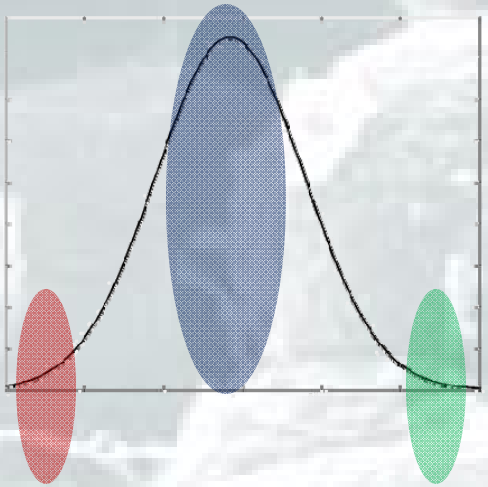
Objective 4.3 Novel Molecular Markers

Exposure → Infection → Disease / Subclinical Infection / Resolution



Are resilient animals doing something quantitatively different to counter clinical disease?

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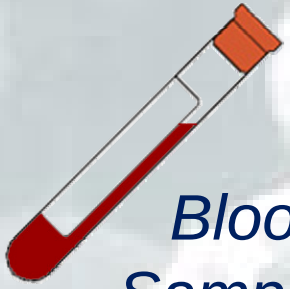
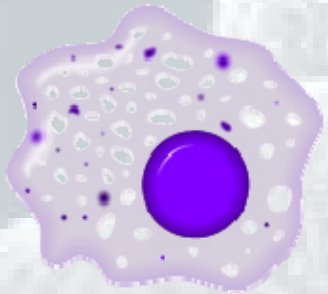
To be truly useful we need to access tissue in the live animal =



Blood Sampling



Macrophage

= Haematological target of most relevance

Immune targets most easily measured via =

Gene Expression

The 4.3 Brief...

- Identify Resilient and Susceptible animals
- Extract and grow cervine macrophages *in vitro.*
- Challenge macrophages with *Map*.
- Compare readouts from R and S types. and identify distinguishing characteristics.

Milestone 4.3 Novel Molecular Markers for Johne's Resistance (Year 3)

The researchers will undertake the following services in Year 3:

a) Select deer with polarised breeding values for resistance or susceptibility to Johne's Disease.

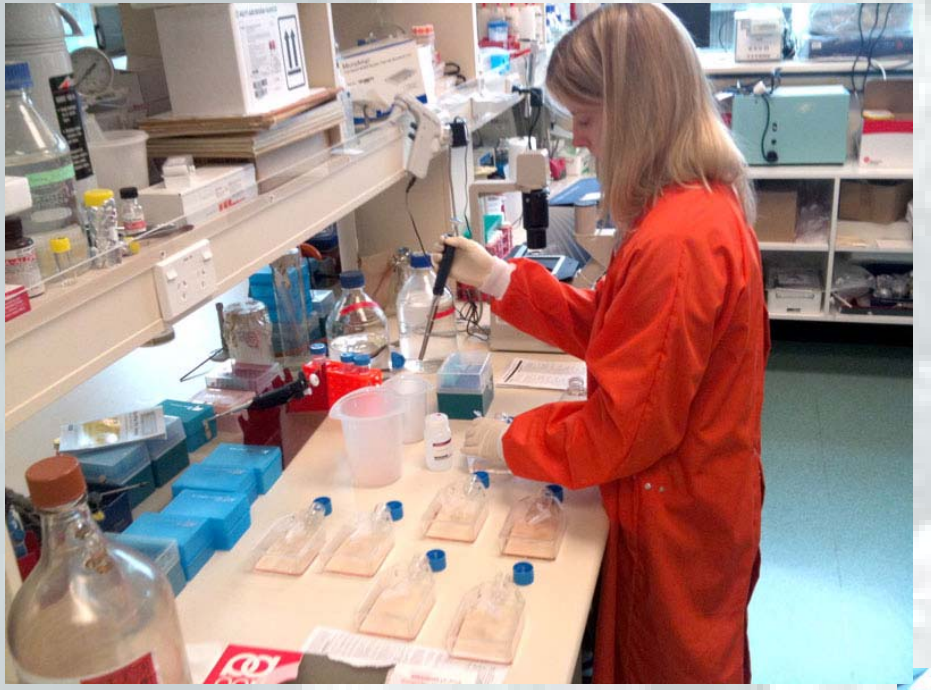
b) Further refine macrophage cultures *in vitro* for increased RNA recovery necessary for (e).

