

New Porcine FAANG project

- ***USDA-NIFA-AFRI- 2018-67015-27501***
- ***Overall goal: catalog functional elements in the porcine genome for many biological states, create Chromatin state map***
- **Aim 1 Adult tissues**
 - extend FAANG pilot of 8 -> 25 tissues
- **Aim 2 Fetal tissues**
 - Correlate allele-specific expression and chromatin modification
 - Parent-of-origin effects (reciprocal crosses Meishan x WhiteCross)
- **Aim 3 Immune System**
 - Circulating Blood Cell types
 - Single cell analysis of blood PMBC and immune tissues
- **Aim 4 Data Integration and Analysis**
 - Develop a higher-order regulatory understanding of the porcine genome, including a chromatin state map

Samples and assays detail

Aim 1 Adult tissues

- extend FAANG pilot of 8 -> ~25 tissues
- **List may change as project proceeds**
- **Collecting additional tissues this Spring- email if you have a favorite not on the list!**
- N=2 for each tissue
- RNAseq, RAMPAGE, Iso-seq
- Histone modifications
 - H3K4me3
 - H3K4me1
 - H3K27me3
 - H3K27ac
- ATAC-seq
- Whole Genome Bisulfite Seq

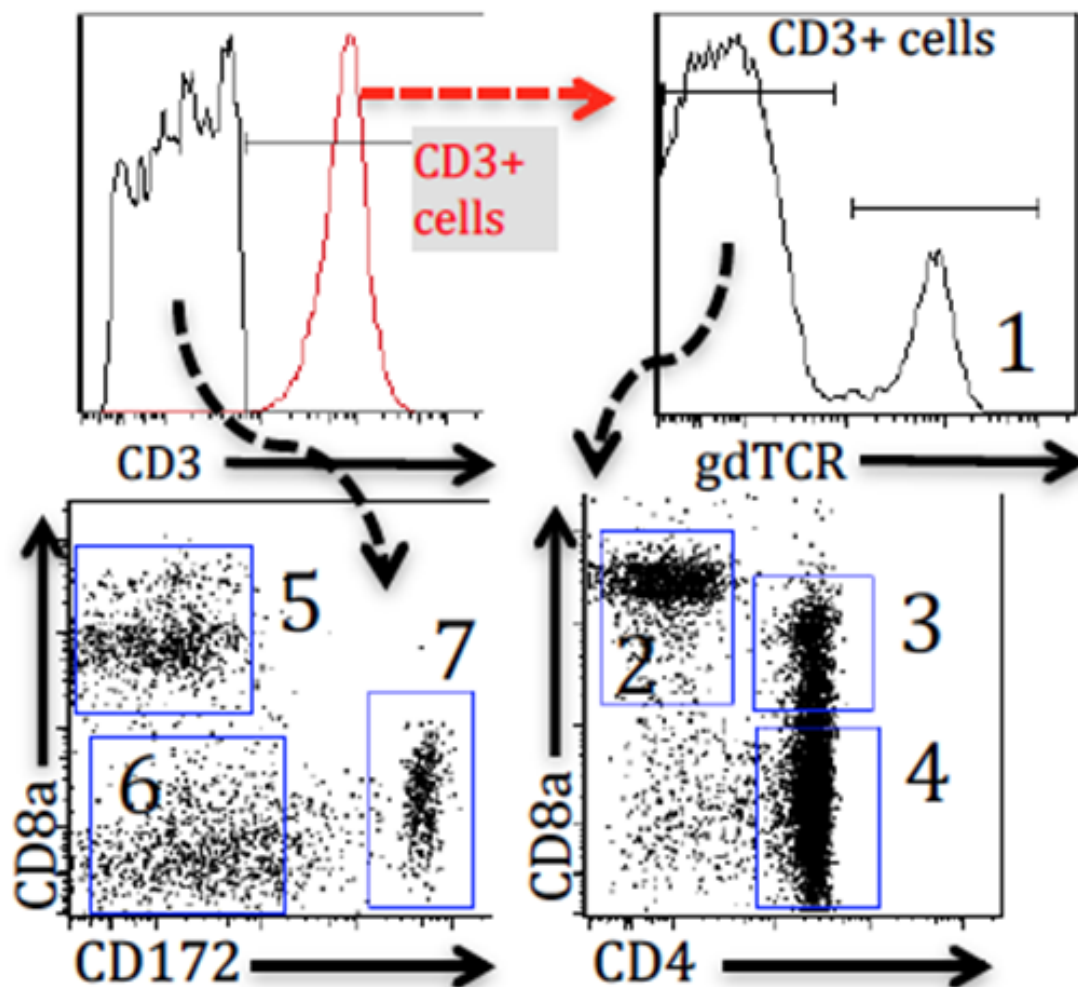
Adult tissues	Objective 1
<i>FAANG Pilot</i>	Liver
<i>FAANG Pilot</i>	Skeletal muscle
	Heart
<i>FAANG Pilot</i>	Adipose- subcutaneous
<i>FAANG Pilot</i>	Brain cortex
<i>FAANG Pilot</i>	Cerebellum
<i>FAANG Pilot</i>	Hypothalamus
	Esophagus
	Stomach
	Jejunum
<i>FAANG Pilot</i> [^]	Ileum
	Colon
	Trachea
<i>FAANG Pilot</i>	Lung
<i>FAANG Pilot</i>	Spleen
	Skin
	Thymus
	Bone marrow
	Testis
	Ovary
	Uterus
	Mammary gland
	Kidney
	Bladder
	Pancreas
	Thyroid gland

