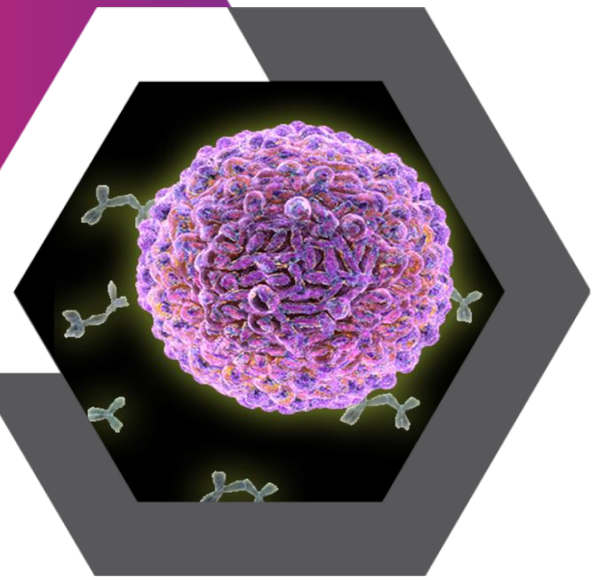

Dissecting polyclonal responses

John Hammond
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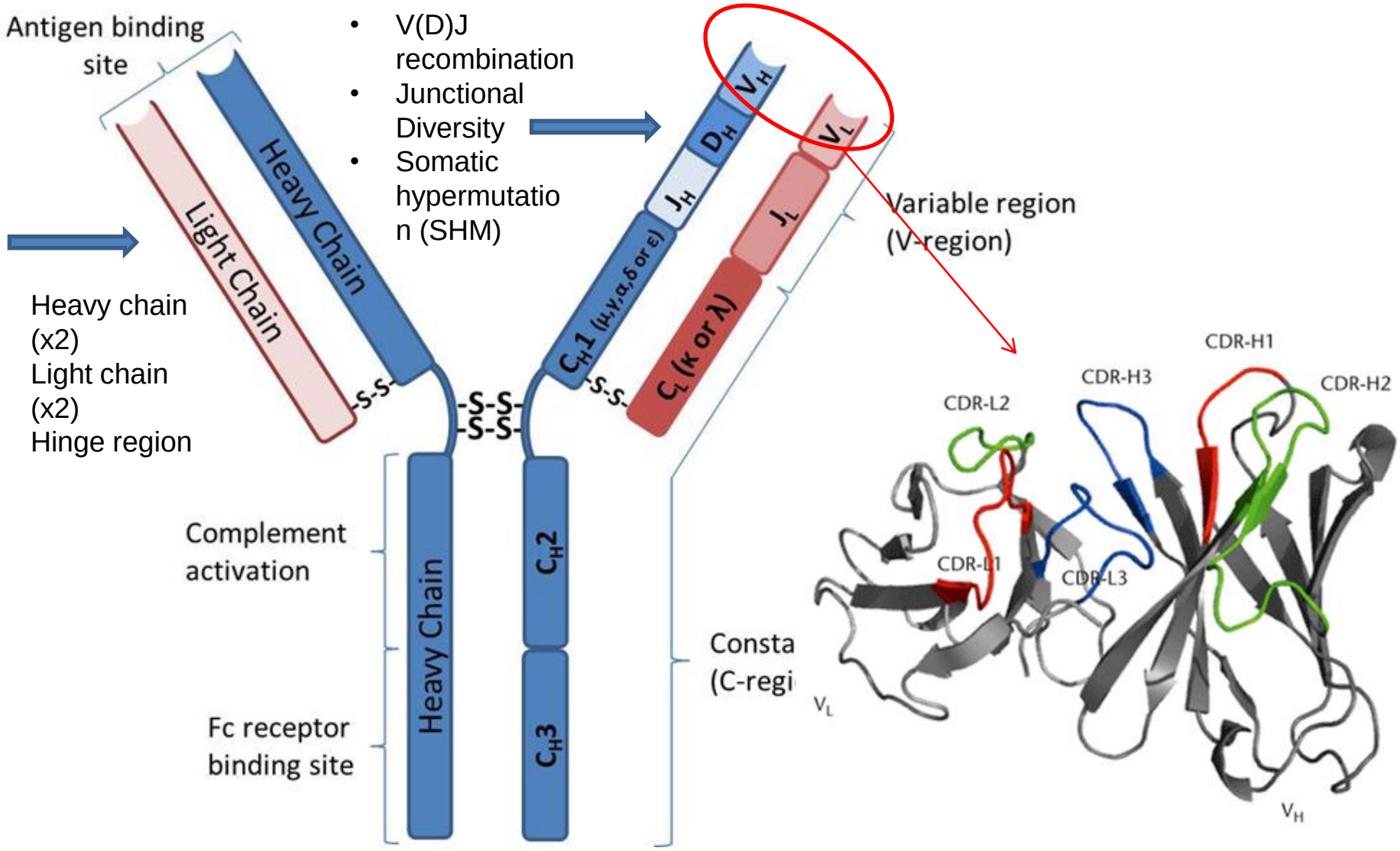
BBSRC bioscience for the future
The Pirbright Institute receives strategic funding from BBSRC.

Antibody structure

- Heavy chain (x2)
- Light chain (x2)
- Hinge region

- V(D)J recombination
- Junctional Diversity
- Somatic hypermutation (SHM)



What are we trying to do?

1. Identify (and quantify) the hallmark of an FMDV specific antibody response in cattle

2. Identify antibodies that are serotype specific or cross reactive for multiple FMDV serotypes

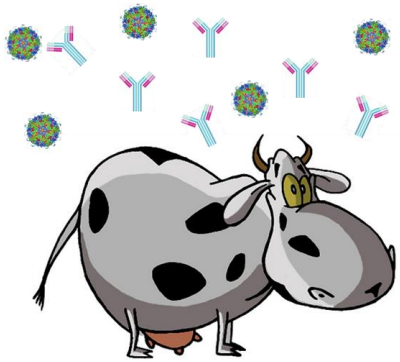
Requirements:

An unbiased characterization of the antibody repertoire

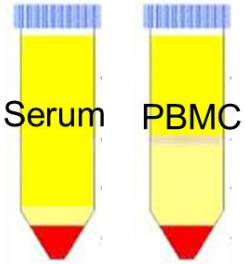
Reference-free repertoire analysis

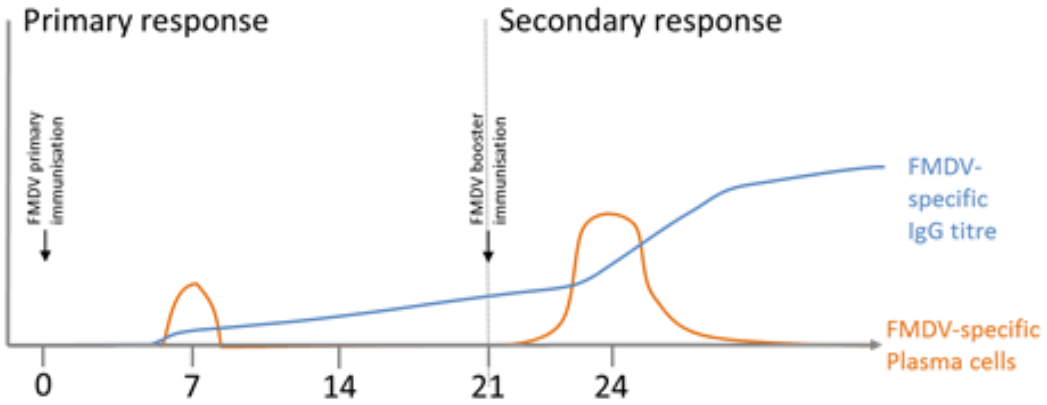
Identification of antigen specificity within the repertoire

How are trying to do it?



Vaccinated cattle





- ELISpot
- Neutralising antibody titre
- Non-neutralising antibody titre

- Heavy and light chain amplification and deep sequencing
- Single B cell sorting and amplification of heavy and light chain
- FMDV capsid pull-down and mass spec of serum antibodies

Vaccination regimes

Single serotype – Prime and boost regime.

- 4 animals per group (4 serotypes) – total 16 animals
- Booster vaccination given a day 21

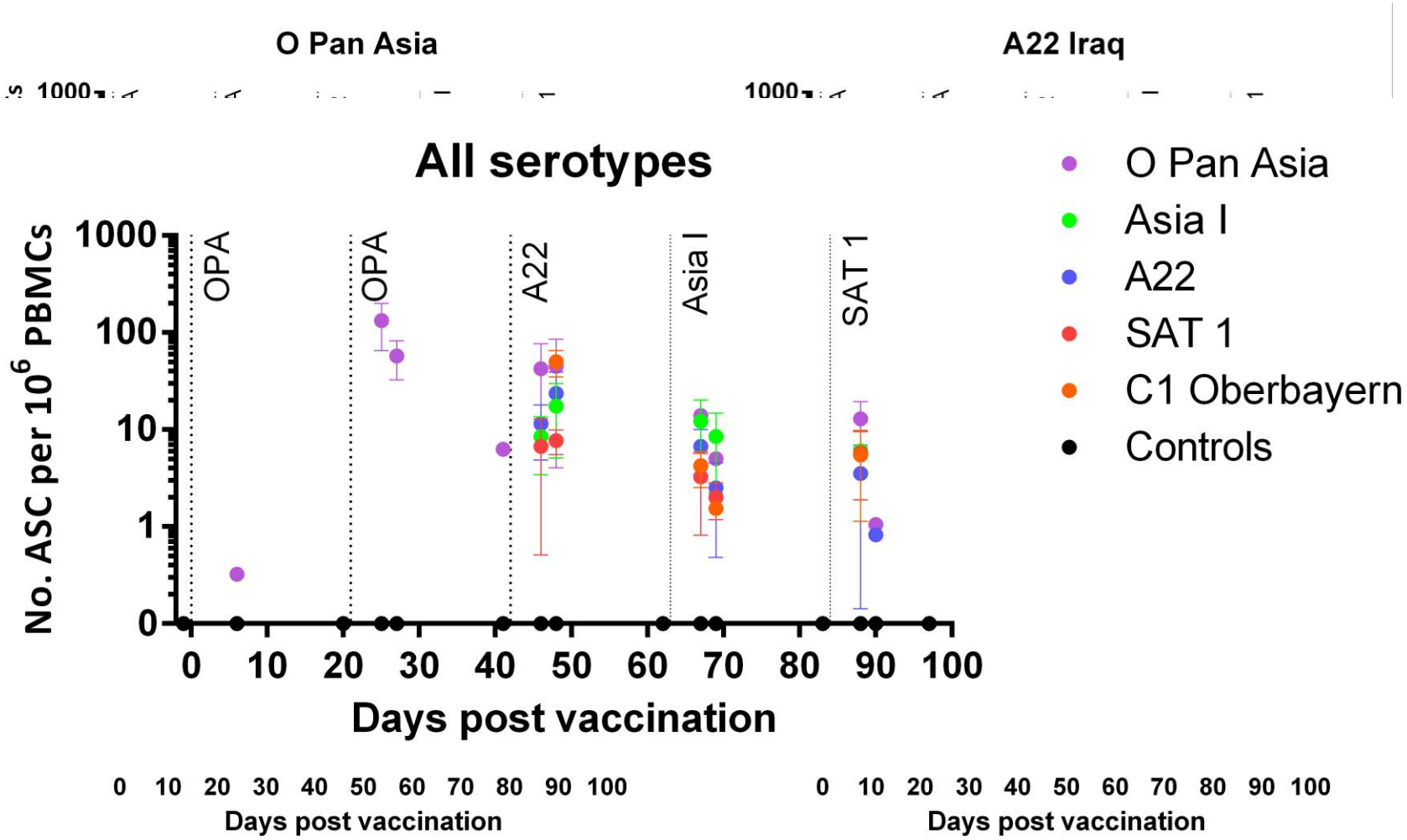
Prime immunisation (O Panasia II, A22 Iraq, Asia I Shamir or SAT I Zim)	D-1, D7, D20
Booster immunisation (O Panasia II, A22 Iraq, Asia I Shamir or SAT I Zim)	D25, D27, D34, D67 (LN only)

Sequential serotype regime

- 4 animals plus 2 control animals (total 6 animals)
- Vaccinations given 21 days apart

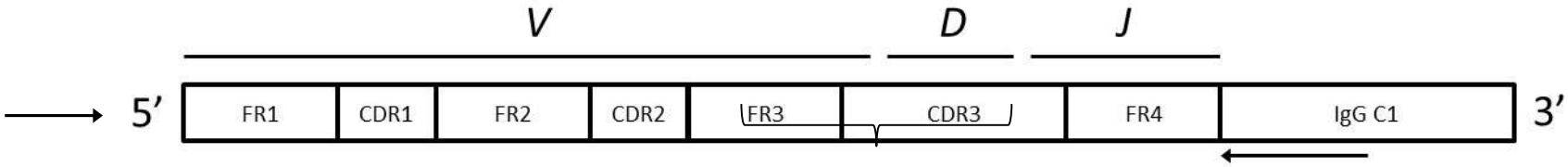
1. D-1, D7, D20
2. D25, D27, D41
3. D46, D48, D62
4. D67, D69, D83
5. D88, D90, D97 and D102 (LN only)

