Hormonal effects of prohomones in bovines

Microarrays: a screening tool for prohormone abuse

Name:	Mans Broekgaarden
Student number:	1172338
Graduate school:	Hogeschool Utrecht
	Faculty of Nature and Technology
	Institute for Life Sciences and Chemistry
	Department of Molecular Biology.
	P.O. Box 13272
	3507 LG Utrecht.
Period:	January 22Th
	August 31Th
Location:	RIKILT, Institute of Food Safety, Wageningen University and Research centre.
	P.O. box 230
	6700 AE Wageningen.
	Cluster Toxicology and Effectmonitoring.
Supervisors:	Jeroen Rijk.
	Geert Hoelen

Preface

Dear reader,

Between Januari and September, I've worked on my bachelor thesis project at RIKILT, Institute of Food Safety, in the cluster Toxicology and Effectmonitoring. Here I've worked under the supervision of Jeroen Rijk on the project "the hormonal effects of prohormones" which focuses on the growth promoting effects of prohormones, as well as detection on the abuse of these compounds. During this time I have learned much and would like to thank several people who made my work a lot easier. First of all, I would like to thank my supervisor Jeroen Rijk, and I would furthermore like to thank Hakan Baykus, Jenneke Poortman and clusterleader Ad van Peijnenburg. I would also like to thank everyone else of the cluster Toxicology and Effectmonitoring for their hospitality, kindness, and of course, the cake.

With kind regards,

Mans Broekgaarden.

Summary.

Since 1988, it has been prohibited in the European Union to use hormonally active compounds in cattle fattening. Reasons for the ban on the use of these compounds are the food quality and the health risks for consumers. During livestock inspections however, the use of so called prohormones turns up more and more. Prohormones are compounds without hormonal action by themselves, but are either metabolized in vivo into hormonal active compounds, or are able to indirectly influence natural hormone levels. Screening on these compounds is difficult due to the lack of detection methods and the metabolites of these compounds are unknown. A prohormone which is suspected to be used as a growth promoter is dehydroepiandrosterone (DHEA). DHEA is a steroid which is endogenously produced in the adrenal gland, and can be converted in peripheral target tissues. Another prohormone is ipriflavone (IF). Ipriflavone is a synthetic isoflavone which has a chemical structure comparable to hormonal active isoflavones and is used in humans to increase bone mass.

In this study we will focus on the feasibility of using microarrays as a new method to screen on prohormone abuse in bovines. Secondly, we will look at the effects of prohormones on gene expression profiles. Thirdly, we will validate a previously conducted and present microarray experiment by means of real-time RT-PCR. Finally, we made a start with testing the in vitro effects of prohormones on human liver carcinoma cell lines.

Animal experiments were conducted by exposure of Frisian bovines to DHEA and IF. Seven bovines from differently conducted experiments were selected as control animals and did not receive any administration. Six animal were exposed to oral or intramuscular administrated DHEA in three separately conducted experiments. Eight animals were treated with different concententration of orally administrated IF. RNA was extracted from the treated and untreated animals, labeled with fluorescent dyes and hybridized onto bovine arrays. Data analysis was done with PCA, gene selection and pathway analysis. Real time RT-PCR was performed to validate a previously conducted microarray experiment and the present microarray experiment studying gene expression profiles in bovine blood tissue. Anti-proliferative effects of DHEA on the human liver carcinoma cell line HepG2, were measured using the dimethylthiazol-diphenyl-tetrazoliumbromide (MTT) test.

Results obtained from the first experiment concluded that no great differences are observed between DHEA treated and untreated animals in blood tissue. This experiment provided insight into the feasibility of microarrays as a screening method on prohormone. The second experiment indicated that the exposure to IF induces large differences between treated and untreated animals. The gene expression profile further exhibits prohormonal action by regulating many steroid metabolism and biosynthesis related genes. RT-PCR validated a previously conducted research to the gene expression profile in liver induced by DHEA but failed to validate the microarray study of DHEA induced gene expression in blood tissue. Finally, a setup was made for future research on in vitro effects of prohormones.

Table of contents.

Preface. 2

Summary. 3

Table of contents. 4

Abbreviations. 6

1. Introduction. 9

1.1. Growth promoters. 9

1.2. Prohormones. 12

1.2.1. DHEA. 12

1.2.2 Ipriflavone. 14

1.3. Microarray technology and real-time RT-PCR technology. 14

1.4. Aims and outline of the research project. 17

1.4.1. Aims 17

1.4.2. Outline of the research project. 18

2. Materials and methods. 19

2.1. Chemicals and solutions. 19

2.2. Microarray experiments. 19

2.2.1. Animal experiments. 19

2.2.2. RNA extractions. 20

2.2.3. Microarray hybridization. 22

2.2.4. Data analysis 23

2.3. Real-time RT-PCR 23

2.4. Setup for in vitro experiments. 24

2.4.1. Cell culturing and exposure. 24

2.4.2. MTT-test. 23

2.4.2. Protein assay. 25

3. Results and discussion. 26

Part 1: Biomarkers for DHEA abuse in bovine blood. 26

3.1. Blood RNA. 26

3.2. DHEA microarray experiment. 28

3.2.1. RNA labeling. 28

3.2.2. Hybridization quality control. 29

3.2.3. Principal components analysis. 31

3.2.4. Gene selection. 33

3.2.5. Pathway analysis. 36

Part 2: Biomarkers for ipriflavone abuse in bovine blood. **39**
3.3. RNA from liver biopsies. **39**
3.4. Ipriflavone microarray experiment. **40**
3.4.1. RNA labeling. **40**
3.4.2. Hybridization quality control. **41**
3.4.3. Principal components analysis. **42**
3.4.4. Gene selection. **43**
3.4.5. Pathway analysis. **44**
Part 3: Real-time RT-PCR. **46**
3.5. Validation: DHEA induced gene expression in bovine livers. **47**
3.6. Validation: DHEA induced gene expression in bovine blood. **49**
Part 4: Setup for in vitro experiments. **51**
3.7. Growth inhibition assay. **51**
3.8. HepG2 exposure to DHEA. **52**
4. Conclusions. **54**
4.1. Biomarkers for DHEA abuse in bovine blood. **54**
4.2. Biomarkers for ipriflavone abuse in bovine liver. **55**
4.3. Real-time RT-PCR. **56**
4.4. Setup for in vitro experiments. **56**
References. **57**
Appendices. **63**
A. Protocols. **64**
B. Microarray scans. **85**
C. DHEA induced gene expression in bovine blood. **93**
1. Total control group compared to exposed group. **93**
2. Control group 1 compared to exposed group. **95**
3. Control group 2 compared to exposed group. **105**
E. Primers designed for RT-PCR validation. **124**

List of abbreviations.

3β-HSD	3-bèta hydroxysteroid dehydrogenase
17β-HSD	17-bèta hydroxysteroid dehydrogenase
µg	microgram
µl	microliter
A	absorbance
ACTH	adrenocorticotropin
AR	androgen receptor
ATCC	American type culture collection
AVP	arginine vasodepressin
BSA	bovine serum albumin
bp	basepairs
°C	degrees Celsius
C	control
C1	control group 1
C2	control group 2
cDNA	copy DNA
CRH	corticotropin releasing hormone
Ct	threshold cycle
Ctot	total control group
Cy3	cyanine-3-cytosinetriphosphate
Cy5	cyanine-5-cytosinetriphosphate
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone-sulphate
DHT	dihydrotestosterone
DMEM	Dulbecco's Eagle's modified medium
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
ds	double stranded
EDTA	ethylenediamine tetraacetic acid
ER	estrogen receptor
Exp	total exposed DHEA group
FBS	fetal bovine serum
FSH	follicle stimulating hormone
g	gram
GH	growth hormone
GHR	growth hormone receptor

h	hour
HBSS	Hank's balanced salts solution
IM	Intramuscular
IF	ipriflavone
IFG1	insulin like growth factor 1
Kg	kilogram
L-Glut	L-glutamine
M1-MV7	ipriflavone metabolites 1 to 7
MGA	melengestrol acetate
Min	minute(s)
Ml	millilitre
MS	mass spectrometry
MTT	dimethylthiazol diphenyl tetrazoliumbromide
ng	nanogram
nm	nanometer
NEAA	non-essential amino acids
O	oral
P450ssc	cytochrome P450 desmolase
PBS	phosphate buffered saline
PCR	polymerase chain reaction
Pmol	picomol
QTOF MS	quadrupole time-of-flight
RNA	ribonucleic acid
rpm	rotations per minute
rRNA	ribosomal RNA
RT	reverse transcriptase
RT-PCR	real-time reverse transcriptase polymerase chain reaction
S	Svedberg
SDS	sodium dodecyl sulphate
ss	single stranded
THG	tetrahydrogestrinone
Tm	melting temperature
UPLC	ultra performance liquid chromatography
V	Volt

1. Introduction.

1.1 Growth promoters.

In the European Union the use of growth promoting compounds is prohibited in cattle fattening according to European Council directive 96/22/EC. Estrogens, androgens, gestagens, thyreostats and bèta-agonists are mentioned specifically in this directive. The illegality of these compounds is based on the account that the residues they leave in meat and other products of animal origin may be dangerous for consumers and may also affect the quality of these foodstuffs [1]. Although the council directive specifically names the use of the previously mentioned compounds, it also mentions that basically all compounds that have hormonal activity are illegal. The general principle of a hormone is that a compound is produced by endocrine cells which release it into the circulatory system. When it reaches a target cell, it binds to its corresponding receptor and induces a signal specific for this hormone [52] (Figure 1).

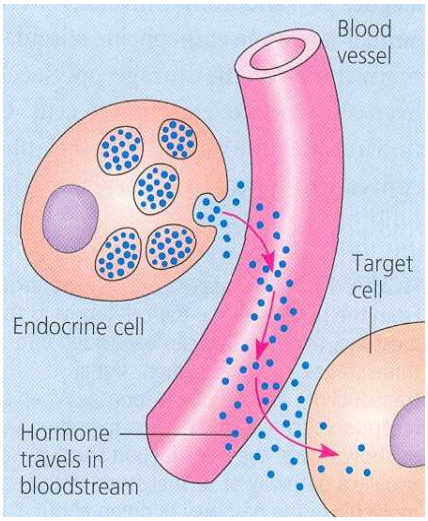


Figure 1: The general principle of a hormonal effect is that a hormone is produced by endocrine cells, which releases the compound in the bloodstream. When the target cell is encountered it binds to the hormone receptor and induces a signal.

Since 1988, when all growth promoting compounds were declared illegal for use in cattle, the number of reported positives became very small [16,18]. However the examination on preparation samples (needles, vials, syringes etc. Figure 2) contrasted on these promising results. The results showed that steroids and beta-agonists are still used, more and more directed to (esters of) natural hormones. The results also show that a large number of products were found that had never been used before in the field of growth promotion [16]. Difficulties in the detection of these compounds are:

- The use of exogenous administration of natural hormones is hard to determine.
- The use of low dosages and combinations of compounds (e.g. different kinds of hormonesin the same cocktail).
- The use of compounds known to be hard to analyze because of strong metabolism and metabolites might be unknown.
- The use of compounds known not to be examined in the involved country.[3]

Screening on synthetic compounds (often much more active than natural compounds) is relatively easy due to the screening on metabolites and residues on compounds that are not found in nature. However, the use of natural compounds as growth promoters is a growing problem. In illegal preparations used for

growth promotion the natural hormones have become the most popular ingredient. Although less effective, exogenous administration of natural hormones leads to the same hormones and metabolites as the endogenous produced hormones. This makes conventional screening difficult, and the determination of natural growth promoter abuse needs to be determined by quantitative measurements. The difficulty is that there is a wide variation in the concentration of these hormones due to animal species, age, sex and physiological state. If no decision levels exist for the concentration levels of natural sex steroid hormones, another quantitative measurement using other parameters has to be performed. This can be done by using a metabolite or biosynthetic precursor of the hormone. For instance, the ratio 17β-testosterone/17α-testosterone increases after administration of 17β-testosterone. It is difficult however, to present absolute evidence of illegal use based upon the quantification of these natural compounds due to biological variability of endogenous hormone levels [16]. So since the use of growth promoting compounds still continues to be a problem, it is therefore necessary to develop new screening methods to determine the use and abuse of growth promoting compounds.

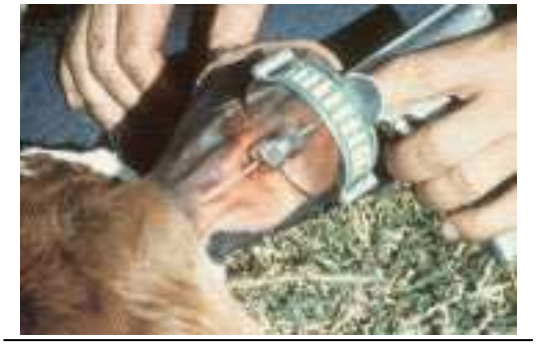


Figure 2: Hormone implants used for cattle fattening in the USA. The implant is attached to the ear which is removed when the animal is slaughtered.

One class of natural hormonal compounds used in cattle fattening are androgens. Androgens are endogenous sex steroid hormones, are mainly synthesized by the testes, and stimulate the development of the male secondary sex characteristics as well as muscle growth and spermatogenesis [2]. Testosterone and other androgens are considered to be classical anabolic agents in humans, and are known for their abuse in sports [12]. The androgen receptor (AR) is stimulated by androgens, and is a nuclear receptor which acts as a transcription factor, regulating gene expression [54]. In bovids like cattle and sheep, androgens are less active corresponding to lower androgen receptor concentrations in muscle, which is, on average, only one quarter of the concentration of estrogen receptor [3,13]. Androgens are known for stimulation of growth hormone (GH) and insulin like growth factor 1 (IFG1) secretion [14]. Residues in meat might lead to unwanted androgenic and anabolic effects, as well an increased risk of prostate and testicular cancer.

Like androgens, estrogens are endogenous sex steroid hormones, mainly synthesized in the ovaries under the influence of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Figure 3), which stimulate the development of the female secondary sex characteristics and fat deposition [2]. Estrogens exert their action by binding to estrogen receptors (ER) alpha and beta. ER α and β are, like ARs, intracellular nuclear hormone receptors which act as a transcription factor when stimulated, regulating gene expression [53]. Activation of the estrogen receptors results in indirect anabolic effects by stimulating growth hormone (GH) secretion [5], growth hormone receptor (GHR) levels in liver, and insulin-like growth factor 1 (IGF1) levels in

blood [6-7]. In bone, liver and muscle tissue, estrogens seem to be active to improve protein anabolism and mineral retention [3, 8,9]. Animals with low endogenous estrogen production (calves, lambs, heifers, steers) respond well to exogenous estrogens and enhance growth performance by 5-15% [3,4]. In cattle fattening, additional application of estrogens in anabolic preparations to increase live-weight gain leaves greater amounts of residues of estrogens in edible tissues [10]. For the consumer, consumption of this tissue might lead to unwanted estrogenic effects as well as an increased risk of cancer, because estrogens, as well as their metabolites, are known to promote different types of cancer [11].

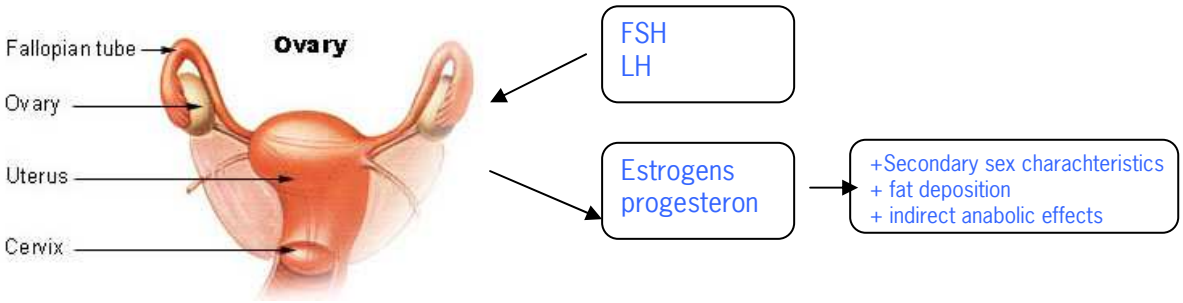


Figure 3: Estrogens are mainly produced by the ovaries influenced by Follicle stimulating hormone(FSH) and luteinizing hormone (LH). Estrogenic effects are the development of the secondary sex characteristics, increased fat deposition and indirect anabolic effects.

Progesterone is the only natural gestagen and is also, like estrogens and androgens, a sex steroid hormone. It is related to pregnancy, prepares the reproductive tract for zygote implantation, maintains the subsequent pregnant state and gestagens are also known to stimulate the basal metabolic rate [2]. Gestagens exert their action by binding to the intracellular progestin receptor. Although the growth promoting effect of progesterone is unclear [3], synthetic gestagens like melengestrol (MGA) acetate are known to stimulate the synthesis of endogenous estradiol [15]. Anabolic effects of MGA are dose dependent and are only observed in non-pregnant heifers with intact ovaries [3].

Thyreostats are a group of compounds that affect the function of the thyroid gland which as a consequence, lower the circulating levels of thyroid hormones. This reduces basal metabolism, lowers gastro-intestinal motility and increases extracellular water retention. Therefore, the consumer is misled (meat contains abnormal quantities of water), and the quality of the meat is inferior [16]. Consumption of residues in meat may also lead to similar effects in the consumer.

Beta-agonists act on the adrenergic receptor causing smooth muscle relaxation and are used by asthma patients. Beta agonists can be used in cattle for several reasons, including the induction of growth. In high doses they can exhibit anabolic effects and can induce hypertrophy by decreasing muscular degradation and fat synthesis [16,17] As a result, the ratio of muscle/fat is increased, with an overall increase in growth [17].

1.2. Prohormones.

During inspection of livestock farms in the Netherlands, feed additives and preparations containing prohormones, synthetic as well as (concentrations of) natural compounds, occasionally turn up. Prohormones are compounds without hormonal activity by themselves, but can influence natural hormone levels. Prohormones are metabolized *in vivo* into more active compounds, or affect endogenous hormone levels indirectly by e.g. regulating enzymes involved in hormone biosynthesis. Several types of prohormones exist, related to their origin and effects. For instance, precursors of endogenous hormones, phytosterols, ecdysteroids, and herbal extracts can all act as prohormones. The difficulty in the screening on prohormones is that the effects of these compounds and their metabolism are practically unknown. For these reasons, prohormones now have a short history in sports and exercise to be used as doping while doping tests remained negative. One of the first prohormones introduced was the endogenous precursor of testosterone, dehydroepiandrosterone (DHEA), which was introduced in 1996 [19].

1.2.1 DHEA.

As mentioned, dehydroepiandrosterone (DHEA) is a prohormone used in sports to enhance performance and promote muscle growth. DHEA is an endogenous precursor of sex steroid hormones and together with its sulfate ester DHEAS it is the most abundant steroid found in humans. DHEA and DHEAS are mainly produced in the zona reticularis of the adrenal gland (Figure 4). It is related to many functions and positive effects on diseases, but in general, the long term effects of DHEA use is unclear [20]. Because of its potential anabolic effects, DHEA is also on the World Anti Doping Agency's list of banned substances [24] and is used as a growth promoter in cattle.

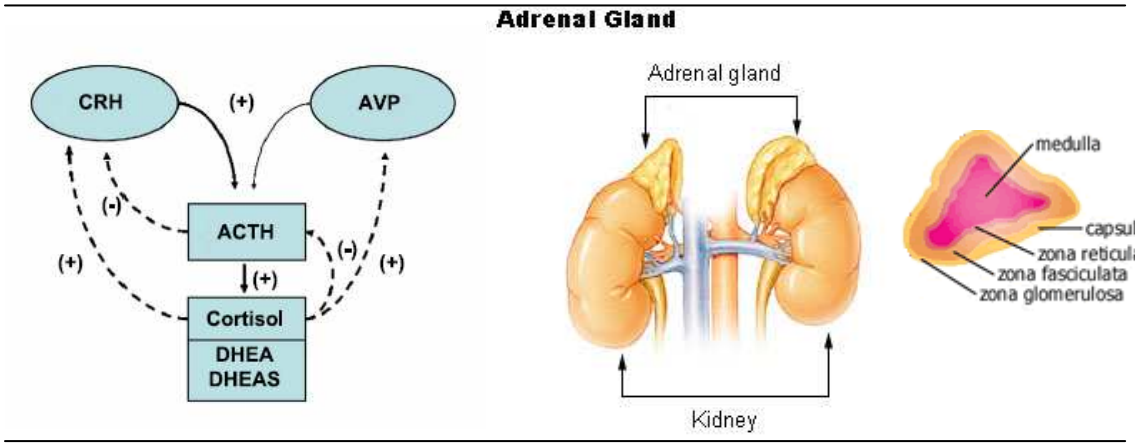


Figure 4: On the left, the endocrine regulation of DHEA biosynthesis is shown. CRH and AVP stimulate the release of ACTH, which in its turn stimulated the release of DHEA, DHEAS and cortisol. Cortisol in its turn regulated the release of CRH, AVP and ACTH. On the right, the adrenal glands, which lie on top of the kidneys and the zona retiularis, where DHEA and DHEAS biosynthesis takes place, are shown.

Corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) stimulate the pituitary gland to release adrenocorticotropin (ACTH), which stimulates the adrenal cortex to secrete DHEA, DHEAS and cortisol, which provides the negative feedback on CRH, AVP and ACTH. After secretion in blood, peripheral target organs metabolize DHEA and DHEAS into more hormonal active compounds [56]. The biosynthesis of DHEA starts with extra mitochondrial cholesterol and mitochondrial desmolase (P450scc). Extra mitochondrial cholesterol binds a StAR protein, which transports it into the mitochondia where it is converted by P450ssc desmolase to pregnenolone. Pregnenolone is transported to the cytosol where further metabolism a takes place. P450 17α-hydroxylase catalyzes the 2-step oxidation reaction of pregnenolone into respectively 17α-OH-pregnenolone and DHEA. Pregenenolone and 17α-OH-pregnenolone can be converted in the adrenal gland by membrane bound 3β-hydroxysteroid dehydrogenases (3β-HSDs) into respectively progesterone and 17α-OH-progesterone [55]. DHEA is partly converted into its sulfate ester DHEAS and these compounds are released into the bloodstream to be converted in peripheral tissue into more hormonal active steroids [20].

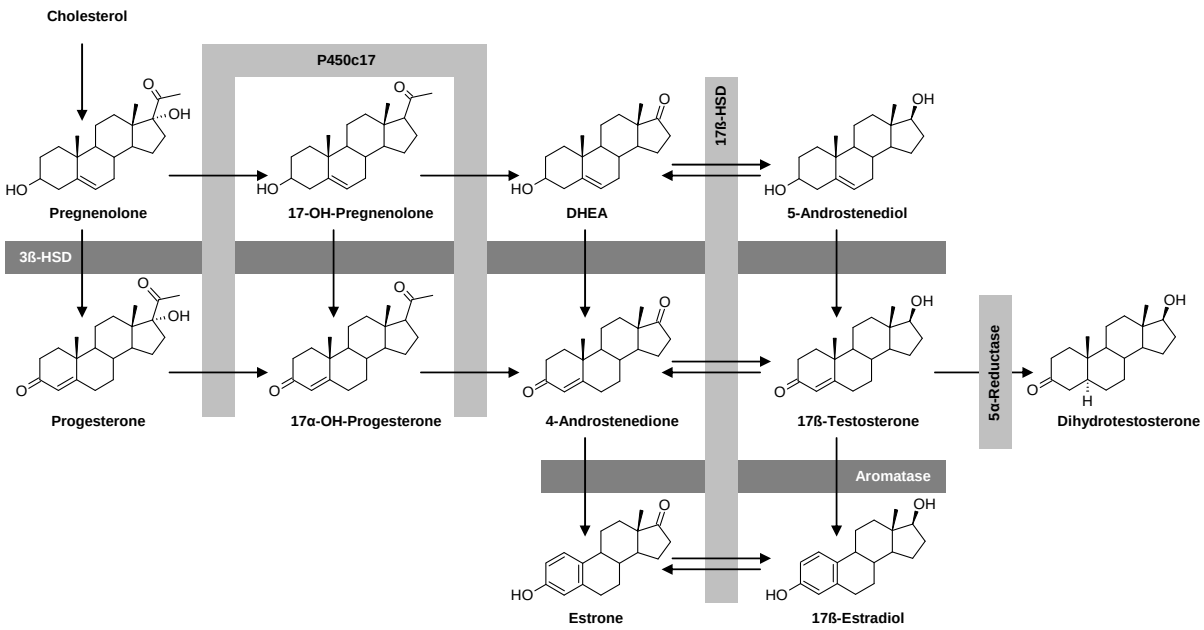


Figure 5: The steroidogenic biochemical pathway, starting with the conversion of cholesterol in pregnenolone. Further downstream the pathway we see DHEA which can be further metabolized into androgens and estrogens.

In these peripheral target organs, mainly the gonads, DHEA can be converted by 3β-HSD into 4-androstenedione, which can be further metabolized by *aromatase* into estrone. DHEA, 4-androstenedione and estrone can be further metabolized by several forms of 17β-Hydroxysteroid dehydrogenases (17β-HSD) into respectively 4-androstenediol, 17β-testosterone and 17β estradiol. 4-androstenediol can also be converted into 17β-testosterone by 3β-HSD and 17β-testosterone can be further aromatized into 17β-estradiol by aromatase. A further 5α-reduction leads to the conversion of 17β-testosterone into dihydrotestosterone (DHT) [55] (Figure 5). The conversion of DHEA into 17β-estradiol and 17β-testosterone in the ovaries and gonads is regulated by the pituitary hormones LH and FSH.

1.2.2 Ipriflavone.

The second prohormone we will discuss is ipriflavone (IF). In the 1930's estrogenic effects observed in cattle were related to the intake of isoflavones. Isoflavones are found in high quantities in soy (*Glycine max*) and possess weak estrogenic activity. In 1969 a research project synthesized a number of isoflavones that could exhibit anabolic but not estrogenic activity. Among these was IF which was selected for its calcium retaining properties [25, 26]. Ipriflavone was later used in research to bone related diseases such as osteoporosis and osteogenesis imperfecta [25, 27]. Presently, ipriflavone is used as a bone building isoflavone for its properties to inhibit bone resorption and promote bone formation [27]. Ipriflavone is metabolized in the liver and seven metabolites have been identified (M1 – M7). Except from metabolite M2, which is also known as daidzein, little is known about the effects of these metabolites. In figure 6 an overview of the metabolites and the involved enzymes is shown.

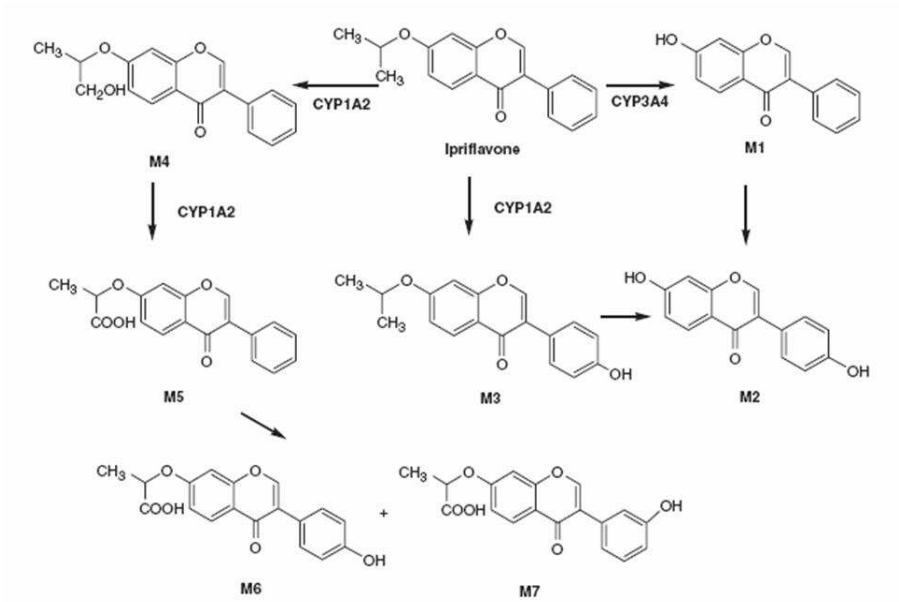


Figure 6: Metabolic pathway of ipriflavone, which is metabolized in human liver. Seven metabolites have been identified. M2 is also known as daidzein and is known to possess estrogenic effects.

In figure 6 we can see that ipriflavone is metabolized by cytochrome p450 enzymes. In humans, metabolites M1, M1 (Daidzein) M3 and M5 seem predominate. Metabolite M2 or daidzein is structurally related to genistein, an isoflavone which is also known for its weak estrogenic effects [25, 28]. The positive effects on bone metabolism and estrogenic effects of its metabolites makes this compound an interesting compound in cattle fattening. Therefore IF is suspected of being used as a growth promoting compound.

1.3 Microarray and real-time RT-PCR technology.

In a microarray study by *Labrie et al (2006)*, DHEA was identified as an anabolic steroid just like dihydrotestosterone (DHT) and tetrahydrogestrinone (THG) [23]. In this experiment, gonadectomized male

mice were exposed to a single high dose of DHEA or DHT. After 24 hours the mice were sacrificed and tissue samples were taken. The gene expression profile was examined and compared to that of DHT. The experiment concluded that DHEA induces a gene expression profile similar to DHT. In a study by *Reiter et al (2007)*, the gene expression profiles of bovines treated with melengestrol acetate, trenbolone acetate and zeranol were studied by using real-time Reverse Transcriptase Polymerase Chain Reaction (real-time RT-PCR) technology. The results of this study provided useful information on the identification of possible biomarkers for alternative screening on hormone abuse [40]. These two studies focused on the changes in gene expression profiles using microarray and RT-PCR technology, and presented results that emphasize the usefulness these technologies to study the effects of hormones and prohormones.

A relatively new tool in gene expression research is the use of DNA microarrays. DNA microarrays are assays for quantifying the types and amounts of mRNA transcripts in a RNA extraction. The amount of mRNA from a specific gene corresponds to the expression of that gene. In practice, RNA is extracted from a specific sample, which is then converted to a form of fluorescently labeled polynucleotides which is hybridized onto the array. The microarray itself consists of a solid surface on which single stranded copy DNA (ss cDNA) is immobilized in specified positions. These probes are basically printed using ink-jet technology and are referred to as probes. The labeled polynucleotides will bind by hybridization to the probes with which they share sufficient sequence complementarity. After hybridization, the unbound polynucleotides are removed by washing. The amount of polynucleotides bound to the probes is measured using a fluorescence microarray scanner and is an estimate of the expression of the gene represented by that probe [29] (see Figure 7).

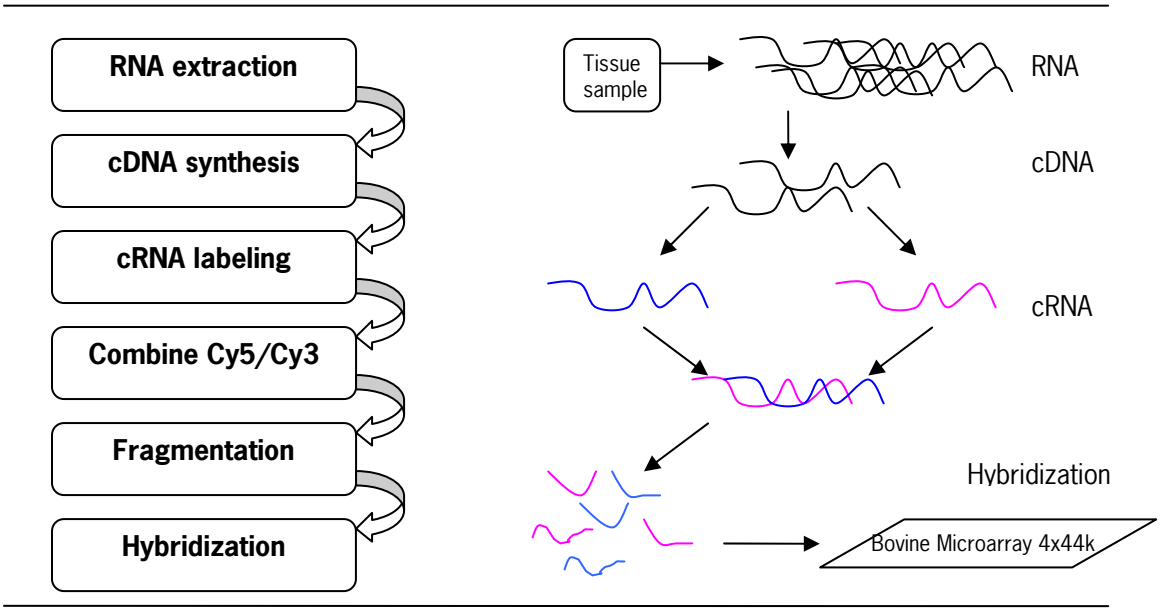


Figure 7: Overview and flow-chart of the microarray procedure. RNA is extracted from tissue, which is reverse transcribed into cDNA. The cDNA is used to synthesize labeled cRNA using labels Cy3 and Cy5. Equal amounts of Cy5/Cy3 cRNA are combined and fragmented prior to hybridization onto the microarray.

Microarray experiments should be carefully planned and the design should be selected for the desired data. Many microarray experiment designs exist, where each design compares samples and groups in a different way. One of the most-used designs is the balanced block design which compares two RNA samples to each other on one array. Another frequently used design is the reference design, where all RNA samples are hybridized together with a random RNA sample, which is the same on each array, and used to normalize the target RNA signals. A third design is the control reference design. This design uses the principle of the reference design to normalize microarray signals using a pool of samples. However the control reference design uses a pool of control samples as a reference to normalize the target (treated and control) samples. This design is used to minimize competition of target and reference RNA for on the array, for regulated genes. This makes this design particularly interesting for screening on exogenous administration of natural compounds. All the discussed designs are shown in Figure 8.

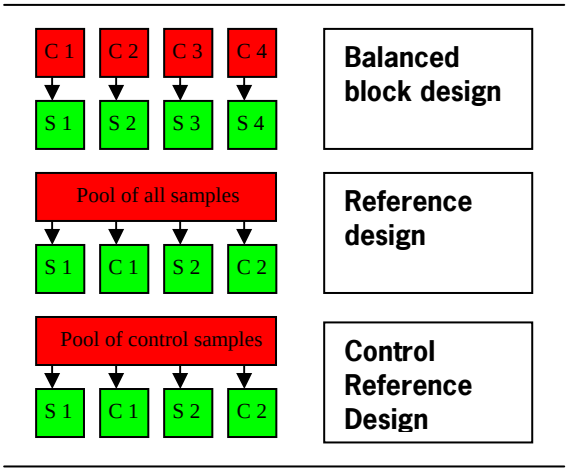


Figure 8: Three types of common microarray designs. C and S indicate respectively controls and samples, red and green indicate the different dyes.

Because many microarray platforms excist, it is often difficult to comparing microarray results. Therefore, real-time Reverse transcriptase Polymerase chain reaction is often used to validate microarray experiments [58].Reverse transcription (RT) is used combination with Polymerase Chain Reaction (PCR) to quantify gene expression. Reverse transcriptase is used to reverse transcribe RNA into copy DNA (cDNA). The cDNA is then added to a reaction mixture containing small oligonucleotide sequences that bind to a specific gene (primers), a DNA polymerase which is stable at high temperatures in a optimal reaction buffer with deoxynucleotides (dNTP's). During a PCR cycle, the cDNA is denatatured in the first step at a high temperature (96°C), during the second step the temperature is lowered (60°C) to induce primer annealing to the ssDNA strands and to activate DNA polymerase to bind to the primer-annealed DNA strand, the DNA polymerase then synthesizes double stranded DNA (dsDNA). The reaction then enters the next cycle, where the dsDNA is denatured again at a high temperature. A PCR reaction usually consists of many cycles, where DNA is amplified during each cycle.

Real-time PCR, according to the SYBR®green method, uses a fluorescent dye which binds specifically to dsDNA. The dye molecules bind to the minor groove of the dsDNA during the amplification cycles, and after each cycle the fluorescence is measured and recorded. For an overview of the practical procedure, see figure 9. Real-time RT-PCR data analysis can be done by measuring the cycle in which a certain threshold is reached. The threshold is usually reached when the DNA amplification reaches the exponential phase of amplification. This Cycle threshold (Ct) value can be used as a quantitative value for DNA concentration. The 2-ΔΔCt method uses target and reference genes which are both amplified using real-time RT-PCR. A

template dilution is made to obtain □Ct values indicating the difference in Ct-value for a certain dilution. A plot of the □Ct values versus the log dilution is used to relatively quantify the target and reference gene concentrations. The □Ct value of the target gene and the reference gene can be used to obtain a □□Ct value which corresponds to a normalized relative DNA concentration. Ratios between control and experimental □□Ct values can be 2-log transformed to obtain fold changes in gene expression [57]. This method is often used to study gene expression and used as a confirmation method of DNA microarray experiments.

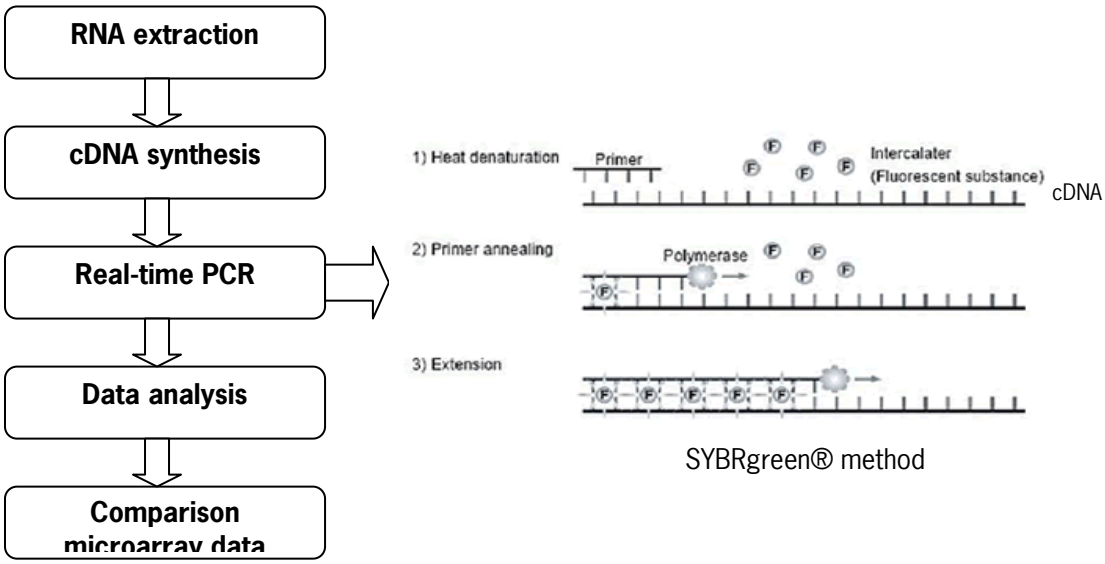


Figure 9: The principle of a two-step SYBRgreen real-time PCR. First, cDNA is made from isolated mRNA. During the PCR, dsDNA is made and the SYBRgreen dye molecules bind to the DNA's minor groove and fluorescence is measured and represents the cDNA concentration.

1.4 Aims and outline of the research project.

1.4.1 Aims.

At Rikilt the Institute of Food Safety, cluster Toxicology and Effect monitoring, the project "The Hormonal effects of prohormones" focuses on the effects and detection of prohormones both *in vivo* and *in vitro*. In this report we will focus on experiments using prohormones DHEA and Ipriflavone and their effects on gene expression using DNA microarray technology. We will also make a setup to study the in vitro effects of prohormones. The overall goals of these experiments are:

- To study the feasibility of DNA microarrays as a screening method for prohormone abuse and to identify biomarkers for prohormone abuse.
- To study the effects of prohormones both *in vivo and* in *vitro*.

1.4.2 Outline of this study.

The feasibility of microarrays as a screening method on prohormone abuse was previously studied (*Rijk et al*, 2007 article submitted) by examining the changes in gene expression profile in bovine livers related to prohormones. In this experiment, bovines were exposed to DHEA by intramuscular and oral administration. The experiment included practical and biological variables as age, weight, background and administration type. The differences in gene expression profiles were studied using Agilent technologies© bovine 4x44k microarrays. This experiment pointed out that a short exposure to DHEA did not result in large effects in gene expression between exposed and control groups. The genes that were found regulated were analyzed with pathway analysis and several were selected for confirmation with real-time PCR. This study revealed that microarrays can be used to provide information on the possible screening on prohomone abuse in bovines. Information obtained from microarray screening might lead to the identification of biomarkers which might be usable for future screening on prohormone abuse. Microarray gene expression analysis may also be a very useful tool to study the effects of prohormones *in vivo* on target organs.

For screening on prohormone abuse in bovines, liver tissue can only be taken *ex vivo*. Blood samples however can be taken *in vivo* and animals do not have to be slaughtered prior to prohormone screening. Therefore, we will focus on the feasibility of microarrays as a screening method by studying DHEA induced gene expression in bovine blood.

Another prohormone will be tested on the feasibility of microarrays as a screening method on prohormone abuse. The second animal experiment will include 8 additional animals treated with different concentrations of ipriflavone per day for four weeks. Control animals will be the same control animals as in the DHEA experiment. This experiment will include practical and biological variables as age, weight, dose and background. Results from this experiment might not only provide us with information about the feasibility of microarrays in prohormone screening, but also elucidate the effects on gene expression of ipriflavone and its usefulness as a growth promoter.

To study the metabolism related to prohormones, the use of animals is unwanted due to ethical reasons. Using cell lines as an alternative is much more convenient. Since prohormones as DHEA and Ipriflavone are metabolized in liver tissue, the metabolite profiles will be studied using liver cell lines. However, since no bovine liver cell lines are available, we will study the metabolism of prohormones on a HepG2 human liver carcinoma cell line. Prior to biochemical analysis, the anti-proliferative effects of DHEA will be determined using the MTT (dimethylthiazol diphenyl tetrazolium bromide) test. Cells are then exposed to non-cytotoxic concentrations and the medium can be analyzed using Ultra Performance Liquid Chromatography coupled to a Quadrupole Time-Of-Flight Mass Spectrometer (UPLC-QTOF-MS).

2. Materials and methods.

2.1. Chemicals and solutions.

Gibco™ RNase free water, Gibco™ Fetal Bovine Serum (FBS), Gibco™ L-Glutamine (L-Glut) and phenol were ordered from Invitrogen life technologies, Breda, The Netherlands. Hanks balanced salts solution (HBSS), phenol red, Bovine serum albimin (BSA), dimethylthiazol diphenyl tetrazolium bromide (MTT), DHEA, 10% SDS and Ipriflavone were obtained from Sigma-Aldrich, St. Louis, MO, USA. Biowhittaker™ Dulbecco's modified Eagle's medium was ordered from Lonza, Verviers, Belgium. Chemicals ethanol, chlorophorm, dimethyl sulfoxide (DMSO), sodiumhydroxide, isoamylalcohol and ethylenediamine tetraacetic acid (EDTA) were from Merck KGaA, Darmstadt, Germany. Chemicals isopropanol, acetonitrile, formic acid were ordered from Biosolve BV, Valkenswaard, The Netherlands. Non-essential amino acids (NEAA) was obtained from MP Biomedicals Inc, Illkirch, France, Trypsin was from Difco International BV,Leeuwarden, The Netherlansd, and PBS was from Oxoid ltd, Hampshire, United Kingdom. MilliQ grade water was obtained from a Rossmark© Elga Purelab ultra filtration system.

2.2. Microarray experiments.

2.2.1. Animal experiments.

Animal experiments were conducted with Frisian bovines purchased at a local market. Animals were 6-13 months old and weighed between 150 and 450 kg. The animals were housed 2-3 weeks prior to the start of the experiment. In total, seven bovines were randomly selected as control animals. Three animals were selected in the first animal experiment and four animals were selected in the third animal experiment (see Table 1). Control animals did not receive any prohormone administration during the experiment. Control animals were the same in both the DHEA and IF experiment.

The DHEA experiment consisted of six additional animals which were treated with DHEA (see Table 1). The six treated animals received a daily dose of 1g DHEA (Sigma-Aldrich), three animals received intramuscular injections (DHEA dissolved in 10 ml Miglyol 812 (Certa SA, Braine-l'Alliud, Belgium)), and three animals received oral administration (DHEA capsules). The animals were treated during 7 days at 24 hour intervals and were sacrificed 24 hours after the last treatment. Blood samples were taken at sacrifice, using PAXgene™ Blood RNA tubes (PreAnalitiX GmbH, Hombrechtikon, CH) and were stored at -80°C.

Experiment	Treatment	Code	Age	Weight
Experiment 1	DHEA intramuscular	IM 1	9 months	290 kg
	DHEA oral	O 1	8 months	253 kg
	Control	C 1.1	6 months	174 kg
	Control	C 1.2	6 months	172 kg
	Control	C 1.3	6 months	153 kg
Experiment 2	DHEA intramuscular	IM 2	9 months	262 kg
	DHEA oral	O 2	8 months	210 kg
Experiment 3	DHEA intramuscular	IM 3	12 months	355 kg
	DHEA oral	O 3	13 months	410 kg
	Control	C 2.1	13 months	350 kg
	Control	C 2.2	14 months	386 kg
	Control	C 2.3	14 months	368 kg
	Control	C 2.4	13 months	432 kg

Table 1: The overview of the animal experiments in the DHEA experiment shows the setup of the experiments, treatments, codes, ages and weights of the animals used in this study.

The IF experiment consisted of eight additional animals (see Table 2). The animals received a daily dose of 0.2 g (n=4) or 2g (n=4) of IF (Sigma-Aldrich) by oral administration at 24 hour intervals. After a total exposure time of four weeks, the animals were sacrificed. Liver samples of approximately 100-200 µg were excised, transferred into cryogenic tubes, snap-frozen in liquid nitrogen and stored at -80°C.

Experiment	Treatment	Code	Age	Weight
Experiment 1	Control	C 1.1	6 months	174 kg
	Control	C 1.2	6 months	172 kg
	Control	C 1.3	6 months	153 kg
Experiment 3	Control	C 2.1	13 months	350 kg
	Control	C 2.2	14 months	386 kg
	Control	C 2.3	14 months	368 kg
	Control	C 2.4	13 months	432 kg
	Ipriflavone 0.2g /day	IF 0.2 – 1	13 months	302 kg
	Ipriflavone 0.2g /day	IF 0.2 – 2	12 months	307 kg
	Ipriflavone 0.2g /day	IF 0.2 – 3	12 months	376 kg
	Ipriflavone 0.2g /day	IF 0.2 – 4	12 months	384 kg
	Ipriflavone 2g /day	IF 2 – 1	12 months	265 kg
	Ipriflavone 2g /day	IF 2 – 2	14 months	342 kg
	Ipriflavone 2g /day	IF 2 – 3	13 months	378 kg
	Ipriflavone 2g /day	IF 2 – 4	14 months	383 kg

Table 2: The overview of the animal experiments shows the setup of the ilpriflavone experiments, treatments, codes, ages and weights of the animals used in this study.

2.2.2. RNA extractions.

Total RNA from blood was isolated using the PAXgene™ Blood RNA kit (PreAnalitiX GmbH, Hombrechtikon, Switzerland). In short, 2.5 ml blood is collected in PAXgene™ Blood RNA tubes, which contains a mix of reagents which stabilizes RNA. Isolation begins with a centrifugation step to pellet nucleic acids in the

PAXgene blood RNA tube. The pellet was washed, resuspended and incubated in optimized buffers containing proteinase K to digest proteins. A second centrifugation step is performed to remove residual cell debris, and the supernatant is transferred to a fresh microcentrifuge tube. Ethanol 75% is added to adjust binding conditions, and the lysate is applied to a PAXgene RNA spin column. During a brief centrifugation, RNA is selectively bound to the silica-gel membrane of the spin column as contaminants pass trough. Remaining contaminants are removed in three wash steps, and pure RNA is then eluted in buffer BR5 [1] protocol see Appendix A 1. Blood extracted RNA was purified by using the RNeasy® MiniElute™ cleanup kit (Qiagen GmbH, Hilden, Germany) following the manufacturers protocol designed for RNA extractions done with the PAXgene™ blood RNA kit (Appendix A 2). The kit is used to clean up low concentration (<45 µg) RNA samples. Lysis buffer containing guanidine-isothiocyanate and ethanol are added to the RNA samples to create conditions that promote selective binding of RNA to the RNeasy MiniElute membrane. The sample is then applied to the RNeasy miniElute spin column. RNA binds to the silica-gel membrane, contaminants are efficiently washed away, and high quality RNA is eluted in buffer BR5 [2].

Quality check of blood extracted RNA was assessed using the Experion™ RNA stdsens analysis kit (Bio-Rad, Hercules, CA, USA), following the manufacturers protocol. In short, a gel solution was filtered using a spin filter tube and a centrifugation of 10 minutes at 1500 rpm. 1 µl of RNA stain was added to 65 µl filtered gel. 2 µl of extracted RNA was transferred into microcentrifuge tubes and incubated at 37°C for 30 minutes. The RNA samples and ladder were denatured by 2 minute incubation at 70°C. The Experion chip was primed by adding 9 µl of stained gel to the according well, and priming was done using the Experion priming station. The samples and ladder were added to the chip and the RNA analysis was runned using an Experion electrophoresis station and data was analyzed using the corresponding software [6] (Appendix A 6).

Total RNA from bovine liver was isolated by homogenization in Trizol® (Invitrogen). In short, 1 ml of Trizol was added to the liver tissue samples, and the samples were homogenized. Addition of one volume of chloroform separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. In the aqueous phase, the RNA is recovered by precipitation with isopropanol [3]. After centrifugation, RNA pellets are washed once with ethanol and air dried. The RNA pellet can now be dissolved in RNase free water (Appendix A 3). Additional cleanup of RNA extractions was done using the RNeasy™ mini kit (Qiagen GmbH, Hilden, Germany) following the manufacturers protocol (Appendix A 4). In short, buffer RLT and ethanol are added to the RNA samples to create conditions that promote selective binding of RNA to the silica-gel membrane. The sample is then applied to the RNeasy Mini spin column. Total RNA binds to the membrane, contaminants are efficiently washes away and high quality RNA is eluted in RNase free water [4].

An RNA quality check of RNA extractions from biopsies was done using molecular biology grade agarose (Eurogentech Nederland BV, Maastricht, The Netherlands) gel electrophoresis (Bio-Rad). One µg of RNA was adjusted to 9 µl using RNase free water and 1 µl of 10x loading buffer (Invitrogen) was added. The +1kb DNA ladder (Invitrogen life sciences) was used for calibration purposes. The RNA samples were brought into the wells of a 1.5% agarose gel. Electrophoresis was done during 90 minutes at 75 V (Appendix A 7).

Quantification of the RNA extractions was measured using the Nanodrop ND-1000 UV-VIS spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and the corresponding software. A total volume of 1.5 µl was used to assess RNA quantity and purity. RNA extractions were used for the microarray experiments when A260/280 and A260/230 ratios were higher than 1.80 (Appendix A 5).

2.2.3. Microarray hybridization.

The microarray experiment was carried out according to the Agilent technologies, "Two-color microarray-based gene expression analysis protocol" [5] and using the Agilent Gene expression analysis kit. The control reference design was used in this experiment. The protocol is summarized and shown in Appendix A 8. All Reagents and the Bovine 4x44k arrays were ordered from Agilent technologies. In short, 1 µg of RNA from all target samples was taken and adjusted to a volume of 8.3 µl. A pool of control samples was used as reference RNA. The reference RNA was made by pooling 1 µg of all control samples and making four 8.3 µl reference samples. Reference and target samples were spiked with internal control RNA which binds specifically to internal control spots on the array. Spike A mix was added to reference RNA samples and spike B mix was added to target RNA samples. Reverse transcriptase was added to all target and reference samples, followed by a reaction protocol where all samples were linearly amplified once. The new cDNA was used in a labeling reaction, where the newly synthesized reference cRNA was labeled with Cyanine 3-CTP (Cy3) and the target cRNA was labeled with Cyanine 5-CTP (Cy5) (PerkinElmer Life and Analytical Sciences, Boston, MA, USA). Samples were cleaned using the RNeasy® mini kit (as described previously) and assessed using the Nanodrop ND-1000 UV-VIS spectrophotometer. The reference samples were pooled and all target and reference samples were approved for hybridization when the cRNA yield was higher than 825 ng and the labeled cRNA concentration was higher than 8.0 pmol/µl. The cRNA was fragmented into 20-25 bp fragments and equal amounts of target and reference cRNA were mixed and dispensed onto the array gasket slide. The microarrays were brought onto the array gasket slides and tightened into an Agilent SureHyb hybridization assembly. The hybridization was done during 17h in a hybridization oven at 65 °C with rotation function (10 rpm). After 17 hours, the arrays were washed with subsequently Agilent Wash buffer 1 (1 minute room temperature), Agilent wash buffer 2(30 seconds at 37°C), acetonitrile (30 seconds, room temperature) and Agilent Drying solution (30 seconds room temperature). After washing, the arrays were scanned using an Agilent G2565AA microarray scanner. Microarray scans were extracted by Agilent Feature Extraction software.

