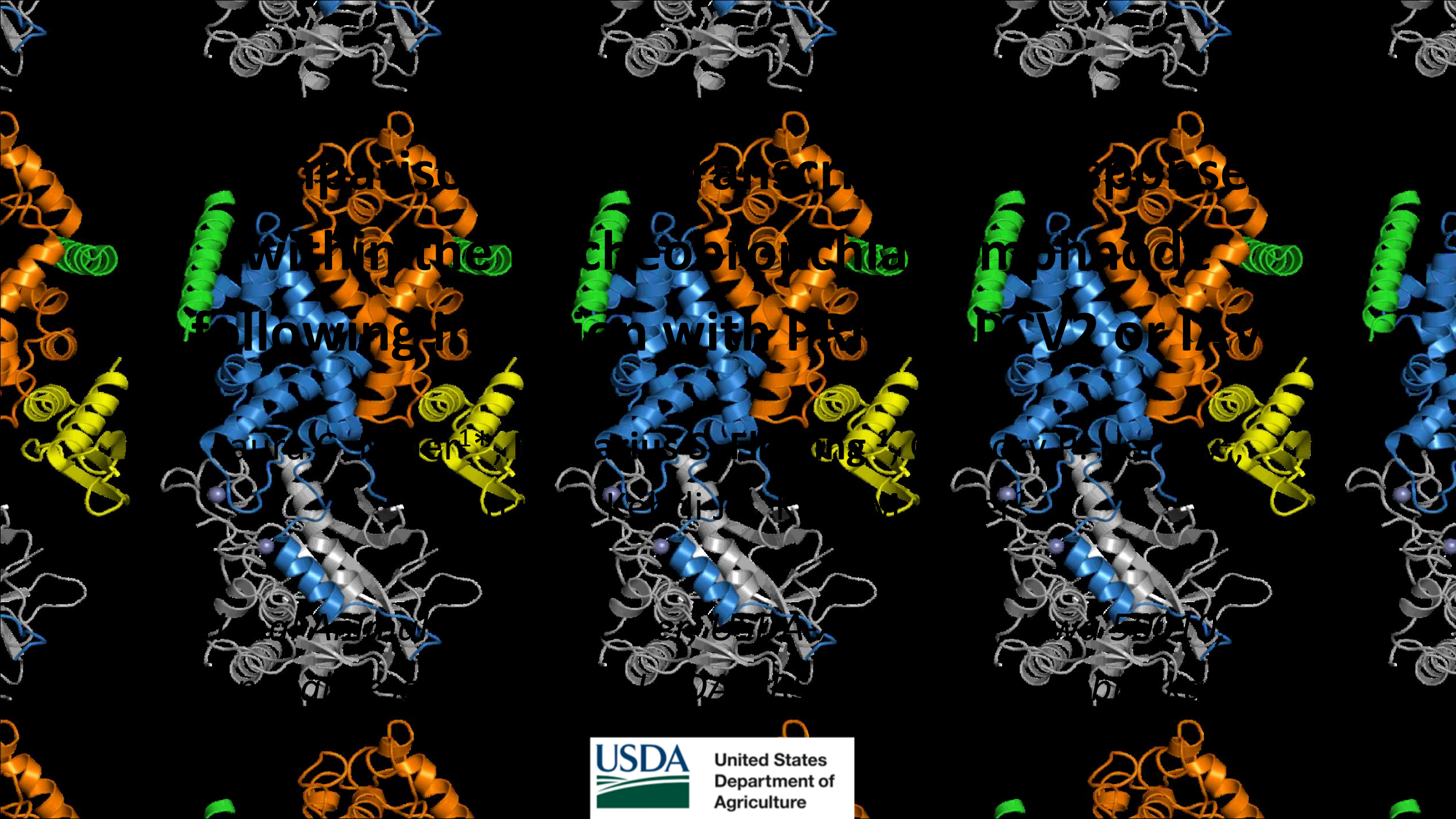
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