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# Improved FMD vaccines for East Africa

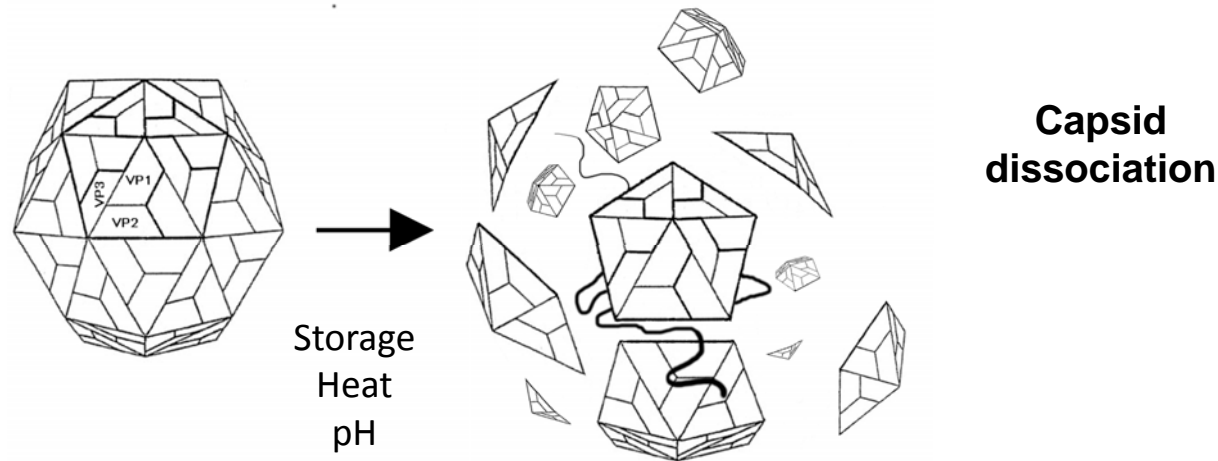
Julian Seago



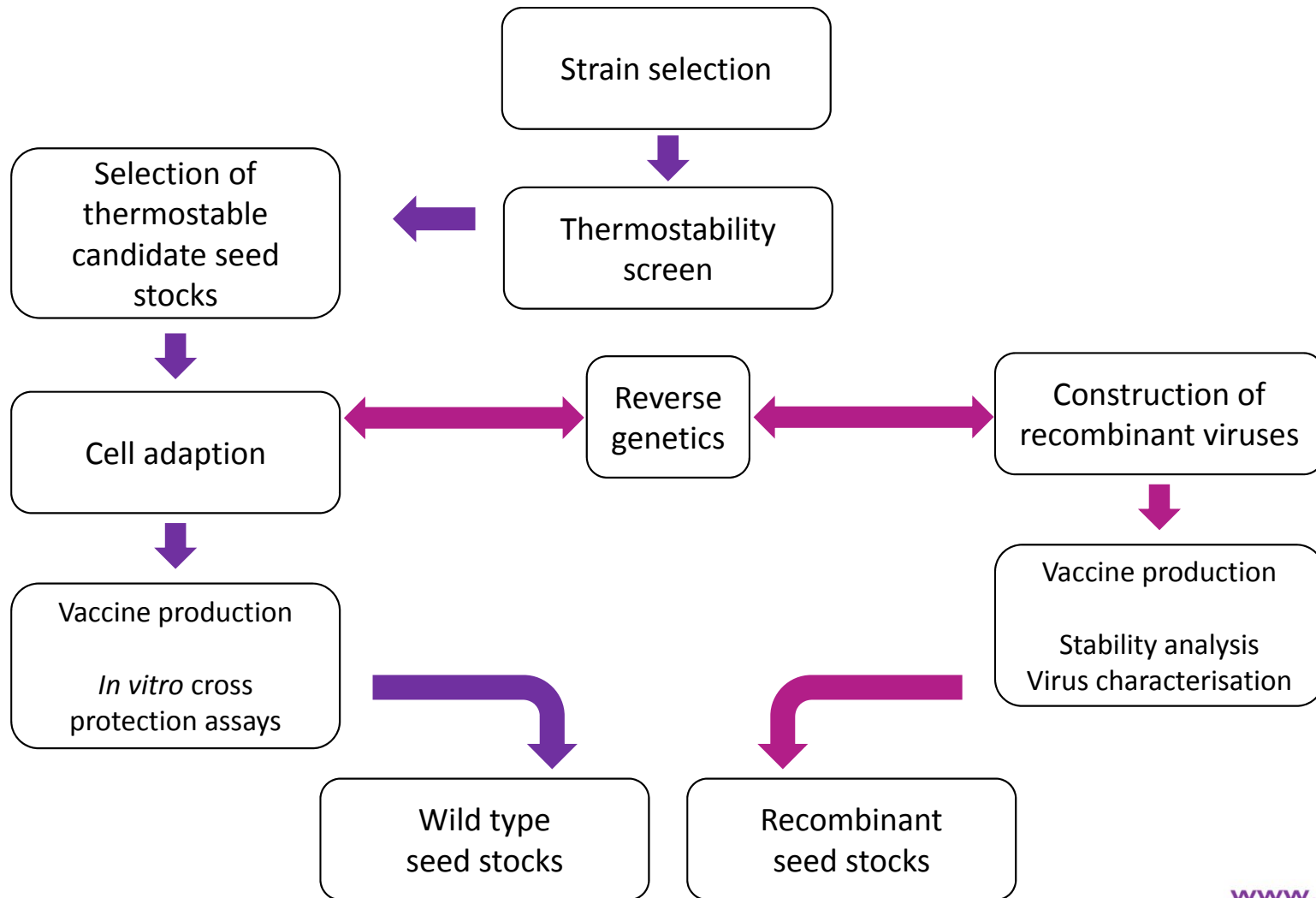
[www.pirbright.ac.uk](http://www.pirbright.ac.uk)