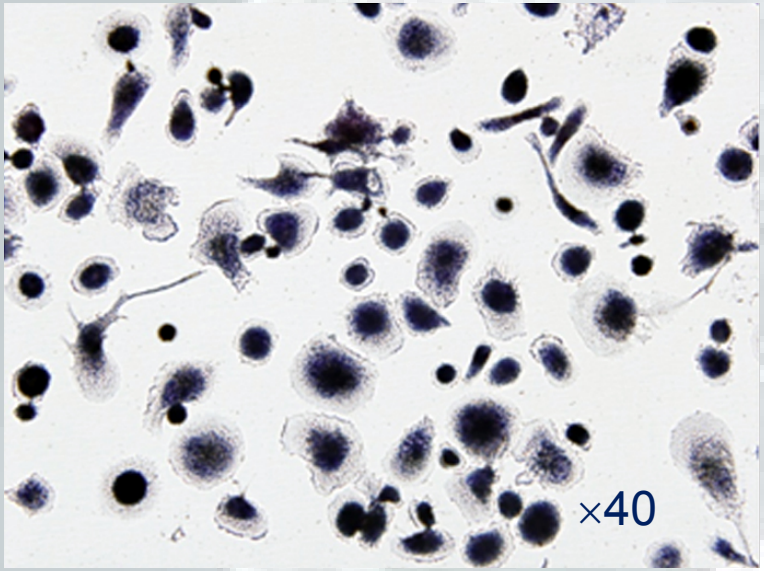
c) Harvest macrophages from at least 6 deer of different genotypes which are known to express either Resistant or Susceptible phenotypes for *Map* infection.

d) Determine gene expression levels for candidate genes known to be associated with basic macrophage function in R and S deer.

e) Informed by the results (c) and (d), perform a primary transcriptome analysis for identification of as yet unknown genes expressed differentially in R/S macrophages

