliQ system from Millipore.

Protein extraction from cultured cells

Cells were seeded in 75-cm^2 tissue culture flasks, grown for 1day prior to exposure to *Bt* or non-*Bt* maize extracts or medium only (negative control) as above. The cells were harvested 48 h after treatment, rinsed with PBS, and trypsinized with a solution of 2.5 g/L trypsin and 0.2 g/L EDTA. After 1 min, media containing FBS were added to terminate the action of trypsin. The resulting suspension was centrifuged at 12000×g for 10 min at 4 ^{0}C. After the supernatant was discarded, the cells were resuspended in ice-cold 1× PBS and centrifuged at 12000×g for 10 min at 4 ^{0}C and the supernatant was removed. This wash-step was repeated thrice. Cells were resuspended in cold PBS and counted. The pelleted cells were stored at -80 ^{0}C until further use. Cells, previously treated with *Bt* maize extract and cells treated with isogenic maize extract were resuspended in lysis buffer (containing 7 M urea, 2 M thiourea, 65 mM DTT, 2% CHAPS, 2 mM PMSF, 0.5 % IPG buffer, and protease inhibitor mixture (GE healthcare)). The extraction mixture was sonicated using Sonic Dismembrator model 100 (Fisher Scientific), and then centrifuged at 12000×g at 4 ^{0}C for 20 minutes, for removal of particulate materials. After the protein solution was collected and transferred to a clean tube, the supernatant was then directly applied to 2D gel electrophoresis or stored at -20 ^{0}C as aliquots until use. Subsequent details for protein separation and identification are as described above for the *in vivo* studies.

2.2.5 Statistical analysis

The significance of the differences was tested using the parametric analysis of variance (one-way ANOVA) and the nonparametric analysis of variance (Kruskal wallis test). The IBM SPSS statistics 20 and Minitab 16 programs were used to perform statistical analyses. Differences were considered to be statistically significant when the *p*-value was <0.05 and highly significant when the *p*-value was <0.001.

Chapter 3

3 *In Vivo* Effects of Transgenic Maize Expressing the δ-Insecticidal Protein Cry 1Ab from *Bacillus thuringiensis* on Weight Gain and Food Consumption of Young Adults Male Wistar Rats

3.1 Effects of different maize lines, including MON810, on weight gain and food consumption of male Wistar rats

3.1.1 Diet formulation for rat feeding trials

All control, test and reference diets were formulated by Purina Mills International (PMI) using corn (maize) grain supplied by Monsanto Company. Approximately 33% (w/w) corn grain was used for all diets, adjusting other components of the diet to provide approximately equal levels of protein, calories and nutrients in all diets as detailed in Table 3.1. Compositional, contaminant, and nutritional content of the experimental diets met the specifications for Certified Rodent LabDiet 5002 established by PMI. PCR analysis confirmed that the test diet contained MON 810 as it tested positive for the Cry1Ab transformation event. The control and reference diets did not test positive for the Cry1Ab transformation event and therefore was considered to be free from contamination by the transgenic line.

3.1.2 Mortality

Feeding trials were carried out over both 7 days and 28 days to investigate the effects of the different maize lines on both rat performance and differential gene expression in the epithelial cells of the small intestine. During these periods no mortality occured.

3.1.3 Behavioural observations

The rats were observed twice daily for well-being. There were no changes noted during the duration of the study in behaviour (no aggressive behaviour or biting), activity, posture, gait, or external appearance for any of the different treatments and all of the 40 animals were healthy and appeared normal during the course of the study.

3.1.4 Absolute body weight measurements for the 7-day rat feeding trial

There was an increase in the absolute body weight for the entire five groups from day 1 to day 8 (Table 3.2 and Figure 3.1). Changes in body weight were noted after 1

week. The average body weight of the rats in the control (MON Conv Corn) and test (MON 810) group were 283.5 g and 266.5 g respectively. In rats belonging to the control group the body weight was greater (283.5 g) as compared to the other four groups, but this showed no significant differences (p =0.603).

3.1.5 Body weight gain for the 7-day rat feeding trial

The data presented in Figure 3.2 show changes in body weight gain of rats fed the five different diets over a period of 7 days. The growth rate was highest with the Purina rodent chow followed by control, reference 2, test and reference 1 diets. Therefore, rats fed on the commercial Purina rodent chow and diet containing approximately 33% control corn grain gained more body weight than rats fed on diet containing 33% transgenic *Bt* corn grain or the ones fed on diet containing 33% reference corns. The body weight gain of the test group compared with the control is 9.9% less than the control where as body weight gain of Reference 1 and Reference 2 groups compared with the control are 10.6% and 7.1% less than the control respectively. In addition, the body weight gain of the commercial rodent diet group compared with the control is 9.2% greater than the control. However, no significant differences in body weight gain between the five diets (p =0.859) (Table 3.3).

3.1.6 Food consumption for the 7-day rat feeding trial

Food intake was calculated on a weekly basis for the rats by group over the treatment period as shown in Table 3.4 and Figure 3.3. The food consumption of the control and test group was 283 g and 267.75 g, respectively. The weekly food intake for the control diet group was higher than in the other groups during the whole treatment period (283 g), but this showed no significant differences between the five diets (p =0.695).

3.1.7 Feed conversion efficiency (FCE) for the 7-day rat feeding trial

Feed conversion efficiency (food intake/Weight gain), which is a measure of an animal's efficiency in converting feed mass into increased body mass, was measured as illustrated below (Table 3.5):

Group mean food intake in week/Group mean weight gain in week

Animals that have a low FCE are considered efficient users of feed. Therefore, the rats fed on the commercial rodent diet are considered efficient users of feed and they have the highest body weight gain (Figure 3.4), but this also showed no significant differences between the five diets (p =0.600).

3.1.8 Absolute body weight measurements for the 28-day rat feeding trial

There was an increase in the absolute body weight for the entire five groups from day 1 to day 29 (Table 3.6 and Figure 3.5). Changes in body weight were noted after four week. The average body weight of the rats in the control and test groups were 325.5 g and 335.25 g respectively, after four weeks. In rats belonging to the test group the body weight was greater (335.25 g) as compared to the other four groups.

There was no significant differences in absolute body weight of rats from the various feed groups after 28 days ($F_{4,15}$=0.33, p=0.852 after week 1, $F_{4,15}$=1.26, p=0.333 after week 2, $F_{4,15}$=0.88, p=0.498 after week 3, $F_{4,15}$=0.78, p=0.558 after week 4). The residuals were not significantly different to a normal distribution (p=0.810). The residual of an observed value is the difference between the observed value and the estimated function value. It can also be stated that the highest mean absolute body weight was attained by rats fed on test diet followed by the control, and the least was for the reference 1 group.

3.1.9 Body weight gain for the 28-day rat feeding trial

There was no significant differences in body weight gain of rats from the various feed groups after 28 days ($F_{4,15}$=1.35, p=0.297 after four weeks). The residuals were not significantly different to a normal distribution (p=0.682). It can also be stated that the highest mean body weight gain was attained by rats fed on diet containing approximately 33% transgenic *Bt* corn grain followed by the commercial diet and the lowest was for the reference 1 group (Figure 3.6).

The body weight gain of the test group compared with the control is 35.7% greater than the control where as body weight gain of Reference 1 and Reference 2 groups compared with the control are 13.6% and 0.23% lower than the control, respectively. In addition, the body weight gain of the commercial rodent diet group compared with the control is 21.6% greater than the control (Table 3.7).

3.1.10 Food consumption for the 28-day rat feeding trial

Food intake was calculated on a weekly basis for the rats by group over the treatment period, as shown in Table 3.8 and Figure 3.7. The food consumption of the control and test group was 332.5 g and 294.75 g respectively, after 28 days. The weekly food intake for the control diet group was higher than in the other groups during the whole treatment period (332.5 g), but this showed no significant differences between the five diets (p =0.450).

3.1.11 Feed conversion efficiency (FCE) for the 28-day rat feeding trial

Feed conversion efficiency (food intake/weight gain), which is a measure of an animal's efficiency in converting feed mass into increased body mass, was measured as shown in section 3.1.7 above (Table 3.9).

Animals that have a low FCE are considered efficient users of feed. Therefore, the rats fed on diet containing approximately 33% transgenic *Bt* corn grain are considered efficient users of feed and they have the highest body weight gain (Figure 3.8), but there was no significant differences in FCE between the five diets (p =0.270).

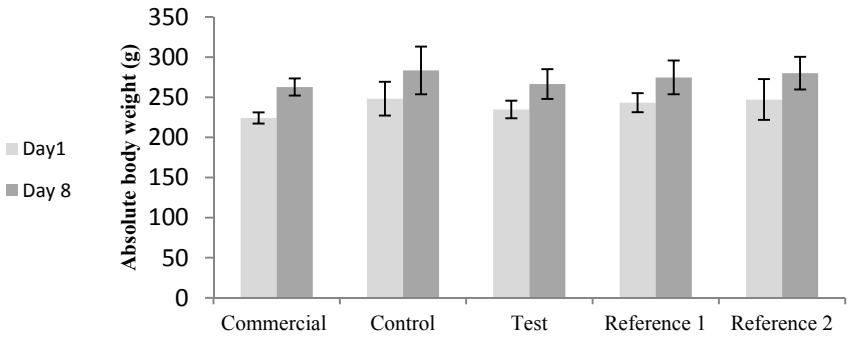
Table 3.1: Composition of experimental diets for rats (%).

ingredients	Test (MON810)	MON Garst (reference 1)	MON Gold (reference 2)	Control (MON Conv Corn)
Cereal products (ground Corn, Ground Oats, Wheat Germ, Ground Wheat, Wheat Middlings)	61	59.4	58.3	58.1
Vegetable Proteins (Dehydrated Alfalfa Meal, Dried Beet Pulp, Ground Soybean Hulls, Dehulled Soybean Meal, Dried Brewers Yeast)	26.6	27.8	30.3	29.7
Animal Proteins (fish Meal, Acid Casein, Dried Whey)	6.1	6.4	5.0	6.0
Energy Sources (Animal Origin)	0.0	0.0	0.0	0.0
Energy Sources-All other (Soybean Oil, Cane Molasses)	4.2	4.3	4.2	4.1
Supplementation (vitamins, Major Minerals, Trace Minerals, Amino Acids)	2.1	2.1	2.2	2.1

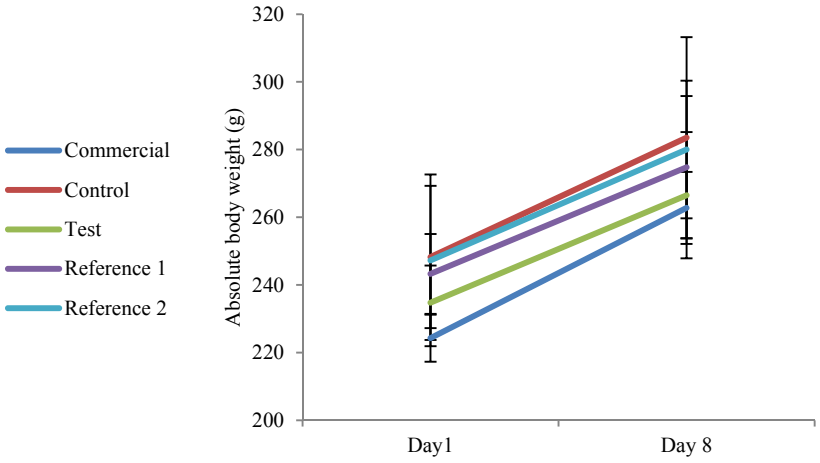
*All of these diets and the commercial chow (not shown) are based very tightly on Certified Rodent LabDiet 5002 which is a proprietary formula so PMI would not be able to provide us with the ingredient percentages for any of the diets. Control and reference grain are from conventional varieties that arenot biotechnology-derived. Only the test (MON810) is biotechnology-derived.

Table 3.2: Change in absolute body weight (grams) using group means ± SD after 1 week for the 7-day rat feeding trial.

Groups of rats (n=4)	Body Weight	
	Day1	Day 8
Commercial	224.25±6.94	262.75±10.65
Control	248.25±21.02	283.5±29.69
Test	234.75±10.99	266.5±18.64
Reference 1	243.25±11.78	274.75±21.04
Reference 2	247.25±25.36	280±20.33



a



b

Figure 3.1: Change in absolute body weight (grams) for 7-day rat feeding studies using group means after 1 week. Values are means ± SD.

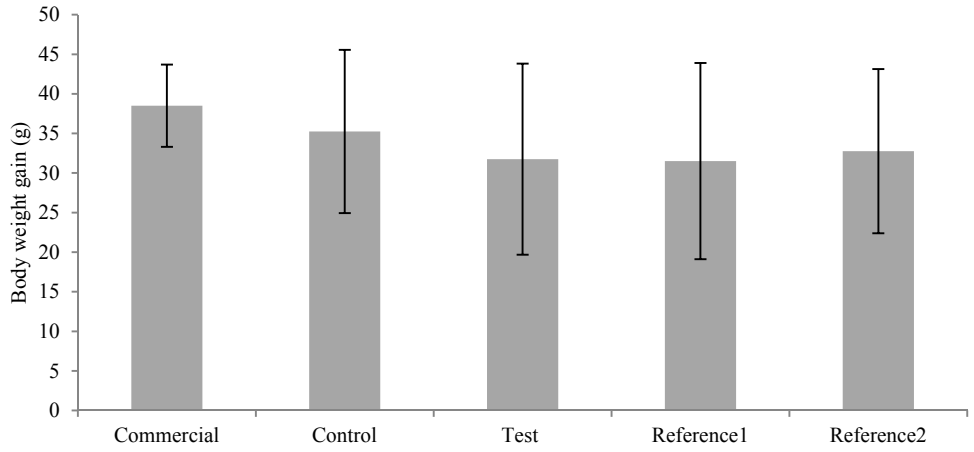


Figure 3.2: Change in body weight gain (grams) for 7-day rat feeding studies using group means after 1 week (on day 8). Values are means ± SD.

Table 3.3: Differences in body weight gain on D8 compared with the control for the 7-day rat feeding studies. Values are means ± SD.

Group (n=4)	Body weight Gain on D8	Difference with control (%)
Commercial	**38.5**±5.19	9.2
Control	**35.25**±10.30	---
Test	**31.75**±12.06	-9.9
Reference1	**31.5**±12.39	-10.6
Reference2	**32.75**±10.37	-7.1

Table 3.4: Weekly food consumption (g) for 7-day rat feeding studies using group means of weekly food intake.

Group (n=4)	week 1
Commercial	**260.25**±7.58
Control	**283**±23.35
Test	**267.75**±13.02
Reference 1	**274.75**±16.09
Reference 2	**279.75**±23.92

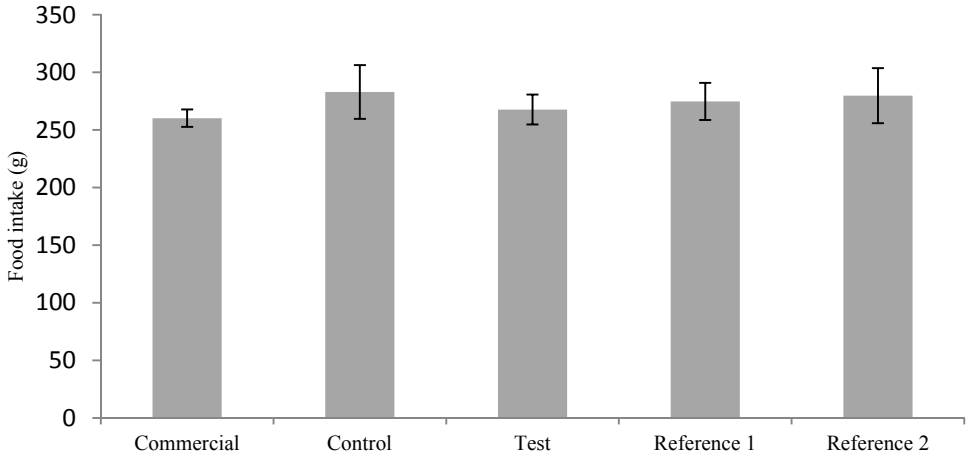


Figure 3.3: Weekly food consumption (in grams) for 7-day rat feeding studies using group means of weekly food intake. The values represented are means ± SD.

Table 3.5: Feed conversion efficiency (FCE) for the 7-day rat feeding trial

Group (n=4)	Group mean food intake in week 1	Group mean weight gain in week 1	Feed conversion efficiency
Commercial	260.25	38.5	6.831±0.72
Control	283	35.25	8.4375±1.89
Test	267.75	31.75	9.28625±3.20
Reference 1	274.75	31.5	9.656±3.35
Reference 2	279.75	32.75	9.278±3.28

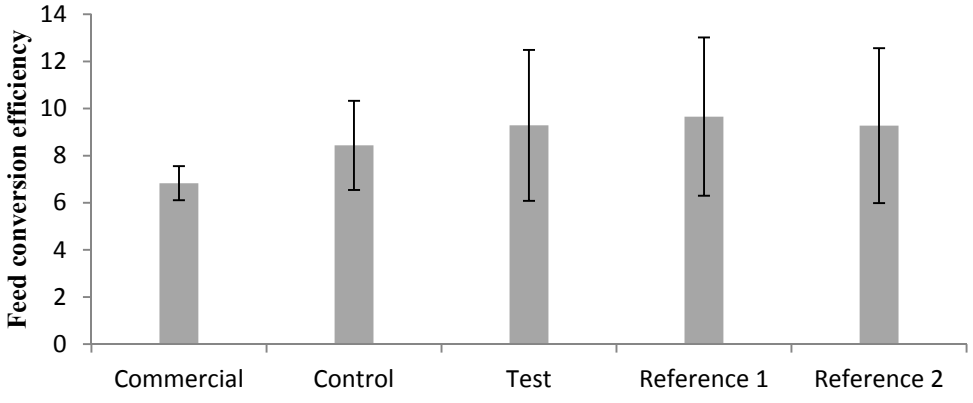


Figure 3.4: Feed conversion efficiency (FCE) for the 7-day rat feeding trial. The values represented are means ± SD.

Table 3.6: Change in absolute body weight (grams) using group means ± SD after 1 week, 2 weeks, 3 weeks and 4 weeks for the 28-day rat feeding trial.

Groups of rats (n=4)	Body weight				
	Day1	Day 8	Day 15	Day 22	Day 29
Commercial	190.25±11.32	228.75±6.84	271±12.80	293.5±22.05	322.25±27.70
Control	217±21.83	252.25±24.30	279.25±29.07	304.75±35.01	325.5±37.56
Test	188±31.05	220.25±34.82	251±37.46	263.5±38.85	335.25±31.43
Reference 1	202.25±21.23	232.5±27.34	257±29.24	276.75±31.82	296±38.68
Reference 2	214.75±14.63	249.75±16.72	276±23.25	303.5±32.41	323±37.37

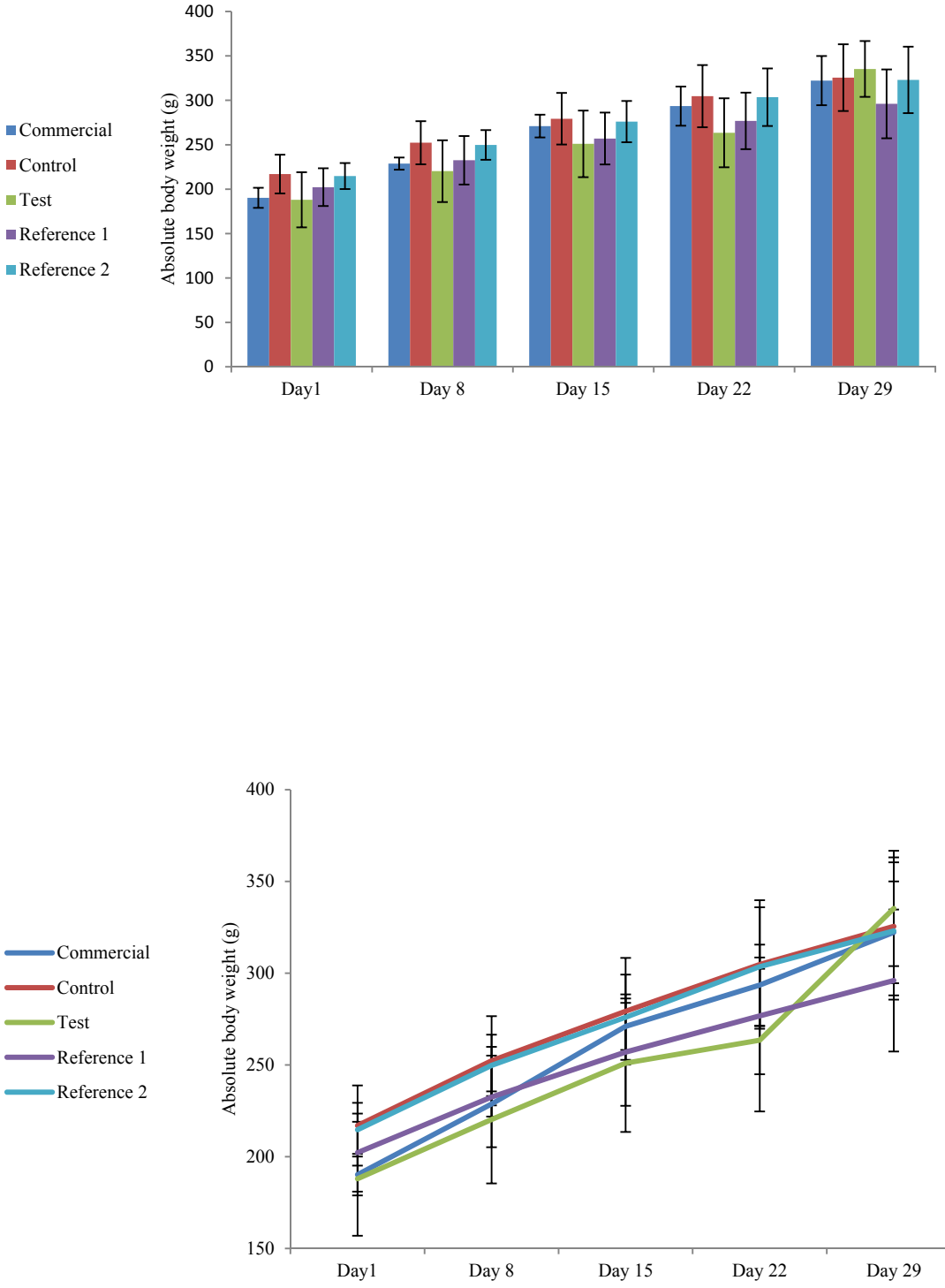


Figure 3.5: Change in absolute body weight (grams) using group means for the 28-day rat feeding trial. Values are means ± SD.

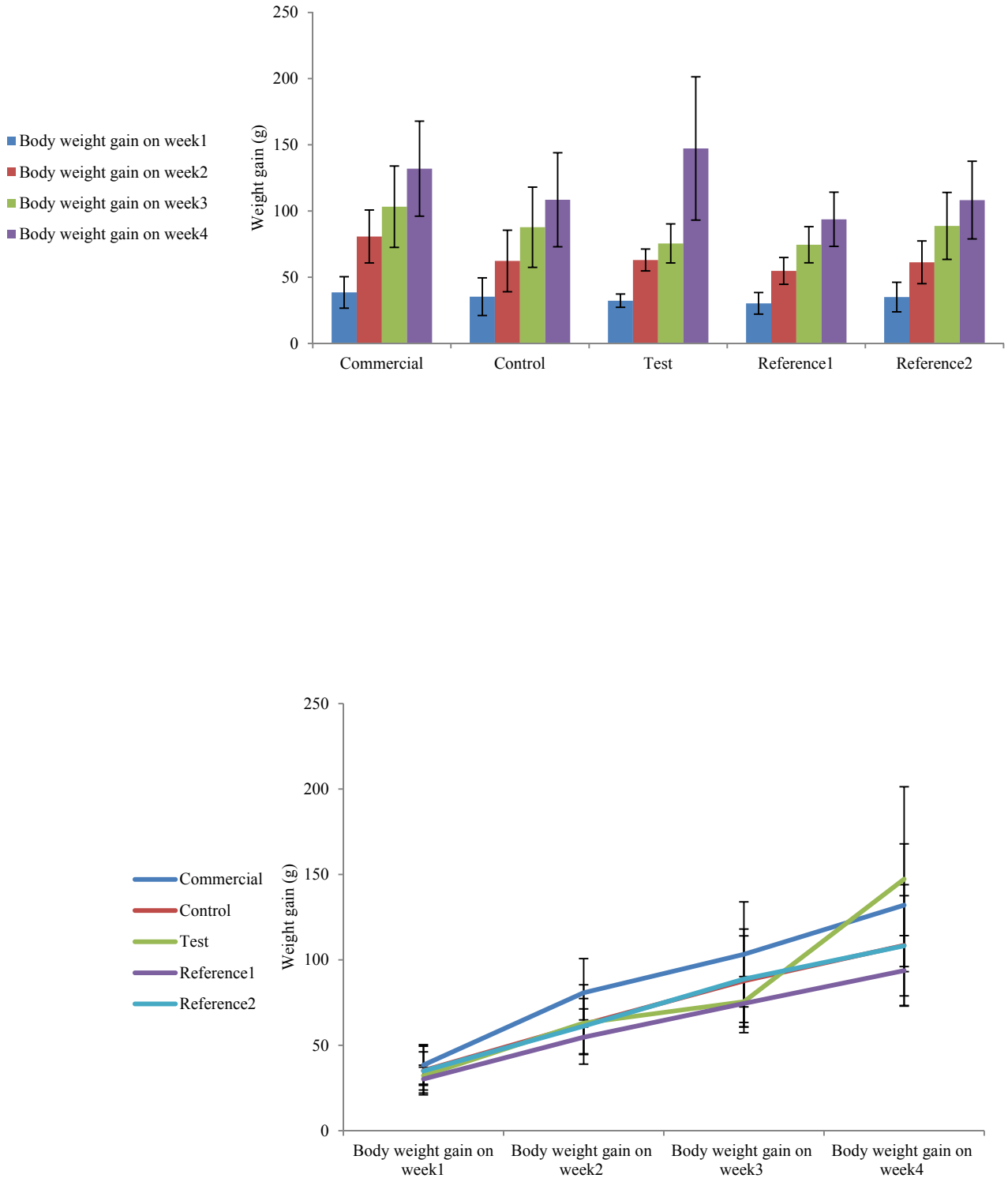


Figure 3.6: Change in body weight gain (grams) using group means for week 1, 2, 3 and 4 for the 28-day rat feeding trial. Values are means ± SD.

Table 3.7: Differences in body weight gain on day 29 (after 4 weeks) compared with the control diet for the 28-day rat feeding trial. Values are means ± SD.

Groups (n=4)	Body weight gain on week1	Body weight gain on week2	Body weight gain on week3	Body weight gain on week4	Difference with control (%)
Commercial	38.5±11.90	80.75±20.02	103.25±30.73	132±35.91	21.6
Control	35.25±14.22	62.25±23.22	87.75±30.31	108.5±35.51	---
Test	32.25±4.99	63±8.28	75.5±14.73	147.25±54.10	35.7
Reference1	30.25±8.18	54.75±10.14	74.5±13.67	93.75±20.43	-13.6
Reference2	35±11.16	61.25±16.15	88.75±25.31	108.25±29.34	-0.23

Table 3.8: Weekly food consumption (g) for the 28-day rat feeding trial using group means of weekly food intake.

Groups (n=4)	**week 1**	**week 2**	**week 3**	**week 4**
Commercial	226.75±8.05	268±10.09	301.5±18.57	330±27.94
Control	251.25±22.94	286.5±27.95	313.5±34.21	332.5±36.23
Test	220.25±35.34	251±36.76	272.25±39.85	294.75±31.85
Reference 1	232±27.01	263.25±32.38	286.75±32.71	303.75±37.16
Reference 2	251.25±17.05	283±21.83	310.5±31.11	329±35.13

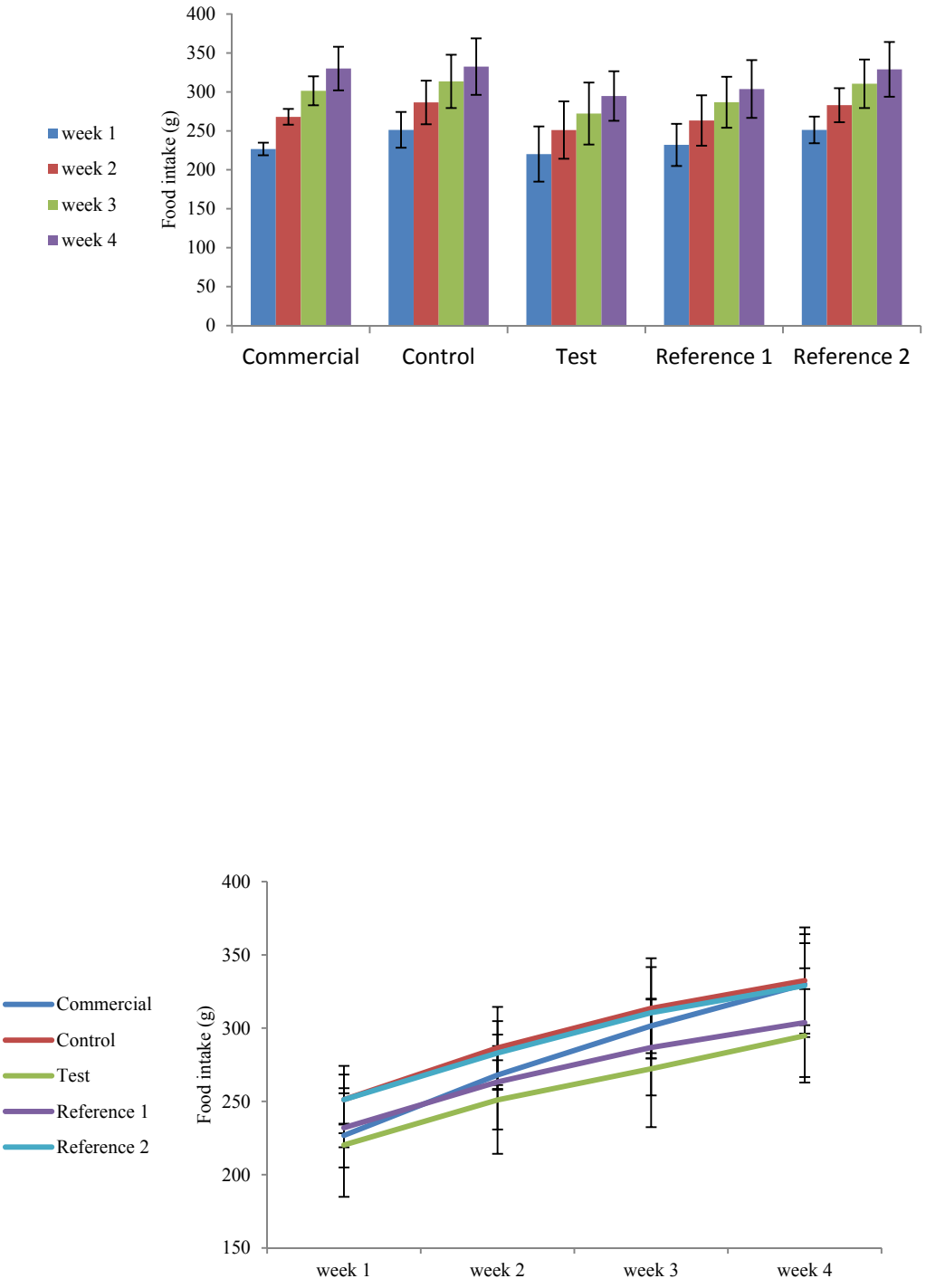


Figure 3.7: Weekly food consumption for the 28-day rat feeding trial using group means of weekly food intake.

Table 3.9: Feed conversion efficiency (FCE) for the 28-day rat feeding trial.

Group (n=4)	**week 1**	**week 2**	**week 3**	**week 4**
Commercial	6.37875±2.14	3.473456±0.83	3.0987563±0.80	2.611311±0.54
Control	8.836±5.66	5.2908617±2.50	3.9635924±1.53	3.322774±1.07
Test	6.856134±0.76	3.9916667±0.38	3.6703348±0.61	2.2794306±1.01
Reference 1	9.6563497±3.35	4.8603603±0.49	3.8945799±0.42	3.2969066±0.39
Reference 2	9.2781944±3.28	4.798677±0.90	3.6562076±0.73	3.1581234±0.58

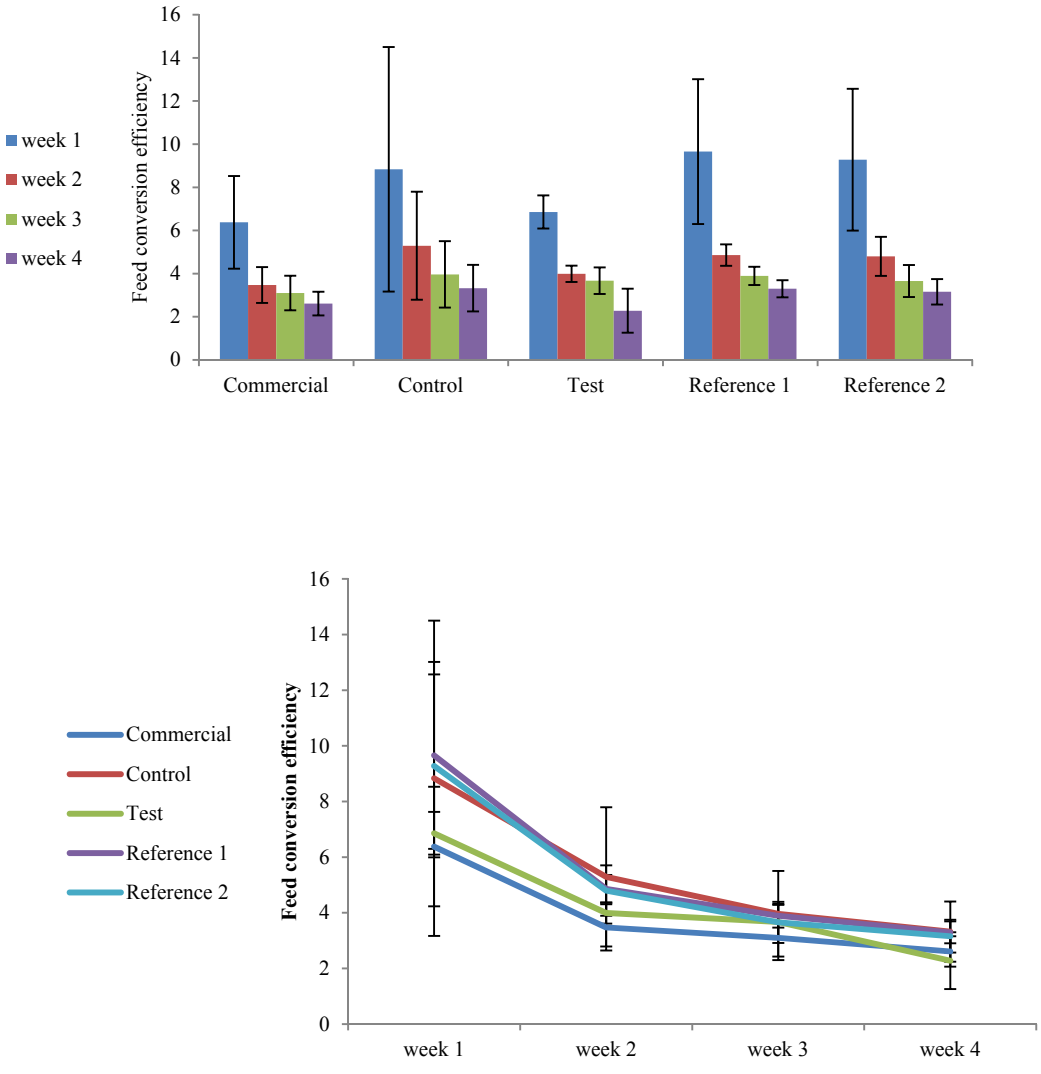


Figure 3.8: Feed conversion efficiency (FCE) for the 28-day rat feeding trial.

Chapter 4

4 *In vivo* Effects of Transgenic Maize Expressing the δ-Insecticidal Protein Cry 1Ab from *Bacillus thuringiensis* on Differential Gene Expression at the Proteome Level in the Epithelial Cells of the Small Intestine of Male Wistar Rats.

4.1 Separation of protein by 2-D gel electrophoresis

For 2-DE it is critical that equal amounts of protein are loaded on the gels. Thus total protein concentrations of the isolated rat small intestinal epithelial cells were determined using the 2-D Quant kit (GE healthcare, Sweden) as illustrated in Figure 4.1 and 4.2 and Appendix 25 and 26 (see Appendices to chapter 4).

All rat samples were initially separated in the first dimension by isoelectric focusing (IEF). Samples were focused successfully until they reached the steady state. The steady state of IEF was indicated by two conditions: 1) Final high voltage (10000V) was reached; 2) current decreased and reached steady state (Figure 4.3).

Following IEF all protein samples were then separated successfully by SDS-PAGE (Figure 4.4 and 4.5).

4.2 2D gel image analysis and spot picking gels

Initial identification of differentially expressed proteins was carried out by Nonlinear Dynamics Ltd (UK). However, before cutting the protein spots from the relevant gels for identification, further analysis was required.

The objectives for refining the analysis were:

1- To confirm the results of the detection of differentially expressed proteins.

2- To exclude false positives from the analysis.

3- To provide required information for picking the protein spots for identification by mass spectroscopy.

BSA µg	Standard
0	0.895
10	0.809
20	0.729
30	0.655
40	0.594
50	0.522

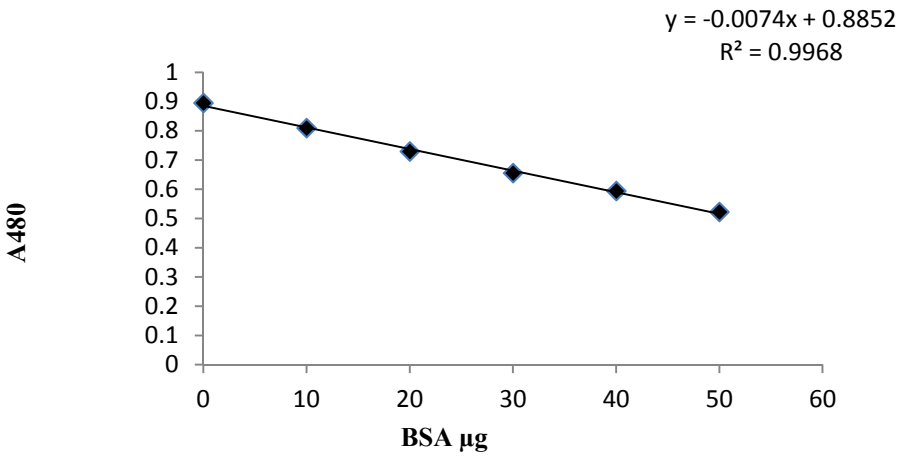


Figure 4.1: Standard calibration curve for protein concentration of the isolated rat small intestinal epithelial cells for the 7-day trial using bovine serum albumin (BSA) as a standard protein..

BSA µg	Standard
0	0.784
10	0.681
20	0.611
30	0.527
40	0.457
50	0.389

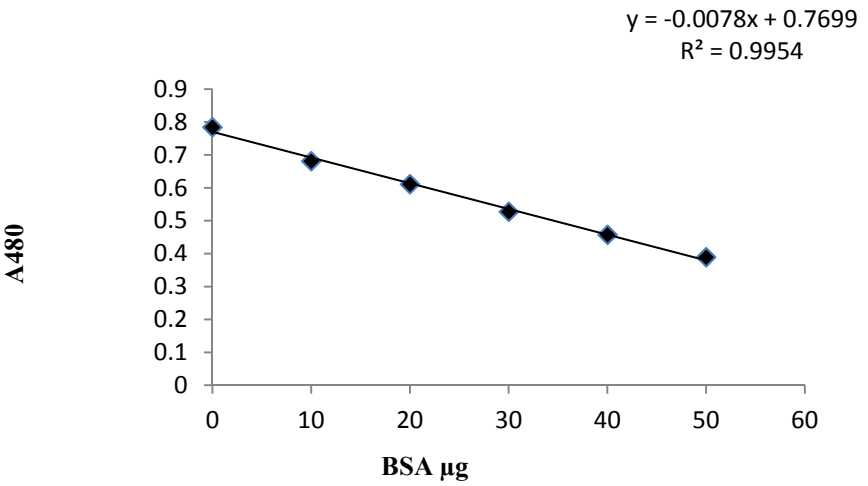


Figure 4.2: Standard calibration curve for protein concentration of the isolated rat small intestinal epithelial cells for the 28-day trial using bovine serum albumin (BSA) as a standard protein.

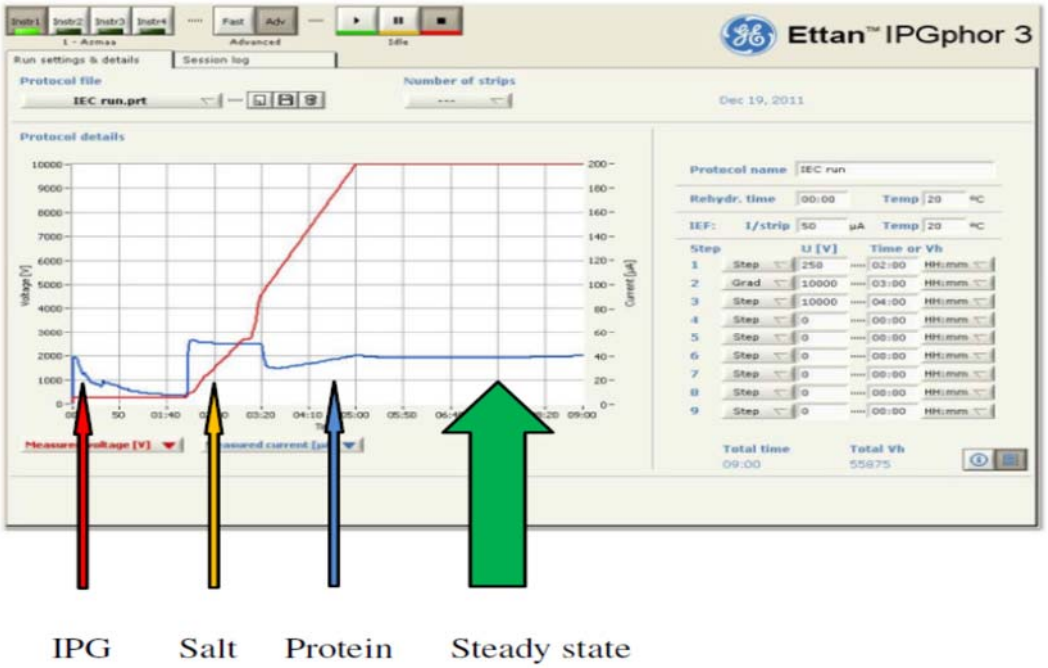


Figure 4.3: IEF run for rat samples showing recorded data for current, voltage and volt-hours (Vh). The first red arrow indicated the IPG buffer movement, the yellow arrow indicated the salt movement and the blue arrow indicated the protein movement. The green arrow indicated that all proteins were focussed and steady state was reached.

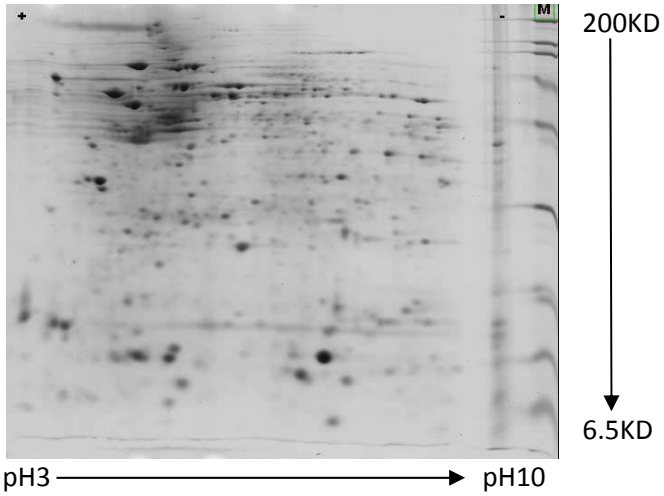


Figure 4.4: Representative image of the colloidal coomassie brilliant blue G-250 stained 2D gel (7-day trial).
2D gels were used to separate small intestinal epithelial cells proteins extracted from rats from the 7-day trial. The first dimension was performed using immobilized pH 3-10 gradients gel strips, followed by SDS-PAGE using 12.5% acrylamide gels.

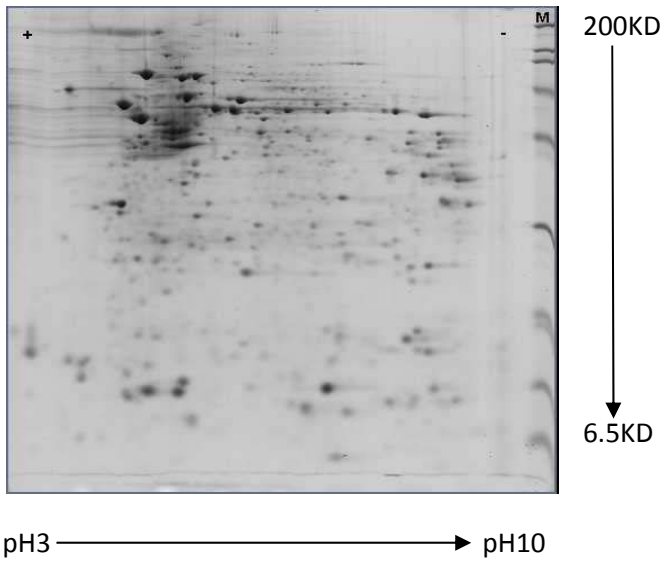


Figure 4.5: Representative image of the colloidal coomassie brilliant blue G-250 stained 2D gel (28-day trial).
2D gels were used to separate small intestinal epithelial cells proteins extracted from rats from the 28-day trial. The first dimension was performed using immobilized pH 3-10 gradients gel strips, followed by SDS-PAGE using 12.5% acrylamide gels.

Progenesis Samespot software version 4.1 was used for refining the analysis. Average normalized volume of 200,000 was selected. In general, the experimental design was not changed from the original analysis which was carried out by Nonlinear Dynamics Ltd (UK); several artefacts and false positives were removed. There were also some spots that were detected as differentially expressed proteins, however they were quite faint on the actual gels and were classified as "unpickable" spots. Some protein spots were considered not to have a sufficiently high protein content for identification by mass spectroscopy. Examples of such proteins are presented in Figure 4.6. Because of the high resolution of the images and magnifying tools in Progenesis software, the number of the observed protein spots by using the software might be higher than the pickable protein spots in actual gels.

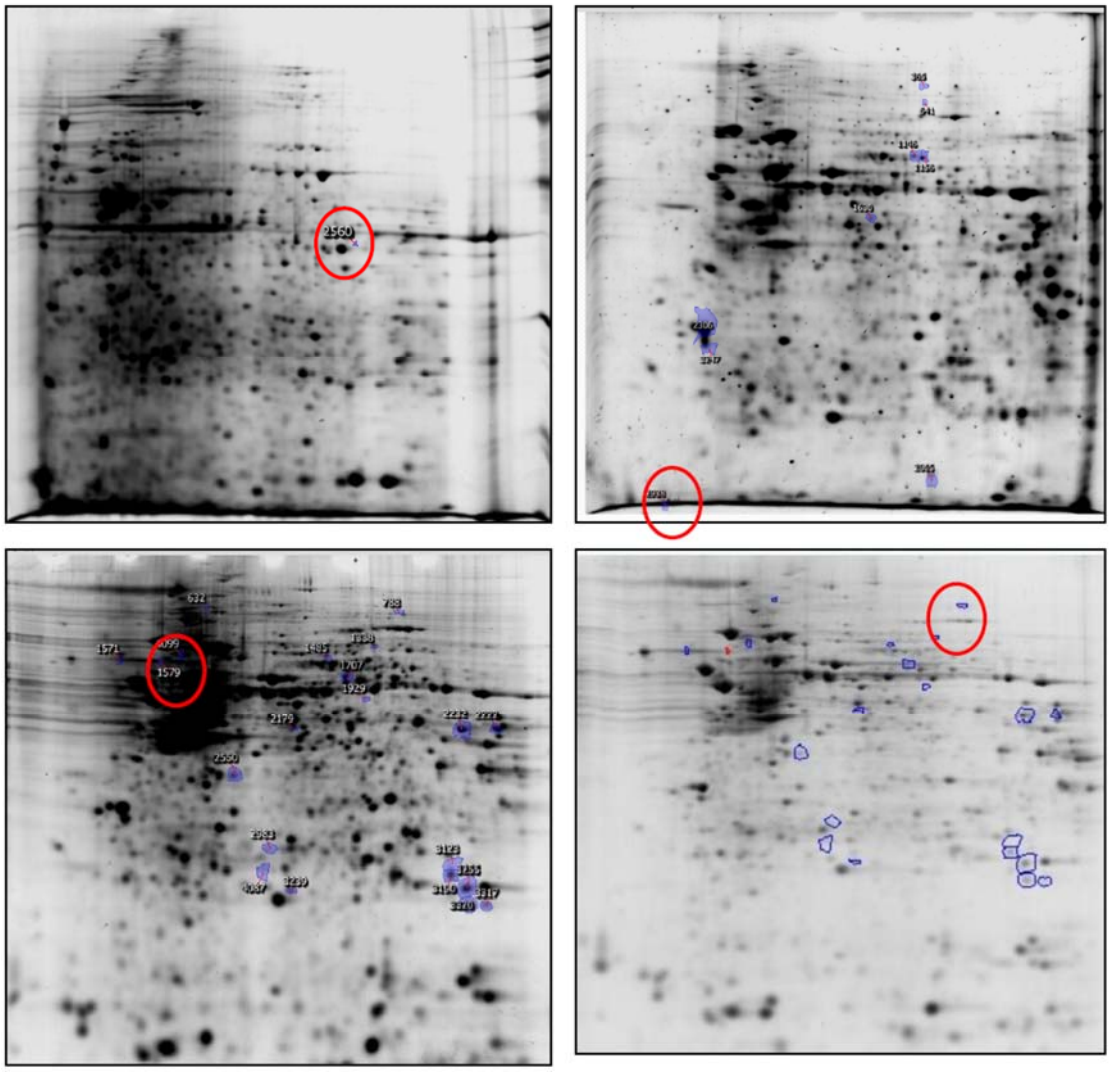


Figure 4.6: Examples of the protein spots that are detected as differentially expressed protein by Progenesis software, but are false positives or not practically pickable.
These spots are indicated in red zones.

4.2.1 7 Day rat feeding trial: gel image analysis

Spots were filtered by an average normalised volume (pixel intensity over spot area) of 200,000. This removed 276 spots, leaving 1226 to be taken forward for analysis. The number of spots to pick for protein identification reduced from 136 to 52. In 'View Results', all filters were removed and all groups selected. The entire complement of 1226 spots were ranked by *p*-value (low to high) then viewed individually to highlight their relevance. From the initial work, there were many spots shown to be significant, yet were actually anomalous results. Spots were discounted from subsequent analysis according to the following:

1-Too close to edge of gel, out of the gel (Figure 4.6).

2- Contained streaks (Figure 4.7).

3- Spots within the area of high protein concentration, acidic, high Mw, problematic separation (Figure 4.8).

With the original experimental design and the original filters in place, the refined data set was placed into the same groupings to show the differentially expressed spots. Whilst the original data gave 136 spots as differentially expressed, the refined data reduced them to 52 spots (Table 4.1). Spot picking gels are illustrated in Appendix 1 to 10 (see Appendices to chapter 4).

4.2.2 28 Day rat feeding trial: gel image analysis

There were 1722 spots in the filtering step. A filter by average normalised volume of 200,000 removed 225 spots. A further 50 spots were removed from the size standard of the gel, thus leaving 1447 (Appendix 11). From the initial work, there were again many spots shown to be significant, yet were actually anomalous results. Spots were removed according to the following:

1- Contained streaks.
2- De-staining issues leading to higher background signal, realising a false positive.
3- Too close to edge of gel, problematic separation.
4- Extra care was taken when assessing spots within the area of high protein concentration; acidic, high Mw.
5- Issues with alignment (Appendix 12).

The original data gave 73 spots as being differentially expressed, however the refined data reduced them to 38 spots (Table 4.2 and Figure 4.9).

Spot picking gels are illustrated in Appendix 13 to 20 (see Appendices to chapter 4). Protein spots that were selected and picked for both 7 and 28-day rat feeding trials are illustrated in Table 4.3.

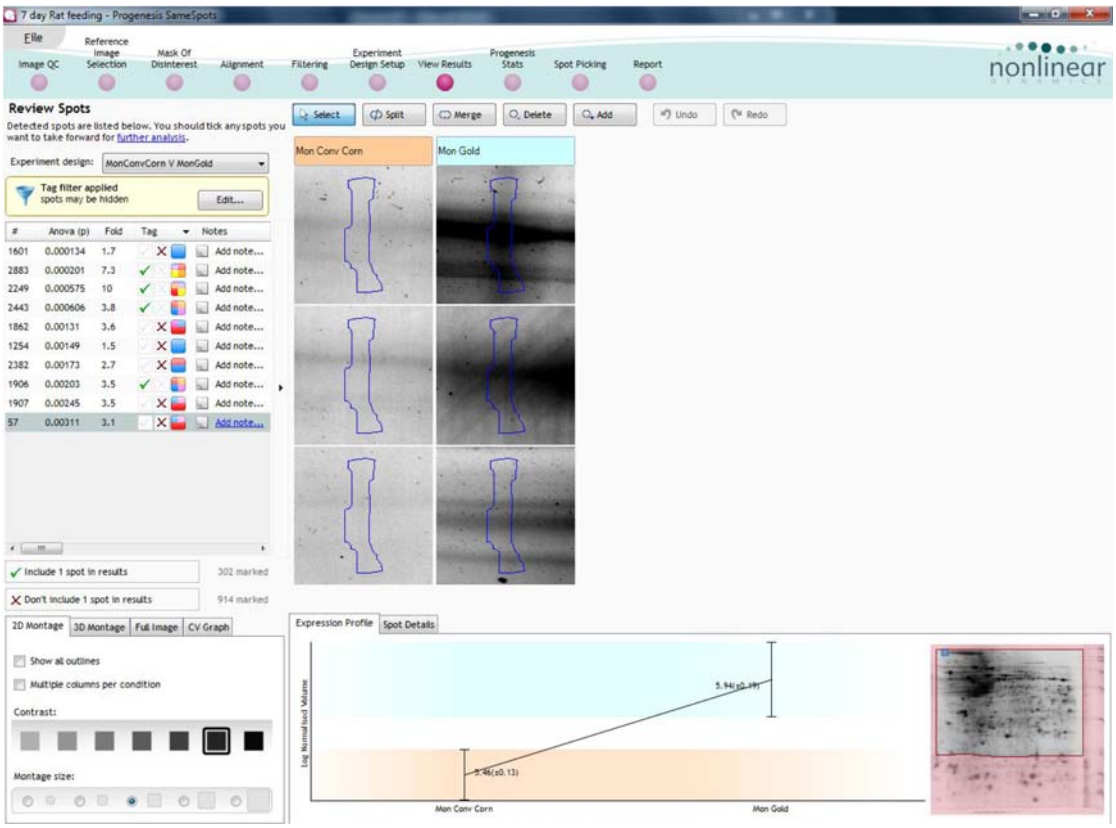


Figure 4.7: Example of Spot streaking at edge of gel.

