

Do old genetics hold the future for mastitis susceptibility?



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Introduction



- Animal Scientist at National Animal Disease Center
 - Started April 2023
- Project: “Genomic and Mitigation Strategies to Control Mastitis”
- John Lippolis



Why Research Mastitis?

- **Costliest dairy cow disease - \$2 billion annually** (Sordillo and Streicher 2002)
 - Treatment cost, discarded milk, death and premature culling, decreased genetic advancement, and reduced milk quality
- **Single clinical case costs \$586** (Overton and Rollin 2014)
 - Treatment and labor costs
- **Subclinical mastitis another \$1 billion annually** (Ott 1999)
 - 70% of loss reduced milk production



Why Research Mastitis Mitigation?

- Antibiotic use for mastitis prevention and treatment is greater by mass than all other antibiotic use in dairy cows combined (Pol and Ruegg 2007a; Pol and Ruegg 2007b)
- 85.6% of cows diagnosed with mastitis were treated with antibiotics (USDA-APHIS 2016)

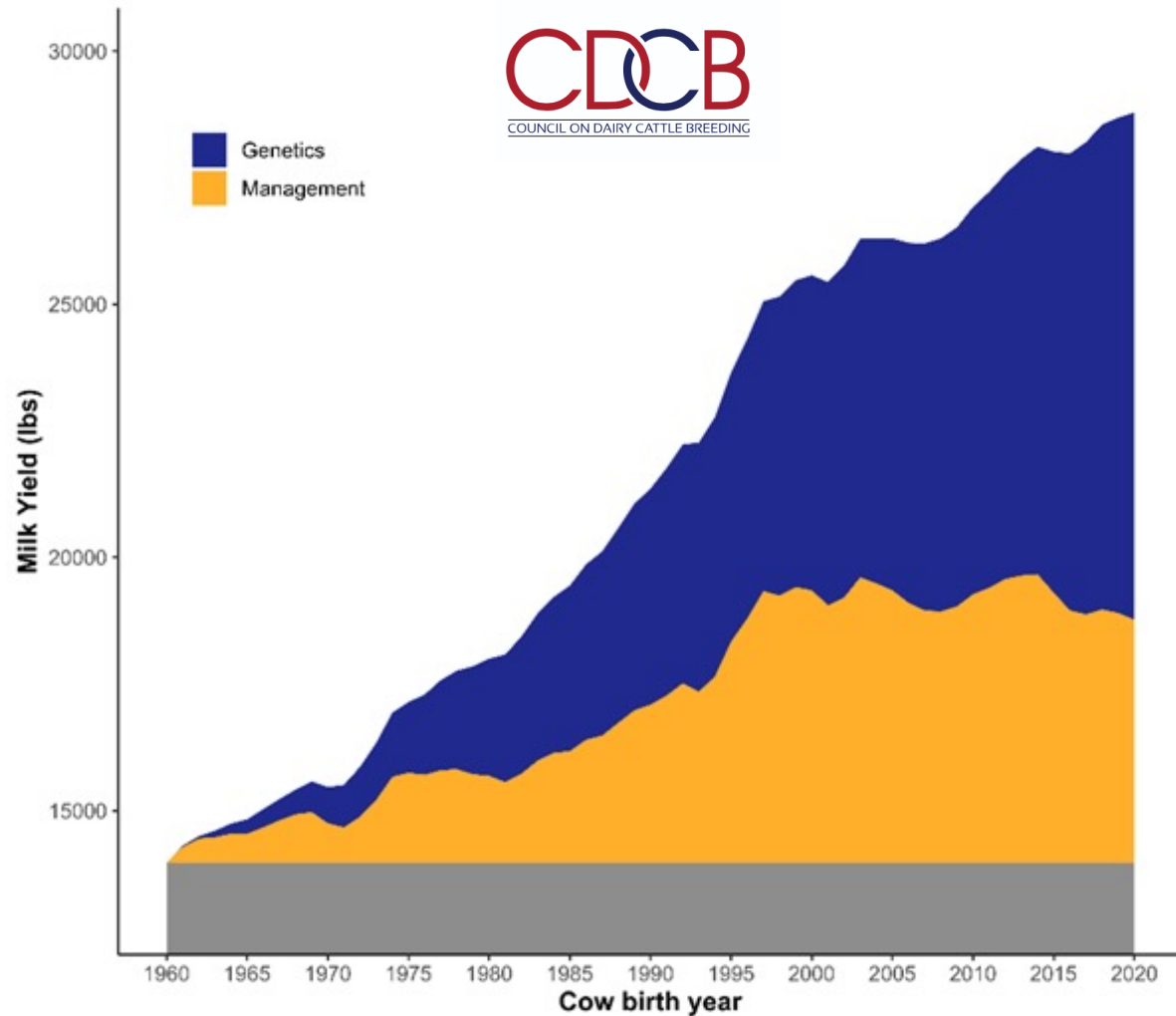


Old Genetics: Unselected Holstein

- Dr. Charles Young, University of Minnesota, established the unselected (UH, control) Holstein herd in 1964
 - 305d milk yield 4500 kg less than contemporary Holstein
 - Closed herd
 - Inbreeding < 6.25%



Unselected vs Contemporary Holsteins



Genome Changes

- Not just milk production
- Also, large immune gene clusters including MHC, T cells and immunoglobins

Research article | [Open access](#) | [Published: 11 February 2019](#)

Genome changes due to artificial selection in U.S. Holstein cattle

[Li Ma](#), [Tad S. Sonstegard](#), [John B. Cole](#), [Curtis P. VanTassell](#), [George R. Wiggans](#), [Brian A. Crooker](#), [Cheng Tan](#), [Dzianis Prakapenka](#), [George E. Liu](#) & [Yang Da](#) 

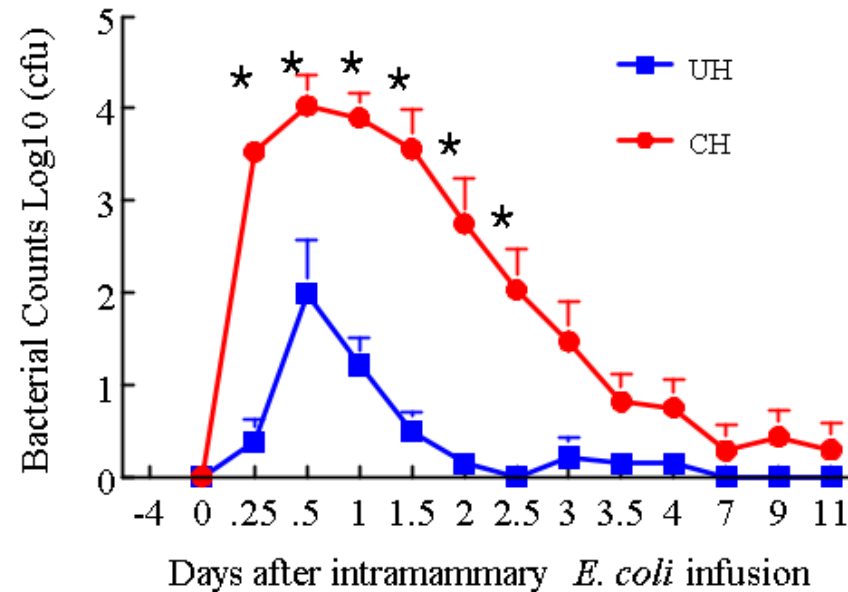
[BMC Genomics](#) **20**, Article number: 128 (2019) | [Cite this article](#)

5655 Accesses | 34 Citations | 12 Altmetric | [Metrics](#)



1964 Immune Response

DIFFERENTIAL RESPONSE TO INTRAMAMMARY *E. coli* CHALLENGE



Bacterial counts were greater and triggered greater systemic responses in CH cows while the infection remained mostly confined within the gland of UH cows.

