



Genetic mechanisms regulating host response during mastitis

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Prevention of Intramammary Infections

- Host's individual immune response to mastitis varies, some cows are more **susceptible** to intramammary infections than others
- Mucus layer
 - Highly hydrated barrier against bacterial and fungal organisms
 - Forms in the **mammary gland** after milking
- **Mucins** are present in the mucus layer, protect epithelial surfaces and aid in immune response

Transcriptomics and RNA-Sequencing

- Transcriptomics to measure gene expression in the **entire transcriptome**
- The transcriptome is the sum of all RNA synthesized in a **specific cell/tissue** at a **specific moment**
- Gene transcription and regulation underlie many biological processes and **phenotypic variation** found in living organisms
- RNA-Sequencing technology allows us to study the transcriptome at a **high-throughput** level
 - Gene expression
 - Structural variants (SNP, indel and splice variants)

Objectives

To study the genetic mechanism of response to infection in the mammary gland in Holstein cows using RNA-Sequencing technology

Specific objectives:

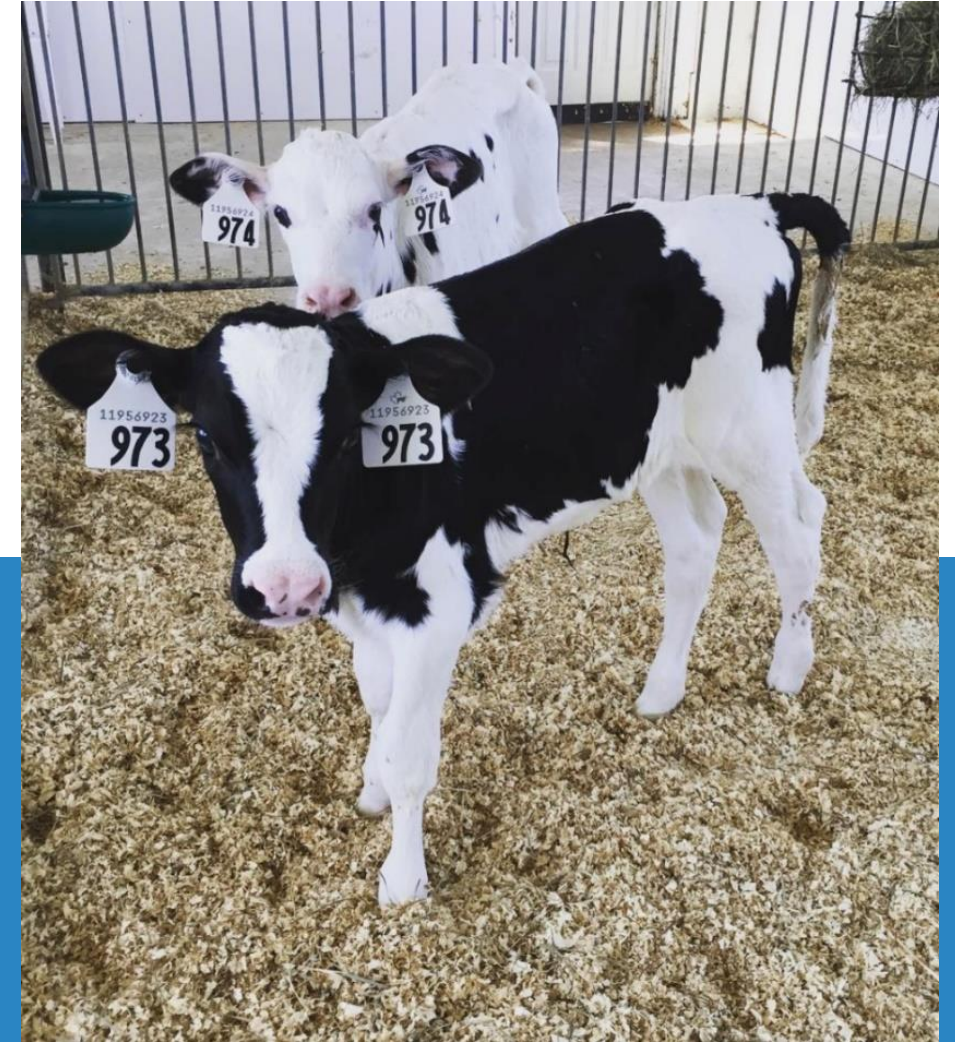
Identify milk SC genes DE between healthy and mastitic samples using RNA-Sequencing



