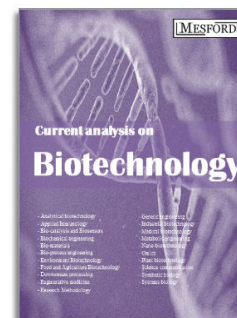


A study of the Global *In Vivo* Gene Expression Pathway Profile in *Mycobacterium bovis* infected cattle from Mexico.

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Abstract:

Bovine tuberculosis is serious animal health and economic problem worldwide. Intense efforts have been invested in the search of biomarkers to define the spectrum of disease progression and also as diagnostic-prognostic. Most of the studies addressing this issue have been made after *in vitro* PBMCs stimulation with *M. bovis*. Furthermore, some data have been found after *M. bovis* infected the calves, followed by *M. bovis* BCG vaccination, with some promising results. But still there are many questions that should be answered from *in vivo* studies. Thus, in the present work we focused to determine the global *in vivo* gene expression pathway of *M. bovis* infected cattle from the north states of Mexico. From the analysis of data, we found that there are a set of genes modulated up (around 280 genes) and down (around 580 genes) (> 2 SD) that were influenced by the *M. bovis* infection. From the functional analysis using Reactome database, with those genes most significantly regulated up and down ($P < 0.05$) ($n = 27$ genes), we found that the pathways relate to innate immune response, such as IFN $\alpha\beta$; IL-6; [Toll-like] receptors; chemokine receptors, TRAF6 mediated induction of NFK β and NOTCH signaling; adaptive immunity (B and T cell signaling); IL17 and IL-10. In addition to these findings, we observed that natural transcriptional printing of *M. bovis* is also in other pathways such as MAP kinases cascade activity (autophagy), metabolism (inositol, lipids, glycogen), cell cycle, signal transduction and apoptosis. By summarizing the result, this is a first analysis of the *in vivo* transcriptional gene pathway profile of bTB in Mexico. We think that might be possible to further develop a scanning chip for diagnostic based on the mostly printed signatures of *M. bovis* in cattle.

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M. bovis, gene expression, signalization, immune, biochemical pathways, transcriptional profile, Th1 type, Th17 induced expression.

INTRODUCTION.

Bovine tuberculosis (bTB) is a chronic disease of cattle, caused by *Mycobacterium bovis*, a gram-positive bacteria, member of the *Mycobacterium tuberculosis complex* and the *Mycobacteriaceae* family [1, 2]. Bovine tuberculosis is of zoonotic importance and constitutes in the reemergence of serious health problem [3-5]. It is estimated that more than 50 million cattle are infected with *M. bovis*, causing economic losses in agriculture and livestock [6,7]. *M. bovis* is transmitted mostly by aerosol (80 - 90%) but it can be transmitted by oral route (meat or unpasteurized milk) [8,9]. The risk of transmitting bTB to humans in Mexico might be explained by the high prevalence of this disease in cattle (16%), the manipulation of infected animals (a common practice in Mexico) and the consumption of the unpasteurized milk. According to a recent report from the Binational Council Organization -EUA (SAGARPPA., 2012); 82% of National territory consist of a prevalence of

0.5% of bTB except in the zones of dairy livestock; while there are other regions where the prevalence is unknown or even higher [10-11]. It is more frequent to find *M. bovis* in humans than *M. tuberculosis* in livestock like cattle [8, 13]. In general, we know that cellular immune response play a key role against pathogenic mycobacteria, characterized by interferon-gamma (IFN- γ) production derived from the CD4+ T cells [14-17]. This cytokine enable to infected macrophages to maintain an activated state [18, 19]. A better understanding of protective anti-mycobacterial cellular immune responses is required in order to develop more effective vaccines and diagnostic tests [17, 20, 21]. In fact, there are very few reports about to find immunological correlates of protection which would improve vaccine development [21, 22]. Vaccination of cattle might offer a long-term solution for the control of the disease and for the development of a bTB vaccine [23, 24]. This development should complement BCG since it has been observed that BCG vaccine can compromise the specificity of the intradermic

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tuberculin tests as well as the blood test based in tuberculin specificity [21, 24]. Upon its introduction as the only official BCG vaccine to human tuberculosis in the 20's and 30's, a great number of experiments and tests were performed with BCG in cattle from different countries such as United States, New Zealand, etc [25]. The reports were promising at the beginning, however as the time passed, it was found that the grade of protection induced by BCG were highly variable from 0 to 0.7%, which also mirrored in a human infected with *M. tuberculosis* [26-31]. The quantitative tools that measure changes in the expression of genes have advanced enormously due to the necessity of finding more effective, sensible and faster methods and that make it possible to determine more integrated response of the interaction of pathogen [32-37]. Two recent studies demonstrated the potential of the microarray technology as a molecular diagnostic method but also it allows to identify biomarkers with therapeutic potential in human TB [38] or in bovine Tuberculosis [39-46]. Aranday-Cortes et al., 2012 [47] using first a mouse model of aerogenically *M. bovis* infection to predict biomarkers of bTB ante-mortem diagnosis of bTB, selected the most strong up-regulated genes expressed from the whole transcriptome of lung and spleen cells. From the 14 genes most upregulated in mice (granzyme A and B, cxcl19, IL-22), evaluated them after antigen-stimulated PMBCs from uninfected cattle and naturally infected cattle. They found that these biomarkers including also cxcl10, are the most expressed after PPD-stimulation in infected cattle versus naïve uninfected cattle cells [47]. Despite of this wealth of data, the spectrum of bovine tuberculosis (active vs latent), the natural transcriptional printing of *M. bovis*, remains to be determined. In the present work, we are reporting a set of genes that are mostly regulated up and down (> 2 SD) and they impact importantly in interferon alpha/bet signaling, cytokine receptor signaling, STAT-2, TLRs signalization cascade and in other aspects of the innate and adaptive immune system. Moreover, it was found that transcriptional printing of *M. bovis* influence also other important pathways such as metabolism (carbohydrates, inositol), proteasome, RNA transcription initiation, caspases cascade activation, FGFR binding and activation. The genes that are involved in each of the mentioned pathways might serve to explore an scanning chip for diagnostic, prognostic in bovine tuberculosis.

MATERIALS AND METHODS

Animals

Cattle for the TST field tests. Negative (no reaction to any of the field test); positive (TST+) to field test (caudal and comparative cervical) and confirmed by bacteriological and histopathological analysis. Reactor (TST+).

Blood from cattle from different regions of the state of Zacatecas were collected based in the selection of negative and positive bovines to the tuberculin skin test (TST). TST- to the caudal anus and the comparative cervical test was considered Negative while TST+ to both, was considered a reactor and later on confirmed by bacteriological and histopathological exams as positive

Blood Collection

Blood from positive and negative animal were collected in tempus blood (Thermofisher Scientific). Briefly, after animal were selected following the inclusion criteria described above. Three ml of blood were collected and mixed vigorously and then stored stored to -86°C until use.

