**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Differential expression of genes related with meat tenderness in
Nellore cattle**

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Dissertation presented to obtain the Master's degree
in Science. Field: Animal Science and Pastures

**Piracicaba
2015**

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Differential expression of genes related with meat tenderness in Nellore cattle
versão revisada de acordo com a resolução CoPGr 6018 de 2011

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DEDICATION

I dedicate this work to my father Mauricio and my mother Silvia who have always given me examples, strength, support, love, and have always encouraged me during all my life.

*and I OFFER this work to:
Those who will always be examples to me: my grandparents
Osana, Eró (in memoriam), Julva and Silvio (in memoriam).*

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“Your time is limited, so don’t waste it living someone else’s life. Don’t be trapped by dogma – which is living with the results of other people’s thinking. Don’t let the noise of other’s opinion drown out your own inner voice. And most important, have the courage to follow your heart and intuition. They somehow already know what you truly want to become. Everything else is secondary.”

Steve Jobs

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RESUMO

Expressão diferencial de genes relacionados com maciez da carne em bovinos da raça Nelore

A qualidade da carne bovina no Brasil é importante tanto para o consumidor, como para a indústria alimentícia devido à alta competitividade e exigência do mercado nacional e internacional. Portanto, é necessário o desenvolvimento de pesquisas para melhorar a qualidade da carne bovina da raça Nelore (*Bos indicus*), principalmente a maciez, que é considerada uma das principais responsáveis por agregar valor à carne. Tecnologias de nova geração proporcionam informações precisas, rápidas e baratas de todo genoma, mostrando grande vantagem em relação aos métodos convencionais de sequenciamento e de estudos de expressão gênica. Essas novas tecnologias geram um grande volume de dados, sendo necessário o uso de ferramentas de bioinformática para realizar as análises de sequenciamento e ter uma maior compreensão de mecanismos biológicos de regulação, controle celular, interações gênicas, entre outras aplicações. Em um estudo prévio, foram coletadas amostras do músculo *Longissimus dorsi* de 790 animais da raça Nelore e foram realizadas avaliações da força de cisalhamento 24 horas após abate, e com sete e 14 dias de maturação. Com o objetivo de identificar genes diferencialmente expressos (DE), foram selecionadas no total 34 amostras de animais da raça Nelore com valores extremos de valor genético estimado (EBV) para força de cisalhamento (SF), e sequenciados pelo método de sequenciamento de RNA (RNA-Seq) (*Illumina HiScanSQ*). Neste estudo foi realizado o processamento dos dados gerados pelo RNA-Seq através dos softwares QuasiSeq e Cuffdiff. Foram encontrados 22 genes DE para as análises do QuasiSeq e 113 genes DE para as análises do Cuffdiff. Para melhor compreensão dos processos biológicos envolvidos na maciez da carne, análises integrativas identificaram possíveis reguladores que podem explicar a atividade de regulação transcricional neste processo utilizando os métodos do Coeficiente de Correlação Parcial com Teoria da Informação (PCIT), Fator de Impacto Fenotípico (PIF) e Fator de Impacto Regulatório (RIF). Os genes encontrados nas análises do PCIT *USP2*, *GBR10*, *ANO1* e *TMBIM4*, assim como os microRNAs encontrados nas análises do RIF, *bta-mir-133a-2* e *bta-mir-22* e os genes de maior valor de PIF *MB*, *ENO3*, *CA3* podem ser fundamentais para desvendar os complexos mecanismos moleculares que controlam a maciez da carne na raça Nelore.

Palavras-chave: *Bos indicus*; Força de cisalhamento; Transcriptoma; RNA-Seq; QuasiSeq; Redes

ABSTRACT

Differential expression of genes related with meat tenderness in Nellore cattle

Beef quality in Brazil is important for both consumers and the food industry due to high demand and competitiveness in the domestic and international markets. Therefore, it is necessary to develop research to improve beef quality of Nellore cattle (*Bos indicus*), mainly tenderness, one of the main features to add value to meat. New-generation technologies provide accurate, rapid and inexpensive information on the entire genome, showing great advantage over conventional methods for sequencing and gene expression. However, these new technologies generate large database, which require the use of bioinformatics tools for data analyses of sequencing and for a better understanding of biological regulation mechanisms, cellular control, gene interactions, among other applications. In a previous study, samples were collected from the *Longissimus dorsi* muscle of 790 animals from Nellore cattle and shear force assessments were made 24 hours after slaughter, with seven and 14 days of aging. Aiming to identify differentially expressed (DE) genes, 34 samples from Nellore animals with extreme levels of estimated breeding value (EBV) for shear force (SF) were selected, sequenced by the method of RNA sequencing (RNA-Seq) (*Illumina HiScanSQ*). This study performed the processing of data generated by RNA-Seq using software QuasiSeq and Cuffdiff. In the QuasiSeq analysis, 22 DE genes were found, while in the Cuffdiff analysis, 113 DE genes were found. To better understand the biological process involved in meat tenderness, integrative analysis identified possible regulators that can explain the activity of transcriptional regulation in this process using partial correlation coefficient with information theory (PCIT), phenotypic impact factor (PIF) and regulatory impact factor (RIF) methods. The genes found in the PCIT analysis *USP2*, *GBR10*, *ANO1* and *TMBIM4*; microRNAs found in RIF analysis *bta-mir-133a-2* and *bta-mir-22*, and the genes with high PIF value *MB*, *ENO3*, *CA3* could be fundamental to unravel the complex molecular mechanisms that control the meat tenderness in Nellore cattle.

Keywords: *Bos indicus*; Shear force; Transcriptome; RNA-Seq; QuasiSeq; Cuffdiff; Networks

1 INTRODUCTION

Meat tenderness is an important economic trait for the meat sector worldwide, mainly to countries such as Brazil, which has the largest commercial herd and is one of the largest beef exporter of the world (CARVALHO et al., 2014). Despite the prominent position of the beef cattle industry in the economic scenario, Brazil still exports low added-value meat (PAZ; LUCHIARI FILHO, 2000). This might be explained because *Bos indicus* (zebu) cattle have different genetic potential for some characteristics, such as meat quality, mainly meat tenderness, compared to *Bos taurus* (CROUSE et al., 1989; CARVALHO et al., 2014).

Approximately 80% of the Brazilian cattle are *Bos indicus* and Nellore breed accounts for 90% of this species (CARVALHO et al., 2014). Nellore is largely used due to its adaptability to tropical environments, namely high heat tolerance, small body, resistance to ectoparasites, endoparasites and to other tropical diseases (CANAVEZ et al., 2012). The Brazilian production of Nellore cattle mostly occurs on pasture because of low costs of beef production (CANAVEZ et al., 2012). Despite the advantages to produce Nellore cattle in Brazil, studies on beef tenderness are still necessary. First, because Zebu meat is not considered as tender as that of *Bos taurus* (CROUSE et al., 1989; CARVALHO et al., 2014). Second, meat tenderness is the most important palatability trait for consumers (PAZ; LUCHIARI FILHO, 2000). Finally, biological mechanisms involved with this trait are not completely understood (OUALI et al., 2013).

For complex traits such as meat tenderness, the next-generation sequencing (NGS) technologies have allowed studies on transcriptome, which might identify differentially expressed (DE) genes and biological pathways involved with the phenotype (METZKER, 2010). RNA Sequencing (RNA-Seq) allows the analysis of differential expression profile through the abundance of transcripts with high sensitivity to transcripts that could not be detectable by microarray (WERNER, 2010). The use of RNA-Seq also improves genome annotation and allows the discovery of new biological functions. This approach provides valuable information on changes and new alternative splices, reflecting in more information about the complex mechanisms for regulating RNA (WANG; GERSTEIN; SNYDER, 2010).

Bioinformatic and statistical tools are used to analyze the large list of genes and differentially expressed genes generated by RNA-Seq. However, biological interpretation of the RNA-Seq results is still a challenge. The integration of

information from genes, proteins, metabolites and cellular processes become complex to connect the molecular and cellular world (BARABÁSI; OLTVAI, 2004).

An integrative analysis allows a better understanding of biological processes involved in meat tenderness. The study on biological interaction networks is called systems biology (BARABÁSI; OLTVAI, 2004). The PCIT (Partial Correlation Information Theory) (REVERTER et al., 2006) is a method that shows the difference in the specific behavior or co-expression of targeted pairs of genes and the quantification of a gene differential connectivity. The PCIT finds connectors and differential magnitude of each connection, even when correlation is weak in the network.

Complementary analyses, based on the transcription factors (TF), play a fundamental regulatory role in the study on the control of gene expression (HUDSON; REVERTER; DALRYMPLE, 2009b; REVERTER et al., 2006). This analysis gives the absolute co-expression correlation averaged for all genes in a given module. HUDSON and colleagues (2009) used this approach and observed that a gene, not differentially expressed, contained the causal mutation. Thus, the regulatory impact factor (RIF) algorithm is a possible effective method to find genes that account for the functional activation of TF rather than differential expression alone. This methods gives a more complete interpretation of gene expression data (REVERTER et al., 2010).

Another method that integrates the results of differential expression with RIF is the phenotypic impact factor (PIF), which allows to evaluate the importance of each differentially expressed gene to the differences related with phenotypes (REVERTER et al., 2010).

This is the first study on meat tenderness using Nellore cattle with a RNA-Seq approach to analyze transcriptome of the *Longissimus dorsi* (LD) muscle from selected animals. Animals were selected according to extreme values for tenderness with estimated breeding values (EBV) for shear force (SF) at seven and 14 days of aging. The aim was to identify DE genes and clarify mechanisms of beef tenderness in LD muscle of Nellore cattle using RNA-Seq. In addition, new methods of systems biology analysis provided a better understanding of the role of other genes by integrating data with DE genes to elucidate the process of meat tenderness.

2 LITERATURE REVIEW

2.1 Importance of cattle

With approximately 211 million heads, according to the Brazilian Institute of Geography and Statistics (IBGE, 2013), Brazil has the largest commercial herd in the world and is the largest exporter of fresh beef, which makes cattle one of the main Brazilian agribusiness sector in the world scenario.

Beef cattle is one of the major productive activities of Brazilian agribusiness, providing the highest income compared to other supply chains. In a study conducted by the Center for Advanced Studies in Applied Economy of ESALQ/USP (CEPEA), agribusiness accounts for 23% of the Brazilian Gross Domestic Product (GDP), contributing to greater income distribution and poverty reduction, reflecting the economic and social importance of cattle in our country (CEPEA, 2015). According to the Ministry of Agriculture, Livestock and Supply (MAPA), 34.4 million heads were slaughtered in 2013, and just in the second quarter of 2014, 8.5 million heads were slaughtered across the country, and the states of Mato Grosso, Mato Grosso do Sul, Goiás, São Paulo, Minas Gerais, Pará and Rondônia are the leaders in slaughters (MAPA, 2014).

MAPA projections 2022/23 show a promising scenario, supplying 200 million of Brazilians annually supplied and Brazil will generate export surplus to approximately 200 countries. In 2014, beef cattle exports were allocated to 65 countries, with Russia as the major importer. These projections for the meat market in Brazil show that the sector should provide strong growth in the few years. Regarding livestock, the higher projections of production growth in 2013-2023 are chicken, with an expected growth of 3.9% annually, and cattle, with projected growth of 2.0% per year for the same period. These rates correspond to increases of 22.5% in beef production between 2013 and 2023. Consumption projections also show the preference of Brazilian consumers for beef. The projected growth for meat consumption is 3.6% per year in the period of 2013-2023, which means an increase of 42.8% in consumption over the next 10 years (MAPA, 2013).

These data and statistical predictions highlight the great importance of cattle sector not only in Brazil, but also in the international market. However, Brazil still needs to improve characteristics related to meat quality to offer products with higher value added in the world market (PAZ; LUCHIARI FILHO, 2000). Zebu breeds have different genetic potential parameters. To improve their recognition in the

international market, the use of genomics and transcriptomics, implemented with an animal breeding program, could be a great strategy to produce tender meat in zebu, using tools to select animals with better performance taking into account the additive genetic effects.

2.2 Meat Tenderness

Because of the prominent position of beef cattle in the national economic scenario, Brazil has to produce high quality meat. Meat quality traits, namely sensory characteristics (tenderness, flavor, juiciness and color), are extremely important, and tenderness is considered the main organoleptic characteristics desired by consumers (PAZ; LUCHIARI FILHO, 2000).

There are two main factors that influence tenderness: amount of connective tissue (mainly collagen), and the myofibrillar constituent. RENAND and colleagues (2001) showed that less than a third to a quarter of the variability in tenderness and flavor could be explained by variability in muscle characteristics in the living animal. In the *post-mortem* the myofibrillar proteolysis process by calpains (*CAPN*) is considered the main biological process involved in muscle conversion in meat (MALTIN et al., 2003).

Calpastatin (*CAST*), calpain inhibitor protein, is fundamental in the proteolytic activity regulation during *post-mortem* (KOOHMARAIE, 1988). High calpastatin action results in reduced protein degradation (MORGAN et al., 1993). The tenderness system and proteolysis inhibition performed by calpain and calpastatin, respectively, are considered the most important targets in research to understand the complex mechanism related to meat tenderness (MORGAN et al., 1993).

The meat aging process is performed by physicochemical factors (pH, osmotic pressure, calcium ions and oxidative processes), enzymes without peptidase activity (glycolytic enzymes, ATPases and glycosidases) and peptidases (cathepsins, calpains, muscle endopeptidases complexes) (KOOHMARAIE et al., 1996), resulting in tenderness and in the development of the flavor trait. The aging time varies from seven to 21 days according to the desired result. Prolonged periods of aging confer tenderness but taste and odor become more pronounced, which may dissatisfy some consumers (KOOHMARAIE et al., 1996).

According to KOOHMARAIE (2003), meat tenderness is a phenotypic trait influenced by genetic factors, environment and genotype-environment interaction,

measured by shear force (WHEELER et al., 2005). The heritability average for beef tenderness is about 0.3 (KOOHMARAIE, 2003), and the use of selection by genetic markers can improve this feature, especially because some of the phenotypes associated with meat quality such as tenderness, taste and color are difficult to be measured directly, but only after the slaughter of animals (REZENDE, 2010).

The transcriptome study identify important genes. BERNARD and colleagues (2007) in a study with microarrays, identified differentially expressed genes related to high and low meat quality in young Charolais. They identified 615 differentially expressed genes for meat tenderness, 1005 genes that differed in expression for juiciness, 799 genes that showed significant differences in expression between the more and less tasty meats and 215 genes were differentially expressed in terms of tenderness, juiciness, and/or taste. The author found that muscle mass in the carcass and oxidative metabolism could be responsible for the variability in tenderness. In addition, the presence of heat shock proteins and anti-apoptotic processes were related to less tenderness.

2.3 Genomic and transcriptomic in cattle

Cattle has an important role in the evolution of agriculture and livestock since the beginning of civilization and are fundamental species of domesticated animals to the economy (CUNNINGHAM; SYRSTAD, 1987). Milk and meat production is the major supply for food industries in the country. There are approximately 800 cattle breeds, but some of them are under threat of extinction because of agricultural practices (CUNNINGHAM; SYRSTAD, 1987).

The cow is a model organism for research on human health, such as obesity and infectious diseases, or in studies on endocrinology, physiology and reproduction (ROSA; FRAGOSO, 2010). The use of genomics and transcriptomics improves the understanding of cattle, such as studies on the digestive system evolution, complex interactions between the microbiota of the digestive system and host, embryonic development, studies on mammary gland, lactation physiology and resistance to diseases, such as mastitis (ROSA; FRAGOSO, 2010).

Animal breeding allows inferring the genetic variability between individuals or breed and identifying superior individuals before they express their characteristics in order to obtain higher genetic gain (ROSA; FRAGOSO, 2010). The use of genomic information as an additional tool in animal breeding generated a new era in animal

science. Previously, most systems used to determine genetic values were based on progeny tests involving phenotypic records analysis in a large number of animals to determine the superiority of male and female reproduction. These studies were very time consuming, each bull should have thousands of offspring and to assess the quality traits such as milk production, it was necessary to wait for daughters of sires to reach puberty to begin the production, which demands time and money. Currently with the use of genomic, there is significant increase in the genetic gain by increasing selection accuracy. Genotyping methods have greatly improved breeding efficiency, producing the double with half of animals used in previous progeny tests (ROSA; FRAGOSO, 2010). In a few years, the bovine genome progressed from mapping protein-coding genes to the complete genome sequencing.

The first cattle genome assembly produced by the Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC) and colleagues was based on a Hereford cow in 2009, with a cover of 7.1 x. This genome increased the number of studies using QTL mapping in cattle associated with genomic characteristics (CHILDERS et al., 2011; ELSIK et al., 2009). The *Bos taurus* genome is about 3000 MB, organized into 29 pairs of autosomes and two sex chromosomes. The whole genome sequencing, including assembly, analysis and annotation had a total cost of US\$ 54 million in six years of work (CHILDERS et al., 2011; ELSIK et al., 2009).

Bovine transcriptome sequencing is relatively new and helps to elucidate complex studies on genes and improve research on genetic and animal breeding. Cattle research developed faster than other species in animal production. Studies on bovine transcriptome have identified 22,000 genes, with high levels of conservation in their structure. Various genetic markers already found for cattle are related to traits such as lactation and metabolism (ROSA; FRAGOSO, 2010), embryo transcriptome (HUANG; KHATIB, 2010) and immune responses to infections (NALPAS et al., 2013).

2.3 RNA-Seq

The mRNA, RNA non-coding, and small RNAs are transcript species present in the cell that form the transcriptome and are involved in gene expression levels under different conditions (WANG; GERSTEIN; SNYDER, 2010).

Microarray is a method based on the hybridization for the study of gene expression that uses labeled cDNA to hybridize to different genome tissues

(CHURCHILL, 2002). Although relatively inexpensive, this method has some limitations, such as prior knowledge of the genome to be studied, limited detection rate due to signal saturation and difficult normalization (OKONIEWSKI; MILLER, 2006; WANG; GERSTEIN; SNYDER, 2010). Other technologies for expression studies have been developed providing accurate information and high yield, however, they are based on Sanger sequencing, which makes the analysis expensive. In addition, many small fragments do not map uniquely into the genome (HARBERS; CARNINCI, 2005; WANG; GERSTEIN; SNYDER, 2010).

Despite being restricted to the analysis of genes represented on the chip, microarray techniques have been successfully used to obtain genes differentially expressed in muscle tissues from various species. Microarray allows monitoring the expression of hundreds to thousands of genes along myogenesis in pigs (SEO; BEEVER, 2001; ERNST et al., 2002; BAI et al., 2003; TE PAS et al., 2005, CAGNAZZO et al., 2006), and elucidate growth control mechanisms and meat quality traits (PONSUKSILI et al., 2008a, 2008b). In cattle, studies on *Longissimus dorsi* transcriptome showed that it is possible to obtain information on biological processes in the muscle and meat aging (BERNARD et al., 2005).

However, microarrays are based on prior knowledge and oligonucleotide design. This technique cannot detect transcripts with few copies or provide complete coverage of the genome due to the lack of available probes. In cattle, probes are based on the sequencing of the *Bos taurus* genome, which means that probe polymorphisms can hinder hybridization with Nellore cDNA. In addition, microarrays do not provide information on alternative splices and new transcripts (MARTIN; WANG, 2011).

RNA sequencing (RNA-Seq) is replacing the microarray methods in gene expression studies. This new technology depends on the combination of sample preparation, sequencing, graphical representation, alignment and transcriptome assembly methods (METZKER, 2010). The ability to sequence the entire transcriptome allows the comparison of many organisms in large scale and evolutionary studies that can be performed and that were unimaginable some years ago.

RNA-Seq improves genome annotation, makes new discoveries of biological functions, and provides valuable information on variations of alternative splices and new splices, resulting in more complex regulatory mechanisms of RNA (MARTIN;

WANG, 2011). It starts with the construction of a cDNA library, mRNA is fragmented and after processing in double-stranded cDNA, adapters are fixed at one end (single-end) or both ends (paired-end). Fragments may range from 30-400 bp depending on the equipment used by the enterprise. Small RNAs can be sequenced after connecting the adapters, but larger RNAs must be fragmented to 200-500 bp, which can be done by sonication (WANG; GERSTEIN; SNYDER, 2010). After sequencing, reads generated are used for alignment against a reference genome or in a new assembly, from own transcripts to generate a new transcriptome and/or see the expression of each gene. The study of transcriptome requires to construct a quality cDNA library with biological replicates to facilitate the analysis of results and avoid PCR artifacts (WANG; GERSTEIN; SNYDER, 2010).

The first step after obtaining the data is the quality control that shows the quality of each base sequenced. The results of quality control allows filtering and removing possible contaminants. The next step is to align reads against a reference genome or assemble them into contigs and align against the sequence obtained. An alignment problem is that many reads map in multiple sequences of the genome. The paired-end strategy, in which both ends of the fragment are sequenced, is a more precise technique that facilitates to reduce the number of alignment errors (WANG; GERSTEIN; SNYDER, 2010).

Another advantage of RNA-Seq is to find splices variants. To examine splicing, the poly-A tails and exon-exon junctions should be observed because they are removed during the process. It is still hard to find methods to detect splicing between two distant sequences or in different genes. To find splicing variants or rare transcripts, it is necessary to increase coverage of sequencing. In addition, the RNA-Seq technique can review annotations of genes, and 5' and 3' limits can be mapped (WANG; GERSTEIN; SNYDER, 2010). The RNA-Seq depth coverage allows detection of gene expression at low cost, if considered the large number of data generated, which potentially facilitates proteomic studies and comparisons of peptides found by mass spectrometry with their respective gene sequence. The RNA-Seq has also been used as an efficient method to identify SNPs in transcribed regions from different species (CHEPELEV et al., 2009; CIRULLI et al., 2010; CLOONAN et al., 2008; MORIN et al., 2008). Many studies were successful using RNA-Seq for differential expression in various applications (DRIVER et al., 2012; HO

et al., 2012; MCCABE et al., 2012; MORTAZAVI et al., 2008). However, there is still a challenge in bioinformatics to analyze and understand these data types.

3 OBJECTIVES

3.1 General Objective

To identify differentially expressed genes related to meat tenderness in a Nellore population.

3.2 Specific Objectives

- 1) To identify differentially expressed genes in the muscle tissue of animals with extreme estimated breeding values for shear force.
- 2) To identify possible regulators involved in the meat tenderness process.

3.3 Hypothesis

Differences in the gene expression pattern between contrasting animals for meat tenderness may indicate genes and biological mechanisms involved with this phenotype.

4 MATERIAL AND METHODS

4.1 Animals and phenotypic data

Three hundred and ten Nellore steers from 34 selected Nellore sires that represent the main breeding lineages used in Brazil according to the National Summary of Nellore (from the Brazilian Association of Zebu Breeders (ABCZ) and Brazilian Agricultural Research Corporation (EMBRAPA)) were used in this study. According to CESAR et al. (2014), animals were raised in feedlots under identical nutrition systems and handling conditions until slaughter at an average age of 25 months (CESAR et al., 2014). Samples of *Longissimus dorsi* (LD) muscle between the 12th and 13th ribs were collected and frozen in liquid nitrogen immediately after slaughter and stored at -80°C for mRNA analysis. For the study of meat quality, 2.54 cm thick steaks were collected 24 hours after slaughter to evaluate traits such as carcass weight, dressing percentage, fat thickness, rib-eye area, carcass length, color, texture and marbling score.

For the study of meat tenderness, the methodology described by WHEELER et al. (1995) was used to obtain values from TA XT2i (Stable Micro Systems Ltd., Surrey, UK) texture analyzer coupled to a Warner-Bratzler blade with 1.016 mm thickness in 24 hours, seven days and 14 days after slaughter. Samples were stored at 2°C (TIZIOTO et al., 2013a) and previously thawed at 4°C for 24 hours before cooking until reaching the internal temperature of 70°C. After cooling, eight cylinders with approximately 1.27 cm in diameter were removed from each steak parallel to the orientation of muscle fiber.