Samples and assays detail

- Aim 2 Fetal tissues
 - Tissues collected from fetuses from reciprocal crosses Meishan x WhiteCross – highly divergent breeds
 - Correlate allele-specific expression and chromatin modification
 - Parent-of-original effects
 - N=2 for each cross
 - Tissues: brain, placenta, muscle, liver
 - Two stages of fetal development: day 30, day 70
 - ***All assays as for Aim 1***

Samples and assays detail

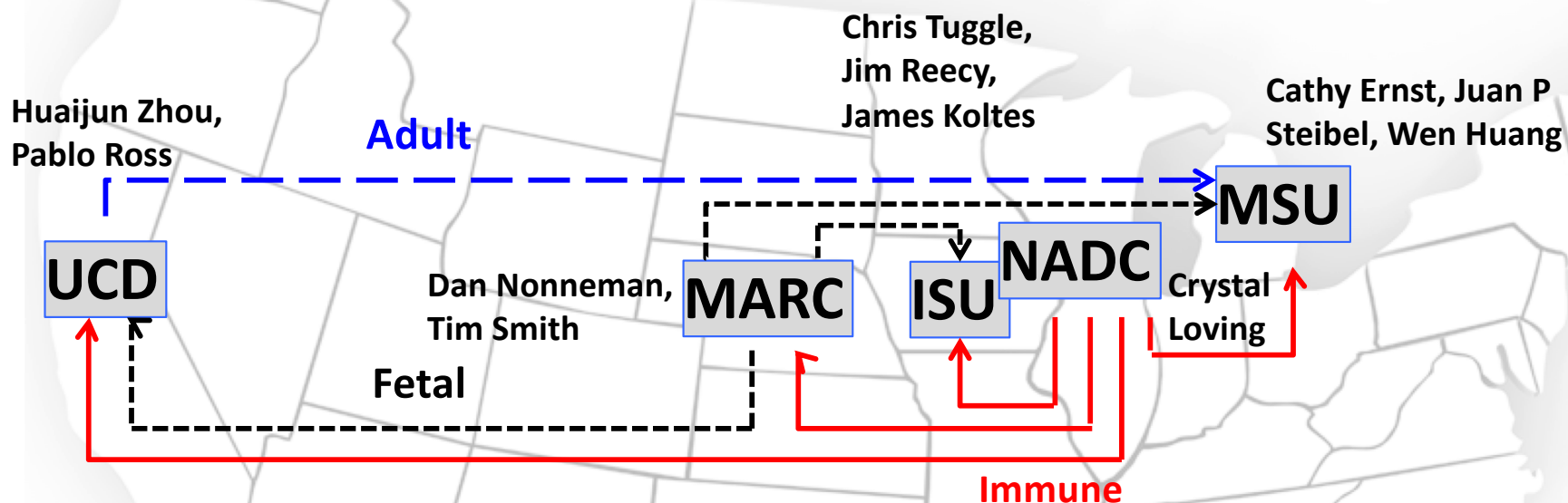
- Aim 3 Immune System
 - Circulating Blood Cell types and tissue macrophages- *all assays listed previously*
 - *Single cell RNAseq analysis* of blood PMBC and immune tissues



