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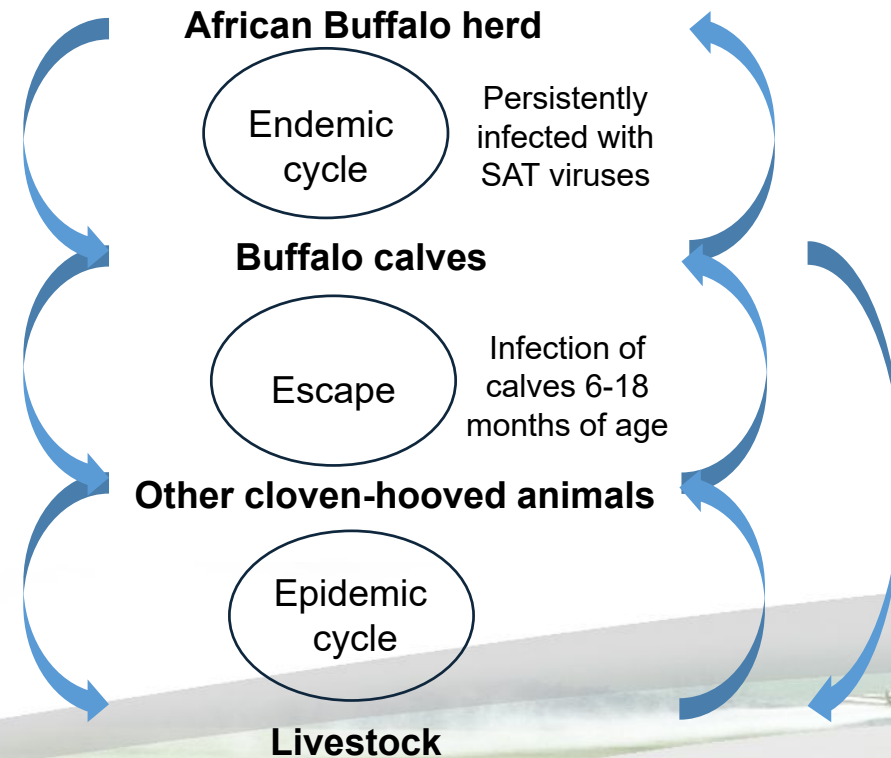


# Construction of a recombinant antibody phage display library derived from the immune repertoire of FMDV – SAT immune buffalo. Potential new diagnostic reagents?

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# Foot-and-Mouth Disease in Southern Africa

- Southern Africa is endowed with an abundance of wildlife
- Neighbouring communities - the livestock/wildlife interface presents unique challenges to livestock disease control
- The effective management of FMD in southern Africa differs radically from elsewhere
- The epidemiology of FMD in Africa is influenced by two different patterns

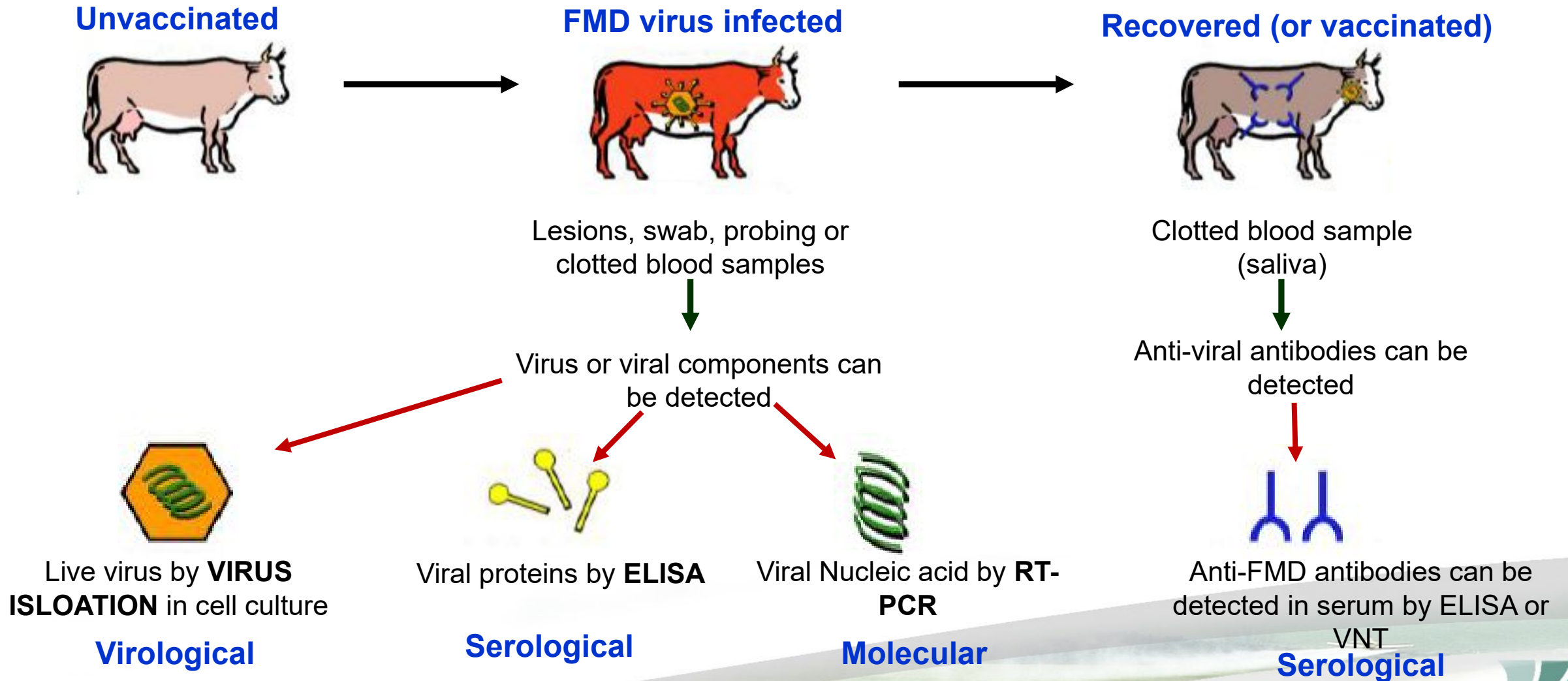


**Complicates the control of FMD in southern African countries**

# Control of Foot-and-Mouth Disease

- To ensure proper control of FMD in southern African countries
  - Regular livestock vaccination programmes
    - Vaccines are matched to circulating field strains
    - Degree of cross protection provided by the vaccine against emerging field strains
  - Accurate diagnosis of FMDV infection is of utmost importance
  
