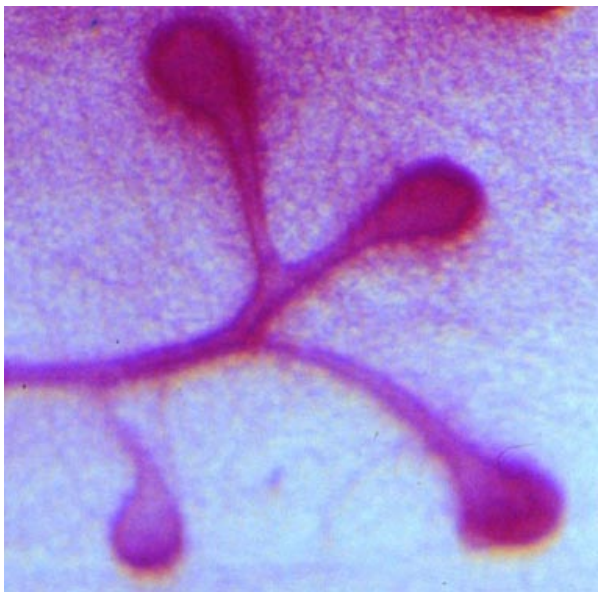
Milk cell transcriptome opens a new dimension in the mammary gland biology research



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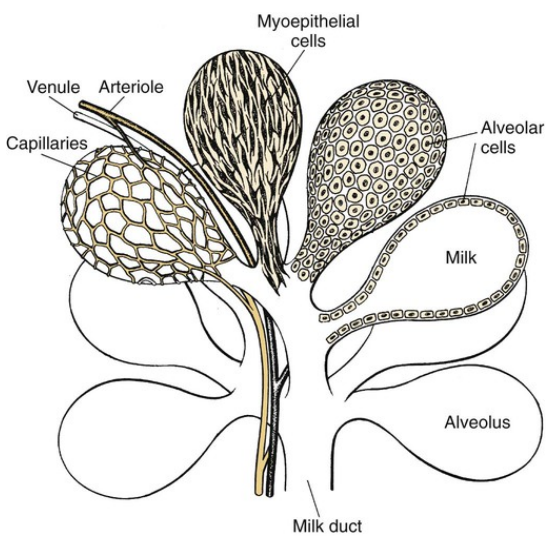
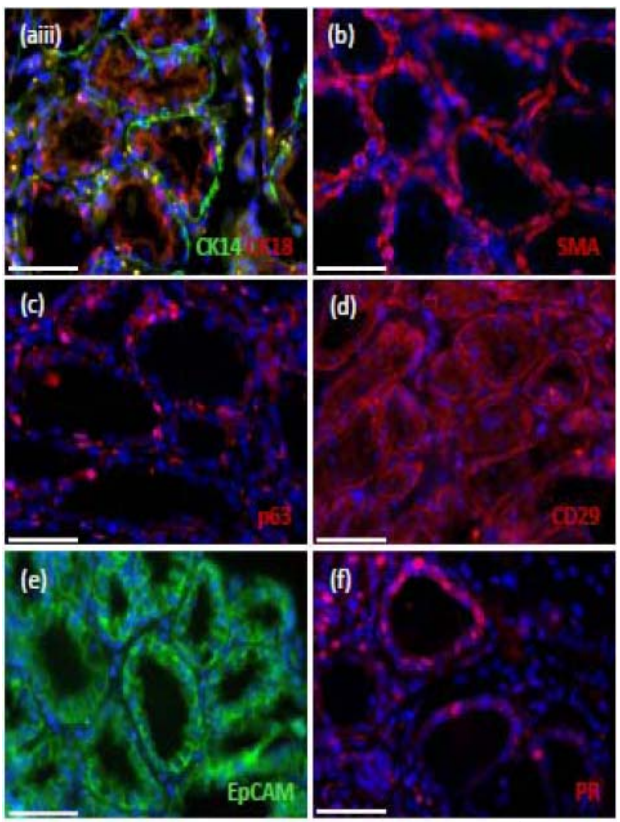
Basic structure of the mammary gland is conserved among species

- Epithelial and myoepithelial cells build alveolar and ductal structures in the mammary gland.





Prpar et al., 2012

Sources of biological material from mammary gland

Invasive collection methods:

•biopsies of the mammary gland tissue,
•laser capture microdissection

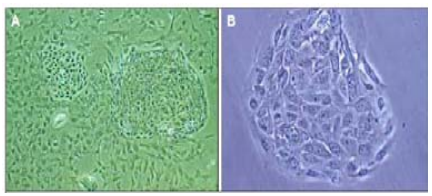
Non-invasive collection methods:

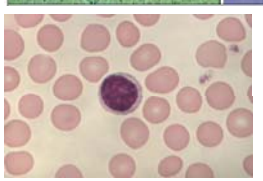
•milk somatic cells,
•milk fat globules
•antibody-captured milk mammary epithelial cells

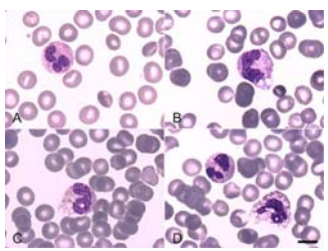
Major somatic cell types in milk

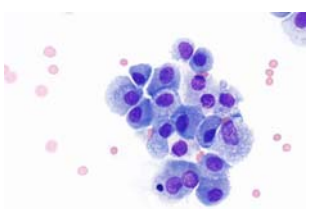
A consequence of the complex function of the mammary gland and intense secretion of milk is also the presence of somatic cells in milk.

- epithelial cells
- lymphocytes
- polymorphonuclear neutrophils (PMN)
- and macrophages.



