



Differential expression of bovine mRNA isoforms with functional consequence on mastitis host defense

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Ghent, Belgium
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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
- Two forms of mastitis:
 1. Clinical mastitis - diagnosed by visual cues
 - On average, costs producers **\$170 USD per cow**
 2. Subclinical mastitis - no clinically detectable abnormalities
 - High somatic cell count (**>150,000 cells/mL**) impacts the quality and hygiene of the milk

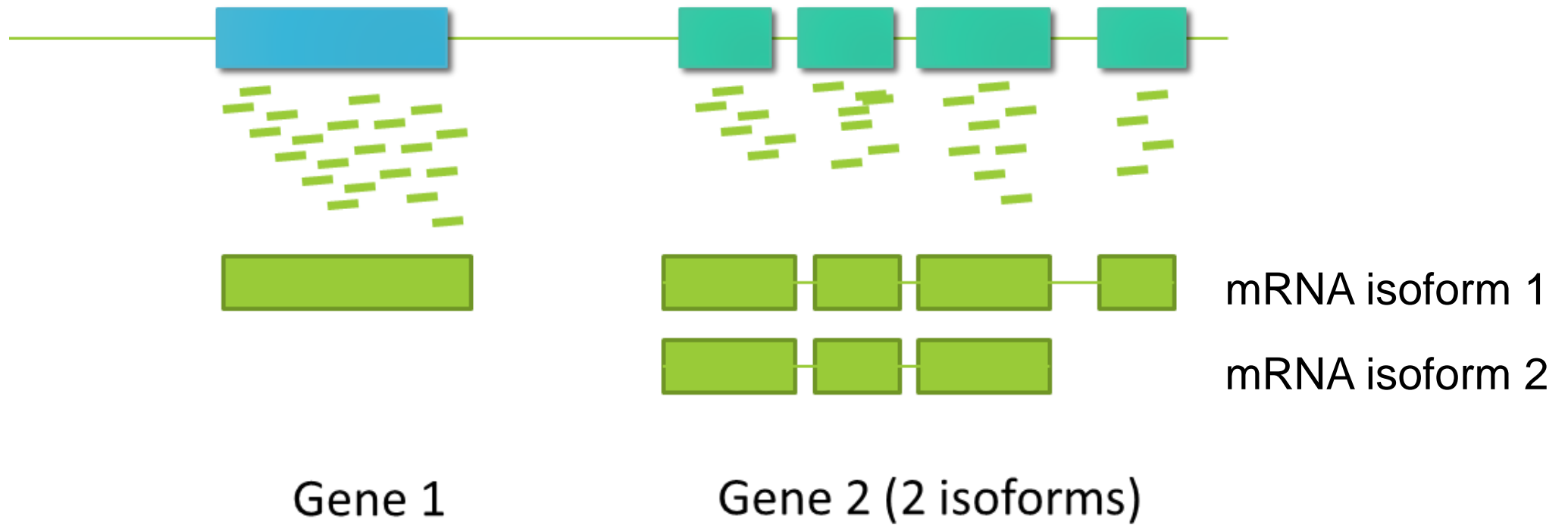
Transcriptomics and RNA-Sequencing

- 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**
- RNA-Sequencing technology allows us to study the transcriptome at a **high-throughput** level
 - Gene expression, mRNA isoform expression
 - Genetic variants (SNP, indel and splice variants)



mRNA Isoforms

What is an mRNA isoform?



Objective

To identify mRNA Isoforms that are differentially expressed (DE) and have functional consequences on host defense to mastitis

Identify mRNA isoform DE between healthy and mastitic quarters



Identify DE mRNA isoforms highly involved in immune processes



Generate a functional candidate mRNA isoform list



Identify genetic variants within candidate mRNA isoforms (SNP, indels)



Materials and Methods

Sample Collection

SCIENTIFIC REPORTS

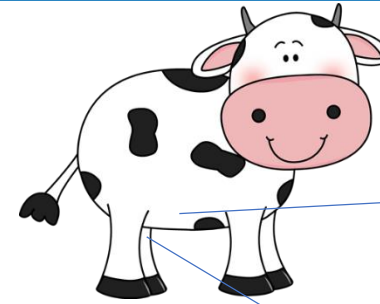
OPEN Comparison of five different RNA sources to examine the lactating bovine mammary gland transcriptome using RNA-Sequencing