Shear force estimated breeding values (EBVSF) were computed using standard BLUP procedures under an animal model (HENDERSON, 1972; MRODE, 2005) using the mixed procedure. Animals were ranked on EBVSF at seven (EBVSF7) and 14 days (EBVSF14) of aging to adjust for fixed effects. Two groups (high (H) and low (L)) of 17 animals with extremes values for EBVSF14 were selected. The mathematical model used in this analysis was $\mathbf{Y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}$ with 284 animals in the matrix of relationship ($\mathbf{A}$). The $\mathbf{Y}$ is the phenotype of interest (dependent variable), $\mathbf{X}$ is the incident matrix of fixed effects in $\mathbf{b}$. The fixed effects were contemporary group (slaughter group and birth season) and age as linear covariate. $\mathbf{Z}$ is the incident matrix of random animal effects in $\mathbf{u}$, where $\mathbf{u} \sim N(0, A\sigma_a^2)$ for SF at seven days (SF7) and SF at 14 days (SF14) of aging. Random animal effects was the additive genetic effect. The $\mathbf{e}$ is the vector of random residuals, $NID(0, I\sigma_e^2)$.

Variance component was conducted by restricted maximum likelihood (REML) method (PATTERSON; THOMPSON, 1971) with an animal model in another Nellore herd (n=2,500) (data not published).

4.2 RNA Extraction, Library and RNA-Seq

Total RNA extraction from 34 samples of *Longissimus dorsi* muscle (100 mg) from animals that presented extreme values of EBV values was performed using 1mL of Trizol reagent (Life Technologies, Carlsbad, CA, U.S.A.). The RNA extracted was quantified using a spectrophotometer (NanoDrop 200 - Thermo Scientific. Wilmington. Delaware USA). The integrity of the material was verified using 1% of agarose gel and 2100 Bioanalyzer (Agilent Technologies - Santa Clara, CA, USA). The integrity of total RNA (RIN) used was greater than or equal to eight.

The average size of cDNA libraries was estimated using the Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA) and quantified using quantitative PCR with the KAPA Library Quantification kit (KAPA Biosystems, Foster City, CA, USA). Then, samples were pooled, diluted to 17 pM and clusterized on the sequencing Flowcell by the cBOT (Illumina - San Diego, USA) using the TruSeq PE Cluster Kit v3-cBOT-HS kit. Samples were sequenced using a TruSeq SBS Kit v3-HS (200 cycles) and the HiScanSQ sequencer (Illumina San Diego, CA, USA) in the University of São Paulo, Genomics Center at ESALQ, Piracicaba, São Paulo, Brazil. The 34 libraries were barcoded, multi-plexed, and sequenced in seven lanes. A read was defined as a 100 bp cDNA fragment sequenced from a paired end.

4.3 Mapping, Counting Reads

After sequencing, data quality was evaluated with FastQC [<http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/>]. The software Seqclean [<https://bitbucket.org/izhbannikov/seqclean/downloads>] was used with 26 Phred quality parameters for maximum average error, followed by removal of less than 65bp. Vector and adaptor sequences from the Univec database [<https://www.ncbi.nlm.nih.gov/tools/vecscreen/univec/>] were used as guide to remove possible contaminants. Three samples had a low number of reads, possibly due to some issues during library preparation; therefore, they were not used in the analyses of differential expression. TopHat 2.0.10 (TRAPNELL; PACHTER; SALZBERG, 2009) and Bowtie2 v2.1.0 (LANGMEAD et al., 2009) was performed to align the

reads against UMD3.1 *Bos taurus* masked genome available at Ensembl [http://www.ensembl.org/Bos_taurus/Info/Index/]. A maximum of one mismatch was allowed and reads with non-unique mapping were discarded. Also, a reference-guided assembly was performed using Cufflinks v2.1.1 (TRAPNELL et al., 2010) using the RABT option to identify novel transcripts and the resulted file was combined with the GTF file. To quantify gene expression, the read counting was made using HTSeq v0.5.4p2 [<http://www-huber.embl.de/users/anders/HTSeq/doc/count.html>], with the model “nonempty intersection” that considers reads aligned in more than one gene such as ambiguous, and were not counted.

4.4 Differentially Expressed Genes using Cuffdiff

A different filtering was used for the Cuffdiff analysis with Python scripts to trim and filter reads and improve read quality using Phred score above 30 and reads with 95pb. Reads were mapped to the reference genome masked (UMD3.1, ENSEMBL) using the same software programs. A maximum of one mismatch was allowed and reads with non-unique mapping were discarded. To calculate the expression levels of each transcript, the Cufflinks software (LANGMEAD et al., 2009) was used for counting the fragments that map each transcript and then normalize this count by the length of each transcript comparing the FPKMs (fragments per kilo base of exon per million fragments mapped). Cufflinks and Cuffdiff implement a linear statistical model to estimate an assignment of abundance for each transcript that explains the observed reads by maximum likelihood (TRAPNELL et al., 2010). The methodology of BENJAMINI-HOCHEBERG (1995) was used to control false discovery rate (FDR) at 10%.

4.5 Differentially Expressed Genes using QuasiSeq

The differential expressed (DE) gene analysis was performed using QuasiSeq, a R statistical package (LUND et al., 2012). Other statistical software programs also support the analysis of these data such as DESeq2 (LOVE; HUBER; ANDERS, 2014), DESeq (ANDERS; HUBER, 2010), edgeR (ROBINSON; MCCARTHY; SMYTH, 2010) and Cuffdiff (TRAPNELL et al., 2010). The QuasiSeq is based on quasi-likelihood methods with estimates of dispersion based on adaptation of SMYTH (2004) for estimating error variation in gene-specific microarray data. The

method is similar to variance analysis and estimate FDR rates by a variety of simulations calculated with actual data. QuasiSeq allows adding effects to the original GLM model and applying the QLSpline method (LUND et al., 2012) to quasi-negative binomial models. The negative binomial distribution was chosen because of the flexibility in modeling variances (LUND et al., 2012).

Prior to analysis, raw count data was processed using three filters. First, all transcripts with zero counts were removed from all samples as these genes were assumed to be unexpressed in skeletal muscle. Second, all transcripts were required to have at least two reads to remove very lowly expressed genes. Finally, transcripts were required to have non-zero read counts in 1/5 of all samples to remove transcripts that may be quantified in error. In this study, transcripts with no reads in six or more samples were removed from the analysis (LUND et al., 2012). Two mathematical models were used, one under the alternative hypothesis and another under the null hypothesis. The definition of models to be used was made after analysis of significant fixed effects using PROC GLM in Statistical Analysis System 9.1 (SAS, 2003) for shear force with 14 days of aging. Significant fixed effects used in the QuasiSeq model, for animals used in the previous analysis, were final age, contemporary group (slaughter group, origin and birth season) and lane. However, lane was confounded with contemporary group in the model, and as both covariates were important, some animals were eliminated, with 24 animals remaining in this analysis.

The normalization method used here was Upper Quartile according to BULLARD et al. (2010) and the BENJAMINI-HOCHEBERG (1995) methodology was used to control false discovery rate (FDR) at 10%.

4.6 Enrichment analysis

The Functional Annotation Clustering of DAVID tools (HUANG; SHERMAN; LEMPICKI, 2009) used the GOTERM, SP_PIR_KEYWORDS and KEGG_PATHWAYS analyses to make clusters that showed decreasing values of enrichment scores for genes. The Benjamini and Hochberg correction applied for DAVID enrichments was P value adjusted (padj.) <0.1 , for genes that were differentially expressed in QuasiSeq and Cuffdiff analysis. The novel transcripts were annotated using the Genome-to-seq and GOanna for GO annotations based on sequence homology by Basic Local Alignment Search Tool (BLAST) at AgBase

(MCCARTHY et al., 2011). Some uncharacterized proteins differentially expressed present in the results were annotated by looking for their gene orthologous at BioMart Ensembl [<http://www.ensembl.org/biomart>].

4.7 PCIT, PIF and RIF

The file used for the PCIT was the FPKM file generated by the Cufflink analysis. From the FPKM file, we performed the residual analysis of FPKM with a script on R environment based in the same covariates used for the QuasiSeq model.

The script used for PCIT (REVERTER; CHAN, 2008) was implemented by KOESTERKE et al. (2013) and created a number of filtered results file based on direct and partial correlation thresholds. The strength of the correlation between regulators can indicate target genes that are changing with the candidate regulator. The differential hubbing (DH) was determined using the number of significant connections a gene had in the H group subtracted by the number of significant connections a gene had in the L group detected by the PCIT algorithm (HUDSON; REVERTER; DALRYMPLE, 2009; REVERTER; CHAN, 2008). These filtered correlations could be tested with gene set enrichment analysis to determine if they have biologically meaningful results in the context of the gene expression experiment (KOESTERKE et al., 2013). For each DH gene, a list of all positive and all negative correlated genes was generated. These PCIT data were visualized by DAVID enrichments ($\text{padj} < 0.1$) in GOTERMS and KEGG_PATHWAYS. Only transcripts with a direct and partial correlation ≥ 0.90 were used for the DH analysis. Graphics with the network resulted from PCIT were visualized by BioLayout program (ENRIGHT; OUZOUNIS, 2001).

To determine the importance of each DE gene with respect to phenotype, the phenotypic impact factor (PIF) was calculated in the R environment using the same residual FPKM files used for PCIT analysis. PIF is calculated using the average of DE genes generated by QuasiSeq coupled with the H group and L group FPKM files.

For the RIF analysis, the PCIT results with correlation ≥ 0.90 and PIF results with no threshold were used to calculate the scores. RIF1 is calculated using the DH file for each H and L group generated by PCIT algorithm combining with the PIF results. RIF2 is calculated using the DH files, the average fitted expression level of each L and H group FPKM file. The algorithms used for each RIF analysis are described by HUDSON et al. (2009b). RIF1 is based in the algorithm that take into

account the average PIF of one regulator weighted by the square of the genes resulted in PCIT (value of differential co-expression between the regulator “x” and the DE gene “y”). Genes with a high RIF1 score predict the abundance of the number of DE genes associated with the trait of interest. RIF2 checks the ability of regulator “x” in predicting the abundance of the gene DE “y”. Genes with high RIF2 score are related to the change in correlation in the network.

5 RESULTS

5.1 Phenotypic analysis and mapping

The animals selected for this work came from a larger study where we evaluated meat quality traits in more than 430 animals (TIZIOTO et al., 2013a). In order to focus on the genetic mechanisms that influence shear force, we selected animals with high and low EBVSF14 for transcriptome analysis. While in the larger study, the average of SF at 0, 7 and 14 days were 8.70, 5.93 and 4.56 kgf/cm² (TIZIOTO et al., 2013a), the animals selected for this study had SF values of 9.24, 7.54 and 6.52 kgf/cm² for the H and 8.06, 3.18 and 2.73 kgf/cm² for the L group, respectively. As seen in Table 1, both groups presented normal maturation process, with a reduction in SF from 0 to 14 days of 2.72 kgf/cm² for the H and 5.33 kgf/cm² for the L group (Table 1).

The phenotype selected to be the covariate tested in QuasiSeq was EBVSF14, but EBVSF7 composed the mathematical model for the BLUP analysis showed before.

Table 1 - Averages of shear force values in kgf/cm², EBVSF values and accuracy calculated for the L group and for the H in seven and 14 days of aging

Sample average	SF0 ¹	SF7 ²	EBVSF7 ³	acc7 ⁴	SF14 ⁵	EBVSF14 ⁶	acc14 ⁷
H group	9.24	7.54	0.62	0.34	6.52	0.73	0.34
L group	8.06	3.18	-0.65	0.33	2.73	-0.5	0.33

¹SF0 – Shear force for samples 24 hours after slaughter (there is no BLUP analysis for SF0).

²SF7 – Shear force for samples with seven days of aging.

³EBVSF7 - Estimated breeding values with seven days of aging.

⁴Acc7 – Accuracy of BLUP analysis for samples with seven days of aging.

⁵SF14 – Shear force for samples with 14 days of aging.

⁶EBVSF14 - Estimated breeding values with 14 days of aging.

⁷Acc14– Accuracy of BLUP analysis for samples with 14 days of aging.

Differential gene expression between the group with high and low EBVSF14 was determined by RNA-Seq. On average, 31 million reads were generated per sample. After quality filtering by the Phyton script used in the Cuffdiff analysis, 21 million reads per sample were aligned to 3.1 UMD *Bos taurus* Genome. The average of reads obtained for each group can be visualized in Table 2. An average of 70% of

the reads were mapped by TopHat, which is similar to other studies, such as PEÑAGARICANO et al. (2013) in a study on embryos that had an average of 75% of the total reads mapped using two mismatches. The uniquely aligned reads by software HTSeq had a mean number of approximately 61% (Table 2).

Table 2 - Average of total alignments and read counting of 31 samples in each group

Group*	Total Reads ¹	Total Aligned Reads	Aligned Reads (%)	Uniquely Aligned Reads	Uniquely Aligned Reads (%)
H group	31,217,927	19,771,997	64.13	12,012,411	60.86
L group	30,506,606	22,425,278	75.86	13,801,683	61.00

*Group starting with L have low EBV values and group starting with H have high EBV values for shear force. ¹Total reads before the filter.

For the QuasiSeq analysis, some animals were eliminated because of confounding factors between two covariates. After filtering with Seqclean, the 24 samples of the QuasiSeq analysis had on average 19 million reads per sample aligned to 3.1 UMD *Bos taurus* Genome. The average obtained for each group can be visualized in Table 3. An average of 65% of the reads was mapped by TopHat and the reads aligned uniquely after HTSeq had a mean number of approximately 76% (Table 3). Even though the average of reads aligned after Phyton script filter (70%) was higher than the average of reads aligned after the Seqclean filter (65%), the last filter gave a higher average value of reads aligned uniquely (76%).

Table 3 – Average of total alignments and read counting of 24 samples in each group

Group*	Total Reads ¹	Total Aligned Reads	Aligned Reads (%)	Uniquely Aligned Reads	Uniquely Aligned Reads (%)
H group	31,248,340	17,764,374	57.68	13,640,713	77
L group	30,694,043	20,859,422	72.19	15,621,070	75

*Group starting with L have low EBV values and group starting with H have high EBV values for shear force. ¹Total reads before filtering.

5.2 Differential expression gene analysis with QuasiSeq

A total of 15,693 expressed genes were identified in this transcriptome study, and among these, 1,743 were CUFFs (short transcripts assembled in this analysis). QuasiSeq statistical package identified 22 DE genes (FDR<10%), where 16 genes were annotated by Biomart Ensembl (<http://www.ensembl.org/biomart/martview/>). From six CUFFs, two were annotated by AgBase tools (McCarthy et al., 2011) and four were unannotated (Table 4). There were eight genes up regulated in the group with high EBV for shear force and 14 genes up regulated in the group with low EBV for shear force based on the total counts for each gene after normalization. This DE

gene analysis used a numeric covariate (shear force with 14 days of aging), which did not allow fold change estimation.

Table 4 - List of 22 differentially expressed genes identified by QuasiSeq annotated by BioMart Ensembl and AgBase

Gene name	Transcript ID	A. Counts ¹ H group	A. Counts ¹ L group	padj. ²
<i>SYT4</i>	ENSBTAT00000002357	50.64	39.23	7.78E-07
<i>DCSTAMP</i>	ENSBTAT00000031443	14.64	3.08	1.16E-06
<i>PRSS2</i>	ENSBTAT00000028731	30.00	17.62	5.77E-06
<i>RPL9</i>	ENSBTAT00000064379	3.18	9.85	4.82E-05
<i>Pseudogene</i>	ENSBTAT00000012115	2.36	3.38	6.39E-05
<i>AP1M1</i>	CUFF.45691	1.36	3.00	6.43E-05
<i>RPL36</i>	ENSBTAT00000050149	4.92	9.07	0.0001
<i>Unannotated</i>	CUFF.1369	0.73	4.00	0.0002
<i>7SK</i>	ENSBTAT00000060649	1.29	3.17	0.0005
<i>RPL31</i>	ENSBTAT00000064020	2.09	2.77	0.0005
<i>RPS14</i>	ENSBTAT00000027356	2.03	3.45	0.0010
<i>ABCC4</i>	ENSBTAT00000010182	22.12	38.12	0.0026
<i>Unannotated</i>	CUFF.20989	1.64	4.92	0.0034
<i>APOL3</i>	ENSBTAT00000016190	4.73	4.69	0.0038
<i>SNORD113</i>	ENSBTAT00000059671	2.81	1.75	0.0039
<i>Unannotated</i>	CUFF.14892	4.36	6.38	0.0047
<i>EEF1A1</i>	ENSBTAT00000030625	0.18	5.69	0.0275
<i>JAM2</i>	CUFF.2483	2.91	3.43	0.0673
<i>B4GALNT2</i>	ENSBTAT00000013010	0.52	3.98	0.0727
<i>Unannotated</i>	CUFF.14879	259.80	138.44	0.0741
<i>EME2</i>	ENSBTAT00000022021	2.93	2.84	0.0818
<i>KIAA1456</i>	ENSBTAT00000011782	3.75	2.47	0.0973

¹ A. counts for H and L group - Average counts for the two groups H and L of extremes values, after normalization; ² padj - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

There were six uncharacterized protein annotated by Biomart ensembl based on the orthologous genes: *RPL9*, *RPL36*, *RPL31*, *RPS14*, *ABCC4* and *APOL3*. The enrichment analysis with the differentially expressed genes by DAVID did not produce significant (FDR<10%) GO terms or pathways considering the small number of DE genes identified by QuasiSeq.

5.3 Differential expression gene analysis with Cuffdiff

From 16,348 expressed genes identified in this transcriptome study, 113 genes were differentially expressed (FDR<10%) by the Cuffdiff approach, and 76 were annotated by BioMart Ensembl (genes and pseudogenes). Table 5 shows the list of all annotated differentially expressed genes. While 53 genes were up regulated in the H group (log2fold-change positive), 60 genes were up regulated in the L group

(log2fold-change negative). In total, 58 genes were annotated by BioMart Ensembl, 25 genes annotated by AgBase, 18 pseudogenes and 12 unannotated genes. (Tables 5 and 6)

Table 5 - List of 58 differentially expressed genes identified by Cuffdiff, annotated by BioMart Ensembl (continues)

Gene name	Gene ID	log2(FC) ¹	padj. ²
<i>RCAN1</i>	ENSBTAG00000020035	0.94	0.01
<i>CBX3</i>	ENSBTAG00000005540	0.94	0.01
<i>BDH1</i>	ENSBTAG00000000448	2.15	0.01
<i>ESRRB</i>	ENSBTAG00000012285	0.96	0.01
<i>GAPDH</i>	ENSBTAG00000018554	-1.67	0.01
<i>ROCK2</i>	ENSBTAG00000005847	0.53	0.01
<i>H4</i>	ENSBTAG00000048065	0.53	0.01
<i>RSAD2</i>	ENSBTAG00000016061	0.64	0.01
<i>SOHLH2</i>	ENSBTAG00000018955	-1.1	0.01
<i>RPL8</i>	ENSBTAG00000003560	-0.76	0.01
<i>RPL9</i>	ENSBTAG00000024608	-0.57	0.01
<i>RPL30</i>	ENSBTAG00000040051	-2.67	0.01
<i>SCN3B</i>	ENSBTAG00000016768	0.85	0.01
<i>RPL27A</i>	ENSBTAG00000005349	-0.7	0.01
<i>SNORA3</i>	ENSBTAG00000042354	-0.7	0.01
<i>SNORA3</i>	ENSBTAG00000042335	-0.7	0.01
<i>CHRD2</i>	ENSBTAG00000021306	1.13	0.01
<i>ESRRG</i>	ENSBTAG00000010392	1.14	0.01
<i>FAM101A</i>	ENSBTAG00000039688	0.87	0.01
<i>RPL26</i>	ENSBTAG00000012344	-0.7	0.01
<i>MYHC-EMBRYONIC</i>	ENSBTAG00000011803	-0.8	0.01
<i>EXTL1</i>	ENSBTAG00000006349	0.69	0.01
<i>CMYA1</i>	ENSBTAG00000046512	0.93	0.01
<i>RPL10A</i>	ENSBTAG00000019494	-0.5	0.01
<i>H2B</i>	ENSBTAG00000031785	0.85	0.01
<i>H4</i>	ENSBTAG00000025391	0.85	0.01
<i>PMF1</i>	ENSBTAG00000009432	-1.3	0.01
<i>PPDPF</i>	ENSBTAG00000009002	-2.2	0.01
<i>ATP5I</i>	ENSBTAG00000047845	-1.42	0.01
<i>RPS28</i>	ENSBTAG00000002468	-1.1	0.01
<i>KANK3</i>	ENSBTAG00000002469	-1.1	0.01
<i>TOMM7</i>	ENSBTAG00000006398	-1.1	0.01
<i>CXHXorf64</i>	ENSBTAG00000008763	1	0.01
<i>RPL36A</i>	ENSBTAG00000019253	-2	0.01
<i>BPGM</i>	ENSBTAG00000037625	0.86	0.02
<i>FAM134B</i>	ENSBTAG00000016444	0.55	0.02
<i>GATM</i>	ENSBTAG00000005586	-0.6	0.03
<i>NNMT</i>	ENSBTAG00000008564	1.33	0.03
<i>U. protein</i>	ENSBTAG00000039486	0.52	0.03
<i>PDE4D</i>	ENSBTAG00000000494	0.52	0.03
<i>ASB5</i>	ENSBTAG00000014143	0.5	0.03

Table 5 - List of 58 differentially expressed genes identified by Cuffdiff, annotated by BioMart Ensembl (conclusion)

Gene name	Gene ID	log2(FC) ¹	padj. ²
<i>LPL</i>	ENSBTAG00000012855	0.58	0.03
<i>ABCA1</i>	ENSBTAG00000020661	0.5	0.03
<i>PTPN3</i>	ENSBTAG00000018841	0.59	0.04
<i>U2</i>	ENSBTAG00000045486	0.59	0.04
<i>GSTM1</i>	ENSBTAG00000031788	-0.5	0.05
<i>FKPB8</i>	ENSBTAG00000045585	-0.89	0.05
<i>MEGF9</i>	ENSBTAG00000013706	0.5	0.06
<i>miRNA</i>	ENSBTAG00000045720	0.67	0.06
<i>PRR5</i>	ENSBTAG00000039784	-0.5	0.06
<i>DIXDC1</i>	ENSBTAG00000008665	0.51	0.07
<i>ODF2L</i>	ENSBTAG00000012907	-0.6	0.07
<i>TP53INP2</i>	ENSBTAG00000005504	0.44	0.08
<i>MAP1A</i>	ENSBTAG00000014001	0.48	0.08
<i>bta-mir-2366</i>	ENSBTAG00000045096	0.48	0.08
<i>PDE7B</i>	ENSBTAG00000015888	0.49	0.08
<i>ARMC2</i>	ENSBTAG00000036087	1.11	0.08
<i>IFI47</i>	ENSBTAG00000015727	0.67	0.09

¹log2(FC) – log2(foldchange); ²padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR); *U. protein – uncharacterized protein.