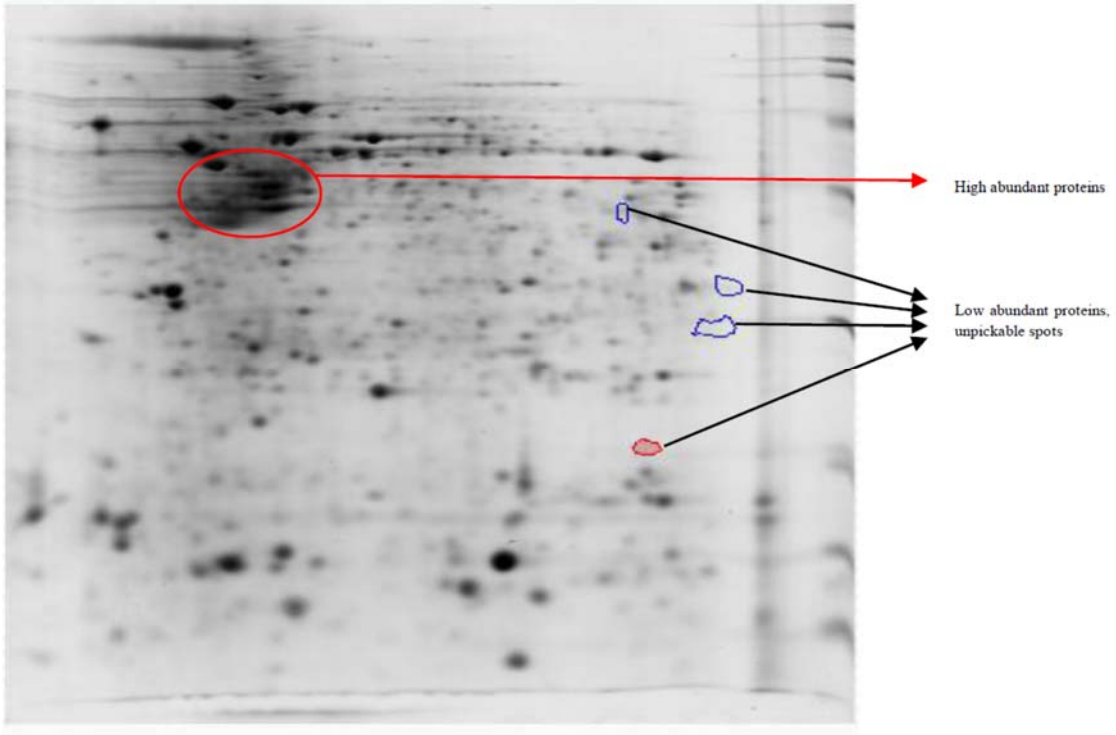


Figure 4.8: Area of high protein concentration, acidic side of gel, high MW.

Table 4.1: Summary of results of the original number of protein spots and the number of spots after refining for the 7-day rat feeding trial.

Treatment	Original number of spots	Spots after refining
MCert V Mon 810	09	04
MCert V Mon Garst	23	07
MCert V Mon Conv Corn	30	12
MCert V Mon Gold	13	01
Mon 810 V Mon Garst	15	07
Mon 810 V Mon Conv Corn	23	10
Mon810 V Mon Gold	03	01
Mon Garst V Mon Conv Corn	16	06
Mon Garst V Mon Gold	01	01
Mon Conv Corn V Mon Gold	03	03
Total	136	52

Table 4.2: Summary of results of the original number of protein spots and the number of spots after refining for the 28-day rat feeding trial.

Treatment	Original number of spots	Spots after refining
MCert V Mon 810	00	00 (20*)
MCert V Mon Garst	02	01
MCert V Mon Conv Corn	10	07
MCert V Mon Gold	03	02
Mon 810 V Mon Garst	15	09
Mon 810 V Mon Conv Corn	21	12
Mon810 V Mon Gold	16	05
Mon Garst V Mon Conv Corn	05	01
Mon Garst V Mon Gold	00	00
Mon Conv Corn V Mon Gold	01	01
Total	73	38

*According to P and Q values there were zero significant spots. By switching from Q-value to fold change of >1.5 these 20 spots were detected and this shows how the software can alter the results by changing some parameters.

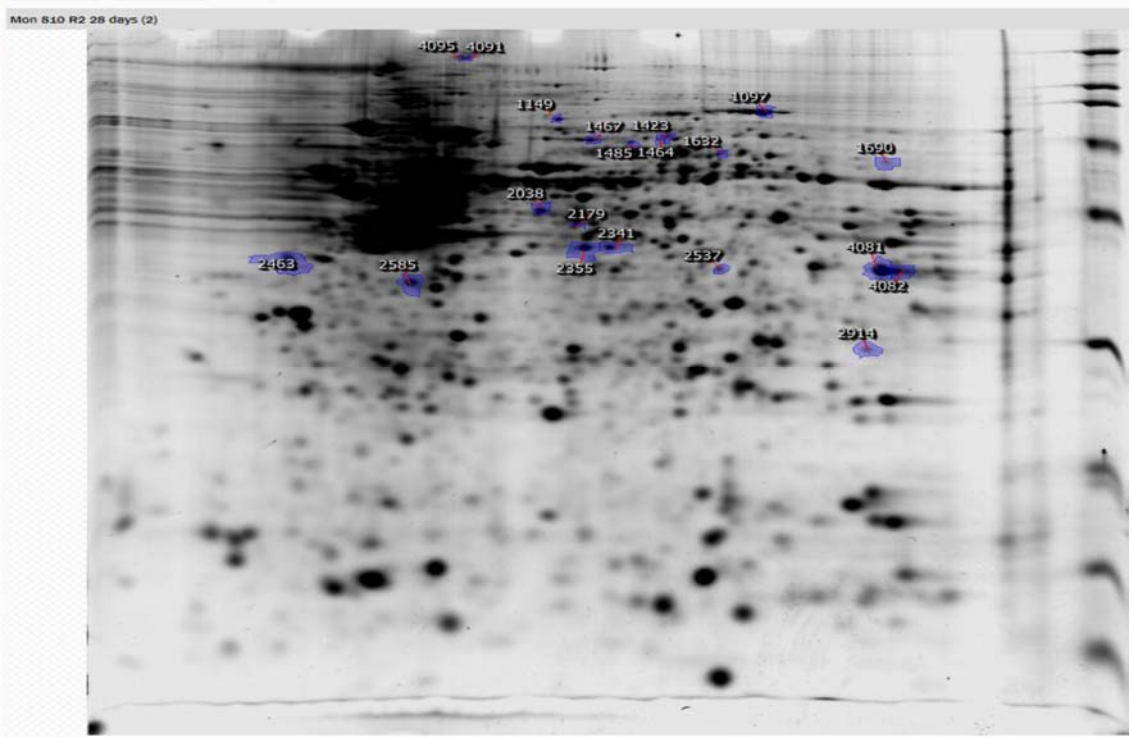


Figure 4.9: According to P and Q values there were zero significant spots when comparing between MCert and MON810 groups for the 28-day rats feeding trial.
By switching from Q-value to fold change of >1.5 the following list of spots was obtained.

Table 4.3: Protein spots numbers that were picked for both 7 and 28-day rat feeding trials.

7-Day rats feeding trial (29 Spots)										28-Day rats feeding trial (25 spots)							
MCertVMon810	MCertVMonGarst	MCertVMonConvCorn	MCertVMonGold	Mon810VMonGarst	Mon810VMonConv Corn	Mon810VMonGold	MonGarstVMonConv Corn	MonGarstVMonGold	MonConvCornVMon Gold	MCertvsMonConvCorn	MCertvsMonGarst	MCertvsMonGold	Mon810vsMonConvCorn	Mon810vsMonGarst	Mon810 vs MonGold	MonConvCorn vs Mon Garst	MonConvCorn vs MonGold
1146 1630 2306 3258	1222 1516 1976 2212 2608 3243 3248	1146 1580 1976 1950 2016 2021 2212 2045 2249 2400 2511 2883	2249	1950 2048 2212 2279 2626 3243 3248	1328 1777 1950 2004 2045 2212 2249 2400 2443 2883	2626	1257 1777 2249 2400 2443 2883	1444	2249 2443 2883	2550 2762 3123 3190 3305 3320 3506	2912	2149 3305	1485 1707 2179 2232 2222 2442 2550 3123 3190 3320 3255 3506	1485 1960 1972 2209 2912 2893 2965 4082 4101	1485 1564 2149 2232 3305	1972	2149

4.3 Protein identification using the global proteome machine (GPM) search engine

Protein IDs were done at the Burchmore lab, Glasgow Polyomics, University of Glasgow. For protein identifications, the analysis of mass spectrometry data were assigned using the Global Proteome Machine (GPM) search engine (www.thegpm.org) to interrogate protein sequences in the ENSEMBL *Rattus Norvegicus* database as illustrated in Appendix 21 to 23. The common Repository of Adventitious Proteins, cRAP, is an attempt to create a list of proteins commonly found in proteomics experiments that are present either by accident or through unavoidable contamination of protein samples. The types of proteins included fall into three general classes:

1. common laboratory proteins;
2. proteins added by accident through dust or physical contact; and
3. proteins used as molecular weight or mass spectrometry quantitation standards.

A list of contaminants can be found at both GPM and Mascot sites, they call it cRAP. The GPM link which was used in this study is: http://www.thegpm.org/crap/
GPM database with over 100 junk proteins is already linked to other databases including rat. That is why human keratin is in the list of identified proteins (Appendix 24).

Most of the proteins from the rat small intestine (s.i.) epithelial cells from both the 7-day and 28-day study were successfully identified as illustrated in Appendix 27 and 28. The LC–MS/MS analysis of 29 colloidal comassie brilliant blue-stained 2-D gel spots for the 7-day rats feeding trial generated a total of 69 protein identifications. Of these 29 spots, 20 were a mixture of two or more proteins, and 9 generated single protein identification for each spot. Some proteins were identified from multiple spots. For example, in the 7-day rat feeding trial, catalase was identified from three spots. Catalase was identified in spots 1146, 1257, and 1328. Similarly, protein disulfide-isomerase A3 was identified from four spots. Protein disulfide-isomerase A3 was identified in spots 1444, 1516, 1580, and 1630 (Table 4.4). LC–MS/MS analysis of 25 colloidal comassie brilliant blue-stained 2-D gel spots for the 28-day rat feeding trial generated a total of 39 protein identifications. Of these 25 spots, 18 were a mixture of two or more proteins, and 7 generated single protein identification for each spot. Some proteins were identified from multiple spots. For example, in the 28-days rat feeding trial, stress-induced-phosphoprotein 1 was identified

from two spots. Stress-induced-phosphoprotein 1 was identified in spots 1485 and 1564 (Table 4.5).

4.3.1 Comparison of differentially expressed proteins from rat small intestine epithelial cells: 7-day study

The venn diagrams illustrated in Figure 4.10 and 4.11 show the number of 2D gel protein spots identified by Progenesis SameSpots software (Nonlinear Dynamics Ltd, Newcastle upon Tyne, UK) between two diet groups (Figure 4.10) and all the five diet groups (Figure 4.11) that were differentially expressed and up-regulated when rats were fed for 7 day on conventional diet or the parental control for MON810 corn diet (Mon Conv Corn) which was formulated to contain approximately 33% control corn grain; test diet (MON 810 group) which was formulated to contain the test corn grain at approximately 33%; reference diets (Mon Garst and Mon Gold Groups) which were formulated to contain the references corn grain at approximately 33% and commercial Purina rodent chow (Mcert) which was purchased from Purina Mills Inc and normally contains approximately 33% corn. The threshold used to qualify differentially expressed spots was a Student t test *P* value less than 0.05 and the false discovery rate (FDR, from q-values).

Comparison of differentially expressed proteins between MON810 and Mon Conv Corn diet groups

The venn diagram illustrated in Figure 4.10 a summarizes the significantly regulated spots between MON810 and Mon Conv Corn groups. One thousand two hundred and sixteen protein spots were identified to be commonly expressed in both MON810 and Mon Conv Corn, whereas 4 up-regulated spots were identified for MON810 fed rats (Spot No. 1950, 2212, 1328, 2045) and 6 up-regulated spots were identified for Mon Conv Corn fed rats (Spot No. 2883, 2443, 2400, 2004, 1777, 2249).

Comparison between differentially expressed proteins between rats fed MON810 and Mon Conv Corn showed that 2 stress-related proteins (catalase and 60 kDa heat shock protein) were up-regulated (2.4 fold) in the former group but no stress related proteins were up-regulated in Mon Conv Corn group.

Comparison of differentially expressed proteins between MON810 and Mcert diet groups

The Venn diagram (Figure.4.10 b) summarizes the significantly regulated spots between Mcert and MON810 groups. One thousand two hundred and twenty two spots were identified to be commonly expressed in both Mcert and MON810, whereas only 2 up-regulated protein spots were identified for Mcert fed rats (Spot No. 2306 and 3258) and 2 up-regulated spots were identified for MON810 fed rats (Spot No. 1146 and 1630).

Comparison between differentially expressed proteins between rats fed MON810 and Mcert showed that 3 stress-related proteins (catalase, 60 kDa heat shock protein and stress-induced phosphoprotein 1) were up-regulated (between 2.2-2.5 fold) in the former group but no stress related proteins were up-regulated in Mcert group.

Comparison of differentially expressed proteins between Mcert and Mon Garst diet groups

The venn diagram illustrated in Figure 4.10 c summarizes the significantly regulated spots between Mcert and Mon Garst groups. One thousand two hundred and nineteen spots were identified to be commonly expressed in both Mcert and Mon Garst, whereas 7 up-regulated spots were identified for Mcert fed rats (Spot No. 3248, 1222, 2212, 3243, 2608, 1976, 1516).

Comparison between differentially expressed proteins between rats fed Mcert and Mon Garst showed that 2 stress-related proteins (thioredoxin-dependent peroxide reductase and protein DJ-1) were up-regulated (between 1.4-2.6 fold) in the former group but no stress related proteins were up-regulated in Mon Garst group.

Comparison of differentially expressed proteins between Mcert and Mon Conv Corn diet groups

The venn diagram illustrated in Figure 4.10 d summarizes the significantly regulated spots between Mcert and Mon Conv Corn groups. One thousand two hundred and fourteen spots were identified to be commonly expressed in both Mcert and Mon Conv Corn, whereas 7 up-regulated spots were identified for Mcert fed rats (Spot No. 1580, 2511, 2016,

2212, 2045, 1976, 1950) and 5 up-regulated spots were identified for Mon Conv corn fed rats (Spot No. 2883, 2021, 2249, 1146, 2400).

Comparison between differentially expressed proteins between rats fed Mcert and Mon Conv Corn showed that 1 stress-related protein (60 kDa heat shock protein) was up-regulated (3 fold) in the former group and 1 stress related protein (stress-induced phosphoprotein 1) was up-regulated in Mon Conv Corn group.

Comparison of differentially expressed proteins between Mcert and Mon Gold diet groups

The venn diagram illustrated in Figure 4.10 e summarizes the significantly regulated spots between Mcert and Mon Gold groups. One thousand two hundred and twenty five spots were identified to be commonly expressed in both Mcert and Mon Gold, whereas only 1 up-regulated spot was identified for Mcert fed rats (Spot No. 2249).

Comparison between differentially expressed proteins between rats fed Mcert and Mon Gold showed that no stress-related proteins were up-regulated.

Comparison of differentially expressed proteins between MON810 and Mon Garst diet groups

The venn diagram illustrated in Figure 4.10 f summarizes the significantly regulated spots between MON810 and Mon Garst groups. One thousand two hundred and nineteen spots were identified to be commonly expressed in both MON810 and Mon Garst, whereas 7 up-regulated spots were identified for MON810 fed rats (Spot No. 2212, 3248, 2279, 3243, 1950, 2626, 2048).

Comparison between differentially expressed proteins between rats fed MON810 and Mon Garst showed that 3 stress-related proteins (thioredoxin-dependent peroxide reductase, peroxiredoxin-6 and LDLR chaperone MESD precursor) were up-regulated (1.8-2.5 fold) in the former group.

Comparison of differentially expressed proteins between MON810 and Mon Gold diet groups

The venn diagram illustrated in Figure 4.10 g summarizes the significantly regulated spots between MON810 and Mon Gold groups. One thousand two hundred and twenty five spots were identified to be commonly expressed in both MON810 and Mon Gold, whereas only 1 up-regulated spotwas identified for MON810 fed rats (Spot No. 2626).

Comparison between differentially expressed proteins between rats fed MON810 and Mon Gold showed that 2 stress-related proteins (peroxiredoxin-6 and LDLR chaperone MESD precursor) were up-regulated (1.2 fold) in the former group.

Comparison of differentially expressed proteins between Mon Garst and Mon Conv Corn diet groups

The venn diagram illustrated in Figure 4.10 h summarizes the significantly regulated spots between Mon Garst and Mon Conv Corn groups. One thousand two hundred and twenty spots were identified to be commonly expressed in both Mon Garst and Mon Conv Corn, whereas 6 up-regulated spots were identified for Mon Conv Corn fed rats (Spot No. 2249, 1257, 1777, 2400, 2883, 2443).

Comparison between differentially expressed proteins between rats fed Mon Conv Corn and Mon Garst showed that 1 stress-related protein (catalase) was up-regulated (2.6 fold) in the former group.

Comparison of differentially expressed proteins between Mon Garst and Mon Gold diet groups

The venn diagram illustrated in Figure 4.10 i summarizes the significantly regulated protein spots between Mon Garst and Mon Gold groups. One thousand two hundred and twenty five spots were identified to be commonly expressed in both Mon Garst and Mon Gold, whereas only 1 up-regulated spot was identified for Mon Garst fed rats (Spot No.1444).

Comparison between differentially expressed proteins between rats fed Mon Garst and Mon Gold showed that 1 stress-related protein (T-complex protein 1 subunit beta) was up-regulated (1.5 fold) in the former group.

Comparison of differentially expressed proteins between Mon Conv Corn and Mon Gold diet groups

The venn diagram illustrated in Figure 4.10 j summarizes the significantly regulated spots between Mon Conv Corn and Mon Gold groups. One thousand two hundred and twenty three spots were identified to be commonly expressed in both Mon Conv Corn and Mon Gold, whereas 3 up-regulated spots were identified for Mon Conv Corn fed rats (Spot No. 2883, 2249, and 2443).

Comparison between differentially expressed proteins between rats fed Mon Conv Corn and Mon Gold showed that no stress-related proteins were up-regulated.

Comparison of differentially expressed proteins between all 5 diet groups: 7-day study

When all five groups were compared there were 5, 4, 3, 0, 0, differentially expressed protein spots for Mcert, Mon Conv Corn, MON810, Mon Garst and MON Gold, respectively (Figure 4.11).

Comparison between differentially expressed proteins between rats fed Mcert, Mon Conv Corn, MON810, Mon Garst and MON Gold showed that only 2 stress-related proteins (LDLR chaperone MESD precursor and peroxiredoxin-6) were up-regulated (2.4 fold) in the MON810 group and 1 stress-related protein (thioredoxin-dependent peroxide reductase) was up-regulated (2.6 fold) in the Mcert group.

4.3.2 Comparison of differentially expressed proteins from rat small intestine epithelial cells: 28-day study

The venn diagrams illustrated in Figure 4.12 and 4.13 show the number of 2D gel protein spots identified by Progenesis SameSpots software (Nonlinear Dynamics Ltd, Newcastle upon Tyne, UK) between two groups (Figure 4.12) and all the five groups (Figure 4.13) that were differentially expressed and up-regulated when rats were fed for 28-days on conventional diet or the parental control for MON810 corn diet (Mon Conv Corn)

which was formulated to contain approximately 33% control corn grain; test diet (MON 810 group) which was formulated to contain the test corn grain at approximately 33%; reference diets (Mon Garst and Mon Gold Groups) which were formulated to contain the references corn grain at approximately 33% and commercial Purina rodent chow (Mcert) which was purchased from Purina Mills Inc and normally contains approximately 33% corn. The threshold used to qualify differentially expressed spots was a Student t test *P* value less than 0.05 and the false discovery rate (FDR, from q-values).

Comparison of differentially expressed proteins between MON810 and Mon Conv Corn diet groups

The venn diagram illustrated in Figure 4.12 a summarizes the significantly regulated spots between MON810 and Mon Conv Corn groups. One thousand four hundred and thirty five spots were identified to be commonly expressed in MON810 and Mon Conv Corn, whereas 9 up-regulated spots were identified for MON810 fed rats (Spot No. 3320, 1485, 2550, 2222, 3190, 2179, 1707, 2232, 2442) and 3 up-regulated spots were identified for Mon Conv Corn fed rats (Spot No. 3123, 3506, 3255).

Comparison between differentially expressed proteins between rats fed MON810 and Mon Conv Corn showed that 2 stress-related proteins (stress-induced phosphoprotein 1 and peroxiredoxin-1) were up-regulated (between 2.5-3.1 fold) in the former group and 1 stress related protein (superoxide dismutase) was up-regulated (1.5 fold) in Mon Conv Corn group.

Comparison of differentially expressed proteins between MON810 and Mcert diet groups

The Venn diagram (Figure 4.12 b) shows that there were no differentially expressed spots between Mcert and MON810 groups. One thousand four hundred and forty seven spots were identified to be commonly expressed in both Mcert and MON810 groups.

Comparison of differentially expressed proteins between Mcert and Mon Garst diet groups

The venn diagram illustrated in Figure 4.12 c summarizes the significantly regulated spots between Mcert and Mon Garst groups. One thousand four hundred and forty six

spots were identified to be commonly expressed in both Mcert and Mon Garst, whereas only 1 up-regulated spot was identified for Mcert fed rats (Spot No. 2912).

Comparison between differentially expressed proteins between rats fed Mcert and Mon Garst showed that no stress-related proteins were up-regulated.

Comparison of differentially expressed proteins between Mcert and Mon Conv Corn diet groups

The venn diagram illustrated in Figure 4.12 d summarizes the significantly regulated spots between Mcert and Mon Conv Corn groups. One thousand four hundred and forty spots were identified to be commonly expressed in both Mcert and Mon Conv Corn, whereas 4 up-regulated spots were identified for Mcert fed rats (Spot No. 3190, 3320, 2550, 2762) and 3 up-regulated spots were identified for Mon Conv corn fed rats (Spot No. 3123, 3506, 3305).

Comparison between differentially expressed proteins between rats fed Mcert and Mon Conv Corn showed that 1 stress-related protein (peroxiredoxin-1) was up-regulated (2.8 fold) in the former group.

Comparison of differentially expressed proteins between Mcert and Mon Gold diet groups

The venn diagram illustrated in Figure 4.12 e summarizes the significantly regulated spots between Mcert and Mon Gold groups. One thousand four hundred and forty five spots were identified to be commonly expressed in both Mcert and Mon Gold, whereas only 1 up-regulated spot was identified for Mcer fed rats (Spot No. 2149) and 1 up-regulated spot was identified for Mon Gold fed rats (Spot No. 3305).

Comparison between differentially expressed proteins between rats fed Mcert and Mon Gold showed that no stress-related proteins were up-regulated.

Comparison of differentially expressed proteins between MON810 and Mon Garst diet groups

The venn diagram illustrated in Figure 4.12 f summarizes the significantly regulated spots between MON810 and Mon Garst groups. One thousand four hundred and thirty

eight spots were identified to be commonly expressed in both MON810 and Mon Garst, whereas 7 up-regulated spots were identified for MON810 fed rats (Spot No. 2209, 1485, 1960, 1972, 2912, 4082, 4101) and 2 up-regulated spots were identified for Mon Garst fed rats (Spot No. 2893, 2965).

Comparison between differentially expressed proteins between rats fed MON810 and Mon Garst showed that 2 stress-related proteins (stress-induced phosphoprotein 1 and 60 kDa heat shock protein) were up-regulated (between 2.2-2.4 fold) in the former group and 1 stress related protein (T-complex protein 1 subunit beta) was up-regulated (3.1 fold) in Mon Garst group.

Comparison of differentially expressed proteins between MON810 and Mon Gold diet groups

The venn diagram illustrated in Figure 4.12 g summarizes the significantly regulated spots between MON810 and Mon Gold groups. One thousand four hundred and forty two spots were identified to be commonly expressed in both MON810 and Mon Gold, whereas 4 up-regulated spots were identified for MON810 fed rats (Spot No. 2232, 2149, 1564, 1485) and 1 up-regulated spot was identified for Mon Gold fed rats (Spot No. 3305).

Comparison between differentially expressed proteins between rats fed MON810 and Mon Gold showed that 2 stress-related proteins (2 stress-induced phosphoprotein 1) were up-regulated (between 1.8-2.4 fold) in the former group.

Comparison of differentially expressed proteins between Mon Garst and Mon Conv Corn diet groups

The venn diagram illustrated in Figure 4.12 h summarizes the significantly regulated spots between Mon Garst and Mon Conv Corn groups. One thousand four hundred and forty six spots were identified to be commonly expressed in both Mon Garst and Mon Conv Corn, whereas only 1 up-regulated spot was identified for Mon Conv Corn fed rats (Spot No. 1972).

Comparison between differentially expressed proteins between rats fed Mon Garst and Mon Conv Corn showed that no stress-related proteins were up-regulated.

Comparison of differentially expressed proteins between Mon Garst and Mon Gold diet groups

The venn diagram illustrated in Figure 4.12 i summarizes the significantly regulated spots between Mon Garst and Mon Gold groups. One thousand four hundred and forty seven spots were identified to be commonly expressed in both Mon Garst and Mon Gold, whereas no differentially expressed spots between Mon Garst and Mon Gold groups were found.

Comparison of differentially expressed proteins between Mon Conv Corn and Mon Gold diet groups

The venn diagram illustrated in Figure 4.12 j summarizes the significantly regulated spots between Mon Conv Corn and Mon Gold groups. One thousand four hundred and forty six spots were identified to be commonly expressed in both Mon Conv Corn and Mon Gold groups, whereas only 1 up-regulated spot was identified for Mon Conv Corn fed rats (Spot No. 2149).

Comparison between differentially expressed proteins between rats fed Mon Conv Corn and Mon Gold showed that no stress-related proteins were up-regulated.

Comparison of differentially expressed proteins between all 5 diet groups: 28-day study

When all five groups were compared, only two spots were differentially expressed, these being in the rats fed MON810 (Figure 4.13). Comparison between differentially expressed proteins between rats fed Mcert, Mon Conv Corn, MON810, Mon Garst and MON Gold showed that 1 stress-related protein (stress-induced phosphoprotein 1) was up-regulated (3.1 fold) in the MON 810 group.

In order to assess functional relevance of changes in the identified differentially expressed proteins, the proteins were aligned into 28 groups according to their molecular functions for the 7-day rat feeding trial and into 21 groups for the 28-day rat feeding trial (Table 4.4 and 4.5; Figure 4.14, 4.15 and 4.16). Categorizations were based on information provided by the online resource UniProt classification system. Some proteins were annotated manually, based on literature searches and closely related homologues.

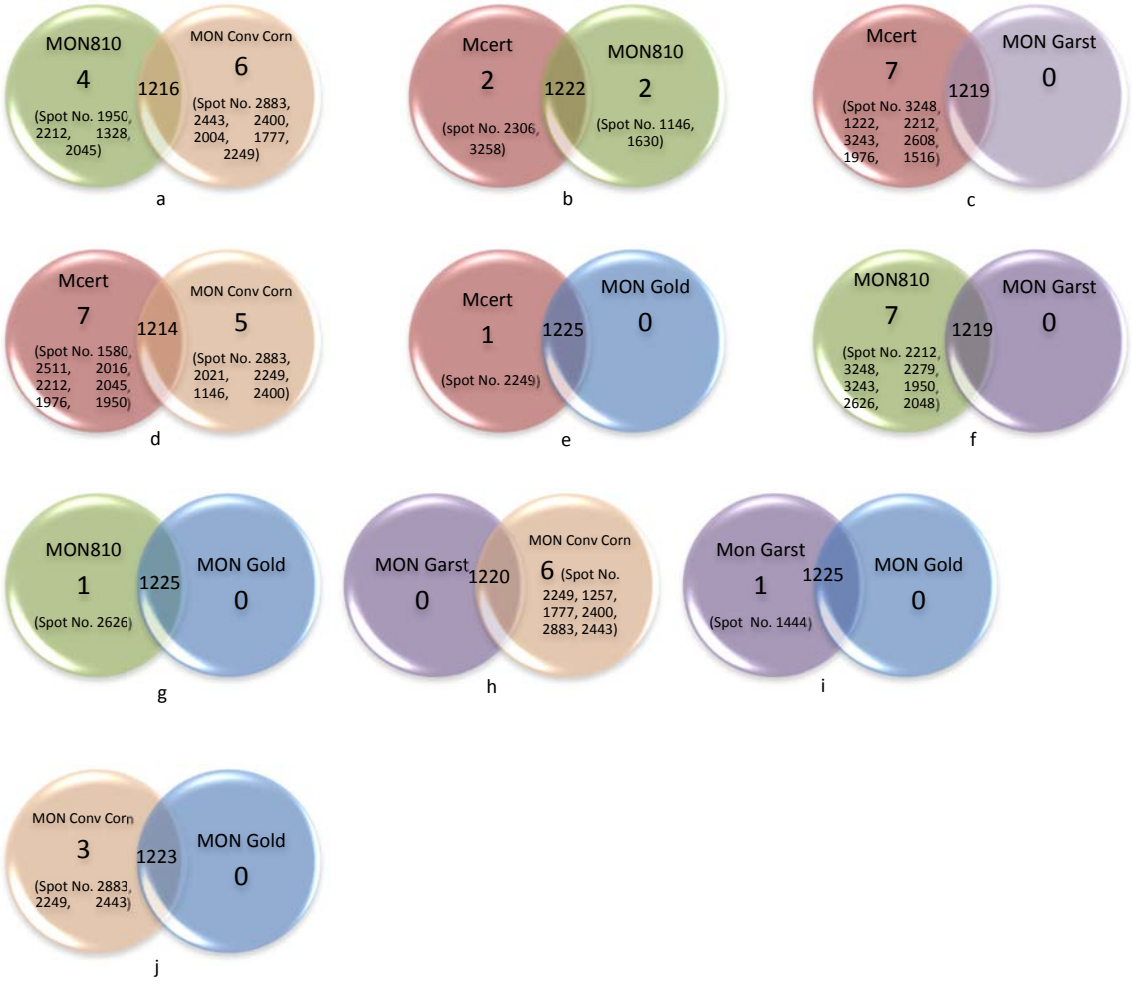


Figure 4.10: Two-set venn diagrams comparing the changes in the differentially expressed protein spots and up-regulated spot number when rats were fed for 7 days on conventional diet (Mon Conv Corn group) which was formulated to contain approximately 33% control corn grain; test diet (MON810 group) which was formulated to contain the test corn grain at approximately 33%; reference diets (Mon Garst and Mon Gold groups) which were formulated to contain the references corn grain at approximately 33% and commercial Purina rodent chow (Mcert group) which was purchased from Purina Mills Inc and normally contains approximately 33% corn.

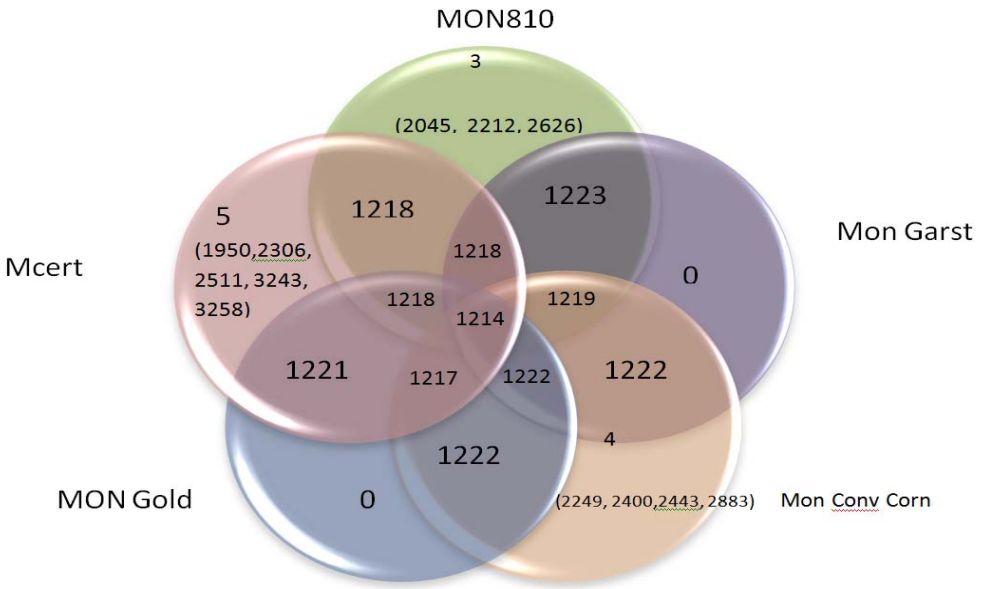


Figure 4.11: Five-set venn diagram analysis of the differentially expressed protein spots showing the up-regulated spots between the five groups for the 7-day rat feeding trial.

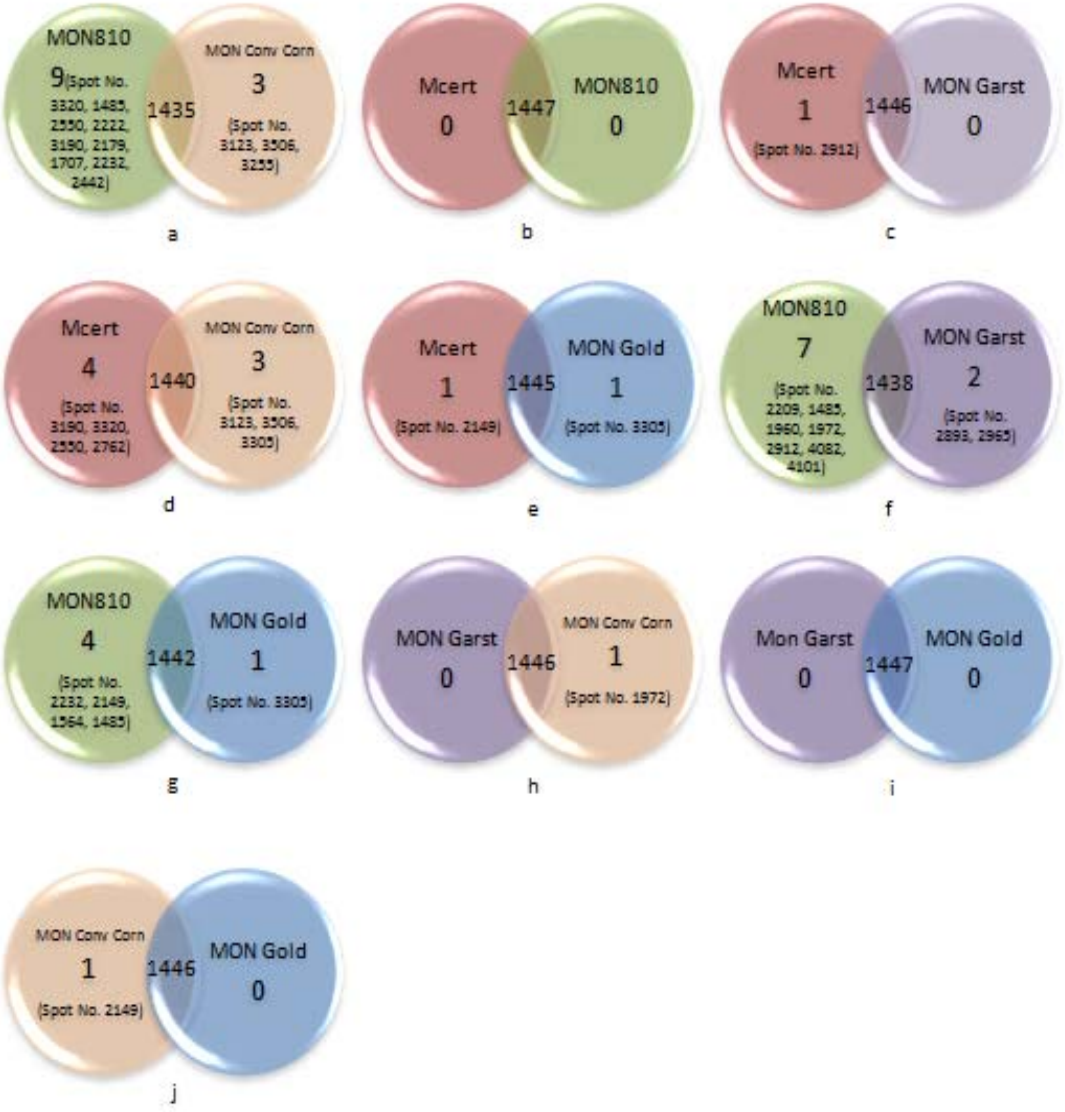


Figure 4.12: Two-set venn diagrams comparing the changes in the differentially expressed protein spots and up-regulated spot number when rats were fed for 28 days on conventional diet (Mon Conv Corn group) which was formulated to contain approximately 33% control corn grain; test diet (MON 810 group) which was formulated to contain the test corn grain at approximately 33%; reference diets (Mon Garst and Mon Gold groups) which were formulated to contain the references corn grain at approximately 33% and commercial Purina rodent chow (Mcert group) which was purchased from Purina Mills Inc and normally contains approximately 33% corn.

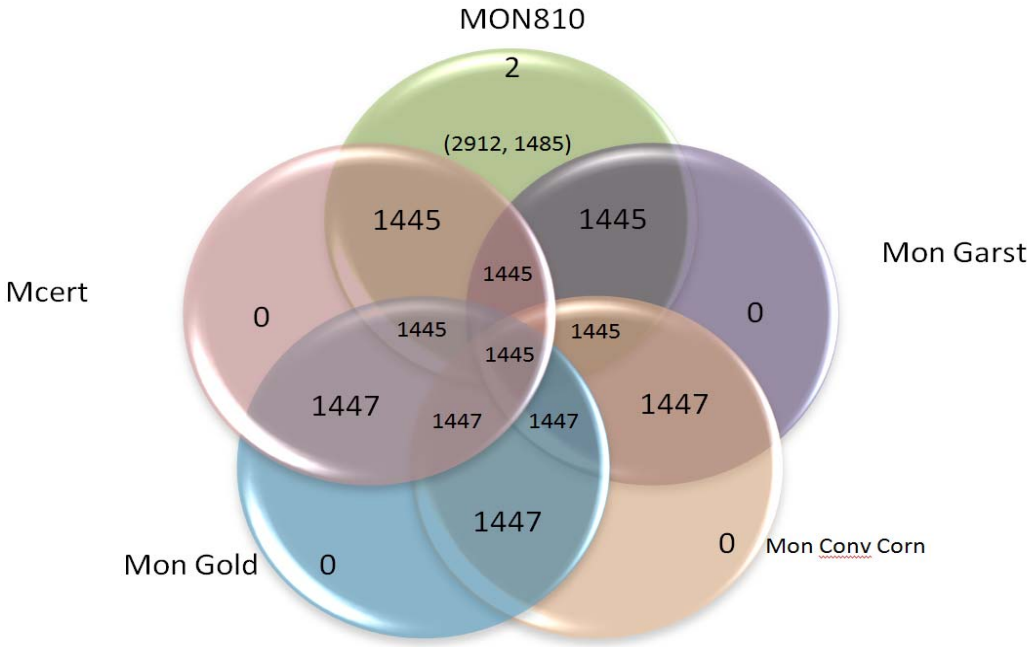


Figure 4.13: Five-set venn diagram analysis of the differentially expressed protein spots showing the up-regulated spots between the five groups for the 28-day rat feeding trial.

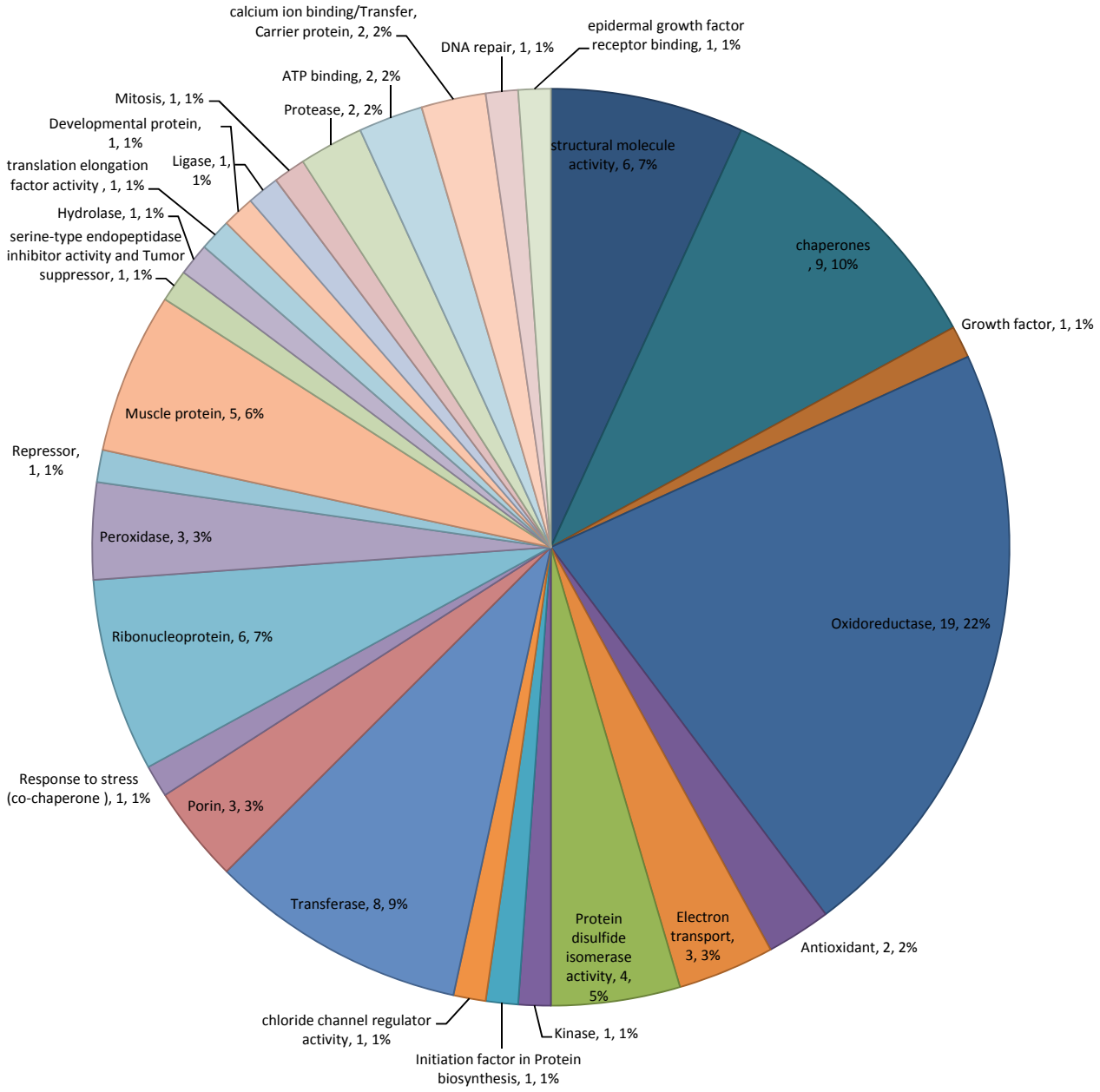


Figure 4.14: Functional classifications of differentially expressed intestinal epithelial cells proteins for the 7-day rat feeding trial.
A total of 88 proteins were identified and these proteins fall within 28 major functional categories. 19 (22%) contain oxidoreductase activity, 9 (10%) are chaperones, 8 (9%) contained transferase activity, 6 (7%) contained structural molecule activity and 6 (7%) are ribonucleoproteins.

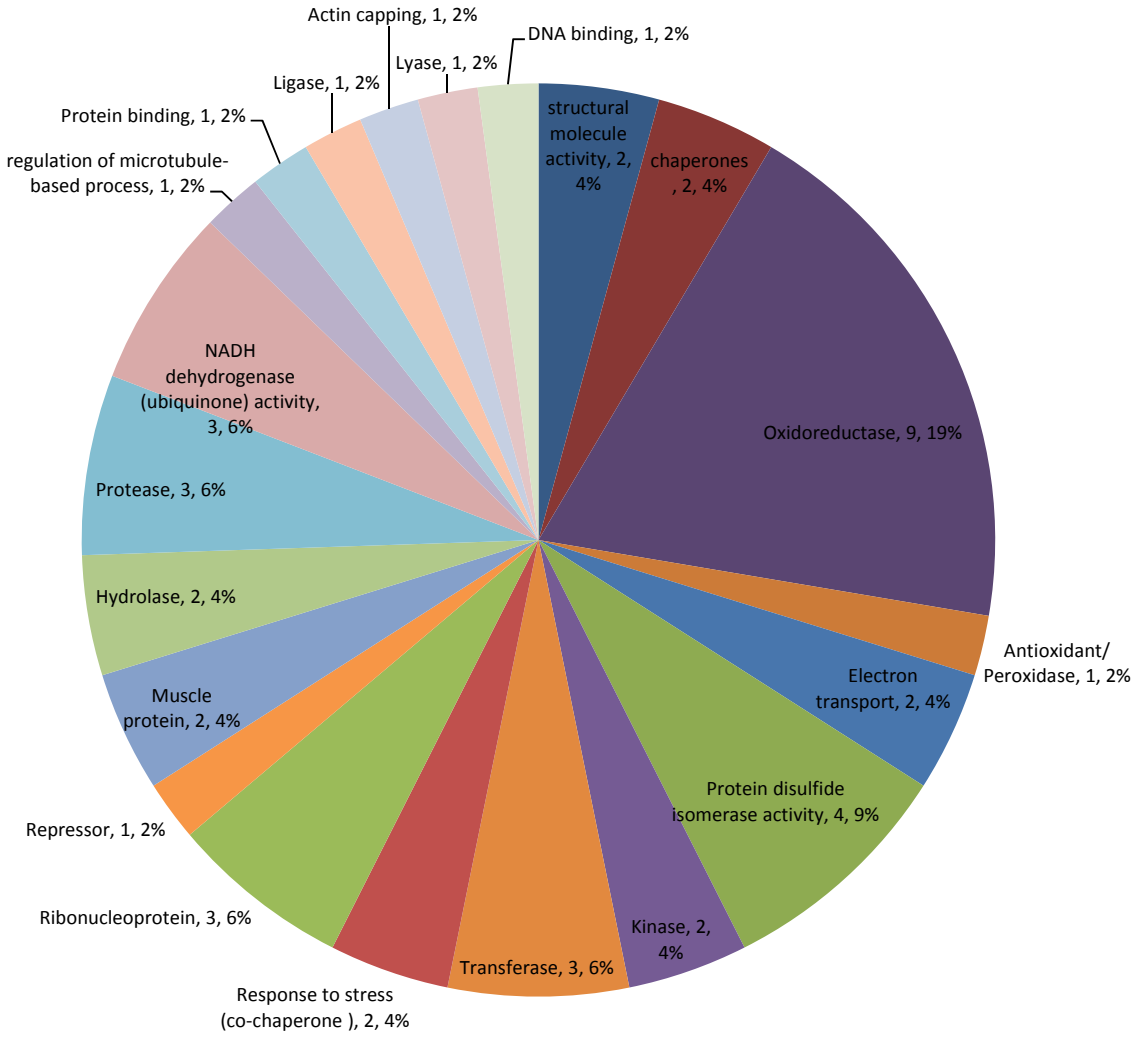


Figure 4.15: Functional classifications of differentially expressed intestinal epithelial cells proteins for the 28-day rat feeding trial.
A total of 47 proteins were identified and these proteins fall within 21 major functional categories. 9 (19%) contain oxidoreductase activity, 2 (4%) are chaperones, 3 (6%) contained transferase activity, 2 (4%) contained structural molecule activity and 3 (6%) are ribonucleoproteins.

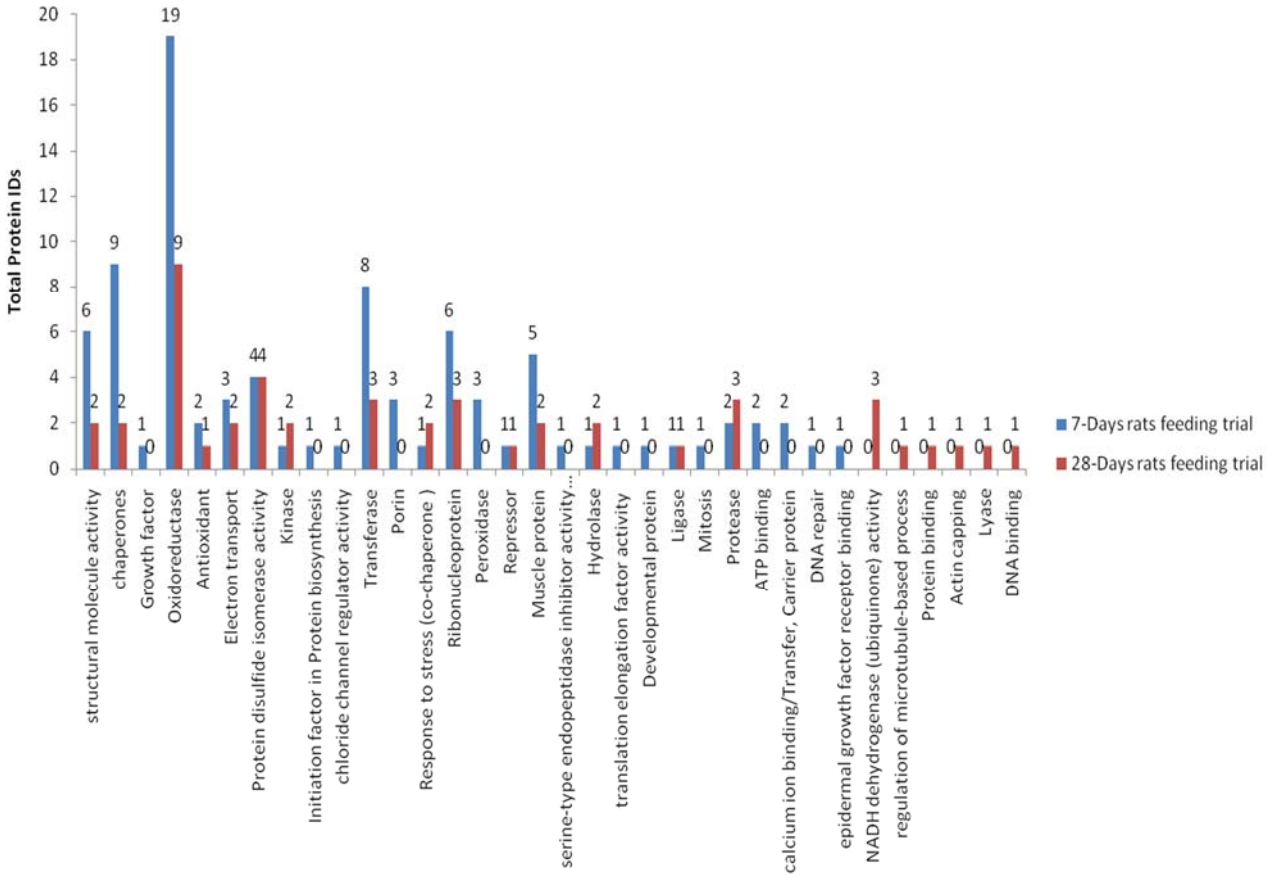


Figure 4.16: Summary of proteins identified by LC-MS/MS from small intestinal epithelial cells for both 7 and 28 day rat feeding trials, representing molecular functions.

Table 4.4: Functional classification of the identified protein spots of the 7-day rat feeding trial.