Perform functional analysis using the list of DE genes to identify biological processes and metabolic pathways



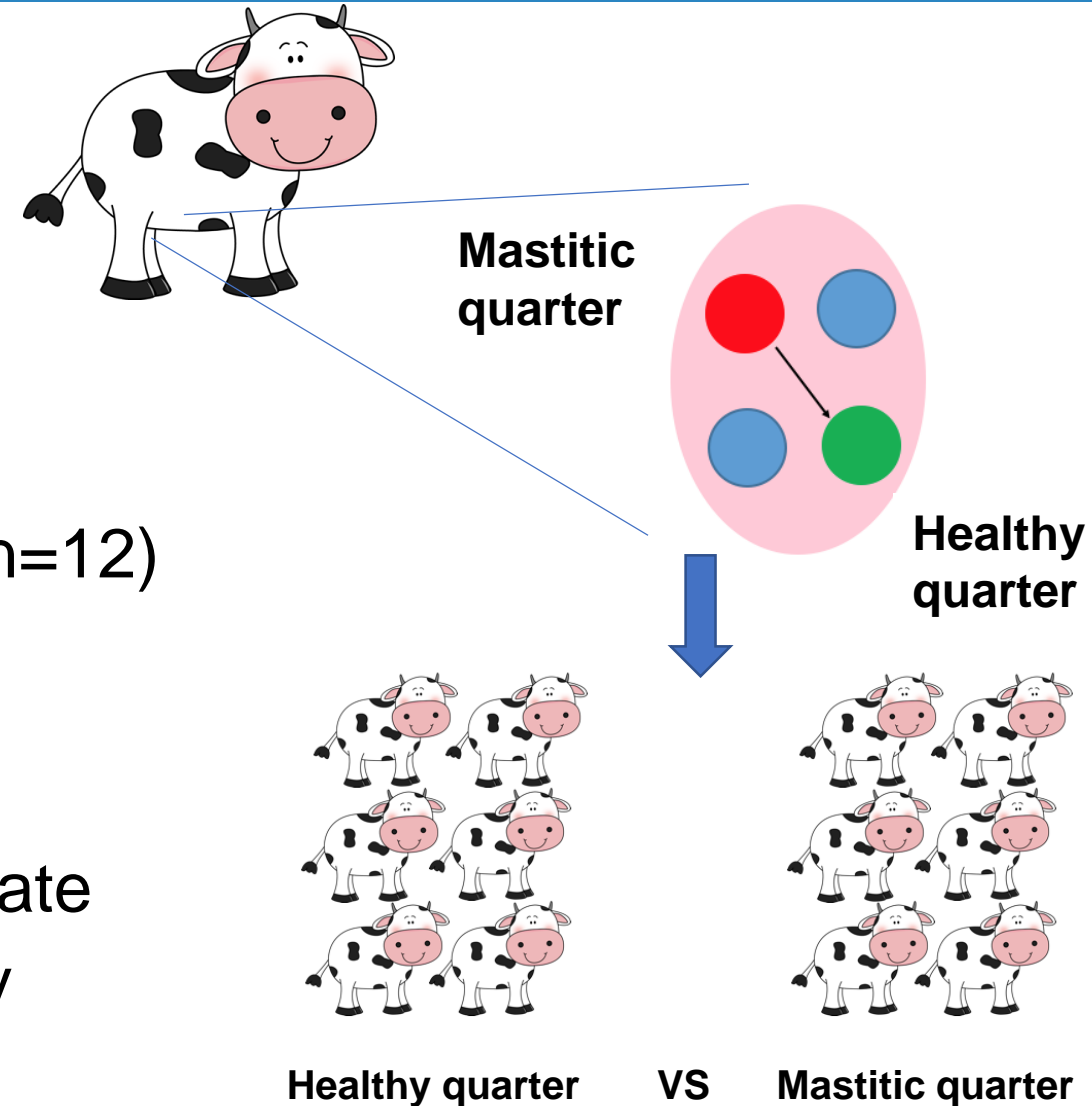
Integrate transcriptomic and functional data using systems biology to identify functional candidate genes