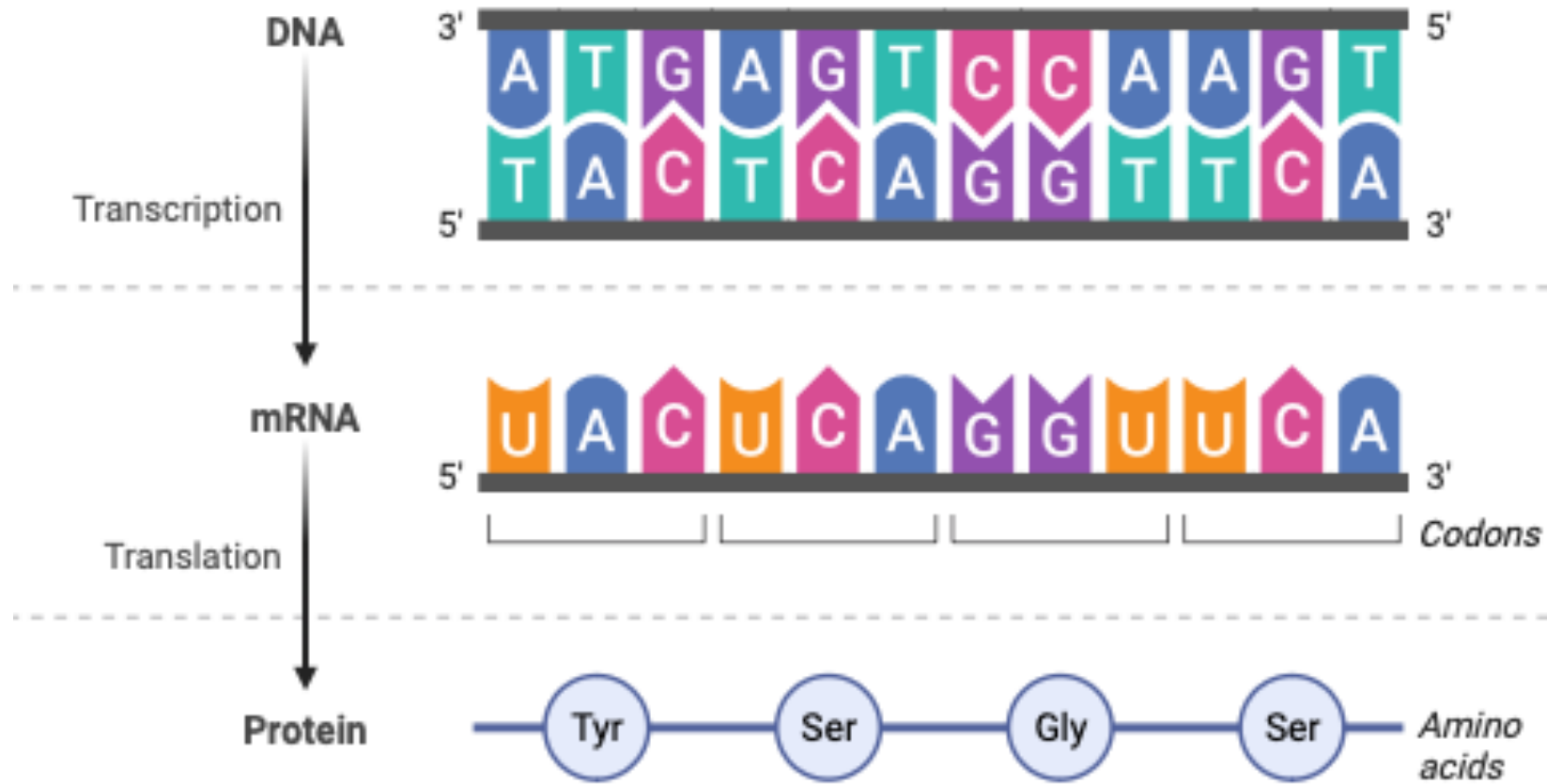
UH cows were more resistant to the *E. coli* mastitis challenge.

Questions to answer

- What it is that makes the 1964 herd mastitis resistant
- How do we get it into modern cows?



Gene Expression

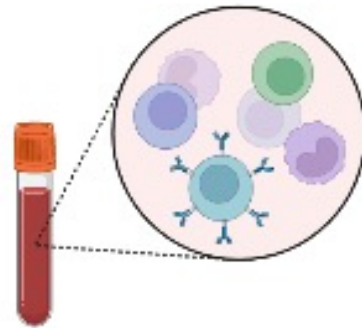
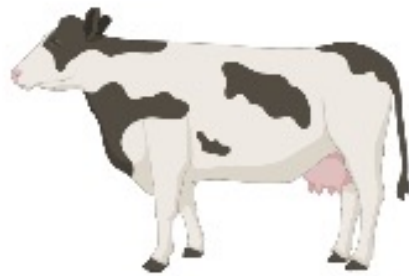


1964 Immune Response

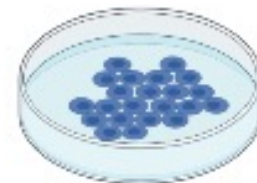
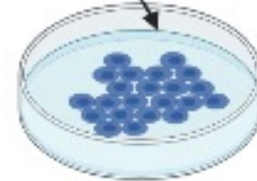
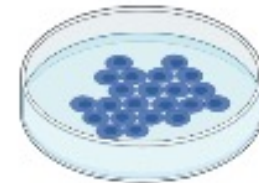
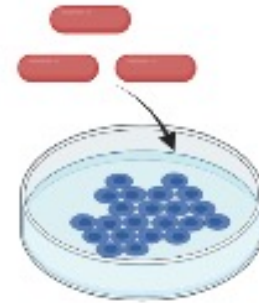
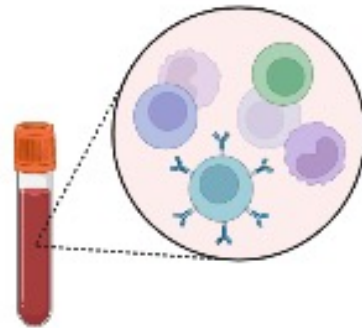
Since the mid 1960s the University of Minnesota has maintained a herd of Holsteins unselected for milk production



Blood was collected from four primiparous, lactating, unselected Holstein heifers and four primiparous, lactating, contemporary Holstein heifers



PBMCs were isolated from the blood



Total RNA was extracted from PBMCs after 24 hours with or without *Brucella abortus* strain RB51 stimulation