RNA Extraction

RNA extraction was made using a KIT and following the manufacturer instructions (AMBION, Life technology). Briefly, the cap of the tube was retired. The blood with the solution was transferred to a falcon tube of 50 ml. 3 ml of buffer phosphate (PBS steril) (Gibco, Co). Then, it was mixed vigorously and centrifuged to $3,000\text{g (rfc)}/4^{\circ}\text{C}/30$ min. Then, the supernatant was discarded. The falcon tubes were inverted for 2 min. $400\ \mu\text{l}$ of solution A (resuspension buffer) was added and resuspended with a tip. Then, a brief vortexing was applied. The samples were then kept on ice. A RNA column (or purification filter)(PF) was set in a Eppendorf tube and it was added $100\ \mu\text{l}$ of solution B (washing buffer 1). Then the $400\ \mu\text{l}$ of the resuspended solution was passed through the RNA column and centrifuged for 30 seconds to $16,000\ \text{g (RFC)}/4^{\circ}\text{C}$. The PF was transferred to a new Eppendorf tube of 2 ml, discarding the supernatant. $500\ \mu\text{l}$ of solution B was added, then centrifugated for $30\ \text{sec}/16,000\ \text{g (RFC)}/4^{\circ}\text{C}$. This step was repeated again but with a new Eppendorf tube and with solution C ($500\ \mu\text{l}$ of washing buffer 2)/ $30\ \text{sec}/16,000\ \text{g (RFC)}/4^{\circ}\text{C}$. This step was repeated, transferring each time to a new Eppendorf tube but then it was incubated for 5 min. After this time, it was centrifuged for $30\ \text{sec}/16,000\ \text{g (RFC)}/4^{\circ}\text{C}$, discarded the supernatant and once more time it is washed with solution C, centrifuged and it discarded the supernatant. The membrane of the PF was dried by centrifugation at $30\ \text{sec}/16,000\ \text{g (RFC)}/4^{\circ}\text{C}$. The PF was transferred to another Eppendorf tube of 2 ml free of RNAsas. The filtrate was discarded. $500\ \mu\text{l}$ of solution D (elution buffer) was added to the PF and then centrifuged to recover the RNA eluted at $30\ \text{sec}/16,000\ \text{g (RFC)}/4^{\circ}\text{C}$. Then the PF was transferred to a 2 ml eppendorf tube free of RNAses. The eluted volume ($100\ 100\ \mu\text{l}$) were passed once again through the same PF and centrifuged for $2\ \text{min}/16,000\ \text{g (RFC)}/4^{\circ}\text{C}$. Finally, the PF was discarded. The recovered RNA is transferred to a new tube of 2 ml free of RNAses, carefully. Storage to -20°C (to be used in days) or -80°C was stored until use. The integrity of the RNA was recorded in agarose gel 1% in TAE (prepared with steril MQ H₂O)/and syber safe ($30\ \text{ml}/2\ \mu\text{l}$) and visualized in the Chemidoc (BioRad, Co).

Microarray Preparation

cDNA was prepared from RNA using the KIT (first strand cDNA labeling KIT (Invitrogen) (Unit of Microarray, IFC-UNAM, Mexico City) incorporating g aminoalil-dUTP, using an alkaline reaction and quantified with Nano-drop (Thermofisher scientific). In general, RNA was with oligo DT ($1,0\ \mu\text{l}$), dH₂O ($15,0\ \mu\text{l}$ total), incubated for 5 min at 70°C followed to put on ice. Thereafter, enzyme buffer ($5,0\ \mu\text{l}$), dNTPs ($1,5\ \mu\text{l}$), dH₂O ($2,5\ \mu\text{l}$) and enzyme M-MLVRT ($1,0\ \mu\text{l}$) were added for a total volume of $25,0\ \mu\text{l}$. The final mixture is

homogenized smoothly with the tip and then spin off to concentrate the sample, Finally, it was incubated for one hour to 42°C and stored at -30°C until use. The microarray preparation was made accordingly to a protocol used by the Unit of Microarray in IFC -UNAM Mexico, City. The chip M32K_10_23 and the hybridization were made with equimolar quantities of label fluophores, -Alexa555- for the control, while -Alexa647- label for the positive sample.

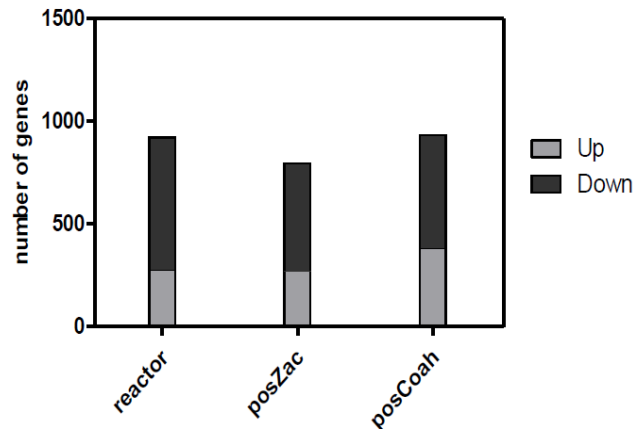


Figure 1. Global gene expression data microarray of the natural infected cattle *M. bovis* infected cattle from Mexico (a-b) analyzed using statistical analysis (Microarray Unit, IFC - UNAM Mexico).

Statistical Analysis

For the microarray analysis, the program Gene arise was used. Up and down regulated genes were filtered for two cutoffs, one for Z-Score higher than 1.5 and the second, with Z – Scores higher than 2 (> 1.5 and 2.0 SD) (P < 0.05).

RESULTS

A Group of Genes are up and down Regulated in *M. bovis* Infected cattle from Mexico

After microarray determination (Materials and Methods), a first statistical analysis from around 23 000 genes), rendered a pool of genes that were modulated (up or down) in the cattle infected with *M. bovis* from Mexico (Figure 1a-b) in agreement with data from in vitro studies [40], we found that effectively, most of the genes are down regulated genes than up- regulated (Figure 1a-b). Thus, in positive *M. bovis* cattle from Zacatecas or from Coahuila State, a total of 589 or 549 genes were down regulated (> 2 fold change, p< 0.05) while reactor (TST +/-) cattle from Zacatecas, 644 genes were down regulated (> 2 fold change, p< 0.05).

Next, we aimed to investigate the most modulated genes from these set of genes among the three groups of animals (Materials and Methods), we choose a range of most up regulated and most down regulated accordingly to the Z scores values. In first term, it was selected a Z score (fold change) from 2.9 to 4.3 (n= 27) equivalent to > 2 fold change (p< 0.05) (Figure 2A-C) whereas most of the genes down regulated were from -4.5 to 6.0 (n= 27)(> 2 fold change, p< 0.05) (Figure 2A-

C). Searching in the NCBI database, it was found the identity of genes added either up or down regulated (Table 1A). In general, these genes encode a wide variety of activities like binding to proteins, carbohydrates, metal-ion, DNA binding as well as for enzymatic activities (Table 2A). Furthermore, we compared these data (Figure 2B) with those obtained after analysis of gene expression pattern from animals that were just TST+/-, named as “reactive” or “suspicious” (Figure 2A, Table 2A). The genes encode also primarily functions of protein and DNA binding, transcription factors, enzyme activities and peptide-ligand binding receptors (Table 2B).

Table 1. Most of genes from cattle infected with *M. bovis* from Mexico are down regulated. Data represent statistical analyses of genes that are significantly (P < 0.05) modulated with a Z score > 2 SD. A, cattle TST (+); B, cattle (+/-) from Zacatecas, State and C, cattle TST(+) from Coahuila, State. Mexico

	C	Up	Down
A	Positive Zac	272	583
B	Suspected	278	644
C	Positive Coah	384	549

Interestingly and in agreement with those data reported in vitro assays, one the most affected pathways are the cytokine and /or interleukin signaling (Figure 2A, table 1A). More importantly, we found that among the list of 27 analyzed genes, a set of genes (FGF 23, Pgm211, Dgat 2, Nudt4, Pde6g, Cpsf1), being the most predominant FGF (Fibroblast growth factor that highly influenced different key pathways (Figure 2A). Likewise, most of the genes strongly up regulated (n= 27) (3 to 4.3 fold change)(Figure 2C) from Coahuila State, Mexico while the genes that are mostly down regulated (n= 27)(-4.5 to 6.0) are those genes that encode also protein, DNA, RNA binding transcription factors, enzyme activities, frizzled binding and peptide-ligand-binding receptors (Figure 2C, Table 2C).

Functional Analysis of the Group of Genes up and down Regulated in *M. bovis* Infected Cattle from Mexico.