Molecular function	number of proteins	name of protein	Spot No.	Groups
structural molecule activity	2	prelamin-A/C	1146, 1630,	Mcert Vs MON810
	2	Actin, cytoplasmic 1 Actin, cytoplasmic 1	2016, 2021	Mcert Vs Mon Conv Corn
	1	Actin, gamma-enteric smooth muscle	2021	Mcert Vs Mon Conv Corn
	1	beta-actin-like protein 2	2021	Mcert Vs Mon Conv Corn
chaperones	3	60 kDa heat shock protein, mitochondrial	1630, 1580, 1328	Mcert Vs MON810, Mcert Vs Mon Conv Corn, MON810 Vs Mon Conv Corn
	1	Calnexin Precursor	1222	Mcert Vs Mon Garst
	1	Calreticulin Precursor	1976	Mcert Vs Mon Garst, Mcert Vs Mon Conv Corn
	1	T-complex protein 1 subunit zeta	1146	Mcert Vs MON810, Mcert Vs Mon Conv Corn
	1	T-complex protein 1 subunit beta (TCP-1-beta) (CCT-beta)	1444	Mon Garst Vs Mon Gold
	1	LDLR chaperone MESD Precursor (Mesoderm development candidate 2) (Mesoderm development protein)	2626	MON810 Vs Mon Garst, MON810 Vs Mon Gold
	1	Protein DJ-1 (Parkinson disease protein 7 homolog)	2608	Mcert Vs Mon Garst
Growth factor	1	Mesencephalic astrocyte-derived neurotrophic factor Precursor (Protein ARMET) (Arginine-rich protein)	2883	Mcert Vs Mon Conv Corn, MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn, Mon Conv Corn Vs Mon Gold
Oxidoreductase	1	aldehyde dehydrogenase X, mitochondrial precursor	1516	Mcert Vs Mon Garst
	1	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	2249	Mcert Vs Mon Conv Corn, Mcert Vs Mon Gold, MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn, Mon Conv Corn Vs Mon Gold

Molecular function	number of proteins	name of protein	Spot No.	Groups
	1	D-beta-hydroxybutyrate dehydrogenase, mitochondrial Precursor (BDH) (EC 1.1.1.30) (3-hydroxybutyrate dehydrogenase)	2249	Mcert Vs Mon Conv Corn, Mcert Vs Mon Gold, MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn, Mon Conv Corn Vs Mon Gold
	1	Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial	1146	Mcert Vs MON810, Mcert Vs Mon Conv Corn
	2	Glyceraldehyde-3-phosphate dehydrogenase	2045, 2048	Mcert Vs Mon Conv Corn, MON810 Vs Mon Conv Corn, MON810 Vs Mon Garst
	1	Malate dehydrogenase, mitochondrial	2004	Mon810 Vs MonConvCorn
	2	Dihydrolipoyl dehydrogenase, mitochondrial Precursor (EC 1.8.1.4) (Dihydrolipoamide dehydrogenase)	1328, 1444	MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Gold
	2	UDP-glucose 6-dehydrogenase (UDP-Glc dehydrogenase) (UDP-GlcDH) (UDPGDH) (EC 1.1.1.22)	1328, 1444	MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Gold
	1	Methylmalonate-semialdehyde dehydrogenase , mitochondrial	1328	MON810 Vs Mon Conv Corn
	1	Pyridine nucleotide-disulfide oxidoreductase domain-containing protein 2	1257	Mon Garst Vs Mon Conv Corn
	1	Succinate dehydrogenase iron-sulfur subunit, mitochondrial Precursor (EC 1.3.5.1) (Iron-sulfur subunit of complex II)	2443	MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn, Mon Conv Corn Vs Mon Gold
	1	Glutamate dehydrogenase 1, mitochondrial	1444	Mon Garst Vs Mon Gold
	1	aldehyde dehydrogenase X, mitochondrial precursor	1516	Mcert Vs Mon Garst
	1	4-trimethylaminobutyraldehyde dehydrogenase (TMABADH) (EC 1.2.1.47)	1516	Mcert Vs Mon Garst

Molecular function	number of proteins	name of protein	Spot No.	Groups
	2	Aldehyde dehydrogenase, mitochondrial Precursor (EC 1.2.1.3) (ALDH class 2) (ALDH1) (ALDH-E2)	1516, 1580	Mcert Vs Mon Garst, Mcert Vs Mon Conv Corn
Antioxidant	1	Thioredoxin-dependent peroxide reductase, mitochondrial Precursor (EC 1.11.1.15) (Peroxiredoxin-3) (PRX-3) (PRx III)	3243	Mcert Vs Mon Garst
	1	Peroxiredoxin-6 (EC 1.11.1.15) (Antioxidant protein 2) (1-Cys peroxiredoxin) (1-Cys PRX)	2626	MON810 Vs Mon Garst, MON810 Vs Mon Gold
Electron transport	1	Cytochrome b-c1 complex subunit 2, mitochondrial Precursor (Ubiquinol-cytochrome-c reductase complex core protein 2) (Core protein II) (Complex III subunit 2)	1777	MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn
	1	Electron transfer flavoprotein subunit beta (Beta-ETF)	2608	Mcert Vs Mon Garst
	1	Electron transfer flavoprotein subunit alpha, mitochondrial Precursor (Alpha-ETF)	2279	MON810 Vs Mon Garst
Protein disulfide isomerase activity	4	Protein disulfide-isomerase A3	1444, 1516, 1580, 1630	Mon Garst Vs Mon Gold, Mcert Vs Mon Garst, Mcert Vs Mon Conv Corn, Mcert Vs MON810
Kinase	1	UMP-CMP kinase (EC 2.7.4.14) (Cytidylate kinase)	2608	Mcert Vs Mon Garst
Initiation factor in Protein biosynthesis	1	Eukaryotic translation initiation factor 3 subunit I (eIF3i) (Eukaryotic translation initiation factor 3 subunit 2) (eIF-3-beta)	2016	Mcert Vs Mon Conv Corn
chloride channel regulator activity	1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	2016	Mcert Vs Mon Conv Corn

Molecular function	number of proteins	name of protein	Spot No.	Groups
Transferase	1	N-acetyl-D-glucosamine kinase (N-acetylglucosamine kinase) (EC 2.7.1.59) (GlcNAc kinase)	2016	Mcert Vs Mon Conv Corn
	2	3-ketoacyl-CoA thiolase, mitochondrial	1777, 3248	MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn, Mcert Vs Mon Garst, MON810 Vs Mon Garst
	1	Phosphoglycerate kinase 1	1777	MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn
	1	Creatine kinase U-type, mitochondrial	1777	MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn
	1	Succinyl-CoA:3-ketoacid-coenzyme A transferase 1, mitochondrial Precursor (EC 2.8.3.5)	1328	MON810 Vs Mon Conv Corn
	1	Pyruvate kinase isozymes M1/M2	1257	Mon Garst Vs Mon Conv Corn
	1	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	2443	MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn, Mon Conv Corn Vs Mon Gold
Porin	2	Voltage-dependent anion-selective channel protein 2 (VDAC-2) (Outer mitochondrial membrane protein porin 2) (B36-VDAC)	2212, 2279	Mcert Vs Mon Garst, Mcert Vs Mon Conv Corn, MON810 Vs Mon Garst, MON810 Vs Mon Conv Corn
	1	Voltage-dependent anion-selective channel protein 1	2249	Mcert Vs Mon Conv Corn, Mcert Vs Mon Gold, MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn, Mon Conv Corn Vs Mon Gold
Response to stress (co-chaperone)	1	Stress-induced-phosphoprotein 1	1146	Mcert Vs MON810, Mcert Vs Mon Conv Corn

Molecular function	number of proteins	name of protein	Spot No.	Groups
Ribonucleoprotein	1	heterogeneous nuclear ribonucleoprotein L isoform b	1146	Mcert Vs MON810, Mcert Vs Mon Conv Corn
	2	Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1)	2279, 2400	MON810 Vs Mon Garst, Mcert Vs Mon Conv Corn, MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn
	1	Heterogeneous nuclear ribonucleoprotein H	1580	Mcert Vs Mon Conv Corn
	1	Heterogeneous nuclear ribonucleoprotein H2 (hnRNP H2)	1580	Mcert Vs Mon Conv Corn
	1	ErbB3-binding protein 1	1630	Mcert Vs MON810
Peroxidase	3	Catalase	1146, 1257, 1328	Mcert Vs MON810, Mcert Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn, MON810 Vs Mon Conv Corn
Repressor	1	coiled-coil-helix-coiled-coil-helix domain containing protein 3	2400	Mcert Vs Mon Conv Corn, MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn
Muscle protein	1	Tropomyosin beta chain	1976	Mcert Vs Mon Garst, Mcert Vs Mon Conv Corn
	3	Tropomyosin alpha-1 chain	1976, 2021, 3258	Mcert Vs Mon Garst, Mcert Vs Mon Conv Corn, Mcert Vs MON810
	1	Tropomyosin alpha-3 chain	2306	Mcert Vs MON810
serine-type endopeptidase inhibitor activity and Tumor suppressor	1	Serpin B5 (Peptidase inhibitor 5) (PI-5) (Maspin)	1950	Mcert Vs Mon Conv Corn
Hydrolase	1	3'(2'),5'-bisphosphate nucleotidase 1 (EC 3.1.3.7)	1950	Mcert Vs Mon Conv Corn
translation elongation factor activity	1	Elongation factor 2	1950	Mcert Vs Mon Conv Corn
Developmental protein	1	THO complex subunit 6 homolog	2048	MON810 Vs Mon Garst
Ligase	1	UPF0027 protein C22 or f28 homolog (p55)	1328	MON810 Vs Mon Conv Corn

Molecular function	number of proteins	name of protein	Spot No.	Groups
Mitosis	1	Centrosomal protein of 55 kDa.	1328	MON810 Vs Mon Conv Corn
Protease	1	Proteasome subunit alpha type-7 (EC 3.4.25.1) (Proteasome subunit RC6-1)	2443	MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn, Mon Conv Corn Vs Mon Gold
	1	Cytosol aminopeptidase (EC 3.4.11.1) (Leucine aminopeptidase 3) (LAP-3) (Leucyl aminopeptidase) (Proline aminopeptidase) (EC 3.4.11.5) (Prolyl aminopeptidase)	1444	Mon Garst Vs Mon Gold
ATP binding	2	ATP synthase subunit alpha, mitochondrial	1444, 2443	Mon Garst Vs Mon Gold, MON810 Vs Mon Conv Corn, Mon Garst Vs Mon Conv Corn, Mon Conv Corn Vs Mon Gold
calcium ion binding/Transfer, Carrier protein	1	annexin A11	1444	Mon Garst Vs Mon Gold
	1	Annexin A2	2045	Mcert Vs Mon Conv Corn, MON810 Vs Mon Conv Corn
DNA repair	1	PAX interacting (with transcription-activation domain) protein 1	2511	Mcert Vs Mon Conv Corn
epidermal growth factor receptor binding	1	Growth factor receptor-bound protein 2	2626	MON810 Vs Mon Garst, MON810 Vs Mon Gold

Table 4.5: Functional classification of the identified protein spots of the 28-day rat feeding trial.

Molecular function	number of proteins	name of protein	Spot No.	Groups
structural molecule activity	2	Lamin-A	1485, 1564	MON810 Vs Mon Conv Corn, MON810 Vs Mon Gold
chaperones	1	60 kDa heat shock protein, mitochondrial	1960	MON810 Vs Mon Garst
	1	T-complex protein 1 subunit beta (TCP-1-beta) (CCT-beta)	2893	MON810 Vs Mon Garst
Oxidoreductase	1	Isocitrate dehydrogenase subunit alpha, mitochondrial Precursor (EC 1.1.1.41)	3190	Mcert Vs Mon Conv Corn
	1	Isocitrate dehydrogenase , mitochondrial	2222	MON810 Vs Mon Conv Corn
	2	Isocitrate dehydrogenase cytoplasmic	2209, 4101	MON810 Vs Mon Garst
	1	Glyceraldehyde-3-phosphate dehydrogenase	4082	MON810 Vs Mon Garst
	1	Malate dehydrogenase, mitochondrial	4082	MON810 Vs Mon Garst
	1	Dihydrolipoyl dehydrogenase, mitochondrial Precursor (EC 1.8.1.4) (Dihydrolipoamide dehydrogenase)	1707	MON810 Vs Mon Conv Corn
	1	Superoxide dismutase , mitochondrial Precursor (EC 1.15.1.1)	3255	MON810 Vs Mon Conv Corn
	1	Alcohol dehydrogenase (EC 1.1.1.2) (Aldehyde reductase) (Aldo-keto reductase family 1 member A1) (3-DG-reducing enzyme)	2442	MON810 Vs Mon Conv Corn
Antioxidant/ Peroxidase	1	Peroxiredoxin-1 (EC 1.11.1.15) (Thioredoxin peroxidase 2) (Thioredoxin-dependent peroxide reductase 2)	3190	Mcert Vs Mon Conv Corn
Electron transport	1	Cytochrome b-c1 complex subunit 2, mitochondrial Precursor (Ubiquinol-cytochrome-c reductase complex core protein 2) (Core protein II)	2232	MON810 Vs Mon Conv Corn

Molecular function	number of proteins	name of protein	Spot No.	Groups
	1	Electron transfer flavoprotein subunit beta (Beta-ETF)	2912	Mcert Vs Mon Garst
Protein disulfide isomerase activity	4	Protein disulfide-isomerase A3	2149, 1707, 1960, 1972	Mcert Vs Mon Gold, MON810 Vs Mon Conv Corn, MON810 Vs Mon Garst
Kinase	1	Adenylate kinase 2, mitochondrial (AK 2) (EC 2.7.4.3) (ATP-AMP transphosphorylase 2)	2912	Mcert Vs Mon Garst
	1	Phosphoglycerate kinase 1	2232	MON810 Vs Mon Conv Corn
Transferase	2	Ornithine aminotransferase, mitochondrial Precursor (EC 2.6.1.13) (Ornithine--oxo-acid aminotransferase)	2550, 2179	Mcert Vs Mon Conv Corn, MON810 Vs Mon Conv Corn
	1	3-ketoacyl-CoA thiolase, mitochondrial	2222	MON810 Vs Mon Conv Corn
Response to stress (co-chaperone)	2	Stress-induced-phosphoprotein 1	1485, 1564	MON810 Vs Mon Conv Corn, MON810 Vs Mon Gold
Ribonucleoprotein	1	Heterogeneous nuclear ribonucleoprotein A3 (hnRNP A3)	4082	MON810 Vs Mon Garst
	1	heterogeneous nuclear ribonucleoprotein U	2550	Mcert Vs Mon Conv Corn
	1	40S ribosomal protein S3	2762	Mcert Vs Mon Conv Corn
Repressor	1	chromobox homolog 3	3305	Mcert Vs Mon Conv Corn, Mcert Vs Mon Gold
Muscle protein	1	Actin, gamma-enteric smooth muscle	3506	Mcert Vs Mon Conv Corn
	1	Actin, cytoplasmic 1 Actin, cytoplasmic 1, N-terminally processed	3506	Mcert Vs Mon Conv Corn
Hydrolase	1	6-phosphogluconolactonase (6PGL) (EC 3.1.1.31)	2965	MON810 Vs Mon Garst
	1	Acyl-coenzyme A thioesterase 2, mitochondrial	4101	MON810 Vs Mon Garst

Molecular function	number of proteins	name of protein	Spot No.	Groups
Protease	1	Proteasome subunit alpha type-4 (EC 3.4.25.1) (Proteasome component C9)	2912	Mcert Vs Mon Garst
	1	Cytosol aminopeptidase (EC 3.4.11.1) (Leucine aminopeptidase 3) (LAP-3) (Leucyl aminopeptidase)(Proline aminopeptidase) (EC 3.4.11.5) (Prolyl aminopeptidase)	2442	MON810 Vs Mon Conv Corn
	1	Lactotransferrin; Lactoferrin; EC 3.4.21.	2893	MON810 Vs Mon Garst
NADH dehydrogenase (ubiquinone) activity	2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	3190, 3123	Mcert Vs Mon Conv Corn
	1	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9	3320	Mcert Vs Mon Conv Corn
regulation of microtubule-based process	1	Protein MEMO1	2762	Mcert Vs Mon Conv Corn
Protein binding	1	Calcyclin-binding protein	2912	Mcert Vs Mon Garst
Ligase	1	Tyrosyl-tRNA synthetase, cytoplasmic (EC 6.1.1.1) (Tyrosyl--tRNA ligase) (TyrRS)	1707	MON810 Vs Mon Conv Corn
Actin capping	1	Gelsolin Precursor (Actin-depolymerizing factor) (ADF) (Brevin)	2209	MON810 Vs Mon Garst
Lyase	1	Alpha-enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Non-neural enolase) (NNE) (Enolase 1)	1960	MON810 Vs Mon Garst
DNA binding	1	DNA-directed RNA polymerases I, II, and III subunit RPABC1 (RNA polymerases I, II, and III subunit ABC1) (DNA-directed RNA polymerase II subunit E)	2965	MON810 Vs Mon Garst

Chapter 5

5 *In vitro* Effects of Transgenic Maize (*Bt* Maize MON810) Expressing Cry1Ab Protein (*Bacillus Thuringiensis* Toxin) on Mammalian Epithelial Cells.

5.1 Characterization of test material

Western blot analyses of the grain extracts from MON810 revealed a band corresponding to the predicted molecular weight of Cry1Ab (65 KDa protein), confirming the presence of Cry1Ab in *Bt* maize. This was absent from its corresponding parental non-transgenic maize (Figure 5.1).

5.2 Establishment in culture of epithelial cells isolated from rat small intestine and the commercial human epithelial cell line HCT116

Rat gut epithelial cells were successfully established in culture. Examination of the morphology suggests that these cells were derived from progenitor cells and have the ability to rapidly proliferate in culture. They do provide a good *in vitro* model for rat small intestine, particularly since they are derived from non-tumour cells. In summary, these cells must be considered to be 'epithelial-like' (IE cells; Figure 5.2). Human epithelial cell line HCT116 was sourced and successfully established in culture (Figure 5.3).

5.3 Calibration of growth curve through the growth cell cycle

A growth curve was plotted when fresh medium was inoculated with a given number of cells (5000 cells), and the growth monitored over a period of time (10 days). Plotting the data yielded a typical growth curve for the normal epithelial-like cells and tumor cells (Figure 5.4 and 5.5; Table 5.1 and 5.2).

When the IE cells were grown in 24-well plates, starting with 5000 cells/well, the cells initially adjusted to the new medium (lag phase) and then started dividing regularly (exponential phase). When their growth became limited, the cells stopt dividing (plateau phase or stationary phase), until eventually they showed loss of viability (death phase). When HCT116 cells were grown in 24-well plate (starting with 5000 cells/well) the cells

initially adjusted to the new medium showing a slow initial phase or lag phase followed by a middle phase characterized by rapid growth (exponential phase). When their growth became limited the cells stopt dividing and showed loss of viability (death phase). However, unlike IECs, no plateau phase or stationary phase was observed.

5.4 Cytotoxicity assessment (MTT assay)

MTT assays were conducted to evaluate the cytotoxicity of *Bt* maize extracts on IE cells and HCT116 cells (Table 5.3 and 5.4).

A non-toxic dose of extracts from both *Bt* maize and its near isogenic line at concentrations that are biologically meaningful but still present an effect, were used on the above cells. The MTT data for the IE cells and HCT cells showed that IE cells are more sensitive than HCT116 cells to the toxicity of *Bt* maize and non-transgenic maize, indicating that these different cells have different sensitivities to *Bt* toxicity. For IE cells, only 6.25 µg/ml produced acceptable low levels of toxicity, i.e. non-cytotoxic dose (<20% toxicity) from exposure to *Bt* maize and non-transgenic maize, with the higher concentrations tested showing unacceptable levels of cytotoxicity. For HCT116 cells, doses of 50 µg/ml (the highest non-cytotoxic dose) were required to cause an acceptable toxic effect.

In vitro toxicological studies with IE cells in the presence of extracts from *Bt* maize and its near isogenic line indicates that there was no significant difference between the transgenic and parental lines and that the isolated gut epithelial cells are a suitable model for *in vivo* studies (Figure 5.6). Similarly, *In vitro* toxicological studies with HCT116 cells in the presence of extracts from *Bt* maize and its near isogenic line indicates that there was no significant differences (Figure 5.7). Statistical analyses suggest that the transgenic line expressing *Bt* in these single dose experiments is not toxic to these cells.

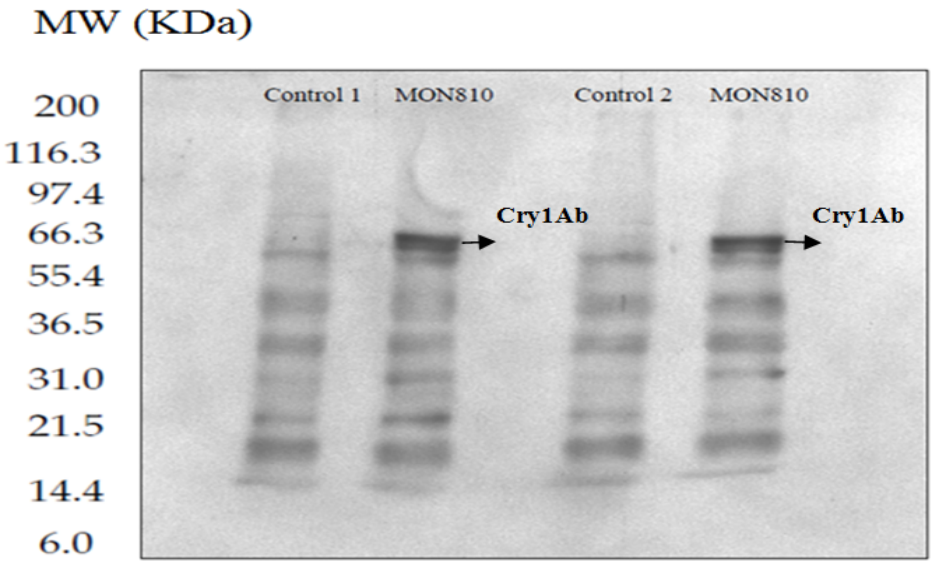


Figure 5.1: Immunoassay by western blot showing the presence of Cry1Ab in protein extracts from the seeds of maize MON810. Cry1Ab was absent in seed extracts of the near-isogenic parental line.

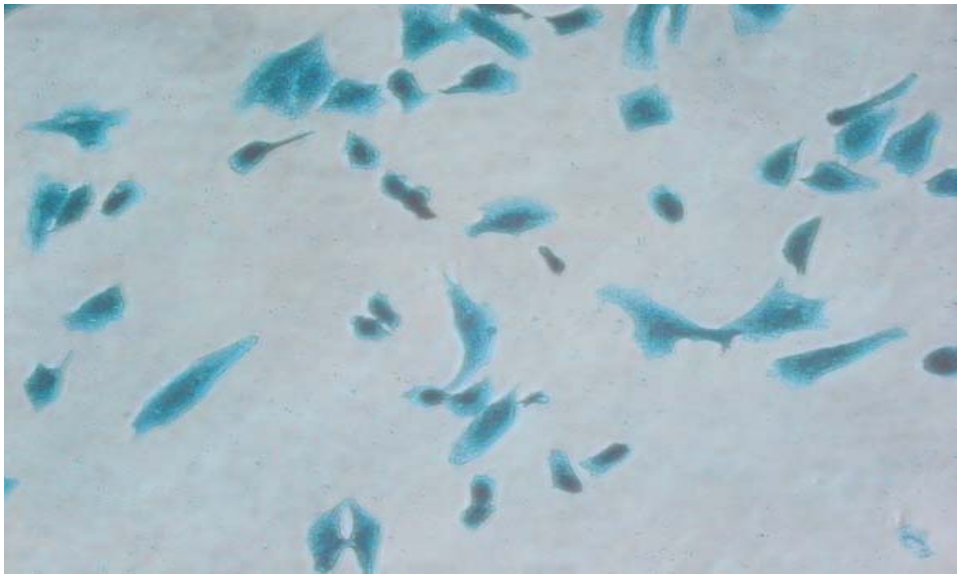


Figure 5.2: Rats small intestinal epithelial-like cells stained with coomassie brilliant blue G-250 (X10).

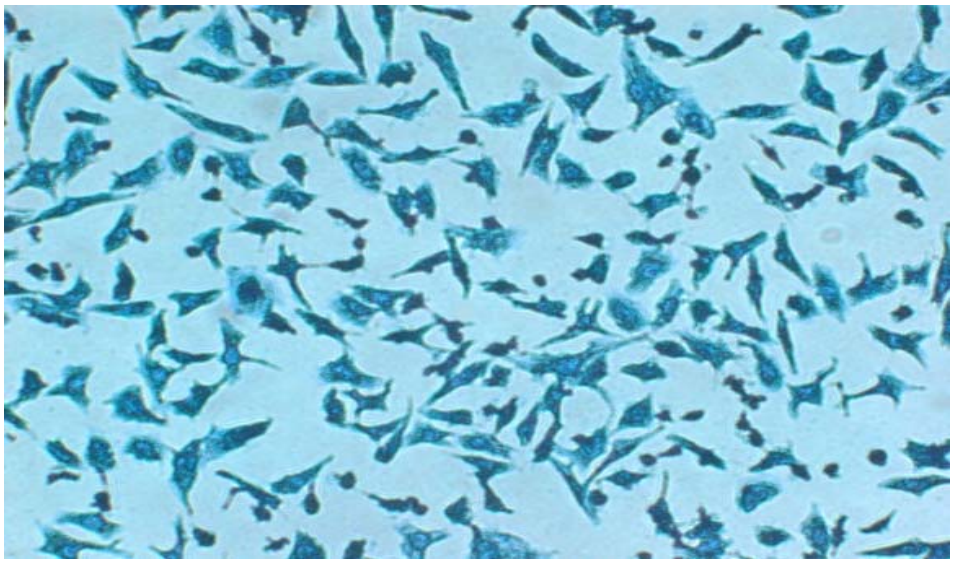


Figure 5.3: HCT116 cells stained with coomassie brilliant blue G-250 (X10).

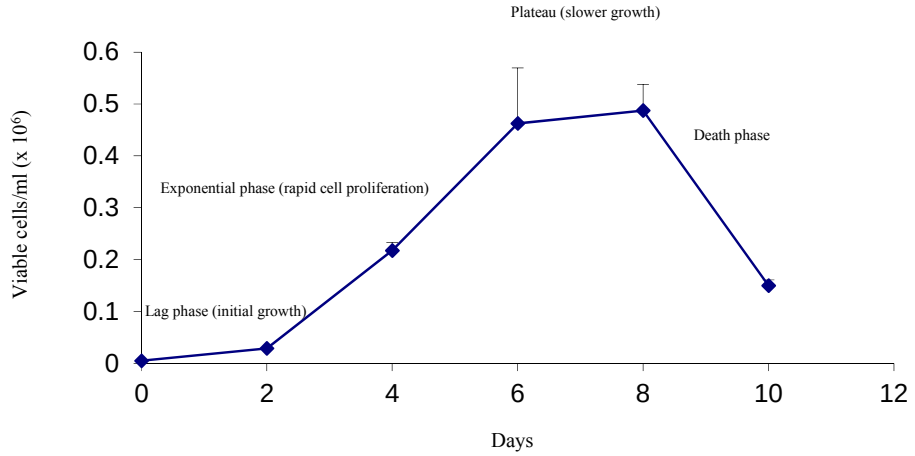


Figure 5.4: Typical growth curve for IE cells showing the different phases.
When IE cells were grown in 24 well plate starting with 5000 cells/well the cells initially adjusted to the new medium (lag phase) until they can start dividing regularly (exponential phase). When their growth becomes limited, the cells stop dividing (plateau phase or stationary phase), until eventually they show loss of viability (death phase). Growth was expressed as change in the number viable cells vs time. Each value represents the mean ±SE.

Table 5.1: Viability of IE cells over a period of 10 days.

Day	No. of viable cells/ml x 10^6 (Mean ±SE)
0	0.005±0.000
2	0.02875±0.002
4	0.2175±0.015
6	0.4625±0.107
8	0.4875±0.050
10	0.15±0.010

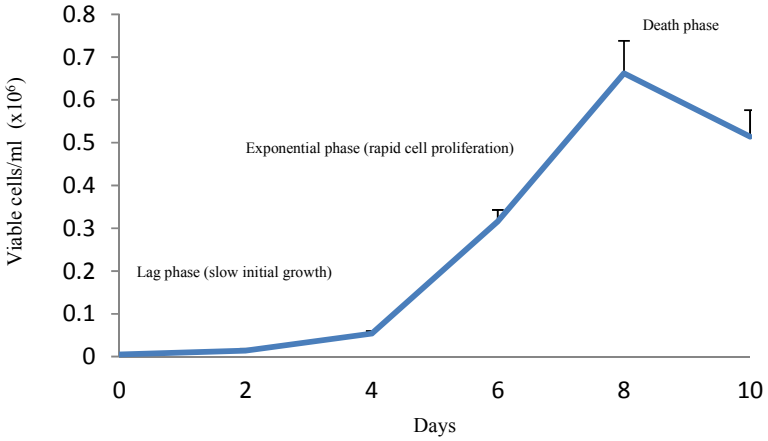


Figure 5.5: Typical growth curve for HCT116 cell line showing the different phases.
When HCT116 cells were grown in 24 well plate starting with 5000 cells/well the cells initially adjusted to the new medium showing a slow initial phase or lag phase until they can start dividing rapidly (exponential phase). When their growth becomes limited the cells stop dividing and they show loss of viability (death phase). Unlike IECs, no plateau phase or stationary phase was observed. Growth was expressed as change in the number viable cells vs time. Each value represents the mean ±SE.

Table 5.2: Viability of HCT116 over a period of 10 days.

Day	No. of viable cells/ml x 10^6 (Mean ±SE)
0	0.005±0.000
2	0.0138±0.004
4	0.0538±0.006
6	0.316±0.027
8	0.662±0.076
10	0.514±0.062

Table 5.3: MTT assay results for evaluation of IE cell viability.
Cells were exposed to the indicated concentration of non-*Bt* maize extract and *Bt* maize extract (6.25-200µg/ml). A dose of 6.25µg/ml of non-transgenic maize and *Bt* maize extract produced acceptable levels of toxicity (<20% toxicity).

IE Cells			
Non-transgenic maize extract (control)		Bt maize extract	
Concentration µg/ml	Viability %	Concentration µg/ml	Viability %
0	100	0	100
6.25	79.16	6.25	77.07
12.5	75.2	12.5	67.28
25	76.26	25	63.88
50	70.85	50	67.81
100	63.16	100	58.77
200	63.16	200	61.46

Table 5.4: MTT assay results for evaluation of HCT116 cell viability.
Cells were exposed to the indicated concentration of non-*Bt* maize extract and *Bt* maize extract (6.25-200µg/ml). A dose of 50ug/ml and less of non-transgenic maize and *Bt* maize extract produced acceptable levels of toxicity (<20% toxicity).

HCT116			
Non-transgenic maize extract (control)		Bt maize extract	
Concentration µg/ml	Viability %	Concentration µg/ml	Viability %
0	100	0	100
6.25	83.39	6.25	84.35
12.5	74.22	12.5	79.87
25	82.53	25	78.98
50	85.54	50	83.24
100	73.28	100	76.63
200	66.49	200	71.74

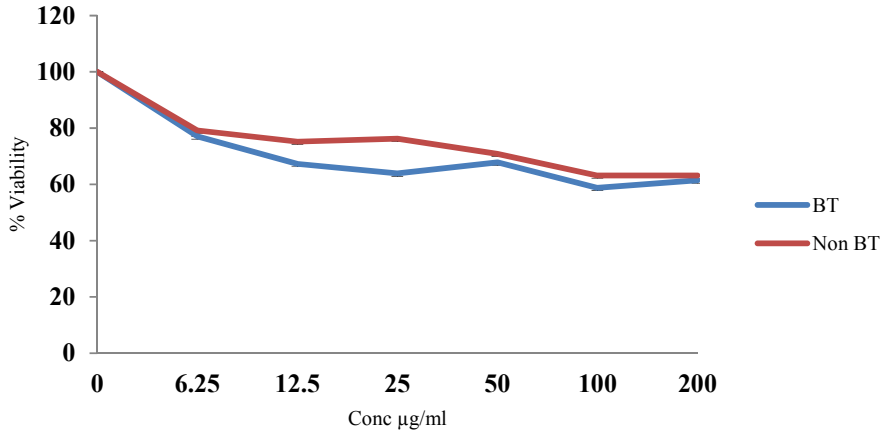


Figure 5.6: Cytotoxic effects of extracts from *Bt* maize and its corresponding parental non-transgenic maize on epithelial cells isolated from rat small intestine.
Cells were exposed to the indicated concentration of non-*Bt* maize extract and *Bt* maize extract (6.25-200µg/ml). As shown in this diagram, doses of 6.25µg/ml of non-transgenic maize and *Bt* maize produced acceptable levels of toxicity (<20% toxicity). Statistical analyses indicate that there was no significant difference (p-value >0.05) between *Bt* and non-*Bt* maize. Each value represents the mean ±SE.

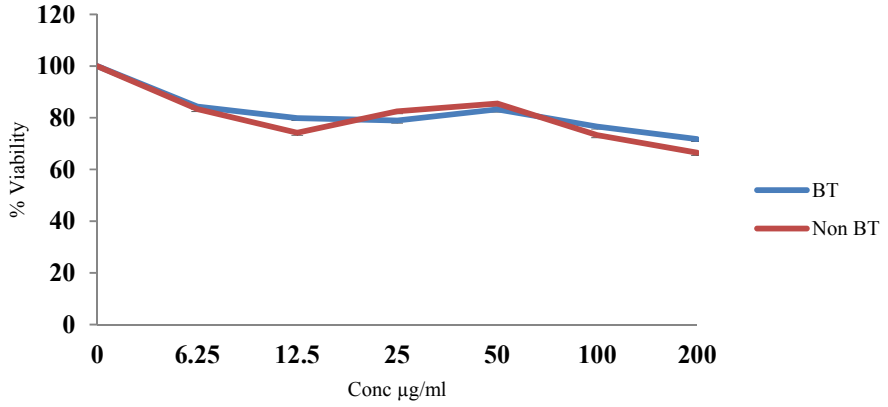


Figure 5.7: Cytotoxic effects of extracts from *Bt* maize and its corresponding parental non-transgenic maize on human epithelial cell line HCT116.
Cells were exposed to the indicated concentration of non-*Bt* maize extract and *Bt* maize extract (6.25-200µg/ml). As shown in this diagram, doses of 50µg/ml and less of non-transgenic maize and *Bt* maize produced acceptable levels of toxicity (<20% toxicity). Statistical analyses indicate that there was no significant difference (*p*-value >0.05) between *Bt* and non-*Bt* maize. Each value represents the mean ±SE.

5.5 Determination of exposure time of *Bt* and non-*Bt* maize extracts for both rat IE cells and the HCT116 cell line

To determine the optimal exposure time of IE cells and the HCT116 cell line to maize extracts, growth curves were constructed using the control medium (without any maize extracts) and medium with non-cytotoxic concentrations of both *Bt* and non-*Bt* maize extracts. For both the control assay and the experimental assay, 5000 cells/well were seeded at day 0 in 24-well plates containing medium supplemented with 10% FBS and incubated for 24 hours. After this duration, for the controls the medium was removed and new medium was added to each well, whereas for the experimental plates the medium was removed and new medium containing non-cytotoxic concentrations of either *Bt* maize extract or non-*Bt* maize extract (based on results from the MTT assay dose of 6.25µg/ml for IECs and dose of 50µg/ml for HCT116 cell line) was added to each well. All plates were incubated for another 24 hours. On day 2, the cells of 5 wells were trypsinised and cell numbers and viability were determined. This was repeated for days 3, 4 and 5 (Table 5.5 and Table 5.6). All experiments were done in triplicate for each treatment (Figure 5.8 and Figure 5.9).

The results suggest that both *Bt*-maize and non-*Bt* maize affect growth of the IE cells (or possibly more cells have died in the treatment groups) as both treatments cause a slight reduction in cell numbers (Table 5.5). However, neither of these maize seed extracts appeared to affect growth of the HCT116 cell line (Table 5.6). However, statistical analyses indicate that there was no significant difference (p-value >0.05) between *Bt* and non-*Bt* maize extract on IE cells and HCT116 cell line. Regarding to the observed results, the cells were incubated with the maize extracts for 4 days after seeding for the protein samples and not more than that period to avoid the cells from entering in a lag phase after 4 days.

5.6 Determination of protein concentration

Total protein concentrations of the IE cells and HCT116 cell line were determined using the 2-D Quant kit (GE healthcare, Sweden) as illustrated in Figure 5.10 and 5.11 and Table 5.7 and 5.8.

Table 5.5: Growth curves for IE cells using control medium (without any maize extracts) and medium containing non-cytotoxic concentration (6.25µg/ml) of either *Bt* maize extract or non-*Bt* maize extract.
Values show mean± standard error.

Treatment (n=5)	No. of viable cells/ml x 10^6 (Mean ±SE)				
	Day 0	Day 2	Day 3	Day 4	Day 5
Control	0.005±0.00	0.03±0.00	0.06±0.02	0.12±0.04	0.27±0.08
Non-Bt maize	0.005±0.00	0.02±0.00	0.05±0.01	0.12±0.02	0.21±0.07
Bt maize	0.005±0.00	0.03±0.01	0.04±0.01	0.09±0.03	0.21±0.05

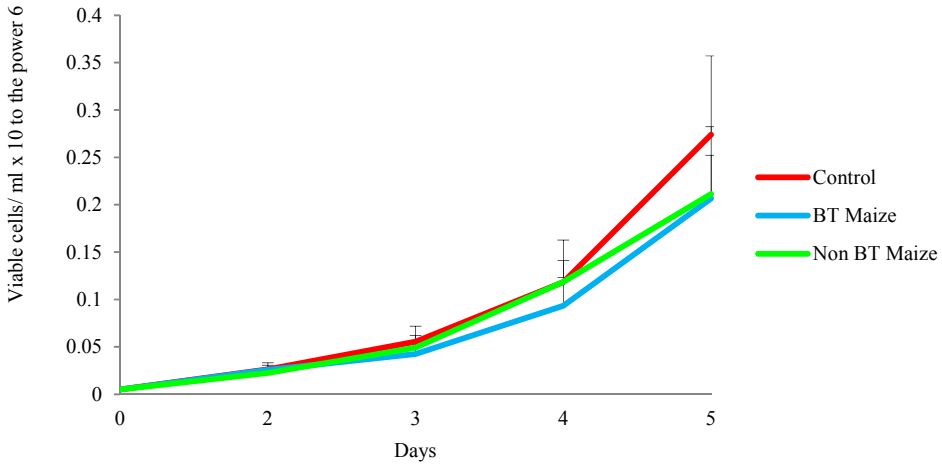


Figure 5.8: Growth curves for rat intestinal epithelial (IE) cells using control medium (without any maize extracts) and medium containing non-cytotoxic concentration of either *Bt* maize extract or non-*Bt* maize extract. Statistical analyses indicate that there was no significant difference (*p*-value >0.05) between *Bt*, non-*Bt* maize and the control.
Values show mean± standard error.

Table 5.6: Growth curves for HCT116 cell line using control medium (without any maize extracts) and medium containing non-cytotoxic concentration (50µg/ml) of either *Bt* maize extract or non-*Bt* maize extract. Values show mean± standard error.

Treatment (n=5)	No. of viable cells/ml x 10^6 (Mean ±SE)				
	Day 0	Day 2	Day 3	Day 4	Day 5
Control	0.005±0.00	0.02±0.00	0.04±0.01	0.13±0.05	0.26±0.07
Non-Bt maize	0.005±0.00	0.02±0.00	0.05±0.01	0.12±0.03	0.28±0.05
Bt maize	0.005±0.00	0.02±0.00	0.05±0.01	0.13±0.05	0.28±0.07

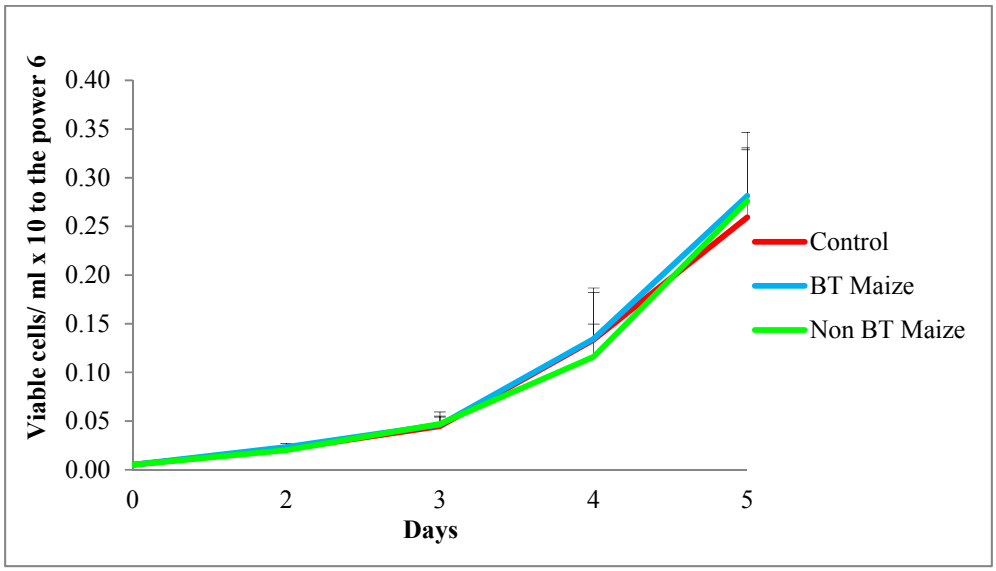


Figure 5.9: Growth curves for HCT116 cell line using control medium (without any maize extracts) and medium containing non-cytotoxic concentration of either *Bt* maize extract or non-*Bt* maize extract. Statistical analyses indicate that there was no significant difference (*p*-value >0.05) between *Bt*, non-*Bt* maize and the control.
Values show mean± standard error.

5.7 First dimensional electrophoresis (Isoelectric Focusing (IEF))

Protein samples were focused successfully until they reached the steady state. The steady state of IEF was indicated by two conditions: 1) Final high voltage (10000V) was reached; 2) current decreased and reached steady state (Figure 5.12).

5.8 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Proteins samples were separated successfully by SDS-PAGE (Figure 5.13 and 5.14).

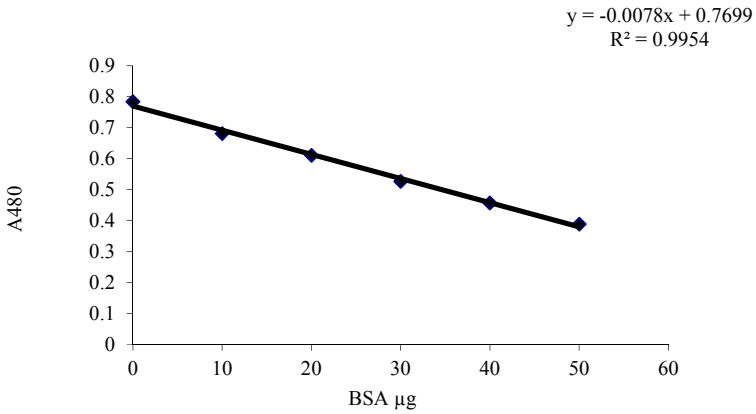
5.9 2D gel image analysis and spot picking gels

Initial identification of differentially expressed proteins was carried out by Nonlinear Dynamics Ltd (UK). However, before cutting the protein spots from the relevant gels for identification, further analysis was required as detailed in chapter 4.

Progenesis Samespot software version 4.1 was used for refining the analysis. Average normalized volume of 200,000 was selected. In general, the experimental design was not changed; several artefacts and false positives were removed. There are also some spots that are readily detected as differentially expressed proteins, however they are quite faint on the actual gels and can be classified as "unpickable" spots or spots with low protein contents that may not contain enough protein for identification by mass spectroscopy. Because of high resolution of the images and magnifying tools in Progenesis software, the number of the observed protein spots by using the software might be higher than the pickable protein spots in actual gels. In the Filtering step there were 1627 spots. These were filtered by average normalised volume of 200,000. This removed 273 features, leaving 1354 for further analysis. The number of protein spots to pick for subsequent analysis was reduced from 90 to 48 (Table 5.9).

Figure 5.10: Standard calibration curve

BSA µg	Standard
0	0.784
10	0.681
20	0.611
30	0.527
40	0.457
50	0.389



for protein concentration of the rat IE cells using bovine serum albumin (BSA) as a standard protein.

Table 5.7: Protein concentrations of the rat IE cells.

	1	2	mean	x µg/5µl	µg/µl	µl/500ug	µl/1300ug	µl/1500ug
IECs Bt maize	0.334	0.353	0.3435	60.785	12.157	41.128568	106.934277	123.385704
IECs non-Bt maize	0.296	0.311	0.3035	66.5	13.3	37.593985	97.7443609	112.781955

BSA µg	Standard
0	0.82
10	0.721
20	0.644
30	0.561
40	0.459
50	0.402

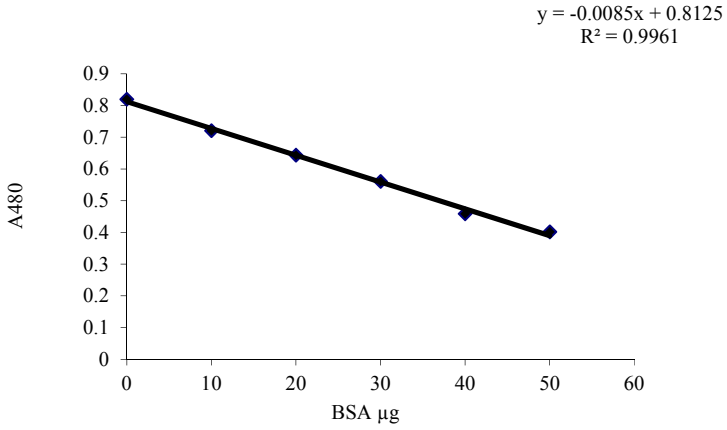


Figure 5.11: Standard calibration curve for protein concentration of the HCT116 cell line using bovine serum albumin (BSA) as a standard protein.

Table 5.8: Protein concentrations of the HCT116 cell line.

	1	2	mean	x µg/5µl	µg/µl	µl/500ug	µl/1300ug	µl/1500
HCT116 Bt maize	0.277	0.267	0.272	67.5	13.5	37.037037	96.2962963	111.111111
HCT116 non Bt maize	0.295	0.286	0.2905	65.1875	13.0375	38.3509108	99.7123682	115.052733

Figure 5.12: A typical IEF run for protein sample of the rat IE cells with recorded data of current, voltage and Vh.
The first red arrow indicated the IPG buffer movement, the yellow arrow indicated the salt movement and the blue arrow indicated the protein movement. The green arrow indicated that all proteins were focussed and steady state was reached.

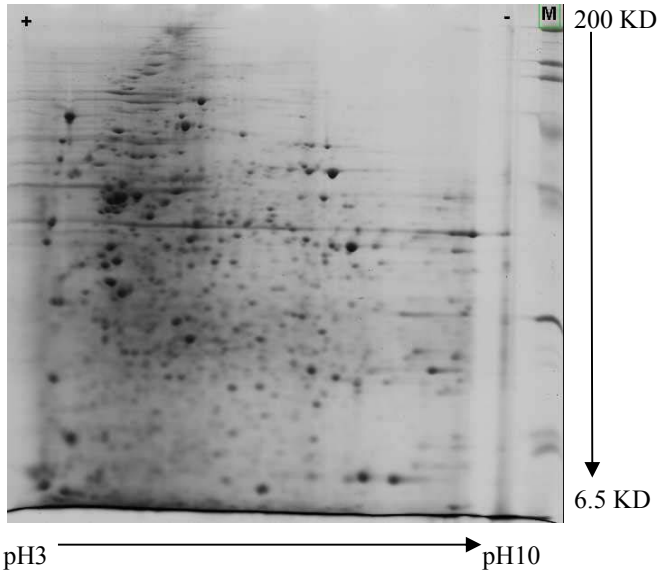


Figure 5.13: Representative image of the colloidal coomassie brilliant blue G-250 stained 2D gel. 2D gel was used to separate small intestinal epithelial-like cells (IE cells) proteins extracted from cultured IE cells exposed to *Bt* maize extract.
The first dimension was performed using immobilized pH 3-10 gradient gel strips, and followed by SDS-PAGE.

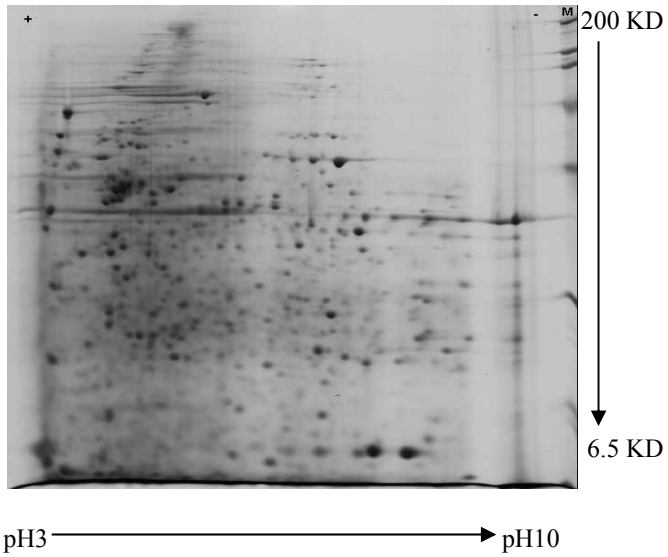


Figure 5.14: Representative image of the colloidal coomassie brilliant blue G-250 stained 2D gel. 2D gel was used to separate proteins extracted from HCT116 cell line exposed to *Bt* maize extract.
The first dimension was performed using immobilized pH 3-10 gradient gel strips, and followed by SDS-PAGE.

In 'View Results', all Filters were removed and all groups selected. The entire complement of features were ranked by *p*-value (low to high), and then viewed individually to highlight their relevance. Spots were removed according to the following:

- Contained a streak.
- De-staining issues leading to higher background signal, realising a false positive.
- Very close to edge of gel, problematic separation.
- Extra care was taken when assessing spots within the area of high protein concentration; acidic, high Mw.
- Issues with alignment.

Spot picking gels are illustrated in Appendix 29 to 31 (see appendices to chapter 5). Protein spots that were picked are illustrated in Table 5.10.

5.10 Protein identification using the global proteome machine (GPM) search engine

Protein IDs were done at the Burchmore lab, Glasgow Polyomics, University of Glasgow. For protein identifications, the analysis of mass spectrometry data were assigned using the Global Proteome Machine (GPM) search engine (www.thegpm.org) to interrogate protein sequences in the ENSEMBL *Rattus Norvegicus* database. The common Repository of Adventitious Proteins, cRAP (pronounced "cee-RAP"), is an attempt to create a list of proteins commonly found in proteomics experiments that are present either by accident or through unavoidable contamination of protein samples. The types of proteins included fall into three general classes as detailed in chapter 4.

Table 5.9: Results summary for both IE cells and HCT116 cell line of the original number of spots and the Spots after refining.

Treatment	Original number of spots	Spots after refining
HCT116 Vs IEC for Bt samples.	56	30
HCT116 Vs IEC for non-Bt samples.	24	12
Bt Vs non-Bt for the IECs	09	06
Bt Vs non-Bt for the HCT116	01	00
Total	90	48

Table 5.10: Protein spots that have been picked from the 2D gels (43 spots).

Bt Vs non *Bt*-maize for IECs samples	HCT116 Vs IECs for *Bt*-maize samples	HCT116 Vs IECs for non-*Bt*-maize samples
1627	814	914
1687	914	1687
2204	1561	1895
2547	1650	2370
2601	1615	2600
2882	1043	3663
	3634	2817
	1706	2877
	1954	3667
	1891	3360
	2098	3120
	2170	3309
	2262	
	2370	
	2393	
	2401	
	2550	
	2547	
	2590	
	2597	
	3657	
	2648	
	2786	
	2817	
	2987	
	3653	
	3137	
	3391	
	3436	
	3524	

There were no differentially expressed protein spots between the HCT116 cell lines when cells were exposed to *Bt* maize and non-*Bt* maize extracts.

Most of the proteins were successfully identified as illustrated in Appendix 32 (Appendices to chapter 5). In general, LC–MS/MS analysis of the 43 colloidal comassie brilliant blue stained 2-D gel protein spots representing all the differentially expressed protein spots between treatments (Table 5.10), generated a total of 152 protein identifications. Of the 43 spots, 36 spots were a mixture of two or more proteins, and 7 spots generated single protein identification for each spot.

5.10.1 Comparison of differentially expressed protein spots from IE cells and HCT116 cell line after exposure to *Bt*-maize extract

When comparing between HCT116 cell line and IE cells following exposure to *Bt*-maize extracts, 30 differentially expressed protein spots were analysed by LC-MS/MS. LC–MS-MS analysis of 30 colloidal comassie brilliant blue stained 2-D gel spots generated a total of 108 protein identifications. Of the 30 spots, 25 spots were a mixture of two or more proteins, whilst only 5 spots generated single protein identification for each spot. The threshold used to qualify differentially expressed spots was a Student t test, *P* value less than 0.05 and the false discovery rate (FDR, from q-values). One thousand three hundred and twenty four spots were identified to be commonly expressed in both HCT116 cell line and IE cells whereas 10 up-regulated spots were identified for HCT116 (Spot No. 2393, 2401, 3436, 1954, 1043, 2648, 2098, 2370, 1650, 3524) and 20 up-regulated spots were identified for IECs (Spot No. 2597, 814, 2547, 3634, 1561, 3653, 1891, 2262, 1615, 914, 3391, 3137,2170, 2817, 2550, 2590, 2987, 3657, 2786, 1706) (Figure 5.15 a).

Comparison between differentially expressed proteins between HCT116 cell line and IE cells exposed to *Bt* maize (MON810) extracts showed that 5 stress-related proteins (3 Stress-70 protein, 60 KDa heat shock protein and peroxiredoxin-1) were up-regulated (between 1.5-2.6 fold) in the former cell line and 19 stress related proteins (5 heat shock protein HSP 90-beta, 4 heat shock protein HSP 90-alpha, 4 stress-70 protein, heat shock protein 90 KDa beta member 1, T-complex protein 1 subunit epsilon, 2 60 KDa heat shock protein, T-complex protein 1 subunit alpha and peroxiredoxin-4) were up-regulated (between 1.5-4.4 fold) in IE cells.

5.10.2 Comparison of differentially expressed protein spots from IE cells and HCT116 cell line after exposure to non-transgenic near isogenic maize line extract

The venn diagram illustrated in Figure 5.15 (b) shows the number of 2D gel protein spots identified when comparing between HCT116 cell line and IE cells that were differentially expressed and up-regulated when cells were exposed to non-*Bt* maize extract. One thousand three hundred and forty two spots were identified to be commonly expressed in both HCT116 cell line and IE cells, whereas 5 up-regulated protein spots were identified for HCT116 (Spot No. 2600, 2877, 3309, 3360, 2370) and 7 up-regulated protein spots were identified for IE cells (Spot No. 914, 3663, 3667, 2817, 1895, 3120, 1687). Comparison between differentially expressed proteins between HCT116 cell line and IE cells exposed to non-*Bt* near isogenic maize extract showed that 8 stress-related proteins (2 Stress-70 protein, heat shock protein 4 like, heat shock protein HSP 90-beta, peroxiredoxin-1, thioredoxin peroxidase 2, 60 KDa heat shock protein and peroxiredoxin-4) were up-regulated (between 1.3-1.9 fold) in the former cell line and 4 stress related proteins (heat shock protein HSP 90-alpha, heat shock protein HSP 90-beta, 60 KDa heat shock protein and heat shock-related 70 KDa protein 2) were up-regulated (between 1.2-1.9 fold) in IE cells.

5.10.3 Comparison of differentially expressed protein spots from IE cells after exposure to *Bt*-maize and non-transgenic near isogenic maize line extract

The venn diagram illustrated in Figure 5.15 (c) shows the number of 2D gel protein spots identified when comparing between IE cells that were differentially expressed and up-regulated when cells were exposed to *Bt* maize and non-*Bt* maize extract. One thousand three hundred and forty eight spots were identified to be commonly expressed in both IE cells exposed to *Bt* maize extract and IE cells exposed to non-*Bt* maize extract whereas 4 up-regulated spots were identified for IE cells exposed to *Bt* maize extract (Spot No. 2547, 1627, 2601, 2882) and 2 up-regulated spots were identified for IE cells exposed to non-*Bt* maize extract (Spot No. 1687, 2204). Comparison between differentially expressed proteins between IE cells exposed to *Bt* maize extract and IE cells exposed to non-*Bt* maize extract showed that 8 stress-related proteins (2 Stress-70 protein, heat shock protein HSP 90-alpha, 2 heat shock protein HSP 90-beta, 2 T-complex protein 1 subunit epsilon and 60 KDa heat shock protein) were up-regulated (between 2.1-3.7 fold) in the IE cells exposed to

Bt maize extract and 1 stress related proteins (heat shock-related 70 KDa protein 2) was up-regulated (2.2 fold) in IE cells exposed to non-*Bt* maize extract.

5.10.4 Comparison of differentially expressed protein spots from HCT116 cell line after exposure to *Bt*-maize and non-transgenic near isogenic maize line extract

The venn diagram illustrated in Figure 5.15 (d) shows the number of 2D gel protein spots identified when comparing between HCT116 cell lines that were differentially expressed and up-regulated when cells were exposed to *Bt* maize and non-*Bt* maize extract. One thousand three hundred and fifty four spots were identified to be commonly expressed in both HCT116 cell line exposed to *Bt* maize extract and HCT116 cell line exposed to non-*Bt* maize extract and shows that there were no differentially expressed protein spots between the cells.

In order to assess functional relevance of changes in the identified differentially expressed proteins of IECs and HCT116, the proteins were aligned into 27 groups according to their molecular functions (Appendix 33 and 34). Categorizations were based on information provided by the online resource UniProt classification system. Some proteins were annotated manually, based on literature searches and closely related homologues. For IECs, proteins were aligned into 14 groups according to their molecular functions (Table 5.11; Figure 5.16).

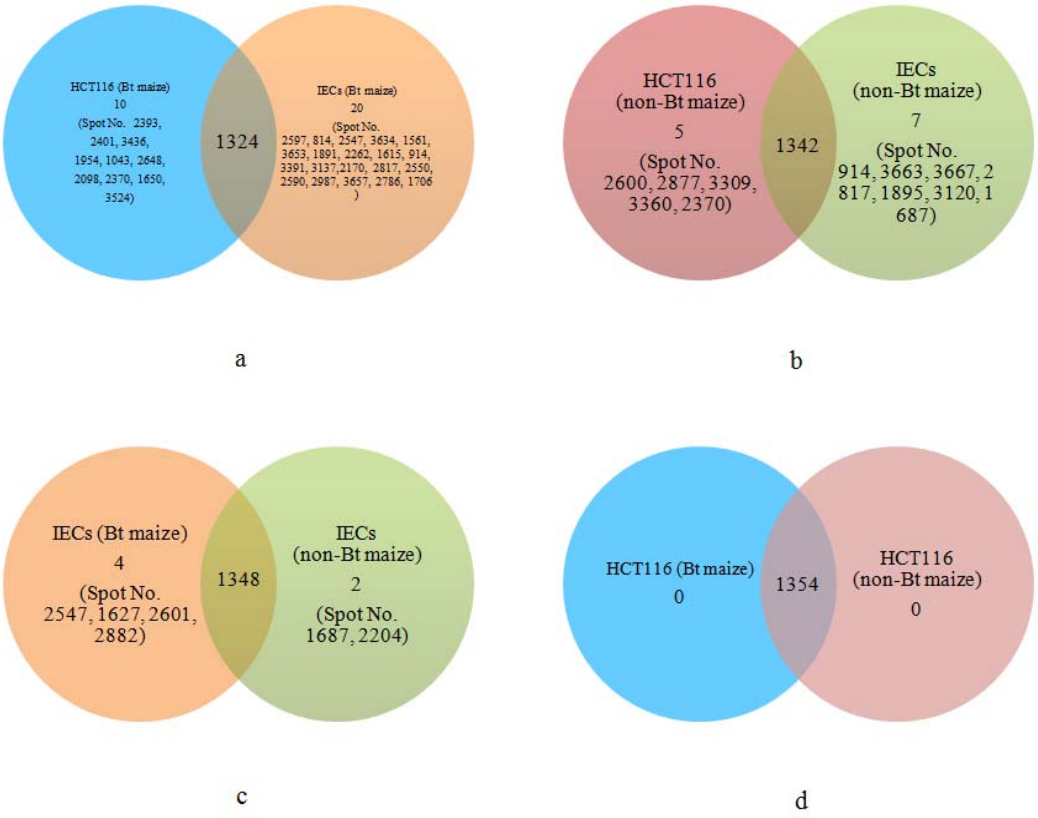


Figure 5.15: Two sets venn diagrams comparing the changes in the differentially expressed and up regulated spot number when cells were exposed to *Bt* and non-*Bt* maize extracts.
a) Venn diagram summarises the number of protein spots with differential expression and up-regulation between HCT116 cell line and IE cells when exposed to dose of 50µg/ml of *Bt* maize extract for HCT116 cell line and 6.25µg/ml of *Bt* maize extract for IECs. b) Venn diagram summarises the number of protein spots with differential expression and up-regulation between HCT116 cell line and IE cells when exposed to dose of 50µg/ml of non-*Bt* maize extract for HCT116 cell line and 6.25µg/ml of non-*Bt* maize extract for IE cells. c) Venn diagram summarises the number of protein spots with differential expression and up-regulation when IE cells were exposed to dose of 6.25µg/ml of *Bt* and non-*Bt* maize extracts. d) Venn diagram summarises the number of protein spots with differential expression and up-regulation when HCT116 cell line was exposed to dose of 50µg/ml of *Bt* and non-*Bt* maize extracts. Overlap between the two sets indicates those protein spots that were identified to be commonly expressed in both treatments.