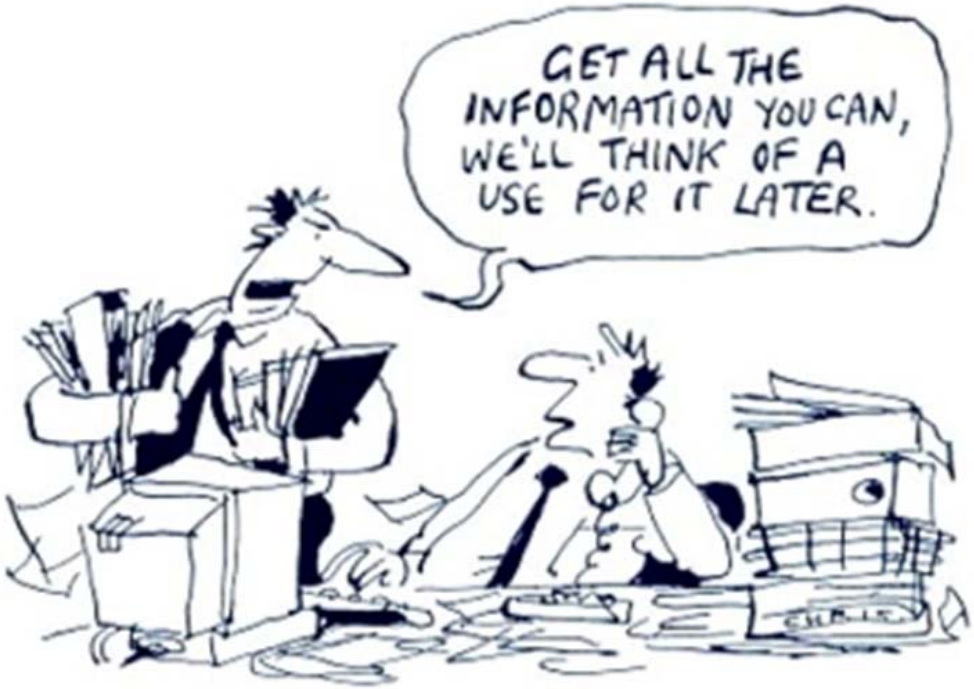
Why Do We Need Bioinformatics?