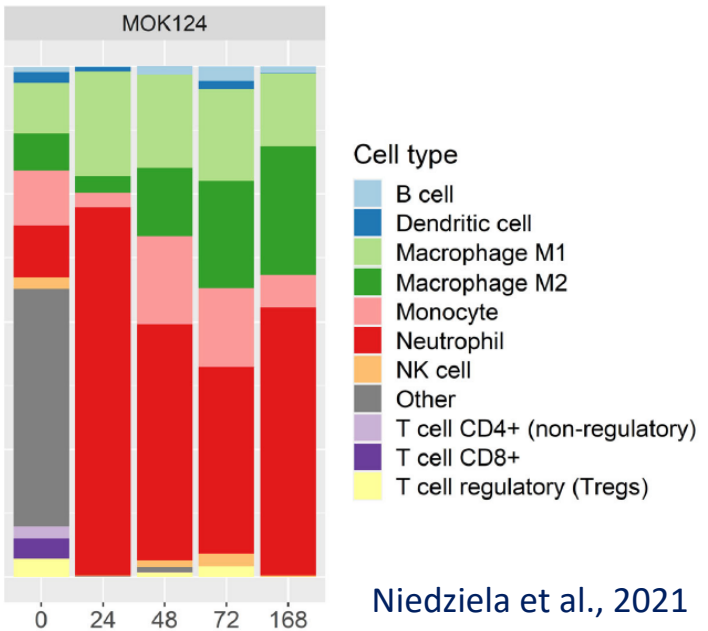






Somatic cell count and differential somatic cell count

- The ratio of epithelial and immune cells differs among species. In cattle and sheep, the epithelial cell fraction represents only a relatively small part of somatic cells in milk, whereas, in porcine and goat milk, epithelial cells are the predominant cell type in milk.
- In the course of infection the proportion of different immune cells is changing.

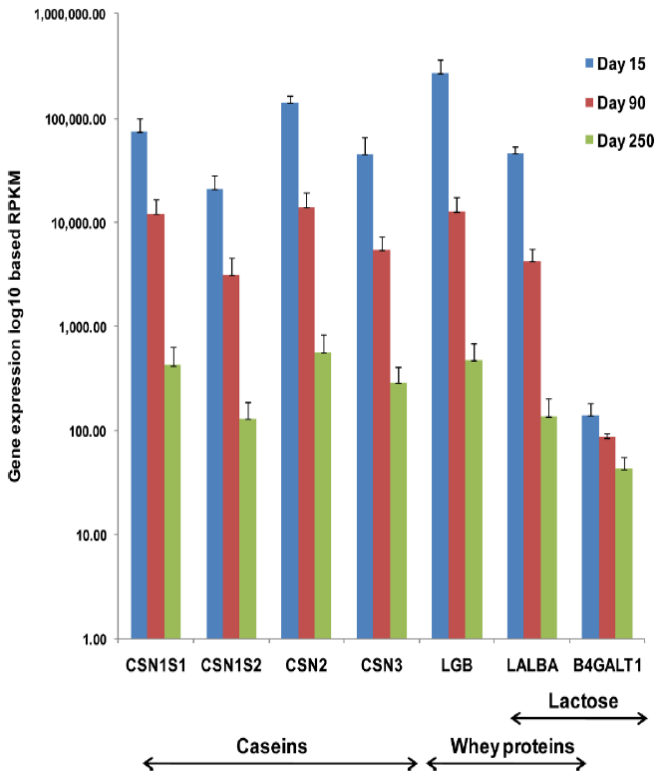


Niedziela et al., 2021

- Somatic cell count (SCC) provides the cumulative number of somatic cells in milk.
- Differential somatic cell count (DSCC) allows differentiation between PMN and lymphocytes versus macrophages.