2.2.4. Data analysis.

After microarray scan extraction a hybridization quality control was done using R, LimmaGUI by assessment of the M-box plot. Filtering, 2log-transformation, normalization (as described by *Pellis et al(2003)* [6] and statistics were done with Genemaths XT (Applied Maths, St Martens-Latem, Belgium). Gene lists were made by calculating ratios using Microsoft Excel. Genes were selected on account of their fold-change and p-value. Gene selections were visualized using Spotfire Decision site® (Tibco software BV, Rotterdam, The Netherlands). Pathway analysis was done using Genego Metacore™ (Genego Inc, St Joseph, MI, USA) pathway analysis combined with literature mining.

2.3. Real-time RT-PCR.

Primers were designed using Beacon Designer software (Premier Biosoft International, Palo Alto, CA, USA). Primers were designed using a Tm of 60±2°C, a primer length of 20-30 bp and a product length of 100-250 bp. Further analysis of primer quality was done by assessing the chance of hairpin structures and homology to other genes (using NCBI online nucleotide BLAST (Basic Local Alignement and Search Tool)) (Appendix A 9).

The Promega RQ1 DNase kit (Promega Benelux BV, Leiden, The Netherlands) was used to treat RNA extractions with DNase to remove genomic DNA and prevent contamination during real time RT-PCR. A total of 10 µg RNA was taken, and 10 units RQ1 RNase Free DNase were added to the RNA together with RQ1 RNase free DNase buffer. After incubation at 37°C for 30 min, the samples were cleaned with successively Phenol/Chloroform/Isoamylalcohol (25:24:1), Chloroform/Isoamylalchol (24:1), 96% and 75% ethanol. Final DNase treated RNA was eluted in nuclease free water (Appendix A 10)

One µg of total RNA was reverse transcribed into cDNA in a 20 µl reaction mixture using the iScript™ cDNA synthesis kit. The reaction was done by incubating 5 min. at 25°C, 30 min at 40°C, 5 min at 85°C and cooling on ice. After the reaction, 80 µl RNase free water was added and cDNA was stored in -20°C (Appendix A 11).

The IQ™ SYBR green supermix (Bio-Rad) was used for real-time RT-PCR. Per reaction, 1 µl cDNA (described above) was added to a 24 µl reaction mix consisting of: 12.5 µl SYBR green, 1µl 10mM anti-sense primer, 1µl 10mM sense primer and 9.5 µl of nuclease free water. The reaction mixtures were applied to 96 wells PCR plates and the plates were sealed with IQ® optical tape (Bio-Rad). The real-time RT-PCR was done in a Bio-Rad iCycler, fluorescence was measured with the Bio-Rad iCycler IQ® multicolor Real-time PCR Detection System and IQ® iCycler software. In each RT-PCR experiment, one sample was diluted 10, 100, 1000, and 10000 times and was used to make a calibration line. All samples were added to the 96 wells PCR plate in duplicate. The reaction was done according to the protocol in table 3. Real time RT-PCR experiments were analyzed by converting the Ct values to relative cDNA concentrations using the calibration

line. These concentrations were normalized by correction for the total amount of cDNA using the reference gene cDNA concentrations and averaging the duplicates. Ratios were calculated by taking the average cDNA concentration. These ratios were compared to microarray data (Appendix A 12). The amplicon sizes were analyzed by agarose gel electrophoresis as described in Appendix A 7.

Cycle No#	Time	Temperature	Description
Cycle 1 (1x)	3 min.	95°C	
Cycle 2 (45x)	15 s.	95°C	
	30 s.	60°C	- Data collection and real-time analysis
Cycle 3 (1x)	1 min.	95°C	
Cycle 4 (1x)	1 min.	55°C	
Cycle 5 (60x)	10 s	65°C - 95°C	- Increased set point temperature after cycle 2 by 0.5°C. - Melt curve data collection and analysis.

Table 3: The reaction protocol used in the real-time PCR analysis is shown in this table. Data was collected during cycle 2 and melt curve data was obtained during cycle 5.

2.4. Setup for In vitro experiments.

2.4.1. Cell culturing and exposure.

In vitro experiments were carried out using the HepG2 human liver carcinoma cell line (ATCC cat. no. 8065, ATCC, Manassas, VA, USA). The cell culture was maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS, 1% NEAA, 1% Penstrep (DMEM+++). Cell cultures were kept in a 37°C, 5% CO^2 atmosphere. Cells were passed when confluence was around 80%. Cells were washed using calcium and magnesium free Hanks balanced salts solution (HBSS-Ca-Mg) and trypsinized during 15 min at room temperature using Trypsin 0.25% and EDTA 0.05% dissolved in HBSS-Ca-Mg without phenol red (Appendix A 13). Exposure was done by were seeding the cells in a 24 wells plate at a density of approximately 7.500 cells/well in DMEM+++. After 24 hours, the medium was removed, and new medium was added which contained various prohormone concentrations dissolved in 1% DMSO. DMSO was added to the cells as a negative control (1% DMSO) and positive control (10% DMSO). At time points 4, 6, 24 and 48 hours, the anti-proliferative effects of DHEA were measured as described below (Appendix A 14).

2.4.2. MTT-test.

Anti-proliferative effects were measured using the MTT (dimethylthiazol diphenyl tetrazolium bromide) test. The medium was removed after exposure, and new Eagle's minimal essential medium (EMEM from Sigma-Aldrich) containing 500 µg/ml MTT (Sigma-Aldrich) and 2mM L-Glutamine (Sigma Aldrich) was added to the cells. After one hour of incubation at 37°C, 5% CO^2, the medium was removed from the cells, and the cells were lysed using 4°C isopropanol. Cells were kept at 4°C for 20 min. After 20 min. the plate was mixed and 100 µl of each well was transferred to a 96 wells plate. Extinction was measured at 562 nm and corrected

at a reference wavelength of 650nm using an Elx808™ absorbance microplate reader and KCJunior™ software (Bio-Tek Instruments Inc, Winooski, VT, USA). Cytotoxicity was determined by calculating the reduction in extinction (Appendix A 15).

2.3.4. Protein assay.

The non-cytotoxic concentrations of 0, 0.1, 1 and 10 uM were used to expose cells. Cells were exposed as described above, to the non-cytotoxic concentrations of 0, 0.1, 1 and 10 uM DHEA for 24h. After 24 hours of exposure, the medium was transferred to microcentrifuge tubes and stored in -80°C. Cells were washed once with 500 µl PBS, and after adding a second volume of 500 µl PBS, the plates containing the cell sample wells were stored until further use in -20°C. Protein concentrations in the cell sample wells were measured using the BioRad DC protein assay kit. A lysis solution was made which contained 0.8 M sodiumhydroxide (NaOH) and 10% SDS. Bovine serum albumin was diluted in lysisbuffer to concentrations 1.5, 1.25, 1, 0.75, 0.5, 0.25, 0.1 and 0 mg/ml. The 24 wells plates containing cells frozen in PBS were defrosted at room temperature and 500 µl lysisbuffer was added to the cell suspensions. The cells were shaken and incubated for 30 min. at room temperature. 20 µl of reagent S was added to each milliliter of reagent A needed. After incubation, 5 µl of all samples (triplicates) and BSA standards were added to a low-protein binding 96 microwellsplate (Greiner cat. no. 655101, Greiner Bio-One BV, Alphen aan de Rijn, The Netherlands). A volume of 25 µl reagent A (containing 20 µl reagent S/ml) was added to the standards and samples. Finally, 200 µl reagent B was added to the wells and the plate was mixed for 5 s. After 15 min of incubation at room temperature, the absorbtion of the wells was measured at 750 nm using the Elx808™ absorbance microplate reader and KCJunior™ software. In the future, the metabolite profiles of the exposed cells can be analysed, using the protein assay data to quantify the relative amounts of metabolites formed (appendix A 16)

3. Results and discussion.

Part 1: Biomarkers for DHEA abuse in bovine blood.

In this experiment, we studied the effects of DHEA administration in bovines on gene expression in bovine blood to search for specific biomarkers for DHEA abuse. The experiment included thirteen bovines which were purchased at a local market. Six animals were treated with 1g DHEA per day either by intramuscular injections or oral administration for seven days. The other seven animals were control animals from different experiments. RNA was isolated from whole blood which was processed and hybridized onto bovine microarrays. The results from the microarray experiment were analyzed and several genes which were found regulated were selected for confirmation using real time RT-PCR.

3.1 Blood RNA quality.

RNA was isolated from 2.5 ml blood using the PAXgene™ blood RNA kit. The isolated RNA samples were analyzed using the Nanodrop ND-1000 UV-VIS spectrophotometer. The results from the spectrophotometric analysis are shown in Table 4. The spectrophotometric analysis determines the RNA concentration by measuring the absorbance at a wavelength of 260 nm, proteins have an optimal absorbance at 280 nm and disturb the absorbance of RNA. Therefore, the ratio 260/280 is taken as an indication of proteins present in the RNA sample. Organic compounds like phenol, ethanol and salts can also interfere with the RNA absorbance and for these compounds; the ratio 260/230 was taken as a parameter for contamination of these compounds in the RNA samples. The measured concentrations range from 47 to 152 ng/µl and all A260/280 and A260/230 ratios are higher than 1.80. Based on the outcome of this analysis, all samples were approved for further analysis.

Description	Concentration ng/µl	Ratio A260/280	Ratio A260/230
Control 1.1	153	1.94	1.98
Control 1.2	107	2.02	2.11
Control 1.3	67	2.05	1.97
Control 2.1	75	1.92	2.06
Control 2.2	128	1.94	2.05
Control 2.3	74	1.95	1.88
Control 2.4	124	2.06	1.99
DHEA Im 1	113	1.99	1.83
DHEA Im 2	110	1.98	2.06
DHEA Im 3	47	1.95	1.99
DHEA O 1	56	2.04	1.97
DHEA O 2	115	2.05	1.99
DHEA O 3	144	1.98	2.07

Table 4: The results obtained from the spectrophotometrical analysis showing the concentrations and A260/280 and A260/230 ratios. Control animals 1.1 to 1.3 and 2.1 to 2.4 are control animals from respectively experiment 1 and 3. DHEA IM are animals treated with intramuscular injections of DHEA in experiments 1, 2 and 3. DHEA O are orally exposed animals from experiments 1, 2 and 3.

To check for presence of RNases in the extracts, RNA was incubated 1h at 37°C and analyzed using the Experion™ electrophoresis system. A small volume of RNA extract was taken and applied to the Experion™ labchip. The electrophoresis system analyzed the samples and generated a digital gel picture. Results from this analysis are shown in Figure 10 and 11. In these figures we can see that there is no breakdown of RNA observed in the RNA samples (an example of RNA breakdown is shown in Figure 12). Clearly visible are the 18S and 28S ribosomal rRNA bands which are the 2 main parts of cellular ribosomes. All samples were approved for the labeling procedure.

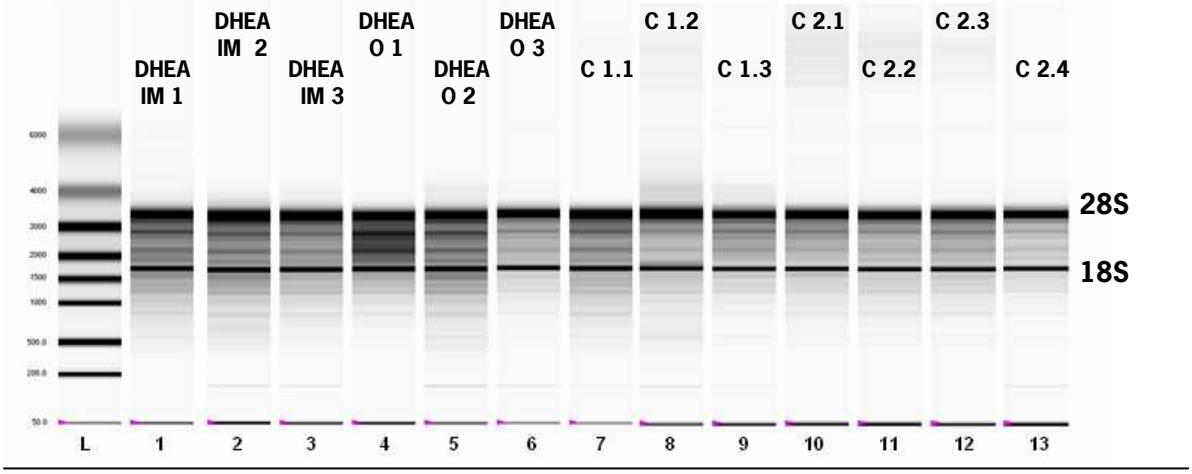


Figure 10: Digital gel used in the quality assessment of the RNA blood samples. No breakdown or genomic DNA contamination is observed. Clearly visible are the 18 and 28S rRNA bands.

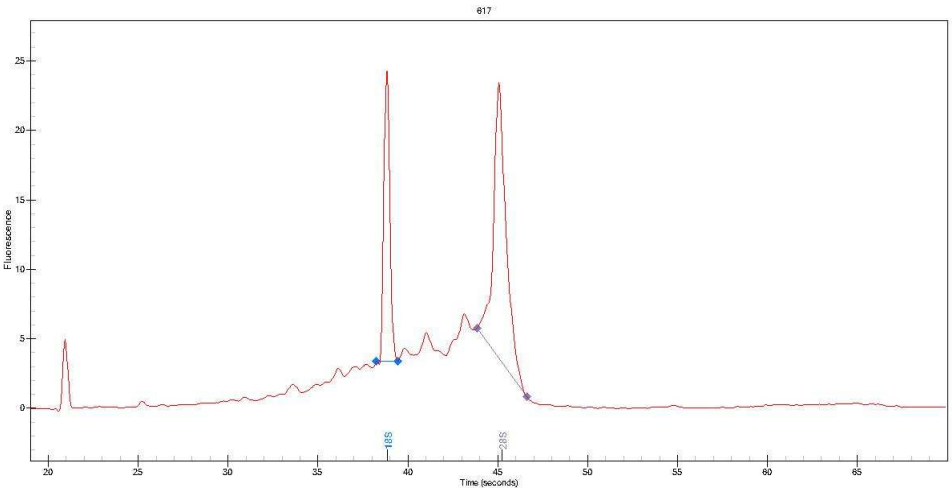


Figure 11: Chromatogram of RNA sample DHEA O3. The two large peaks are respectively the 18S and 28S rRNA bands, the smaller peaks between the three peaks represent the mRNA of various lengths.

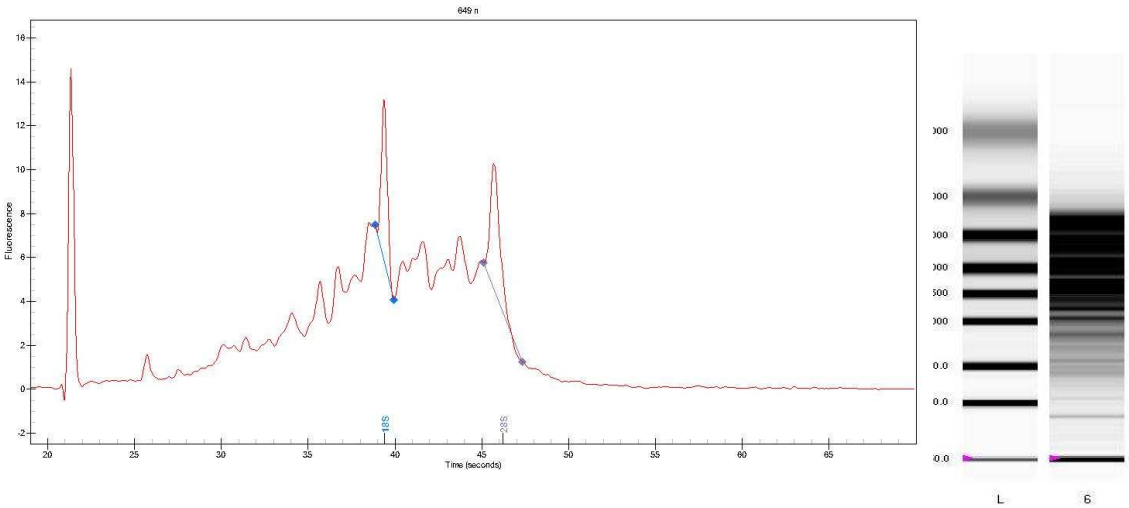


Figure 12: In this figure, an example of RNA breakdown is given. The smear represents the large amounts of rRNA which are broken down by the 1h incubation at 37°C due to RNases present in the sample.

3.2 DHEA Microarray experiment.

3.2.1 RNA labeling.

During the preparation of the microarray hybridization, mRNA was converted into labeled RNA. This was done by reverse transcription of mRNA and the newly synthesized cDNA was used in a labeling reaction resulting in newly synthesized labeled cRNA. Table 5 shows the results from the spectrophotometrical analysis of the labeling reaction where we can see that the labeling reaction was performed successfully. According to the manufactures protocol, all labeled yields should be higher than 8.0 pmol/µl. Although the Cy3 labeled pool of controls does not meet the criteria, it was decided to continue with the 7.90 pmol/µl of labeled cRNA in the Cy3 reference pool due to the minimal deviation from this criteria.

Description	Cy3 RNA pmol	Cy5 RNA pmol	Total concentration ng/µl	Labeled yield pmol/µl	Total yield µg
Control 1.1	-	0.89	74	12.06	2.21
Control 1.2	-	0.96	90	10.72	2.69
Control 1.3	-	1.49	116	12.82	3.49
Control 2.1	-	2.57	195	13.20	5.84
Control 2.2	-	2.38	182	13.06	5.47
Control 2.3	-	1.49	123	12.09	3.70
Control 2.4	-	2.05	155	13.27	4.63
DHEA IM 1	-	1.17	99	11.80	2.97
DHEA IM 2	-	1.59	121	13.14	3.63
DHEA IM 3	-	0.73	73	10.05	2.18
DHEA O 1	-	2.18	176	12.36	5.29
DHEA O 2	-	2.00	158	12.67	4.74
DHEA O 3	-	0.99	87	11.39	2.61
Cy3 pool	1.84	-	232	7.94	27.8

Table 5: The results from the labeling reaction performed during the preparation of the microarray experiment are shown. The concentration Cy3 and Cy5 labeled RNA is shown as well as the total concentration. Using this data, the labeled yields and total yields were calculated.

After the labeling reaction, 825 ng of each target cRNA samples was transferred into a new tube, and 825 ng of reference cRNA was added to each target sample. The hybridization samples were fragmented into fragments around 20-25 bp (corresponding to the length of the probes on the microarray). Although this might introduce false positive probe binding, it allows a much more stringent hybridization procedure. After the fragmentation, hybridization samples were brought onto the array and the microarrays were hybridized for 17h at 65°C and 10 rpm. After the hybridization, the arrays were washed and scanned.

3.2.2 Hybridization quality control.

The quality of the microarrays and the hybridization procedure was first determined by visual assessment. Two scans of the arrays are shown in Figure 13, where a normal hybridized array and an array where leakage was observed are shown. All other arrays are shown in Appendix B. All arrays were further analyzed to obtain more information on array and hybridization quality.

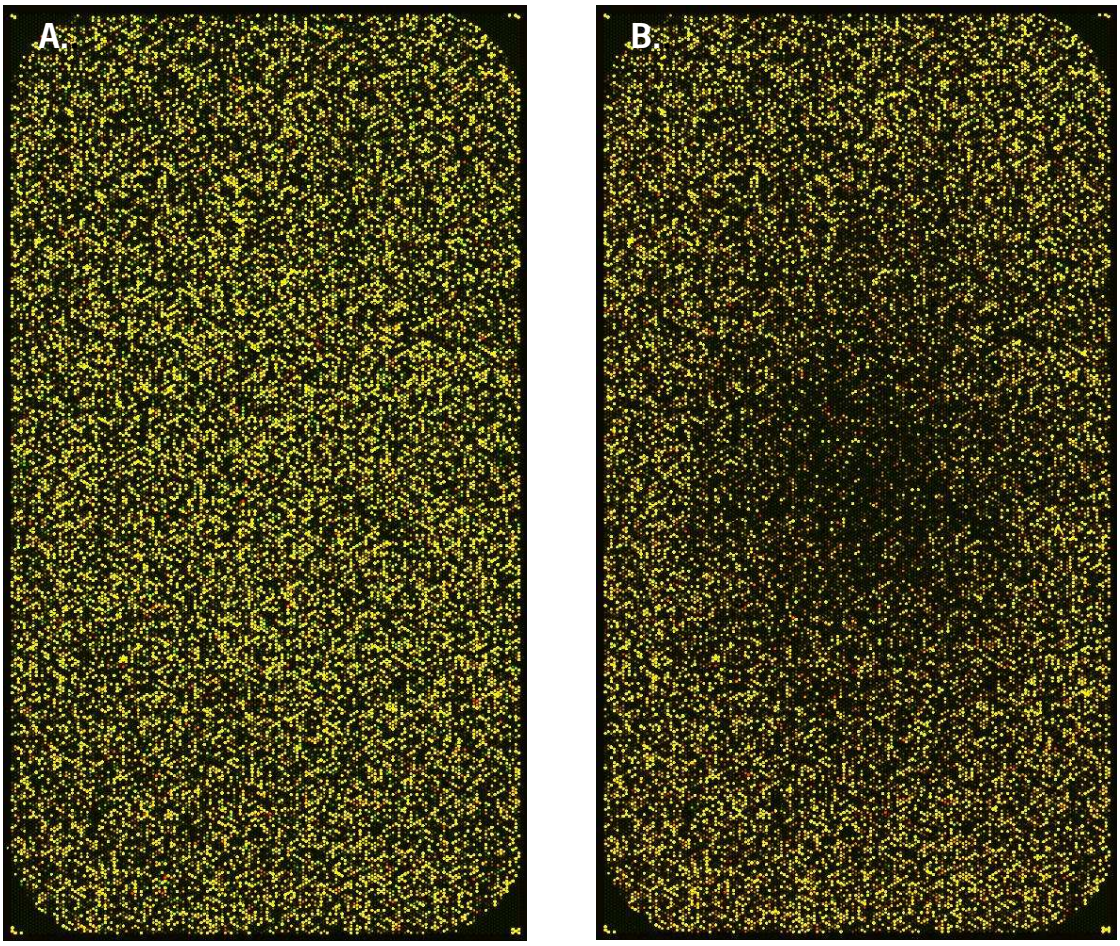


Figure 13: Two of the thirteen array scans are shown, which shows variations in hybridization quality. Control sample 1.1 is hybridized onto array A and DHEA treated sample IM 3 is hybridizd onto array B. After hybridization, a leakage was observed in array B.

All arrays were further analyzed on quality using the programming statistical language R, and the software specifically designed for microarray data analysis LimmaGUI. The M-Box plot of the statistical analysis done in limmaGUI is shown in Figure 14. Analysis was done before filtering and normalization. The thick black line of each array is the mean log2 ratio of the Cy3/Cy5 value of each spot, and should theoretically be zero. The box around the mean ratio is the standard deviation of all these ratios of all spots. The dotted lines represent the 95% reliability interval and the dots outside the 95% reliability interval are outliers which could be internal control spots which have not been filtered, but can also be strongly regulated genes. This form of quality assessment is used to determine that microarrays have hybridized in a comparable way. In this case, all arrays seem to be comparable, and all arrays were further analyzed.

Slide 1	C 1.1
Slide 2	C 1.2
Slide 3	C 1.3
Slide 4	C 2.1
Slide 5	C 2.2
Slide 6	C 2.3
Slide 7	C 2.4
Slide 8	DHEA IM 1
Slide 9	DHEA IM 2
Slide 10	DHEA IM 3
Slide 11	DHEA O 1
Slide 12	DHEA O 2
Slide 13	DHEA O 3

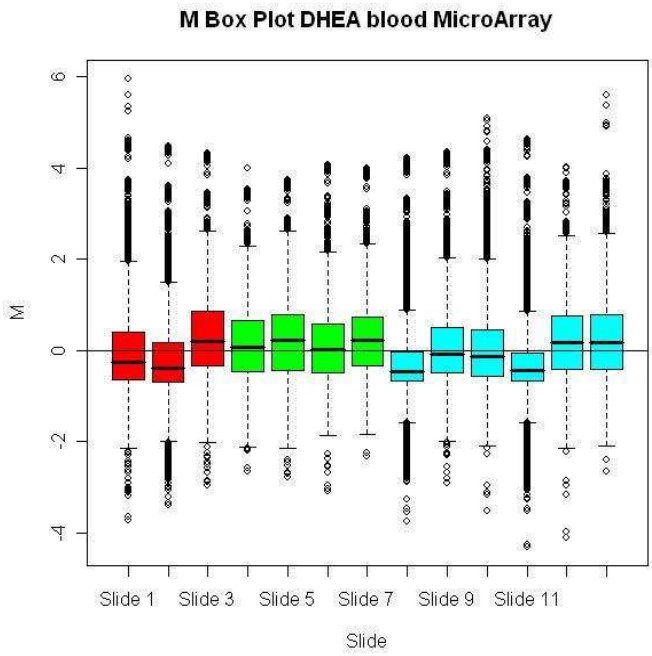


Figure 14: M-box plot which presents the quality of the microarrays and the hybridization procedure. The M-box plot before filtering and normalization is shown. Red boxes represent Control samples 1.1 to 1.3, green boxes represent control samples 2.1 to 2.4 and blue boxes represent the DHEA treated animals.

3.2.3 Principal Components Analysis.

Further data analysis was done after filtering the microarray data from internal control spots (Spike A and B) and signals lower than two times the background signal. After filtering, all signals were 2-log transformed and normalized using the normalization described by *Pellis et al,* (2004) [37] of which an overview is shown in figure 15. This normalization included correction for the random error, using the median Cy3 signal for each individual spot. A second correction was done for the systematic error using the median value of the overall Cy5 signal. First, the mean of the Cy3 values for each spot are averaged and placed onto a virtual slide. The Cy5 value of a specific spot was multiplied by the mean the virtual Cy3 value for that spot and divided by the actual Cy3 value for that spot. Secondly, the ratio between the mean of all Cy5 for every spot on every array by signals by the mean of the Cy5 signal for the individual array was calculated, and the second correction was done by multiplying the spot-specific corrected Cy5 value by this ratio. After normalization, arrays were analyzed using Principal Components Analysis (PCA). In each microarray experiment, all arrays were compared to all other arrays, therefore creating, in this case, 12 (N-1) dimensions. The Genemaths XT software calculates the differences between the arrays for each dimension and determines the percentage of differences between the arrays. The three dimensions in which the largest differences can be observed are visualized in a 3D plot. This plot only visualizes the differences in gene expression between arrays and provides information on how to compare prohormone treated animals with control animals.

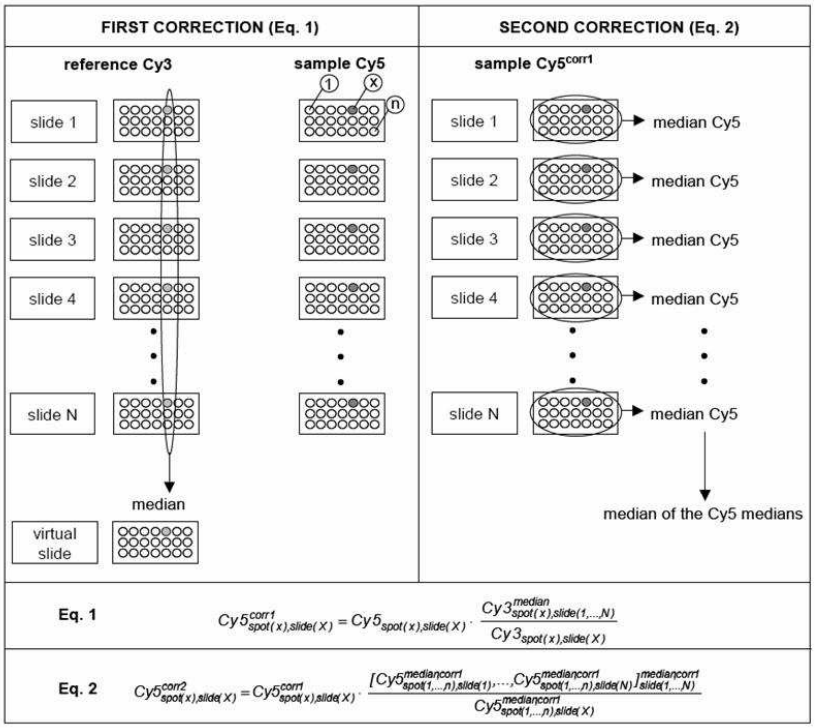


Figure 15: Normalization procedure as described by Pellis et al. The normalized values were used for further processing.

The PCA 3D plot of this experiment is shown in Figure 16. This plot only visualizes the differences in gene expression between arrays and provides information on how to compare prohormone treated animals with control animals. In Figure 16, a total difference between arrays of 74% is observed. Furthermore, a large spreading of all samples and hardly any clustering with exception of control group 2 can be seen. From this analysis we can conclude that there are no great differences between DHEA treated animals and control animals. The small differences can be explained by the short exposure of animals to low doses of DHEA, which also is an endogenously produced compound in addition to the biological variance between the animals. Decided was to pool all DHEA treated animals into an exposed group (Exp) and to pool the control groups separately into control group 1 (C 1) and control group 2 (C 2) and together in a total control group (Ctot). Further analysis was done by comparing the control groups separately to the DHEA treated group.

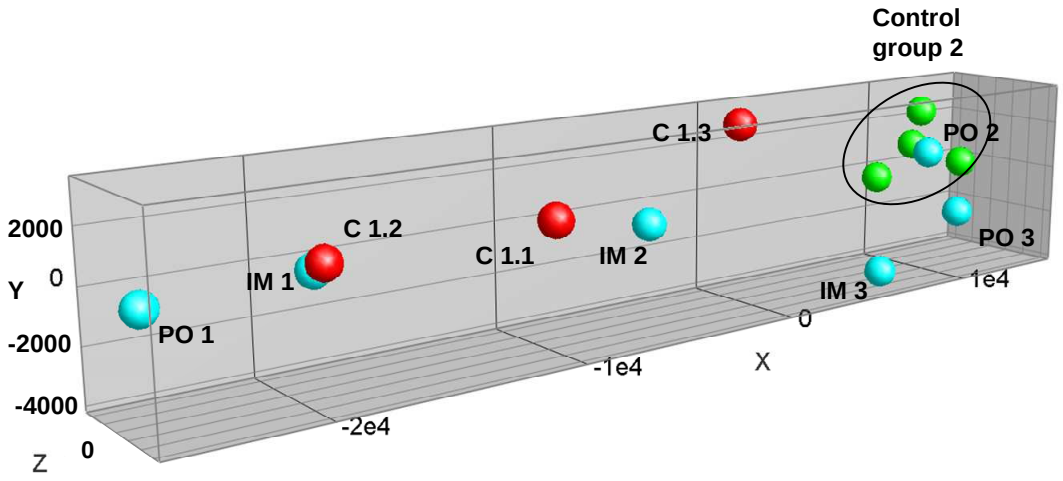


Figure 16: PCA 3D plot in which the three dimensions are plotted which accounted for 74% of the total differences between the individual arrays. Red represents control group 1, green represents control group 2 and blue represent the DHEA treated group.

3.2.4 Gene selection.

Genes were found regulated when its expression was more than 1.5 times up- or downregulated and when the p-value from the independent sample T-test was lower than 0.05. The fold-change in regulation was calculated as shown in Table 5. Fluorescence values were 2-log transformed and means were calculated. The mean of the control animals was divided by the mean of the exposed animals which result in ratios. A ratio below 1 indicates that the gene is upregulated, a ratio above 1 indicates that the gene is downregulated. To obtain a fold change in expression, 1 was divided by the downregulated ratios to obtain a fold-change for this gene. Downregulated genes were multiplied by -1 to obtain a similar fold-change value for that gene. An example of this calculation is shown in table 5. As explained before, DHEA treated animals were compared to the total control group, control group 1 and control group 2 which results in three gene selections. The selection of genes is visualized in figure 16 using Spotfire decisionsite® software. The absolute gene lists are shown in Appendix C. In Figure 17, the fold-change is shown on the x-axis and is plotted against the p-values on the y-axis. Higher numbers of significantly regulated genes were found when the individual control groups are compared to the exposed group, in comparison to the numbers of significantly regulated genes found when the total control group is compared to the exposed group. This observation can be explained by the PCA results shown in Figure 16. The Venn-diagrams in figure 18 show a comparison of the significantly regulated genes. In this figure we can see that there is very little overlap between the two control groups. Due to the small similarity between control groups, it is difficult to select genes which are specifically regulated by DHEA treatment. The gene list which results when the total control group is compared to the exposed group does show genes which are specifically regulated by DHEA.

Gene and Sample type Gene 1: FIBP Gene 2: SC5DL	2-log transformed signal value	Average signal (2^value, average)	Ratio (value C/ value Exp)	Calculate fold change (1/value or -1*value)
1. Control 1.3	14,48	22737.03	0,37	1/0.37= 2,68
1. Exposed IM 2	15,90	61040.32		
2. Control 2.2	10,50	1450,67	2.04	2.04*-1= -2.04
2. Exposed IM 2	9,47	710,65		

Table 5: Calculation of the fold change gene expression was done by calculating the 2-log transformed signal to the original signal. Two example genes are shown, one upregulated (green) and one downregulated (red). Control values are shown in orange, exposed values are shown in blue. FIBP was used to compare control sample C 1.3 to exposed sample IM2, the same was done using gene SC5DL in which control sample 2.2 was compared to exposed sample IM2.

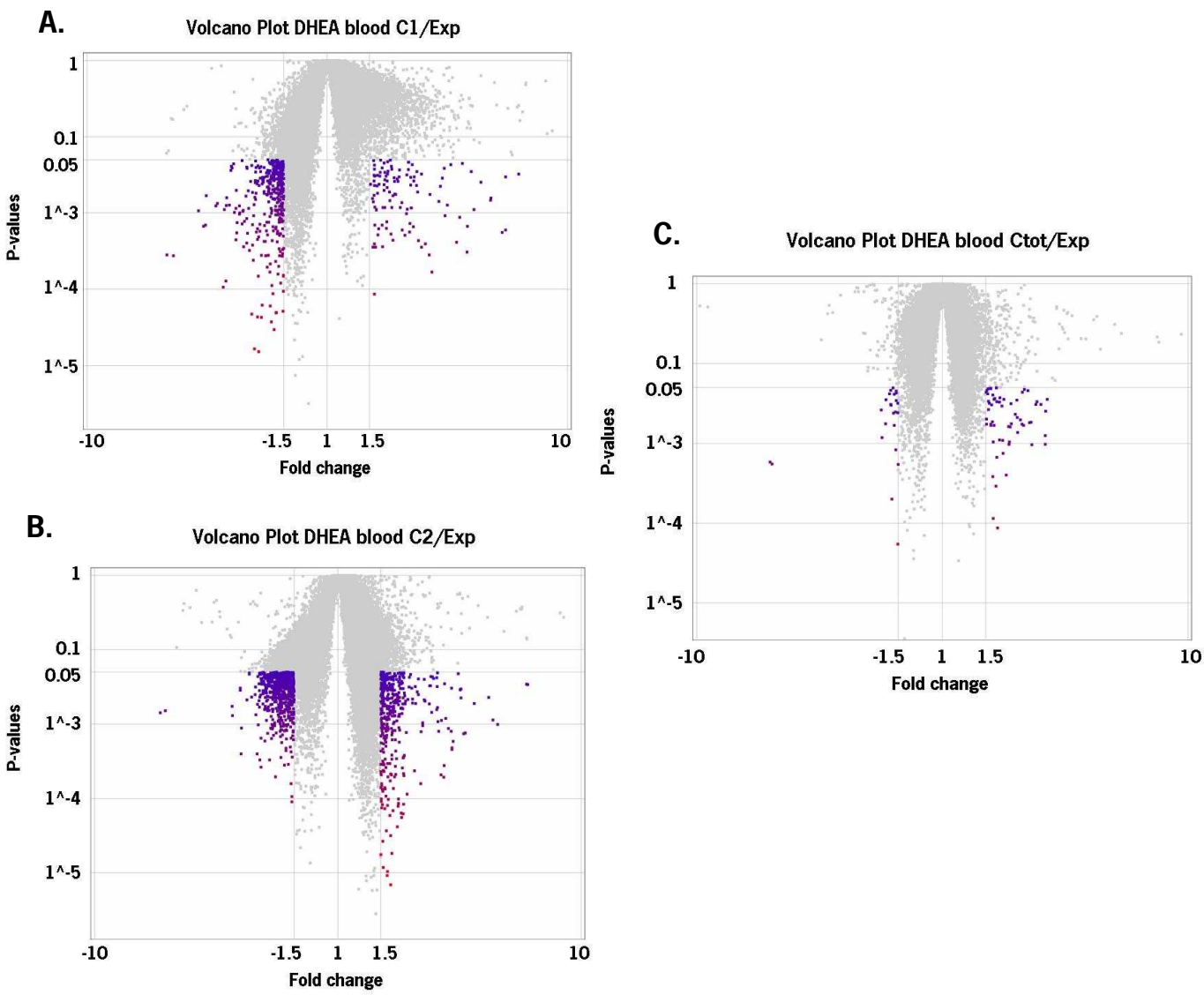


Figure 17: Volcano plots of the significantly regulated genes in the exposed group, when compared to A: control group 1, B: control group 2 and C: the total control group. Genes were found significantly regulated when the fond change was higher than 1.5 or lower than 0.66 combined with a p-value lower than 0.05.

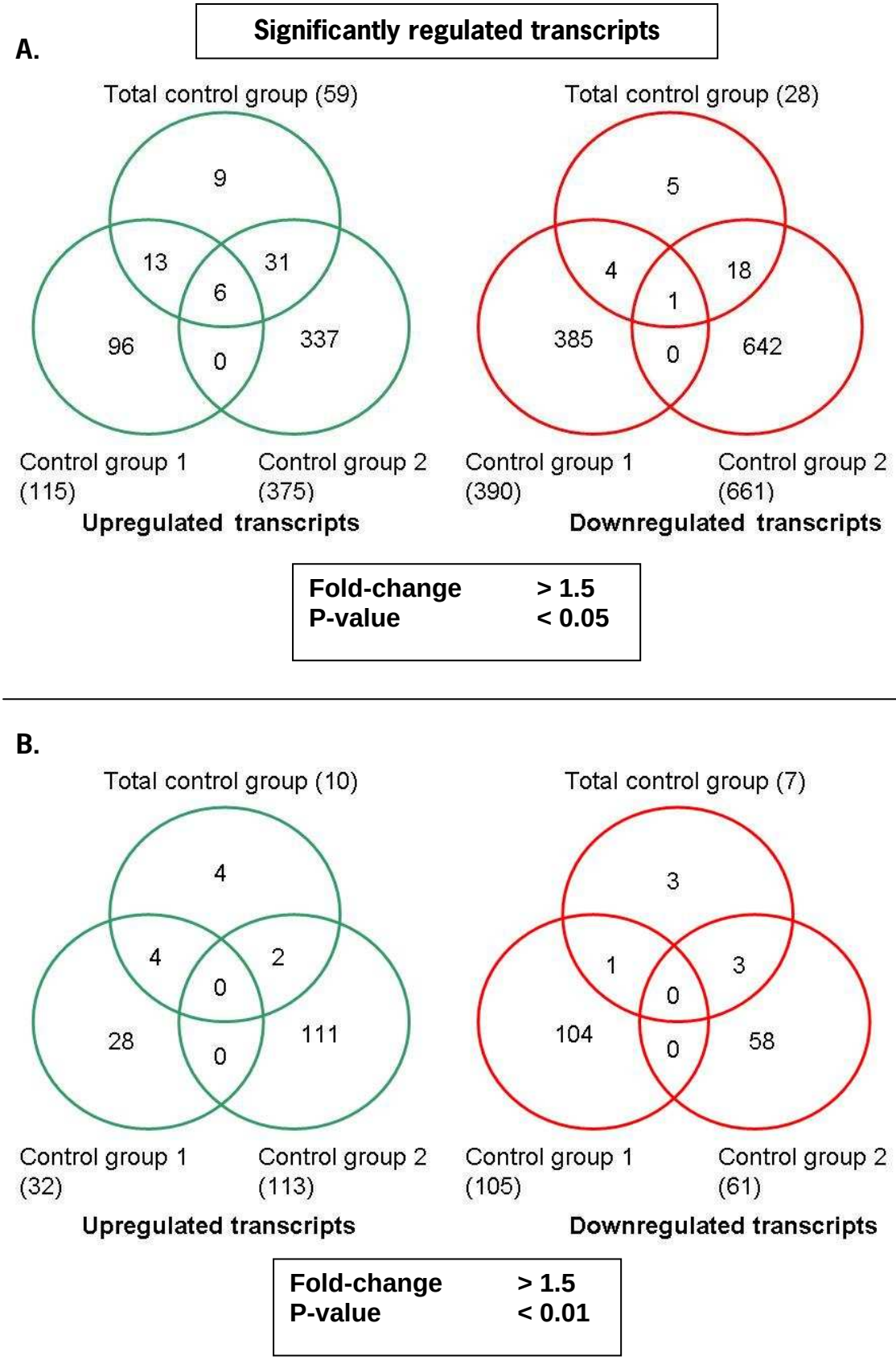


Figure 18: Venn diagrams showing the amounts of regulated genes and the overlap between comparisons. Upregulated genes are shown in the green diagram on the left, downregulated genes are shown in the red diagram on the right. Genes were selected when the fold change was higher than 1.5 or lower than -1.5combined with a p-value lower than 0.05 in upper part A. The selected genes in B were used for pathway analysis and were more than 1.5 up or downregulated with a p-value lower than 0.01.

3.2.5 Pathway analysis

In total, three lists of regulated transcripts were generated. The annotations of these gene transcripts are bovine, which cannot be recognized by online pathway analysis. Most pathway analysis programs are focused on human, mouse and rat genomes and therefore these programs only recognize human, mouse or rat gene annotations. The gene transcripts were translated into their human annotations using the Unigene search function on the NCBI website (http://www.ncbi.nlm.nih.gov). The newly acquired gene annotations were used to perform the pathway analysis. Tables 7, 8 and 9 show the top 10 of pathways which were found regulated by DHEA compared to Control group 1, control group 2 and the total control group. We see several pathways regulated by DHEA, however not many gene transcripts were significantly regulated in these pathways. It should be noted that many transcripts were unable to be translated, and not all genes are recognized by pathway analysis (shown in Table 6).

Gene list	Number of regulated transcripts	Number of transcripts after translation	Number of genes	Number of genes recognized by Metacore
C1 compared to Exp	138	124	86	77
C2 compared to Exp	175	146	114	108
Ctot compared to Exp	86	73	46	40

Table 6: Number of transcripts and genes during the translation and pathway analysis procedure. An amount of genes was lost due to translation, double gene transcripts, and the recognition by pathway analysis software.

Nr#	Pathway influenced in C1/Exp	Regulated genes	Amount of genes regulated	p-value of pathway.
1	dGTP metabolism	PNPH, ACP2, DCK	3/39	9.141e-04
2	dATP/dITP mwtabolism	NP, ACP2, DCK	3/52	2.112e-03
3	Leukotriene 4 biosynthesis and metabolism	MPO, ALOX15	2/16	2.731e-03
4	HETE and HPETE biosynthesis and metabolism	MPO ,ALOX15	2/17	3.085e-03
5	Cholesterol biosynthesis	DHCR24, DHCR7	2/21	4.704e-03
6	Heme metabolism	HMOX1, HMOX2	2/28	8.282e-03
7	Glycolysis and gluconeogenesis	ENO3, FBP1	2/36	1.346e-02
8	Saturated fatty acid synthesis	FASN	1/3	1.467e-02
9	Lectin induced complement pathway	C2, C5AR1	2/39	1.568e-02
10	GTP-XTP metabolism	NP	2/61	3.165e-02

Table 7: Top 10 of pathways influenced by the regulated genes found in the comparison between C1 and Exp are shown in this tale. Upregulated genes are shown in green, downregulated genes are shown in red. Per pathway, the amount of genes regulated and the p-value are also shown.

Nr#	Pathway influenced in C2/Exp	Regulated genes	Amount of total genes	P-value of pathway.
1	FAS signaling pathway	FADD, PAK2, BAX	3/44	2.701e-03
2	TNFR signaling pathway	FADD, BAX, MAP4K2	3/47	3.262e-03
3	Neurofilaments	CDK5R1, ACTB, ACTR10	3/50	3.890e-03
4	Cytoplasm/mitochondrial transport of Bid, BMF and Bim	FADD, BAX	2/33	1.846e-02
5	Caspase cascade	FADD, BAX	2/34	1.954e-02
6	Role of IAP proteins in apoptosis	FADD, BAX	2/36	2.176e-02
7	Vitamin K metabolism	VKGK	1/4	2.511e-02
8	Apoptotic TNF-family pathway	FADD, BAX	2/40	2.651e-02
9	PAC1 in cellular process	PAR2, ACTB	2/46	3.433e-02
10	RAB3A regulation pathway	RABGGTA	1/9	5.563e-02
11	Cholesterol biosynthesis	IDI1	1/21	1.139e-01

Table 8: Ttop 10 of pathways influenced by the regulated genes fomr the comparison between C2 and Exp. Cholesterol biosynthesis was added due to a possible addition in the pathway seen in table 7. Upregulated genes are shown in green, downregulated genes are shown in red. Per pathway, the amount of genes regulated and the p-value are also shown.

Nr#	Map	Involved genes	Amount of total genes	p-value
1	Glycolysis and gluconeogenesis 1	FBP1	1/20	4.736e-02
2	GABA biosynthesis and metabolism	ALD5A1	1/20	4.736e-02
3	MIF-mediated glucocorticoid regulation	VCAM1	1/28	6.568e-02
4	Triacylglycerol metabolism	AGPAT2	1/29	6.795e-02
5	Brca1 as transcription factor	MSH2	1/30	7.021e-02
6	Fructose metabolism	FBP1	1/32	7.472e-02
7	Role of TLRs 3 and 4 in cell antiviral response: TICAM1 specific signaling pathways	TLR3	1/34	7.920-02
8	Plasmalogen biosyntheis	AGPAT2	1/35	8.144e-02
9	Glutathione metabolism	GSTK1	1/36	8.367e-02
10	Glycolysis and gluconeogenesis 2	FBP1	1/36	8.367e-02

Table 9: Maps found during metacore pathway analysis of the significantly regulated genes in the exposed group compared to the total control group, are shown. The involved gene(s), the amount of total gene regulated in that map and the significance of the regulation of the maps are also shown. Upregulated genes are shown in green, downregulated genes are shown in red.

Pathway analysis was done with the gene list shown in appendix C 1, 2 and 3. For gene lists 2 and 3 (C1/Exp and C2/exp), all transcripts were selected with a p-value lower than 0.01, due to the size of the transcript list which has to be manually translated into human annotations. From pathway analysis we can see that not many genes are regulated in a pathway, and several genes are responsible for the regulation of many pathways. Due to the difficulties discussed earlier during the discussion of the PCA and gene selection results, the little effects seen during pathway analysis can be related to the administration of low doses of an endogenously produced compound, in combination with the short exposure time and the

biological variance induced by the three separately performed animal experiments. Several pathways can be related to cholesterol biosynthes including genes like DHCR7 and DHCR24 which are responsible to the conversion of 7-dehydrocholesterol to cholesterol [59], both genes are downregulated. Genes related to hormonal effects were found in the gene lists. The genes Fibroblast growth factor Intracellular Binding Protein (FIBP), Insulin-like Growth Factor 2, Binding Protein 3 (IGF2BP3), Estrogen receptor Binding site Associated antigen 9 (EBAG9) and Adrenergic Receptor Bèta 3 (ADRB3), were found regulated and several of these genes were selected for RT-PCR confirmation.

Part 2: Biomarkers for Ipriflavone abuse in bovine liver.

The gene expression profile of another prohormone was studied *in vivo* in Frisian bovines. The second prohormone studied is the phytosterol ipriflavone (IF). In this microarray experiment, the main goals are to study the effects of IF on gene expression in bovine liver tissue, to identify biomarkers specific for IF and to gain insight into the action and metabolism of IF. The animal experiment included fifteen bovines, using the same control animals as described in the DHEA experiment. Eight animals were divided in two groups of 4 animals exposed by orally to either 0.2 g/day or 2 g/day ipriflavone. After the IF treatment, the animals were sacrificed and liver tissue samples were taken from which RNA was extracted. The RNA was processed and hybridized onto 44k bovine microarrays. Microarray data was analyzed and several genes were selected for RT-PCR confirmation.

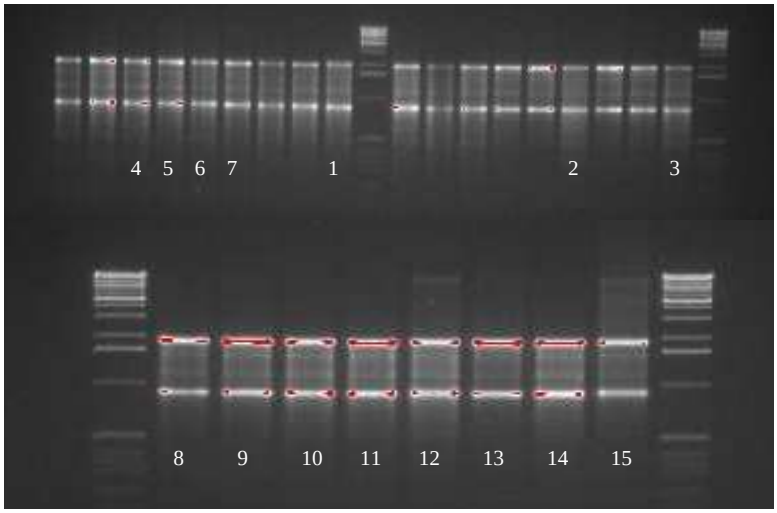
3.3 RNA from liver biopsies.

RNA was extracted from liver tissue samples using homogenization in Trizol® and the RNeasy™ mini kit. Samples were assessed on purity using the Nanodrop ND-1000 UV-VIS spectrophotometer as described in paragraph 3.1.1. The results from the spectrophotometric analysis are shown in Table 10. RNA samples were approved when ratios 260/280 (proteins) and 260/230 (organic compounds) were higher than 1.80. The concentrations range from 191 to 990 ng/µl and all samples have 260/280 and 260/230 ratios higher than 1.80 and therefore all samples were approved for further analysis.

Description	Concentration RNA ng/ µl	Ratio A260/280	Ratio A260/230
Control 1.1	191.5	2.06	1.81
Control 1.2	990.6	2.08	2.16
Control 1.3	445.1	2.05	2.02
Control 2.1	352.8	2.01	1.89
Control 2.2	176.9	2.03	1.89
Control 2.3	263.3	2.03	1.85
Control 2.4	468.0	2.00	1.86
IF 0.2 – 1	243.3	2.09	1.91
IF 0.2 – 2	281.8	2.05	1.98
IF 0.2 – 3	470.9	2.03	1.97
IF 0.2 – 4	244.0	2.11	1.93
IF 2 – 1	537.0	2.06	2.00
IF 2 – 2	589.2	2.09	2.22
IF 2 – 3	312.8	2.05	2.07
IF 2 – 4	256.1	2.04	2.00

Table 10: Spectrophotometric analysis of the RNA samples in the IF experiment. All samples were approved for further analysis.