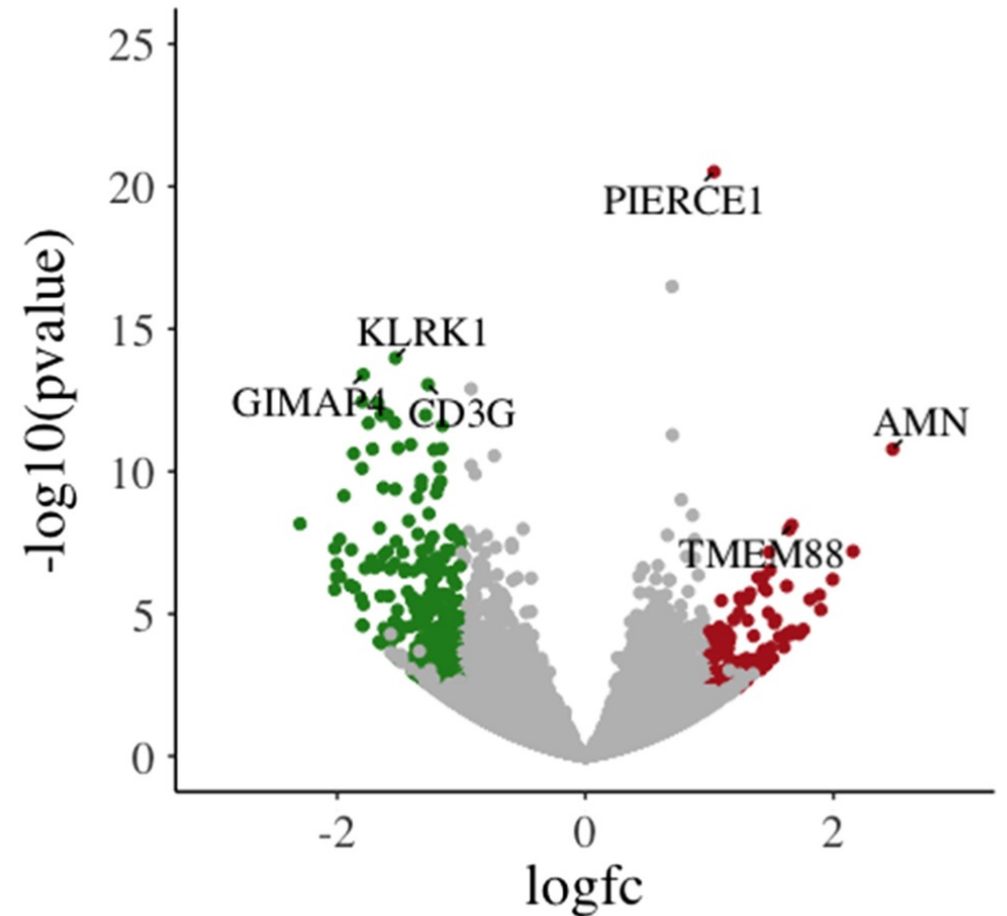


100 bp paired end reads were generated using the Illumina HiSeq 6000

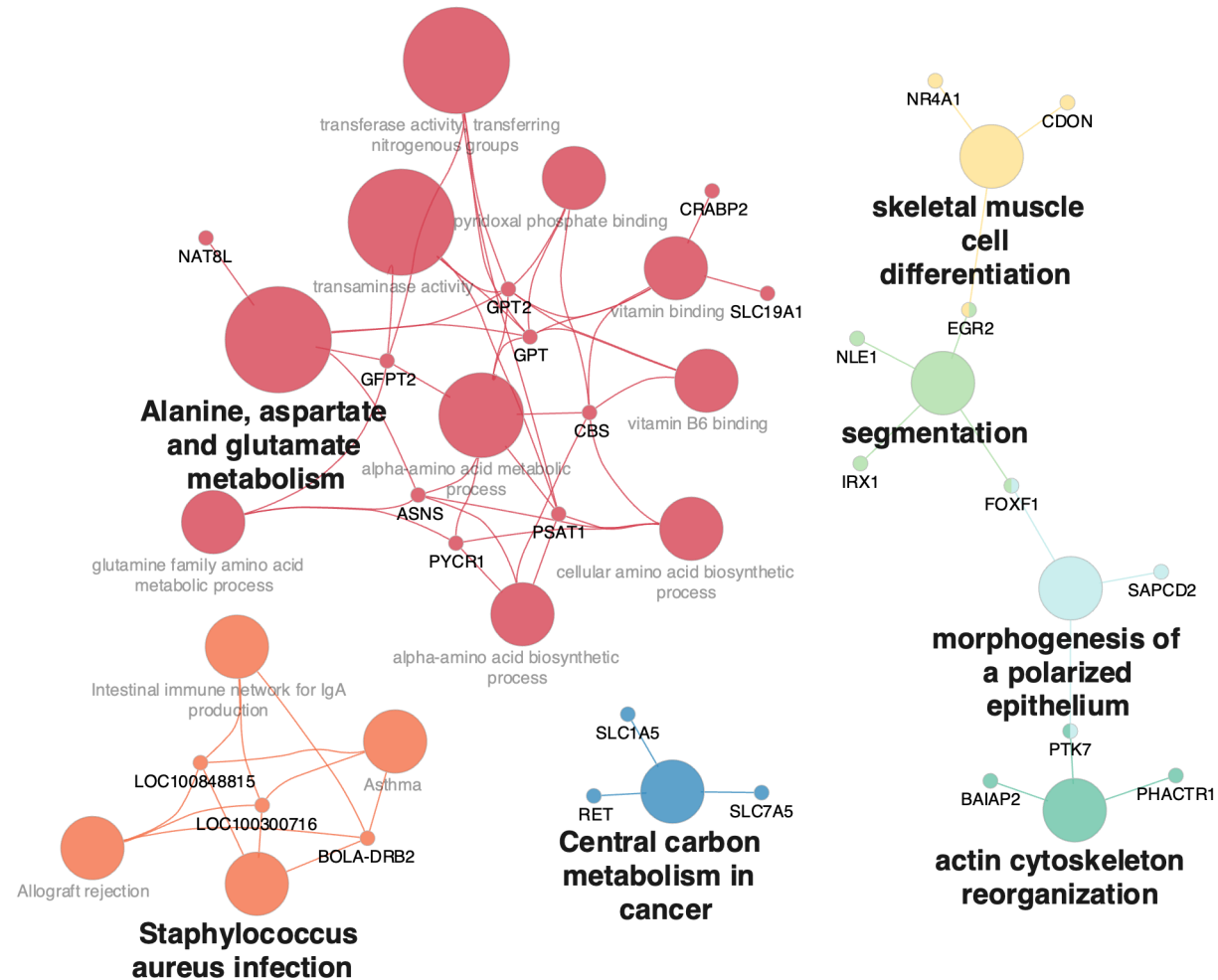


1964 Immune Response