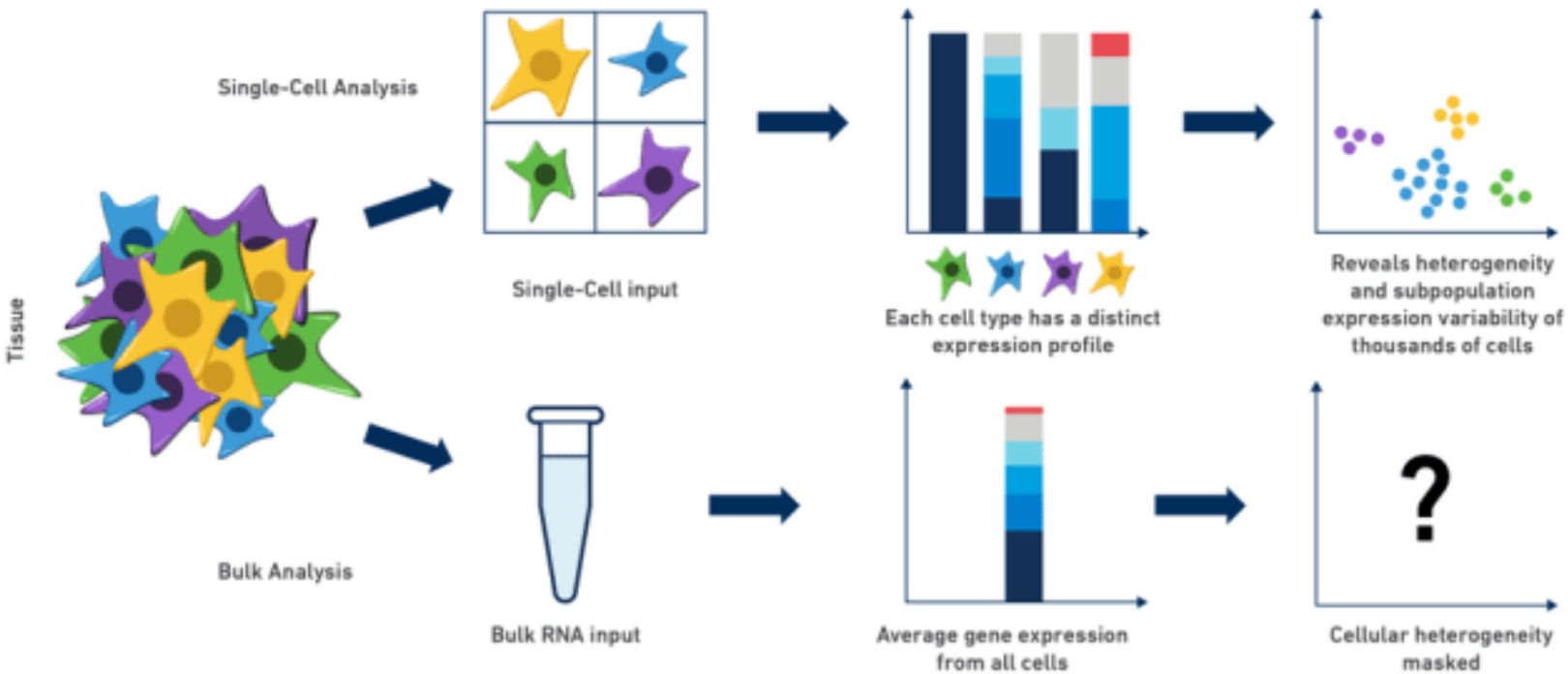
Milk somatic cell transcriptome

- A total number of genes expressed in different lactation stages: 16,892 - 19,094
- 69% of annotated genes are expressed in milk somatic cells
- Approx. 9,000 genes expressed in all lactation stages
- 6,930 genes have a significant change in expression with the stage of lactation
- Genes encoding caseins, whey proteins and enzymes in lactose synthesis pathway show higher expression in early lactation.
- The majority of genes in the fat metabolism pathway have high expression in transition and peak lactation