- Effectively manage FMD and consequently participate in international and regional trade of livestock and livestock products

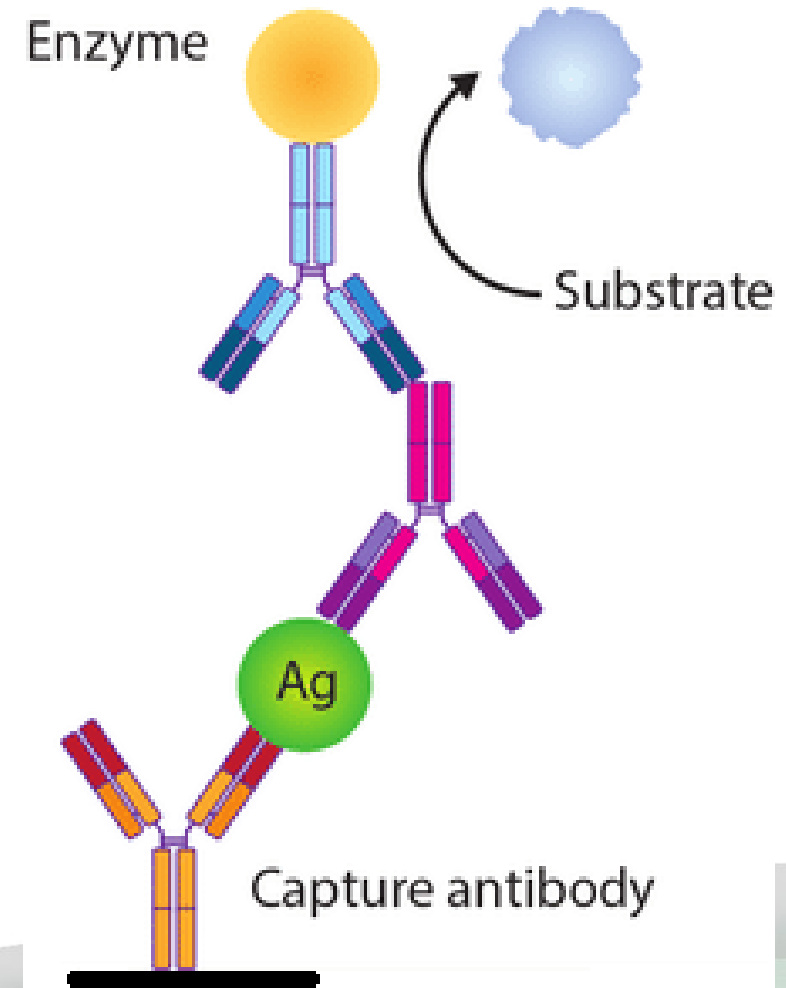
# Diagnosis of Foot-and-Mouth Disease





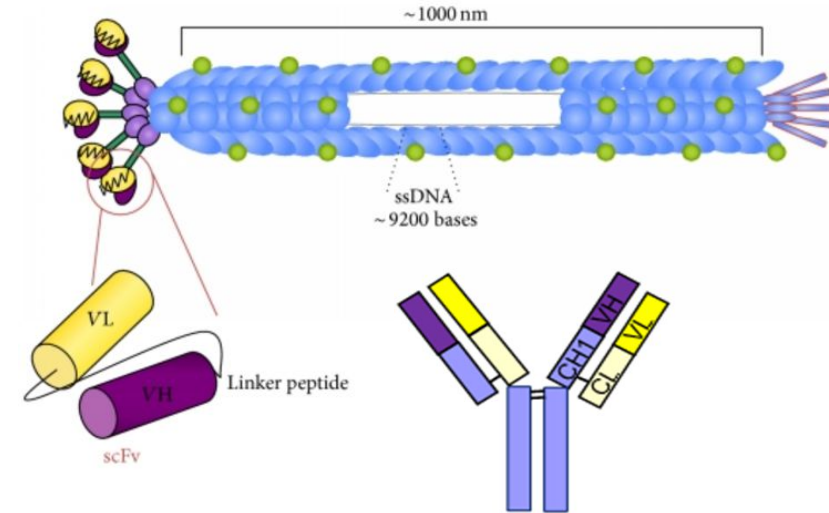
# Liquid Phase Blocking ELISA

- Liquid phase blocking ELISA
  - Well established for SAT1, SAT2 and SAT3 viruses
  - Prevalence and genetic diversity of the SAT-type viruses
- Plagued with inadequacies
  - Cross-reactivity is noted between the SAT1, SAT2 and SAT3 LPBEs
  - **Polyclonal** capture and detection antibodies
    - bind to multiple epitopes on the same antigen
    - higher potential for cross-reactivity
    - good as a capture reagent but not as a detecting reagent
- **Need to improve the sensitivity and specificity of the SAT ELISA's**