Differentially Expressed Genes

Materials and Methods: Sample Collection

- Six Holstein dairy cows
- **Natural cases** of mastitis, diagnosed by California Mastitis Test
- **Two samples** collected from each cow (n=12)
 - Mastitic quarter** (n=6)
 - Healthy quarter** (n=6)
- **Milk somatic cells** were used to investigate the transcriptome in the bovine mammary gland using RNA-Seq (Cánovas et al., 2014)



RNA-Sequencing

RNA extraction from bovine milk SC (n=12)



Sequencing Library (Illumina TruSeq kit)

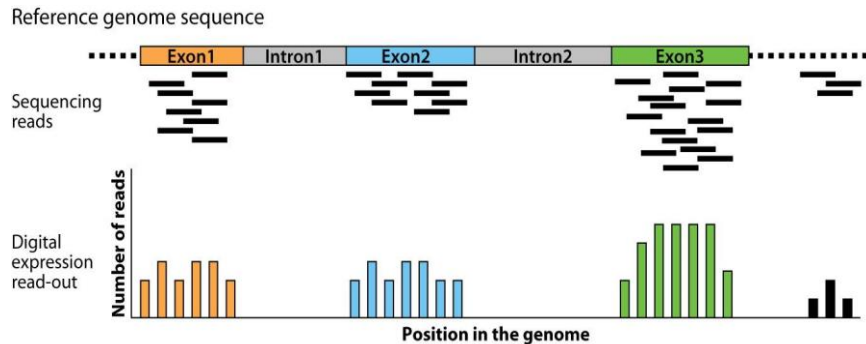


Illumina HiSeq 2000 analyzer

```
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ATTGATCGATTTTCAGAAATCATCAGATTC 18000 1 chr2:113004813 F
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AAAAATTTAAGACTATATAGAAATGACACATC 18000 1 chr1:47332993 F
ATTGAGCTTTTACCTGACAGAGGCTGACAA 18000 1 chr6:15024 F ADI
ATAATCGATGATTCGCAAGATTTGAAATGCTT 18000 1 chr15:7089029 F
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ATGATTTTCAAGAGGAGGAGGAGGAGGAGG 18000 1 chr1:13841939 F
AAAGGTTAAGACTAAGAGGAGGAGGAGGAGG 18000 1 chr19:50748240 I
GTCCCTCCAGTTTAAAGAGGAGGAGGAGGAG 18000 2
AAACAGGCTTCCCAACTGACAGAGGAGGAGG 14993 1 chr19:42825147 F
ATTGCAAGATATATATATGATATGATTTCTA 14993 1 chr5:6992292 R
AAACATTTCTTCCGATAGGCTTGGCAGATTA 14993 1 chr6:2332 F AAM
```

Millions of reads

Mapping to reference genome



Assembly to reference genome

(Bovine reference genome UMD3.1 release 90)



Transformed and normalized the data

Gene expression

(reads per kilo base per million mapped reads (RPKM) ≥ 0.2)

