

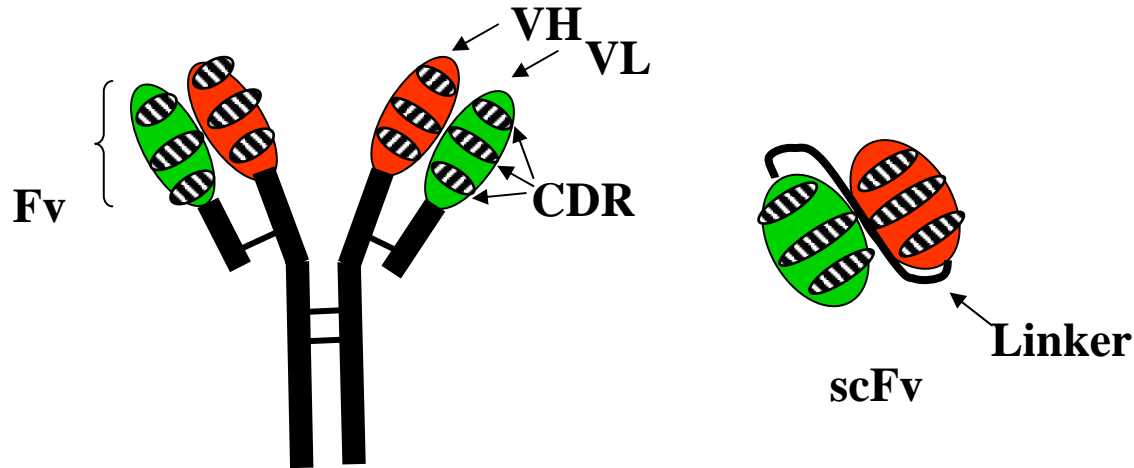
Recombinant Antibodies for Development of an FMDV DIVA Assay

Hans Heine
CSIRO Livestock Industries
Australian Animal Health Laboratory
13 April 2010

Why recombinant antibodies for FMDV?

- Save and economical production of quality assured diagnostic reagents is crucial for the implementation of a strategy to differentiate FMDV infected from vaccinated animals (DIVA).
- RecAbs are stable immortalized antibody clones, eliminating the need for antibody production in animals or the maintenance of hybridoma cell lines.
- A DIVA assay entirely based on recombinant proteins, 3ABC non-structural antigen and 3ABC-specific recAb, may provide cost effective quality reagents in nearly unlimited quantities.

What are recombinant antibodies?

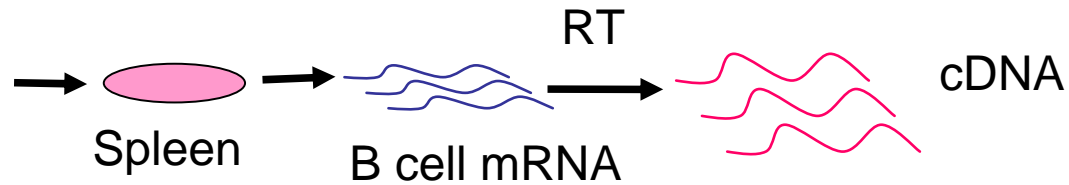


- Recombinant antibodies are genetic constructs that containing antigen-binding properties.
- Single chain variable fragment (scFv) antibody are the smallest recombinant antibodies containing only the Fv region of immunoglobulin.
- Recombinant antibodies can be genetically modified to add new properties such as fusion to alkaline phosphatase reporter gene to enable a single-step assay.
- Recombinant antibodies can be produced using various recombinant expression systems (bacteria, yeast, plants).

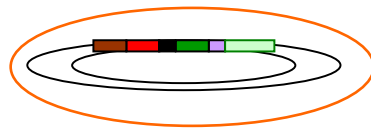
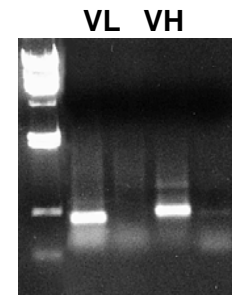
Recombinant antibody library construction