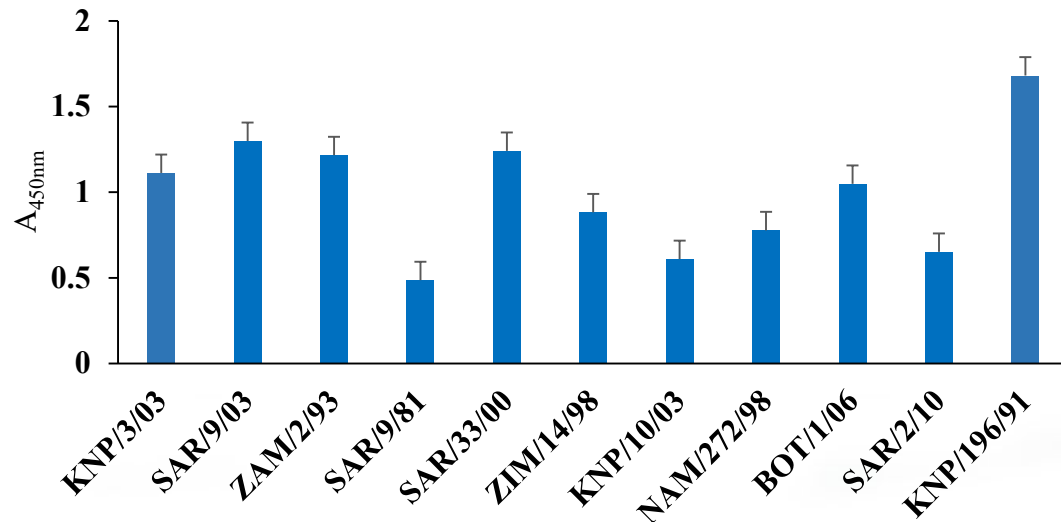
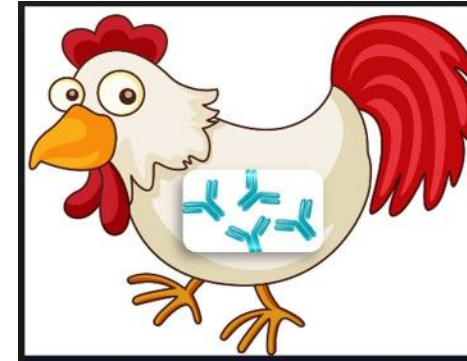
# Monoclonal Antibody Production

- Monoclonal antibodies - improve specificity and sensitivity - bind to one unique epitope
  - Hybridoma technology – expensive, lengthy, expertise, laboratory animals
- **Recombinant reagents for ELISA - Antibody phage display technology**
  - *In vitro* selection technique
  - Robust immunoreagents with high affinity and specificity
- Antibody libraries of Fab
  - Genes coding for the VH and VL antibody fragments (scFv) displayed on the surface of a filamentous phage
    - Immune library - animals immunized with a target antigen
    - Naïve library - non-immunized animals
  - Advantages: stable, rapid and inexpensive to produce and easily manipulated

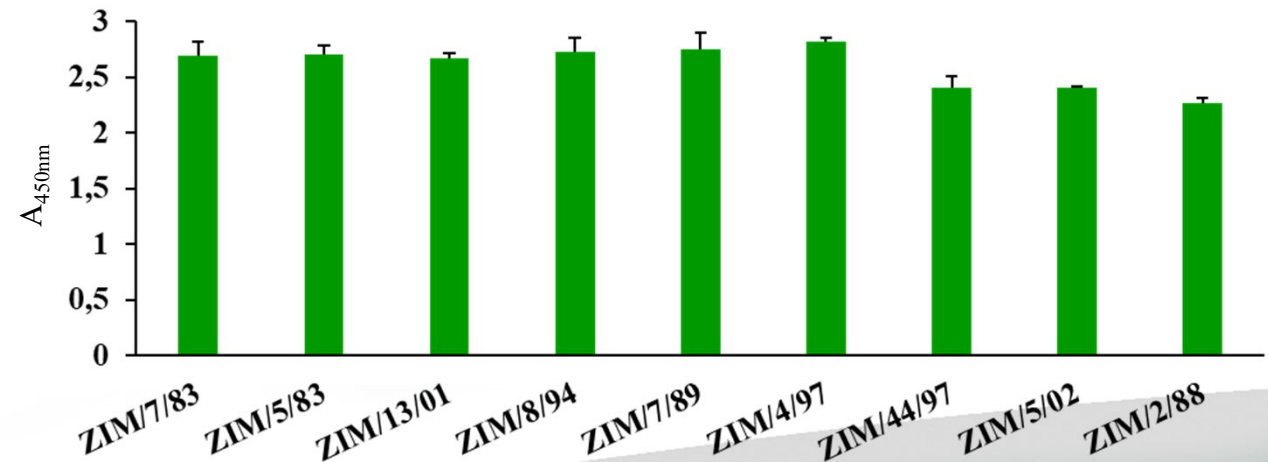


# Proof of Concept as Diagnostic Reagents

- Nkuku<sup>®</sup> phage display library
  - Large phagemid-based chicken scFv library
  - Naïve immunoglobulin repertoire of the chicken
- Selected SAT1 and SAT2-specific scFvs
- Tested in an ELISA format as capturing reagents