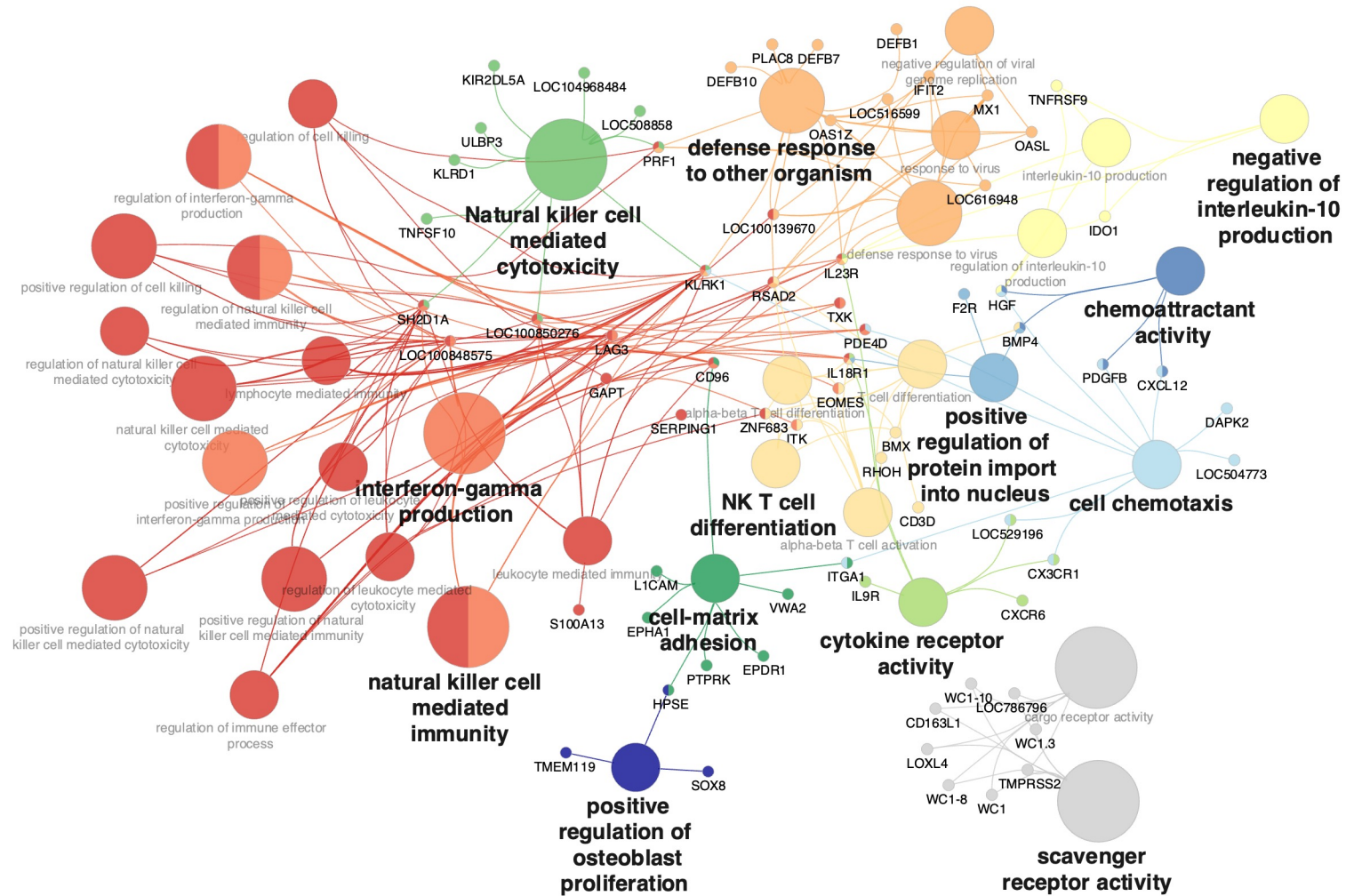
- 128 genes with higher expression in contemporary Holsteins (red)
- 284 genes with higher expression in unselected Holsteins (green)