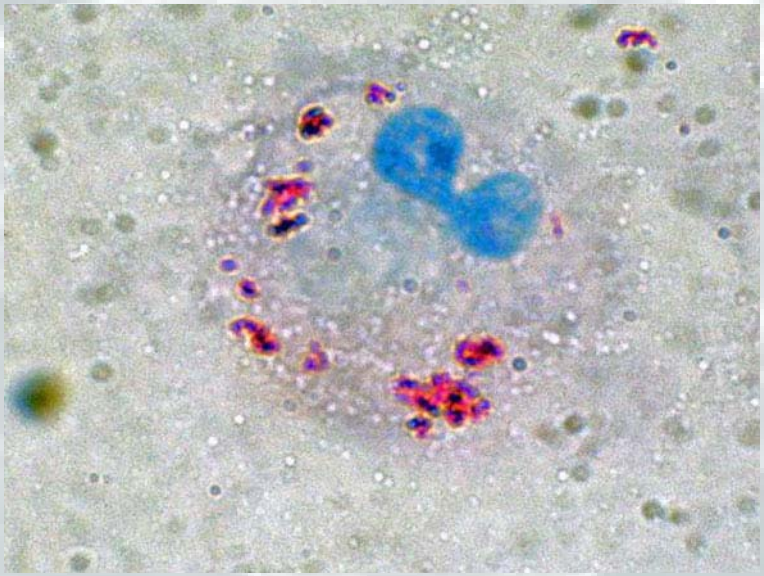

Compoflex
2011.0g
Fresenius Kabi
REF
T2118
LOT
40CG2048

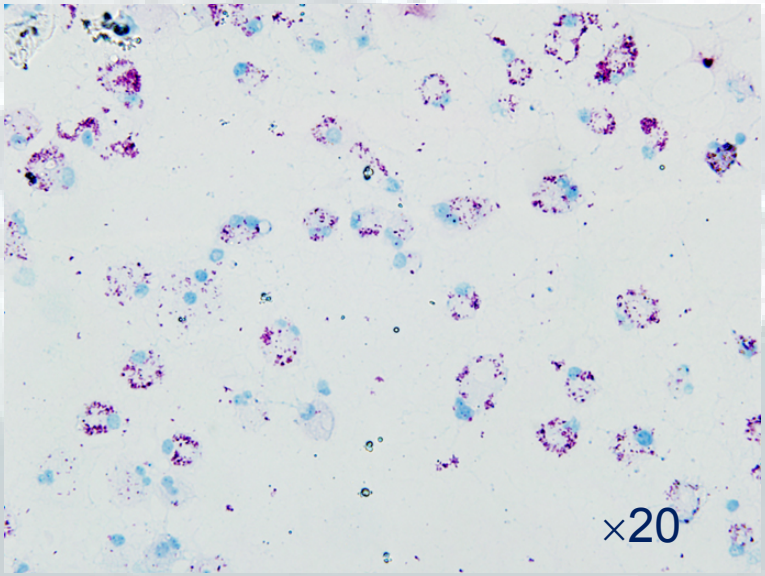




Mature MDMsstained
for α-Naphthyl acetate esterase

MDMs infected with *Map*

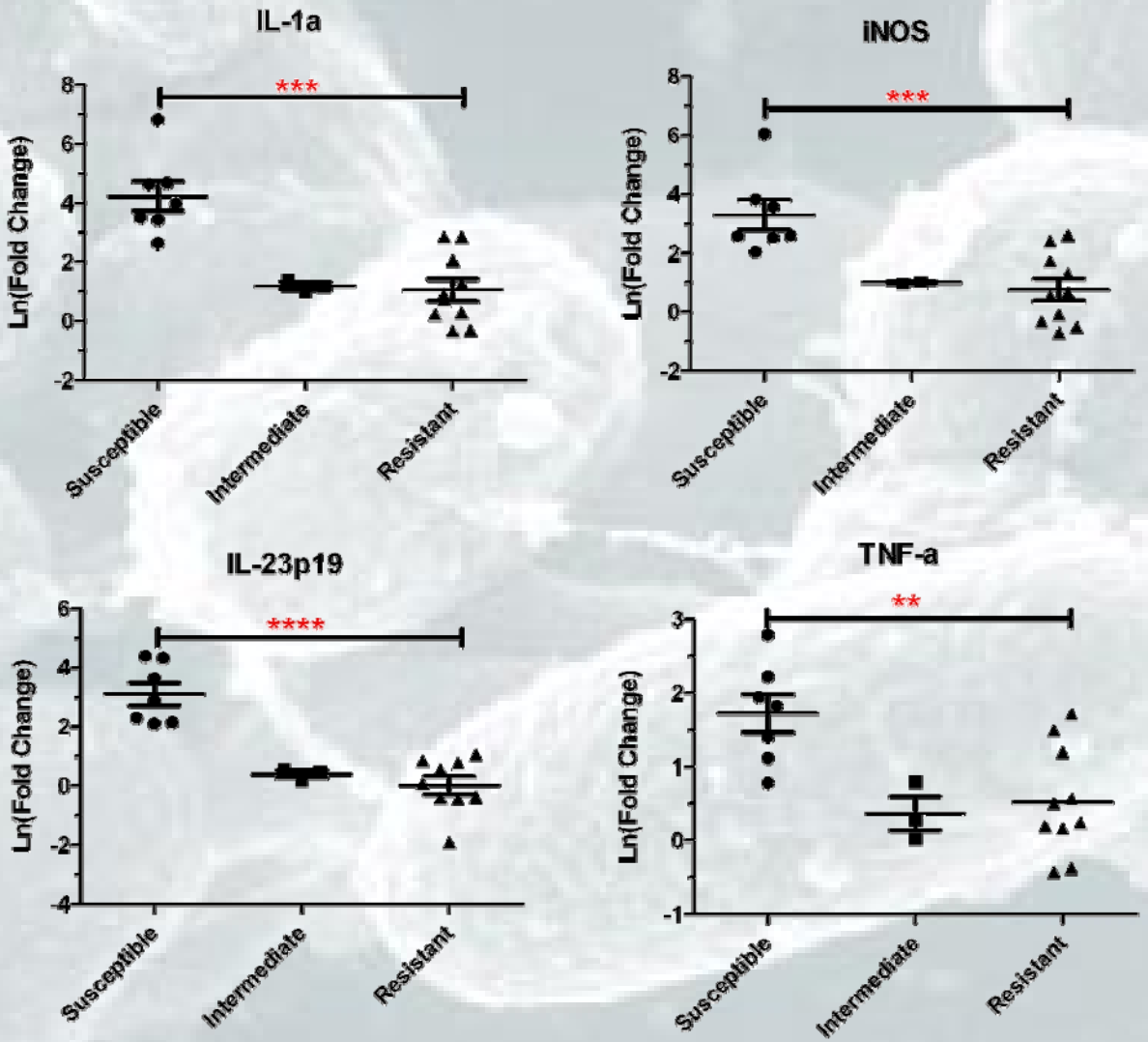




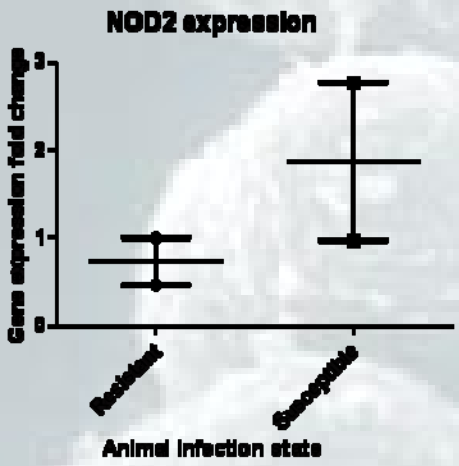
The 4.3 Brief...

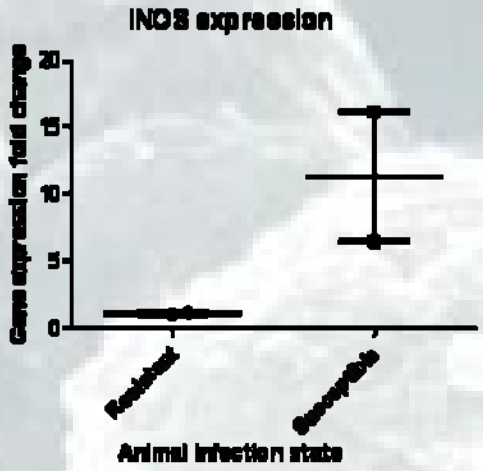
- Identify Resilient and Susceptible animals
- Extract and grow cervine macrophages *in vitro.*
- Challenge macrophages with *Map*.
- Compare readouts from R and S types. and identify distinguishing characteristics.