# FMDV capsid stability

- FMDV capsids are unstable - serotypes O & SAT have been reported to be less stable than A & Asia1.
- Capsid dissociates into pentameric subunits at  $< \text{pH}7$ ,  $> 30^\circ\text{C}$  and during storage.
- Vaccine efficacy requires intact capsids.



# Project workflow



# Candidate selection criteria

➤ **Region**

East Africa – Egypt, Sudan, Ethiopia, Kenya, Tanzania

➤ **Livestock**

Cattle

➤ **Date of collection**

2005 - 2013

➤ **Serotype**

SAT1, SAT2, O, A

➤ **Subtype**

SAT1: topotype I, IX; SAT2: topotype IV, VII, IX, XIII, O: East Africa 2, 3, 4; A: genotype I, IV, VII.

Viruses purchased  
from WRL, Pirbright



# Candidate selection criteria - region

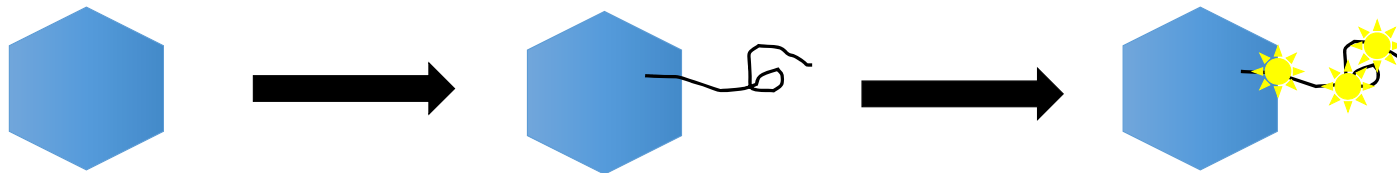
## 42 FMDV strains

- Serotype O (15 strains)
- Serotype A (9 strains)
- Serotype SAT1 (8 strains)
- Serotype SAT2 (10 strains)

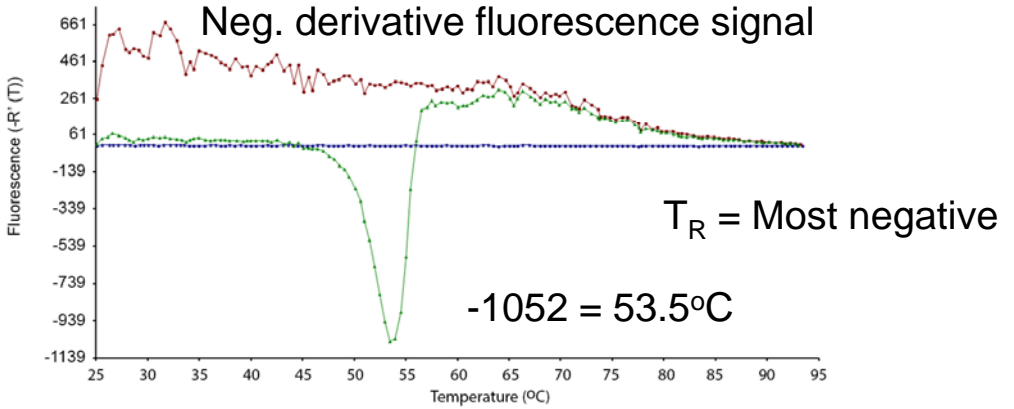
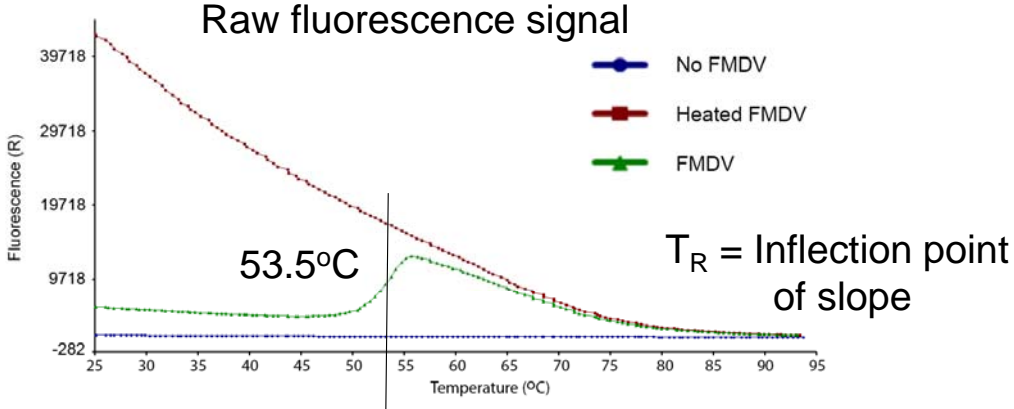