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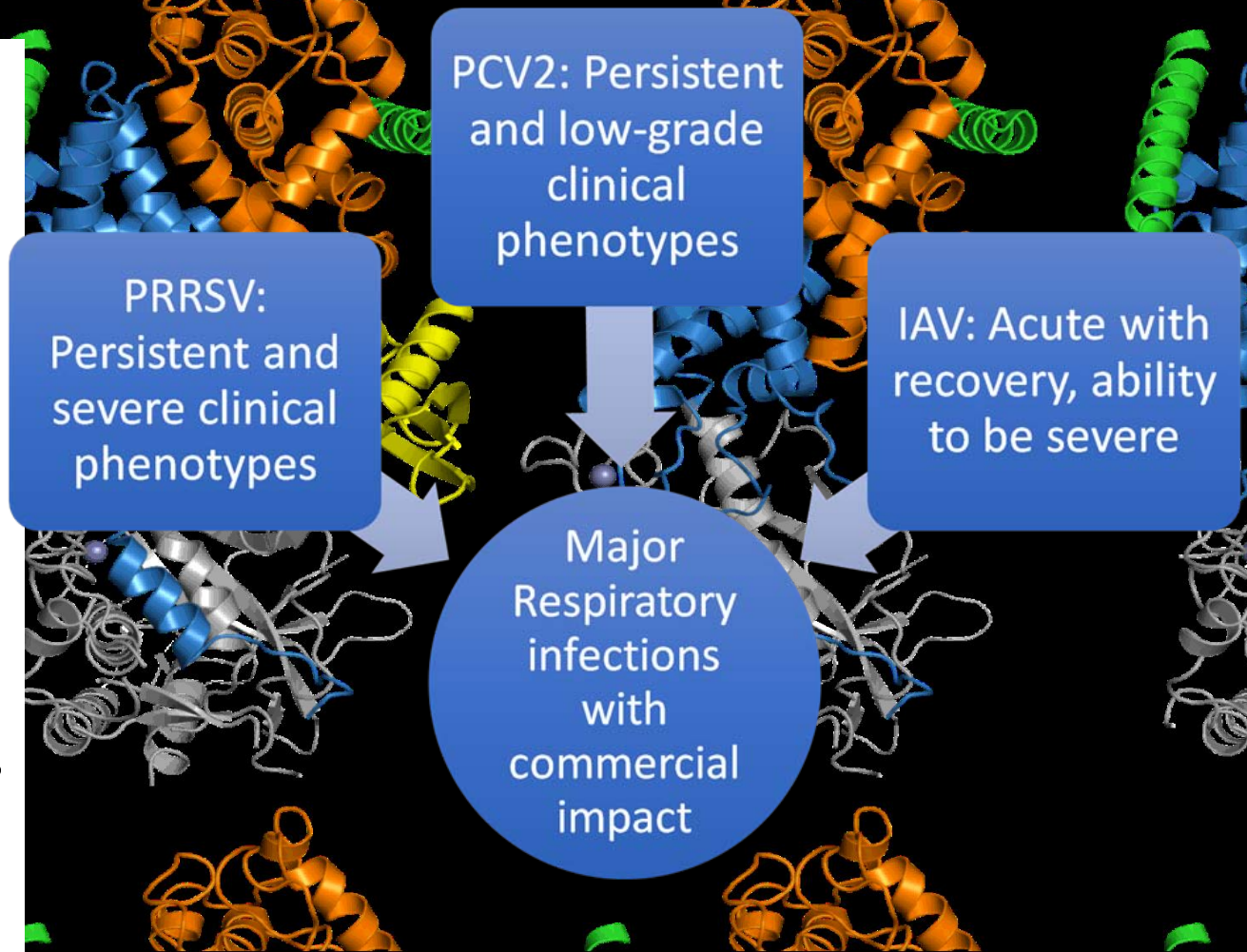
- This investigation was a first in a series of *in vivo* comparative studies into the swine host immune response to several major porcine respiratory infections.
  - Porcine reproductive and respiratory syndrome virus (PRRSV)
  - Porcine circovirus type 2 (PCV2)
  - Swine influenza virus (IAV)