RNA quality was assessed by agarose gel electrophoresis. A 1.5% agarose gel was made, RNA samples were incubated for 1h at 37°C. The samples were loaded onto the gel and electrophoresis was performed. Pictures of the gel were taken in a Bio-Rad geldoc and these pictures are shown in Figure 19. On the gel, no breakdown of RNA is observed which is seen as a smear. Based upon the results of the spectrophotometric analysis and agarose gel electrphoresis analysis, all samples were approved for further processing.



Sample #	Animal
1	Control 1.1
2	Control 1.2
3	Control 1.3
4	Control 2.1
5	Control 2.2
6	Control 2.3
7	Control 2.4
8	IF 0.2 – 1
9	IF 0.2 – 2
10	IF 0.2 – 3
11	IF 0.2 – 4
12	IF 2 – 1
13	IF 2 – 2
14	IF 2 – 3
15	IF 2 – 4

Figure 19: The RNA samples obtained from liver tissue of animals treated with ipriflavone. Again, the 18 and 28S rRNA bands are clearly visible.

3.4 Ipriflavone Microarray experiment.

3.4.1 RNA labeling

As described previously, the RNA samples were reverse transcribed into cDNA, and the cDNA was translated into labeled cRNA. The results from the labeling reaction were spectrophotometrically analyzed and the results are shown in Table 11. With the use of this data, the labeled yields and the total yields were calculated. Although the Cy3 pool does not meet the required 8.00 pmol/µl of labeled yield required for hybridization, decided was to continue with this Cy3 pool. After the labeling procedure, equal amounts of target RNA and reference RNA were combined and fragmented. Hybridization was done under the stringent conditions of 65°C in a hybridization oven with rotation function (10 rpm). After hybridization the arrays were washed and scanned.

Description	Cy3 RNA pmol	Cy5 RNA pmol	Total concentration ng/µl	Labeled yield pmol/µl	Total yield µg
C 1.1	-	1.52	179	8.5	5.36
C 1.2	-	1.30	149	8.7	4.47
C 1.3	-	1.73	188	9.5	5.48
C 2.1	-	1.35	138	9.70	4.15
C 2.2	-	1.86	183	10.2	5.50
C 2.3	-	1.71	171	10.0	5.13
C 2.4	-	2.02	187	10.8	5.60
IF 1	-	2.83	253	11.2	7.58
IF 2	-	4.05	342	11.8	10.23
IF 3	-	3.14	290	10.8	8.69
IF 4	-	3.01	257	11.7	7.71
IF 5	-	2.42	237	10.2	7.12
IF 6	-	3.59	305	11.8	9.16
IF 7	-	3.84	334	11.5	10.03
IF 8	-	3.13	268	11.7	8.03
Cy3 pool	1.55	-	204	7.6	6.12

Table 11: Results from the labeling reaction including the measured Cy3 and Cy5 and total concentrations are shown. With the use of this data, the labeled yields and the total yields were calculated.

3.4.2 Hybridization quality control

The microarray hybridization quality control after hybridization, washing, scanning and feature extraction was done was described previously in paragraph 3.1.3. Microarray scans were visually checked and are shown in Appendix B. The M-boxplot is shown in Figure 20, shows the results before and after filtering and normalization. This figure shows that array 14 was not hybridized in a comparable way to all other arrays. This slide shows large numbers of outliers as well as a broad 95% reliability interval. Decided was to remove array 14 (sample IF 2 - 3) from further analysis.

Slide 1	Control 1.1
Slide 2	Control 1.2
Slide 3	Control 1.3
Slide 4	Control 2.1
Slide 5	Control 2.2
Slide 6	Control 2.3
Slide 7	Control 2.4
Slide 8	IF 0.2 - 1
Slide 9	IF 0.2 - 2
Slide 10	IF 0.2 - 3
Slide 11	IF 0.2 - 4
Slide 12	IF 2 - 1
Slide 13	IF 2 - 2
Slide 14	IF 2 - 3
Slide 15	IF 2 - 4

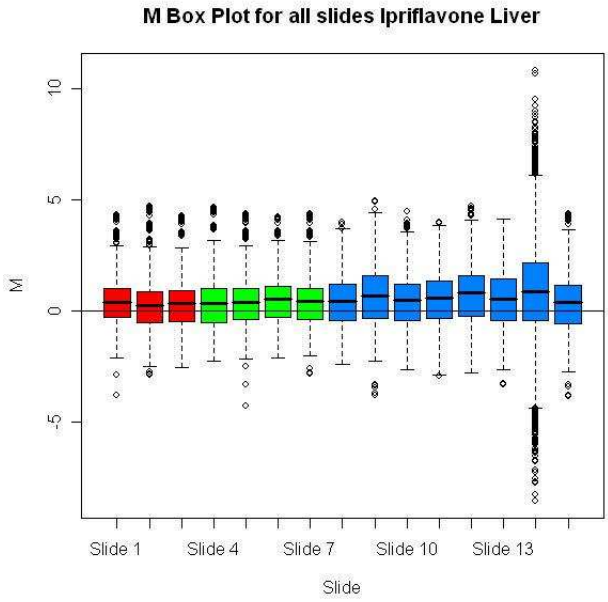


Figure 20: M-box plot used to assess the hybridization quality control of the ipriflavone microarray experiment. All. Red represents control group 1, green represents control group 2 and blue represents the IF treated group.

3.4.3 Principal components analysis

Principle components analysis was performed as described previously in paragraph 3.1.4. The PCA was performed after filtering, 2-log transformation and normalization. The PCA 3D plot showing the three dimensions in which the largest differences can be seen is shown in Figure 21. Large differences between the ipriflavone and total control group are observed. The large differences can be explained due to the long exposure time of the animals to IF which in addition is not an endogenous produced compound. The differences between the two groups of control animals can also be observed in this PCA plot, however the differences between the IF treated animals and control animals are larger than the differences between the two control groups. The dosage of IF administration does not induce differences between the high and low dose treated animals, according to the PCA. For further comparisons, all control animals were pooled into one control group and compared to the pooled IF group.

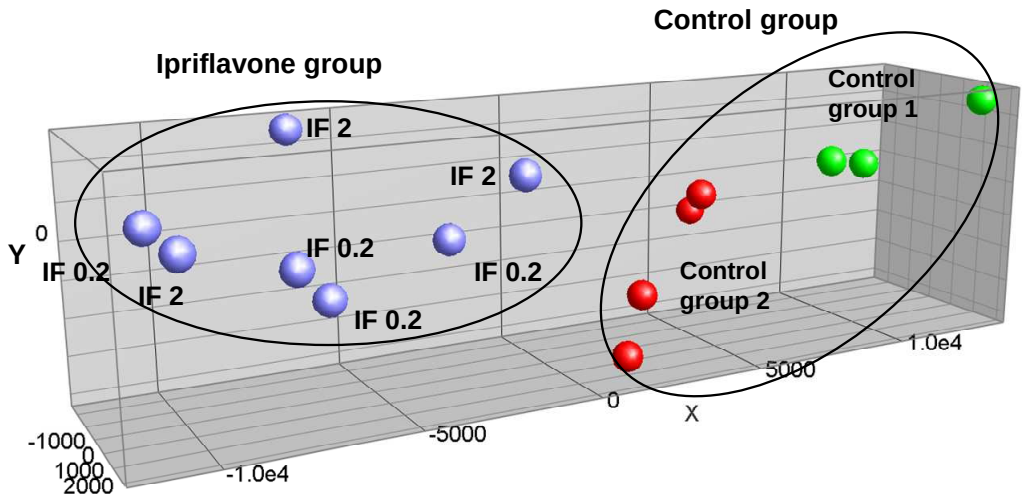


Figure 21: Priniciple Components Analysis of the ipriflavone microarray experiment. The three dimensions where the largest differences (62.1 % of total differences) can be observed are plotted. Red, green and blue dots respectively represent control group 1, 2 and the ipriflavone group.

3.4.4 Gene selection.

Gene selection from the data analysis was done in a similar way as described in paragraph 3.1.5. Transcripts were selected when the fold change was higher than 2 with a significance lower than 0.01, these transcripts are visualized in figure a and the amounts of up- and downregulated transcripts are shown in Figure 22. Higher selection criteria were selected because the differences between the exposed and control groups are greater than in the DHEA experiment.

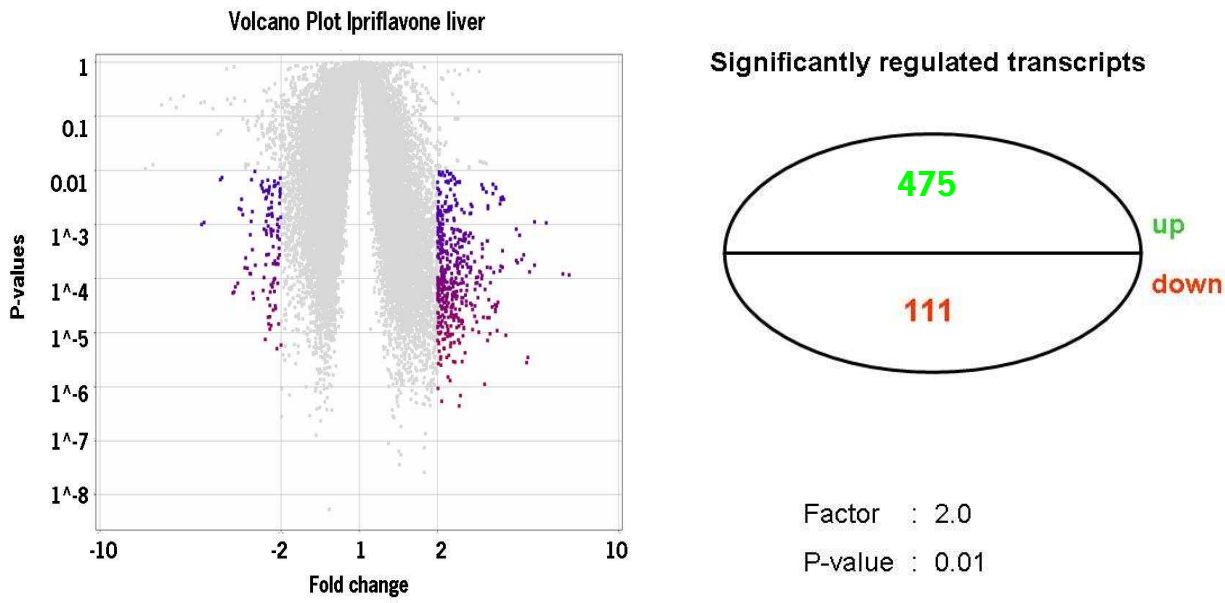


Figure 22: The Volcano plot on the right shows the significantly regulated transcripts found in the comparison between the Control group and the IF group. Transcripts were selected when the fold-change was higher than 2 and the p-value obtained from the individual sample t-test was lower than 0.01.The diagram on the left shows the amounts of transcripts significantly regulated by IF.

3.4.5 Pathway analysis

As described previously, the bovine gene annotations first have to be translated into their human annotations in order to perform pathway analysis. The translation and pathway analysis were done as described in paragraph 3.1.6. Table 12 shows the top 20 of pathways which were found affected by IF induced gene regulation.

Nr#	Map	Involved genes	Amount of transcripts in pathway	p-value
1	Role of AKT in hypoxia induced HIF1 activation	CCT,HSP70, PGK1	9/50	6.403e-08
2	Glucocorticoid receptor signaling	HSP70, HSP90, NFKBIA	7/40	2.360e-06
3	Role of ASK1 under oxidative stress	HSP70,HSP90	6/32	8.449e-06
4	Role of IAP proteins in apoptosis	CYCS, HSP70,	6/36	1.724e-05
5	Putative ubiquitin pathway	HSP70	5/31	1.053e-05
6	n-3 polyunsaturated fatty acid biosynthesis	FADS2, ACOX1, EHHAD, ACSL1,	4/22	3.318e-04
7	n-6 polyunsaturated fatty acid biosynthesis	FADS2, ACOX1, EHHAD, ACSL1,	4/22	3.318e-04
8	Fatty acid omega oxidation	ACSL1, ACOX1, EHHADH	3/14	1.184e-03
9	Parkin disorder under parkinson's disease	HSP70	5/52	1.237e-03
10	Role of Parkin in the ubiquitin-proteasomal pathway	HSP70	5/52	1.237e-03
11	Androstenedione and Testosterone biosynthesis and metabolism	AKR1D1, UGT2A1, UGT1A4	3/17	2.136e-04
12	Vitamin E (alfa-tocopherol) metabolism	ACSL1, ACOX1, EHHADH	3/17	2.316e-03
13	Unsaturated fatty acid metabolism	ACSL1, FADS2, SCD	3/18	2.535e-03
14	Lymphotoxin-beta receptor signalling	LTB, CYCS, CHUK, NFKBIA	4/41	3.649e-03
15	Role of AP-1 in regulation of cellular metabolism	NQO1, CDKN1A, GCLM	4/43	4.341e-03
16	ERBB-family signaling	ERBB2, ERBB3, CHUK, NFKB1A	4/51	7.985e-03
17	PPAR regulation of lipid metabolism	FABP5, ACSL1, EHHADH	3/28	9.081e-03
18	Peroxysomal straight chain fatty acid beta-oxidation	ACOX1, EHHADH	2/10	9.944e-03
19	AKT signaling	CHUK, NFKB1A, CDKN1A	4/57	1.175-e02
20	Anti-apoptotic TNFs/NF-kB/IAP pathway	CHUK, NFKB1A	3/31	1.204e-03

Table 12: Top 20 of pathways regulated by IF administration accompanied by the amount of regulated genes, the total amount of genes in the pathway and the p-value. Upregulated genes are shown in blue, downregulated genes are shown in red.

From the pathway analysis, we can see many regulated pathways. Due to the relatively large set of genes, decided was to focus on the possible growth promoting effects of IF administration. The maps shown in Table 13 presents some of the pathways in which the genes are regulated. In the glucocorticoid receptor signaling pathway, genes coding for proteins in the HSP70 and HSP90 complex are upregulated while NFkBIA is downregulated. Heat shock proteins HSP70 and HSP90 complexes are related to glucocorticoid receptor signaling [45], indicating that the glucocorticoid receptor (GR) is stimulated. The activation of GR leads to an upregulation of the glucocorticoid responsive promoter IkBα, which in its turn, downregulates the nuclear transcription factor NFkB which regulates many immune response related genes [46, 47, 48].

This downregulation was observed during pathway analysis. Another map shows genes related to androstenedione and testosterone metabolism. Genes UGT2A1 and UGT1A4 are involved in the glucuronidation of testosterone and androstenedione [49]. Aldo-keto reductase 1 D 1 (AKR1D1) is involved in many steroid related reactions and is mainly responsible for the 5β-reduction of steroids like progesterone, androstendedione, 17α-hydroxyprogesterone and testosterone [50]. CYP2C8 is a cytochrome P450 enzyme which is related to the biosynthesis and metabolism of estrone and 17β-estradiol [51], which in its turn, affects the regulation of ERBB2 and ERBB3 [52], which is shown in two maps of the pathway analysis. In the gene selection, but not in pathway analysys we can see more steroid metabolism and biosynthesis related genes such as HSD17B13 and other UGT genes. The induction of these genes show that IF regulated steroid related genes and stimulates the activation of the glucocortcoid receptor. It confirms that IF has hormonal action and influences natural hormone levels. Metabolite research is required to find out in which way these levels are affected, but it is clear that IF might induce growth promoting effects in bovines.

Nr#	Map	Amount of transcripts in pathway	Involved genes	p-value pathway
1	Glucocorticoid receptor signaling	7/40	HSP70, HSP90, NFkBIA	6.040e-06
2	Androstenedione and testosterone metabolism	3/17	AKR1D1, UGT2A1, UGT1A4	3.187e-02
3	MIF-mediated glucocorticoid regulation	3/28	CHUK, NFkB1A, VCAM1	1.329e-02
4	Aldosterone biosynthesis and metabolism	1/8	AKR1D1	1.344e-01
5	ESR1: interaction with growth factors signalling	2/39	ERBB2, ERBB3	1.726e-01
6	Estrogen biosynthesis	1/11	CYP2C8	1.800e-01
7	Cortisone metabolism and biosynthesis	1/17	AKR1D1	2.643e-01
8	Androstenedione and testosterone metabolism 2	1/17	AKR1D1	2.642e-01
9	ER1/ER2 activation	2/56	ERBB2, ERBB3	2.646e-01

Table 13: Steroid and growth promoter related maps in which the genesare regulated. The involved downregulated genes are shown in red, upregulated genes are shown in green.

Part 3: Real-Time RT-PCR.

Real-time Reverse transcriptase-PCR (RT-PCR) was used to confirm the expression of several genes on the array. First, RNA was treated with DNase to remove genomic DNA from the sample. After the DNase treatment, cDNA was synthesized from mRNA by reverse transcription. After reverse transcription, cDNA was added to a SYBRgreen® reaction mix, all samples were applied to the plate in duplicate for the target gene and in duplicate for the reference gene. The sample with highest expression of the target gene was diluted to 1/10, 1/100, 1/1000 and 1/10000, and these sample standards were used to create a calibration curve as seen in Figure 23.

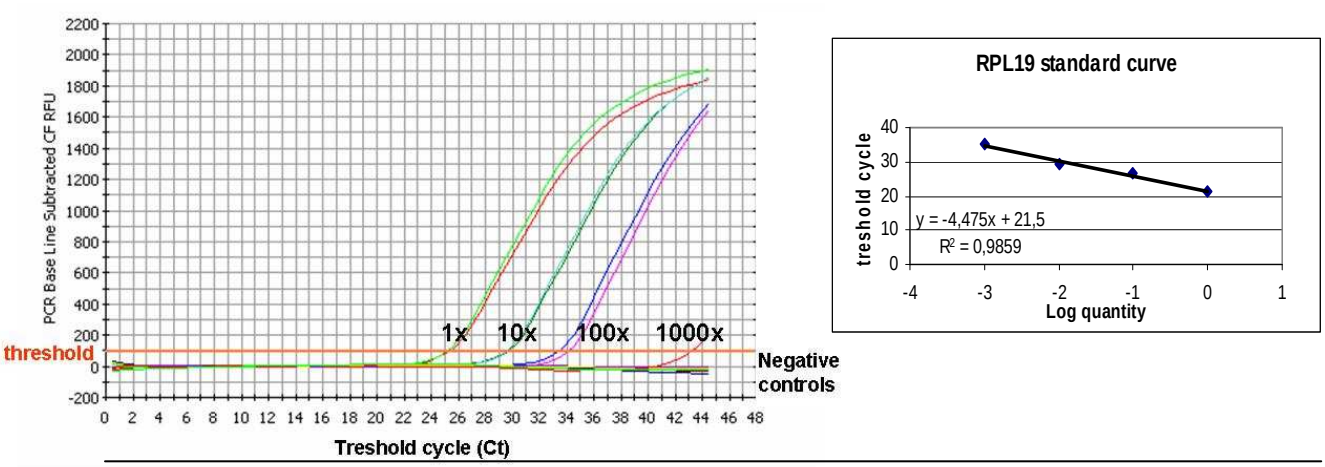


Figure 23: An example of a calibration curve, which was used to calculate relative reference gene cDNA concentrations. The curves are respectively the undiluted (log 0),10 times diluted (log-1), 100 times diluted (log-2), 1000 times diluted (log-3) and 10000 times siluted (log-4) samples of control sample 1.1. The log-4 dilution is does not reach the threshold value and the log-3 dilution comes up very late. Therefore, log 0, log-1, and log-2 values can be used to calculate a calibration curve, which is shown on the right..

The cycle in which the fluorescence of the sample reached the threshold fluorescence signal, was recorded as the Ct-value. The calibration curves for target and reference genes were used to calculate the relative cDNA concentration in each sample well. The relative concentrations were normalized by averaging the duplicates and dividing the relative target concentrations by the relative reference concentrations. Gene expression ratios were calculated by deviding the target quantity by the reference quantity. Standard deiations for these ratios were calculated according to the following formula:

$$\text{Stdev}^{new} = \text{Value}^{new} \sqrt{\left(\left(\frac{\text{Stdev ref}}{\text{Quantity Ref}}\right)^2 + \frac{\text{stdev Target}}{\text{Quantity Target}}\right)^2}$$

An example of this calculation is shown in table 14. From these normalized data, ratios were calculated as well as their corresponding standard deviations.

Gene sample	Ct value	Loq Quantity	Quantity	Average	Standard deviation	Normalized quantity	New standard deviation
MARCO PO3	33.6	-0.741	0.18	0.23	0.06	0.055	0.02
MARCO PO3	33.1	-0.569	0.27				
MRPL53 PO3	23.7	0.629	4.25	4.12	0.19		
MRPL53 PO3	23.8	0.601	3.99				

Table 14: RT-PCR analysis was done by using the calibration curve formula to calculate relative cDNA quantities. The table shows the calculated values from the Ct value to the normalized quantity.

The products formed after RT-PCR were checked on length, using the melt-curve data and agarose gel electrophoresis. Melt-curve data was obtained during the RT-PCR, and an example of a melt curve is shown in figure 24. If the meltcurve shows more than two products (Target and reference gene), other curves can be seen as contamination, or when more products are formed, the primers could be unspecific of for the target gene. additional and definitive confirmarion of the amlicon was done by agarose gel electrophoresis to determine the size of the amplicon.

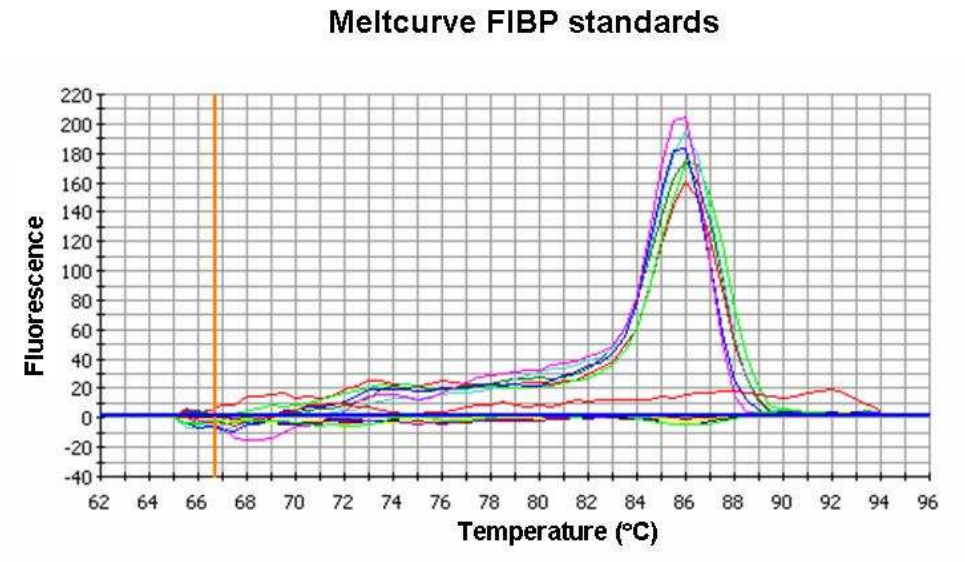


Figure 24: The meltcurve data of FIBP shows the amounts of amplicons formed. This curve shows only one kind amplicon formed during the real-time RT-PCR procedure

3.5 DHEA liver microarray confirmation

A selection of genes which were found regulated in the liver of DHEA treated animals compared to control group 1, 2 and total. Genes were selected for RT-PCR confirmation based on their expression level, regulation and overlap between control groups. The selected genes are CYP1A1, CYP11A1, HSD17B7, PRLR, IGF1, GHR and CYP2C9. RPL19 shows a constant gene expression in all samples and was therefore used as a reference gene during each RT-PCR. The primers used for real-time RT-PCR analysis are shown in appendix D 1. Figure 25 shows the total overview of microarray and RT-PCR gene expression analysis.

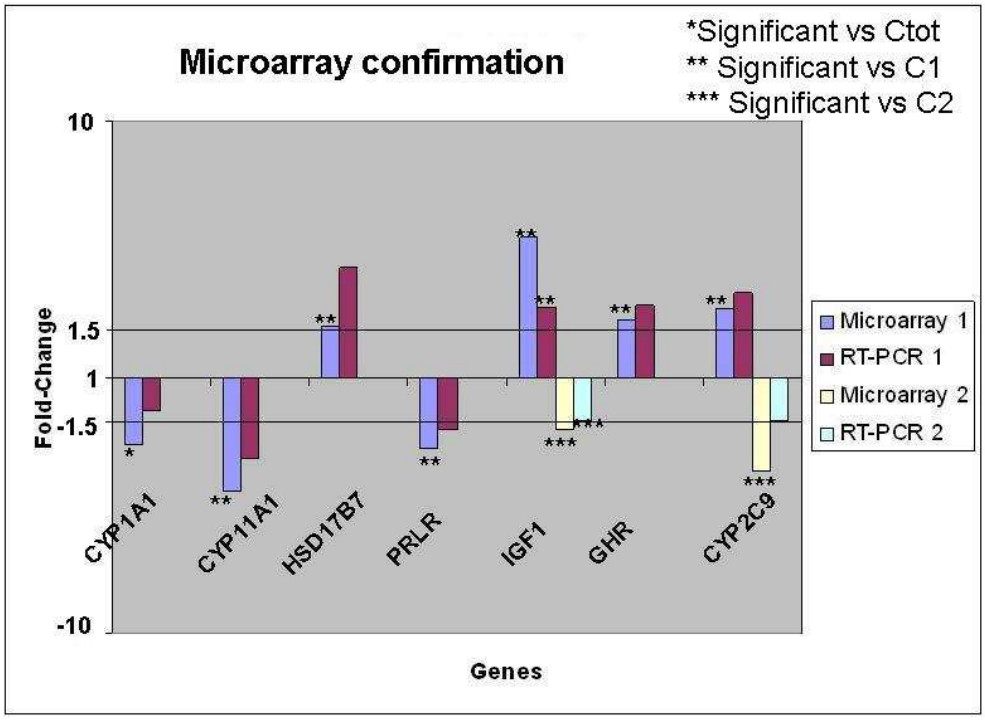


Figure 25: All gene expressions found by analyzing the microarray results were confirmed with RT-PCR, except for CYP1A1. Microarray results are shown in blue, RT-PCR results are shown in red, addition microarray and RT-PCRs (from two comparisons) are shown in respectively yellow and green. The asterixes indicate the significance versus respectively Ctot (1 asterix), C1 (two asterixes) and C2 (three asterixes).

The results presented in figure 24 shows that the fold-change in expression of almost all genes are confirmed by RT-PCR. Not all RT-PCR values were found significant by the individual sample T-test (P-value lower than 0.1) which is due to the spreading in expression between the animals in one group, which causes a high standard deviation, which as a result results in a high p-value. On the agarose gel, we see that all reactions have resulted in one product, except for CYP11A1, where a second product is found on the gel (< 50bp). All products were checked on length shown in the table next to the gel. The product of RPL19 is not shown but was checked previously and was determined to have the right length. All products shown in Figure 25 have the correct length and therefore, the RT-PCR validation for the DHEA liver microarray experiment was successful.

Nr	Gene	Amplicon size	Correct size
1	CYP1A1	180	yes
2	CYP11A1	166	yes
3	HSD17B7	128	yes
4	PRLR	203	yes
5	IGF1	188	yes
6	GHR	136	yes
7	CYP2C9	201	yes

Figure 26: The agarose gel on which the amplicon sizes in the RT-PCR reactions of the DHEA liver microarray validation are electrophorized. The table in this figure shows the theoretical amplicon size, and if the amplicon on the gel has the right length. In this case.

3.6 DHEA blood microarray confirmation.

Based upon their regulation in gene list, significance and relationship to hormonal effects, the genes FIBP, DHCR24, and IGF2BP3 were selected for real-time RT-PCR confirmation. The primers designed for these genes and additional information about the primers and its amplicon are shown inAappendix D 2.

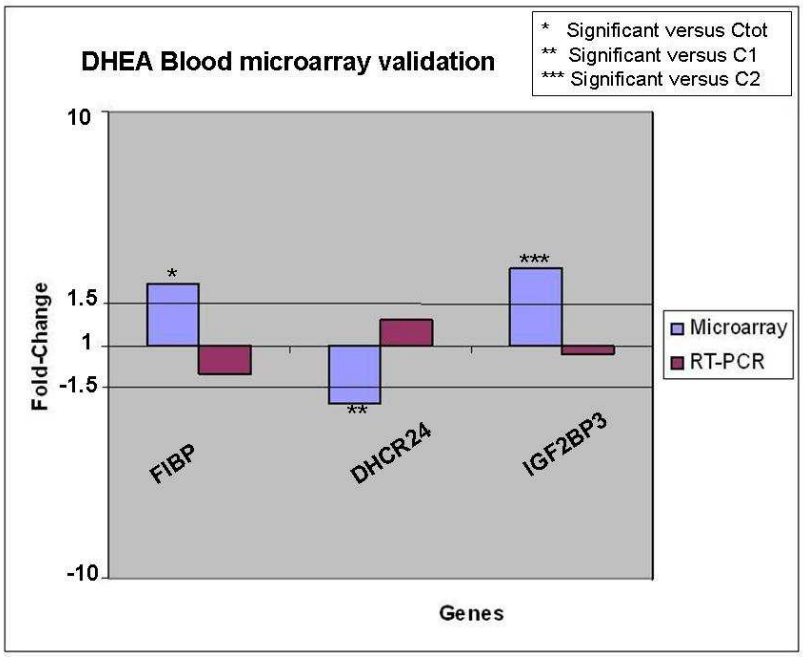
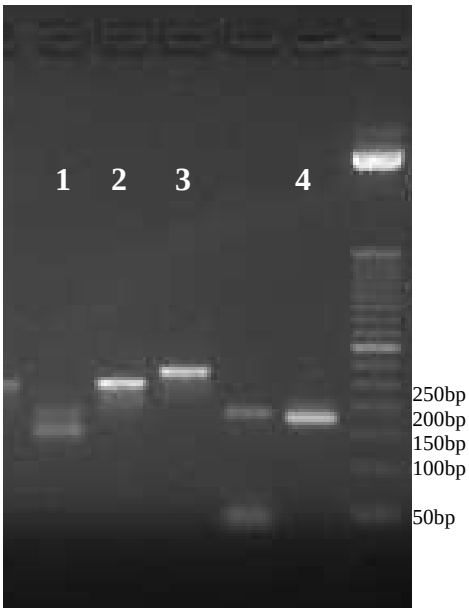


Figure 27: Gene expression of the individual genes determined by microarray (blue) and RT-PCR (red). Asterixes present significance versus Ctot (1 asterix), C1 (two asterixes) and C2 (3 asterixes). Shown are genes FIBP, DHCR24 and IGF2BP3, no gene expressions were confirmed with RT-PCR.



Nr	Gene	Amplicon size	Correct size
1	FIBP	148	yes
2	IGF2BP3	243	yes
3	DHCR24	272	Yes
4	MRPL53	173	Yes

Figure 28: Agarose gel on which the amplicon sizes in the RT-PCR reactions of the DHEA blood microarray validation are electrophorized. The table in this figure shows the theoretical amplicon size, and if the amplicon on the gel has the right length. In this plot, the gel shows that many products have formed, therefore most of the RT-PCR results are not reliable.

Figure 27, we can see that the expression of the selected genes is not confirmed by real-time RT-PCR. When we look at the agarose gel in Figure 28, we also see that there is more than one product formed which makes the real time RT-PCR validation of the microarray experiment unreliable and, so far, unsuccessful. The expressions of the genes measured by RT-PCR are more sensitive to minor deviations in cDNA concentrations. The low concentrations of initial RNA extractions might contribute to this deviation and in combination with the previously discussed variations between animals inside the groups, this might lead to complications during the RT-PCR analysis. Another possibility can be that primer pairs are unspecific, which were designed according to the reaction procedure, and when no primers were found, the primers were allowed to contain cross-homology. However, the primer pairs as well as the probe on the microarray were checked on sequence homology using nucleotide BLAST (Basic Logical Alignment Statistical Tool, NCBI-website) where no other *Bos Taurus* genes were found. On the gel in figure 27 we can see multiple products which indicates that the primers are indeed unspecific although the correct size of the product is also shown. In addition, the meltcurves (not shown) also indicate the presence of multiple products. It is recoomended for future validation of the DHEA blood microarray experiment to select other genes for confirmation, or to design new primer pairs avoiding cross homology.

Part 4: *In vitro* experiments.

3.7 Growth inhibition assay.

For an effective exposure of HepG2 cells to DHEA, it is important to know which concentrations inhibit growth and can therefore interfere with further research. The growth inhibition properties of various DHEA concentrations were measured using the MTT-test. The MTT-test is a colorometric assay first developed by *Mossman* in 1983 [41]. Theorettically, cells are exposed to a compound. After a certain exposure time, the medium is removed from the cells and new medium is added which contains dimethylthiazol-diphenyl-tetrazoliumbromide (MTT). During a one hour incubation with MTT, the cells convert the MTT into blue formazan crystals (reaction in Figure 29). Absorbtion can be measured at 562 nm to determine the amount metabolic active cells in the sample.

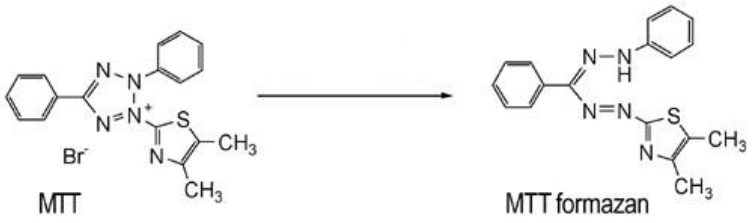
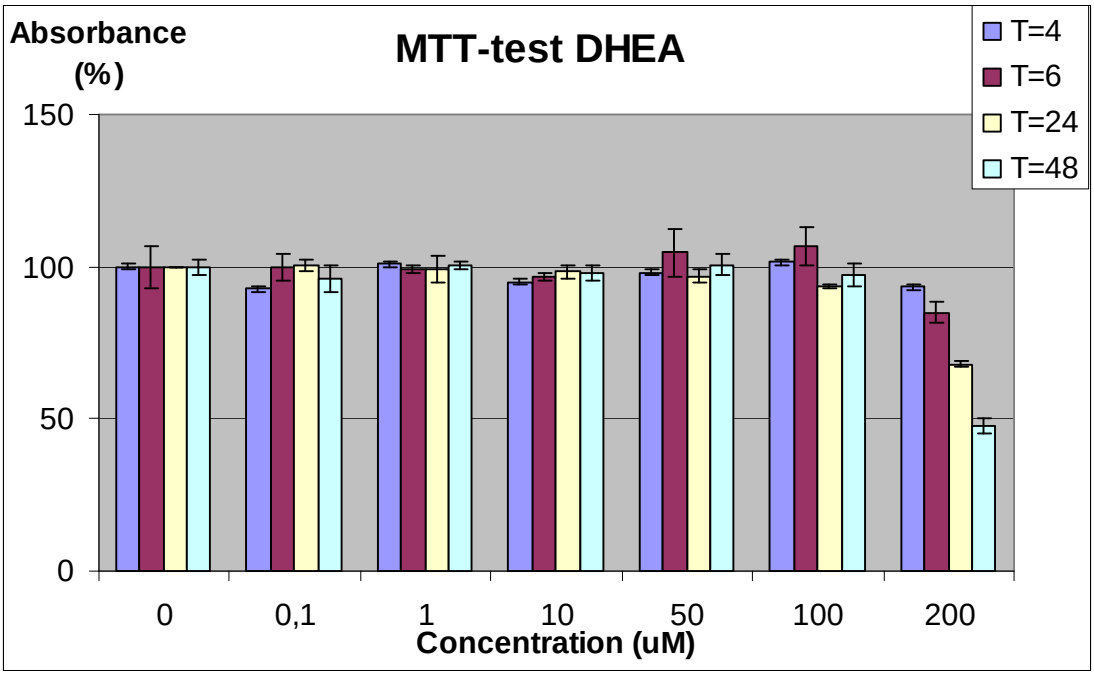


Figure 29: MTT is converted in the mitochondria of metabolic active cells into blue formazan which forms crystals.

The concentration range was taken and expanded from a study by *Yohsida et al (2003) [42]*, in which the human liver carcinoma cell line HepG2 was exposed to a concentration range of 0,1,10 and 100 µM DHEA. In our experiment, we used a concentration range of 0, 0.1, 1, 10, 50, 100 and 200 µM DHEA. DHEA was dissolved in DMSO which was diluted in culture medium to a total volume of 1%. Cells were seeded at a density of 7.5x10^4 cells per well in 24 multi wells plates. The seeded cells were exposed by adding DHEA containing medium (containing 1% DMSO) to the cells. After exposure times of 4h, 6h, 24h and 48h, the medium was removed from the cells and the MTT-test was performed. The absorbance of the wells containing 0 µM DHEA were set to 100% and the percentage of absorbances in the other wells were calculated using the 100% absorbance. The experiment was done in triplicate and the results are shown in Figure 30. This figure shows the column chart which visualizes the data in table below. After 24 hours the absorbance of the 200 µM DHEA wells are reduced to 69% of that of the 0 µM wells, which means that the concentration of 200 µM DHEA inhibits the growth of HepG2 cells, while other the other concentrations do not seem to have any effects on HepG2 growth. *Yoshida et al* found that a concentration of 100 µM was growth inhibiting, however the amount of cells used in their experiments were much lower and different medium was used during exposure, their results are therefore hard to compare to this experiment. A concentration range between 0 and 100 µM can be taken to use in furthers research for an exposure using and exposure time of 24h according to the exposure procedure described in this study.



Concentration DHEA	T=4	Standard deviation	T=6	Standard deviation	T=24	Standard deviation	T=48	Standard deviation
0	100.00	4.11	100.00	5.35	100.00	3.50	100.00	1.92
0.1	96.83	5.31	94.07	1.91	95.81	3.73	96.07	5.66
1	97.56	3.33	89.61	5.62	97.76	13.44	98.02	5.48
10	92.80	8.02	89.55	6.73	94.58	1.19	89.00	2.32
50	101.04	3.33	95.34	5.93	98.14	0.18	98.91	5.56
100	92.38	1.75	89.55	6.49	94.71	2.11	88.06	4.32
200	92.99	6.95	85.22	3.02	69.23	4.81	37.25	4.44

Figure 30: Column chart and table representing the data from the MTT-test. The columns represent the absorbance of the wells after 4h (blue), 6h (red), 24(yellow) and 48h(green) of exposure to DHEA.

3.8 HepG2 exosure to DHEA

For a new exposure to DHEA, HepG2 cells were seeded and exposed during 24h to DHEA in the same density as described in the MTT-test, using the concentration that had no anti-proliferative effects. After exposure cell culture medium was transferred to a fresh tube, which was used for biochemical analysis, and the cells were washed and stored in PBS. The protein concentration was measured to quantify the amount of cells in the cell sample well. To achieve this, the BioRad DC protein assay kit was used, which is a colorimetric protein assay according to the Lowry method [43, 44]. In this reaction, copper sulphate is added to a protein solution in a strong alkaline environment, a purplish-violet colour is produced, which is the result from a complex formation between the cupric ions and the peptide bond [44]. After the reaction, the absorbance of the cell sample wells was measured using the Elx808™ absorbance microplate reader

and its corresponding software KCJunior™. Protein concentrations in the cell sample wells are calculated using a BSA standard curve. The results of the protein assay are shown in figure 30 and table 15. The concentrations seem to be quite stable ranging from 0.444 to 0.559 mg protein per ml which determines that no anti-proliferative effects of DHEA are seen. For further analysis, the metabolite profile of DHEA has to be studied, which can be done using UPLC-QTOF-MS.

Exposure	Sample #	Protein concentration mg/ml	Standard deviation
0 µM DHEA	1	0,546	0,005
	2	0,509	0,017
	3	0,449	0,017
	4	0,464	0,027
	5	0,469	0,01
	6	0,472	0,027
0.1 µM DHEA	1	0,518	0,045
	2	0,492	0,065
	3	0,469	0,005
	4	0,446	0,049
	5	0,444	0,042
	6	0,446	0,025
1 µM DHEA	1	0,541	0,037
	2	0,515	0,025
	3	0,523	0,05
	4	0,473	0,018
	5	0,494	0,04
	6	0,473	0,018
10 µM DHEA	1	0,5	0,013
	2	0,559	0,082
	3	0,556	0,031
	4	0,465	0,028
	5	0,494	0,032
	6	0,488	0,06

Table 15: The protein concentrations in the cell suspension samples are indicative for the amount of cells in the sample well.

4. Conclusions.

4.1 Part one: Biomarkers for DHEA abuse in bovine blood.

The first part focussed ont the identification of biomarkers for DHEA abuse in bovine blood. In the results section, we showed that RNA extraction from bovine blood was successful, and RNA was of high quality prior to the labeling procedure. The labeling procedure was successful for Cy5- and adequate for Cy3 labeling. Microarray hybridization was carried out, checked on quality and all arrays were approved for further data analysis. Further data analysis was done by PCA, gene selection based on regulation and significance and finally the regulated transcripts were analyzed using pathway analysis. Prior to pathway analysis bovine gene annotations where translated into human gene annotations. The results from the PCA showed that that there were no large differences between treated and untreated animals. Furthermore a large spreading between all animals was observed, except for the control animals from experiment 3, which were clustered on the PCA plot. This resulted in a comparison between the exposed animals and the different groups of control animals. Lists of regulated transcripts between these groups show very little overlap. And only a small list of transcripts were found significantly regulated when all control animals were compared to all exposed animals.

These results show basically that the experimental setup is essential in the screening feasibility of microarrays on DHEA abuse. The short exposure time in combination with the administration of low doses of endogenous compounds does not result in many gene regulations in bovine blood. Furthermore, the animals were treated in three different experiments, and are therefore hard to compare to each other which is shown in the PCA plot where large differences can be seen between the control groups. However, the setup of the experiment does simulate the practice situation, where animals have differences in age, weight, background and treatment type. The dose of administration was extrapolated from human dosage to bovine dosages which resulted in an administration dosage of 1g/day. In the comparisons between the individual control groups, there were several genes regulated related to steroids and growth promoting effects, like Insulin like growth factor 2 binding protein 3 (IGF2BP3), estrogen receptor binding site associated antigen 9 (EBAG9) and cholesterol biosynthesis related genes like dehydrocholesterolreductase DHCR24 and DHCR7. These genes might be used as biomarkers in future research and were selected for validation of the microarray experiment. Pathway analysis did not reveal many strongly regulated pathways, mainly because of the small differences between the treated and untreated animals resulting in small numbers of significantly regulated genes. However despite all the negative points discussed, there were 46 significantly regulated genes found when all untreated animals were compared to all treated animals. These findings make the concept of microarray technology interesting in the field of prohormone screening using blood as a target tissue.

4.2 Part two: Biomarkers and effects of Ipriflavone abuse in bovine liver.

In the second part, we focussed on the effects of IF administration on liver tissue in bovines. In the results section we discussed the quality of the RNA extractions from liver biopsies, and concluded that the RNA extractions were approved for the labeling procedure. The labeling procedure using Cy5 dye was successful and the Cy3 dye labeling did not precisely meet the requirements but was approved for the hybridization procedure. The hybridization procedure was carried out and checked on quality where one array was excluded for further analysis. Data analysis was done by PCA, transcript selection based on high regulation and high significance, and by pathway analysis. From the PCA analysis, where a very clear separation between treated and untreated animals was seen, decided was to pool the treated group and to compare this group to the total untreated group. Although differences were also observed between control groups, the differences were smaller when compared to the IF treated group. The comparison between treated and untreated animals resulted in a large set of strongly regulated transcripts. However, to obtain a transcript list more suited for data analysis, decided was to increase the criteria for selection. Prior to the pathway analysis, the bovine gene annotations were once again translated to human gene annotations.

The results revealed that the treatment with IF results in large differences between treated and untreated animals, which furthermore results in a large set of significantly regulated transcripts. It should be noted that, when this experiment is compared to the DHEA experiment, the duration of the treatment was much longer, and furthermore, IF is an exogenous compound, while DHEA is an endogenous produced compound. So, larger differences than in the DHEA experiment were expected, however, the setup of the experiment is nonetheless comparable to the practice situation and the results are therefore of great value for further research. A total of 486 regulated transcripts were found significant and were analyzed using pathway analysis. The pathway analysis revieled many genes involved in the glucocorticoid receptor acticvation pathways and genes related to steroid biosynthesis and metabolism. Transcripts related to the formation of HSP70 and HSP90 complexes were upregulated while NFKB1A was downregulated. These effects can directly be coupled to glucocorticoid receptor activation [46]. The genes aldo-keto reductase 1D1 (AKR1D1), many UDP-glutathione transferases (UGTs), cytochrome P450 2C8 (CYPIIC8) are all upregulated and influence both the androgen or estrogen biosynthesis and metabolism. This study therefore proves that ipriflavone is indeed a potent prohormone which affects natural hormone levels, and can be seen as a compound which induces growth and anabolism in bovines. For more insight in the actual effects of IF on natural hormone and metabolite levels, the metabolite profile of bovines treated with IF should be studied. For publishing purposes, it is recommended to validate this microarray study by real-time RT-PCR comfirmation.

4.3 Part three: Gene expression confirmation.

In the study on DHEA induced gene expression in liver tissue described by *Rijk et al (2007 article submitted)*, several lists of significantly regulated transcripts were obtained. These genes were selected for the validation of the microarray study using real-time RT-PCR according to the SYBR®green method. The same was done for the DHEA microarray experiment on blood tissue. All genes were selected based on their biological function, regulation and overlap between different comparison of the exposed group to the different control groups.

The results of the microarray validation of the DHEA microarray experiment on liver tissue have confirmed several genes that were significantly regulated on the microarray. Furthermore, the amplified products had the correct length from which we can conclude that this validation was successful. The genes selected in the DHEA microarray experiment in blood tissue study, no genes were confirmed using RT-PCR. These negative results can be related to the low concentrations of RNA extracted from blood, which induces an increased sensitivity for experimental procedures. This, in combination with the experimental setup and the variances in gene expression between treated animals, leads to difficulties in a successful validation. In addition, the reaction resulted in multiple amplified products per reaction which strongly indicates the inspecificity of the primers. For future validation, it is recommended to make a new gene selection for the validation of this microarray experiment or to design new primers for the currently selected genes.

4.4 Part four: Setup for in vitro experiments.

A setup was made for future in vitro experiments. The setup includes the culturing of human liver carcinoma cells and the exposure to various concentrations of prohormones. Anti-proliferative effects of, in this case, DHEA were determined for this method of exposure using the MTT-test as described by *Mossman (1983)* [41] which was adapted as described in this study. DHEA induces anti-proliferative effects at a concentration of 200 µM, and therefore a concentration range of 0 to 100 µM DHEA can be used to expose human liver carcinoma cells. This method can be applied for future experiments using other prohormones like, for instance, IF. The human liver carcinoma cells were exposed to the non-cytotoxic concentrations of DHEA as described in this study and the concentration of proteins was measured using the protein assay, also described in this study, to quantify the metabolites formed by the cells. The exposed cells can also be used for future transcriptomics research, studying the gene expression profiles in human liver cells.

References.

1. **European Union.**
   European union Council directive 96/22/EC.
   Off. J. Eur. Commun. 1996, L 125: 3-9, 1996.
2. **Hadley ME.**
   Endocrinology, fifth edition.
   Prentice Hall 2000.
3. **Meyer HD.**
   Biochemistry and physiology of anabolic hormones used for improvement of meat production.
   APMIS 109: 1-8, 2001.
4. **Meisonnier E.**
   Anabolics in animal production.
   Soregraph, Levallois, 1983.
5. **Davis SL, Ohlson DL, Klindt J, Anfinsobn NS.**
   Episodic growth hormone secretory patterns in sheep: relationship to gonadal steroids.
   Am. J. Physiol. 233: E519-23, 1977.
6. **Breier BH, Gluckmann PD, Bass JJ.**
   The somatotropic axis in young steers: influence of nutritional status and oestradiol-17B on hepatic high- and low affinity somatotropic binding sites.
   J. Endocrinol. 116: 169:177, 1988.
7. **Sauerwein H, Meyer HHD, Schams D.**
   Divergent effects of oestrogens on the somatotropic axis in male and female calves.
   J. Reprod. Develop. 38: 271-278, 1992.
8. **Meyer HHD, Stoffel B, Hagen-Mann K.**
   B-agonists, anabolic steroids and their receptors: new ascpets in growth regulation.
   In: Von Engelhart W, Leonhard-Marek S, Breves G, Giesecke D, eds
   Ruminant physiology: digestion, metabolism, growth and reproduction.
   Proc. Of the eight international symposium on ruminant physiology.
   Ferdinand Enke verlag, Stuttgart. 1995: 475-482. 1995.
9. **Sauerwein H, Pfaffl M, Hagen-Mann K, Malucelli A, Meyer HHD.**
   Expression of estrogen and androgen receptor in the bovine gestrointestinal tract.
   Deutsche Tierarztl Wochenschr 102:143-178, 1995.
10. **Daxenberger A, Ibarreta D, Meyer HDD.**
    Possible health impacts of animal oestrogens in food.
    Human Reprod. Upd. Vol.7, 3: 430-355. 2001.
11. **Service RF.**

New role for estrogen in cancer?
Science 279:1631-1633, 1998.

12 **Bahrke MS, Yesalis CE.**
Abuse of anabolic steroids and related substances in sport and excersise.
Curr. Op. Pharm. 4: 614-620. 2004.

13 **Sauerwein H, Meyer HDD.**
Androgen and estrogen receptors in bovine skeletal muscle: relation to steroid induced allometric muscle growth.
J. Anim. Sci. 67: 206-212. 1989.

14. **Sauerwein H.**
Die Bedeutung der somatotropen Achse für Muskelwachstum und Reproduktion bei landwirtschaftlichen Nutztieren.
Verlag Dr Markus Hänsel-Hohenhausen, Egelsbach, 1994:1-244. 1994.

15. **Henricks DM, Brandt RT, Titgemeyer EC, Milton CT.**
Serum concentration of trenbolone-17B and estradiol-17B and performance of heifers treated wtih trenbolone-acetate, melengestrol acetate or estradiol-17B.
J. Anim. Sci. 75:2627-2633. 1997.

16. **Coutheyn D, Le Bizec B, Brambilla G, De Branabnder HF, Cobbeart E, Van de Wiele M, Vercammen J, De Wasch K.**
Recent deveopments in the use and abuse of growth promoters.
Analyt. Chim. Act. 473:71-82. 2002.

17. **Serratosa J, Blass A, Rigau B, Mongrell B, Rigau T, Tortadès M, Tolosa E, Aguilar C, Ribó O, Balagué J.**
Residues from veterinary medicinal products, growth promoters and performance enhancers in food producing animals: a European Union perspective.
Rev. Sci. Tech. Off. Epiz. 25(2): 637-653. 2006.

18. **Sabbe J, Vander Beken, T.**
BUFALAW-2001, the illegal use of growth promoters in Europe.
Comparative report, Brussels, Belgium, 15-16. Nov 2001.

19. **Delbeke FT, Van Eenoo P, Van Thuyne W, Desmet N.**
Prohormones and sport.
J. Steroid. Biochem. Mol. Biol. 83:245-251. 2003.

20 **Binello E, Gordon CM.**
Clinical uses and misuses for dehydroepiandrosterone.
Curr. Op. Pharm. 3: 635-641. 2003.

21. **Labrie F, Bélanger A, Luu-The V, Labrie C, Simard J, Cusan L, Gomez JL, Candas B.**

DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: Its role during aging.
Steroids. 63: 322-328. 1998.

22. **Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R.**
The key role of 17□-HSDs in sex steroid biology.
Steroids. 62: 148-158. 1997.

23. **Labrie F, Luu-The V, Martel C, Chernomoretz A, Calvo E, Morisette J, Labrie C.**
Dehydroepiandrosterone(DHEA) is an anabolic sterroid like dihydrotestosterone(DHT), the most potent natural androgen and tetrahydrogestrinone (THG).
J. Steroid. Biochem. Mol. Biol. 100: 52-58. 2006.

24. **World Anti Doping Agency (WADA)**
The world anti doping code, the 2007 prohibited list, international standard (03-08-2007)
http://www.wada-ama.org/rtecontent/document/2007_List_En.pdf

25. **Gennari C.**
Ipriflavone: Background.
Calcif. Tissue Int. 61: s3-s4. 1997.