Table 6 - List of 25 differentially gene expressed identified by Cuffdiff, annotated by AgBase and 12 unannotated genes

Gene name	Locus	log2(FC) ¹	padj. ²
<i>Unannotated</i>	10:39350419-39350934	-0.814175	0.01
<i>ESRRG</i>	16:20592147-20595301	0.823283	0.01
<i>HSPA8</i>	16:22391200-22391421	-325.156	0.01
<i>LOC101906520</i>	16:73001980-73002542	318.721	0.01
<i>ALPK3</i>	21:22938779-22939188	0.987333	0.01
<i>ALPK3</i>	21:22939372-22939635	101.973	0.01
<i>Unannotated</i>	21:69620556-69621110	-149.553	0.01
<i>TRAK1</i>	22:15161382-15161565	-126.713	0.01
<i>LOC100296311</i>	23:8427234-8427431	-0.687121	0.01
<i>Unannotated</i>	29:44291297-44292678	144.081	0.01
<i>TMEM178B/LOC785954</i>	4:105475768-105476029	-349.617	0.01
<i>RPS26</i>	6:81188576-81188998	-0.732473	0.01
<i>ITGB1BP3</i>	7:21288690-21289703	-0.910571	0.01
<i>LOC101906684</i>	7:22798051-22798599	-0.621404	0.01
<i>Unannotated</i>	9:6631173-6631334	-0.9583	0.01
<i>Unannotated</i>	X:114171052-114171181	0.594646	0.01
<i>KY</i>	1:135852164-135852692	-0.912214	0.02
<i>Unannotated</i>	12:17740699-17740846	-120.103	0.02
<i>LOC101907593</i>	16:74930545-74930813	-0.976537	0.02
<i>TMEM233</i>	17:58032822-58079621	0.536705	0.02
<i>CWC15</i>	18:48776273-48776627	-0.878163	0.02
<i>LOC100295294/ELOVL7</i>	20:18483889-18484016	-213.307	0.02
<i>LOC101906520</i>	16:73000531-73001584	45.586	0.05
<i>CCNG1</i>	19:43186080-43187061	-0.633005	0.05
<i>Unannotated</i>	29:44289549-44290832	134.902	0.05
<i>ZNF608</i>	7:30344585-30345574	111.805	0.05
<i>Unannotated</i>	15:34765966-34766984	0.748966	0.05
<i>Unannotated</i>	21:23264706-23265202	-0.727968	0.05
<i>Unannotated</i>	4:86129251-86131130	0.514505	0.05
<i>LOC101903901</i>	29:44338388-44344979	0.527624	0.06
<i>UBAP1L/LOC101906923</i>	10:11997416-11997826	-0.497706	0.07
<i>EIF3F</i>	13:48173136-48173532	-282.325	0.07
<i>NDUFB4/LOC100847355</i>	22:44213091-44213522	-0.905264	0.07
<i>Unannotated</i>	2:92720746-92720837	-231.699	0.09
<i>ALPK3</i>	21:22939946-22940259	0.80226	0.09
<i>Unannotated</i>	4:54948658-54950970	-0.830784	0.09
<i>COXP1</i>	5:111053318-111053478	-0.781664	0.09

¹log2(FC) – log2(foldchange); ²padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

The enrichment analysis is determined by over-represented terms and had a score of 1.92, including GO terms related with acetylation, phosphoprotein, translation, structural molecule activity, structural constituent of ribosome, etc.

However, the significant pathway was associated with ribosome (adjusted value $7.5E-3$ with the Benjamini-Hochberg procedure for DE genes with $FDR < 10\%$) (Table 7).

Table 7 - Functional Annotation Clustering analyzed by DAVID tools. These annotation clusters had a group of enrichment score of 1.92

Annotation Cluster	Representative Annotation Terms	Count	P_Value	padj. ¹
KEGG_PATHWAY	Ribosome	6	0.00023	0.007
GOTERM_MF_FAT	Structural constituent of ribosome	6	0.0018	0.19
SP_PIR_KEYWORDS	Ribosomal protein	6	0.0028	0.27
GOTERM_CC_FAT	Ribosome	6	0.0054	0.45
SP_PIR_KEYWORDS	Ribonucleoprotein	6	0.0076	0.34
GOTERM_MF_FAT	Structural molecule activity	6	0.010	0.47
GOTERM_CC_FAT	Non-membrane-bounded organelle	12	0.013	0.51
GOTERM_CC_FAT	Intracellular non-membrane-bounded organelle	12	0.013	0.51
GOTERM_BP_FAT	Translation	6	0.020	1.0
GOTERM_CC_FAT	Ribonucleoprotein complex	6	0.055	0.79

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

The Functional Annotation Clustering showed that only the ribosomal pathway was significant ($padj.<0.1$) (Table 7), and the six genes that represent this pathway were up regulated in the L group.

5.4 PCIT and differential hubbings

When a gene has a significant number of connections determined by the PCIT algorithm (REVERTER; CHAN, 2008) it could be considered a differential hubbing (DH) (HUDSON; REVERTER; DALRYMPLE, 2009). For the PCIT results the differential hubbing (DH) was identified for transcripts with correlation ≥ 0.9 . Tables 8 and 9 showed the top 10 for the negative and positive differentially hubbed genes comparing the H group minus the L group.

Table 8 - Top 10 genes presenting negative differentially hubbed (DH) comparing the H group minus the L group

Gene name	Gene ID	DH	A. FPKM H group ¹	A. FPKM L group ¹
<i>Pseudogene</i>	ENSBTAT00000052414	-1412	290.66	522.08
<i>PTPN3</i>	ENSBTAT00000025085	-1276	336.87	200.38
<i>TRIM45</i>	ENSBTAT00000002293	-1035	295.02	369.59
<i>USP2</i>	ENSBTAT00000012857	-1021	433.97	396.01
<i>RTP4</i>	ENSBTAT00000045760	-1013	161.854	122.27
<i>NT5C2</i>	ENSBTAT00000017090	-982	242.99	172.50
<i>GRB10</i>	CUFF.37260	-959	1.14	69.62
<i>HECTD4</i>	ENSBTAT00000014551	-953	217.46	326.87
<i>AKTIP</i>	ENSBTAT00000021617	-923	177.94	191.91
<i>ALPK3</i>	ENSBTAT00000002122	-922	462.14	548.35

¹ A. FPKM L and H group – Average of fragments per kilobase of exon per million fragments mapped for each group;

Table 9 - Top 10 presenting genes for positive differentially hubbed (DH) comparing the H group minus the L group

Gene name	Gene ID	DH	A. FPKM H group ¹	A. FPKM L group ¹
<i>TMBIM4</i>	ENSBTAT00000008243	3975	323.59	387.80
<i>STXBP6</i>	ENSBTAT00000028368	3832	375.14	400.10
<i>XRCC2</i>	ENSBTAT00000043719	3805	2.28	2.51
<i>TMEM150A</i>	ENSBTAT00000024342	3791	186.29	153.55
<i>HAUS6</i>	ENSBTAT00000031479	3729	417.22	416.19
<i>EIF2B1</i>	ENSBTAT00000009560	3709	630.41	349.28
<i>ENPP4</i>	ENSBTAT00000004547	3692	326.29	349.93
<i>ANO1</i>	ENSBTAT00000013189	3670	140.53	111.47
<i>SLC25A44</i>	ENSBTAT00000012410	3663	588.48	506.67
<i>CDK5RAP3</i>	ENSBTAT00000018878	3653	512.39	602.99

¹ A. FPKM L and H group – Average of fragments per kilobase of exon per million fragments mapped for each group;

For each top gene with negative DH, there were positively and negatively genes correlated for the L and H groups that were enriched with gene ontology terms and visualized by DAVID tools. ANNEX A shows tables from 10 to 29 with all enrichments for each DH.

Figure 1 and 2 were constructed using the BioLayout program to visualize some negative and positive differential hubbings (DH) between the high and low groups for EBVSF14.

H group

L group

TRIM45

USP2

NT5C2

GRB10

H group**L group***HECTD4* *ALPK3*

Figure 1 - Negative differential hubbing (DH) between the high and low groups for EBVSF14. The center spot represents the gene with high value of DH, red lines represent positive DH and blue lines represent negative DH

H group**L group***TMBIM4*

H group**L group***TMEM150A* *EIF2B1* *ENPP4* *ANO1*

H group*SLC25A44* **L group**

Figure 2 - Positive differential hubbing (DH) between the high and low groups for EBVSF14. The center spot represents the gene with high value of DH, red lines represent positive DH and blue lines represent negative DH

5.5 RIF and PIF

The RIF analysis shows genes that could be important to meat tenderness, since they are regulators present in the network based on the difference of correlations (Hudson et al., 2009). The genes identified by RIF1 and RIF2 show the candidate genes for the two groups H and L (Tables 30 and 31), respectively.

Table 30 - Top negative and positive genes identified by Regulatory Impact Factor 1 (RIF1) scores

Gene name	Transcript ID	RIF1	A. FPKM H group ¹	A. FPKM L group ¹
Top Negative RIF1				
<i>HIST1H2AC</i>	ENSBTAT00000056983	-0,038758	463.18	324.46
<i>SNCG</i>	ENSBTAT00000004571	-0,038758	0.19	21.28
<i>ABHD8</i>	CUFF.45316	-0,038758	0.50	0.52
<i>MKX</i>	ENSBTAT00000010101	-0,038758	23.41	0.22
<i>EIF2C2</i>	CUFF.13100	-0,038758	0.44	0.37
Top Positive RIF1				
<i>RPS15</i>	ENSBTAT00000012115	28,77981	0	9.69
<i>bta-mir-133a-2</i>	ENSBTAT00000042285	28,77121	1,023	14,934
<i>Unannotated</i>	CUFF.21214	28,33927	0.02	0.61
<i>U6</i>	ENSBTAT00000060288	28,18287	0	257.23
<i>UBE2D1</i>	CUFF.31054	27,44942	11.26	31.87

¹ A. FPKM L and H group – average of fragments per kilobase of exon per million fragments mapped for each group.

Table 31 - Top negative and positive genes identified by Regulatory Impact Factor 2 (RIF2) scores

Gene name	Transcript ID	RIF2	A. FPKM H group	A. FPKM L group
Top Negative RIF2				
<i>RPS15</i>	ENSBTAT00000012115	-16.949	0	9.69
<i>bta-mir-133a-2</i>	ENSBTAT00000042285	-16.9442	1,023	14,934
<i>LOC618123</i>	CUFF.17073	-16.9202	0	42,185
<i>bta-mir-22</i>	ENSBTAT00000042309	-16.8998	196.38	9,263
<i>SIMC1</i>	CUFF.4368	-16.8972	0.07	2.17
Top Positive RIF2				
<i>ATAD3A</i>	ENSBTAT00000012676	0.15098	334.24	347.83
<i>HINT2</i>	ENSBTAT00000015208	0.14754	181.95	192.13
<i>MED26</i>	CUFF.45514	0.14573	1.96	2.45
<i>IFITM1</i>	ENSBTAT00000045242	0.06096	0	22.3
<i>TAF5L</i>	CUFF.31983	0.06079	1.94	4.06

¹ A. FPKM L and H group – average of fragments per kilobase of exon per million fragments mapped for each group.

The PIF analysis identified 995 genes ($p_{adj} < 0.05$) that could be regulators of gene expression and responsible for the observed phenotype. Among the top 10 genes identified with PIF (Table 32), there are interesting genes that could be potential regulators such as *MB* (myoglobin), *ENO3* (enolase 3 (beta, muscle)) and *CA3* (carbonic anhydrase III, muscle specific). The enrichment analysis of these genes was made by DAVID ($p_{adj} < 0.1$) and showed some important pathways involved in cellular metabolic process, cellular process, metabolic process, intracellular, cellular macromolecule metabolic process, nitrogen compound metabolic process, intracellular part, gene expression, biosynthetic process and primary metabolic process (Table 33).

Table 32 - Top 10 genes with the highest value for Phenotypic Impact factor (PIF) analysis

Gene name	Gene ID	PIF
<i>MB</i>	ENSBTAT00000007014	772,383.72
<i>ENO3</i>	ENSBTAT00000007278	323,207.66
<i>CA3</i>	ENSBTAT00000020243	48,453.50
<i>MYBPC2</i>	ENSBTAT00000026756	47,278.23
<i>RPL19</i>	ENSBTAT00000002666	12,856.74
<i>COX71A</i>	ENSBTAT00000019808	9,308.63
<i>HS3ST6</i>	CUFF.29715	5,936.79
<i>MAP4K4</i>	CUFF.8544	5,879.58
<i>COX7B</i>	ENSBTAT00000053293	5,398.79
<i>PDLIM5</i>	ENSBTAT00000017030	4,735.79

Table 33 - List of terms enriched by DAVID for all Phenotypic Impact factor (PIF) scores

Category	Term	Count	%	P-Value	padj. ¹
GOTERM_BP_ALL	cellular metabolic process	186	21.6	4.90E-08	8.30E-05
GOTERM_BP_ALL	cellular process	267	30.9	3.00E-07	2.60E-04
GOTERM_BP_ALL	metabolic process	214	24.8	4.30E-05	2.40E-02
GOTERM_CC_ALL	intracellular	288	33.4	5.30E-05	1.50E-02
GOTERM_BP_ALL	cellular biosynthetic process	83	9.6	1.60E-04	6.40E-02
GOTERM_BP_ALL	cellular macromolecule metabolic process	130	15.1	1.70E-04	5.60E-02
GOTERM_BP_ALL	cellular nitrogen compound metabolic process	84	9.7	1.80E-04	5.00E-02
GOTERM_BP_ALL	nitrogen compound metabolic process	86	10	2.30E-04	5.40E-02
GOTERM_CC_ALL	intracellular part	256	29.7	2.60E-04	3.70E-02
GOTERM_BP_ALL	gene expression	66	7.6	3.60E-04	7.40E-02
GOTERM_BP_ALL	biosynthetic process	85	9.8	3.70E-04	6.70E-02
GOTERM_BP_ALL	primary metabolic process	179	20.7	4.10E-04	6.80E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

6 DISCUSSION

6.1 Differential expression gene analysis with QuasiSeq

Two different approaches were used to investigate the genetic mechanisms associated with shear force. A more conservative, significance depending approach (QuasiSeq) identified a small list of genes differentially expressed between H and L groups. A second approach, significance independent (PCIT) used all the genes measured to rank the more relevant ones associated with SF14.

The model used in QuasiSeq showed genes related with ribosome as differentially expressed. In general, these genes are related with ribosome constituent: *RPL9*, *RPL36*, *RPL31*, *RPS14* and all they are up regulated in the L group. Protein synthesis was not measured in this study; however, based on weight gain observed during feedlot period, there is no indication of difference in protein accretion between the L and H groups. The balance between synthesis and degradation of proteins is called protein turnover, an essential process for cell survival, providing amino acids to cells when there is no dietary protein source available, eliminating polypeptides that contain transcription/translation errors, degrading proteins, among other functions (VOET; VOET; PRATT, 2013).

OKSBJERG and THERKILDSEN (2012) observed that high protein turnover in slaughter time is important because it shows whether proteolytic potential of the animal is good, which is important to meat tenderization. De JAGER and colleagues (2010), reported on a study that involved two crossed breeds and Brahman in which a ribosomal module of genes (network) had a slightly higher expression level in their tender genotype (as determined by SNP markers in *calpain* and *calpastatin*) (CAFE et al., 2010).

Protein turnover could be higher in the L group before the slaughter because of the ribosomal DE genes and because of the presence of *EEF1A1* (eukaryotic translation elongation factor 1 alpha 1) as a DE gene, also up regulated in the L group. Ribosomal proteins interact with elongation factor *EEF1* promoting the GTP-dependent binding of aminoacyl-tRNA to site A in the ternary complex with GTP during protein biosynthesis (GRAIFER; KARPOVA, 2014). As most of these genes here are up regulated in the L group, they could be showing that the animals have great proteolytic potential or that there is a high protein turnover on their muscle.

Since 1917, some authors have believed that meat tenderness is a result of proteolysis (KOOHMARAIE, 1994). There are three proteolytic enzymes considered highly important to proteolysis: calpains, proteasome and cathepsins (OKSBJERG; THERKILDSEN, 2012). Here, calpain and calpastatin were not differentially expressed. The system calpain-calpastatin is distinct for *Bos indicus* and *Bos taurus* because there is greater action of calpastatin in *Bos indicus* (CAFE et al., 2010; SHACKELFORD; WHEELER; KOOHMARAIE, 1995; FERGUSON et al., 2001). Besides CAFE et al., (2010b) concluded that selection based on gene markers of calpain and calpastatin were efficient for Brahman cattle, here the results show that tenderization in Nellore cattle have different genes responsible for this process, such as *PRSS2*, *SYT4*, *APOL3* and *ABCC4*.

Gene *PRSS2* (protease, serine, 2 (trypsin 2)) is a serine protease in the collagen metabolic process and related to calcium ion binding, and was up regulated in the H group. Calcium is directly involved with *PRSS2* in other tissues such as pancreas, intestines and bones; however, in the muscle, its relationship is still not clear. BOUDIDA and colleagues (2014) showed that serine proteases could make meat tenderer and possibly participate in muscle proteolysis (ALARCON-ROJO; DRANSFIELD, 1995; UYTTERHAEGEN; CLAEYS; DEMEYER, 1994). Inhibitors of serine proteases (serpins) are also present in the muscle and they degrade a large set of muscle proteins, cysteine proteases such as calpains, and inhibit the effect of caspases, responsible for programmed cell death (GOLL et al., 2008; BOUDIDA et al., 2014). LUKE et al. (2007) showed in a study on *Caenorhabditis elegans* that an intracellular serpin could block death induced by heat shock, oxidative stress and hypoxia providing survival function to the cell. The action of serine proteases and their inhibitors are wide, but here, gene *PRSS2* was up regulated in the H group, which could mean that this gene could be involved in many complex mechanisms making the meat less tender. TIZIOTO et al. (2013a), in a study of quantitative trait loci (QTL) with the same animals and phenotype used in this study, identified *SERPIN2* in a QTL region located near associated SNPs on BTA2.

The differentially expressed *SYT4* (synaptotagmin IV) was up regulated in the H group. Synaptotagmins are transmembrane proteins involved in the regulation of neurotransmitter levels, generation of a signal in cell-cell signaling, cell communication, and transmission of nerve impulse. In general, they act as calcium regulators of exocytosis, binding to C2 calcium-dependent membrane targeting

(SUDHOF, 2002; JOHNSON et al., 2010). Calcium is important to muscle contraction and to activate proteolytic enzymes (PRINGLE et al., 1999) that are responsible for protein degradation in muscle fibers. The meat tenderization process is enzymatic and related with intracellular proteolytic systems (OUALI et al., 2006, 2013).

Calpains and synaptotagmins have similar actions in the pancreas and could work in combination or alternation (AGANNA et al., 2006). *SYT4* is the only one of this family that is ubiquitous and has different functions related with calcium, which are still uncertain (SUDHOF, 2002; JOHNSON et al., 2010) mainly in the muscle. NONNEMAN and colleagues (2013) in a study on swine found a SNP in *SYT9* (synaptotagmin IX) related with meat quality. Some mutant forms of *SYT4* in the murine brain acts as dominant-negative inhibitors of calcium and affects the glutamate release by other molecules (ZHANG et al., 2004). *SYT4* inhibits calcium in the brain but more studies are necessary before stating that this gene inhibits calcium also in the muscle, providing less tender meat.

Gene *APOL3* (apolipoprotein L, 3) is up regulated in the H group. Apolipoprotein L proteins come from the high-density lipoprotein family and are involved with lipid and cholesterol transportation and location (SADKOWSKI et al., 2006). There are apolipoproteins (*ApoLs* 1, 2, 4 and 5) that act in the reticulum endoplasmic; however, *APOL3* is present in the cytoplasm and is considered ancestor of other apolipoproteins, and is highly conserved among species. Apolipoproteins have been implicated with meat tenderness in pigs as described by HUI and colleagues (2013). Low expression levels of genes involved in lipid metabolism in Qinchuan cattle could be associated with more muscles (ZHANG; ZAN; WANG, 2011). However, more studies are necessary to understand why this gene is up regulated in the H group in Nellore cattle.

Gene *ABCC4* (ATP-binding cassette, sub-family C (*CFTR/MRP*), member 4) was differentially expressed and up regulated in the L group. This gene participates in the purine nucleoside binding, chloride channel activity, adenyly nucleotide binding, ATPase activity and it is also known as *MRP4* (multidrug resistance-associated protein) that are ATP-dependent-export and anion transporters (HAMMOND et al., 2007). One substrate of the differentially expressed *ABCC4* is glutathione (*GSH*), which is anionic and its excretion is greater in the presence of *ABCC4* (BAI et al., 2003; LAI; TAN 2002). The export of *GSH* by *ABCC4* is observed in the cell during apoptosis, and the decrease of *GSH* can increase the reactive oxygen species

(ROS) or accelerate mitochondrial damage. If *GSH* is not exported, cell survival will increase (HAMMOND et al., 2007). D'ALESSANDRO and colleagues (2012), in a study on Chianina cattle, described that the presence of ROS can induce protein fragmentation that indicate meat tenderness. The presence of *GSH* in the tender group showed that the oxidative stress is related with glycolysis. They also showed that accumulation of *GSH* is proportional to *HSPB1* (heat shock protein beta 1) that has anti-apoptotic activities. TIZIOTO et al. (2013a), in a study with the same animals and phenotype used in this study, identified large QTL for SF in 24 hours located in BTA23 at 24Mb that accounts for 0.11% of the additive genetic variance, which comprises the glutathione S-transferase alpha gene family.

6.2 Comparison with Cuffdiff results

Similar to the QuasiSeq results, the Cuffdiff results showed genes related with ribosome constituents as differentially expressed also in the L group: *RPL8*, *RPL9*, *RPL30*, *RPL27A*, *RPL26*, *RPL10A*, *RPS28*, *RPL36A*. The significant pathway was associated with ribosome (adjusted value 7.5E-3) in DAVID enrichment. *RPL9* and *RPL36* were differentially expressed for both methods. As both methods were consistent, it could be proving that the animals have great proteolytic potential or there is a high protein turnover in the muscle of L group.

The enrichment analysis of DAVID Functional Annotation Table showed some genes that could be important for meat tenderness such as *BDH1* (3-hidroxybutyrate dehydrogenase, type 1), related with synthesis and degradation of ketone bodies and butyrate metabolism (BIONAZ; LOOR, 2008). Other genes that could also be important are *LPL* (lipoprotein lipase), related with glycerolipid metabolism and *PPAR* signaling pathway. The *MYHC-EMBRYONIC* (myosin, heavy chain 3, skeletal muscle, embryonic), involved in tight junction. *GSTM1* (Glutathione S-transferases Mu1), detoxification enzymes that play a role in the oxidative stress and *GATM* (L-arginine: glycine amidinotransferase), related with amino acids metabolism.

Some genes that were described in the Cuffdiff results were also described in other studies related with meat tenderness. D'ALESSANDRO and colleagues (2012), in a study on Chianina cattle, observed that creatine, which helps to supply energy to muscle, were higher in their tenderness group. We found gene *GATM*, precursor of creatine, up regulated in the L group. It happened because creatine could be used as energy reservoirs, and if it were high in the muscle, it could mean that the lack of

energy in the sarcomere contraction was delayed and the glycolytic rate was still high (D'ALESSANDRO et al., 2012).

In the mitochondrion matrix, the oxidative energy metabolism finishes after oxidation of degraded products of lipids, amino acids and polysaccharides that provide substrates to the electron transport chain to produce ATP (OUALI et al., 2013). This leads to cell deprivation of oxygen, and energy is necessary to metabolic activities, ATP synthesis occurs via anaerobic conditions, with glycolytic phosphorylation by creatine phosphate and by muscle adenylate kinase, increasing glycolytic enzymes and producing CO₂, HCO₃⁻, NH₄ and lactic acid that are transported to the liver to be recycled (OUALI et al., 2006, 2013). Toxic metabolites can be produced in the conversion of pyruvate into lactate, cell detoxification can catalyse them by lactoylglutathione lyase and hydroxyacylglutathione hydrolase, the former was recognized as a marker for meat tenderness (OUALI et al., 2013).