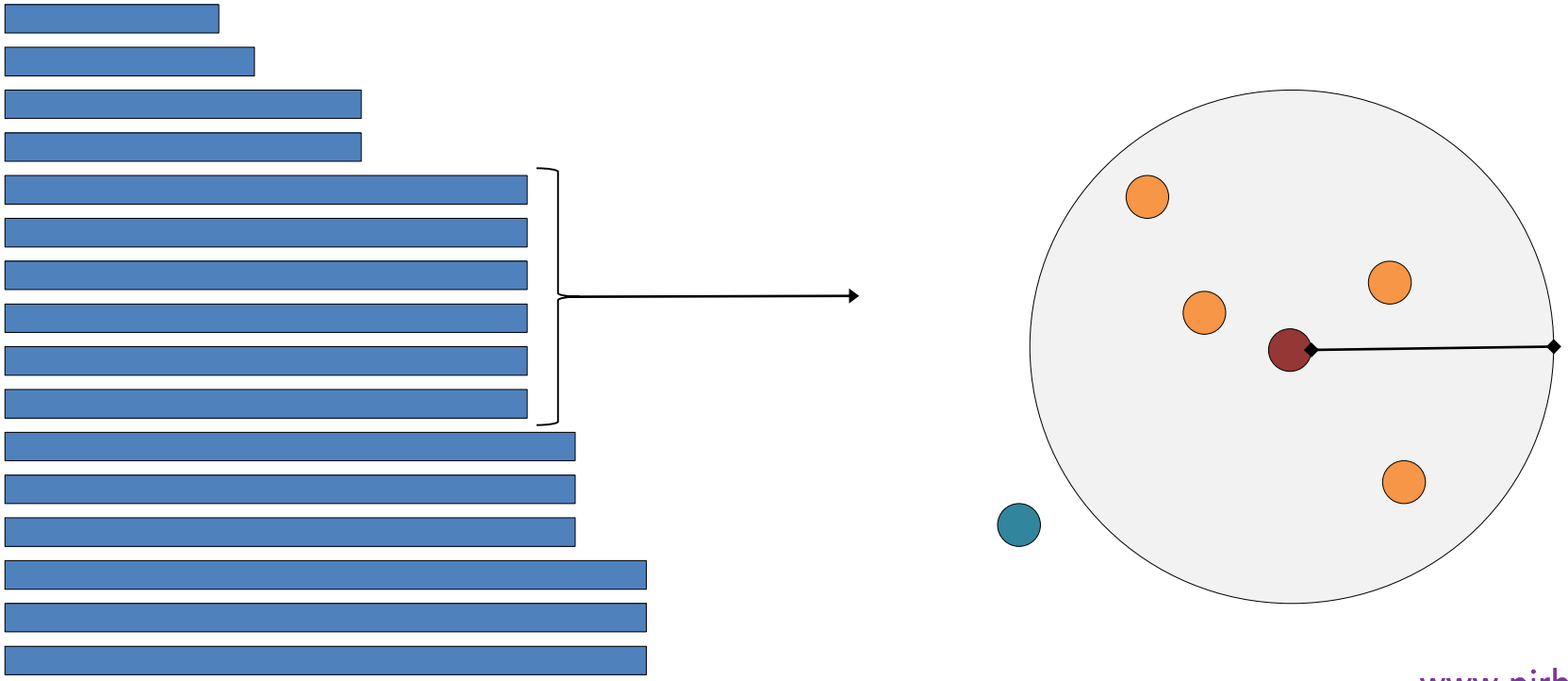
Kinetics of the FMDV-specific plasma cell response following sequential immunisation