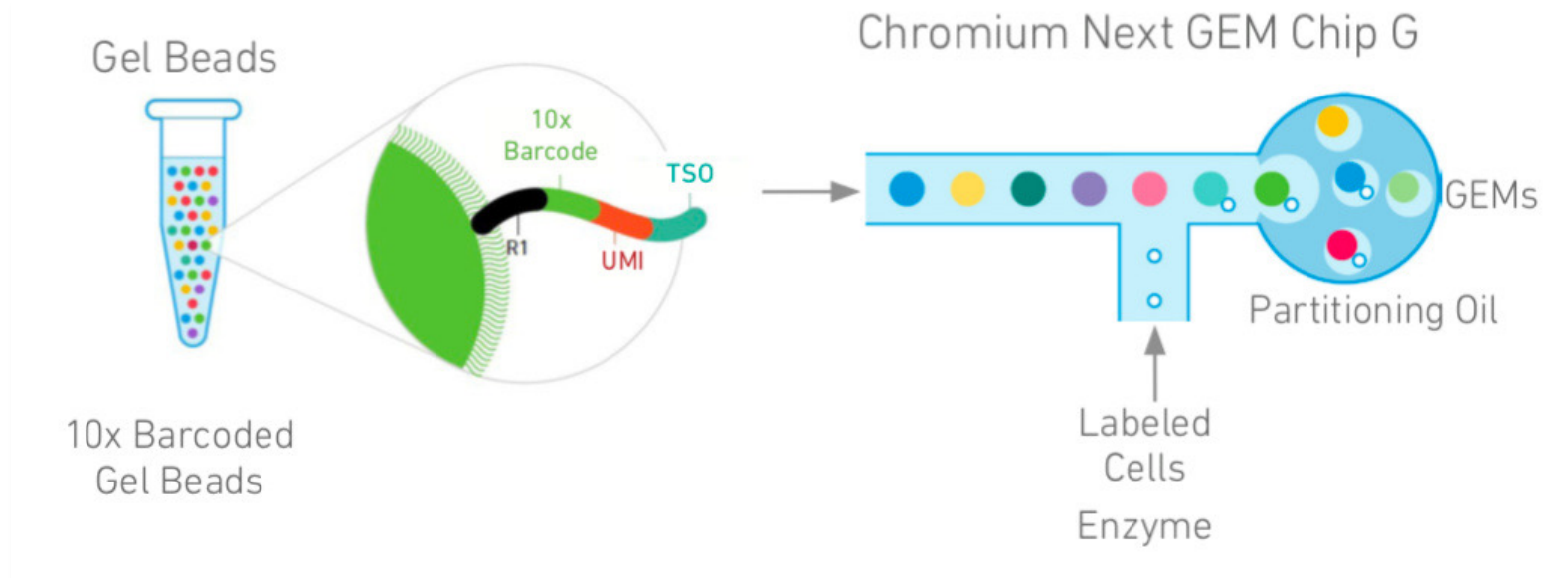
Contemporary Holsteins



Unselected Holsteins



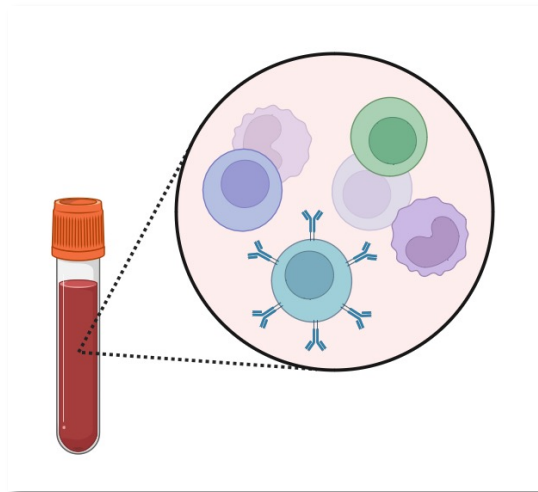
Bulk to Single Cell RNA



Single Cell RNA

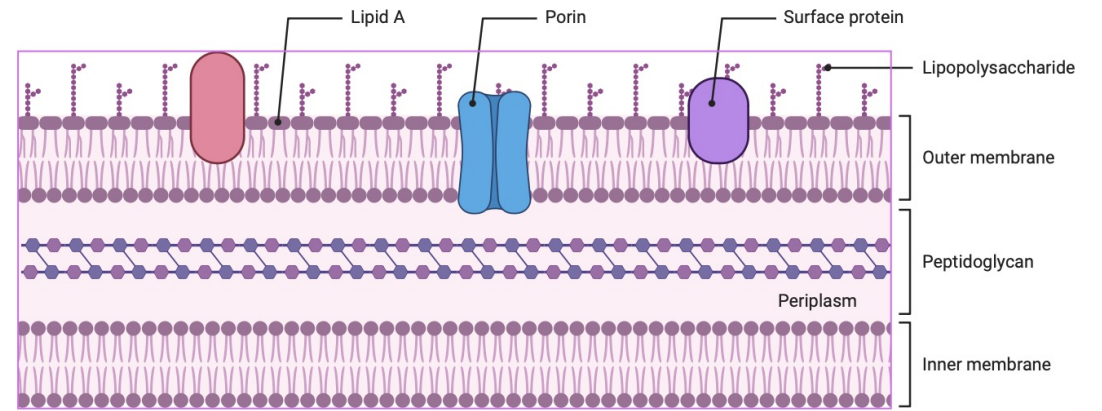
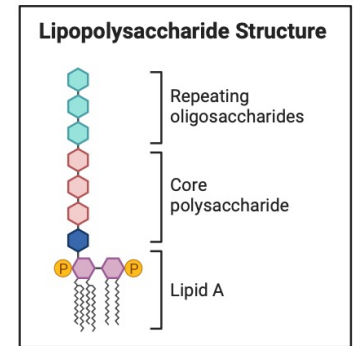
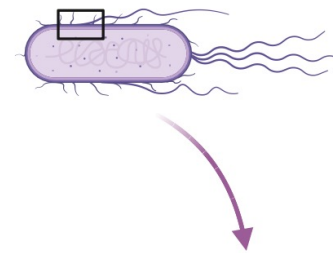
Blood was collected from three primiparous, lactating, unselected Holstein heifers and three primiparous, lactating, contemporary Holstein heifers

PBMCs were isolated from the blood

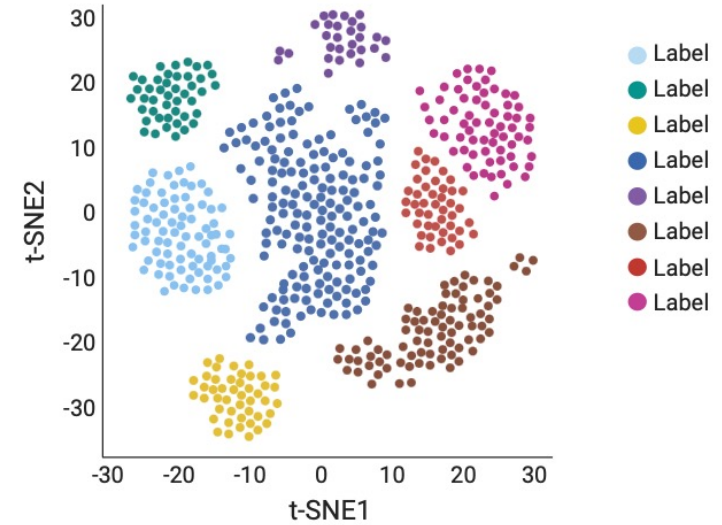
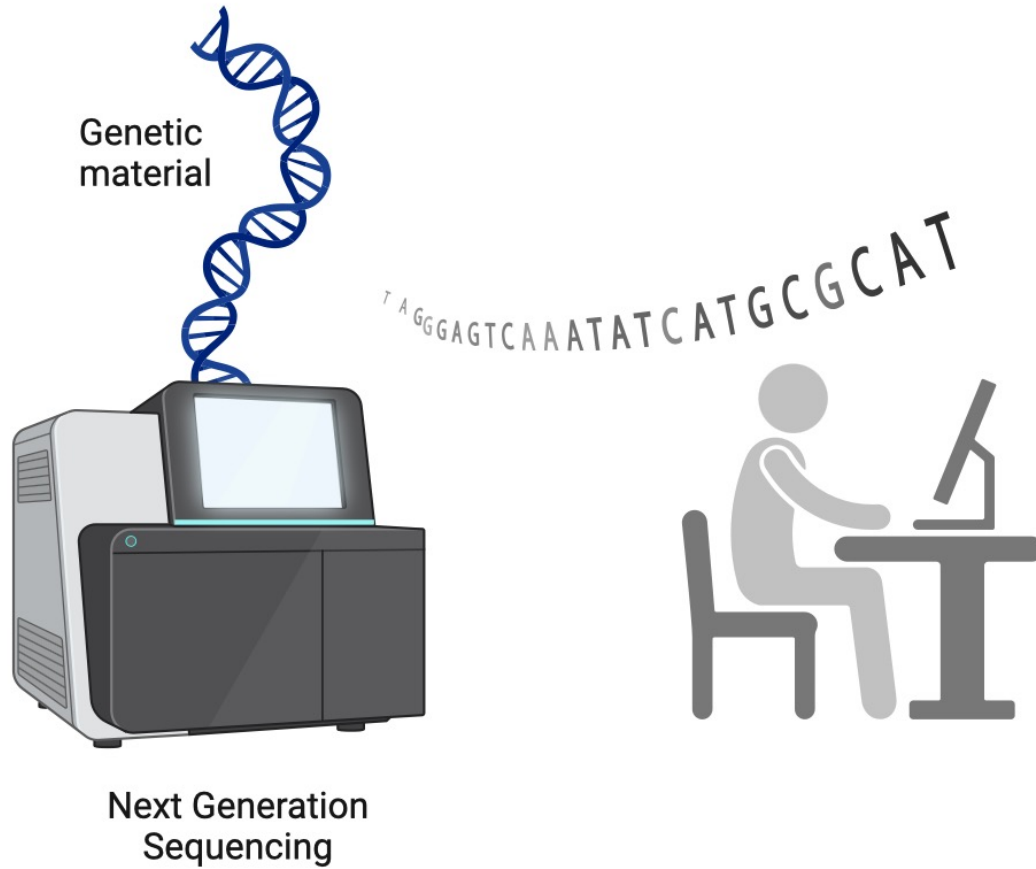