26. **Nogradi M.**
The ipriflavone story (1969-1993).
Kem Kozl. 77: 55-58. 1993.

27. **Kathleen A, Head ND.**
Ipriflavone: an important bone building isoflavone.
Altern Med Rev 4(1): 10-22 1999

28. **Kuiper GJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, Van der Saag PT, Van der Burg B,Gustafsson JA.**
Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta.
Endocrin. 139(10):4252-4263, 1998.

29. **Simon RM, Korn EL, McShane LM, Radmacher MD, Wright GW, Zhao Y.**
Statistics for biology and health: Design and analyis of DNA microarray investigations.
Springer, 2003.

30. **Zipper H, Brunner H, Bernhagen J, Vitzthum F.**
Investigations on DNA intercalation and surface binding by SYBR green I, its structure determination and methodological implications.
Nucl. Ac. Res. 32(12): 1-10. 2004.

31. **PreAnalytix GmbH.**
PAXgene™ Blood RNA Kit Handbook.
Feb. 2004.

32. **Qiagen GmbH.**

RNeasy ® MiniElute™ Cleanup Handbook.
March 2003.

33. **Invitrogen life technologies.**
Trizol® Reagent manual.
http://www.invitrogen.com/content/sfs/manuals/15596026.pdf (31-7-2007).

34. **Qiagen GmbH.**
RNeasy® Mini Handbook.
Fourth edition April 2006.

35. **Agilent Technologies Inc.**
Two-Color Micro array-based gene expression analysis protocol.
Version 5.0.1. August 2006.

36. **Bio-Rad laboratories Inc.**
Experion™ RNA StdSens analysis kit Instruction manual.

37. **Pellis L, Franssen-van Hal NLW, Burema J, Keijer J.**
The intraclass correlation coefficient applied for evaluation of data correction, labeling methods, and rectal biopsy sampling in DNA micro array experiments.
Phys Gen 16:99-106. 2003.

38. **Lukacik P, Kavanagh KL, Oppermann U.**
Structure and function of human 17-B hydroxysteroid dehydrogenases.
Mol. Cel. Endo. 248: 61-71. 2006.

39. **Labrie F, Luu-The V, Calvo E, Martel C, Cloutier J, Gauthier S, Belleau P, Boucher H, Morisette J, Levesque MH, Labrie C.**
Tetrahydrogestrinone (THG) induces a genomic signature typical to a potent androgenic steroid.
J. Endocrinol. 184(2): 427-433. 2005

40. **Reiter M, Walf VM, Christians A, Pfaffl MW, Meyer HHD.**
Modification of mRNA expression after treatment with anabolic agents and the usefulness for gene expression biomarkers.
Anal. Chim. Act. 586: 73-81. 2007.

41. **Mossman T.**
Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays.
J. Immunol. Methods 65: 55-63. 1983

42. **Yoshida S, Honda A, Matsuzaki Y, Fukushima S, Tanaka N, Takagiwa A, Fujimoto Y, Miyazaki H, Salen G.**
Anti-proliferative action of endogenous dehydroepiandrosterone metabolites on human cancer cell lines.
Steroids. 68: 73-83. 2003.

43. **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.**
J. Biol. Chem. 193: 265-275. 1951.

44. **Sapan CV, Lundblad RL, Price NC.**
Colorimetric assay techniques.
Biotechnol. Appl. Biochem. 29: 99-108. 1999.

45. **Dittmar KD, Banach M, Galigniana MD, Pratt WB.**
The role of DnaJ-like proteins in glucocorticoid receptor-hsp90 heterocomplex assembly by the reconstituted hsp90-p60-hsp70 foldosome complex.
J. Biol. Chem. 273(13) 7358-7366. 1998.

46. **Deroo BJ, Archer TK.**
Glucocorticoid receptor activation of the IkBa promotor within chromatin.
Mol. Biol. Cell. 12 3365-3374. 2001.

47. **May MJ, Ghosh S.**
Rel/NF-kappa B and I kappa B proteins: an overview.
Semin. Cancer Biol. 8: 63-73. 1997.

48. **Ghosh S.**
Regulation of inducible gene expression by the transcription factor NF-kappaB.
Immunol. Res. 19:183-189. 1999.

49. **Mackenzie PI, Walter Block K, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, Miners JO, Owens IS, Nebert DW.**
Nomenclature for the mammalian UDP glycosyltransferases (UGT) gene superfamily.
Pharmacogen. Genom. 15: 677-685. 2005

50. **Charbonneau A, The VL.**
Genomic organization of a human 5B-reductase and its pseudogene and substrate selectivity of the expressed enzyme.
Biochim. Biophys. Acta. 1517:228-235. 2001

51. **Delaforge M, Pruvost A, Perrin L, André F.**
Cytochrome P450-mediated oxidation of glucuronide derivatives: example of estradiol 17B-glucuronide oxidation to 2-hydroxy-estradiol-17B-glucuronide by CYP 2C8.
Drug Metab. Disp. 33(5):466:473. 2005

52. **Campbell NA, Reece JB.**
Biology.
Pearson education inc, sixth edition. 2002.

53. **Lever ER.**
Integration of the extranuclear and nuclear actions of estrogen.
Mol. Endocrinol. 19(8): 1951-1959. 2005.

54. **Mooradian AD, Morkley JE, Koreman SG.**

Biological functions of androgens.
Endocr. Rev. 8(1): 1-28. 1987

55. **Labrie F.**
Intracrinology.
Mol. Cel. Endocrin. 78: C118-C118. 1991.

56. **Payne AH, Hales DB.**
Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones.
Endocr. Rev. 25:947-970. 2004.

57. **Livak KJ, Schmittgen TD.**
Analysis of relative gene expression data using real-time quantitative PCR and the 2-□□Ct method.
Methods 25:402-408. 2001.

58. **Bosotti R, Locatelli G, Healy S, Scacheri E, Sartori L, Mercurio C, Calogero R, Isacchi A.**
Cross platform microarray analysis for robust identification of differentially expressed genes.
BMC Bioinformatics. 8(suppl I):S5. 2007.

59. **Moebius FF, Fitzky BU, Lee JN, Paik YK, Glossman H.**
Molecular cloning and expression of the human Δ7-sterol reductase.
Proc. Nat. Acad. Sci. USA. 95(40: 1899-1902. 1998.

Appendices.

A: Protocols.

B: Microarray scans.

C: Gene list DHEA blood microarray experiment.

D: Gene list IF liver microarray experiment.

E: Primer list for RT-PCR validation.

Appendix A: Protocols.

1. RNA extraction from Blood

Using the PAXgene™ Blood RNA tubes and the PAXgene™ Blood RNA kit.

- Before starting, the PAXgene blood RNA tube must be incubated for at least 2h at room temperature prior to centrifugation.

1. Centrifuge the Pax gene Blood RNA tube for 10 min at 4000x g using a swing out rotor with adapters for round bottom tubes.
2. Remove the supernatant by decanting or pipetting, and discard. Clean the rim of the tube with a clean paper towel. Add 5 ml RNase free water to the pellet and close the tube using a fresh secondary Hemogard closure (supplied).
3. Thoroughly resuspend the pellet by vortexing and centrifuge for 10 min at 4000x g. Remove the entire supernatant by decanting or pipetting and discard.
4. Add 360 ul buffer BR1 and vortex until the pellet is visibly dissolved.
5. Pipet the sample into a fresh 1.5 microcentrifuge tube. Add 300 ul buffer BR2 and 40 ul Proteinase K. Mix by vortexing, and incubate for 10 min at 55' C using a shaker-incubator at 800 rpm.
6. Centrifuge the sample for 3 min at maximum speed in a microcentrifuge (Beckman Coulter™ Microfuge18® centrifuge), and transfer the supernatant to a fresh 1.5 ml microcentrifuge tube.
7. Add 350 ul 96% Ethanol. Mix by vortexing, and centrifuge briefly (1-2 s at 1000x g) to remove drops from the inside of the tube lid.
8. Add 700 ul sample to the PAXgene spin column placed in a 2ml processing tube. Centrifuge for 1 min at 10.000 rpm. Place the PAXgene spin column in a new processing tube, and discard the old processing tube containing the flow through.
9. Add the remaining sample to the PAXgene spin column and centrifuge for 1 min at 10.000 rpm for 1 min. Place the Paxgene spin column in a new processing tube, and discard the old.
10. Add 700 ul buffer BR3 to the PAXgene spin column and centrifuge for 1 min at 10.000 rpm. Place the column in a new processing tube, and discard the old processing tube.
11. Add 500 ul buffer BR4 to the PAXgene spin column and centrifuge 1 min at 10.000 rpm. Place the column in a new processing tube, and discard the old processing tube.
12. Add another 500 ul buffer BR4 to the PAXgene spin column. Centrifuge for 3 min at maximum speed to dry the PAXgene spin column membrane.
13. Discard the old processing tube containing the flow through. And place the PAXgene spin column in a new processing tube (not supplied).

14. Discard the old processing tube containing the flow-through from step 13. Place the PAXgene spin column into a 1.5ml collection tube, and add 40 ul buffer BR5 directly onto the PAXgene spin column. Centrifuge for 1 min at 10.000 rpm to elute the RNA. Do not discard the eluate.

15. Add another 40 ul buffer BR5 onto the PAXgene spin column membrane. Centrifuge for 1 min at 10.000rpm to elute further RNA. Do not discard the eluate.

16. Discard the PAXgene spin column. Incubate the RNA eluate for 5 min at 65' C in a heating block. After incubation, chill directly on ice.

2. Blood extracted RNA Cleanup

Using the Qiagen RNeasy® MiniElute™ cleanup kit.

1. Heat a heating block to 65°C for use in step 9.

2. Adjust sample to a volume of 100 µl with RNase free water. Add 350 µl Buffer RLT and mix thouroughly.

3. Add 250 µl of 96% ethanol to the diluted RNA and mix thouroughly by pipetting. Do not centrifuge, continue immediately with step 4.

4. Apply the 700 µl sample to an RNeasy MiniElute spin column in a 2 ml collection tube. Close the tube gently and centrifuge for 15s at 10.000 rpm in a microcentrifuge. Discard the flow-through.

5. Transfer the spin column into a new 2 ml collection tube. Pipet 500 µl Buffer RPE onto the spin column. Close the tube gently and centrifuge for 15 s at 10.000 rpm to wash te column. Discard the flow-through.

6. Add 500 µl of 80% ethanol to the spin column. Close the tube gently and centrifuge for 2 min at 10.000 rpm to dry the silica gel membrane. Discard the flow-through and collection tube.

7. Transfer the spin column to a new 2ml collection tube, open the cap of the spin column and centrifuge in a microcentrifuge at 14000 rpm speed for 5 min. Discard the flow-through and collection tube.

8. To elute, transfer the spin column to a new 1.5 ml microcentrifuge tube. Pipet 14 ul Buffer BR5 onto the center of the silica gel membrane. Close the tube gently, wait one minute and centrifuge for 1 min at 14.000 rpm to elute. Repeat this step.

9. Incubate the eluate for 5 min at 65°C in a heating block or water bath. Following incubation, chill immediately on ice and/or store at -80°C.

3. RNA extraction from biopsies.

Using Invitrogen Trizol®

1. Put homogenizer in fume hood.

2. Homogenize biopsies in Trizol® on ice with tissue homogenizer (put cryovial on ice in small beaker while homogenizing.

3. Incubate the homogenized sample for 5 min at room temperature

4. Separate the sample into two fresh tubes. Proceed with both tubes

5. Add 0,2 ml of Chloroform per 1 ml of Trizol and shake tubes vigorously by hand for 15 sec

6. Incubate at RT for 3 min.

7. Centrifuge at < 12000xg for 15 min at 2-8°C and Transfer upper aqueous phase (containing RNA) to a fresh tube

8. Add 0,5 ml of isopropanol per 1 ml of Trizol

9. Mix and incubate samples for 10 minutes at RT and centrifuge at < 12000xg for 10 min at 2-8°C. Remove supernatant.

10. Wash pellet once with 75% EtOH (at least 1 ml of EtOH per 1 ml of Trizol) and vortex.

11. Centrifuge at < 7500xg for 5 min at 2-8°C. Dry RNA pellet (air dry or vacuum-dry for 2-5 min).

12. Dissolve RNA in 40 µl of RNase-free water, and pool the two tubes of one sample.

13. 10 min at 60°C

4. Biopsy extracted RNA cleanup

Using the Qiagen RNeasy™ Mini kit

- ß-Mercaptoethanol (ßME) must be added to Buffer RLT before use. Add 10 µl ß-ME per 1 ml RLT Buffer.
- Buffer RPE is supplied as a concentrate. Add 4 volumes of ethanol (96-100%).

1. Adjust sample to a volume of 100 µl with RNase-free water. Add 350 µl Buffer RLT (with ß-ME) and mix / pipet thoroughly.
2. Add 250 µl ethanol (96-100%) to the diluted RNA, and mix thoroughly by pipetting (do not centrifuge).
3. Apply sample (700 µl) to an RNeasy mini column placed in a 2 ml collection tube. Close tube gently, and centrifuge for 15 sec at ≥8000 xg (= 10000 rpm). Discard the flow-through and collection tube.
4. Transfer the RNeasy column into a new 2 ml collenction tube. Pipet 500 µl Buffer RPE onto the RNeasy column. Close the tube gently, and centrifuge for 15 sec at ≥ 8000 xg (= 10000 rpm) to wash the column. Discard the flow-through.
5. Add another 500 µl Buffer RPE onto the RNeasy column. Close the tube gently, and centrifuge for 2 min at ≥ 8000 xg (= 10000 rpm) to dry the RNeasy silica-gel membrane.
6. Place the RNeasy column in a new 2 ml collection tube, and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.
7. To elute, transfer the RNeasy column to a new 1,5 ml collection tube. Pipet 50µl RNase-free water directly onto the RNeay sillica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥8000 xg (= 10000 rpm) to elute.
8. Store in -80°C.

5. RNA Quantification

Using the Nanodrop ND-1000 UV-VIS spectrophotometer.

1. Denature RNA samples for 5 min at 65°C, vortex, and spin down.

2. Launch the Nanodrop software and select Nucleic Acid. Load 1.5 µl of RNase free water onto the nanodrop , close the lid and initialize the spectrophotometer. Select RNA-40 as sample type in the drop-down menu and make a blank measurement with RNase free water (or buffer BR5 for Blood RNA samples) using a volume of 1.5 µl.

3. Clean the nanodrop and measure all samples using a volume of 1.5 µl sample, clean the nanodrop after each sample using laboratory wipe.

4. Select samples with ratios A260/280 and A260/230 lower than 1.80 for additional cleanup.

6. Experion™ RNA Quality analysis.

Using the Bio-Rad Experion™ StdSens analysis kit.

- A maximum of 12 RNA samples can be measure per chip.
- Clean the electrophoresis station before use. Place a cleaning chip filled with 800 µl Experion Electrode cleaner into the electrophoresis station, close the lid for 2 minutes and remove the chip. Place another cleaning chip with 800 µl RNase free water into the electrophoresis station, close the lid for 5 minutes, replace the water in the chip and repeat the rinse step. Remove the cleaning chip

1. Prepare the gel stain solution. Centrifuge 600 ul of in a spin filter at 1500 g for 10 min. Pipet 65 µl filtered gel into an RNase free microcentrifuge tube and add 1 µl RNA stain to the filterd gel. Vortex.
2. Prepare the RNA samples and RNA ladder. Pipet at least 3 µl RNA ladder and RNA samples into RNase free microcentrifuge tubes. Denature the ladder and samples for 2 min at 72°C. Cool the ladder and samples on ice for 5 min. Briefly spin down the tubes and mix before use. Store on ice.
3. Remove an RNA StdSens chip from its packaging and place it in the Experion Priming station. Prime the chip by adding 9 µl of gel-stain solution (from step 1) into the well labeled GS (orange well). Close lid of priming station, set pressure to B and the time to 1. Press Start. After priming is finished, remove the chip from the priming station and visually inspect the microchannels for air bubbles or incomplete priming.
4. Pipet 9 µl gel-stain solution into the other well labeled GS (not orange). Pipet 9 µl filtered gel in the well labeled G. Pipet 5 µl of loading buffer into each sample well (1-12) and into the ladder well (labeled L) do not leave any sample well empty.
5. Pipet 1 µl denatured RNA ladder into the well labeled L. Load 1 µl of denatured RNA samples into each of the 12 wells. Pipet 1 µl RNase free water in any unused sample wells.
6. Place the chip in the Experion vortex station. Vortex for 1 min. Run the chip in the Experion electrophoresis station within 5 min of loading.
7. Turn on the power to the electrophoresis station and launch the Experion software. Place the loaded chip into the electrophoresis station chip platform and close the lid. Select New Run and then RNA StdSens. Select the desired RNA assay (mRNA) and click the Start button.
8. After the run is complete, remove the chip and clean the electrophoresis station using a cleaning chip filled with 800 µl of RNase free water. Close the lid for 60 seconds and open it for 30 seconds to allow the electrodes to dry completely. Remove the cleaning chip.

7. Agarose gel electrophoresis

1. Weigh 1.5 g of Molecular biology grade Agarose per 100 ml gel for a 1.5% gel.

2. Add the appropriate volume of 0.5x TBE buffer (containing 5.4g Tris, 2.75g Boric acid and 2 ml 0.5M EDTA, pH 8.0). Mix and heat the gel until the agarose is completely dissolved. Cool down the gel solution and add 5 µl of ethidiumbromide per 100 ml gel. Mix well and pour the gel into the tray with comb. Let the gel cool and harden.

3. Prepare samples by taking 1 µg of RNA and adjusting the volume to 9 µl using RNase free water. Add 1 µl 10x loading buffer, vortex and spin down.

4. Remove the comb from the hardened gel. Add the +1kb DNA ladder to the gel, as well as the RNA samples.

5. Run gel at 75 V for 1.5 hour.

6. Transfer the gel to a Bio-Rad Gel doc. Make a photo using Quantity One software and assess the RNA samples on the presence of the 2 ribosomal bands (18 and 22S) RNA breakdown is seen as a smear on the gel and genomic DNA leaves a residue in the sample well.

8. Micro array.

According to the Agilent technologies "Two color Micro array-based gene expression analysis" protocol and the Agilent Gene expression hybridization kit.

Step 1, RNA amplification.

1. Prepare spike A and B mix. Mix stock solutions vigorously on a vortex mixer and spin down. Dilute the spike A and B mix stocks, make a first dilution of 1:20, dilute further 1:40 and a final dilution of 1:4.
2. Add 500 ng of all individual RNA samples to a microcentrifuge tube and adjust the volume to 8.3 µl either by adding RNase free water or using the Savant Speed Vac® Plus SC 110A.
3. Pool 500 ng of every control sample into 1 tube and prepare 4 tubes of 8.3 µl of reference RNA.
4. Add 1.2 µl T7 Promoter primer and 2 µl Spike A or B mix (Spike A for reference samples, Spike B for target samples). This will bring the total volume to 11.5 µl.
5. Denature primer and template RNA by incubating at 65°C in a circulating water bath for 10 minutes. Place the reactions on ice and incubate 5 min.
6. Prior to use, mix the components for the cDNA master mix in step 7. Preheat the 5x First strand buffer at 80°C for 3 to 4 minutes and keep at room temperature.
7. Add per reaction, the following components:
4 µl First strand buffer
2 µl 0.1M DTT
1 µl 10mM dNTP mix
1 µl MMLV-RT
0.5 µl RNaseOut
Total volume: 8.5 µl.

8. Add the 8.5 µl cDNA master mix to the 11.5 µl spiked RNA samples. Mix by vortexing and spin down.
9. Amplify the RNA samples into cDNA by incubating the reactions at 40°C in a circulating water bath for 2h. Move samples to a 65°C circulating water bath and incubate for 15 min. Move samples to ice, incubate for 5 min.
10. Split samples, store 1 sample in -80°C and proceed with the other sample. Use the -80°C sample as a back up.

Step 2: Labeling cRNA.

11. Prior to use, mix the components for the Transcription master mix in step 12. Prewarm the 5-% PEG solution at 40°C for 1 min.
12. Add per reaction the following components:

Reference RNA+ spike A			Target RNA +Spike B		
7.65	µl	Nuclease free water	7.65	µl	Nuclease free water
10	µl	Transcription buffer	10	µl	Transcription buffer
3	µl	0.1M DTT	3	µl	0.1M DTT
4	µl	NTP mix	4	µl	NTP mix
3.2	µl	50% PEG	3.2	µl	50% PEG
0.25	µl	RNaseOut	0.25	µl	RNaseOut
0.3	µl	Inorganic Phosphatase	0.3	µl	Inorganic Phosphatase
0.4	µl	T7 RNA Polymerase	0.4	µl	T7 RNA Polymerase
1.2	µl	Cyanine 3-CTP	1.2	µl	Cyanine 5-CTP

Total volume: 30 µl.

13. Add 30 µl of Transcription master mix to each sample tube, gently mix by pipetting.

14. Incubate at 40°C in a circulating water bath for 2h to obtain labeled cRNA.

Step 3: Purify the labeled and amplified RNA.

15. Add 60 µl of RNase free water to your cRNA sample, for a total volume of 100 µl.

16. Purify the RNA as described in B 4: Biopsy extracted RNA Cleanup. Elute using 30 µl RNase free water.

Step 4: Quantify the cRNA.

17. Quantify the cRNA using the Nanodrop ND-1000 UV-VIS spectrophotometer. Launch the Nanodrop software, select Microarray Measurement. Clean the spectrophotometer and load 1.5 µl RNase free water to initialize the spectrophotometer. Select RNA-40 as sample type in the drop-down menu and make a blank measurement with 1.5 µl RNase free water.

18. Measure all Cy5 labeled cRNA samples (using 1.5 µl)and clean the spectrophotometer after each sample using laboratory wipes.

19. Pool the reference Cy3 labeled cRNA into one tube, vortex and spin down. Measure the sample on the Nanodrop (using 1.5 µl).

20. Determine the total cRNA yield.

21. Determine the total labeled yield.

$$\text{Yield in µg RNA} = \frac{\text{Concentration cRNA x 30 (µl)}}{1000}$$

$$\text{Labeled yield in pmol RNA} = \frac{\text{Concentration labeled cRNA}}{\text{Concentration of total cRNA}} \times 1000$$

If the yield is <825 ng and the labeled yield is less than <8.0 pmol per µg cRNA, do not proceed to the hybridization step. Repeat cRNA preparation.

Step 5: Fragmentation of cRNA.

22. Prepare 10x blocking agent by adding 500 µl of RNase free water to vial containing lyophilized 10x blocking agent. Mix gently by vortexing and spin down.

23. Mix the following components in new microcentrifuge tubes.

Cy3 labeled, linearly amplified cRNA	825 ng
Cy5 labeled, linearly amplified cRNA	825 ng
10x Blocking agent.	11 µl
RNase free water	bring to volume of 52.8 µl
25x fragmentation buffer	2.2 µl
Total volume:	55 µl

24. To fragmentate the cRNA incubate at 60°C for exactly 30 min. spin down the tubes.

25. Add 55 µl 2x GEx hybridization buffer HI-RPM to stop the fragmentation reaction. Mix well by careful pipetting. Avoid air bubbles and spin briefly. Place sample on ice and use immediately.

Step 6: Micro array hybridization.

26. Prepare the hybridization assembly. Load a clean gasket slide into the Agilent SureHyb chamber base with the label facing up and aligned with the rectangular section of the chamber base.

27. Slowly dispense 100 µl of hybridization sample onto the gasket well in a drag and dispense manner, being sure not to touch the gasket walls.

28. Place an array 'Active side down onto the sureHyb gasket slide, so that the "Agilent"-labeled barcode is facing down and the numeric code is facing up. Verify that the sandwich pair is properly aligned.

29. Place the SureHyb cover onto the sandwiched slides and slide the clamp assembly onto both pieces. Hand tighten the clamp to the chamber.

30. Vertically rotate the assembled chamber to wet the gasket and assess the mobility of the bubbles. Tap the assembly on a hard surface, if necessary to move stationary bubbles.

31. Place the assembled silde chamber in a hybridization oven with a rotation function. Set the temperature to 65°C and the rotation speed to 10 rpm.

32. Hybridize at 65°C for 17h.

Step 7. Micro array wash.

33. Warm the Agilent wash buffer 2 to 37°C

34. Fill 3 staining dishes with subsequently Agilent wash buffer 1(1), Acetonitrile(3) and agilent drying solution(4). Reserve 1 dish for Agilent wash buffer2 (2). Add magnetic stir bars to the dishes.

35. Remove the micro array sandwich from the SureHyb chamber. Submerge the micro array in Agilent Wash buffer 1 and disassemble the micro array sandwich.

36. Place the arrays in a transferable slide rack and place the rack into the dish containing Agilent wash buffer 1, transfer the dish containing the array to a magnetic stir plate and incubate for 1 minute at room temperature.

37. Fill dish 2 with Agilent wash buffer 2 at 37°C and transfer the slide rack to dish 2. Place the dish on the magnetic stir plate and incubate for 1 minute.

38. Transfer the slide rack to dish 3 containing acetonitrile, don't transfer to magnetic stir plate. Incubate for 1 minute.

39. Transfer the slide rack to dish 4 containing Agilent drying solution. Incubate 30 seconds and slowly remove the slide rack from the dish.

40. Scan slides immediately to minimize the impact of environmental oxidants on signal intensities. Store slides in slide boxes in the dark.

Step 8. Scanning the micro array slides.

41. Assemble the slides into an appropriate slide holder. Slides should be placed into the slide holder such that when the holders containing the slide are oriented into the scanner carousel the numeric barcode is visible.

42. Place assembles slide holders into the scanner carousel.

43. Verify scan settings for two-color scans.

Scan region	Scan area (61 x 21.6 mm)
Scan resolution (µm)	5
5 µm scanning mode	Single Pass
eXtended Dynamic range	(selected)
Dye channel	Red&Green
Green PMT	XDR Hi 100%
	XDR Lo 10%
Red PMT	XDR Hi 100%
	XDR Lo 10%

44. Verify the scanner status is ready.

45. Click "scan slot m-n" on the scan control main window where the letter "m" represent the start slot and "n" the end slot.

9. Primer Design

Using Beacon designer 7.

1. Select the genes for confirmation and get the RefSeqID, expression signals of each sample, ratios and probe sequence. Save file

2. Open Beacon Designer. Open new project if necessary.

3. Upload the RefSeqID mRNA sequence by pasting them in File, Open, Sequence, Entrez.

4. Check mRNA sequences on structure by Template structure search. Set search parameters to 1-1200 bp.

5. Blast mRNA sequence for information on intron and exon positions, set the search parameters to Eukaryotic, Cow and RefSeq RNA.

6. Make sure primer search is set to SYBR®green primer design.

7. Search primers, Set search parameters to avoid cross homology and template structure.

8. Check the designed primers on rating, length, melttemperature, GC content and hairpin structures.

9. Export the results to a new file.

10. Check whether primers and probes code for the same gene by using NCBI nucleotide BLAST.

10. Removal of genomic DNA from RNA samples

- **Using the Promega RQ1 DNase kit.**

1. Take 10 µg of each RNA sample, add 10 µl of 10x RQ1 DNase Buffer and 10 µl of RQ1 DNase. Adjust volume to 100 µl using RNase free water.

2. Incubate 30 min at 37°C.

3. Add 100 µl Phenol/Chlorophorm/Isoamylalcohol (P/C/IAA 25:24:1).

4. Centrifuge 5 min at 14000 rpm and transfer upper aqueous phase to a fresh microcentrifuge tube.

5. Add 100 µl Chlorophorm/Isoamylalcohol (C/IAA 24:1).

6. Centrifuge 5 min at 14000 rpm and transfer upper aqueous phase to a new microcentrifuge tube.

7. Add 10 µl 2.5M NatriumAcetate pH 5.3, mix and add 250 µl 96% ethanol. Mix.

8. Incubate 5 min on ice and centrifuge at 14000 rpm and 4°C for 30 min.

9. Remove supernatant, and wash pellet with 75% ethanol.

10. Air dry pellet at room temperature for 10 min.

11. Dissolve pellet in 20 µl RNase free water.

12. Vortex, spin down, incubate 5 min at 65°C, vortex and spin down again.

13. Quantify using the Nanodrop ND-1000 as described in B V: RNA Quantification.

11. Reverse transcriptase

- **Using the Bio-Rad iScript cDNA synthesis kit.**

1. Take 1 µg of DNase treated RNA sample. Adjust volume to 15 µl using RNase free water.

2. Add 4 µl 5x iScript Reaction mix and 1 µl iScript Reverse Transcriptase. This brings the total volume to 20 µl.

3. Perform the reaction using the following reaction protocol:

5 min	room temperature
30 min	42°C in a circulating water bath
5 min	58°C in a heat-block.
5 min	4°C on ice.

4. Add 80 µl RNase free water to the cDNA samples and store at -20°C.

12. Real time PCR

-Using the Bio-Rad iCycler™ and IQ™ SYBRgreen.

1. Determine the gene to be confirmed and select an appropriate house-keeping gene.

2. Determine in which sample the gene is expressed the highest. Use this sample to make a calibration curve.

3. Dilute the sample using 10 µl sample and adding 90 µl RNase free water. Dilute the sample successively 10 times, 100 times, 1000 times and 10000 times. Use these four dilutions to make the calibration curve.

4. Dilute the primers if necessary to a concentration of 10 µM.

5. Make a PCR master mix for 40 reactions for the target gene and a mix for 40 reactions for the reference gene. Mix the following components:

	Target gene		Reference gene	
	1 reaction	*40 reactions*	*1 reaction*	*40 reactions*
SYBRgreen mix	12.5	500	12.5	500
Primer sence Target	1	40	-	-
Primer antisense Target	1	40	-	-
Primer sense Reference	-	-	1	40
Primer antisense Reference	-	-	1	40
RNase free water	9.5	380	9.5	380

6. Add the 24 µl PCR master mix and 1 µl sample cDNA to a Bio-Rad IQ 96 wells PCR plate according to the following schedule.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample1 1X	Sample1 1X	Sample 4	Sample 4	Sample 12	Sample 12	Sample1 1X	Sample1 1X	Sample 4	Sample 4	Sample 12	Sample 12
B	10X	10X	Sample 5	Sample 5	Sample 13	Sample 13	10X	10X	Sample 5	Sample 5	Sample 13	Sample 13
C	100X	100X	Sample 6	Sample 6			100X	100X	Sample 6	Sample 6		
D	1000X	1000X	Sample 7	Sample 7			1000X	1000X	Sample 7	Sample 7		
E	10000X	10000X	Sample 8	Sample 8			10000X	10000X	Sample 8	Sample 8		
F	-RT	H2O	Sample 9	Sample 9			-RT	H2O	Sample 9	Sample 9		
G	Sample 2	Sample 2	Sample 10	Sample 10			Sample 2	Sample 2	Sample 10	Sample 10		
H	Sample 3	Sample 3	Sample 11	Sample 11			Sample 3	Sample 3	Sample 11	Sample 11		

7. Seal the plate using Bio-Rad IQ optical tape and place the plate in the Bio-Rad iCycler PCR system.

8. Launch iCycler software and apply the appropriate real-time PCR protocol and plate setup and specify the reaction volume to 25 µl.

Real time PCR protocol:

- Cycle 1 (1x)	95°C	3 min
- Cycle 2 (45x)	95°C	15 s
	60°C	45 s

Data collection and real-time analysis

- Cycle 3 (1x)	95°C	1 min
- Cycle 4 (1x)	55°C	1 min
- Cycle 5 (60x)	65-95°C	10 s

Increased set point temperature after cycle 2 by 0.5°C.

Melt curve data collection and analysis.

9. After the PCR run, print the quantification and melt-curve report. Make a calibration line using the calibration samples for the target and reference gene. Calculate relative cDNA concentrations, normalize by pooling the duplicates and dividing the average concentrations by the reference gene concentrations. Pool the groups and calculate ratios.

13. Cell culturing.

1. Assess the cell culture flask (Costar®, Corning Inc., ,)containing HepG2 cells on confluence. Pass the cells when confluence is high (90%) 1:4

2. Warm up the culture media (DMEM+++), calcium and magnesium free Hank's Balanced Salts Solution (HBSS-Ca-Mg) (Sigma-Aldrich)and Trypsine conatining 0.2 M trypsin and 0.25 mM EDTA (Sigma-Aldrich). Warm to 37°C.

3. Remove the old medium from the cells. Wash the cells using 10 ml HBSS-Ca-Mg. Remove the HBSS-Ca-Mg.

4. Add 2.5 ml Trypsine, spread the trypsine over the flask surface, and incubate 15 min at room temperature to trypsinise the cells. Do not move the flask to avoid cell clumps.

5. Add 10 ml DMEM+++, Resuspend the cells and remove 8 ml medium from the flask. Add another 8 ml DMEM+++, resuspend and close the flask. Incubate the passed cells in a 37°C, 5% CO^2 stove.

14. Exposure of cells to DHEA

1. Remove flask from stove. Assess the confluency (target >80%).

2. Warm up the DMEM+++, HBSS-Ca-Mg and the trypsine.

3. Remove the old medium from the cells and wash once with HBSS-Ca-Mg. Remove the HBSS-Ca-Mg.

4. Add 2.5 ml Trypsine and incubate 15 min at room temperature. Do not move the flask.

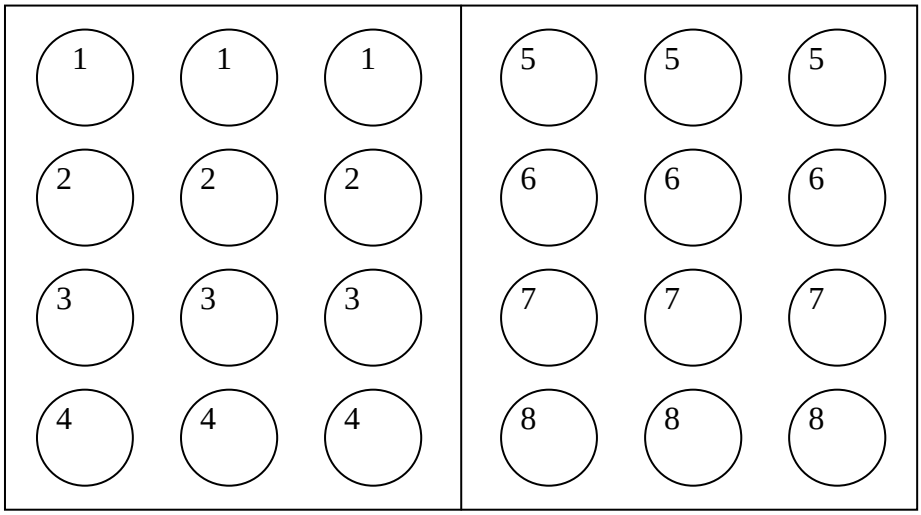
5. Add 10 ml DMEM+++, and determine the amount of cells using a Bürker cell count chamber. Take 0.5 ml cell suspension, add 0.5 ml DMEM+++ and add 1 ml tryptan blue solution (0.4%) (Sigma-Aldrich). Incubate 5 minutes and distribute the cells in the counting chamber. Count cells under a microscope. Count cells in a 4x4 box and determine the amount of cells per ml. Target amount of cells: 15*10^4 cells/ml.

6. Dilute the cell suspension if necessary and count the cells again.

7. Plate cells in a 24-wells plate (Costar®) at 0.5 ml cell suspension per well (7.5*10^4 cells/well).

8. Incubate cells for 48 hours

9. Remove old medium from the 24 wells plate, add new medium containing 1% DMSO and DHEA in several concentrations. Add according to the following schedule.



1		1% DMSO
2	0.1 µM DHEA	1%DMSO
3	1 µM DHEA	1%DMSO
4	10 µM DHEA	1%DMSO
5	50 µM DHEA	1%DMSO
6	100 µM DHEA	1%DMSO
7	200 µM DHEA	1%DMSO
8		10% DMSO

15. Cytotoxicity test.

Using the MTT (dimethylthiazol diphenyl tetrazolium bromide) test

1. Remove medium from cells after the timepoints 4h, 6h, 24h and 48h.

2. Wash cells once using Eagle's minimal essential medium (EMEM), containing 200 mM L-Glutamine (EMEM+) of 37°C.

3. Add 0.5 ml of 5mg/ml MTT (in EMEM+) of 37°C. Incubate 1 hour.

4. Remove the MTT medium and wash cells once using EMEM+ of 37°C.

5. Add 0.5 ml of 4°C Isopropanol. Incubate 20 min at 4°C.

6. Shake the plate to homogenize the suspension in the wells. Transfer 100 µl from the 24 wells plates into a flat bottom 96 wells plate (Corning).

7. Measure the plate using a Elx808™ absorbance microplate reader and KCJunior™ software (Bio-Tek Instruments Inc). Set the target wavelength at 562 nm and the reference wavelength to 650 nm. Measure and print results.

8. Determine the amount of active cells in the well by calculating the percentage of extinction, by setting the blanc (0 µM DHEA, 1%DMSO) on 100%.

16. Protein assay

- Using the Biorad DC Protein assay

Step 1: sample preparations.

1. Expose cells as described in B XIII: Exposure of cells to DHEA. Expose the cels to 0, 0.1, 1 and 10 µM DHEA.

2. After 24 hours, transfer the medium from the cells into labeled microcentrifuge tubes. Store the media in -80°C

3. Wash cells using 0.5 ml, 37°C PBS, remove the PBS and add another 0.5 ml 37°C PBS. Store cells in -20°C.

Step 2: Protein assay.

1. Prepare the lysisbuffer by Adding 1 volume of 0.8 M NaOH to 1 volume of 10% SDS.

2. Make BSA standards of 1.5, 1.25, 1, 0.75,0.5, 0.25, 0.1 and 0 mg/ml.

3. Defrost the 24 wells plate containing the cell suspensions at room temperature.

4. Add 500 µl of lysisbuffer to each sample well and incubate 30 min. on a microplate shaker.

5. Add 5 µl of all standards and samples onto a Greiner 96 multiwellsplate (Greiner lotnr. 655101).

6. Add 20 µl of Reagent S to each ml of Reagent A (Reagent A')

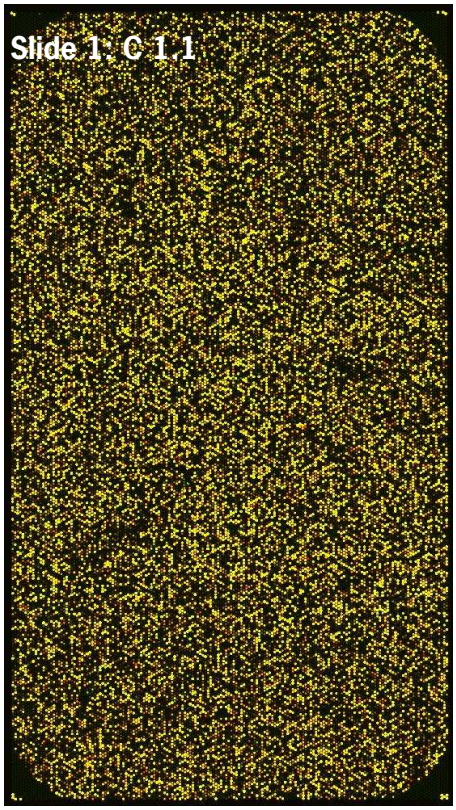
7. Add 25 µl of Reagent A' to the sample wells.

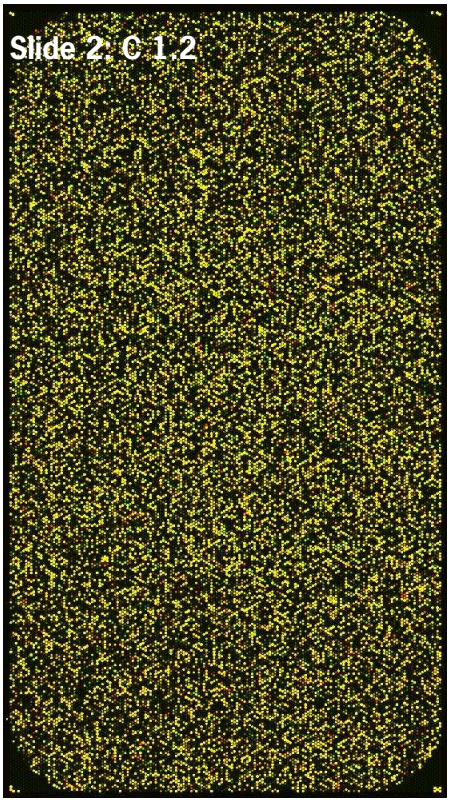
8. Add 200 µl Reagent B to each sample well and mix the plate for 5 s.

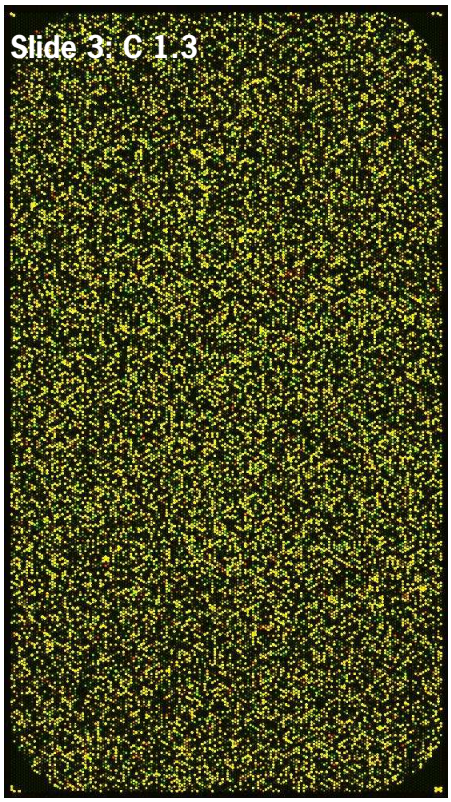
9. Incubate the plate for 15 min. at room temperature, and measure the absorbance at 750 nm on the Elx808™ absorbance microplate reader and use the KCJunior™ software.

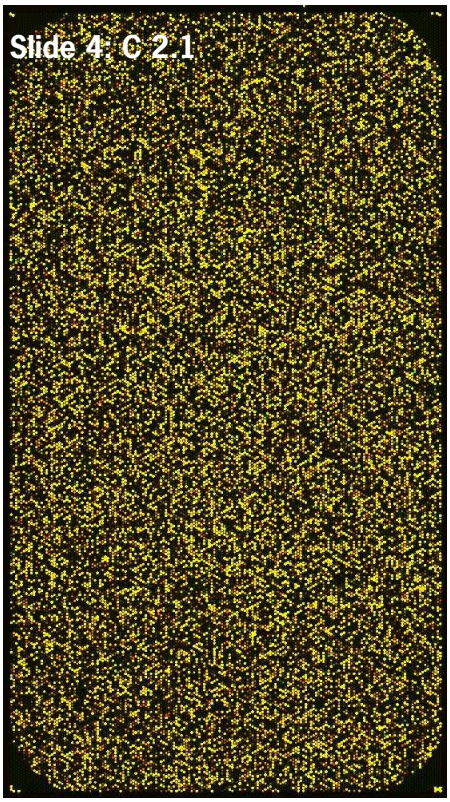
Appendix B.

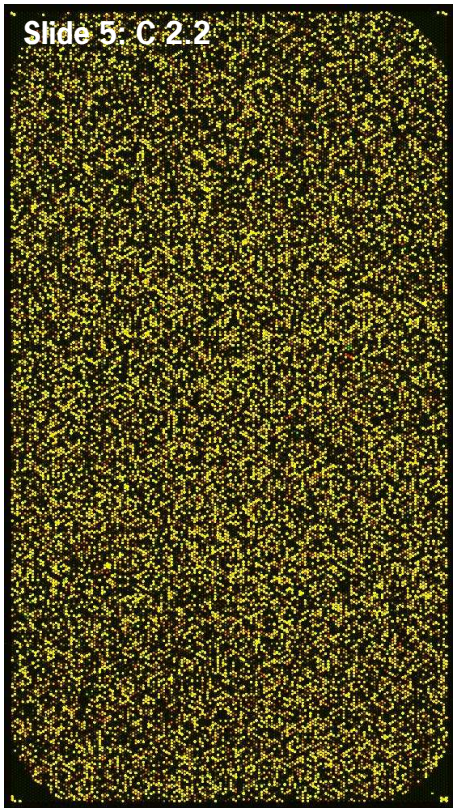
1. Micro array scans: DHEA induced gene expression in bovine bood.