To even further analyze the functionality of these genes, we used Reactome database [48] in order to pinpoint the different pathways that are influenced by the modulation of these genes in the *M. Bovis* transcriptional printing (Figure 3I-III, A-B). First, as shown in Figure 3I (reactor, TST (+/-) cattle from Zacatecas State, the most influenced pathways were inositol phosphate (p-value of 0,007), photo-transduced cascade (0,028); glycogen synthesis (p-value of 0,041); triglyceride biosynthesis (p-value of 0,36); PI-3K cascade (p-value of 0,048; 0,031) (Figure 3I, A-B). Interestingly, most down-regulated genes represented by Egr1, Ip6k2 and Kpna1 influenced key host immune response such as interferon alpha-beta signaling, interferon signaling, cytokine signaling in immune system, while other important pathways was also printed by *M. Bovis* infection like Fah (Phenylalanine and tyrosine metabolism)(p-value of 0,084); Ngb (intracellular oxygen transport); Cul4a (DNA damage record, neddylation)(p-value of 0,05; 0,089), Lims1 (Regulation of cytoskeleton)(p-value of 0,016) (Figure

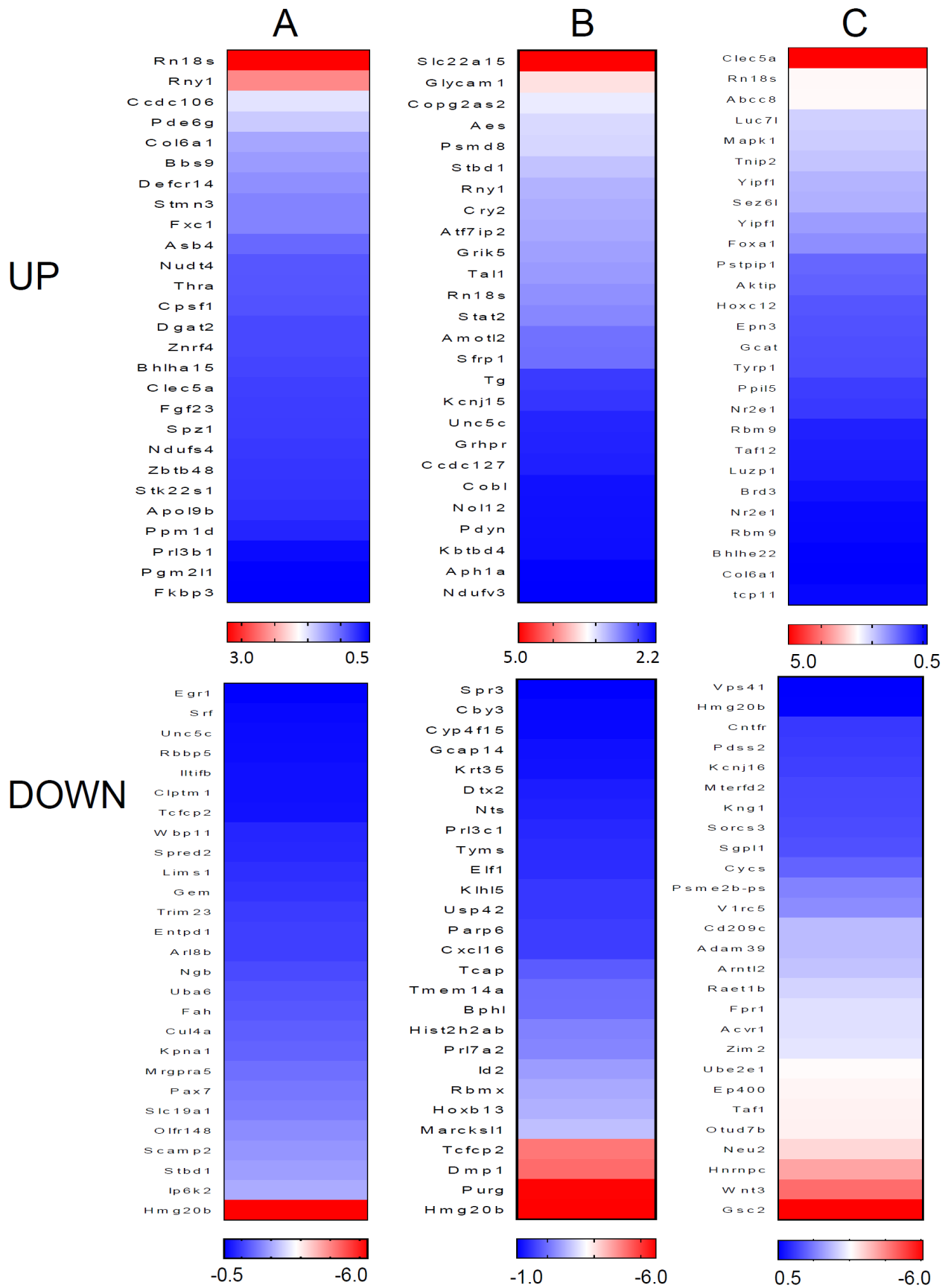


Fig. (2). Heat maps constructed (Graph Pad 7-0 software) from the Z scores of the genes most Up and Down regulated (< 2 SD)($P < 0.05$) from TST (+/-) (A), positive (B) cattle from Zacatecas and positive cattle from Coahuila State (C) Mexico.

Table 2. Data from NCBI bank of genes most Up and Down regulated accordingly to Z scores and significant ($P < 0.05$) as described in material and methods. in reactor cattle TST(+/-) (A) and positive cattle TST(+) from Zacatecas State, Mexico (B); positive cattle TST(+) from Coahuila State, Mexico (C).