SUBJECT AREAS:
RNA SEQUENCING
GENE EXPRESSION

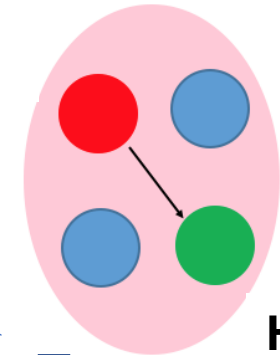
Received
10 October 2013

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23 April 2014

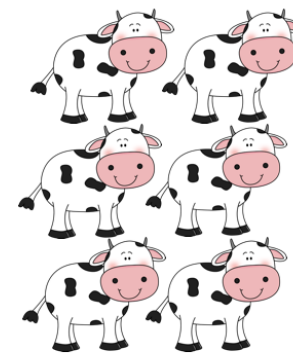
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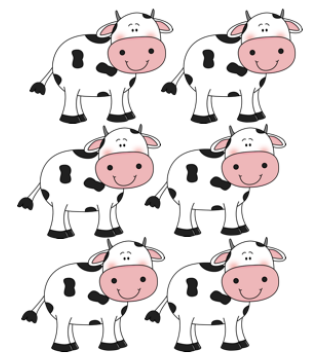
Mastitic quarter



Healthy quarter



Healthy Quarter



Mastitic Quarter

VS

RNA-Sequencing

- Six Holstein dairy cows, **natural cases** of mastitis
- Two samples collected from each cow (n=12)
 - Mastitic quarter** (n=6)
 - Healthy quarter** (n=6)
- **Milk somatic cells** used to investigate the transcriptome in the bovine mammary gland

RNA-Sequencing

RNA extraction from bovine milk SC (n=12)