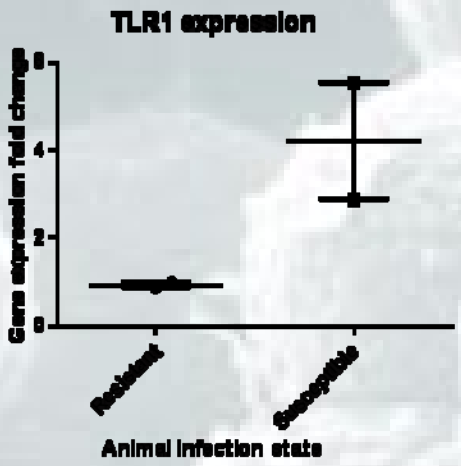
- 20 yearling animals of mixed breed/sex.
- JBVs ranging between +0.48 (S) and -0.57 (R).
- All tested Paralisa negative.
- Macrophages infected with *Map* at 10:1 for 24 hrs.
- Tested for iNOS, IL-1α, TNFα, IL-10, IL12-p35 and IL-23p19.

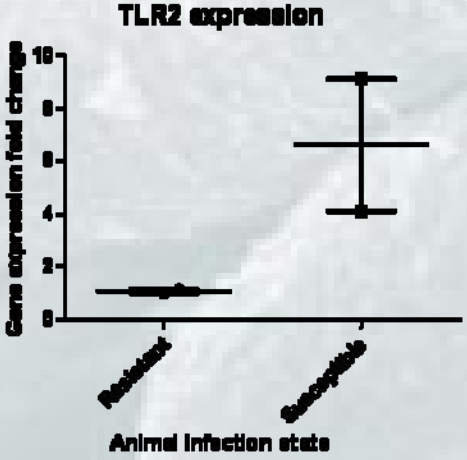


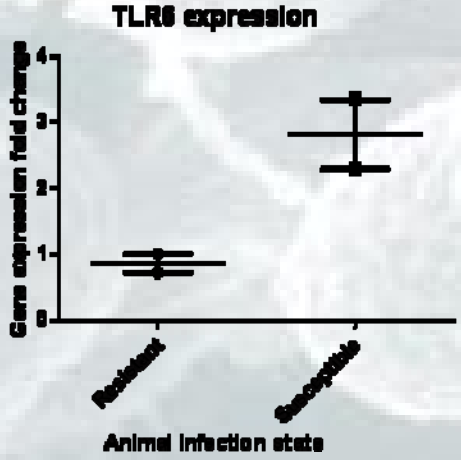
Polarised R/S outcomes from Colin's Biopsy Challenge exhibit similar trends -