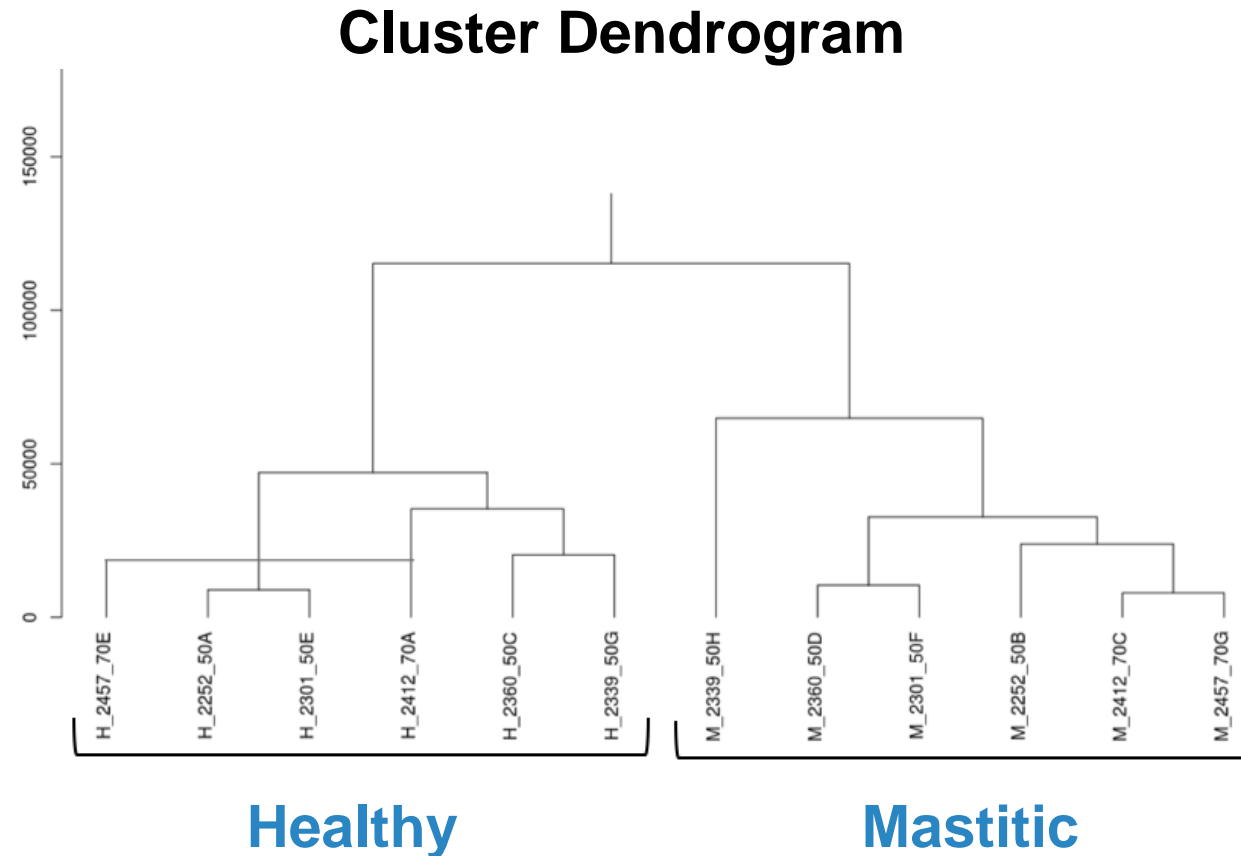
Differentially expressed genes between healthy and mastitic samples (P-value < 0.01, FDR < 0.05, FC > ± 2)

Functional analysis and metabolic pathway analysis



Results: Mapping & Cluster Dendrogram

- **226 million** total reads produced from milk somatic cells
 - On average, each sample generated **19 million** reads
- **78%** of reads mapped to the annotated UMD3.1 bovine reference genome release 90
- Cluster dendrogram created using distance matrix using the whole gene expression data
 - Samples **clustered perfectly** between healthy and mastitis quarters



Differential Gene Expression

449 genes were differentially expressed between healthy and mastitic quarters

- P-value < 0.01
- False discover rate (**FDR**) < 0.05
- Fold change (**FC**) > ± 2

