

Investigation of pleiotropic effects influencing feed efficiency and immunity through the study of milk somatic cell transcriptome in Holstein dairy cows

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Introduction



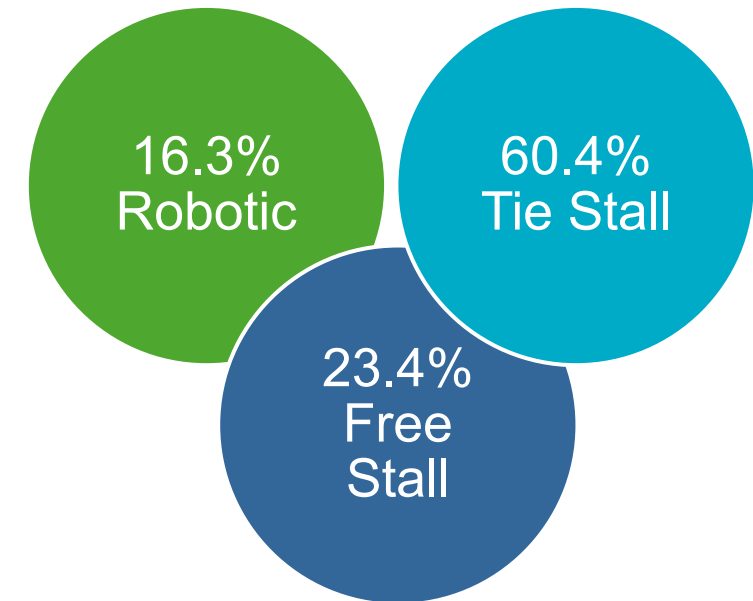
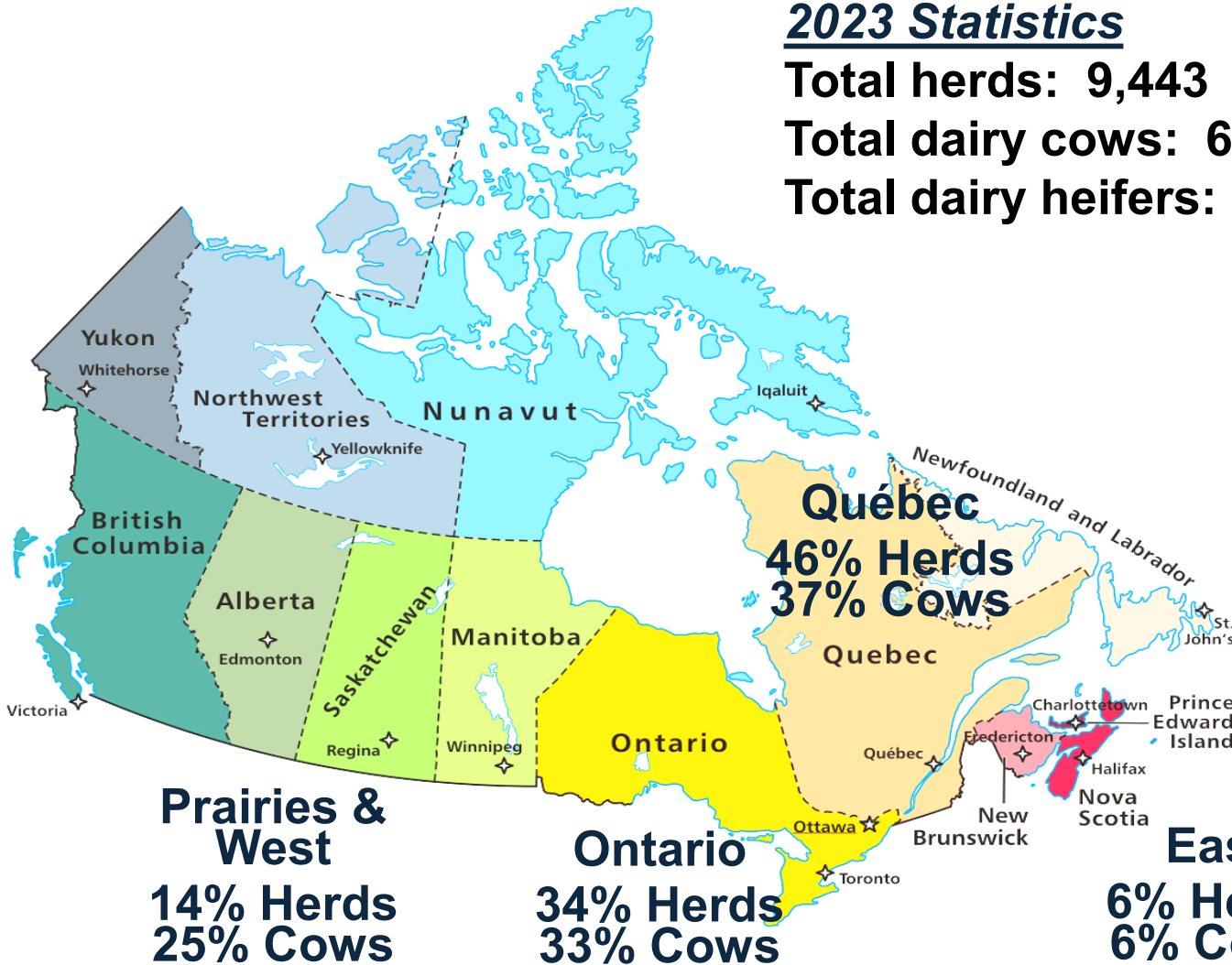
Canadian Dairy Industry