**FMDV SAT1 serotype viruses**



**FMDV SAT2 serotype viruses**

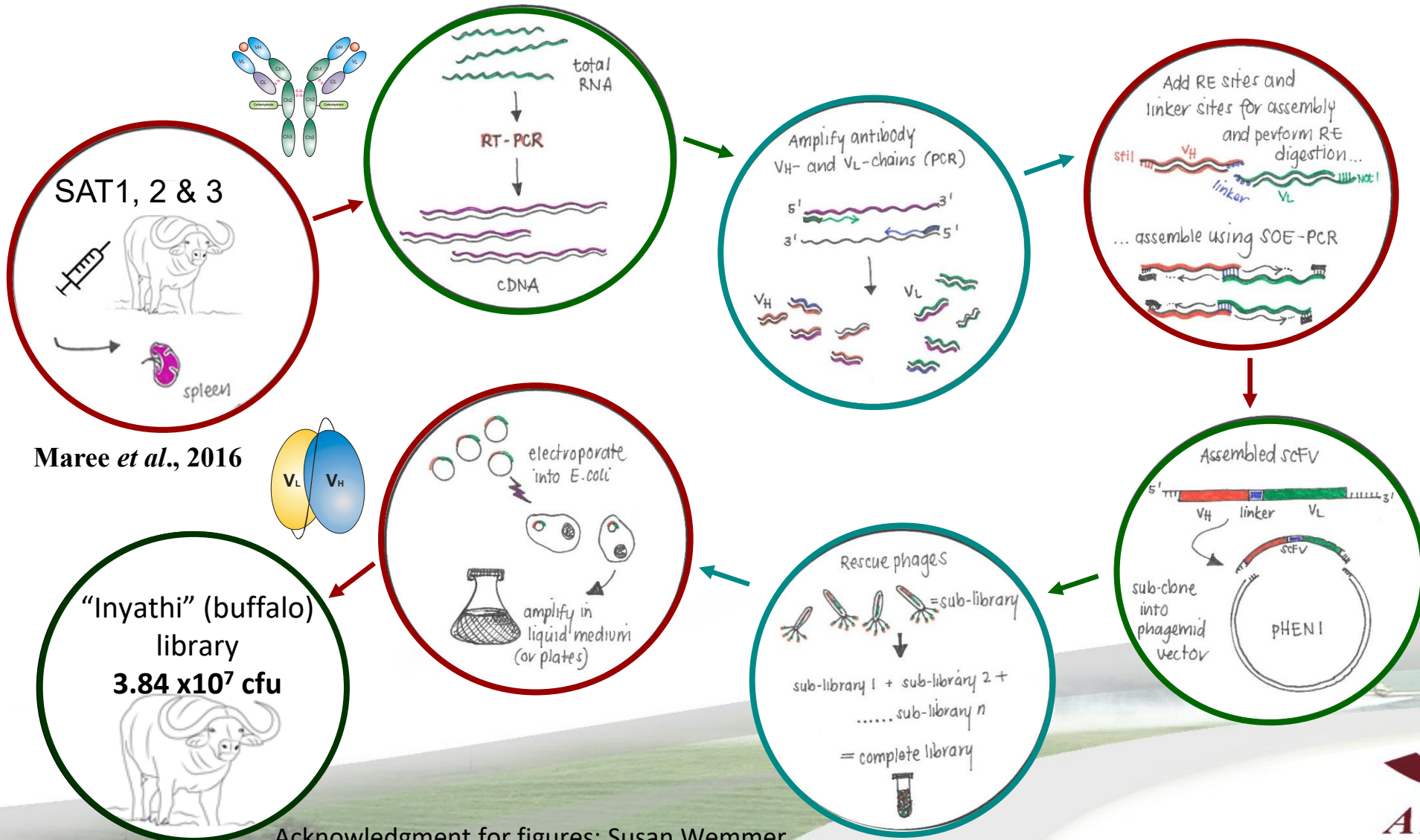
# AIM

**Construct a FMD SAT serotype-specific immune phage display library,  
derived from infected buffalo spleen, with the intentions to utilize the  
scFvs to achieve improved FMD diagnostic tests**

Immune libraries are derived from animals infected or immunized with a target antigen (FMDV) and are therefore pre-biased towards antibody fragments with desirable affinities and specificities