449 DE genes

200 genes

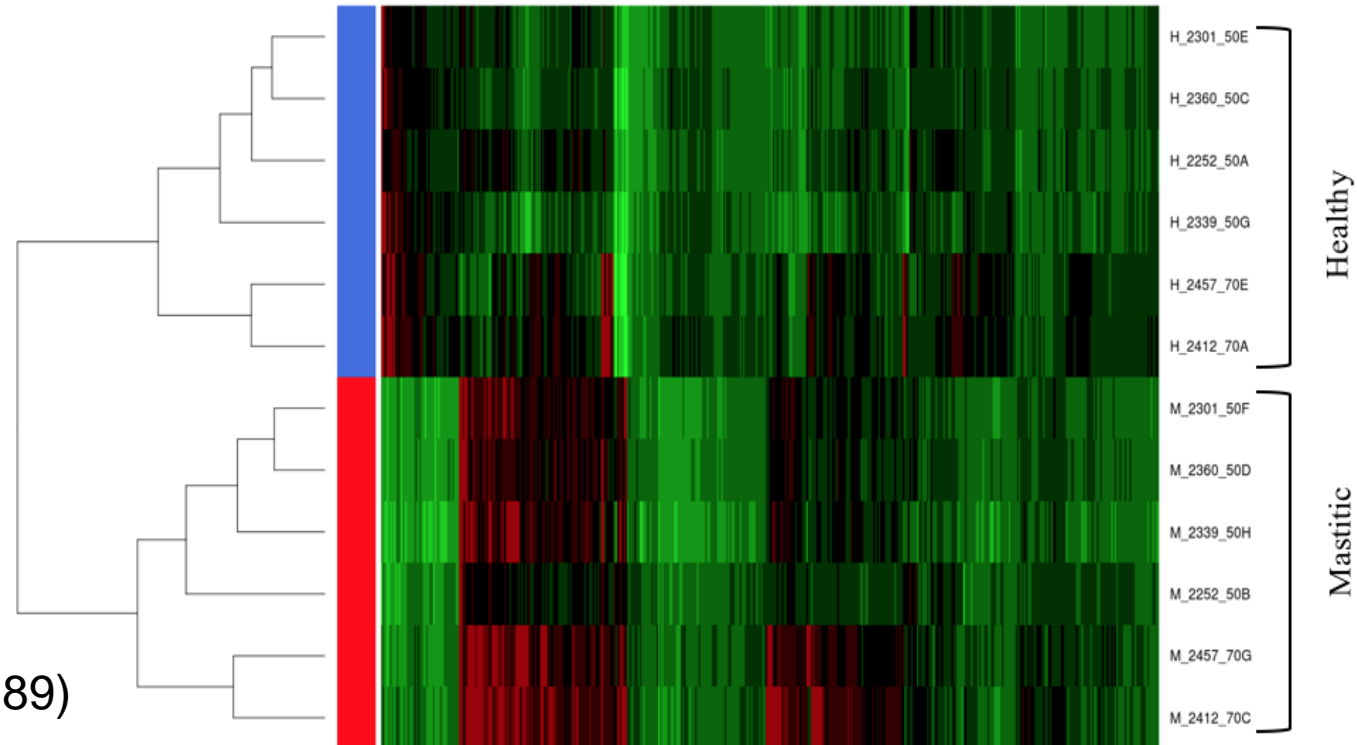
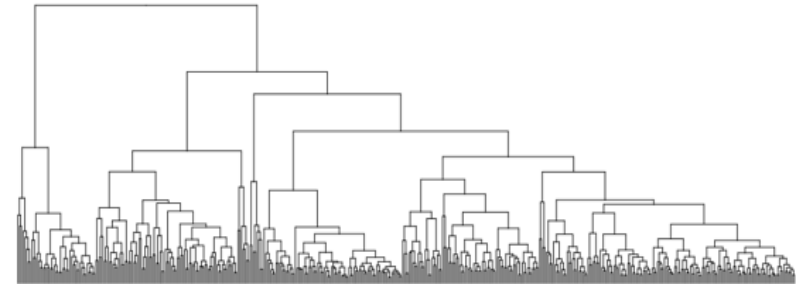
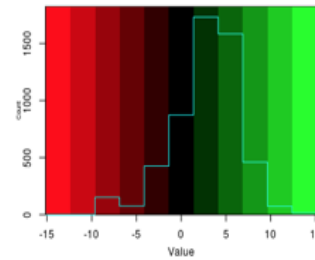
under expressed

SLC34A2 gene (FC = -84.28)

249 genes

over expressed

SAA gene (FC = 117.89)



Highly Expressed Genes (≥ 500 RPKM)

- Differentially expressed genes were categorized into three different groups (Wickramasinghe et al., 2012):
 - High** expressed genes (≥ 500 RPKM)
 - Medium** expressed genes (≥ 10 to 500 RPKM)
 - Low** expressed genes (< 10 RPKM)