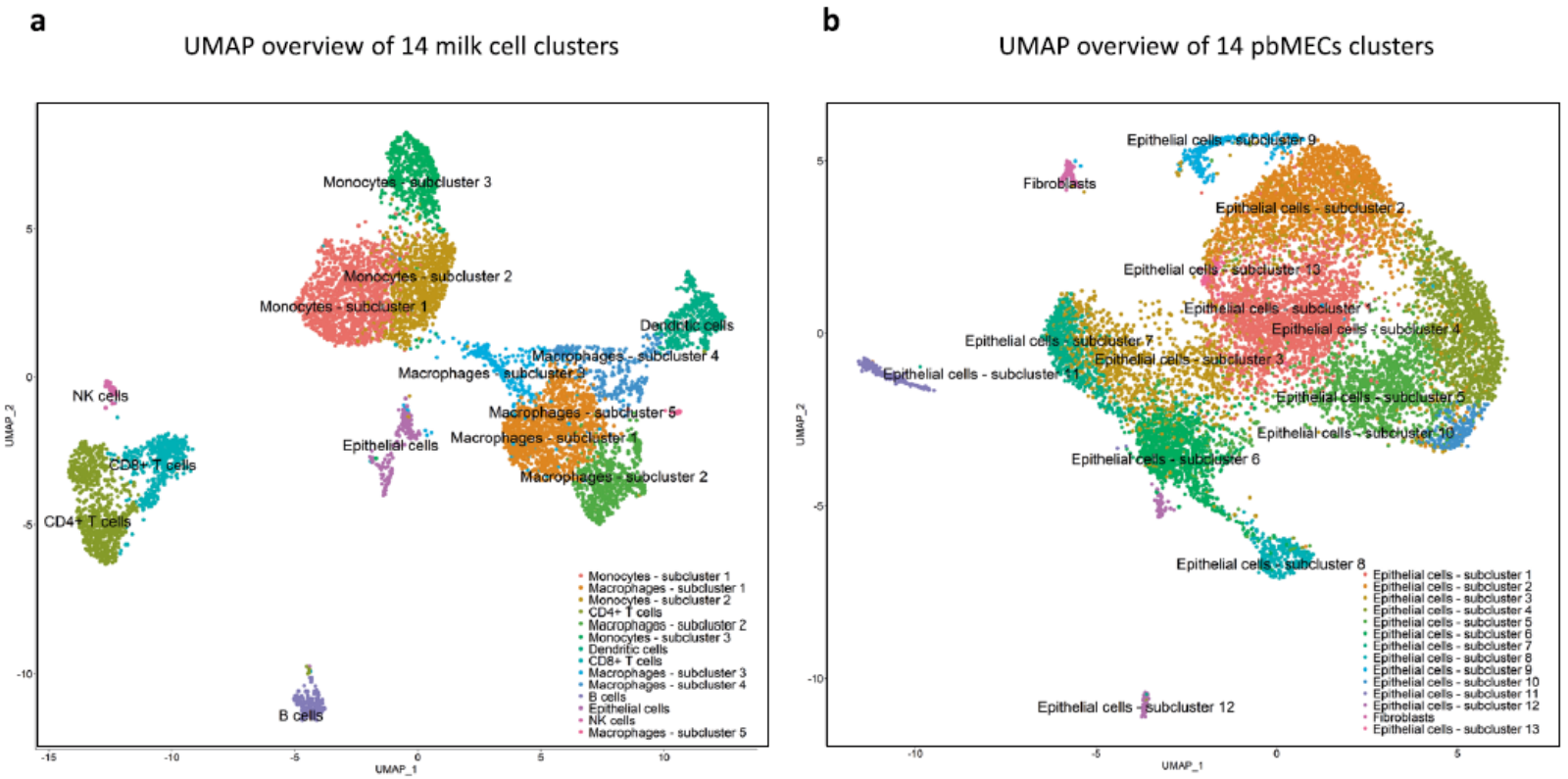
Wickramasinghe et al., 2012

Bulk vs. single cell transcriptome analysis



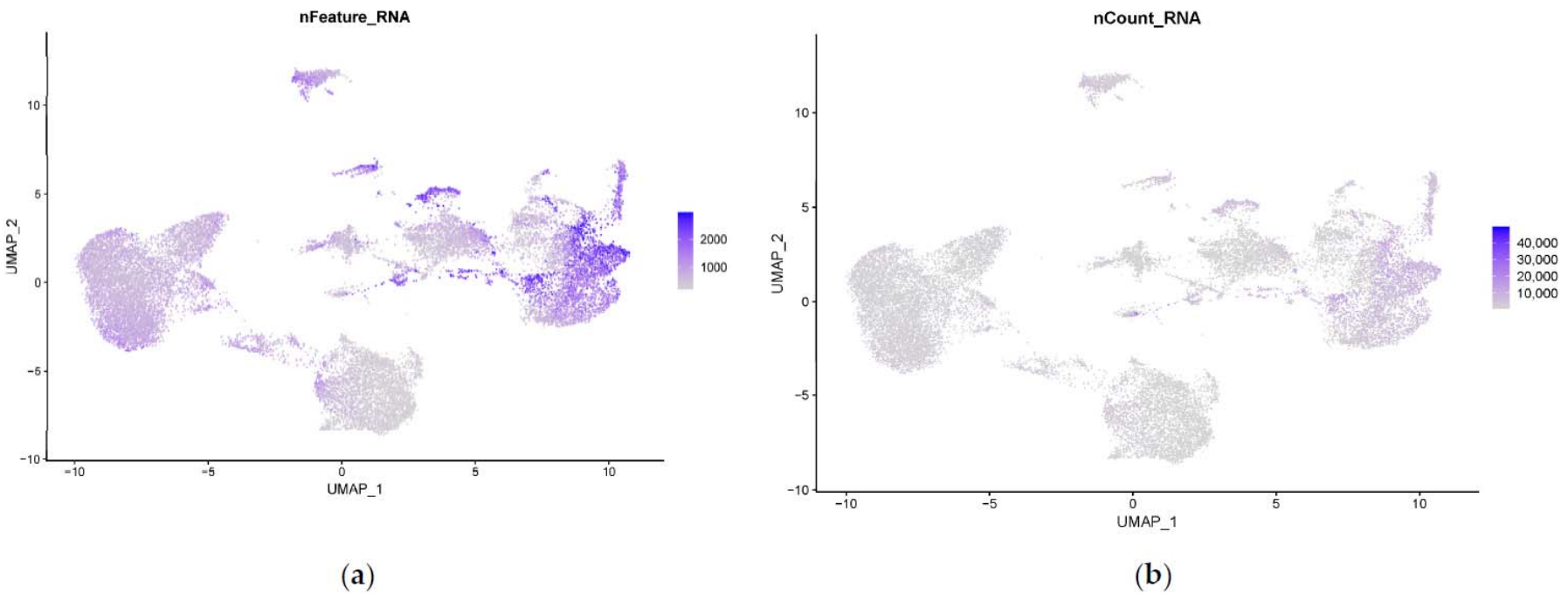
https://www.10xgenomics.com/

Comparison of milk and pbMEC cell type clusters



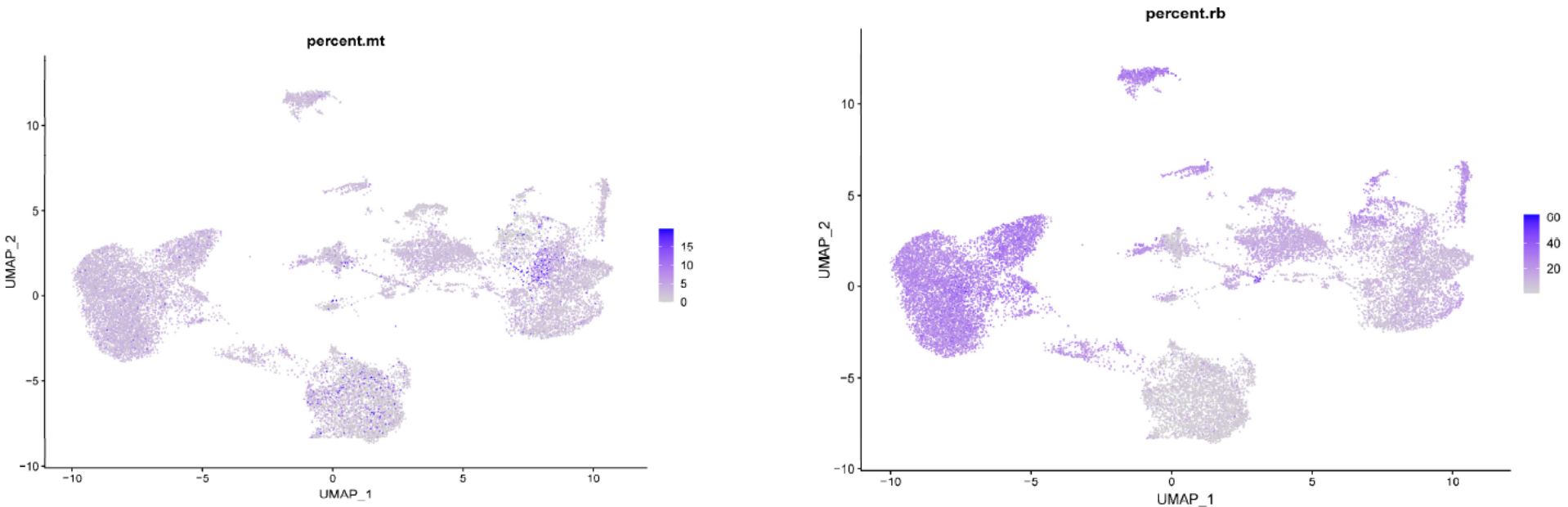
Becker et al., 2021

ScRNA−seq quality control analysis



ScRNA−seq QC analysis of two somatic milk cell datasets after integration:
a) number of genes per cell
b) number of UMI reads per cell