Chicken immunized with FMDV 3ABC



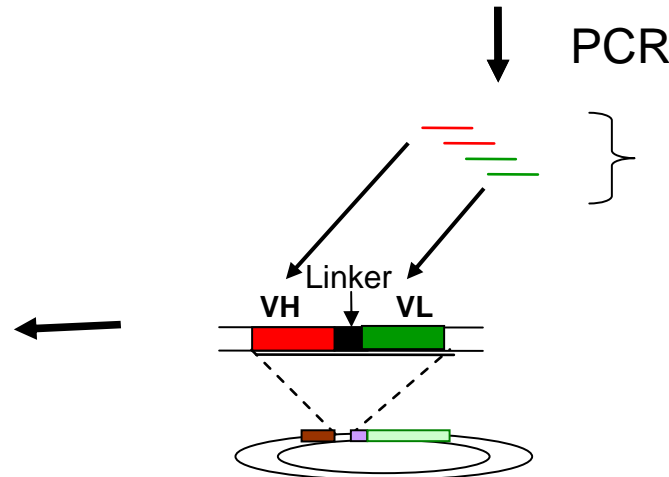
PCR



Transform library into *E. coli* and rescue recombinant phage

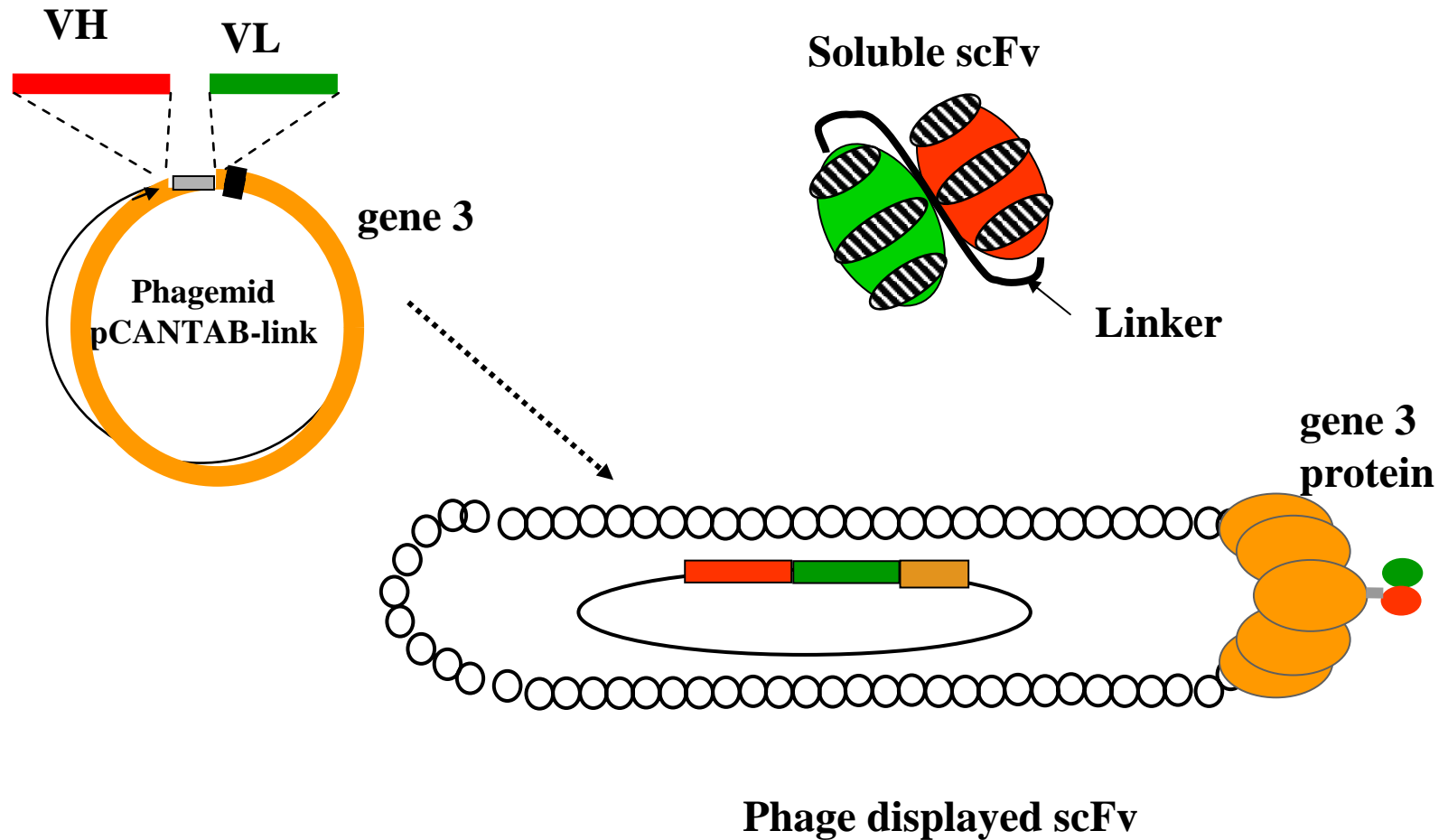


' Bio-Panning '