Table 5.11: Functional classification of the identified protein spots for IE cells.
Spot No. 2547, 1627, 2601, 2882, 1687, 2204.

Molecular function	number of proteins	name of protein	Spot No.	Groups
structural molecule activity	1	Actin, cytoplasmic 1 Actin, cytoplasmic 1	2601	IECs Bt vs IECs non-Bt
	1	Lamin-B1	2547	IECs Bt vs IECs non-Bt
	1	Nuclear migration protein nudC	2547	IECs Bt vs IECs non-Bt
	1	Tubulin beta-5 chain	2547	IECs Bt vs IECs non-Bt
	2	Moesin	2547, 2601	IECs Bt vs IECs non-Bt
	1	Vimentin	1687	IECs Bt vs IECs non-Bt
chaperones	1	Endoplasmin Precursor (Heat shock protein 90 kDa beta member 1)	2547	IECs Bt vs IECs non-Bt
	2	Heat shock protein HSP 90-beta	2547, 2601	IECs Bt vs IECs non-Bt
	1	Heat shock protein HSP 90-alpha	2547	IECs Bt vs IECs non-Bt
	2	T-complex protein 1 subunit epsilon	2547, 1627	IECs Bt vs IECs non-Bt
	1	60 kDa heat shock protein, mitochondrial	1627	IECs Bt vs IECs non-Bt
	1	Heat shock-related 70 kDa protein 2	1687	IECs Bt vs IECs non-Bt
	2	Stress-70 protein, mitochondrial Precursor	2547, 1627	IECs Bt vs IECs non-Bt
Oxidoreductase	1	L-lactate dehydrogenase B chain	2547	IECs Bt vs IECs non-Bt
	1	Aldose reductase	2547	IECs Bt vs IECs non-Bt
	1	aldo-keto reductase family 1, member B8	2547	IECs Bt vs IECs non-Bt
Protein disulfide isomerase activity	1	Protein disulfide-isomerase	1687	IECs Bt vs IECs non-Bt
Kinase	1	Pyruvate kinase isozymes M1/M2	2547	IECs Bt vs IECs non-Bt
Transferase	1	S-adenosylmethionine synthetase isoform type-2	2547	IECs Bt vs IECs non-Bt
Hydrolase	1	Transitional endoplasmic reticulum ATPase	2601	IECs Bt vs IECs non-Bt
ATP binding	1	78 kDa glucose-regulated protein	1687	IECs Bt vs IECs non-Bt
calcium ion binding	1	Annexin A2	2204	IECs Bt vs IECs non-Bt
mitochondrial calcium ion homeostasis	1	inner membrane protein, mitochondrial	1627	IECs Bt vs IECs non-Bt
nucleotide binding	1	Nucleolin-like protein	2601	IECs Bt vs IECs non-Bt
protein domain specific binding	1	14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1)	2882	IECs Bt vs IECs non-Bt
	1	14-3-3 protein theta	2882	IECs Bt vs IECs non-Bt
actin binding	1	14-3-3 protein gamma	2882	IECs Bt vs IECs non-Bt
sodium channel regulator activity	1	14-3-3 protein eta	2882	IECs Bt vs IECs non-Bt

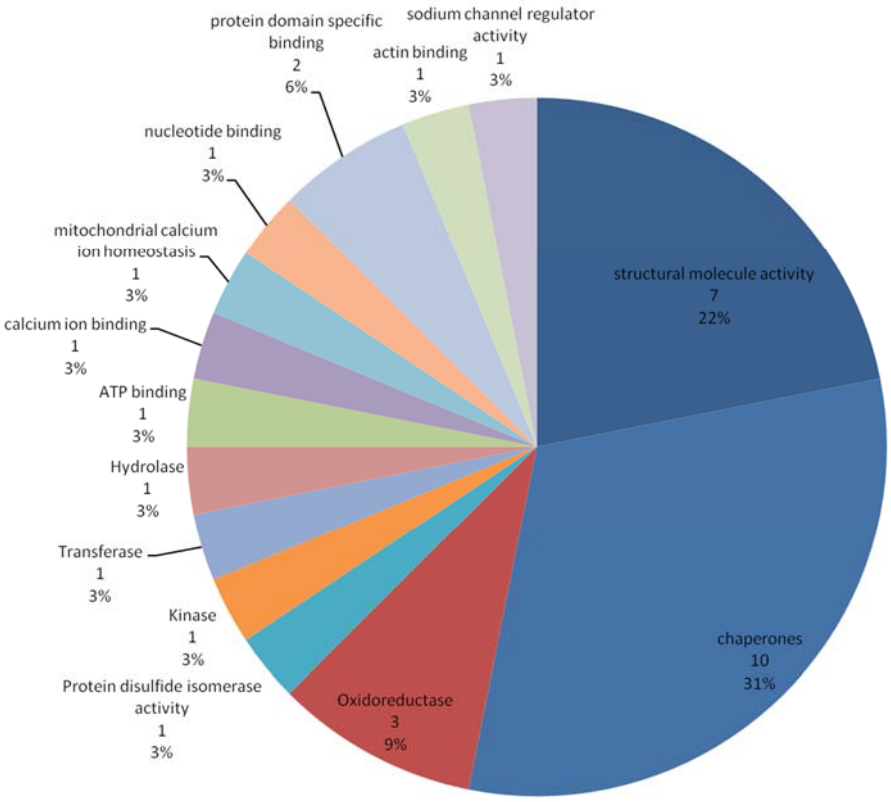


Figure 5.16: Functional classifications of differentially expressed intestinal epithelial cells proteins (IECs *Bt* and IECs non-*Bt*).
A total of 32 proteins were identified and these proteins fall within 14 major functional categories. 3 (9%) contain oxidoreductase activity, 10 (31%) are chaperones, 1 (3%) contained transferase activity and 7 (22%) contained structural molecule activity.

Chapter 6

6 Discussion

The present study investigated the effects of MON810, a commercial insect-resistant GM maize line expressing the δ-insecticidal protein Cry1Ab from *Bacillus thuringiensis*, on both the feed conversion efficiency and differential gene expression at the proteome level in the epithelial cells of the small intestine of rat as a non-target model system. In addition, comparative *in vitro* studies of Cry1Ab on mammalian epithelial cells were conducted as a possible alternative to animal studies. The potential cellular effects of Cry1Ab on mammalian epithelial cells are unknown. Therefore, these cellular effects were investigated via changes in the proteome as a first stage, to look for possible changes in major metabolic/biosynthetic pathways. Molecular proteomic profiling techniques were included for both the *in vivo* and *in vitro* approaches for the safety evaluation of MON810. For this study the maize lines used were grown simultaneously in neighbouring fields, using the same agricultural techniques and had therefore the same external climatic conditions, which eliminates or at least reduces environmental variables. Three reference lines were used to determine whether the changes that may occur with the consumption of MON810 maize lay within the expected range for several different unmodified reference varieties or whether the change lies outside of it. In addition, the compositional analysis indicated that both the transgenic and non-transgenic maize had similar nutritional composition, and thus the diets given to the animals were similarly balanced.

6.1 Physiological effects of MON 810 in rats

In the present study potential effects of expression of Cry1Ab protein were limited to the measurements of the absolute body weight, body weight gain, food consumption and feed conversion efficiency. Monitoring body weight gain and food consumption in feeding studies with rats, ruminants, pigs and poultry can be a sensitive indicator of overall animal health. The absence of any meaningful differences in these measured parameters across a variety of animal species provides further support for the comparability of MON810 to conventional corn varieties (Borzelleca, 1996). However, other studies have also investigated the effects of Cry1Ab on blood chemistry, hematology, urine chemistry, organ weights, gross and microscopic appearance of

tissues, peripheral immune response and digestive fate of the Cry1Ab gene as discussed by below. This was because previous extensive studies had already been carried out and was outside the scope of the present study. Although it was necessary to verify the lack of toxicity of Cry1Ab protein in rats, the focus of the present study was to investigate the effects of MON810 at the subcellular level by evaluating the effects on the proteome of the epithelial cells from the small intestine of young male rates.

During the course of the 7 and 28-day rat feeding studies, no adverse behavioural effects on rats were observed and there were no significant differences in absolute body weights, body weight gains, food consumption and feed conversion efficiency between animals fed MON 810 in the diet when compared to animals fed diets containing grain from conventional control, reference varieties and the commercial rodent control. Whilst a small number of animals may affect the statistical power regarding the detection of subtle differences, in line with requirements governing the use of animals for experimentation (The three Rs, Balls and Fentem, 2008), four rats per group is considered to be the minimum, but acceptable, number used by the industry in such studies (Cockburn, pers. comm.).

The comparable responses of rats fed MON810 to rats fed control grain supports the absence of untoward pleiotropic effects in MON810 corn as confirmed in comprehensive composition studies as well as feeding studies in swine, poultry and ruminants with MON 810 and related *Bt* corn varieties that produce Cry1Ab protein *in planta* (Weber *et al.*, 2000; Hammond *et al.*, 2002; Donkin *et al.*, 2003; Taylor *et al.*, 2003).

Hammond *et al.* (2006) have carried out a 90-day rat feeding study with grain from MON 810 corn (YieldGard®Cornborer – YieldGard Cornborer is a registered trademark of Monsanto Technology, LLC) that is protected against feeding damage from Corn borer insects. Corn borer protection was accomplished through the introduction of Cry1Ab coding sequences into the corn genome for *in planta* production of a bioactive form of Cry1Ab protein. In the study by Hammond *et al.* (2006), grain from MON810 and its near-isogenic control were separately formulated into rodent diets at levels of 11% and 33% (w/w) by Purina Mills, Inc. (PMI). A total of 400 rats were used, these being divided into 10 groups of 20 rats/sex/group. The responses of rats fed diets containing MON810 grain were compared to those of rats fed grain from conventional corn varieties. Overall health, body weight, food consumption, clinical

pathology parameters (blood chemistry, hematology, and urine chemistry), organ weights, and gross and microscopic appearance of tissues were comparable between groups fed diets containing MON810 and conventional corn varieties. The ‘‘no observed effect level’’ is equal to the highest dietary level of MON810 (33%) fed to rats. From their studies, these authors hypothesized that substantial safety margins exist for human consumption of MON810 grain based on the rat feeding study and are supported by a lack of adverse effects in numerous farm animal feeding studies. Consistent with agronomic, compositional and farm animal feeding studies, the 90-day rat feeding study did not detect detrimental effects in the MON810 grain. Thus, MON810 is considered to be substantially equivalent to, and as safe and nutritious as, conventional corn varieties (Hammond *et al.*, 2006).

In agreement with these findings, a study was done by Kılıç and Akay (2008) on Wistar albino rats that were fed through three generations with either 20% *Bt* corn or its conventional counterpart corn (below the safety margin 33%) containing diets in order to maintain a balanced rodent diet in this long-term feeding study. The *Bt* corn and its reference line that were used by Kılıç and Akay were obtained from the agency of Turkish Ministry of Agriculture and Rural Affairs. No statistically significant differences were found in final body weights and relative organ weights (Liver and kidney) of rats within groups but there were some minimal histopathological changes in the liver and kidney. They concluded that although the results obtained from their study showed minor histopathological effects in rats fed with *Bt* corn, long-term consumption of transgenic *Bt* corn throughout three generations did not cause severe health concerns on rats.

In addition, Onose *et al.* (2008) have evaluated the sub-chronic toxicity of dietary administered Cry1Ab protein from *Bacillus thuringiensis* in male rats with chemically induced gastrointestinal impairment. They investigated the possible toxicity of *Bt* proteins under conditions of small intestinal damage and/or reduced gastricacid secretion. Gastrointestinal impairment (GI) alone and GI with a purified *Bt* protein Cry1Ab fed (GI + Bt) groups were given intra-peritoneal injections of famotidine to decrease gastric acid secretion twice a day at 30 mg/kg body weight in weeks two and four. GI and GI + *Bt* groups were additionally fed diets containing 80 ppm indomethacin for chemical induction of intestinal damage during weeks one and three. *Bt* alone and GI + *Bt* groups were also fed diet containing *Bt* protein Cry1Ab at a

concentration of 10 ppm in weeks two and four. A no treatment control group was also included in their study. At the end of week four, all rats were euthanized under ether anesthesia and blood samples were collected for serum biochemistry and hematology and a complete necropsy was performed. No significant changes indicative of toxicity of the *Bt* protein Cry1Ab were found with any of the parameters investigated. From their study, they concluded that no significant toxicological effects were detected in this sub-chronic gastrointestinal impairment rat model.

The summary prepared by the GMO Panel of the European Food Safety Authority (EFSA) also supports the conclusion that Cry1Ab is not toxic to mammals. Their conducted 90-day sub-chronic rodent studies and the analysis was performed on feed consumption, body weight, clinically observable adverse effects, clinical pathology during life, as well as organ weights and histopathology after study termination. Following their review of MON810 rat feeding studies for 90-day, they concluded that the results of that study do not indicate adverse effects from consumption of maize line MON810 (EFSA, 2004).

It is noteworthy that similar studies with pigs, as a human model, failed to demonstrate any toxic effects on either growth or the intestinal flora as a consequence of feeding on MON810 in either short-term or long-term studies (Walsh *et al.*, 2011; Buzoianu *et al.*, 2012a). Furthermore, this group also showed that feeding sows MON810 did not cause any inflammation or allergy or effects on the peripheral immune response (Walsh *et al.,* 2012; Buzoianu *et al.,* 2012b). Interestingly it would appear that Cry 1Ab is readily digested.

In addition to the effects of *Bt* maize, several studies have been carried out to investigate the effects of other *Bt* crops in rats. One of these studies was a 90 day feeding trial on Wistar rats to assess the safety of *Bt* rice consumption. Blood samples collected one week prior to sacrifice were analyzed and compared for biochemical and standard haematological parameters and the results showed that only a few parameters were significantly different, but all within the normal reference intervals for rats of this breed and age and not in relation to any other findings, thus these differences were not considered treatment related. Upon sacrifice a large number of organs were weighed, histopathological and macroscopic examinations were carried out; the results showed no adverse or toxic effects of *Bt* rice in rats (Schrøder *et al.*, 2007). Another study performed by Kroghsbo *et al.* (2008) to examine the immunmodulating effect of *Bt* rice

expressing Cry1Ab protein from *Bacillus thuringiensis (Bt)* in 28- and 90-day Wistar rats feeding studies. Similarly, no detrimental effects of Cry1Ab protein were found. Also Yuan *et al.* (2013) constructed a study to verify the safety of the consumption of *Bt* rice on gastrointestinal (GI) health of rats after 90 days by measuring a range of parameters including intestinal immunity, intestinal permeability, bacterial activity, fecal enzymes, epithelial structure, and microflora composition; again, no significant differences were found between genetically modified and non-genetically modified groups and no detrimental effects were found on GI health resulting from exposure to genetically modified *Bt* rice. From their study, these authors hypothesized that this study may offer a systematic safety assessment model for GM crops with respect to the effects on GI health.

6.1.1 Effects of consumption of MON810 on gene expression in rat small intestinal epithelial cells at the proteome level: *In vivo* effects

To the best of our knowledge, this study is the first to evaluate the potential effects of transgenic maize (MON810) expressing Cry1Ab on differential gene expression in the epithelial cells of the small intestine (s.i.) of rats at the level of the proteome. Thus, there are no direct comparative studies investigating effects of *Bt* Cry proteins on small intestine proteome. The present study shows that many of the differentially expressed proteins are defense/stress-related proteins. The focus of this study was on expression of stress-related proteins because these proteins act as indicators that the cells are exposed to different kinds of stress conditions, such as infection, inflammation, exercise, exposure of the cell to toxins, starvation, hypoxia, or water deprivation. Initially the study was conducted for 7 days to see if there is an acute effect and then for 28 days to see if there is a sub-chronic effect of MON810 maize. The time points selected in the present study, i.e. 7 days and 28 days, were selected on the basis of the OECD guidelines in respect of oral toxicity studies in rodents. Whilst it is acknowledged that changes in the proteome are dynamic and will therefore change with time, what is important is to make a direct correlation between the proteome profile with potential toxicity at the same given time point. Whilst changes to the epithelial small intestinal cells are likely to be detected within a much shorter time frame, physiological effects will take much longer to be detectable. No positive control was used in this study, because, to the best of my knowledge, there are no appropriate molecules that could be used which exhibit an identical mode of action to that of Cry1Ab.

7-Day rat feeding trial: Acute effects

As mentioned above, rat body weights were similar in all animals during the whole experiment. At sacrifice, body weight gain for the five different groups varied from 31.5 to 38.5 g. However, there were no significant differences between control and GM maize-fed animals as well as to the other rats fed on reference maize diets and commercial rodent diet.

Comparison between MON810 and Mon Conv Corn fed rats

Proteome analysis demonstrated that in MON810 maize-fed rats there were a number of differentially expressed proteins, mostly related to metabolic pathways (i.e. protein biosynthesis, lipid and carbohydrate metabolism) when compared to its parental control maize-fed rats (Mon Conv Corn group). Interestingly, similar results were found with rats fed other maize varieties.

Comparison between differentially expressed proteins between rats fed MON810 maize diet and its parental control diet (Mon Conv Corn group) showed that 2 stress-related proteins (catalase and 60 kDa heat shock protein) were up-regulated in rats fed MON810 maize diet but no stress related proteins were up-regulated in Mon Conv Corn group.

This 60 kDa heat shock protein is known to be expressed in response to a wide variety of physiologicaland environmental insults, acting as molecular chaperones for nascent and stress-accumulated misfolded proteins, or mediating immunological functions, thus exerting a protective function. Cells from virtually all organisms respond to a variety of stresses by the rapid synthesis of a highly conserved set of polypeptides termed heat shock proteins (HSPs). The precise functions of HSPs are unknown, but there is considerable evidence that these stress proteins are essential for survival at both normal and elevated temperatures. HSPs also appear to play a critical role in the development of thermotolerance and protection from cellular damage associated with stresses such as ischemia, cytokines, and energy depletion. These observations suggest that HSPs play an important role in both normal cellular homeostasis and the stress response suggesting that the HSPs may be important modifying factors in cellular responses to a variety of physiologically relevant conditions such as hyperthermia, exercise, oxidative stress, metabolic challenge, and aging (Kregel, 2002). Expression of HSPs occurs contributing to the higher capability

of young rats to cope against xenobiotics and stress conditions (Malatesta *et al.*, 2008). Previous studies have shown that heat shock proteins (HSPs) are involved in cytoprotection mediated by their function as a molecular chaperone (Nakamura *et al.*, 1991; Schoeniger *et al.*, 1994; Otaka *et al.*, 1997). Takada *et al.* (2010) have examined the effect of HSP60 or HSP70 over expression on hydrogen peroxide induced (H_2O_2) cell damage in the small intestinal epithelial cells. They have established HSP60- or HSP70-overexpressing small intestinal epithelial cells by transfecting full length of human HSP60 or HSP70 cDNA to rat small intestinal (IEC-6) cells, and investigated the cytoprotective ability of these cells to withstand hydrogen peroxide-induced small intestinal epithelial cell injury. These cells were treated with H_2O_2 (0–0.14 mM) and the viability of these cells was determined by MTT-assay, whilst cell necrosis was evaluated by LDH-release assay. Furthermore, they evaluate apoptosis by caspases-3/7 activity assay. They found that the viability was significantly higher in both HSP60-overexpressing cells and HSP70-overexpressing cells compared with that in control cells after H_2O_2 treatment; they also found that the apoptotic cells were also reduced in HSP60-overexpressing. From their study, they concluded that HSP60 plays an important role in protecting small intestinal epithelialcells from H_2O_2-induced cell injury. However, they found that HSP70-overexpressing cells did not show anti-apoptotic ability. From their findings, they suggested that the function of each HSP is different in the small intestine. Therefore, they suggested that for the therapy of small intestinal mucosal lesion, HSP60-induction therapy could be a new therapeutic strategy.

The other stress-related protein which was up regulated in rats fed MON810 maize diet is catalase. Tissues are normally protected from oxidative damage by the presence of enzymes such as catalase, which is an oxidative stress response protein which occurs in almost all aerobically respiring organisms and serves to remove the hydrogen peroxide formed in the cells to protect them from the toxic effects of the hydrogen peroxide (Takeuchi *et al.*, 1995).

Comparison between MON810 and Mcert maize fed rats

Proteome analysis revealed an up-regulation of three stress-related proteins in MON810 maize-fed rats when compared to Mcert maize-fed rats, whereas no stress related proteins were up-regulated in Mcert maize-fed rats. These stress-related proteins were catalase, 60 kDa heat shock protein and stress-induced phosphoprotein 1. Stress-induced phosphoprotein 1 (STIP1) is a 62.6 kDa protein, also known as heat shock

protein (HSP)-organizing protein (HOP). STIP1 acts primarily as an adapter that directs HSP 90 to HSP 70-client protein complexes in the cytoplasm, but recent evidence suggests that STIP1 can also modulate the chaperone activities of these HSPs (Odunuga *et al.*, 2004). Overexpression of this protein may confer protective reactions or mediate anti-apoptosis signaling in the epithelial cells (Zhang *et al.*, 2006).

Comparison between MON810 and Mon Garst maize fed rats

Again, proteome analysis revealed an up-regulation of three stress-related proteins in MON810 maize-fed rats when compared to Mon Garst maize-fed rats, whereas no stress related proteins were up-regulated in Mon Garst maize-fed rats. These stress-related proteins were thioredoxin-dependent peroxide reductase, peroxiredoxin-6 and LDLR chaperone MESD precursor. Thioredoxin-dependent peroxide reductase enzyme protects radical-sensitive enzymes from oxidative damage by a radical-generating system (Masaki *et al.*, 2003). The antioxidant enzyme peroxiredoxin 6 (Prdx6) is a key regulator of the cellular redox balance, particularly under stress conditions and overexpressed Prdx6 reduced oxidative stress as reflected by the lower levels of oxidized phospholipids in the protumorigenic skin of Prdx6 transgenic mice (Rolfs *et al.*, 2013). In addition, there is increasing evidence that the peroxiredoxin plays a role in cell signaling, by controlling and/or sensing hydrogen peroxide and peroxynitrite levels (Bertoletto *et al.*, 2012). Prdx6 has important roles in both antioxidant defense based on its ability to reduce peroxidized membrane phospholipids and in phospholipid homeostasis based on its ability to generate lysophospholipid substrate for the remodeling pathway of phospholipid synthesis (Fisher, 2011). LDLR chaperone MESD (Mesoderm development protein) precursor is a chaperone specifically assisting the folding of beta-propeller/EGF (epidermal growth factor) modules within the family of low-density lipoprotein receptors (LDLRs). Members of the low-density lipoprotein receptor (LDLR) family require the chaperone called Mesoderm development, in the mouse. All LDLRs have at least one six-bladed beta-propeller domain, which is immediately followed by an epidermal growth factor (EGF) repeat (Koduri and Blacklow, 2007). In the present study, the LDLR chaperone MESD precursor and peroxiredoxin-6 were uniquely differentially expressed in MON810 maize fed rats.

Comparison between MON810 and Mon Gold maize fed rats

In addition, proteome analysis revealed an up-regulation of two stress-related proteins in MON810 maize-fed rats when compared to Mon Gold maize-fed rats,

whereas no stress related proteins were up-regulated in Mon Gold maize-fed rats. These two stress-related proteins were peroxiredoxin-6 and LDLR chaperone MESD precursor, as discussed above.

Furthermore, comparison between differentially expressed proteins between rats fed Mcert maize, Mon Conv Corn, MON810 maize, Mon Garst maize and MON Gold maize showed that 2 stress-related proteins (LDLR chaperone MESD precursor and peroxiredoxin-6) were up-regulated in MON810 maize-fed rats and 1 stress-related protein (thioredoxin-dependent peroxide reductase) was up-regulated in the Mcert maize-fed rats. These results highlight the importance of including other conventional varieties when evaluating the effects of a GM ingredient.

Comparison between rats fed non-transgenic maize lines

Expression of HSPs and other stress-related proteins have been found with other maize varieties. Comparison between differentially expressed proteins between rats fed Mcert maize diet and Mon Garst maize diet showed that 2 stress-related proteins (Thioredoxin-dependent peroxide reductase and protein DJ-1) were up-regulated in the Mcert maize-fed rats, but no stress related proteins were up-regulated in Mon Garst maize-fed rats. DJ-1 is an antioxidant protein that participates in anti-oxidative stress reactions to protect cells against oxidative stress and cell death (Ariga *et al.*, 2013). Comparison between differentially expressed proteins between Mcert maize-fed rats and Mon Conv Corn-fed rats showed that 1 stress-related protein (60 kDa heat shock protein) was up-regulated in the Mcert maize-fed rats and 1 stress related protein (stress-induced phosphoprotein 1) was up-regulated in Mon Conv Corn-fed rats. Comparison between differentially expressed proteins between Mon Conv Corn-fed rats and Mon Garst maize-fed rats showed that 1 stress-related protein (catalase) was up-regulated in the Mon Conv Corn-fed rats. Comparison between differentially expressed proteins between Mon Garst maize-fed rats and Mon Gold maize-fed maize showed that 1 stress-related protein (T-complex protein 1 subunit beta) was up-regulated in the Mon Garst maize-fed rats. T-complex protein 1 subunit beta is a molecular chaperone which assists the folding of proteins upon ATP hydrolysis (UniProtKB, 2013).

Importantly, the 7-day rat feeding trial data confirmed the up-regulation of a few stress-related proteins not only in MON810 maize-fed rats but also in its parental control maize-fed rats, reference maize variety-fed rats (Mon Garst group) and

commercial maize-fed rats. Thus, the subsequent up-regulation of these stress-related proteins in the rat small intestine is not unique to the GM maize diet, but was also shown to occur in the non-transgenic maize lines. Thus, *in vivo* effects of these different maize varieties on the proteome of the epithelial cells of the small intestine were negligible, with only 2 stress-related proteins were up-regulated in MON810 maize-fed rats and 1 stress-related protein was up-regulated in the Mcert maize-fed rats when the five different groups were compared to each other. The difference in the number of these stress-related proteins is likely to be due to the physiological differences between rats. However, there were no other studies to support this observation. The only other study to have used proteomics to investigate the stress response in rodents was that of Zhang *et al.* (2003), who studied the differentially expressed proteins of gamma-ray irradiated mouse intestinal epithelial cells. They compared the proteomics of mouse intestinal epithelial cells with its irradiated counterparts *in vivo* to provide an efficient resolution to analyze radiation related proteins directly at the protein level. The intestinal epithelia were isolated from mice grouped as sham-irradiation, 3 h and 72 h irradiation and the mice were exposed to 9.0 Gy single dose of γ-irradiation. The irradiated mice were then killed 3 h and 72 h post-radiation, and intestines were removed. A series of methods, including two dimensional electrophoresis, PDQuest 2-DE software analysis, peptide mass fingerprinting based on matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) and SWISS-PROT database searching, were used to separate and identify the differential proteins. They also used western blotting and RT-PCR to validate the differentially expressed proteins. From their study, they found that the mouse intestine was severely damaged by 9.0 Gy γ-irradiation. A total of 19 spots matched known proteins after database searching. These proteins were mainly involved in metabolism, anti-oxidation, protein post-translational processes, and signal transduction. They found that among the 19 preliminarily identified proteins, three spots belonged to oxidative stress response proteins, i.e., peroxiredoxin I, glutathione S-transferase P2, and antioxidant protein 2. From their study, they concluded that these differentially expressed proteins might play important roles when mouse intestine was severely injured by γ-irradiation and they suggested that differential proteomic analysis may be a useful tool to study the proteins involved in radiation damage of mouse intestinal epithelia.

In conclusion, the observed differences in the number of stress-related proteins identified in the 7-day feeding study are consistent with normal physiological variation between rats and are not related to the consumption of MON810 maize. Therefore, the results of this study do not indicate adverse effects on the proteome of the small intestinal epithelial cells of rats from the consumption of the transgenic variety MON 810 maize.

28-Day rat feeding trial: Sub-chronic effects

The study design is an adaptation of the Organization for Economic Cooperation and Development (OECD) guideline No. 407 for a repeated dose 28-day oral toxicity study in rodents (OECD, 2008). In the present study, rats were fed diets containing 33% (w/w, high dose) maize. Rats were fed diets formulated with either MON810 maize or the parental control for MON810 corn (Mon Conv Corn group) or commercially available maize based rodent diet (Mcert group). Two reference control groups (Mon Garst and Mon Gold groups) were included to establish a normal range of values for all the parameters measured. The rats in these reference groups were fed on diets containing non-genetically modified maize varieties.

It is important to note that the study, while based on the protocol for a sub-chronic toxicity study, is a comparative feeding study, with different varieties of maize. In this particular study, the highest level of incorporation in the diet of MON810 maize was 33%. The ability of feeding studies to detect adverse effects from a constituent present in a food product will be largely dependent on the intrinsic toxicity of any such constituent and whether it is present in the food in a sufficient amount to induce toxicity under the conditions of the study. The study has been well designed and executed and the absence of any adverse effects in this study may however provide additional assurances of safety.

No treatment-related deaths or adverse effects were observed during the study; body weight gain, food consumption, feed conversion efficiency were similar across all groups. At sacrifice, the body weight gain varied from 93.75 to 147.25 g. As seen in the 7-day study, there were no significant differences between control and GM maize-fed animals, or indeed between rats fed on reference maize diets and commercial rodent diet.

Comparison between MON810 and Mon Conv Corn fed rats

Proteome analysis demonstrated that in MON810 maize-fed rats the majority of the differentially expressed proteins were related to metabolic pathways when compared to its parental control maize-fed rats (Mon Conv Corn group). Interestingly, the same results were found with the other maize varieties fed rats.

As with the 7-day study, proteome analysis revealed the up-regulation of several stress-related proteins in MON810 maize-fed rats when compared to its parental control maize-fed rats. Comparison between differentially expressed proteins between rats fed MON810 maize diet and its parental control diet (Mon Conv Corn group) showed that 2 stress-related proteins (stress-induced phosphoprotein 1 and peroxiredoxin-1) were up-regulated in rats fed MON810 maize diet and 1 stress related protein (superoxide dismutase) was up-regulated in Mon Conv Corn group. Peroxiredoxin-1 and superoxide dismutase are antioxidant defense enzymes. Peroxiredoxin-1 is involved in the redox regulation of cells, such as reducing peroxides with reducing equivalents throughthe thioredoxin system (Zhang *et al.*, 2003). The peroxiredoxin proteins have conserved cysteine residues that participate in oxidoreductive reactions and protect macromolecules from the oxidative damage. Therefore, peroxiredoxin I is known to protect the cells from apoptosis by reducing oxidative damage (Ishii *et al.*, 2000). Ishii *et al.* (2000) investigated the induction of murine intestinal peroxiredoxin I by the dietary butylated hydroxyanisole (BHA), which is a synthetic phenolic antioxidant that is widely used as a food preservative. The mice were fed a diet containing 0.7% (w/w) BHA for 7 days and they have found that dietary BHA induces intestinal peroxiredoxin I. From their study, they found that the induction of peroxiredoxin I may be important to protect the cells and tissues against toxic electrophiles and reactive oxygen species. Superoxide dismutase destroys radicals which are normally produced within the cells and which are toxic to biological systems (MacMillan-Crow and Thompson, 1999).

Comparison between MON810 and Mon Garst maize fed rats

Comparison between differentially expressed proteins between rats fed MON810 maize and rats fed Mon Garst maize showed that 2 stress-related protein (stress-induced phosphoprotein 1 and 60 kDa heat shock protein) were up-regulated in rats fed MON810 maize and 1 stress related protein (T-complex protein 1 subunit beta) was up-regulated in rats fed Mon Garst maize.

Comparison between MON810 and Mon Gold maize fed rats

Similarly, comparison between differentially expressed proteins between rats fed MON810 maize and rats fed Mon Gold maize showed that 2 stress-related proteins (2 stress-induced phosphoprotein 1) were up-regulated in rats fed MON 810 maize. Furthermore, comparison between differentially expressed proteins between all five groups (rats fed Mcert, Mon Conv Corn, MON810, Mon Garst and MON Gold maize) showed that 1 stress-related protein (stress-induced phosphoprotein 1) was up-regulated in rats fed MON810 maize.

Comparison between rats fed non-transgenic maize lines

In the present study, expression of a stress-related protein was also found in rats fed the commercial maize based rodent diet (Mcert group). Comparison between differentially expressed proteins between rats fed Mcert maize and Mon Conv Corn showed that 1 stress-related protein (peroxiredoxin-1) was up-regulated in rats fed Mcert maize.

Importantly, our 28-day rat feeding trial data confirmed the up-regulation of stress-related proteins both in rats fed the GM (MON810) maize and those fed the non-transgenic maize varieties. These results highlight the importance of including other conventional varieties when evaluating the effects of a GM ingredient and these data suggest that these stress-related proteins found in MON810 maize-fed rats are not directly related to the consumption of MON810 maize diet, but are due to normal physiological variation. Thus, *in vivo* effects of these different maize varieties on the proteome of the epithelial cells of the small intestine were negligible, with only 1 stress-related protein being up-regulated in MON810 maize-fed rats when the five different groups were compared to each other.

Psychological stress is a common event in normal daily life and a mild stress can be beneficial because it improves physiological functions in the body, such as a mild increase in noradrenalin in the blood improves circulation; a little more secretions of thyrotrophic releasing hormone helps basic metabolism in the body (Gardner Jr, 2001).

The intestine is one of the common targets of stress. The intestinal barrier consists of a single layer of epithelial cells and the tight junctions connect the epithelial cells to each other so as to form a barrier between the external environment and the internal

environment. The intestinal epithelial barrier allows nutrients and water to be absorbed and prevents noxious substances from absorption (Nagler-Anderson, 2001).

As previously discussed, heat shock proteins (HSPs), also called stress proteins, are a group of proteins that are present in all cells and are induced when a cell undergoes various types of environmental stresses like heat, cold and oxygen deprivation (Trautinger, 2001). HSPs are also present in cells under perfectly normal conditions acting like 'chaperones,' to make sure that the cell's proteins are in the right shape and in the right place at the right time. For example, HSPs help new or distorted proteins fold into shape, which is essential for their function. They also shuttle proteins from one compartment to another inside the cell, and transport old proteins to 'garbage disposals' inside the cell. Heat shock proteins are also believed to play a role in the trafficking of peptides to the cell surface to help the immune system recognize diseased cells (Fehrenbach and Northoff, 2000; Latchman, 2001). Based on the information above, we suggest that HSPs in the intestinal epithelium can be affected by psychological stress and normal rat intestinal epithelial cells express HSPs. Therefore, the expression of these stress-related proteins in the epithelial cells of the rat small intestine does not indicate any adverse effects, which can be directly related to MON810 maize. The most compelling evidence for this is the comparison of results for test group individuals against those for the parental, references and commercial controls. This clearly demonstrates that the observed differences in the number of stress-related proteins represent normal biological variability.

Similarly, Yang *et al.* (2009) have indicated that the normal rat intestinal epithelial cells express HSPs. The rats in the Yang *et al.* study were subjected to chronic water deficient stress. They found that the intestinal epithelial heat shock protein (HSP) 70 was detected in normal intestinal epithelial cells and the chronic stress inhibited intestinal epithelial cell HSP70 expression at both mRNA and protein levels that correlated with the stress-induced intestinal epithelial hyperpermeability. Also, they found that the pretreatment with HSP70 abrogated stress-induced intestinal barrier dysfunction. They suggest that these findings provide evidence in support of the concept that chronic stress is involved in many chronic disorders such as inflammatory bowel diseases. Furthermore, that HSP70 inhibited chronic stress induced intestinal barrier dysfunction may prevent stress induced intestinal epithelial cell apoptosis.

Wang *et al.* (2013) assessed the safety of transgenic *Bt* rice expressing Cry1Ab protein by establishing 30 and 90 day safety studies on female Swiss rats. Their studies revealed that *Bt* rice had no significant effect on hematological indicators, calcium ion concentration of blood lymphocytes and apoptosis rate of blood lymphocytes, indicating that Cry1Ab protein could not affect the blood lymph of Swiss rat. Similarly, *Bt* rice had no effect on enzyme activities including catalase (CAT), acetylcholine esterase (AChE), superoxide dismutase (SOD) and glutathione (GSH) in heart, liver, spleen, brain, kidney and ovary of Swiss rat. These particular enzymes were selected for study as they are known to be involved in the stress response. In addition to thses enzyme studies quantitative real-time PCR (qPCR) was used to analyze the transcript levels of 6 genes encoding for these major detoxification enzymes in liver (AchE, CAT, SOD, alkaline phosphatase (ALP), glutamic oxalacetictransaminase (AST) and alanine aminotransferase (ALT). QPCR analysis of these genes revealed that Cry1Ab rice exerted no influences on the levels of these transcripts in liver of Swiss rat. No comparable studies with *Bt* maize appeare to have been carried out with rats. Several studies have been carried out to investigate the effects of Cry1Ab protein on gene expression at the transcriptome level. Guertler *et al.* (2012) carried out a long-term study with 36 lactating dairy cows fed GM maize (N = 18, MON810) or the near-isogenic counterpart (N = 18) for a time period of 25 months to study the effect of feeding MON810 to cows and to investigate the fate of both the recombinant DNA and protein. After a period of 25 months, tissues of the gastrointestinal tract (small intestine, large intestine and appendix) and samples from liver were used for gene expression analysis of major genes of the inflammation, cell cycle and apoptosis pathways. These tissues were chosen due to the fact that immunactive Cry1Ab protein and its fragments are detectable in the gastrointestinal tract and therefore get in contact with the epithelial cells of the gastrointestinal tract. The results indicate, that feeding GM maize (event MON810), compared to feeding the near-isogenic maize variety, does not influence the gene expression of biomarkers for apoptosis, inflammation and cell cycle in liver and in the gastrointestinal tract of cows. Statistical analysis of the examined gene expression pattern revealed no significant difference in the gene expression profile of cows fed transgenic or near-isogenic feed ration. Therefore, Guertler *et al.* (2012) assumed that compared to near-isogenic feed, GM maize MON810 does not have any effect on major genes involved in apoptosis, inflammation and cell cycle in the gastrointestinal tract and in the liver of dairy cows.

In conclusion, the observed differences in the differentially expressed proteins are consistent with normal physiological variation and are not related to the consumption of MON810 maize *per se*. The observed proteomic changes are similarly unremarkable for rats of this strain and age. Therefore, the results of this study do not indicate adverse effects on the proteome of the small intestinal epithelial cells of rats from the consumption of the transgenic variety MON810 maize. The differences observed are not believed to be of major biological importance and were not associated with any adverse health effects. These data can potentially be extrapolated to the humans, considering the suitability of rats as a model for humans.

6.2 Effect of *Bt*-maize extracts on cultured epithelial cells at the proteome level: *In vitro* effects

The increasing presence of GM food and feed on the market has provoked a strong demand for a comprehensive risk assessment by use of rat feeding studies. Currently, there is an emphasis on reducing the number of animal experimentations. The present study follows the OECD guidance document in terms of the recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (OECD, 2002). The purpose of this guidance document was to apply the principles of the Three Rs (Replacement, Reduction, Refinement) to the use of animals in toxicity tests. The principles of the 3Rs, Replacement, Reduction, Refinement, were defined as:

• Replacement – Using non-animals methods which avoid or replace the use of animals such as cell culture.

• Reduction – Minimising the numbers of animals used by improving the experimental design and statistical analysis used in a study.

• Refinement – improving experimental procedures and other factors affecting animals such as their housing and care, to reduce suffering and improve welfare throughout the animals' lives. Alternative *in vitro* cell culture assays may contribute to risk assessment, yielding supplementary information on cellular physiological and biochemical modes of action of novel feed and food compounds (Bondzio *et al.*, 2013).

The present study presents an *in vitro* model system that is useful as a supplementary tool in toxicity screening to study the effects of the insect-specific *Bt*-toxin Cry1Ab on the rat intestinal epithelial cells before testing substances on animals *in vivo*.

The present study was performed to investigate the effects of transgenic maize (MON810) expressing Cry1Ab on the viability of rat intestinal epithelial cells (IECs) extracted from male Wistar rats and its potential impact on the proteome. In addition, we investigated whether MON810 maize has any effect on the viability of the human transformed colon cancer cell line (HCT116) and its potential impact on the proteome. This is, to the best of our knowledge, only the second proteomic study performed on mammalian epithelial cells exposed to commercially available GMO. The first study was done by Bondzio *et al.* (2013), as detailed below.

The Intestinal epithelial primary cells (IECs) used in the present study is a non-transformed intestinal cell line originally derived from the small intestine, isolated from immature male Wistar rats. IECs were selected for this study because these primary cells maintain differentiated characteristics and exhibit strong similarities to primary intestinal epithelial cells used in the rats feeding trial. Therefore, these cells represent a potentially more suitable model than most commonly used transformed cell lines.

The MTT assay was conducted to evaluate the cytotoxicity of *Bt* maize extracts on IECs and HCT116 cells. Extracts from both *Bt* maize and its near isogenic line (non-transgenic maize), at non-toxic doses that are biologically meaningful but still see an effect, were used one the above cells. For IECs, only the lowest concentration, 6.25µg/ml, produced acceptable levels of toxicity (<20% toxicity) following exposure to *Bt* maize and non-transgenic maize. For HCT116 cells, doses of 50µg/ml (the highest non-cytotoxic dose) and less, produced acceptable toxicity (<20% toxicity) following exposure to *Bt* maize and non-transgenic maize. The MTT data for IEC cells and HCT cells showed that IECs are more sensitive than HCT116 cells to the toxicity of *Bt* maize and non-transgenic maize because different cells have different sensitivity to toxicity. However, *in vitro* toxicological studies with IECs in the presence of extracts from *Bt* maize and its near isogenic line indicates that there was no significant differences. Similarly, *in vitro* toxicological studies with HCT116 cells in the presence of extracts from *Bt* maize and its near isogenic line indicates that there was no significant differences. Based on the statistical results of MTT assays of cell viability, we were able to suggest that the transgenic line expressing *Bt* in this single dose experiments is not toxic to these cells.

Similarly, Bondzio *et al.* (2013) investigated the effects of Cry1Ab on porcine intestinal cells (IPEC-J2 cell line). Different endpoint assays were performed. Based

on three different assays (WST-1 conversion using the Cell Proliferation Reagent WST-1 (Roche); the measurement of ATP content which indicates the energy metabolism of the cells; and the measurement of LDH release, which reflects the membrane integrity of the cells) a more comprehensive picture of the impact of Cry1Ab on IPEC J2 cells was obtained. Moreover, Bondzio and colleagues used transepithelial electrical resistance (TEER) as a functional parameter on differentiated IPEC-J2 cells grown on collagen-coated membranes in order to characterize the barrier function. Based on the results of these different parameters of cell viability they were able to exclude a short-term toxic effect of Cry1Ab on cultured IPEC-J2 cells. Furthermore, a novel electronic cell sensor array technology, the real-time cell analysis (RTCA) system, was used for dynamic monitoring of cellular events of the Cry1Ab response. No cytotoxic effects were found, either from the use of the endpoint assays or by real-time monitoring.

Furthermore, Bondzio *et al.* (2008) have also introduced a new sheep rumen epithelial cell culture as an *in vitro* cell system to evaluate cytotoxicity of Cry1Ab, using lactate dehydrogenase (LDH) release, WST-1 conversion, ATP content and caspase 3/7 activity to evaluate cytotoxicity of Cry1Ab. Neither cytotoxic effects nor signs of apoptosis were detected in rumen epithelial cells and no evidence for Cry1Ab mediated cytotoxicity was found.

However, Mesnage *et al.* (2013) have recently performed a study on the non-target effects of natural *Bt* toxins. The mechanisms of action of insect resistance of natural *Bt* toxins are not fully understood and the metabolism of these proteins in mammals is currently unknown. Mesnage *et al.* exposed human embryonic kidney 293 cell line to Cry1Ab. Mitochondrial respiration assay (MTT) through the succinate dehydrogenase activity measurement and other cytotoxicity tests were used. Mesnage *et al.* (2013) found that Cry1Ab led to respiration inhibition and plasma membrane alterations. This could be consistent with the fact that the consumption of MON810 maize producing Cry1Ab (in the ppm range) induced signs of hepatorenal alterations in a subchronic feeding study on rats (De Vendômois *et al.*, 2009). This occurred at relatively high concentrations (100 ppm) in comparison to the concentrations normally produced in GM plants (1–20 ppm) (Székács *et al.*, 2010). However, the content can differ greatly according to the GM variety and environmental conditions under which the plants are grown (Then and Lorch, 2008).

In an earlier study carried out by Shimada *et al.* (2003), primary cultured bovine hapatocytes were used as a model system to determine if Cry1Ab protein affects mammalian cells. There were no significant changes in the secretion of albumin or the morphology of the Cry1Ab-treated cells. The LDH release method showed a tendency to increase after the administration of Cry1Ab, but not significantly. Shimada *et al.* (2003) have taken these results on bovine hepatocytes into consideration suggesting that Cry1Ab has little acute toxicity on mammalian cells.

The similarity of amino acid sequence of novel protein with proteins that could have potential safety concerns, i.e., known toxins or allergens, is one of the key endpoints for assessing the safety of a novel protein. For this reason, an amino acid sequence comparison was done on Cry1Ab/Ac protein. The immunoglobulin E (IgE) cross-reactivity between a newly expressed novel protein and a known allergen should be considered a possibility when there is more than 35% identity in a segment of 80 or more amino acids and it is reasonable to assume that only matches of eight contiguous and identical amino acids have relevance for identifying potential allergens. The recent results of the *in silico* analysis showed no evidence for any similarity between the Cry1Ab protein and any known allergenic or toxic proteins, i.e., less than 35% identity with known allergens or toxins across a length of 80 amino acids and no continuous eight amino acids identity with known allergens. However, theoretically the bioinformatics analysis of the Cry1Ab protein show that it is safe but the *in silico* analysis had to be verified by *in vitro* assays (Xu *et al.*, 2009). The bioinformatics analysis of the Cry1Ab protein shows no amino acid sequence similarity to known protein toxins or allergens (Delaney *et al.*, 2008).

In the present *in vitro* approach, molecular proteomic techniques (2-DE) for investigating the effects of MON810 extracts were included. Comparison between differentially expressed proteins between IECs exposed to *Bt* maize extract and IECs exposed to non-*Bt* maize extracts showed that 8 stress-related proteins (2 Stress-70 protein, heat shock protein HSP 90-alpha, 2 heat shock protein HSP 90-beta, 2 T-complex protein 1 subunit epsilon and 60 KDa heat shock protein) were up-regulated in the IECs exposed to *Bt* maize extract and 1 stress related protein (heat shock-related 70 KDa protein 2) was up-regulatedin IECs exposed to the non-*Bt* maize extract. However, the majority of differentially expressed proteins were related to metabolic pathways. Comparison between differentially expressed proteins between HCT116 cell

lines exposed to *Bt* maize and non-*Bt* maize extract showed that there were no differentially expressed protein spots between the cells. Interestingly, we observed from the proteomic analyses that IECs are more sensitive than HCT116 cell line to the *Bt* maize extract and these results support the MTT assay results. The identified stress proteins are multifunctional and involved in important cellular processes as discussed in the following section.

One protein which was up-regulated in the IECs exposed to *Bt* maize extracts is the stress-70 protein, which is implicated in the control of cell proliferation and cellular aging. It also acts as a chaperone that is required for essential cellular functions, such as, protein folding and assembly or reassembly (UniProtKB, 2014e). Two other proteins that were up-regulated are the heat shock protein HSP 90-alpha and heat shock protein HSP 90-beta. These are both molecular chaperones that promote the maturation, structural maintenance and proper regulation of specific target proteins involved in cell cycle control and signal transduction. These two molecular chaperones undergo a functional cycle that is linked to their ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. They also are known to interact dynamically with various co-chaperones that modulate their substrate recognition, the ATPase cycle and the function of other chaperones (UniProtKB, 2014c; UniProtKB, 2014d). A third protein that was found to be up-regulated is T-complex protein 1 subunit epsilon which is a molecular chaperone that assists the folding of proteins upon ATP hydrolysis. It is also known to play a role, *in vitro*, in the folding of actin and tubulin (Seo *et al.*, 2010). A 60 KDa heat shock protein was also identified, which is implicated in mitochondrial protein import and macromolecular assembly. It is thought that this protein may facilitate the correct folding of imported proteins and also prevent misfolding. Furthermore, it may promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix (UniProtKB, 2014a).

The only stress related protein which was up-regulated in the IECs exposed to the non-*Bt* maize extract is heat shock-related 70 KDa protein 2; Hsp70s is known to stabilize preexistent proteins against aggregation and mediate the folding of newly translated polypeptides in the cytosol as well as within organelles. The Hsp70s participate in all these processes through their ability to recognize nonnative conformations of other proteins. They bind extended peptide segments with a net

hydrophobic character exposed by polypeptides during translation and membrane translocation, or following stress-induced damage (UniProtKB, 2014b).

Similarly, Bondzio *et al.* (2013) included a molecular proteomic profiling study based on 2-DE to evaluate the *in vitro* effects of Cry1Ab on porcine intestinal cells (IPEC-J2 cell line). Only three proteins were differentially expressed in Bondzio's study. Whilst these three proteins are multifunctional and involved in important cellular processes they are also associated with responses to stress. They were identified as heat shock 70 kD protein, type II Keratin cytoskeletal 8 and heterogeneous nuclear ribonucleoprotein (HnRNP) H2-like. This Hsp70 stress protein has been used to monitor the impact of environmental factors on various animal species, including pig as well as in *in-vitro* models (Bondzio *et al.*, 2013).

There were no other proteomic studies on mammalian intestinal cells for comparison with the present study results as far as I am aware.

In the present study, heat shock proteins were found to be up-regulated in IE cells following exposure to *Bt* maize extracts. This finding suggests that they may have a protective function and may represent an adaptive response for the maintaining of cellular homeostasis under stress. By comparing the *in vitro* study with the *in vivo* study, we found that the 60 kDa heat shock protein was expressed in IECs exposed to *Bt* maize extracts as well as in rats fed MON810 maize in both 7-day and 28-day rat feeding trials and in rats fed commercial rodent diet for 7-day rat feeding trial.

These findings suggest that the combination of *in vitro* testing and proteomic analysis may serve as a promising tool for mechanism based safety assessment because the MTT cytotoxicity assay results agreed with the results obtained from the proteomic analysis of IECs and HCT116 cell line. Further studies, particularly long-term investigations are needed to determine whether increased expression of heat shock proteins is only a transient short-term adaptive response to Cry1Ab or may be the cause of further unintended side effects of this protein.

Finally, the reason for doing the *in vitro* study was to see whether there is a viable alternative for animal studies by comparing the *in vitro* study with the results obtained from in the *in vivo* study. In this particular system the effects both in the cytotoxicity assay and the proteomic results are in broad agreement. Therefore, in my opinion this

particular system has potential to be a viable alternative for animal studies because the same epithelial cells were used in both the *in vitro* and *in vivo* studies. However, we are a long way from being able to substitute animal studies.

Conclusions

- The results clearly demonstrated that there were no adverse behavioural effects on rats and no significant differences between any of the maize varieties tested on rat weight gain or food consumption in either 7-day or 28-day feeding studies. Thus the transgenic variety MON810 had no adverse effects on these parameters.
- The *in vivo* effects of these different maize varieties on the proteome of the epithelial cells of the small intestine were negligible, with only 3 proteins spots being up regulated in the small intestinal epithelial cells of rats fed MON810 for 7 days and only 2 proteins spots being up regulated in the small intestinal epithelial cells of rats fed MON810 for 28 days, when compared to other maize varieties and to the commercial rodent diet.
- The *in vitro* effects of MON810 and its near isogenic line on the proteome of the epithelial cell lines were negligible, with only 4 protein spots being up regulated in the small intestinal primary epithelial cells (IECs) when exposed to *Bt* maize extracts and 2 protein spots being up regulated when exposed to non-*Bt* maize extracts. There were no differentially expressed spots in the HCT116 cell lines when exposed to either the *Bt* or non-*Bt* maize extracts.
- The findings from the *in vivo* and *in vitro* studies were moderately similar and the studies reveal the absence of toxicity, with no adverse effects. Considering the low concentrations of Cry1Ab protein in maize, it is highly unlikely to result in any toxic effects. Digestibility of Cry1Ab expressed protein was demonstrated by the rapid degradation in simulated gastric or enteric juices. This is an additional guarantee of lack of absorption of the whole protein and absence of systemic absorption and is already an indication of the low allergenic potential of the protein. Thus, MON810 is considered to be substantially equivalent to, and as safe and nutritious as, conventional corn varieties.

Future studies

- Further studies to investigate the *in vivo* effects of the commercial GM crop (MON810), an insect resistant maize variety expressing the δ-insecticidal protein Cry1Ab from *Bacillus thuringiensis*, on differential gene expression at the proteome level in the liver of rat as a non-target model system using molecular proteomic profiling techniques (2-DE) will be useful in confirming the safety of MON810 maize. The liver will be a suitable organ to choose because of the detoxification function of the liver.
- Conducting comparative *in vitro* studies of Cry1Ab on liver cells may also be useful as a possible alternative to animal studies to assess the safety of MON810 maize.