# Confirming stability - Thermofluor Assay

- A simple, fast method to analyse capsid stability
- Minimal requirements:
  - qPCR machine
  - Fluorescent dye that binds RNA genome – SYBR green II
  - Virus sample
- Methodology:
- Temperature increases, the FMDV capsid dissociates releasing the viral genome which is bound by the Fluorescent dye.



# Thermofluor Assay



# Summary of thermostability screens

40/42 FMDV strains

## Thermofluor stability assays:

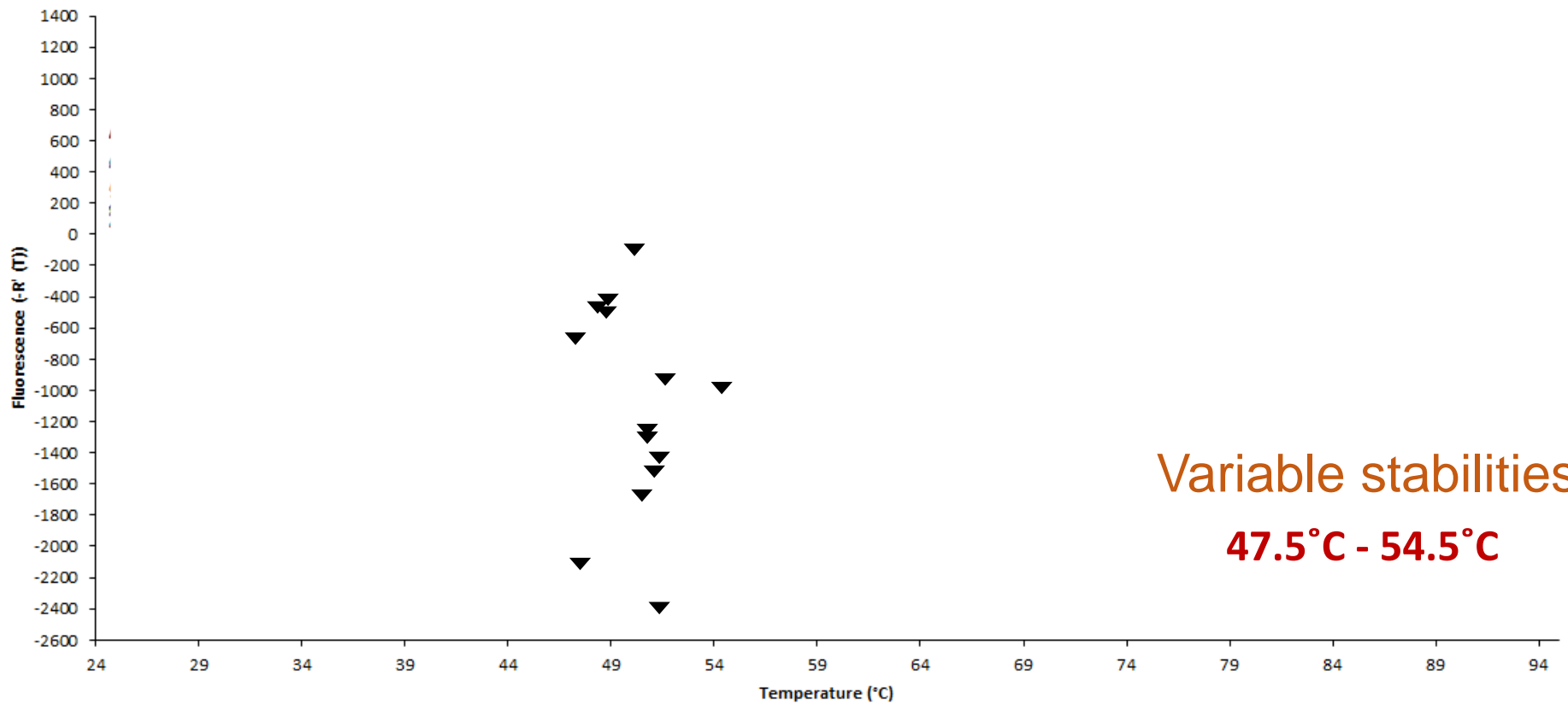
- 9/9 A serotypes (+ 5 control A serotypes)
- 14/15 O serotypes
- 8/8 SAT 1 serotypes
- 9/10 SAT 2 serotypes