In the QuasiSeq results, there is a transporter of glutathione (*ABCC4*) differentially expressed in the L group. Gene *GSTM1* was up regulated in the L group with Cuffdiff analysis, which is in agreement with what D'ALESSANDRO and colleagues (2012) found and was already described here.

Gene *GAPDH* was up regulated in the L group. According to OUALI et al. (2013), glyceraldehyde-3-phosphate dehydrogenase is involved in the second phase of glycolysis and was described as a good marker for meat tenderness because it play a multifunctional role in the cell and could have a pro-apoptotic or pro-survival function (TARZE et al., 2007; COLELL et al., 2007).

RCAN1 was up regulated in the H group and it is linked with the response of cells to stress stimuli and is essential to diverse processes, including physiological and pathological mechanisms (BAEK et al., 2009). MÉNDEZ-BARBERO and colleagues (2013) showed that *RCAN1* action is mediated by the oxidized *LDL* uptake in human atherosclerosis and the presence of *RCAN1* could be associated with the increase of collagen in stable plaques. Both *RCAN1* and *LDL* genes were up regulated in the H group.

As described by ZHANG and colleagues (2011), we found a gene related with muscle development, differentiation and striated muscle contraction, *MyHC-EMBRYONIC* was up regulated in the L group. Myosin is an important structural protein of the thick filament of sarcomere, responsible for contraction velocity and power. Muscle fibers cause acto-myosin hardness, which is directly related with beef

quality. ZHAO and colleagues (2012) explained that myosin heavy chains (*MyH3*) are present in their tender meat because of the proteolytic activity of the muscle that continues in the *post-mortem*. The composition of *MyHC* isoform can affect meat quality in bovine muscles and is important to determine phenotypic properties of the muscles. CARVALHO and colleagues (2014) found that myosin regulatory light chain (*MLC2*) and myosin binding protein H (*MyBP-H*) expressions are associated with meat tenderness.

The Rho-associated, coiled-coil containing protein kinase (*ROCK*) pathway, responsible for the rearrangement of actin cytoskeleton inhibiting Ca^{2+} -independent MLC phosphatase (*MLCP*), can be activated by oxidized low-density lipoproteins (*oxLDLs*) (ABURIMA et al., 2013). In this study, *ROCK2* is expressed in H group. *ROCK2* is also related with Wnt signaling pathway. During the myogenesis, Wnt uses protein kinase A (PKA) and cAMP response element-binding protein (*CREB*) to activate *MyoD* (myogenic differentiation) and *Myf5* genes (KURODA et al., 2013). PKA phosphorylates various proteins involved in coupling excitation-contraction of channels L-type Ca^{2+} (BERS, 2002). SHI et al. (2013) showed in a study on mouse embryonic fibroblast that *ROCK2* deletion can inhibit both *MLC2* and cofilin phosphorylation, which regulates actin filaments.

The presence of two miRNAs (ENSBTAG00000045720 and ENSBTAG00000045096) as differentially expressed genes could be related with the regulation of the meat tenderness process. In this study, according to the TargetScanHuman database (<http://www.targetscan.org/>), the *bta-miR-2366* had 139 conserved sites and 50 poorly conserved sites. In the conserved sites, there are transcripts related with many functions for example in membrane, cytoplasm, zinc ion binding, phosphoprotein, and potassium and sodium channel. This miRNA was upregulated in the H group, and some sites are also related with growth factor as *MYRIP* (NM_015460 myosin VIIA and Rab interacting protein), *IGF1R* (NM_000800 insulin-like growth factor receptor) and *FGF1* (fibroblast growth factor 1 (acidic)). Other target genes are related with calcium: *SYT13* (NM_020826 synaptotagmin XIII), *CAMTA1* (NM_001195563 calmodulin binding transcription activator 1), *CAMK2N2* (NM_033259 calcium/calmodulin-dependent protein kinase II inhibitor 2). However, one interesting site was the *PXN* (NM_001080855 paxillin). Paxillin is degraded by calpains during proteolysis, however, the exact paxillin function is still unknown (CARRAGHER et al., 1999; FRANCO; PERRIN; HUTTENLOCHER, 2004;

CORTESIO et al., 2011) (Figure 6).

Figure 6– Calpain and calpastatin interactions with paxillin in cell motility (Pathway by Biocarta)

6.3 PCIT and Differential Hubbing

Partial Correlation with Information Theory (PCIT) was used to evaluate the specific behavior or co-expression of genes and quantification of a gene differential connectivity. The total number of connections determines which genes can be considered a hub (REVERTER; CHAN, 2008).

Among the negative DH genes, one gene was already associated with meat tenderness in the literature - gene *USP2* (ubiquitin specific peptidase 2) that was strongly correlated with tenderness in a study with Wagyu combined breed as described in the annual report of CRC for Beef Genetic Technologies, Australia (2008/2009). Here, this gene was up regulated in the H group. Gene *TRIM45* (tripartite motif containing 45), up regulated in the L group, is a component of the ubiquitin proteasome and could be associated with tenderness. DAVID enrichment allowed observing that both genes were in the proteasome KEGG pathway.

There are some other interesting correlations, for example, gene *PTPN3* (protein tyrosine phosphatase, non-receptor type 3), a member of the protein tyrosine phosphatase (*PTP*), which had the cellular process as the most enriched pathway, for

negative and positive correlated, respectively. This gene was differentially expressed in a research with non-diabetic insulin-resistant humans in both adipose and muscle tissues showing that this gene could be related with insulin regulation (ELBEIN et al., 2011). *RTP4* (receptor (chemosensory) transporter protein 4) is a protein-coding gene and had starch and sucrose metabolism as enriched KEGG pathway for negative correlated and proteasome as KEGG pathway for positive correlated. *NT5CP* (5'-nucleotidase, cytosolic II) is related to inosine 5'-monophosphate and other purine nucleotides. This gene showed cellular metabolic process and intracellular as the most enriched pathway, for negative and positive correlated, respectively. *GBR10* (growth factor receptor-bound protein 10) had proteasome and pyrimidine metabolism as KEGG pathway for negative correlated and endocytosis and ubiquitin mediated proteolysis as KEGG enriched pathway for positive correlated. *HECTDA* (HECT domain containing E3 ubiquitin protein ligase 4) presented proteasome as KEGG pathway enriched for negative correlated and spliceosome for positive correlated. *AKTIP* (AKT interacting protein) interact with serine/threonine kinase protein kinase B and could be related with apoptosis, in addition, it is similar to ubiquitin ligase domain. There was a KEGG enriched pathway just for the positive correlated: Alanine, aspartate and glutamate metabolism.

Among the positive genes DH, gene *ANO1* (anoctamin 1, calcium activated chloride channel) although up regulated in the H group, could be a potential regulator for meat tenderness because it is involved with Ca^{2+} -activated chloride channel that is essential for numerous physiological functions. In the presence of calcium chloride, there was an increase in myofibrils fragmentation of sheep meat, reducing calpain activity but keeping the lysosomal cathepsins activity (KOOHMARAIE, 1994). In this study, the infusion of calcium chloride in meat increased tenderness, while the activity of cathepsins remained unchanged (KOOHMARAIE, 1994). Another positive DH gene is *TMBIM4* (transmembrane protein 150A), associated with apoptosis and modulate both capacitance of Ca^{2+} entry and inositol 1,4,5-trisphosphate (IP3)-mediated Ca^{2+} release. Gene *TMBIM4* is also known as Golgi antiapoptotic protein (GAAP) in virus and hGAAP in humans and related to cell survival (GUBSER et al., 2007).

6.4 RIF and PIF

RIF 1 and RIF 2 results showed two microRNAs with a large impact in the meat

tenderness process. Both *bta-mir-133a-2* and gene *RPS15* appeared in RIF1 and in RIF2. A study showed that miRNA could be responsible for regulation of skeletal muscle differentiation and with carcass and meat quality in swine (WILLIAMS et al., 2009). PONSUKSILI and colleagues (2013) in a study of swine meat found some miRNAs that are related with adult fast myosin heavy chain (*MyH*), lipid metabolism and glucose homeostasis.

The *bta-mir-133a-2* is a great potential as regulator of meat tenderness because it is up regulated in the L group and appeared in both RIF1 and RIF2 top results. According to the TargetScanHuman database (<http://www.targetscan.org/>) and miRBase (<http://www.mirbase.org/>), this miRNA has 579 transcripts with conserved sites, with 621 conserved sites and 93 poorly conserved sites.

The *bta-mir-133a-2* had target genes related with calcium as *CADPS2* (NM_001009571; Ca⁺⁺-dependent secretion activator 2), *CAMKK2* (NM_006549; calcium/calmodulin-dependent protein kinase kinase 2, beta), *CHP* (NM_007236; calcium binding protein P22), *SYT1* and 9 (NM_001135805; synaptotagmin I and NM_175733; IX) and *CAPN5* (NM_004055; calpain 5). This miRNA also had many target genes involved with apoptosis such as *BCL2L1* and *L2* (NM_001191; BCL2-like 1 and NM_001199839; BCL2-like 2) that play the role of mitochondria in apoptotic signalling, in the apoptotic signalling in the response to DNA damage and in the opposing roles of AIF (apoptosis inducing factor) in apoptosis and cell survival pathways. Other target genes are *ABCC1* (ATP-binding cassette, sub-family C (CFTR/MRP), member 1), the *KCNJ12* (potassium inwardly-rectifying channel, subfamily J, member 12) and *DNAJC3* (NM_006145; DnaJ (Hsp40) homolog, subfamily C, member 3). Some target genes are present in pathways such as vascular smooth muscle contraction, regulation of actin cytoskeleton, MAPK signalling pathway, ubiquitin-mediated proteolysis and some collagen genes are present in the ECM-receptor interaction. WESTON (2002), observed that collagen maturity in muscle and the amount of crosslinking are more important for meat tenderness than their content.

Figures 3, 4 and 5 show the calcium-signaling pathway, apoptosis and TGF-beta signaling pathways, respectively, showing the target regulated genes by *bta-mir-133a-2* in red.

Figure 3 – Calcium signalling pathway has eight target genes and one cycle (in red) regulated by bta-mir-133a-2

Figure 4 - Apoptosis pathway has four target genes and three cycles (in red) regulated by bta-mir-133a-2

Figure 5 – TGF-beta signalling pathway has eight target genes and four cycles (in red) regulated by bta-mir-133a-2

The *bta-mir-22* has 252 transcripts with conserved sites with 262 conserved sites and 90 poorly conserved sites. The *bta-mir-22* is also up regulated in the L group, present in the top negative RIF2. It has different and less target genes present in the same pathways than the *bta-mir-133a-2*. While the latter has target genes more related with apoptosis, *bta-mir-22* has target genes more related with cell survival. An interesting fact is that *bta-mir-22* has *SYT4* as a target gene, a DE gene already found by the QuasiSeq analysis.

The *bta-mir-22* had DE gene *SYT4* (NM_020783) as a target gene and many genes related with potassium channel such as *KCNJ11* (NM_000525; potassium inwardly-rectifying channel, subfamily J, member 11) already described by TIZIOTO et al. (2013b) as a functional candidate for meat tenderness in the same population of Nellore. Some target genes are also related with calcium such as *CACNA1B* (NM_000718; calcium channel, voltage-dependent, N type, alpha 1B subunit), *ATP2B2* (NM_001001331; ATPase, Ca⁺⁺ transporting, plasma membrane 2), *CAMTA1* (NM_000718; calmodulin binding transcription activator 1), *SYTI* and related with apoptosis such as *PDCD6IP* (NM_001162429; programmed cell death 6 interacting protein). Both miRNAs have important target genes related with meat tenderness.

For RIF 1 there are other potential regulators as *HIST1H2AC* (histone cluster 1, H2ac) related with chromatin organization and DNA packaging, the *SNCG* (synuclein, gamma (breast cancer-specific protein 1) related with alpha and beta tubulin binding and regulation of nerve impulse. Gene *MKX* (mohawk homeobox) is related with cell adhesion and transcription regulation. *UBE2D1* (ubiquitin-conjugating enzyme E2D 1) is related with protein degradation.

RIF 2 showed interesting possible regulators such as *ATAD3A* (ATPase family, AAA domain containing 3A), which is important for mitochondrial metabolism and necessary for cholesterol channel. *IFITM1* (interferon induced transmembrane protein 1) related to cell adhesion and control of cell growth. Gene *TAF5L* (TAF5-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65kDa) is involved with myogenic transcription (GeneCards, 2014).

Genes with highest phenotypic impact (*MB*, *ENO3* and *CA3*) are coherent with phenotype and can explain changes in the shear force for both groups.

PIF results showed in the enrichment analysis that genes involved with cellular

metabolic process are the most important for the regulation of shear force after 14 days of aging. Among the top 10 genes of PIF, there is the *MB* (myoglobin), present in skeletal and cardiac muscle, responsible for oxygen storage and transport. There is high content of myoglobin and cytochrome in muscle red fibers, generating energy through oxidative phosphorylation process due to large number of mitochondria (CHOI; KIM, 2009). Type I fibers support the aerobic metabolic capacity because of mitochondria content, myoglobin and iron-containing (NEMETH; LOWRY, 1984; CHOI; KIM, 2009). CHOI and KIM (2009) reported some studies that associate type I fiber with meat tenderness (STRYDOM et al., 2000; RENAND et al., 2001). Myoglobin is related with iron ion binding, iron presence was described as important for meat tenderness. CASAS et al. (2014) observed that markers in the genes of calpastatin (*CAST*) and mu-calpain (*CAPN4751*) had association with iron levels.

Gene *ENO3* participates in muscle development, regeneration and glycogen storage. In addition, enolase was already described as a potential marker of tenderness (CHOI et al., 2010; LAVILLE et al., 2009). *CA3* is also a potential regulator because it is important for several functions such as gluconeogenesis and lipogenesis (BAYRAM et al., 2008). D'ALESSANDRO and colleagues (2012) observed high presence of the glycolytic enzyme *CA3* in tender meat, a high-speed enzyme that makes proton homeostasis converting carbohydrate into carbon dioxide. Changes in the proton homeostasis decrease the electrostatic repulsion of myofibrillar proteins and facilitate the lateral shrinkage of the muscle fibers (sarcomere length) (PEARCE et al., 2011).

7 CONCLUSION

Many metabolic pathways including apoptosis, calcium pathway, proteolysis and ribosomal synthesis can regulate the complex mechanism of meat tenderness. The QuasiSeq and Cuffdiff results showed that different genes from *Bos taurus* could be responsible for meat tenderness in Nellore cattle. The analysis of systems biology using PCIT, PIF and RIF helped to better understand the differential expressed gene behavior and showed interesting correlations as the top 10 negative and positive DH genes. The microRNAs that appeared in RIF analysis and the genes with highest phenotypic impact could be potential regulators to tenderness. Genes from different analyses showed the same pathways. After comparing the results of analyses, we conclude that the results were consistent and interconnected, helping to elucidate some important processes involved in meat tenderness in Nellore cattle.

REFERENCES

- ABURIMA, A.; WRAITH, K. S.; RASLAN, Z.; LAW, R.; MAGWENZI, S.; NASEEM, K. M. cAMP signaling regulates platelet myosin light chain (MLC) phosphorylation and shape change through targeting the RhoA-Rho kinase-MLC phosphatase signaling pathway. **Blood**, New York, v. 122, n. 20, p. 3533–3545, 2013.
- AGANNA, E.; BURRIN, J.M.; HITMAN, G.A; TURNER, M.D. Involvement of calpain and synaptotagmin Ca²⁺ sensors in hormone secretion from excitable endocrine cells. **The Journal of endocrinology**, Bristol, v. 190, n. 3, p. R1–7, 2006.
- ALARCON-ROJO, A. D.; DRANSFIELD, E. Alteration of post-mortem ageing in beef by the addition of enzyme inhibitors and activators. **Meat Science**, Amsterdam, v. 41, n. 2, p. 163–178, 1995.
- ANDERS, S.; HUBER, W. Differential expression analysis for sequence count data. **Genome Biology**, London, v. 11, 2010. Available in: <http://genomebiology.com/content/pdf/gb-2010-11-10-r106.pdf>. Accessed on: 04 Dec. 2014.
- BAEK, K.-H.; ZASLAVSKY, A.; LYNCH, R. C.; BRITT, C.; OKADA, Y.; SIAREY, R. J.; LENSCH, M. W.; PARK, I.-H.; YOON, S. S.; MINAMI, T.; KORENBERG, J. R.; FOLKMAN, J.; DALEY, G. Q.; AIRD, W. C.; GALDZICKI, Z.; RYEOM, S. Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1. **Nature**, London, v. 459, n. 7250, p. 1126–1130, 2009.
- BAI, J.; LAI, L.; YEO, H. C.; GOH, B.C.; TAN, T.M. Multidrug resistance protein 4 (MRP4/ABCC4) mediates efflux of bimane-glutathione. **The International Journal of Biochemistry & Cell Biology**, Exeter, v. 36, n. 2, p. 247–257, 2004.
- BAI, Q.; MCGILLIVRAY, C.; DA COSTA, N.; DORNAN, S.; EVANS, G.; STEAR, M. J.; CHANG, K.-C. Development of a porcine skeletal muscle cDNA microarray: analysis of differential transcript expression in phenotypically distinct muscles. **BMC Genomics**, London, v. 4, n. 1, p. 8, 2003.
- BARABÁSI, A.-L.; OLTVAI, Z. N. Network biology: understanding the cell's functional organization. **Nature Reviews. Genetics**, London, v. 5, n. 2, p. 101–113, 2004.
- BENJAMINI, Y.; HOCHBERG, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. **Journal of the Royal Statistical Society Serie B (Methodological)**, London, v. 57, p. 289-300, 1995.
- BERNARD, C.; CASSAR-MALEK, I.; LE CUNFF, M.; DUBROEUCQ, H.; RENAND, G.; HOCQUETTE, J.-F. New indicators of beef sensory quality revealed by expression of specific genes. **Journal of Agricultural and Food Chemistry**, Easton, v. 55, n. 13, p. 5229–52237, 2007.

BERNARD, C.; DEGRELLE, S.; OLLIER, S.; CAMPION, E.; CASSAR-MALEK, I.; CHARPIGNY, G.; DHORNE-POLLET, S.; HUE, I.; HOCQUETTE, J.-F.; LE PROVOST, F.; LEROUX, C.; PIUMI, F.; ROLLAND, G.; UZBEKOVA, S.; ZALACHAS, E.; MARTIN, P. A cDNA macroarray resource for gene expression profiling in ruminant tissues involved in reproduction and production (milk and beef) traits. **Journal of Physiology and Pharmacology** : an official journal of the Polish Physiological Society, Krakow, v. 56 Suppl 3, p. 215–224, 2005.

BERS, D.M. Cardiac excitation-contraction coupling. **Nature**, London, v. 415, n. 6868, p. 198–205, 2002.

BIONAZ, M.; LOOR, J.J. Gene networks driving bovine milk fat synthesis during the lactation cycle. **BMC Genomics**, London, v. 9, n. 1, p. 366, 2008.

BOUDIDA, Y.; GAGAOUA, M.; BECILA, S.; PICARD, B.; BOUDJELLAL, A.; HERRERA-MENDEZ, C. H.; SENTANDREU, M.; OUALI, A. Serine protease inhibitors as good predictors of meat tenderness: which are they and what are their functions? **Critical reviews in food science and nutrition**, London, 2014. Available in: < http://www.tandfonline.com/doi/abs/10.1080/10408398.2012.741630?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed > Accessed on: 20 Dec. 2014.

BROWN, H.; MEISTER, B.; DEENEY, J.; CORKEY, B.E.; YANG, S.N.; LARSSON, O.; RHODES, C.J.; SEINO, S.; BERGGREN, P.O.; FRIED, G. Synaptotagmin III isoform is compartmentalized in pancreatic beta-cells and has a functional role in exocytosis. **Diabetes**, New York, v. 49, n. 3, p. 383–391, 2000.

BULLARD, J.H.; PURDOM, E.; HANSEN, K.D.; DUDOIT, S. Evaluation of statistical methods for normalization and differential expression in mRNA-Seq experiments. **BMC bioinformatics**, London, v. 11, n. 1, p. 94, 2010.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Available in: <<http://www.agricultura.gov.br/>>. Accessed on: 02 Jan. 2015.

CAFE, L.M.; MCINTYRE, B.L.; ROBINSON, D.L.; GEESINK, G.H.; BARENDSE, W.; PETHICK, D.W.; THOMPSON, M.; GREENWOOD, P.L. Production and processing studies on calpain-system gene markers for tenderness in Brahman cattle : 2. Objective meat quality. **Journal of Animal Science**, Savoy, v. 88, p. 3059–3069, 2010a.

CAFE, L.M.; MCINTYRE, B.L.; ROBINSON, D.L.; GEESINK, G.H.; BARENDSE, W.; GREENWOOD, P.L. Production and processing studies on calpain-system gene markers for tenderness in Brahman cattle: 1. Growth, efficiency, temperament, and carcass characteristics. **Journal of Animal Science**, Savoy, v. 88, n. 9, p. 3047–3058, Sept. 2010b.

CAGNAZZO, M.; TE PAS, M.F.W.; PRIEM, J.; DE WIT, A.A.C.; POOL, M.H.; DAVOLI, R.; RUSSO, V. Comparison of prenatal muscle tissue expression profiles of two pig breeds differing in muscle characteristics. **Journal of Animal Science**, Savoy, v. 84, n. 1, p. 1–10, 2006.

CANAVEZ, F.C.; LUCHE, D.D.; STOTHARD, P.; LEITE, K.R.M.; SOUSA-CANAVEZ, J.M.; PLASTOW, G.; MEIDANIS, J.; SOUZA, M.A.; FEIJAO, P.; MOORE, S.S.; CAMARA-LOPES, L.H. Genome sequence and assembly of *Bos indicus*. **Journal of Heredity**, Washington, doi:10.1093/jhered/esr153, 2012.

CARRAGHER, N.O.; LEVKAU, B.; ROSS, R.; RAINES, E.W. Degraded collagen fragments promote rapid disassembly of smooth muscle focal adhesions that correlates with cleavage of pp125 (FAK), paxillin, and talin. **The Journal of Cell Biology**, New York, v. 147, n. 3, p. 619–630, 1999.

CARVALHO, M.E.; GASPARIN, G.; POLETI, M.D.; ROSA, A.F.; BALIEIRO, J.C. C.; LABATE, C.A.; NASSU, R.T.; TULLIO, R.R.; REGITANO, L.C.D.A.; MOURÃO, G.B.; COUTINHO, L.L. Heat shock and structural proteins associated with meat tenderness in Nellore beef cattle, a *Bos indicus* breed. **Meat Science**, Amsterdam, v. 96, n. 3, p. 1318–13124, 2014.

CASAS, E.; DUAN, Q.; SCHNEIDER, M.J.; SHACKELFORD, S.D.; WHEELER, T. L.; CUNDIFF, L.V.; REECY, J.M. Polymorphisms in calpastatin and mu-calpain genes are associated with beef iron content. **Animal Genetics**, Oxford, v. 45, n. 2, p. 283–284, 2014.

CEPEA - CENTRO DE ESTUDOS AVANÇADOS EM ECONOMIA APLICADA DA ESALQ/USP. **Relatório PIB Agro – Brasil**. Available in <
<http://cepea.esalq.usp.br/pib/>> Accessed on 5 Dec. 2015.

CESAR, A. S.M.; REGITANO, L.C.A; MOURÃO, G.B.; TULLIO, R.R.; LANNA, D. P.D.; NASSU, R.T.; MUDADO, M.A; OLIVEIRA, P.S.N.; DO NASCIMENTO, M.L.; CHAVES, A.S.; ALENCAR, M.M.; SONSTEGARD, T.S.; GARRICK, D.J.; REECY, J.M.; COUTINHO, L.L. Genome-wide association study for intramuscular fat deposition and composition in Nellore cattle. **BMC genetics**, London, v. 15, p. 39, 2014.