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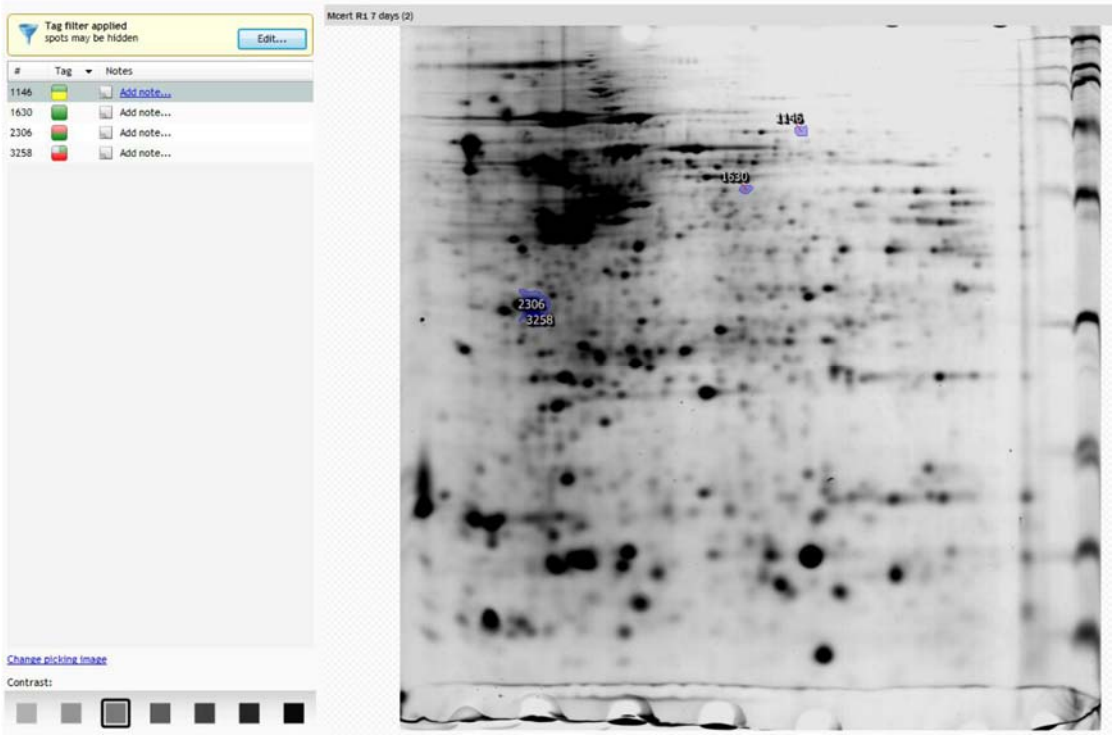
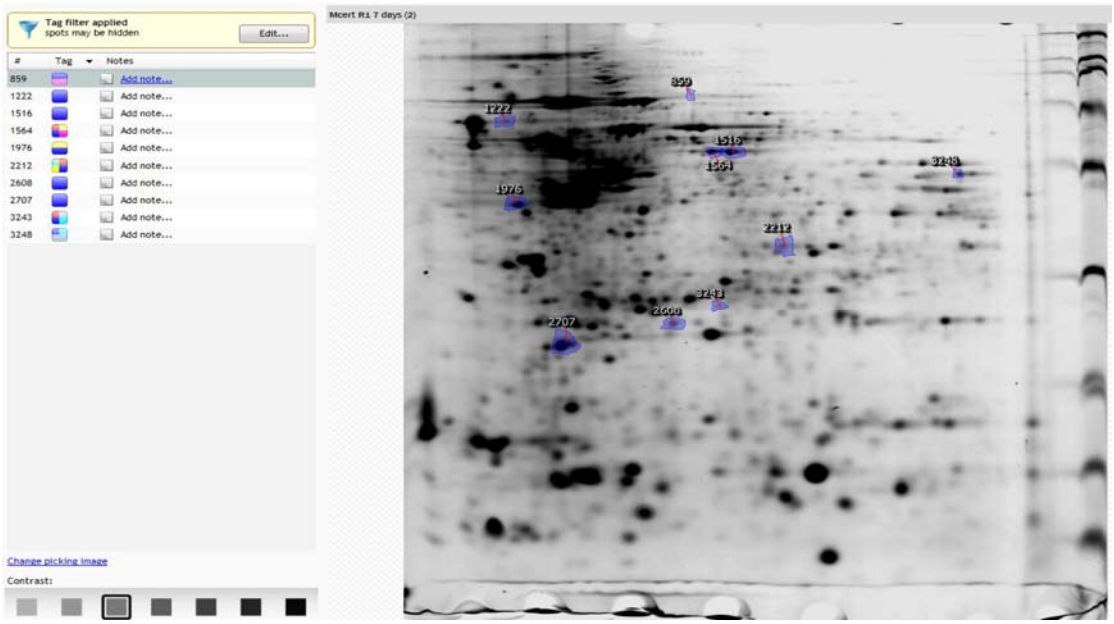
Appendices

Appendices to chapter 4

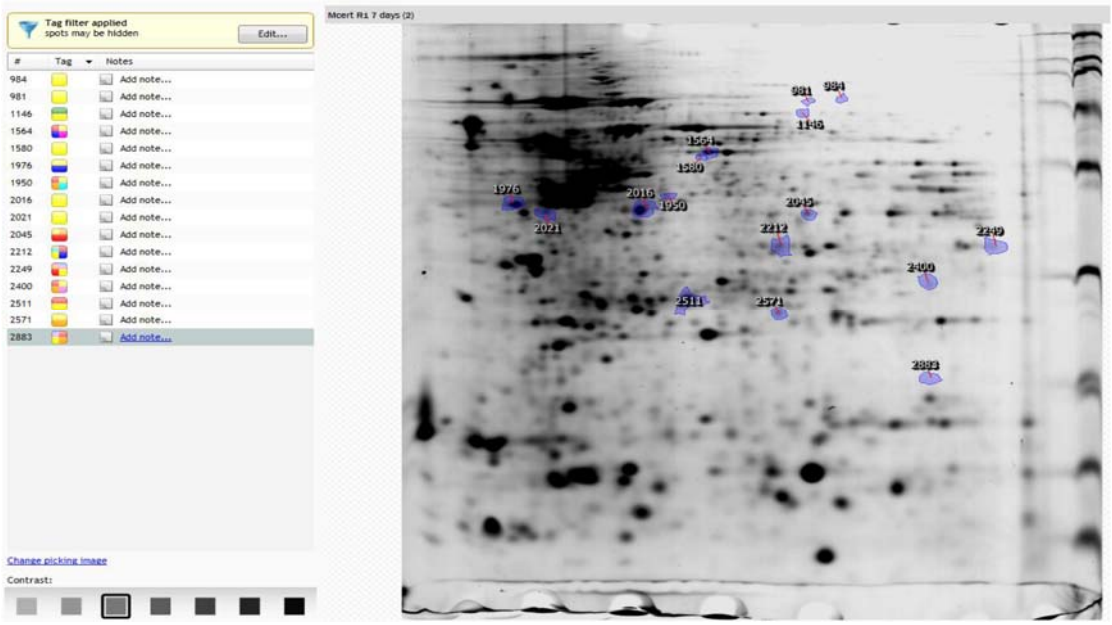
Note: Supplementary information of the respective chapters. The first numeral in the caption indicates the chapter, where as the second numeral represents the increasing number of the Figure/Table within the same chapter.



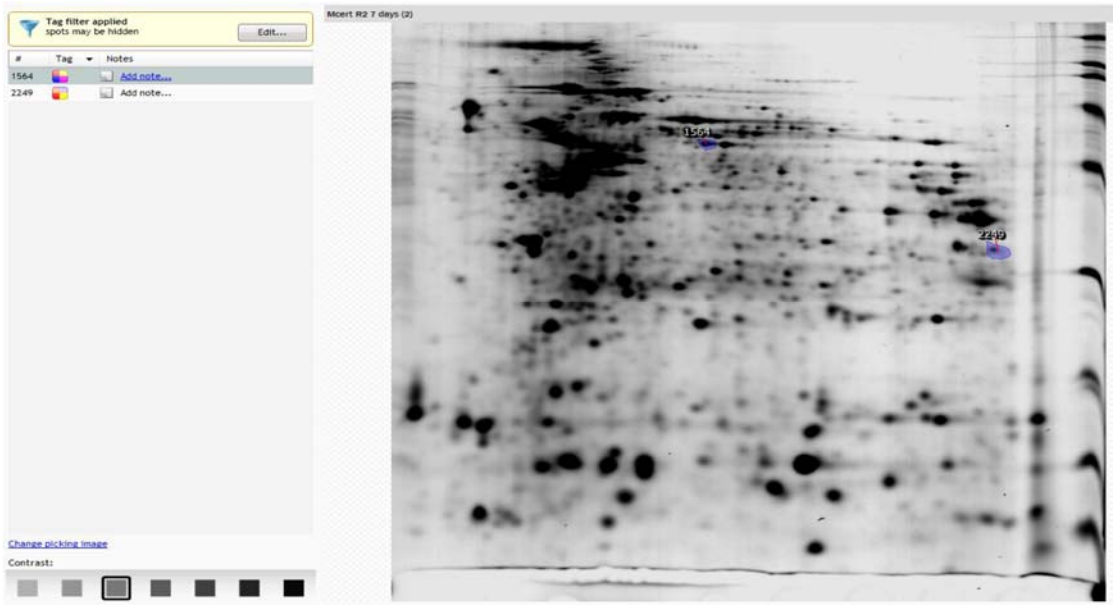
Appendix 1: 2DE of the proteome of the epithelial cells from the small intestine of rats fed Mcert diet for 7 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on MON810 diet for 7 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 1146, 1630, 2306, and 3258 (as indicated on the proteome map).



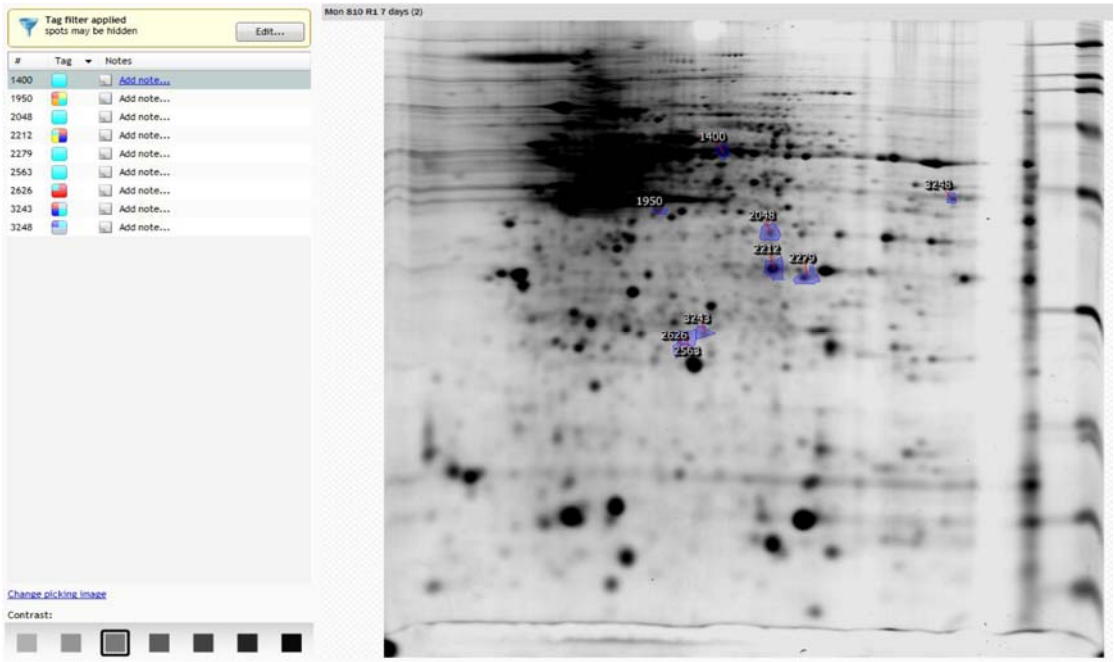
Appendix 2: 2DE of the proteome of the epithelial cells from the small intestine of rats fed Mcert diet for 7 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Garst diet for 7 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 1222, 1516, 1976, 2212, 2608, 3243, and 3248 (as indicated on the proteome map).



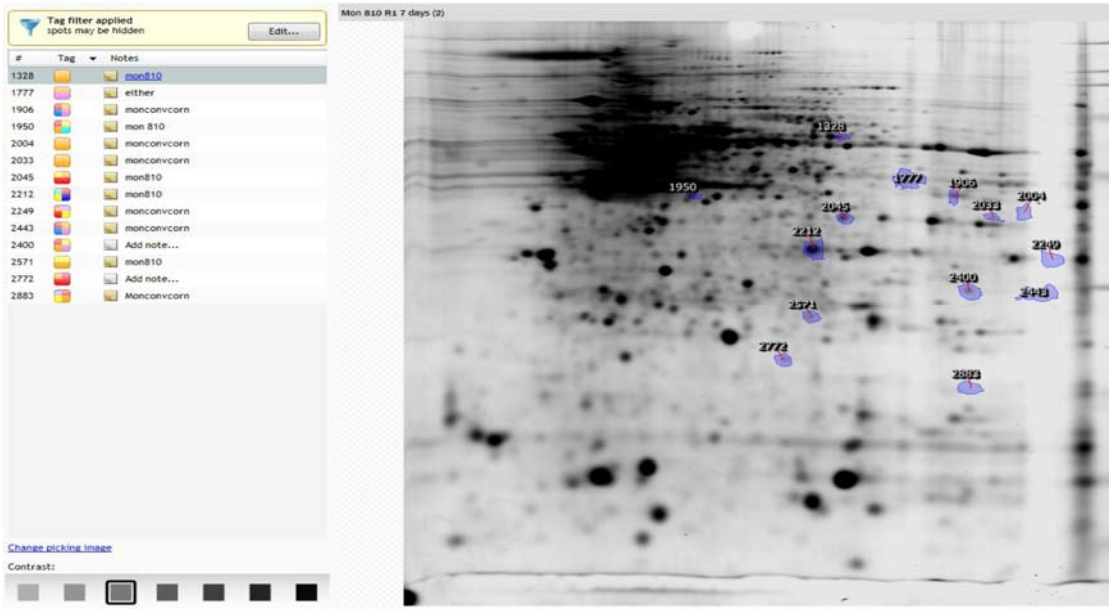
Appendix 3: 2DE of the proteome of the epithelial cells from the small intestine of rats fed Mcert diet for 7 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Conv Corn diet for 7 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 1146, 1580, 1976, 1950, 2016, 2021, 2045, 2212, 2249, 2400, 2511, and 2883 (as indicated on the proteome map).



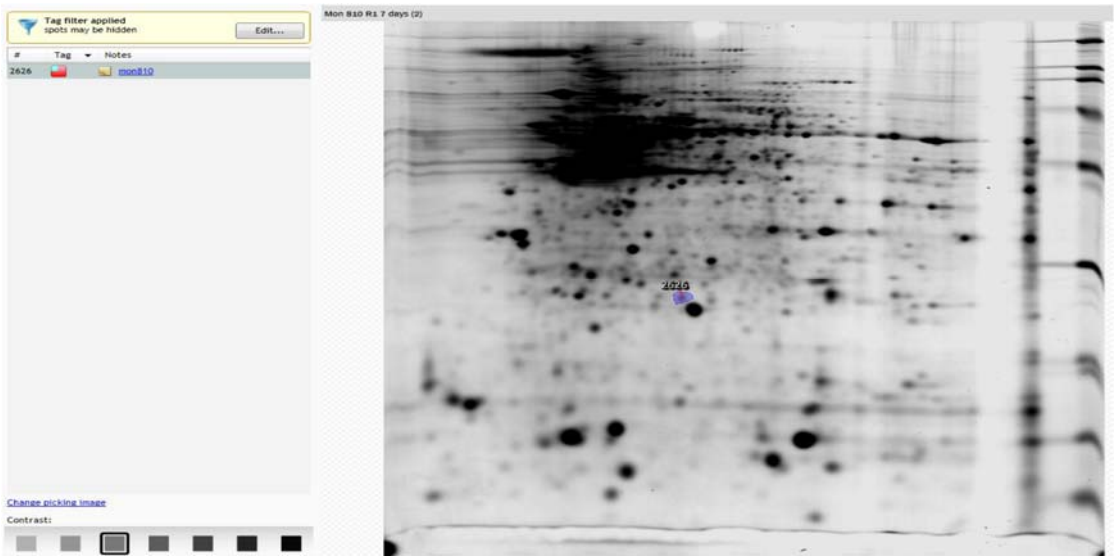
Appendix 4: 2DE of the proteome of the epithelial cells from the small intestine of rats fed Mcert diet for 7 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Gold diet for 7 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spot that was picked for subsequent identification by LC-MS/MS was 2249 (as indicated on the proteome map).



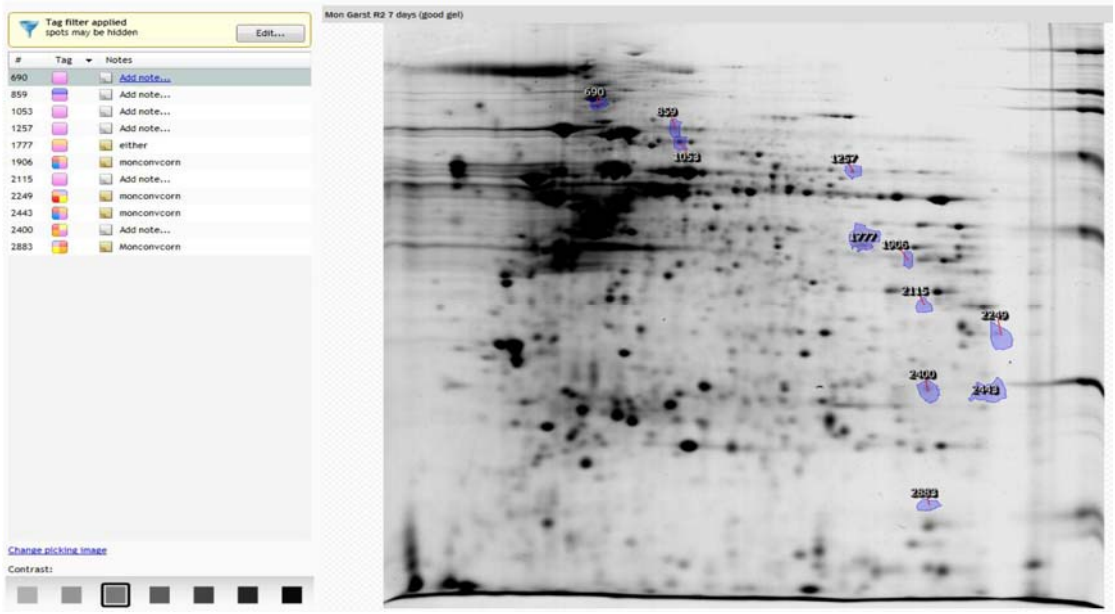
Appendix 5: 2DE of the proteome of the epithelial cells from the small intestine of rats fed MON810 diet for 7 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Garst diet for 7 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 1950, 2048, 2212, 2279, 2626, 3243, and 3248 (as indicated on the proteome map).



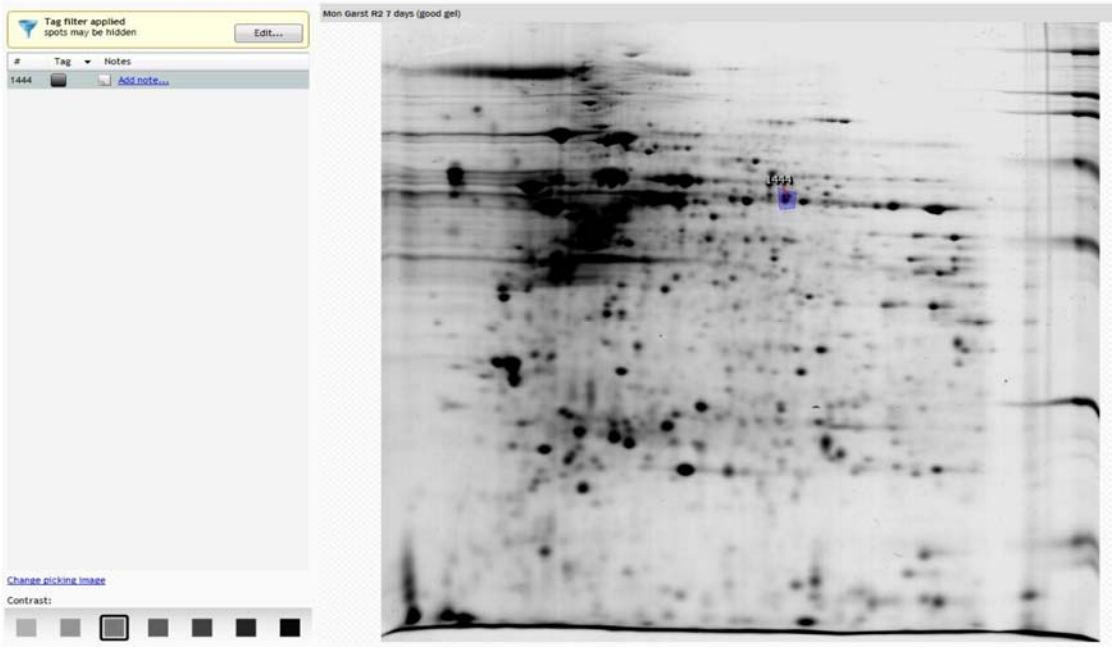
Appendix 6: 2DE of the proteome of the epithelial cells from the small intestine of rats fed MON810 diet for 7 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Conv Corn diet for 7 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 1328, 1777, 1950, 2004, 2045, 2212, 2249, 2443, 2400, and 2883 (as indicated on the proteome map).



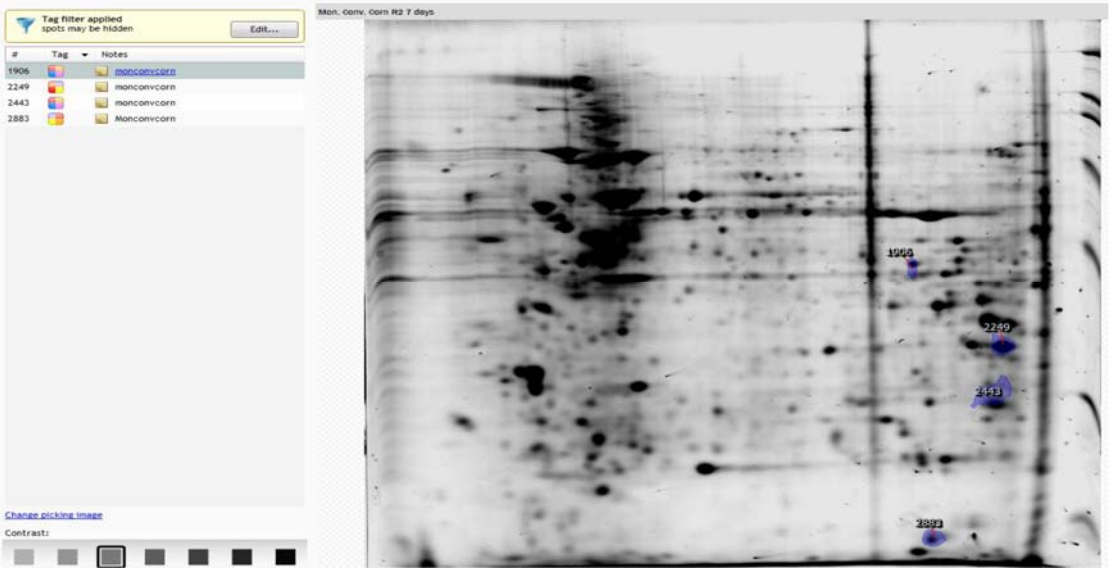
Appendix 7: 2DE of the proteome of the epithelial cells from the small intestine of rats fed MON810 diet for 7 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Gold diet for 7 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spot that was picked for subsequent identification by LC-MS/MS was 2626 (as indicated on the proteome map).



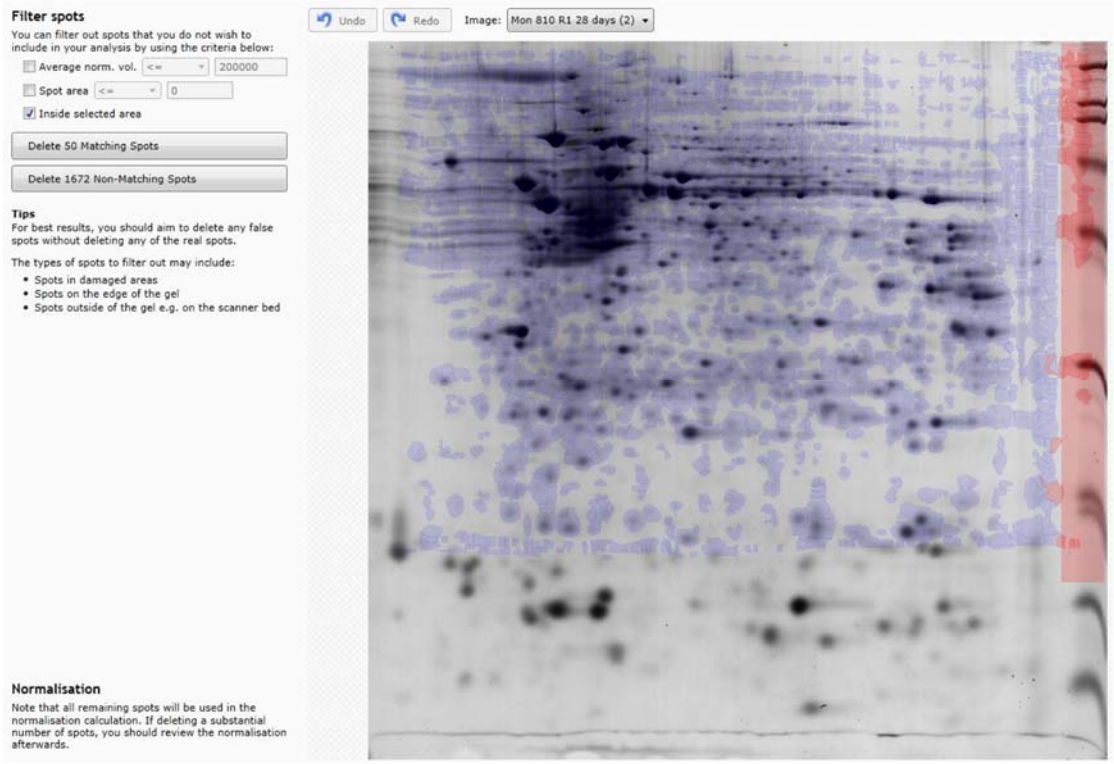
Appendix 8: 2DE of the proteome of the epithelial cells from the small intestine of rats fed Mon Garst diet for 7 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Conv Corn diet for 7 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 1257, 1777, 2249, 2443, 2400, and 2883 (as indicated on the proteome map).



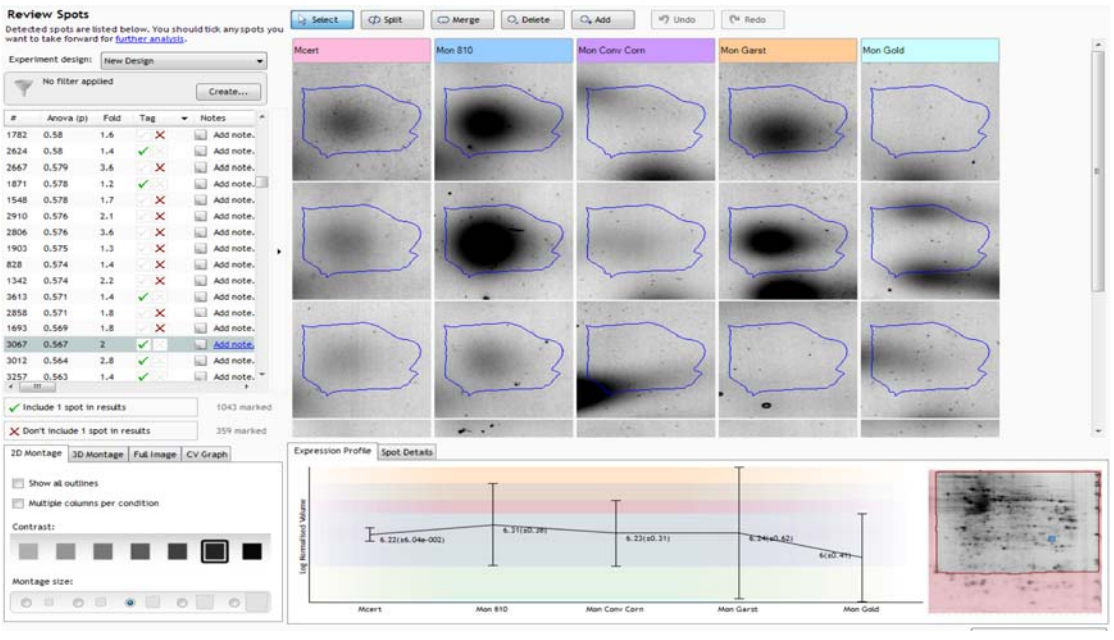
Appendix 10: 2DE of the proteome of the epithelial cells from the small intestine of rats fed Mon Garst diet for 7 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Gold diet for 7 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spot that was picked for subsequent identification by LC-MS/MS was 1444 (as indicated on the proteome map).



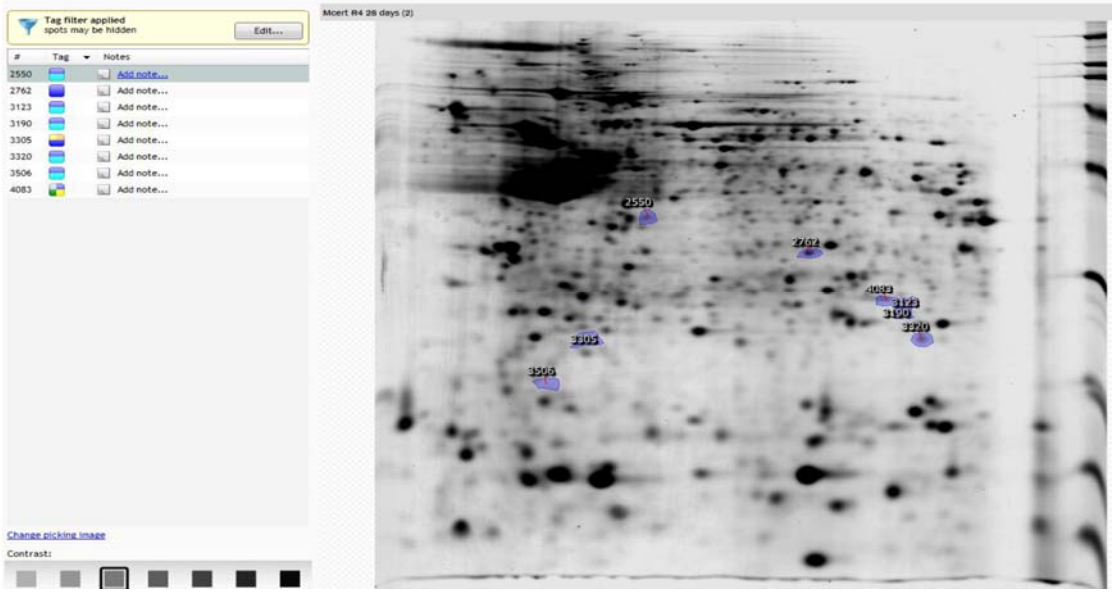
Appendix 9: 2DE of the proteome of the epithelial cells from the small intestine of rats fed Mon Conv Corn diet for 7 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Gold diet for 7 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 2249, 2443, and 2883 (as indicated on the proteome map).



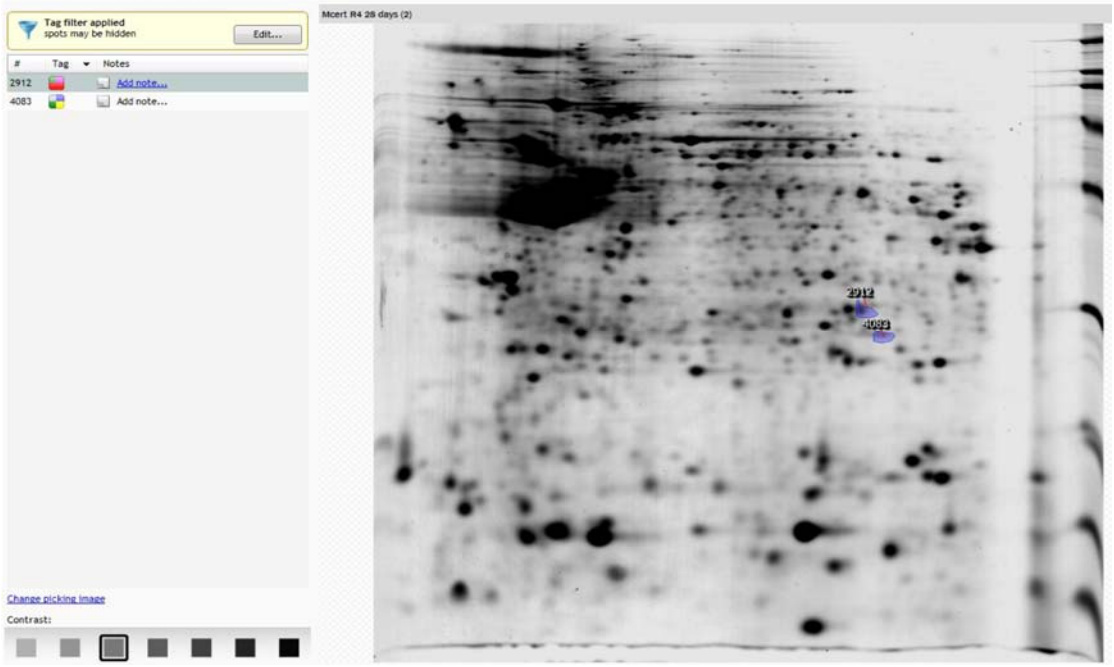
Appendix 11: Removal of 50 features from the size standard of the aggregate gel image (28-day rat feeding trial).



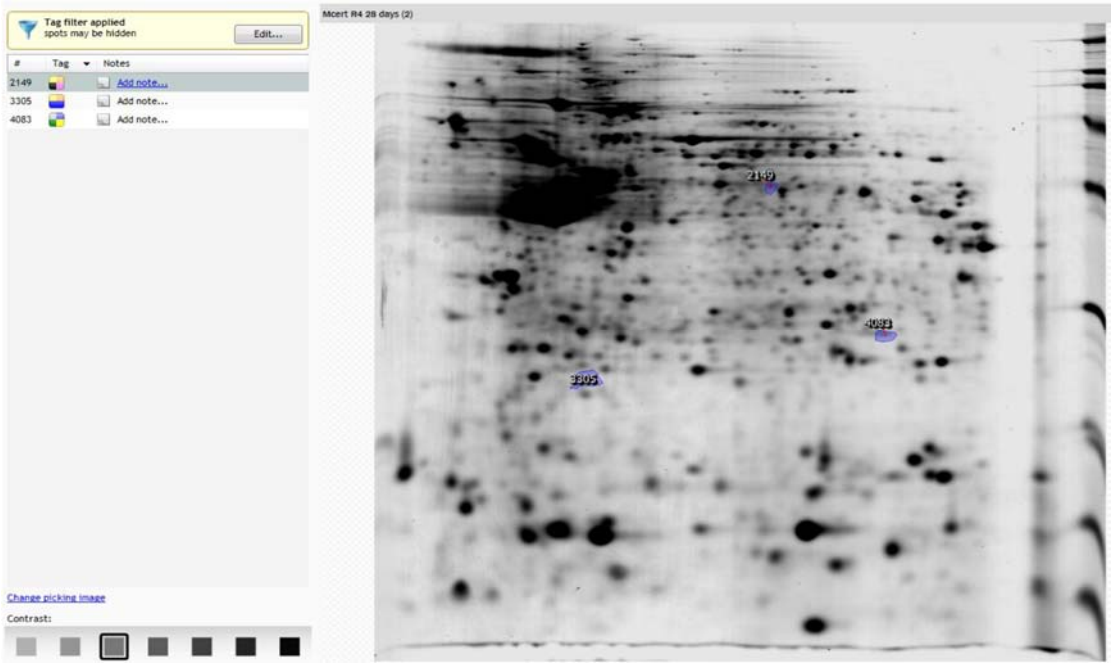
Appendix 12: Example of alignment issue.
This spot was removed as the incorrect spot had been aligned in Mon Garst replicate 2 (28-day rat feeding trial).



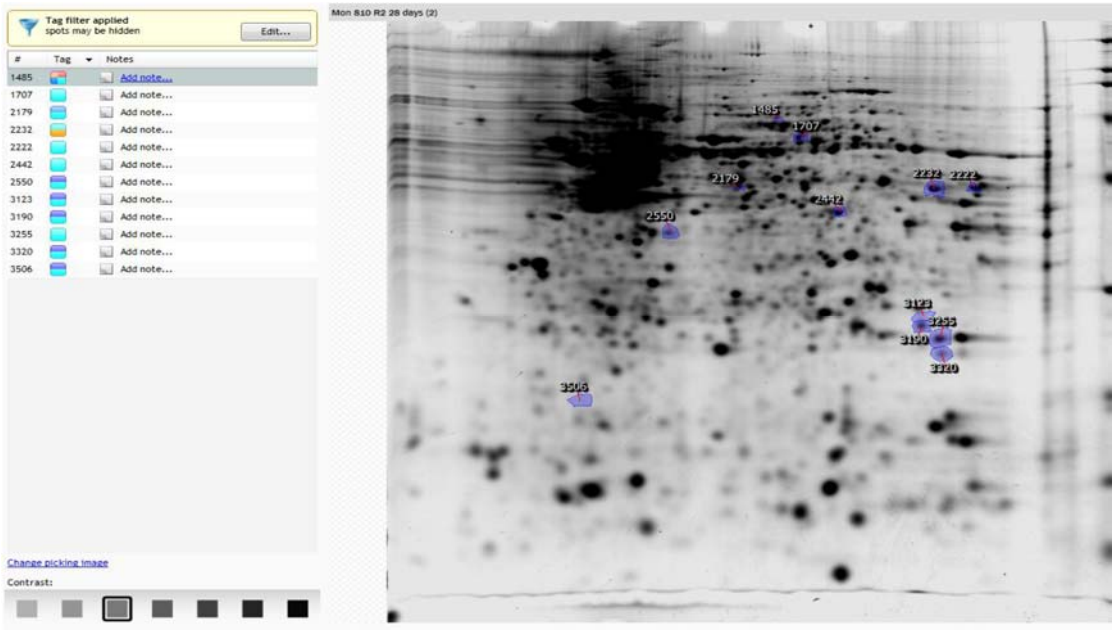
Appendix 13: 2DE of the proteome of the epithelial cells from the small intestine of rats fed MCert diet for 28 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Conv Corn diet for 28 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 2550, 2762, 3123, 3190, 3305, 3320, and 3506 (as indicated on the proteome map).



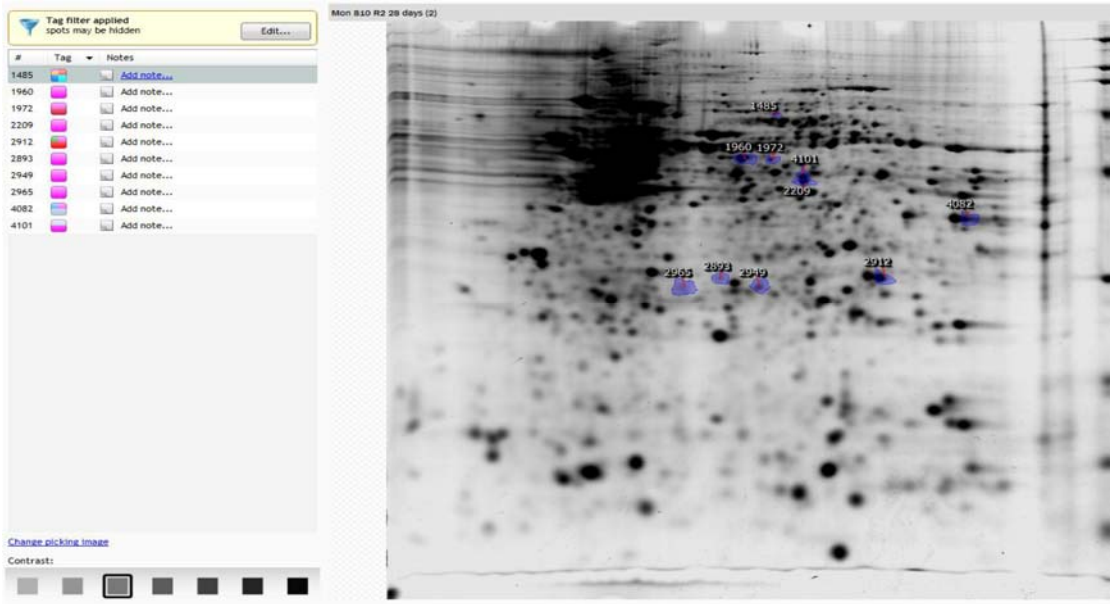
Appendix 14: 2DE of the proteome of the epithelial cells from the small intestine of rats fed MCert diet for 28 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Garst diet for 28 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spot that was picked for subsequent identification by LC-MS/MS was 2912 (as indicated on the proteome map).



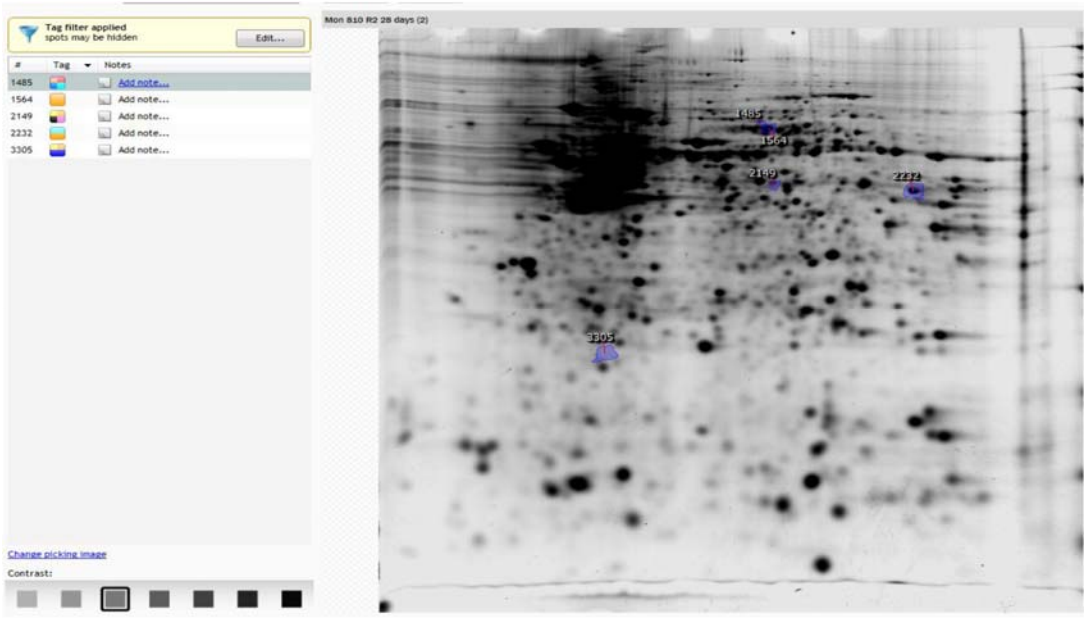
Appendix 15: 2DE of the proteome of the epithelial cells from the small intestine of rats fed MCert diet for 28 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Gold diet for 28 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 2149, and 3305 (as indicated on the proteome map).



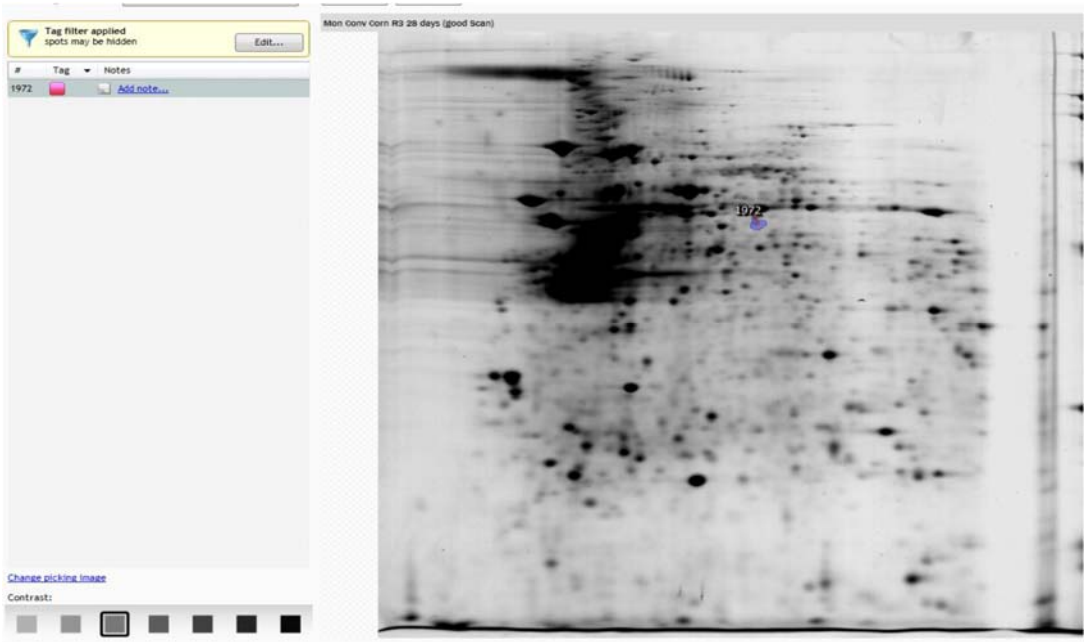
Appendix 16: 2DE of the proteome of the epithelial cells from the small intestine of rats fed MON810 diet for 28 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Conv Corn diet for 28 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 1485, 1707, 2179, 2232, 2222, 2442, 2550, 3123, 3190, 3255, 3320, and 3506 (as indicated on the proteome map).



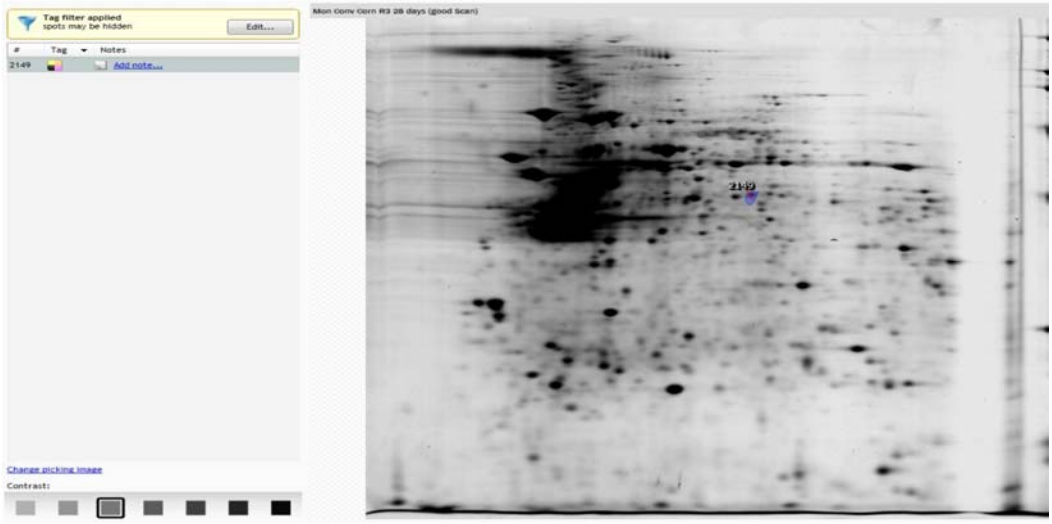
Appendix 17: 2DE of the proteome of the epithelial cells from the small intestine of rats fed MON810 diet for 28 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Garst diet for 28 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 1485, 1960, 1972, 2209, 2912, 2893, 2965, 4082, and 4101(as indicated on the proteome map).



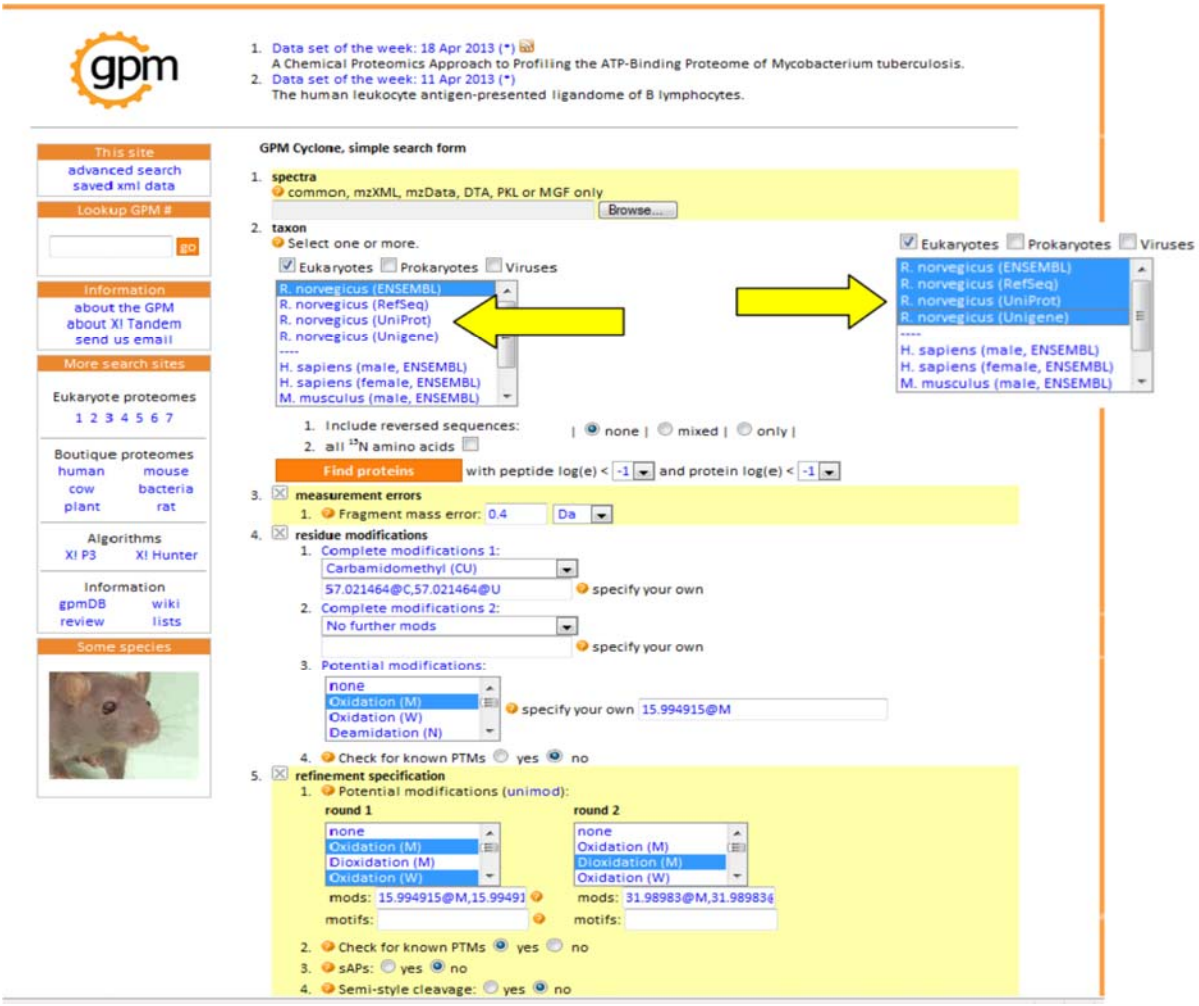
Appendix 18: 2DE of the proteome of the epithelial cells from the small intestine of rats fed MON810 diet for 28 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Gold diet for 28 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 1485, 1564, 2149, 2232, and 3305 (as indicated on the proteome map).



Appendix 19: 2DE of the proteome of the epithelial cells from the small intestine of rats fed Mon Conv Corn diet for 28 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Garst diet for 28 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spot that was picked for subsequent identification by LC-MS/MS was 1972 (as indicated on the proteome map).

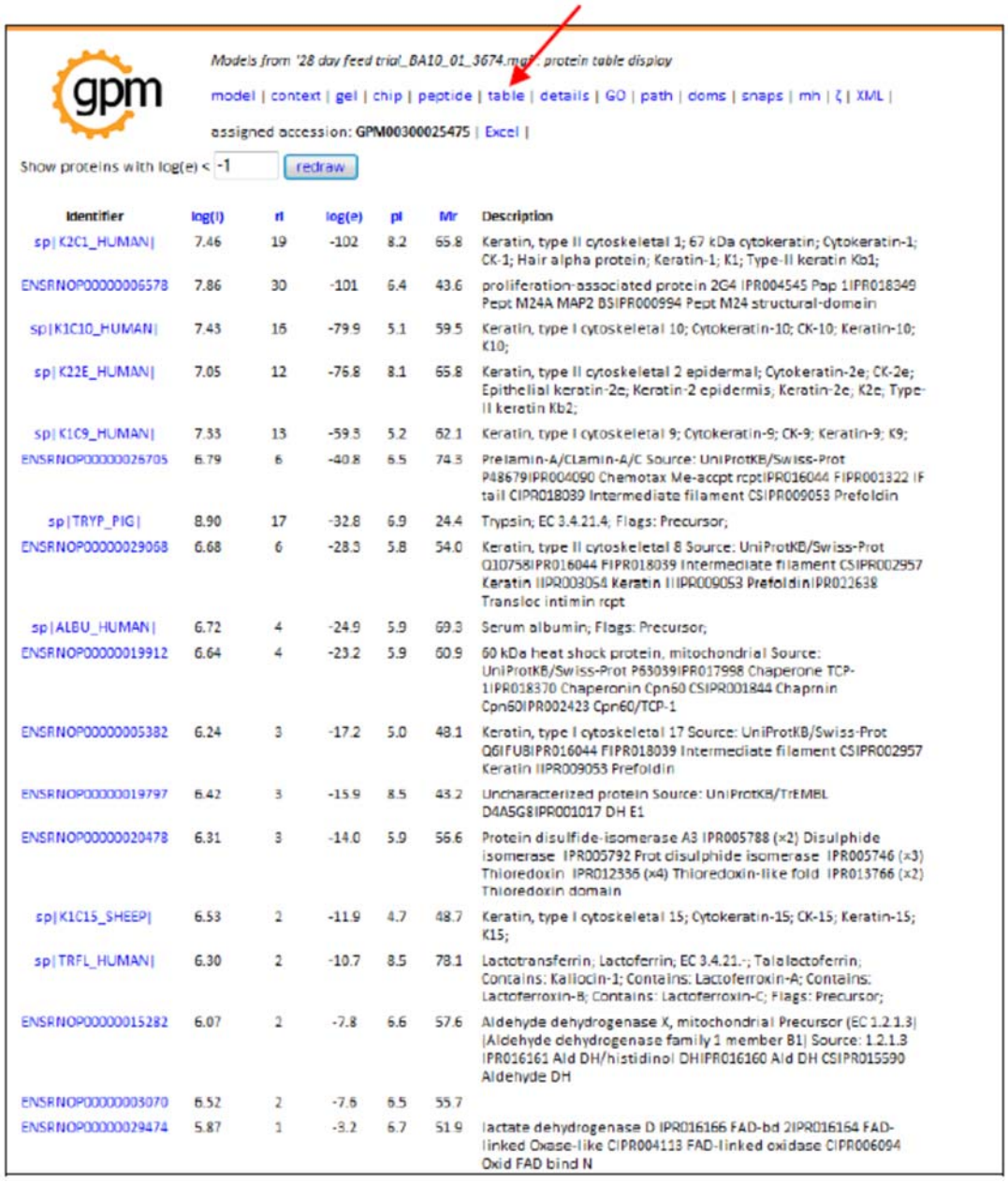


Appendix 20: 2DE of the proteome of the epithelial cells from the small intestine of rats fed Mon Conv Corn diet for 28 days. Protein spots from this gel were compared to those from comparator gels where rats were feds on Mon Gold diet for 28 days.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spot that was picked for subsequent identification by LC-MS/MS was 2149 (as indicated on the proteome map).



Appendix 21: The Global Proteome Machine (GPM) search engine.
Any rat database (e.g. UniProt or ENSEMBL) or all of them can be selected. The following settings were applied: method, ion trap (4Da); complete modifications, carbamidomethyl (CU); variable modification, oxidation (M).

Appendix 22: The search engine Global Proteome Machine (GPM) was used to putatively identify differentially expressed proteins. The following settings were also applied: fragment mass error, 0.4 Da; parent mass error, ±0.3 Da; fragment type, monoisotopic; maximum parent charge, 4.
Only identification with log(e) values ≤ -3 were regarded as significant hits regardless of the number of peptides.