Symbol gene	Gene name	Localization	A	Function	Process	Fold change
Rn18s	18 S ribosomal RNA	nucleolus/ribosome		Serves as the precursor for the 18 S, 5.8S and 28S rRNA	Serves as the precursor for the 18 S, 5.8S and 28S rRNA	4,712421
Rny1	Rna Y1 small cytoplasmic Ro associated	nucleus/chromosome		DNA binding	DNA replication initiation; nuclear DNA replicaton	4,137724
Ccdc106	Coiled coil domain containing 106	cytosol/nucleus		molecular function	biological process	3,516355
Pde6g	Phosphodiesterase 6 G	cytosol/membrane		Hydrolase activity	Signaling by Wnt; pos reg of G protein and EGF receptor sig	3,411287
Col6a1	collagen alpha 1(VI) chain	collagen trimmer/ext matrix/extracell space		protein heterodimerization	extracellular matrix organization: collagen formation	3,256749
Bbs9	Bordet Biedl Syndrome 9	cytoplasm/cytoskeleton/membrane		protein binding	cargo transport	3,211959
Defcr14	Defensin alpha 14	extracellular space/extracellular region		protein binding	antimicrobial peptide; response to Gram(+) and Gram(-)	3,160262
Stmn3	Stathmin 3	Golgi apparatus/perinuclear region of cytoplasm		Protein domain specific binding	Reg of GTPase act and microtubule org	3,110613
Trimm108	Fxc1 Translocase of inner mitochondrial membrane 10B	mitochondrial import inner memb complex		metal ion binding	protein transport mitochondrial	3,109248
Asb4	Ankrin repeat and SOCS box containing 4 protein	cytosol /cytoplasm		ubiquitin protein transferase activity	protein autoubiquitination	2,996994
Nudt4	Nudix hydrolase	cytosol /cytoplasm		catalytic function	inositol phosphate metabolism	2,91962
Thra	Thyroid hormone receptor alpha	cytoplasm/cytosol/RNA pol II trans factor complex		DNA binding transcription factor activity	neg reg of transcription by RNA pol II and Wnt sig pathway	2,913547
Cpsf1	Cleavage and polyadenylation specific factor 1	mRNA cleavage and polyadenilation/specificity complex		enzyme binding;mRNA 3' UTR AU rich region binding	mRNA processing	2,902818
Dgat2	diacyl glycerol O acyl transf 2	endoplasmic reticulum/int comp membrane		diacylglycerol O acyl transferase act	Triglyceride biosynthetic process;lipid metabolism	2,861424
Znrf4	zinc and ring finger 4	cytoplasm/memb/endoplasmic reticulum	UP	transferase and ubiquitin protein ligase activity	ubiquitin dependent protein catabolic process	2,860493
Bhlha15	basic helix loop helix	cytoplasm/membrane/int comp memb		DNA binding transcript activator act. RNA pol II specific	regulation of transcription DNA replicaton	2,844749
Clec5a	C type lectin domain containing 5A	int part of membrane/nucleus		carbohydrate, protein binding	protein binding	2,820821
Fgf23	Fibroblast growth factor 23	extracell region/extracell space		fibroblast growth factor receptor, protein binding	signaling by ILs(IL 2,3,5); MAPK sig pathway	2,812453
Spz1	spermatogenic leucine zipper 1	cytoplasm/nucleus		DNA binding	DNA binding transcription factor activity	2,809266
Ndufs4	NADH ubiquinone oxidorrreductae core subunit 54	mitochondrial resp chain complex I		NADH dehydrogenase (ubiquinone) activity)	cAMP mediated signaling; Oxidation Reduction process	2,792578
Zbtb48	telomere Zinc finger associated domain cont 48	chromosome/nucleus/cytosol		DNA binding, metal ion binding	Transcription, DNA templated	2,782274
Stk22s1	testis specific serine kinase substrate serine	centriole/cytoplasm/cytoskeleton	protein kinase binding	negative regulator of protein kinase signalization	2,77109	
ApoJ9b	apolipoprotein L 9b	cellular component	lipid binding	biological process	2,755473	
Ppm1d	protein phosphatase 1D	cytoplasm/cytosol/nucleus		catalytic act; cation binding; p53 signaling	DNA methylation; signal transduction by p53 mediator	2,710728
Pr13b1	prolactin family 3, subfamily b	extracellular region/extracellular space		hormone activity, prolactin receptor binding	positive regulation of JAK STAT signaling	2,598878
Pgm211	Phosphoglucomutase 2	cytoplasm		phosphoglucomutase; transferase activity	carbohydrate metabolic process	2,562214
Fkbp3	peptidyl prolyl cis trans FKBP3	nucleus		protein binding; isomerase activity	protein peptidyl prolyl isomerization	2,558778
Hmg20b	HMG box containing protein 20	nucleus		DNA binding transcription factor 4 way DNA junction	Chromatin org; neg reg of protein sumoylation	5,633444
Ipk6k2	inositol hexaphosphate kinase 2	cytoplasm/cytosol/nucleus		3'kinase; transferase act; nucleotide binding	inositol phosphate biosynthetic, post reg of apoptosis	4,025772
Stbd1	Starch binding domain 1/glycophagy cargo	cytoplasm/cytosol		Carbohydrate, enzyme binding	autophagy; carbohydrate met process	3,963144
Scamp2		ext cellular matrix/membrane		carrier/protein transport	protein transport	3,917525
Olf148	Olfactory receptor 14	int comp membrane		G protein coupled receptor, olfactory receptor activity	G protein coupled recept signaling pathway	3,878472
Slc19a1	solute carrier family 19 member 3	Golgi apparatus/cytoplasm/endorosome		folate anion transporter activity	vitamin transport	3,80426
Pax7	paired box transcription factor	intr comp membrane		DNA binding	regulation of transcription; positive reg of histone methylation	3,777645
Mrgpra5	member A5/G protein coupled receptor	lysosome/vacuolar memb/intracellular		nuclear import signal receptor, G protein coupled receptor act.	Signal transduction; G protein coupled receptor	3,740153
Kpna1	Karyopherin subunit alpha 3	cytoplasm/mitochondrion	DOWN	protein binding; protein transp activity	postsynapse to nucleus signaling pathway	3,680486
Cullin4A	Cullin 4A	cytosol		ubiquitin protein ligase binding; ubiquitin mediated proteolysis	cellular response to DNA damage	3,658435
Fah	Fumarylacetoacetato hydrolase	cytosol		nuclear import signal receptor activity	metabolic pathways, metabolism of aminoacids	3,625094
Uba6	ubiquitin activating enzyme E2 like 2/	cytosol/cytoplasm		ubiquitin activity;ATP binding	protein ubiquitination; cellular protein mod process	3,598864
Ngb	neuroglobin	cytoplasm/cytosol/nucleus/nuclear pore		heme binding; metal ion binding	apoptotic process; pos reg of peroxide induced cell death	3,563347
Ar18b	ADP ribosylation factor/ like GTPase 8B	intr comp memb/plasma memb/membrane		GTP binding	cell cycle; cell division	3,510195
Entpd1	ectonucleoside triphosphate/diphosphohydrolase 1	nucleus/trans factor complex		Nucleotide phosphohydrolase activity	lymphoid cell antigen	3,508237
Trim23	tripartite motif containing 3	apical plasma memb/membrane/plasma memb		ubiquitin, metal ion binding; transferase activity	proteasome mediated ubiquitin dependent catabolic process	3,492411
Gem	GTP binding protein/overexpressed in skeletal muscle	memb/plasma memb/int com memb		GTP binding	chromosome organization; signal transduction	3,45063
Lims1	LIM zinc finger domain/containing 1	cytoplasm		protein binding;protein kinase activity	cell matriz adhesion; cell cell adhesion	3,427456
Spred2	sprouty related, EVH1/domain containing 2	cytosol/membrane/plasma membrane		protein kinase binding	inactivation of MAPK; reg ulation of signal transduction	3,395787
Wbp211	WW domain binding protein 11	cytosol/cytoplasm		WW doman binding	mRNA, rRNA processing	3,388238
Tcfcp2	transcription alpha globin factor	nucleus/chromosome		DNA binding transcription factor	positive regulation of transcription by RNA pol II	3,28972
C1ptm1	Cleft lip palate transmembrane protein 1 homolog	ext int component of plasm membrane		molecular function	regulation of T cell diff in thymus; IL 19,20,22, 24 signaling	3,283702
Il1f1b	interleukin 22b	extracellular region/extracellular space		cytokine activity	signaling by cytokines in immune system	3,283623
Rbbp5	retinoblastoma binding protein 5	histonye methylation ransferase/ nucleolus/nucleus		methylated histone, protein binding	transcription DNA templated; cellular response to DNA damage	3,264214
Unc5c	netrin receptor UNC5C	int comp memb/plasma memb/ cell junction		netrin receptor act; protein binding	netrin activated signaling pathway; pos reg of apoptotic process	3,258664
Srf	serum response factor	cytoplasm/nucleus/nuclear chromatin		DNA binding; chromatin binding	cell adhesion; actin cytoskeleton organization	3,241575
Egr1	early growth response 1	cytoplasm/nucleus		metal ion, transcription factor binding	neg reg canonical Wnt path; T cell diff; IL 8; IL 1 signalization pathway to mycophenolic acid	3,208