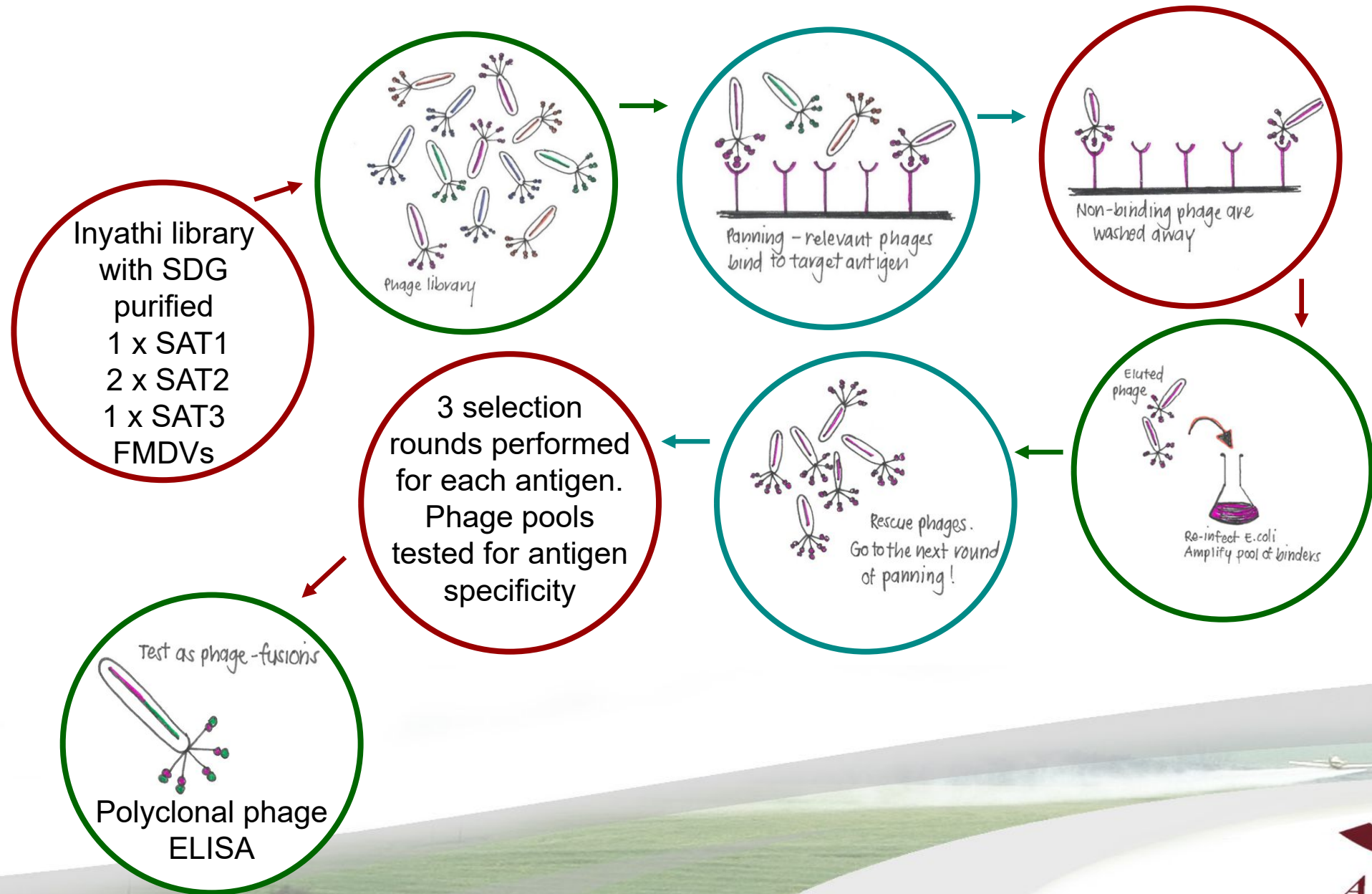


# Construction of a Recombinant Antibody Phage Display Library

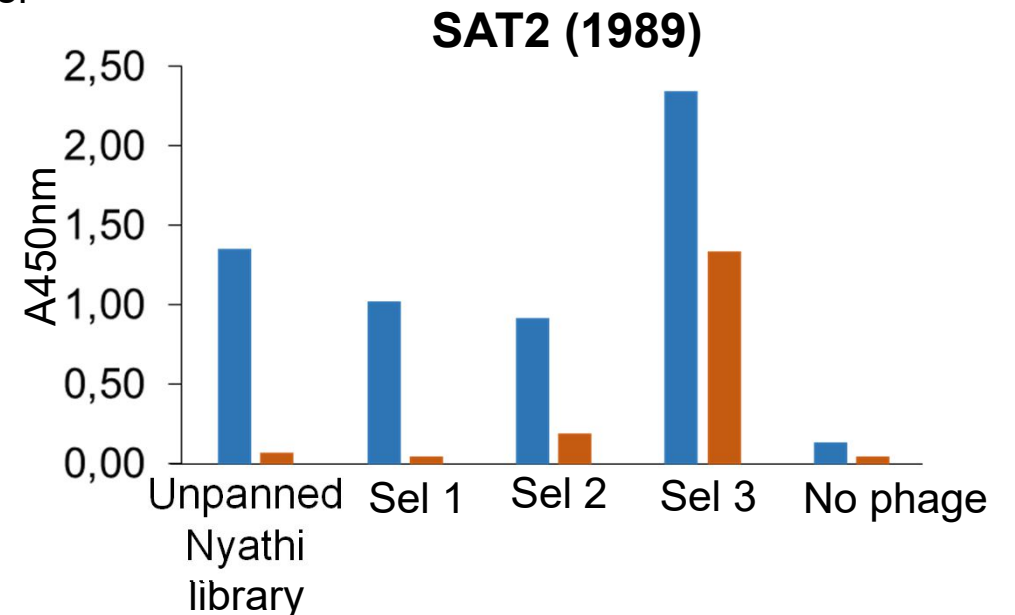