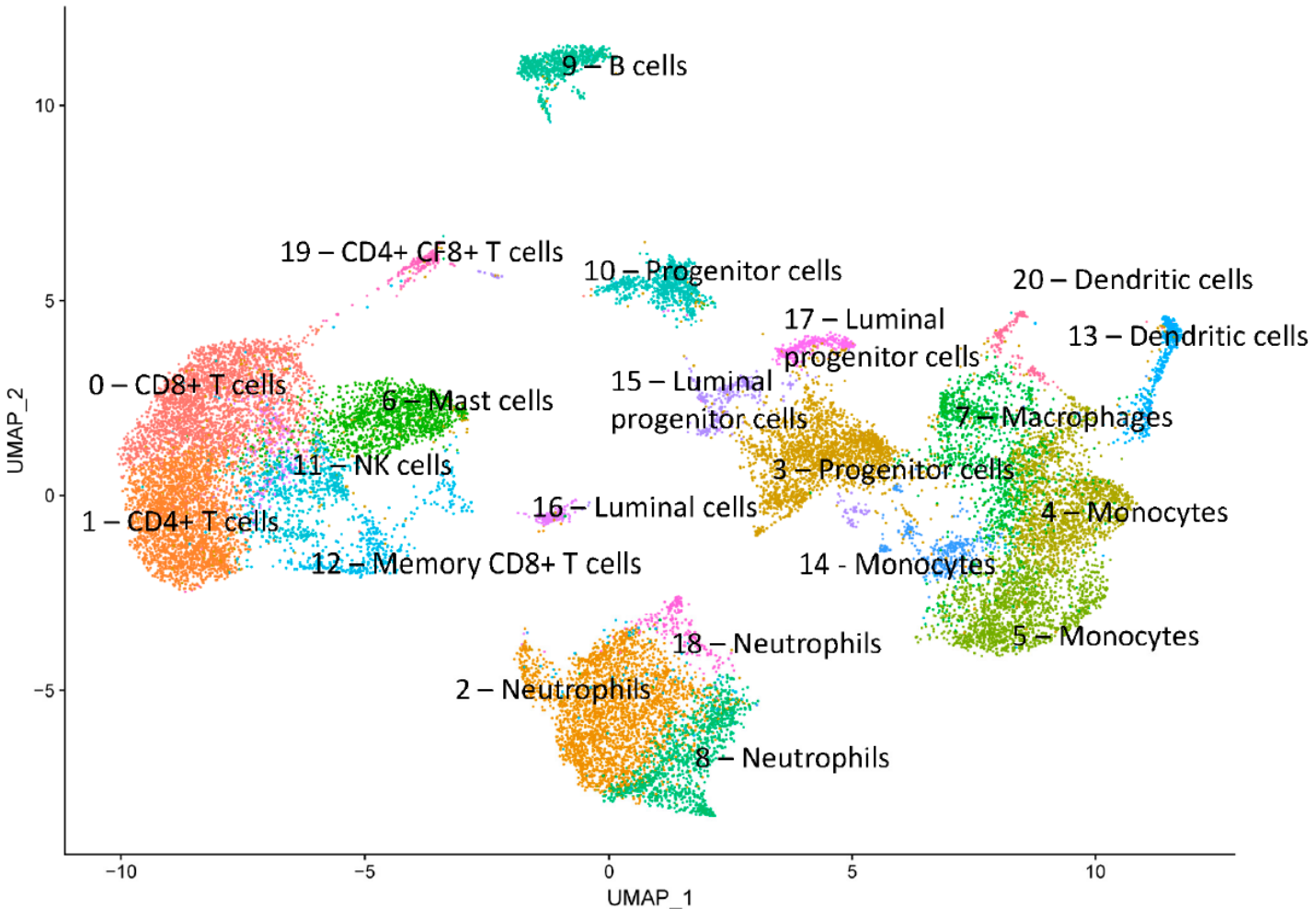
ScRNA−seq quality control analysis



(c) (d)

ScRNA−seq QC analysis of two somatic milk cell datasets after integration:
c) percentage of cell counts mapping to mtDNA genes,
d) percentage of cell counts mapping to ribosomal protein transcripts