Models from '28 day feed trial_BA10_01_3674.mgf': protein table display

model | context | gel | chip | peptide | table | details | GO | path | doms | snaps | mh | ζ | XML |

assigned accession: GPM00300025475 | Excel |

Show proteins with log(e) < -1 redraw

Identifier	log(I)	rl	log(e)	pI	Mr	Description
sp|K2C1_HUMAN|	7.46	19	-102	8.2	65.8	Keratin, type II cytoskeletal 1; 67 kDa cytokeratin; Cytokeratin-1; CK-1; Hair alpha protein; Keratin-1; K1; Type-II keratin Kb1;
ENSRNOP00000006578	7.86	30	-101	6.4	43.6	proliferation-associated protein 2G4 IPR004545 Pap 1IPR018349 Pept M24A MAP2 BSIPR000994 Pept M24 structural-domain
sp|K1C10_HUMAN|	7.43	16	-79.9	5.1	59.5	Keratin, type I cytoskeletal 10; Cytokeratin-10; CK-10; Keratin-10; K10;
sp|K22E_HUMAN|	7.05	12	-76.8	8.1	65.8	Keratin, type II cytoskeletal 2 epidermal; Cytokeratin-2e; CK-2e; Epithelial keratin-2e; Keratin-2 epidermis; Keratin-2e; K2e; Type-II keratin Kb2;
sp|K1C9_HUMAN|	7.33	13	-59.5	5.2	62.1	Keratin, type I cytoskeletal 9; Cytokeratin-9; CK-9; Keratin-9; K9;
ENSRNOP00000026705	6.79	6	-40.8	6.5	74.3	Prelamin-A/C;Lamin-A/C Source: UniProtKB/Swiss-Prot P48679IPR004090 Chemotax Me-accpt rcptIPR016044 FIPR001322 IF tail CIPR018039 Intermediate filament CSIPR009053 Prefoldin
sp|TRYP_PIG|	8.90	17	-32.8	6.9	24.4	Trypsin; EC 3.4.21.4; Flags: Precursor;
ENSRNOP00000029068	6.68	6	-28.3	5.8	54.0	Keratin, type II cytoskeletal 8 Source: UniProtKB/Swiss-Prot Q10758IPR016044 FIPR018039 Intermediate filament CSIPR002957 Keratin IIPR003054 Keratin II IIPR009053 PrefoldinIPR022638 Transloc intimin rcpt
sp|ALBU_HUMAN|	6.72	4	-24.9	5.9	69.3	Serum albumin; Flags: Precursor;
ENSRNOP00000019912	6.64	4	-23.2	5.9	60.9	60 kDa heat shock protein, mitochondrial Source: UniProtKB/Swiss-Prot P63039IPR017998 Chaperone TCP-1IPR018370 Chaperonin Cpn60 CSIPR001844 Chaprnin Cpn60IPR002423 Cpn60/TCP-1
ENSRNOP00000005382	6.24	3	-17.2	5.0	48.1	Keratin, type I cytoskeletal 17 Source: UniProtKB/Swiss-Prot Q6IFU8IPR016044 FIPR018039 Intermediate filament CSIPR002957 Keratin IIPR009053 Prefoldin
ENSRNOP00000019797	6.42	3	-15.9	8.5	43.2	Uncharacterized protein Source: UniProtKB/TrEMBL D4A5G8IPR001017 DH E1
ENSRNOP00000020478	6.31	3	-14.0	5.9	56.6	Protein disulfide-isomerase A3 IPR005788 (x2) Disulphide isomerase IPR005792 Prot disulphide isomerase IPR005746 (x3) Thioredoxin IPR012336 (x4) Thioredoxin-like fold IPR013766 (x2) Thioredoxin domain
sp|K1C15_SHEEP|	6.53	2	-11.9	4.7	48.7	Keratin, type I cytoskeletal 15; Cytokeratin-15; CK-15; Keratin-15; K15;
sp|TRFL_HUMAN|	6.30	2	-10.7	8.5	78.1	Lactotransferrin; Lactoferrin; EC 3.4.21.-; Talalactoferrin; Contains: Kallocin-1; Contains: Lactoferroxin-A; Contains: Lactoferroxin-B; Contains: Lactoferroxin-C; Flags: Precursor;
ENSRNOP00000015282	6.07	2	-7.8	6.6	57.6	Aldehyde dehydrogenase X, mitochondrial Precursor (EC 1.2.1.3) (Aldehyde dehydrogenase family 1 member B1) Source: 1.2.1.3 IPR016161 Ald DH/histidinol DHIPR016160 Ald DH CSIPR015590 Aldehyde DH
ENSRNOP00000003070	6.52	2	-7.6	6.5	55.7	
ENSRNOP00000029474	5.87	1	-3.2	6.7	51.9	lactate dehydrogenase D IPR016166 FAD-bd 2IPR016164 FAD-linked Oxase-like CIPR004113 FAD-linked oxidase CIPR006094 Oxid FAD bind N

Appendix 23: Clicking on table tab gave the list of identified proteins in one 2D gel spot.
The lower the log(e) value means more confidence in protein ID. rl indicates the number of peptides involved in ID of that protein.

2. Dust/contact proteins:

	Id	Description	Reason
1.	AMYS_HUMAN	(P04745) Salivary alpha-amylase precursor (EC 3.2.1.1) (1,4-alpha-D-glucan glucanohydrolase)	Human saliva
2.	K1C10_HUMAN	(P13645) Keratin, type I cytoskeletal 10 (Cytokeratin-10) (CK-10) (Keratin-10) (K10)	Human skin
3.	K1C9_HUMAN	(P35527) Keratin, type I cytoskeletal 9 (Cytokeratin-9) (CK-9) (Keratin-9) (K9)	Human skin
4.	K2C1_HUMAN	(P04264) Keratin, type II cytoskeletal 1 (Cytokeratin-1) (CK-1) (Keratin-1) (K1) (67 kDa cytokeratin) (Hair alpha protein)	Human skin
5.	K22E_HUMAN	(P35908) Keratin, type II cytoskeletal 2 epidermal (Cytokeratin-2e) (K2e) (CK 2e)	Human skin
6.	K1H1_HUMAN	(Q15323) Keratin, type I cuticular Ha1 (Hair keratin, type I Ha1)	Human hair
7.	K1H2_HUMAN	(Q14532) Keratin, type I cuticular Ha2 (Hair keratin, type I Ha2)	Human hair
8.	K1H4_HUMAN	(O76011) Keratin, type I cuticular Ha4 (Hair keratin, type I Ha4)	Human hair
9.	K1H5_HUMAN	(Q92764) Keratin, type I cuticular Ha5 (Hair keratin, type I Ha5)	Human hair
10.	K1H6_HUMAN	(O76013) Keratin, type I cuticular Ha6 (Hair keratin, type I Ha6)	Human hair
11.	K1H7_HUMAN	(O76014) Keratin, type I cuticular Ha7 (Hair keratin, type I Ha7)	Human hair

Appendix 24: Some of the list of proteins commonly found in proteomics experiments that are present either by accident or through unavoidable contamination of protein samples. Human keratin is in the list of identified proteins as contaminants.

Appendix 25: Protein concentrations of the rats epithelial cells for the 7-day trial.

	Replicate 1	Replicate 2	mean	x µg/5µl	µg/µl	µl/1300µg
Mcert R1	0.675	0.702	0.6885	28.071	5.6142	231.555698
Mcert R2	0.687	0.641	0.664	31.571	6.3142	205.885148
Mcert R3	0.616	0.619	0.6175	38.214	7.6428	170.09473
Mcert R4	0.536	0.561	0.5485	48.071	9.6142	135.216659
Mon810R1	0.484	0.488	0.486	57	11.4	114.035088
Mon810R2	0.656	0.571	0.6135	38.785	7.757	167.590563
Mon810R3	0.579	0.585	0.582	43.285	8.657	150.167495
Mon810R4	0.499	0.543	0.521	52	10.4	125
MonGarstR1	0.61	0.623	0.6165	38.357	7.6714	169.460594
MonGarstR2	0.544	0.521	0.5325	50.357	10.0714	129.07838
MonGarstR3	0.541	0.505	0.523	51.714	10.3428	125.691302
MonGarstR4	0.652	0.699	0.6755	29.928	5.9856	217.187918
MonGoldR1	0.556	0.547	0.5515	47.642	9.5284	136.434239
MonGoldR2	0.599	0.597	0.598	41	8.2	158.536585
MonGoldR3	0.596	0.554	0.575	44.285	8.857	146.776561
MonGoldR4	0.514	0.52	0.517	52.571	10.5142	123.642312
ConvCornR1	0.492	0.481	0.4865	56.928	11.3856	114.179314
ConvCornR2	0.479	0.514	0.4965	55.5	11.1	117.117117
ConvCornR3	0.554	0.558	0.556	47	9.4	138.297872
ConvCornR4	0.669	0.635	0.652	33.285	6.657	195.283161

Appendix 26: Protein concentrations of the rats epithelial cells for the 28-day trial.

	Replicate 1	Replicate 2	mean	x µg/5µl	µg/µl	µl/1300ug
Mcert R1	0.337	0.348	0.3425	60.928	12.1856	106.683298
Mcert R2	0.435	0.398	0.4165	50.214	10.0428	129.445971
Mcert R3	0.485	0.507	0.496	39	7.8	166.666667
Mcert R4	0.413	0.422	0.4175	50.214	10.0428	129.445971
Mon810R1	0.509	0.506	0.5075	37.357	7.4714	173.996841
Mon810R2	0.501	0.508	0.5045	37.785	7.557	172.025936
Mon810R3	0.455	0.361	0.408	51.571	10.3142	126.039829
Mon810R4	0.397	0.408	0.4025	52.357	10.4714	124.147678
MonGarstR1	0.595	0.597	0.596	24.714	4.9428	263.008821
MonGarstR2	0.538	0.531	0.5345	33.5	6.7	194.029851
MonGarstR3	0.482	0.51	0.496	39	7.8	166.666667
MonGarstR4	0.45	0.437	0.4435	46.5	9.3	139.784946
MonGoldR1	0.402	0.365	0.3835	55.071	11.0142	118.029453
MonGoldR2	0.502	0.501	0.5015	38.214	7.6428	170.09473
MonGoldR3	0.386	0.356	0.371	56.857	11.3714	114.321895
MonGoldR4	0.437	0.437	0.437	47.428	9.4856	137.049844
ConvCornR1	0.319	0.309	0.314	65	13	100
ConvCornR2	0.307	0.39	0.3485	60.071	12.0142	108.20529
ConvCornR3	0.455	0.437	0.446	46.142	9.2284	140.86949
ConvCornR4	0.399	0.417	0.408	51.571	10.3142	126.039829

Appendix 27: Identification of differentially expressed proteins from the epithelial cells of the small intestine of rats fed different maize based diets for 7-day.
The data represent two way comparisons as follows (Mcert vs Mon810, Mcert vs Mon Garst, Mcert vs Mon Conv Corn, Mcert vs Mon Gold, Mon810 vs Mon Garst, Mon810 vs Mon Conv Corn, Mon Garst vs Mon Conv Corn, Mon810 vs Mon Gold, Mon Garst vs Mon Gold, Mon Conv Corn vs Mon Gold) and five way comparisons.

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon 810
1146	2.5	7.44	63,795	prelamin-A/C isoform C2	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin. Lamin A and C are present in equal amounts in the lamina of mammals. Plays an important role in nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics. Prelamin-A/C can accelerate smooth muscle cell senescence. It acts to disrupt mitosis and induce DNA damage in vascular smooth muscle cells (VSMCs), leading to mitotic failure, genomic instability, and premature senescence	37	-165	6.5	74.3	6.29E+05	1.59E+06
				Stress-induced-phosphoprotein 1	Mediates the association of the molecular chaperones HSC70 and HSP90 (HSPCA and HSPCB).	11	-62.9	6.4	62.5		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon 810
				heterogeneous nuclear ribonucleoprotein L isoform b	This protein is a component of the heterogeneous nuclear ribonucleoprotein (hnRNP) complexes which provide the substrate for the processing events that pre-mRNAs undergo before becoming functional, translatable mRNAs in the cytoplasm. Is associated with most nascent transcripts including those of the landmark giant loops of amphibian lampbrush chromosomes. Associates, together with APEX1, to the negative calcium responsive element (nCaRE) B2 of the APEX2 promoter (Homo sapiens (Human)).	10	-38.2	7.9	64.3		
				T-complex protein 1 subunit zeta	Protein which is transiently involved in the noncovalent folding, assembly and/or disassembly of other polypeptides or RNA molecules, including any transport and oligomerisation processes they may undergo, and the refolding and reassembly of protein and RNA molecules denatured by stress. Though involved in these processes, chaperones are not an integral part of these functioning molecules. Also used for metallochaperones, which function to provide a metal directly to target proteins while protecting this metal from scavengers.	5	-31.7	6.6	58		
				Catalase	Occurs in almost all aerobically respiring organisms and serves to protect cells from the toxic effects of hydrogen peroxide. Promotes growth of cells.	5	-31.4	7.1	59.7		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon 810
				Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial	Accepts electrons from ETF and reduces ubiquinone	3	-14.6	7.3	68.1		
1630	2.2	6.83	47,989	ErbB3-binding protein 1	May play a role in a ERBB3-regulated signal transduction pathway. Seems be involved in growth regulation. Acts a corepressor of the androgen receptor and is regulated by the ERBB3 ligand neuregulin-1/heregulin (HRG). Inhibits transcription of some E2F1-regulated promoters, probably by recruiting histone acetylase (HAT) activity. Binds RNA. Associates with 28S, 18S and 5.8S mature rRNAs, several rRNA precursors and probably U3 small nucleolar RNA. May be involved in regulation of intermediate and late steps of rRNA processing. May be involved in ribosome assembly. Mediates cap-independent translation of specific viral IRESs (internal ribosomal entry site) (Human)	30	-101	6.4	43.6	5.69E+05	1.24E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon 810
				prelamin-A/C isoform C2	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin. Lamin A and C are present in equal amounts in the lamina of mammals. Plays an important role in nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics. Prelamin-A/C can accelerate smooth muscle cell senescence. It acts to disrupt mitosis and induce DNA damage in vascular smooth muscle cells (VSMCs), leading to mitotic failure, genomic instability, and premature senescence	6	-40.8	6.5	74.3		
				60 kDa heat shock protein, mitochondrial	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix.	4	-23.2	5.9	60.9		
				Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	3	-14	5.9	56.6		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon 810
2306	1.7	4.34	33,859	Tropomyosin alpha-3 chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	63	-170	4.7	29	5.65E+07	3.25E+07
3258	1.6	4.35	32,615	Tropomyosin alpha-1 chain (Tropomyosin-1)(Alpha-tropomyosin)	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	51	-109	4.7	28.5	2.22E+07	1.36E+07

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	r.I	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Garst
3248	6	9.22	43,593	3-ketoacyl-CoA thiolase, mitochondrial	Pathway: Lipid metabolism; fatty acid metabolism.	4	-19.5	8.4	41.9	3.93E+06	6.58E+05
1222	2.4	4.1	63,450	Calnexin Precursor	Calcium-binding protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum. It may act in assisting protein assembly and/or in the retention within the ER of unassembled protein subunits. It seems to play a major role in the quality control apparatus of the ER by the retention of incorrectly folded proteins. Associated with partial T-cell antigen receptor complexes that escape the ER of immature thymocytes, it may function as a signaling complex regulating thymocyte maturation. Additionally it may play a role in receptor-mediated endocytosis at the synapse. Calnexin is a chaperone, characterized by assisting protein folding and quality control, ensuring that only properly folded and assembled proteins proceed further along the secretory pathway. Calnexin acts to retain unfolded or unassembled N-linked glycoproteins in the ER.	4	-17.6	4.6	67.4	4.45E+06	1.83E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Garst
2212	1.9	7.18	35,193	Voltage-dependent anion-selective channel protein 2 (VDAC-2)(Outer mitochondrial membrane protein porin 2)(B36-VDAC)	Forms a channel through the mitochondrial outer membrane that allows diffusion of small hydrophilic molecules. The channel adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective	17	-72.9	7.4	31.8	7.26E+06	3.77E+06
3243	2.6	6.34	28,275	Thioredoxin-dependent peroxide reductase, mitochondrial Precursor (EC 1.11.1.15)(Peroxiredoxin-3)(PRX-3)(PRx III)	Involved in redox regulation of the cell. Protects radical-sensitive enzymes from oxidative damage by a radical-generating system. Acts synergistically with MAP3K13 to regulate the activation of NF-kappa-B in the cytosol. (Human)	9	-15.7	7.1	28.3	2.86E+06	1.12E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Garst
2608	1.4	5.91	27,277	UMP-CMP kinase (EC 2.7.4.14)(Cytidylate kinase)	Catalyzes the phosphorylation of pyrimidine nucleoside monophosphates at the expense of ATP. Plays an important role in de novo pyrimidine nucleotide biosynthesis. Has preference for UMP and CMP as phosphate acceptors. (Human)	10	-27.7	8.2	25.8	5.28E+06	3.80E+06
				Electron transfer flavoprotein subunit beta (Beta-ETF)	The electron transfer flavoprotein serves as a specific electron acceptor for several dehydrogenases, including five acyl-CoA dehydrogenases, glutaryl-CoA and sarcosine dehydrogenase. It transfers the electrons to the main mitochondrial respiratory chain via ETF-ubiquinone oxidoreductase (ETF dehydrogenase). (Human)	3	-9	7.6	27.7		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Garst
				Protein DJ-1 (Parkinson disease protein 7 homolog)I	Protects cells against oxidative stress and cell death. Plays a role in regulating expression or stability of the mitochondrial uncoupling proteins SLC25A14 and SLC25A27 in dopaminergic neurons of the substantia nigra pars compacta and attenuates the oxidative stress induced by calcium entry into the neurons via L-type channels during pacemaking. Eliminates hydrogen peroxide and protects cells against hydrogen peroxide-induced cell death. May act as an atypical peroxiredoxin-like peroxidase that scavenges hydrogen peroxide. Following removal of a C-terminal peptide, displays protease activity and enhanced cytoprotective action against oxidative stress-induced apoptosis. Stabilizes NFE2L2 by preventing its association with KEAP1 and its subsequent ubiquitination. Binds to OTUD7B and inhibits its deubiquitinating activity. Enhances RELA nuclear translocation. Binds to a number of mRNAs containing multiple copies of GG or CC motifs and partially inhibits their translation but dissociates following oxidative stress. Required for correct mitochondrial morphology and function and for autophagy of dysfunctional mitochondria. Regulates astrocyte inflammatory responses. Acts as a positive regulator of androgen receptor-dependent transcription. Prevents aggregation of SNCA. Plays a role in fertilization. Has no proteolytic activity. Has cell-growth promoting activity and transforming activity. May function as a redox-sensitive chaperone	4	-7.2	6.3	20		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Garst
1976	2.7	4.14	41,109	Tropomyosin beta chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments. The non-muscle isoform may have a role in agonist-mediated receptor internalization	23	-81.9	4.5	28.6	1.22E+07	4.54E+06
				Tropomyosin alpha-1 chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	12	-48.8	4.7	32.7		
				Calreticulin Precursor (CRP55)(Calregulin)(HACBP)(Endoplasmic reticulum resident protein 60)(ERp60)	Calcium-binding chaperone that promotes folding, oligomeric assembly and quality control in the endoplasmic reticulum (ER) via the calreticulin/calnexin cycle. This lectin interacts transiently with almost all of the monoglucosylated glycoproteins that are synthesized in the ER. Interacts with the DNA-binding domain of NR3C1 and mediates its nuclear export. Involved in maternal gene expression regulation. May participate in oocyte maturation via the regulation of calcium homeostasis	17	-39.2	4.3	48		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Garst
1516	1.8	6.51	51,934	aldehyde dehydrogenase X, mitochondrial precursor	ALDHs play a major role in the detoxification of alcohol-derived acetaldehyde. They are involved in the metabolism of corticosteroids, biogenic amines, neurotransmitters, and lipid peroxidation	66	-288	6.6	57.6	1.09E+07	5.95E+06
				4-trimethylaminobutyraldehyde dehydrogenase (TMABADH)(EC 1.2.1.47)(Aldehyde dehydrogenase family 9 member A1)(EC 1.2.1.3)	Converts gamma-trimethylaminobutyraldehyde into gamma-butyrobetaine.	14	-73.9	6.9	56.4		
				Aldehyde dehydrogenase, mitochondrial Precursor (EC 1.2.1.3)(ALDH class 2)(ALDH1)(ALDH-E2)	catalytic activity: An aldehyde + NAD+ + H2O = a carboxylate + NADH.	12	-67.2	7	56.4		
				Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	4	-26.4	5.9	56.6		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	r'l	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
2883	6.9	8.89	21,929	Mesencephalic astrocyte-derived neurotrophic factor Precursor (Protein ARMET)(Arginine-rich protein)	Selectively promotes the survival of dopaminergic neurons of the ventral mid-brain. Modulates GABAergic transmission to the dopaminergic neurons of the substantia nigra. Enhances spontaneous, as well as evoked, GABAergic inhibitory postsynaptic currents in dopaminergic neurons. Inhibits cell proliferation and endoplasmic reticulum (ER) stress-induced cell death.	4	-18.3	8.5	20.4	1.41E+06	9.70E+06
1580	3	6.27	49,144	Aldehyde dehydrogenase, mitochondrial Precursor (EC 1.2.1.3)	Catalytic acyivity: An aldehyde + NAD+ + H2O = a carboxylate + NADH.	20	-118	7	56.4	1.18E+06	3.96E+05
				60 kDa heat shock protein, mitochondrial	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix.	21	-110	5.9	60.9		
				Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	8	-62.2	5.9	56.6		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
				Heterogeneous nuclear ribonucleoprotein HHeterogeneous nuclear ribonucleoprotein H, N-terminally processed	This protein is a component of the heterogeneous nuclear ribonucleoprotein (hnRNP) complexes which provide the substrate for the processing events that pre-mRNAs undergo before becoming functional, translatable mRNAs in the cytoplasm. Mediates pre-mRNA alternative splicing regulation. Inhibits, together with CUGBP1, insulin receptor pre-mRNA exon 11 inclusion in myoblast. Binds to the IR RNA. Binds poly(RG)	5	-36.5	6.2	51.2		
				Heterogeneous nuclear ribonucleoprotein H2 (hnRNP H2)(Heterogeneous nuclear ribonucleoprotein H')(hnRNP H')	This protein is a component of the heterogeneous nuclear ribonucleoprotein (hnRNP) complexes which provide the substrate for the processing events that pre-mRNAs undergo before becoming functional, translatable mRNAs in the cytoplasm. Binds poly(RG)	4	-15.9	5.9	49.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
2511	3.2	6.09	29,404	PAX interacting (with transcription-activation domain) protein 1	Involved in DNA damage response and in transcriptional regulation through histone methyltransferase (HMT) complexes. Plays a role in early development. In DNA damage response is required for cell survival after ionizing radiation. In vitro shown to be involved in the homologous recombination mechanism for the repair of double-strand breaks (DSBs). Its localization to DNA damage foci requires RNF8 and UBE2N. Recruits TP53BP1 to DNA damage foci and, at least in particular repair processes, effective DNA damage response appears to require the association with TP53BP1 phosphorylated by ATM at 'Ser-25'. Together with TP53BP1 regulates ATM association. Recruits PAGR1 to sites of DNA damage and the PAGR1:PAXIP1 complex is required for cell survival in response to DNA damage; the function is probbaly independent of MLL-containing histone methyltransferase (HMT) complexes. Promotes ubiquitination of PCNA following UV irradiation and may regulate recruitment of polymerase eta and RAD51 to chromatin after DNA damage. Proposed to be involved in transcriptional regulation by linking MLL-containing histone methyltransferase (HMT) complexes to gene promoters by interacting with promoter-bound transcription factors such as PAX2. Associates with gene promoters that are known to be regulated by MLL2. During immunoglobulin class switching in activated B-cells is involved in trimethylation of histone H3 at 'Lys-4' and in transcription initiation of downstream switch regions at the immunoglobulin heavy-chain locus; this function appears to involve the recruitment of MLL-containing HMT complexes. (Human)	1	-1.2	8.8	93.9	1.90E+07	5.90E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
2021	2.9	4.5	39,794	Actin, cytoplasmic 1 Actin, cytoplasmic 1, N-terminally processed	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.	17	-75.6	5.3	41.7	4.08E+06	1.18E+07
				Actin, gamma-enteric smooth muscle	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.	13	-51.1	5.3	41.8		
				Tropomyosin alpha-1 chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	9	-31.2	4.7	32.7		
				beta-actin-like protein 2	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells	6	-29.8	5.3	41.9		
2016	2.1	5.56	39,900	Actin, cytoplasmic 1 Actin, cytoplasmic 1, N-terminally processed	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.	23	-86.9	5.3	41.7	1.89E+07	9.20E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
				Eukaryotic translation initiation factor 3 subunit I (eIF3i)(Eukaryotic translation initiation factor 3 subunit 2)(eIF-3-beta)	Component of the eukaryotic translation initiation factor 3 (eIF-3) complex, which is required for several steps in the initiation of protein synthesis. The eIF-3 complex associates with the 40S ribosome and facilitates the recruitment of eIF-1, eIF-1A, eIF-2:GTP:methionyl-tRNAi and eIF-5 to form the 43S preinitiation complex (43S PIC). The eIF-3 complex stimulates mRNA recruitment to the 43S PIC and scanning of the mRNA for AUG recognition. The eIF-3 complex is also required for disassembly and recycling of post-termination ribosomal complexes and subsequently prevents premature joining of the 40S and 60S ribosomal subunits prior to initiation	10	-35.8	5.3	19.4		
				Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Scaffold protein that connects plasma membrane proteins with members of the ezrin/moesin/radixin family and thereby helps to link them to the actin cytoskeleton and to regulate their surface expression. Necessary for recycling of internalized ADRB2. Was first known to play a role in the regulation of the activity and subcellular location of SLC9A3. Necessary for cAMP-mediated phosphorylation and inhibition of SLC9A3. May enhance Wnt signaling. May participate in HTR4 targeting to microvilli. Involved in the regulation of phosphate reabsorption in the renal proximal tubules	6	-34.3	5.7	38.8		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
				N-acetyl-D-glucosamine kinase (N-acetylglucosamine kinase)(EC 2.7.1.59)(GlcNAc kinase)	Converts endogenous N-acetylglucosamine (GlcNAc), a major component of complex carbohydrates, from lysosomal degradation or nutritional sources into GlcNAc 6-phosphate. Involved in the N-glycolylneuraminic acid (Neu5Gc) degradation pathway. Also has ManNAc kinase activity.	3	-18.1	5.6	37.2		
2212	2.2	7.18	35,193	Voltage-dependent anion-selective channel protein 2 (VDAC-2)(Outer mitochondrial membrane protein porin 2)(B36-VDAC)	Forms a channel through the mitochondrial outer membrane that allows diffusion of small hydrophilic molecules. The channel adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective	17	-72.9	7.4	31.8	7.26E+06	3.77E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
2249	4.4	9.64	34,773	Voltage-dependent anion-selective channel protein 1	Forms a channel through the mitochondrial outer membrane and also the plasma membrane. The channel at the outer mitochondrial membrane allows diffusion of small hydrophilic molecules; in the plasma membrane it is involved in cell volume regulation and apoptosis. It adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective. May participate in the formation of the permeability transition pore complex (PTPC) responsible for the release of mitochondrial products that triggers apoptosis	10	-44.3	8.6	30.7	4.41E+06	1.92E+07
				Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	Plays an essential role in the mitochondrial beta-oxidation of short chain fatty acids. Exerts it highest activity toward 3-hydroxybutyryl-CoA. Pathway: Lipid metabolism; fatty acid beta-oxidation	4	-12.3	8.8	34.4		
				D-beta-hydroxybutyrate dehydrogenase, mitochondrial Precursor (BDH)(EC 1.1.1.30)(3-hydroxybutyrate dehydrogenase)	Catalytic activity: (R)-3-hydroxybutanoate + NAD+ = acetoacetate + NADH.	3	-7.3	9	38.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
2045	3.3	7.49	38,983	Glyceraldehyde-3-phosphate dehydrogenase	Has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby playing a role in glycolysis and nuclear functions, respectively. Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme in glycolysis that catalyzes the first step of the pathway by converting D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate. Modulates the organization and assembly of the cytoskeleton. Facilitates the CHP1-dependent microtubule and membrane associations through its ability to stimulate the binding of CHP1 to microtubules. Also participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. Nuclear functions are probably due to the nitrosylase activity that mediates cysteine S-nitrosylation of nuclear target proteins such as SIRT1, HDAC2 and PRKDC. Component of the GAIT (gamma interferon-activated inhibitor of translation) complex which mediates interferon-gamma-induced transcript-selective translation inhibition in inflammation processes. Upon interferon-gamma treatment assembles into the GAIT complex which binds to stem loop-containing GAIT elements in the 3'-UTR of diverse inflammatory mRNAs (such as ceruplasmin) and suppresses their translation. Pathway: carbohydrate degradation; Glycolysis	28	-119	8.1	35.8	2.90E+06	8.87E+05

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
				Annexin A2	Calcium-regulated membrane-binding protein whose affinity for calcium is greatly enhanced by anionic phospholipids. It binds two calcium ions with high affinity.	4	-27.4	7.5	38.9		
1146	3.3	7.45	65	prelamin-A/C isoform C2	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin. Lamin A and C are present in equal amounts in the lamina of mammals. Plays an important role in nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics. Prelamin-A/C can accelerate smooth muscle cell senescence. It acts to disrupt mitosis and induce DNA damage in vascular smooth muscle cells (VSMCs), leading to mitotic failure, genomic instability, and premature senescence	37	-165	6.5	74.3	6.29E+05	2.08E+06
				Stress-induced-phosphoprotein 1	Mediates the association of the molecular chaperones HSC70 and HSP90 (HSPCA and HSPCB).	11	-62.9	6.4	62.5		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
				heterogeneous nuclear ribonucleoprotein L isoform b	This protein is a component of the heterogeneous nuclear ribonucleoprotein (hnRNP) complexes which provide the substrate for the processing events that pre-mRNAs undergo before becoming functional, translatable mRNAs in the cytoplasm. Is associated with most nascent transcripts including those of the landmark giant loops of amphibian lampbrush chromosomes. Associates, together with APEX1, to the negative calcium responsive element (nCaRE) B2 of the APEX2 promoter (Homo sapiens (Human)).	10	-38.2	7.9	64.3		
				T-complex protein 1 subunit zeta	Protein which is transiently involved in the noncovalent folding, assembly and/or disassembly of other polypeptides or RNA molecules, including any transport and oligomerisation processes they may undergo, and the refolding and reassembly of protein and RNA molecules denatured by stress. Though involved in these processes, chaperones are not an integral part of these functioning molecules. Also used for metallochaperones, which function to provide a metal directly to target proteins while protecting this metal from scavengers.	5	-31.7	6.6	58		
				Catalase	Occurs in almost all aerobically respiring organisms and serves to protect cells from the toxic effects of hydrogen peroxide. Promotes growth of cells.	5	-31.4	7.1	59.7		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
				Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial	Accepts electrons from ETF and reduces ubiquinone	3	-14.6	7.3	68.1		
2400	2.5	8.85	31,500	coiled-coil-helix-coiled-coil-helix domain containing protein 3	Required for maintenance of mitochondrial crista integrity and mitochondrial function. May act as a scaffolding protein that stabilizes protein complexes involved in crista architecture and protein import. Has also been shown to function as a transcription factor which binds to the BAG1 promoter and represses BAG1 transcription.	11	-46.7	8.3	26.4	2.55E+06	6.35E+06
				Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1)	nvolved with pre-mRNA processing. Forms complexes (ribonucleosomes) with at least 20 other different hnRNP and heterogeneous nuclear RNA in the nucleus. (Human)	3	-14	9.1	37.3		
1976	3.5	4.14	41,109	Tropomyosin beta chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments. The non-muscle isoform may have a role in agonist-mediated receptor internalization	23	-81.9	4.5	28.6	1.22E+07	3.44E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
				Tropomyosin alpha-1 chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	12	-48.8	4.7	32.7		
				Calreticulin Precursor (CRP55)(Calregulin)(HA CBP)(Endoplasmic reticulum resident protein 60)(ERp60)	Calcium-binding chaperone that promotes folding, oligomeric assembly and quality control in the endoplasmic reticulum (ER) via the calreticulin/calnexin cycle. This lectin interacts transiently with almost all of the monoglucosylated glycoproteins that are synthesized in the ER. Interacts with the DNA-binding domain of NR3C1 and mediates its nuclear export. Involved in maternal gene expression regulation. May participate in oocyte maturation via the regulation of calcium homeostasis	17	-39.2	4.3	48		
1950	2.1	5.89	41,582	Serpin B5 (Peptidase inhibitor 5)(PI-5)(Maspin)	Tumor suppressor. It blocks the growth, invasion, and metastatic properties of mammary tumors. As it does not undergo the S (stressed) to R (relaxed) conformational transition characteristic of active serpins, it exhibits no serine protease inhibitory activity	12	-77.1	5.7	42	1.82E+06	8.67E+05

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
				3'(2'),5'-bisphosphate nucleotidase 1 (EC 3.1.3.7)(Bisphosphate 3'-nucleotidase 1)(PAP-inositol-1,4-phosphatase)(PIP)(scHAL2 analogous 3)	Converts adenosine 3'-phosphate 5'-phosphosulfate (PAPS) to adenosine 5'-phosphosulfate (APS) and 3'(2')-phosphoadenosine 5'- phosphate (Bravo *et al.*) to AMP. Has 1000-fold lower activity towards inositol 1,4-bisphosphate (Ins(1,4)P2) and inositol 1,3,4-trisphosphate (Ins(1,3,4)P3), but does not hydrolyze Ins1P, Ins(3,4)P2, Ins(1,3,4,5)P4 or InsP6.	12	-57.1	5.6	29		
				Elongation factor 2	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation. During this step, the ribosome changes from the pre-translocational (PRE) to the post-translocational state as the newly formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively. Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome.	17	-56.7	6.4	95.2		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Gold
2249	2.3	9.64	34,773	Voltage-dependent anion-selective channel protein 1	Forms a channel through the mitochondrial outer membrane and also the plasma membrane. The channel at the outer mitochondrial membrane allows diffusion of small hydrophilic molecules; in the plasma membrane it is involved in cell volume regulation and apoptosis. It adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective. May participate in the formation of the permeability transition pore complex (PTPC) responsible for the release of mitochondrial products that triggers apoptosis	33	-116	8.6	30.7	4.41E+06	1.93E+06
				Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	Plays an essential role in the mitochondrial beta-oxidation of short chain fatty acids. Exerts it highest activity toward 3-hydroxybutyryl-CoA. Pathway: Lipid metabolism; fatty acid beta-oxidation	4	-12	8.8	34.4		
				D-beta-hydroxybutyrate dehydrogenase, mitochondrial Precursor	Catalytic activity: (R)-3-hydroxybutanoate + NAD+ = acetoacetate + NADH.	3	-7.3	9	38.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
2212	3.4	7.18	35,193	Voltage-dependent anion-selective channel protein 2 (VDAC-2)(Outer mitochondrial membrane protein porin 2)(B36-VDAC)	Forms a channel through the mitochondrial outer membrane that allows diffusion of small hydrophilic molecules. The channel adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective	17	-72.9	7.4	31.8	1.28E+07	3.77E+06
3248	3.7	9.22	43,593	3-ketoacyl-CoA thiolase, mitochondrial	Pathway: Lipid metabolism; fatty acid metabolism. Abolishes BNIP3-mediated apoptosis and mitochondrial damage. (Human)	4	-19.5	8.4	41.9	2.43E+06	6.58E+05
2279	3.7	7.53	34,221	Electron transfer flavoprotein subunit alpha, mitochondrial Precursor (Alpha-ETF)	The electron transfer flavoprotein serves as a specific electron acceptor for several dehydrogenases, including five acyl-CoA dehydrogenases, glutaryl-CoA and sarcosine dehydrogenase. It transfers the electrons to the main mitochondrial respiratory chain via ETF-ubiquinone oxidoreductase (ETF dehydrogenase).	6	-39.9	8.6	34.9	8.58E+06	2.34E+06
				Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1)	Involved with pre-mRNA processing. Forms complexes (ribonucleosomes) with at least 20 other different hnRNP and heterogeneous nuclear RNA in the nucleus	5	-23.9	9.1	37.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
				Voltage-dependent anion-selective channel protein 2 (VDAC-2)(Outer mitochondrial membrane protein porin 2)(B36-VDAC)	Forms a channel through the mitochondrial outer membrane that allows diffusion of small hydrophilic molecules. The channel adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective	3	-19.6	7.4	31.8		
3243	2.5	6.34	28,275	Thioredoxin-dependent peroxide reductase, mitochondrial Precursor (EC 1.11.1.15)(Peroxiredoxin-3)(PRX-3)(PRx III)	Involved in redox regulation of the cell. Protects radical-sensitive enzymes from oxidative damage by a radical-generating system. Acts synergistically with MAP3K13 to regulate the activation of NF-kappa-B in the cytosol. (Human)	9	-15.7	7.1	28.3	2.83E+06	1.12E+06
1950	1.6	5.89	41,582	Serpin B5 (Peptidase inhibitor 5)(PI-5)(Maspin)	Tumor suppressor. It blocks the growth, invasion, and metastatic properties of mammary tumors. As it does not undergo the S (stressed) to R (relaxed) conformational transition characteristic of active serpins, it exhibits no serine protease inhibitory activity	12	-77.1	5.7	42	1.76E+06	1.09E+06
				3'(2'),5'-bisphosphate nucleotidase 1 (EC 3.1.3.7)(Bisphosphate 3'-nucleotidase 1)(PAP-inositol-1,4-phosphatase)(PIP)(scHAL2 analogous 3)	Converts adenosine 3'-phosphate 5'-phosphosulfate (PAPS) to adenosine 5'-phosphosulfate (APS) and 3'(2')-phosphoadenosine 5'- phosphate (Bravo *et al.*) to AMP. Has 1000-fold lower activity towards inositol 1,4-bisphosphate (Ins(1,4)P2) and inositol 1,3,4-trisphosphate (Ins(1,3,4)P3), but does not hydrolyze Ins1P, Ins(3,4)P2, Ins(1,3,4,5)P4 or InsP6.	12	-57.1	5.6	29		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
				Elongation factor 2	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation. During this step, the ribosome changes from the pre-translocational (PRE) to the post-translocational state as the newly formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively. Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome.	17	-56.7	6.4	95.2		
2626	1.8	6.19	27,015	LDLR chaperone MESD Precursor (Mesoderm development candidate 2)(Mesoderm development protein)	Chaperone specifically assisting the folding of beta-propeller/EGF modules within the family of low-density lipoprotein receptors (LDLRs). Acts as a modulator of the Wnt pathway through chaperoning the coreceptors of the canonical Wnt pathway, LRP5 and LRP6, to the plasma membrane. Essential for specification of embryonic polarity and mesoderm induction. (Human)	10	-46.6	5.5	25.2	3.75E+06	2.05E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
				Growth factor receptor-bound protein 2	Adapter protein that provides a critical link between cell surface growth factor receptors and the Ras signaling pathway. Isoform 2 does not bind to phosphorylated epidermal growth factor receptor but inhibits EGF-induced transactivation of a RAS-responsive element. Isoform 2 acts as a dominant negative protein over GRB2 and by suppressing proliferative signals, may trigger active programmed cell death. (human)	9	-36.4	5.9	25.2		
				Peroxiredoxin-6 (EC 1.11.1.15)(Antioxidant protein 2)(1-Cys peroxiredoxin)(1-Cys PRX)	Involved in redox regulation of the cell. Can reduce H2O2 and short chain organic, fatty acid, and phospholipid hydroperoxides. May play a role in the regulation of phospholipid turnover as well as in protection against oxidative injury	7	-33.4	5.6	24.8		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
2048	1.7	7.12	39,058	Glyceraldehyde-3-phosphate dehydrogenase	Has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby playing a role in glycolysis and nuclear functions, respectively. Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme in glycolysis that catalyzes the first step of the pathway by converting D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate. Modulates the organization and assembly of the cytoskeleton. Facilitates the CHP1-dependent microtubule and membrane associations through its ability to stimulate the binding of CHP1 to microtubules. Also participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. Nuclear functions are probably due to the nitrosylase activity that mediates cysteine S-nitrosylation of nuclear target proteins such as SIRT1, HDAC2 and PRKDC. Component of the GAIT (gamma interferon-activated inhibitor of translation) complex which mediates interferon-gamma-induced transcript-selective translation inhibition in inflammation processes. Upon interferon-gamma treatment assembles into the GAIT complex which binds to stem loop-containing GAIT elements in the 3'-UTR of diverse inflammatory mRNAs (such as ceruplasmin) and suppresses their translation	4	-26.1	8.1	35.8	3.76E+06	2.23E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
				THO complex subunit 6 homolog (WD repeat-containing protein 58)	Component of the THO subcomplex of the TREX complex. The TREX complex specifically associates with spliced mRNA and not with unspliced pre-mRNA. It is recruited to spliced mRNAs by a transcription-independent mechanism. Binds to mRNA upstream of the exon-junction complex (EJC) and is recruited in a splicing- and cap-dependent manner to a region near the 5' end of the mRNA where it functions in mRNA export. The recruitment occurs via an interaction between ALYREF/THOC4 and the cap-binding protein NCBP1. DDX39B functions as a bridge between ALYREF/THOC4 and the THO complex	3	-14.9	6.7	37.4		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Conv Corn
2883	7.1	8.89	21,929	Mesencephalic astrocyte-derived neurotrophic factor Precursor (Protein ARMET)(Arginine-rich protein)	Selectively promotes the survival of dopaminergic neurons of the ventral mid-brain. Modulates GABAergic transmission to the dopaminergic neurons of the substantia nigra. Enhances spontaneous, as well as evoked, GABAergic inhibitory postsynaptic currents in dopaminergic neurons. Inhibits cell proliferation and endoplasmic reticulum (ER) stress-induced cell death. (Mouse)	4	-18.3	8.5	20.4	1.36E+06	9.70E+06
1950	2	5.89	41,582	Serpin B5 (Peptidase inhibitor 5)(PI-5)(Maspin)	Tumor suppressor. It blocks the growth, invasion, and metastatic properties of mammary tumors. As it does not undergo the S (stressed) to R (relaxed) conformational transition characteristic of active serpins, it exhibits no serine protease inhibitory activity	12	-77.1	5.7	42	1.76E+06	8.67E+05
				3'(2'),5'-bisphosphate nucleotidase 1 (EC 3.1.3.7)(Bisphosphate 3'-nucleotidase 1)(PAP-inositol-1,4-phosphatase)(PIP)(scHAL2 analogous 3)	Converts adenosine 3'-phosphate 5'-phosphosulfate (PAPS) to adenosine 5'-phosphosulfate (APS) and 3'(2')-phosphoadenosine 5'- phosphate (Bravo *et al.*) to AMP. Has 1000-fold lower activity towards inositol 1,4-bisphosphate (Ins(1,4)P2) and inositol 1,3,4-trisphosphate (Ins(1,3,4)P3), but does not hydrolyze Ins1P, Ins(3,4)P2, Ins(1,3,4,5)P4 or InsP6.	12	-57.1	5.6	29		

				Elongation factor 2	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation. During this step, the ribosome changes from the pre-translocational (PRE) to the post-translocational state as the newly formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively. Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome.	17	-56.7	6.4	95.2		
2443	5.7	9.53	30,665	Succinate dehydrogenase iron-sulfur subunit, mitochondrial Precursor (EC 1.3.5.1)(Iron-sulfur subunit of complex II)	Iron-sulfur protein subunit of succinate dehydrogenase (SDH) that is involved in complex II of the mitochondrial electron transport chain and is responsible for transferring electrons from succinate to ubiquinone (coenzyme Q). Pathway: Carbohydrate metabolism; tricarboxylic acid cycle; fumarate from succinate (eukaryal route): step 1/1. (Human).	23	-65.3	9	31.8	2.64E+06	1.50E+07

				Proteasome subunit alpha type-7 (EC 3.4.25.1)(Proteasome subunit RC6-1)	The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity. Plays an important role in the regulation of cell proliferation or cell cycle control, transcriptional regulation, immune and stress response, cell differentiation, and apoptosis. Interacts with some important proteins involved in transcription factor regulation, cell cycle transition, viral replication and even tumor initiation and progression. Inhibits the transactivation function of HIF-1A under both normoxic and hypoxia-mimicking conditions. The interaction with EMAP2 increases the proteasome-mediated HIF-1A degradation under the hypoxic conditions. Plays a role in hepatitis C virus internal ribosome entry site-mediated translation. Mediates nuclear translocation of the androgen receptor and thereby enhances androgen-mediated transactivation. Promotes MAVS degradation and thereby negatively regulates MAVS-mediated innate immune response (Human).	14	-52.2	8.6	28.4		

				ATP synthase subunit alpha, mitochondrial	Mitochondrial membrane ATP synthase (F1F0 ATP synthase or Complex V) produces ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain. F-type ATPases consist of two structural domains, F1 - containing the extramembraneous catalytic core, and F0 - containing the membrane proton channel, linked together by a central stalk and a peripheral stalk. During catalysis, ATP synthesis in the catalytic domain of F1 is coupled via a rotary mechanism of the central stalk subunits to proton translocation. Subunits alpha and beta form the catalytic core in F1. Rotation of the central stalk against the surrounding alpha3beta3 subunits leads to hydrolysis of ATP in three separate catalytic sites on the beta subunits. Subunit alpha does not bear the catalytic high-affinity ATP-binding sites (Human)	6	-33.8	9.3	59.8		
				Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	Pathway: Amino-acid degradation; L-lysine degradation via saccharopine pathway; glutaryl-CoA from L-lysine: step 6/6. The 2-oxoglutarate dehydrogenase complex catalyzes the overall conversion of 2-oxoglutarate to succinyl-CoA and CO2. It contains multiple copies of 3 enzymatic components: 2-oxoglutarate dehydrogenase (E1), dihydrolipoamide succinyltransferase (E2) and lipoamide dehydrogenase (E3).	4	-19	8.9	48.9		
2400	2.1	8.85	31,500	coiled-coil-helix-coiled-coil-helix domain containing 3	Required for maintenance of mitochondrial crista integrity and mitochondrial function. May act as a scaffolding protein that stabilizes protein complexes involved in crista architecture and protein import. Has also been shown to function as a transcription factor which binds to the BAG1 promoter and represses BAG1 transcription.	11	-46.7	8.3	26.4	2.95E+06	6.35E+06

				Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1)	Nvolved with pre-mRNA processing. Forms complexes (ribonucleosomes) with at least 20 other different hnRNP and heterogeneous nuclear RNA in the nucleus. (Human)	3	-14	9.1	37.3		
2212	3.9	7.18	35,193	Voltage-dependent anion-selective channel protein 2 (VDAC-2)(Outer mitochondrial membrane protein porin 2)(B36-VDAC)	Forms a channel through the mitochondrial outer membrane that allows diffusion of small hydrophilic molecules. The channel adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective	17	-72.9	7.4	31.8	1.28E+07	3.32E+06
2004	4.8	9.42	40,215	Malate dehydrogenase, mitochondrial	Catalytic activity: (S)-malate + NAD+ = oxaloacetate + NADH.	4	-21.6	8.9	35.7	1.27E+06	6.05E+06
1777	2.7	8.15	43,948	Phosphoglycerate kinase 1	Catalytic activity: ATP + 3-phospho-D-glycerate = ADP + 3-phospho-D-glyceroyl phosphate.	27	-129	8	44.5	6.02E+06	1.63E+07
				Creatine kinase U-type, mitochondrial	Reversibly catalyzes the transfer of phosphate between ATP and various phosphogens (e.g. creatine phosphate). Creatine kinase isoenzymes play a central role in energy transduction in tissues with large, fluctuating energy demands, such as skeletal muscle, heart, brain and spermatozoa.	14	-70	8.6	46.9		
				3-ketoacyl-CoA thiolase, mitochondrial	Abolishes BNIP3-mediated apoptosis and mitochondrial damage	11	-67.3	8.4	41.9		
				Cytochrome b-c1 complex subunit 2, mitochondrial Precursor (Ubiquinol-cytochrome-c reductase complex core protein 2)(Core protein II)(Complex III subunit 2)	This is a component of the ubiquinol-cytochrome c reductase complex (complex III or cytochrome b-c1 complex), which is part of the mitochondrial respiratory chain. The core protein 2 is required for the assembly of the complex.	5	-34.4	9.2	48.4		

224	1328	2.4	7.55	57,752	Succinyl-CoA:3-ketoacid-coenzyme A transferase 1, mitochondrial Precursor (EC 2.8.3.5)	Key enzyme for ketone body catabolism. Transfers the CoA moiety from succinate to acetoacetate. Formation of the enzyme-CoA intermediate proceeds via an unstable anhydride species formed between the carboxylate groups of the enzyme and substrate	24	-122	8.9	48	2.17E+06	8.89E+05
					Dihydrolipoyl dehydrogenase, mitochondrial Precursor (EC 1.8.1.4)(Dihydrolipoamide dehydrogenase)	Lipoamide dehydrogenase is a component of the glycine cleavage system as well as of the alpha-ketoacid dehydrogenase complexes. Involved in the hyperactivation of spermatazoa during capacitation and in the spermatazoal acrosome reaction	17	-100	8	54		
					UDP-glucose 6-dehydrogenase (UDP-Glc dehydrogenase)(UDP-GlcDH)(UDPGDH)(EC 1.1.1.22)	Involved in the biosynthesis of glycosaminoglycans; hyaluronan, chondroitin sulfate, and heparan sulfate.	13	-74.7	7.1	54.9		
					UPF0027 protein C22orf28 homolog (p55)	Catalytic subunit of the tRNA-splicing ligase complex that acts by directly joining spliced tRNA halves to mature-sized tRNAs by incorporating the precursor-derived splice junction phosphate into the mature tRNA as a canonical 3',5'-phosphodiester. May act as a RNA ligase with broad substrate specificity, and may function toward other RNAs	9	-58.3	6.8	55.2		
					Methylmalonate-semialdehyde dehydrogenase , mitochondrial	Plays a role in valine and pyrimidine metabolism. Binds fatty acyl-CoA.	9	-35.7	8.4	57.7		
					Catalase	Occurs in almost all aerobically respiring organisms and serves to protect cells from the toxic effects of hydrogen peroxide. Promotes growth of cells.	4	-24.6	7.1	59.7		

				Centrosomal protein of 55 kDa.	Plays a role in mitotic exit and cytokinesis. Not required for microtubule nucleation. Recruits PDCD6IP and TSG101 to midbody during cytokinesis	4	-23.1	7.6	54		
				60 kDa heat shock protein, mitochondrial	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix.	4	-22.4	5.9	60.9		
225											
2249	8.1	9.64	34,773	Voltage-dependent anion-selective channel protein 1	Forms a channel through the mitochondrial outer membrane and also the plasma membrane. The channel at the outer mitochondrial membrane allows diffusion of small hydrophilic molecules; in the plasma membrane it is involved in cell volume regulation and apoptosis. It adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective. May participate in the formation of the permeability transition pore complex (PTPC) responsible for the release of mitochondrial products that triggers apoptosis	10	-44.3	8.6	30.7	2.36E+06	1.92E+07
				Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	Plays an essential role in the mitochondrial beta-oxidation of short chain fatty acids. Exerts it highest activity toward 3-hydroxybutyryl-CoA. Pathway: Lipid metabolism; fatty acid beta-oxidation	4	-12.3	8.8	34.4		
				D-beta-hydroxybutyrate dehydrogenase, mitochondrial Precursor (BDH)(EC 1.1.1.30)(3-hydroxybutyrate dehydrogenase)	Catalytic activity: (R)-3-hydroxybutanoate + NAD+ = acetoacetate + NADH.	3	-7.3	9	38.3		

226	2045	4	7.49	38,983	Glyceraldehyde-3-phosphate dehydrogenase	Has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby playing a role in glycolysis and nuclear functions, respectively. Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme in glycolysis that catalyzes the first step of the pathway by converting D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate. Modulates the organization and assembly of the cytoskeleton. Facilitates the CHP1-dependent microtubule and membrane associations through its ability to stimulate the binding of CHP1 to microtubules. Also participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. Nuclear functions are probably due to the nitrosylase activity that mediates cysteine S-nitrosylation of nuclear target proteins such as SIRT1, HDAC2 and PRKDC. Component of the GAIT (gamma interferon-activated inhibitor of translation) complex which mediates interferon-gamma-induced transcript-selective translation inhibition in inflammation processes. Upon interferon-gamma treatment assembles into the GAIT complex which binds to stem loop-containing GAIT elements in the 3'-UTR of diverse inflammatory mRNAs (such as ceruplasmin) and suppresses their translation. Pathway: carbohydrate degradation; Glycolysis	28	-119	8.1	35.8	3.55E+06	8.87E+05
					Annexin A2	Calcium-regulated membrane-binding protein whose affinity for calcium is greatly enhanced by anionic phospholipids. It binds two calcium ions with high affinity.	4	-27.4	7.5	38.9		

Spot No	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Gold
2626	1.2	6.19	27,015	LDLR chaperone MESD Precursor (Mesoderm development candidate 2)(Mesoderm development protein)	Chaperone specifically assisting the folding of beta-propeller/EGF modules within the family of low-density lipoprotein receptors (LDLRs). Acts as a modulator of the Wnt pathway through chaperoning the coreceptors of the canonical Wnt pathway, LRP5 and LRP6, to the plasma membrane. Essential for specification of embryonic polarity and mesoderm induction. (Human)	10	-46.6	5.5	25.2	3.75E+06	3.14E+06
				Growth factor receptor-bound protein 2	Adapter protein that provides a critical link between cell surface growth factor receptors and the Ras signaling pathway. Isoform 2 does not bind to phosphorylated epidermal growth factor receptor but inhibits EGF-induced transactivation of a RAS-responsive element. Isoform 2 acts as a dominant negative protein over GRB2 and by suppressing proliferative signals, may trigger active programmed cell death. (human)	9	-36.4	5.9	25.2		
				Peroxiredoxin-6 (EC 1.11.1.15)(Antioxidant protein 2)(1-Cys peroxiredoxin)(1-Cys PRX)	Involved in redox regulation of the cell. Can reduce H2O2 and short chain organic, fatty acid, and phospholipid hydroperoxides. May play a role in the regulation of phospholipid turnover as well as in protection against oxidative injury	7	-33.4	5.6	24.8		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Garst	Mon Conv Corn
2249	11.6	9.64	34,773	Voltage-dependent anion-selective channel protein 1	Forms a channel through the mitochondrial outer membrane and also the plasma membrane. The channel at the outer mitochondrial membrane allows diffusion of small hydrophilic molecules; in the plasma membrane it is involved in cell volume regulation and apoptosis. It adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective. May participate in the formation of the permeability transition pore complex (PTPC) responsible for the release of mitochondrial products that triggers apoptosis	10	-44.3	8.6	30.7	1.66E+06	1.92E+07
				Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	Plays an essential role in the mitochondrial beta-oxidation of short chain fatty acids. Exerts it highest activity toward 3-hydroxybutyryl-CoA. Pathway: Lipid metabolism; fatty acid beta-oxidation	4	-12.3	8.8	34.4		
				D-beta-hydroxybutyrate dehydrogenase, mitochondrial Precursor (BDH)(EC 1.1.1.30)(3-hydroxybutyrate dehydrogenase)	Catalytic activity: (R)-3-hydroxybutanoate + NAD+ = acetoacetate + NADH.	3	-7.3	9	38.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Garst	Mon Conv Corn
1257	2.6	8.06	61,099	Catalase	Occurs in almost all aerobically respiring organisms and serves to protect cells from the toxic effects of hydrogen peroxide. Promotes growth of cells.	34	-134	7.1	59.7	1.18E+06	3.02E+06
				Pyruvate kinase isozymes M1/M2	Glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP. Stimulates POU5F1-mediated transcriptional activation	18	-110	7.1	57.7		
				Pyridine nucleotide-disulfide oxidoreductase domain-containing protein 2	Probable oxidoreductase	4	-24.1	8.4	62.8		
1777	2.9	8.15	43,948	Phosphoglycerate kinase 1	Catalytic activity: ATP + 3-phospho-D-glycerate = ADP + 3-phospho-D-glyceroyl phosphate.	27	-129	8	44.5	5.54E+06	1.63E+07
				Creatine kinase U-type, mitochondrial	Reversibly catalyzes the transfer of phosphate between ATP and various phosphogens (e.g. creatine phosphate). Creatine kinase isoenzymes play a central role in energy transduction in tissues with large, fluctuating energy demands, such as skeletal muscle, heart, brain and spermatozoa.	14	-70	8.6	46.9		
				3-ketoacyl-CoA thiolase, mitochondrial	Abolishes BNIP3-mediated apoptosis and mitochondrial damage	11	-67.3	8.4	41.9		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Garst	Mon Conv Corn
				Cytochrome b-c1 complex subunit 2, mitochondrial Precursor (Ubiquinol-cytochrome-c reductase complex core protein 2)(Core protein II)(Complex III subunit 2)	This is a component of the ubiquinol-cytochrome c reductase complex (complex III or cytochrome b-c1 complex), which is part of the mitochondrial respiratory chain. The core protein 2 is required for the assembly of the complex.	5	-34.4	9.2	48.4		
2400	2.8	8.85	31,500	coiled-coil-helix-coiled-coil-helix domain containing 3	Required for maintenance of mitochondrial crista integrity and mitochondrial function. May act as a scaffolding protein that stabilizes protein complexes involved in crista architecture and protein import. Has also been shown to function as a transcription factor which binds to the BAG1 promoter and represses BAG1 transcription.	11	-46.7	8.3	26.4	2.28E+06	6.35E+06
				Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1)	nvolved with pre-mRNA processing. Forms complexes (ribonucleosomes) with at least 20 other different hnRNP and heterogeneous nuclear RNA in the nucleus. (Human)	3	-14	9.1	37.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Garst	Mon Conv Corn
2883	5.4	8.89	21,929	Mesencephalic astrocyte-derived neurotrophic factor Precursor (Protein ARMET)(Arginine-rich protein)	Selectively promotes the survival of dopaminergic neurons of the ventral mid-brain. Modulates GABAergic transmission to the dopaminergic neurons of the substantia nigra. Enhances spontaneous, as well as evoked, GABAergic inhibitory postsynaptic currents in dopaminergic neurons. Inhibits cell proliferation and endoplasmic reticulum (ER) stress-induced cell death. (Mouse)	4	-18.3	8.5	20.4	1.79E+06	9.70E+06
2443	5.7	9.53	30,665	Succinate dehydrogenase iron-sulfur subunit, mitochondrial Precursor (EC 1.3.5.1)(Iron-sulfur subunit of complex II)	Iron-sulfur protein subunit of succinate dehydrogenase (SDH) that is involved in complex II of the mitochondrial electron transport chain and is responsible for transferring electrons from succinate to ubiquinone (coenzyme Q). Pathway: Carbohydrate metabolism; tricarboxylic acid cycle; fumarate from succinate (eukaryal route): step 1/1. (Human).	23	-65.3	9	31.8	2.64E+06	1.50E+07