- Aim of this study was to acquire a better understanding of PRRS disease by comparing gene expression changes that occur in tracheobronchial lymph nodes (TBLN) of pigs infected these viruses.



- Experimental Design
- Pigs were allotted to one of 4 treatment groups:
  - sham inoculated control,
  - PRRSV-challenge (SDSU strain),
  - PCV2-challenge,
  - IAV-challenge.
- Pigs received an intranasal challenge with 2 ml of either sham or virus inoculum. Control pigs were sham inoculated with tissue culture supernatant.
- Five pigs from each group were euthanized and necropsied on 1, 3, 6, and 14 dpi.
- TBLN were homogenized and aliquots used for RNA extraction.
- Total RNA was pooled for each group within time point to make 16 libraries, for DGETP SAGE sequencing.

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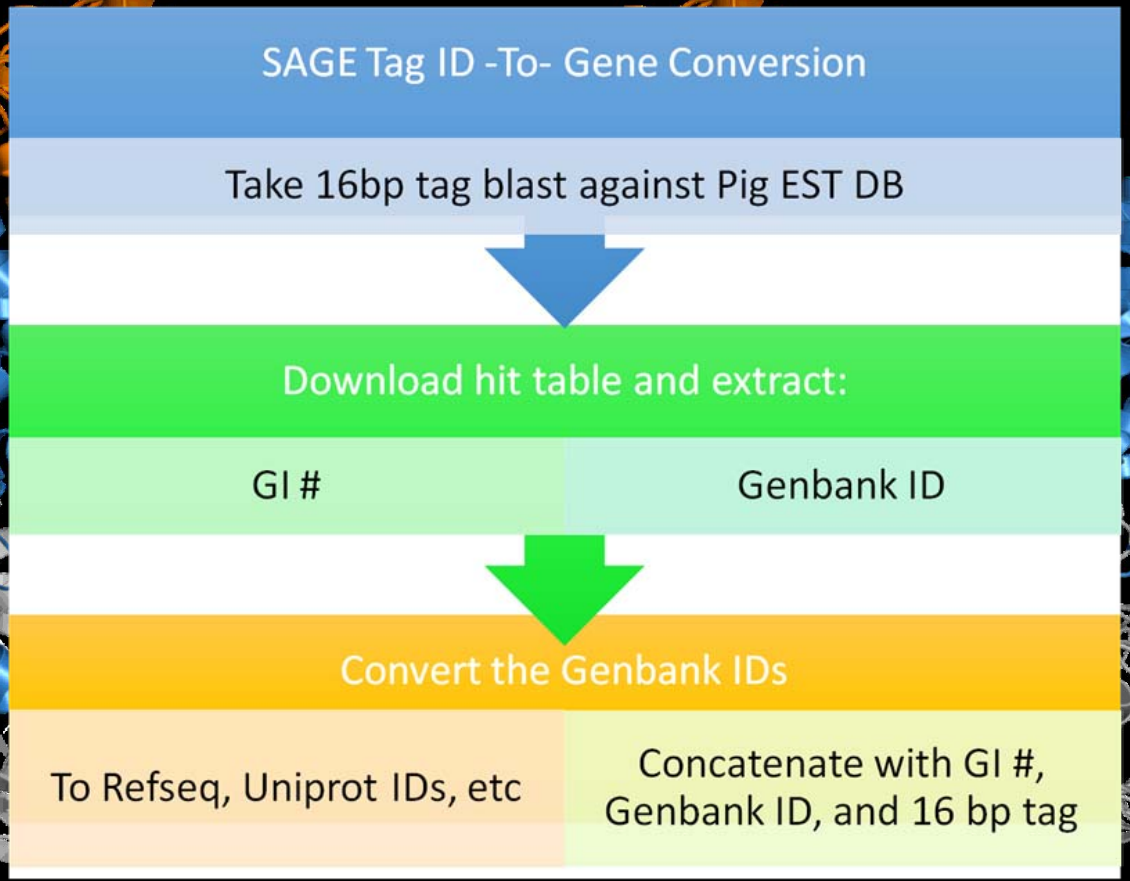
- SAGE (Serial analysis of gene expression)
  - Tag sequences obtained from **3' end within cDNA that are long enough to *uniquely identify*** each transcript.
  - Sensitive to low-abundant transcripts and small changes in **gene counts.**

- Caveats
  - *However, **older method** of sequencing reliant on genome annotation.*
  - Sequence tags ***need to be linked to known ID***.
  - Identified tags can be ***normalized as counts***.



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- 1 main effect: Treatment (n=4)
  - ***Control, PRRSV(SDSU), PCV2, IAV (H1N1)***
- 1 cofactor: Time (n=4)
  - ***1 DPI, 3 DPI, 6DPI, 14 DPI***
- Based on reduced model *Y= ~Treatment +Time + E*
- 3 separate runs of Control vs. Virus
- Fit-type parametric; FDR applied ***Q ≤ 0.1***
  - Counts over 3000 genes
  - Tag sequences with **5 counts or less removed**
  - Dispersion fit type: parametric