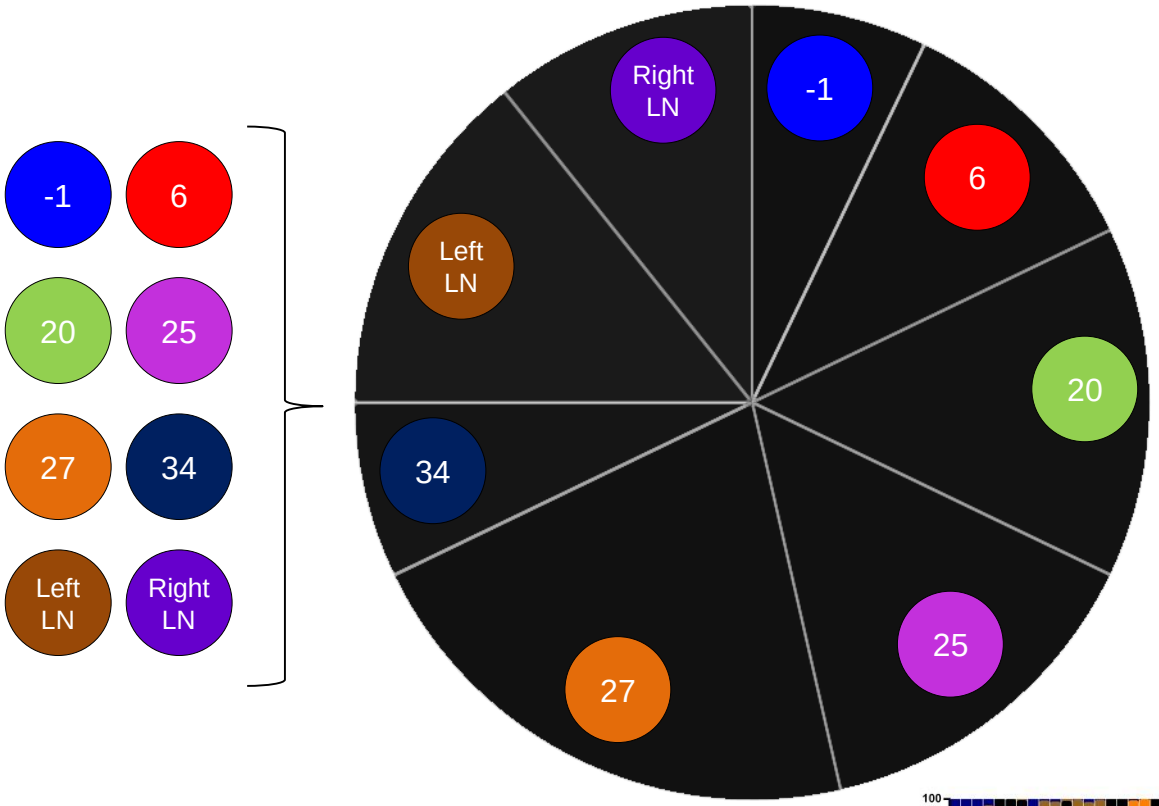
HCDR3 clustering

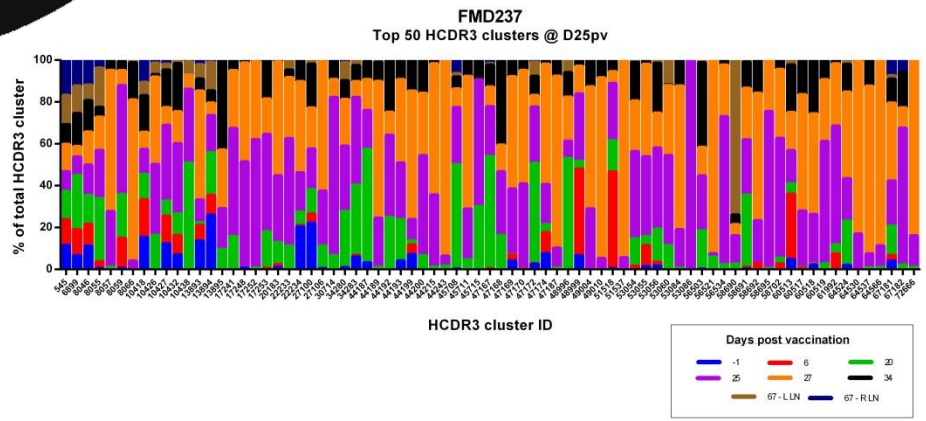
218 samples ~250000 reads per sample





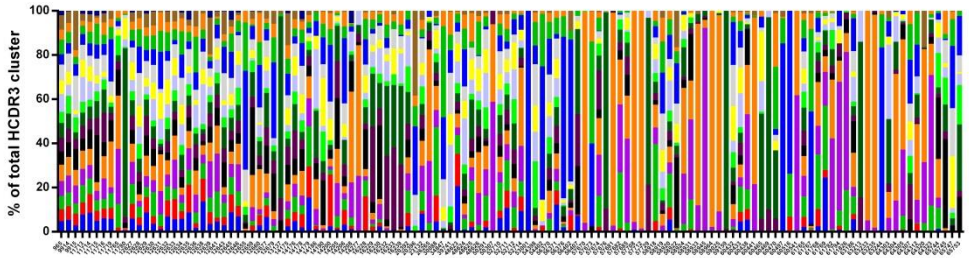
HCDR3 clustering by animal over time



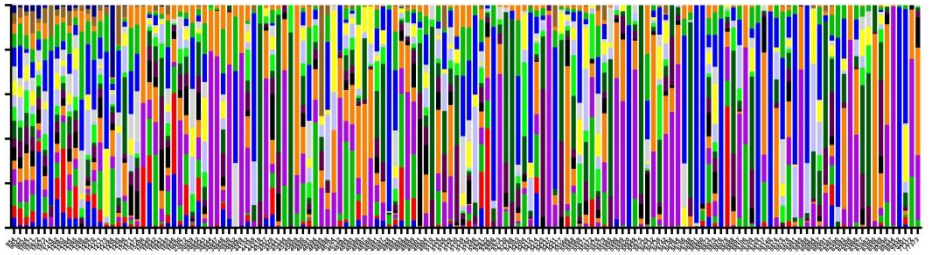


HCDR3 clustering – Sequential serotype vaccinates

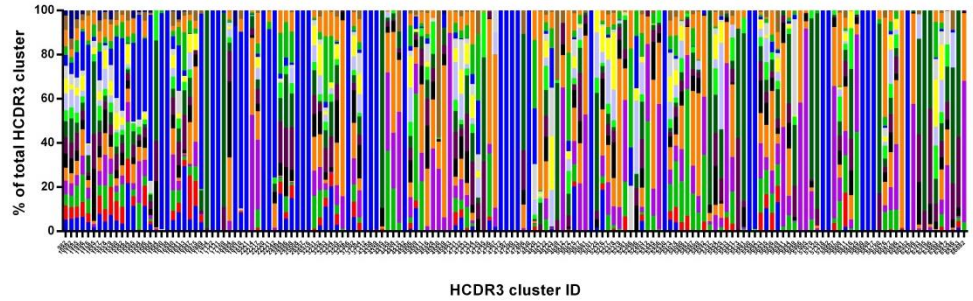
FMD251



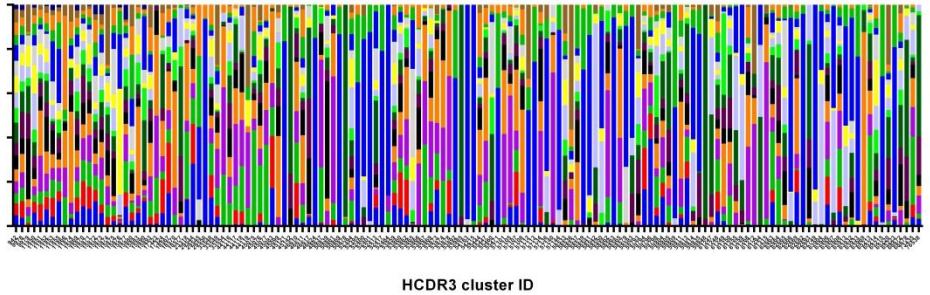
FMD253



FMD252

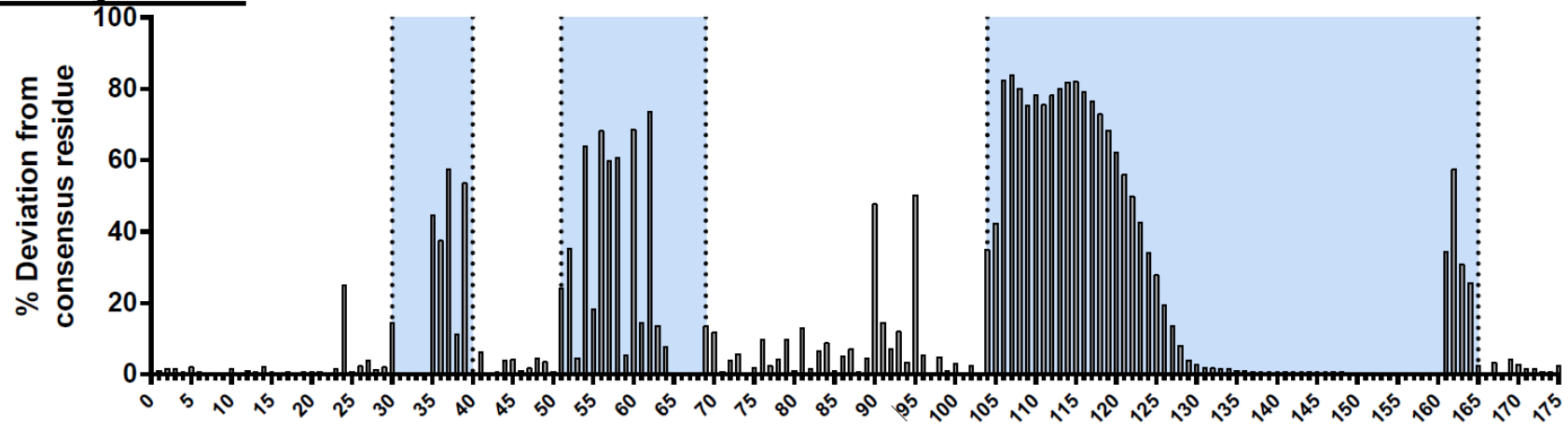


FMD254

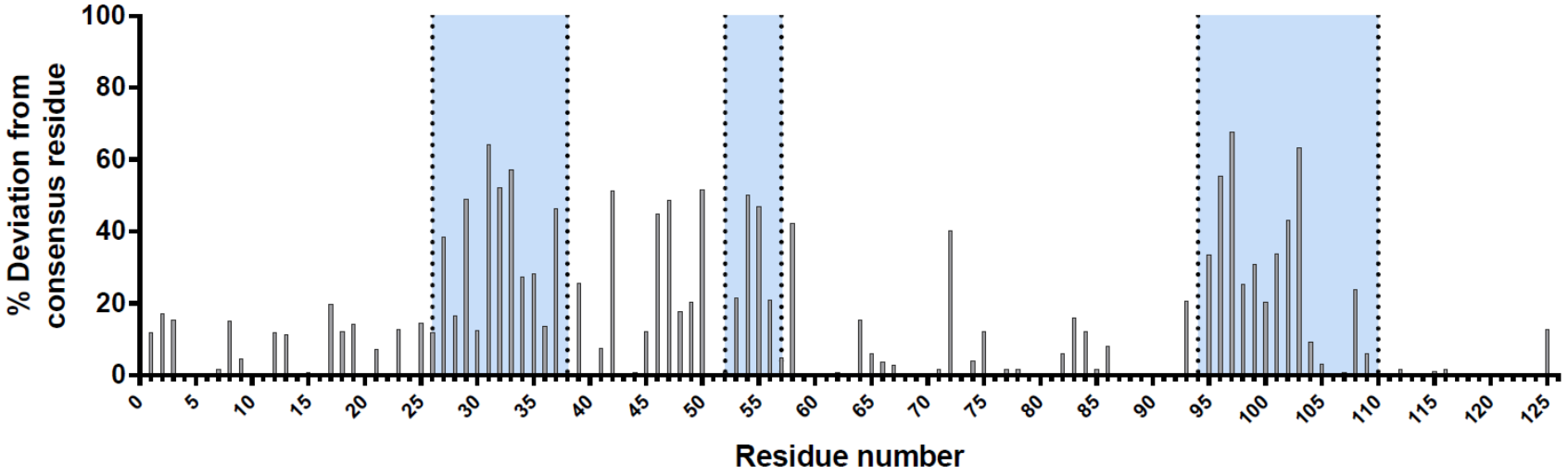


Amino acid variation in IgH and IgL V regions

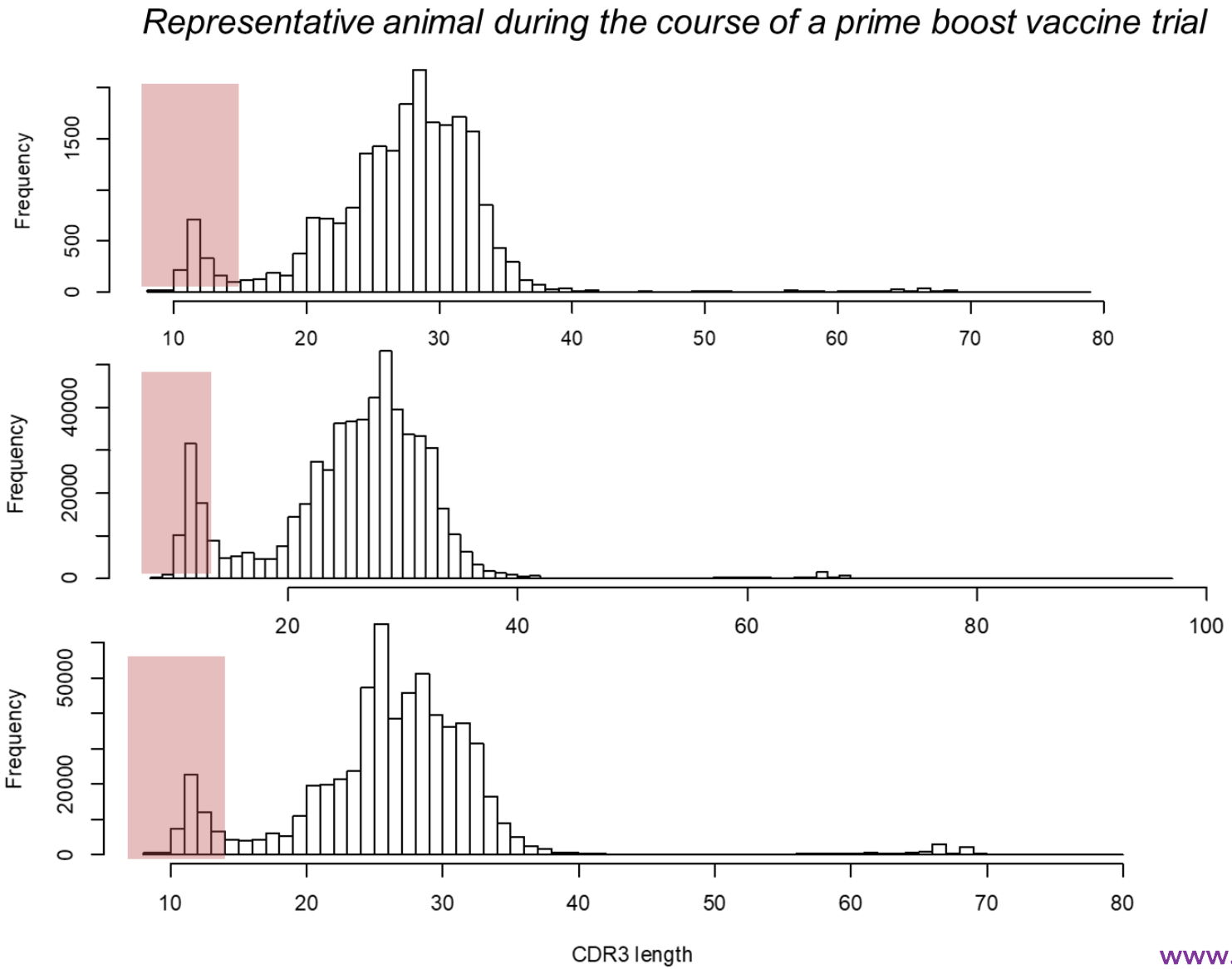
Heavy chain



Light chain

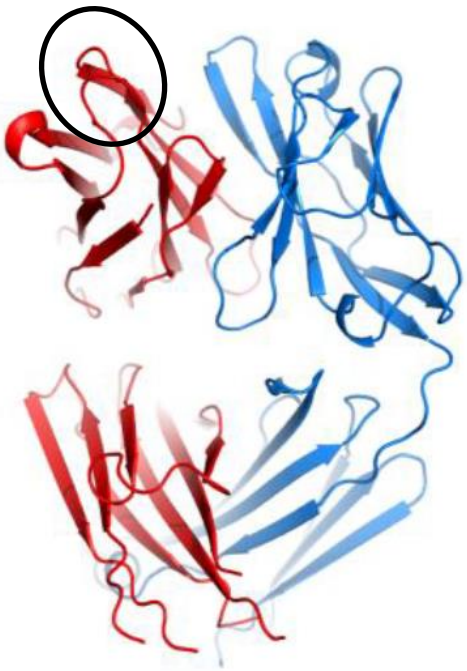


Long and consistent cattle CDR3 length compared to human and mouse (shaded)

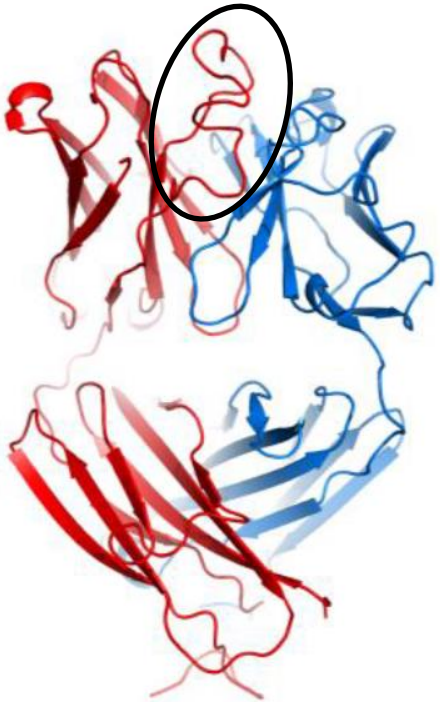


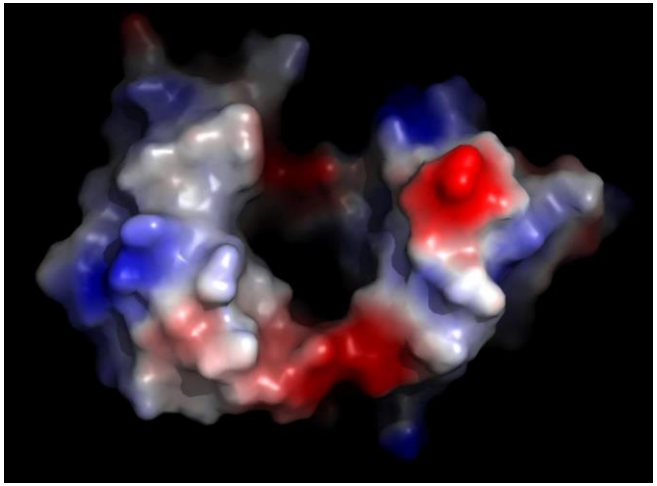
Bovine IgH CDR3 region structural diversity

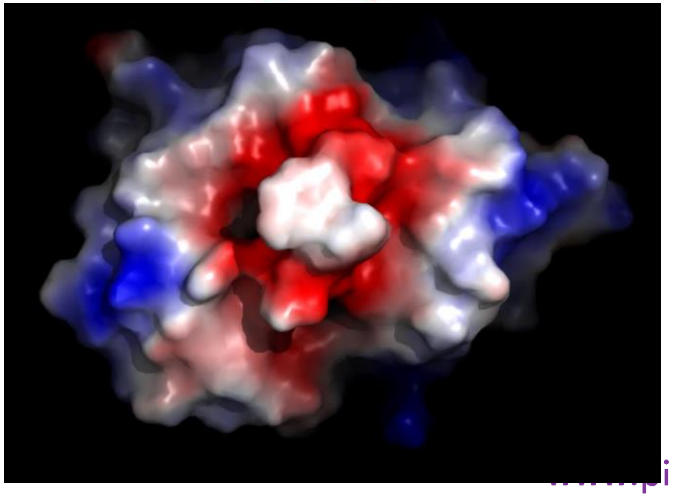
CDR3 = 8aa



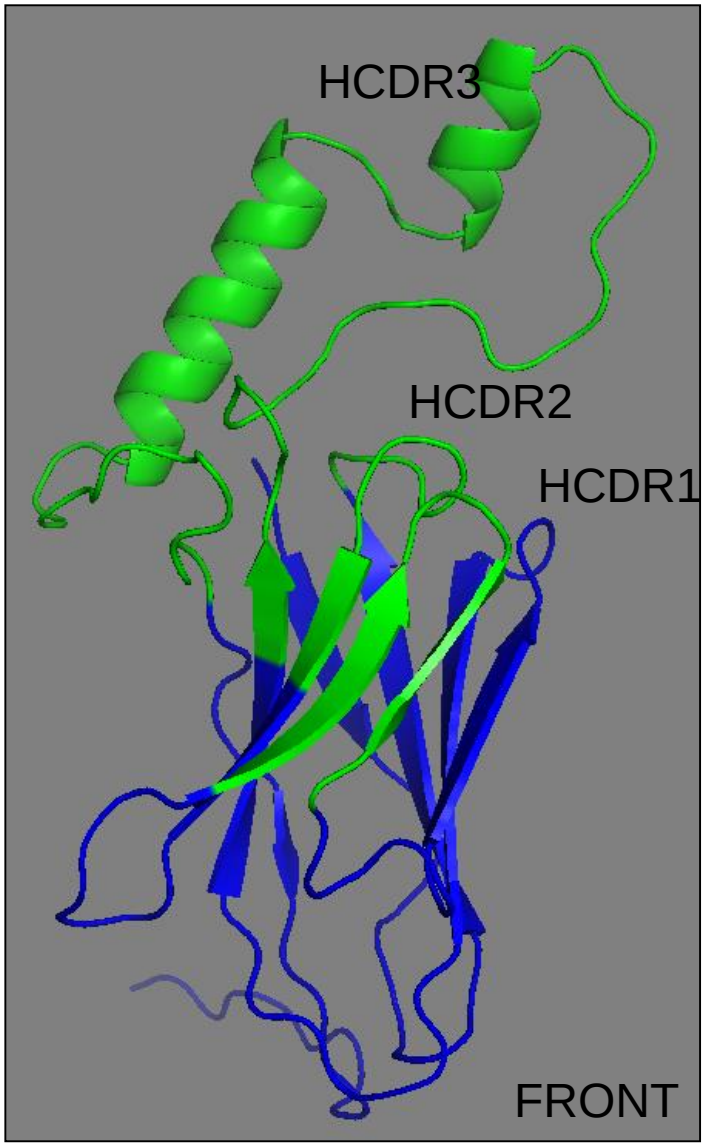
CDR3 = 26aa

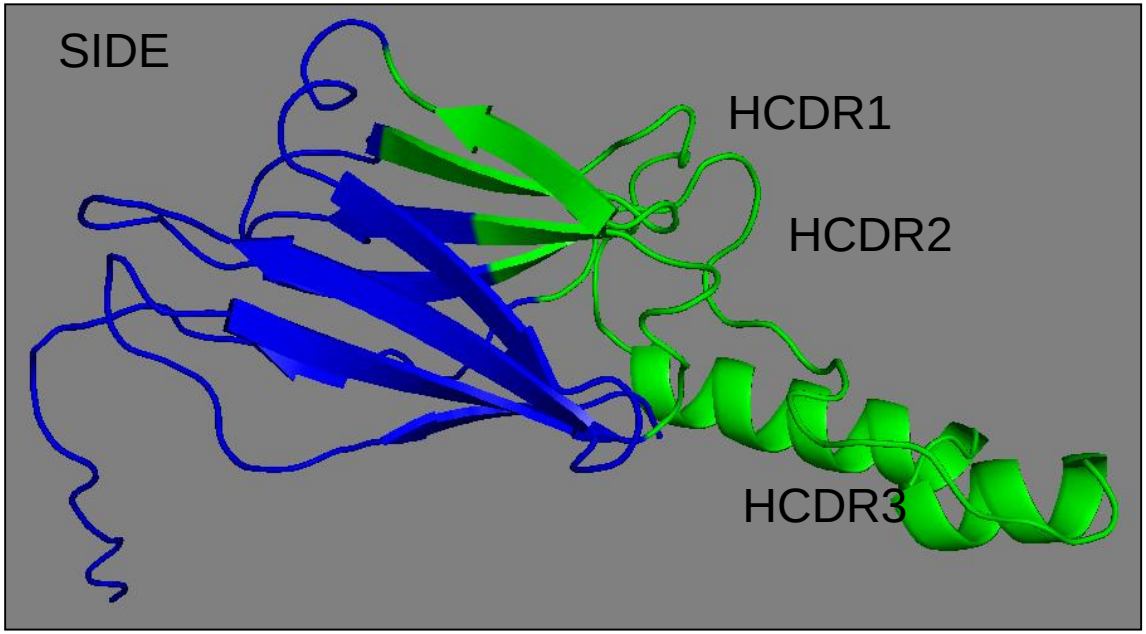






Predicted structures: bovine super long CDR3 region (60aa)





Reshaping antibody diversity.