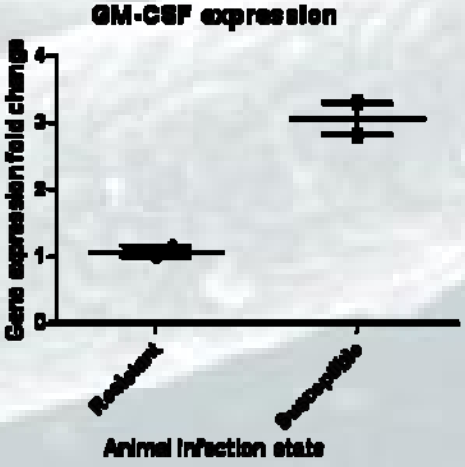












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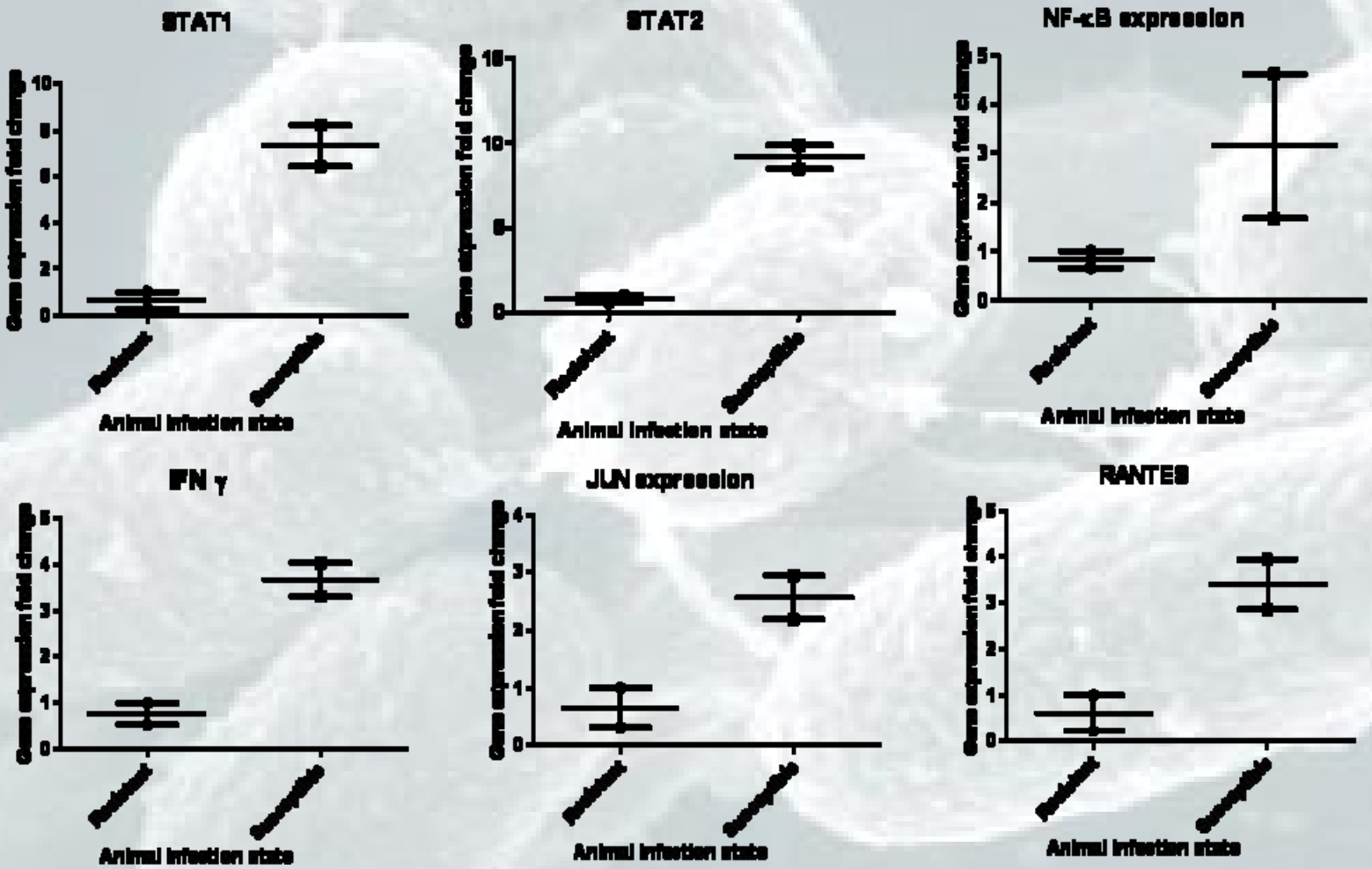
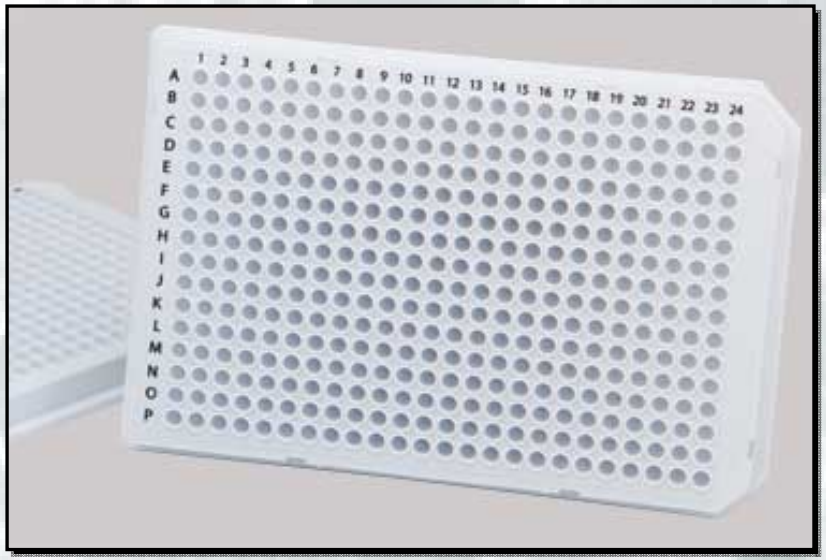