Symbol gene	Gene name	Localization	B	Function	Process	Fold change
Slc22a15	solute carrier family 22 member 15	membrane/int component membrane		transmembrane transporter activity	ion transport	4,407453
Inka1	inka box actin regulator 1	cytoplasm / nucleus		protein kinase binding	negative regulation of protein serine threonine kinase inhibitor activity	3,694247
Glycam2	glycosylation dependent cell adhesion molecule 1	membrane		cell adhesion molecule binding	cell adhesion; response to bacterium	3,534364
Copg2as2	coatomer protein complex subunit gamma 2	cytoplasm / nucleus		molecular function	molecular function/biological process	3,47754
Aes	amino terminal enhancer of split	nucleus / transcription factor complex		transcription corepressor activity	cell fate determination; neurological development	3,46834
Psm8	ATPase 8	cytosol / nucleoplasm / nucleus / proteasome regulatory particle		molecular function	proteasome mediated ubiquitin dependent proteolysis	3,403699
Stbd1	starch binding domain 1	cytosol / plasma membrane / endoplasmic reticulum		protein binding; enzyme binding; glycogen binding	autophagy; glycolysis; carbohydrate metabolism	3,352752
Rny1	Rna Y1 small cytoplasmic associated	nucleus/chromosome		DNA binding	DNA replication initiation; nuclear DNA replication	3,333531
Cry2	cryptochrome circadian regulator 2	cytosol / extracellular region / nuclear speck / nucleus		protein binding; phosphatase binding; DNA binding	Circadian rhythm; transcription, DNA templated	3,323612
Atf7ip2	activating transcription factor 7 interacting protein 2	nucleus		molecular function	biological process	3,296941
Grik5	glutamate ionotropic receptor kainate type subunit 5	cell junction / dendrite / endoplasmic reticulum		glutamate receptor; signaling receptor act/ion transport	ion transport; receptor clustering	3,278964
Tal1	TAL bHLH transcription factor 1, erythroid diff. factor	nucleus / nucleoplasm / nuclear chromatin		E box binding; protein binding	Erythrocyte, myeloid differentiation; negative regulation of transcription	3,24721
Rn18s	18 S ribosomal RNA	nucleolus/ribosome		Serves as the precursor for the 18 S, 5.8S and 28S rRNA	Serves as the precursor for the 18 S, 5.8S and 28S rRNA	3,216225
Stat2	signal transducer and activator of transcription 2	cytosol / nucleoplasm / plasma membrane		DNA binding transcription factor act/ubiquitin protein binding	Type I IFN signaling pathway, defense response to virus	3,147885
Amotl2	angiomotin like 2	apical plasma membrane / cytoplasmic vesicle	UP	protein binding	Wnt signaling pathway	3,142668
Sfrp1	secreted frizzled related protein 1	extracellular space / plasma membrane		Wnt protein binding heparin binding	Wnt signaling pathway; actin cytoskeleton org	2,974467
Tg	Thyroglobulin	extracellular region		hormone biosynthetic process; receptor signaling receptor act.	Transcytosis; hormone biosynthetic process	2,95789
Kcnj15	ATP sensitive inward rectifier potassium channel 15	integral comp membrane/plasma membrane		potassium channel act; protein binding	ion transport; regulation of ion transport	2,904019
Unsc5c	netrin receptor UNSC	cell junction / integral comp memb/plasma memb		netrin receptor act; protein binding	apoptotic process; netrin signaling pathway	2,896831
Grhr	glyoxylate reductase; hydroxypyruvate reductase	cytosol/cytoplasm		NAD binding; oxidoreductase activity	dicarboxylic acid met; Oxidoreduction process	2,889236
Ccdc127	coiledcoil domain containing protein 127	cellular		molecular function	Biological process	2,840705
Cobl	protein cordon blue	colocalizes with actin filament/cell cortex/plasma memb		actin binding; protein binding	actin filament process	2,838225
Nol12	nucleolar protein 12	nucleolus		rRNA binding	positive regulation of cell proliferation; neg reg of apoptotic process	2,835455
Pdyn	Prodynorphin/proenkephalin B	extracellular region cytoplasm/plasma membrane/synaptic vesicle		neuropeptide activity; opioid peptide activity	neuropeptide signaling pathway	2,829673
Kbtbd4	Kelch repeat and BTB(PO2) domain containing 4	dendrites/membranes/extracellular region cytoplasm		molecular function	cell differentiation	2,79219
Aph1a	gamma secretase subunit APH1A	Golgi apparatus/endoplasmic reticulum / mitochondrion		endopeptidase activity; protein binding	Notch signaling pathway; protein processing	2,791426
Ndufv3	NADH dehydrogenase (ubiquinone) flavoprotein 3	membrane/mitochondrial inner memb/nucleoplasm		catalytic activity	mitochondrial ATPs synthesis coupled electron transport; OxRed	2,739045
	mitochondrial	mitochondrion				
Hmg20b	high mobility group (HMG) 20B	nucleus		DNA binding	cell cycle; chromatin org; neg reg of protein sumoylation	6,317998
Purg	purine rich element binding protein G	cytoplasm / extracellular region / nucleus		DNA binding; purine rich neg reg element binding	biological process	6,308604
Dmp1	dentin matrix acidic phosphoprotein	cytoplasm / membrane / mitochondrion / nucleus		Hsp70 protein binding; extracellular matrix binding	extracellular matrix organization	5,699881
Tcfcp2	transcription factor CP2like 1	cytoplasm / plasma membrane		DNA binding; transcription factor binding	mRNA transcrip by RNA pol II, Transcrip DNA templated	5,629522
Marcks1	MARCKS like	nucleus / nucleoplasm		actin binding; calmodulin binding	positive regulation of cell proliferation	4,47293
Hoxb13	homeobox B13	nucleus / nucleoplasm / catalytic step 2 spliceosome		DNA binding methyl CpG binding	mRNA of transcription DNA templated: regulation of cell growth	4,385222
Rbmx	RNA binding motif protein Xlinked	cytoplasm / cytosol / nucleus		RNA binding; mRNA binding	regulation of transcription; cellular response to IL 1	4,34581
Id2	inhibitor of DNA binding 2	extracellular region / extracellular space		ion channel binding; protein dimerization activity	Peyer's patch development: NK cell diff; neg reg of B cell diff	4,271447
Prl7a2	prolactin family 7, subfamily a, member 2	nuclear chromatin / nucleosome / nucleus		hormone act; prolactin receptor binding	positive regulation of JAK STAT cascade; pos reg of cell proliferation	4,135262
Hist2h2ab	histone cluster 2, H2ab	mitochondrion / mitochondrial outer membrane		DNA binding; protein dimerization	Chromatin organization	4,1102
Bphl	biphenyl hydrolase like	endoplasmic reticulum and integral component of membrane		hydrolase activity	Metabolism; biological oxidation	3,994193
Tmem14a	transmembrane protein 14A	cellular component		molecular function	negative regulation of apoptotic process;	3,992781
Tcap	titin cap	Z disc		FAT2, channel and titin Z domain binding	sarcomere organization; skeletal muscle contraction	3,894461
Cxcl16	chemokine ligand 16	integral component of membrane	DOWN	chemokine activity; LDL and scavenger receptor activity	T cell chemotaxis; cellular response to LPS	3,720884
Parp6	poly(ADP ribose) polymerase family member 6	intracellular		NAD+ ADP ribosyltransferase activity	protein ADP ribosylation	3,717596
Usp42	ubiquitin carboxyl terminal hydrolase 42	cellular component		cysteine type endopeptidase activity	regulation of apoptotic process; protein deubiquitination	3,683714
Klhl5	kelch like family member 5	cytoplasm / cytosol / cytoskeleton		molecular function	peptide crosslinking	3,682623
Elf1	E74 like ETS transcription factor 1	nucleoplasm / nucleoplasm		DNA binding transcription factor activity	regulation of cytokine production; neg reg of T cell receptor signaling path	3,620549
Tyms	thymidylate synthetase	cytoplasm / mitochondrial inner membrane / nucleus		thymidylate synthase activity	dTMP dUMP biosynthetic process	3,613085
Prl3c1	prolactin family 3, subfamily c, member 1	extracellular region / extracellular space		hormone activity; prolactin receptor binding	positive regulation of JAK STAT cascade and cell proliferation	3,593588
Nts	neurotensin	transport vesicle / extracellular region		neuropeptide binding; hormone activity	regulation of signaling receptor activity	3,552457
Dtx2	deltex E3 ubiquitin ligase 2	cytoplasm / nuclear membrane / nucleoplasm		protein binding and metal ion binding; transferase activity	NOTCH signaling pathway	3,529861
Krt35	keratin type cuticular Ha5	intermediate filament		structural molecular activity	keratinization and keratinocyte differentiation; peptide cross linking	3,464097
Gcap14	Ccser2 serine rich coiledcoil domain containing protein 2	microtubule cytoskeleton		microtubule binding	microtubule bundle formation	3,454172
Cyp4f15	cytochrome P450, family 4, subfamily f, polypeptide 5	cytoplasm, membrane / int comp membrane		heme binding	Eicosanoid; leukotriene and Cytochrome P450 pathways	3,406029
Cby3	protein chibby homolog 3	colocalizes with centriole/Not ciliary basal body		molecular function	NOT neg reg of Wnt Signaling pathway	3,402938
Spr3	small proline rich protein 3	Golgi apparatus cytoplasmic formed envelope		structural molecular activity	keratinization; keratinocyte diff and peptide cross linking	3,362478