Library preparation and sequencing



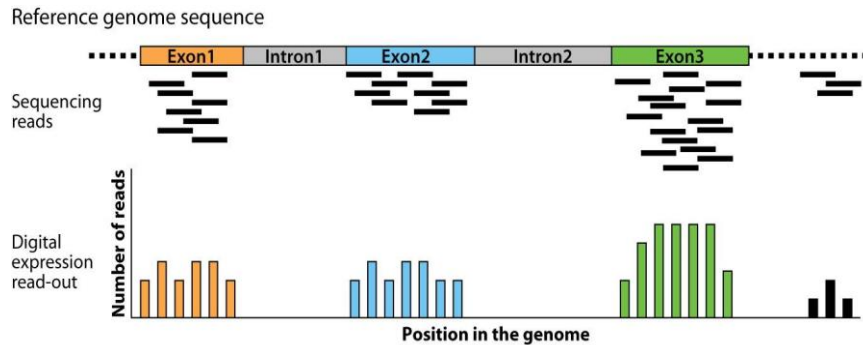
Illumina HiSeq 2000 analyzer

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AAACCTACTGTTTCTTCTTCTTCTTAAAGTTTA 16950 1 chr2:113004913 I
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ATTGCGCTTTTACCTCTACAGAGGCGCCAGAA 18000 1 chr2:150244 F ADI
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ATTGCAAGATATATATATGCAATATGTTCTA 16953 1 chr2:16982292 R
AAACGATTCCTTCCGATAGGCTTCCGATTA 16953 1 chr2:17320 F AAM
```

On average ≥ 26 million / per sample

Millions of reads

Mapping to reference genome



Assembly to reference genome
Bovine reference genomes (UMD3.1 and ARS.UCD1.2)

Transformed and normalized the data

mRNA isoform expression
(reads per kilo base per million mapped reads (RPKM) ≥ 0.2)

Differentially expressed mRNA isoform
healthy X mastitic
(P-value < 0.01 , FDR < 0.05 , FC $> \pm 2$)

Functional analysis
(metabolic pathways and gene network analysis)



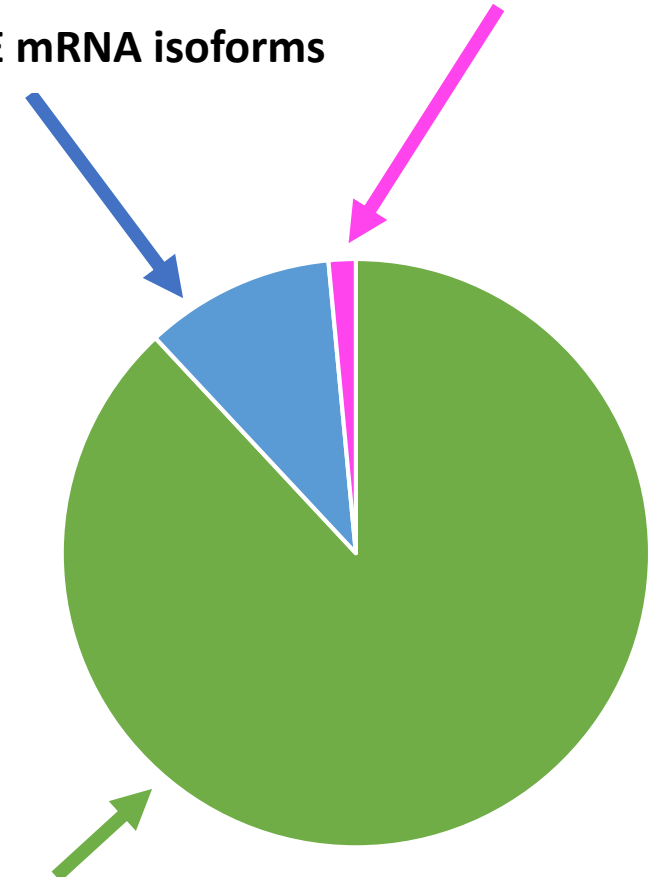
Results and Discussion

Differentially Expressed mRNA Isoforms

- **22,104** transcripts expressed in the bovine milk somatic cell transcriptome (release 90: 26,740 genes)
- Differential mRNA isoform expression analysis
 - **515 DE mRNA isoforms** between the healthy and mastitic samples, corresponding to **502 genes**
 - **67 genes** had two or more mRNA isoforms, with at least one being DE (**80 DE mRNA isoforms**)

11 genes with 2 DE mRNA isoforms

1 gene with 3 DE mRNA isoforms

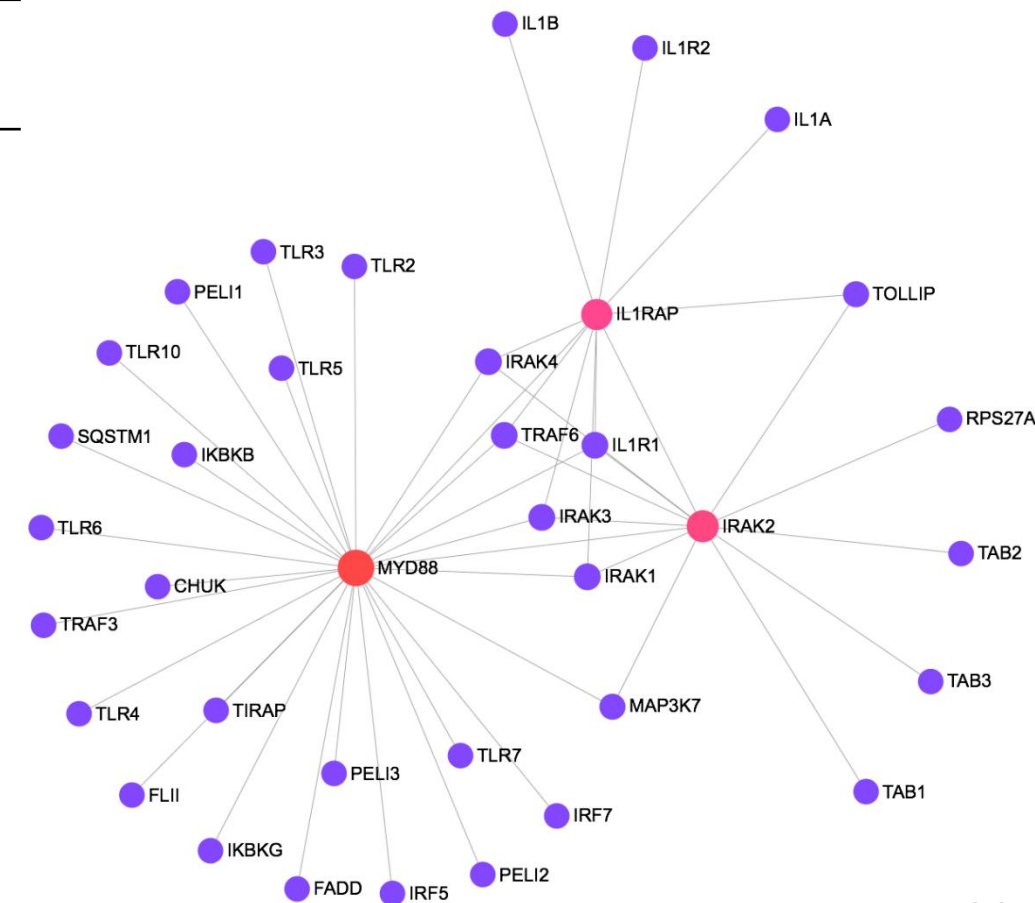


55 genes with 1 DE mRNA isoforms

Metabolic Pathways and Gene Network

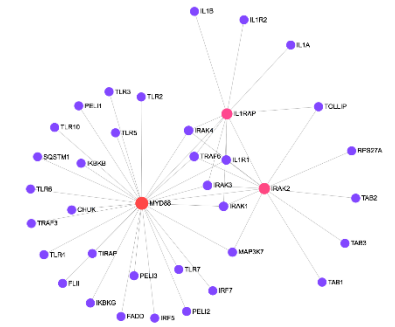
- Using the list of **80 DE mRNA isoforms**
 - 33 significant metabolic pathways** identified

Pathway	Number of DE mRNA isoforms	P-value	FDR
positive regulation of immune response	15	1.53E-20	1.02E-17
regulation of immune response	13	4.07E-19	1.35E-16
regulation of cytokine biosynthetic process	11	7.64E-06	0.000506