PBMCs were challenged with LPS for 2 hours

Gram negative bacteria cell wall structure

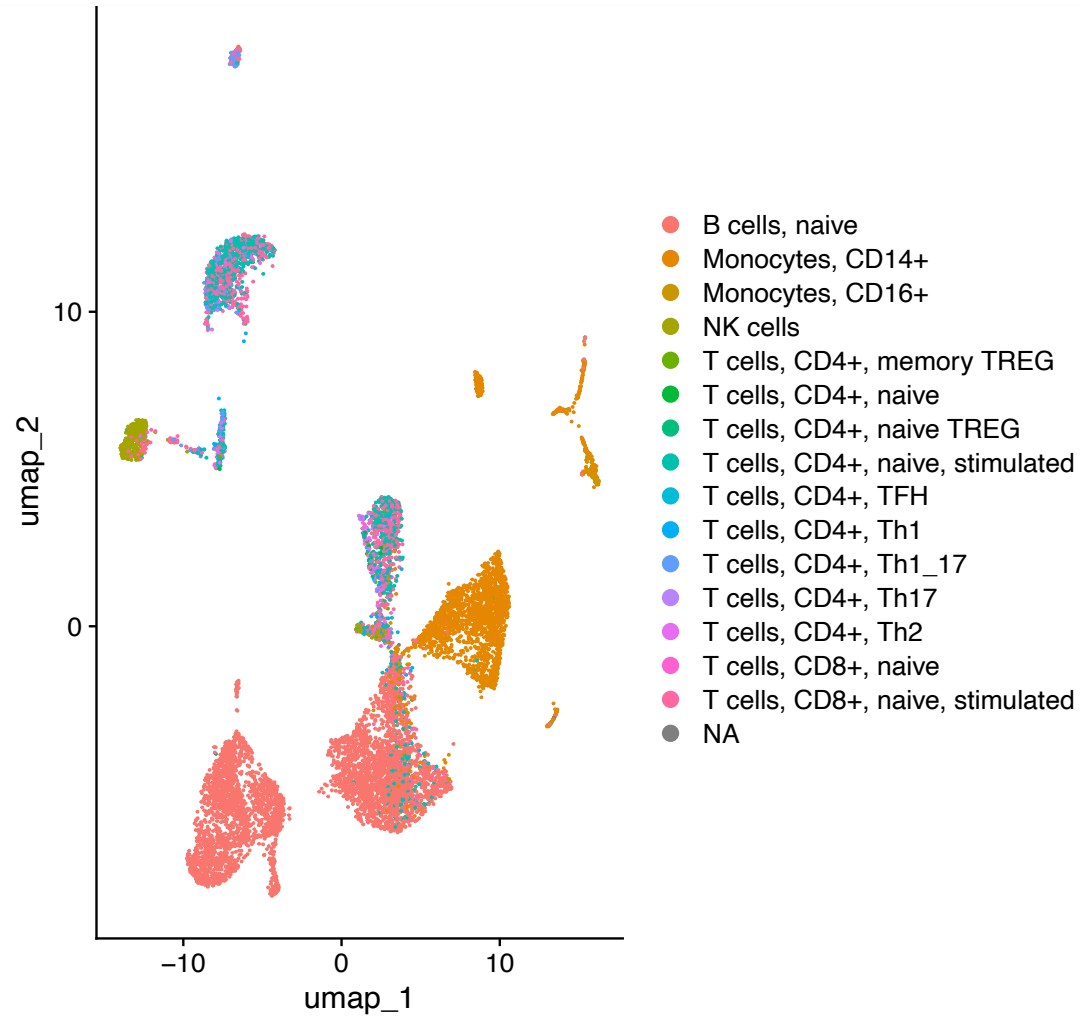


Single Cell Analysis

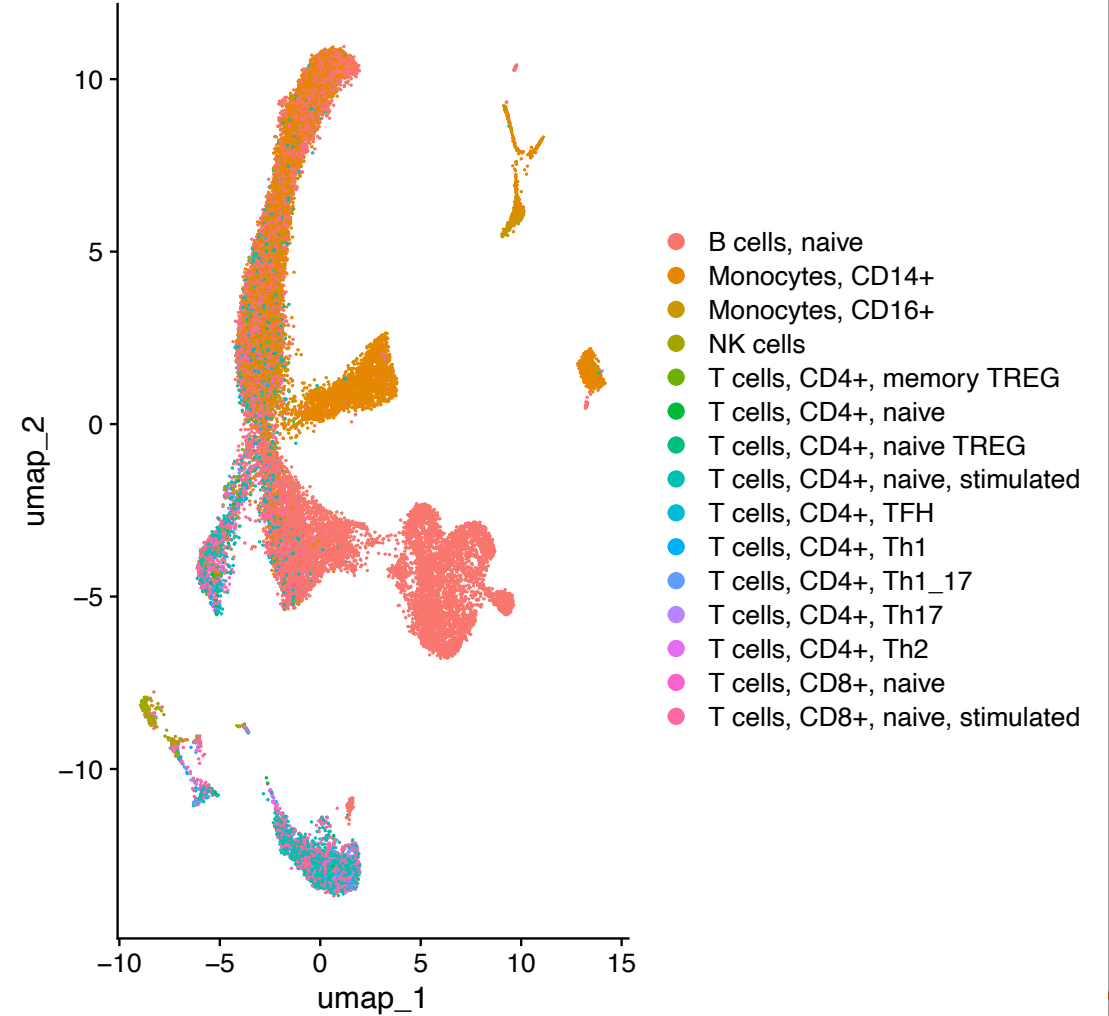


Single Cell Results

Contemporary



Unselected



B cells

Lymphocytes

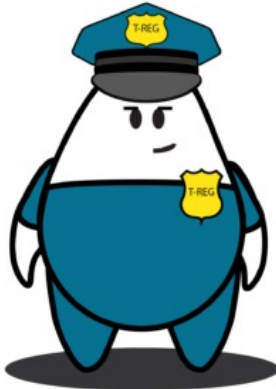
CD8 T Cell



B Cell



Regulatory
T Cell



CD4 T Cell



CellCartoons.net

B

B Cell

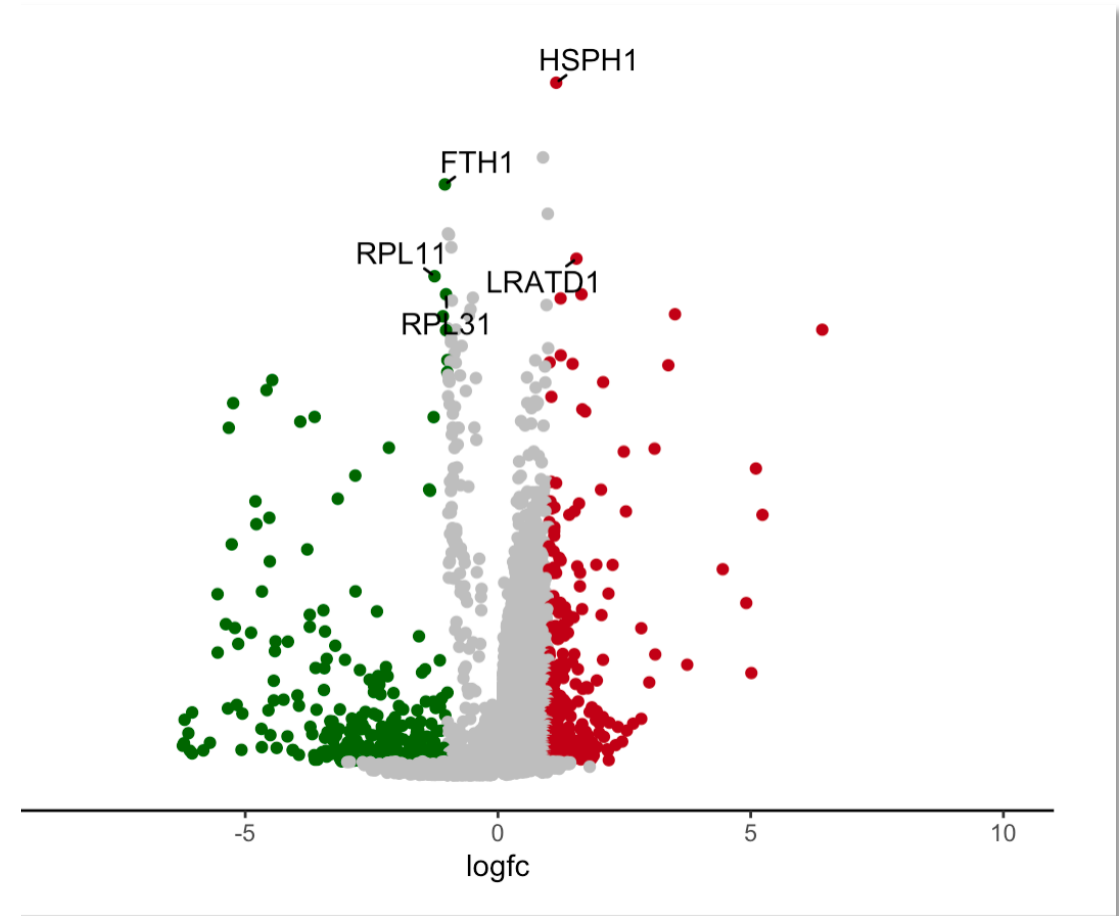


CellCartoons.net



B cells

- 266 genes with higher expression in contemporary Holsteins (red)
- 377 genes with higher expression in unselected Holsteins (green)



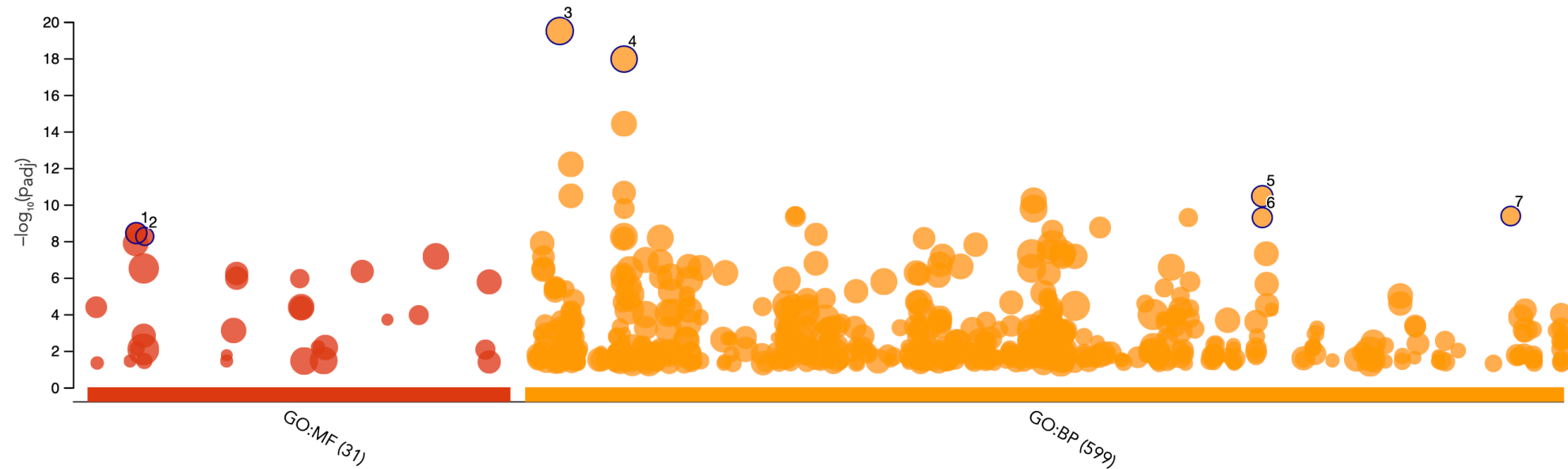
Contemporary Holsteins B cells