2023 Statistics

Total herds: 9,443

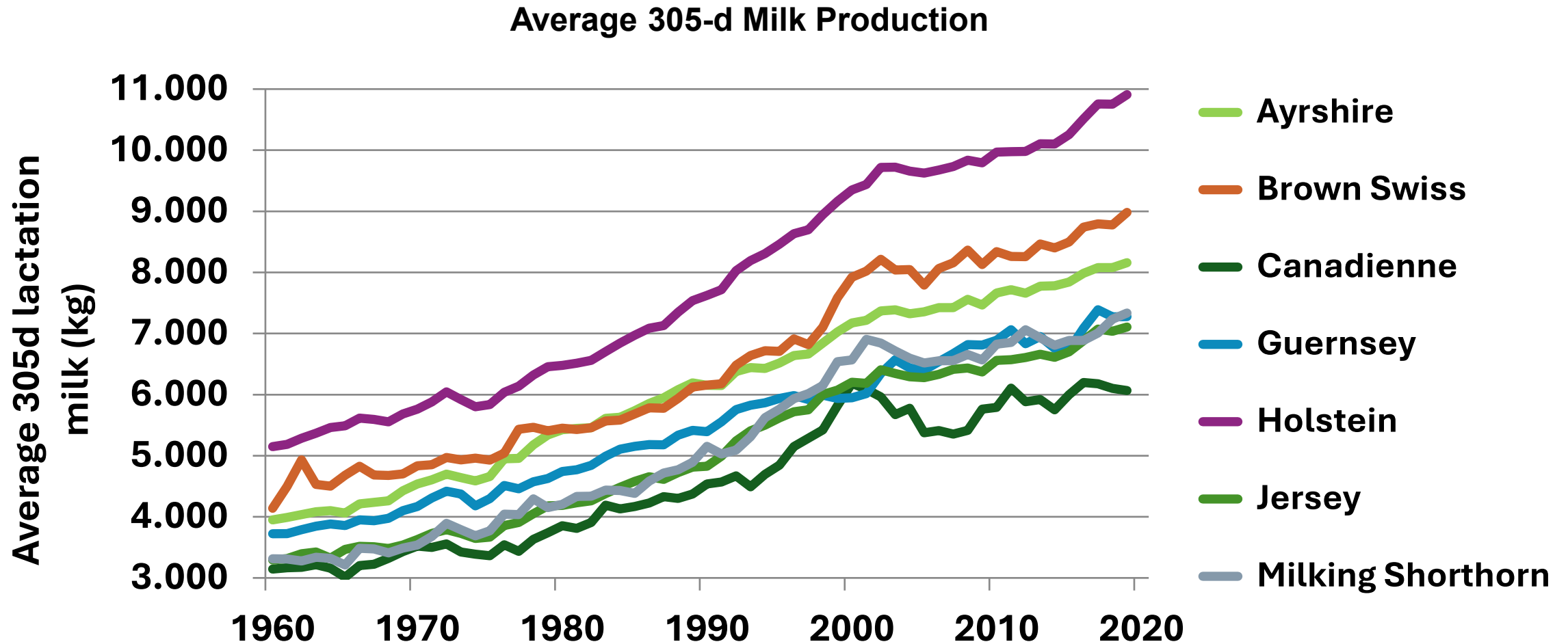
Total dairy cows: 69,500

Total dairy heifers: 412,500



***Many regional differences**

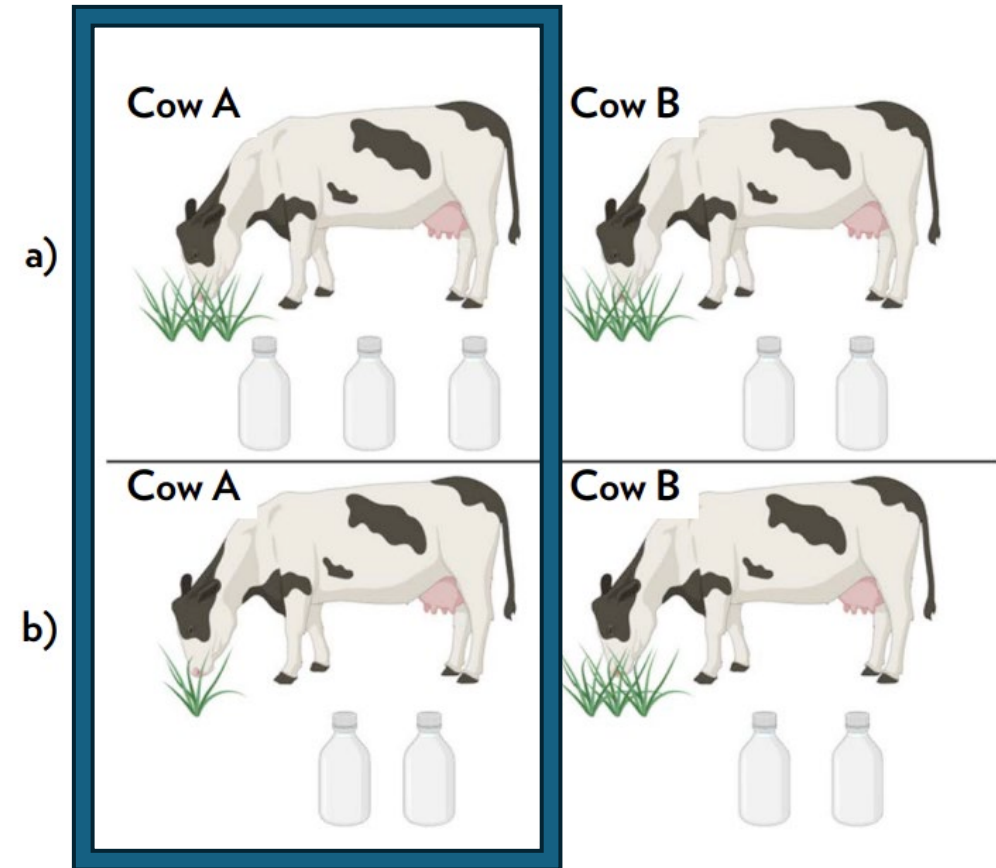
Genetic Improvement in Canadian Dairy Cattle



- Since 1970, dairy farms and cows have reduced by 1/3
- All breeds have doubled their lactation yields in 50 years

Feed Costs and Feed Efficiency

- Feed accounts for the largest expense on dairy farms (**43% total cost** on Canadian dairy farms)
- Improving the feed efficiency (**FE**) of herds, cows need to **convert feed into milk** → very energy demanding process
- Residual feed intake (**RFI**; kg/d) can be used to identify cows that have superior **FE**
RFI = actual feed intake – expected feed requirements
- Feed efficiency is a pleiotropic trait
 - Genes associated with FE might **impact other important traits**, including immunity, reproduction and production



Transcriptomics using RNA-Sequencing Technology

- Transcriptomics to measure gene expression in the **entire transcriptome** using **RNA-Sequencing** technology
- Gene transcription and regulation underlie many biological processes and **phenotypic variation**
- RNA-Sequencing technology allows us to study the transcriptome at a **high-throughput level**
 - Gene expression, mRNA isoforms / alternative splicing and other regulatory elements (i.e. lncRNA expression)
 - Structural variants (SNP, indel and splice variants)

Objective

Use RNA – Seq from **milk somatic cells** from Holstein dairy cows with extreme RFI phenotype to identify **functional candidate genes** with specific functions related to **FE** and potential **pleiotropic effect on immunity**.