Healthy Group	Mastitic Group
Top 5 highly expressed genes:	Top 5 highly expressed genes:
Casein beta (<i>CSN2</i>)	β -2-microglobulin (<i>B2M</i>)
Casein kappa (<i>CSN3</i>)	CD74 molecule (<i>CD74</i>)
Casein- α -s1 (<i>CSN1S1</i>)	Major histocompatibility complex class II, DR alpha (<i>BoLA DR- alpha</i>)
Alpha-S2-caesin Casocidin-1 (<i>CSN1S2</i>)	Serine dehydratase (<i>SDS</i>)
β - lactoglobulin (<i>BLG</i>)	L-Lactate dehydrogenase A chain (<i>LDHA</i>)
Functions associated with: milk components	Functions associated with: immunity

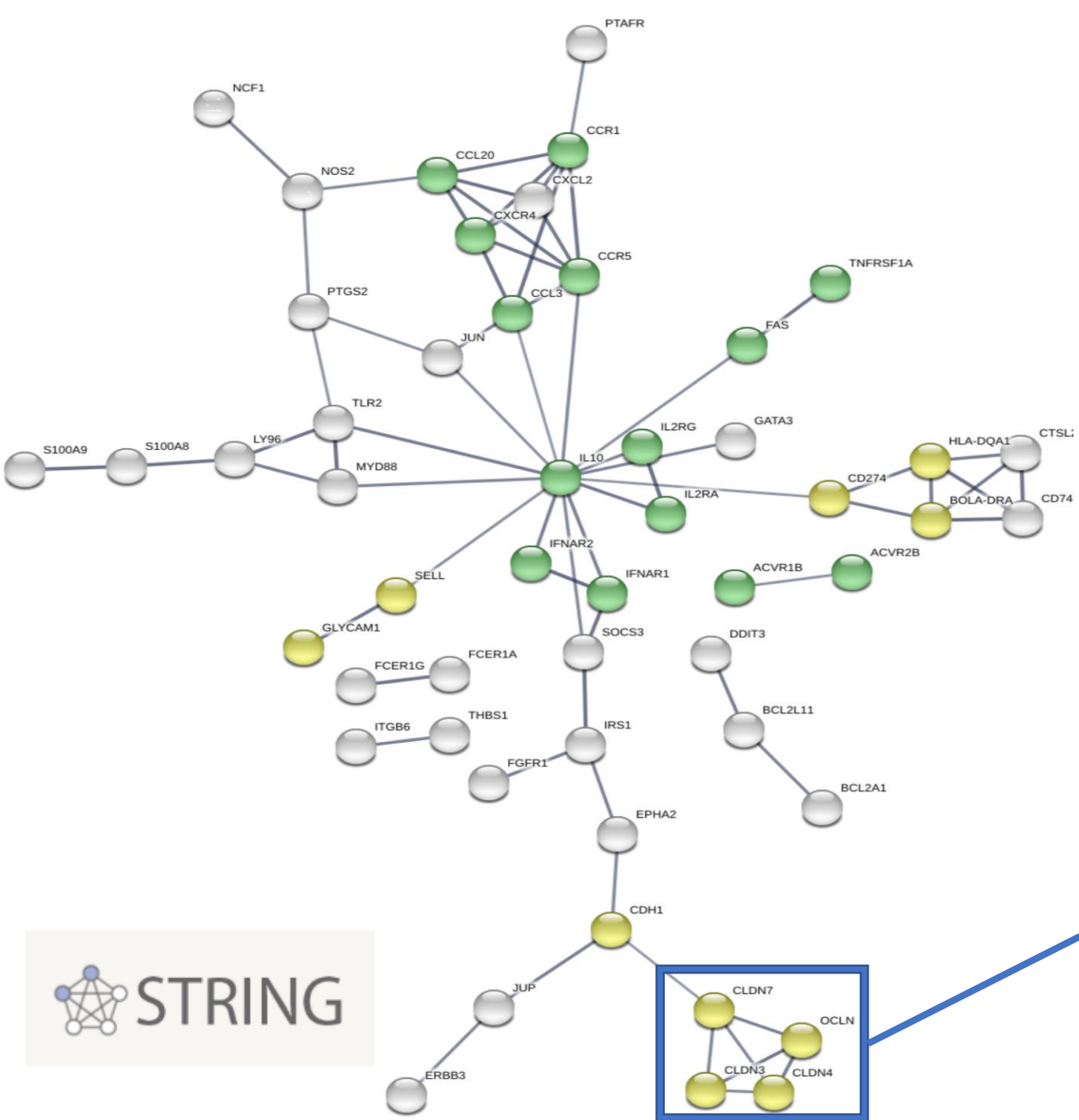