Symbol gene	Gene name	C	Localization	Function	Process	Fold change
Clec5a	C		cell surface/cytosol/plasma memb	protein coding	rRNA	4,319691
Rn18s	RNA ribosomal 18 S		nucleo/nucleolus	structural	signaling	3,657197
Abcc8	ATP binding cassette sub family C member 8		synaptic ves mem/plasma mem/ion channel	transporter	mRNA splice selection	3,656106
Luc7l	putative RNA binding protein Luc7 like 1		nucleus/U1 snRNP	RNA binding	B;T cell/ERK1;ERK2 signaig pathway	3,514455
Mapk1	mitogen activated protein kinase 1		Cytoplasm/endosome/nucleus	transferase act	immune system process	3,504118
Tnip2	TNFAIP3 interacting protein 2		cytoplasm/cytosol/nucleus	protein coding	immune system process	3,480367
Yipf1	protein YIPF2 domain family		endosome/int comp memb/transport vesicular	protein coding	Rab GTPase binding	3,438103
Foxa1	hepatocyte nuclear factor 3 alpha		nucleus/microvillus/fibrilar center	protein coding	response to stimulus	3,42924
Pstpip1	proline serine threonine phosphatase interacting protein 1		plasma memb/cytoplasm/cytoskeleton	transferase act	signaling	3,375711
Aktip	AKT interacting protein		cytoplasm/plasma memb/FHH complex	transcription	immune system process	3,338202
Hoxc12	homeobox protein Hox C12		cellular component/nucleus	cytoskeletal	cell death	3,231466
Epn3	epsin 3		clathrin coated pit/cytoplasm/nucleus	protein coding	nucleic acid templated transcription	3,218709
Gcat	2 amino 3 ketobutyrate coenzyme A ligase, mitochondrial		mitochondrial memb/nucleus/mitochondrion	DNA binding	biological process	3,18704
Typr1	5,6 dihydroxyindole 2 carboxylic acid oxidase		Clathrin coated/endosome/int comp memb	lipid binding	metabolic process	3,175419
Ppil5 (Lrr1)	leucine rich repeat protein 1		difs cellular locations	glycine C acetyl	cell differentiation	3,166818
Nr2e1	nuclear receptor subfamily 2 group E member 1		nucleus	oxidoreductase	molecular function	3,162646
Rbm9	RNA binding protein fox 1 homolog 2		cytoplasm/cytosol/nucleus	protein coding	negative regulation of apoptotic process	3,121603
Taf12	transcription initiation factor TFIID subunit 12		nucleus/transcript factor TFIID	DNA binding	RNA splicing	3,111475
Luzp1	leucine zipper protein 1		nucleus	RNA binding	protein metabolic process	3,045866
Brd3	bromodomain containing protein 3		cellular component nucleus	transcription	biological process	3,034095
Bhlhe22	Basic helix loop helix family member 22		cytoplasm/membrane/int comp memb	DNA binding	system development	3,028504
Col6a1	collagen alpha 1(VI) chain		extracellular matrix/collagen trimer	molecular function	chromatin organization	3,00171
Rbmj1a1	RNA binding motif protein, family 1 member A1		RNA nucleus/ribonucleoprotein complex	molecular function	system development	2,977725
Acaa1a	3 ketoacyl CoA thiolase A, peroxisomal		peroxisome/mitochondrion/int mem bound	chromatin binding	chromatin organization	2,975673
Rcan2	calcipressin 2		cytoplasm	protein binding	cell differentiation	2,962502
R3hdm2	R3H domain containing 2		cellular component/nucleus	transcription	Ras protein (SMAD protein signaling)	2,960735
Jun	transcription factor AP 1		cytosol/nucleus/transcript factor AP1 complex	transcription	monocyte differentiation	2,975673
Gsc2	homeobox protein gooseoid 2		nucleo	transcription	nucleic acid templated transcription	6,03057
Wnt3	proto oncogene Wnt 3		cytoplasm/extra matrix/ext space	signaling receptor binding	signaling	5,606744
Hnrnpc	heterogeneous nuclear ribonucleoproteins C1/C2		actin cytoskeleton/nucleus/cytosol	phosphatase binding	biological process	5,384634
Neu2	sialidase 2		cytoplasm/cytosol/intracellular mem	hydrolase act/sialidase act.	carbohydrate, lipid metabolism	5,182497
Otud7b	OTU domain containing protein 7B		cytoplasm/nucleus	hydrolase	lipid metabolic process	5,078586
Taf1	transcription initiation factor TFIID subunit 1		nucleus/transc factorcomplex	DNA binding	biological process	5,07364
Ep400	E1A binding protein p400		nucleus(nuclear speck)	hydrolase	DNA repair	5,062969
Ube2e1	ubiquitin conjugating enzyme E2 E1		nucleus/ubiquitin ligase complex	transferase	immune system process	5,039832
Zim2	Zim2 zinc finger imprinted 2		Cellular component	molecular function	response to stimulus	4,921464
Acvr1	activin receptor type 1		membrane/int comp plasma membrane	signaling receptor activity	protein metabolic process	4,903474
Fpr1	fMet Leu Phe receptor		int comp mamemb/membrane	signaling receptor activity	inflammation	4,900078
Raet1b	retinoic acid early inducible protein 1 beta		Golgi apparaturs/caveola/cytoske	signaling receptor binding	biological process	4,8499
Arntl2	aryl hydrocarbon receptor nuclear translocator like protein 2		cytoplasm/nucleus/nucleolo	DNA binding	biological process	4,784523
Adam39	a disintegrin and metallopeptidase domain 39		Cellular component	molecular function	signaling	4,755927
Cd209c	CD209 antigen like protein C		Cellular component	carbohydrate binding	homeostatic process	4,755472
V1rc5	vomeronasal 1 receptor, C5		Cellular component	signaling function	immune system process	4,570314
Psme2b ps	protease (prosome, macropain) 28 subunit beta B		Cellular component	molecular function	nucleic acid templated transcription	4,528281
Cycs	Cycs cytochrome C, somatic		cytosol/nucleus/mitochondrial	oxidoreductase	biological process	4,408696
Sgpl1	sphingosine 1 phosphate lyase 1		endoplasmic ret/membrane/int membrane	protein coding	biological process	4,331022
Sorcs3	VPS10 domain containing receptor SorCS3		membrane/int com of memb	protein binding	signal transduction	4,311266
Kng1	kininogen 1		extracellular region /ext space	enzyme regulator	homeostatic process	4,294935
Mterfd2	transcription termination factor 4, mitochondrial		cytosol/mitochondrion	RNA binding	establishment of localization	4,294131
Kcrj16	inward rectifier potassium channel 16		int comp memb/plasma memb	transporter	establishment of localization	4,265176
Pdss2	decaprenyl diphosphate synthase subunit 2		cytosol/mitochondrion/trans complex	transferase	lipid metabolic process	4,247797
Cntfr	ciliary neurotrophic factor receptor subunit alpha		membrane/plasma memb/recep com	signaling receptor activity	response to stimulus	4,237354
Hmg20b	high mobility group (HMG) 20B		nucleus/nucleoplasm/nuclear bodies	DNA binding	nucleic acid templated transcription	4,021251
Vps41	vacuolar protein sorting associated protein 41 homolog		golgi app/colocalizes with clathrin	cytoskeletal protein binding	cellular component organization	4,013891