Materials and Methods



Sample Collection



Population of 200 milking cows

Only first lactation cows in range of 60 – 150 DIM eligible

Low-RFI
n = 22

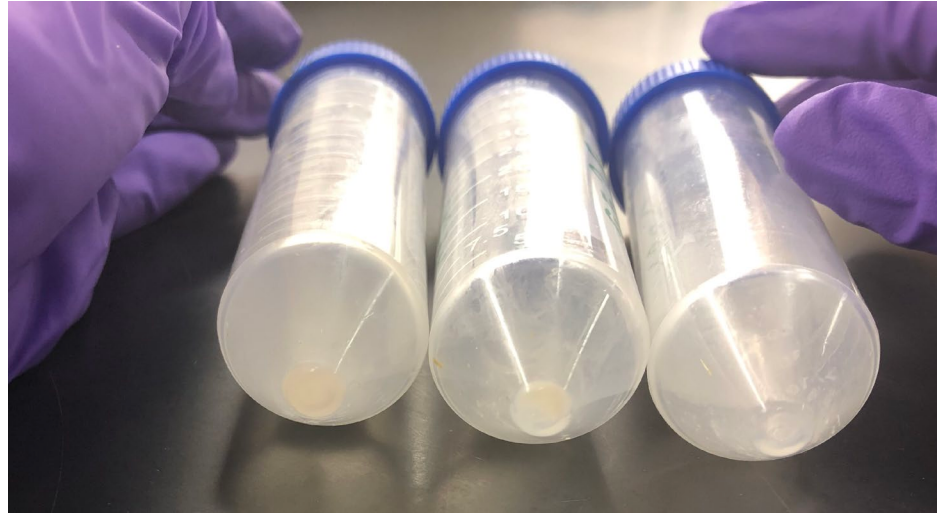
High-RFI
n = 17



Milk collected to extract RNA from milk somatic cells

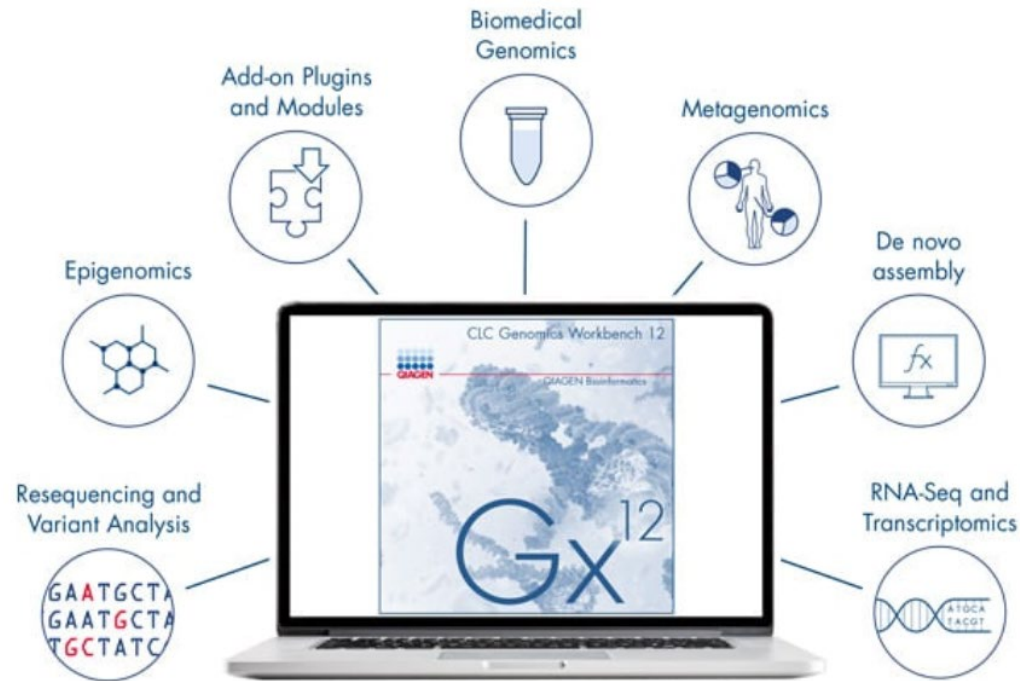
RNA Extraction and Sequencing

1. RNA extracted from 50mL **bovine milk somatic cells** (N = 39) Direct-zol RNA Miniprep Kits following the manufacturer's protocol