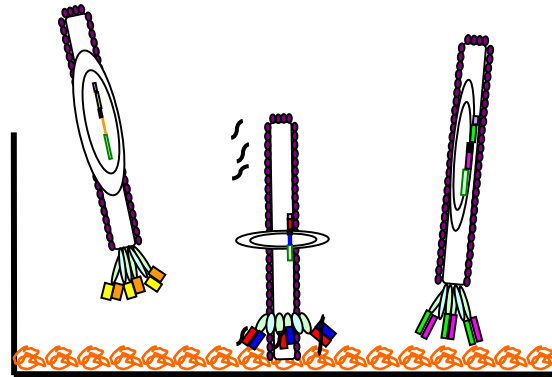


Ligation of VH and VL fragments into phagemid vector to generate library

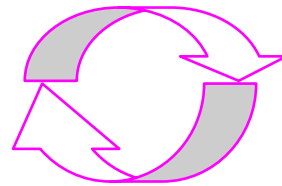
Phage-displayed and soluble scFv antibodies



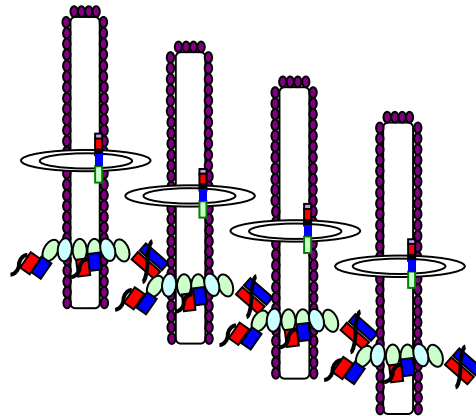
Affinity Selection – ‘Bio-Panning’



Binding of phage-displayed scFv to antigen coated on vessel



Multiple rounds of selection

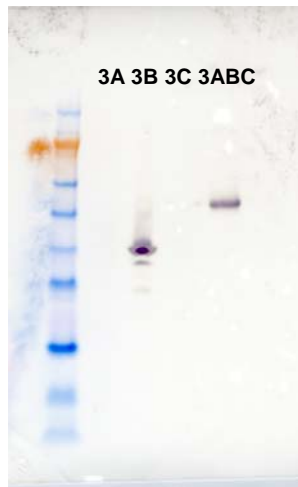


Non-binders are washed away and binders are eluted

Amplification of selected library in *E.coli* and enrichment of specific binders

Results (1): Characterisation of scFv's

- **Sequence analysis (FM26, FM27)**
- **Epitope mapping (3ABC, 3B; repeat region)**
- **Biacore analysis (FM26, FM27)**



Western blot analysis:
FM26 and FM27 (shown)
antibodies mapped to 3B.

26-02	APIYADLLEPRS	27-B4	LETRIYPDQLEL
26-04	NDMILFTDWLDN	27-B6	QLFLDPLEPTPM
26-07	FPSWL YFDPLEA	27-B8	HMLPYYDPLNPV
26-09	MYPDPLDGRHLF	27-C10	APIYADLLEPRS
26-10	TPWLYPDLLETG	27-E4	LLYDDPLEPRLS
26-11	IQPQLYDPLEY	27-F6	WANNAPYRDYLD
26-12	ATTPYDPLDTF	27-G6	WSSVFIWPDYLE
26-13	KHLYMDPLESRP		
26-14	ADNIYMDPLEYY		
26-15	NSTIMYDPLER		
3B-1	GPYAGPLE	3B-1	GPYAGPLE
3B-2	GPYAGPME	3B-2	GPYAGPME
3B-3	GPYEGPVK	3B-3	GPYEGPVK
	FM26		FM27

Fine mapping using phage display **random 12-mer peptide libraries** revealed consensus sequences that matched sequences in repeat regions of the 3B peptide.