Flow-sorting to Isolate Eight Blood Cell types

0. Neutrophils (gradient separation)
1. $\gamma\delta$ T (CD3+gdTCR+)
2. CD4- CD8a+ T
3. CD4+ CD8a+ T
4. CD4+ CD8a- T
5. NK (CD3-CD8a+)
6. B (CD3-CD8a-)
7. Monocytes (CD172+)

Community Sharing of Samples and Expertise



<u>Location</u>	<u>Tissue collection</u>	<u>Sample Analyses</u>
ISU	None	Histone CHIP, ATAC-Seq
MARC	Fetal*	RNAseq, IsoSeq
MSU	None#	DNA Methylation (WG-BS)
NADC	Immune	None
UCD	Adult#	Histone CHIP, ATAC-Seq, RAMPAGE

*Fetal Tissue already collected
 # Some adult tissues to be collected at MSU through 2016 AFRI project to UCD

DELIVERABLES

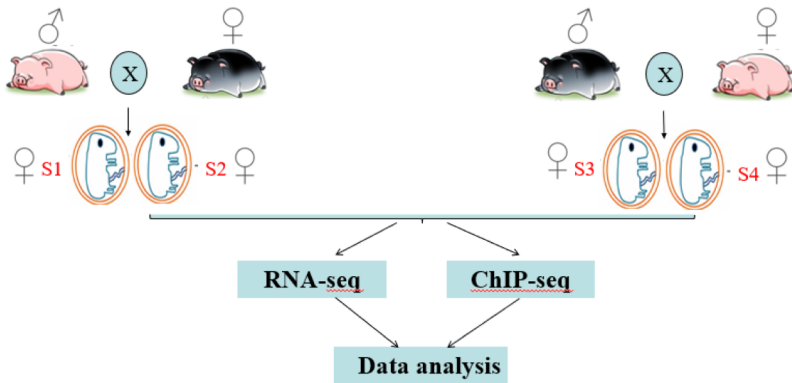
1. Novel porcine **transcriptomic and epigenetic information on the genes and regulators** controlling expression in **~40 biological states** (~18 adult, four fetal tissues at two stages of development, eight blood cell types during healthy growth and macrophages during responses to two models of infection at two times post-stimulation).
2. **Prediction of the function of genomic regions** across tissues and cells relevant for prioritizing SNPs associated with specific phenotypes related to these tissues/cells