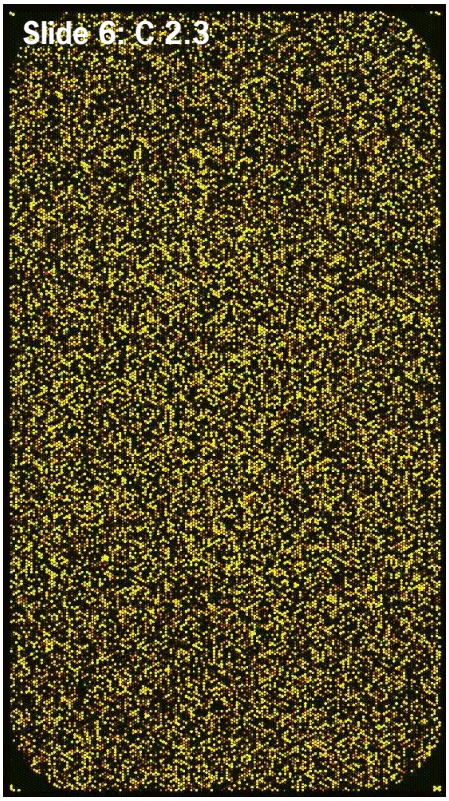


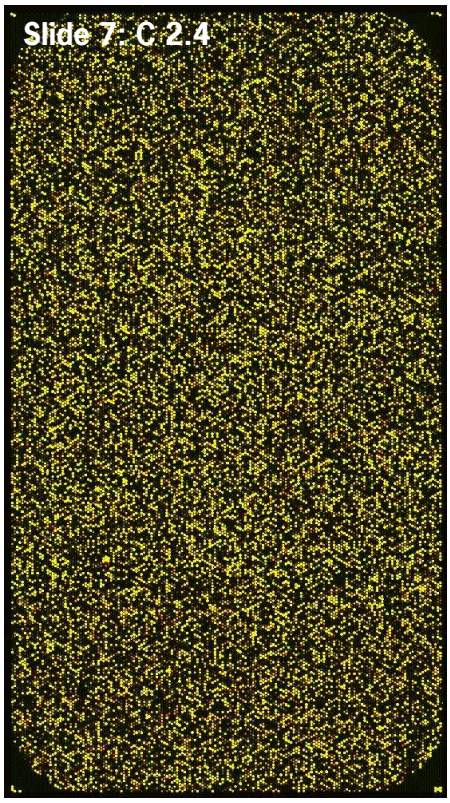


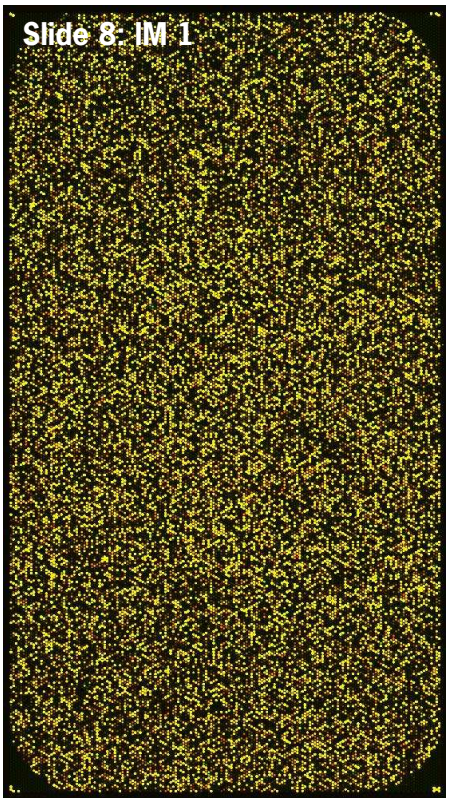


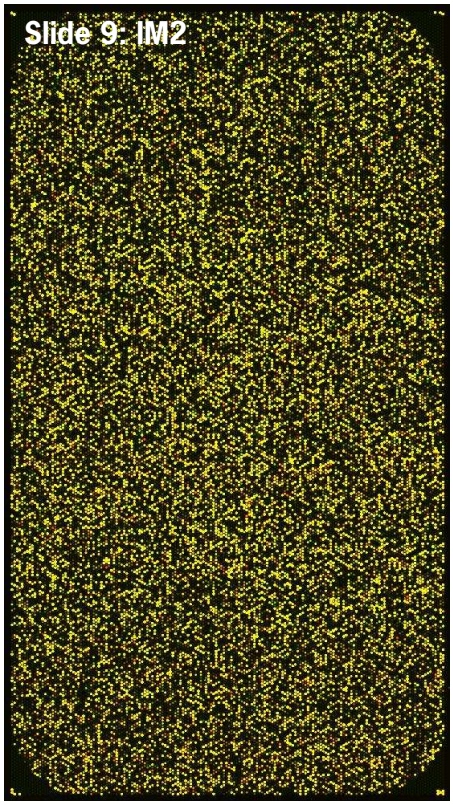


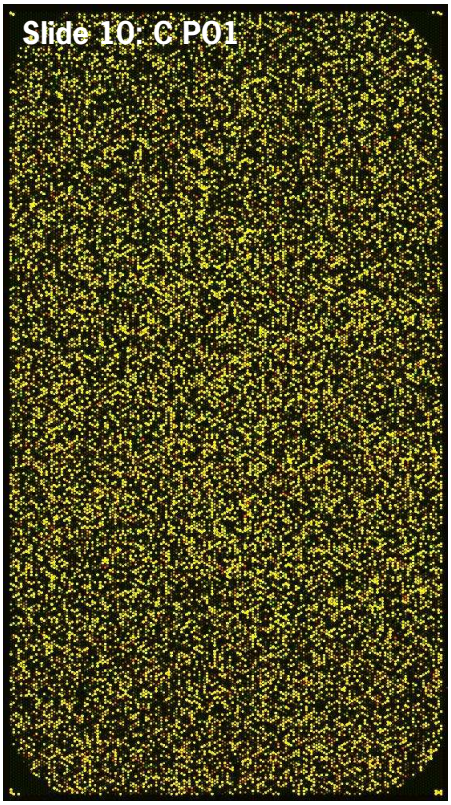

Slide 5: C 2.2

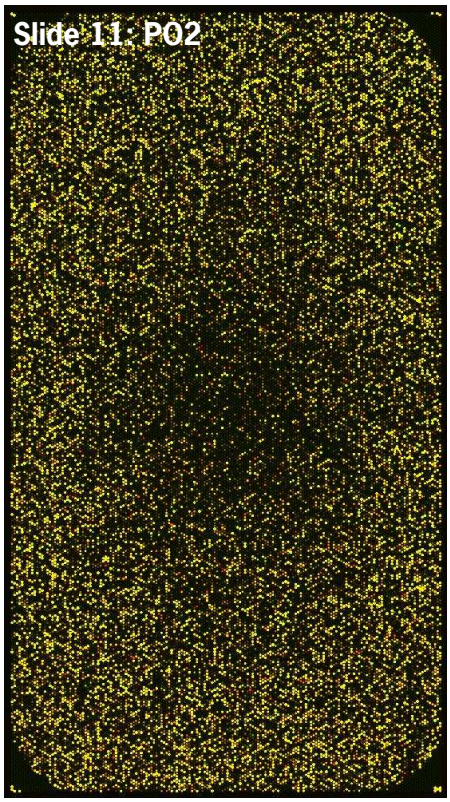

Slide 6: C 2.3

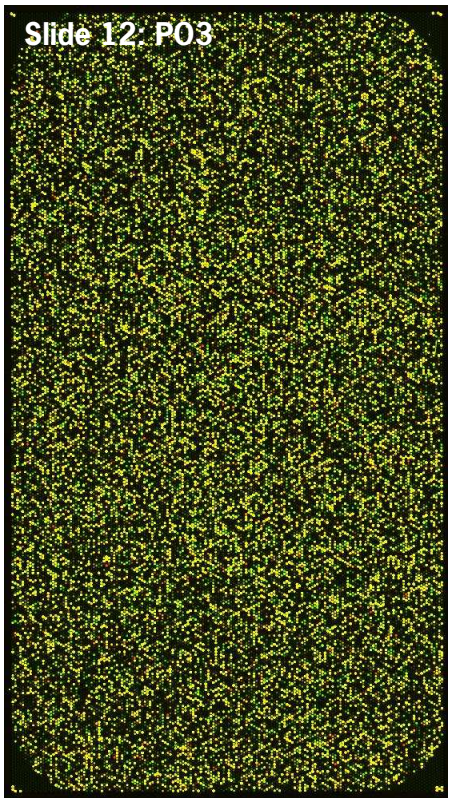

Slide 7: C 2.4

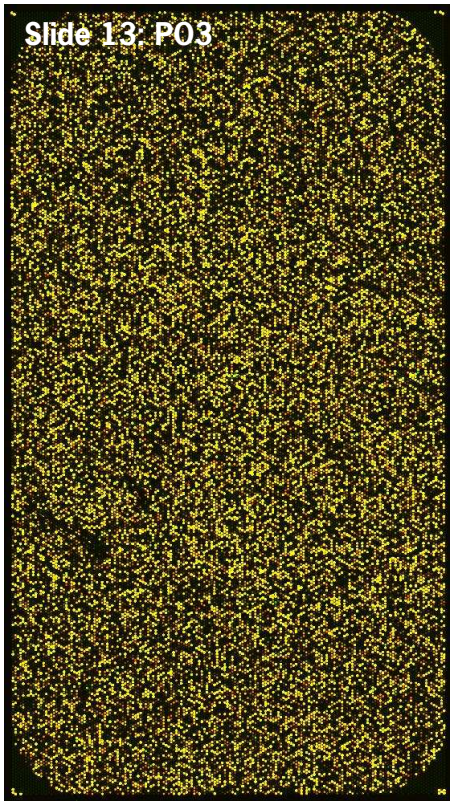

Slide 8: IM 1


Slide 9: IM2

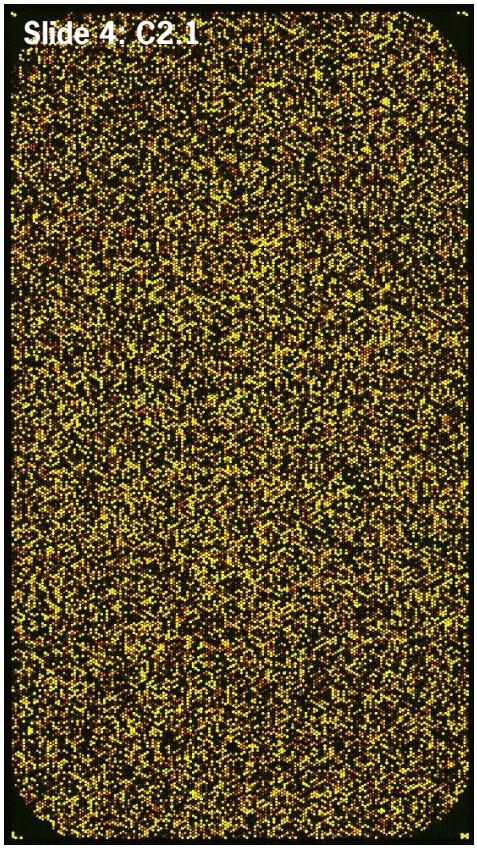

Slide 10: C PO1


Slide 11: PO2

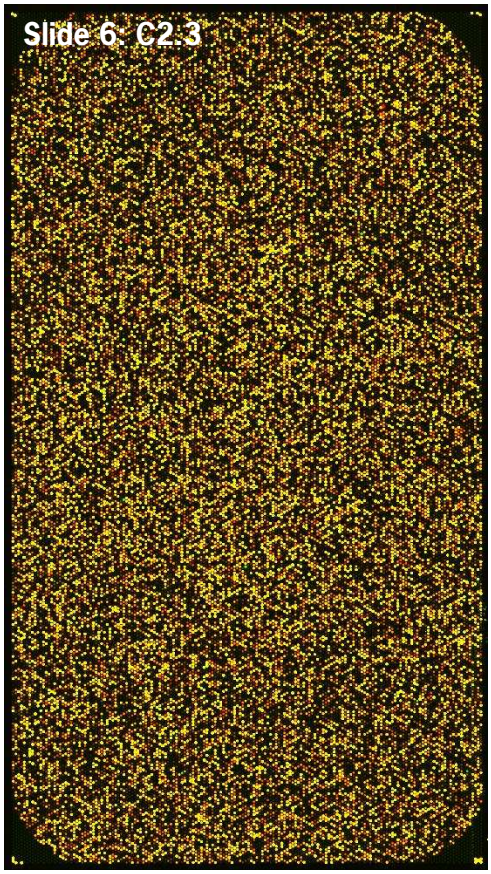

Slide 12: PO3

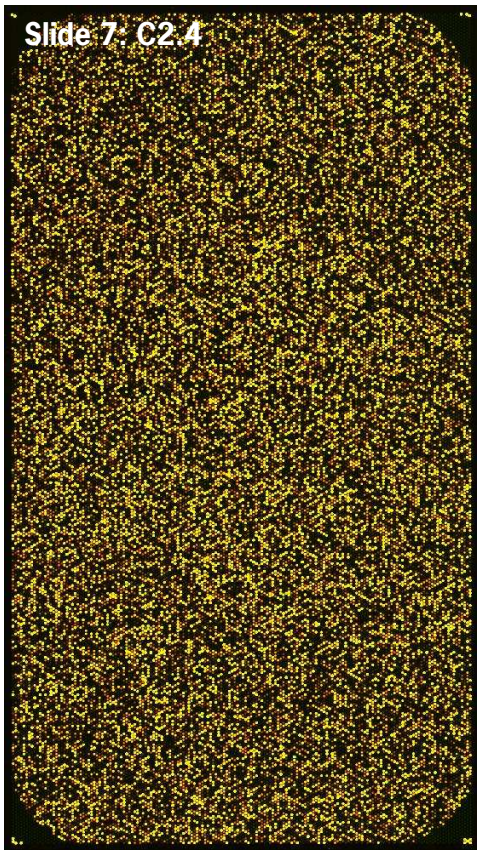

Slide 13: PO3

2. Micro array scans: IF induced gene expression in bovine livers.



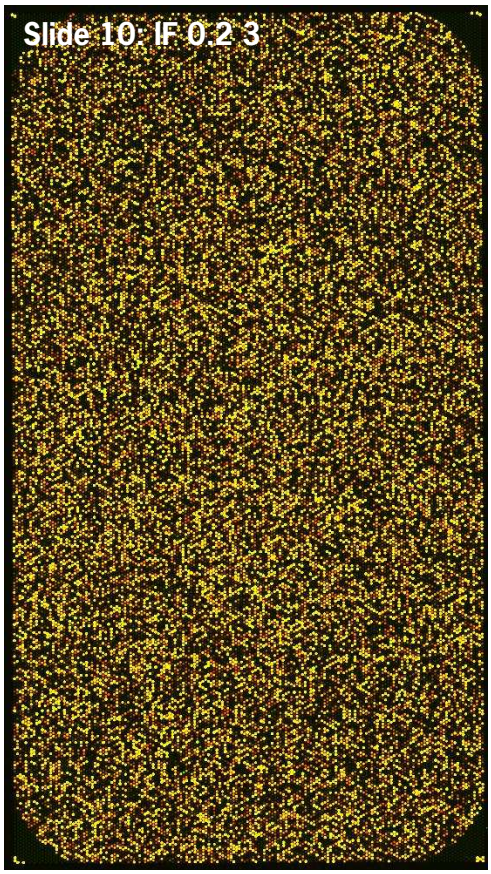

Slide 5: C2.2

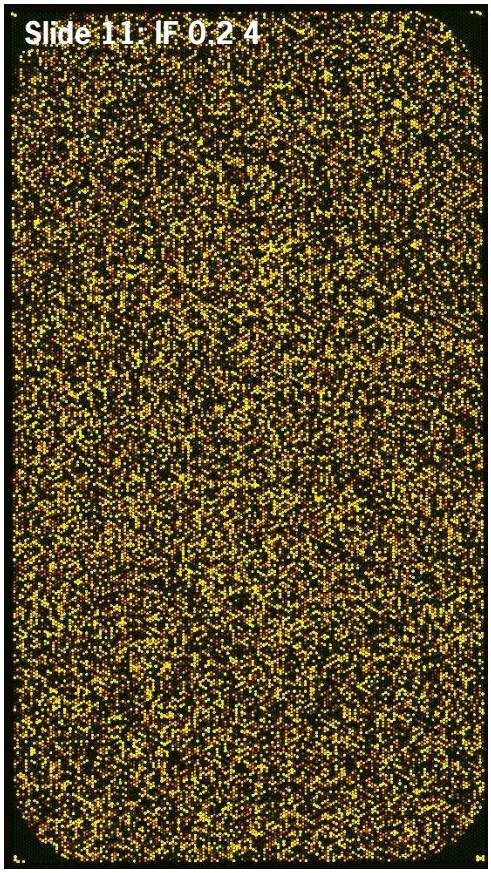

Slide 6: C2.3

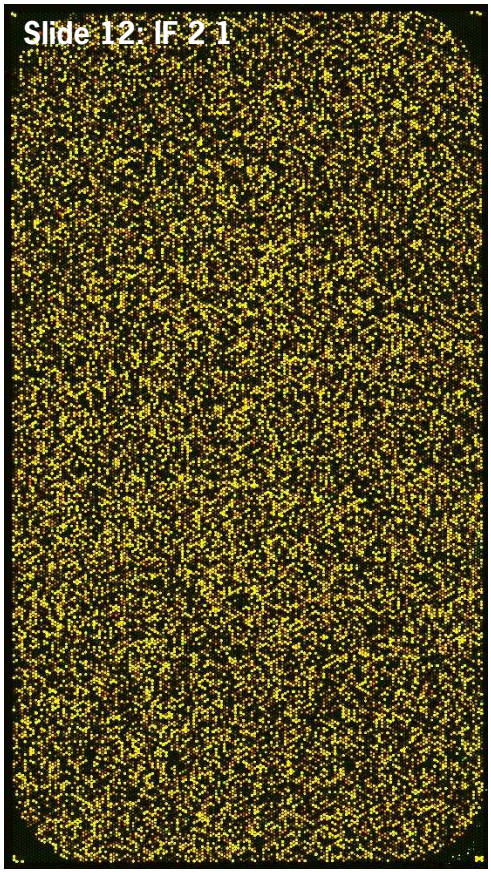

Slide 7: C2.4

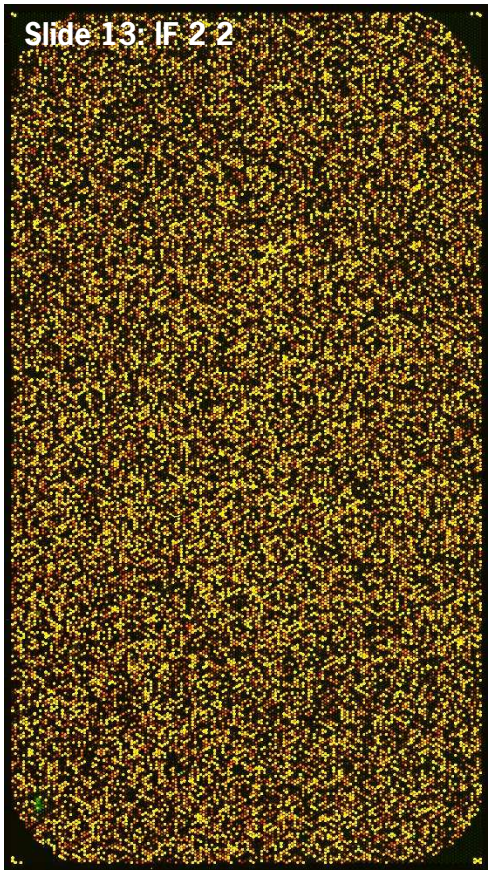

Slide 8: IF 0.2 1

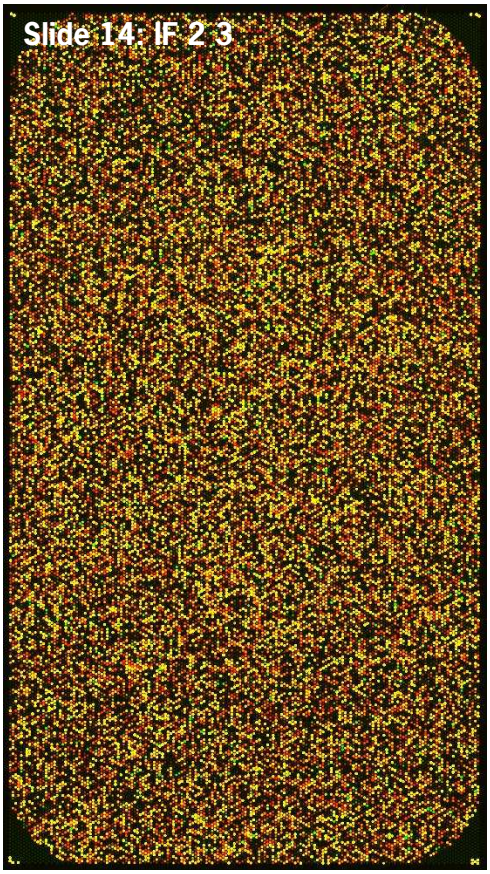

Slide 9: IF 0.2 2


Slide 10: IF 0.2 3


Slide 11: IF 0.2 4


Slide 12: IF 2 1


Slide 13: IF 2 2


Slide 14: IF 2 3


Slide 15: IF 2 4

Appendix C

1: Regulated transcripts in DHEA exposed animals versus total control group.

Upregulated transcripts.

Transcript description	Fold-change	Significance (p-value)
Bos taurus similar to Homo sapiens hematopoietic SH2 domain containing (HSH2D)	1,54	3,36E-02
Bos taurus vascular cell adhesion molecule 1 (VCAM1)	1,57	1,67E-02
Bos taurus similar to Homo sapiens peptidyl arginine deiminase, type III (PADI3)	1,65	2,91E-03
Bos taurus alkaline phosphatase, liver/bone/kidney (ALPL)	1,60	1,85E-02
Bos taurus similar to Homo sapiens potassium voltage-gated channel, subfamily H (eag-related), member 3 (KCNH3)	2,29	1,83E-02
Bos taurus solute carrier family 38, member 5 (SLC38A5)	1,86	1,63E-02
Bos taurus similar to Homo sapiens SLIT-ROBO Rho GTPase activating protein 1 (SRGAP1)	1,60	1,87E-02
Bos taurus alpha-1 acid glycoprotein (AGP)	1,87	9,70E-03
Bos taurus similar to Homo sapiens Williams Beuren syndrome chromosome region 27 (WBSCR27)	2,52	3,06E-02
Unidentified transcripts on BTA27 position 6402112-6401623	2,11	1,74E-02
Unidentified transcripts on BTA6 position 19437124-19436469	1,58	4,89E-02
Unidentified transcripts on BTA23 position 10577825-10576852	1,71	3,72E-02
Bos taurus similar to Homo sapiens heat shock 27kDa protein 2 (HSPB2)	1,66	4,96E-02
Bos taurus similar to Homo sapiens CD300 molecule-like family member f (CD300LF)	1,61	1,04E-02
Bos taurus similar to Homo sapiens fructose-1,6-bisphosphatase 1 (FBP1)	2,23	4,57E-02
Bos taurus similar to Homo sapiens fructose-1,6-bisphosphatase 1 (FBP1)	2,16	4,75E-02
Bos taurus tachykinin 3, neurokinin beta (TAC3)	2,31	9,63E-03
PREDICTED: Bos taurus similar to Squamous cell carcinoma antigen 1 (SCCA-1) (Protein T4-A) (LOC615636)	2,62	9,71E-03
Bos taurus similar to Homo sapiens 1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta) (AGPAT2), transcript variant 1	1,63	3,63E-02
Bos taurus similar to Homo sapiens fibroblast growth factor (acidic) intracellular binding protein (FIBP), transcript variant 2	1,98	2,31E-02
Bos taurus similar to Homo sapiens 1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta) (AGPAT2), transcript variant 1	1,60	4,06E-02
Bos taurus lingual antimicrobial peptide (LAP)	1,71	1,10E-02
Bos taurus capping protein (actin filament), gelsolin-like (CAPG)	2,01	3,47E-02
Bos taurus tachykinin 3, neurokinin beta (TAC3)	2,03	1,36E-02
Bos taurus alkaline phosphatase, liver/bone/kidney (ALPL)	1,55	3,16E-02
Bos taurus similar to Homo sapiens potassium voltage-gated channel, subfamily H (eag-related), member 3 (KCNH3)	2,27	1,82E-02
Bos taurus putative beta-defensin (BBD(C7))	1,76	1,10E-02
Bos taurus similar to Homo sapiens chromosome 1 open reading frame 178 (C1orf178), transcript variant a	1,62	3,57E-02
Bos taurus bactericidal/permeability-increasing protein (BPI)	2,40	3,06E-02
Bos taurus transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase) (TGM2)	1,61	1,14E-03
Unidentified transcripts on BTA25 position 31093369-31094201	1,89	1,60E-02
Bos taurus solute carrier family 38, member 5 (SLC38A5)	2,02	2,10E-02
PREDICTED: Bos taurus similar to nanos homolog 3 (LOC523375)	2,67	3,56E-02
Bos taurus putative beta-defensin (BBD(C7))	1,77	7,50E-03
PREDICTED: Bos taurus similar to zinc finger protein 16 (LOC510613)	1,75	2,95E-02
Bos taurus similar to Homo sapiens DEAH (Asp-Glu-Ala-His) box polypeptide 34 (DHX34), transcript variant 2	1,63	1,68E-02
Bos taurus similar to Homo sapiens fibroblast growth factor (acidic) intracellular binding protein (FIBP), transcript variant 2	2,08	1,97E-02
Bos taurus similar to Homo sapiens CD300 molecule-like family member f (CD300LF)	1,67	6,67E-03
PREDICTED: Bos taurus similar to Squamous cell carcinoma antigen 1 (SCCA-1) (Protein T4-A) (LOC615636)	2,63	1,25E-02
PREDICTED: Bos taurus similar to nanos homolog 3 (LOC523375)	2,42	3,33E-02
Bos taurus similar to Homo sapiens DEAH (Asp-Glu-Ala-His) box polypeptide 34 (DHX34), transcript variant 2	1,58	1,74E-02
Bos taurus similar to Homo sapiens Williams Beuren syndrome chromosome region 27 (WBSCR27)	2,64	2,54E-02
Unidentified transcripts on BTA25 position 31093369-31094201	1,91	1,76E-02
Bos taurus transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase) (TGM2)	1,68	8,65E-04
Unidentified transcripts on BTA27 position 6402112-6401623	2,14	1,69E-02

Bos taurus similar to Homo sapiens tripartite motif-containing 2 (TRIM2)	1,54	4,84E-02
Unidentified transcripts on BTA23 position 10577825-10576852	1,78	3,55E-02
Bos taurus similar to Homo sapiens CCAAT/enhancer binding protein (C/EBP), epsilon (CEBPE)	1,90	3,13E-02
Bos taurus alpha-1 acid glycoprotein (AGP)	1,87	1,04E-02
Bos taurus capping protein (actin filament), gelsolin-like (CAPG)	1,71	3,29E-02
Bos taurus similar to Homo sapiens peptidyl arginine deiminase, type III (PADI3)	1,60	3,80E-03
Bos taurus similar to Homo sapiens chromosome 1 open reading frame 178 (C1orf178), transcript variant a	1,53	4,16E-02
Bos taurus similar to Homo sapiens CD82 molecule (CD82), transcript variant 1	1,57	4,87E-02
PREDICTED: Bos taurus similar to PTPRF interacting protein alpha 1 isoform a (LOC616932)	1,52	4,64E-02
Bos taurus vascular cell adhesion molecule 1 (VCAM1)	1,59	3,03E-02
Bos taurus similar to Homo sapiens hematopoietic SH2 domain containing (HSH2D)	1,54	2,66E-02
Unidentified transcripts on BTA6 position 19437124-19436469	1,57	4,85E-02
Bos taurus lingual antimicrobial peptide (LAP)	1,82	3,99E-03
Bos taurus similar to Homo sapiens heat shock 27kDa protein 2 (HSPB2)	1,67	3,34E-02
Unidentified transcripts	2,12	4,38E-02

Downregulated transcripts

Transcript description	Fold-change	Significance (p-value)
Bos taurus similar to Homo sapiens aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase) (ALDH5A1), nuclear gene encoding mitochondrial protein, transcript variant 2	-1,76	1,18E-02
Unidentified transcripts	-1,55	2,47E-02
Bos taurus similar to Homo sapiens peptidylprolyl isomerase G (cyclophilin G) (PPIG)	-1,64	4,20E-02
Unidentified transcripts on BTA10 position 16405353-16406182	-1,57	4,13E-02
Bos taurus similar to Homo sapiens ubiquitin D (UBD)	-1,55	8,29E-03
Unidentified transcripts	-1,54	4,26E-02
Bos taurus similar to Homo sapiens ERGIC and golgi 2 (ERGIC2)	-1,51	4,25E-02
Bos taurus leucine aminopeptidase 3 (LAP3)	-1,62	3,06E-02
Bos taurus similar to Homo sapiens lactamase, beta 2 (LACTB2)	-1,51	4,65E-03
Bos taurus similar to Homo sapiens zinc ribbon domain containing 1 (ZNRD1), transcript variant a	-5,04	5,83E-03
Bos taurus interleukin 18 (interferon-gamma-inducing factor) (IL18)	-1,57	1,68E-02
Bos taurus similar to Homo sapiens establishment of cohesion 1 homolog 2 (S. cerevisiae) (ESCO2)	-1,62	2,99E-02
Bos taurus similar to Homo sapiens serologically defined colon cancer antigen 1 (SDCCAG1)	-1,50	3,42E-02
Bos taurus similar to Homo sapiens mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) (MSH2)	-1,59	4,94E-02
Bos taurus toll-like receptor 3 (TLR3)	-1,52	5,44E-03
Unidentified transcripts on BTA29 position 29030660-29031722	-1,77	2,63E-02
Bos taurus similar to Homo sapiens TXK tyrosine kinase (TXK)	-1,60	1,99E-03
Bos taurus similar to Homo sapiens hypothetical protein LOC55610, isoform b (FLJ20097), transcript variant 1	-1,51	3,78E-02
Bos taurus similar to Homo sapiens prefoldin subunit 4 (PFDN4)	-1,58	2,39E-02
Bos taurus similar to Homo sapiens tetraspanin 6 (TSPAN6)	-1,52	5,45E-04
Bos taurus similar to Homo sapiens prefoldin subunit 4 (PFDN4)	-1,54	3,45E-02
Bos taurus chromosome 14 open reading frame 10 (C14orf10)	-1,54	4,55E-02
Bos taurus similar to Homo sapiens geminin, DNA replication inhibitor (GMNN)	-1,69	3,53E-02
Bos taurus similar to Homo sapiens zinc ribbon domain containing 1 (ZNRD1), transcript variant a	-4,93	5,52E-03
Bos taurus similar to Homo sapiens ubiquitin D (UBD)	-1,51	1,26E-02
Bos taurus similar to Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32 family, member E (ANP32E)	-1,52	2,43E-02
Bos taurus similar to Homo sapiens aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase) (ALDH5A1), nuclear gene encoding mitochondrial protein, transcript variant 2	-1,70	1,75E-02
Bos taurus similar to Homo sapiens geminin, DNA replication inhibitor (GMNN)	-1,62	4,64E-02
Bos taurus similar to PREDICTED: Homo sapiens chromosome 6 open reading frame 115 (C6orf115)	-1,52	3,19E-02

2: Regulated transcripts in DHEA exposed animals versus control Group 1.

Upregulated transcripts:

Transcript description	Fold change	Significance (p-value)
PREDICTED: Bos taurus similar to [Segment 1 of 2] Traf2 and NCK interacting kinase (LOC537013)	2,00	3,65E-02
Bos taurus similar to Homo sapiens peptidyl arginine deiminase, type III (PADI3)	1,64	2,38E-02
Bos taurus similar to Homo sapiens solute carrier family 7, (cationic amino acid transporter, y+ system) member 11 (SLC7A11)	1,92	1,78E-02
Bos taurus similar to Homo sapiens ankyrin repeat and SOCS box-containing 2 (ASB2)	1,93	1,75E-02
Bos taurus similar to Homo sapiens ATPase, Class II, type 9A (ATP9A)	1,86	1,18E-02
PREDICTED: Bos taurus CD80 anitgen (LOC407131)	1,79	3,25E-02
Bos taurus neutrophil beta-defensin-9 like peptide precursor	2,06	6,00E-03
Bos taurus similar to Homo sapiens dual specificity phosphatase 26 (putative) (DUSP26)	1,60	1,09E-02
Unidentified transcripts	2,23	2,34E-02
Bos taurus similar to Homo sapiens hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD)	5,53	2,97E-02
Bos taurus neutrophil beta-defensin-9 like peptide precursor	2,11	5,11E-03
Bos taurus similar to Homo sapiens cannabinoid receptor 2 (macrophage) (CNR2)	1,91	2,60E-02
PREDICTED: Bos taurus complement receptor type 2 (cd21)	1,62	3,12E-02
Unidentified transcripts on BTA6 position 19437124-19436469	2,66	2,83E-03
Bos taurus similar to Homo sapiens ATP-binding cassette, sub-family C (CFTR/MRP), member 4 (ABCC4)	1,99	2,98E-02
PREDICTED: Bos taurus similar to NACHT, leucine rich repeat and PYD containing 7 isoform 1 (LOC507335)	1,55	1,55E-02
Unidentified transcripts	1,97	2,59E-02
Bos taurus similar to Homo sapiens homeobox B6 (HOXB6)	2,14	9,80E-03
Unidentified transcripts on BTA9 position 34156534-34157135	2,15	3,16E-02
Bos taurus similar to Homo sapiens serpin peptidase inhibitor, clade B (ovalbumin), member 2 (SERPINB2)	3,98	3,47E-02
Unidentified transcripts on BTA1 position 54621464-54620666	1,73	2,36E-02
Unidentified transcripts on BTA11 position 65387188-65388011	2,47	3,57E-03
Bos taurus similar to Homo sapiens CD300 molecule-like family member f (CD300LF)	1,74	2,74E-02
Bos taurus similar to Homo sapiens fructose-1,6-bisphosphatase 1 (FBP1)	5,53	6,01E-03
Bos taurus similar to Homo sapiens fructose-1,6-bisphosphatase 1 (FBP1)	5,36	5,57E-03
Bos taurus tachykinin 3, neurokinin beta (TAC3)	2,96	2,23E-02
PREDICTED: Bos taurus similar to zinc finger protein 16 (LOC510613)	2,30	2,64E-02
Bos taurus arachidonate 15-lipoxygenase (ALOX15)	13,60	1,02E-02
Bos taurus similar to Homo sapiens BMP and activin membrane-bound inhibitor homolog (Xenopus laevis) (BAMBI)	1,60	1,11E-02
Bos taurus similar to Homo sapiens cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4) (CDKN2B), transcript variant 1	1,83	4,95E-02
Unidentified transcripts on BTA5 position 52999741-53000171	1,57	3,94E-03
Bos taurus similar to PREDICTED: Homo sapiens hypothetical protein LOC646431 (LOC646431)	2,19	4,66E-02
Bos taurus intercellular adhesion molecule 3 (ICAM3)	2,36	2,30E-02
Bos taurus lingual antimicrobial peptide (LAP)	1,86	3,75E-02
Bos taurus tachykinin 3, neurokinin beta (TAC3)	2,69	1,76E-02
Bos taurus similar to Homo sapiens aminolevulinate, delta-, synthase 2 (sideroblastic/hypochromic anemia) (ALAS2), nuclear gene encoding mitochondrial protein, transcript variant 1	3,17	1,26E-02
Bos taurus similar to Homo sapiens adhesion molecule, interacts with CXADR antigen 1 (AMICA1)	1,79	4,68E-02
Bos taurus putative beta-defensin (BBD(C7))	2,15	1,12E-02
Bos taurus arachidonate 15-lipoxygenase (ALOX15)	13,24	9,97E-03
Bos taurus similar to PREDICTED: Homo sapiens hypothetical protein LOC646431 (LOC646431)	2,18	4,65E-02
Bos taurus similar to Homo sapiens dual specificity phosphatase 26 (putative) (DUSP26)	1,76	6,32E-03
Bos taurus similar to Homo sapiens cannabinoid receptor 2 (macrophage) (CNR2)	1,96	2,79E-02
PREDICTED: Bos taurus similar to [Segment 1 of 2] Traf2 and NCK interacting kinase (LOC537013)	2,26	4,30E-02
Bos taurus similar to Homo sapiens arachidonate 5-lipoxygenase (ALOX5)	4,80	1,48E-02
Bos taurus similar to Homo sapiens ankyrin repeat and SOCS box-containing 2 (ASB2)	1,88	1,70E-02
Bos taurus similar to Homo sapiens BMP and activin membrane-bound inhibitor homolog (Xenopus laevis) (BAMBI)	1,55	3,56E-03
Bos taurus similar to Homo sapiens tachykinin receptor 1 (TACR1), transcript variant long	1,57	4,27E-02
Bos taurus NADP-dependent retinol dehydrogenase/reductase (RDHL)	2,24	1,17E-02
Bos taurus transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase) (TGM2)	1,77	7,63E-03

Bos taurus similar to Homo sapiens arachidonate 5-lipoxygenase (ALOX5)	4,37	2,54E-02
Bos taurus similar to Homo sapiens FAT tumor suppressor homolog 1 (Drosophila) (FAT)	1,57	2,64E-02
Bos taurus immunoglobulin A Fc receptor (FCAR)	1,78	2,62E-02
Bos taurus C5a anaphylatoxin receptor (C5R1)	1,63	5,20E-03
PREDICTED: Bos taurus similar to tumor necrosis factor receptor superfamily, member 26 (LOC510798)	1,66	3,04E-02
PREDICTED: Bos taurus similar to Adenosine A3 receptor (LOC617432)	1,57	3,78E-02
Unidentified transcripts on BTA9 position 34156534-34157135	1,57	1,72E-02
Bos taurus similar to Homo sapiens chromosome 4 open reading frame 19 (C4orf19)	3,84	6,73E-03
Bos taurus similar to Homo sapiens solute carrier family 7, (cationic amino acid transporter, y+ system) member 11 (SLC7A11)	2,29	6,61E-03
Bos taurus similar to Homo sapiens tumor protein p53 inducible nuclear protein 2 (TP53INP2)	1,74	3,40E-02
Bos taurus similar to Homo sapiens CCAAT/enhancer binding protein (C/EBP), epsilon (CEBPE)	3,02	2,86E-02
Bos taurus similar to Homo sapiens sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II) (SMPD3)	4,81	1,58E-02
Bos taurus C-type lectin domain family 6 member A (CLEC6A)	2,31	2,98E-02
PREDICTED: Bos taurus similar to SUMO1/sentrin/SMT3 specific protease 3 (LOC509705)	2,24	1,16E-02
Bos taurus putative beta-defensin (BBD(C7))	2,20	5,62E-03
Bos taurus similar to Homo sapiens deoxycytidine kinase (DCK)	1,70	3,62E-03
PREDICTED: Bos taurus similar to zinc finger protein 16 (LOC510613)	2,38	1,90E-02
Bos taurus similar to Homo sapiens homeobox B6 (HOXB6)	2,40	4,35E-03
Bos taurus similar to Homo sapiens CD300 molecule-like family member f (CD300LF)	1,82	1,89E-02
Bos taurus similar to Homo sapiens acyloxyacyl hydrolase (neutrophil) (AOAH)	1,52	2,39E-02
Bos taurus similar to Homo sapiens ATPase, Class II, type 9A (ATP9A)	1,82	1,46E-02
Bos taurus C-type lectin domain family 6 member A (CLEC6A)	2,48	3,24E-02
Unidentified transcripts	3,27	4,30E-02
Bos taurus elastase 2 (Ela2)	1,70	3,88E-02
Bos taurus similar to Homo sapiens zinc finger protein 161 (ZNF161)	1,72	1,99E-02
Bos taurus similar to Homo sapiens synaptic vesicle glycoprotein 2B (SV2B)	1,69	6,97E-03
Bos taurus similar to Homo sapiens adhesion molecule, interacts with CXADR antigen 1 (AMICA1)	1,85	4,08E-02
Unidentified transcripts on BTA11 position 65387188-65388011	2,57	3,95E-03
Bos taurus similar to Homo sapiens deoxycytidine kinase (DCK)	1,76	3,06E-02
Bos taurus mucin 15 (MUC15)	2,02	1,18E-02
Bos taurus similar to Homo sapiens myeloid/lymphoid or mixed-lineage leukemia 5 (trithorax homolog, Drosophila) (MLL5), transcript variant 1	1,62	1,83E-02
Bos taurus similar to Homo sapiens serpin peptidase inhibitor, clade B (ovalbumin), member 2 (SERPINB2)	3,65	4,49E-02
Bos taurus transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase) (TGM2)	1,88	4,42E-03
Unidentified transcripts on BTA18 position 48974183-48974679	1,57	2,30E-02
Unidentified transcripts on BTA15 position 53073257-53072563	1,66	3,42E-02
PREDICTED: Bos taurus similar to SUMO1/sentrin/SMT3 specific protease 3 (LOC509705)	2,23	2,95E-02
Bos taurus similar to Homo sapiens hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD)	6,28	3,24E-02
Bos taurus similar to Homo sapiens chromosome 4 open reading frame 19 (C4orf19)	3,57	8,76E-03
Bos taurus similar to Homo sapiens synaptic vesicle glycoprotein 2B (SV2B)	1,59	3,58E-03
PREDICTED: Bos taurus similar to Multiple EGF-like-domain protein 5 precursor (Multiple epidermal growth factor-like domains 9) (LOC533820)	1,90	2,91E-02
Bos taurus similar to Homo sapiens sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II) (SMPD3)	4,08	1,11E-02
PREDICTED: Bos taurus similar to Multiple EGF-like-domain protein 5 precursor (Multiple epidermal growth factor-like domains 9) (LOC533820)	1,78	2,17E-02
Bos taurus similar to Homo sapiens CCAAT/enhancer binding protein (C/EBP), epsilon (CEBPE)	3,10	9,27E-03
PREDICTED: Bos taurus similar to egf-like module-containing mucin-like receptor 3 isoform a (LOC530259), partial mRNA.	1,58	3,05E-02
PREDICTED: Bos taurus complement receptor type 2 (cd21)	1,67	4,91E-02
Unidentified transcripts on BTA16 position 18486451-18487440	1,61	2,26E-02
Unidentified transcripts on BTA5 position 69928003-69929166	1,64	6,23E-03
Bos taurus immunoglobulin A Fc receptor (FCAR)	1,73	4,53E-02
Bos taurus similar to Homo sapiens aminolevulinate, delta-, synthase 2 (sideroblastic/hypochromic anemia) (ALAS2), nuclear gene encoding mitochondrial protein, transcript variant 1	2,92	1,42E-02
Bos taurus similar to Homo sapiens peptidyl arginine deiminase, type III (PADI3)	1,57	3,14E-02
Bos taurus intercellular adhesion molecule 3 (ICAM3)	2,36	3,51E-02
Bos taurus NADP-dependent retinol dehydrogenase/reductase (RDHL)	2,21	7,30E-03
PREDICTED: Bos taurus similar to NACHT, leucine rich repeat and PYD containing 7 isoform 1 (LOC507335)	1,56	1,40E-02
Bos taurus similar to Homo sapiens myeloperoxidase (MPO), nuclear gene encoding mitochondrial protein	3,46	4,12E-03

Bos taurus similar to PREDICTED: Homo sapiens egf-like module containing, mucin-like, hormone receptor-like 4 (EMR4)	1,58	6,98E-03
Bos taurus similar to Homo sapiens myotubularin related protein 9 (MTMR9)	1,58	8,68E-04
Unidentified transcripts on BTA18 position 48974183-48974679	1,58	1,20E-02
Unidentified transcripts on BTA15 position 22474110-22475022	1,56	4,87E-02
Bos taurus similar to Homo sapiens myeloperoxidase (MPO), nuclear gene encoding mitochondrial protein	3,82	3,09E-03
Unidentified transcripts on BTA6 position 19437124-19436469	2,73	1,69E-03
Bos taurus similar to Homo sapiens zinc finger protein 161 (ZNF161)	1,85	5,33E-03
Unidentified transcripts on BTA16 position 18486451-18487440	1,71	1,17E-02
Bos taurus lingual antimicrobial peptide (LAP)	1,79	3,54E-02
Bos taurus similar to Homo sapiens EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1), transcript variant 1	2,18	3,71E-02
Bos taurus mucin 15 (MUC15)	2,05	1,37E-02
Unidentified transcripts on BTA1 position 54621464-54620666	1,74	1,19E-02
Unidentified transcripts	3,08	3,87E-02

Downregulated transcripts.

Transcript description	Fold change	Significance (p-value)
Bos taurus similar to Homo sapiens dual specificity phosphatase 10 (DUSP10), transcript variant 3	-1,70	1,12E-03
Bos taurus similar to Homo sapiens LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae) (LSM3)	-1,56	2,01E-02
Unidentified transcripts	-2,12	1,15E-02
Bos taurus similar to Homo sapiens adenine phosphoribosyltransferase (APRT), transcript variant 1	-1,53	2,49E-02
Bos taurus mevalonate kinase (mevalonic aciduria) (MVK)	-1,61	2,79E-02
Bos taurus similar to Homo sapiens globoside alpha-1,3-N-acetylgalactosaminyltransferase 1 (GBGT1)	-1,55	1,80E-02
Bos taurus similar to Homo sapiens leucine zipper protein 5 (LUZP5)	-1,55	1,33E-02
Bos taurus similar to Homo sapiens complement component 1, q subcomponent, C chain (C1QC)	-2,62	1,58E-02
PREDICTED: Bos taurus similar to nasal embryonic LHRH factor, transcript variant 2 (LOC615854)	-1,53	3,68E-02
Bos taurus similar to Homo sapiens chromosome 7 open reading frame 30 (C7orf30)	-1,55	2,16E-02
Bos taurus proteasome (prosome, macropain) subunit, beta type, 10 (PSMB10)	-1,66	1,92E-02
Bos taurus similar to Homo sapiens tudor domain containing 9 (TDRD9)	-2,56	1,27E-02
Bos taurus similar to Homo sapiens dUTP pyrophosphatase (DUT), nuclear gene encoding mitochondrial protein, transcript variant 2	-1,50	2,30E-02
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 153 (C14orf153)	-1,68	3,39E-02
Bos taurus laminin receptor 1 (ribosomal protein SA, 67 kDA) (LAMR1)	-1,60	4,44E-02
Bos taurus similar to Homo sapiens glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group) (GCNT2), transcript variant 2	-1,63	3,79E-02
Bos taurus similar to Homo sapiens kleisin beta (hCAP-H2), transcript variant 2	-1,50	3,44E-02
Bos taurus similar to Homo sapiens Epstein-Barr virus induced gene 3 (EBI3)	-1,55	1,30E-02
Bos taurus similar to Homo sapiens cyclin D2 (CCND2)	-1,72	4,40E-02
Bos taurus similar to Homo sapiens Tar (HIV-1) RNA binding protein 2 (TARBP2), transcript variant 3	-1,57	3,65E-02
Bos taurus similar to Homo sapiens membrane-spanning 4-domains, subfamily A, member 7 (MS4A7), transcript variant 1	-2,31	1,01E-02
PREDICTED: Bos taurus pyrophosphatase (inorganic), transcript variant 1 (PP)	-1,69	4,33E-03
Bos taurus similar to Homo sapiens meiotic nuclear divisions 1 homolog (S. cerevisiae) (MND1)	-1,82	9,72E-03
Bos taurus similar to Homo sapiens SLC2A4 regulator (SLC2A4RG)	-1,51	4,14E-02
Bos taurus similar to Homo sapiens ribosomal protein S23 (RPS23)	-1,60	1,58E-03
Bos taurus MAPA protein (MAPA)	-2,26	7,19E-03
Bos taurus heat shock 10kDa protein 1 (chaperonin 10) (HSPE1)	-1,51	3,83E-02
Unidentified transcripts on BTA5 position 67775861-67777575	-1,55	2,22E-02
Bos taurus 7-dehydrocholesterol reductase (DHCR7)	-1,51	1,56E-02
Bos taurus similar to Homo sapiens tetraspanin 33 (TSPAN33)	-1,79	5,86E-03
Bos taurus similar to Homo sapiens FLJ43339 protein (FLJ43339)	-1,64	2,32E-03
Unidentified transcripts	-1,58	4,52E-02
Bos taurus similar to Homo sapiens protein phosphatase 1, regulatory (inhibitor) subunit 14B (PPP1R14B)	-1,56	3,38E-02
Bos taurus similar to Homo sapiens proteasome (prosome, macropain) subunit, alpha type, 4 (PSMA4)	-1,73	1,79E-02
Bos taurus similar to Homo sapiens pleckstrin homology domain containing, family H (with MyTH4 domain) member 3 (PLEKHH3)	-1,54	1,84E-02
Unidentified transcripts on BTA26 position 22364440-22365005	-1,95	4,52E-03
Bos taurus similar to Homo sapiens dual specificity phosphatase 7 (DUSP7)	-1,57	1,22E-02

Bos taurus acetylserotonin O-methyltransferase (ASMT)	-1,86	2,93E-02
Bos taurus similar to Homo sapiens eukaryotic translation initiation factor 1A, Y-linked (EIF1AY)	-1,53	1,63E-02
Bos taurus similar to Homo sapiens chromosome 9 open reading frame 98 (C9orf98)	-1,67	1,54E-02
Bos taurus similar to Homo sapiens amino-terminal enhancer of split (AES), transcript variant 2	-1,79	7,62E-03
Bos taurus mRNA decapping enzyme (DCPS)	-1,59	3,39E-03
Unidentified transcripts on BTA17 position 45028945-45027762	-1,56	9,61E-03
Bos taurus similar to Homo sapiens BCL2-related protein A1 (BCL2A1)	-1,72	2,53E-02
Bos taurus similar to Homo sapiens NDRG family member 2 (NDRG2), transcript variant 2	-1,53	3,67E-02
Bos taurus eukaryotic translation elongation factor 1 beta 2 (EEF1B2)	-1,69	2,81E-02
Bos taurus similar to Homo sapiens asparagine synthetase (ASNS), transcript variant 3	-1,51	1,58E-02
Bos taurus similar to Homo sapiens optineurin (OPTN), transcript variant 2	-1,60	1,99E-02
Bos taurus similar to Homo sapiens peptidyl-tRNA hydrolase 2 (PTRH2), nuclear gene encoding mitochondrial protein, transcript variant 2	-1,51	2,47E-02
Bos taurus similar to Homo sapiens protein tyrosine phosphatase, receptor type, F (PTPRF), transcript variant 2	-1,50	4,36E-02
PREDICTED: Bos taurus annexin A6 (ANXA6)	-1,51	1,67E-02
Bos taurus cytoskeleton associated protein 1 (CKAP1)	-1,53	6,20E-03
Bos taurus fatty acid synthase (FASN)	-1,60	9,66E-03
Bos taurus similar to Homo sapiens transmembrane protein 132B (TMEM132B)	-1,60	2,76E-02
Bos taurus mitochondrial ribosomal protein L12 (MRPL12)	-1,83	1,83E-02
Bos taurus similar to Homo sapiens acid phosphatase 2, lysosomal (ACP2)	-1,55	8,35E-03
Bos taurus NADH dehydrogenase (ubiquinone) Fe-S protein 6, 13kDa (NADH-coenzyme Q reductase) (NDUFS6)	-1,66	2,67E-02
Bos taurus similar to Homo sapiens Notch homolog 3 (Drosophila) (NOTCH3)	-1,63	6,58E-03
Bos taurus similar to Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble) (HMGCS1)	-1,53	4,61E-02
Unidentified transcripts on BTA26 position 22364440-22365005	-2,04	7,06E-03
Bos taurus similar to Homo sapiens vesicle-associated membrane protein 5 (myobrevin) (VAMP5)	-1,77	2,62E-02
Bos taurus similar to Homo sapiens isochorismatase domain containing 2 (ISOC2)	-1,53	4,76E-02
Bos taurus similar to Homo sapiens killer cell lectin-like receptor subfamily B, member 1 (KLRB1)	-1,61	1,37E-02
Bos taurus similar to Homo sapiens SH2 domain protein 2A (SH2D2A)	-1,53	1,36E-02
Bos taurus mitochondrial ribosomal protein L12 (MRPL12)	-1,74	3,32E-02
Bos taurus similar to Homo sapiens megakaryocyte-associated tyrosine kinase (MATK), transcript variant 1	-1,55	3,12E-02
PREDICTED: Bos taurus hypothetical LOC511765, transcript variant 3 (LOC511765)	-1,55	3,84E-02
Bos taurus similar to Homo sapiens tumor necrosis factor receptor superfamily, member 25 (TNFRSF25)	-1,59	1,51E-02
Bos taurus similar to Homo sapiens checkpoint suppressor 1 (CHES1)	-2,32	9,66E-03
Bos taurus similar to Homo sapiens PALM2-AKAP2 protein (PALM2-AKAP2), transcript variant 1	-1,69	3,19E-02
Bos taurus similar to Homo sapiens sodium channel, voltage-gated, type I, beta (SCN1B), transcript variant a	-1,69	2,03E-02
Bos taurus similar to Homo sapiens meiotic nuclear divisions 1 homolog (S. cerevisiae) (MND1)	-1,75	1,92E-02
Bos taurus MAPA protein (MAPA)	-2,21	7,76E-03
Unidentified transcripts on BTA28 position 34687548-34688131	-1,86	2,74E-02
Bos taurus similar to Homo sapiens armadillo repeat gene deletes in velocardiofacial syndrome (ARVCF)	-1,73	1,68E-03
Bos taurus similar to Homo sapiens transcription factor 7-like 2 (T-cell specific, HMG-box) (TCF7L2)	-1,65	1,10E-02
Bos taurus heme oxygenase (decyclizing) 1 (HMOX1)	-2,16	8,47E-03
Bos taurus similar to Homo sapiens LSM2 homolog, U6 small nuclear RNA associated (S. cerevisiae) (LSM2)	-1,74	2,48E-02
PREDICTED: Bos taurus hypothetical LOC511765, transcript variant 3 (LOC511765)	-1,56	4,52E-02
Bos taurus similar to Homo sapiens lymphotoxin beta (TNF superfamily, member 3) (LTB), transcript variant 2	-1,54	2,79E-02
Bos taurus similar to Homo sapiens hypothetical protein BC007540 (LOC144097)	-1,50	3,87E-02
Bos taurus ribosomal protein L10 (RPL10)	-1,66	3,49E-02
Bos taurus similar to Homo sapiens centromere protein B, 80kDa (CENPB)	-1,56	2,64E-02
Bos taurus similar to Homo sapiens GTP cyclohydrolase I feedback regulator (GCHFR)	-1,55	2,59E-02
Bos taurus similar to Homo sapiens splicing factor 1 (SF1), transcript variant 1	-1,52	2,84E-02
Bos taurus similar to Homo sapiens amidohydrolase domain containing 2 (AMDHD2)	-1,88	2,80E-03
Bos taurus similar to Homo sapiens amidohydrolase domain containing 2 (AMDHD2)	-1,80	2,77E-03
Bos taurus similar to Homo sapiens proteasome (prosome, macropain) 26S subunit, non-ATPase, 11 (PSMD11)	-1,50	2,94E-02
Bos taurus similar to Homo sapiens epoxide hydrolase 2, cytoplasmic (EPHX2)	-2,44	1,01E-02
Bos taurus G protein-coupled receptor TYMSTR (CXCR6)	-1,56	3,11E-03
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 153 (C14orf153)	-1,70	2,92E-02
Bos taurus similar to Homo sapiens tumor necrosis factor (TNF superfamily, member 2) (TNF)	-1,52	4,27E-02

Bos taurus similar to Homo sapiens rhomboid 5 homolog 1 (Drosophila) (RHBDF1)	-1,60	3,25E-02
Bos taurus similar to PREDICTED: Homo sapiens similar to amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 16, transcript variant 1 (LOC653539)	-1,68	4,21E-02
Bos taurus profilin 1 (PFN1)	-1,68	2,72E-02
Bos taurus similar to Homo sapiens kleisin beta (hCAP-H2), transcript variant 2	-1,54	2,47E-02
Bos taurus similar to Homo sapiens glioma tumor suppressor candidate region gene 2 (GLTSCR2)	-1,60	2,47E-02
Bos taurus chemokine (C-C motif) ligand 3-like 1 (CCL3L1)	-1,52	7,82E-03
Unidentified transcripts	-1,67	2,87E-02
PREDICTED: Bos taurus similar to ribosomal protein L37 (LOC616464)	-2,16	4,02E-02
Bos taurus similar to Homo sapiens Notch homolog 3 (Drosophila) (NOTCH3)	-1,61	5,75E-03
Bos taurus similar to Homo sapiens microsomal glutathione S-transferase 3 (MGST3)	-1,57	3,98E-02
Bos taurus similar to Homo sapiens cysteine and glycine-rich protein 2 (CSRP2)	-1,59	1,73E-02
Bos taurus allograft inflammatory factor 1 (AIF1)	-1,95	6,22E-03
Bos taurus similar to Homo sapiens ribosomal protein L30 (RPL30)	-1,73	2,54E-02
Bos taurus similar to Homo sapiens coiled-coil-helix-coiled-coil-helix domain containing 1 (CHCHD1)	-1,67	3,42E-02
Bos taurus similar to Homo sapiens chromosome 21 open reading frame 70 (C21orf70)	-1,60	3,83E-02
Bos taurus T-cell receptor alpha chain, constant and 3' untranslated region (TCR@)	-1,68	2,28E-02
Bos taurus similar to Homo sapiens complement component 1, q subcomponent, B chain (C1QB)	-3,27	6,74E-03
PREDICTED: Bos taurus similar to SAM domain and HD domain-containing protein 1 (Dendritic cell-derived IFNG-induced protein) (DCIP) (Monocyte protein 5) (MOP-5) (LOC615523)	-1,59	3,00E-02
Unidentified transcripts on BTA25 position 33239816-33238720	-1,65	1,52E-02
Bos taurus similar to Homo sapiens syndecan 3 (N-syndecan) (SDC3)	-1,98	4,80E-02
Bos taurus similar to Homo sapiens acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain (ACADS), nuclear gene encoding mitochondrial protein	-1,58	1,22E-02
Bos taurus similar to Homo sapiens oxysterol binding protein-like 3 (OSBPL3), transcript variant 1	-1,56	1,48E-02
Bos taurus similar to Homo sapiens THO complex 4 (THOC4)	-1,59	8,20E-03
Bos taurus similar to Homo sapiens centaurin, gamma 1 (CENTG1)	-1,61	4,95E-02
Bos taurus similar to Homo sapiens nucleolar protein 12 (NOL12)	-1,73	8,96E-03
Bos taurus 13kDa differentiation-associated protein (DAP13)	-1,64	4,06E-02
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 166 (C14orf166)	-1,61	9,49E-03
Unidentified transcripts	-1,53	3,93E-02
Bos taurus similar to Homo sapiens fatty acid desaturase domain family, member 6 (FADS6)	-2,51	3,63E-02
Bos taurus similar to PREDICTED: Homo sapiens similar to amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 16, transcript variant 1 (LOC653539)	-1,64	4,64E-02
Bos taurus similar to Homo sapiens epoxide hydrolase 2, cytoplasmic (EPHX2)	-2,23	1,08E-02
Bos taurus similar to Homo sapiens uridine-cytidine kinase 2 (UCK2)	-1,52	4,29E-02
Bos taurus mevalonate kinase (mevalonic aciduria) (MVK)	-1,58	3,86E-02
Bos taurus similar to Homo sapiens megakaryocyte-associated tyrosine kinase (MATK), transcript variant 1	-1,55	3,94E-02
Bos taurus cytoskeleton associated protein 1 (CKAP1)	-1,60	1,81E-02
Bos taurus similar to Homo sapiens transcription factor 7-like 2 (T-cell specific, HMG-box) (TCF7L2)	-1,82	6,91E-03
Bos taurus similar to Homo sapiens neuron derived neurotrophic factor (NENF)	-1,57	8,11E-03
Bos taurus similar to Homo sapiens Fc fragment of IgG, low affinity IIIa, receptor (CD16a) (FCGR3A)	-2,49	3,88E-02
Bos taurus similar to Homo sapiens ABI gene family, member 3 (ABI3)	-1,68	3,31E-02
Bos taurus similar to Homo sapiens carbohydrate kinase-like (CARKL)	-1,59	1,11E-02
Bos taurus nucleoside phosphorylase (NP)	-1,72	6,07E-04
Bos taurus similar to Homo sapiens hypothetical protein FLJ11151 (FLJ11151)	-2,02	9,25E-03
Bos taurus similar to Homo sapiens armadillo repeat gene deletes in velocardiofacial syndrome (ARVCF)	-1,66	3,46E-02
Bos taurus similar to Homo sapiens natural killer cell group 7 sequence (NKG7)	-2,08	2,58E-02
Bos taurus similar to Homo sapiens ribosomal protein S13 (RPS13)	-1,70	1,82E-02
Bos taurus similar to Homo sapiens chromosome 1 open reading frame 50 (C1orf50)	-1,51	9,03E-03
Bos taurus similar to Homo sapiens zinc finger protein 205 (ZNF205), transcript variant 1	-2,17	1,67E-02
Bos taurus similar to Homo sapiens Fc fragment of IgG, low affinity IIIa, receptor (CD16a) (FCGR3A)	-2,51	4,08E-02
Bos taurus similar to Homo sapiens ribosomal protein S23 (RPS23)	-1,58	5,38E-03
Bos taurus similar to Homo sapiens tubulin, alpha, ubiquitous (K-ALPHA-1)	-1,51	4,46E-02
Bos taurus similar to Homo sapiens nucleophosmin/nucleoplasmin, 3 (NPM3)	-1,90	2,87E-02
Bos taurus similar to Homo sapiens ZW10, kinetochore associated, homolog (Drosophila) (ZW10)	-1,52	1,53E-03
Bos taurus similar to Homo sapiens dual specificity phosphatase 10 (DUSP10), transcript variant 3	-1,76	2,81E-03
Bos taurus similar to Homo sapiens ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative function) (ENPP4)	-1,59	4,14E-02
Bos taurus similar to Homo sapiens nucleolar protein 5A (56kDa with KKE/D repeat) (NOL5A)	-1,63	6,36E-03
Unidentified transcripts	-1,78	1,73E-02
Unidentified transcripts on BTA5 position 67775861-67777575	-1,56	2,97E-02
Bos taurus chemokine (C-C motif) ligand 5 (CCL5)	-1,77	3,63E-02
Bos taurus similar to Homo sapiens transmembrane protein 132B (TMEM132B)	-1,57	2,58E-02