ID	Source	Term ID	Term Name	padj (query_1)
1	GO:MF	GO:0004510	tryptophan 5-monooxygenase activity	4.553×10^{-2}
2	GO:MF	GO:0043167	ion binding	4.913×10^{-2}



Unselected Holsteins B Cells

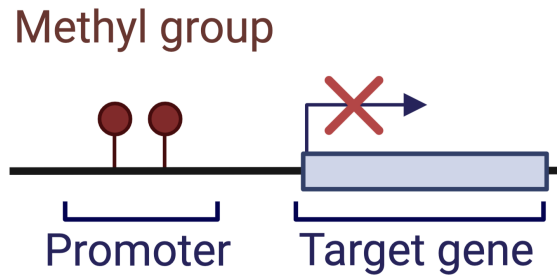


ID	Source	Term ID	Term Name	Padj (query_1)
1	GO:MF	GO:0005125	cytokine activity	3.687×10^{-9}
2	GO:MF	GO:0008009	chemokine activity	5.582×10^{-9}
3	GO:BP	GO:0002376	immune system process	3.234×10^{-20}
4	GO:BP	GO:0006955	immune response	1.115×10^{-18}
5	GO:BP	GO:0097529	myeloid leukocyte migration	3.547×10^{-11}
6	GO:BP	GO:0097530	granulocyte migration	5.264×10^{-10}
7	GO:BP	GO:1990266	neutrophil migration	4.309×10^{-10}