Annotated cell clusters from two bovine milk samples

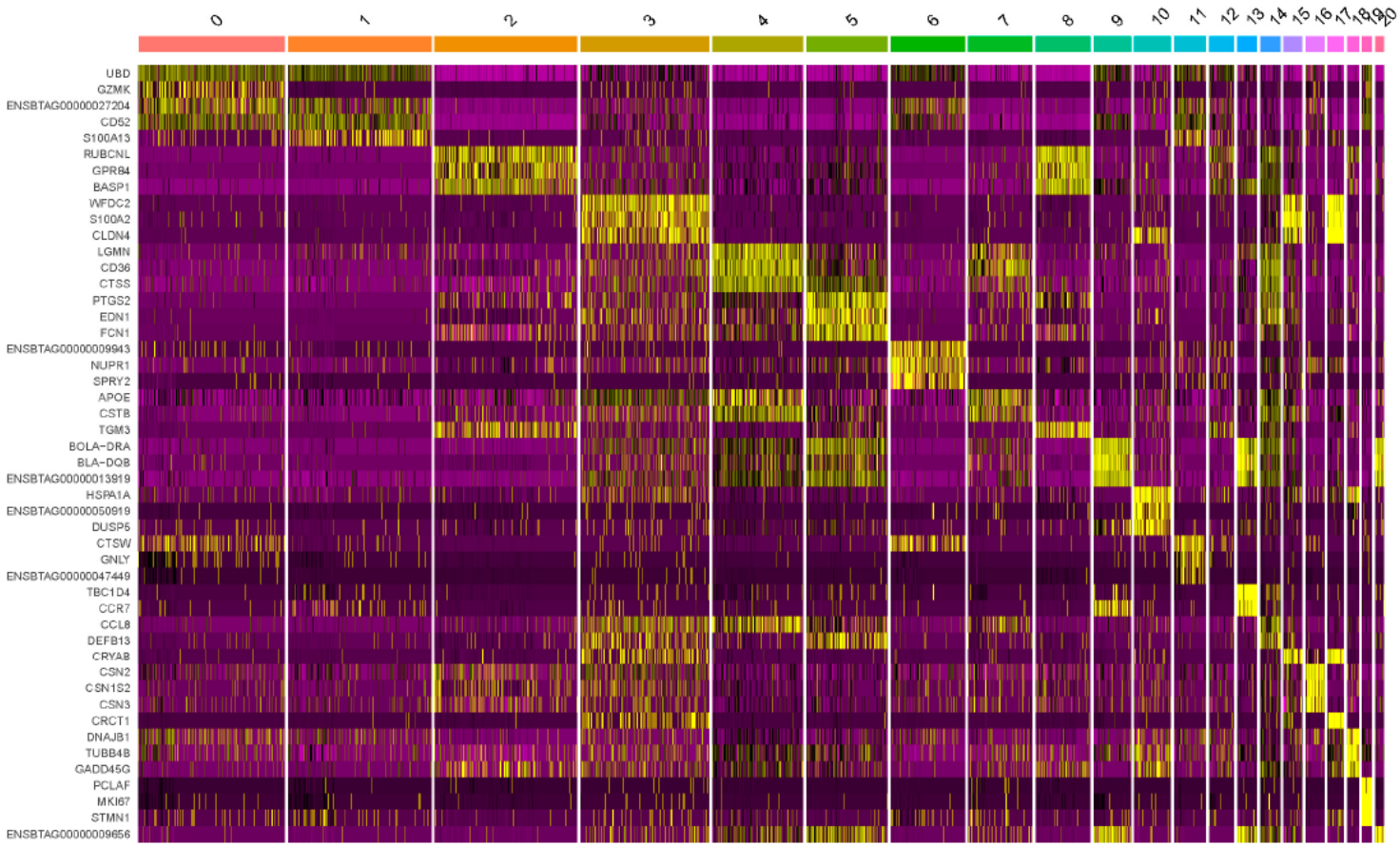


Zorc et al., 2024

Annotation of milk somatic cell clusters

Cluster	Cluster Annotation	Marker Gene
0	CD8+ T cells	*GZMK, ENSBTAG00000027204, CD52, ENSBTAG00000010828, FAM162A, RGS1, CCL5, ENSBTAG00000034609, ENSBTAG00000000432*
1	CD4+ T cells	*UBD, ICOS, ENSBTAG00000055140, GUCY1B1, ENSBTAG00000027204, CD4, S100A13, NCR3, ENSBTAG00000034609*
2	Neutrophils	*GPR84, BASP1, PLAU, PLEK, MARCKS, IL1B, DMXL2, IL1RN, BATF3*
3	Progenitor cells	*CCL2, APOE, S100A2, CLDN4, CD9, CCL8, CST6, CRYAB, CRCT1*
4	Monocytes	*CD36, CTSS, CTSB, LIPA, CCL8, GRN, CNDP2, TREM2, CD9*
5	Monocytes	*EDN1, FCN1, LYZ, CD14, ARAF, TNF, CXCL3, BOLA-DRA, DEFB13*
6	Mast cells	*NUPR1, KIT, ENSBTAG00000055197, SPRY2, CTSW, ENSBTAG00000000144, CD7, TNFRSF9, ENSBTAG00000034609*
7	Macrophages	*FABP5, CD36, CTSB, APOE, CSTB, CNDP2, CTSZ, ATOX1, CD9*
8	Neutrophils	*BASP1, ENSBTAG00000048980 (Chemokine interleukin-8-like domain-containing protein), IFITM3, CXCR1, GPR84, SELL, ENSBTAG00000034366, TGM3, S100A9*
9	B cells	*BLA-DQB, ENSBTAG00000013919, CD74, MS4A1, ENSBTAG00000055240, TNFRSF13C, CCR7, ENSBTAG00000009656, IRF4*
10	Progenitor cells	*TACSTD2, RASD1, ENSBTAG00000050919, DUSP5, EFNB2, ARC, KLF4, HSPA2, MAFB*
11	NK cells	*GNLY, ENSBTAG00000047449 (Saposin B-type domain-containing protein), CD52, UBD, S100A13, PRF1, GPR183, ENSBTAG00000000144 (Ig-like domain-containing protein), ENSBTAG00000055197 (Immunoglobulin C1-set domain-containing protein)*
12	Memory CD8+ T cells	*RUBCNL, UBD, CD52, BASP1, PLAU, ENSBTAG00000027204, PLEK, IL1RN, ENSBTAG00000034609*
13	Dendritic cells	*CCR7, GPR183, LY75, BLA-DQB, TAMALIN, PKIB, ENSBTAG00000013919, BOLA-DRA, FSCN1*
14	Monocytes	*PTGS2, CCL2, CD36, CCL8, CTSS, RUBCNL, EDN1, CXCL5, DEFB13*
15	Luminal progenitor cells	*CLU, CLDN3, CLDN4, CRYAB, KRT7, DSTN, WFDC2, KRT19, LTF*
16	Luminal cells	*CSN1S1, PAEP, CSN1S2, CSN3, GLYCAM1, LALBA, HSTN, SCGB1D, FABP3*
17	Luminal progenitor cells	*CRCT1, AGPAT2, CLDN3, KRT7, CLDN4, DSTN, S100A2, CRYAB, WFDC2*
18	Neutrophils	*TUBB4B, GADD45G, DDIT4, GADD45A, HSPH1, HSPA1A, IER5L, ZFAND2A, LRIF1*
19	CD4+ CD8+ T cells	*PCLAF, MKI67, STMN1, TOP2A, DUT, HMGB2, CENPF, TMPO, DNMT1, UBE2C*
20	Dendritic cells	*BOLA-DRA, ENSBTAG00000013919 (BOLA-DRB3), ENSBTAG00000009656 (BOLA-DQA2), CD74, C3H1orf54, BLA-DQB, CST3, ENSBTAG00000037605 (BOLA-DQA1), BOLA-DMA, PLAC8A*