Cervine Innate & Adaptive Immune Targets Currently Developed-

IL-10	Anti-inflammatory Responses	**NOD1**	Intracellular PAMP	**RORC**	Transcription Factor
IL-19	Anti-inflammatory Responses	**NOD2**	Intracellular PAMP	**STAT1**	Transcription Factor
IL-4	Anti-inflammatory Responses	**RANTES**	Leucocyte Recruitment	**STAT2**	Transcription Factor
HLA-DMB	Assists MHC	**B2M**	Normalisation Gene	**STAT3**	Transcription Factor
IL-17	Autoimmunity	**IL-18**	Proinflammatory Cytokine	**STAT4**	Transcription Factor
P53	Cell Cycle Regulator	**IL-6**	Proinflammatory Cytokine	**STAT5A**	Transcription Factor
MAPK11	Cell Differentiation	**IRF3**	Regulatory Factor	**STAT5B**	Transcription Factor
MAPK14	Cell Differentiation	**MyD88**	Signal Transduction	**STAT6**	Transcription Factor
MAPK8	Cell Differentiation	**TRAF6**	Signal Transduction	**Tbet**	Transcription Factor
SOCS1	Cytokine Suppression	**TRAF1**	Signal Transduction	**IL-1b**	Inflammatory Responses
SOCS2	Cytokine Suppression	**IL-12p35**	Stimulation of T cells	**NF-kB**	Inflammatory Responses
SOCS3	Cytokine Suppression	**IL1-α**	Stimulation of T cells	**NLRP3**	Inflammatory Responses
SOCS4	Cytokine Suppression	**IL-23p19**	Stimulation of T cells	**TNFα**	Inflammatory Responses
TLR1	Environmental Sensing	**IL-2**	T Cell Signalling	**PIAS1**	Inhibition of STAT Signaling
TLR2	Environmental Sensing	**FoxP3**	T Reg Cells	**PIAS2**	Inhibition of STAT Signaling
TLR4	Environmental Sensing	**CREB**	Transcription Factor	**PIAS4**	Inhibition of STAT Signaling
TLR6	Environmental Sensing	**FOS**	Transcription Factor	**IFNβ**	Innate immunity
GM-CSF	Growth Factor	**GATA3**	Transcription Factor	**IFNγ**	Innate/Adaptive immunity
RANK	Immune Signaling	**JUN**	Transcription Factor	**iNOS**	Intracellular killing

JDRC Strategic Goals
(development and delivery of cost effective tools for farmers)

- Array expression targets in 384 well format
- A tool for breeders to assess immunological responses to mycobacterial challenge in *naïve* animals?