Work presented at PAG 2019

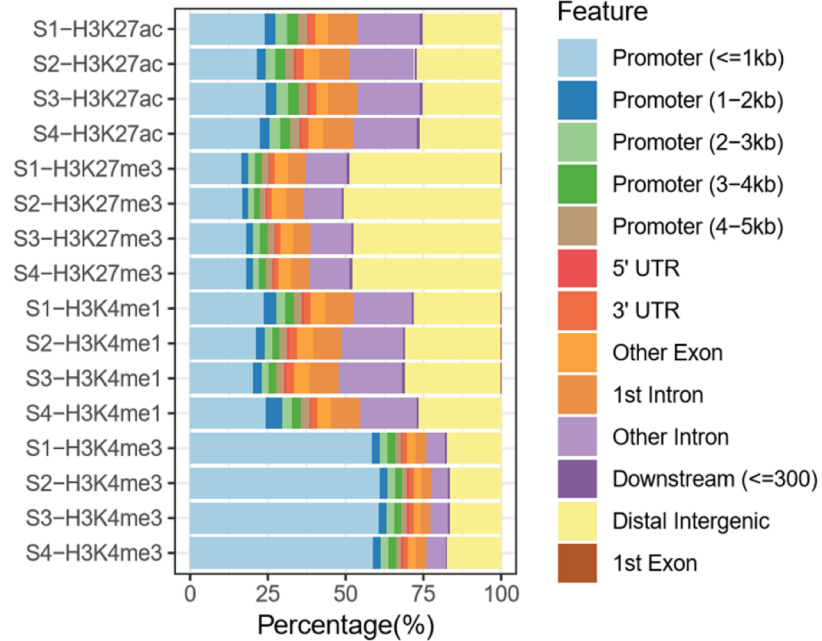
Niu et al. Poster # PE0420

Aim 2 Fetal tissues

Approach

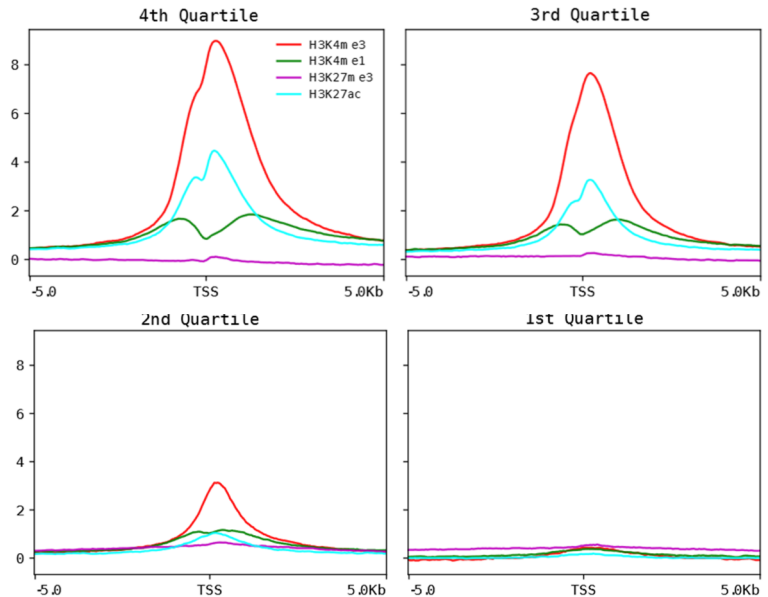


Feature Distribution



Results and Conclusion

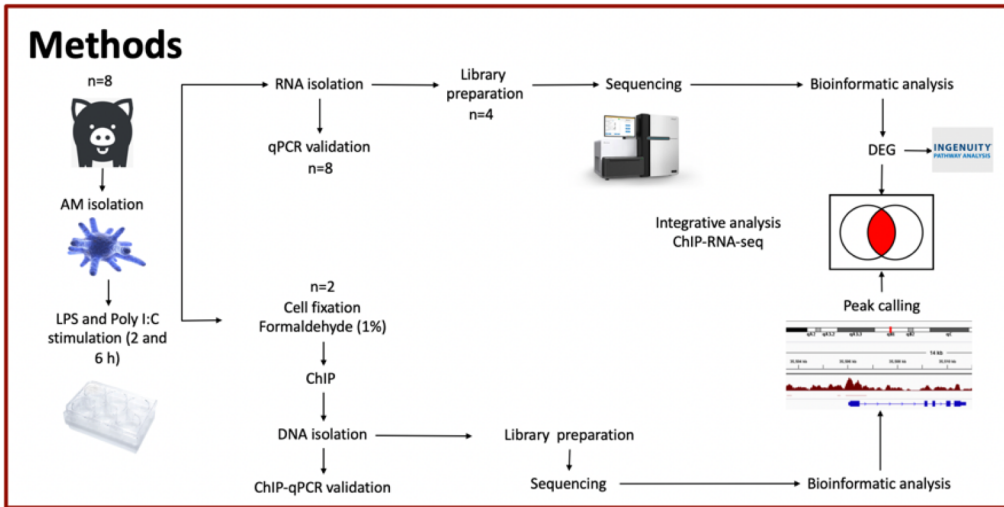
- ChIP-seq data has high quality for all 4 histone marks.
- Expected correlation of gene expression with marks H3K4me3 and H3K27ac was found.
- In-depth analysis are ongoing to correlate allele-specific expression and chromatin modification.