CHEPELEV, I.; WEI, G.; TANG, Q.; ZHAO, K. Detection of single nucleotide variations in expressed exons of the human genome using RNA-Seq. **Nucleic Acids Research**, Oxford, v. 37, n. 16, p. e106, 2009.

CHILDERS, C.P.; REESE, J.T.; SUNDARAM, J.P.; VILE, D.C.; DICKENS, C M.; CHILDS, K.L.; SALIH, H.; BENNETT, A.K.; HAGEN, D.E.; ADELSON, D.L.; ELSIK, C. G. Bovine Genome Database: integrated tools for genome annotation and discovery. **Nucleic Acids Research**, Oxford, v. 39, n. Database issue, p. D830–4, 2011.

CHOI, Y.M.; KIM, B.C. Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. **Livestock Science**, Amsterdam, v. 122, n. 2/3, p. 105–118, 2009.

CHOI, Y.M.; LEE, S.H.; CHOE, J.H.; RHEE, M.S.; LEE, S.K.; JOO, S.T.; KIM, B. C. Protein solubility is related to myosin isoforms, muscle fiber types, meat quality traits, and postmortem protein changes in porcine longissimus dorsi muscle. **Livestock Science**, Amsterdam, v. 127, n. 2/3, p. 183–191, 2010.

CHURCHILL, G.A. Fundamentals of experimental design for cDNA microarrays. **Nature Genetics**, London, v. 32 Suppl, p. 490–495, 2002.

CIRULLI, E.T.; SINGH, A.; SHIANN, K.V.; GE, D.; SMITH, J.P.; MAIA, J.M.; HEINZEN, E.L.; GOEDERT, J.J.; GOLDSTEIN, D.B. Screening the human exome: a comparison of whole genome and whole transcriptome sequencing. **Genome Biology**, London, v. 11, n. 5, p. R57, 2010.

CLOONAN, N.; FORREST, A.R.R.; KOLLE, G.; GARDINER, B.B.A.; FAULKNER, G.J.; BROWN, M.K.; TAYLOR, D.F.; STEPTOE, A.L.; WANI, S.; BETHEL, G.; ROBERTSON, A.J.; PERKINS, A.C.; BRUCE, S.J.; LEE, C.C.; RANADE, S.S.; PECKHAM, H.E.; MANNING, J.M.; MCKERNAN, K.J.; GRIMMOND, S M. Stem cell transcriptome profiling via massive-scale mRNA sequencing. **Nature Methods**, New York, v. 5, n. 7, p. 613–619, 2008.

COLELL, A.; RICCI, J.-E.; TAIT, S.; MILASTA, S.; MAURER, U.; BOUCHIER-HAYES, L.; FITZGERALD, P.; GUIO-CARRION, A.; WATERHOUSE, N.J.; LI, C.W.; MARI, B.; BARBRY, P.; NEWMAYER, D.D.; BEERE, H.M.; GREEN, D.R. GAPDH and autophagy preserve survival after apoptotic cytochrome c release in the absence of caspase activation. **Cell**, Cambridge, v. 129, n. 5, p. 983–997, 2007.

CRC- Cooperative Research Centre for Beef Genetic Technologies (n.d.) Annual report of CRC for Beef Genetic Technologies (2008/2009). High Quality Beef for Global Consumers. Armidale, Australia

CORTESIO, C.L.; BOATENG, L.R.; PIAZZA, T.M.; BENNIN, D.A.; HUTTENLOCHER, A. Calpain-mediated proteolysis of paxillin negatively regulates focal adhesion dynamics and cell migration. **The Journal of Biological Chemistry**, Baltimore, v. 286, n. 12, p. 9998–10006, 2011.

CROUSE, J.D.; CUNDIFF, L.V.; KOCH, R.M.; KOOHMARAIE, M.; SEIDEMAN, S.C. Comparisons of *Bos indicus* and *Bos Taurus* inheritance for carcass beef characteristics and meat palatability. **Journal Animal Science**, Albany, v. 67, p. 2661-2668, 1989.

CUNNINGHAM, E.P.; SYRSTAD, O. Crossbreeding bos indicus and bos taurus for milk production in the tropics: Zebu cattle. **FAO animal production and health paper**, Rome, n.68, 1987. Available in: <<http://www.fao.org/docrep/009/t0095e/t0095e00.HTM>>. Accessed on: 26 Dec. 2014.

D'ALESSANDRO, A.; MARROCCO, C.; RINALDUCCI, S.; MIRASOLE, C.; FAILLA, S.; ZOLLA, L. Chianina beef tenderness investigated through integrated Omics. **Journal of proteomics**, New York, v. 75, n. 14, p. 4381-4398, 2012.

DE JAGER, N.; HUDSON, N.; REVERTER-GOMEZ, T.; CAFE, L.; GREENWOOD, P.; WANG, Y.; PETHICK, D.; BARNARD, R.; DALRYMPLE, B. Effects of Tenderness Genotypes and of Experimental Site on the Expression of Fat and Ribosomal Module Genes. In: WORLD CONGRESS ON GENETICS APPLIED TO LIVESTOCK PRODUCTION, 9., 2010. Leipzig, Germany. **Anais...** Leipzig, Germany. 2010

DRIVER, A.M.; PEÑAGARICANO, F.; HUANG, W.; AHMAD, K.R.; HACKBART, K. S.; WILTBANK, M.C.; KHATIB, H. RNA-Seq analysis uncovers transcriptomic variations between morphologically similar in vivo- and in vitro-derived bovine blastocysts. **BMC genomics**, London, v. 13, n. 1, p. 118, 2012.

ELBEIN, S.C.; KERN, P.A.; RASOULI, N.; YAO-BORENGASSER, A.; SHARMA, N. K.; DAS, S.K. Global gene expression profiles of subcutaneous adipose and muscle from glucose-tolerant, insulin-sensitive, and insulin-resistant individuals matched for BMI. **Diabetes**, New York, v. 60, n. 3, p. 1019–1029, 2011.

ELSIK, C.G.; TELLAM, R.L.; WORLEY, K.C.; GIBBS, R.A.; MUZNY, D.M.; WEINSTOCK, G.M.; ADELSON, D.L.; EICHLER, E.E.; ELNITSKI, L.; GUIGÓ, R.; HAMERNIK, D.L.; KAPPES, S.M.; LEWIN, H.A.; LYNN, D.J.; NICHOLAS, F.W.; REYMOND, A.; RIJNKELS, M.; SKOW, L.C.; ZDOBNOV, E.M.; SCHOOK, L.; WOMACK, J.; ALIOTO, T.; ANTONARAKIS, S.E.; ASTASHYN, A.; CHAPPLE, C.E.; CHEN, H.-C.; CHRAST, J.; CÂMARA, F.; ERMOLAEVA, O.; HENRICHSEN, C.N.; HLAVINA, W.; KAPUSTIN, Y.; KIRYUTIN, B.; KITTS, P.; KOKOCINSKI, F.; LANDRUM, M.; MAGLOTT, D.; PRUITT, K.; SAPOJNIKOV, V.; SEARLE, S.M.; SOLOVYEV, V.; SOUVOROV, A.; UCLA, C.; WYSS, C.; ANZOLA, J.M.; GERLACH, D.; ELHAIK, E.; GRAUR, D.; REESE, J.T.; EDGAR, R.C.; MCEWAN, J.C.; PAYNE, G. M.; RAISON, J. M.; JUNIER, T.; KRIVENTSEVA, E. V.; EYRAS, E.; PLASS, M.; DONTU, R.; LARKIN, D. M.; REECY, J.; YANG, M. Q.; CHEN, L.; CHENG, Z.; CHITKO-MCKOWN, C. G.; LIU, G. E.; MATUKUMALLI, L. K.; SONG, J.; ZHU, B.; BRADLEY, D. G.; BRINKMAN, F. S. L.; LAU, L. P. L.; WHITESIDE, M. D.; WALKER, A.; WHEELER, T. T.; CASEY, T.; GERMAN, J. B.; LEMAY, D. G.; MAQBOOL, N. J.; MOLENAAR, A. J.; SEO, S.; STOTHARD, P.; BALDWIN, C. L.; BAXTER, R.; BRINKMEYER-LANGFORD, C. L.; BROWN, W. C.; CHILDERS, C. P.; CONNELLEY, T.; ELLIS, S. A.; FRITZ, K.; GLASS, E. J.; HERZIG, C. T. A.; IIVANAINEN, A.; LAHMERS, K. K.; BENNETT, A. K.; DICKENS, C. M.; GILBERT, J. G. R.; HAGEN, D. E.; SALIH, H.; AERTS, J.; CAETANO, A. R.; DALRYMPLE, B.; GARCIA, J. F.; GILL, C. A.; HIENDLEDER, S. G.; MEMILI, E.; SPURLOCK, D.; WILLIAMS, J. L.; ALEXANDER, L.; BROWNSTEIN, M. J.; GUAN, L.; HOLT, R. A.; JONES, S. J. M.; MARRA, M. A.; MOORE, R.; MOORE, S. S.; ROBERTS, A.; TANIGUCHI, M.; WATERMAN, R. C.; CHACKO, J.; CHANDRABOSE, M. M.; CREE, A.; DAO, M. D.; DINH, H. H.; GABISI, R. A.; HINES, S.; HUME, J.; JHANGIANI, S. N.; JOSHI, V.; KOVAR, C. L.; LEWIS, L. R.; LIU, Y.-S.; LOPEZ, J.; MORGAN, M. B.; NGUYEN, N. B.; OKWUONU, G. O.; RUIZ, S. J.; SANTIBANEZ, J.; WRIGHT, R. A.; BUHAY, C.; DING, Y.; DUGAN-ROCHA, S.; HERDANDEZ, J.; HOLDER, M.; SABO, A.; EGAN, A.; GOODELL, J.; WILCZEK-BONEY, K.; FOWLER, G. R.; HITCHENS, M. E.; LOZADO, R. J.; MOEN, C.; STEFFEN, D.; WARREN, J. T.; ZHANG, J.; CHIU, R.; SCHEIN, J. E.; DURBIN, K. J.; HAVLAK, P.; JIANG, H.; LIU, Y.; QIN, X.; REN, Y.; SHEN, Y.; SONG, H.; BELL, S. N.; DAVIS, C.; JOHNSON, A. J.; LEE, S.; NAZARETH, L. V.; PATEL, B. M.; PU, L.-L.; VATTATHIL, S.; WILLIAMS, R. L.;

CURRY, S.; HAMILTON, C.; SODERGREN, E.; WHEELER, D. A.; BARRIS, W.; BENNETT, G. L.; EGGEN, A.; GREEN, R. D.; HARHAY, G. P.; HOBBS, M.; JANN, O.; KEELE, J. W.; KENT, M. P.; LIEN, S.; MCKAY, S. D.; MCWILLIAM, S.; RATNAKUMAR, A.; SCHNABEL, R. D.; SMITH, T.; SNELLING, W. M.; SONSTEGARD, T. S.; STONE, R. T.; SUGIMOTO, Y.; TAKASUGA, A.; TAYLOR, J. F.; VAN TASSELL, C. P.; MACNEIL, M. D.; ABATEPAULO, A. R. R.; ABBEY, C. A.; AHOLA, V.; ALMEIDA, I. G.; AMADIO, A. F.; ANATRIELLO, E.; BAHADUE, S. M.; BIASE, F. H.; BOLDT, C. R.; CARROLL, J. A.; CARVALHO, W. A.; CERVELATTI, E. P.; CHACKO, E.; CHAPIN, J. E.; CHENG, Y.; CHOI, J.; COLLEY, A. J.; DE CAMPOS, T. A.; DE DONATO, M.; SANTOS, I. K. F. DE M.; DE OLIVEIRA, C. J. F.; DEOBALD, H.; DEVINOY, E.; DONOHUE, K. E.; DOVC, P.; EBERLEIN, A.; FITZSIMMONS, C. J.; FRANZIN, A. M.; GARCIA, G. R.; GENINI, S.; GLADNEY, C. J.; GRANT, J. R.; GREASER, M. L.; GREEN, J. A.; HADSELL, D. L.; HAKIMOV, H. A.; HALGREN, R.; HARROW, J. L.; HART, E. A.; HASTINGS, N.; HERNANDEZ, M.; HU, Z.-L.; INGHAM, A.; ISO-TOURU, T.; JAMIS, C.; JENSEN, K.; KAPETIS, D.; KERR, T.; KHALIL, S. S.; KHATIB, H.; KOLBEHDARI, D.; KUMAR, C. G.; KUMAR, D.; LEACH, R.; LEE, J. C.-M.; LI, C.; LOGAN, K. M.; MALINVERNI, R.; MARQUES, E.; MARTIN, W. F.; MARTINS, N. F.; MARUYAMA, S. R.; MAZZA, R.; MCLEAN, K. L.; MEDRANO, J. F.; MORENO, B. T.; MORÉ, D. D.; MUNTEAN, C. T.; NANDAKUMAR, H. P.; NOGUEIRA, M. F. G.; OLSAKER, I.; PANT, S. D.; PANZITTA, F.; PASTOR, R. C. P.; POLI, M. A.; POSLUSNY, N.; RACHAGANI, S.; RANGANATHAN, S.; RAZPET, A.; RIGGS, P. K.; RINCON, G.; RODRIGUEZ-OSORIO, N.; RODRIGUEZ-ZAS, S. L.; ROMERO, N. E.; ROSENWALD, A.; SANDO, L.; SCHMUTZ, S. M.; SHEN, L.; SHERMAN, L.; SOUTHEY, B. R.; LUTZOW, Y. S.; SWEEDLER, J. V.; TAMMEN, I.; TELUGU, B. P. V. L.; URBANSKI, J. M.; UTSUNOMIYA, Y. T.; VERSCHOOR, C. P.; WAARDENBERG, A. J.; WANG, Z.; WARD, R.; WEIKARD, R.; WELSH, T. H.; WHITE, S. N.; WILMING, L. G.; WUNDERLICH, K. R.; YANG, J.; ZHAO, F.-Q. The genome sequence of taurine cattle: a window to ruminant biology and evolution. **Science** (New York, N.Y.), v. 324, n. 5926, p. 522–528, 2009.

ENRIGHT, A.J.; OUZOUNIS, C.A. BioLayout- an automatic graph layout algorithm for similarity visualization. **Bioinformatics** (Oxford, England), v. 17, n. 9, p. 853–854, 2001.

ERNST C.W.; RILINGTON V.D.; RANEY N.E.; YAO J.; SIPKOWSKY S.S. SAAMA P.M.; TEMPELMAN R.J.; COUSSENS, P.M. **Use of cdna microarrays to detect differentially expressed genes in developing pig skeletal muscle**. In: PLANT AND ANIMAL GENOMES X CONFERENCE, 2002, San Diego, CA. **Abstracts...** p.9. San Diego: Scherago International, 2002, v.10, 702p.

FERGUSON, D.M.; BRUCE, H.L.; THOMPSON, J.M.; EGAN, A.F.; PERRY, D.; SHORTHORSE, W.R. Factors affecting beef palatability — farmgate to chilled carcass. **Animal Production Science**, Melbourne, v. 41, n. 7, p. 879–891, 2001.

FRANCO, S.; PERRIN, B.; HUTTENLOCHER, A. Isoform specific function of calpain 2 in regulating membrane protrusion. **Experimental Cell Research**, Amsterdam, v. 299, n. 1, p. 179–187, 2004.

FUENTES, J.J.; GENESCÀ, L.; KINGSBURY, T.J.; CUNNINGHAM, K.W.; PÉREZ-RIBA, M.; ESTIVILL, X.; DE LA LUNA, S. DSCR1, overexpressed in Down syndrome, is an inhibitor of calcineurin-mediated signaling pathways. **Human Molecular Genetics**, Oxford, v. 9, n. 11, p. 1681–1690, 2000.

GOLL, D.E.; NETI, G.; MARES, S.W.; THOMPSON, V. F. Myofibrillar protein turnover: the proteasome and the calpains. **Journal of Animal Science**, Savoy, v. 86, n. 14 Suppl, p. E19–35, 2008.

GOLL, D.E.; THOMPSON, V.F.; LI, H.; WEI, W.; CONG, J. The calpain system. **Physiological Reviews**, Bethesda, v.83, p.731–801, 2003.

GRAIFER, D.; KARPOVA, G. Roles of ribosomal proteins in the functioning of translational machinery of eukaryotes. **Biochimie**, Paris, v. 109, p.1-17, 2014.

GUBSER, C.; BERGAMASCHI, D.; HOLLINSHEAD, M.; LU, X.; VAN KUPPEVELD, F.J.M.; SMITH, G.L. A new inhibitor of apoptosis from vaccinia virus and eukaryotes. **PloS Pathogens**, San Francisco, v. 3, n. 2, p. e17, 2007.

HAMMOND, C.L.; MARCHAN, R.; KRANCE, S.M.; BALLATORI, N. Glutathione export during apoptosis requires functional multidrug resistance-associated proteins. **The Journal of Biological Chemistry**, Baltimore, v. 282, n. 19, p. 14337–14347, 2007.

HARBERS, M.; CARNINCI, P. Tag-based approaches for transcriptome research and genome annotation. **Nature Methods**, New York, v. 2, n. 7, p. 495–502, 2005.

HENDERSON, C.R. Sire evaluation and genetics trends. In: ANIMAL BREEDING AND GENETIC SYMPOSIUM, 1972, Blacksburg, VA: American Society of Animal Science; Champaign: American Dairy Science Association, 1972, p. 10.

HO, D.W.Y.; YANG, Z.F.; YI, K.; LAM, C.T.; NG, M.N.P.; YU, W.C.; LAU, J.; WAN, T.; WANG, X.; YAN, Z.; LIU, H.; ZHANG, Y.; FAN, S.T. Gene expression profiling of liver cancer stem cells by RNA-sequencing. **PloS one**, San Francisco, v. 7, n. 5, p. e37159, 2012.

HUANG, D.W.; SHERMAN, B.T.; LEMPICKI, R.A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. **Nature Protocols**, London, v. 4, n. 1, p. 44–57, 2009.

HUANG, W.; KHATIB, H. Comparison of transcriptomic landscapes of bovine embryos using RNA-Seq. **BMC Genomics**, London, v. 11, p. 711, 2010. Available in: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3019235/pdf/1471-2164-11-711.pdf>> Accessed on: 12 Dec. 2014.

HUDSON, N.J.; REVERTER, A.; DALRYMPLE, B.P. A differential wiring analysis of expression data correctly identifies the gene containing the causal mutation. **PLoS Computational Biology**, London, v. 5, 2009b. Available in: <<http://www.ploscompbiol.org/article/fetchObject.action?uri=info%3Adoi%2F10.1371%2Fjournal.pcbi.1000382&representation=PDF>>. Accessed on: 12 Dec. 2014.

HUDSON, N.J.; REVERTER, A.; WANG, Y.; GREENWOOD, P.L.; DALRYMPLE, B. P. Inferring the transcriptional landscape of bovine skeletal muscle by integrating co-expression networks. **PloS one**, San Francisco, v. 4, n. 10, p. e7249, 2009a.

HUI, Y. T.; YANG, Y. Q.; LIU, R. Y.; ZHANG, Y. Y.; XIANG, C. J.; LIU, Z. Z.; DING, Y. H.; ZHANG, Y. L.; WANG, B. R. Significant association of APOA5 and APOC3 polymorphisms with meat quality traits in Kele pigs. **Genetics and Molecular Research**, v. 12, n. 3, p. 3643–3650, 2013.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. Estatística da produção pecuária. Available in: <http://www.ibge.gov.br/home/estatistica/indicadores/agropecuaria/producaoagropecuaria/abate-leite-couro-ovos_201403_publ_completa.pdf>. Accessed on: 26 Dec. 2014.

JOHNSON, S.L.; FRANZ, C.; KUHN, S.; FURNESS, D.N.; RÜTTIGER, L.; MÜNKNER, S.; RIVOLTA, M.N.; SEWARD, E.P.; HERSCHMAN, H.R.; ENGEL, J.; KNIPPER, M.; MARCOTTI, W. Synaptotagmin IV determines the linear Ca²⁺ dependence of vesicle fusion at auditory ribbon synapses. **Nature Neuroscience**, New York, v. 13, n. 1, p. 45–52, 2010.

KANEHISA, M.; GOTO, S.; SATO. KEGG: Kyoto Encyclopedia of Genes and Genomes. **Nucleic Acids Research**, Oxford, v.28, p.27-30, 2000.

KANEHISA, M.; GOTO, S.; SATO, Y., KAWASHIMA, M.; FURUMICHI, M.; TANABE, M. Data, information, knowledge and principle: back to metabolism in KEGG. **Nucleic Acids Research**, Oxford v.42, p.199–205, 2014.

KOESTERKE, L.; MILFELD, K.; VAUGHN, M.W.; STANZIONE, D.; KOLTES, J.E.; WEEKS, N.T.; REECY, J.M. Optimizing the PCIT algorithm on stampede's Xeon and Xeon Phi processors for faster discovery of biological networks. In: CONFERENCE ON EXTREME SCIENCE AND ENGINEERING DISCOVERY ENVIRONMENT: GATEWAY TO DISCOVERY, 2013, San Diego. **Proceedings...** San Diego: ACM, 2013. p. 1-8.

KOOHMARAIE, M. Biochemical factors regulations the toughening and tenderization process of meat. **Journal of Animal Science**, Albany. v. 43, p. S193-S201, 1996.

KOOHMARAIE, M. Case study: Understanding and Managing Variation in Meat Tenderness. 1 CD-ROM. In: Veiseth, E., Kent, M. P.; Shackelford, S.D., eds. 2003. **Proceeding of Annual Meeting of the Brazilian Society of Animal Sciences**, 40. SBZ, Santa Maria, RS, Brasil, 2003.

KOOHMARAIE, M. Muscle Proteinases and Meat Aging. **Meat science**, Amsterdam, v. 36, p. 93–104, 1994.

KOOHMARAIE, M. The role of endogenous proteases in meat tenderness. In: RECIPROCAL MEAT CONFERENCE, 41, 1988, Wyoming. **Proceedings...** Champaign: AMSA, 1988. p. 89-100.

KOOHMARAIE, M.; SHACKELFORD, S. D.; WHEELER, T. L.; LONERGAN, S. M.; DOUMIT, M. E. A Muscle Hypertrophy Condition in Lamb (Callipyge): Characterization of Effects on Muscle Growth and Meat Quality Traits. **Journal of Animal Science**, Albany, v. 73, p. 3596–3607, 1995.

KURODA, K.; KUANG, S.; TAKETO, M.M.; RUDNICKI, M.A. Canonical Wnt signaling induces BMP-4 to specify slow myofibrogenesis of fetal myoblasts. **Skeletal Muscle**, London, v. 3, n. 1, p. 5, 2013.

LAI, L.; TAN, T.M.C. Role of glutathione in the multidrug resistance protein 4 (MRP4/ABCC4)-mediated efflux of cAMP and resistance to purine analogues. **The Biochemical Journal**, London, v. 361, n. 3, p. 497–503, 2002.

LANGMEAD, B.; TRAPNELL, C.; POP, M.; SALZBERG, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. **Genome Biology**, London, v. 10, n. 3, p. R25, 2009.

LAVILLE, E.; SAYD, T.; MORZEL, M.; BLINET, S.; CHAMBON, C.; LEPETIT, J.; RENAND, G.; HOCQUETTE, J.F. Proteome changes during meat aging in tough and tender beef suggest the importance of apoptosis and protein solubility for beef aging and tenderization. **Journal of Agricultural and Food Chemistry**, Easton, v. 57, n. 22, p. 10755–10764, 2009.