2. Library preparation and sequencing using the **Illumina NovaSeq 6000** analyzer
 - Yielded **150bp paired-end** sequences

RNA-Seq: Transcript Detection and Functional Analysis



Raw RNA-Seq data

Quality Control

Alignment to bovine reference genome ARS_UCD1.2.1

Transformed and Normalized data

Gene expression

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

Genes differentially expressed between High- and Low- RFI animals (FDR < 0.05, |Fold Change| > 2)

Functional analysis (gene ontology, metabolic pathways, QTL annotation)

Identification of functional candidate genes

Results and Discussion



Differential Gene Expression

Mapping Statistics

- **>92% of reads** uniquely mapped for both Low- and High- RFI animals

Differential Expression Analysis

- **36 genes were** DE between Low- and High- RFI animals

Categorizing the 36 DE genes between High – and Low - RFI animals based on RPKM* expression

Category	High - RFI genes (n)	Low - RFI genes (n)
Low expressed ^a	1	0
Medium expressed ^b	4	9
Highly expressed ^c	31	27

*RPKM = reads per kilobase per million mapped reads; ^a = < 10 RPKM; ^b = 10 to 500 RPKM; ^c = >500 RPKM

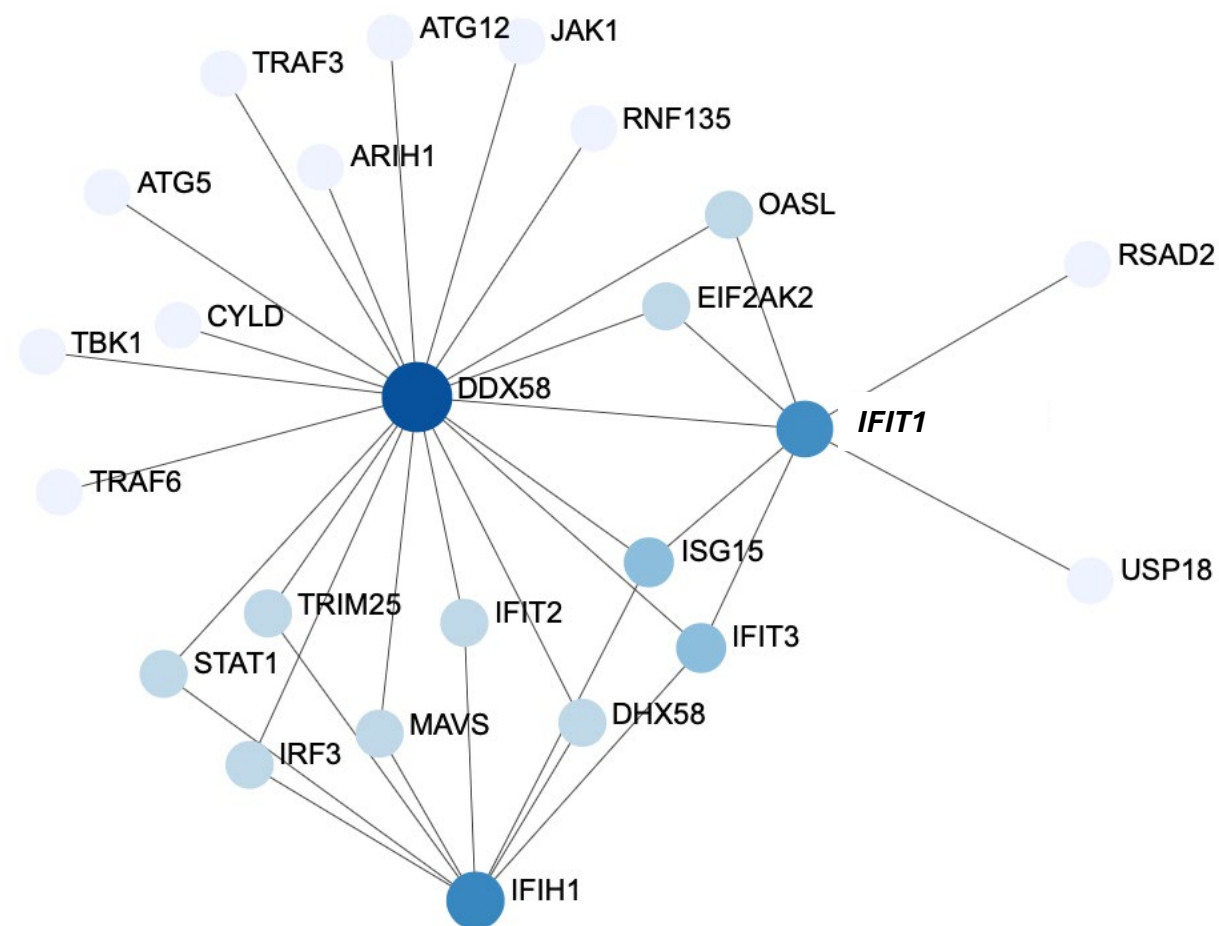
Functional Metabolic Pathway Analysis

- DE genes were **significantly enriched** in 29 metabolic pathways (FDR<0.01)
 - **Network 1** - 28 significantly enriched metabolic pathways, majority of genes associated with host immune response
 - **Network 2**, 1 significantly enriched pathway for hormone signaling

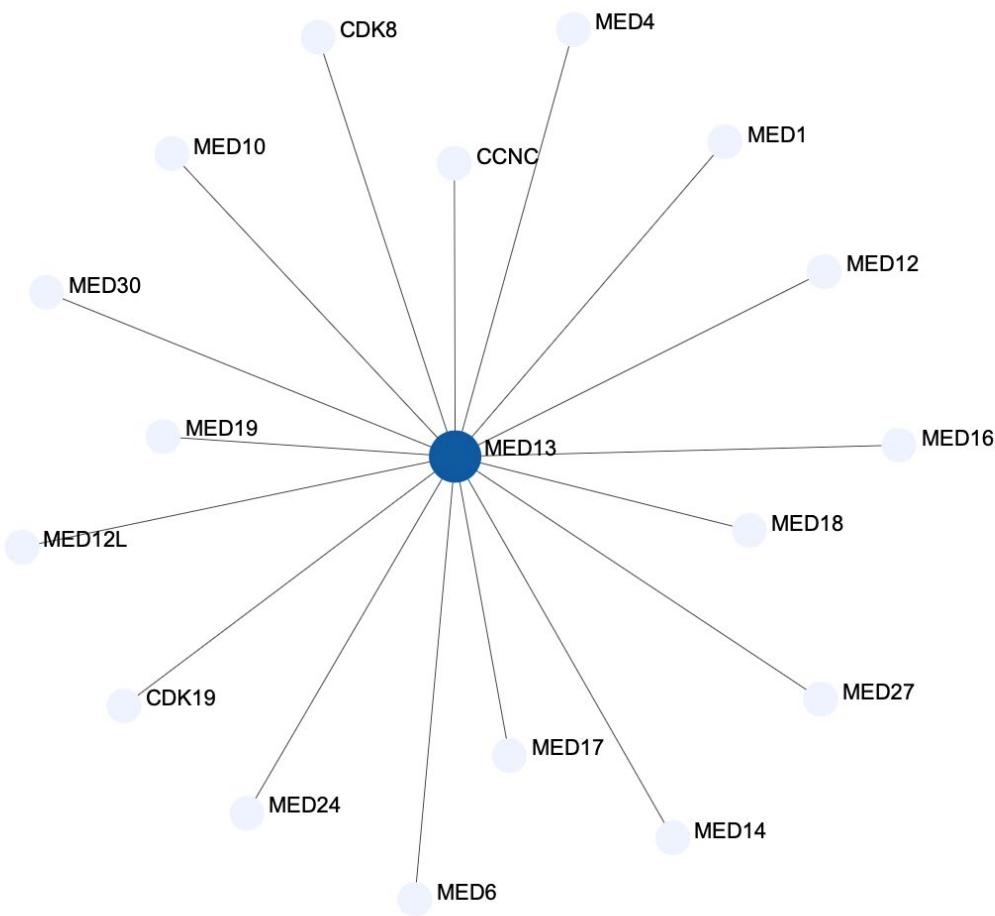
Network	Pathway	Number DE genes	FDR
1	RIG-1 like receptor signaling pathway	13	1.23E-19
	NOD-like receptor signaling pathway	9	1.35E-09
	NF-kappa B signaling pathway	5	564E-05
	Toll-like receptor signaling pathway	5	7.75E-05
	IL-17 signaling pathway	3	1.69E-02
2	Thyroid hormone signaling pathway	11	4.33E-19