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Garst	Mon Conv Corn
				Proteasome subunit alpha type-7 (EC 3.4.25.1)(Proteasome subunit RC6-1)	The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity. Plays an important role in the regulation of cell proliferation or cell cycle control, transcriptional regulation, immune and stress response, cell differentiation, and apoptosis. Interacts with some important proteins involved in transcription factor regulation, cell cycle transition, viral replication and even tumor initiation and progression. Inhibits the transactivation function of HIF-1A under both normoxic and hypoxia-mimicking conditions. The interaction with EMAP2 increases the proteasome-mediated HIF-1A degradation under the hypoxic conditions. Plays a role in hepatitis C virus internal ribosome entry site-mediated translation. Mediates nuclear translocation of the androgen receptor and thereby enhances androgen-mediated transactivation. Promotes MAVS degradation and thereby negatively regulates MAVS-mediated innate immune response (Human).	14	-52.2	8.6	28.4		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Garst	Mon Conv Corn
				ATP synthase subunit alpha, mitochondrial	Mitochondrial membrane ATP synthase (F1F0 ATP synthase or Complex V) produces ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain. F-type ATPases consist of two structural domains, F1 - containing the extramembraneous catalytic core, and F0 - containing the membrane proton channel, linked together by a central stalk and a peripheral stalk. During catalysis, ATP synthesis in the catalytic domain of F1 is coupled via a rotary mechanism of the central stalk subunits to proton translocation. Subunits alpha and beta form the catalytic core in F1. Rotation of the central stalk against the surrounding alpha3beta3 subunits leads to hydrolysis of ATP in three separate catalytic sites on the beta subunits. Subunit alpha does not bear the catalytic high-affinity ATP-binding sites (Human)	6	-33.8	9.3	59.8		
				Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	Pathway: Amino-acid degradation; L-lysine degradation via saccharopine pathway; glutaryl-CoA from L-lysine: step 6/6. The 2-oxoglutarate dehydrogenase complex catalyzes the overall conversion of 2-oxoglutarate to succinyl-CoA and CO2. It contains multiple copies of 3 enzymatic components: 2-oxoglutarate dehydrogenase (E1), dihydrolipoamide succinyltransferase (E2) and lipoamide dehydrogenase (E3).	4	-19	8.9	48.9		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Garst	Mon Gold
1444	1.5	7.43	54,604	Dihydrolipoyl dehydrogenase, mitochondrial Precursor (EC 1.8.1.4)(Dihydrolipoamide dehydrogenase) 2	Lipoamide dehydrogenase is a component of the glycine cleavage system as well as of the alpha-ketoacid dehydrogenase complexes. Involved in the hyperactivation of spermatazoa during capacitation and in the spermatazoal acrosome reaction	34	-153	8	54	7.70E+06	4.97E+06
				Glutamate dehydrogenase 1, mitochondrial	May be involved in learning and memory reactions by increasing the turnover of the excitatory neurotransmitter glutamate.	24	-99	8.1	61.4		
				Cytosol aminopeptidase (EC 3.4.11.1)(Leucine aminopeptidase 3)(LAP-3)(Leucyl aminopeptidase)(Proline aminopeptidase)(EC 3.4.11.5)(Prolyl aminopeptidase)	Involved in the biosynthesis of glycosaminoglycans; hyaluronan, chondroitin sulfate, and heparan sulfate.	8	-52	6.8	56.1		
				Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	7	-46	5.9	56.6		
				annexin A11	Binds specifically to calcyclin in a calcium-dependent manner. Required for midbody formation and completion of the terminal phase of cytokinesis. (Human)	6	-39	7.5	54.1		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Garst	Mon Gold
				T-complex protein 1 subunit beta (TCP-1-beta)(CCT-beta)	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin	4	-24	6	57.4		
				ATP synthase subunit alpha, mitochondrial	Mitochondrial membrane ATP synthase (F1F0 ATP synthase or Complex V) produces ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain. F-type ATPases consist of two structural domains, F1 - containing the extramembraneous catalytic core, and F0 - containing the membrane proton channel, linked together by a central stalk and a peripheral stalk. During catalysis, ATP synthesis in the catalytic domain of F1 is coupled via a rotary mechanism of the central stalk subunits to proton translocation. Subunits alpha and beta form the catalytic core in F1. Rotation of the central stalk against the surrounding alpha3beta3 subunits leads to hydrolysis of ATP in three separate catalytic sites on the beta subunits. Subunit alpha does not bear the catalytic high-affinity ATP-binding sites	4	-17	9.3	59.8		
				UDP-glucose 6-dehydrogenase (UDP-Glc dehydrogenase)(UDP-GlcDH)(UDPGDH) (EC 1.1.1.22)	Involved in the biosynthesis of glycosaminoglycans; hyaluronan, chondroitin sulfate, and heparan sulfate.	3	-17	7.1	54.9		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Conv Corn	Mon Gold
2883	7.2	8.89	21,929	Mesencephalic astrocyte-derived neurotrophic factor Precursor (Protein ARMET)(Arginine-rich protein)	Selectively promotes the survival of dopaminergic neurons of the ventral mid-brain. Modulates GABAergic transmission to the dopaminergic neurons of the substantia nigra. Enhances spontaneous, as well as evoked, GABAergic inhibitory postsynaptic currents in dopaminergic neurons. Inhibits cell proliferation and endoplasmic reticulum (ER) stress-induced cell death. (Mouse)	4	-18.3	8.5	20.4	9.70E+06	1.35E+06
2249	10	9.64	34,773	Voltage-dependent anion-selective channel protein 1	Forms a channel through the mitochondrial outer membrane and also the plasma membrane. The channel at the outer mitochondrial membrane allows diffusion of small hydrophilic molecules; in the plasma membrane it is involved in cell volume regulation and apoptosis. It adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective. May participate in the formation of the permeability transition pore complex (PTPC) responsible for the release of mitochondrial products that triggers apoptosis	10	-44.3	8.6	30.7	1.50E+07	3.99E+06
				Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	Plays an essential role in the mitochondrial beta-oxidation of short chain fatty acids. Exerts it highest activity toward 3-hydroxybutyryl-CoA. Pathway: Lipid metabolism; fatty acid beta-oxidation	4	-12.3	8.8	34.4		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Conv Corn	Mon Gold
				D-beta-hydroxybutyrate dehydrogenase, mitochondrial Precursor (BDH)(EC 1.1.1.30)(3-hydroxybutyrate dehydrogenase)	Catalytic activity: (R)-3-hydroxybutanoate + NAD+ = acetoacetate + NADH.	3	-7.3	9	38.3		
2443	3.8	9.53	30,665	Succinate dehydrogenase iron-sulfur subunit, mitochondrial Precursor (EC 1.3.5.1)(Iron-sulfur subunit of complex II)	Iron-sulfur protein subunit of succinate dehydrogenase (SDH) that is involved in complex II of the mitochondrial electron transport chain and is responsible for transferring electrons from succinate to ubiquinone (coenzyme Q). Pathway: Carbohydrate metabolism; tricarboxylic acid cycle; fumarate from succinate (eukaryal route): step 1/1. (Human).	23	-65.3	9	31.8	1.50E+07	3.99E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Conv Corn	Mon Gold
				Proteasome subunit alpha type-7 (EC 3.4.25.1)(Proteasome subunit RC6-1)	The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity. Plays an important role in the regulation of cell proliferation or cell cycle control, transcriptional regulation, immune and stress response, cell differentiation, and apoptosis. Interacts with some important proteins involved in transcription factor regulation, cell cycle transition, viral replication and even tumor initiation and progression. Inhibits the transactivation function of HIF-1A under both normoxic and hypoxia-mimicking conditions. The interaction with EMAP2 increases the proteasome-mediated HIF-1A degradation under the hypoxic conditions. Plays a role in hepatitis C virus internal ribosome entry site-mediated translation. Mediates nuclear translocation of the androgen receptor and thereby enhances androgen-mediated transactivation. Promotes MAVS degradation and thereby negatively regulates MAVS-mediated innate immune response (Human).	14	-52.2	8.6	28.4		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Conv Corn	Mon Gold
				ATP synthase subunit alpha, mitochondrial	Mitochondrial membrane ATP synthase (F1F0 ATP synthase or Complex V) produces ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain. F-type ATPases consist of two structural domains, F1 - containing the extramembraneous catalytic core, and F0 - containing the membrane proton channel, linked together by a central stalk and a peripheral stalk. During catalysis, ATP synthesis in the catalytic domain of F1 is coupled via a rotary mechanism of the central stalk subunits to proton translocation. Subunits alpha and beta form the catalytic core in F1. Rotation of the central stalk against the surrounding alpha3beta3 subunits leads to hydrolysis of ATP in three separate catalytic sites on the beta subunits. Subunit alpha does not bear the catalytic high-affinity ATP-binding sites (Human)	6	-33.8	9.3	59.8		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Conv Corn	Mon Gold
				Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	Pathway: Amino-acid degradation; L-lysine degradation via saccharopine pathway; glutaryl-CoA from L-lysine: step 6/6. The 2-oxoglutarate dehydrogenase complex catalyzes the overall conversion of 2-oxoglutarate to succinyl-CoA and CO2. It contains multiple copies of 3 enzymatic components: 2-oxoglutarate dehydrogenase (E1), dihydrolipoamide succinyltransferase (E2) and lipoamide dehydrogenase (E3).	4	-19	8.9	48.9		

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
1950	2.23E-04	2.1	5.89/41.582	Serpin B5 (Peptidase inhibitor 5)(PI-5)(Maspin)	Tumor suppressor. It blocks the growth, invasion, and metastatic properties of mammary tumors. As it does not undergo the S (stressed) to R (relaxed) conformational transition characteristic of active serpins, it exhibits no serine protease inhibitory activity	12	-77	5.7/42	1.82E+06	1.76E+06	1.09E+06	8.67E+05	1.26E+06
				3'(2'),5'-bisphosphate nucleotidase 1 (EC 3.1.3.7)(Bisphosphate 3'-nucleotidase 1)(PAP-inositol-1,4-phosphatase)(PIP)(scHAL2 analogous 3)	Converts adenosine 3'-phosphate 5'-phosphosulfate (PAPS) to adenosine 5'-phosphosulfate (APS) and 3'(2')-phosphoadenosine 5'- phosphate (Bravo *et al.*) to	12	-57	5.6/29					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					AMP. Has 1000-fold lower activity towards inositol 1,4-bisphosphate (Ins(1,4)P2) and inositol 1,3,4-trisphosphate (Ins(1,3,4)P3), but does not hydrolyze Ins1P, Ins(3,4)P2, Ins(1,3,4,5)P4 or InsP6.								
				Elongation factor 2	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation. During this step, the ribosome changes from the pre-translocational (PRE) to the post-translocational state as the newly	17	-57	6.4/95.2					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively. Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome.								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
2045	2.45E-04	4	7.49/38.983	Glyceraldehyde-3-phosphate dehydrogenase	Has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby playing a role in glycolysis and nuclear functions, respectively. Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme in glycolysis that catalyzes the first step of the pathway by converting D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate. Modulates the organization and assembly of the cytoskeleton.	28	-119	8.1/35.8	2.90E+06	3.55E+06	2.00E+06	8.87E+05	1.35E+06

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					Facilitates the CHP1-dependent microtubule and membrane associations through its ability to stimulate the binding of CHP1 to microtubules. Also participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. Nuclear functions are probably due to the nitrosylase activity that mediates cysteine S-nitrosylation of nuclear target proteins such as SIRT1, HDAC2 and PRKDC. Component of the								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					GAIT (gamma interferon-activated inhibitor of translation) complex which mediates interferon-gamma-induced transcript-selective translation inhibition in inflammation processes. Upon interferon-gamma treatment assembles into the GAIT complex which binds to stem loop-containing GAIT elements in the 3'-UTR of diverse inflammatory mRNAs (such as ceruplasmin) and suppresses their translation. Pathway: carbohydrate								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					degradation; Glycolysis								
				Annexin A2	Calcium-regulated membrane-binding protein whose affinity for calcium is greatly enhanced by anionic phospholipids. It binds two calcium ions with high	4	-27	7.5/38.9					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					affinity.								
2212	7.30E-07	3.9	7.18/35.102	Voltage-dependent anion-selective channel protein 2 (VDAC-2)(Outer mitochondrial membrane protein porin 2)(B36-VDAC)	Forms a channel through the mitochondrial outer membrane that allows diffusion of small hydrophilic molecules. The channel adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is cation-selective	17	-73	7.4/31.8	7.26E+06	1.28E+07	3.77E+06	3.32E+06	4.81E+06

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
2249	6.68E-06	11.6	9.65/34.686	Voltage-dependent anion-selective channel protein 1	Forms a channel through the mitochondrial outer membrane and also the plasma membrane. The channel at the outer mitochondrial membrane allows diffusion of small hydrophilic molecules; in the plasma membrane it is involved in cell volume regulation and apoptosis. It adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. The open state has a weak anion selectivity whereas the closed state is	10	-44	8.6/30.7	4.41E+06	2.36E+06	1.66E+06	1.92E+07	1.93E+06

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					cation-selective. May participate in the formation of the permeability transition pore complex (PTPC) responsible for the release of mitochondrial products that triggers apoptosis								
				Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	Plays an essential role in the mitochondrial beta-oxidation of short chain fatty acids. Exerts it highest activity toward 3-hydroxybutyryl-CoA. Pathway: Lipid metabolism; fatty acid beta-oxidation	4	-12	8.8/34.4					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
				D-beta-hydroxybutyrate dehydrogenase, mitochondrial Precursor (BDH)(EC 1.1.1.30)(3-hydroxybutyrate dehydrogenase)	Catalytic activity: (R)-3-hydroxybutanoate + NAD+ = acetoacetate + NADH.	3	-7.3	9/38.3					
2306	0.001	1.7	4.32/33.852	Tropomyosin alpha-3 chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	63	-170	4.7/29	5.65E+07	3.25E+07	3.42E+07	4.30E+07	3.49E+07

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
2400	0.002	2.8	8.85/31.443	coiled-coil-helix-coiled-coil-helix domain containing 3	Required for maintenance of mitochondrial crista integrity and mitochondrial function. May act as a scaffolding protein that stabilizes protein complexes involved in crista architecture and protein import. Has also been shown to function as a transcription factor which binds to the BAG1 promoter and represses BAG1 transcription.	11	-47	8.3/26.4	2.55E+06	2.95E+06	2.28E+06	6.35E+06	3.31E+06
				Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1)	nvolved with pre-mRNA processing. Forms complexes (ribonucleosomes) with at least 20 other different hnRNP and	3	-14	9.1/37.3					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					heterogeneous nuclear RNA in the nucleus. (Human)								
2443	3.84E-04	5.7	9.53/30.665	Succinate dehydrogenase iron-sulfur subunit, mitochondrial Precursor (EC 1.3.5.1)(Iron-sulfur subunit of complex II)	Iron-sulfur protein subunit of succinate dehydrogenase (SDH) that is involved in complex II of the mitochondrial electron transport chain and is responsible for transferring electrons from succinate to ubiquinone (coenzyme Q). Pathway: Carbohydrate metabolism; tricarboxylic acid cycle; fumarate from succinate	23	-65	9/31.8	1.16E+07	2.81E+06	2.64E+06	1.50E+07	3.99E+06

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					(eukaryal route): step 1/1. (Human).								
				Proteasome subunit alpha type-7 (EC 3.4.25.1)(Proteasome subunit RC6-1)	The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity. Plays an important role in the regulation of cell proliferation or cell cycle control, transcriptional regulation, immune and stress	14	-52	8.6/28.4					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					response, cell differentiation, and apoptosis. Interacts with some important proteins involved in transcription factor regulation, cell cycle transition, viral replication and even tumor initiation and progression. Inhibits the transactivation function of HIF-1A under both normoxic and hypoxia-mimicking conditions. The interaction with EMAP2 increases the proteasome-mediated HIF-1A degradation under								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					the hypoxic conditions. Plays a role in hepatitis C virus internal ribosome entry site-mediated translation. Mediates nuclear translocation of the androgen receptor and thereby enhances androgen-mediated transactivation. Promotes MAVS degradation and thereby negatively regulates MAVS-mediated innate immune response (Human).								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
				ATP synthase subunit alpha, mitochondrial	Mitochondrial membrane ATP synthase (F1F0 ATP synthase or Complex V) produces ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain. F-type ATPases consist of two structural domains, F1 - containing the extramembraneous catalytic core, and F0 - containing the membrane proton channel, linked together by a	6	-34	9.3/59.8					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					central stalk and a peripheral stalk. During catalysis, ATP synthesis in the catalytic domain of F1 is coupled via a rotary mechanism of the central stalk subunits to proton translocation. Subunits alpha and beta form the catalytic core in F1. Rotation of the central stalk against the surrounding alpha3beta3 subunits leads to hydrolysis of ATP in three separate catalytic sites on the beta subunits. Subunit alpha does not bear the catalytic high-								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					affinity ATP-binding sites (Human)								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
				Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	Pathway: Amino-acid degradation; L-lysine degradation via saccharopine pathway; glutaryl-CoA from L-lysine: step 6/6. The 2-oxoglutarate dehydrogenase complex catalyzes the overall conversion of 2-oxoglutarate to succinyl-CoA and CO2. It contains multiple copies of 3 enzymatic components: 2-oxoglutarate dehydrogenase (E1), dihydrolipoamide succinyltransferase (E2) and lipoamide dehydrogenase	4	-19	8.9/48.9					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					(E3).								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
2511	2.34E-04	3.2	6.09/29.372	PAX interacting (with transcription-activation domain) protein 1	Involved in DNA damage response and in transcriptional regulation through histone methyltransferase (HMT) complexes. Plays a role in early development. In DNA damage response is required for cell survival after ionizing radiation. In vitro shown to be involved in the homologous recombination mechanism for the repair of double-strand breaks (DSBs). Its localization to DNA damage foci requires RNF8 and UBE2N. Recruits TP53BP1 to DNA damage foci and, at least in particular repair processes, effective DNA damage response appears to require the	1	-1.2	8.8/93.9	1.90E+07	1.33E+07	1.69E+07	5.90E+06	1.42E+07

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					association with TP53BP1 phosphorylated by ATM at 'Ser-25'. Together with TP53BP1 regulates ATM association. Recruits PAGR1 to sites of DNA damage and the PAGR1:PAXIP1 complex is required for cell survival in response to DNA damage; the function is probbaly independent of MLL-containing histone methyltransferase (HMT) complexes. Promotes ubiquitination of PCNA following UV irradiation and may regulate recruitment of polymerase eta and RAD51 to chromatin after DNA damage. Proposed to be involved in								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					transcriptional regulation by linking MLL-containing histone methyltransferase (HMT) complexes to gene promoters by interacting with promoter-bound transcription factors such as PAX2. Associates with gene promoters that are known to be regulated by MLL2. During immunoglobulin class switching in activated B-cells is involved in trimethylation of histone H3 at 'Lys-4' and in transcription initiation of downstream switch regions at the immunoglobulin heavy-chain locus; this function appears to involve the recruitment of MLL-containing HMT								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					complexes. (Human)								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
2626	4.58E-04	2.4	6.18/26.990	LDLR chaperone MESD Precursor (Mesoderm development candidate 2)(Mesoderm development protein)	Chaperone specifically assisting the folding of beta-propeller/EGF modules within the family of low-density lipoprotein receptors (LDLRs). Acts as a modulator of the Wnt pathway through chaperoning the coreceptors of the canonical Wnt pathway, LRP5 and LRP6, to the plasma membrane. Essential for specification of embryonic polarity and mesoderm induction. (Human)	10	-47	5.5/25.2	3.62E+06	3.75E+06	2.05E+06	1.55E+06	3.14E+06
				Growth factor receptor-bound protein 2	Adapter protein that provides a critical link between cell surface growth	9	-36	5.9/25.2					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					factor receptors and the Ras signaling pathway. Isoform 2 does not bind to phosphorylated epidermal growth factor receptor but inhibits EGF-induced transactivation of a RAS-responsive element. Isoform 2 acts as a dominant negative protein over GRB2 and by suppressing proliferative signals, may trigger active programmed cell death. (human)								

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
				Peroxiredoxin-6 (EC 1.11.1.15)(Antioxidant protein 2)(1-Cys peroxiredoxin)(1-Cys PRX)	Involved in redox regulation of the cell. Can reduce H2O2 and short chain organic, fatty acid, and phospholipid hydroperoxides. May play a role in the regulation of phospholipid turnover as well as in protection against oxidative injury	7	-33	5.6/24.8					
2883	1.82E-06	7.2	8.89/21.919	Mesencephalic astrocyte-derived neurotrophic factor Precursor (Protein ARMET)(Arginine-rich protein)	Selectively promotes the survival of dopaminergic neurons of the ventral mid-brain. Modulates GABAergic transmission to the dopaminergic neurons of the substantia nigra. Enhances	4	-18	8.5/20.4	1.41E+06	1.36E+06	1.79E+06	9.70E+06	1.35E+06

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					spontaneous, as well as evoked, GABAergic inhibitory postsynaptic currents in dopaminergic neurons. Inhibits cell proliferation and endoplasmic reticulum (ER) stress-induced cell death. (Mouse)								
3243	0.002	2.6	6.33/28.246	Thioredoxin-dependent peroxide reductase, mitochondrial Precursor (EC 1.11.1.15)(Peroxiredoxin-3)(PRX-3)(PRx III)	Involved in redox regulation of the cell. Protects radical-sensitive enzymes from oxidative damage by a radical-generating system. Acts synergistically with MAP3K13 to regulate the activation of NF-kappa-B in the cytosol. (Human)	9	-16	7.1/28.3	2.86E+06	2.83E+06	1.12E+06	1.13E+06	1.64E+06

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
3258	9.31E-04	1.6	4.35/32.615	Tropomyosin alpha-1 chain (Tropomyosin-1)(Alpha-tropomyosin)	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin	51	-109	4.7/28.5	2.22E+07	1.36E+07	1.35E+07	1.89E+07	1.42E+07

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW in KiloDaltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Garst	Mon Conv Corn	Mon Gold
					filaments.								

Appendix 28: Identification of differentially expressed proteins from the epithelial cells of the small intestine of rats fed different maize based diets for 28-Day.
The data represent two way comparisons as follows (Mcert vs Mon Garst, Mcert vs Mon Conv Corn, Mcert vs Mon Gold, Mon810 vs Mon Garst, Mon810 vs Mon Conv Corn, Mon Garst vs Mon Conv Corn, Mon810 vs Mon Gold, Mon Conv Corn vs Mon Gold) and five way comparisons.

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
3190	2.8	8.55	40,202	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), that is believed not to be involved in catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone	36	-113	7.6	20.8	3.94E+06	1.42E+06
				Peroxiredoxin-1 (EC 1.11.1.15)(Thioredoxin peroxidase 2)(Thioredoxin-dependent peroxide reductase 2)(Heme-binding 23 kDa protein)(HBP23)	Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system but not from glutaredoxin. May play an important role in eliminating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and tumor necrosis factor-alpha by regulating the intracellular concentrations of H2O2. Reduces an intramolecular disulfide bond in GDPD5 that gates the ability to GDPD5 to drive postmitotic motor neuron differentiation	3	-16.8	8.3	22.1		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
				Isocitrate dehydrogenase subunit alpha, mitochondrial Precursor (EC 1.1.1.41)(Isocitric dehydrogenase subunit alpha)(NAD(+)-specific ICDH subunit alpha)	catalytic activity: Isocitrate + NAD+ = 2-oxoglutarate + CO2 + NADH.	3	-10.4	6.5	39.6		
3123	3.1	8.56	41,617	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), that is believed not to be involved in catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone	10	-70.1	7.6	20.8	1.50E+06	4.65E+06
3320	2.9	8.77	36,807	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9	Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), that is believed to be not involved in catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone	1	-4.5	8.2	21.9	3.77E+06	1.32E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
2550	1.6	5.7	56,979	Ornithine aminotransferase, mitochondrial Precursor (EC 2.6.1.13)(Ornithine--oxo-acid aminotransferase)	catalytic activity: L-ornithine + a 2-oxo acid = L-glutamate 5-semialdehyde + an L-amino acid.	1	-2.6	6.5	48.3	2.76E+06	1.71E+06
				heterogeneous nuclear ribonucleoprotein U	Component of the CRD-mediated complex that promotes MYC mRNA stabilization. Binds to pre-mRNA. Has high affinity for scaffold-attached region (Otaka *et al.*) DNA. Binds to double- and single-stranded DNA and RNA.	1	-2.6	5.9	87.7		
3506	3.1	4.69	32,253	Actin, cytoplasmic 1 Actin, cytoplasmic 1, N-terminally processed	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.	4	-27.6	5.3	41.7	1.52E+06	4.68E+06
				Actin, gamma-enteric smooth muscle	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.	4	-26.4	5.3	41.8		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Conv Corn
2762	2.4	7.5	50,171	Protein MEMO1	May control cell migration by relaying extracellular chemotactic signals to the microtubule cytoskeleton. Mediator of ERBB2 signaling. The MEMO1-RHOA-DIAPH1 signaling pathway plays an important role in ERBB2-dependent stabilization of microtubules at the cell cortex. It controls the localization of APC and CLASP2 to the cell membrane, via the regulation of GSK3B activity. In turn, membrane-bound APC allows the localization of the MACF1 to the cell membrane, which is required for microtubule capture and stabilization. Is required for breast carcinoma cell migration.	3	-8.6	6.4	32.1	2.81E+06	1.18E+06
				40S ribosomal protein S3	Ribosomal protein S3 (rpS3) is a component of the 40S ribosomal subunit and is involved in translation.	2	-6	9.7	26.7		
3305	2.3	5.07	37,180	chromobox homolog 3	Seems to be involved in transcriptional silencing in heterochromatin-like complexes. Recognizes and binds histone H3 tails methylated at 'Lys-9', leading to epigenetic repression. May contribute to the association of the heterochromatin with the inner nuclear membrane through its interaction with lamin B receptor (LBR). Involved in the formation of functional kinetochore through interaction with MIS12 complex proteins.	2	-9.4	5.2	20.8	2.62E+06	6.06E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Garst
2912	3.5	8.12	45,065	Electron transfer flavoprotein subunit beta (Beta-ETF)	The electron transfer flavoprotein serves as a specific electron acceptor for several dehydrogenases, including five acyl-CoA dehydrogenases, glutaryl-CoA and sarcosine dehydrogenase. It transfers the electrons to the main mitochondrial respiratory chain via ETF-ubiquinone oxidoreductase (ETF dehydrogenase)	66	-149	7.6	27.7	5.13E+06	1.48E+06
				Proteasome subunit alpha type-4 (EC 3.4.25.1)(Proteasome component C9)	The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity.	12	-49.3	7.6	29.5		
				Adenylate kinase 2, mitochondrial (AK 2)(EC 2.7.4.3)(ATP-AMP transphosphorylase 2)	Catalyzes the reversible transfer of the terminal phosphate group between ATP and AMP. Plays an important role in cellular energy homeostasis and in adenine nucleotide metabolism. Adenylate kinase activity is critical for regulation of the phosphate utilization and the AMP de novo biosynthesis pathways. Plays a key role in hematopoiesis	12	-44.6	7	25.5		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Garst
				Calcyclin-binding protein	May be involved in calcium-dependent ubiquitination and subsequent proteasomal degradation of target proteins. Probably serves as a molecular bridge in ubiquitin E3 complexes. Participates in the ubiquitin-mediated degradation of beta-catenin (CTNNB1)	10	-34	7.6	26.5		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Gold
2149	2.5	7.09	66,200	Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	4	-20.3	5.9	56.6	1.33E+06	5.32E+05

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mcert	Mon Gold
3305	2.7	5.07	37,180	chromobox homolog 3	Seems to be involved in transcriptional silencing in heterochromatin-like complexes. Recognizes and binds histone H3 tails methylated at 'Lys-9', leading to epigenetic repression. May contribute to the association of the heterochromatin with the inner nuclear membrane through its interaction with lamin B receptor (LBR). Involved in the formation of functional kinetochore through interaction with MIS12 complex proteins.	2	-9.4	5.2	20.8	2.62E+06	7.05E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Conv Corn
3123	3.4	8.56	41,617	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), that is believed not to be involved in catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone	10	-70.1	7.6	20.8	1.35E+06	4.65E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Conv Corn
3506	5.1	4.7	32,253	Actin, cytoplasmic 1 Actin, cytoplasmic 1, N-terminally processed	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.	4	-27.6	5.3	41.7	9.19E+05	4.68E+06
				Actin, gamma-enteric smooth muscle	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.	4	-26.4	5.3	41.8		
3320	3.2	8.77	36,807	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9	Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), that is believed to be not involved in catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone	1	-4.5	8.2	21.9	4.29E+06	1.32E+06
1485	3.1	6.92	73,807	Lamin-A	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin.	22	-110	6.5	74.3	5.72E+05	1.83E+05
				Stress-induced-phosphoprotein 1 (STI1) (Hsc70/Hsp90-organizing protein)(Guertler *et al.*)	Mediates the association of the molecular chaperones HSC70 and HSP90 (HSPCA and HSPCB).	3	-14.2	6.4	62.5		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Conv Corn
2550	1.5	5.7	56,979	Ornithine aminotransferase, mitochondrial Precursor (EC 2.6.1.13)	catalytic activity: L-ornithine + a 2-oxo acid = L-glutamate 5-semialdehyde + an L-amino acid.	1	-2.6	6.5	48.3	2.53E+06	1.71E+06
				heterogeneous nuclear ribonucleoprotein U	Component of the CRD-mediated complex that promotes MYC mRNA stabilization. Binds to pre-mRNA. Has high affinity for scaffold-attached region (Otaka *et al.*) DNA. Binds to double- and single-stranded DNA and RNA.	1	-2.6	5.9	87.7		
2222	3.3	9.2	64,993	3-ketoacyl-CoA thiolase, mitochondrial	Abolishes BNIP3-mediated apoptosis and mitochondrial damage	14	-63.5	8.4	41.9	2.63E+06	8.07E+05
				Isocitrate dehydrogenase , mitochondrial	Plays a role in intermediary metabolism and energy production. It may tightly associate or interact with the pyruvate dehydrogenase complex	6	-23.1	8.9	50.9		
3190	2.5	8.6	40,202	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), that is believed not to be involved in catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone	36	-113	7.6	20.8	3.52E+06	1.42E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Conv Corn
				Peroxiredoxin-1 (EC 1.11.1.15)(Thioredoxin peroxidase 2)(Thioredoxin-dependent peroxide reductase 2)(Heme-binding 23 kDa protein)(HBP23)	Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system but not from glutaredoxin. May play an important role in eliminating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and tumor necrosis factor-alpha by regulating the intracellular concentrations of H2O2. Reduces an intramolecular disulfide bond in GDPD5 that gates the ability to GDPD5 to drive postmitotic motor neuron differentiation	3	-16.8	8.3	22.1		
				Isocitrate dehydrogenase subunit alpha, mitochondrial Precursor (EC 1.1.1.41)(Isocitric dehydrogenase subunit alpha)(NAD(+)-specific ICDH subunit alpha)	catalytic activity: Isocitrate + NAD+ = 2-oxoglutarate + CO2 + NADH.	3	-10.4	6.5	39.6		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Conv Corn
2179	2.5	6.49	66,200	Ornithine aminotransferase, mitochondrial Precursor (EC 2.6.1.13)(Ornithine--oxo-acid aminotransferase)	catalytic activity: L-ornithine + a 2-oxo acid = L-glutamate 5-semialdehyde + an L-amino acid.	5	-22.2	6.5	48.3	9.67E+05	3.91E+05
1707	1.9	7.15	66,200	Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	71	-192	5.9	56.6	3.34E+06	1.73E+06
				Tyrosyl-tRNA synthetase, cytoplasmic (EC 6.1.1.1)(Tyrosyl--tRNA ligase)(TyrRS)	Catalyzes the attachment of tyrosine to tRNA(Tyr) in a two-step reaction: tyrosine is first activated by ATP to form Tyr-AMP and then transferred to the acceptor end of tRNA(Tyr)	7	-35	8.6	63		
				Dihydrolipoyl dehydrogenase, mitochondrial Precursor (EC 1.8.1.4)(Dihydrolipoamide dehydrogenase)	Lipoamide dehydrogenase is a component of the glycine cleavage system as well as of the alpha-ketoacid dehydrogenase complexes. Involved in the hyperactivation of spermatazoa during capacitation and in the spermatazoal acrosome reaction	4	-25.2	8	54		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Conv Corn
3255	1.5	8.77	38,762	Superoxide dismutase , mitochondrial Precursor (EC 1.15.1.1)	Destroys superoxide anion radicals which are normally produced within the cells and which are toxic to biological systems.	5	-30	9	24.7	6.78E+06	9.84E+06
2232	1.8	8.72	64,735	Phosphoglycerate kinase 1	Catalytic activity: ATP + 3-phospho-D-glycerate = ADP + 3-phospho-D-glyceroyl phosphate.	39	-152	8	44.5	8.40E+06	4.73E+06
				Cytochrome b-c1 complex subunit 2, mitochondrial Precursor (Ubiquinol-cytochrome-c reductase complex core protein 2)(Core protein II)	This is a component of the ubiquinol-cytochrome c reductase complex (complex III or cytochrome b-c1 complex), which is part of the mitochondrial respiratory chain. The core protein 2 is required for the assembly of the complex.	8	-52.6	9.2	48.4		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Conv Corn
2442	1.6	6.7	30,665	Alcohol dehydrogenase (EC 1.1.1.2)(Aldehyde reductase)(Aldo-keto reductase family 1 member A1)(3-DG-reducing enzyme)	Catalyzes the NADPH-dependent reduction of a variety of aromatic and aliphatic aldehydes to their corresponding alcohols. Catalyzes the reduction of mevaldate to mevalonic acid and of glyceraldehyde to glycerol. Has broad substrate specificity. Plays a role in the activation of procarcinogens, such as polycyclic aromatic hydrocarbon trans-dihydrodiols, and in the metabolism of various xenobiotics and drugs. Catalyzes the NADPH-dependent reduction of 3-deoxyglucosone (3-DG).	33	-85.4	6.8	36.5	4.43E+06	3.56E+06
				Cytosol aminopeptidase (EC 3.4.11.1)(Leucine aminopeptidase 3)(LAP-3)(Leucyl aminopeptidase)(Proline aminopeptidase)(EC 3.4.11.5)(Prolyl aminopeptidase)	Presumably involved in the processing and regular turnover of intracellular proteins. Catalyzes the removal of unsubstituted N-terminal amino acids from various peptides	6	-40.7	6.8	56.1		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
2893	3.1	6.27	45,776	Lactotransferrin; Lactoferrin; EC 3.4.21.	Transferrins are iron binding transport proteins which can bind two Fe3+ ions in association with the binding of an anion, usually bicarbonate.Lactotransferrin has antimicrobial activity which depends on the extracellular cation concentration.Lactoferroxins A, B and C have opioid antagonist activity. Lactoferroxin A shows preference for mu-receptors, while lactoferroxin B and C have somewhat higher degrees of preference for kappa-receptors than for mu-receptors.The lactotransferrin transferrin-like domain 1 functions as a serine protease of the peptidase S60 family that cuts arginine rich regions. This function contributes to the antimicrobial activity.Isoform DeltaLf: transcription factor with antiproliferative properties and inducing cell cycle arrest. Binds to DeltaLf response element found in the SKP1, BAX, DCPS, and SELH promoters.	21	-147	8.5	78.1	1.15E+06	3.54E+06
				T-complex protein 1 subunit beta (TCP-1-beta)(CCT-beta)	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin	3	-11.7	6	57.4		
2209	1.6	7.22	65,424	Gelsolin Precursor (Actin-depolymerizing factor)(ADF)(Brevin)	Calcium-regulated, actin-modulating protein that binds to the plus (or barbed) ends of actin monomers or filaments, preventing monomer exchange (end-blocking or capping). It can promote the assembly of monomers into filaments (nucleation) as well as sever filaments already formed. Plays a role in ciliogenesis	12	-55.7	5.9	86.2	2.73E+06	1.70E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
				Isocitrate dehydrogenase cytoplasmic	catalise the reactiom: Isocitrate + NADP+ = 2-oxoglutarate + CO2 + NADPH.	7	-24.2	6.5	46.7		
1485	2.4	6.92	73,807	Lamin-A	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin.	22	-110	6.5	74.3	5.72E+05	2.35E+05
				Stress-induced-phosphoprotein 1 (STI1) (Hsc70/Hsp90-organizing protein)(Guertler *et al.*)	Mediates the association of the molecular chaperones HSC70 and HSP90 (HSPCA and HSPCB).	3	-14.2	6.4	62.5		
1960	2.2	6.53	66,200	Alpha-enolase (EC 4.2.1.11)(2-phospho-D-glycerate hydro-lyase)(Non-neural enolase)(Enolase 1)	Multifunctional enzyme that, as well as its role in glycolysis, plays a part in various processes such as growth control, hypoxia tolerance and allergic responses. May also function in the intravascular and pericellular fibrinolytic system due to its ability to serve as a receptor and activator of plasminogen on the cell surface of several cell-types such as leukocytes and neurons. Stimulates immunoglobulin production.	32	-149	6.2	47.1	8.88E+06	4.11E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
				60 kDa heat shock protein, mitochondrial	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix.	6	-29.7	5.9	60.9		
				Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	3	-14.3	5.9	56.6		
1972	2.6	6.86	66,200	Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	2	-6.9	5.9	56.6	1.80E+06	6.86E+05
2965	1.9	5.85	44,112	6-phosphogluconolactonase (6PGL)(EC 3.1.1.31)	Hydrolysis of 6-phosphogluconolactone to 6-phosphogluconate	5	-28.5	6.4	30.8	2.52E+06	4.68E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
				DNA-directed RNA polymerases I, II, and III subunit RPABC1 (RNA polymerases I, II, and III subunit ABC1)(DNA-directed RNA polymerase II subunit E)	DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates. Common component of RNA polymerases I, II and III which synthesize ribosomal RNA precursors, mRNA precursors and many functional non-coding RNAs, and small RNAs, such as 5S rRNA and tRNAs, respectively. Pol II is the central component of the basal RNA polymerase II transcription machinery. Pols are composed of mobile elements that move relative to each other. In Pol II, POLR2E/RPB5 is part of the lower jaw surrounding the central large cleft and thought to grab the incoming DNA template. Seems to be the major component in this process	5	-24.5	5.7	24.6		
2912	3.8	8.12	31,042	Electron transfer flavoprotein subunit beta (Beta-ETF)	The electron transfer flavoprotein serves as a specific electron acceptor for several dehydrogenases, including five acyl-CoA dehydrogenases, glutaryl-CoA and sarcosine dehydrogenase. It transfers the electrons to the main mitochondrial respiratory chain via ETF-ubiquinone oxidoreductase (ETF dehydrogenase)	66	-149	7.6	27.7	5.58E+06	1.48E+06
				Proteasome subunit alpha type-4 (EC 3.4.25.1)(Proteasome component C9)	The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity.	12	-49.3	7.6	29.5		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
				Adenylate kinase 2, mitochondrial (AK 2)(EC 2.7.4.3)(ATP-AMP transphosphorylase 2)	Catalyzes the reversible transfer of the terminal phosphate group between ATP and AMP. Plays an important role in cellular energy homeostasis and in adenine nucleotide metabolism. Adenylate kinase activity is critical for regulation of the phosphate utilization and the AMP de novo biosynthesis pathways. Plays a key role in hematopoiesis	12	-44.6	7	25.5		
				Calcyclin-binding protein	May be involved in calcium-dependent ubiquitination and subsequent proteasomal degradation of target proteins. Probably serves as a molecular bridge in ubiquitin E3 complexes. Participates in the ubiquitin-mediated degradation of beta-catenin (CTNNB1)	10	-34	7.6	26.5		
4082	2	9.09	57,000	Malate dehydrogenase, mitochondrial	Catalytic activity: (S)-malate + NAD+ = oxaloacetate + NADH.	23	-125	8.9	35.7	5.19E+06	2.65E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
				Glyceraldehyde-3-phosphate dehydrogenase	Has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby playing a role in glycolysis and nuclear functions, respectively. Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme in glycolysis that catalyzes the first step of the pathway by converting D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate. Modulates the organization and assembly of the cytoskeleton. Facilitates the CHP1-dependent microtubule and membrane associations through its ability to stimulate the binding of CHP1 to microtubules. Also participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. Nuclear functions are probably due to the nitrosylase activity that mediates cysteine S-nitrosylation of nuclear target proteins such as SIRT1, HDAC2 and PRKDC. Component of the GAIT (gamma interferon-activated inhibitor of translation) complex which mediates interferon-gamma-induced transcript-selective translation inhibition in inflammation processes. Upon interferon-gamma treatment assembles into the GAIT complex which binds to stem loop-containing GAIT elements in the 3'-UTR of diverse inflammatory mRNAs (such as ceruplasmin) and suppresses their translation	15	-64.1	8.1	35.8		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Garst
				Heterogeneous nuclear ribonucleoprotein A3 (hnRNP A3)	Plays a role in cytoplasmic trafficking of RNA. Binds to the cis-acting response element, A2RE. May be involved in pre-mRNA splicing.	5	-26.1	8.5	37.1		
4101	1.7	7.21	44,497	Isocitrate dehydrogenase cytoplasmic	catalytic activity: Isocitrate + NADP+ = 2-oxoglutarate + CO2 + NADPH.	24	-108	6.5	46.7	2.59E+06	1.50E+06
				Acyl-coenzyme A thioesterase 2, mitochondrial	Acyl-CoA thioesterases are a group of enzymes that catalyze the hydrolysis of acyl-CoAs to the free fatty acid and coenzyme A (CoASH), providing the potential to regulate intracellular levels of acyl-CoAs, free fatty acids and CoASH. Most active on substrates with chain lengths ranging from C14-C20.	9	-64.7	8.2	49.6		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Gold
2232	2.3	8.72	64,735	Phosphoglycerate kinase 1	Catalytic activity: ATP + 3-phospho-D-glycerate = ADP + 3-phospho-D-glyceroyl phosphate.	39	-152	8	44.5	8.40E+06	3.73E+06
				Cytochrome b-c1 complex subunit 2, mitochondrial Precursor (Ubiquinol-cytochrome-c reductase complex core protein 2)(Core protein II)	This is a component of the ubiquinol-cytochrome c reductase complex (complex III or cytochrome b-c1 complex), which is part of the mitochondrial respiratory chain. The core protein 2 is required for the assembly of the complex.	8	-53	9.2	48.4		
2149	2.7	7.09	66,200	Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	4	-20	5.9	56.6	1.44E+06	5.32E+05
1564	1.8	6.99	67,644	Stress-induced-phosphoprotein 1 (STI1)(Hsc70/Hsp90-organizing protein)	Mediates the association of the molecular chaperones HSC70 and HSP90 (HSPCA and HSPCB).	40	-131	6.4	62.5	5.44E+06	3.09E+06
				Lamin-A	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin.	32	-125	6.5	74.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon 810	Mon Gold
1485	2.4	6.92	73,807	Lamin-A	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin.	22	-110	6.5	74.3	5.72E+05	2.40E+05
				Stress-induced-phosphoprotein 1 (STI1) (Hsc70/Hsp90-organizing protein)(Guertler *et al.*)	Mediates the association of the molecular chaperones HSC70 and HSP90 (HSPCA and HSPCB).	3	-14	6.4	62.5		
3305	2.8	5.07	37,180	chromobox homolog 3	Seems to be involved in transcriptional silencing in heterochromatin-like complexes. Recognizes and binds histone H3 tails methylated at 'Lys-9', leading to epigenetic repression. May contribute to the association of the heterochromatin with the inner nuclear membrane through its interaction with lamin B receptor (LBR). Involved in the formation of functional kinetochore through interaction with MIS12 complex proteins.	2	-9.4	5.2	20.8	2.51E+06	7.05E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Conv Corn	Mon Garst
1972	2.5	6.86	66,200	Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	2	-6.9	5.9	56.6	1.73E+06	6.86E+05

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										Mon Conv Corn	Mon Gold
2149	2.7	7.09	66,200	Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	4	-20	5.9	56.6	1.45E+06	5.32E+05

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW Kilo in Daltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Conv Corn	Mon Garst	Mon Gold
2912	7.67E-05	3.8	8.12/31.042	Electron transfer flavoprotein subunit beta (Beta-ETF)	The electron transfer flavoprotein serves as a specific electron acceptor for several dehydrogenases, including five acyl-CoA dehydrogenases, glutaryl-CoA and sarcosine dehydrogenase. It transfers the electrons to the main mitochondrial respiratory chain via ETF-ubiquinone oxidoreductase (ETF dehydrogenase)	66	-149	7.6/27.7	5.13E+06	5.58E+06	3.33E+06	1.48E+06	3.60E+06

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW Kilo in Daltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Conv Corn	Mon Garst	Mon Gold
				Proteasome subunit alpha type-4 (EC 3.4.25.1)(Proteasome component C9)	The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity.	12	-49.3	7.6/29.5					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW Kilo in Daltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Conv Corn	Mon Garst	Mon Gold
				Adenylate kinase 2, mitochondrial (AK 2)(EC 2.7.4.3)(ATP-AMP transphosphorylase 2)	Catalyzes the reversible transfer of the terminal phosphate group between ATP and AMP. Plays an important role in cellular energy homeostasis and in adenine nucleotide metabolism. Adenylate kinase activity is critical for regulation of the phosphate utilization and the AMP de novo biosynthesis pathways. Plays a key role in hematopoiesis	12	-44.6	7/25.5					

Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW Kilo in Daltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Conv Corn	Mon Garst	Mon Gold
				Calcyclin-binding protein	May be involved in calcium-dependent ubiquitination and subsequent proteasomal degradation of target proteins. Probably serves as a molecular bridge in ubiquitin E3 complexes. Participates in the ubiquitin-mediated degradation of beta-catenin (CTNNB1)	10	-34	7.6/26.5					

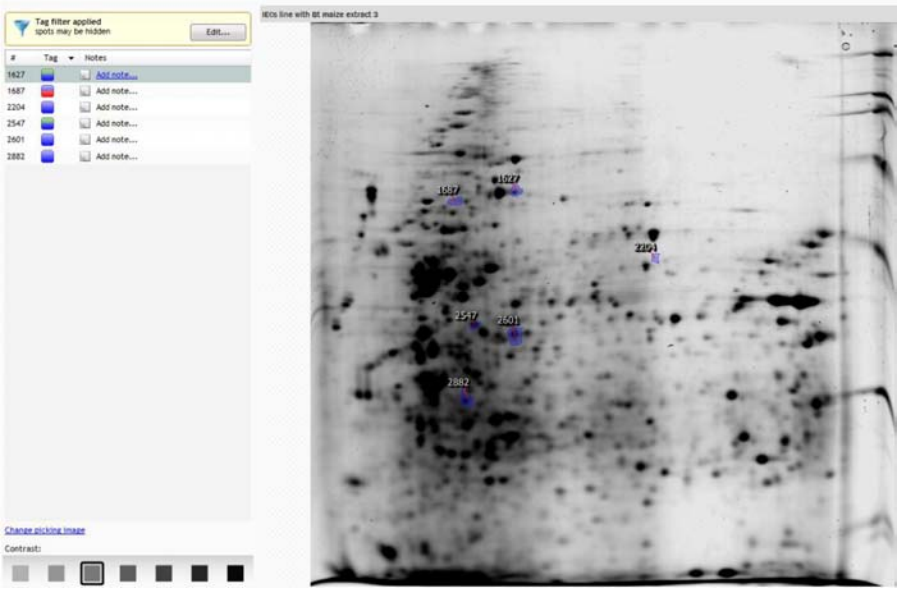
Spot No.	Anova (p)	Fold	Experimental Ip/MW (MW Kilo in Daltons)	Protein Description	Function	rI	log(e)	Theoritical Ip/MW (MW in KiloDaltons)	Average Normalised Volumes				
									Mcert	Mon 810	Mon Conv Corn	Mon Garst	Mon Gold
1485	3.10E-05	3.1	6.91/72.759	Lamin-A	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin.	22	-110	6.5/74.3	3.32E+05	5.72E+05	1.83E+05	2.35E+05	2.40E+05
				Stress-induced-phosphoprotein 1 (STI1) (Hsc70/Hsp90-organizing protein)	Mediates the association of the molecular chaperones HSC70 and HSP90 (HSPCA and HSPCB).	3	-14.2	6.4/62.5					

log(e): the base-10 log of the expectation that any particular protein assignment was made at random (E-value).

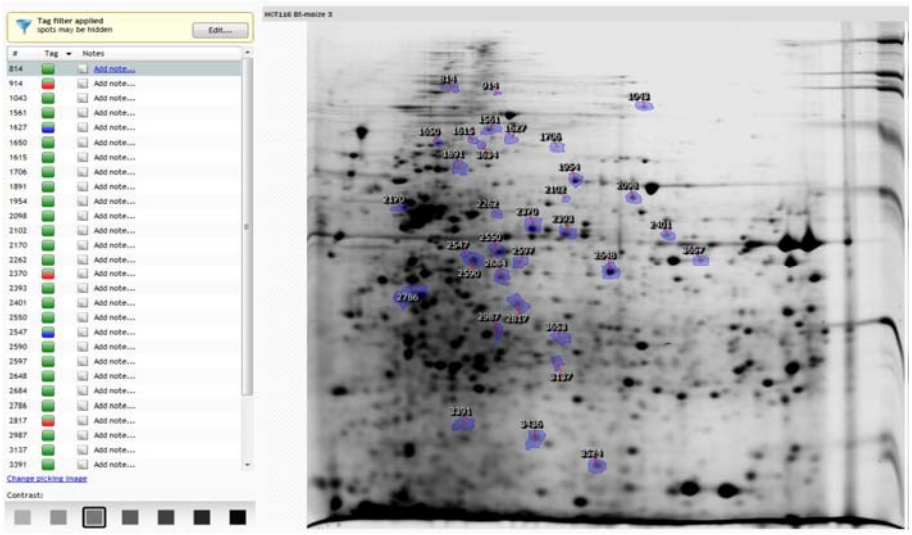
rl: the number of unique peptide sequences associated with this protein assignment.

pI: isoelectric point for the intact gene product.

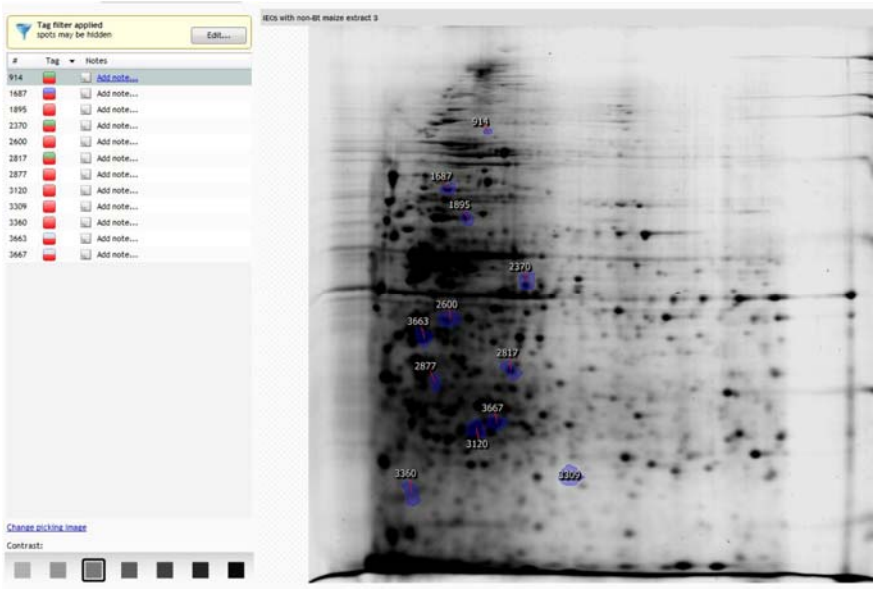
Appendices to chapter 5



Appendix 29: 2DE of the proteome of the primary epithelial-like cells from the small intestine of rats exposed to *Bt* maize extract. Protein spots from this gel were compared to those from comparator gels where intestinal primary epithelial-like cells were exposed to non-*Bt* maize extract.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 1627, 1687, 2204, 2547, 2601, and 2882 (as indicated on the proteome map).



Appendix 30: 2DE of the proteome of HCT116 cell line exposed to *Bt* maize extract. Protein spots from this gel were compared to those from comparator gels where intestinal primary epithelial-like cells were exposed to *Bt* maize extract.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 814, 914, 1561, 1650, 1615, 1043, 3634, 1706, 1891, 1954, 2098, 2170, 2262, 2370, 2393, 2401, 2550, 2547, 2590, 2597, 3657, 2648, 2786, 2817, 2987, 3653, 3137, 3391, 3436, and 3524 (as indicated on the proteome map).



Appendix 31: 2DE of the proteome of the primary epithelial-like cells from the small intestine of rats exposed to non-*Bt* maize extract. Protein spots from this gel were compared to those from comparator gels where HCT116 cell line was exposed to non-*Bt* maize extract.
Differentially expressed protein spots were identified by Progenesis SameSpot software. Protein spots that were picked for subsequent identification by LC-MS/MS were 914, 1687, 1895, 2370, 2600, 3663, 2817, 2877, 3667, 3120, 3360, and 3309 (as indicated on the proteome map).

Appendix 32: Identification of differentially expressed proteins from the primary epithelial cells of the small intestine of rats and from HCT116 cell line exposed to *Bt* and non-*Bt* maize extracts.
The data represent two way comparisons as follows (HCT116 *Bt* vs IECs *Bt*, IECs *Bt* vs IECs non-*Bt*, HCT116 non-*Bt* vs IECs non-*Bt*).
Spots were ranked by p-value (from low to high).