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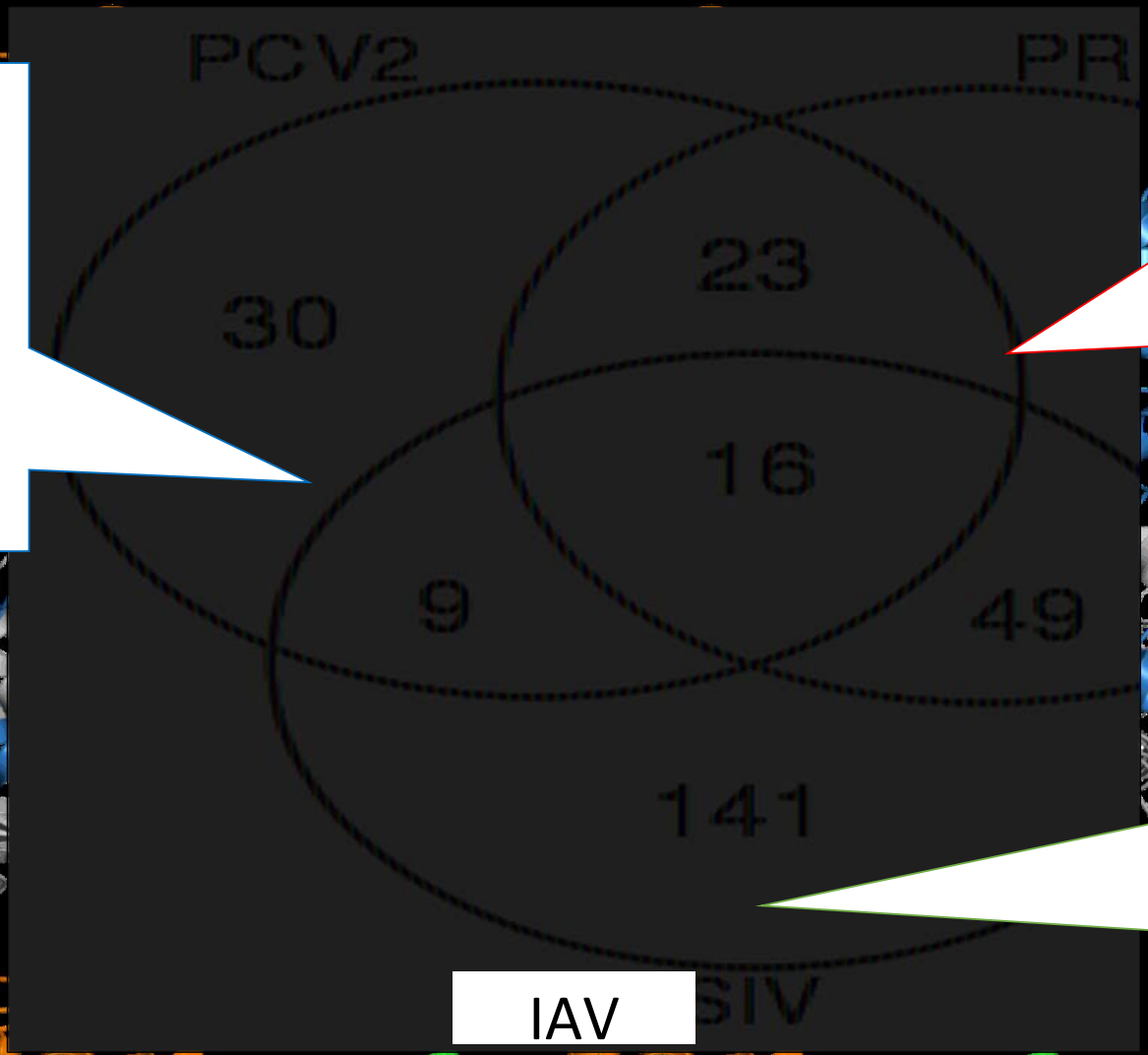
- **PCV2**
  - only 78 genes significant
  - 44% downregulated
  - 56% upregulated



- **PRRSV**
  - Total of 308 DEG
  - 43% down regulated
  - 57% upregulated

- **IAV**
  - Total of 215 DEG
  - 35% downregulated
  - 65% upregulated

-1.3	-0.91	Endocytosis of glycoproteins by macrophages	Phagosome, Adaptive Immune System
-1.62	-0.79	Reactive oxygen species metabolic process	Signal Transduction
-1.73	-0.51	Extracellular matrix organization	ECM proteoglycans, Integrin cell surface interactions
1.21	0.67	Exocytotic and endocytotic pathway regulation	AMPK signaling pathway
0.95	0.73	Oxidation-reduction process	Pyruvate metabolism
2.21	0.79	Extracellular matrix disassembly	Degradation of the extracellular matrix