Work presented at PAG 2019

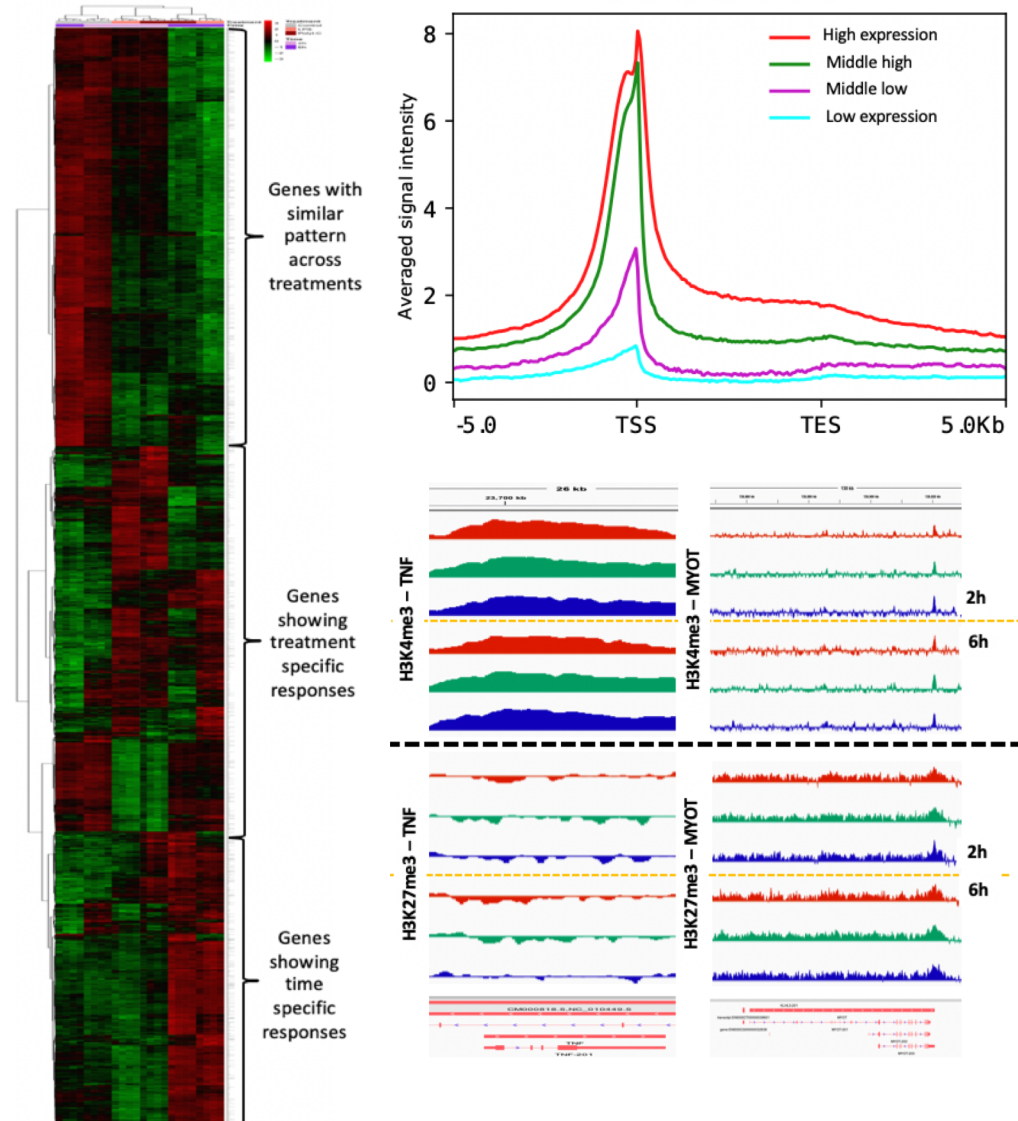
Herrera Uribe et al. Poster #PO0417

Aim 3 Macrophage stimulation



Conclusions and future perspectives

- Stimulation by bacterial and viral mimics produced three clusters of gene expression.
- H3K4me3 marks at promoter is associated with expression level of genes, but few H3K4me3 and H3K27me3 changes at inflammatory gene promoters at 2 and 6 h after pathogen-mimic stimulation.
- Further histone marks (H3K4me1 and H3K27ac) and ATAC-seq will be included in the study to clarify the epigenetic regulation in response to these mimics.