UP

DOWN

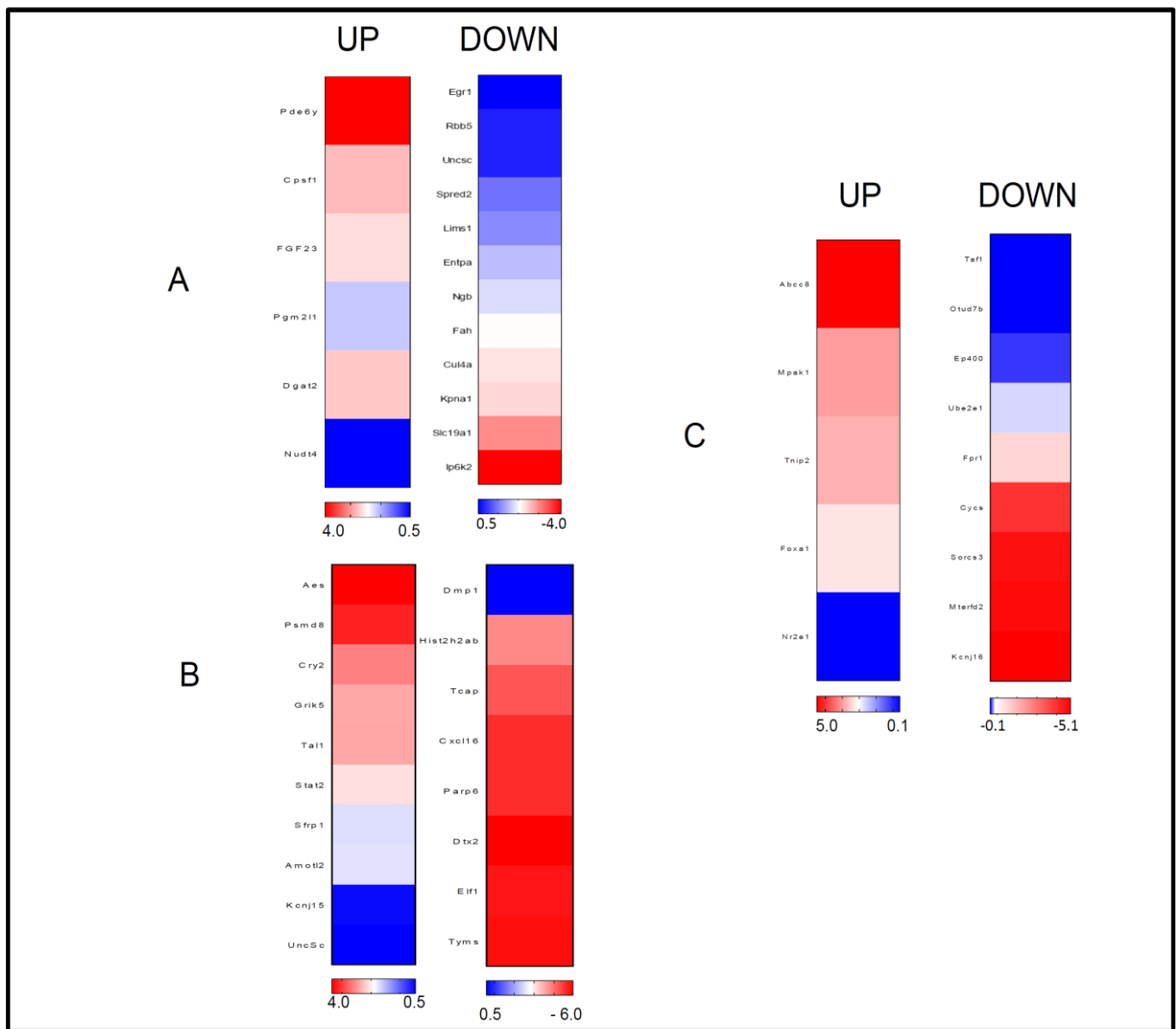


Fig. (3). Functional analysis of the most up and down regulated genes using Reactome data base (A) and expressed also as heat maps constructed (B) from the Z scores of the genes most up and down regulated (< 2 SD)($P < 0.05$) from TST (+/-) (A), positive (B) cattle from Zacatecas and Positive from Coahuila State (C) Mexico.

3A). Second, in the cattle infected with *M. bovis* (TST+) from Zacatecas, Mexico (Figure 3B), we found that these genes impact for example in NOTCH 4 (p-value of 0,025) and/or Wnt signaling (p-value of 0,03); regulation of IFN-alpha/beta (STAT2)(p-value of 0,056); G-protein gated potassium channels (p-value of 0,055) (Figure 3II, A-B). While most of the down-regulated genes also influence important pathways such as, RUNX-1 transcription of genes involved in interleukin (p-value of 0,015); of BCG signaling (p-value of 0,015); activity of NOTCH 4 transmits signal to the nucleus (p-value of 0,066); chemokine receptor bind chemokines (p-value of 0,098); chromatin organization (p-value of 0,017) (Figure 3II, A-B). In third place, from the set of genes of infected cattle with *M. bovis* (TST +) from Coahuila Mexico (Figure 3III A-B) that are most up and down-regulated were also performed using the Reactome bioinformatics database [48](Figure 3III,

A-B). From this analysis, we found that a small set of genes, Mapk1, Tnfp2, Abcc8, Nr2e1, Foxa1 have also an impact in key host immune response pathways; IL-17 signaling (p-value of 6.84e-4), Myd88 cascade in endosome (p-value of 0,02), and in plasma membrane (p-value of 0,001); all the TLR 1, 2, 3 -10; TRAF 6 mediated induction of NFK β and MAP kinase (p-value of 0,001); MAP kinase activity (p-value of 4.84 e-4); activity of the AP-1 family of transcription factors (p-value of 2.81 e-4) (Figure 3III, upper panel) Furthermore, and in marked contrast a more larger set of genes were most down-regulated, like Taf1, Cycs, Ep400, Fpr, Mterfd2, Otud7b. Interestingly, IL10 (p-value of 0,016) and/or IL-6 (p-value of 0,031) as well as the TNFR-1 produced pro-apoptotic signaling (p-value of 0,031); cellular response to stress (p-value of 0,026); apoptotic mediated response (p-value of 0,02); RNA

Table 3. Functionality analyses of genes most up and down regulated accordingly to Reactome data base and p values which express the probability that the extent of change of a gene (up or down) be affected in determined reaction (pathway step) in reactor TST (+/-) (A), positive TST+ cattle (B) from Zacatecas and TST (+) from Coahuila State (C) Mexico.