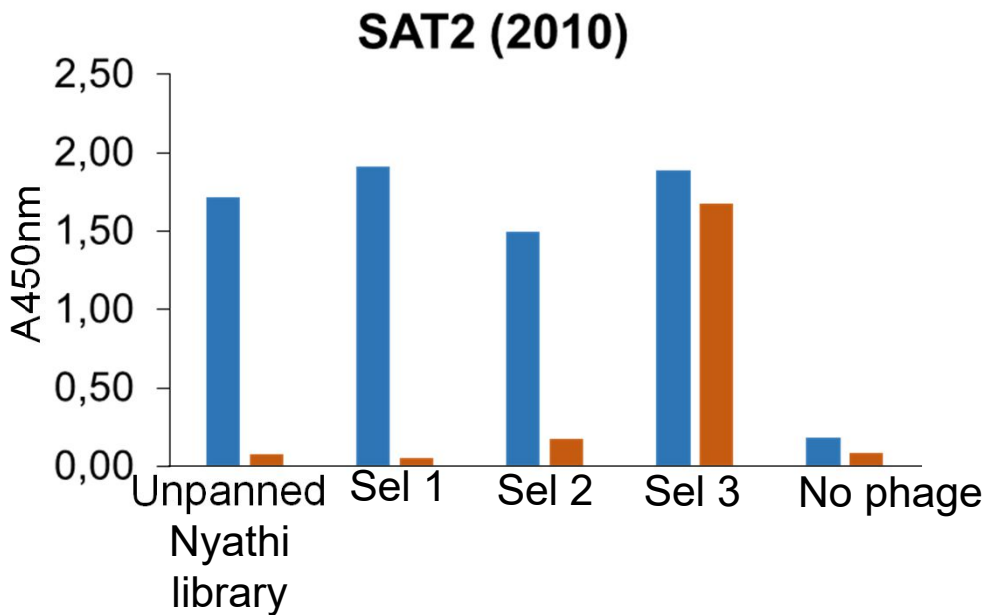
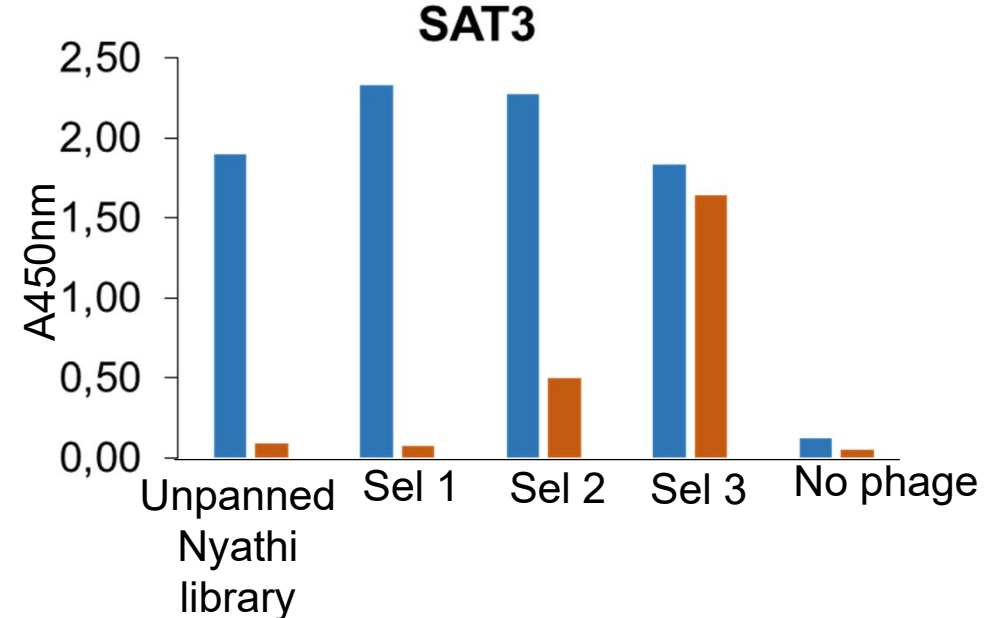
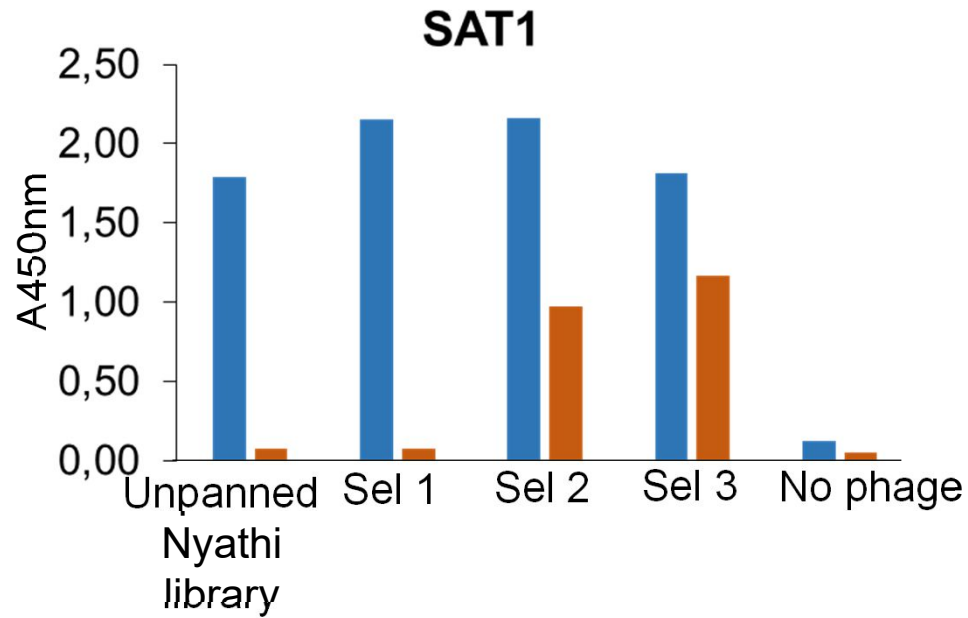