Work presented at PAG 2019

Liu. et al. Poster #P00421

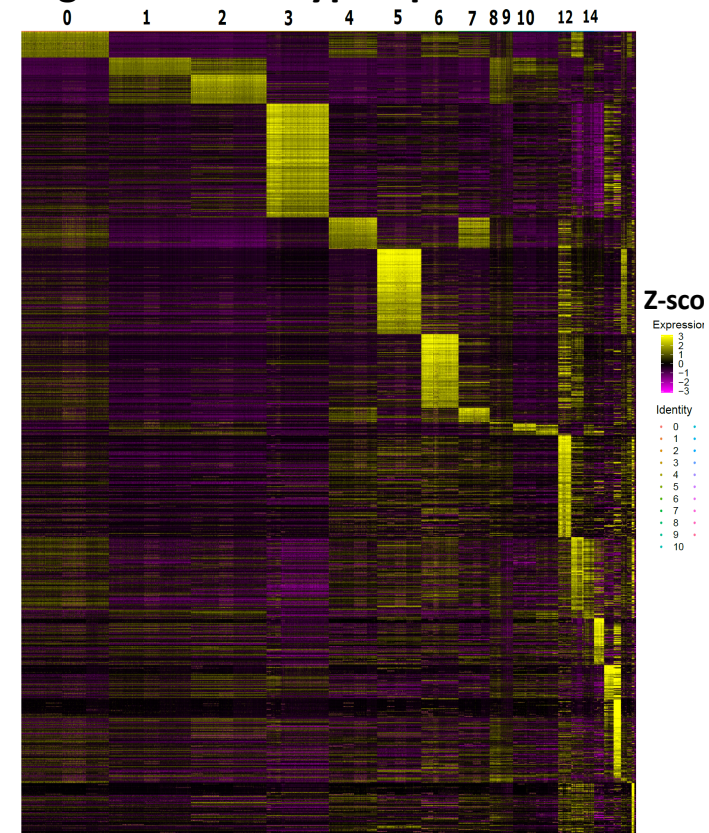
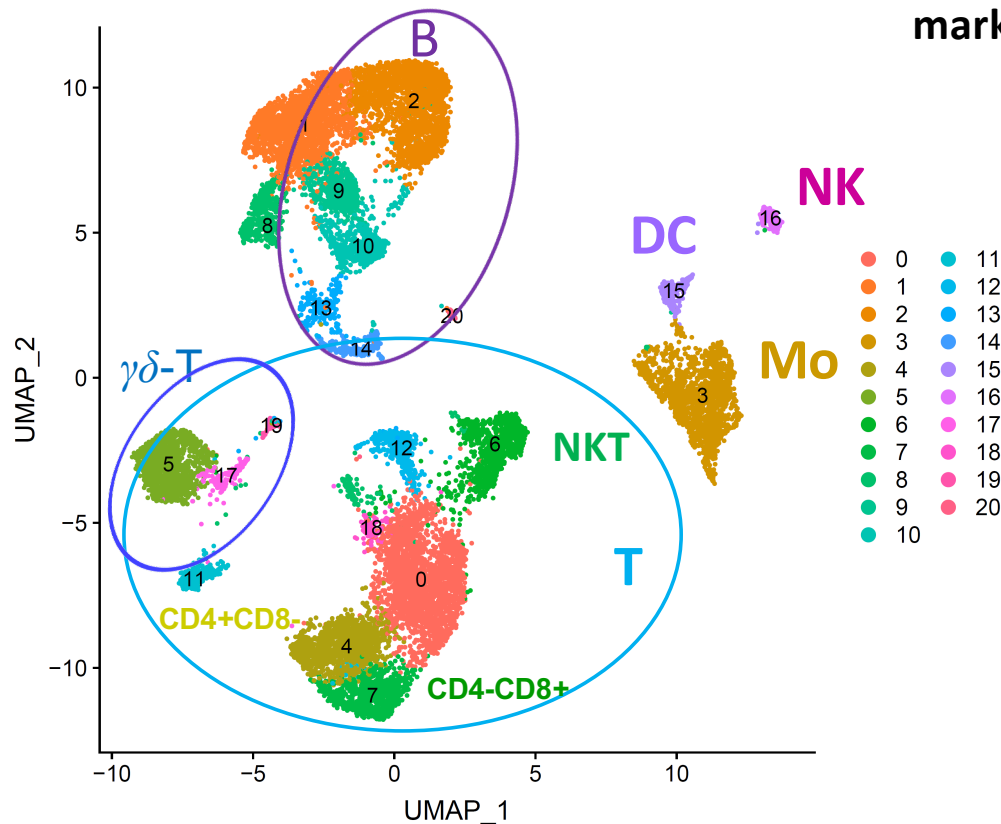
Aim 3 Single Cell analysis of resting PBMC