interleukin-1 secretion	2	0.00262	0.0965
response to other organism	1	0.019	0.485
positive regulation of cytokine biosynthetic process	1	0.0346	0.764



Functional Candidate mRNA Isoforms

- There are 80 DE mRNA isoforms within the gene networks
 - Three genes with mRNA isoforms from this list explain the **majority of the topology**



Associated gene name	mRNA isoform annotated	mRNA isoform	Length (bp)	P-value	Fold change	FDR	RPKM	
							Healthy	Mastitic
<i>MyD88</i>	2	mRNA isoform 1	2558	1.40E-04	3.56	0.0117	50.95	124.86
		mRNA isoform 2	2423	0.13561368	2.74	0.8482	0.43	1.11
<i>IL1RAP</i>	2	mRNA isoform 1	2064	2.05E-04	5.43	0.0151	4.17	15.61
		mRNA isoform 2	1921	1.56E-03	5.24	0.0600	10.06	36.92
<i>IRAK2</i>	2	mRNA isoform 1	2799	4.62E-04	3.68	0.0272	6.58	20.13
		mRNA isoform 2	1982	0.524189657	-2.28	1.0000	1.37	0.40

Mycoid differentiation-associated protein 88 (*MyD88*)

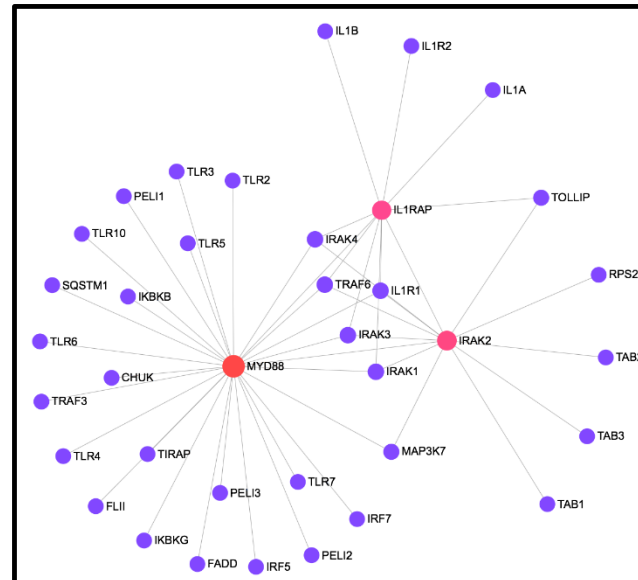
Responsible in the regulation of innate immune response (Schroeder et al., 1997; Beutler, 2004)

SNP Discovery within DE mRNA Isoforms

80 DE mRNA isoforms
(corresponding to 67 genes)



59 mRNA isoforms
with variants fixed in
healthy and mastitic
groups



2 mRNA isoforms with
fixed variants

- MyD88
- IRAK2



Amino Acid change
caused by:

- SNP (n= 9)
- Indel (n=4)

Mamm Genome (2010) 21:592-598
DOI 10.1007/s00335-010-9297-z

SNP discovery in the bovine milk transcriptome using RNA-Seq technology

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Alma Islas-Trejo · Saumya Wickramasinghe ·
Juan F. Medrano

SNP Discovery within DE Genes



J. Dairy Sci. TBC:1–17
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Genetic mechanisms regulating the host response during mastitis

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7 DE genes



2 DE genes with
fixed variants in
healthy and mastitic
groups

- SDS
- NFKBIA



Amino Acid change
caused by:

- SNP (n=32)