Specificity of marker gene expression



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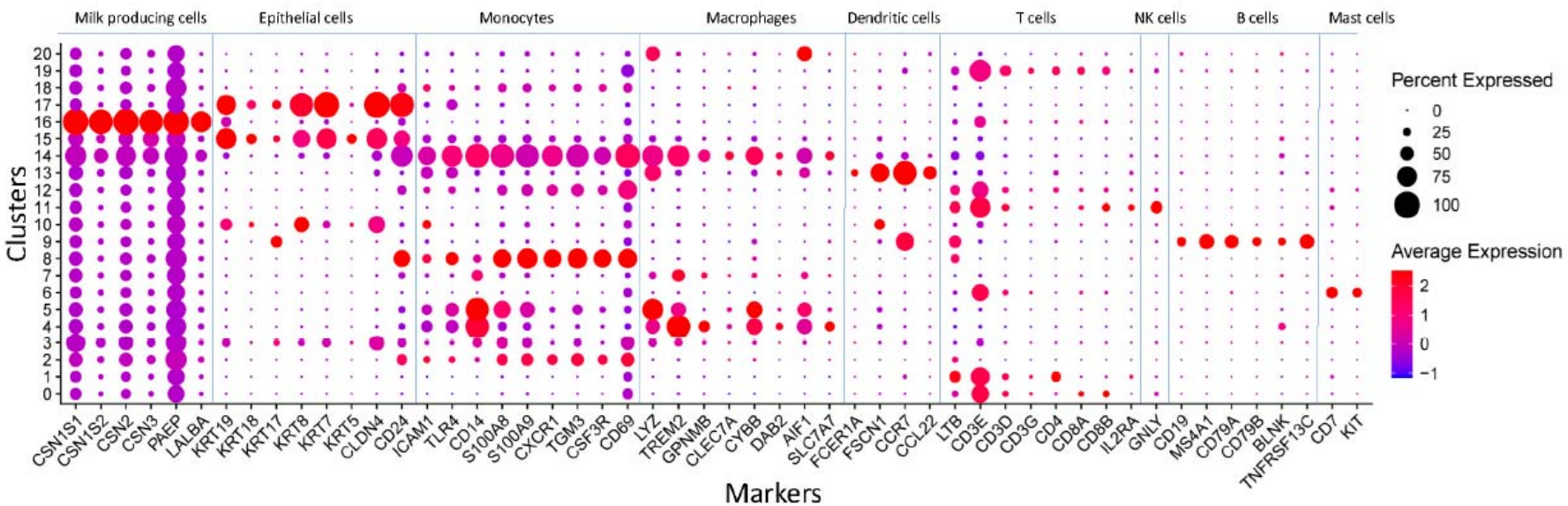
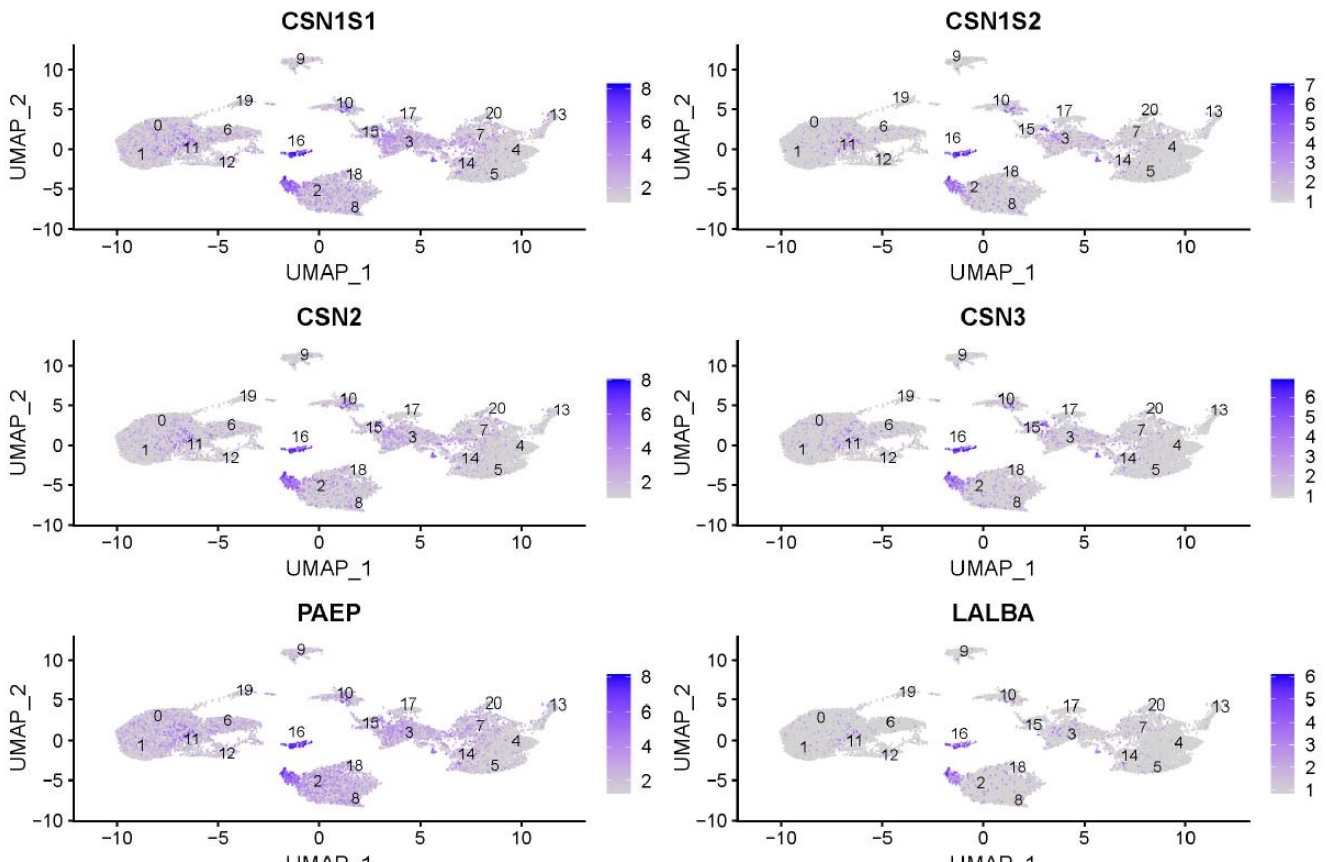
Standardized expression of three major marker genes identified for each cell cluster.
High expression of a particular gene is labeled with yellow and low expression with purple.