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
2393	1.8	6.43	40,646	Elongation factor 2	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation. During this step, the ribosome changes from the pre-translocational (PRE) to the post-translocational state as the newly formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively. Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome. Biological process: Protein biosynthesis	32	-112	6.4	95.2	7.87E+06	4.40E+06
				inner membrane protein, mitochondrial	involved in the maintenance of an internal steady state of calcium ions within the cytoplasm of a cell or between mitochondria and their surroundings	8	-25.3	5.6	87.1		
				Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)(GRP-75)(Heat shock 70 kDa protein 9)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	3	-17.3	5.9	73.7		
2597	1.6	5.8	37,634	Actin, cytoplasmic 1 Actin, cytoplasmic 1, N-terminally processed	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.	12	-62.3	5.3	41.7	4.36E+06	6.97E+06
814	3.2	5	98,898	filamin-A	Promotes orthogonal branching of actin filaments and links actin filaments to membrane glycoproteins. Anchors various transmembrane proteins to the actin cytoskeleton and serves as a scaffold for a wide range of cytoplasmic signaling proteins. Interaction with FLNA may allow neuroblast migration from the ventricular zone into the cortical plate. Tethers cell surface-localized furin, modulates its rate of internalization and directs its intracellular trafficking. Involved in ciliogenesis.	33	-168	5.7	281.1	1.27E+06	4.03E+06
				Myosin-9	Cellular myosin that appears to play a role in cytokinesis, cell shape, and specialized functions such as secretion and capping.	20	-115	5.5	226.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
				Fibronectin Precursor (FN)	Fibronectins bind cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin. Fibronectins are involved in cell adhesion, cell motility, opsonization, wound healing, and maintenance of cell shape. Anastellin binds fibronectin and induces fibril formation. This fibronectin polymer, named superfibronectin, exhibits enhanced adhesive properties. Both anastellin and superfibronectin inhibit tumor growth, angiogenesis and metastasis. Anastellin activates p38 MAPK and inhibits lysophospholipid signaling	5	-32.7	5.4	272.3		
				Vinculin (Metavinculin)	Actin filament (F-actin)-binding protein involved in cell-matrix adhesion and cell-cell adhesion. Regulates cell-surface E-cadherin expression and potentiates mechanosensing by the E-cadherin complex. May also play important roles in cell morphology and locomotion	4	-28.4	5.8	116.5		
				Spectrin alpha chain, brain	Fodrin, which seems to be involved in secretion, interacts with calmodulin in a calcium-dependent manner and is thus candidate for the calcium-dependent movement of the cytoskeleton at the membrane	3	-17.3	5.2	285.3		
				78 kDa glucose-regulated protein	Probably plays a role in facilitating the assembly of multimeric protein complexes inside the ER.	3	-15.6	5.1	72.3		
				filamin, beta	Connects cell membrane constituents to the actin cytoskeleton. May promote orthogonal branching of actin filaments and links actin filaments to membrane glycoproteins. Anchors various transmembrane proteins to the actin cytoskeleton. Interaction with FLNA may allow neuroblast migration from the ventricular zone into the cortical plate. Various interactions and localizations of isoforms affect myotube morphology and myogenesis. Isoform 6 accelerates muscle differentiation in vitro.	3	-14.3	5.4	277.7		
				integrin alpha V Gene	The alpha-V integrins are receptors for vitronectin, cytotactin, fibronectin, fibrinogen, laminin, matrix metalloproteinase-2, osteopontin, osteomodulin, prothrombin, thrombospondin and vWF. They recognize the sequence R-G-D in a wide array of ligands. In case of HIV-1 infection, the interaction with extracellular viral Tat protein seems to enhance angiogenesis in Kaposi's sarcoma lesions.	4	-13.6	5.4	108.9		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
2547	4.4	5.13	38,583	Heat shock protein HSP 90-beta (Heat shock 84 kDa)(HSP 84)(HSP84)	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	24	-73.2	5	83.2	4.16E+05	1.82E+06
				Heat shock protein HSP 90-alpha	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	9	-39.9	4.9	84.8		
				L-lactate dehydrogenase B chain	Catalytic activity: (S)-lactate + NAD+ = pyruvate + NADH. Pathway: Fermentation; pyruvate fermentation to lactate; (S)-lactate from pyruvate: step 1/1. Biological process: Glycolysis	5	-38.6	5.7	36.6		
				Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)(GRP-75)(Heat shock 70 kDa protein 9)(Peptide-binding protein 74)(PBP74)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	7	-30.7	5.9	73.7		
				Pyruvate kinase isozymes M1/M2	Glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP. Stimulates POU5F1-mediated transcriptional activation. Pathway: Carbohydrate degradation; glycolysis; pyruvate from D-glyceraldehyde 3-phosphate: step 5/5	4	-24.3	6.6	57.8		
				Lamin-B1	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin	4	-24.2	5.1	66.6		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
				S-adenosylmethionine synthetase isoform type-2 (AdoMet synthetase 2)	Catalyzes the formation of S-adenosylmethionine from methionine and ATP. Pathway: Amino-acid biosynthesis; S-adenosyl-L-methionine biosynthesis; S-adenosyl-L-methionine from L-methionine: step 1/1	5	-17.4	5.7	42.7		
				Nuclear migration protein nudC (Nuclear distribution protein C homolog)	Plays a role in neurogenesis and neuronal migration. Necessary for correct formation of mitotic spindles and chromosome separation during mitosis	3	-17.2	5.3	38.4		
				Endoplasmin Precursor (Heat shock protein 90 kDa beta member 1)(94 kDa glucose-regulated protein)(GRP-94)	Molecular chaperone that functions in the processing and transport of secreted proteins. When associated with CNPY3, required for proper folding of Toll-like receptors. Functions in endoplasmic reticulum associated degradation (ERAD). Has ATPase activity	3	-16.3	4.7	92.7		
				Moesin	Probably involved in connections of major cytoskeletal structures to the plasma membrane.	3	-15.8	6.1	67.6		
				Tubulin beta-5 chain	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.	3	-10.6	4.8	49.6		
				T-complex protein 1 subunit epsilon (TCP-1-epsilon)(CCT-epsilon)	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin	4	-9.5	5.5	59.5		
				Aldose reductase	Catalyzes the NADPH-dependent reduction of a wide variety of carbonyl-containing compounds to their corresponding alcohols with a broad range of catalytic efficiencies.	6	-8.7	6.3	35.8		
				aldo-keto reductase family 1, member B8	Biological process: oxidation-reduction process	5	-8.2	7.1	36.1		
3634	3	5.33	63,881	Plectin-1	Interlinks intermediate filaments with microtubules and microfilaments and anchors intermediate filaments to desmosomes or hemidesmosomes. May be involved not only in the cross-linking and stabilization of cytoskeletal intermediate filaments network, but also in the regulation of their dynamics.	12	-80.3	5.6	521.7	7.73E+05	2.30E+06
				Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	17	-65	5.9	73.7		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
				60 kDa heat shock protein, mitochondrial	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix.	6	-41.1	5.9	60.9		
1561	1.9	5.46	72,629	Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)(GRP-75)(Heat shock 70 kDa protein 9)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	71	-236	5.9	73.7	4.04E+06	7.52E+06
				78 kDa glucose-regulated protein	Probably plays a role in facilitating the assembly of multimeric protein complexes inside the ER.	8	-52.3	5.1	72.3		
				prelamin-A/C isoform C2	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin. Lamin A and C are present in equal amounts in the lamina of mammals. Plays an important role in nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics. Prelamin-A/C can accelerate smooth muscle cell senescence. It acts to disrupt mitosis and induce DNA damage in vascular smooth muscle cells (VSMCs), leading to mitotic failure, genomic instability, and premature senescence.	4	-23.8	6.5	74.3		
				Lactotransferrin; Lactoferrin	Transferrins are iron binding transport proteins which can bind two Fe^{3+} ions in association with the binding of an anion, usually bicarbonate. Lactotransferrin has antimicrobial activity which depends on the extracellular cation concentration. Lactoferroxins A, B and C have opioid antagonist activity. Lactoferroxin A shows preference for mu-receptors, while lactoferroxin B and C have somewhat higher degrees of preference for kappa-receptors than for mu-receptors. The lactotransferrin transferrin-like domain 1 functions as a serine protease of the peptidase S60 family that cuts arginine rich regions. This function contributes to the antimicrobial activity.	3	-7.6	8.5	78.1		
3653	2.3	6.34	27,447	Pyruvate kinase isozymes M1/M2	Glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP. Stimulates POU5F1-mediated transcriptional activation	5	-33.4	6.6	57.8	2.78E+06	6.27E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
				Proteasome subunit alpha type-6	The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity.	4	-18.2	6.3	27.4		
				Annexin A2	Calcium-regulated membrane-binding protein whose affinity for calcium is greatly enhanced by anionic phospholipids. It binds two calcium ions with high affinity.	4	-17.9	7.5	38.9		
1891	2.5	5.05	54,196	Vimentin	Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally. Involved with LARP6 in the stabilization of type I collagen mRNAs for CO1A1 and CO1A2	16	-86.2	5.1	53.7	2.71E+06	6.73E+06
				ATP synthase subunit beta, mitochondrial	Mitochondrial membrane ATP synthase (F_1F_0 ATP synthase or Complex V) produces ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain. F-type ATPases consist of two structural domains, F_1 - containing the extramembraneous catalytic core, and F_0 - containing the membrane proton channel, linked together by a central stalk and a peripheral stalk. During catalysis, ATP synthesis in the catalytic domain of F_1 is coupled via a rotary mechanism of the central stalk subunits to proton translocation. Subunits alpha and beta form the catalytic core in F_1. Rotation of the central stalk against the surrounding $alpha_3beta_3$ subunits leads to hydrolysis of ATP in three separate catalytic sites on the beta subunits.	8	-56.8	5.1	56.3		
2262	2.2	5.55	42,710	Vimentin	Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally. Involved with LARP6 in the stabilization of type I collagen mRNAs for CO1A1 and CO1A2	20	-102	5.1	53.7	9.03E+05	2.02E+06
				60 kDa heat shock protein, mitochondrial	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix.	12	-82.2	5.9	60.9		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
				Heat shock protein HSP 90-beta (Heat shock 84 kDa)(HSP 84)	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	8	-49.5	5	83.2		
				Heat shock protein HSP 90-alpha	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	6	-39.7	4.9	84.8		
				tubulin, alpha 1B (Tuba1b), mRNA	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.	3	-19.8	4.9	50.2		
				Nuclear migration protein nudC (Nuclear distribution protein C homolog)(c15)	Plays a role in neurogenesis and neuronal migration. Necessary for correct formation of mitotic spindles and chromosome separation during mitosis	4	-17.7	5.3	38.4		
				Pyruvate kinase isozymes M1/M2	Glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP. Stimulates POU5F1-mediated transcriptional activation. Pathway: Carbohydrate degradation; glycolysis; pyruvate from D-glyceraldehyde 3-phosphate: step 5/5	3	-11.8	6.6	57.8		
1615	2.2	5.24	67,203	Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)(GRP-75)(Heat shock 70 kDa protein 9)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	24	-87.3	5.9	73.7	6.69E+05	1.49E+06
				78 kDa glucose-regulated protein	Probably plays a role in facilitating the assembly of multimeric protein complexes inside the ER.	4	-25.9	5.1	72.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
2401	1.8	7.76	40,564	Transketolase (UniProtKB)	Catalyzes the transfer of a two-carbon ketol group from a ketose donor to an aldose acceptor, via a covalent intermediate with the cofactor thiamine pyrophosphate	9	-41.9	7.5	71.1	2.35E+06	1.28E+06
				Elongation factor 2	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation. During this step, the ribosome changes from the pre-translocational (PRE) to the post-translocational state as the newly formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively. Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome. Biological process: Protein biosynthesis	5	-28.3	6.4	95.2		
3436	1.4	6	14,785	Non-POU domain-containing octamer-binding protein	DNA- and RNA binding protein, involved in several nuclear processes. Binds the conventional octamer sequence in double stranded DNA. Also binds single-stranded DNA and RNA at a site independent of the duplex site. Involved in pre-mRNA splicing, probably as a heterodimer with SFPQ. Interacts with U5 snRNA, probably by binding to a purine-rich sequence located on the 3' side of U5 snRNA stem 1b. The SFPQ-NONO heteromer associated with MATR3 may play a role in nuclear retention of defective RNAs. The SFPQ-NONO heteromer may be involved in DNA unwinding by modulating the function of topoisomerase I/TOP1. The SFPQ-NONO heteromer may be involved in DNA nonhomologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination and may stabilize paired DNA ends. In vitro, the complex strongly stimulates DNA end joining, binds directly to the DNA substrates and cooperates with the Ku70/G22P1-Ku80/XRCC5 dimer to establish a functional preligation complex. NONO is involved in transcriptional regulation. The SFPQ-NONO-NR5A1 complex binds to the CYP17 promoter and regulates basal and cAMP-dependent transcriptional avtivity. NONO binds to an enhancer element in long terminal repeats of endogenous intracisternal A particles (IAPs) and activates transcription. Together with PSPC1, required for the formation of nuclear paraspeckles.	7	-29.8	9	54.9	7.70E+06	5.64E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
				ErbB3-binding protein 1	May play a role in a ERBB3-regulated signal transduction pathway. Seems be involved in growth regulation. Acts a corepressor of the androgen receptor and is regulated by the ERBB3 ligand neuregulin-1/heregulin (HRG). Inhibits transcription of some E2F1-regulated promoters, probably by recruiting histone acetylase (HAT) activity. Binds RNA. Associates with 28S, 18S and 5.8S mature rRNAs, several rRNA precursors and probably U3 small nucleolar RNA. May be involved in regulation of intermediate and late steps of rRNA processing. May be involved in ribosome assembly. Mediates cap-independent translation of specific viral IRESs (internal ribosomal entry site)	4	-11.4	6.4	43.6		
914	2.7	5.48	92,918	Heat shock protein HSP 90-alpha	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	9	-73.1	4.9	84.8	2.52E+05	6.84E+05
				Heat shock protein HSP 90-beta	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	9	-57.1	5	83.2		
				Vinculin (Metavinculin)	Actin filament (F-actin)-binding protein involved in cell-matrix adhesion and cell-cell adhesion. Regulates cell-surface E-cadherin expression and potentiates mechanosensing by the E-cadherin complex. May also play important roles in cell morphology and locomotion	7	-56.2	5.8	116.5		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
1954	2.3	6.56	49,964	Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)(GRP-75)(Heat shock 70 kDa protein 9)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	59	-156	5.9	73.7	6.12E+06	2.71E+06
3391	1.9	5.1	15,950	Ezrin	Probably involved in connections of major cytoskeletal structures to the plasma membrane. In epithelial cells, required for the formation of microvilli and membrane ruffles on the apical pole. Along with PLEKHG6, required for normal macropinocytosis	7	-41.6	5.8	69.3	2.79E+06	5.21E+06
				Nucleolin-like protein	Molecular function: nuclic acid binding and nucleotide binding	9	-30.8	5.3	61.7		
				T-complex protein 1 subunit alpha	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin	5	-30.4	5.9	60.3		
				Heat shock protein HSP 90-beta	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	5	-28.3	5	83.2		
				40S ribosomal protein SA	Required for the assembly and/or stability of the 40S ribosomal subunit. Required for the processing of the 20S rRNA-precursor to mature 18S rRNA in a late step of the maturation of 40S ribosomal subunits. Also functions as a cell surface receptor for laminin. Plays a role in cell adhesion to the basement membrane and in the consequent activation of signaling transduction pathways. May play a role in cell fate determination and tissue morphogenesis. Also acts as a receptor for several other ligands, including the pathogenic prion protein, viruses, and bacteria. Acts as a PPP1R16B-dependent substrate of PPP1CA	5	-21.7	4.8	32.8		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
				Eukaryotic translation initiation factor 3 subunit I (eIF3i)	Component of the eukaryotic translation initiation factor 3 (eIF-3) complex, which is required for several steps in the initiation of protein synthesis. The eIF-3 complex associates with the 40S ribosome and facilitates the recruitment of eIF-1, eIF-1A, eIF-2:GTP:methionyl-tRNAi and eIF-5 to form the 43S preinitiation complex (43S PIC). The eIF-3 complex stimulates mRNA recruitment to the 43S PIC and scanning of the mRNA for AUG recognition. The eIF-3 complex is also required for disassembly and recycling of post-termination ribosomal complexes and subsequently prevents premature joining of the 40S and 60S ribosomal subunits prior to initiation.	3	-9.2	5.3	19.4		
1043	2	7.5	87,256	Lactotransferrin	Transferrins are iron binding transport proteins which can bind two Fe^{3+} ions in association with the binding of an anion, usually bicarbonate. Lactotransferrin has antimicrobial activity which depends on the extracellular cation concentration. Lactoferroxins A, B and C have opioid antagonist activity. Lactoferroxin A shows preference for mu-receptors, while lactoferroxin B and C have somewhat higher degrees of preference for kappa-receptors than for mu-receptors. The lactotransferrin transferrin-like domain 1 functions as a serine protease of the peptidase S60 family that cuts arginine rich regions. This function contributes to the antimicrobial activity.	3	-18.2	8.5	78.1	2.12E+06	1.09E+06
3137	1.4	6.24	21,626	Annexin A1	Calcium/phospholipid-binding protein which promotes membrane fusion and is involved in exocytosis. This protein regulates phospholipase A2 activity. It seems to bind from two to four calcium ions with high affinity.	14	-41.2	7	38.8	1.90E+06	2.69E+06
				Ras-related protein Rab-11B	GTPase that modulates endosomal trafficking. Acts as a major regulator of membrane delivery during cytokinesis	4	-25.6	5.6	24.5		
				NADH dehydrogenase flavoprotein 2, mitochondrial	Core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I) that is believed to belong to the minimal assembly required for catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone	5	-22.9	6.2	27.4		
2170	1.6	4.36	43,576	Vimentin	Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally. Involved with LARP6 in the stabilization of type I	41	-127	5.1	53.7	2.77E+06	4.40E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
					collagen mRNAs for CO1A1 and CO1A2						
				Heat shock protein HSP 90-beta (Heat shock 84 kDa)	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	9	-61.5	5	83.2		
				beta-actin-like protein 2	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells	3	-19.9	5.3	41.9		
2817	1.4	5.8	33,218	Protein disulfide-isomerase A3	Catalytic activity: Catalyzes the rearrangement of -S-S- bonds in proteins.	20	-61.6	5.9	56.6	5.64E+06	7.98E+06
				Serine-threonine kinase receptor-associated protein	The SMN complex plays an essential role in spliceosomal snRNP assembly in the cytoplasm and is required for pre-mRNA splicing in the nucleus. STRAP may play a role in the cellular distribution of the SMN complex. Negatively regulates TGF-beta signaling but positively regulates the PDPK1 kinase activity by enhancing its autophosphorylation and by significantly reducing the association of PDPK1 with 14-3-3 protein. Biological process: mRNA processing and mRNA splicing	5	-32.8	5	38.4		
				14-3-3 protein epsilon (14-3-3E)(Mitochondrial import stimulation factor L subunit)	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	5	-24.3	4.6	29.2		
				Chloride intracellular channel protein 1	Can insert into membranes and form chloride ion channels. Channel activity depends on the pH. Membrane insertion seems to be redox-regulated and may occur only under oxydizing conditions	5	-18	5.1	27		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
2550	1.7	5.58	38,542	Tubulin beta-5 chain	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.	27	-106	4.8	49.6	5.72E+06	9.78E+06
				Tubulin beta-2C chain	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain	30	-94.6	4.8	49.8		
				60S acidic ribosomal protein P0 (60S ribosomal protein L10E)	Ribosomal protein P0 is the functional equivalent of E.coli protein L10.	7	-38	5.9	34.2		
				Pyruvate dehydrogenase E1 component subunit beta, mitochondrial Precursor	The pyruvate dehydrogenase complex catalyzes the overall conversion of pyruvate to acetyl-CoA and CO_2, and thereby links the glycolytic pathway to the tricarboxylic cycle. Biological process: Glucose metabolism	7	-29.6	6.2	39		
				L-lactate dehydrogenase B chain	catalytic activity: (S)-lactate + NAD+ = pyruvate + NADH. Pathway: Fermentation; pyruvate fermentation to lactate; (S)-lactate from pyruvate: step 1/1. Biological process: Glycolysis	4	-25.6	5.7	36.6		
				Aldose reductase (EC 1.1.1.21)(Aldehyde reductase)	Catalyzes the NADPH-dependent reduction of a wide variety of carbonyl-containing compounds to their corresponding alcohols with a broad range of catalytic efficiencies.	4	-9	6.3	35.8		
2590	1.5	5.26	37,799	Heat shock protein HSP 90-alpha	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function. Biological process: Stress response	28	-88.6	4.9	84.8	6.04E+06	9.34E+06
				Tubulin beta-5 chain	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain. Biological process: protein polymerization	8	-34.4	4.8	49.6		
				S-adenosylmethionine synthetase isoform type-2 (AdoMet synthetase 2)	Catalyzes the formation of S-adenosylmethionine from methionine and ATP. Pathway:Amino-acid biosynthesis; S-adenosyl-L-methionine biosynthesis; S-adenosyl-L-methionine from L-methionine: step 1/1.	10	-33.5	5.7	42.7		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
				Tubulin beta-2C chain	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain	5	-26.6	4.8	49.8		
2648	2.8	7.05	36,520	Elongation factor 2	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation. During this step, the ribosome changes from the pre-translocational (PRE) to the post-translocational state as the newly formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively. Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome.	43	-128	6.4	95.2	9.14E+06	3.24E+06
2987	2.5	5.54	29,186	Pyruvate kinase isozymes M1/M2	Glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP. Stimulates POU5F1-mediated transcriptional activation	11	-61.3	6.6	57.8	1.89E+06	4.70E+06
				Annexin A1	Calcium/phospholipid-binding protein which promotes membrane fusion and is involved in exocytosis. This protein regulates phospholipase A2 activity. It seems to bind from two to four calcium ions with high affinity.	14	-49.6	7	38.8		
				Alpha-enolase (EC 4.2.1.11)	Multifunctional enzyme that, as well as its role in glycolysis, plays a part in various processes such as growth control, hypoxia tolerance and allergic responses. May also function in the intravascular and pericellular fibrinolytic system due to its ability to serve as a receptor and activator of plasminogen on the cell surface of several cell-types such as leukocytes and neurons. Stimulates immunoglobulin production.	6	-29.9	6.2	47.1		
				Chloride intracellular channel protein 1	Can insert into membranes and form chloride ion channels. Channel activity depends on the pH. Membrane insertion seems to be redox-regulated and may occur only under oxydizing conditions	6	-23.8	5.1	27		
				14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1)	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	4	-17.7	4.7	27.8		
				Peroxiredoxin-4	Probably involved in redox regulation of the cell. Regulates the activation of NF-kappa-B in the cytosol by a modulation of I-kappa-B-alpha phosphorylation	3	-7.8	5.7	29.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
2098	2.6	7.35	44,484	Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	3	-17.1	5.9	73.7	3.55E+06	1.36E+06
				Lactotransferrin	Transferrins are iron binding transport proteins which can bind two Fe3+ ions in association with the binding of an anion, usually bicarbonate. Lactotransferrin has antimicrobial activity which depends on the extracellular cation concentration. Lactoferroxins A, B and C have opioid antagonist activity. Lactoferroxin A shows preference for mu-receptors, while lactoferroxin B and C have somewhat higher degrees of preference for kappa-receptors than for mu-receptors. The lactotransferrin transferrin-like domain 1 functions as a serine protease of the peptidase S60 family that cuts arginine rich regions. This function contributes to the antimicrobial activity.	3	-16.6	8.5	78.1		
3657	3.2	8.21	37,716	aldolase A-like 1	Plays a key role in glycolysis and gluconeogenesis. In addition, may also function as scaffolding protein	10	-38.8	7.1	39.9	2.44E+06	7.85E+06
				Malate dehydrogenase, mitochondrial	catalytic activity: (S)-malate + NAD+ = oxaloacetate + NADH.	3	-16.8	8.9	35.7		
2786	2.2	4.48	33,961	Tropomyosin alpha-3 chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	58	-187	4.7	29	1.84E+07	4.12E+07
				Tropomyosin alpha-3 chain (Tropomyosin-3)	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	48	-139	4.7	33.1		
				Tropomyosin alpha-3 chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	36	-111	4.7	32.8		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
				Tropomyosin alpha-4 chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments. Binds calcium.	46	-101	4.7	28.5		
				Tropomyosin alpha-1 chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	27	-76.6	4.7	32.7		
2370	1.5	6.01	41,224	Elongation factor 2	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation. During this step, the ribosome changes from the pre-translocational (PRE) to the post-translocational state as the newly formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively. Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome. Biological process: Protein biosynthesis	69	-189	6.4	95.2	1.06E+07	7.27E+06
				60 kDa heat shock protein, mitochondrial	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix.	3	-17.5	5.9	60.9		
				Moesin	Probably involved in connections of major cytoskeletal structures to the plasma membrane.	3	-16.5	6.1	67.6		
1650	2	4.82	65,915	Vimentin	Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally.	15	-86.6	5.1	53.7	2.69E+06	1.32E+06
				78 kDa glucose-regulated protein	Probably plays a role in facilitating the assembly of multimeric protein complexes inside the ER.	5	-30.3	5.1	72.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT Bt	IEC Bt
				Protein disulfide-isomerase	This multifunctional protein catalyzes the formation, breakage and rearrangement of disulfide bonds. At the cell surface, seems to act as a reductase that cleaves disulfide bonds of proteins attached to the cell. May therefore cause structural modifications of exofacial proteins. Inside the cell, seems to form/rearrange disulfide bonds of nascent proteins. At high concentrations, functions as a chaperone that inhibits aggregation of misfolded proteins. At low concentrations, facilitates aggregation (anti-chaperone activity). May be involved with other chaperones in the structural modification of the TG precursor in hormone biogenesis. Also acts a structural subunit of various enzymes such as prolyl 4-hydroxylase and microsomal triacylglycerol transfer protein MTTP. Catalyzes the rearrangement of -S-S- bonds in proteins.	4	-21.6	4.8	56.9		
1706	2.9	6.32	62,985	Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins.	36	-141	5.9	56.6	2.01E+06	5.80E+06
				Lactotransferrin	Transferrins are iron binding transport proteins which can bind two Fe3+ ions in association with the binding of an anion, usually bicarbonate. Lactotransferrin has antimicrobial activity which depends on the extracellular cation concentration. Lactoferroxins A, B and C have opioid antagonist activity. Lactoferroxin A shows preference for mu-receptors, while lactoferroxin B and C have somewhat higher degrees of preference for kappa-receptors than for mu-receptors. The lactotransferrin transferrin-like domain 1 functions as a serine protease of the peptidase S60 family that cuts arginine rich regions. This function contributes to the antimicrobial activity.	5	-26.2	8.5	78.1		
				tubulin, alpha 1B (Tuba1b)	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.	4	-17.2	4.9	50.2		
3524	1.9	6.83	6,534	Peroxiredoxin-1; EC 1.11.1.15	Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system but not from glutaredoxin. May play an important role in eliminating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and tumor necrosis factor-alpha by regulating the intracellular concentrations of H_2O_2. Reduces an intramolecular disulfide bond in GDPD5 that gates the ability to GDPD5 to drive postmitotic motor neuron differentiation	24	-62.8	8.3	22.1	5.12E+06	2.69E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										HCT non-Bt	IEC non-Bt
2600	1.3	4.95	37,592	Lamin-B1	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin	6	-36.7	5.1	66.6	5.94E+06	4.51E+06
				S-adenosylmethionine synthetase isoform type-2 (AdoMet synthetase 2)(EC 2.5.1.6)	Catalyzes the formation of S-adenosylmethionine from methionine and ATP. Pathway: Amino-acid biosynthesis; S-adenosyl-L-methionine biosynthesis; S-adenosyl-L-methionine from L-methionine: step 1/1.	4	-23.6	5.7	42.7		
				Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)(GRP-75)(Heat shock 70 kDa protein 9)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	8	-23.5	5.9	73.7		
				heat shock protein 4 like	Biological process: Stress response. Molecular function: ATP binding	3	-16.8	5.5	94.2		
				Aldose reductase	Catalyzes the NADPH-dependent reduction of a wide variety of carbonyl-containing compounds to their corresponding alcohols with a broad range of catalytic efficiencies.	3	-9.3	6.3	35.8		
2877	1.9	4.78	31,743	Heat shock protein HSP 90-beta (Heat shock 84 kDa)	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	7	-55.6	5	83.2	2.66E+06	1.37E+06
				Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)(GRP-75)(Heat shock 70 kDa protein 9)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	23	-52	5.9	73.7		

				14-3-3 protein gamma	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	4	-25.8	4.8	28.3		
				Tubulin beta-5 chain	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.	4	-19.1	4.8	49.6		
				Tubulin beta-2C chain	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain	4	-15.1	4.8	49.8		
3309	1.8	6.55	18,177	Peroxiredoxin-1; EC 1.11.1.15; Natural killer cell-enhancing factor A	Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system but not from glutaredoxin. May play an important role in eliminating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and tumor necrosis factor-alpha by regulating the intracellular concentrations of H_2O_2. Reduces an intramolecular disulfide bond in GDPD5 that gates the ability to GDPD5 to drive postmitotic motor neuron differentiation	66	-109	8.3	22.1	1.32E+07	7.28E+06
				Peroxiredoxin-1 (EC 1.11.1.15)(Thioredoxin peroxidase 2)	Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system but not from glutaredoxin. May play an important role in eliminating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and tumor necrosis factor-alpha by regulating the intracellular concentrations of H_2O_2. Reduces an intramolecular disulfide bond in GDPD5 that gates the ability to GDPD5 to drive postmitotic motor neuron differentiation	57	-98	8.3	22.1		
				Peroxiredoxin-4	Probably involved in redox regulation of the cell. Regulates the activation of NF-kappa-B in the cytosol by a modulation of I-kappa-B-alpha phosphorylation	8	-15.4	5.7	29.3		
				14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1)	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	4	-14.1	4.7	27.8		
914	1.9	5.48	92,918	Heat shock protein HSP 90-alpha	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	9	-73.1	4.9	84.8	1.74E+05	3.31E+05

				Heat shock protein HSP 90-beta (Heat shock 84 kDa)	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	9	-57.1	5	83.2		
3663	1.7	4.55	36,189	Tropomyosin alpha-1 chain (Tropomyosin-1)(Alpha-tropomyosin)	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	116	-227	4.7	32.8	9.83E+06	1.72E+07
				Tropomyosin alpha-3 chain (Tropomyosin-3)(Gamma-tropomyosin)	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	61	-117	4.7	33.1		
				Tropomyosin alpha-1 chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.	67	-114	4.7	28.5		
3667	1.9	5.58	23,692	Ubiquitin carboxyl-terminal hydrolase isozyme L1	Ubiquitin-protein hydrolase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins. This enzyme is a thiol protease that recognizes and hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin. Also binds to free monoubiquitin and may prevent its degradation in lysosomes. The homodimer may have ATP-independent ubiquitin ligase activity	19	-45.3	5.1	24.8	4.83E+06	9.30E+06
				14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1)(KCIP-1)(Mitochondrial import stimulation factor S1 subunit)	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	4	-24.3	4.7	27.8		
				Ras-related protein Rab-11B	GTPase that modulates endosomal trafficking. Acts as a major regulator of membrane delivery during cytokinesis	3	-18.3	5.6	24.5		
				Tropomyosin beta chain	Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments. The non-muscle isoform may have a role in agonist-mediated receptor internalization	4	-3.6	4.5	28.6		

2817	1.4	5.8	33,218	Protein disulfide-isomerase A3	Catalytic activity: Catalyzes the rearrangement of -S-S- bonds in proteins.	20	-61.6	5.9	56.6	5.53E+06	7.60E+06
				prelamin-A/C isoform C2	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin. Lamin A and C are present in equal amounts in the lamina of mammals. Plays an important role in nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics. Prelamin-A/C can accelerate smooth muscle cell senescence. It acts to disrupt mitosis and induce DNA damage in vascular smooth muscle cells (VSMCs), leading to mitotic failure, genomic instability, and premature senescence	8	-48.2	6.5	74.3		
				Serine-threonine kinase receptor-associated protein	The SMN complex plays an essential role in spliceosomal snRNP assembly in the cytoplasm and is required for pre-mRNA splicing in the nucleus. STRAP may play a role in the cellular distribution of the SMN complex. Negatively regulates TGF-beta signaling but positively regulates the PDPK1 kinase activity by enhancing its autophosphorylation and by significantly reducing the association of PDPK1 with 14-3-3 protein. Biological process: mRNA processing and mRNA splicing	5	-32.8	5	38.4		
				14-3-3 protein epsilon (14-3-3E)(Mitochondrial import stimulation factor L subunit)(MSF L)	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	5	-24.3	4.6	29.2		
				14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1)(KCIP-1)(Mitochondrial import stimulation factor S1 subunit)	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	5	-23	4.7	27.8		
				Chloride intracellular channel protein 1	Can insert into membranes and form chloride ion channels. Channel activity depends on the pH. Membrane insertion seems to be redox-regulated and may occur only under oxydizing conditions	5	-18	5.1	27		
				Nucleolin-like protein	Molecular function: nuclic acid binding and nucleotide binding	4	-17.7	5.3	61.7		
1895	2.3	5.21	54,033	ATP synthase subunit beta, mitochondrial	Mitochondrial membrane ATP synthase (F_1F_0 ATP synthase or Complex V) produces ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain. F-type ATPases consist of two structural domains, F_1 - containing the extramembraneous catalytic core, and F_0 - containing the membrane proton channel, linked together by a central stalk and a peripheral stalk. During catalysis, ATP synthesis in the catalytic domain of F_1 is coupled via a rotary	19	-72.6	5.1	56.3	1.59E+06	3.59E+06

					mechanism of the central stalk subunits to proton translocation. Subunits alpha and beta form the catalytic core in F_1. Rotation of the central stalk against the surrounding $alpha_3beta_3$ subunits leads to hydrolysis of ATP in three separate catalytic sites on the beta subunits.						
				Vimentin	Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally. Involved with LARP6 in the stabilization of type I collagen mRNAs for CO1A1 and CO1A2	4	-22.6	5.1	53.7		
3120	1.2	5.3	22,508	60 kDa heat shock protein, mitochondrial	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix.	13	-61.4	5.9	60.9		
				Ubiquitin carboxyl-terminal hydrolase isozyme L1	Ubiquitin-protein hydrolase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins. This enzyme is a thiol protease that recognizes and hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin. Also binds to free monoubiquitin and may prevent its degradation in lysosomes. The homodimer may have ATP-independent ubiquitin ligase activity	4	-20	5.1	24.8	8.25E+06	1.00E+07
				Nucleolin-like protein	Molecular function: nuclic acid binding and nucleotide binding	3	-15.4	5.3	61.7		
3360	1.6	4.38	16,884	Tubulin beta-5 chain	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.	2	-10.7	4.8	49.6	7.95E+06	4.83E+06
2370	1.5	6.01	41,224	Elongation factor 2	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation. During this step, the ribosome changes from the pre-translocational (PRE) to the post-translocational state as the newly formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively. Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome. Biological process: Protein biosynthesis	69	-189	6.4	95.2	1.07E+07	7.27E+06
				60 kDa heat shock protein, mitochondrial	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix.	3	-17.5	5.9	60.9		
				Moesin	Probably involved in connections of major cytoskeletal structures to the plasma membrane.	3	-16.5	6.1	67.6		

1687	1.8	4.93	64,125	Protein disulfide-isomerase	This multifunctional protein catalyzes the formation, breakage and rearrangement of disulfide bonds. At the cell surface, seems to act as a reductase that cleaves disulfide bonds of proteins attached to the cell. May therefore cause structural modifications of exofacial proteins. Inside the cell, seems to form/rearrange disulfide bonds of nascent proteins. At high concentrations, functions as a chaperone that inhibits aggregation of misfolded proteins. At low concentrations, facilitates aggregation (anti-chaperone activity). May be involved with other chaperones in the structural modification of the TG precursor in hormone biogenesis. Also acts a structural subunit of various enzymes such as prolyl 4-hydroxylase and microsomal triacylglycerol transfer protein MTTP. Catalyzes the rearrangement of -S-S- bonds in proteins.	36	-129	4.8	56.9	2.06E+06	3.61E+06
				78 kDa glucose-regulated protein	Probably plays a role in facilitating the assembly of multimeric protein complexes inside the ER.	10	-65.5	5.1	72.3		
				Heat shock-related 70 kDa protein 2 (Heat shock protein 70.2)(Testis-specific heat shock protein-related)	In cooperation with other chaperones, Hsp70s stabilize preexistent proteins against aggregation and mediate the folding of newly translated polypeptides in the cytosol as well as within organelles. These chaperones participate in all these processes through their ability to recognize nonnative conformations of other proteins. They bind extended peptide segments with a net hydrophobic character exposed by polypeptides during translation and membrane translocation, or following stress-induced damage.	5	-34.9	5.5	69.6		
				Vimentin	Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally. Involved with LARP6 in the stabilization of type I collagen mRNAs for CO1A1 and CO1A2	6	-32.9	5.1	53.7		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										IEC Bt	IEC non-Bt
2547	3.7	5.13	38,583	Heat shock protein HSP 90-beta (Heat shock 84 kDa)(HSP 84)(HSP84)	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	24	-73.2	5	83.2	1.82E+06	4.86E+05
				Heat shock protein HSP 90-alpha	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	9	-39.9	4.9	84.8		
				L-lactate dehydrogenase B chain	Catalytic activity: (S)-lactate + NAD+ = pyruvate + NADH. Pathway: Fermentation; pyruvate fermentation to lactate; (S)-lactate from pyruvate: step 1/1. Biological process: Glycolysis	5	-38.6	5.7	36.6		
				Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)(GRP-75)(Heat shock 70 kDa protein 9)(Peptide-binding protein 74)(PBP74)(mtHSP70) (Mortalin)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	7	-30.7	5.9	73.7		

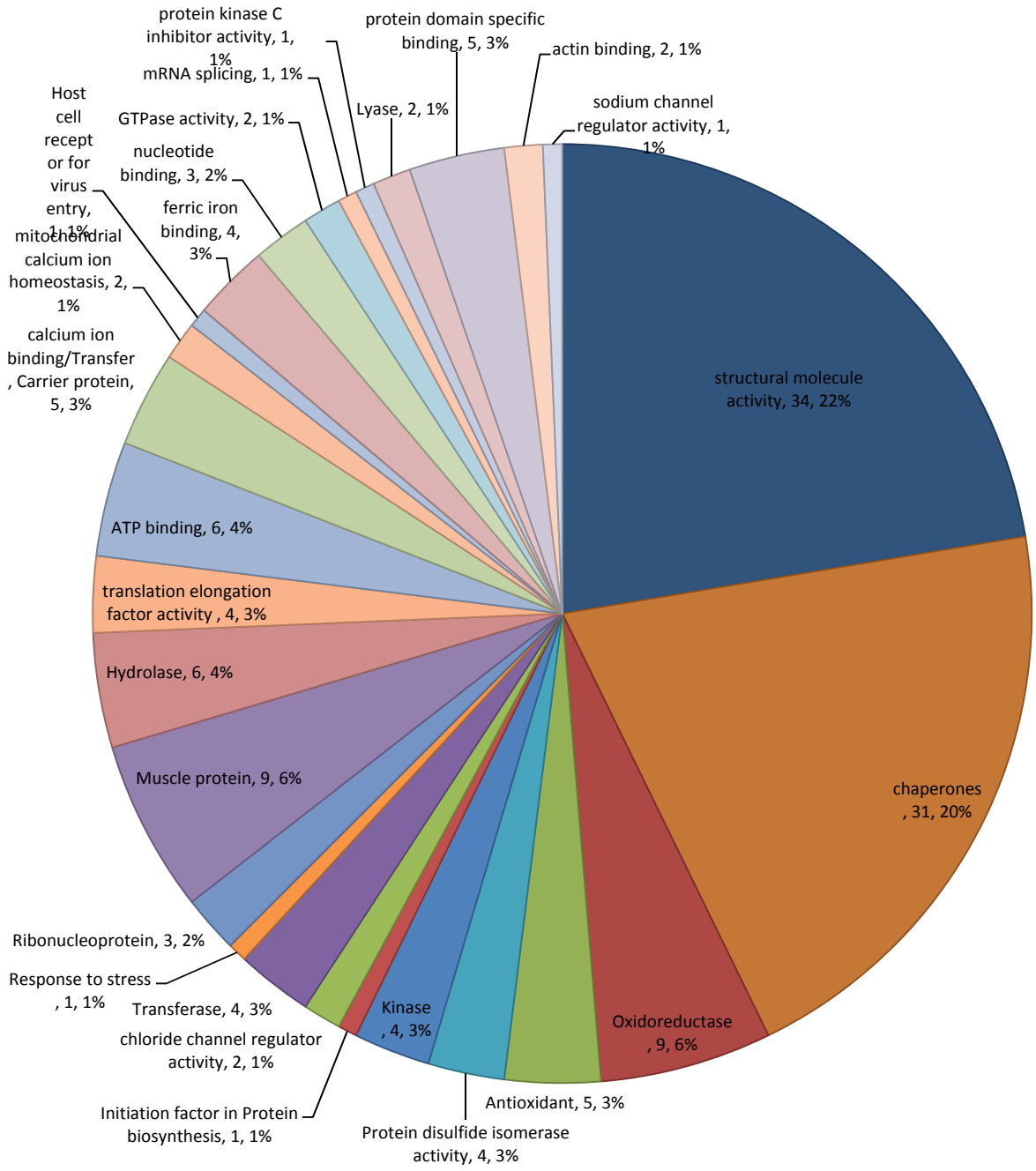
Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										IEC Bt	IEC non-Bt
				Pyruvate kinase isozymes M1/M2	Glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP. Stimulates POU5F1-mediated transcriptional activation. Pathway: Carbohydrate degradation; glycolysis; pyruvate from D-glyceraldehyde 3-phosphate: step 5/5	4	-24.3	6.6	57.8		
				Lamin-B1	Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin	4	-24.2	5.1	66.6		
				S-adenosylmethionine synthetase isoform type-2 (AdoMet synthetase 2)(EC 2.5.1.6)	Catalyzes the formation of S-adenosylmethionine from methionine and ATP. Pathway: Amino-acid biosynthesis; S-adenosyl-L-methionine biosynthesis; S-adenosyl-L-methionine from L-methionine: step 1/1	5	-17.4	5.7	42.7		
				Nuclear migration protein nudC (Nuclear distribution protein C homolog)(c15)	Plays a role in neurogenesis and neuronal migration. Necessary for correct formation of mitotic spindles and chromosome separation during mitosis	3	-17.2	5.3	38.4		
				Endoplasmin Precursor (Heat shock protein 90 kDa beta member 1)(94 kDa glucose-regulated protein)(GRP-94)	Molecular chaperone that functions in the processing and transport of secreted proteins. When associated with CNPY3, required for proper folding of Toll-like receptors. Functions in endoplasmic reticulum associated degradation (ERAD). Has ATPase activity	3	-16.3	4.7	92.7		
				Moesin	Probably involved in connections of major cytoskeletal structures to the plasma membrane.	3	-15.8	6.1	67.6		
				Tubulin beta-5 chain	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.	3	-10.6	4.8	49.6		
				T-complex protein 1 subunit epsilon (TCP-1-epsilon)(CCT-epsilon)	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin	4	-9.5	5.5	59.5		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										IEC Bt	IEC non-Bt
				Aldose reductase (EC 1.1.1.21)(Aldehyde reductase)	Catalyzes the NADPH-dependent reduction of a wide variety of carbonyl-containing compounds to their corresponding alcohols with a broad range of catalytic efficiencies.	6	-8.7	6.3	35.8		
				aldo-keto reductase family 1, member B8	Biological process: oxidation-reduction process	5	-8.2	7.1	36.1		
1627	2.1	5.72	66,495	60 kDa heat shock protein, mitochondrial	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix.	21	-113	5.9	60.9	3.81E+06	1.81E+06
				Stress-70 protein, mitochondrial Precursor (75 kDa glucose-regulated protein)(GRP-75)(Heat shock 70 kDa protein 9)(Peptide-binding protein 74)(PBP74)(mtHSP70)(Mortalin)	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	16	-74.5	5.9	73.7		
				T-complex protein 1 subunit epsilon (TCP-1-epsilon)(CCT-epsilon)	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin	20	-56.5	5.5	59.5		
				inner membrane protein, mitochondrial	Interacts with OPA1, preferentially with the soluble OPA1 form. Component of the MINOS/MitOS complex, that includes IMMT, HSPA9 and CHCHD3 and associates with mitochondrial outer membrane proteins SAMM50, MTX1 and MTX2. Interacts with CHCHD6; the interaction is direct	4	-17.3	5.6	87.1		
2601	2.3	5.64	37,592	Actin, cytoplasmic 1 Actin, cytoplasmic 1	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.	20	-70.3	5.3	41.7	6.29E+06	2.79E+06
				Nucleolin-like protein	Molecular function: nuclic acid binding and nucleotide binding	7	-25.4	5.3	61.7		
				Moesin	Probably involved in connections of major cytoskeletal structures to the plasma membrane.	5	-23.4	6.1	67.6		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										IEC Bt	IEC non-Bt
				Transitional endoplasmic reticulum ATPase	Necessary for the fragmentation of Golgi stacks during mitosis and for their reassembly after mitosis. Involved in the formation of the transitional endoplasmic reticulum . The transfer of membranes from the endoplasmic reticulum to the Golgi apparatus occurs via 50-70 nm transition vesicles which derive from part-rough, part-smooth transitional elements of the endoplasmic reticulum . Vesicle budding from the tER is an ATP-dependent process. The ternary complex containing UFD1L, VCP and NPLOC4 binds ubiquitinated proteins and is necessary for the export of misfolded proteins from the ER to the cytoplasm, where they are degraded by the proteasome. The NPLOC4-UFD1L-VCP complex regulates spindle disassembly at the end of mitosis and is necessary for the formation of a closed nuclear envelope. Regulates E3 ubiquitin-protein ligase activity of RNF19A. Also involved in DNA damage response: recruited to double-strand breaks (DSBs) sites in a RNF8- and RNF168-dependent manner and promotes the recruitment of TP53BP1 at DNA damage sites. Recruited to stalled replication forks by SPRTN: may act by mediating extraction of DNA polymerase eta (POLH) to prevent excessive translesion DNA synthesis and limit the incidence of mutations induced by DNA damage. Component of the VCP/p97-AMFR/gp78 complex that participates in the final step of the sterol-mediated ubiquitination and endoplasmic reticulum-associated degradation (ERAD) of HMGCR.	3	-19.6	5.1	89.3		
				Heat shock protein HSP 90-beta (Heat shock 84 kDa)(HSP 84)(HSP84)	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function	3	-18.2	5	83.2		
1687	2.2	4.93	64,125	Protein disulfide-isomerase	This multifunctional protein catalyzes the formation, breakage and rearrangement of disulfide bonds. At the cell surface, seems to act as a reductase that cleaves disulfide bonds of proteins attached to the cell. May therefore cause structural modifications of exofacial proteins. Inside the cell, seems to form/rearrange disulfide bonds of nascent	36	-129	4.8	56.9	1.62E+06	3.61E+06

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										IEC Bt	IEC non-Bt
					proteins. At high concentrations, functions as a chaperone that inhibits aggregation of misfolded proteins. At low concentrations, facilitates aggregation (anti-chaperone activity). May be involved with other chaperones in the structural modification of the TG precursor in hormone biogenesis. Also acts a structural subunit of various enzymes such as prolyl 4-hydroxylase and microsomal triacylglycerol transfer protein MTTP. Catalyzes the rearrangement of -S-S- bonds in proteins.						
				78 kDa glucose-regulated protein	Probably plays a role in facilitating the assembly of multimeric protein complexes inside the ER.	10	-65.5	5.1	72.3		
				Heat shock-related 70 kDa protein 2 (Heat shock protein 70.2)(Testis-specific heat shock protein-related)	In cooperation with other chaperones, Hsp70s stabilize preexistent proteins against aggregation and mediate the folding of newly translated polypeptides in the cytosol as well as within organelles. These chaperones participate in all these processes through their ability to recognize nonnative conformations of other proteins. They bind extended peptide segments with a net hydrophobic character exposed by polypeptides during translation and membrane translocation, or following stress-induced damage.	5	-34.9	5.5	69.6		
				Vimentin	Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally. Involved with LARP6 in the stabilization of type I collagen mRNAs for CO1A1 and CO1A2	6	-32.9	5.1	53.7		
2204	1.9	7.56	43,287	Annexin A2	Calcium-regulated membrane-binding protein whose affinity for calcium is greatly enhanced by anionic phospholipids. It binds two calcium ions with high affinity.	3	-18	7.5	38.9	6.20E+05	1.15E+06
2882	1.4	5.01	31,609	14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1)(KCIP-1)(Mitochondrial import stimulation factor S1 subunit)	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	14	-58.1	4.7	27.8	2.41E+06	1.72E+06
				14-3-3 protein gamma	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	6	-43.2	4.8	28.3		

Spot No.	Fold	Experimental pI	Experimental MW	Protein Description	Function	rI	log(e)	Theoritical pI	Theoritical MW (KiloDaltons)	Average Normalised Volumes	
										IEC Bt	IEC non-Bt
				14-3-3 protein eta	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner. Negatively regulates the kinase activity of PDPK1	6	-39.6	4.8	28.2		
				14-3-3 protein theta (14-3-3 protein tau)	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner. Negatively regulates the kinase activity of PDPK1	9	-39.2	4.7	27.8		



Appendix 33: Functional classifications of differentially expressed intestinal epithelial cells proteins (IECs and HCT116).
A total of 152 proteins were identified and these proteins fall within 27 major functional categories. 9 (6%) contain oxidoreductase activity, 31 (20%) are chaperones, 4 (3%) contained transferase activity, 34 (22%) contained structural molecule activity and 3 (2%) are ribonucleoproteins.

Appendix 34: Functional classification of the identified protein spots when comparing IE cells and HCT116 cell line.

Molecular function	number of proteins	name of protein	Spot No.	Groups
structural molecule activity	1	Fibronectin Precursor (FN)	814	HCT116 Bt vs IECs Bt
	2	Actin, cytoplasmic 1 Actin, cytoplasmic 1	2597, 2601	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt
	1	filamin-A	814	HCT116 Bt vs IECs Bt
	2	Vinculin (Metavinculin)	814, 914	HCT116 Bt vs IECs Bt
	1	filamin, beta	814	HCT116 Bt vs IECs Bt
	1	prelamin-A/C isoform C2	1561	HCT116 Bt vs IECs Bt
	2	Lamin-B1	2547, 2600	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt, IECs Bt vs IECs non-Bt
	2	Nuclear migration protein nudC	2547, 2262	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt
	5	Tubulin beta-5 chain	2547, 2550, 2590, 2877, 3360	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt, IECs Bt vs IECs non-Bt
	1	tubulin, alpha 1B (Tuba1b)	1706	HCT116 Bt vs IECs Bt
	3	Tubulin beta-2C chain	2550, 2590, 2877	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt
	3	Moesin	2547, 2370, 2601	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt
	1	Plectin-1	3634	HCT116 Bt vs IECs Bt
	1	tubulin, alpha 1B (Tuba1b), mRNA	2262	HCT116 Bt vs IECs Bt
	1	Ezrin	3391	HCT116 Bt vs IECs Bt
	6	Vimentin	1891, 2262, 2170, 1650, 1895, 1687	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt, IECs Bt vs IECs non-Bt
	1	Myosin-9	814	HCT116 Bt vs IECs Bt
chaperones	1	Endoplasmin Precursor (Heat shock protein 90 kDa beta member 1)	2547	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt
	7	Heat shock protein HSP 90-beta	2547, 2262, 914, 3391, 2170, 2877, 2601	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt, IECs Bt vs IECs non-Bt
	4	Heat shock protein HSP 90-alpha	2547, 2262, 914, 2590	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt
	2	T-complex protein 1 subunit epsilon	2547, 1627	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt
	1	T-complex protein 1 subunit alpha	3391	HCT116 Bt vs IECs Bt

	5	60 kDa heat shock protein, mitochondrial	3634, 2262, 2370,3120, 1627	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt, IECs Bt vs IECs non-Bt
	1	Heat shock-related 70 kDa protein 2	1687	IECs Bt vs IECs non-Bt
	10	Stress-70 protein, mitochondrial Precursor	2393, 2547, 3634, 1561, 1615, 1954, 2098, 2600, 2877, 1627	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt, IECs Bt vs IECs non-Bt
Oxidoreductase	1	L-lactate dehydrogenase B chain	2547	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt
	1	aldo-keto reductase family 1, member B8	2547	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt
	1	NADH dehydrogenase flavoprotein 2, mitochondrial	3137	HCT116 Bt vs IECs Bt
	1	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial Precursor	2550	HCT116 Bt vs IECs Bt
	1	L-lactate dehydrogenase B chain	2550	HCT116 Bt vs IECs Bt
	3	Aldose reductase	2547, 2550, 2600	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt , IECs Bt vs IECs non-Bt
	1	Malate dehydrogenase, mitochondrial	3657	HCT116 Bt vs IECs Bt
Antioxidant	2	Peroxiredoxin-4	2987, 3309	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt
	3	Peroxiredoxin-1	3524, 3309	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt
Protein disulfide isomerase activity	2	Protein disulfide-isomerase A3	2817, 1706	HCT116 Bt vs IECs Bt
	2	Protein disulfide-isomerase	1650, 1687	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt , IECs Bt vs IECs non-Bt
Kinase	4	Pyruvate kinase isozymes M1/M2	2547, 3653, 2262, 2987	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt
Initiation factor in Protein biosynthesis	1	Eukaryotic translation initiation factor 3 subunit I (eIF3i)	3391	HCT116 Bt vs IECs Bt

chloride channel activity	2	Chloride intracellular channel protein 1	2817, 2987	HCT116 Bt vs IECs Bt
Transferase	3	S-adenosylmethionine synthetase isoform type-2	2547, 2590, 2600	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt, IECs Bt vs IECs non-Bt
	1	Transketolase	2401	HCT116 Bt vs IECs Bt
Response to stress	1	heat shock protein 4 like	2600	HCT116 non-Bt vs IECs non-Bt
Ribonucleoprotein	1	40S ribosomal protein SA	3391	HCT116 Bt vs IECs Bt
	1	60S acidic ribosomal protein P0	2550	HCT116 Bt vs IECs Bt
	1	ErbB3-binding protein 1	3436	HCT116 Bt vs IECs Bt
Muscle protein	1	Tropomyosin alpha-4 chain	2786	HCT116 Bt vs IECs Bt
	3	Tropomyosin alpha-1 chain	2786, 3663	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt
	1	Tropomyosin beta chain	3667	HCT116 non-Bt vs IECs non-Bt
	4	Tropomyosin alpha-3 chain	2786, 3663	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt
Hydrolase	1	Proteasome subunit alpha type-6	3653	HCT116 Bt vs IECs Bt
	1	Transitional endoplasmic reticulum ATPase	2601	IECs Bt vs IECs non-Bt
	2	Ubiquitin carboxyl-terminal hydrolase isozyme L1	3667, 3120	HCT116 non-Bt vs IECs non-Bt
	2	ATP synthase subunit beta, mitochondrial	1891, 1895	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt
translation elongation factor activity	4	Elongation factor 2	2393, 2401, 2648, 2370	HCT116 Bt vs IECs Bt
ATP binding	5	78 kDa glucose-regulated protein	814, 1561, 1615, 1650, 1687	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt, IECs Bt vs IECs non-Bt
	1	beta-actin-like protein 2	2170	HCT116 Bt vs IECs Bt
calcium ion binding	1	Spectrin alpha chain, brain	814	HCT116 Bt vs IECs Bt
	2	Annexin A1	3137, 2987	HCT116 Bt vs IECs Bt
	2	Annexin A2	3653, 2204	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt
mitochondrial calcium ion homeostasis	2	inner membrane protein, mitochondrial	2393, 1627	HCT116 Bt vs IECs Bt, IECs Bt vs IECs non-Bt

Host cell receptor for virus entry	1	integrin alpha V Gene	814	HCT116 Bt vs IECs Bt
ferric iron binding	4	Lactotransferrin	1561, 1043, 2098, 1706	HCT116 Bt vs IECs Bt
nucleotide binding	1	Non-POU domain-containing octamer-binding protein	3436	HCT116 Bt vs IECs Bt
	2	Nucleolin-like protein	3120, 2601	HCT116 non-Bt vs IECs non-Bt , IECs Bt vs IECs non-Bt
GTPase activity	2	Ras-related protein Rab-11B	3137, 3667	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt
mRNA splicing	1	Serine-threonine kinase receptor-associated protein	2817	HCT116 Bt vs IECs Bt
protein kinase C inhibitor activity	1	14-3-3 protein epsilon	2817	HCT116 Bt vs IECs Bt
Lyase	1	Alpha-enolase	2987	HCT116 Bt vs IECs Bt
	1	aldolase A-like 1	3657	HCT116 Bt vs IECs Bt
protein domain specific binding	4	14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1)	2987, 3309, 3667, 2882	HCT116 Bt vs IECs Bt, HCT116 non-Bt vs IECs non-Bt, IECs Bt vs IECs non-Bt
	1	14-3-3 protein theta	2882	IECs Bt vs IECs non-Bt
actin binding	2	14-3-3 protein gamma	2877, 2882	HCT116 non-Bt vs IECs non-Bt, IECs Bt vs IECs non-Bt
sodium channel regulator activity	1	14-3-3 protein eta	2882	IECs Bt vs IECs non-Bt