Acknowledgment for figures: Susan Wemmer

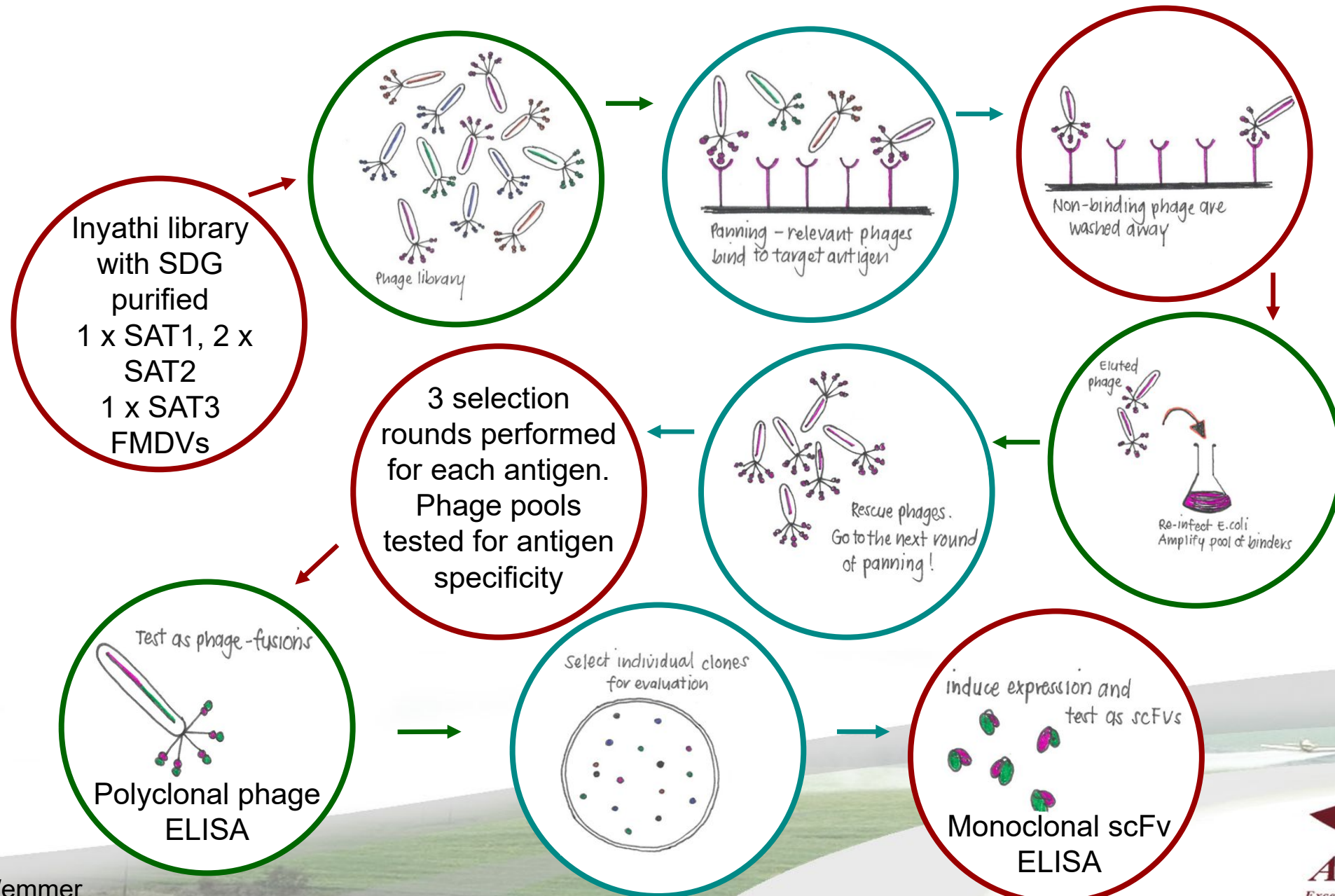
# Bio-Panning of SAT Antigens



# Bio-Panning Enrichment



# Bio-Panning of SAT Antigens

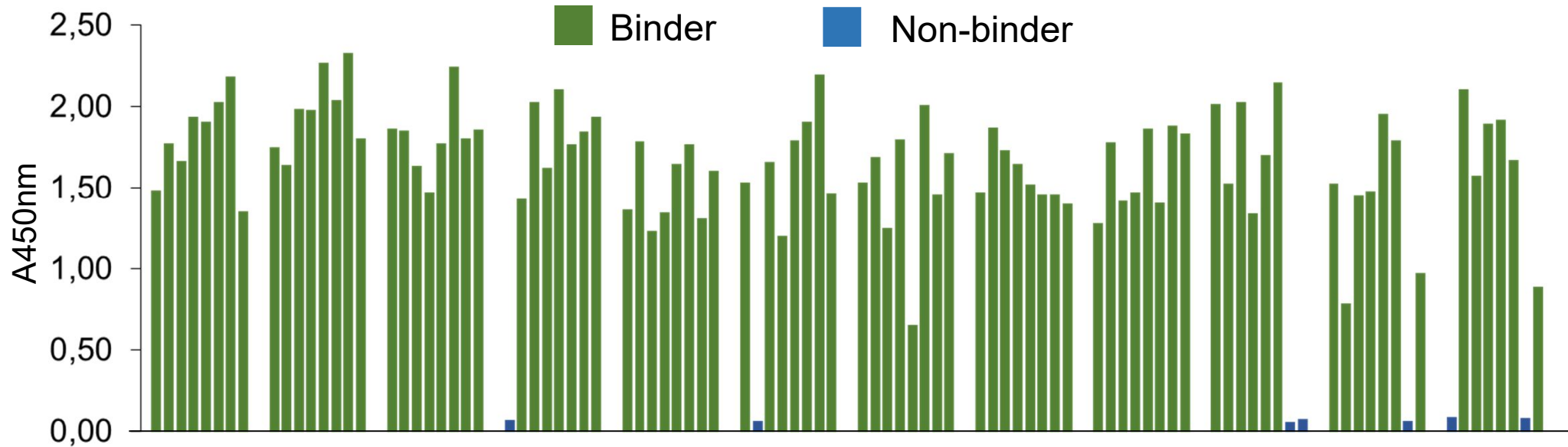




# Screening for Unique SAT Specific Binders

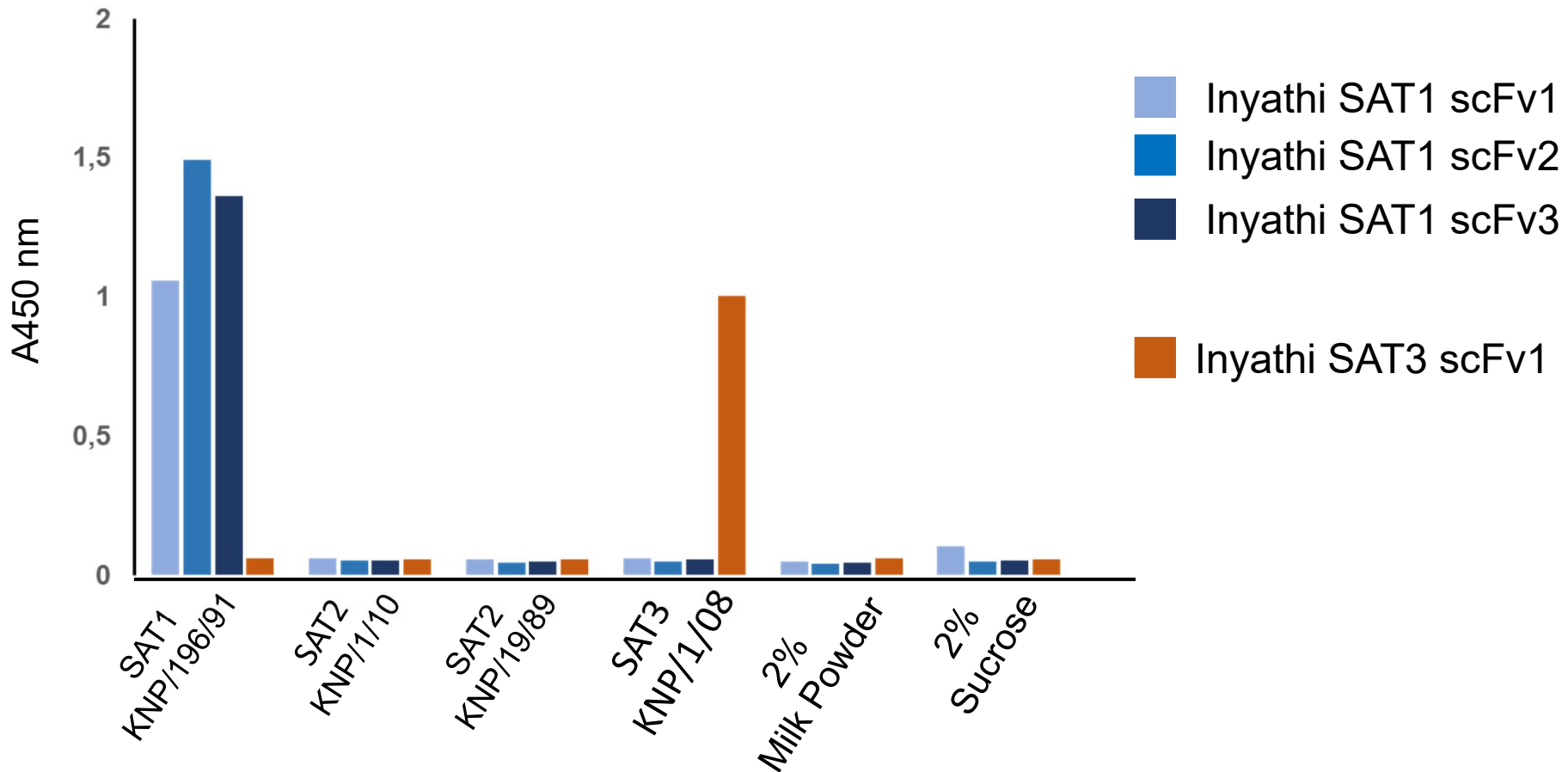
Individual SAT1/KNP/196/91 clones screened in the monoclonal scFv ELISA