Marker genes, used to determine cluster identity

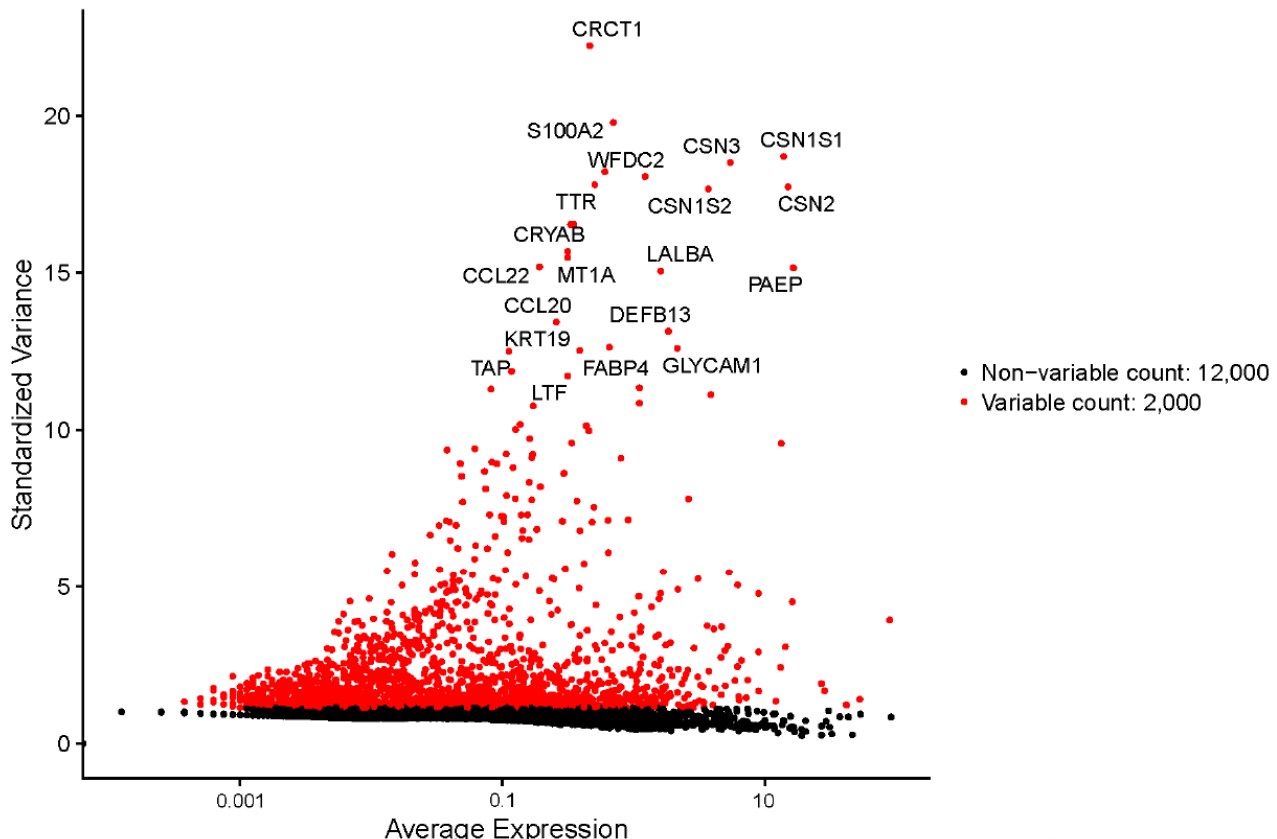


- The size of the circle corresponds to the percentage of cells in the cluster expressing the marker.
- Shading corresponds to the extent of expression.

Expression of casein and whey protein genes in bovine somatic milk cell clusters



Highly variable expressed genes in bovine milk somatic cells



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Conclusions

- Our study aimed to capture a snapshot of cellular mechanisms driving milk synthesis and secretion at mid-lactation.
- Detection of highly variable expressed genes allowed identification of genes that strongly contribute to cell-to-cell variation within the cell population.
- Animal welfare arguments and the possibility of obtaining multiple samples from the same animal in the course of lactation are strong arguments for a non-invasive sampling approach.
- The identification of a considerably higher number of cell types in the milk somatic cell fraction compared to traditional expectations opens a new horizon for a more complex interpretation of the biological processes in the mammary gland.

Thank you for your attention!