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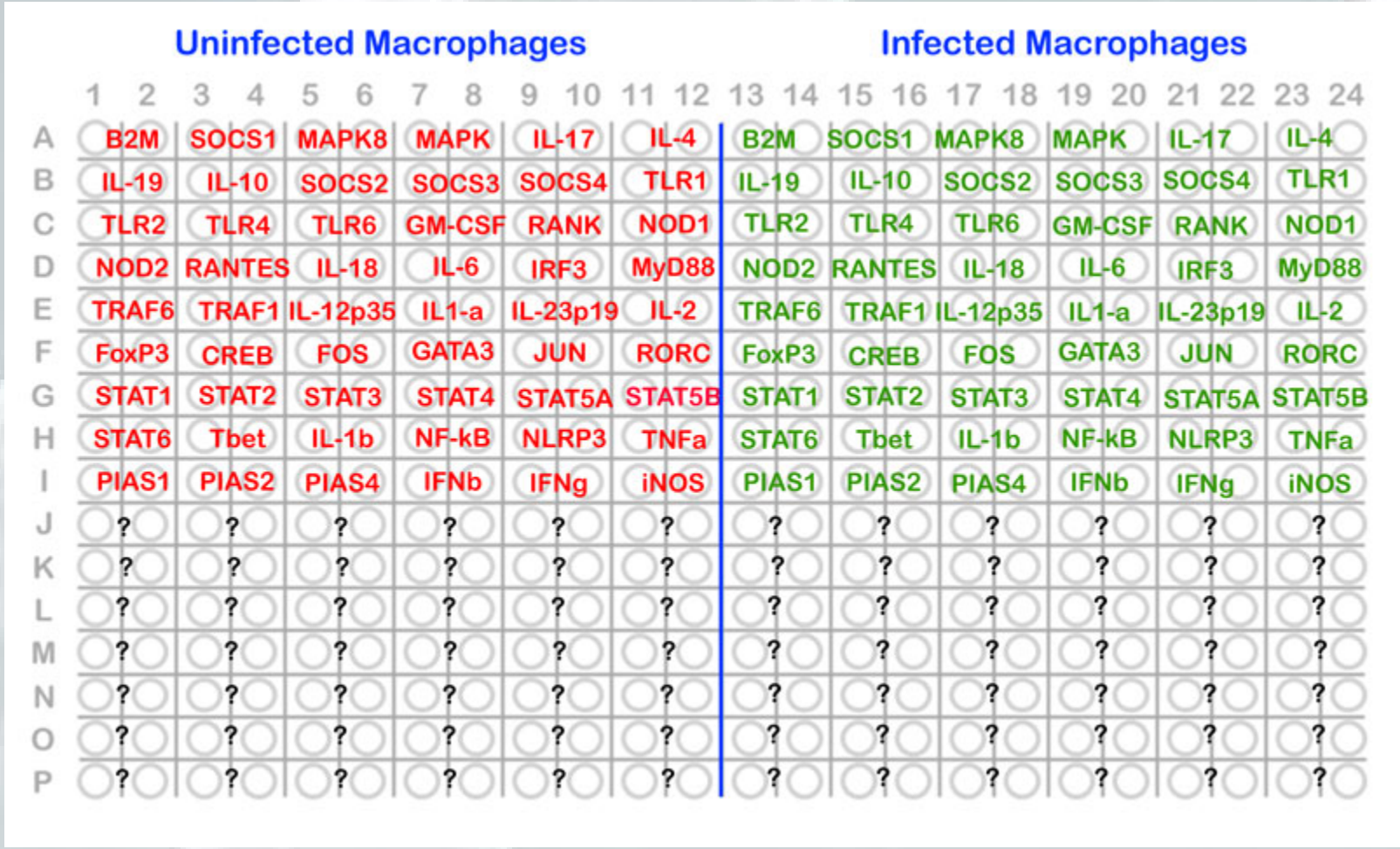

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Transciptomics

- Candidate genes can only tell us what we already *think* to look for; we don't know what we don't know.
- Next Generation Sequencing has made dramatic strides in recent years in terms of cost and amount of RNA necessary.
- 454 Sequencing (Roche) preferred technology.
  - Longer read lengths (~500bp) assist assembly and transcript ID.
  - Requires greater amounts of input RNA.
  - Currently no service provider in NZ.

Acknowledgements

- Ms Brooke Dobson
  PhD Candidate
  University of Otago
- Johne's Disease
  Research Consortium
- Dr Colin Mackintosh
  AgResearch Invermay.
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- All the Vets and Farmers who have contributed to these efforts through their submissions and ongoing support.

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