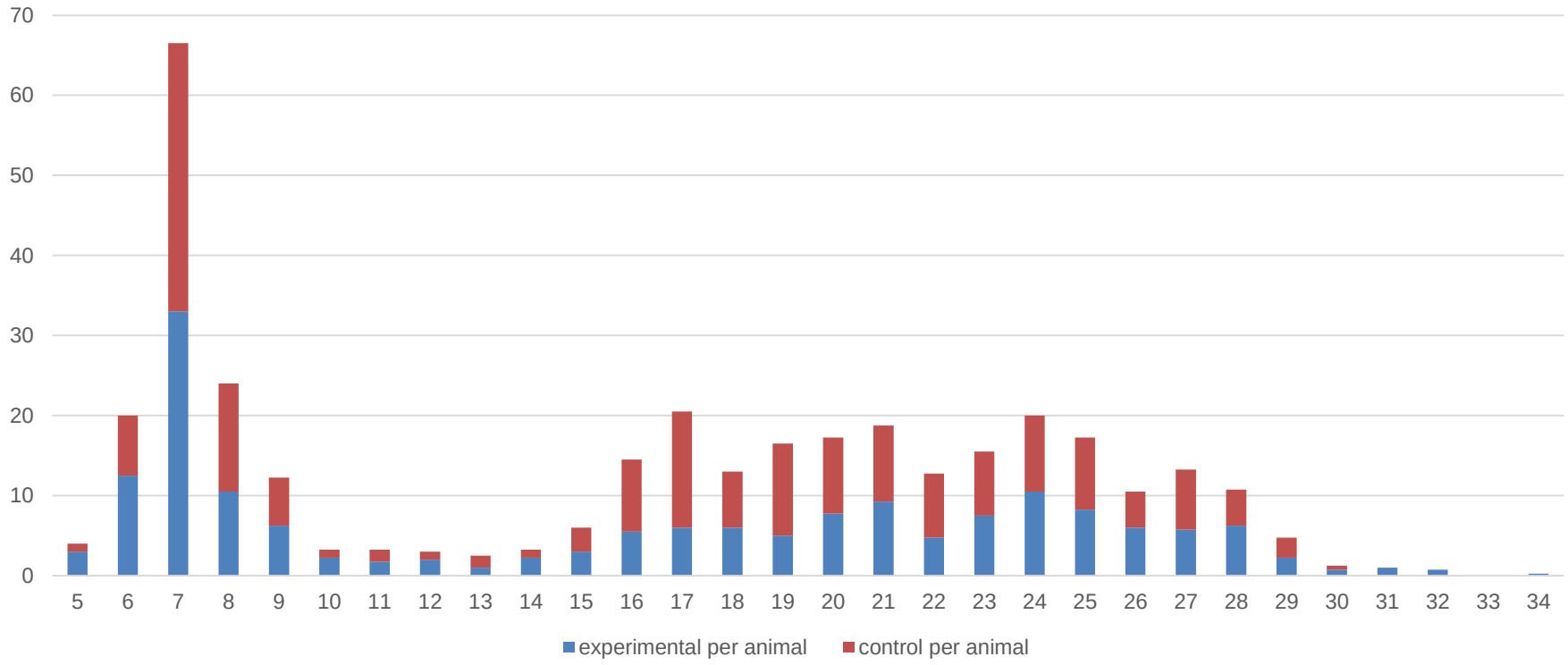
Wang F, Ekiert DC, Ahmad I, Yu W, Zhang Y, Bazirgan O, Torkamani A, Raudsepp T, Mwangi W, Criscitiello MF, Wilson IA, Schultz PG, Smider VV.

Cell. 2013 Jun 6;153(6):1379-93. doi: 10.1016/j.cell.2013.04.049.

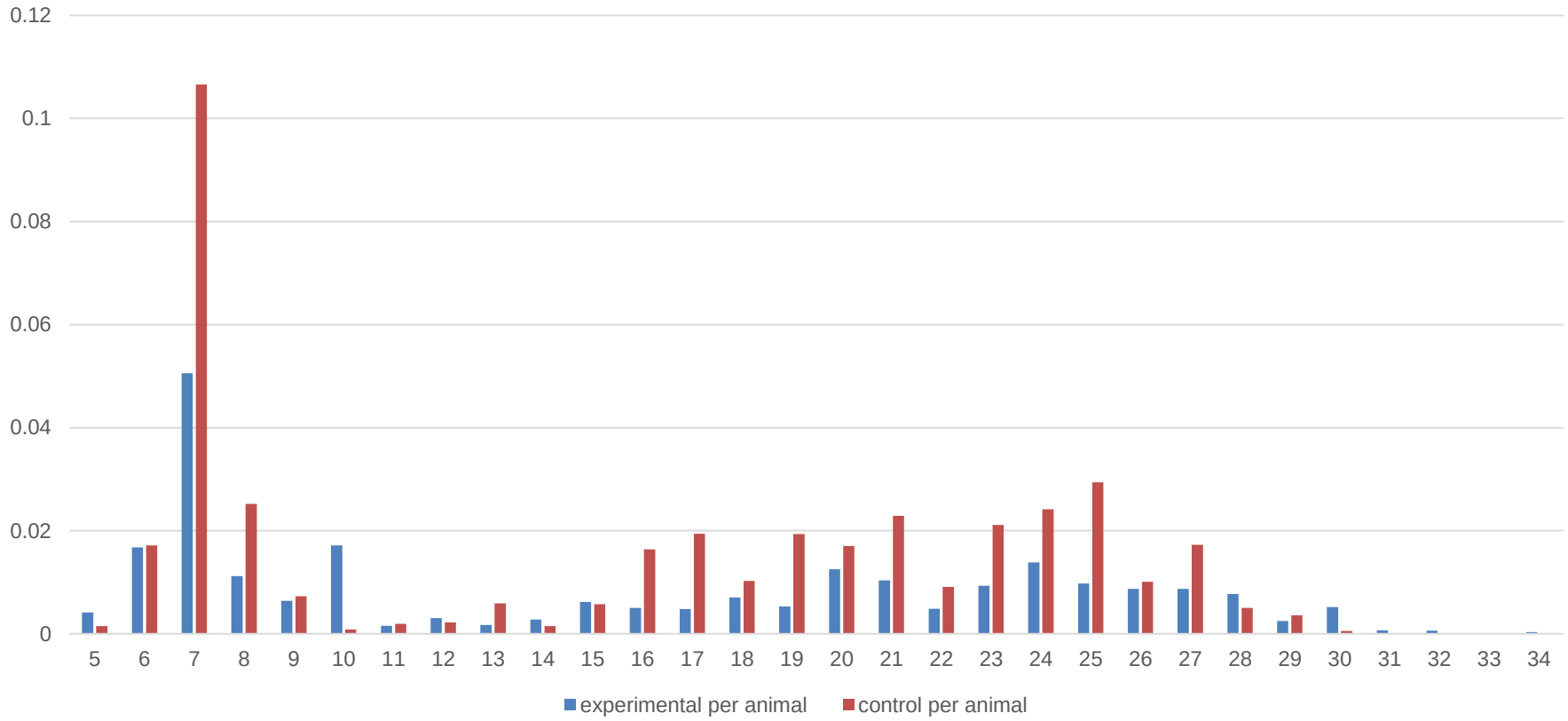
PMID: 23746848 Free PMC Article

Similar articles

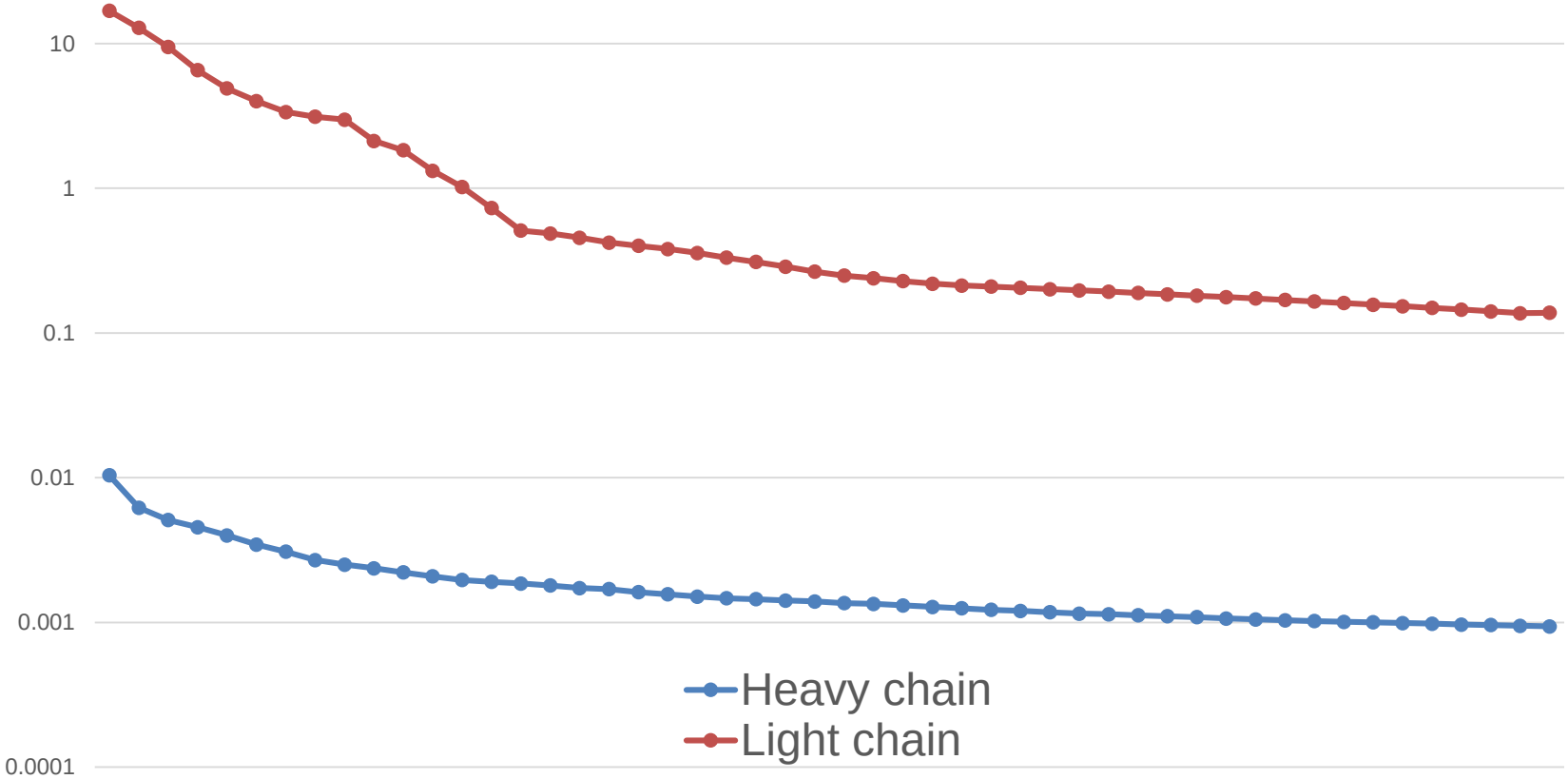
CDR3 length does not vary between experimental and control groups



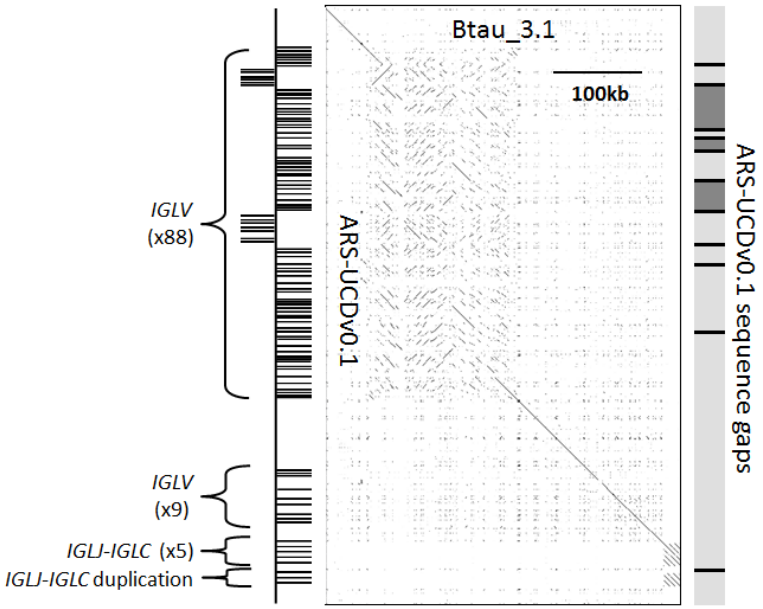
There are fewer high frequency clusters in experimental animals