A. Reactor (TST(+/-). Zacatecas, Zac. Mexico)							
Up regulated	symbol gene	fold change	pvalue	Down regulated	symbol gene	fold change	pvalue
Inositol phosphate metabolism	Nudt4	2,02	0,007	Interferon alpha/beta signaling	Egr1/Ip6k2/Kpna1	-3,21	0,0005
Tryglycerides biosynthesis	Dgat2	2,86	0,036	interferon signaling	Egr1/Ip6k2/Kpna1	-3,21	0,001
Glycogen synthesis	Pgm2l1	2,56	0,041	Cytokine signaling in immune response	Egr1/Ip6k2/Kpna1	-3,21	0,064
Glycogenolysis	Pgm2l2	2,56	0,041	intracellular oxygen transport	Ngb	-3,56	0,01
Phospholipase C mediated cascade FGFR1	FGF23	2,81	0,046	FGF1.1 modulation of FCFR1 signaling	Spred2	-3,39	0,029
FGFR1c ligand binding and activation	FGF23	2,81	0,046	Cell extracellular matrix interactions	Lims1	-3,43	0,039
FG FR1.1 modulation of FGR1 signaling	FGF23	2,81	0,031	Netrin mediated repulsion signal	Uncsc	-3,26	0,021
PI-3K cascade FGFR3	FGF23	2,81	0,045	Apoptosis induced DNA fragmentation	Kpna1	-3,68	0,027
Processing of intron less Pre-mRNA	Cpsf1	2,9	0,045	Phenylalanine and Tyrosine catabolism	Fah	-3,62	0,084
Activity of phototransduced cascade	Pde6y	3,41	0,028	Neddylation	Cul4a/Rbb5/Kpna1	-3,66	0,089
B. Rositive (TST(+). Zacatecas, Zac. Mexico)							
Regulation of IFN α/β signaling	Stat 2	3,147	0,056	RUNX1 regulates transcription of genes in IL-signaling	Elf1	-3,62	0,015
Signaling by WNT	Aes	3,468	0,02	RUNX1 regulates transcription of genes in BCR signaling	Elf1	-3,62	0,015
NOTCH 2 activity/transmission nucleus	Aph1a	2,791	0,047	Act NOTCH 1 transmits signal to the nucleus	Dtx2	-3,53	0,066
NOTCH 4 activity transmission nucleus	Aph1a	2,791	0,025	Signaling by NOTCH 1	Dtx2	-3,53	0,164
Signaling by NOTCH 4	Aph1a	2,791	0,016	Peptide signal receptors	Cxcl16	-3,72	0,07
Non Canonical act of Notch 3	Aph1a	2,791	0,016	Chemokine receptor bind chemokine	Cxcl16	-3,72	0,098
G-protein gated potassium channel	Kcnj15	2,904	0,055	Metalloprotease DB5	Hist2h2ab	-4,11	0,066
Act of Ca+2 permeable Kainate receptors	Grik5	3,278	0,027	Deubiquitination	Hist2h2ab	-4,11	0,066
Netrin mediated repulsion signals	Unc5c	2,894	0,021	ECM proteoglycans	Dmp1	-5,7	0,126
Circadian clock	Cry2	3,323	0,019	Chromatin organization	Hist2h2ab	-4,11	0,126
C. Rositive (TST(+). Coahuila, Mexico)							
Toll like receptor cascade (3-10)	Mapk1	4,504	0,001	IL-6 type cytokine receptor ligand interaction	Cyts/Fpr1	-4,41	0,037
IL-17 signaling	Mapk1/Tnip2	3,504	0,0007	IL-10 signaling	Fpr1	-4,9	0,016
TRIP (TICAM1) mediated TLR4 signaling	Mapk1/Tnip2	3,504	0,002	TNFR induction of pro-apoptotic signaling	Otud7b	-5,07	0,031
TRAF6 mediated induction of NFK β	Mapk1/Tnip2	3,504	0,001	RNA polymerase II transcription initiation	Cyts	-4,41	0,006
MAP Kinase activation	Mapk1/Tnip2	3,504	0,0005	RNA polymerase II transcription initiation and promoter clearance	Taf1	-5,07	0,006
Myd88 cascade initiation on endosome	Mapk1/Tnip2	3,504	0,001	Cytochrome c mediated apoptotic response	Cyts	-4,41	0,015
Myd88 cascade initiation on plasma memb	Mapk1/Tnip	3,504	0,002	apoptosis factor-mediated response	Cyts	-4,41	0,02
Act of the AP-1 family of transcription factors	Mapk1	4,504	0,0003	Formyl sulfide receptors bind formyl sulphids and many other ligands	Fpr1	-4,9	0,034
Toll like receptor TLR1: TLR2 cascade	Mapk1/Tnip2	3,504	0,004	Potassium channel transport	Kcnj16	-4,26	0,009
Myd88 independent TLR4 cascade	Mapk1/Tnip2	3,504	0,002	Cyts/Fpr1/Ep400/Ub	e2e1	-4,41	0,026
				Cellular response to stress			

polymerase II transcription initiation and promoter clearance (p-value of 0,007) (Figure 3III, A B).

DISCUSIÓN.

The *in vivo* gene expression pathway profile from naturally infected cattle with *M. bovis* from Mexico, two close geographical states (Zacatecas, and Coahuila State) by using the microarray technology, have allowed to determined a set of signaling pathways that are printed through the host-pathogen interaction. We propose that the data obtained in this study might be used to further explore a scanning chip for diagnostic in bovine tuberculosis.

In human tuberculosis, it has been shown that transcriptional signature is printed in the type I IFNs signalization pathways at the level of innate immune response (neutrophils) [49]. Furthermore, it was shown that Eicosanoid and lipid metabolism are down regulated by type I IFNs leading to exacerbation of disease but at the same time could be used as host-therapy [49]. Therefore, this study is noteworthy since it revealed that biomarkers of disease progression can impact in different pathways and these can be used as multi-target therapy [38] instead of a dominant single target gene. In bovine

tuberculosis, the scenary is different. Most of the studies have been focused in the determination of expression profiles after PBMCs stimulation with PPD_b and/or infection followed by BCG treatment [36, 38, 43, 45, 47]. In these studies by using an extensive bioinformatics study, it was demonstrated that canonical pathways and genes are also involved in *M. bovis* infection, and for each pathways it was selected genes that were up or down regulated [46]. In another set of data, it was reported that IL-22 is a dominant biomarker [40]. Not further studies were made to explore the potential use of this dominant IL-22 as a predictor of protection [40]. We think that *in vitro* studies [41-46] are remarkable and have given clues that potentially can be extrapolated to cattle [47], but still are necessary to approach the disease from direct *in vivo* studies. Therefore, in this work, we focused precisely to determine *in vivo* gene expression pathway profile of cattle infected with *M. bovis* from Mexico. From the data obtained using microarray and functional analysis (Figure 2-3 A-C)(Table 2-3 A-C)(Reactome Database)[48](Figures 3A-C), we found that a set of genes (n= 27) modulated up and down (P < 0.05) are printed by *M. bovis* infection of cattle from Mexico. Among them, it can be mentioned, genes that encode transcription factors, binding (DNA, RNA, proteins, carbohydrates, metal-

ions, lipids, peptides), enzymes activities, catalytic roles, carriers, co-factors and molecular function (Figures 2A-C; Table 2A-C) Genes that participate in key host pathways such as innate and adaptive immune responses, Wnt and NOTCH signaling, TLR cascade (1-10); Myd88, MAPK kinase activity, autophagy, glycolysis, metabolism (inositol, triglycerides, glycogen, carbohydrates, eicosanoids, arachidonic acids, aminoacids), apoptotic mediated response, G-protein receptor signalization, RNA Pol II initiation, chromatin organization, cell cycle, oxido-reduction, G-mediated potassium channels process (Table 2A-C) cell matrix interactions. This *in vivo* study without *in vitro* Ag stimulation of PBMCs from uninfected and infected cattle with *M. bovis* is highly coincident with those data from *in vitro* studies [41 -46], meaning that it is possible to develop a ante-mortem molecular diagnostic under these experimental settings reported in this study. Furthermore, we think that is noteworthy that we found that interferon alpha/beta signaling (STAT2) (p value of 0,056) as well as IL-17 signaling (p value of 6.8 e-4) pathways were printed from *M. bovis* *in vivo* (Tables 2 A -C)(Figures 2 A-C) Autophagy process as well as the eicosanoid pathways were importantly impacted by this pathogen giving rise to a set of pathways-encoded by genes that might represent candidate biomarkers of the natural *M. bovis* infection (Figure 3A-C) Thus, the data obtained from this analysis highlight a proposal of a pattern based in the pathway set of the transcriptional printing of *M. bovis*. Therefore, it could be translated in the development of 22 genes most up regulated (representing key pathways of the host response to *M. bovis* infection) while 27 genes might represent the most down regulated (Figure 3A-C; Table 3A-C). We think that even it would possible to potentially define the spectrum of the infectious disease. In summary, until our knowledge, this a first report of the *in vivo* pathway profile printed by *M. bovis* in cattle from Mexico.

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CONFLICT OF INTEREST

There is no conflict of interest.

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