Functional Pathway Analysis

Functional Metabolic Pathway Analysis

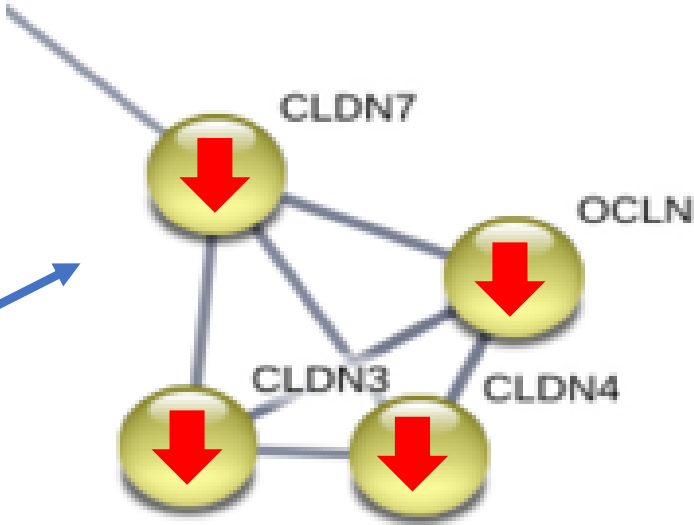
- Using the list of 449 DE genes between healthy and mastitic samples
 - **55 significant metabolic pathways** identified (FDR < 0.05)
 - Top two significantly enriched pathways:

Pathway	P-value	DE genes mapped in pathway (n)	Total genes in pathway (n)
Cytokine-cytokine receptor interaction	2.38E-07	25	297
Cell adhesion molecules	0.00017895	14	168



Legend

- = cytokine-cytokine interaction pathway
- = cell adhesion molecules pathway
- = not involved in cytokine or cell adhesion pathways