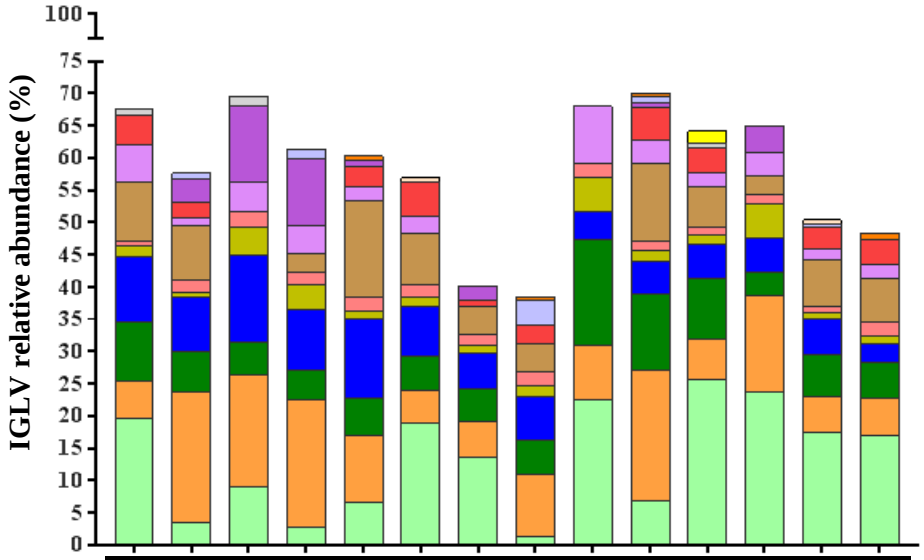


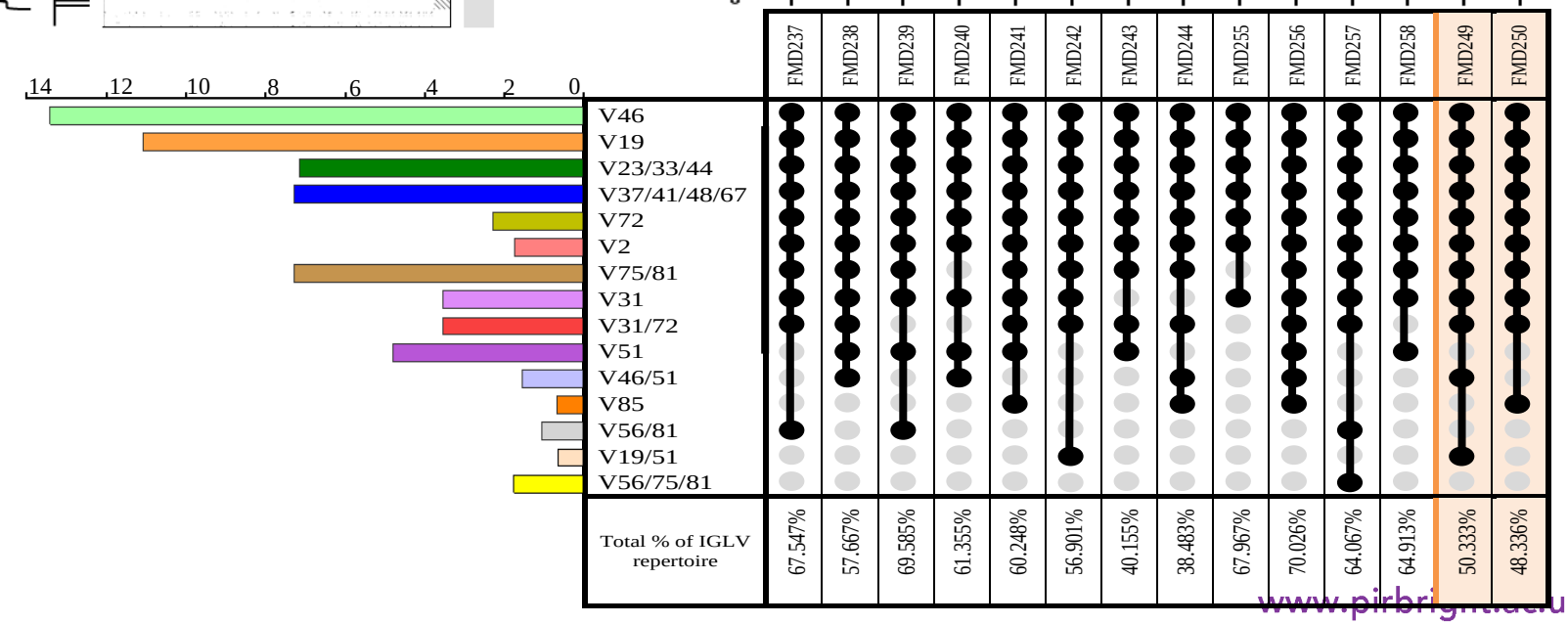
Average proportion of the most abundant 50 heavy and light chain antibody clusters from 18 cattle



The IgL repertoire is dominated by relatively few genes

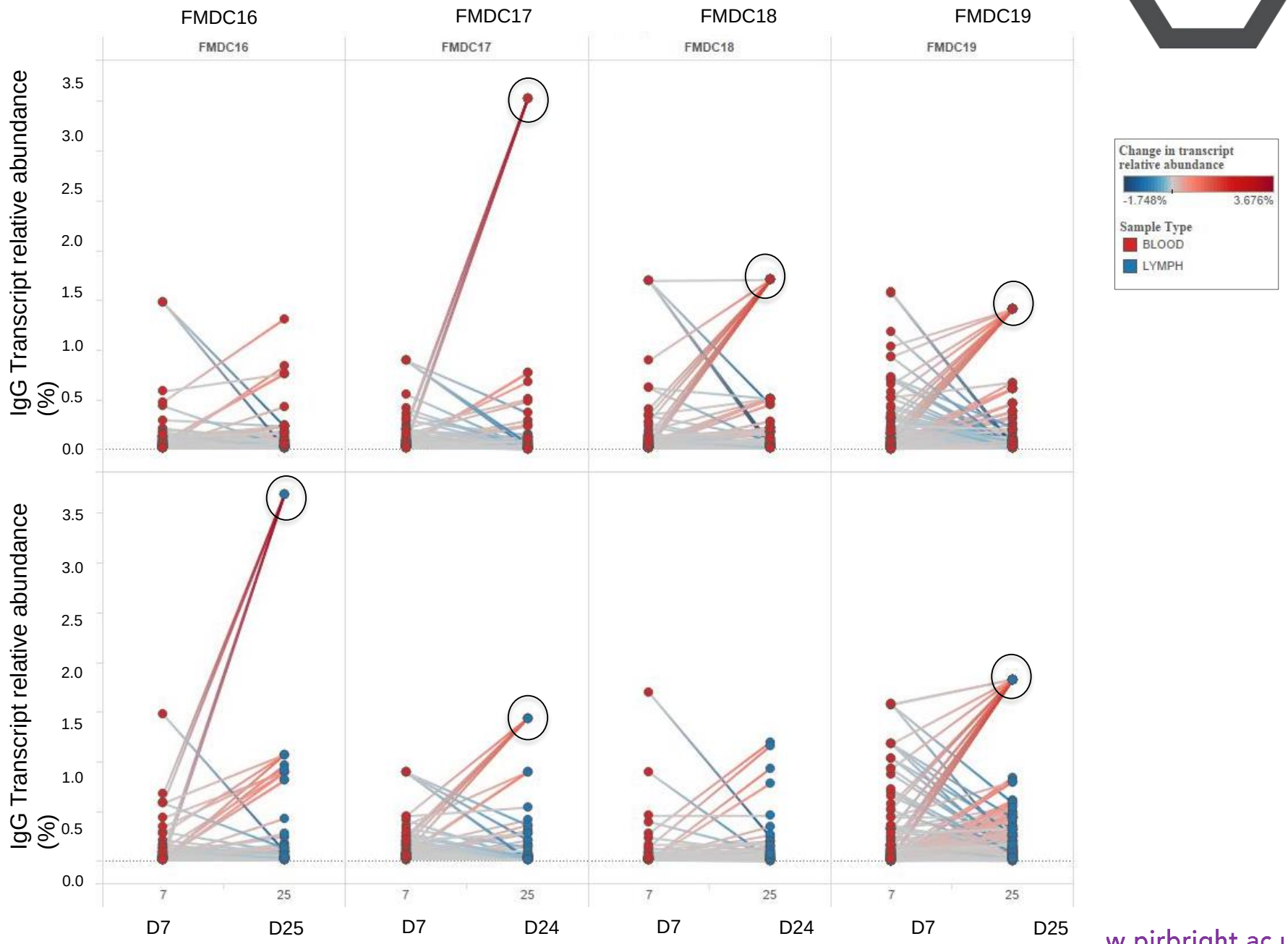




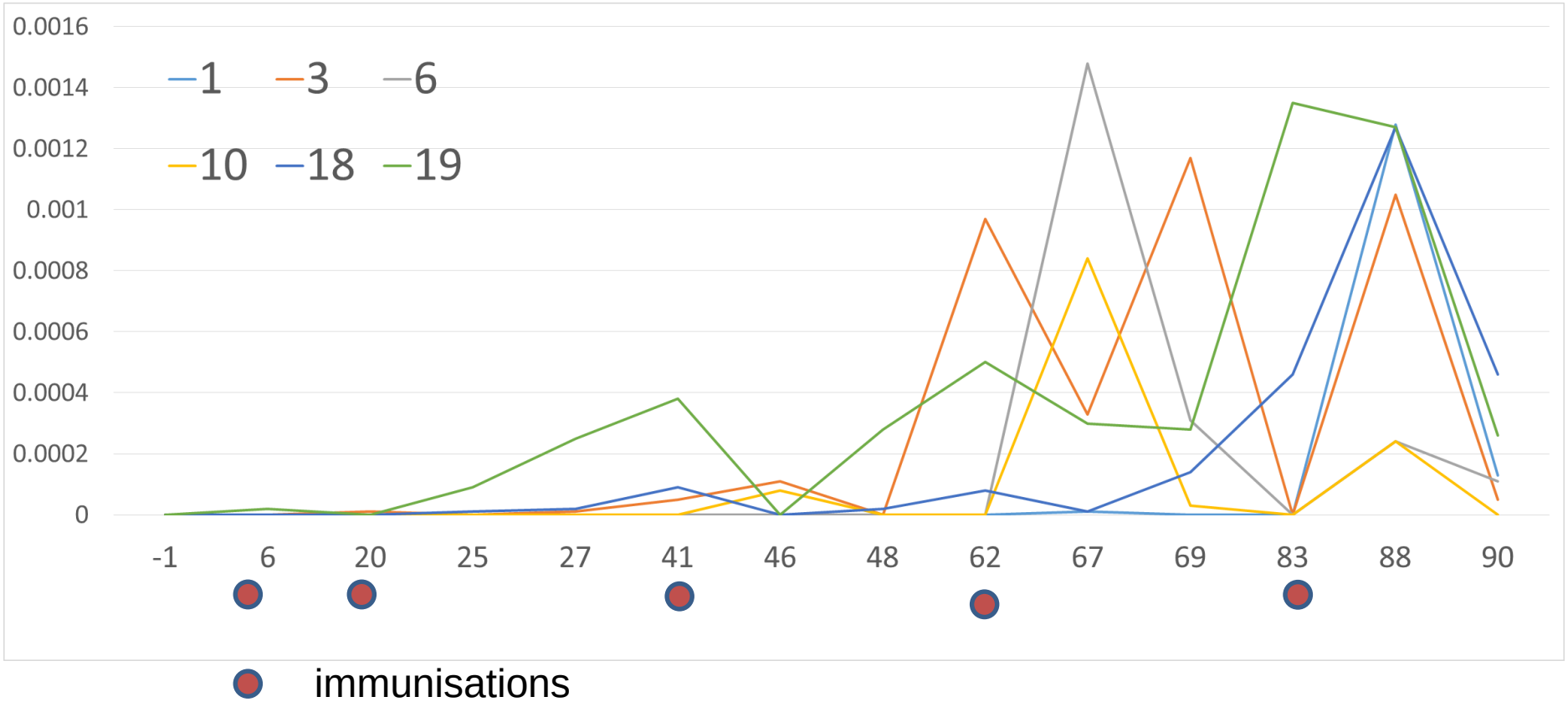


The IgL repertoire is dominated by relatively few genes

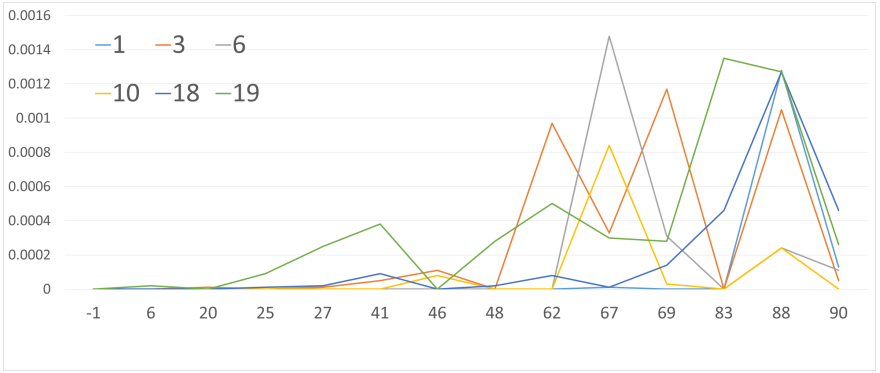
The antibody response becomes focussed during the immune response