LOVE, M.I.; HUBER, W.; ANDERS, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. **Genome Biology**, London, v. 15, n. 12, p. 550, 2014.

LUKE, C.J.; PAK, S. C.; ASKEW, Y.S.; NAVIGLIA, T.L.; ASKEW, D.J.; NOBAR, S. M.; VETICA, A.C.; LONG, O.S.; WATKINS, S.C.; STOLZ, D.B.; BARSTEAD, R.J.; MOULDER, G.L.; BRÖMME, D.; SILVERMAN, G.A. An intracellular serpin regulates necrosis by inhibiting the induction and sequelae of lysosomal injury. **Cell**, Cambridge, v. 130, n. 6, p. 1108–1119, 2007.

LUND, S.P.; NETTLETON, D.; MCCARTHY, D.J.; SMYTH, G.K. Detecting differential expression in RNA-sequence data using quasi-likelihood with shrunken dispersion estimates. **Statistical Applications in Genetics and Molecular Biology**, Berkeley, v. 11, n. 5, p. 1-42, 2012.

MALTIN, C.; BALCERZAK, D.; TILLEY, R.; DELDAY, M. Determinants of meat quality: tenderness. **The Proceedings of the Nutrition Society**, London, v. 62, n. 2, p. 337–347, 2003.

MARTIN, J.A; WANG, Z. Next-generation transcriptome assembly. **Nature reviews. Genetics**, London. v. 12, n. 10, p. 671–682, 2011.

MCCABE, M.; WATERS, S.; MORRIS, D.; KENNY, D.; LYNN, D.; CREEVEY, C. RNA-seq analysis of differential gene expression in liver from lactating dairy cows divergent in negative energy balance. **BMC genomics**, London, v. 13, n. 1, p. 193, 2012.

MCCARTHY, F.M.; GRESHAM, C.R.; BUZA, T.J.; CHOUVARINE, P.; PILLAI, L.R.; KUMAR, R.; OZKAN, S.; WANG, H.; MANDA, P.; ARICK, T.; BRIDGES, S.M.; BURGESS, S.C. AgBase: supporting functional modeling in agricultural organisms. **Nucleic Acids Research**, Oxford, v. 39, p. 497-506, 2011.

MÉNDEZ-BARBERO, N.; ESTEBAN, V.; VILLAHOZ, S.; ESCOLANO, A.; URSO, K.; ALFRANCA, A.; RODRÍGUEZ, C.; SÁNCHEZ, S.A.; OSAWA, T.; ANDRÉS, V.; MARTÍNEZ-GONZÁLEZ, J.; MINAMI, T.; REDONDO, J.M.; CAMPANERO, M.R. A major role for RCAN1 in atherosclerosis progression. **EMBO Molecular Medicine**, Hoboken, v. 5, n. 12, p. 1901–1917, 2013.

METZKER, M.L. Sequencing technologies - the next generation. **Nature Reviews. Genetics**, London, v. 11, n. 1, p. 31–46, 2010.

MORGAN, J.B.; WHEELER, T.L.; KOOHMARAIE, M.; SAVELL, J.W.; CROUSE, J.D. Meat tenderness and the calpain proteolytic system in the *Longissimus* muscle of young bulls and steers. **Journal of Animal Science**, Albany, v. 71, p. 1471, 1993.

MORIN, R.D.; O'CONNOR, M.D.; GRIFFITH, M.; KUCHENBAUER, F.; DELANEY, A.; PRABHU, A.-L.; ZHAO, Y.; MCDONALD, H.; ZENG, T.; HIRST, M.; EAVES, C.J.; MARRA, M. A. Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells. **Genome Research**, New York, v. 18, n. 4, p. 610–621, 2008.

MORTAZAVI, A.; WILLIAMS, B.A.; MCCUE, K.; SCHAEFFER, L.; WOLD, B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. **Nature Methods**, New York, v. 5, n. 7, p. 1–8, 2008.

MRODE R.A. In: _____. **Linear models for the prediction of animal breeding values**. 2nd ed. Wallingford, Oxon: CAB International, 2005, 344p.

NALPAS, N.C.; PARK, S.D.; MAGEE, D.A.; TARAKTSOGLU, M.; BROWNE, J. A.; CONLON, K.M.; RUE-ALBRECHT, K.; KILLICK, K.E.; HOKAMP, K.; LOHAN, A. J.; LOFTUS, B.J.; GORMLEY, E.; GORDON, S.V; MACHUGH, D E. Whole-transcriptome, high-throughput RNA sequence analysis of the bovine macrophage response to *Mycobacterium bovis* infection in vitro. **BMC Genomics**, London, v. 14, n. 1, p. 230, 2013.

NEMETH, P.M.; LOWRY, O.H. Myoglobin levels in individual human skeletal muscle fibers of different types. **The journal of histochemistry and cytochemistry** : official journal of the Histochemistry Society, Seattle, v. 32, n. 11, p. 1211–1216, 1984.

NONNEMAN, D.J.; SHACKELFORD, S.D.; KING, D.A.; ROHRER, G.A. Genome-wide association of meat quality traits and tenderness in swine. **Journal of Animal Science**, Savoy, v. 91, p. 4043–4050, 2013.

OKONIEWSKI, M.J.; MILLER, C.J. Hybridization interactions between probesets in short oligo microarrays lead to spurious correlations. **BMC Bioinformatics**, London, v. 7, p. 276, 2006.

OKSBJERG, N.; THERKILDSEN, M. Importance of muscle development and growth for animal production and meat quality. **Muscle development, growth and muscle function of meat animal**. Wallingford, Oxfordshire, UK ; Cambridge, MA : CABI Pub., 2004. Chapter 15 p. 1–22, [s.d.], 2012. Available in: [http://vsp.lf.dk/%7E/media/Files/Laerebog_fysiologi/Chapter_15.ashx]. Accessed on 20 Dec. 2014.

OUALI, A.; GAGAOUA, M.; BOUDIDA, Y.; BECILA, S.; BOUDJELLAL, A.; HERRERA-MENDEZ, C. H.; SENTANDREU, M. A. Biomarkers of meat tenderness: present knowledge and perspectives in regards to our current understanding of the mechanisms involved. **Meat Science**, Amsterdam, v. 95, n. 4, p. 854–870, 2013.

OUALI, A.; HERRERA-MENDEZ, C.H.; COULIS, G.; BECILA, S.; BOUDJELLAL, A.; AUBRY, L.; SENTANDREU, M.A. Revisiting the conversion of muscle into meat and the underlying mechanisms. **Meat Science**, Amsterdam, v. 74, n. 1, p. 44–58, 2006.

PATTERSON, A.H.D.; THOMPSON, R. Recovery of Inter-Block Information when Block Sizes are Unequal. **Biometrika Trust**, London, v. 58, n. 3, p. 545–554, 1971.

PAZ, C.C.P.; LUCHIARI FILHO, A. Melhoramento genético e diferenças de raças com relação à qualidade da carne bovina. **Pecuária de Corte**, São Paulo, n.101, p. 58-63, 2000.

PEARCE, K.L.; ROSENVOLD, K.; ANDERSEN, H.J.; HOPKINS, D.L. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes--a review. **Meat Science**, Amsterdam, v. 89, n. 2, p. 111–124, 2011.

PEÑAGARICANO, F.; SOUZA, A.H.; CARVALHO, P.D.; DRIVER, A.M.; GAMBRA, R.; KROPP, J.; HACKBART, K. S.; LUCHINI, D.; SHAVER, R.D.; WILTBANK, M.C.; KHATIB, H. Effect of maternal methionine supplementation on the transcriptome of bovine preimplantation embryos. **PloS one**, London, v. 8, n. 8, p. e72302, 2013.

PONSUKSILI, S.; DU, Y.; HADLICH, F.; SIENGDEE, P.; MURANI, E.; SCHWERIN, M.; WIMMERS, K. Correlated mRNAs and miRNAs from co-expression and regulatory networks affect porcine muscle and finally meat properties. **BMC genomics**, London, v. 14, p. 533, 2013.

PONSUKSILI, S.; JONAS, E.; MURANI, E.; PHATSARA, C.; SRIKANCHAI, T.; WALZ, C.; SCHWERIN, M.; SCHELLANDER, K.; WIMMERS, K. Trait correlated expression combined with expression QTL analysis reveals biological pathways and candidate genes affecting water holding capacity of muscle. **BMC genomics**, London, v. 9, p. 367, 2008a.

PONSUKSILI, S.; MURANI, E.; PHATSARA, C.; JONAS, E.; WALZ, C.; SCHWERIN, M.; SCHELLANDER, K.; WIMMERS, K. Expression profiling of muscle reveals transcripts differentially expressed in muscle that affect water-holding capacity of pork. **Journal of Agricultural and Food Chemistry**, Easton, v. 56, n. 21, p. 10311–10317, 2008b.

PRINGLE, T.D.; HARRELSON, J.M.; WEST, R.L.; WILLIAMS, S.E.; JOHNSON, D. D. Calcium-activated tenderization of strip loin, top sirloin , and top round steaks in diverse genotypes of cattle. **Journal of Animal Science**, Savoy, n. 77, p. 3230–3237, 1999.

RENAND, G.; PICARD, B.; TOURAILLE, C.; BERGE, P.. LEPETIT, J. Relationships between muscle characteristics and meat quality traits of young Charolais bulls. **Meat Science**, Amsterdam, v. 59, p. 49–60, 2001.

REVERTER, A.; CHAN, E.K.F. Combining partial correlation and an information theory approach to the reversed engineering of gene co-expression networks. **Bioinformatics** Oxford, v. 24, n. 21, p. 2491–2497, 2008.

REVERTER, A.; HUDSON, N.J.; NAGARAJ, S.H.; PÉREZ-ENCISO, M.; DALRYMPLE, B.P. Regulatory impact factors: unraveling the transcriptional regulation of complex traits from expression data. **Bioinformatics** Oxford, , v. 26, n. 7, p. 896–904, 2010.

REVERTER, A.; HUDSON, N.J.; WANG, Y.; TAN, S.-H.; BARRIS, W.; BYRNE, K. A.; MCWILLIAM, S.M.; BOTTEMA, C.D.K.; KISTER, A.; GREENWOOD, P.L.; HARPER, G.S.; LEHNERT, S.A.; DALRYMPLE, B P. A gene coexpression network for bovine skeletal muscle inferred from microarray data. **Physiological genomics**, Bethesda, v. 28, n. 1, p. 76–83, 2006.

REZENDE, L.R. **Expressão de genes relacionados ao metabolismo de vitamina D3 mediante suplementação e estudo de associação com a maciez da carne em bovinos da raça Nelore**. 2010. 89 p. Dissertação de Mestrado (Ciência Animal e Pastagens) - Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, Piracicaba, 2010.

ROBINSON, M.D.; MCCARTHY, D.J.; SMYTH, G.K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. **Bioinformatics** Oxford, v. 26, n. 1, p. 139–140, 2010.

ROSA, A.J. de M.; FRAGOSO, R. da R. **Análise Genômica em Bovinos**. Planaltina, DF:Embrapa Cerrados. 2010. p 1-3.

SADKOWSKI, T.; JANK, M.; MOTYL, T.; AGRICULTURAL, W. Age-dependent changes in bovine skeletal muscle transcriptomic profile 1. **Journal of Physiology and Pharmacology**: an official journal of the Polish Physiological Society, Krakow, v. 57, n. 7, p. 95–110, 2006.

SAEKI, M.; IRIE, Y.; NI, L.; ITSUKI, Y.; TERAOKA, Y.; KAWABATA, S.; KAMISAKI, Y. Calcineurin potentiates the activation of procaspase-3 by accelerating its proteolytic maturation. **The Journal of Biological Chemistry**, Baltimore, v. 282, n. 16, p. 11786–11794, 2007.

SAS INSTITUTE. **Statistical Analysis System**: user's guide; version 9.1. ed. Cary, 2003. 5136 p.

SEO, K.; BEEVER, J.E. **Monitoring gene expression in swine skeletal muscle**. In: PLANT AND ANIMAL GENOMES '99, CONFERENCE, 2001, San Diego, CA. **Abstracts...** p.9. San Diego: Scherago International, 2001, v.9. p. 207.

SHACKELFORD, S.D.; WHEELER, T.L.; KOOHMARAIE, M. Relationship between shear force and trained sensory panel tenderness ratings of 10 major muscles from *Bos indicus* and *Bos taurus* cattle. **Journal of Animal Science**, Savoy, v. 73, n. 11, p. 3333–3340, 1995.

SHI, J.; WU, X.; SURMA, M.; VEMULA, S.; ZHANG, L.; YANG, Y.; KAPUR, R.; WEI, L. Distinct roles for ROCK1 and ROCK2 in the regulation of cell detachment. **Cell Death & Disease**, New York, v. 4, p. e483, 2013.

SMYTH, G.K. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. **Statistical applications in Genetics and Molecular Biology**, Berkeley, v. 3, p. Article 3, 2004.

STRYDOM, P.E.; NAUDE, R.T.; SMITH, M.F.; SCHOLTZ, M.M.; VAN WYK, J.B. Characterisation of indigenous African cattle breeds in relation to meat quality traits. **Meat Science**, Amsterdam, v. 55, n. 1, p. 79–88, 2000.

SÜDHOF, T.C. Synaptotagmins: why so many? **The Journal of Biological Chemistry**, Baltimore, v. 277, n. 10, p. 7629–7632, 2002.

TARZE, A.; DENIAUD, A.; LE BRAS, M.; MAILLIER, E.; MOLLE, D.; LAROCLETTE, N.; ZAMZAMI, N.; JAN, G.; KROEMER, G.; BRENNER, C. GAPDH, a novel regulator of the pro-apoptotic mitochondrial membrane permeabilization. **Oncogene**, New York, v. 26, n. 18, p. 2606–2620, 2007.

TE PAS, M.F.W.; DE WIT, A.A.W.; PRIEM, J.; CAGNAZZO, M.; DAVOLI, R.; RUSSO, V.; POOL, M.H. Transcriptome expression profiles in prenatal pigs in relation to myogenesis. **Journal of Muscle Research and Cell Motility**, London, v. 26, n. 2/3, p. 157–165, 2005.

TIZIOTO, P.C.; DECKER, J.E.; TAYLOR, J.F.; SCHNABEL, R.D.; MUDADU, M.A.; SILVA, F.L.; MOURÃO, G.B.; COUTINHO, L.L.; THOLON, P.; SONSTEGARD, T. S.; ROSA, A.N.; ALENCAR, M.M.; TULLIO, R.R.; MEDEIROS, S.R.; NASSU, R. T.; FEIJÓ, G.L.D.; SILVA, L.O.C.; TORRES, R.A; SIQUEIRA, F.; HIGA, R.H.; REGITANO, L.C.A. Genome scan for meat quality traits in Nelore beef cattle. **Physiological Genomics**, Bethesda, v. 45, n. 21, p. 1012–1020, 2013a.

TIZIOTO, P.C.; GASPARIN, G.; SOUZA, M.M.; MUDADU, M.A; COUTINHO, L.L.; MOURÃO, G.B.; THOLON, P.; MEIRELLES, S.L.C.; TULLIO, R.R.; ROSA, A.N.; ALENCAR, M.M.; MEDEIROS, S.R.; SIQUEIRA, F.; FEIJÓ, G.L.D.; NASSU, R.T.; REGITANO, L.C.A. Identification of KCNJ11 as a functional candidate gene for bovine meat tenderness. **Physiological Genomics**, Bethesda, v. 45, n. 24, p. 1215–21, 2013b.

TRAPNELL, C.; PACHTER, L.; SALZBERG, S. L. TopHat: discovering splice junctions with RNA-Seq. **Bioinformatics** Oxford, v. 25, n. 9, p. 1105–1111, 2009.

TRAPNELL, C.; WILLIAMS, B.A.; PERTEA, G.; MORTAZAVI, A.; KWAN, G.; VAN BAREN, M.J.; SALZBERG, S.L.; WOLD, B.J.; PACHTER, L. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. **Nature Biotechnology**, London, v. 28, p. 511-515, 2010.

UYTTERHAEGEN, L.; CLAEYS, E.; DEMEYER, D. Effects of exogenous protease effectors on beef tenderness development and myofibrillar degradation and solubility. **Journal of Animal Science**, Savoy, v. 72, n. 5, p. 1209–1223, 1994.

VOET, D.; VOET, J.G.; PRATT, C.W. **Fundamentals of Biochemistry**: Live at the molecular level. 4th Ed. In: _____. Hoboken, NJ: Wiley, 2013. 1200p.

WANG, Z.; GERSTEIN, M.; SNYDER, M. RNA-Seq : a revolutionary tool for transcriptomics **Nature Reviews. Genetics**, London, v. 10, n. 1, p. 57–63, 2010.

WERNER, T. Next generation sequencing in functional genomics. **Briefings in Bioinformatics**, London, v. 11, n. 5, p. 499–511, 2010.

WESTON, A.R.; ROGERS, R.W.; ALTHEN, T.G. REVIEW: The Role of Collagen in Meat Tenderness. **Professional Animal Scientist**, Savoy, v. 18, n. 2, p. 107–111, 2002.

WHEELER, T.L.; CUNDIFF, L.V; SHACKELFORD, S.D.; KOOHMARAIE, M. Characterization of biological types of cattle (Cycle VII): Carcass, yield, and longissimus palatability traits. **Journal of Animal Science**, Savoy, v. 83, n. 1, p. 196–207, 2005.

WHEELER, T.L.; KOOHMARAIE, M.; SHACKELFORD, S.D. **Standardized warner bratzler shear force procedures for meat tenderness measurement**. Clay Center: Romam L. Hruska U. S. MARC, USDA, p. 7, 1995. p. 7.

WILLIAMS, A.H.; LIU, N.; VAN ROOIJ, E.; OLSON, E.N. MicroRNA control of muscle development and disease. **Current Opinion in Cell Biology**, Philadelphia, v. 21, n. 3, p. 461–469, 2009.

WU, Y.; SONG, W. Regulation of RCAN1 translation and its role in oxidative stress-induced apoptosis. **FASEB journal** : official publication of the Federation of American Societies for Experimental Biology, Bethesda, v. 27, n. 1, p. 208–221, 2013.

ZAMPIERI, M.; SORANZO, N.; ALTAFINI, C. Discerning static and causal interactions in genome-wide reverse engineering problems. **Bioinformatics**, Oxford, v. 24, n. 13, p. 1510–1515, 2008.

ZHANG, Q.; FUKUDA, M.; VAN BOCKSTAELE, E.; PASCUAL, O.; HAYDON, P. G. Synaptotagmin IV regulates glial glutamate release. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 101, n. 25, p. 9441–9446, 2004.

ZHANG, Y.; ZAN, L.; WANG, H. Screening candidate genes related to tenderness trait in Qinchuan cattle by genome array. **Molecular Biology Reports**, New York, v. 38, n. 3, p. 2007–2014, 2011.

ZHAO, C.; TIAN, F.; YU, Y.; LUO, J.; HU, Q.; BEQUETTE, B. J.; BALDWIN VI, R. L.; LIU, G.; ZAN, L.; SCOTT UPDIKE, M.; SONG, J. Muscle transcriptomic analyses in Angus cattle with divergent tenderness. **Molecular Biology Reports**, New York, v. 39, n. 4, p. 4185–4193, 2012.

ANNEX

ANNEX A - Tables from 10 to 29 with all enrichments for each DH.

Table 10 shows the results of the top enrichments for all negative and all positive genes correlated with *Pseudogene* for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 10 - Top enrichments for all negative and positive genes correlated with *Pseudogene* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	387	36.4	4.80E-15	9.20E-12
GOTERM_BP_ALL	cellular metabolic process	270	25.4	1.80E-14	1.70E-11
GOTERM_BP_ALL	metabolic process	316	29.7	2.50E-11	1.60E-08
GOTERM_BP_ALL	cellular macromolecule metabolic process	198	18.6	5.20E-10	2.50E-07
GOTERM_BP_ALL	primary metabolic process	269	25.3	9.80E-10	3.70E-07
GOTERM_MF_ALL	protein binding	299	28.1	3.10E-09	2.10E-06
GOTERM_BP_ALL	cellular protein metabolic process	129	12.1	1.40E-08	4.50E-06
GOTERM_BP_ALL	macromolecule metabolic process	214	20.1	1.10E-07	3.00E-05
GOTERM_BP_ALL	cellular catabolic process	50	4.7	3.20E-06	7.70E-04
GOTERM_BP_ALL	protein metabolic process	143	13.5	8.10E-06	1.70E-03
KEGG_PATHWAY	Proteasome	11	1	9.80E-05	1.60E-02
Enrichments for all positive genes correlated with DH					
GOTERM_BP_ALL	cellular process	183	26.3	1.20E-06	1.60E-03
GOTERM_MF_ALL	binding	258	37.1	3.50E-05	1.60E-02
GOTERM_BP_ALL	cellular macromolecule metabolic process	94	13.5	5.30E-05	3.40E-02
KEGG_PATHWAY	Insulin signaling pathway	12	1.7	6.60E-04	4.20E-02
KEGG_PATHWAY	Neurotrophin signaling pathway	12	1.7	4.00E-04	5.10E-02
GOTERM_BP_ALL	cellular metabolic process	119	17.1	1.30E-04	5.50E-02
GOTERM_MF_ALL	phosphatase activity	15	2.2	1.10E-03	9.50E-02
GOTERM_MF_ALL	nucleotide binding	67	9.6	1.10E-03	8.10E-02
GOTERM_MF_ALL	zinc ion binding	60	8.6	5.50E-04	1.20E-01
GOTERM_MF_ALL	RNA binding	22	3.2	7.80E-04	1.20E-01
GOTERM_MF_ALL	nucleic acid binding	79	11.4	9.00E-04	1.00E-01

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 11 shows the results of the top enrichments for all negative and all positive genes correlated with *PTPN3* (protein tyrosine phosphatase, non-receptor type 3) for significant GO terms for biologic process (BP) and molecular function (MF).