Gene Network 1

Associated gene name	Fold change	FDR	RPKM	
			High – RFI	Low – RFI
<i>DDX58</i>	-2.43	4.92E-02	1411.35	648.86
<i>IFIH1</i>	-2.27	4.56E-02	2410.18	1157.77
<i>IFIT1</i>	-4.74	1.65E-02	3124.59	1007.86



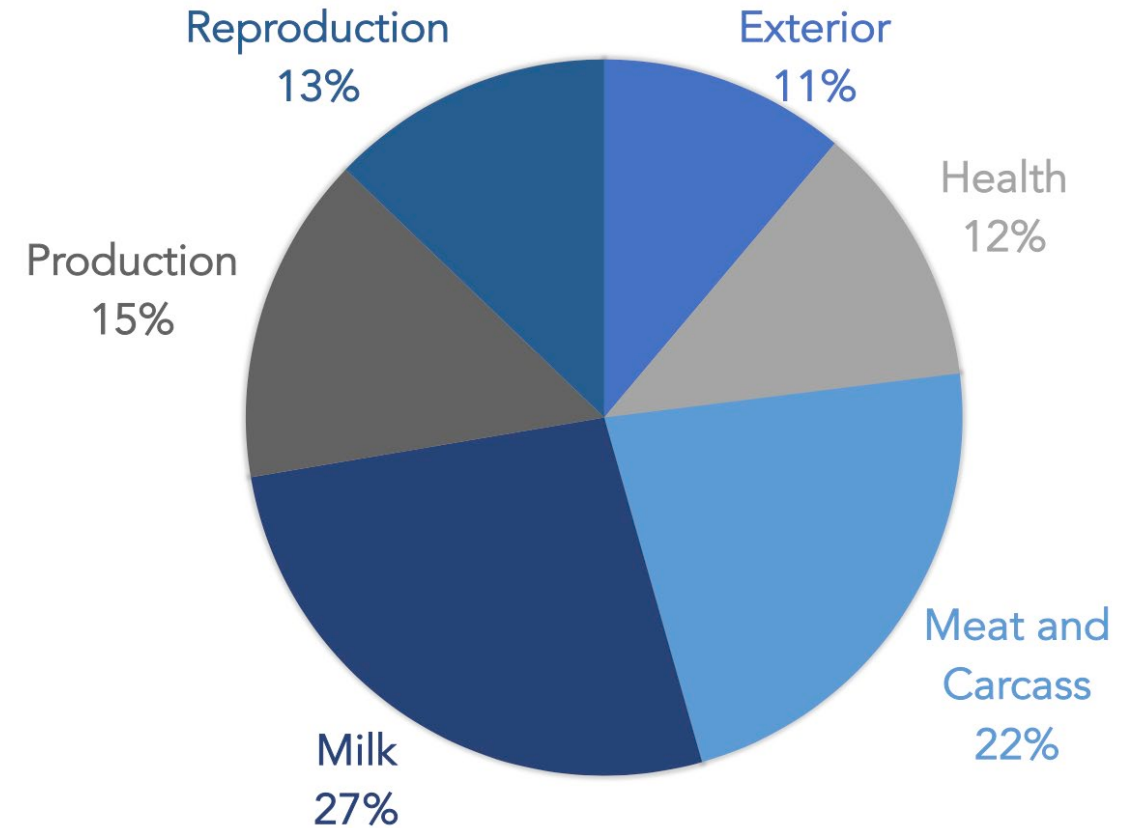
Gene Network 2



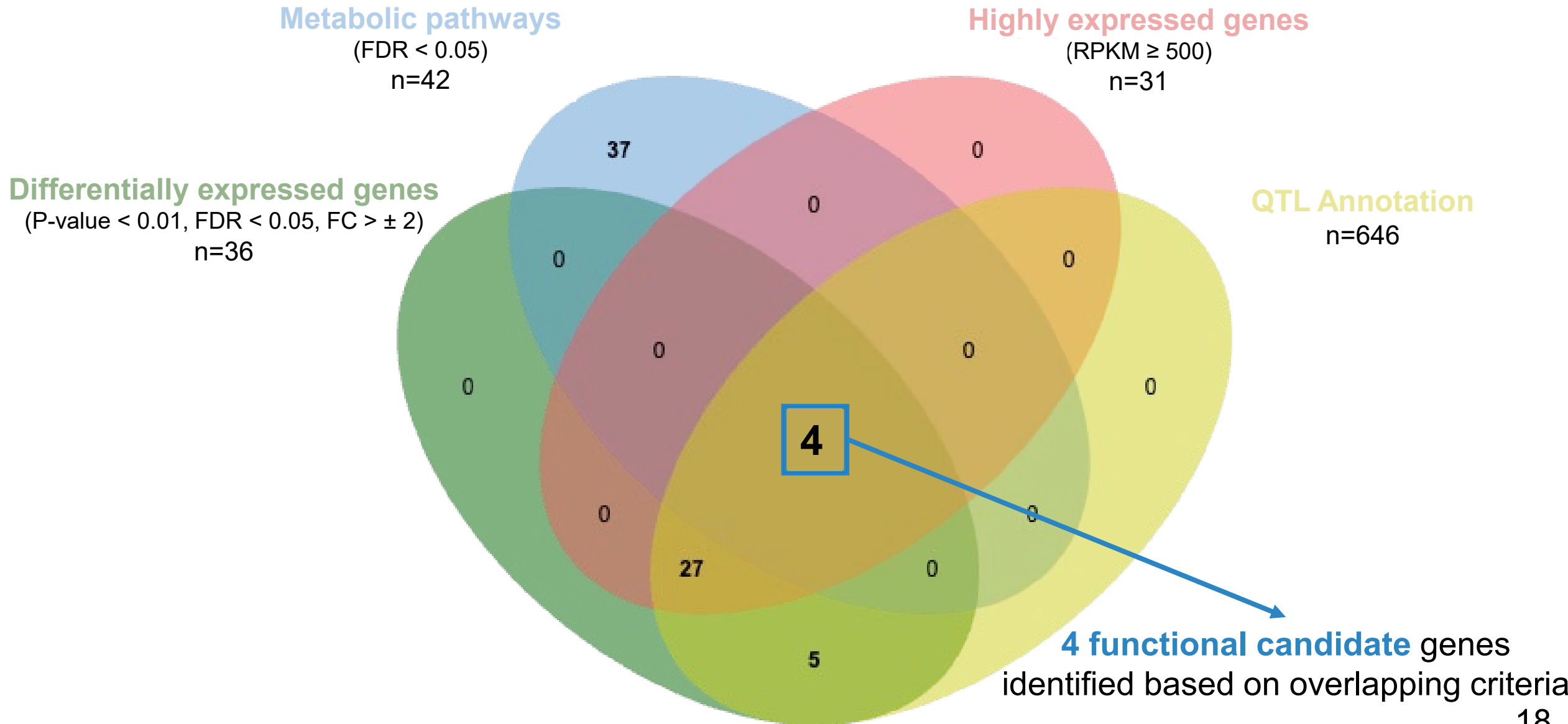
Associated gene name	Fold change	FDR	RPKM	
			High – RFI	Low – RFI
MED13	-2.25	4.56E-02	3944.00	1877.45

QTL Annotation

- Using the **genomic regions of the 36 DE genes** using the **GALLO** R package, 646 previously annotated QTLs
 - More QTLs annotated for economically important traits



Identification of Functional Candidate Genes



List of Functional Candidate Genes

List of **4 functional candidate genes** among the overlapping criteria

Gene	P-value	FDR	Fold Change	High- RFI RPKM	Low- RFI RPKM
<i>DDX58</i>	6.93E-05	4.92E-02	-2.43	1411.35	648.86
<i>IFIH1</i>	2.90E-05	4.56E-02	-2.27	2410.18	1157.77
<i>IFIT1</i>	3.05E-06	1.65E-02	-4.74	3124.59	1007.86
<i>MED13</i>	3.70E-05	4.56E-02	-2.25	3944.00	1877.45

DDX58— Part of glycoprotein interferon signaling complex, plays a role in antiviral responses, fat metabolism and feeding, dysregulated in obesity and insulin

Conclusions

- **36 genes were differentially expressed** (DE) between High- and Low- RFI milk somatic cells from Holstein dairy cows
- **31 unique highly DE genes** were identified in High- and Low-RFI groups, the majority of which were associated with immunity
- **29 significant metabolic pathways** were significantly enriched in the list of DE genes, including: toll like receptor and IL-17 signaling pathways
- **4 functional candidate genes** that may impact the variability in **feed efficiency** of Holstein dairy cows while also having a **pleiotropic effect on immunity** were identified

Acknowledgements



Acknowledgements



Thank You



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