Ongoing work: Epigenomics

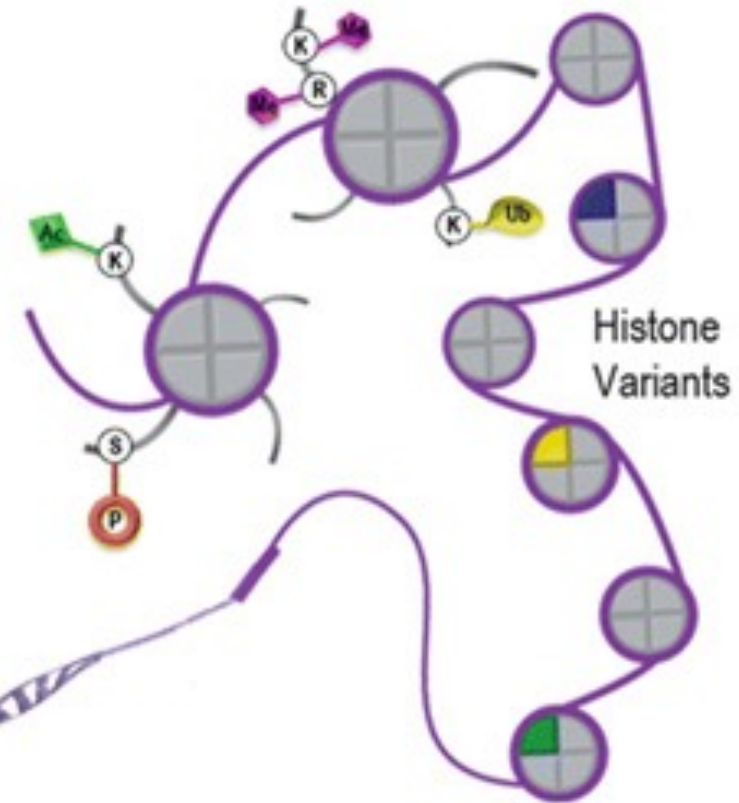
- Marks that change gene expression but not DNA sequence



DNA Methylation



Histone Modifications



Upcoming disease challenges

- *Streptococcus uberis* – gram positive
- *Klebsiella pneumoniae*

Dairy Management Inc.

The dairy research and promotion program is here for farmers and dairy importers across the nation, supporting programs and innovation so their hard work gets the sales and trust it deserves.



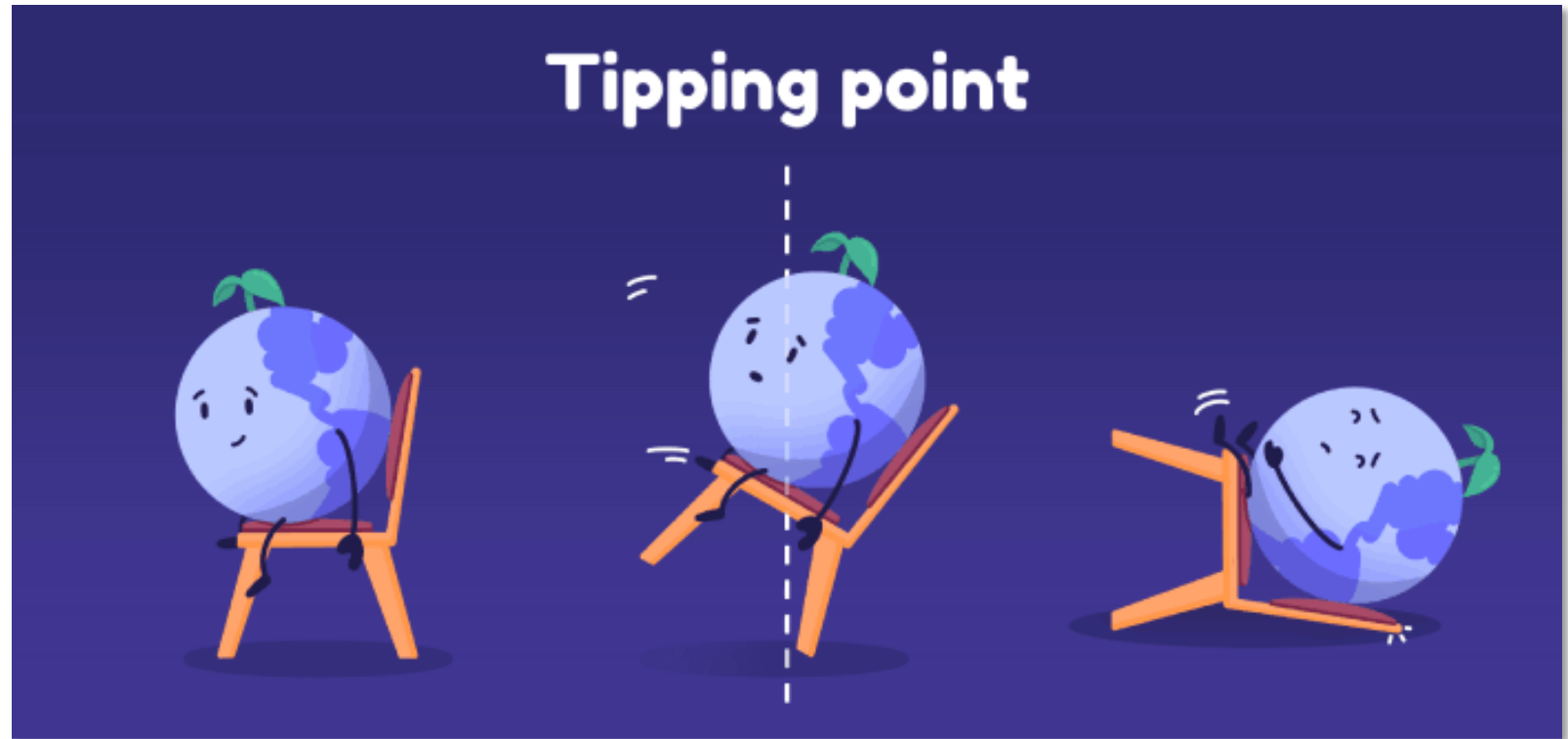
Seeking Input & Advocacy

Kaitlyn.sarlodavila@usda.gov



Challenges

- Rising input & labor costs
- Stagnant budget
 - Underfunded



Advocacy

Research Project: Genomic and Mitigation Strategies to Control Mastitis

Location: [Ruminant Diseases and Immunology Research](#)

Project Number: 5030-32000-238-000-D

Project Type: In-House Appropriated

- Talk to you congressional representatives
- Kaitlyn.sarlodavila@usda.gov
 - Animal Scientist
- Roxann.motroni@usda.gov
 - National Program Leader for Animal Health
- Benjamin.bell@usda.gov
 - Legislative analyst