Table 11 - Top enrichments for all negative and positive genes correlated with *PTPN3* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_MF_ALL	binding	555	47.6	2.00E-11	1.40E-08
GOTERM_MF_ALL	protein binding	316	27.1	5.40E-09	1.90E-06
GOTERM_BP_ALL	cellular process	376	32.2	8.50E-07	1.70E-03
GOTERM_BP_ALL	macromolecule localization	61	5.2	1.20E-06	1.20E-03
GOTERM_BP_ALL	protein transport	49	4.2	2.00E-06	1.30E-03
GOTERM_BP_ALL	establishment of protein localization	49	4.2	2.10E-06	1.00E-03
GOTERM_BP_ALL	protein localization	52	4.5	3.10E-06	1.20E-03
GOTERM_BP_ALL	intracellular transport	36	3.1	1.90E-05	6.30E-03
GOTERM_BP_ALL	regulation of biological process	229	19.6	3.60E-05	1.00E-02
GOTERM_BP_ALL	regulation of cellular process	217	18.6	4.60E-05	1.10E-02
Enrichments for all positive genes correlated with DH					
GOTERM_BP_ALL	cellular process	53	40.5	2.70E-05	1.70E-02
GOTERM_BP_ALL	cellular macromolecule metabolic process	30	22.9	4.00E-04	8.40E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 12 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *TRIM45* (tripartite motif containing 45) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 12 - Top enrichments for all negative and positive genes correlated with *TRIM45* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	373	35	2.80E-13	5.60E-10
GOTERM_MF_ALL	protein binding	309	29	4.90E-13	3.30E-10
GOTERM_BP_ALL	cellular metabolic process	255	23.9	9.60E-12	9.60E-09
GOTERM_BP_ALL	cellular macromolecule metabolic process	191	17.9	3.00E-09	2.00E-06
GOTERM_BP_ALL	metabolic process	294	27.6	1.10E-07	5.40E-05
GOTERM_BP_ALL	primary metabolic process	252	23.6	3.30E-07	1.30E-04
GOTERM_BP_ALL	macromolecule metabolic process	204	19.1	1.50E-06	5.10E-04
GOTERM_BP_ALL	cellular protein metabolic process	117	11	3.80E-06	1.10E-03
GOTERM_MF_ALL	binding	488	45.7	6.10E-06	2.10E-03
GOTERM_BP_ALL	protein localization	48	4.5	7.20E-06	1.80E-03
KEGG_PATHWAY	Proteasome	11	1	6.90E-05	1.10E-02
Enrichments for all positive genes correlated with DH					
GOTERM_MF_ALL	zinc ion binding	40	12.3	2.00E-06	6.60E-04
GOTERM_BP_ALL	cellular metabolic process	67	20.6	9.70E-06	8.60E-03
GOTERM_BP_ALL	cellular process	93	28.5	1.10E-05	5.00E-03
GOTERM_MF_ALL	binding	129	39.6	1.20E-05	1.90E-03
GOTERM_MF_ALL	transition metal ion binding	42	12.9	6.30E-05	6.70E-03
GOTERM_BP_ALL	nitrogen compound metabolic process	35	10.7	2.60E-04	7.30E-02
	cellular nitrogen compound metabolic process	34	10.4	2.90E-04	6.30E-02
GOTERM_MF_ALL	nucleic acid binding	44	13.5	4.10E-04	3.30E-02
GOTERM_BP_ALL	regulation of RNA metabolic process	24	7.4	4.60E-04	7.70E-02
GOTERM_MF_ALL	DNA binding	28	8.6	4.80E-04	3.10E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 13 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *USP2* (ubiquitin specific peptidase 2) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 13 - Top enrichments for all negative and positive genes correlated with *USP2* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	354	37.1	1.10E-13	2.20E-10
GOTERM_MF_ALL	protein binding	285	29.8	1.10E-11	7.40E-09
GOTERM_BP_ALL	cellular metabolic process	231	24.2	6.80E-09	6.60E-06
GOTERM_BP_ALL	cellular macromolecule metabolic process	177	18.5	3.80E-08	2.50E-05
GOTERM_BP_ALL	macromolecule localization	55	5.8	1.20E-06	5.70E-04
GOTERM_BP_ALL	protein localization	48	5	1.30E-06	4.90E-04
GOTERM_BP_ALL	protein transport	44	4.6	2.70E-06	8.70E-04
GOTERM_BP_ALL	establishment of protein localization	44	4.6	2.80E-06	8.00E-04
GOTERM_BP_ALL	cellular process	191	20	4.30E-06	9.50E-04
GOTERM_BP_ALL	primary metabolic process	233	24.4	4.20E-06	1.00E-03
KEGG_PATHWAY	Proteasome	11	1.2	2.30E-05	3.60E-03
Enrichments for all positive genes correlated with DH					
GOTERM_BP_ALL	cellular metabolic process	67	22	3.10E-06	3.00E-03
GOTERM_BP_ALL	primary metabolic process	67	22	6.30E-05	3.00E-02
GOTERM_BP_ALL	cellular macromolecule metabolic process	50	16.4	1.00E-04	3.20E-02
GOTERM_BP_ALL	cellular process	88	28.9	1.40E-04	3.30E-02
GOTERM_MF_ALL	binding	127	41.6	2.60E-04	7.90E-02
GOTERM_BP_ALL	regulation of RNA metabolic process	24	7.9	3.10E-04	5.90E-02
GOTERM_BP_ALL	regulation of gene expression	31	10.2	3.60E-04	5.60E-02
GOTERM_BP_ALL	regulation of transcription	29	9.5	3.80E-04	5.10E-02
GOTERM_BP_ALL	regulation of nucleobase. nucleoside. nucleotide and nucleic acid metabolic process	30	9.8	4.10E-04	4.80E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 14 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *RTP4* (receptor (chemosensory) transporter protein 4) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 14 - Top enrichments for all negative and positive genes correlated with *RTP4* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular metabolic process	111	27.9	2.40E-08	2.80E-05
GOTERM_BP_ALL	glucose metabolic process	14	3.5	6.90E-08	4.00E-05
GOTERM_BP_ALL	catabolic process	34	8.5	4.60E-07	1.80E-04
GOTERM_BP_ALL	cellular process	151	37.9	7.10E-07	2.10E-04
GOTERM_BP_ALL	hexose metabolic process	14	3.5	7.20E-07	1.70E-04
GOTERM_BP_ALL	metabolic process	128	32.2	1.10E-06	2.10E-04
GOTERM_BP_ALL	monosaccharide metabolic process	14	3.5	2.30E-06	3.80E-04
GOTERM_MF_ALL	binding	208	52.3	2.30E-06	1.00E-03
GOTERM_MF_ALL	protein binding	123	30.9	1.10E-05	2.50E-03
GOTERM_BP_ALL	macromolecule catabolic process	21	5.3	2.30E-05	3.30E-03
KEGG_PATHWAY	Starch and sucrose metabolism	6	1.5	7.70E-04	8.60E-02
KEGG_PATHWAY	Purine metabolism	11	2.8	1.10E-03	6.50E-02
Enrichments for all positive genes correlated with DH					
GOTERM_BP_ALL	cellular process	434	33.7	2.90E-10	6.20E-07
GOTERM_MF_ALL	protein binding	352	27.3	4.70E-10	3.40E-07
GOTERM_MF_ALL	binding	598	46.4	6.40E-08	2.40E-05
GOTERM_BP_ALL	establishment of protein localization	53	4.1	2.10E-06	9.10E-04
GOTERM_BP_ALL	cellular metabolic process	278	21.6	1.20E-06	1.30E-03
GOTERM_BP_ALL	protein localization	57	4.4	1.90E-06	1.30E-03
GOTERM_BP_ALL	protein transport	53	4.1	2.00E-06	1.10E-03
GOTERM_BP_ALL	macromolecule localization	64	5	5.70E-06	2.10E-03
GOTERM_BP_ALL	cellular catabolic process	53	4.1	4.10E-05	1.20E-02
GOTERM_BP_ALL	cellular macromolecule metabolic process	202	15.7	9.90E-05	2.60E-02
KEGG_PATHWAY	Proteasome	11	0.9	3.70E-04	5.90E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 15 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *NT5C2* (5'-nucleotidase, cytosolic II) for significant GO terms for biologic process (BP), molecular function (MF) and cellular component (CC).

Table 15 - Top enrichments for all negative and positive genes correlated with *NT5C2* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular metabolic process	231	24.8	8.30E-13	1.50E-09
GOTERM_BP_ALL	cellular process	328	35.3	1.80E-12	1.60E-09
GOTERM_BP_ALL	cellular macromolecule metabolic process	175	18.8	2.00E-10	1.20E-07
GOTERM_BP_ALL	primary metabolic process	234	25.2	1.10E-09	4.70E-07
GOTERM_BP_ALL	metabolic process	266	28.6	5.00E-09	1.80E-06
GOTERM_BP_ALL	macromolecule metabolic process	189	20.3	2.80E-08	8.30E-06
GOTERM_BP_ALL	cellular protein metabolic process	111	11.9	8.90E-08	2.30E-05
GOTERM_BP_ALL	cellular catabolic process	47	5.1	4.40E-07	9.80E-05
GOTERM_MF_ALL	protein binding	250	26.9	2.30E-07	1.40E-04
GOTERM_BP_ALL	protein transport	42	4.5	2.40E-06	4.80E-04
Enrichments for all positive genes correlated with DH					
GOTERM_CC_ALL	intracellular	100	33.4	3.20E-04	5.20E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 16 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *GRB10* (growth factor receptor-bound protein 10) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 16 - Top enrichments for all negative and positive genes correlated with *GRB10* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	489	35.2	4.10E-16	9.60E-13
GOTERM_BP_ALL	cellular metabolic process	328	23.6	6.10E-13	6.60E-10
GOTERM_MF_ALL	protein binding	384	27.7	8.60E-12	6.50E-09
GOTERM_BP_ALL	cellular macromolecule metabolic process	243	17.5	1.10E-09	7.70E-07
GOTERM_BP_ALL	primary metabolic process	333	24	3.10E-09	1.70E-06
GOTERM_BP_ALL	metabolic process	384	27.7	6.30E-09	2.70E-06
GOTERM_BP_ALL	protein transport	60	4.3	7.30E-08	2.60E-05
GOTERM_BP_ALL	establishment of protein localization	60	4.3	7.90E-08	2.40E-05
GOTERM_BP_ALL	protein localization	64	4.6	9.90E-08	2.70E-05
GOTERM_BP_ALL	macromolecule localization	73	5.3	1.40E-07	3.40E-05
KEGG_PATHWAY	Proteasome	11	0.8	5.90E-04	9.50E-02
KEGG_PATHWAY	Pyrimidine metabolism	16	1.2	1.10E-03	8.90E-02
Enrichments for all positive genes correlated with DH					
GOTERM_MF_ALL	binding	586	41.7	1.50E-15	1.00E-12
GOTERM_BP_ALL	cellular macromolecule metabolic process	219	15.6	1.40E-12	2.80E-09
GOTERM_BP_ALL	cellular process	403	28.7	1.50E-12	1.50E-09
GOTERM_BP_ALL	post-translational protein modification	90	6.4	2.90E-11	2.00E-08
GOTERM_BP_ALL	regulation of cellular metabolic process	147	10.5	5.30E-11	2.70E-08
GOTERM_BP_ALL	cellular metabolic process	273	19.4	5.60E-11	2.30E-08
GOTERM_BP_ALL	regulation of primary metabolic process	142	10.1	9.40E-11	3.20E-08
GOTERM_BP_ALL	biopolymer modification	103	7.3	1.60E-10	4.70E-08
GOTERM_BP_ALL	regulation of metabolic process	152	10.8	1.70E-10	4.40E-08
GOTERM_BP_ALL	regulation of biological process	256	18.2	2.50E-10	5.70E-08
KEGG_PATHWAY	Endocytosis	28	2	3.30E-05	5.00E-03
KEGG_PATHWAY	Ubiquitin mediated proteolysis	23	1.6	5.00E-05	3.70E-03
KEGG_PATHWAY	TGF-beta signaling pathway	16	1.1	1.50E-04	7.60E-03
KEGG_PATHWAY	Pathways in cancer	34	2.4	2.50E-03	8.80E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 17 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *HECTD4* (HECT domain containing E3 ubiquitin protein ligase 4) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 17 - Top enrichments for all negative and positive genes correlated with *HECTD4* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	302	36.3	2.30E-09	4.10E-06
GOTERM_MF_ALL	protein binding	249	29.9	4.80E-09	2.90E-06
GOTERM_BP_ALL	cellular metabolic process	201	24.2	1.80E-07	1.70E-04
GOTERM_BP_ALL	metabolic process	239	28.7	8.80E-06	5.30E-03
GOTERM_BP_ALL	cellular macromolecule metabolic process	146	17.5	3.50E-05	1.50E-02
GOTERM_BP_ALL	primary metabolic process	202	24.3	5.50E-05	2.00E-02
GOTERM_BP_ALL	translation	29	3.5	6.30E-05	1.80E-02
GOTERM_BP_ALL	cellular protein metabolic process	95	11.4	6.80E-05	1.70E-02
GOTERM_BP_ALL	macromolecule localization	44	5.3	1.60E-04	3.40E-02
KEGG_PATHWAY	Proteasome	10	1.2	8.90E-05	1.30E-02
Enrichments for all positive genes correlated with DH					
GOTERM_BP_ALL	cellular macromolecule metabolic process	88	15	1.70E-08	1.80E-05
GOTERM_BP_ALL	cellular process	152	26	3.50E-08	1.80E-05
GOTERM_BP_ALL	cellular metabolic process	108	18.5	6.50E-08	2.20E-05
GOTERM_MF_ALL	binding	219	37.4	1.80E-07	7.30E-05
GOTERM_BP_ALL	macromolecule metabolic process	90	15.4	7.60E-06	1.90E-03
GOTERM_MF_ALL	zinc ion binding	57	9.7	8.60E-06	1.80E-03
GOTERM_BP_ALL	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	50	8.5	9.60E-06	2.00E-03
GOTERM_BP_ALL	cellular nitrogen compound metabolic process	54	9.2	1.20E-05	2.00E-03
GOTERM_MF_ALL	transition metal ion binding	65	11.1	2.50E-05	3.50E-03
KEGG_PATHWAY	Spliceosome	12	2.1	1.70E-04	1.80E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 18 shows the results of the enrichment for all positive genes correlated with *AKTIP* (AKT interacting protein) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway. There was no significant enrichment for negative genes correlated with *AKTIP*.

Table 18 - Top enrichments for all positive genes correlated with *AKTIP* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all positive genes correlated with DH					
GOTERM_BP_ALL	cellular process	220	35.4	1.50E-08	2.10E-05
GOTERM_MF_ALL	binding	287	46.1	1.70E-07	8.20E-05
GOTERM_BP_ALL	cellular metabolic process	149	24	3.80E-07	2.70E-04
GOTERM_MF_ALL	nucleotide binding	83	13.3	1.70E-06	4.10E-04
GOTERM_MF_ALL	catalytic activity	155	24.9	3.50E-05	5.70E-03
GOTERM_MF_ALL	ligase activity	20	3.2	7.70E-05	9.50E-03
GOTERM_MF_ALL	purine nucleotide binding	67	10.8	9.80E-05	9.60E-03
GOTERM_BP_ALL	primary metabolic process	148	23.8	9.90E-05	3.40E-02
GOTERM_BP_ALL	metabolic process	172	27.7	7.00E-05	3.20E-02
KEGG_PATHWAY	Alanine, aspartate, glutamate metabolism	7	1.1	2.30E-04	3.20E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 19 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *ALPK3* (alpha-kinase 3) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 19 - Top enrichments for all negative and positive genes correlated with *ALPK3* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular metabolic process	247	24.2	5.60E-10	1.10E-06
GOTERM_BP_ALL	cellular process	358	35.1	1.40E-09	1.40E-06
GOTERM_BP_ALL	metabolic process	299	29.3	3.60E-09	2.40E-06
GOTERM_BP_ALL	primary metabolic process	251	24.6	2.80E-07	1.40E-04
GOTERM_MF_ALL	protein binding	278	27.2	4.60E-07	3.00E-04
KEGG_PATHWAY	Proteasome	13	1.3	3.90E-06	6.50E-04
GOTERM_MF_ALL	catalytic activity	171	16.7	4.10E-05	1.60E-02
GOTERM_BP_ALL	cellular macromolecule metabolic process	108	10.6	4.80E-05	1.60E-02
GOTERM_BP_ALL	cellular biosynthetic process	86	8.4	1.10E-04	3.00E-02
GOTERM_BP_ALL	gene expression	78	7.6	2.60E-04	6.20E-02
GOTERM_BP_ALL	cellular macromolecule biosynthetic process	171	16.7	4.10E-05	1.60E-02
Enrichments for all positive genes correlated with DH					
GOTERM_MF_ALL	binding	331	38.5	2.10E-08	1.10E-05
GOTERM_MF_ALL	zinc ion binding	85	9.9	2.10E-07	5.70E-05
GOTERM_BP_ALL	regulation of cellular process	137	15.9	1.10E-07	1.40E-04
GOTERM_BP_ALL	regulation of biological process	142	16.5	2.50E-07	1.60E-04
GOTERM_MF_ALL	cation binding	128	14.9	6.00E-07	1.10E-04
GOTERM_MF_ALL	metal ion binding	126	14.7	9.80E-07	1.30E-04
GOTERM_MF_ALL	ion binding	128	14.9	1.00E-06	1.10E-04
GOTERM_MF_ALL	transition metal ion binding	97	11.3	1.10E-06	1.00E-04
GOTERM_BP_ALL	biological regulation	146	17	2.30E-06	9.90E-04
GOTERM_BP_ALL	cellular macromolecule metabolic process	113	13.2	4.30E-06	1.40E-03
KEGG_PATHWAY	Adipocytokine signaling pathway	10	1.2	5.30E-04	6.70E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

These were the enrichments for each negative genes DH. The next enrichments are to the positive genes DH. Table 20 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *TMBIM4* (transmembrane BAX inhibitor motif containing 4) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 20 - Top enrichments for all negative and positive genes correlated with *TMBIM4* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	651	33.3	1.10E-18	2.80E-15
GOTERM_BP_ALL	cellular metabolic process	433	22.2	1.50E-14	1.90E-11
GOTERM_BP_ALL	cellular macromolecule metabolic process	324	16.6	1.10E-11	8.80E-09
GOTERM_BP_ALL	metabolic process	519	26.6	1.60E-11	1.00E-08
GOTERM_BP_ALL	primary metabolic process	445	22.8	6.00E-11	3.00E-08
GOTERM_MF_ALL	binding	864	44.2	8.30E-10	7.30E-07
GOTERM_MF_ALL	protein binding	486	24.9	2.60E-09	1.10E-06
GOTERM_BP_ALL	cellular protein metabolic process	201	10.3	2.10E-08	8.80E-06
GOTERM_BP_ALL	macromolecule metabolic process	350	17.9	4.50E-08	1.60E-05
GOTERM_BP_ALL	protein localization	77	3.9	5.30E-07	1.70E-04
Enrichments for all positive genes correlated with DH					
GOTERM_MF_ALL	binding	786	49.2	8.60E-27	7.00E-24
GOTERM_BP_ALL	cellular process	560	35	3.10E-23	7.60E-20
GOTERM_MF_ALL	protein binding	470	29.4	1.50E-22	6.10E-20
GOTERM_BP_ALL	cellular macromolecule metabolic process	295	18.4	2.00E-17	2.40E-14
GOTERM_BP_ALL	cellular localization	80	5	1.10E-14	8.60E-12
GOTERM_BP_ALL	macromolecule metabolic process	319	19.9	1.80E-13	1.10E-10
GOTERM_BP_ALL	cellular protein metabolic process	189	11.8	3.80E-13	1.80E-10
GOTERM_BP_ALL	establishment of localization in cell	72	4.5	1.50E-12	6.10E-10
GOTERM_BP_ALL	protein localization	80	5	5.00E-12	1.70E-09
Top 10 KEGG Pathways for all positive genes correlated with DH					
KEGG_PATHWAY	Ubiquitin mediated proteolysis	29	1.8	2.60E-06	4.50E-04
KEGG_PATHWAY	Neurotrophin signaling pathway	26	1.6	7.10E-06	6.00E-04
KEGG_PATHWAY	Chronic myeloid leukemia	19	1.2	2.00E-05	1.10E-03
KEGG_PATHWAY	Pathways in cancer	46	2.9	4.90E-05	2.10E-03
KEGG_PATHWAY	Aminoacyl-tRNA biosynthesis	12	0.8	2.60E-04	8.80E-03
KEGG_PATHWAY	Pancreatic cancer	16	1	2.60E-04	7.30E-03
KEGG_PATHWAY	Colorectal cancer	18	1.1	3.80E-04	9.00E-03
KEGG_PATHWAY	Renal cell carcinoma	15	0.9	6.40E-04	1.30E-02
KEGG_PATHWAY	Proteasome	12	0.8	6.40E-04	1.20E-02
KEGG_PATHWAY	Focal adhesion	29	1.8	7.80E-04	1.30E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 21 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *STXBP6* (syntaxin binding protein 6 (amisyn)), for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 21 - Top enrichments for all negative and positive genes correlated with *STXBP6* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	579	33.2	8.60E-16	2.20E-12
GOTERM_BP_ALL	cellular metabolic process	394	22.6	8.30E-15	1.00E-11
GOTERM_BP_ALL	cellular macromolecule metabolic process	297	17	2.80E-12	2.20E-09
GOTERM_BP_ALL	metabolic process	469	26.9	1.60E-11	9.60E-09
GOTERM_BP_ALL	primary metabolic process	399	22.9	3.50E-10	1.70E-07
GOTERM_BP_ALL	cellular protein metabolic process	187	10.7	2.60E-09	1.10E-06
GOTERM_MF_ALL	binding	763	43.8	3.80E-09	3.10E-06
GOTERM_MF_ALL	protein binding	430	24.7	1.30E-08	5.10E-06
GOTERM_BP_ALL	macromolecule metabolic process	317	18.2	4.30E-08	1.50E-05
GOTERM_MF_ALL	catalytic activity	403	23.1	4.00E-07	1.10E-04
Enrichments for all positive genes correlated with DH					
GOTERM_MF_ALL	binding	835	49.3	3.20E-27	2.70E-24
GOTERM_BP_ALL	cellular process	598	35.3	2.40E-24	5.80E-21
GOTERM_MF_ALL	protein binding	486	28.7	8.70E-20	3.60E-17
GOTERM_BP_ALL	cellular macromolecule metabolic process	303	17.9	1.00E-14	1.20E-11
GOTERM_BP_ALL	vesicle-mediated transport	58	3.4	5.30E-12	4.30E-09
GOTERM_BP_ALL	macromolecule localization	95	5.6	8.20E-12	5.00E-09
GOTERM_BP_ALL	cellular localization	77	4.5	9.60E-12	4.70E-09
GOTERM_BP_ALL	protein localization	82	4.8	2.40E-11	9.90E-09
GOTERM_BP_ALL	macromolecule metabolic process	328	19.4	3.50E-11	1.20E-08
GOTERM_BP_ALL	cellular metabolic process	373	22	4.80E-11	1.50E-08
Top 10 KEGG Pathways for all positive genes correlated with DH					
KEGG_PATHWAY	Ubiquitin mediated proteolysis	30	1.8	5.50E-06	9.50E-04
KEGG_PATHWAY	Neurotrophin signaling pathway	26	1.5	3.60E-05	3.10E-03
KEGG_PATHWAY	Pathways in cancer	48	2.8	1.20E-04	6.70E-03
KEGG_PATHWAY	Fc gamma R-mediated phagocytosis	19	1.1	5.20E-04	2.20E-02
KEGG_PATHWAY	Chronic myeloid leukemia	17	1	7.30E-04	2.50E-02
KEGG_PATHWAY	Glioma	14	0.8	2.20E-03	6.10E-02
KEGG_PATHWAY	Aminoacyl-tRNA biosynthesis	11	0.6	2.30E-03	5.50E-02
KEGG_PATHWAY	Endocytosis	29	1.7	2.50E-03	5.30E-02
KEGG_PATHWAY	Focal adhesion	29	1.7	3.20E-03	6.00E-02
KEGG_PATHWAY	Proteasome	11	0.6	4.90E-03	8.00E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 22 shows the results of the enrichment for all negative genes correlated with *XRCC2* (X-ray repair complementing defective repair in Chinese hamster cells 2) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway. There was no significant enrichment for positive genes correlated with *XRCC2*.