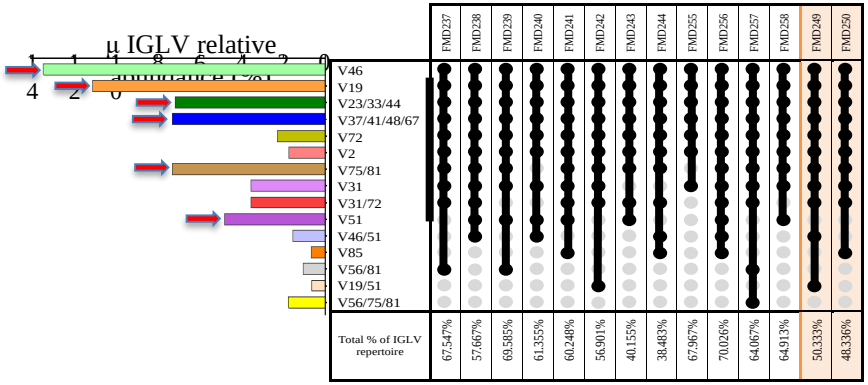


The abundance pattern of several high-abundance clusters correlates with vaccine boosting



Transient expression of Fabs to assess potential binding



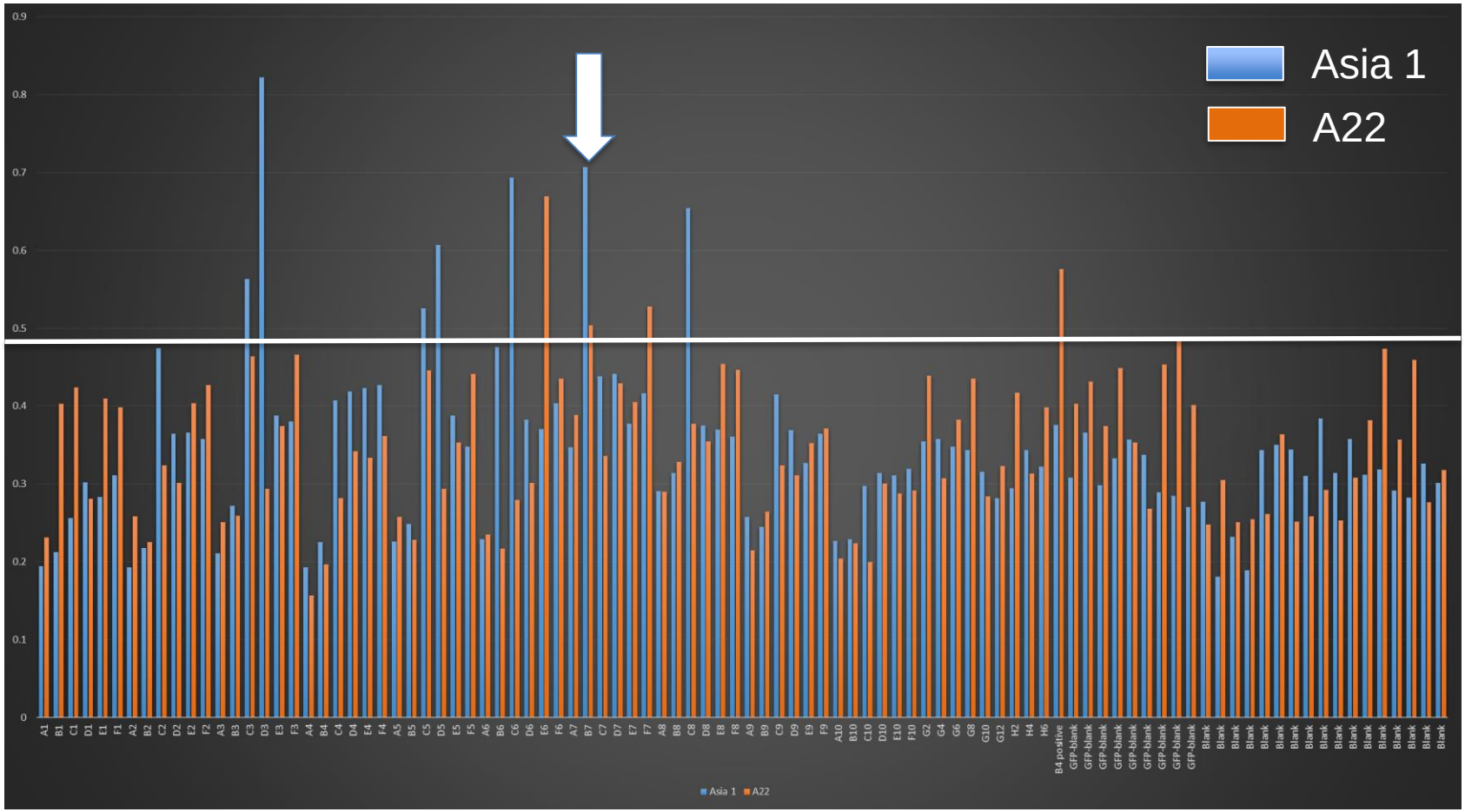


HEK cell transient transfection
-HIS tagged Fabs
-whole cattle Ig

Protein expression measured
Assembly ELISA developed

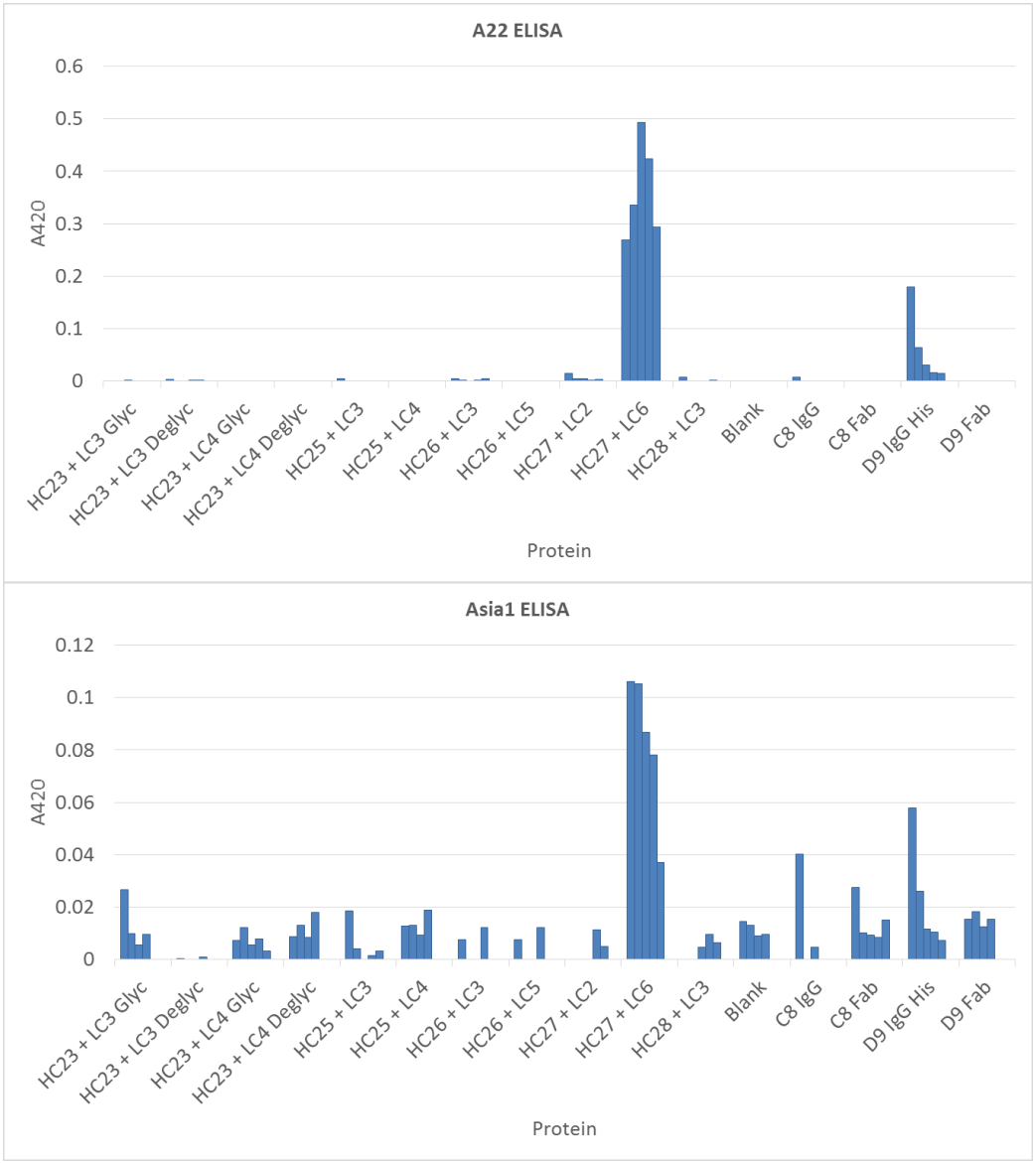
Cryo EM

Crude supernatant used in ELISA with capsid coated plates to measure potential binding

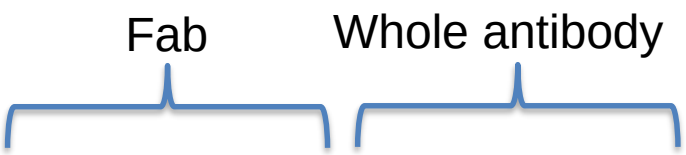


One IgH and IgL pair shows binding to two FMDV serotypes

Immunisation:
O Pan Asia
Asia 1
A22
SAT 1

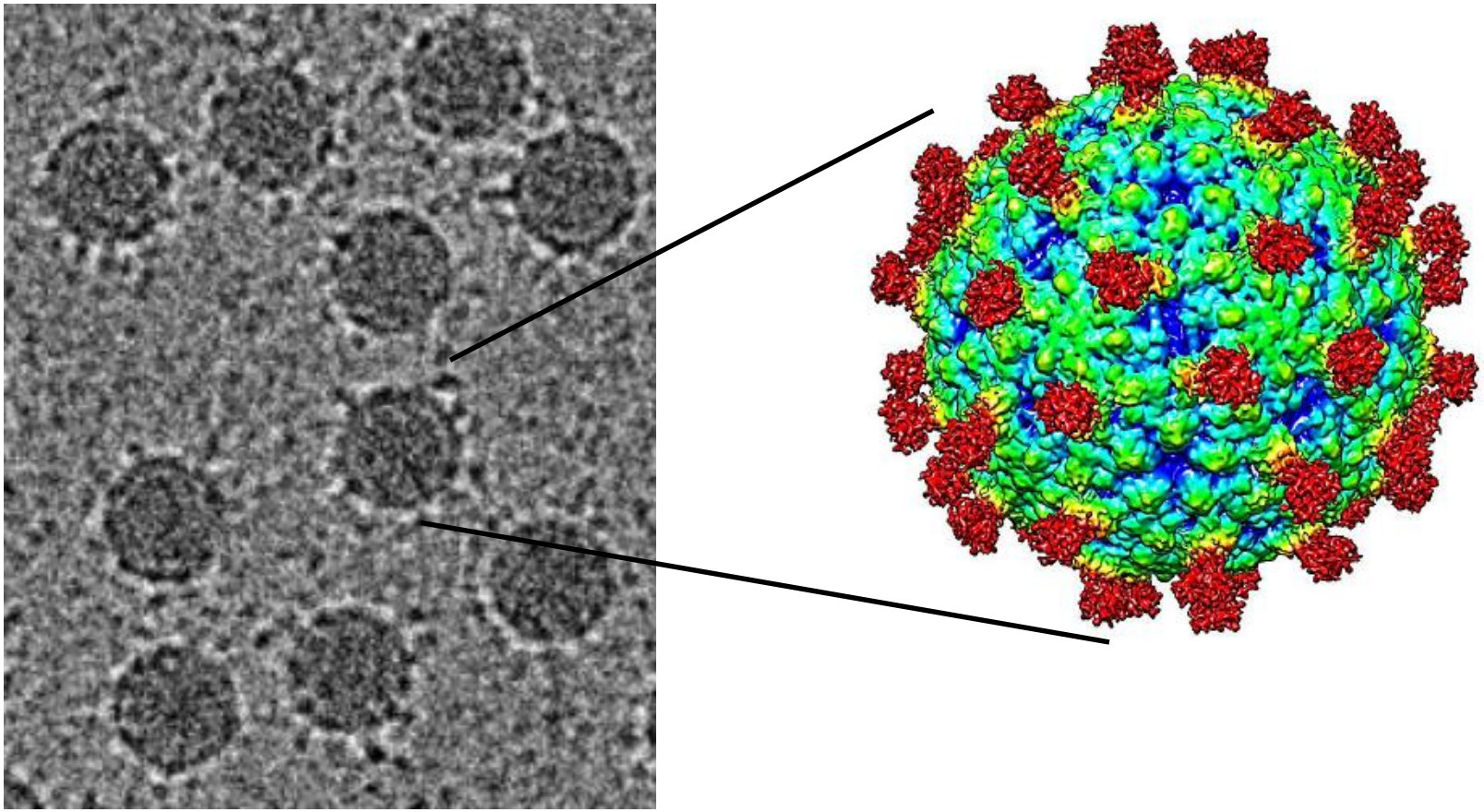


Whole antibody expression increases avidity.