Bos taurus similar to Homo sapiens CD83 molecule (CD83), transcript variant 1	-1,62	4,20E-03
Bos taurus similar to Homo sapiens carbonyl reductase 3 (CBR3)	-1,51	2,33E-02
Bos taurus similar to Homo sapiens chromosome 6 open reading frame 64 (C6orf64)	-1,75	3,56E-02
Bos taurus similar to Homo sapiens interleukin 20 receptor, alpha (IL20RA)	-1,66	2,97E-04
Bos taurus similar to Homo sapiens ribosomal protein L17 (RPL17)	-1,79	3,36E-02
Bos taurus similar to Homo sapiens ribosomal protein L36a (RPL36A)	-1,96	2,40E-02
Unidentified transcripts on BTA11 position 69156901-69156291	-1,64	2,08E-02
Bos taurus similar to Homo sapiens chromosome 18 open reading frame 24 (C18orf24), transcript variant 2	-1,51	1,83E-02
Bos taurus similar to Homo sapiens rhomboid 5 homolog 1 (Drosophila) (RHBDF1)	-1,70	4,58E-02
Unidentified transcripts on BTA10 position 16405353-16406182	-1,94	2,64E-02
PREDICTED: Bos taurus similar to [Pyruvate dehydrogenase [lipoamide]] kinase isozyme 1, mitochondrial precursor (Pyruvate dehydrogenase kinase isoform 1) (LOC614996)	-1,51	8,12E-03
Bos taurus similar to Homo sapiens protein tyrosine phosphatase, receptor type, F (PTPRF), transcript variant 2	-1,52	3,22E-02
Bos taurus similar to Homo sapiens ubiquitin D (UBD)	-1,57	4,43E-02
Bos taurus NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa (NADH-coenzyme Q reductase) (NDUFS3)	-1,55	3,03E-02
Bos taurus similar to Homo sapiens catenin (cadherin-associated protein), alpha-like 1 (CTNNAL1)	-1,95	4,35E-04
Bos taurus similar to Homo sapiens fer-1-like 3, myoferlin (C. elegans) (FER1L3), transcript variant 2	-1,59	3,42E-02
Bos taurus similar to Homo sapiens actin related protein 2/3 complex, subunit 3, 21kDa (ARPC3)	-1,98	3,95E-02
Bos taurus similar to Homo sapiens nucleolar protein 12 (NOL12)	-1,70	1,08E-02
Bos taurus similar to Homo sapiens chromosome 11 open reading frame 31 (C11orf31)	-1,53	1,96E-02
Bos taurus similar to Homo sapiens enhancer of rudimentary homolog (Drosophila) (ERH)	-1,52	4,96E-03
Bos taurus similar to Homo sapiens c-mer proto-oncogene tyrosine kinase (MERTK)	-1,91	7,12E-03
Bos taurus similar to Homo sapiens tumor necrosis factor, alpha-induced protein 8-like 3 (TNFAIP8L3)	-1,57	1,79E-02
Bos taurus neurolysin (EP24.16)	-1,78	1,96E-03
Bos taurus similar to Homo sapiens chromosome 21 open reading frame 70 (C21orf70)	-1,64	3,25E-02
Bos taurus similar to Homo sapiens acyl-CoA synthetase long-chain family member 5 (ACSL5), transcript variant 1	-1,56	1,20E-03
Bos taurus similar to Homo sapiens checkpoint suppressor 1 (CHES1)	-2,40	1,15E-02
Bos taurus similar to Homo sapiens chromosome 11 open reading frame 67 (C11orf67)	-1,78	1,75E-02
PREDICTED: Bos taurus similar to High mobility group protein 1 (HMG-1) (High mobility group protein B1) (Amphoterin) (Heparin-binding protein p30) (LOC539878)	-1,52	3,43E-03
Bos taurus similar to Homo sapiens chromosome X open reading frame 26 (CXorf26)	-1,52	2,38E-02
Bos taurus similar to Homo sapiens ribosomal protein S13 (RPS13)	-1,67	2,95E-02
Bos taurus similar to Homo sapiens hepatitis B virus x interacting protein (HBXIP)	-1,57	8,71E-03
Bos taurus similar to Homo sapiens enolase 3 (beta, muscle) (ENO3), transcript variant 2	-2,38	5,48E-03
Unidentified transcripts	-1,53	4,21E-02
Bos taurus similar to Homo sapiens copper chaperone for superoxide dismutase (CCS)	-1,68	4,38E-02
Bos taurus NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase) (NDUFS4)	-2,05	3,80E-03
Bos taurus similar to Homo sapiens G protein-coupled receptor 56 (GPR56), transcript variant 3	-1,64	3,48E-02
Bos taurus similar to Homo sapiens ribosomal protein L27a (RPL27A)	-1,99	2,51E-02
Bos taurus NADH dehydrogenase (ubiquinone) Fe-S protein 6, 13kDa (NADH-coenzyme Q reductase) (NDUFS6)	-1,59	3,20E-02
PREDICTED: Bos taurus cathepsin D (lysosomal aspartyl protease), transcript variant 2 (CTSD)	-1,53	1,91E-02
Bos taurus similar to Homo sapiens BCL2-related protein A1 (BCL2A1)	-1,86	2,42E-02
Bos taurus nuclear receptor subfamily 1, group H, member 3 (NR1H3)	-2,00	1,61E-02
Bos taurus similar to Homo sapiens carbonyl reductase 3 (CBR3)	-1,54	1,62E-02
Bos taurus STK25 protein (STK25)	-1,50	9,14E-03
Bos taurus similar to Homo sapiens synaptotagmin XI (SYT11)	-1,71	2,84E-02
Bos taurus similar to Homo sapiens jun D proto-oncogene (JUND)	-1,60	4,20E-02
Bos taurus similar to Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble) (HMGCS1)	-1,56	4,13E-02
Bos taurus similar to Homo sapiens optineurin (OPTN), transcript variant 2	-1,58	2,68E-02
Bos taurus similar to Homo sapiens enolase 3 (beta, muscle) (ENO3), transcript variant 2	-2,37	7,52E-03
Unidentified transcripts on BTA16 position 10941576-10940643	-1,88	4,31E-04
Bos taurus ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein) (ATP5O)	-1,57	2,90E-02
Unidentified transcripts on BTA25 position 33239816-33238720	-1,73	2,70E-02
Unidentified transcripts on BTA28 position 34687548-34688131	-1,98	1,51E-02
PREDICTED: Bos taurus similar to ribosomal protein L37 (LOC616464)	-2,15	3,84E-02
Bos taurus fatty acid synthase (FASN)	-1,56	1,11E-02
Bos taurus similar to Homo sapiens vesicle-associated membrane protein 5 (myobrevin) (VAMP5)	-1,73	3,58E-02
Bos taurus similar to Homo sapiens chromosome 9 open reading frame 98 (C9orf98)	-1,64	9,45E-03

Bos taurus similar to Homo sapiens pleckstrin homology domain containing, family H (with MyTH4 domain) member 3 (PLEKHH3)	-1,54	3,52E-02
Bos taurus similar to Homo sapiens indoleamine-pyrrole 2,3 dioxygenase (INDO)	-1,78	2,18E-02
Bos taurus similar to Homo sapiens C-type lectin domain family 14, member A (CLEC14A)	-2,04	1,87E-02
Bos taurus similar to Homo sapiens ribosomal protein S25 (RPS25)	-1,91	3,91E-02
Bos taurus similar to Homo sapiens heterogeneous nuclear ribonucleoprotein L (HNRPL), transcript variant 1	-1,61	1,06E-02
Bos taurus similar to Homo sapiens translocase of outer mitochondrial membrane 7 homolog (yeast) (TOMM7)	-1,51	1,88E-02
Bos taurus similar to Homo sapiens chromosome 11 open reading frame 67 (C11orf67)	-1,68	3,80E-02
Bos taurus similar to Homo sapiens ring finger protein 4 (RNF4)	-1,54	2,40E-02
Bos taurus similar to Homo sapiens ribosomal protein L17 (RPL17)	-1,79	3,02E-02
Bos taurus STK25 protein (STK25)	-1,54	1,19E-02
Bos taurus similar to Homo sapiens chromosome 11 open reading frame 31 (C11orf31)	-1,54	2,37E-02
Bos taurus similar to Homo sapiens janus kinase and microtubule interacting protein 1 (JAKMIP1)	-1,84	2,55E-02
Bos taurus similar to Homo sapiens HRAS-like suppressor 3 (HRASLS3)	-1,62	1,09E-02
Bos taurus similar to Homo sapiens chromosome X open reading frame 26 (CXorf26)	-1,51	3,11E-02
Bos taurus endozepine (putative ligand of benzodiazepine receptor) mRNA, complete cds	-1,58	2,78E-03
Bos taurus similar to Homo sapiens immediate early response 5-like (IER5L)	-1,68	1,54E-02
Bos taurus similar to Homo sapiens R3H domain containing 1 (R3HDM1)	-1,71	1,63E-02
Bos taurus similar to Homo sapiens leucine zipper protein 5 (LUZP5)	-1,60	2,09E-02
Bos taurus similar to Homo sapiens complement component 1, r subcomponent (C1R)	-1,62	3,53E-02
Bos taurus similar to Homo sapiens eukaryotic translation initiation factor 1A, Y-linked (EIF1AY)	-1,50	1,30E-02
Bos taurus similar to Homo sapiens cathepsin Z (CTSZ)	-1,76	1,92E-02
Bos taurus similar to Homo sapiens tudor domain containing 9 (TDRD9)	-2,68	1,11E-02
Bos taurus nucleoside phosphorylase (NP)	-1,68	8,79E-04
Bos taurus similar to Homo sapiens enhancer of rudimentary homolog (Drosophila) (ERH)	-1,51	5,91E-03
Bos taurus similar to Homo sapiens TNFAIP3 interacting protein 2 (TNIP2)	-1,54	2,05E-02
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 166 (C14orf166)	-1,61	5,36E-03
Bos taurus similar to Homo sapiens ribosomal protein L27a (RPL27A)	-1,98	3,45E-02
Bos taurus similar to Homo sapiens kinesin family member 1C (KIF1C)	-1,56	5,34E-03
PREDICTED: Bos taurus similar to interleukin 27 (LOC614927)	-1,55	2,62E-02
Bos taurus similar to Homo sapiens PALM2-AKAP2 protein (PALM2-AKAP2), transcript variant 1	-1,65	4,82E-02
Bos taurus similar to Homo sapiens peptidyl-tRNA hydrolase 2 (PTRH2), nuclear gene encoding mitochondrial protein, transcript variant 2	-1,58	4,03E-02
Bos taurus membrane cofactor protein (MCP)	-1,60	4,69E-02
Bos taurus similar to Homo sapiens calumenin (CALU)	-1,51	1,23E-02
Bos taurus similar to PREDICTED: Homo sapiens region containing F-box protein 10; chromosome 9 open reading frame 105, transcript variant 2 (LOC644560)	-1,54	2,95E-02
Bos taurus adrenergic, beta 3, receptor (ADRB3)	-4,65	2,82E-03
Bos taurus NADH dehydrogenase 1 beta subcomplex 8 (NDUFB8)	-1,54	4,33E-02
Bos taurus ribosomal protein L26 (RPL26)	-1,89	3,51E-02
Bos taurus allograft inflammatory factor 1 (AIF1)	-1,97	5,67E-03
Bos taurus similar to Homo sapiens zinc finger protein 696 (ZNF696)	-2,40	4,46E-03
Bos taurus similar to Homo sapiens similar to mouse 2310016A09Rik gene (LOC134147)	-1,53	9,85E-03
Bos taurus similar to Homo sapiens indoleamine-pyrrole 2,3 dioxygenase (INDO)	-1,73	2,42E-02
Unidentified transcripts on BTA17 position 45028945-45027762	-1,68	6,65E-03
Bos taurus similar to Homo sapiens ribosomal protein L34 (RPL34), transcript variant 2	-2,39	1,99E-02
Bos taurus fatty acid synthase (FASN)	-1,57	8,50E-03
Bos taurus similar to Homo sapiens ribosomal protein S15a (RPS15A), transcript variant 2	-2,06	4,19E-02
Bos taurus similar to Homo sapiens replication factor C (activator 1) 3, 38kDa (RFC3), transcript variant 1	-1,52	1,09E-02
Bos taurus similar to Homo sapiens TXK tyrosine kinase (TXK)	-1,86	6,19E-04
Bos taurus complement component 1, q subcomponent, alpha polypeptide (C1QA)	-2,90	1,23E-02
Bos taurus similar to Homo sapiens perforin 1 (pore forming protein) (PRF1)	-1,51	2,66E-02
Bos taurus similar to Homo sapiens kinetochore associated 2 (KNTC2)	-1,63	2,05E-02
Bos taurus similar to Homo sapiens phosphatidylglycerophosphate synthase (PGS1)	-2,45	4,42E-02
Bos taurus ribosomal protein L3 (Rpl3)	-1,71	3,03E-02
Bos taurus similar to Homo sapiens immediate early response 5-like (IER5L)	-1,65	1,52E-02
Bos taurus similar to Homo sapiens interleukin 20 receptor, alpha (IL20RA)	-1,63	4,93E-04
Bos taurus similar to Homo sapiens adaptor-related protein complex 1, sigma 1 subunit (AP1S1), transcript variant 1	-1,58	2,32E-02
Bos taurus similar to Homo sapiens janus kinase and microtubule interacting protein 1 (JAKMIP1)	-1,83	1,90E-02
Bos taurus endozepine (putative ligand of benzodiazepine receptor) mRNA, complete cds	-1,54	2,74E-03
Bos taurus similar to Homo sapiens transmembrane emp24 protein transport domain containing 1 (TMED1)	-1,52	1,68E-02

Bos taurus similar to Homo sapiens membrane-spanning 4-domains, subfamily A, member 7 (MS4A7), transcript variant 1	-2,25	1,06E-02
Unidentified transcripts	-1,70	2,82E-02
Bos taurus similar to Homo sapiens methylmalonyl CoA epimerase (MCEE)	-1,52	1,53E-02
Bos taurus similar to Homo sapiens chromosome 9 open reading frame 138 (C9orf138)	-1,67	4,63E-02
Bos taurus similar to Homo sapiens killer cell lectin-like receptor subfamily B, member 1 (KLRB1)	-1,90	5,80E-03
PREDICTED: Bos taurus similar to nasal embryonic LHRH factor, transcript variant 2 (LOC615854)	-1,55	4,45E-02
Bos taurus similar to Homo sapiens acid phosphatase 2, lysosomal (ACP2)	-1,53	2,41E-02
Bos taurus similar to Homo sapiens LSM2 homolog, U6 small nuclear RNA associated (S. cerevisiae) (LSM2)	-1,67	3,70E-02
Bos taurus similar to Homo sapiens transmembrane protein 106C (TMEM106C)	-1,70	1,79E-02
Bos taurus similar to Homo sapiens TNFAIP3 interacting protein 2 (TNIP2)	-1,54	2,10E-02
Bos taurus similar to Homo sapiens Epstein-Barr virus induced gene 3 (EBI3)	-1,61	1,59E-02
PREDICTED: Bos taurus similar to Calcium/calmodulin-dependent protein kinase type IV (CAM kinase-GR) (CaMK IV) (LOC537677), partial mRNA.	-1,62	4,95E-02
Bos taurus similar to Homo sapiens ribosomal protein S24 (RPS24), transcript variant 1	-1,85	2,53E-02
Bos taurus follistatin (FST)	-1,65	4,13E-02
Bos taurus similar to Homo sapiens ZW10, kinetochore associated, homolog (Drosophila) (ZW10)	-1,52	1,49E-03
Unidentified transcripts on BTA1 position 31857905-31858881	-1,68	4,86E-02
Bos taurus similar to Homo sapiens chromosome 7 open reading frame 30 (C7orf30)	-1,56	2,52E-02
Unidentified transcripts on BTA12 position 1633365-1634048	-1,56	1,63E-02
Bos taurus similar to Homo sapiens small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating) (SCYE1)	-1,53	2,95E-03
Bos taurus similar to Homo sapiens adaptor-related protein complex 1, sigma 1 subunit (AP1S1), transcript variant 1	-1,54	3,21E-02
Bos taurus neurolysin (EP24.16)	-1,73	2,15E-03
Bos taurus similar to Homo sapiens hypothetical protein FLJ11151 (FLJ11151)	-2,01	1,28E-02
Bos taurus similar to Homo sapiens ribosomal protein S25 (RPS25)	-1,76	4,97E-02
Bos taurus chemokine (C-C motif) ligand 3-like 1 (CCL3L1)	-1,56	2,44E-02
Unidentified transcripts	-2,13	1,95E-02
Bos taurus similar to Homo sapiens ribosomal protein L36a (RPL36A)	-1,92	2,28E-02
Bos taurus similar to Homo sapiens LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae) (LSM3)	-1,57	3,10E-02
Bos taurus nuclear receptor subfamily 1, group H, member 3 (NR1H3)	-1,98	1,81E-02
Bos taurus similar to Homo sapiens 24-dehydrocholesterol reductase (DHCR24)	-1,75	1,41E-02
Bos taurus NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase) (NDUFS4)	-1,93	4,88E-03
Bos taurus similar to Homo sapiens ribosomal protein L13a (RPL13A)	-1,71	1,15E-02
Bos taurus similar to Homo sapiens methylmalonyl CoA epimerase (MCEE)	-1,60	2,29E-02
Bos taurus similar to Homo sapiens cathepsin Z (CTSZ)	-1,75	2,05E-02
Unidentified transcripts	-1,64	3,67E-02
Bos taurus similar to Homo sapiens LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae) (LSM6)	-1,71	3,76E-04
Bos taurus similar to Homo sapiens sodium channel, voltage-gated, type I, beta (SCN1B), transcript variant a	-1,75	2,07E-02
Bos taurus high-mobility group box 1 (HMGB1)	-1,56	1,95E-02
Bos taurus similar to Homo sapiens synaptotagmin XI (SYT11)	-1,67	3,57E-02
Bos taurus similar to Homo sapiens checkpoint suppressor 1 (CHES1)	-2,32	1,24E-02
Bos taurus similar to Homo sapiens guanylate binding protein 1, interferon-inducible, 67kDa (GBP1)	-1,83	2,93E-02
Bos taurus similar to Homo sapiens TBC1 domain family, member 8 (with GRAM domain) (TBC1D8)	-1,50	1,74E-02
Bos taurus similar to Homo sapiens unc-119 homolog (C. elegans) (UNC119), transcript variant 1	-1,52	2,08E-02
Bos taurus adrenergic, beta 3, receptor (ADRB3)	-4,36	2,75E-03
Bos taurus similar to Homo sapiens checkpoint suppressor 1 (CHES1)	-2,71	1,08E-03
Bos taurus eukaryotic translation elongation factor 1 beta 2 (EEF1B2)	-1,59	4,45E-02
Bos taurus similar to Homo sapiens tetraspanin 33 (TSPAN33)	-1,83	4,27E-03
Bos taurus similar to Homo sapiens dual specificity phosphatase 7 (DUSP7)	-1,55	1,04E-02
PREDICTED: Bos taurus pyrophosphatase (inorganic), transcript variant 1 (PP)	-1,59	1,42E-02
Bos taurus similar to Homo sapiens inhibitor of DNA binding 1, dominant negative helix-loop-helix protein (ID1), transcript variant 1	-3,44	1,07E-02
Bos taurus similar to Homo sapiens proteasome (prosome, macropain) subunit, alpha type, 4 (PSMA4)	-1,62	2,11E-02
Bos taurus similar to Homo sapiens coiled-coil-helix-coiled-coil-helix domain containing 1 (CHCHD1)	-1,60	4,76E-02
PREDICTED: Bos taurus similar to sarcalumenin (LOC513912)	-2,22	3,63E-03
Bos taurus similar to Homo sapiens C-type lectin domain family 14, member A (CLEC14A)	-1,93	1,49E-03
Bos taurus ribosomal protein L26 (RPL26)	-1,89	4,05E-02
Bos taurus similar to Homo sapiens geminin, DNA replication inhibitor (GMNN)	-1,52	4,21E-02

Bos taurus similar to Homo sapiens catenin (cadherin-associated protein), alpha-like 1 (CTNNAL1)	-1,93	1,52E-04
Unidentified transcripts	-1,64	1,85E-02
Bos taurus similar to Homo sapiens nuclear cap binding protein subunit 2, 20kDa (NCBP2), transcript variant 1	-1,53	3,26E-02
Bos taurus similar to Homo sapiens complement component 1, r subcomponent (C1R)	-1,57	4,01E-02
Bos taurus similar to Homo sapiens zinc finger protein 696 (ZNF696)	-2,64	1,28E-03
Bos taurus similar to Homo sapiens inhibitor of DNA binding 1, dominant negative helix-loop-helix protein (ID1), transcript variant 1	-3,19	1,69E-02
Bos taurus similar to Homo sapiens solute carrier family 2 (facilitated glucose transporter), member 6 (SLC2A6)	-2,03	2,40E-03
Bos taurus similar to Homo sapiens FLJ43339 protein (FLJ43339)	-1,64	6,19E-03
Bos taurus similar to Homo sapiens hepatitis B virus x interacting protein (HBXIP)	-1,52	5,18E-04
Bos taurus Fc fragment of IgG, low affinity IIIa, receptor for (CD16) (FCGR3A)	-2,26	4,85E-02
Bos taurus nucleoside-diphosphate kinase NBR-A (NBR-A)	-1,53	3,75E-02
Bos taurus similar to Homo sapiens guanylate binding protein 1, interferon-inducible, 67kDa (GBP1)	-1,84	2,71E-02
Bos taurus similar to Homo sapiens transmembrane protein 106C (TMEM106C)	-1,54	1,24E-02
Unidentified transcripts	-1,59	4,31E-02
Bos taurus similar to Homo sapiens fer-1-like 3, myoferlin (C. elegans) (FER1L3), transcript variant 2	-1,55	2,55E-02
Bos taurus similar to SH2 domain protein 1A (MGC128725)	-1,55	4,71E-02
Bos taurus similar to Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32 family, member E (ANP32E)	-1,70	4,82E-02
Bos taurus similar to Homo sapiens R3H domain containing 1 (R3HDM1)	-1,51	7,37E-03
Bos taurus similar to Homo sapiens centromere protein B, 80kDa (CENPB)	-1,56	2,65E-02
Bos taurus similar to Homo sapiens complement component 2 (C2)	-2,16	4,06E-03
Bos taurus similar to Homo sapiens natural killer cell group 7 sequence (NKG7)	-1,94	4,17E-02
Bos taurus similar to Homo sapiens 24-dehydrocholesterol reductase (DHCR24)	-1,77	5,16E-03
Bos taurus similar to Homo sapiens complement component 1, q subcomponent, C chain (C1QC)	-2,63	1,26E-02
Bos taurus similar to Homo sapiens complement component 2 (C2)	-2,15	5,42E-03
Bos taurus similar to Homo sapiens THO complex 4 (THOC4)	-1,59	7,56E-03
Bos taurus similar to Homo sapiens carbohydrate kinase-like (CARKL)	-1,51	2,16E-02
Unidentified transcripts on BTA10 position 16405353-16406182	-1,83	3,97E-02
Bos taurus fatty acid synthase (FASN)	-1,52	1,57E-02
Bos taurus similar to Homo sapiens HRAS-like suppressor 3 (HRASLS3)	-1,63	1,07E-02
Bos taurus similar to Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32 family, member E (ANP32E)	-1,67	2,89E-02
Bos taurus NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa (NDUFB4)	-1,57	3,09E-02
Bos taurus similar to Homo sapiens heterogeneous nuclear ribonucleoprotein L (HNRPL), transcript variant 1	-1,67	5,85E-03
Bos taurus similar to Homo sapiens COX17 cytochrome c oxidase assembly homolog (S. cerevisiae) (COX17), nuclear gene encoding mitochondrial protein	-1,76	2,21E-02
Bos taurus similar to Homo sapiens solute carrier family 2 (facilitated glucose transporter), member 6 (SLC2A6)	-2,06	4,76E-04
Bos taurus similar to Homo sapiens ribosomal protein L13a (RPL13A)	-1,71	9,95E-03
Bos taurus similar to Homo sapiens La ribonucleoprotein domain family, member 7 (LARP7), transcript variant 1	-1,52	2,01E-02
Bos taurus acetylserotonin O-methyltransferase (ASMT)	-1,74	4,56E-02
Bos taurus similar to Homo sapiens asparagine synthetase (ASNS), transcript variant 3	-1,54	2,15E-02
Bos taurus proteasome (prosome, macropain) subunit, beta type, 10 (PSMB10)	-1,61	2,83E-02
Unidentified transcripts on BTA16 position 10941576-10940643	-2,01	1,67E-04
Bos taurus complement component 1, q subcomponent, alpha polypeptide (C1QA)	-2,86	1,34E-02
Bos taurus similar to Homo sapiens G protein-coupled receptor 171 (GPR171)	-1,62	4,42E-02
Bos taurus similar to Homo sapiens GTP cyclohydrolase I feedback regulator (GCHFR)	-1,53	3,22E-02
Bos taurus similar to Homo sapiens proteasome maturation protein (POMP)	-1,52	9,42E-04
Bos taurus high-mobility group box 1 (HMGB1)	-1,58	5,11E-03
Bos taurus similar to Homo sapiens ubiquinol-cytochrome c reductase hinge protein (UQCRH)	-1,59	2,01E-03
Bos taurus similar to Homo sapiens amino-terminal enhancer of split (AES), transcript variant 2	-1,75	7,59E-03
Bos taurus mRNA decapping enzyme (DCPS)	-1,54	7,69E-03
Unidentified transcripts on BTA12 position 14880498-14882169	-1,51	2,79E-02
Bos taurus ribosomal protein L10 (RPL10)	-1,72	2,53E-02
PREDICTED: Bos taurus similar to sarcalumenin (LOC513912)		4,26E-03
Bos taurus aldehyde dehydrogenase 1 family, member A1 (ALDH1A1)		3,98E-02
Bos taurus 7-dehydrocholesterol reductase (DHCR7)		9,31E-03
Bos taurus similar to Homo sapiens myosin IG (MYO1G)		4,02E-02
Bos taurus heme oxygenase (decyclizing) 1 (HMOX1)		3,22E-03
Bos taurus similar to Homo sapiens complement component 1, q subcomponent, B chain (C1QB)		6,95E-03
Bos taurus similar to PREDICTED: Homo sapiens hypothetical LOC388789 (LOC388789)		2,48E-02

Bos taurus ribosomal protein L3 (Rpl3)	2,78E-02
Unidentified transcripts	1,22E-02
Bos taurus similar to Homo sapiens COX17 cytochrome c oxidase assembly homolog (S. cerevisiae) (COX17), nuclear gene encoding mitochondrial protein	3,62E-02
Bos taurus similar to Homo sapiens CD83 molecule (CD83), transcript variant 1	3,31E-03
Bos taurus chemokine (C-C motif) ligand 5 (CCL5)	4,38E-02
Bos taurus aldehyde dehydrogenase 1 family, member A1 (ALDH1A1)	3,16E-02
Bos taurus similar to Homo sapiens ribosomal protein S24 (RPS24), transcript variant 1	3,05E-02
Bos taurus similar to Homo sapiens inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon (IKBKE)	1,16E-02
Bos taurus similar to Homo sapiens glioma tumor suppressor candidate region gene 2 (GLTSCR2)	3,44E-02
Bos taurus similar to Homo sapiens zinc finger protein 205 (ZNF205), transcript variant 1	2,67E-02
Unidentified transcripts	3,00E-02
Bos taurus similar to Homo sapiens ribosomal protein S14 (RPS14), transcript variant 3	4,83E-02
Bos taurus similar to Homo sapiens LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae) (LSM6)	5,00E-04

3: Regulated transcripts in DHEA exposed animals versus control Group 2.

Upregulated transcripts.

Transcript description	Fold-change	Significance (p-value)
Bos taurus similar to Homo sapiens arylsulfatase A (ARSA)	1,54	2,36E-03
Bos taurus similar to Homo sapiens macrophage receptor with collagenous structure (MARCO)	1,79	3,27E-02
Bos taurus similar to Homo sapiens phospholipase D family, member 4 (PLD4)	1,70	1,07E-02
Bos taurus similar to Homo sapiens cysteine and glycine-rich protein 1 (CSRP1)	1,53	3,96E-02
Unidentified transcripts on BTA27 position 20976875-20977773	1,60	4,13E-02
Bos taurus similar to Homo sapiens complement component 1, q subcomponent, C chain (C1QC)	2,08	1,89E-02
Bos taurus similar to Homo sapiens discs, large (Drosophila) homolog-associated protein 4 (DLGAP4), transcript variant 2	1,60	9,20E-05
Bos taurus vascular cell adhesion molecule 1 (VCAM1)	1,75	1,93E-02
Bos taurus similar to Homo sapiens peptidyl arginine deiminase, type III (PADI3)	1,66	1,41E-02
Unidentified transcripts on BTA3 position 83299919-83298818	1,59	7,06E-03
Bos taurus similar to Homo sapiens RAS protein activator like 2 (RASAL2), transcript variant 2	1,60	2,82E-02
Bos taurus alkaline phosphatase, liver/bone/kidney (ALPL)	1,91	7,74E-03
PREDICTED: Bos taurus similar to thymic stromal lymphopoietin isoform 1 (LOC617637)	1,77	4,79E-02
Bos taurus similar to Homo sapiens Kruppel-like factor 13 (KLF13)	1,60	8,84E-03
PREDICTED: Bos taurus similar to tumor stroma and activated macrophage protein DLM-1 (LOC508333)	2,56	4,89E-02
Bos taurus similar to Homo sapiens potassium voltage-gated channel, subfamily H (eag-related), member 3 (KCNH3)	3,34	7,62E-03
Bos taurus similar to Homo sapiens cysteine-rich protein 2 (CRIP2)	1,67	7,13E-03
Bos taurus similar to Homo sapiens dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenzyme A isomerase) (DCI), nuclear gene encoding mitochondrial protein	1,52	4,49E-02
Bos taurus major histocompatibility complex, class II, DM alpha-chain, expressed (BOLA-DMA)	1,55	3,40E-02
Bos taurus similar to Homo sapiens hypothetical protein MGC42174 (MGC42174)	1,77	9,85E-04
Bos taurus chemokine (C-X-C motif) ligand 2 (CXCL2)	1,66	3,37E-02
Bos taurus similar to Homo sapiens interferon, gamma-inducible protein 30 (IFI30)	1,86	2,99E-02
PREDICTED: Bos taurus similar to torsin family 2, member A (LOC618444)	1,60	1,39E-02
Bos taurus myxovirus (influenza) resistance 1, (murine homolog) (MX1)	3,20	2,96E-02
Bos taurus similar to Homo sapiens timeless homolog (Drosophila) (TIMELESS)	1,62	1,16E-02
Bos taurus similar to Homo sapiens DENN/MADD domain containing 2A (DENND2A)	1,75	2,45E-02
Bos taurus similar to Homo sapiens chromosome 21 open reading frame 62 (C21orf62)	1,52	9,46E-03
Unidentified transcripts on BTA19 position 6414965-6414376	2,48	4,11E-02
Bos taurus similar to Homo sapiens membrane-associated ring finger (C3HC4) 2 (MARCH2), transcript variant 1	1,68	2,56E-02
Bos taurus similar to Homo sapiens Cas-Br-M (murine) ecotropic retroviral transforming sequence b (CBLB)	1,99	4,65E-02
Bos taurus chemokine (C-X-C motif) ligand 2 (CXCL2)	1,71	8,14E-03
Bos taurus similar to Homo sapiens H1 histone family, member 0 (H1F0)	1,68	3,12E-02
Bos taurus solute carrier family 38, member 5 (SLC38A5)	2,65	2,07E-03
Unidentified transcripts	1,73	4,46E-02
Bos taurus similar to Homo sapiens BTB (POZ) domain containing 14A (BTBD14A)	1,61	2,51E-02
PREDICTED: Bos taurus similar to torsin family 2, member A (LOC618444)	1,55	1,19E-02
PREDICTED: Bos taurus similar to Ras-related protein M-Ras (Ras-related protein R-Ras3) (LOC522180)	1,76	2,43E-02
Bos taurus similar to Homo sapiens neurexophilin 3 (NXPH3)	1,84	4,86E-02
Bos taurus similar to Homo sapiens engulfment and cell motility 2 (ELMO2), transcript variant 1	1,77	3,72E-02
Bos taurus similar to Homo sapiens SLIT-ROBO Rho GTPase activating protein 1 (SRGAP1)	1,69	3,88E-02
Unidentified transcripts on BTA5 position 60071724-60072132	1,56	2,20E-02
Bos taurus alpha-1 acid glycoprotein (AGP)	1,86	4,37E-02
Bos taurus similar to Homo sapiens lectin, galactoside-binding, soluble, 3 binding protein (LGALS3BP)	1,84	1,61E-02
Bos taurus Fas-associated via death domain (FADD)	1,66	3,83E-03
Unidentified transcripts on BTA14 position 24818403-24817135	2,20	7,84E-03
Bos taurus similar to Homo sapiens isochorismatase domain containing 2 (ISOC2)	1,52	3,12E-02
Bos taurus retinal outer segment membrane protein 1 (ROM1)	1,54	2,80E-02
Bos taurus similar to Homo sapiens ubiquitin-conjugating enzyme E2L 6 (UBE2L6), transcript variant 1	2,01	3,03E-02
Bos taurus similar to Homo sapiens adenosine deaminase, RNA-specific, B1 (RED1 homolog rat) (ADARB1), transcript variant 1	1,60	1,95E-03
Bos taurus similar to Homo sapiens transmembrane protein 88 (TMEM88)	1,57	3,89E-02
Bos taurus similar to Homo sapiens Williams Beuren syndrome chromosome region 27 (WBSCR27)	4,34	1,14E-02

Bos taurus myosin, light polypeptide kinase (MYLK)	1,79	2,03E-02
Unidentified transcripts on BTA27 position 6402112-6401623	2,84	8,70E-03
Bos taurus mucosal addressin cellular adhesion molecule-1 (LOC527275)	1,52	8,76E-03
Bos taurus similar to Homo sapiens selenoprotein X, 1 (SEPX1)	2,57	1,08E-02
Bos taurus apoptosis-associated speck-like protein containing a CARD (ASC)	1,54	1,87E-02
Bos taurus similar to Homo sapiens zinc finger protein 710 (ZNF710)	1,86	3,60E-03
Bos taurus similar to Homo sapiens dachsous 1 (Drosophila) (DCHS1)	1,84	2,57E-03
Bos taurus similar to Homo sapiens interferon-induced protein 35 (IFI35)	1,75	2,23E-02
Bos taurus similar to Homo sapiens cysteine rich transmembrane BMP regulator 1 (chordin-like) (CRIM1)	1,62	1,72E-02
Bos taurus microfibrillar-associated protein 2 (MFAP2)	1,70	2,43E-02
Bos taurus upstream stimulatory factor 1 (USF1)	1,69	6,81E-04
Bos taurus similar to Homo sapiens breast carcinoma amplified sequence 3 (BCAS3)	1,70	1,60E-02
Bos taurus similar to Homo sapiens polymerase (DNA-directed), delta interacting protein 2 (POLDIP2)	1,52	8,38E-03
Bos taurus similar to Homo sapiens p21 (CDKN1A)-activated kinase 2 (PAK2)	1,55	6,88E-03
Bos taurus integrin alpha 2b (ITGA2B)	1,71	1,47E-02
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 43 (C14orf43)	1,61	4,04E-02
Bos taurus similar to Homo sapiens adenosine deaminase, RNA-specific, B1 (RED1 homolog rat) (ADARB1), transcript variant 1	1,58	2,61E-03
Bos taurus Fas-associated via death domain (FADD)	1,73	4,34E-03
Bos taurus profilin 1 (PFN1)	1,60	2,11E-02
Bos taurus similar to Homo sapiens golgi apparatus protein 1 (GLG1)	1,61	1,26E-02
Bos taurus similar to Homo sapiens immunoglobulin superfamily containing leucine-rich repeat 2 (ISLR2)	1,53	3,21E-02
Bos taurus similar to Homo sapiens complement component 3 (C3)	1,57	5,05E-03
Bos taurus similar to Homo sapiens transmembrane protein 153 (TMEM153)	1,67	2,23E-02
Bos taurus retinal outer segment membrane protein 1 (ROM1)	1,54	1,40E-02
Bos taurus similar to Homo sapiens leukocyte-derived arginine aminopeptidase (LRAP)	1,88	2,62E-02
Bos taurus similar to Homo sapiens LUC7-like (S. cerevisiae) (LUC7L), transcript variant 1	1,55	3,76E-02
Bos taurus similar to Homo sapiens complement component 1, r subcomponent-like (C1RL)	1,79	8,34E-03
Bos taurus similar to Homo sapiens cysteine and glycine-rich protein 1 (CSRP1)	1,54	3,60E-02
Bos taurus similar to Homo sapiens PWWP domain containing 2 (PWWP2)	1,56	3,45E-02
Bos taurus coiled-coil alpha-helical rod protein 1 (CCHCR1)	1,52	3,00E-02
Bos taurus similar to Homo sapiens complement component 1, q subcomponent, B chain (C1QB)	2,38	1,86E-02
Bos taurus similar to Homo sapiens CD300 molecule-like family member f (CD300LF)	1,52	4,92E-02
PREDICTED: Bos taurus similar to Zinc finger and BTB domain containing protein 34 (LOC518084)	1,72	2,04E-02
Bos taurus similar to Homo sapiens transcription elongation factor B (SIII), polypeptide 3 (110kDa, elongin A) (TCEB3)	1,68	8,15E-03
Bos taurus similar to Homo sapiens solute carrier family 29 (nucleoside transporters), member 2 (SLC29A2)	1,54	3,42E-02
Bos taurus similar to Homo sapiens arginyl aminopeptidase (aminopeptidase B)-like 1 (RNPEPL1)	1,56	3,38E-03
Bos taurus similar to Homo sapiens F-box and WD-40 domain protein 7 (archipelago homolog, Drosophila) (FBXW7), transcript variant 2	1,82	4,73E-02
PREDICTED: Bos taurus similar to ADP-ribosyltransferase 5 precursor (LOC618836)	1,97	4,23E-02
Bos taurus similar to Homo sapiens chromodomain helicase DNA binding protein 9 (CHD9)	1,60	3,98E-02
Bos taurus similar to Homo sapiens complement component 3 (C3)	1,60	8,19E-03
Bos taurus similar to Homo sapiens transmembrane protein 88 (TMEM88)	1,56	4,06E-02
Bos taurus similar to Homo sapiens myosin, light polypeptide 9, regulatory (MYL9), transcript variant 1	1,81	3,01E-02
Bos taurus similar to Homo sapiens triggering receptor expressed on myeloid cells-like 2 (TREML2)	1,68	1,42E-02
Bos taurus similar to Homo sapiens zinc finger protein 483 (ZNF483), transcript variant 1	1,57	3,17E-02
Bos taurus similar to Homo sapiens guanosine monophosphate reductase (GMPR)	1,57	3,79E-02
Bos taurus similar to Homo sapiens plexin B3 (PLXNB3)	1,66	2,07E-03
Bos taurus similar to Homo sapiens nebulin-related anchoring protein (NRAP), transcript variant 1	6,03	3,40E-02
Bos taurus similar to Homo sapiens phosphoglucomutase 5 (PGM5)	1,77	7,83E-04
Bos taurus similar to Homo sapiens complement factor B (CFB)	1,63	3,27E-02
Bos taurus similar to Homo sapiens cytochrome P450, family 2, subfamily J, polypeptide 2 (CYP2J2)	1,54	3,23E-02
Bos taurus similar to Homo sapiens breast carcinoma amplified sequence 3 (BCAS3)	1,70	1,93E-02
Bos taurus PDZ and LIM domain 7 (PDLIM7)	1,60	9,03E-03
PREDICTED: Bos taurus similar to CYFIP2, transcript variant 1 (LOC518833)	1,66	1,51E-02
PREDICTED: Bos taurus phosducin (PDC)	1,61	3,97E-02
Bos taurus NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase) (NDUFS8)	1,69	2,00E-02
PREDICTED: Bos taurus similar to epithelial cell transforming sequence 2 oncogene protein (LOC504746)	1,52	1,48E-02
Bos taurus similar to Homo sapiens short stature homeobox (SHOX), transcript variant SHOXb	1,52	2,81E-02
Bos taurus similar to Homo sapiens timeless homolog (Drosophila) (TIMELESS)	1,55	9,98E-03

Bos taurus similar to Homo sapiens meteorin, glial cell differentiation regulator (METRN)	1,57	3,09E-02
Bos taurus similar to Homo sapiens chromosome 1 open reading frame 24 (C1orf24), transcript variant 2	1,54	4,92E-02
Bos taurus similar to Homo sapiens 1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta) (AGPAT2), transcript variant 1	1,83	3,76E-02
PREDICTED: Bos taurus similar to CYFIP2, transcript variant 1 (LOC518833)	1,64	3,15E-02
Bos taurus similar to Homo sapiens arginyl aminopeptidase (aminopeptidase B)-like 1 (RNPEPL1)	1,54	2,94E-03
PREDICTED: Bos taurus similar to mitochondrial hepatocellular carcinoma-downregulated carrier protein (LOC517425)	1,53	2,26E-02
Bos taurus similar to Homo sapiens fibroblast growth factor (acidic) intracellular binding protein (FIBP), transcript variant 2	2,94	4,58E-03
Bos taurus similar to Homo sapiens transmembrane protein 153 (TMEM153)	1,68	2,80E-02
Bos taurus BCL2-associated X protein (BAX)	1,60	1,74E-02
Bos taurus similar to Homo sapiens 1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta) (AGPAT2), transcript variant 1	1,79	4,04E-02
Bos taurus similar to Homo sapiens triggering receptor expressed on myeloid cells-like 2 (TREML2)	1,67	1,29E-02
Bos taurus similar to PREDICTED: Homo sapiens similar to hypothetical protein MGC21881, transcript variant 4 (LOC653444)	1,69	1,01E-02
Bos taurus similar to Homo sapiens S100 calcium binding protein A1 (S100A1)	1,89	1,86E-02
Bos taurus cartilage linking protein 1 (CRTL1)	1,56	1,83E-02
Region 3' of Bos taurus guanylate cyclase 1, soluble, alpha 3 (GUCY1A3)	1,74	2,52E-02
Bos taurus similar to Homo sapiens SERTA domain containing 1 (SERTAD1)	1,80	4,90E-02
PREDICTED: Bos taurus similar to guanine nucleotide binding protein (G protein), alpha 13 (LOC505611)	1,56	4,29E-02
Unidentified transcripts on BTA18 position 55465996-55465662	1,52	4,23E-02
Bos taurus similar to Homo sapiens poliovirus receptor-related 1 (herpesvirus entry mediator C; nectin) (PVRL1), transcript variant 1	1,82	6,32E-04
Bos taurus similar to Homo sapiens lymphocyte-specific protein 1 (LSP1), transcript variant 1	1,52	2,27E-02
Bos taurus alkaline phosphatase, liver/bone/kidney (ALPL)	1,81	1,63E-02
Bos taurus similar to Homo sapiens potassium voltage-gated channel, subfamily H (eag-related), member 3 (KCNH3)	3,29	7,38E-03
Bos taurus similar to Homo sapiens enolase 3 (beta, muscle) (ENO3), transcript variant 2	1,52	2,97E-02
Bos taurus PDZ and LIM domain 7 (PDLIM7)	1,59	9,40E-03
Bos taurus tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) (TIMP1)	1,60	2,93E-03
Bos taurus lysozyme 3 (LYZ3)	1,58	4,39E-02
Bos taurus similar to PREDICTED: Homo sapiens lupus brain antigen 1 (LBA1)	2,74	3,50E-02
Region 3' of Bos taurus guanylate cyclase 1, soluble, alpha 3 (GUCY1A3)	1,77	1,82E-02
Bos taurus AE binding protein 1 (AEBP1)	1,79	4,01E-02
Bos taurus similar to Homo sapiens putative neuronal cell adhesion molecule (PUNC)	1,83	1,65E-02
Bos taurus integrin alpha 2b (ITGA2B)	1,66	8,67E-03
Bos taurus actin, beta (ACTB)	1,63	2,95E-03
Bos taurus microfibrillar-associated protein 2 (MFAP2)	1,78	8,92E-03
Bos taurus similar to Homo sapiens solute carrier family 44, member 4 (SLC44A4), transcript variant 1	1,55	4,06E-02
Bos taurus similar to Homo sapiens enolase 3 (beta, muscle) (ENO3), transcript variant 2	1,52	4,74E-02
Bos taurus similar to Homo sapiens poliovirus receptor-related 1 (herpesvirus entry mediator C; nectin) (PVRL1), transcript variant 1	1,83	5,52E-04
Bos taurus similar to hypothetical protein MGC13102 (MGC13102)	1,57	5,73E-03
Bos taurus similar to Homo sapiens dipeptidyl-peptidase 7 (DPP7)	1,53	3,19E-03
Bos taurus similar to Homo sapiens growth differentiation factor 1 (GDF1)	1,58	4,47E-02
Bos taurus similar to hypothetical protein MGC13102 (MGC13102)	1,56	1,40E-03
Bos taurus similar to Homo sapiens glucosidase, alpha; neutral AB (GANAB), transcript variant 2	1,61	8,64E-03
Bos taurus similar to Homo sapiens oxoeicosanoid (OXE) receptor 1 (OXER1)	1,52	8,30E-04
Bos taurus similar to Homo sapiens nebulin-related anchoring protein (NRAP), transcript variant 1	5,97	3,45E-02
Bos taurus similar to Homo sapiens complement component 1, r subcomponent-like (C1RL)	1,75	1,11E-02
Bos taurus solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2 (SLC3A2)	1,58	3,67E-04
Bos taurus AE binding protein 1 (AEBP1)	1,88	2,41E-02
Bos taurus similar to Homo sapiens KIAA0664 (KIAA0664)	1,62	2,19E-02
Bos taurus similar to Homo sapiens ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8)	1,74	3,43E-03
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 43 (C14orf43)	1,71	2,84E-02
PREDICTED: Bos taurus similar to Zinc finger and BTB domain containing protein 4 (KAISO-like zinc finger protein 1) (KAISO-L1) (LOC613607)	1,56	3,34E-02
Bos taurus similar to Homo sapiens E1A binding protein p400 (EP400)	1,62	4,23E-02
Bos taurus similar to Homo sapiens hypothetical protein FLJ14346 (FLJ14346)	1,58	4,58E-02