- Indel (n=12)

Conclusions

- In total **80 DE mRNA isoforms**, corresponding to **67 genes**, were significantly identified between healthy and mastitic samples
- Using the list of 80 DE mRNA isoforms, **33 significant metabolic pathways** with functional relevance were identified, including *IL-1* secretion and response to other organisms
- **3 genes (*MyD88*, *IRAK2* and *IL1RAP*)** with at least two mRNA isoforms, with at least one being DE were identified integrating structural and functional genomic data, adding them to the candidate gene list
- **Putative functional variants** were identified in candidate mRNA isoforms, but these need to be further validated

Future Direction

- Alignment statistics comparison between bovine reference genomes (**UCD3.1** and **ARS.UCD1.2**)
 - We expect the increased percentage of mapped reads will result in better identification of DE genes, DE mRNA isoforms and variants

Group	Sample ID	Total reads mapped	UMD3.1			ARS-UCD2.1		
			Uniquely mapped %	Non-specifically mapped %	Unmapped %	Uniquely mapped %	Non-specifically mapped %	Unmapped %
Healthy	50A	25,759,763	80.05	4.26	15.69	87.79	4.85	7.36
	50C	15,164,354	66.14	7.22	26.64	75.41	8.59	16.01
	50E	13,312,747	66.91	4.27	28.83	78.41	5.08	16.51
	50G	11,364,349	51.08	3.03	45.89	67.33	4.15	28.52
	70A	18,248,489	73.65	20.30	6.05	86.51	12.28	1.21
	70E	24,674,327	90.58	3.90	3.90	94.39	4.48	1.13
	Total	108,524,029	74.78	7.53	17.69	81.64	6.57	11.79
Mastitic	50B	20,917,517	86.83	4.63	8.53	91.17	5.07	3.76
	50D	28,919,278	89.00	6.20	4.81	91.83	6.41	1.75
	50F	16,394,480	81.82	4.89	13.29	87.66	5.47	6.87
	50H	16,970,255	81.03	6.02	12.95	87.49	6.56	5.95
	70C	16,254,989	91.15	4.70	4.14	94.51	4.34	1.15
	70G	18,846,584	92.01	4.70	3.30	95.01	4.10	0.89
	Total	118,303,103	87.25	5.27	7.48	91.28	5.33	3.40



Acknowledgements

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Collaborators

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