Methods:

- Fresh or frozen **PBMCs** from **5** adult pigs
- scRNA-seq using **10X Genomics Chromium** platform/Hiseq3000

Results:

- Useful data matrix: 15K cells X 11.7K genes
- Identified 20 clusters including subtypes for **B**, **T**, **NK**, **NKT**, **DC**, and **Monocytes**
- Identified interesting **lists of subtype-specific marker genes**



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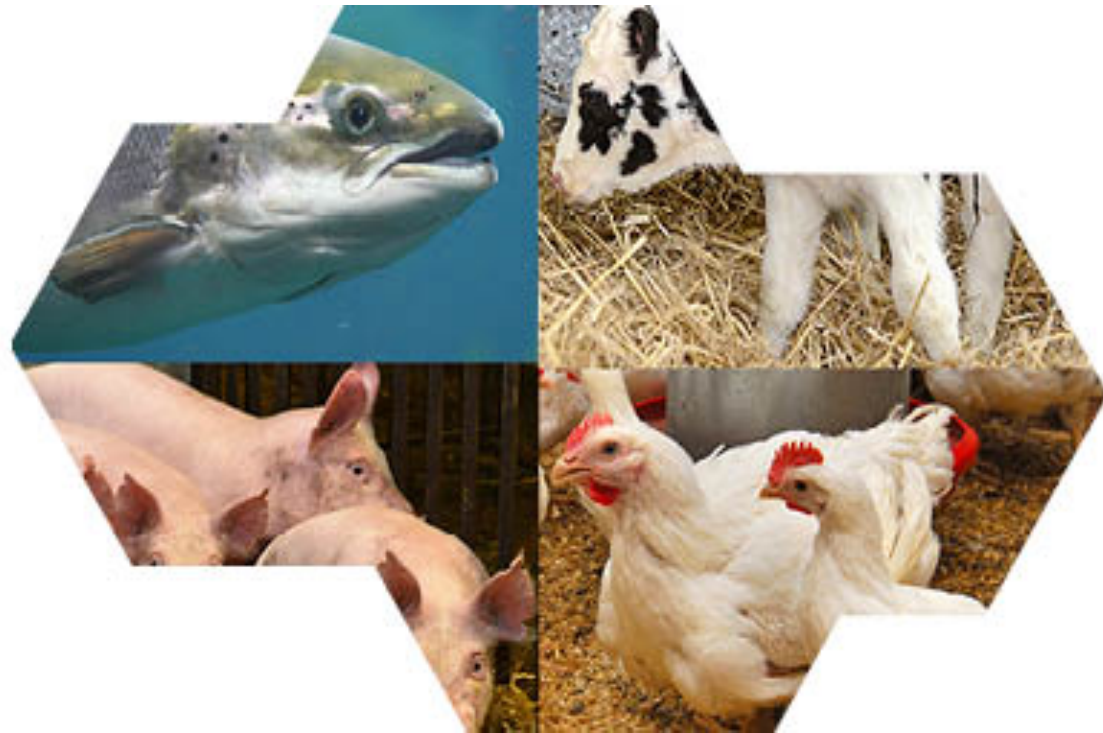
KEY DEADLINES:

Early Bird: 12 February

Bursary: 26 February

Abstract: 12 March

Registration: 09 April



For full details: bit.ly/AGD2019