share ~ 1/3

twice the amount

•*Overlap*

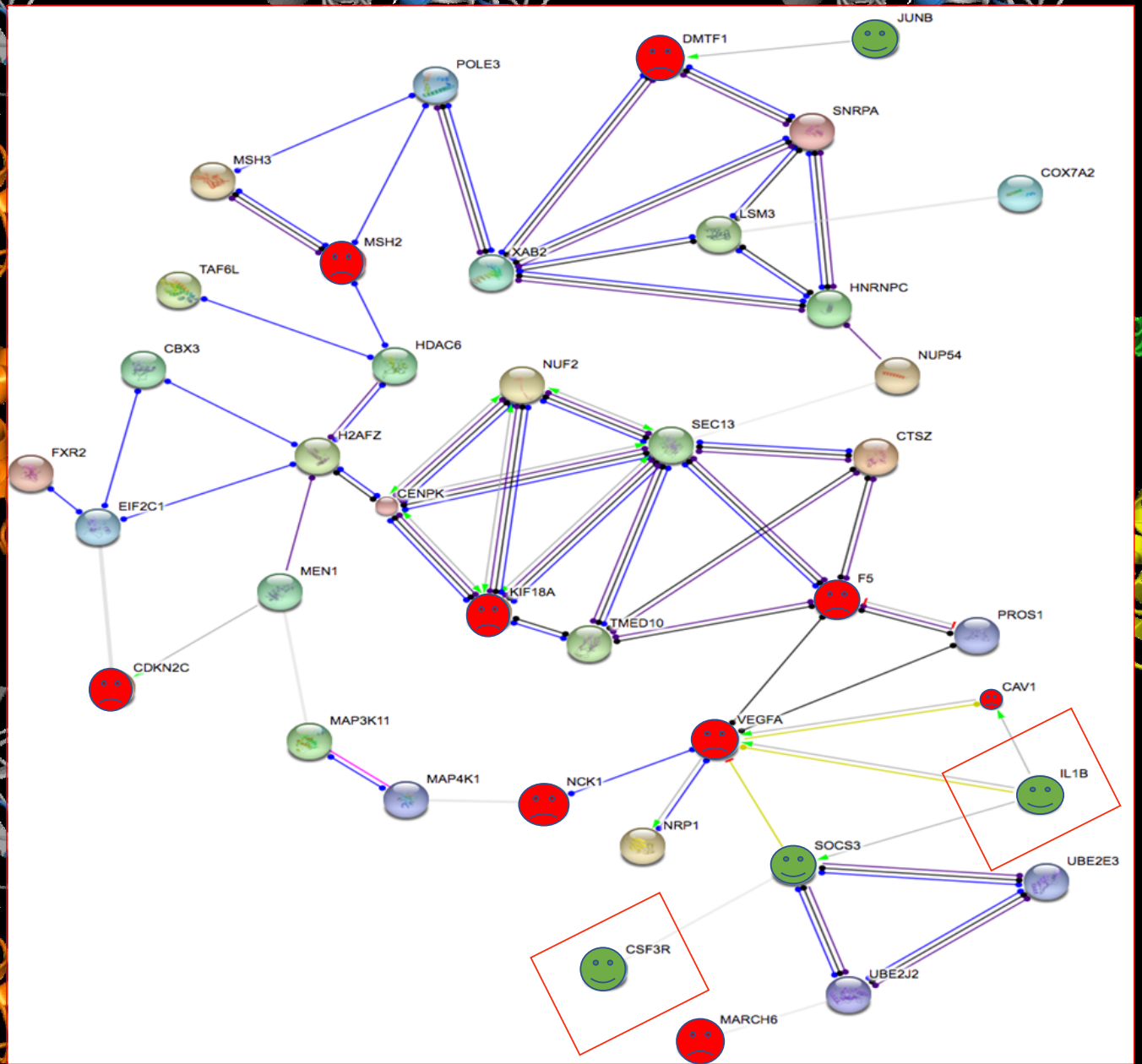
PRRSV & PCV2

ECM matrix, ECM receptor, and cytokine induction pathways

-2.83	GO:0019221 cytokine-mediated signaling pathway
-2.64	GO:0071345 cellular response to cytokine stimulus, GO:0002250 adaptive immune response
-2.34	GO:0010467 gene expression
-1.70	GO:0002684 positive regulation of immune system process
-1.44	GO:0045321 leukocyte activation
-0.95	GO:0045321 leukocyte activation, GO:0002250 adaptive immune response
1.60	GO:0019221 cytokine-mediated signaling pathway
1.44	GO:0019221 cytokine-mediated signaling pathway
1.45	GO:0045321 leukocyte activation, GO:0002250 adaptive immune response
1.94	GO:0045087 innate immune response

CXCL13 helps initiate the switch from innate to adaptive

SOCS3 a suppressor of cytokine signaling viral mediated anti-inflammatory induction



The **red box** containing the immune response genes is likely trying to increase inflammatory signaling.