Bos taurus bactericidal/permeability-increasing protein (BPI)	3,20	2,24E-02
Bos taurus similar to Homo sapiens cathepsin Z (CTSZ)	1,52	8,24E-03
Bos taurus similar to Homo sapiens carbonyl reductase 1 (CBR1)	1,54	4,74E-02
PREDICTED: Bos taurus similar to zinc finger, DHHC domain containing 3 (LOC539973)	1,75	4,17E-04
Bos taurus similar to Homo sapiens epsin 1 (EPN1)	1,63	4,20E-02
Bos taurus similar to Homo sapiens unknown protein LOC51035 (LOC51035)	1,58	1,20E-02
Bos taurus similar to Homo sapiens dipeptidyl-peptidase 7 (DPP7)	1,54	2,84E-03
Bos taurus similar to Homo sapiens SERTA domain containing 1 (SERTAD1)	1,87	4,17E-02
Bos taurus adrenergic, beta 3, receptor (ADRB3)	2,77	1,19E-02
Bos taurus similar to Homo sapiens msh homeobox homolog 2 (Drosophila) (MSX2)	1,53	3,42E-02
Bos taurus profilin 1 (PFN1)	1,65	1,70E-02
Bos taurus similar to Homo sapiens keratin 35 (KRT35)	1,67	1,83E-04
Bos taurus bactericidal/permeability-increasing protein (BPI)	2,93	3,04E-02
Bos taurus peroxiredoxin 5 (PRDX5)	1,58	1,07E-03
Bos taurus pancreatic trypsin inhibitor (PTI)	2,02	2,16E-02
PREDICTED: Bos taurus similar to ADP-ribosyltransferase 5 precursor (LOC618836)	1,85	4,54E-02
Bos taurus CD79A antigen (immunoglobulin-associated alpha) (CD79A)	1,66	4,28E-02
Bos taurus similar to Homo sapiens damage-specific DNA binding protein 1, 127kDa (DDB1)	1,57	3,23E-02
Bos taurus similar to Homo sapiens Kruppel-like factor 13 (KLF13)	1,71	1,47E-02
Bos taurus complement component 1, q subcomponent, alpha polypeptide (C1QA)	2,54	1,57E-02
Bos taurus similar to Homo sapiens myosin, light polypeptide 9, regulatory (MYL9), transcript variant 1	1,84	3,55E-02
Bos taurus similar to Homo sapiens ATP-binding cassette, sub-family A (ABC1), member 12 (ABCA12), transcript variant 1	1,52	8,33E-03
Bos taurus similar to Homo sapiens zinc finger protein 575 (ZNF575)	1,56	3,03E-02
PREDICTED: Bos taurus similar to Musashi homolog 2, transcript variant 3 (LOC505542)	1,79	3,56E-02
Bos taurus cyclin-dependent kinase 5, regulatory subunit 1 (p35) (CDK5R1)	1,83	1,39E-02
Bos taurus similar to Homo sapiens transmembrane protein 92 (TMEM92)	1,58	3,19E-02
Unidentified transcripts on BTA25 position 31093369-31094201	2,72	1,94E-03
PREDICTED: Bos taurus similar to Butyrophilin precursor (BT) (Butyrophilin subfamily 1 member A1) (LOC615257)	1,52	2,04E-02
Bos taurus similar to Homo sapiens amyloid beta (A4) precursor-like protein 2 (APLP2)	1,52	1,55E-02
Bos taurus similar to Homo sapiens KIAA1324 (KIAA1324)	1,56	1,22E-02
Bos taurus similar to Homo sapiens Ran GTPase activating protein 1 (RANGAP1)	1,65	1,43E-02
Bos taurus similar to Homo sapiens insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3)	2,14	4,04E-02
Unidentified transcripts	1,69	3,43E-02
Bos taurus solute carrier family 38, member 5 (SLC38A5)	2,94	4,77E-03
Bos taurus similar to Homo sapiens polymerase (DNA-directed), delta interacting protein 2 (POLDIP2)	1,53	2,07E-02
Bos taurus similar to PREDICTED: Homo sapiens hypothetical protein LOC146517 (LOC146517)	1,55	3,01E-02
Bos taurus similar to Homo sapiens lymphocyte antigen 86 (LY86)	1,56	1,77E-02
Bos taurus similar to Homo sapiens damage-specific DNA binding protein 1, 127kDa (DDB1)	1,59	3,26E-02
Bos taurus similar to Homo sapiens lymphocyte antigen 86 (LY86)	1,54	2,12E-02
Bos taurus similar to Homo sapiens keratin 35 (KRT35)	1,63	5,93E-04
Bos taurus similar to Homo sapiens olfactory receptor, family 4, subfamily D, member 10 (OR4D10)	1,52	2,02E-02
Bos taurus clusterin (CLU)	1,96	3,16E-02
Bos taurus similar to Homo sapiens KIAA0664 (KIAA0664)	1,60	2,21E-02
Bos taurus cytochrome b-245, alpha polypeptide (CYBA)	1,54	1,98E-02
Bos taurus similar to Homo sapiens protocadherin 17 (PCDH17)	1,52	2,25E-02
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 172 (C14orf172)	1,53	2,65E-02
Unidentified transcripts on BTA5 position 69203747-69204483	2,26	4,01E-02
Bos taurus similar to Homo sapiens S100 calcium binding protein A1 (S100A1)	1,90	2,05E-02
Bos taurus similar to Homo sapiens eukaryotic elongation factor, selenocysteine-tRNA-specific (EEFSEC)	1,56	3,95E-02
PREDICTED: Bos taurus similar to nanos homolog 3 (LOC523375)	4,16	2,26E-02
Bos taurus similar to PREDICTED: Homo sapiens lupus brain antigen 1 (LBA1)	2,55	3,20E-02
PREDICTED: Bos taurus similar to double C2-like domains, alpha (LOC528120)	1,52	2,71E-02
PREDICTED: Bos taurus similar to Cbl-interacting protein Sts-1, transcript variant 3 (LOC523809)	1,65	1,92E-02
Bos taurus similar to Homo sapiens von Willebrand factor (VWF)	2,28	2,04E-02
Bos taurus similar to Homo sapiens phosphoglucomutase 5 (PGM5)	1,72	2,69E-03
Bos taurus similar to Homo sapiens eukaryotic elongation factor, selenocysteine-tRNA-specific (EEFSEC)	1,56	4,31E-02
Bos taurus similar to Homo sapiens leukocyte-derived arginine aminopeptidase (LRAP)	1,85	2,24E-02
Bos taurus similar to Homo sapiens cathepsin Z (CTSZ)	1,53	7,54E-03
Bos taurus similar to Homo sapiens rhomboid domain containing 3 (RHBDD3)	1,52	8,12E-03
Bos taurus similar to Homo sapiens leucine-rich repeat-containing G protein-coupled receptor 6 (LGR6), transcript variant 2	1,60	3,07E-02
PREDICTED: Bos taurus similar to Ornithine decarboxylase antizyme 3 (ODC-Az 3) (AZ3) (LOC531865)	1,52	4,97E-02

Bos taurus similar to Homo sapiens aminolevulinate, delta-, synthase 1 (ALAS1), transcript variant 2	1,53	4,44E-02
Bos taurus similar to Homo sapiens chromosome 6 open reading frame 149 (C6orf149)	1,53	3,58E-02
Bos taurus similar to Homo sapiens DENN/MADD domain containing 2A (DENND2A)	1,73	2,12E-02
Bos taurus similar to Homo sapiens dachsous 1 (Drosophila) (DCHS1)	1,92	1,14E-03
Bos taurus myosin, light polypeptide kinase (MYLK)	1,78	2,66E-02
Bos taurus TRAF3 interacting protein 3 (TRAF3IP3)	1,55	2,83E-02
Bos taurus similar to Homo sapiens growth arrest-specific 7 (GAS7), transcript variant a	1,52	3,52E-02
Bos taurus clusterin (CLU)	2,08	3,17E-02
Bos taurus similar to Homo sapiens mitogen-activated protein-binding protein-interacting protein (MAPBPIP)	1,62	2,95E-02
Bos taurus similar to Homo sapiens KIAA0523 protein (KIAA0523)	1,77	2,26E-02
Bos taurus similar to Homo sapiens erythrocyte membrane protein band 4.9 (dematin) (EPB49)	1,59	2,19E-02
PREDICTED: Bos taurus similar to Zinc finger and BTB domain containing protein 34 (LOC518084)	1,71	2,50E-02
Bos taurus similar to Homo sapiens chromosome 1 open reading frame 21 (C1orf21)	1,56	1,35E-03
Bos taurus adrenergic, beta 3, receptor (ADRB3)	2,66	1,12E-02
Bos taurus similar to Homo sapiens fibroblast growth factor (acidic) intracellular binding protein (FIBP), transcript variant 2	2,98	5,88E-03
Bos taurus similar to Homo sapiens plasticity related gene 3 (PRG-3), transcript variant 2	1,53	1,74E-02
Bos taurus similar to Homo sapiens docking protein 2, 56kDa (DOK2)	1,68	4,33E-02
Bos taurus similar to Homo sapiens selenoprotein X, 1 (SEPX1)	2,59	7,97E-03
Bos taurus similar to Homo sapiens CD300 molecule-like family member f (CD300LF)	1,57	2,96E-02
Bos taurus similar to Homo sapiens zinc finger, DHHC-type containing 3 (ZDHHC3)	1,59	1,13E-02
PREDICTED: Bos taurus similar to Rho-GTPase-activating protein 6 (Rho-type GTPase-activating protein RhoGAPX-1) (LOC522082), partial mRNA.	1,63	4,16E-02
Bos taurus similar to Homo sapiens colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog (CSF1R)	1,52	2,90E-02
Unidentified transcripts	1,53	2,70E-02
Bos taurus similar to Homo sapiens Mov10, Moloney leukemia virus 10, homolog (mouse) (MOV10)	1,75	2,48E-02
Bos taurus similar to Homo sapiens ADP-ribosyltransferase 5 (ART5)	2,41	4,33E-02
PREDICTED: Bos taurus similar to zinc finger, DHHC domain containing 3 (LOC539973)	1,85	3,50E-03
Bos taurus similar to Homo sapiens epsin 1 (EPN1)	1,66	4,01E-02
Bos taurus similar to Homo sapiens interferon, gamma-inducible protein 30 (IFI30)	1,87	4,84E-02
Bos taurus similar to Homo sapiens arylsulfatase A (ARSA)	1,53	4,61E-03
PREDICTED: Bos taurus similar to protein tyrosine phosphatase, receptor type, T (LOC516749)	1,64	6,90E-05
Unidentified transcripts on BTA21 position 1960345-1959112	2,06	2,36E-03
Bos taurus trophinin associated protein (tastin) (TROAP)	1,52	2,51E-02
PREDICTED: Bos taurus similar to Cbl-interacting protein Sts-1, transcript variant 3 (LOC523809)	1,61	3,03E-02
Bos taurus myxovirus (influenza) resistance 1, (murine homolog) (MX1)	3,18	2,87E-02
Bos taurus similar to Homo sapiens solute carrier family 44, member 4 (SLC44A4), transcript variant 1	1,59	4,31E-02
Bos taurus similar to Homo sapiens hypothetical protein MGC42174 (MGC42174)	1,77	8,52E-04
Bos taurus similar to Homo sapiens SH3 domain binding glutamic acid-rich protein like 3 (SH3BGRL3)	1,52	7,28E-03
Bos taurus similar to Homo sapiens glucosidase, alpha; neutral AB (GANAB), transcript variant 2	1,59	1,61E-02
Bos taurus apoptosis-associated speck-like protein containing a CARD (ASC)	1,53	1,78E-02
Bos taurus similar to Homo sapiens SMAD, mothers against DPP homolog 9 (Drosophila) (SMAD9)	1,63	1,46E-02
Bos taurus similar to Homo sapiens potassium channel tetramerisation domain containing 11 (KCTD11)	1,53	1,24E-03
Bos taurus similar to Homo sapiens Thy-1 cell surface antigen (THY1)	1,71	1,30E-02
Bos taurus similar to Homo sapiens recombining binding protein suppressor of hairless (Drosophila) (RBPSUH), transcript variant 2	1,86	8,34E-04
Bos taurus similar to Homo sapiens zinc finger, DHHC-type containing 3 (ZDHHC3)	1,60	1,22E-02
Unidentified transcripts on BTA6 position 69133663-69134794	1,55	6,48E-03
PREDICTED: Bos taurus similar to nanos homolog 3 (LOC523375)	3,63	1,88E-02
Bos taurus advanced glycosylation end product-specific receptor (AGER)	1,69	1,35E-03
Unidentified transcripts on BTA3 position 83299919-83298818	1,67	7,77E-03
Bos taurus similar to Homo sapiens potassium channel, subfamily K, member 9 (KCNK9)	1,58	4,07E-02
Bos taurus TRAF3 interacting protein 3 (TRAF3IP3)	1,56	3,02E-02
Bos taurus upstream stimulatory factor 1 (USF1)	1,65	3,16E-04
Bos taurus similar to Homo sapiens complement component 1, r subcomponent (C1R)	1,54	2,82E-02
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 172 (C14orf172)	1,55	3,22E-02
Bos taurus similar to Homo sapiens neuropeptide FF receptor 1 (NPFFR1)	1,55	3,82E-02
Bos taurus tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) (TIMP1)	1,67	3,36E-03
Bos taurus similar to Homo sapiens Ran GTPase activating protein 1 (RANGAP1)	1,69	1,52E-02
Bos taurus similar to Homo sapiens solute carrier family 29 (nucleoside transporters), member 2 (SLC29A2)	1,54	3,33E-02

Bos taurus similar to Homo sapiens cysteine rich transmembrane BMP regulator 1 (chordin-like) (CRIM1)	1,68	1,52E-02
Unidentified transcripts on BTA29 position 44827758-44828236	1,65	3,31E-02
Bos taurus similar to Homo sapiens Mov10, Moloney leukemia virus 10, homolog (mouse) (MOV10)	1,74	3,97E-02
Bos taurus cytochrome b-245, alpha polypeptide (CYBA)	1,56	1,75E-02
Bos taurus similar to Homo sapiens rhomboid domain containing 3 (RHBDD3)	1,54	9,01E-03
Bos taurus similar to Homo sapiens 1-acylglycerol-3-phosphate O-acyltransferase 6 (lysophosphatidic acid acyltransferase, zeta) (AGPAT6)	1,53	1,32E-03
Bos taurus similar to Homo sapiens RNA binding motif protein 19 (RBM19)	1,52	2,46E-02
Bos taurus similar to Homo sapiens abhydrolase domain containing 12 (ABHD12), transcript variant 2	1,87	2,02E-03
Bos taurus similar to Homo sapiens ADP-ribosyltransferase 5 (ART5)	2,40	3,25E-02
Bos taurus similar to Homo sapiens Williams Beuren syndrome chromosome region 27 (WBSCR27)	4,53	9,82E-03
PREDICTED: Bos taurus similar to regulating synaptic membrane exocytosis 4 (LOC539592)	1,72	2,09E-03
Bos taurus similar to Homo sapiens cysteine-rich protein 2 (CRIP2)	1,72	6,13E-03
Bos taurus similar to Homo sapiens glutamate receptor, ionotropic, N-methyl D-asparate-associated protein 1 (glutamate binding) (GRINA), transcript variant 1	1,54	5,41E-03
Bos taurus solute carrier family 39 (zinc transporter), member 3 (SLC39A3)	1,59	3,27E-02
Bos taurus similar to Homo sapiens sorting nexin 8 (SNX8)	1,80	4,33E-02
Unidentified transcripts on BTA25 position 31093369-31094201	2,73	2,75E-03
Bos taurus similar to Homo sapiens BTB (POZ) domain containing 14A (BTBD14A)	1,72	1,91E-02
Bos taurus transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase) (TGM2)	1,56	1,25E-02
Bos taurus similar to Homo sapiens mitogen-activated protein kinase kinase kinase 11 (MAP3K11)	1,53	1,18E-04
PREDICTED: Bos taurus similar to germ cell-less (LOC618666)	1,56	8,75E-03
Bos taurus similar to Homo sapiens complement component 1, q subcomponent, C chain (C1QC)	2,23	9,47E-03
Bos taurus similar to Homo sapiens p21 (CDKN1A)-activated kinase 2 (PAK2)	1,58	7,97E-03
Bos taurus HYAL2 protein (HYAL2)	1,52	1,55E-02
PREDICTED: Bos taurus trophoblast Kunitz domain protein 5 (TKDP5)	1,55	3,61E-02
Bos taurus similar to Homo sapiens BAI1-associated protein 2-like 2 (BAIAP2L2)	1,65	2,87E-02
Bos taurus similar to Homo sapiens lymphocyte-activation gene 3 (LAG3)	1,60	4,18E-02
Bos taurus similar to Homo sapiens mitogen-activated protein-binding protein-interacting protein (MAPBPIP)	1,66	2,21E-02
Unidentified transcripts on BTA17 position 44885452-44884801	1,61	9,75E-04
Bos taurus similar to Homo sapiens ubiquitin-conjugating enzyme E2L 6 (UBE2L6), transcript variant 1	2,14	2,47E-02
Unidentified transcripts on BTA5 position 69203747-69204483	2,38	3,28E-02
Bos taurus Rab geranylgeranyltransferase, alpha subunit (RABGGTA)	1,60	8,08E-04
Unidentified transcripts on BTA27 position 6402112-6401623	2,92	7,98E-03
Bos taurus cyclin-dependent kinase 5, regulatory subunit 1 (p35) (CDK5R1)	1,86	2,16E-03
Bos taurus similar to Homo sapiens phospholipase D family, member 4 (PLD4)	1,70	1,28E-02
Bos taurus similar to Homo sapiens transcription elongation factor B (SIII), polypeptide 3 (110kDa, elongin A) (TCEB3)	1,67	6,12E-03
Bos taurus similar to Homo sapiens insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3)	2,12	2,71E-02
Bos taurus similar to Homo sapiens chromodomain helicase DNA binding protein 9 (CHD9)	1,59	4,66E-02
Bos taurus similar to Homo sapiens macrophage receptor with collagenous structure (MARCO)	1,80	2,29E-02
Bos taurus proteasome (prosome, macropain) subunit, beta type, 10 (PSMB10)	1,53	2,41E-02
Bos taurus complement component 1, q subcomponent, alpha polypeptide (C1QA)	2,57	1,67E-02
Bos taurus advanced glycosylation end product-specific receptor (AGER)	1,64	1,72E-03
Bos taurus similar to Homo sapiens zinc finger protein 710 (ZNF710)	1,79	5,70E-03
Bos taurus lectin, galactoside-binding, soluble, 9 (galectin 9) (LGALS9), transcript variant 2	1,52	2,92E-02
Bos taurus similar to Homo sapiens ectonucleotide pyrophosphatase/phosphodiesterase 6 (ENPP6)	1,56	7,26E-04
Bos taurus similar to Homo sapiens BCL2-related ovarian killer (BOK)	1,68	1,49E-02
Bos taurus cAMP responsive element binding protein 3-like 3 (CREB3L3)	1,62	3,52E-02
Bos taurus similar to Homo sapiens putative neuronal cell adhesion molecule (PUNC)	1,67	7,58E-03
Bos taurus alpha-1 acid glycoprotein (AGP)	1,85	4,58E-02
Bos taurus similar to Homo sapiens nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 2 (NFKBIL2)	1,52	1,96E-02
Bos taurus ribosomal protein L10 (RPL10)	1,52	4,04E-02
Bos taurus similar to Homo sapiens E1A binding protein p400 (EP400)	1,59	4,70E-02
Bos taurus similar to Homo sapiens lectin, galactoside-binding, soluble, 3 binding protein (LGALS3BP)	1,78	1,26E-02
Bos taurus similar to Homo sapiens hyaluronan and proteoglycan link protein 4 (HAPLN4)	1,58	3,95E-02
Bos taurus similar to Homo sapiens interferon-induced protein 35 (IFI35)	1,69	3,99E-02
Bos taurus aldehyde oxidase 1 (AOX1)	3,12	4,74E-02
Bos taurus similar to Homo sapiens peptidyl arginine deiminase, type III (PADI3)	1,63	1,34E-02
Bos taurus similar to Homo sapiens RanBP-type and C3HC4-type zinc finger containing 1 (RBCK1), transcript variant 2	1,55	2,30E-03
Unidentified transcripts on BTA14 position 24818403-24817135	2,23	1,04E-02
Bos taurus pancreatic trypsin inhibitor (PTI)	2,03	1,92E-02

Bos taurus similar to Homo sapiens potassium voltage-gated channel, subfamily H (eag-related), member 4 (KCNH4)	1,55	3,15E-02
Bos taurus similar to Homo sapiens opiate receptor-like 1 (OPRL1), transcript variant 2	1,53	2,65E-04
Bos taurus similar to Homo sapiens abhydrolase domain containing 12 (ABHD12), transcript variant 2	1,88	3,77E-03
Bos taurus similar to Homo sapiens complement component 1, q subcomponent, B chain (C1QB)	2,43	1,43E-02
Bos taurus murine retrovirus integration site 1 homolog (MRVI1), transcript variant 1	1,52	4,50E-02
Bos taurus similar to Homo sapiens membrane-associated ring finger (C3HC4) 2 (MARCH2), transcript variant 1	1,70	1,73E-02
Bos taurus similar to Homo sapiens recombining binding protein suppressor of hairless (Drosophila) (RBPSUH), transcript variant 2	1,85	6,23E-04
Bos taurus similar to Homo sapiens von Willebrand factor (VWF)	2,26	2,10E-02
PREDICTED: Bos taurus similar to tumor stroma and activated macrophage protein DLM-1 (LOC508333)	2,55	4,20E-02
Bos taurus similar to Homo sapiens ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8)	1,71	1,94E-03
Bos taurus BCL2-associated X protein (BAX)	1,57	6,04E-03
Bos taurus POU domain, class 5, transcription factor 1 (POU5F1)	1,58	1,74E-02
Bos taurus similar to Homo sapiens alpha-kinase 3 (ALPK3)	1,67	3,04E-02
Bos taurus solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2 (SLC3A2)	1,60	1,04E-04
Bos taurus similar to Homo sapiens isochorismatase domain containing 2 (ISOC2)	1,54	4,61E-02
Bos taurus similar to PREDICTED: Homo sapiens pecanex-like 3 (Drosophila), transcript variant 1 (PCNXL3)	1,54	4,15E-03
Unidentified transcripts on BTA19 position 6414965-6414376	2,44	4,32E-02
Bos taurus vascular cell adhesion molecule 1 (VCAM1)	1,72	4,39E-02
Bos taurus similar to Homo sapiens hematopoietic SH2 domain containing (HSH2D)	1,64	4,79E-02
Bos taurus similar to Homo sapiens plexin B3 (PLXNB3)	1,59	1,01E-02
Bos taurus similar to Homo sapiens neurexophilin 3 (NXPH3)	1,93	2,13E-02
Bos taurus similar to Homo sapiens complement factor B (CFB)	1,76	2,05E-02
Bos taurus similar to Homo sapiens methyltransferase like 2A (METTL2A)	1,71	4,78E-02
Bos taurus similar to Homo sapiens Thy-1 cell surface antigen (THY1)	1,82	3,48E-02
Bos taurus similar to Homo sapiens solute carrier family 19 (folate transporter), member 1 (SLC19A1), transcript variant 2	1,60	4,75E-02
Bos taurus pregnancy-associated glycoprotein 7 (PAG7)	1,52	9,93E-03
Bos taurus similar to Homo sapiens RanBP-type and C3HC4-type zinc finger containing 1 (RBCK1), transcript variant 2	1,64	2,13E-03
Bos taurus similar to PREDICTED: Homo sapiens pecanex-like 3 (Drosophila), transcript variant 1 (PCNXL3)	1,55	2,84E-03
Bos taurus similar to Homo sapiens golgi apparatus protein 1 (GLG1)	1,62	1,59E-02
Bos taurus similar to Homo sapiens meteorin, glial cell differentiation regulator (METRN)	1,68	1,61E-02
Bos taurus lingual antimicrobial peptide (LAP)	1,85	1,86E-02
Bos taurus similar to Homo sapiens protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform (PPP2R1A)	1,58	6,52E-03
Bos taurus similar to Homo sapiens endoglin (Osler-Rendu-Weber syndrome 1) (ENG)	1,60	2,32E-02
Bos taurus similar to Homo sapiens BCL2-related ovarian killer (BOK)	1,77	1,27E-02
Unidentified transcripts on BTA21 position 1960345-1959112	2,19	1,58E-03
Bos taurus similar to Homo sapiens olfactory receptor, family 3, subfamily A, member 1 (OR3A1)	1,53	2,39E-03
Bos taurus similar to Homo sapiens KIAA0523 protein (KIAA0523)	1,78	1,34E-02

Downregulated transcripts,

Transcript description	Fold-change	Significance (p-value)
PREDICTED: Bos taurus similar to zona pellucida binding protein 2 isoform 2 (LOC514334)	-1,84	7,05E-03
Unidentified transcripts on BTA12 position 33236616-33235396	-1,78	3,64E-02
Unidentified transcripts on BTA23 position 30527455-30526185	-2,06	4,78E-02
Unidentified transcripts on BTA3 position 65338070-65335903	-1,59	4,66E-02
Bos taurus similar to Homo sapiens NGFI-A binding protein 1 (EGR1 binding protein 1) (NAB1)	-1,68	3,58E-02
PREDICTED: Bos taurus similar to Germ cell-less protein-like 1 (mGcl-1) (DP-interacting protein) (DIP) (LOC513934)	-1,76	3,13E-02
Unidentified transcripts on BTA4 position 22178234-22177508	-1,92	3,34E-02
Bos taurus similar to Homo sapiens KIAA1764 protein (KIAA1764)	-2,06	3,42E-02
Bos taurus similar to Homo sapiens pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1 (PLEKHA1), transcript variant 1	-1,72	3,09E-02
Bos taurus Sjogren syndrome antigen B (autoantigen La) (SSB)	-1,55	4,58E-02

Bos taurus stearoyl-coenzyme A desaturase (SCD)	-1,64	4,06E-02
Bos taurus similar to Homo sapiens hepatoma-derived growth factor, related protein 3 (HDGFRP3)	-1,52	4,68E-02
Bos taurus similar to Homo sapiens SNF2 histone linker PHD RING helicase (SHPRH)	-1,60	3,04E-02
PREDICTED: Bos taurus lipoprotein lipase, transcript variant 2 (LPL)	-1,79	4,98E-02
Bos taurus similar to Homo sapiens centrosomal protein 290kDa (CEP290)	-1,64	4,40E-02
Bos taurus similar to Homo sapiens KIAA1764 protein (KIAA1764)	-1,99	4,37E-02
Bos taurus similar to Homo sapiens churchill domain containing 1 (CHURC1)	-1,58	4,21E-02
Bos taurus similar to Homo sapiens hypothetical protein FLJ13611 (FLJ13611)	-1,52	4,72E-02
Unidentified transcripts on BTA16 position 55177278-55176043	-1,65	4,43E-02
Bos taurus similar to Homo sapiens centrosomal protein 290kDa (CEP290)	-1,52	4,56E-02
PREDICTED: Bos taurus similar to COMM domain containing protein 8 (LOC507292)	-1,63	4,25E-02
Bos taurus similar to Homo sapiens colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage) (CSF2RB)	-1,58	3,88E-02
Unidentified transcripts	-1,88	4,46E-02
PREDICTED: Bos taurus similar to splicing factor 3b, subunit 1 isoform 1, transcript variant 1 (LOC510177)	-1,51	4,44E-02
PREDICTED: Bos taurus similar to zinc finger protein 177 (LOC614473)	-1,52	2,14E-02
Bos taurus similar to PREDICTED: Homo sapiens hypothetical protein LOC116068 (LOC116068)	-1,99	4,62E-02
Bos taurus similar to Homo sapiens centrin, EF-hand protein, 3 (CDC31 homolog, yeast) (CETN3)	-1,88	3,24E-02
Bos taurus similar to Homo sapiens KIAA1799 protein (KIAA1799)	-1,56	1,46E-02
Bos taurus similar to Homo sapiens TatD DNase domain containing 1 (TATDN1)	-1,51	3,46E-02
Bos taurus similar to Homo sapiens small nuclear RNA activating complex, polypeptide 1, 43kDa (SNAPC1)	-1,71	4,40E-02
Bos taurus similar to Homo sapiens zinc finger protein 12 (ZNF12), transcript variant 1	-1,77	2,26E-02
Bos taurus stearoyl-coenzyme A desaturase (SCD)	-1,70	3,94E-02
Bos taurus dihydrolipoamide branched chain transacylase E2 (DBT)	-1,82	2,89E-02
Bos taurus similar to Homo sapiens zinc finger protein 711 (ZNF711)	-1,70	3,86E-02
Bos taurus similar to Homo sapiens epithelial cell transforming sequence 2 oncogene (ECT2)	-1,60	3,59E-02
Bos taurus similar to Homo sapiens WD repeat domain 51B (WDR51B)	-1,53	3,45E-02
Unidentified transcripts	-1,77	1,49E-02
Unidentified transcripts on BTA8 position 12524872-12525400	-1,66	1,48E-02
Bos taurus similar to Homo sapiens timeless-interacting protein (FLJ20516)	-1,64	3,51E-02
PREDICTED: Bos taurus similar to COMM domain containing protein 8 (LOC507292)	-1,75	3,07E-02
Unidentified transcripts on BTA18 position 53608650-53607745	-1,72	3,15E-02
Bos taurus similar to Homo sapiens putative homeodomain transcription factor 2 (PHTF2)	-1,84	3,66E-02
Unidentified transcripts	-1,56	4,75E-02
Bos taurus similar to Homo sapiens O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase) (OGT), transcript variant 2	-1,50	3,23E-02
Bos taurus RAP1A, member of RAS oncogene family (RAP1A)	-1,62	4,63E-02
PREDICTED: Bos taurus similar to zona pellucida binding protein 2 isoform 2 (LOC514334)	-1,94	7,47E-03
Bos taurus guanylate cyclase 1, soluble, beta 3 (GUCY1B3)	-1,94	4,61E-02
Unidentified transcripts on BTA1 position 2846963-2845909	-1,78	2,54E-02
Bos taurus similar to Homo sapiens NMD3 homolog (S. cerevisiae) (NMD3)	-1,51	3,02E-02
Bos taurus similar to Homo sapiens similar to metallo-beta-lactamase superfamily protein (LOC153364)	-1,53	1,20E-02
PREDICTED: Bos taurus similar to TATA box-binding protein-associated factor, RNA polymerase II, K, 18kD, transcript variant 1 (LOC513065)	-1,98	4,07E-02
Unidentified transcripts on BTA28 position 22915476-22914689	-1,89	3,32E-02
Bos taurus similar to Homo sapiens aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase) (ALDH5A1), nuclear gene encoding mitochondrial protein, transcript variant 2	-2,15	3,98E-03
Bos taurus similar to Homo sapiens tubulin, gamma complex associated protein 5 (TUBGCP5)	-1,70	5,66E-03
Bos taurus similar to Homo sapiens G protein-coupled receptor associated sorting protein 2 (GPRASP2), transcript variant 2	-1,72	6,81E-03
Bos taurus similar to Homo sapiens NudC domain containing 2 (NUDCD2)	-1,65	4,50E-02
Bos taurus similar to Homo sapiens pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 3 (PLEKHA3)	-1,67	3,20E-02
Bos taurus similar to Homo sapiens myoneurin (MYNN)	-1,55	3,43E-02
Bos taurus IgA regulatory protein (IGIP)	-1,77	3,93E-02
Bos taurus similar to Homo sapiens adenosine kinase (ADK), transcript variant ADK-long	-1,53	2,99E-02
Bos taurus similar to Homo sapiens BET1 homolog (S. cerevisiae) (BET1)	-1,75	3,03E-02
Bos taurus similar to Homo sapiens mbt domain containing 1 (MBTD1)	-1,70	4,59E-02
Bos taurus similar to Homo sapiens heat shock 70kDa protein 14 (HSPA14), transcript variant 1	-1,51	4,01E-02
Bos taurus similar to Homo sapiens related RAS viral (r-ras) oncogene homolog 2 (RRAS2)	-1,71	4,14E-02
PREDICTED: Bos taurus similar to TATA box-binding protein-associated factor, RNA polymerase II, K, 18kD, transcript variant 1 (LOC513065)	-2,06	3,90E-02

Bos taurus similar to Homo sapiens CAS1 domain containing 1 (CASD1)	-2,00	4,81E-02
Bos taurus cyclin G1 (CCNG1)	-1,58	4,44E-02
PREDICTED: Bos taurus similar to breast cancer metastasis-suppressor 1-like (LOC514432)	-1,61	2,39E-02
Bos taurus similar to Homo sapiens dual specificity phosphatase 19 (DUSP19)	-1,60	1,51E-02
Bos taurus ATP synthase, H+ transporting, mitochondrial F0 complex, subunit s (ATP5S)	-1,52	2,11E-02
Bos taurus similar to Homo sapiens transmembrane protein 128 (TMEM128)	-1,98	3,02E-02
Bos taurus similar to Homo sapiens centrin, EF-hand protein, 3 (CDC31 homolog, yeast) (CETN3)	-1,96	2,37E-02
Bos taurus similar to Homo sapiens chromosome 1 open reading frame 19 (C1orf19)	-1,60	2,22E-02
Bos taurus similar to Homo sapiens thioredoxin-like 1 (TXNL1)	-1,63	3,74E-02
Bos taurus similar to Homo sapiens LSM8 homolog, U6 small nuclear RNA associated (S. cerevisiae) (LSM8)	-1,60	1,20E-02
Bos taurus similar to Homo sapiens dual specificity phosphatase 19 (DUSP19)	-1,59	1,27E-02
Unidentified transcripts	-1,71	4,85E-02
Bos taurus similar to PREDICTED: Homo sapiens WD repeat domain 43, transcript variant 1 (WDR43)	-1,59	4,73E-02
Bos taurus similar to Homo sapiens WD repeat domain 51B (WDR51B)	-1,53	2,99E-02
Bos taurus similar to Homo sapiens tubulin, gamma complex associated protein 5 (TUBGCP5)	-1,70	8,42E-03
Bos taurus similar to Homo sapiens DCN1, defective in cullin neddylation 1, domain containing 1 (S. cerevisiae) (DCUN1D1)	-1,68	3,34E-02
Bos taurus similar to Homo sapiens helicase, lymphoid-specific (HELLS)	-1,53	1,05E-02
Bos taurus similar to Homo sapiens splicing factor, arginine/serine-rich 10 (transformer 2 homolog, Drosophila) (SFRS10)	-1,59	2,80E-02
Bos taurus similar to Homo sapiens jerky homolog-like (mouse) (JRKL)	-1,52	2,11E-02
Bos taurus protein phosphatase 1, catalytic subunit, gamma isoform (PPP1CC)	-1,76	3,46E-02
Bos taurus similar to Homo sapiens FERM domain containing 6 (FRMD6), transcript variant 2	-1,75	2,45E-02
Unidentified transcripts on BTA5 position 17285425-17284735	-1,76	1,10E-02
Bos taurus similar to Homo sapiens GUF1 GTPase homolog (S. cerevisiae) (GUF1)	-1,65	1,88E-02
Bos taurus similar to Homo sapiens hepatitis A virus cellular receptor 2 (HAVCR2)	-1,62	1,70E-02
Bos taurus similar to Homo sapiens ADP-ribosylation factor-like 4A (ARL4A), transcript variant 1	-1,75	4,67E-02
Bos taurus similar to Homo sapiens cyclin C (CCNC), transcript variant 1	-2,04	4,88E-02
Bos taurus similar to Homo sapiens chromosome 9 open reading frame 82 (C9orf82)	-1,82	4,90E-02
Bos taurus similar to Homo sapiens chromosome 2 open reading frame 3 (C2orf3)	-1,98	3,11E-02
Bos taurus similar to Homo sapiens KRR1, small subunit (SSU) processome component, homolog (yeast) (KRR1)	-1,86	4,26E-02
PREDICTED: Bos taurus similar to putative transcription factor ZNF131, transcript variant 5 (LOC541143)	-1,76	4,02E-02
Unidentified transcripts on BTA15 position 41748266-41746550	-1,62	4,66E-02
Bos taurus similar to Homo sapiens ATG12 autophagy related 12 homolog (S. cerevisiae) (ATG12)	-1,87	4,80E-02
Bos taurus similar to Homo sapiens tigger transposable element derived 2 (TIGD2)	-1,67	4,04E-02
Bos taurus similar to Homo sapiens TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 32kDa (TAF9), transcript variant 1	-1,51	3,78E-02
Bos taurus similar to Homo sapiens retinoblastoma-like 1 (p107) (RBL1), transcript variant 1	-1,69	4,73E-02
Unidentified transcripts on BTA10 position 38875547-38874641	-1,63	4,74E-02
Bos taurus similar to Homo sapiens kinesin family member 3A (KIF3A)	-1,61	3,30E-02
Bos taurus similar to Homo sapiens PCI domain containing 1 (herpesvirus entry mediator) (PCID1)	-1,52	4,55E-02
PREDICTED: Bos taurus similar to NOL1/NOP2/Sun domain family, member 3, transcript variant 2 (LOC533791)	-1,55	1,21E-02
Unidentified transcripts on BTA18 position 53608650-53607745	-1,79	2,85E-02
Bos taurus similar to Homo sapiens zinc finger protein 639 (ZNF639)	-1,73	1,01E-02
Bos taurus similar to Homo sapiens transmembrane protein 106B (TMEM106B)	-2,08	4,68E-02
Bos taurus similar to Homo sapiens Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1), transcript variant short	-1,52	2,76E-02
Bos taurus similar to Homo sapiens epithelial cell transforming sequence 2 oncogene (ECT2)	-1,71	1,45E-02
Bos taurus similar to Homo sapiens leptin receptor overlapping transcript (LEPROT)	-1,89	1,82E-02
Bos taurus similar to Homo sapiens serologically defined colon cancer antigen 1 (SDCCAG1)	-1,59	3,36E-02
PREDICTED: Bos taurus similar to Mdm4, transformed 3T3 cell double minute 1, p53 binding protein isoform 1 (LOC614357)	-1,52	1,45E-02
Bos taurus similar to Homo sapiens CDC14 cell division cycle 14 homolog A (S. cerevisiae) (CDC14A), transcript variant 1	-1,78	4,40E-02
Bos taurus similar to Homo sapiens cyclin-dependent kinase 7 (MO15 homolog, Xenopus laevis, cdk-activating kinase) (CDK7)	-1,52	2,40E-02
Bos taurus similar to Homo sapiens sprouty homolog 2 (Drosophila) (SPRY2)	-1,72	1,71E-02
Bos taurus peroxiredoxin 3 (PRDX3)	-1,63	4,28E-02
Bos taurus similar to Homo sapiens YEATS domain containing 4 (YEATS4)	-1,76	2,64E-02
Bos taurus similar to Homo sapiens short coiled-coil protein (SCOC)	-2,03	2,76E-02
Bos taurus similar to Homo sapiens ERBB receptor feedback inhibitor 1 (ERRFI1)	-1,59	4,17E-02
Bos taurus similar to Homo sapiens selenoprotein K (SELK)	-1,54	4,50E-02

Bos taurus similar to PREDICTED: Homo sapiens KIAA1935 protein (KIAA1935)	-1,53	2,32E-02
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 100 (C14orf100)	-1,66	2,19E-02
Bos taurus similar to Homo sapiens COMM domain containing 8 (COMMD8)	-1,76	3,90E-02
Bos taurus similar to Homo sapiens zinc finger, BED-type containing 5 (ZBED5)	-1,54	1,86E-02
Bos taurus similar to Homo sapiens KIAA1008 (KIAA1008)	-1,67	1,93E-02
Unidentified transcripts on BTA16 position 36342621-36343363	-1,83	3,02E-02
Bos taurus similar to Homo sapiens DnaJ (Hsp40) homolog, subfamily B, member 9 (DNAJB9)	-1,96	3,34E-02
Bos taurus similar to Homo sapiens DCN1, defective in cullin neddylation 1, domain containing 4 (S. cerevisiae) (DCUN1D4), transcript variant 2	-1,59	4,83E-02
Bos taurus similar to Homo sapiens zinc finger protein 570 (ZNF570)	-1,82	1,04E-02
Bos taurus similar to Homo sapiens CD3g molecule, gamma (CD3-TCR complex) (CD3G)	-1,60	4,97E-02
Unidentified transcripts on BTA11 position 75855413-75854644	-1,86	3,12E-02
Bos taurus similar to Homo sapiens transmembrane protein 161B (TMEM161B)	-1,99	1,74E-02
Bos taurus similar to Homo sapiens armadillo repeat containing, X-linked 5 (ARMCX5)	-1,79	2,71E-02
PREDICTED: Bos taurus ETAA16 protein (ETAA16)	-1,80	2,02E-02
Bos taurus stearoyl-coenzyme A desaturase (SCD)	-1,71	3,84E-02
Bos taurus similar to Homo sapiens transmembrane emp24 protein transport domain containing 7 (TMED7)	-1,76	3,83E-02
PREDICTED: Bos taurus similar to Germ cell-less protein-like 1 (mGcl-1) (DP-interacting protein) (DIP) (LOC513934)	-1,85	2,32E-02
Bos taurus similar to Homo sapiens pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 3 (PLEKHA3)	-1,68	2,53E-02
Unidentified transcripts on BTA19 position 37193021-37193996	-2,72	1,74E-02
PREDICTED: Bos taurus similar to non-imprinted in Prader-Willi/Angelman syndrome 2 (LOC539757)	-1,51	6,34E-03
Bos taurus similar to Homo sapiens RAB11 family interacting protein 2 (class I) (RAB11FIP2)	-1,71	1,93E-02
Bos taurus similar to Homo sapiens claudin domain containing 1 (CLDND1), transcript variant 2	-1,73	1,38E-02
PREDICTED: Bos taurus similar to transmembrane protein 64 (LOC536822)	-1,81	4,90E-02
Bos taurus similar to Homo sapiens PRP39 pre-mRNA processing factor 39 homolog (S. cerevisiae) (PRPF39)	-2,02	4,17E-02
Bos taurus similar to Homo sapiens tetratricopeptide repeat domain 14 (TTC14), transcript variant 1	-1,83	2,94E-02
Bos taurus similar to Homo sapiens killer cell lectin-like receptor subfamily K, member 1 (KLRK1)	-1,87	3,20E-02
PREDICTED: Bos taurus similar to transmembrane protein 64 (LOC536822)	-1,88	4,48E-02
Bos taurus similar to PREDICTED: Homo sapiens hypothetical protein LOC116068 (LOC116068)	-2,09	4,85E-02
Unidentified transcripts on BTA29 position 29030660-29031722	-1,91	4,40E-02
Bos taurus similar to Homo sapiens mitochondrial ribosomal protein S36 (MRPS36), nuclear gene encoding mitochondrial protein	-1,60	3,86E-02
PREDICTED: Bos taurus similar to thymopoietin isoform gamma, transcript variant 3 (LOC510267)	-1,68	2,33E-02
Bos taurus follistatin (FST)	-1,54	3,59E-03
Bos taurus similar to Homo sapiens centrosome and spindle pole associated protein 1 (CSPP1)	-1,93	1,70E-02
Bos taurus DEC-205/CD205 protein (LOC407236)	-1,73	2,16E-02
Bos taurus similar to Homo sapiens HBS1-like (S. cerevisiae) (HBS1L)	-1,79	4,71E-02
Bos taurus similar to Homo sapiens adhesion molecule with Ig-like domain 2 (AMIGO2)	-1,83	3,10E-02
Bos taurus similar to Homo sapiens DCN1, defective in cullin neddylation 1, domain containing 5 (S. cerevisiae) (DCUN1D5)	-1,63	4,41E-03
Unidentified transcripts	-1,74	2,38E-02
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 104 (C14orf104)	-1,59	2,43E-02
Bos taurus similar to Homo sapiens general transcription factor IIF, polypeptide 2 (30kD subunit) (GTF2F2)	-1,66	2,82E-03
Bos taurus krit1 protein (CCM1)	-1,65	4,52E-02
Bos taurus similar to Homo sapiens RAP1 interacting factor homolog (yeast) (RIF1)	-1,66	2,24E-02
Bos taurus similar to Homo sapiens nipsnap homolog 3A (C. elegans) (NIPSNAP3A)	-1,63	3,30E-02
Bos taurus similar to Homo sapiens ERGIC and golgi 2 (ERGIC2)	-1,55	3,94E-02
Bos taurus similar to Homo sapiens echinoderm microtubule associated protein like 4 (EML4)	-1,61	3,57E-02
Bos taurus similar to Homo sapiens succinate-CoA ligase, ADP-forming, beta subunit (SUCLA2)	-1,62	4,69E-02
Unidentified transcripts on BTA9 position 52850360-52849929	-1,85	3,64E-02
Unidentified transcripts on BTA7 position 42089840-42090545	-1,52	3,66E-02
Bos taurus similar to Homo sapiens regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1 (RCBTB1)	-1,59	3,85E-02
Bos taurus UDP-glucose dehydrogenase (UGDH)	-1,77	2,25E-02
PREDICTED: Bos taurus similar to serine/threonine protein kinase MASK (LOC539445), partial mRNA.	-1,60	3,89E-02
Bos taurus similar to Homo sapiens mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) (MSH2)	-1,75	1,80E-02
Bos taurus similar to Homo sapiens transmembrane protein 128 (TMEM128)	-1,95	3,27E-02
Bos taurus similar to Homo sapiens zinc finger and BTB domain containing 24 (ZBTB24)	-1,64	4,72E-02
Bos taurus similar to Homo sapiens ras homolog gene family, member U (RHOU)	-1,50	5,16E-03
Bos taurus similar to Homo sapiens N-glycanase 1 (NGLY1)	-1,66	4,59E-02

Bos taurus similar to Homo sapiens gamma-glutamyl carboxylase (GGCX)	-2,49	8,87E-03
Bos taurus RAP1A, member of RAS oncogene family (RAP1A)	-1,69	4,14E-02
Bos taurus similar to Homo sapiens sex comb on midleg-like 2 (Drosophila) (SCML2)	-1,51	6,81E-03
PREDICTED: Bos taurus similar to putative transcription factor ZNF131, transcript variant 5 (LOC541143)	-1,84	2,52E-02
PREDICTED: Bos taurus similar to transmembrane 9 superfamily member 2 (LOC509946)	-1,62	2,97E-02
Bos taurus similar to Homo sapiens family with sequence similarity 96, member A (FAM96A), transcript variant 1	-1,53	2,68E-02
PREDICTED: Bos taurus similar to DnaJ (Hsp40) homolog, subfamily B, member 9 (LOC614588)	-1,84	2,66E-02
Bos taurus similar to Homo sapiens KIAA0776 (KIAA0776)	-1,84	3,09E-02
Bos taurus similar to Homo sapiens DCN1, defective in cullin neddylation 1, domain containing 4 (S. cerevisiae) (DCUN1D4), transcript variant 2	-1,59	4,30E-02
Bos taurus similar to Homo sapiens solute carrier family 33 (acetyl-CoA transporter), member 1 (SLC33A1)	-1,75	4,27E-02
Unidentified transcripts on BTA19 position 8148504-8148058	-1,58	1,60E-02
Bos taurus similar to Homo sapiens sex comb on midleg-like 2 (Drosophila) (SCML2)	-1,56	4,24E-03
Bos taurus similar to Homo sapiens PTD016 protein (LOC51136)	-1,87	3,26E-02
Bos taurus similar to Homo sapiens inhibitor of Bruton agammaglobulinemia tyrosine kinase (IBTK)	-1,79	2,83E-02
Bos taurus similar to Homo sapiens neurogenin 2 (NEUROG2)	-1,52	2,86E-02
Bos taurus similar to Homo sapiens ubiquitin D (UBD)	-1,53	4,95E-02
Bos taurus similar to Homo sapiens cytoskeleton associated protein 2 (CKAP2)	-1,80	2,94E-02
Bos taurus similar to Homo sapiens coiled-coil domain containing 66 (CCDC66)	-1,85	1,58E-02
Bos taurus similar to Homo sapiens zinc finger protein 90 homolog (mouse) (ZFP90)	-1,50	9,28E-03
PREDICTED: Bos taurus similar to Zinc finger protein 90 (Zfp-90) (Zinc finger protein NK10) (LOC616663)	-1,55	2,05E-02
Bos taurus similar to Homo sapiens fibroblast growth factor receptor substrate 2 (FRS2), transcript variant 1	-1,51	4,64E-02
Bos taurus similar to Homo sapiens RAN binding protein 6 (RANBP6)	-1,86	3,84E-02
Unidentified transcripts on BTA17 position 9673289-9671965	-1,53	3,77E-02
Bos taurus similar to Homo sapiens density-regulated protein (DENR)	-1,99	1,59E-02
Unidentified transcripts on BTA9 position 52850360-52849929	-1,88	2,67E-02
Unidentified transcripts on BTA3 position 16783764-16784497	-1,67	4,62E-02
Bos taurus similar to Homo sapiens zinc finger and BTB domain containing 41 (ZBTB41)	-1,73	4,48E-02
Bos taurus similar to Homo sapiens mitochondrial ribosomal protein L32 (MRPL32), nuclear gene encoding mitochondrial protein	-1,52	7,96E-03
Bos taurus similar to Homo sapiens ankyrin repeat domain 32 (ANKRD32)	-1,71	9,30E-03
Bos taurus similar to Homo sapiens ubiquitously transcribed tetratricopeptide repeat gene, Y-linked (UTY), transcript variant 3	-1,74	1,73E-02
Bos taurus guanylate cyclase 1, soluble, beta 3 (GUCY1B3)	-1,94	4,11E-02
PREDICTED: Bos taurus similar to NOL1/NOP2/Sun domain family, member 3, transcript variant 2 (LOC533791)	-1,56	1,58E-03
Bos taurus IgA regulatory protein (IGIP)	-1,97	2,83E-02
Bos taurus similar to Homo sapiens protein kinase, cAMP-dependent, regulatory, type II, beta (PRKAR2B)	-1,74	1,64E-02
Bos taurus similar to Homo sapiens N-glycanase 1 (NGLY1)	-1,71	3,65E-02
Unidentified transcripts on BTA3 position 56083439-56083161	-1,81	4,76E-02
Bos taurus similar to Homo sapiens AT rich interactive domain 4A (RBP1-like) (ARID4A), transcript variant 1	-1,62	4,23E-02
Unidentified transcripts	-1,64	8,36E-03
Bos taurus similar to Homo sapiens ERGIC and golgi 2 (ERGIC2)	-1,64	1,56E-02
Bos taurus similar to T-cell immunomodulatory protein (MGC128970)	-1,65	4,82E-02
Bos taurus similar to Homo sapiens G protein-coupled receptor associated sorting protein 2 (GPRASP2), transcript variant 2	-1,66	8,10E-03
Bos taurus similar to Homo sapiens trafficking protein particle complex 6B (TRAPPC6B)	-1,68	3,16E-02
Bos taurus similar to Homo sapiens ubiquitin protein ligase E3A (human papilloma virus E6-associated protein, Angelman syndrome) (UBE3A), transcript variant 1	-1,53	4,75E-02
Bos taurus similar to Homo sapiens zinc finger protein 23 (KOX 16) (ZNF23)	-1,53	8,09E-03
Bos taurus similar to Homo sapiens RWD domain containing 3 (RWDD3)	-1,56	6,43E-03
Bos taurus similar to Homo sapiens ankyrin repeat domain 46 (ANKRD46)	-1,98	3,10E-02
Bos taurus similar to Homo sapiens ankyrin repeat domain 46 (ANKRD46)	-1,98	2,90E-02
PREDICTED: Bos taurus similar to WD repeat domain 76 (LOC515302)	-1,53	3,22E-02
Bos taurus similar to Homo sapiens protein kinase, cAMP-dependent, regulatory, type II, beta (PRKAR2B)	-1,81	2,44E-02
Bos taurus similar to Homo sapiens B-cell receptor-associated protein 29 (BCAP29), transcript variant 2	-1,82	9,53E-03
Bos taurus similar to Homo sapiens bromodomain and WD repeat domain containing 1 (BRWD1), transcript variant 1	-1,60	4,31E-02