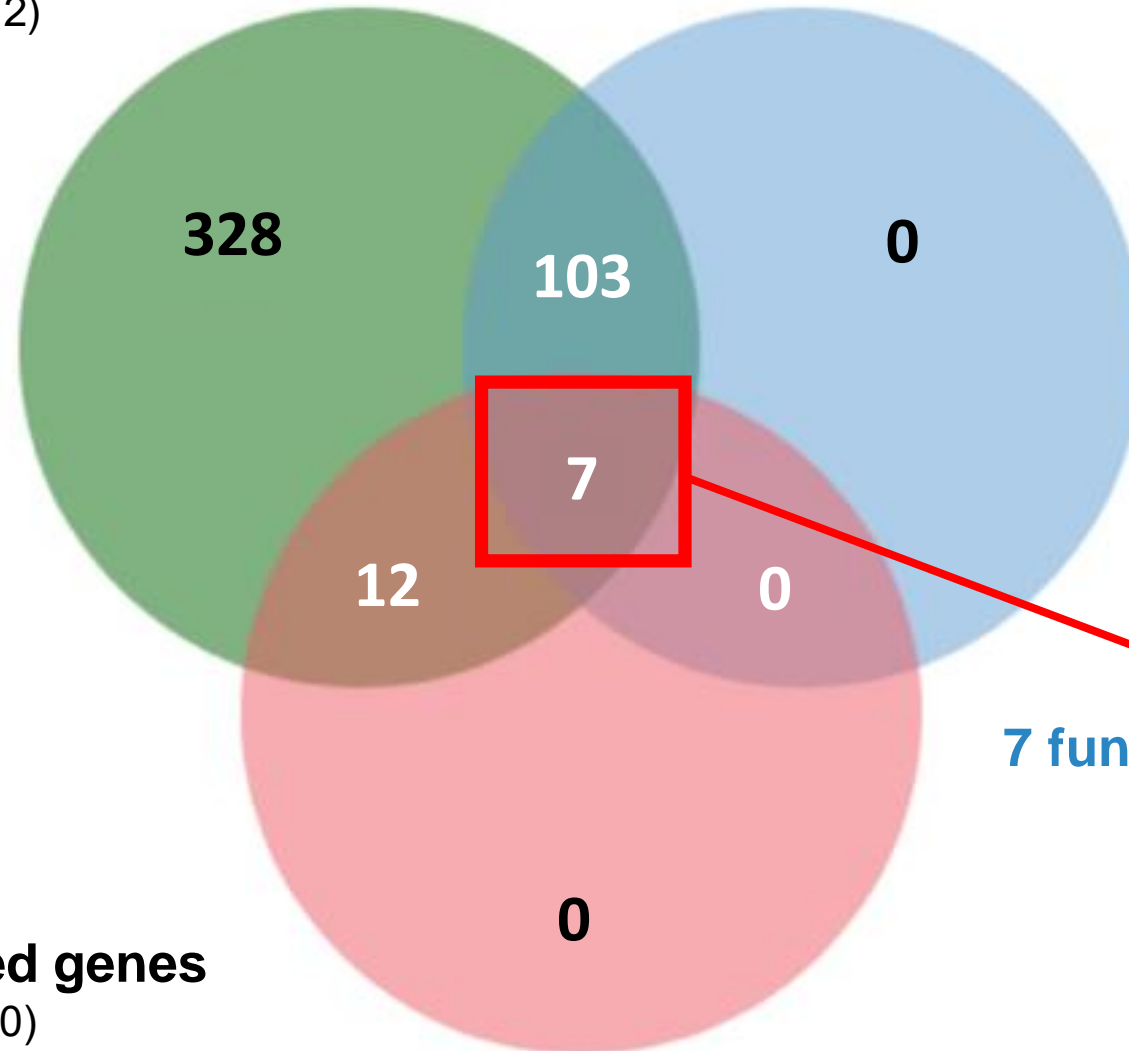
Functional Candidate Genes

Identification of Functional Candidate Genes

Differentially expressed genes
(P-value < 0.01, FDR < 0.05, FC > ± 2)
n=449

Metabolic pathways
(FDR < 0.05)
n=117

Highly expressed genes
(RPKM ≥ 500)
n=19



7 functional candidate genes identified

List of Functional Candidate Genes

- List of **7 functional candidate genes** among the overlapping criteria

Gene	P-value	FDR	Fold Change	Healthy RPKM	Mastitic RPKM
<i>BoLA DR-Alpha</i>	1.51E-04	0.0142	6.404	157.16	926.42
<i>B2M</i>	1.92E-04	0.0164	2.615	1,213.07	2,707.40
<i>CD74</i>	5.72E-04	0.0370	4.107	289.93	1,029.08
<i>FCER1G</i>	1.80E-05	3.75E-03	3.432	221.18	546.31
<i>GLYCAM1</i>	1.87E-08	1.15E-04	-55.59	3,444.66	110.34
<i>NFKBIA</i>	1.42E-04	0.0136	3.38	212.31	570.08
<i>SDS</i>	1.40E-04	0.0136	6.27	182.72	905.28

Conclusions

- **449 genes were differentially expressed** (DE) between healthy and mastitic bovine milk somatic cell samples
- **19 highly DE genes** were identified in healthy and mastitic groups, the highly expressed genes in the healthy group were associated with **milk components**, while in the mastitic group they were associated with **immunity**
- **55 significant metabolic pathways** were significantly enriched in the list of DE genes, including: **cell adhesion molecule pathway** and the **cytokine-cytokine interaction pathway**
- **7 functional candidate** genes identified with potential relevance to immunity or mastitis resistance (*BoLA – DR Alpha, B2M, CD74, FCER1G, GLYCAM1, NFKBIA and SDS*)



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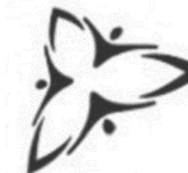


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Thank you!

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