Downregulation of the "hub" genes (red circles) is likely preventing downstream signaling to increase inflammation and/or signal adaptive immunity.

-1.68	GO:0006955 immune response
-1.51	GO:0006955 immune response
-1.45	GO:0038093 Fc receptor signaling pathway
-1.41	GO:0007155 cell adhesion
-1.11	GO:0019058 viral life cycle
1.11	GO:0007155 cell adhesion
1.15	GO:0002443 leukocyte mediated immunity, GO:0080134 regulation of response to stress
1.23	GO:0002443 leukocyte mediated immunity, GO:0080134 regulation of response to stress
1.26	GO:0007159 leukocyte cell-cell adhesion, GO:0080134 regulation of response to stress
2.05	GO:0071345 cellular response to cytokine stimulus, GO:0080134 regulation of response to stress
2.14	GO:0038093 Fc receptor signaling pathway
2.21	GO:0071345 cellular response to cytokine stimulus

innate immune

responses *cellular-*

mediated immune

72, and ELMO1

CD 247, CD

cellular disadvantages to recovery

Conclusion

- The results showed that *PRRSV, IAV and PCV2* viral infections followed a clinical course in the pigs typical of experimental infection of young pigs with these viruses.
- The overlap of expressed genes between PRRSV and PCV2 uncovered an expanse of molecules that play roles in *immune, redox, and structural functions* that may *elucidate co-infections*.
- For the PRRSV infected pigs, we mostly witnessed *downregulation* of genes related to *signaling processes* that can initiate *adaptive immunity*.
- For IAV infected pigs, it is likely the differential expression observed is more closely related to *oxidative and nutritive stress recovery* of the host at later time points.

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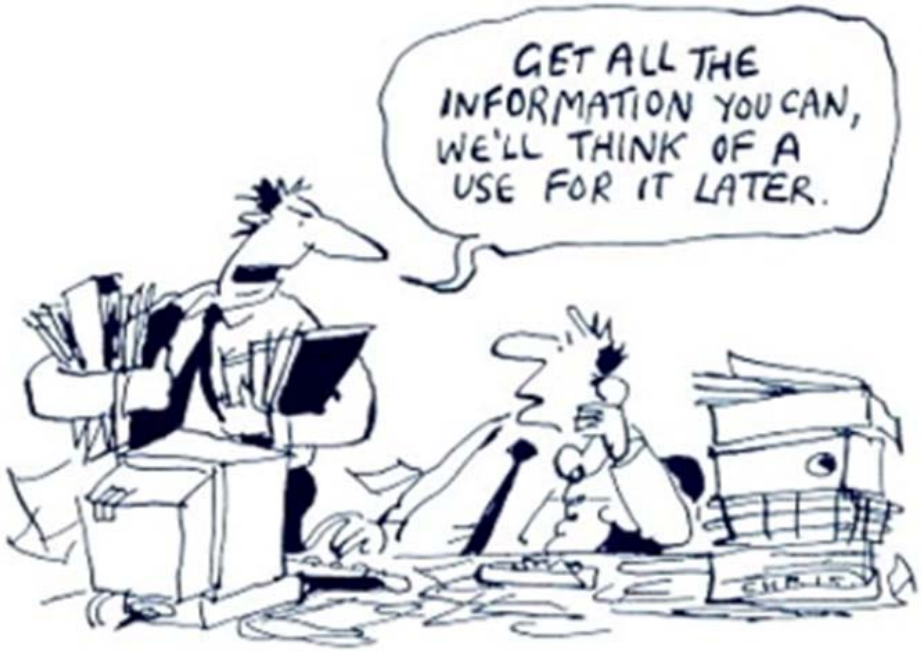

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