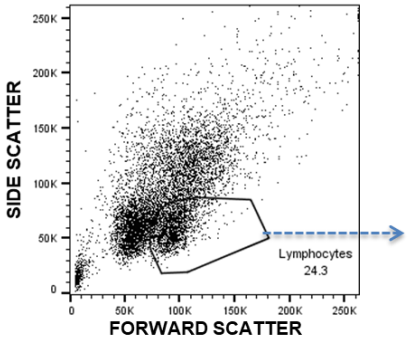
D9 IgG binding to Polio?
D9 IgG is a different control to previous (D9 IgG His)

Three expressed bovine Fabs bind FMDV

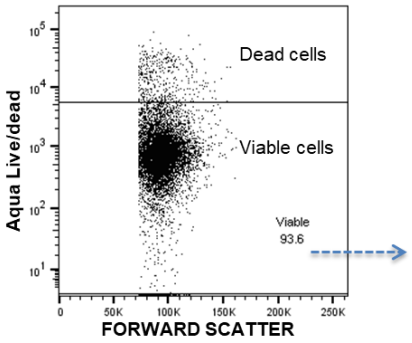


But…….. low avidity binding. Selection of natural heavy and light chain pairs is essential.

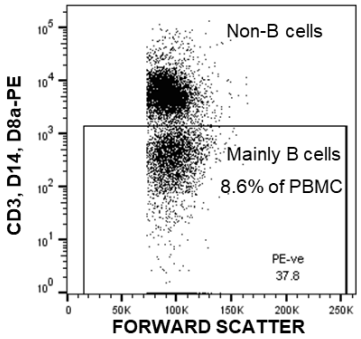
Identification of putative bovine plasma cells



Lymphocytes gated based on light scattering properties; Forward (size) and side (granularity)

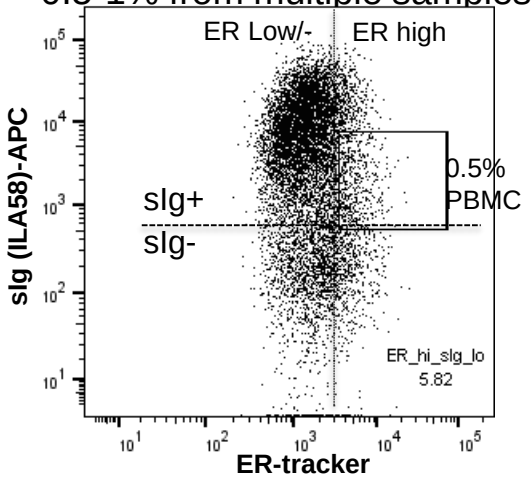


Staining with Aqua live/dead dye used to distinguish between live and dead cells

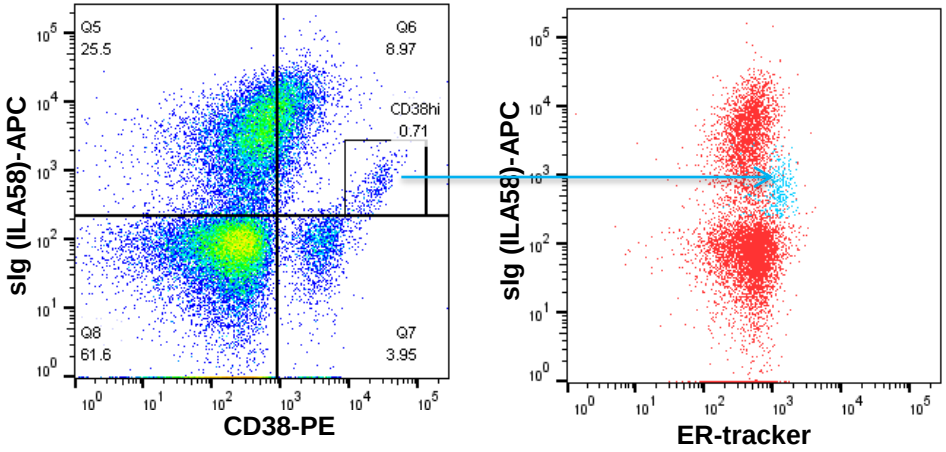


A cocktail of antibodies against T cells and monocytes used to exclude non-B cells in a non-B cell dump channel

Observed ER-tracker^{hi} sIg^{low} levels of 0.5-1% from multiple samples



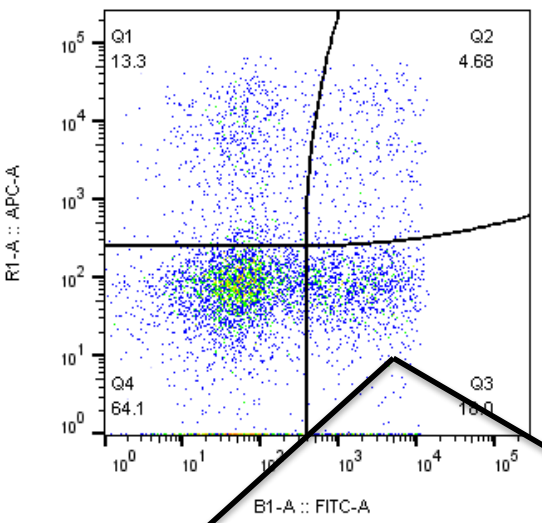
Anti-CD38 stains the same ER tracker high/IgL low population.



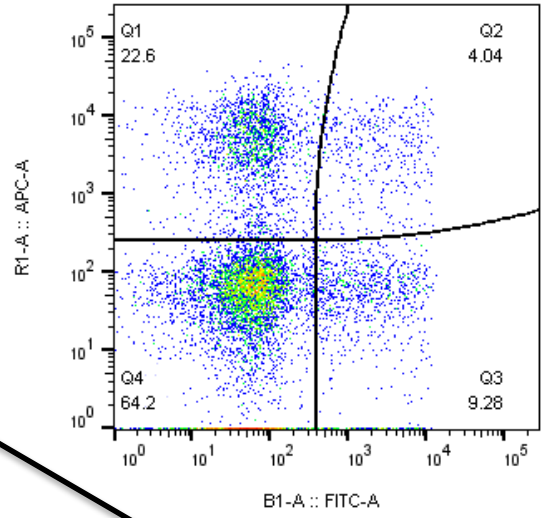
Kurosawa et al. BMC Biology 2012

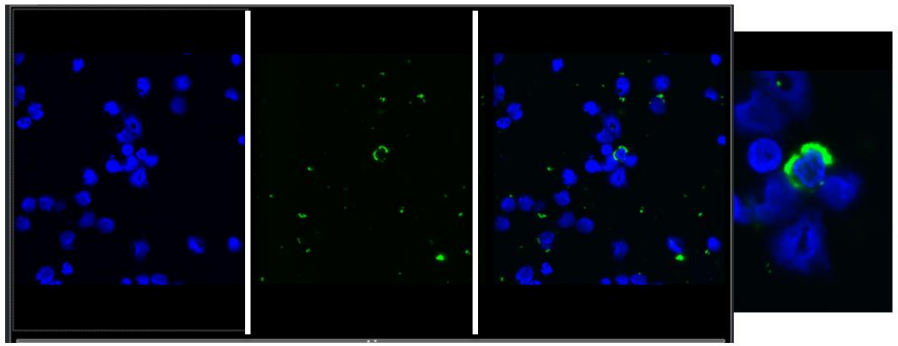
Identification of FMDV specific plasma cells

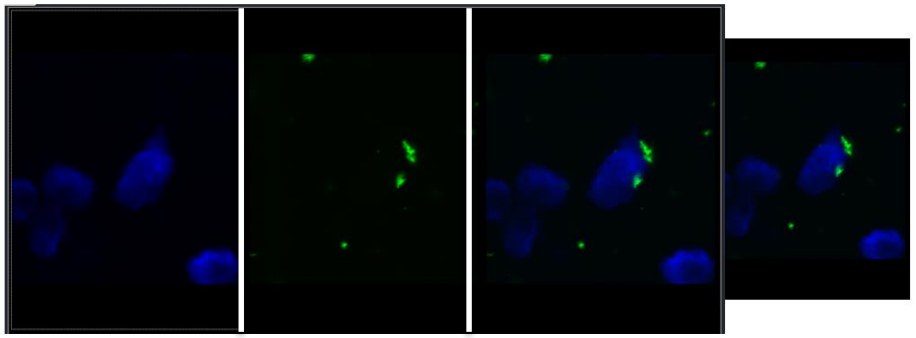
FMD sample permeabilized



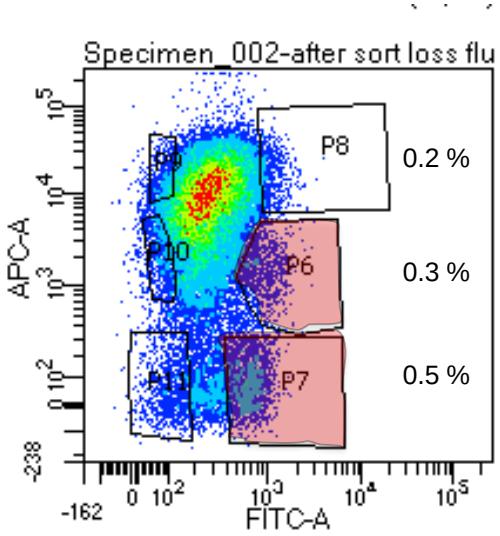
PBMC sample permeabilized







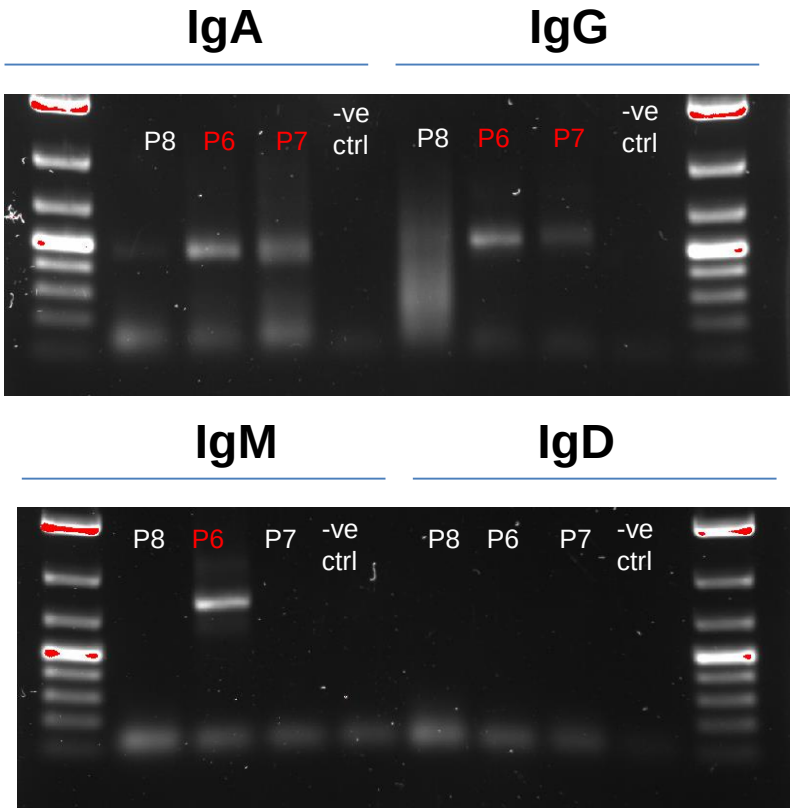
PCR of bovine Ig using one-step RT-PCR



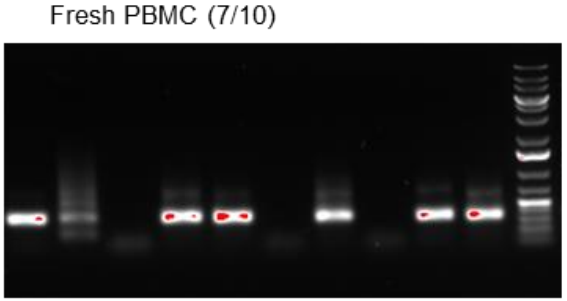
0.2 % $ER^{hi}Ig^{hi}$

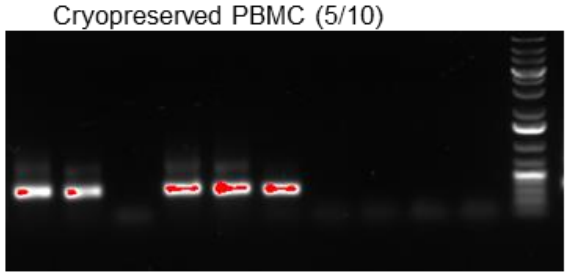
0.3 % $ER^{hi}Ig^{lo}$

0.5 % $ER^{hi}Ig^{-ve}$

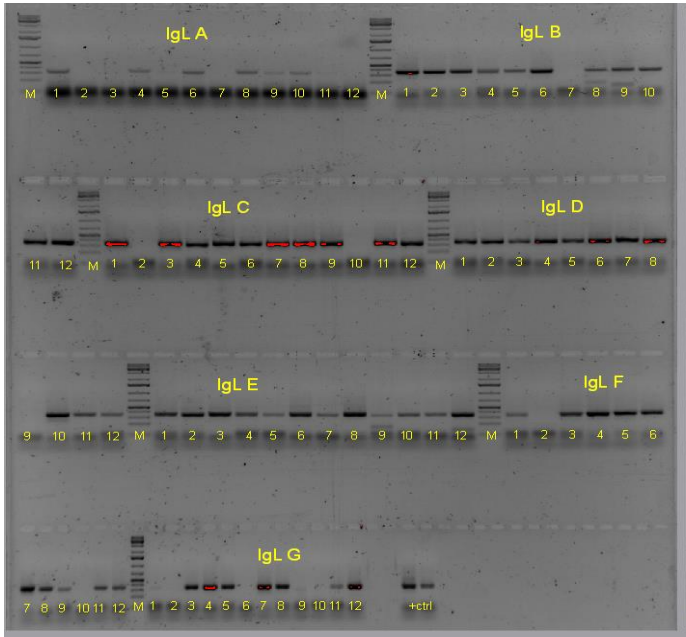


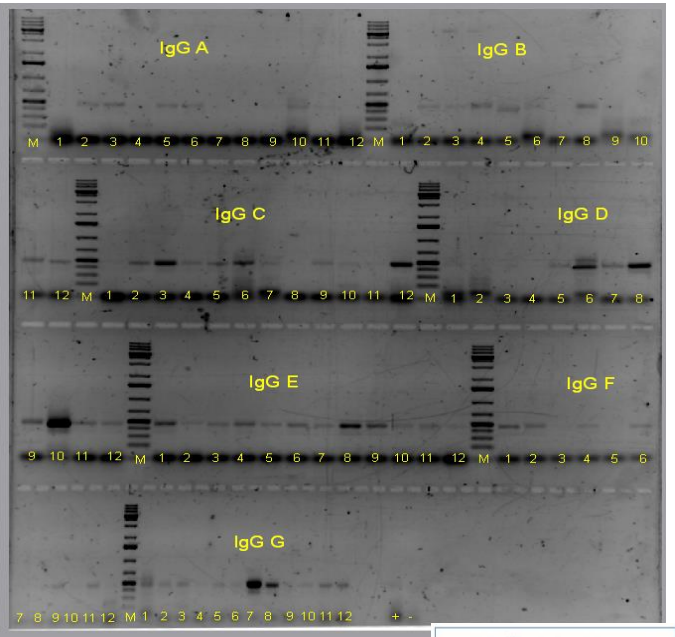
Now enables us to do single cell sorting and sequencing