**SAT1/KNP/196/91**



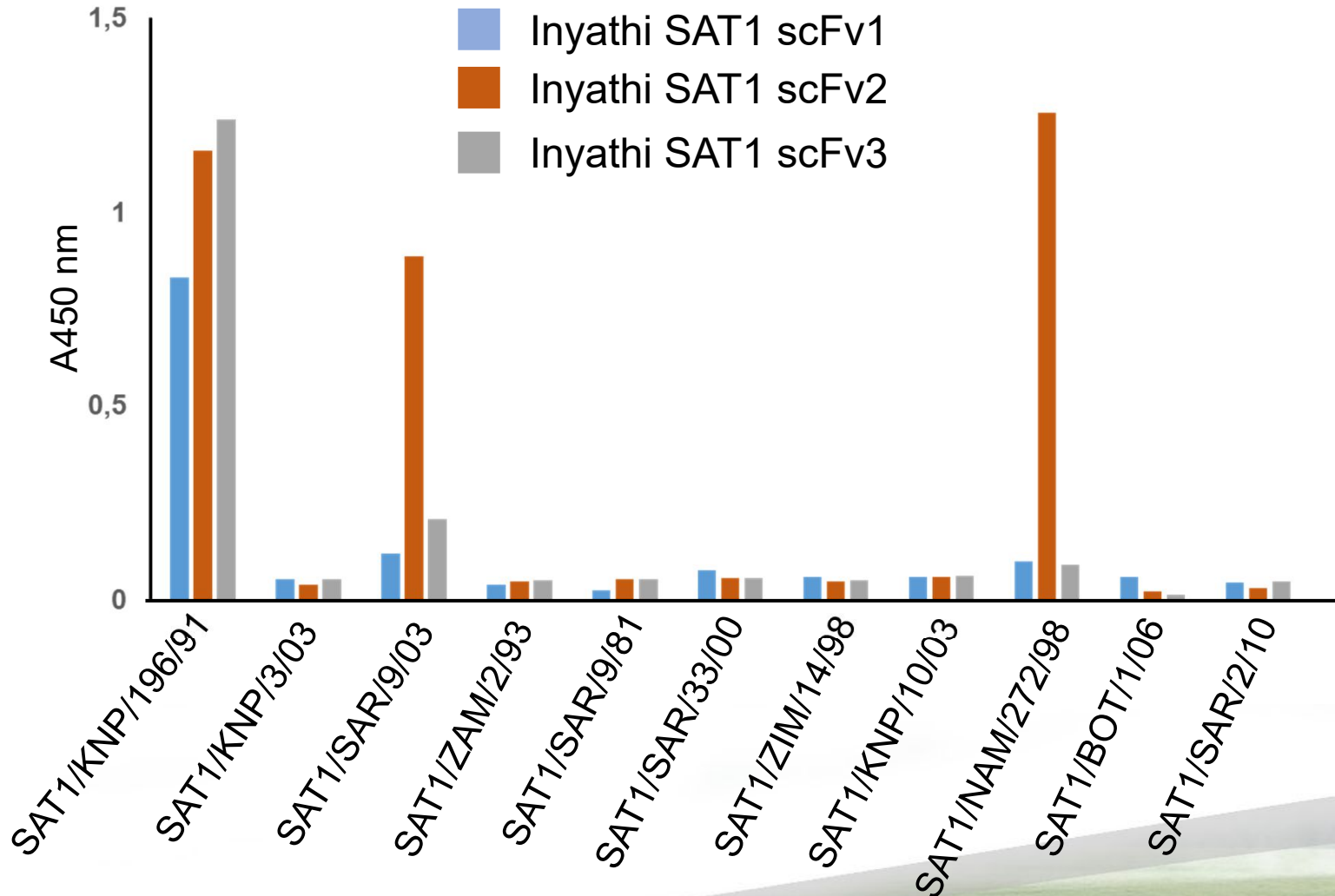
	# Binders screened	# Positive scFv binders	# Inserts sequenced	# Unique binders
SAT1	1440	295	68	3
SAT2 (2010)	270	0	0	0
SAT2 (1989)	270	0	0	0
SAT3	540	1	1	1

# Intra-Serotype Specificity ELISA



- Serotype – specific
  - scFv binder only bound to the virus to which it was bio-panned
- Intra-specific – differentiate between serotypes

# ELISA : SAT1 Detecting Antibody



- ELISA plate coated with SAT1 specific rabbit antiserum
- Inyathi **SAT1** scFv 1, 2 and 3 bound to SAT1/KNP/196/91
- Inyathi **SAT1** scFv 2 bound to SAT1/SAR/9/03 & SAT1/NAM/272/98
- Inyathi **SAT1** scFv 3 showed reduce reactivity to SAT1/SAR/9/03
- Inyathi **SAT3** scFv 1 binder did not work as a detecting reagent

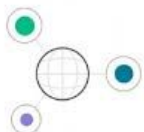
# Conclusions

- We successfully constructed a novel FMD immune phage display library (*Inyathi* (buffalo) library)
- Enrichment occurred for the SAT1 and SAT3 antigens panned
- Three SAT1-specific and one SAT3-specific binder obtained
- All the binders were specific to the virus to which it was panned against
- The SAT1 binders did not show great potential as detecting antibody in an ELISA
  - Test the binders as capturing antibodies
- Immune libraries may not be the best library to select scFv's for diagnostic reagents
  - Too specific
  - Host-specific naïve library may be better
- **These binders show great potential to be used in epitope mapping studies**



# Future Work

- Test the Inyathi SAT3 scFv 1 against a wider panel of SAT3 viruses
  - Potential as a detecting reagent
- Purify the scFvs and test them as capturing antibodies
- Construction of an expanded immune buffalo library (IVVN funded 2019)
  - Identify critical antigenic determinants within the FMDV capsid from strains that circulate in different parts of the world that could be used in the rational design of more effective vaccines for FMD control
  - Target additional organs: Spleen and lymph nodes which are highly stimulated after infection with FMDV



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# Acknowledgements

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Foot-and-Mouth Disease can affect **cloven-hooved** animals, which includes pigs, sheep, goats and cattle.