PREDICTED: Bos taurus similar to arginine/serine-rich coiled-coil 1 (LOC509437)	-1,60	2,43E-02
Unidentified transcripts on BTA2 position 646865-647650	-1,60	1,44E-02
Bos taurus similar to Homo sapiens zinc finger protein 570 (ZNF570)	-2,00	5,91E-03
Bos taurus similar to Homo sapiens zinc finger protein 14 homolog (mouse) (ZFP14)	-1,55	1,12E-02
Bos taurus similar to Homo sapiens matrin 3 (MATR3), transcript variant 2	-1,98	4,42E-02
Bos taurus similar to Homo sapiens claudin domain containing 1 (CLDND1), transcript variant 2	-1,80	7,58E-03
Bos taurus similar to Homo sapiens centromere protein C 1 (CENPC1)	-1,76	4,04E-02
Unidentified transcripts on BTA1 position 34676433-34677199	-1,74	2,60E-02
Bos taurus similar to Homo sapiens TATA box binding protein (TBP)-associated factor, RNA polymerase I, A, 48kDa (TAF1A), transcript variant 1	-1,62	1,67E-02
Bos taurus similar to Homo sapiens ERBB receptor feedback inhibitor 1 (ERRFI1)	-1,61	4,70E-02
Bos taurus cAMP-regulated phosphoprotein (ARPP-19)	-1,95	3,83E-02
Bos taurus fetal globin inducing factor (FGIF)	-1,56	2,74E-02
Unidentified transcripts on BTA18 position 1807133-1809382	-1,53	3,26E-02
Bos taurus similar to Homo sapiens GA binding protein transcription factor, alpha subunit 60kDa (GABPA)	-1,60	2,19E-02
Bos taurus similar to Homo sapiens cofactor required for Sp1 transcriptional activation, subunit 3, 130kDa (CRSP3), transcript variant 1	-1,80	2,09E-02
Bos taurus similar to Homo sapiens solute carrier family 35 (CMP-sialic acid transporter), member A1 (SLC35A1)	-1,66	2,33E-02
Bos taurus similar to Homo sapiens tetratricopeptide repeat domain 8 (TTC8), transcript variant 3	-1,55	1,20E-02
PREDICTED: Bos taurus similar to Protein C9orf55 (LOC536760)	-1,55	3,07E-02
Unidentified transcripts on BTA10 position 4736924-4737874	-1,64	1,78E-02
Bos taurus DDHD domain containing 1 (DDHD1)	-1,55	2,54E-02
Bos taurus similar to Homo sapiens GUF1 GTPase homolog (S. cerevisiae) (GUF1)	-1,87	9,92E-03
Bos taurus similar to Homo sapiens COX18 cytochrome c oxidase assembly homolog (S. cerevisiae) (COX18)	-1,56	3,08E-02
Unidentified transcripts	-2,52	3,02E-02
Bos taurus similar to Homo sapiens LSM14 homolog A (SCD6, S. cerevisiae) (LSM14A)	-1,73	3,66E-02
Bos taurus similar to Homo sapiens GPI-anchored membrane protein 1 (GPIAP1), transcript variant 1	-1,57	2,73E-02
PREDICTED: Bos taurus similar to non-imprinted in Prader-Willi/Angelman syndrome 2 (LOC539757)	-1,64	1,13E-02
Bos taurus similar to Homo sapiens chromosome 2 open reading frame 3 (C2orf3)	-1,93	4,58E-02
Bos taurus similar to Homo sapiens heat shock 70kDa protein 14 (HSPA14), transcript variant 1	-1,54	3,36E-02
PREDICTED: Bos taurus similar to Feline leukemia virus subgroup C receptor-related protein 1 (Feline leukemia virus subgroup C receptor) (hFLVCR) (LOC533317)	-2,10	4,74E-02
Bos taurus similar to Homo sapiens preimplantation protein 3 (PREI3), transcript variant 1	-1,67	4,91E-02
Bos taurus toll-like receptor 3 (TLR3)	-1,55	1,41E-02
Unidentified transcripts	-1,86	3,18E-02
Unidentified transcripts on BTA26 position 8588986-8588099	-1,85	4,24E-02
Bos taurus similar to Homo sapiens lin-7 homolog C (C. elegans) (LIN7C)	-2,01	3,42E-02
Bos taurus similar to Homo sapiens glycosyltransferase 28 domain containing 1 (GLT28D1)	-1,52	1,98E-02
Unidentified transcripts on BTA24 position 6453162-6452122	-1,53	4,44E-02
Bos taurus similar to Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 6 (PSMC6)	-1,93	3,66E-02
Bos taurus similar to PREDICTED: Homo sapiens SEC24 related gene family, member A (S. cerevisiae) (SEC24A)	-2,02	3,76E-02
Bos taurus similar to Homo sapiens nucleosome assembly protein 1-like 5 (NAP1L5)	-1,88	8,67E-03
Bos taurus lipoic acid synthetase (LIAS)	-1,58	1,73E-02
Bos taurus similar to Homo sapiens amidohydrolase domain containing 1 (AMDHD1)	-1,61	3,26E-02
Bos taurus similar to Homo sapiens chromosome 15 open reading frame 29 (C15orf29)	-1,52	6,11E-03
Bos taurus similar to Homo sapiens RNA binding motif protein 7 (RBM7)	-1,97	3,09E-02
Bos taurus similar to Homo sapiens hypothetical protein MGC23909 (MGC23909)	-2,14	1,83E-02
Bos taurus similar to Homo sapiens DEP domain containing 7 (DEPDC7)	-1,65	4,70E-02
Bos taurus similar to Homo sapiens chromosome 1 open reading frame 103 (C1orf103), transcript variant 1	-1,51	1,25E-02
Bos taurus similar to Homo sapiens glycerophosphodiester phosphodiesterase domain containing 1 (GDPD1)	-1,95	1,52E-02
Bos taurus stearoyl-coenzyme A desaturase (SCD)	-1,82	1,98E-02
Bos taurus similar to Homo sapiens chromosome 6 open reading frame 211 (C6orf211)	-1,75	1,14E-02
Bos taurus similar to Homo sapiens fibronectin type III domain containing 3A (FNDC3A)	-2,05	3,71E-02
Bos taurus similar to Homo sapiens lactamase, beta 2 (LACTB2)	-1,62	9,34E-03
Bos taurus similar to Homo sapiens THUMP domain containing 2 (THUMPD2)	-1,51	2,54E-02
Bos taurus similar to Homo sapiens zinc ribbon domain containing 1 (ZNRD1), transcript variant a	-5,37	1,41E-02
Bos taurus similar to Homo sapiens restin-like 2 (RSNL2)	-1,92	1,54E-02
Bos taurus similar to Homo sapiens THUMP domain containing 2 (THUMPD2)	-1,51	2,06E-02
Bos taurus similar to Homo sapiens protein phosphatase 3 (formerly 2B), catalytic subunit, beta	-1,68	2,69E-02

isoform (calcineurin A beta) (PPP3CB)		
Bos taurus KIAA0101 protein (KIAA0101)	-1,73	4,06E-02
Bos taurus interleukin 18 (interferon-gamma-inducing factor) (IL18)	-1,71	2,05E-02
Bos taurus similar to Homo sapiens isopentenyl-diphosphate delta isomerase 1 (IDI1)	-1,89	8,85E-03
Bos taurus similar to Homo sapiens ZW10 interactor (ZWINT), transcript variant 2	-1,69	7,17E-03
Bos taurus similar to Homo sapiens suppressor of hairy wing homolog 4 (Drosophila) (SUHW4), transcript variant 3	-1,99	2,29E-02
Bos taurus Sjogren syndrome antigen B (autoantigen La) (SSB)	-1,73	1,91E-02
Bos taurus similar to Homo sapiens transmembrane protein 41B (TMEM41B)	-2,07	2,63E-03
Bos taurus similar to Homo sapiens establishment of cohesion 1 homolog 2 (S. cerevisiae) (ESCO2)	-1,91	3,31E-03
Unidentified transcripts on BTA5 position 17285425-17284735	-1,96	1,13E-02
Unidentified transcripts on BTA3 position 16783764-16784497	-1,71	4,21E-02
Bos taurus similar to Homo sapiens special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's) (SATB1)	-2,02	3,45E-02
Unidentified transcripts on BTA29 position 43301253-43300539	-1,63	4,66E-02
Bos taurus similar to Homo sapiens TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 32kDa (TAF9), transcript variant 1	-1,63	1,99E-02
Bos taurus similar to Homo sapiens ATPase, Cu++ transporting, alpha polypeptide (Menkes syndrome) (ATP7A)	-1,75	4,42E-02
PREDICTED: Bos taurus similar to Mdm4, transformed 3T3 cell double minute 1, p53 binding protein isoform 1 (LOC614357)	-1,67	9,34E-03
Bos taurus similar to Homo sapiens myotubularin related protein 12 (MTMR12)	-1,64	3,27E-02
Bos taurus similar to Homo sapiens zinc finger, DHHC-type containing 23 (ZDHHC23)	-1,57	4,50E-02
Bos taurus similar to Homo sapiens ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease) (RET)	-1,66	1,44E-02
Unidentified transcripts on BTA19 position 28206662-28204512	-1,83	4,93E-02
Unidentified transcripts	-2,03	1,85E-02
Bos taurus similar to Homo sapiens CASP8 associated protein 2 (CASP8AP2)	-1,55	1,32E-02
Bos taurus similar to Homo sapiens NMD3 homolog (S. cerevisiae) (NMD3)	-1,57	3,01E-02
Bos taurus similar to Homo sapiens transcription elongation factor A (SII)-like 1 (TCEAL1), transcript variant 1	-1,50	1,93E-02
Bos taurus similar to Homo sapiens pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1 (PLEKHA1), transcript variant 1	-1,83	2,77E-02
Bos taurus similar to Homo sapiens SET and MYND domain containing 2 (SMYD2)	-1,54	1,17E-02
Bos taurus similar to Homo sapiens family with sequence similarity 96, member A (FAM96A), transcript variant 1	-1,55	1,79E-02
Bos taurus similar to Homo sapiens abhydrolase domain containing 3 (ABHD3)	-1,70	1,79E-02
Bos taurus similar to Homo sapiens serologically defined colon cancer antigen 1 (SDCCAG1)	-1,70	2,25E-02
Bos taurus similar to Homo sapiens ATP binding domain 1 family, member C (ATPBD1C)	-1,71	1,22E-02
Unidentified transcripts on BTA13 position 27906023-27906738	-1,53	4,73E-02
Bos taurus CD69 antigen (p60, early T-cell activation antigen) (CD69)	-1,89	4,03E-02
Bos taurus similar to Homo sapiens colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage) (CSF2RB)	-1,75	1,25E-02
Bos taurus similar to Homo sapiens RAB, member of RAS oncogene family-like 3 (RABL3)	-1,51	2,78E-02
Bos taurus similar to Homo sapiens LSM8 homolog, U6 small nuclear RNA associated (S. cerevisiae) (LSM8)	-1,73	7,94E-03
Bos taurus similar to Homo sapiens solute carrier family 17 (anion/sugar transporter), member 5 (SLC17A5)	-1,61	2,22E-02
PREDICTED: Bos taurus similar to zinc finger protein 258 (LOC514264)	-1,63	4,44E-02
PREDICTED: Bos taurus similar to translin-associated factor X (LOC533927)	-1,62	8,17E-03
Bos taurus similar to Homo sapiens ClpX caseinolytic peptidase X homolog (E. coli) (CLPX)	-1,59	4,20E-02
Unidentified transcripts	-2,04	2,82E-02
Bos taurus similar to Homo sapiens adaptor-related protein complex 3, mu 1 subunit (AP3M1), transcript variant 2	-1,51	8,50E-03
Bos taurus similar to Homo sapiens mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) (MSH2)	-1,85	9,07E-03
Bos taurus similar to Homo sapiens HCF-binding transcription factor Zhangfei (ZF)	-2,11	2,07E-02
Bos taurus similar to Homo sapiens coiled-coil domain containing 66 (CCDC66)	-1,88	1,27E-02
Bos taurus similar to Homo sapiens estrogen receptor binding site associated, antigen, 9 (EBAG9), transcript variant 1	-2,13	9,73E-03
Bos taurus similar to Homo sapiens phosphatidylinositol glycan anchor biosynthesis, class Y (PIGY)	-1,62	4,37E-02
Bos taurus similar to Homo sapiens signal transducing adaptor molecule (SH3 domain and ITAM motif) 2 (STAM2)	-1,83	4,37E-02
Bos taurus similar to Homo sapiens heterogeneous nuclear ribonucleoprotein L-like (HNRPLL)	-1,74	4,05E-02
Bos taurus similar to Homo sapiens IQ motif containing B1 (IQCB1), transcript variant 3	-1,58	3,50E-02
Bos taurus similar to Homo sapiens KIAA1008 (KIAA1008)	-1,52	3,82E-02
Bos taurus similar to Homo sapiens zinc finger protein 12 (ZNF12), transcript variant 1	-1,86	2,13E-02

Bos taurus toll-like receptor 3 (TLR3)	-1,72	3,55E-03
Bos taurus similar to Homo sapiens tetratricopeptide repeat domain 14 (TTC14), transcript variant 1	-1,83	3,27E-02
Bos taurus similar to PREDICTED: Homo sapiens TAF4b RNA polymerase II, TATA box binding protein (TBP)-associated factor, 105kDa (TAF4B)	-1,55	4,37E-02
Unidentified transcripts on BTA29 position 29030660-29031722	-2,17	1,34E-02
Bos taurus similar to Homo sapiens cryptochrome 1 (photolyase-like) (CRY1)	-1,82	2,54E-02
PREDICTED: Bos taurus similar to ELL associated factor 2 (LOC613536)	-2,32	4,70E-02
Unidentified transcripts on BTA23 position 24643013-24643567	-1,51	3,28E-02
Bos taurus similar to Homo sapiens COMM domain containing 8 (COMMD8)	-2,01	1,57E-02
Bos taurus similar to Homo sapiens hypothetical protein MGC14376 (MGC14376), transcript variant 1	-1,78	4,81E-02
Bos taurus similar to Homo sapiens ubiquitin-activating enzyme E1-like 2 (UBE1L2)	-1,67	2,73E-02
Bos taurus similar to Homo sapiens FUS interacting protein (serine/arginine-rich) 1 (FUSIP1), transcript variant 2	-1,93	2,33E-02
Bos taurus similar to Homo sapiens hypothetical protein FLJ40432 (FLJ40432)	-1,57	2,12E-02
Unidentified transcripts on BTA2 position 69284865-69285705	-1,53	3,26E-02
Bos taurus similar to Homo sapiens preimplantation protein 3 (PREI3), transcript variant 1	-1,68	4,69E-02
Bos taurus similar to Homo sapiens queuine tRNA-ribosyltransferase domain containing 1 (QTRTD1)	-1,56	2,35E-02
Bos taurus similar to Homo sapiens CD3g molecule, gamma (CD3-TCR complex) (CD3G)	-1,63	4,47E-02
Bos taurus similar to Homo sapiens sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, fungal)-like (SC5DL), transcript variant 1	-1,83	4,21E-02
Bos taurus similar to PREDICTED: Homo sapiens myosin VB, transcript variant 1 (MYO5B)	-1,52	1,60E-02
Bos taurus similar to Homo sapiens NudC domain containing 1 (NUDCD1)	-1,71	8,39E-03
Bos taurus similar to Homo sapiens tetratricopeptide repeat domain 21B (TTC21B)	-1,59	2,60E-02
Bos taurus similar to Homo sapiens RNA U, small nuclear RNA export adaptor (phosphorylation regulated) (RNUXA)	-1,92	4,61E-02
Bos taurus similar to Homo sapiens mitochondrial ribosomal protein L33 (MRPL33), nuclear gene encoding mitochondrial protein, transcript variant 1	-1,66	4,84E-02
Bos taurus similar to Homo sapiens mitochondrial ribosomal protein L32 (MRPL32), nuclear gene encoding mitochondrial protein	-1,54	1,34E-02
Unidentified transcripts	-1,79	4,08E-02
Bos taurus similar to Homo sapiens helicase, lymphoid-specific (HELLS)	-1,57	1,89E-02
Bos taurus similar to Homo sapiens zinc finger, RAN-binding domain containing 2 (ZRANB2), transcript variant 2	-2,10	2,82E-02
Unidentified transcripts on BTA1 position 21948023-21948922	-1,50	2,09E-03
Bos taurus similar to Homo sapiens translocase of outer mitochondrial membrane 20 homolog (yeast) (TOMM20)	-1,60	1,90E-02
Bos taurus similar to Homo sapiens ERO1-like (S. cerevisiae) (ERO1L)	-1,54	3,06E-02
Bos taurus similar to Homo sapiens FLJ32363 protein (FLJ32363)	-1,51	2,70E-02
Bos taurus RasGEF domain family, member 1A (ARMC1)	-1,59	4,69E-02
Bos taurus similar to Homo sapiens DEAH (Asp-Glu-Ala-His) box polypeptide 29 (DHX29)	-1,66	1,27E-02
Bos taurus similar to Homo sapiens myotubularin related protein 12 (MTMR12)	-1,56	4,45E-02
Bos taurus similar to Homo sapiens amylo-1, 6-glucosidase, 4-alpha-glucanotransferase (glycogen debranching enzyme, glycogen storage disease type III) (AGL), transcript variant 6	-1,68	2,15E-02
Bos taurus similar to Homo sapiens casein kinase 1, gamma 3 (CSNK1G3), transcript variant 1	-1,56	4,89E-02
Bos taurus similar to Homo sapiens zinc finger protein 354A (ZNF354A)	-2,08	2,46E-02
Unidentified transcripts on BTA2 position 62168988-62168316	-2,07	4,07E-02
Bos taurus similar to Homo sapiens HIG1 domain family, member 1A (HIGD1A)	-1,60	4,38E-02
Bos taurus similar to Homo sapiens zinc finger protein 197 (ZNF197), transcript variant 1	-1,52	3,47E-02
Bos taurus similar to Homo sapiens purinergic receptor P2Y, G-protein coupled, 5 (P2RY5)	-1,78	3,38E-02
Bos taurus similar to Homo sapiens serpin peptidase inhibitor, clade I (neuroserpin), member 1 (SERPINI1)	-1,65	3,00E-02
Bos taurus similar to Homo sapiens RAB11 family interacting protein 2 (class I) (RAB11FIP2)	-2,09	3,30E-03
Bos taurus similar to Homo sapiens CD2-associated protein (CD2AP)	-1,77	4,49E-02
PREDICTED: Bos taurus similar to DnaJ (Hsp40) homolog, subfamily B, member 9 (LOC614588)	-1,81	2,21E-02
Bos taurus similar to Homo sapiens signal recognition particle 9kDa (SRP9)	-2,06	4,75E-02
PREDICTED: Bos taurus lipoprotein lipase, transcript variant 2 (LPL)	-1,84	4,67E-02
PREDICTED: Bos taurus similar to IMMUNE-RESPONSIVE PROTEIN 1 (LOC504806)	-1,79	4,81E-02
PREDICTED: Bos taurus similar to nuclear transcription factor-Y beta (LOC614382)	-2,50	3,95E-03
Bos taurus similar to Homo sapiens hypothetical protein LOC55610, isoform b (FLJ20097), transcript variant 1	-1,81	1,94E-03
Bos taurus similar to Homo sapiens NGFI-A binding protein 1 (EGR1 binding protein 1) (NAB1)	-1,81	2,40E-02
Bos taurus similar to Homo sapiens M-phase phosphoprotein 1 (MPHOSPH1)	-1,60	9,84E-03
Bos taurus similar to Homo sapiens ring finger protein (C3H2C3 type) 6 (RNF6), transcript variant 1	-1,60	4,81E-02
PREDICTED: Bos taurus similar to ankyrin 3, epithelial isoform b (LOC511229)	-1,55	1,05E-03
Unidentified transcripts on BTA23 position 12882640-12883381	-1,51	4,99E-02
Bos taurus similar to Homo sapiens nuclear transcription factor Y, beta (NFYB)	-2,18	3,15E-02

Bos taurus similar to Homo sapiens splicing factor, arginine/serine-rich 3 (SFRS3)	-1,50	4,72E-02
Bos taurus similar to Homo sapiens ADP-ribosylation-like factor 6 interacting protein 6 (ARL6IP6)	-2,04	3,79E-02
Bos taurus similar to Homo sapiens prefoldin subunit 4 (PFDN4)	-1,76	2,77E-02
Bos taurus similar to Homo sapiens splicing factor, arginine/serine-rich 10 (transformer 2 homolog, Drosophila) (SFRS10)	-1,55	4,61E-02
PREDICTED: Bos taurus similar to Y17G7B.10b (LOC614702)	-1,90	2,71E-02
Unidentified transcripts on BTA26 position 30148850-30149954	-1,63	3,42E-02
Bos taurus similar to Homo sapiens cytidylate kinase (CMPK)	-2,19	2,47E-02
Unidentified transcripts	-1,96	2,02E-02
Unidentified transcripts on BTA9 position 34156534-34157135	-1,53	1,35E-02
Bos taurus similar to Homo sapiens CCAAT/enhancer binding protein zeta (CEBPZ)	-1,83	2,61E-02
Bos taurus similar to Homo sapiens chromosome 3 open reading frame 59 (C3orf59)	-1,57	4,12E-02
Bos taurus similar to Homo sapiens zinc finger protein 146 (ZNF146)	-1,74	3,17E-02
Bos taurus similar to Homo sapiens HBS1-like (S. cerevisiae) (HBS1L)	-1,79	4,42E-02
Unidentified transcripts on BTA1 position 20031494-20030818	-1,58	4,11E-02
Bos taurus similar to Homo sapiens DnaJ (Hsp40) homolog, subfamily C, member 10 (DNAJC10)	-1,50	2,75E-02
Bos taurus similar to Homo sapiens cell growth regulator with ring finger domain 1 (CGRRF1)	-1,60	1,65E-02
Bos taurus similar to Homo sapiens deoxynucleotidyltransferase, terminal, interacting protein 2 (DNTTIP2)	-1,51	2,71E-02
Bos taurus similar to Homo sapiens nuclear transcription factor Y, beta (NFYB)	-2,36	2,11E-02
Bos taurus similar to Homo sapiens ubiquitin protein ligase E3A (human papilloma virus E6-associated protein, Angelman syndrome) (UBE3A), transcript variant 1	-1,60	3,61E-02
Bos taurus similar to Homo sapiens zinc finger protein 354A (ZNF354A)	-1,99	3,34E-02
Bos taurus similar to Homo sapiens cornichon homolog (Drosophila) (CNIH), transcript variant 1	-1,60	1,68E-02
Bos taurus similar to Homo sapiens cornichon homolog (Drosophila) (CNIH), transcript variant 1	-1,57	2,36E-02
Bos taurus similar to Homo sapiens hepatoma-derived growth factor, related protein 3 (HDGFRP3)	-1,58	3,16E-02
Bos taurus similar to Homo sapiens component of oligomeric golgi complex 6 (COG6)	-1,67	3,02E-02
Bos taurus similar to Homo sapiens kinectin 1 (kinesin receptor) (KTN1)	-1,77	4,75E-02
Unidentified transcripts on BTA12 position 33236616-33235396	-1,80	4,65E-02
Bos taurus similar to Homo sapiens tetraspanin 6 (TSPAN6)	-1,60	2,88E-03
Bos taurus similar to Homo sapiens reticulocalbin 2, EF-hand calcium binding domain (RCN2)	-2,03	2,56E-02
PREDICTED: Bos taurus similar to Vesicle-associated membrane protein 4 (VAMP-4) (LOC616923)	-1,52	1,01E-02
Bos taurus similar to Homo sapiens chromosome 21 open reading frame 66 (C21orf66), transcript variant 1	-1,94	4,32E-02
PREDICTED: Bos taurus similar to thymopoietin isoform gamma, transcript variant 3 (LOC510267)	-1,63	2,19E-02
Bos taurus similar to Homo sapiens DTW domain containing 1 (DTWD1)	-1,56	3,16E-02
PREDICTED: Bos taurus similar to nuclear transcription factor-Y beta (LOC614382)	-1,97	2,61E-02
Bos taurus similar to Homo sapiens synaptophysin-like 1 (SYPL1), transcript variant 1	-1,59	3,50E-02
Bos taurus similar to Homo sapiens suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein) (ST13)	-1,53	3,83E-02
Bos taurus similar to Homo sapiens eukaryotic translation initiation factor 5 (EIF5), transcript variant 1	-1,55	2,32E-02
Bos taurus similar to Homo sapiens caspase 6, apoptosis-related cysteine peptidase (CASP6), transcript variant alpha	-1,57	1,83E-02
Bos taurus similar to Homo sapiens trafficking protein particle complex 6B (TRAPPC6B)	-1,88	6,37E-03
Bos taurus similar to Homo sapiens transmembrane protein 68 (TMEM68)	-1,52	4,16E-02
Bos taurus similar to Homo sapiens leptin receptor overlapping transcript (LEPROT)	-1,82	3,66E-02
Bos taurus similar to Homo sapiens KIAA1143 (KIAA1143)	-1,69	2,76E-02
Bos taurus similar to Homo sapiens hypothetical protein LOC339745 (LOC339745)	-1,68	4,59E-02
Unidentified transcripts on BTA14 position 49967356-49968097	-1,74	1,57E-02
Unidentified transcripts	-1,63	4,33E-02
Bos taurus similar to Homo sapiens ribosomal protein S3A (RPS3A)	-2,31	4,88E-02
Bos taurus DDHD domain containing 1 (DDHD1)	-1,51	2,91E-02
Bos taurus similar to Homo sapiens O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase) (OGT), transcript variant 2	-1,51	2,53E-02
Bos taurus similar to Homo sapiens TATA box binding protein (TBP)-associated factor, RNA polymerase I, A, 48kDa (TAF1A), transcript variant 1	-1,63	1,67E-02
Bos taurus similar to Homo sapiens prefoldin subunit 4 (PFDN4)	-1,74	3,32E-02
Bos taurus similar to Homo sapiens short coiled-coil protein (SCOC)	-2,26	2,30E-02
Unidentified transcripts	-1,64	3,47E-02
PREDICTED: Bos taurus similar to Host cell factor 2 (HCF-2) (C2 factor), transcript variant 2 (LOC505341)	-1,72	2,45E-02
Unidentified transcripts	-1,58	4,72E-02
Bos taurus similar to Homo sapiens transmembrane protein 27 (TMEM27)	-1,52	2,93E-02
PREDICTED: Bos taurus similar to splicing factor 3b, subunit 1 isoform 1, transcript variant 1 (LOC510177)	-1,52	4,55E-02
Bos taurus similar to Homo sapiens F-box protein 5 (FBXO5)	-1,70	3,22E-02

Bos taurus similar to Homo sapiens glomulin, FKBP associated protein (GLMN)	-1,58	1,82E-02
Bos taurus similar to Homo sapiens cytidylate kinase (CMPK)	-2,06	3,68E-02
Bos taurus similar to Homo sapiens general transcription factor IIF, polypeptide 2 (30kD subunit) (GTF2F2)	-1,61	1,69E-02
Bos taurus similar to Homo sapiens CDC14 cell division cycle 14 homolog A (S. cerevisiae) (CDC14A), transcript variant 1	-1,84	2,77E-02
Unidentified transcripts	-2,52	2,86E-02
Bos taurus similar to Homo sapiens RNA binding motif protein 7 (RBM7)	-2,10	1,69E-02
Unidentified transcripts	-1,61	3,55E-02
Unidentified transcripts	-1,75	2,36E-02
Bos taurus similar to Homo sapiens FGFR1 oncogene partner 2 (FGFR1OP2)	-1,75	3,59E-02
Bos taurus similar to Homo sapiens chromosome 16 open reading frame 69 (C16orf69)	-1,89	2,29E-02
Bos taurus similar to Homo sapiens thioredoxin-like 1 (TXNL1)	-1,75	1,81E-02
Bos taurus similar to Homo sapiens hypothetical protein FLJ38482 (FLJ38482)	-1,51	4,38E-02
PREDICTED: Bos taurus similar to translin-associated factor X (LOC533927)	-1,56	1,21E-02
Bos taurus similar to Homo sapiens zinc finger and BTB domain containing 24 (ZBTB24)	-1,65	4,57E-02
Unidentified transcripts on BTA1 position 2846963-2845909	-1,94	1,61E-02
Bos taurus similar to Homo sapiens chromosome 10 open reading frame 88 (C10orf88)	-1,65	3,37E-02
Bos taurus Rab geranylgeranyltransferase, beta subunit (RABGGTB)	-2,04	2,57E-02
Bos taurus similar to PREDICTED: Homo sapiens similar to mitochondrial translational release factor 1-like (LOC285442)	-1,57	2,49E-02
Bos taurus similar to Homo sapiens zinc finger, X-linked, duplicated B (ZXDB)	-1,58	2,12E-02
Bos taurus interleukin 18 (interferon-gamma-inducing factor) (IL18)	-1,56	4,49E-02
Bos taurus similar to Homo sapiens asparagine-linked glycosylation 6 homolog (S. cerevisiae, alpha-1,3-glucosyltransferase) (ALG6)	-1,62	1,58E-02
Bos taurus similar to Homo sapiens centrosomal protein 170kDa (CEP170), transcript variant alpha	-1,90	2,65E-02
Unidentified transcripts	-1,69	3,12E-02
Bos taurus similar to Homo sapiens LSM14 homolog A (SCD6, S. cerevisiae) (LSM14A)	-1,65	4,45E-02
Bos taurus similar to Homo sapiens hypothetical protein FLJ38482 (FLJ38482)	-1,55	3,47E-02
Unidentified transcripts on BTA26 position 8588986-8588099	-1,95	2,11E-02
Bos taurus similar to Homo sapiens centrosome and spindle pole associated protein 1 (CSPP1)	-1,82	3,14E-02
Bos taurus similar to Homo sapiens zinc finger and BTB domain containing 11 (ZBTB11)	-1,65	1,56E-02
Bos taurus similar to Homo sapiens centromere protein C 1 (CENPC1)	-1,78	3,49E-02
Unidentified transcripts	-1,63	9,74E-03
Bos taurus similar to Homo sapiens polo-like kinase 4 (Drosophila) (PLK4)	-1,51	1,54E-02
Bos taurus similar to Homo sapiens protein tyrosine phosphatase, non-receptor type 12 (PTPN12)	-1,78	2,98E-02
Bos taurus similar to Homo sapiens HIG1 domain family, member 1A (HIGD1A)	-1,66	3,30E-02
Unidentified transcripts on BTA1 position 34676433-34677199	-1,69	3,11E-02
Bos taurus similar to Homo sapiens ubiquitin-activating enzyme E1-like 2 (UBE1L2)	-1,72	1,49E-02
Bos taurus similar to Homo sapiens FUS interacting protein (serine/arginine-rich) 1 (FUSIP1), transcript variant 1	-1,61	1,65E-02
Bos taurus similar to Homo sapiens zinc finger and BTB domain containing 11 (ZBTB11)	-1,58	2,04E-02
Unidentified transcripts	-1,75	3,42E-02
Bos taurus similar to Homo sapiens melanoma antigen family H, 1 (MAGEH1)	-1,64	4,74E-03
Bos taurus similar to Homo sapiens cyclin G2 (CCNG2)	-1,59	3,74E-02
Unidentified transcripts on BTA19 position 28077465-28078450	-1,68	3,88E-02
Bos taurus similar to Homo sapiens suppressor of hairy wing homolog 4 (Drosophila) (SUHW4), transcript variant 3	-1,82	3,47E-02
Bos taurus similar to Homo sapiens succinate-CoA ligase, ADP-forming, beta subunit (SUCLA2)	-1,64	3,95E-02
Unidentified transcripts	-2,01	2,63E-02
Unidentified transcripts on BTA2 position 62168988-62168316	-2,08	4,64E-02
Bos taurus similar to Homo sapiens metaxin 2 (MTX2), transcript variant 1	-1,60	1,56E-02
Unidentified transcripts on BTA12 position 8440118-8440859	-1,74	1,12E-02
Bos taurus similar to Homo sapiens chromosome 1 open reading frame 19 (C1orf19)	-1,53	4,40E-02
Bos taurus similar to Homo sapiens jerky homolog-like (mouse) (JRKL)	-1,70	1,11E-02
Bos taurus similar to Homo sapiens family with sequence similarity 105, member A (FAM105A)	-1,51	5,44E-03
Bos taurus similar to Homo sapiens tetraspanin 13 (TSPAN13)	-1,88	4,28E-02
Bos taurus similar to Homo sapiens splicing factor, arginine/serine-rich 7, 35kDa (SFRS7), transcript variant 2	-1,80	2,89E-02
Unidentified transcripts on BTA24 position 6453162-6452122	-1,51	4,84E-02
Bos taurus similar to Homo sapiens YY1 associated factor 2 (YAF2), transcript variant 1	-1,53	4,63E-02
Bos taurus similar to Homo sapiens armadillo repeat containing, X-linked 2 (ARMCX2)	-1,52	2,73E-02
Bos taurus similar to Homo sapiens nucleoporin like 1 (NUPL1), transcript variant 3	-1,69	4,14E-02
Unidentified transcripts on BTA16 position 37783839-37785410	-1,53	7,81E-03
Bos taurus similar to Homo sapiens protein kinase, interferon-inducible double stranded RNA dependent activator (PRKRA)	-1,52	2,04E-02

Bos taurus similar to Homo sapiens La ribonucleoprotein domain family, member 4 (LARP4), transcript variant 1	-1,54	6,94E-03
Bos taurus similar to Homo sapiens RAS guanyl releasing protein 1 (calcium and DAG-regulated) (RASGRP1)	-1,69	2,31E-02
Bos taurus similar to Homo sapiens solute carrier family 35 (CMP-sialic acid transporter), member A1 (SLC35A1)	-1,59	3,92E-02
PREDICTED: Bos taurus methionyl-tRNA formyltransferase, mitochondrial, transcript variant 1 (MtFMT)	-1,63	6,30E-03
Bos taurus similar to Homo sapiens FGFR1 oncogene partner 2 (FGFR1OP2)	-1,56	4,93E-02
Bos taurus similar to Homo sapiens estrogen receptor binding site associated, antigen, 9 (EBAG9), transcript variant 1	-1,97	1,63E-02
Bos taurus similar to Homo sapiens adhesion molecule with Ig-like domain 2 (AMIGO2)	-1,79	3,71E-02
Bos taurus similar to Homo sapiens N-acetyltransferase 13 (NAT13)	-1,68	4,27E-02
Bos taurus similar to Homo sapiens actin-related protein 10 homolog (S. cerevisiae) (ACTR10)	-1,56	9,12E-03
Bos taurus similar to Homo sapiens metaxin 2 (MTX2), transcript variant 1	-1,53	2,54E-02
PREDICTED: Bos taurus similar to F-box and WD-40 domain protein 12 (LOC528919)	-1,72	1,72E-02
Bos taurus similar to Homo sapiens cytoskeleton associated protein 2 (CKAP2)	-1,74	2,18E-02
Bos taurus similar to Homo sapiens melanoma antigen family H, 1 (MAGEH1)	-1,52	1,35E-02
Unidentified transcripts on BTA23 position 30527455-30526185	-2,14	4,38E-02
Bos taurus similar to Homo sapiens ubiquitously transcribed tetratricopeptide repeat gene, Y-linked (UTY), transcript variant 3	-1,65	1,89E-02
Bos taurus similar to Homo sapiens PTD016 protein (LOC51136)	-1,90	3,77E-02
Bos taurus similar to Homo sapiens small nuclear RNA activating complex, polypeptide 1, 43kDa (SNAPC1)	-1,82	3,19E-02
Bos taurus similar to Homo sapiens ring finger protein (C3H2C3 type) 6 (RNF6), transcript variant 1	-1,66	3,33E-02
Bos taurus similar to Homo sapiens KIAA1715 (KIAA1715)	-1,77	3,66E-02
Bos taurus similar to Homo sapiens family with sequence similarity 76, member B (FAM76B)	-2,01	4,58E-02
Bos taurus similar to Homo sapiens solute carrier family 24, member 5 (SLC24A5)	-1,51	2,92E-02
PREDICTED: Bos taurus similar to glomulin isoform FAP68 (LOC504211)	-1,66	2,38E-02
Unidentified transcripts	-1,62	3,69E-02
Bos taurus similar to Homo sapiens LEM domain containing 3 (LEMD3)	-1,96	1,62E-02
Bos taurus similar to Homo sapiens putative homeodomain transcription factor 2 (PHTF2)	-1,93	3,23E-02
Bos taurus similar to Homo sapiens caspase 6, apoptosis-related cysteine peptidase (CASP6), transcript variant alpha	-1,60	1,48E-02
Unidentified transcripts on BTA19 position 28077465-28078450	-1,68	4,24E-02
Bos taurus similar to Homo sapiens zinc finger protein 639 (ZNF639)	-1,72	1,57E-02
Bos taurus similar to Homo sapiens SNF2 histone linker PHD RING helicase (SHPRH)	-1,79	1,63E-02
Unidentified transcripts	-1,86	1,58E-02
Bos taurus similar to Homo sapiens zinc finger, DHHC-type containing 23 (ZDHHC23)	-1,59	4,06E-02
Bos taurus similar to Homo sapiens zinc finger, BED-type containing 5 (ZBED5)	-1,60	1,31E-02
Bos taurus similar to Homo sapiens SHC SH2-domain binding protein 1 (SHCBP1)	-1,86	8,65E-03
Bos taurus similar to Homo sapiens 15 kDa selenoprotein (SEP15), transcript variant 1	-1,53	4,81E-02
Bos taurus similar to Homo sapiens zinc ribbon domain containing 1 (ZNRD1), transcript variant a	-5,12	1,51E-02
Bos taurus similar to Homo sapiens ADP-ribosylation factor-like 4A (ARL4A), transcript variant 1	-1,80	3,45E-02
Bos taurus ATP synthase, H+ transporting, mitochondrial F0 complex, subunit s (ATP5S)	-1,54	1,96E-02
Bos taurus similar to Homo sapiens B-cell receptor-associated protein 29 (BCAP29), transcript variant 2	-1,62	2,47E-02
Bos taurus similar to Homo sapiens KIAA1799 protein (KIAA1799)	-1,68	1,49E-02
Bos taurus similar to Homo sapiens retinoblastoma binding protein 8 (RBBP8), transcript variant 3	-1,65	4,70E-02
Bos taurus similar to Homo sapiens YEATS domain containing 4 (YEATS4)	-1,87	1,84E-02
Bos taurus similar to Homo sapiens reticulocalbin 2, EF-hand calcium binding domain (RCN2)	-1,92	3,71E-02
Bos taurus similar to Homo sapiens restin-like 2 (RSNL2)	-1,86	1,47E-02
Bos taurus similar to Homo sapiens FERM domain containing 6 (FRMD6), transcript variant 2	-1,79	1,99E-02
Bos taurus similar to Homo sapiens cryptochrome 1 (photolyase-like) (CRY1)	-1,72	2,98E-02
Bos taurus similar to Homo sapiens hypothetical protein LOC552891 (LOC552891)	-1,91	4,30E-02
Bos taurus similar to Homo sapiens chromosome 17 open reading frame 42 (C17orf42)	-1,60	2,77E-02
Bos taurus eukaryotic translation initiation factor 4E (EIF4E)	-2,06	4,02E-02
Unidentified transcripts on BTA19 position 37193021-37193996	-2,72	1,29E-02
Bos taurus similar to Homo sapiens glucosamine-phosphate N-acetyltransferase 1 (GNPNAT1)	-1,76	1,68E-02
Bos taurus similar to Homo sapiens basic leucine zipper and W2 domains 1 (BZW1)	-1,58	4,60E-02
Bos taurus similar to Homo sapiens chromosome 13 open reading frame 1 (C13orf1)	-1,72	2,81E-02
Bos taurus similar to Homo sapiens asparagine-linked glycosylation 6 homolog (S. cerevisiae, alpha-1,3-glucosyltransferase) (ALG6)	-1,61	1,62E-02
Bos taurus similar to Homo sapiens chromobox homolog 3 (HP1 gamma homolog, Drosophila) (CBX3), transcript variant 1	-1,84	4,50E-02
Bos taurus protein phosphatase 1, catalytic subunit, gamma isoform (PPP1CC)	-1,67	4,71E-02
Bos taurus similar to Homo sapiens zinc finger, CCHC domain containing 8 (ZCCHC8)	-1,51	4,07E-02

Bos taurus similar to Homo sapiens RMI1, RecQ mediated genome instability 1, homolog (S. cerevisiae) (RMI1)	-1,70	4,86E-02
Bos taurus similar to Homo sapiens zinc finger protein 555 (ZNF555)	-1,57	4,88E-03
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 135 (C14orf135)	-1,55	3,60E-02
Bos taurus similar to Homo sapiens zinc finger protein 677 (ZNF677)	-1,73	2,33E-02
Bos taurus similar to Homo sapiens ubiquitin specific peptidase 1 (USP1), transcript variant 1	-2,11	3,48E-02
Bos taurus similar to Homo sapiens Nedd4 family interacting protein 1 (NDFIP1)	-1,74	4,74E-02
Bos taurus similar to Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 6 (PSMC6)	-1,79	4,83E-02
Bos taurus similar to Homo sapiens RAS guanyl releasing protein 1 (calcium and DAG-regulated) (RASGRP1)	-1,61	3,72E-02
Bos taurus similar to Homo sapiens 2-aminoadipic 6-semialdehyde dehydrogenase (AASDH)	-1,74	4,37E-02
Bos taurus similar to Homo sapiens WD repeat domain 47 (WDR47)	-1,59	4,84E-02
Bos taurus peroxiredoxin 3 (PRDX3)	-1,61	4,62E-02
Bos taurus similar to Homo sapiens hypothetical protein LOC552891 (LOC552891)	-1,87	4,76E-02
Bos taurus similar to Homo sapiens sprouty homolog 2 (Drosophila) (SPRY2)	-1,57	4,11E-02
Bos taurus similar to Homo sapiens KIAA0776 (KIAA0776)	-1,80	3,07E-02
PREDICTED: Bos taurus similar to breast cancer metastasis-suppressor 1-like (LOC514432)	-1,66	2,57E-02
Bos taurus similar to Homo sapiens tribbles homolog 2 (Drosophila) (TRIB2)	-1,70	4,23E-02
Bos taurus similar to Homo sapiens chromosome 10 open reading frame 78 (C10orf78), transcript variant 2	-1,62	1,66E-02
PREDICTED: Bos taurus similar to glomulin isoform FAP68 (LOC504211)	-1,71	1,70E-02
Bos taurus putative 40-2-3 protein (LOC407149)	-1,78	4,46E-02
Unidentified transcripts on BTA11 position 75855413-75854644	-1,88	3,85E-02
Unidentified transcripts on BTA2 position 646865-647650	-1,50	1,23E-02
Bos taurus similar to Homo sapiens zinc finger protein 354B (ZNF354B)	-1,88	3,70E-02
Bos taurus similar to Homo sapiens zinc finger, RAN-binding domain containing 2 (ZRANB2), transcript variant 2	-1,96	4,48E-02
Bos taurus similar to Homo sapiens nucleosome assembly protein 1-like 5 (NAP1L5)	-1,71	1,67E-02
Bos taurus similar to Homo sapiens cell growth regulator with ring finger domain 1 (CGRRF1)	-1,50	3,72E-02
Bos taurus similar to PREDICTED: Homo sapiens chromosome 6 open reading frame 115 (C6orf115)	-1,61	4,31E-02
Bos taurus similar to Homo sapiens aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase) (ALDH5A1), nuclear gene encoding mitochondrial protein, transcript variant 2	-2,07	6,47E-03
Bos taurus similar to Homo sapiens ADP-ribosylation factor-like 1 (ARL1)	-1,76	1,34E-02
Bos taurus similar to Homo sapiens KRR1, small subunit (SSU) processome component, homolog (yeast) (KRR1)	-1,93	3,53E-02
Bos taurus similar to Homo sapiens HCV F-transactivated protein 1 (LOC401152)	-1,64	8,31E-03
Bos taurus similar to Homo sapiens translin-associated factor X (TSNAX)	-1,85	4,53E-02
Bos taurus similar to Homo sapiens splicing factor, arginine/serine-rich 7, 35kDa (SFRS7), transcript variant 2	-1,76	2,88E-02
Bos taurus similar to Homo sapiens establishment of cohesion 1 homolog 2 (S. cerevisiae) (ESCO2)	-1,72	2,47E-02
PREDICTED: Bos taurus similar to Metal-response element-binding transcription factor 2 (Metal-response element DNA-binding protein M96) (LOC615211), partial mRNA.	-1,96	3,03E-02
PREDICTED: Bos taurus ETAA16 protein (ETAA16)	-1,74	3,18E-02
Bos taurus similar to Homo sapiens zinc finger protein 677 (ZNF677)	-1,75	1,68E-02
Bos taurus similar to Homo sapiens riboflavin kinase (RFK)	-1,83	3,14E-02
Bos taurus similar to Homo sapiens M-phase phosphoprotein 1 (MPHOSPH1)	-1,51	1,21E-02
Unidentified transcripts on BTA8 position 12524872-12525400	-1,68	1,02E-02
Bos taurus similar to Homo sapiens CCAAT/enhancer binding protein zeta (CEBPZ)	-1,82	3,12E-02
Bos taurus similar to Homo sapiens ubiquitin specific peptidase 1 (USP1), transcript variant 1	-2,07	4,77E-02
PREDICTED: Bos taurus leucine-rich glycoprotein homolog, transcript variant 1 (LOC407186)	-1,57	1,12E-02
Unidentified transcripts on BTA12 position 8440118-8440859	-1,60	2,48E-02
Bos taurus similar to Homo sapiens chromosome 14 open reading frame 100 (C14orf100)	-1,61	3,31E-02
Bos taurus similar to Homo sapiens chromosome 10 open reading frame 88 (C10orf88)	-1,57	4,78E-02
Unidentified transcripts on BTA28 position 22915476-22914689	-1,81	3,21E-02
Bos taurus similar to Homo sapiens SMT3 suppressor of mif two 3 homolog 1 (S. cerevisiae) (SUMO1), transcript variant 1	-1,63	4,96E-02
Bos taurus similar to Homo sapiens pyruvate dehydrogenase kinase, isozyme 1 (PDK1), nuclear gene encoding mitochondrial protein	-1,79	3,61E-02
Bos taurus similar to Homo sapiens chromosome 17 open reading frame 42 (C17orf42)	-1,61	3,46E-02
Unidentified transcripts	-1,76	4,82E-02
Bos taurus similar to Homo sapiens adenosine kinase (ADK), transcript variant ADK-long	-1,62	2,41E-02
Bos taurus similar to Homo sapiens SHC SH2-domain binding protein 1 (SHCBP1)	-1,69	1,40E-02
Bos taurus similar to Homo sapiens amidohydrolase domain containing 1 (AMDHD1)	-1,60	1,74E-02
Bos taurus similar to Homo sapiens glomulin, FKBP associated protein (GLMN)	-1,61	1,72E-02

Unidentified transcripts	-1,65	4,90E-02
Bos taurus stearoyl-coenzyme A desaturase (SCD)	-1,71	3,94E-02
Bos taurus similar to Homo sapiens implantation-associated protein (DKFZp564K142)	-1,80	3,70E-02
Bos taurus similar to Homo sapiens selenoprotein K (SELK)	-1,51	4,56E-02
Bos taurus similar to Homo sapiens abhydrolase domain containing 3 (ABHD3)	-1,61	3,69E-02
Bos taurus similar to Homo sapiens cyclin C (CCNC), transcript variant 1	-2,03	4,95E-02
Bos taurus similar to PREDICTED: Homo sapiens chromosome 6 open reading frame 115 (C6orf115)	-1,67	3,76E-02
Bos taurus similar to Homo sapiens phosphoglucomutase 3 (PGM3)	-1,51	2,60E-02
Bos taurus similar to Homo sapiens gamma-glutamyl carboxylase (GGCX)	-2,10	2,30E-02
Bos taurus similar to PREDICTED: Homo sapiens leucine rich repeat containing 58 (LRRC58)	-1,70	4,85E-02
Bos taurus similar to Homo sapiens ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease) (RET)	-1,59	1,72E-02
Unidentified transcripts on BTA10 position 38875547-38874641	-1,59	4,93E-02
Bos taurus similar to Homo sapiens centromere protein A (CENPA), transcript variant 1	-1,54	4,80E-02
Unidentified transcripts	-1,63	4,50E-02
PREDICTED: Bos taurus similar to F-box and WD-40 domain protein 12 (LOC528919)	-1,73	2,06E-02
Bos taurus similar to Homo sapiens zinc finger protein 354B (ZNF354B)	-1,89	4,02E-02
Bos taurus similar to Homo sapiens chromosome 16 open reading frame 69 (C16orf69)	-1,71	3,57E-02
Bos taurus similar to Homo sapiens RWD domain containing 3 (RWDD3)	-1,50	1,45E-02
Bos taurus similar to Homo sapiens Bardet-Biedl syndrome 7 (BBS7), transcript variant 1	-1,54	9,03E-04
Bos taurus similar to Homo sapiens ATP binding domain 1 family, member C (ATPBD1C)	-1,62	3,14E-02
Bos taurus similar to Homo sapiens tigger transposable element derived 2 (TIGD2)	-1,66	4,38E-02
Unidentified transcripts on BTA16 position 55177278-55176043	-1,77	3,79E-02
Bos taurus similar to Homo sapiens armadillo repeat containing, X-linked 5 (ARMCX5)	-1,85	1,14E-02
Bos taurus similar to Homo sapiens KIAA1143 (KIAA1143)	-1,59	4,65E-02
Bos taurus similar to Homo sapiens chromosome 10 open reading frame 78 (C10orf78), transcript variant 2	-1,56	3,84E-02
Bos taurus UDP-glucose dehydrogenase (UGDH)	-1,72	2,97E-02
Unidentified transcripts on BTA16 position 36342621-36343363	-1,79	3,49E-02
Bos taurus similar to Homo sapiens BET1 homolog (S. cerevisiae) (BET1)	-1,69	3,29E-02
Bos taurus similar to Homo sapiens transmembrane protein 41B (TMEM41B)	-1,74	1,07E-02
Unidentified transcripts	-1,68	3,58E-02
Unidentified transcripts	-1,59	3,77E-02
Bos taurus similar to Homo sapiens density-regulated protein (DENR)	-1,78	3,11E-02
Bos taurus similar to Homo sapiens HCF-binding transcription factor Zhangfei (ZF)	-1,89	3,67E-02
Unidentified transcripts on BTA4 position 22178234-22177508	-1,86	3,08E-02
Bos taurus similar to Homo sapiens KIAA1715 (KIAA1715)	-1,69	4,64E-02
Bos taurus similar to Homo sapiens MIS12 homolog (yeast) (MIS12)	-1,50	2,99E-02
Bos taurus similar to Homo sapiens quiescin Q6-like 1 (QSCN6L1)	-1,60	4,44E-02
Bos taurus similar to Homo sapiens family with sequence similarity 76, member B (FAM76B)	-1,89	4,89E-02
Bos taurus similar to Homo sapiens glucosamine-phosphate N-acetyltransferase 1 (GNPNAT1)	-1,71	1,69E-02
Bos taurus similar to Homo sapiens ankyrin repeat domain 32 (ANKRD32)	-1,57	2,35E-02
Bos taurus similar to Homo sapiens inhibitor of Bruton agammaglobulinemia tyrosine kinase (IBTK)	-1,68	3,75E-02
Bos taurus similar to Homo sapiens tetraspanin 13 (TSPAN13)	-1,86	4,78E-02
Bos taurus similar to Homo sapiens DEAH (Asp-Glu-Ala-His) box polypeptide 29 (DHX29)	-1,51	3,32E-02
Bos taurus similar to Homo sapiens NIMA (never in mitosis gene a)-related kinase 1 (NEK1)	-1,58	1,93E-02
Unidentified transcripts	-1,63	2,33E-02
Bos taurus similar to Homo sapiens NIMA (never in mitosis gene a)-related kinase 1 (NEK1)	-1,53	2,32E-02
Unidentified transcripts on BTA10 position 32441205-32440485	-1,76	2,92E-02
Bos taurus similar to Homo sapiens ADP-ribosylation factor-like 1 (ARL1)	-1,68	2,60E-02
Bos taurus similar to Homo sapiens hepatitis A virus cellular receptor 2 (HAVCR2)	-1,61	2,17E-02

Appendix D.

1: List of primers used to validate the DHEA induced gene expression in bovine livers microarray study.

Gene	RPL19
Target / reference	Reference
Sense primer sequence	AAGCCTGTGACTGTCCATTCCC
Anti-sense primer sequence	TGCGCGCCTTGTCTGCCTTC
Amplicon length (bp)	298

Gene	CYP1A1
Target / reference	Target
Sense primer sequence	CCGACCTCTACAGCTTCAC
Anti-sense primer sequence	GCCTCCTTGTTCACATGCTC
Amplicon length (bp)	180

Gene	CYP11A1
Target / reference	Target
Sense primer sequence	CCCTGGCTGGCCTATCACC
Anti-sense primer sequence	TGACGAAGTCCTGAGACACTGG
Amplicon length (bp)	166

Gene	PRLR
Target / reference	Target
Sense primer sequence	AAGGAAGGAGAAACACTCATCCA
Anti-sense primer sequence	AGGTTTGCAGGAGGCTCTG
Amplicon length (bp)	

Gene	HSD17B7
Target / reference	Reference
Sense primer sequence	AGTGTGCCCAGGTACGATGC
Anti-sense primer sequence	TGTGAGGTGTCAACGTGAAAGC
Amplicon length (bp)	128

Gene	IGF1
Target / reference	Target
Sense primer sequence	TGTCCACCCTTGAGAATCCTTCC
Anti-sense primer sequence	ACAATAGCACCACATTGGCATAGC
Amplicon length (bp)	188

Gene	GHR
Target / reference	Target
Sense primer sequence	CCAGTTTCCATGGTTCTTAATTAT
Anti-sense primer sequence	TTCCTTTAATCTTTGGAACTGG
Amplicon length (bp)	136

Gene	CYP2C9
Target / reference	Target
Sense primer sequence	AGGAAGAAATTGATCGTGTGGTTGG
Anti-sense primer sequence	GAGATGTTAATATGACTGTGCCCTTGG
Amplicon length (bp)	201

2: List of primers used to validate the DHEA induced gene expression in bovine blood microarray study.

Gene	MRPL53
Target / reference	Reference
Sense primer sequence	GTCTTACCATCACTCACTGCTTAACC
Anti-sense primer sequence	GACAAGGGACCCGAGAGGAAG
Amplicon length (bp)	172

Gene	FIBP
Target / reference	Target
Sense primer sequence	TCGTCTTCTTTGCCAACAATCGC
Anti-sense primer sequence	TCCATGTCCATGTCATCCACCTG
Amplicon length (bp)	148

Gene	DHCR24
Target / reference	Target
Sense primer sequence	TGTGCCTCTTCCTCCTACCTCTC
Anti-sense primer sequence	GTCCACCTCCAGAATGTCCATCAG
Amplicon length (bp)	272

Gene	IGF2BP3
Target / reference	Target
Sense primer sequence	GAACACTGACTCGGAAACTGCTG
Anti-sense primer sequence	ACGGCTTCTGCTTGGACACTG
Amplicon length (bp)	243