Results (2): Genetic modification of scFv

Fusion of scFv to reporter gene:

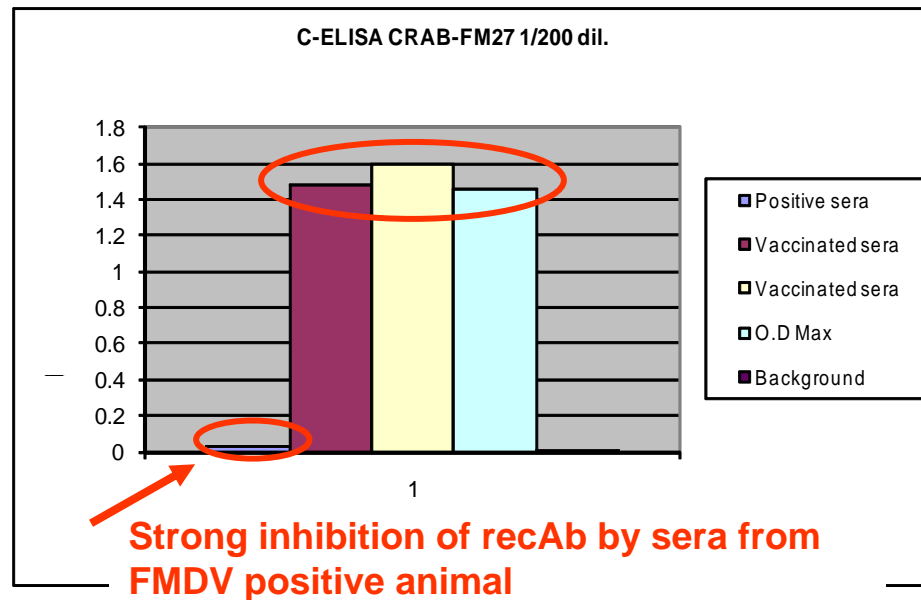
Alkaline phosphatase (AP) reporter gene was genetically fused to the scFv to generate a new FM27-AP construct that showed:

- **Alkaline phosphatase enzyme activity**
- **Binding to FMDV 3B protein**
- **Potential for use in a single-step assay.**

Results (3): Potential use in DIVA assay

Evaluation in a 3ABC competitive ELISA:

All scFv's were able to differentiate infected from vaccinated animals, but the FM27-AP had the advantage of ease of use in a single step assay format.



Conclusions

Recombinant antibodies can be produced in virtual unlimited quantities at low cost to guarantee the supply of a consistent and well-characterised reagent for an FMDV DIVA assay.

The application of an antibody-enzyme fusion protein in the DIVA test has realized the potential of a one-antibody one-step assay format. This will greatly facilitate its use in both developed and developing countries.

Next step: assay validation

References

- Muller JD, Wilkins M, Foord AJ, Dolezal O, Yu M, Heine HG, Wang LF (2010). Improvement of a recombinant antibody-based serological assay for foot-and-mouth disease virus. *J. Immunol. Meth.* 352: 81–88
- Foord AJ, Muller JD, Yu M, Wang LF, Heine HG (2007). Production and application of recombinant antibodies to foot-and-mouth disease virus non-structural protein 3ABC. *J. Immunol. Meth.* 321: 142-151
- Sapats SI, Trinidad L, Gould G, Heine HG, van den Berg TP, Eterradossi N, Jackwood D, Parede L, Toquin D, Ignjatovic J (2006). Chicken recombinant antibodies specific for very virulent infectious bursal disease virus. *Archives of Virology.* 151: 1551–1566
- Sapats SI, Heine HG, Trinidad L, Gould GJ, Foord AJ, Doolan SG, Prowse SJ, Ignjatovic J (2003). Generation of chicken single chain antibody variable fragments (scFv) that differentiate and neutralize infectious bursal disease virus (IBDV). *Archives of Virology.* 148: 497-515

Acknowledgements

CSIRO Livestock Industries - AAHL

Adam J. Foord

Janine D. Muller

Meng Yu

Michelle Wilkins

Lin-Fa Wang

DSR group

Olan Dolezal, CSIRO Molecular and Health Technologies, Parkville

Randolf Kerschbaumer, Baxter, Austria

Australian Biosecurity CRC for Emerging Disease