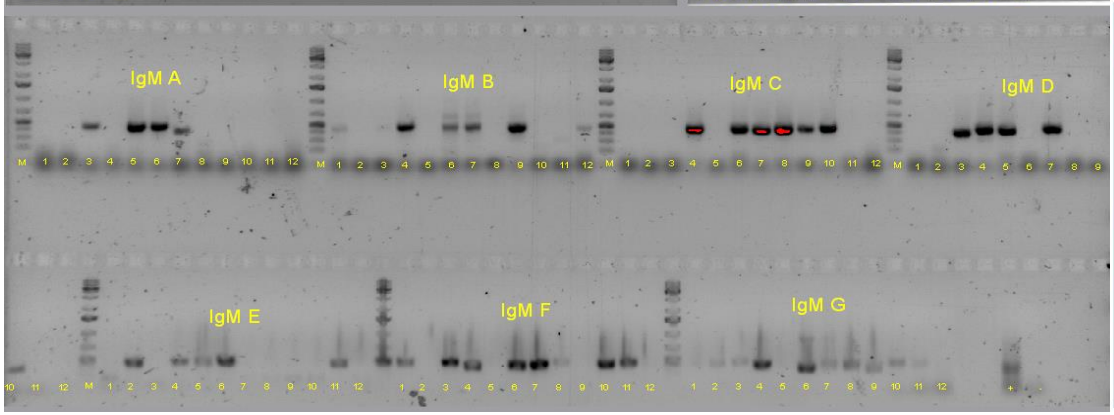


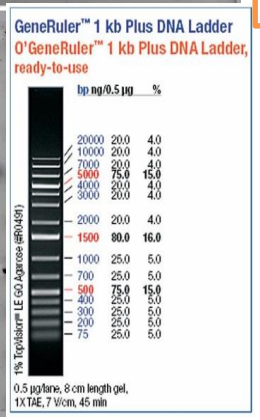


Single cell cDNA amplification and sequencing





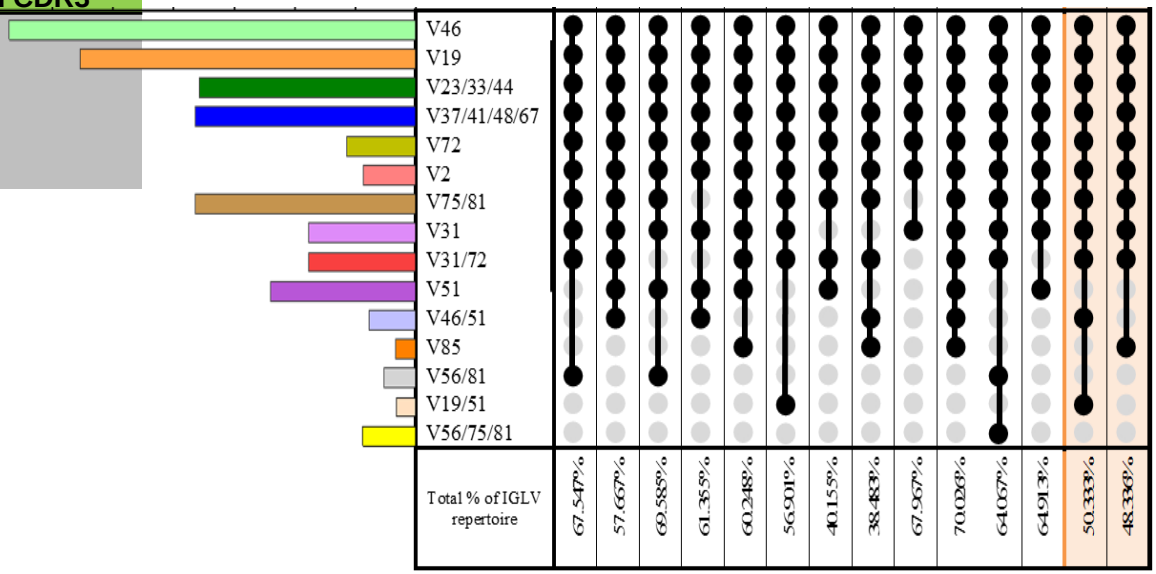


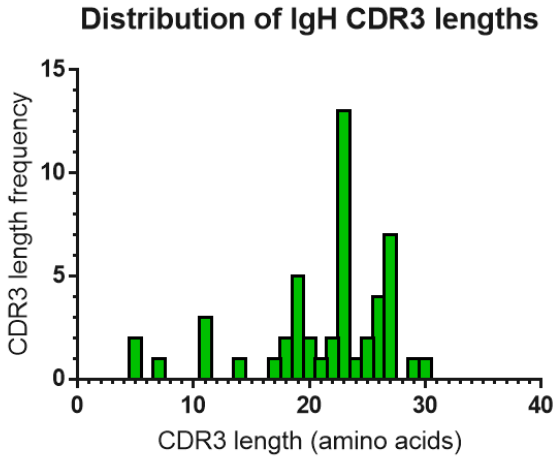


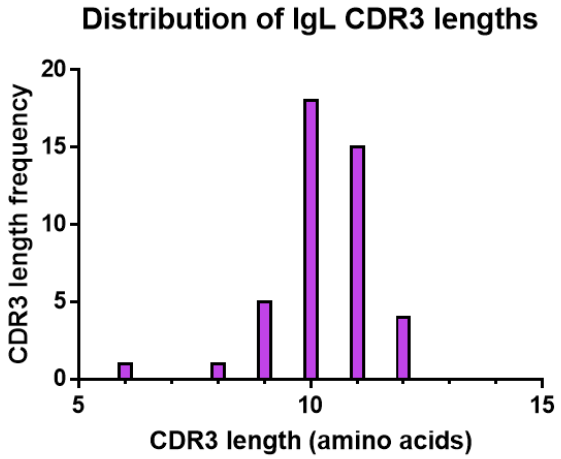
- Gel extraction of amplicons
- Ligation in pGEMTe
- JM109 transformation and Sanger seq

Abundant IgL cluster from single cell sequencing match the whole repertoire data

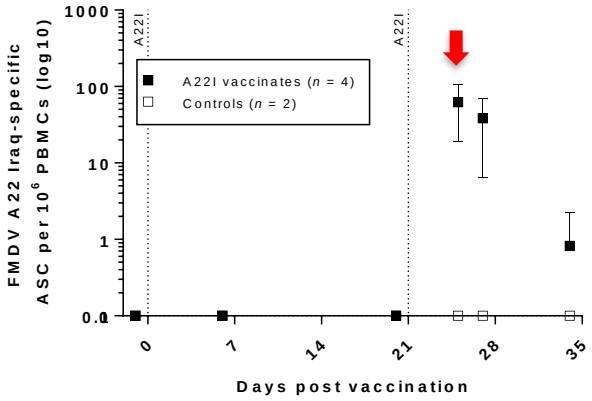
IgL cluster	Previously abundant	Number of IgH CDR3
Cluster1	Yes	1
Cluster2	Yes	3
Cluster3&4	Yes	7
Cluster5	Yes	5
Cluster6	Yes	1
Cluster7	No	3
Cluster8	No	2
Cluster9	No	5
Cluster10	No	2
Cluster11	No	1
Cluster12	No	4
Cluster13	No	1
Cluster14	No	1
Cluster15	No	3
Cluster16	No	1
Cluster17	No	1
Cluster18	No	1
Cluster19	No	1
Cluster20	No	1

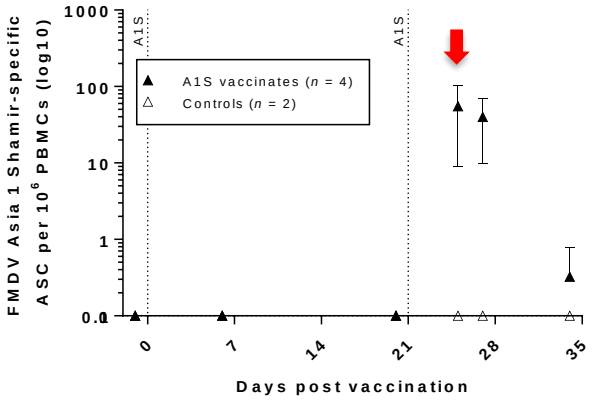


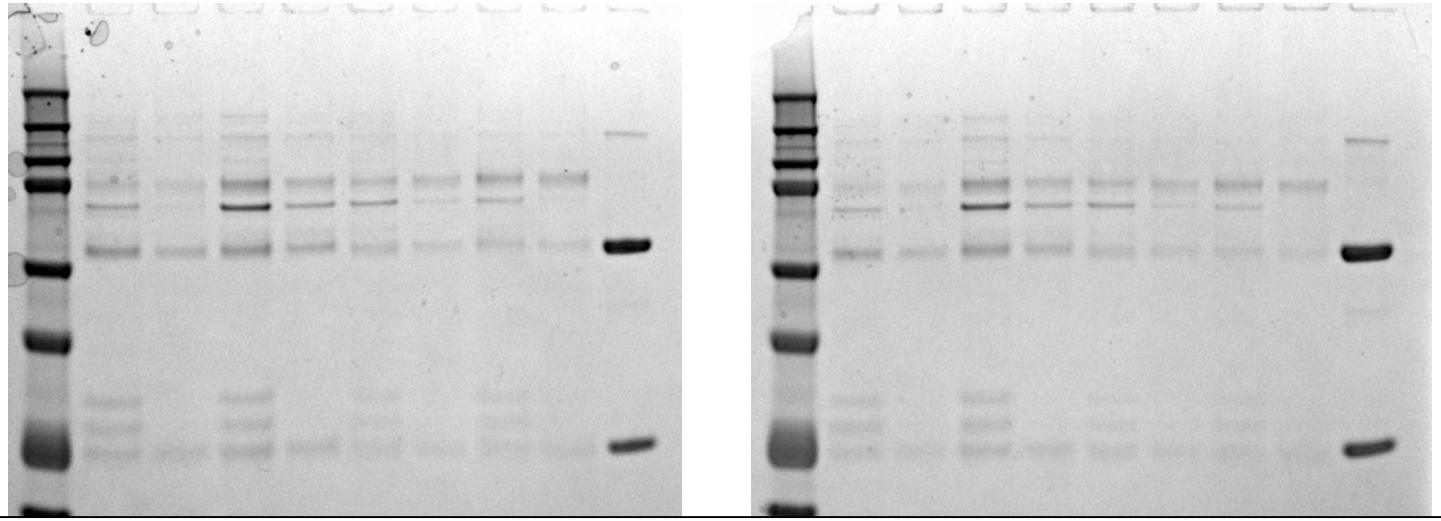




Pulling out FMDV antibodies from serum samples for identification with mass spectrometry





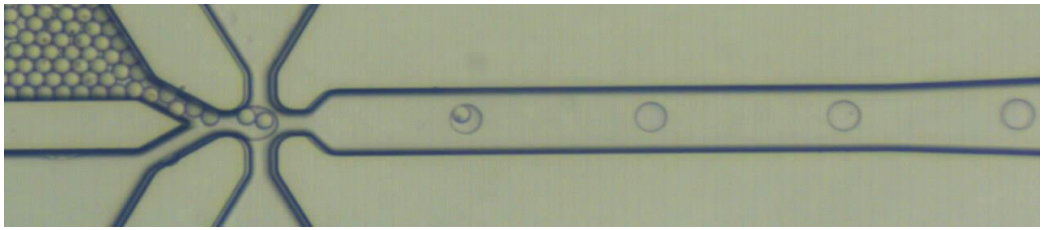


IgG2340

wtllfvlsaprgvlsqmqlr**esGpslVK**aSqtL**sltctVsgf**slstqnvnw**vrqapgkalewvggvgsg**
gstaYnpAlKSRLSITKDNSKSqVSLSVrsVTpEDTATYYCiRCYaS**WGQGLLLTVSSASTTAPKVY**

Current and future work

- Focusing on paired Ig reads and single cell amplification
- Adding labelled FMDV capsids into high-throughput cell sorting protocols
- Studying structural constraints of heavy and light chain pairing
- RNA-seq to define cattle B cell/plasma cell markers
- Refining the analysis of mass spec data
- Studying the mechanisms that diversify the cattle antibody repertoire



BILL & MELINDA GATES foundation

Defining the fine specificity of antibody responses in cattle to inform vaccine design

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OPPF-Ray Owens

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