Table 22 - Top enrichments for all negative genes correlated with *XRCC2* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	614	34.2	2.80E-19	7.00E-16
GOTERM_BP_ALL	cellular metabolic process	413	23	9.60E-16	1.30E-12
GOTERM_BP_ALL	cellular macromolecule metabolic process	311	17.3	5.90E-13	5.00E-10
GOTERM_BP_ALL	metabolic process	491	27.3	3.40E-12	2.20E-09
GOTERM_BP_ALL	primary metabolic process	422	23.5	1.20E-11	6.30E-09
GOTERM_MF_ALL	protein binding	460	25.6	1.80E-10	1.50E-07
GOTERM_BP_ALL	cellular protein metabolic process	196	10.9	8.00E-10	3.40E-07
GOTERM_MF_ALL	binding	802	44.6	1.40E-09	5.80E-07
GOTERM_BP_ALL	macromolecule metabolic process	332	18.5	1.50E-08	5.40E-06
KEGG_PATHWAY	Ribosome	19	1.1	3.40E-04	5.80E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 23 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *TMEM150A* (transmembrane protein 150A) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 23 - Top enrichments for all negative and positive genes correlated with *TMEM150A* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	571	35.9	2.80E-26	6.70E-23
GOTERM_MF_ALL	binding	764	48.1	2.20E-21	1.70E-18
GOTERM_BP_ALL	cellular macromolecule metabolic process	302	19	3.70E-19	4.40E-16
GOTERM_MF_ALL	protein binding	452	28.4	1.00E-18	4.00E-16
GOTERM_BP_ALL	macromolecule metabolic process	325	20.5	1.20E-14	9.30E-12
GOTERM_BP_ALL	cellular metabolic process	368	23.2	1.40E-14	8.10E-12
GOTERM_BP_ALL	cellular protein metabolic process	193	12.1	4.10E-14	1.90E-11
GOTERM_BP_ALL	cellular localization	76	4.8	1.10E-12	4.50E-10
GOTERM_BP_ALL	establishment of localization in cell	71	4.5	5.70E-12	1.90E-09
GOTERM_BP_ALL	primary metabolic process	378	23.8	2.40E-11	7.30E-09
Top 10 KEGG Pathways for all negative genes correlated with DH					
KEGG_PATHWAY	Ubiquitin mediated proteolysis	28	1.8	1.10E-05	1.80E-03
KEGG_PATHWAY	Pathways in cancer	47	3	3.30E-05	2.80E-03
KEGG_PATHWAY	Chronic myeloid leukemia	17	1.1	3.00E-04	1.70E-02
KEGG_PATHWAY	Proteasome	12	0.8	7.30E-04	3.00E-02
KEGG_PATHWAY	Endocytosis	29	1.8	7.50E-04	2.50E-02
KEGG_PATHWAY	Focal adhesion	29	1.8	9.80E-04	2.70E-02
KEGG_PATHWAY	Neurotrophin signaling pathway	21	1.3	1.60E-03	3.80E-02
KEGG_PATHWAY	Toll-like receptor signaling pathway	18	1.1	1.70E-03	3.50E-02
KEGG_PATHWAY	Renal cell carcinoma	14	0.9	2.30E-03	4.30E-02
KEGG_PATHWAY	VEGF signaling pathway	15	0.9	2.40E-03	4.00E-02
Enrichments for all positive genes correlated with DH					
GOTERM_BP_ALL	cellular process	623	33.1	2.40E-16	5.50E-13
GOTERM_BP_ALL	cellular metabolic process	419	22.3	2.60E-14	3.20E-11
GOTERM_BP_ALL	cellular macromolecule metabolic process	316	16.8	4.60E-12	3.80E-09
GOTERM_BP_ALL	metabolic process	502	26.7	2.20E-11	1.40E-08
GOTERM_MF_ALL	binding	842	44.7	3.20E-11	2.70E-08
GOTERM_BP_ALL	primary metabolic process	426	22.6	6.50E-10	3.20E-07
GOTERM_MF_ALL	protein binding	473	25.1	1.10E-09	4.60E-07
GOTERM_BP_ALL	macromolecule metabolic process	339	18	4.90E-08	2.00E-05
GOTERM_BP_ALL	cellular protein metabolic process	193	10.2	6.30E-08	2.20E-05
GOTERM_BP_ALL	protein localization	74	3.9	1.10E-06	3.50E-04

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 24 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *HAUS6* (HAUS augmin-like complex, subunit 6) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 24 - Top enrichments for all negative and positive genes correlated with *HAUS6* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	634	33.7	7.30E-18	1.80E-14
GOTERM_BP_ALL	cellular metabolic process	432	23	1.80E-16	2.80E-13
GOTERM_BP_ALL	metabolic process	515	27.4	4.50E-13	3.80E-10
GOTERM_BP_ALL	cellular macromolecule metabolic process	323	17.2	5.30E-13	3.40E-10
GOTERM_BP_ALL	primary metabolic process	439	23.3	1.20E-11	5.80E-09
GOTERM_MF_ALL	binding	841	44.7	2.40E-10	2.10E-07
GOTERM_MF_ALL	protein binding	476	25.3	6.00E-10	2.60E-07
GOTERM_BP_ALL	cellular protein metabolic process	200	10.6	4.50E-09	1.90E-06
GOTERM_BP_ALL	macromolecule metabolic process	346	18.4	1.10E-08	3.80E-06
GOTERM_MF_ALL	catalytic activity	438	23.3	6.00E-07	1.70E-04
Enrichments for all positive genes correlated with DH					
GOTERM_MF_ALL	binding	776	45.9	3.00E-26	2.40E-23
GOTERM_BP_ALL	cellular process	547	32.4	1.40E-22	3.30E-19
GOTERM_MF_ALL	protein binding	449	26.6	4.70E-18	1.80E-15
GOTERM_BP_ALL	cellular macromolecule metabolic process	291	17.2	7.70E-18	9.30E-15
GOTERM_BP_ALL	cellular metabolic process	354	20.9	3.00E-13	2.40E-10
GOTERM_BP_ALL	cellular localization	74	4.4	2.30E-12	1.40E-09
GOTERM_BP_ALL	macromolecule metabolic process	306	18.1	9.30E-12	4.50E-09
GOTERM_BP_ALL	intracellular transport	58	3.4	9.80E-12	4.00E-09
GOTERM_BP_ALL	protein localization	77	4.6	3.00E-11	1.00E-08
GOTERM_BP_ALL	cellular protein metabolic process	179	10.6	3.30E-11	1.00E-08
Top 10 KEGG Pathways for all positive genes correlated with DH					
KEGG_PATHWAY	Neurotrophin signaling pathway	27	1.6	2.30E-06	4.00E-04
KEGG_PATHWAY	Ubiquitin mediated proteolysis	29	1.7	2.90E-06	2.50E-04
KEGG_PATHWAY	Toll-like receptor signaling pathway	22	1.3	1.80E-05	1.00E-03
KEGG_PATHWAY	Pathways in cancer	47	2.8	2.60E-05	1.10E-03
KEGG_PATHWAY	Renal cell carcinoma	17	1	5.20E-05	1.80E-03
KEGG_PATHWAY	Aminoacyl-tRNA biosynthesis	13	0.8	5.70E-05	1.60E-03
KEGG_PATHWAY	Pancreatic cancer	17	1	7.70E-05	1.90E-03
KEGG_PATHWAY	Apoptosis	19	1.1	9.10E-05	1.90E-03
KEGG_PATHWAY	B cell receptor signaling pathway	17	1	1.10E-04	2.10E-03
KEGG_PATHWAY	VEGF signaling pathway	17	1	2.30E-04	3.90E-03

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR)

Table 25 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *EIF2B1* (eukaryotic translation initiation factor 2B, subunit 1 alpha, 26kDa) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 25 - Top enrichments for all negative and positive genes correlated with *EIF2B1* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	667	33.5	3.60E-18	9.00E-15
GOTERM_BP_ALL	cellular metabolic process	436	21.9	1.20E-12	1.50E-09
GOTERM_MF_ALL	binding	896	45	3.80E-11	3.40E-08
GOTERM_MF_ALL	protein binding	507	25.5	1.30E-10	5.80E-08
GOTERM_BP_ALL	cellular macromolecule metabolic process	324	16.3	6.10E-10	5.10E-07
GOTERM_BP_ALL	metabolic process	522	26.2	2.30E-09	1.40E-06
GOTERM_BP_ALL	primary metabolic process	446	22.4	6.80E-09	3.40E-06
GOTERM_BP_ALL	cellular protein metabolic process	204	10.2	5.70E-08	2.40E-05
GOTERM_BP_ALL	protein localization	80	4	2.10E-07	7.50E-05
GOTERM_BP_ALL	protein transport	73	3.7	5.30E-07	1.70E-04
Enrichments for all positive genes correlated with DH					
GOTERM_MF_ALL	binding	676	45.9	2.50E-20	1.80E-17
GOTERM_BP_ALL	cellular process	481	32.6	3.20E-20	7.10E-17
GOTERM_BP_ALL	cellular macromolecule metabolic process	256	17.4	1.30E-15	1.50E-12
GOTERM_MF_ALL	protein binding	394	26.7	2.80E-15	9.80E-13
GOTERM_BP_ALL	cellular localization	72	4.9	2.90E-14	2.20E-11
GOTERM_BP_ALL	establishment of localization in cell	67	4.5	2.60E-13	1.40E-10
GOTERM_BP_ALL	cellular metabolic process	316	21.4	4.70E-13	2.10E-10
GOTERM_BP_ALL	cellular protein metabolic process	166	11.3	1.60E-12	6.10E-10
GOTERM_BP_ALL	macromolecule metabolic process	272	18.5	3.10E-11	9.80E-09
GOTERM_BP_ALL	intracellular transport	52	3.5	7.40E-11	2.10E-08
Top 10 KEGG Pathways for all positive genes correlated with DH					
KEGG_PATHWAY	Chronic myeloid leukemia	19	1.3	3.50E-06	5.40E-04
KEGG_PATHWAY	ErbB signaling pathway	19	1.3	7.80E-06	6.10E-04
KEGG_PATHWAY	Neurotrophin signaling pathway	22	1.5	9.30E-05	4.80E-03
KEGG_PATHWAY	Insulin signaling pathway	22	1.5	2.20E-04	8.40E-03
KEGG_PATHWAY	Ubiquitin mediated proteolysis	23	1.6	2.20E-04	6.70E-03
KEGG_PATHWAY	Pathways in cancer	40	2.7	2.50E-04	6.50E-03
KEGG_PATHWAY	Proteasome	11	0.7	9.60E-04	2.10E-02
KEGG_PATHWAY	Glioma	13	0.9	1.00E-03	2.00E-02
KEGG_PATHWAY	Wnt signaling pathway	22	1.5	1.30E-03	2.10E-02
KEGG_PATHWAY	Acute myeloid leukemia	12	0.8	1.70E-03	2.60E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 26 show the results of the enrichment for all negative genes correlated and all positive genes correlated with *ENPP4* (ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative)) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 26 - Top enrichments for all negative and positive genes correlated with *ENPP4* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	654	33.8	1.30E-18	3.30E-15
GOTERM_BP_ALL	cellular metabolic process	429	22.2	3.80E-13	4.90E-10
GOTERM_BP_ALL	metabolic process	519	26.8	5.10E-11	4.40E-08
GOTERM_MF_ALL	binding	874	45.2	2.60E-10	2.30E-07
GOTERM_BP_ALL	cellular macromolecule metabolic process	319	16.5	2.60E-10	1.70E-07
GOTERM_MF_ALL	protein binding	495	25.6	3.50E-10	1.60E-07
GOTERM_BP_ALL	primary metabolic process	441	22.8	9.30E-10	4.80E-07
GOTERM_BP_ALL	cellular protein metabolic process	201	10.4	3.30E-08	1.40E-05
GOTERM_BP_ALL	macromolecule metabolic process	345	17.8	5.90E-07	2.20E-04
GOTERM_MF_ALL	catalytic activity	452	23.4	1.30E-06	3.90E-04
KEGG_PATHWAY	Ribosome	20	1	2.80E-04	4.90E-02
KEGG_PATHWAY	Proteasome	13	0.7	6.40E-04	5.50E-02
Enrichments for all positive genes correlated with DH					
GOTERM_MF_ALL	binding	835	45.4	1.10E-25	8.40E-23
GOTERM_BP_ALL	cellular macromolecule metabolic process	314	17.1	1.00E-20	2.40E-17
GOTERM_BP_ALL	cellular process	568	30.9	3.10E-20	3.70E-17
GOTERM_BP_ALL	cellular metabolic process	379	20.6	4.30E-15	3.40E-12
GOTERM_BP_ALL	macromolecule metabolic process	334	18.2	5.90E-15	3.50E-12
GOTERM_MF_ALL	protein binding	468	25.5	1.70E-14	6.50E-12
GOTERM_BP_ALL	cellular protein metabolic process	196	10.7	9.80E-14	4.70E-11
GOTERM_BP_ALL	primary metabolic process	392	21.3	2.50E-12	9.90E-10
GOTERM_MF_ALL	nucleic acid binding	255	13.9	8.00E-12	2.10E-09
GOTERM_MF_ALL	nucleotide binding	213	11.6	1.50E-10	2.80E-08
Top 10 KEGG Pathways for all positive genes correlated with DH					
KEGG_PATHWAY	Neurotrophin signaling pathway	26	1.4	2.60E-05	4.20E-03
KEGG_PATHWAY	Ubiquitin mediated proteolysis	28	1.5	3.20E-05	2.60E-03
KEGG_PATHWAY	Pathways in cancer	49	2.7	3.30E-05	1.80E-03
KEGG_PATHWAY	Chronic myeloid leukemia	19	1	5.30E-05	2.20E-03
KEGG_PATHWAY	Adipocytokine signaling pathway	17	0.9	7.90E-05	2.60E-03
KEGG_PATHWAY	Aminoacyl-tRNA biosynthesis	13	0.7	1.10E-04	3.00E-03
KEGG_PATHWAY	Pancreatic cancer	17	0.9	1.70E-04	4.10E-03
KEGG_PATHWAY	ErbB signaling pathway	18	1	3.60E-04	7.30E-03
KEGG_PATHWAY	Glioma	15	0.8	5.60E-04	1.00E-02
KEGG_PATHWAY	B cell receptor signaling pathway	16	0.9	8.10E-04	1.30E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR).

Table 27 show the results of the enrichment for all negative genes correlated and all positive genes correlated with *ANO1* (anoctamin 1, calcium activated chloride channel) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 27 - Top enrichments for all negative and positive genes correlated with *ANO1* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	536	34	3.80E-18	8.30E-15
GOTERM_BP_ALL	cellular metabolic process	361	22.9	1.30E-14	1.40E-11
GOTERM_BP_ALL	cellular macromolecule metabolic process	277	17.6	3.00E-13	2.20E-10
GOTERM_BP_ALL	cellular protein metabolic process	177	11.2	1.80E-10	1.00E-07
GOTERM_BP_ALL	metabolic process	423	26.8	3.10E-10	1.40E-07
GOTERM_BP_ALL	primary metabolic process	363	23	1.00E-09	3.80E-07
GOTERM_MF_ALL	binding	692	43.9	5.80E-09	4.60E-06
GOTERM_BP_ALL	macromolecule metabolic process	293	18.6	1.30E-08	4.10E-06
GOTERM_MF_ALL	protein binding	387	24.6	1.20E-07	4.70E-05
GOTERM_BP_ALL	biopolymer modification	111	7	2.40E-06	6.40E-04
Enrichments for all positive genes correlated with DH					
GOTERM_MF_ALL	binding	860	48.7	1.10E-25	9.40E-23
GOTERM_BP_ALL	cellular process	611	34.6	3.30E-23	8.50E-20
GOTERM_MF_ALL	protein binding	495	28	4.10E-18	1.70E-15
GOTERM_BP_ALL	cellular macromolecule metabolic process	317	18	2.10E-16	2.90E-13
GOTERM_BP_ALL	macromolecule metabolic process	349	19.8	4.60E-14	4.00E-11
GOTERM_BP_ALL	cellular metabolic process	394	22.3	1.70E-13	1.10E-10
GOTERM_BP_ALL	primary metabolic process	415	23.5	2.20E-12	1.10E-09
GOTERM_BP_ALL	protein localization	83	4.7	4.50E-11	2.00E-08
GOTERM_BP_ALL	cellular localization	76	4.3	1.10E-10	4.10E-08
GOTERM_BP_ALL	vesicle-mediated transport	56	3.2	1.80E-10	5.80E-08
Top 10 KEGG Pathways for all positive genes correlated with DH					
KEGG_PATHWAY	Pathways in cancer	50	2.8	4.70E-05	8.10E-03
KEGG_PATHWAY	Ubiquitin mediated proteolysis	28	1.6	6.50E-05	5.60E-03
KEGG_PATHWAY	Neurotrophin signaling pathway	24	1.4	3.70E-04	2.10E-02
KEGG_PATHWAY	Spliceosome	24	1.4	6.10E-04	2.60E-02
KEGG_PATHWAY	Chronic myeloid leukemia	17	1	9.00E-04	3.10E-02
KEGG_PATHWAY	Prostate cancer	18	1	1.40E-03	3.90E-02
KEGG_PATHWAY	Fc gamma R-mediated phagocytosis	18	1	1.80E-03	4.40E-02
KEGG_PATHWAY	Adherens junction	15	0.8	2.60E-03	5.60E-02
KEGG_PATHWAY	Lysosome	21	1.2	3.00E-03	5.70E-02
KEGG_PATHWAY	Adipocytokine signaling pathway	14	0.8	4.10E-03	7.00E-02

Table 28 shows the results of the enrichment for all negative genes correlated and all positive genes correlated with *SLC25A44* (solute carrier family 25, member 44) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 28 - Top enrichments for all negative and positive genes correlated with *SLC25A44* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	653	34.2	3.60E-18	9.10E-15
GOTERM_BP_ALL	cellular metabolic process	432	22.6	9.50E-14	1.20E-10
GOTERM_BP_ALL	metabolic process	520	27.2	4.10E-11	3.50E-08
GOTERM_BP_ALL	cellular macromolecule metabolic process	321	16.8	1.20E-10	7.50E-08
GOTERM_MF_ALL	protein binding	485	25.4	4.80E-10	4.30E-07
GOTERM_BP_ALL	primary metabolic process	442	23.2	7.30E-10	3.70E-07
GOTERM_MF_ALL	binding	853	44.7	1.50E-09	6.50E-07
GOTERM_BP_ALL	cellular protein metabolic process	201	10.5	3.60E-08	1.50E-05
GOTERM_BP_ALL	macromolecule metabolic process	346	18.1	4.60E-07	1.70E-04
GOTERM_MF_ALL	catalytic activity	444	23.3	1.10E-06	3.30E-04
KEGG_PATHWAY	Ribosome	20	1	2.70E-04	4.60E-02
KEGG_PATHWAY	Proteasome	13	0.7	6.10E-04	5.30E-02
Enrichments for all positive genes correlated with DH					
GOTERM_MF_ALL	binding	857	46.2	7.50E-28	6.10E-25
GOTERM_BP_ALL	cellular process	590	31.8	9.80E-23	2.30E-19
GOTERM_BP_ALL	cellular macromolecule metabolic process	325	17.5	5.40E-22	6.30E-19
GOTERM_BP_ALL	macromolecule metabolic process	347	18.7	2.30E-16	1.70E-13
GOTERM_BP_ALL	cellular metabolic process	390	21	1.40E-15	8.40E-13
GOTERM_MF_ALL	protein binding	475	25.6	4.00E-14	1.60E-11
GOTERM_MF_ALL	nucleic acid binding	264	14.2	6.10E-13	1.70E-10
GOTERM_BP_ALL	cellular protein metabolic process	195	10.5	3.20E-12	1.50E-09
GOTERM_BP_ALL	primary metabolic process	400	21.6	5.80E-12	2.30E-09
GOTERM_BP_ALL	cellular localization	74	4	1.30E-10	4.40E-08
Top 10 KEGG Pathways for all positive genes correlated with DH					
KEGG_PATHWAY	Ubiquitin mediated proteolysis	29	1.6	1.10E-05	1.70E-03
KEGG_PATHWAY	Pathways in cancer	49	2.6	3.20E-05	2.60E-03
KEGG_PATHWAY	Neurotrophin signaling pathway	25	1.3	7.20E-05	3.90E-03
KEGG_PATHWAY	Aminoacyl-tRNA biosynthesis	13	0.7	1.10E-04	4.40E-03
KEGG_PATHWAY	Glioma	16	0.9	1.60E-04	5.10E-03
KEGG_PATHWAY	Chronic myeloid leukemia	18	1	1.80E-04	4.80E-03
KEGG_PATHWAY	Focal adhesion	31	1.7	5.10E-04	1.20E-02
KEGG_PATHWAY	Adherens junction	16	0.9	5.70E-04	1.20E-02
KEGG_PATHWAY	Pancreatic cancer	16	0.9	5.70E-04	1.20E-02
KEGG_PATHWAY	RNA degradation	14	0.8	6.30E-04	1.10E-02

Table 29 show the results of the enrichment for all negative genes correlated and all positive genes correlated with *CDK5RAP3* (CDK5 regulatory subunit associated protein 3) for significant GO terms for biologic process (BP), molecular function (MF), and KEGG pathway.

Table 29 - Top 10 of enrichment for all negative genes correlated with *CDK5RAP3* for groups H and L

Category	Term	Count	%	P-Value	padj. ¹
Enrichments for all negative genes correlated with DH					
GOTERM_BP_ALL	cellular process	598	33.2	1.00E-15	2.40E-12
GOTERM_BP_ALL	cellular metabolic process	391	21.7	2.80E-11	3.30E-08
GOTERM_MF_ALL	binding	806	44.8	4.10E-10	3.50E-07
GOTERM_BP_ALL	cellular macromolecule metabolic process	296	16.4	5.20E-10	4.00E-07
GOTERM_BP_ALL	primary metabolic process	407	22.6	3.20E-09	1.90E-06
GOTERM_BP_ALL	metabolic process	469	26.1	1.60E-08	7.60E-06
GOTERM_MF_ALL	protein binding	449	24.9	2.10E-08	9.10E-06
GOTERM_BP_ALL	cellular protein metabolic process	184	10.2	2.20E-07	8.60E-05
GOTERM_BP_ALL	macromolecule metabolic process	320	17.8	7.40E-07	2.50E-04
GOTERM_BP_ALL	protein transport	64	3.6	7.30E-06	2.20E-03
KEGG_PATHWAY	Ribosome	18	1	7.50E-04	1.20E-01
Enrichments for all positive genes correlated with DH					
GOTERM_BP_ALL	cellular process	568	35.4	1.10E-24	2.60E-21
GOTERM_MF_ALL	binding	764	47.6	8.90E-20	7.20E-17
GOTERM_BP_ALL	cellular macromolecule metabolic process	294	18.3	2.50E-16	2.60E-13
GOTERM_MF_ALL	protein binding	442	27.5	3.30E-15	1.30E-12
GOTERM_BP_ALL	macromolecule metabolic process	321	20	1.90E-13	1.50E-10
GOTERM_BP_ALL	cellular metabolic process	362	22.6	6.80E-13	4.00E-10
GOTERM_BP_ALL	cellular localization	75	4.7	3.70E-12	1.70E-09
GOTERM_BP_ALL	vesicle-mediated transport	56	3.5	5.20E-12	2.00E-09
GOTERM_BP_ALL	cellular protein metabolic process	185	11.5	1.20E-11	3.90E-09
GOTERM_BP_ALL	primary metabolic process	378	23.6	3.80E-11	1.10E-08
Top 10 KEGG Pathways for all positive genes correlated with DH					
KEGG_PATHWAY	Proteasome	16	1	1.30E-06	2.20E-04
KEGG_PATHWAY	Ubiquitin mediated proteolysis	27	1.7	3.30E-05	2.90E-03
KEGG_PATHWAY	Aminoacyl-tRNA biosynthesis	13	0.8	6.40E-05	3.70E-03
KEGG_PATHWAY	Renal cell carcinoma	15	0.9	7.50E-04	3.20E-02
KEGG_PATHWAY	Chronic myeloid leukemia	16	1	9.70E-04	3.30E-02
KEGG_PATHWAY	Spliceosome	22	1.4	1.00E-03	2.90E-02
KEGG_PATHWAY	Neurotrophin signaling pathway	21	1.3	1.60E-03	4.00E-02
KEGG_PATHWAY	ErbB signaling pathway	16	1	1.70E-03	3.60E-02
KEGG_PATHWAY	Insulin signaling pathway	21	1.3	3.30E-03	6.20E-02
KEGG_PATHWAY	Endocytosis	27	1.7	3.50E-03	5.90E-02

¹padj. - Adjusted p value for multiple testing with the Benjamini-Hochberg procedure (FDR)