# Candidate selection - Stability analysis

## O serotype strains



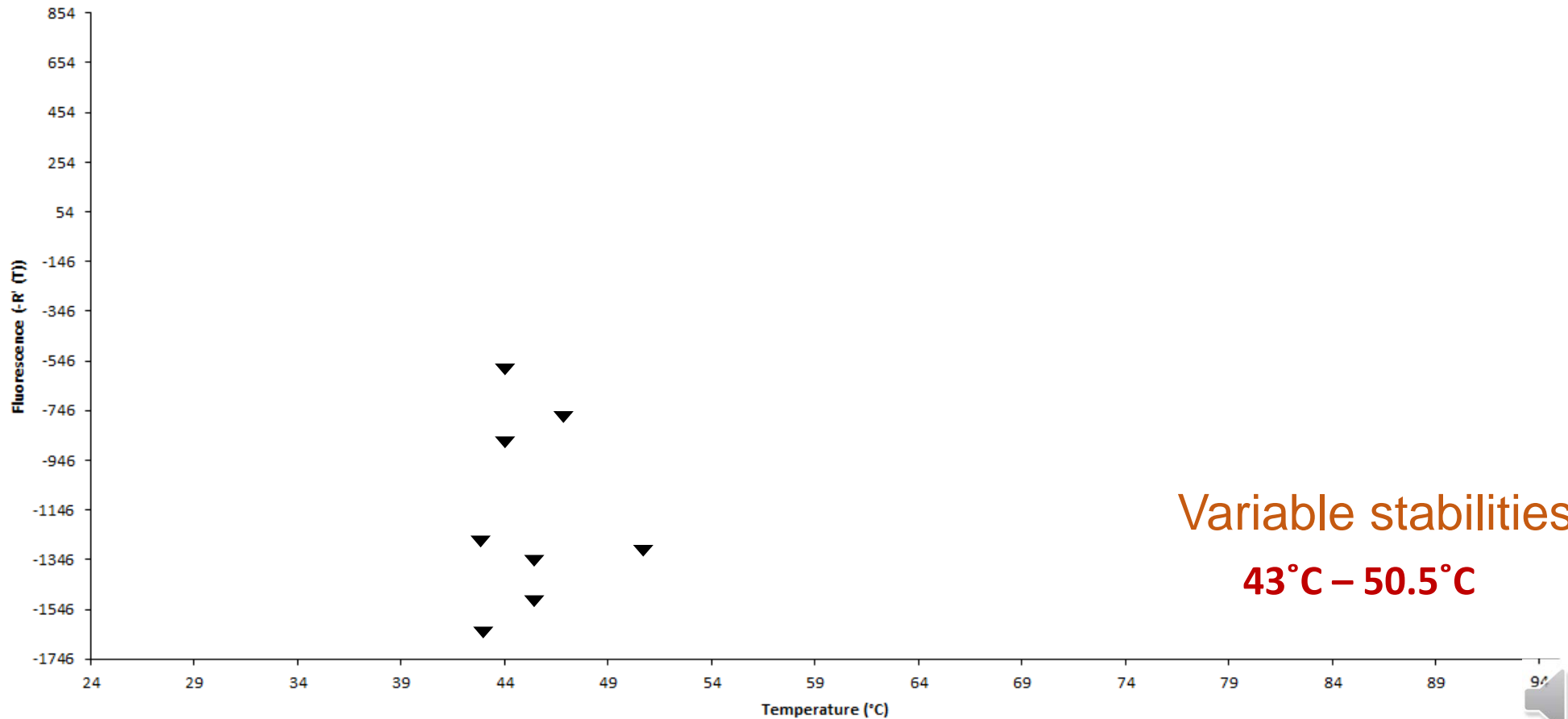
Variable stabilities

47.5°C - 54.5°C



# Candidate selection - Stability analysis

## SAT1 serotype strains



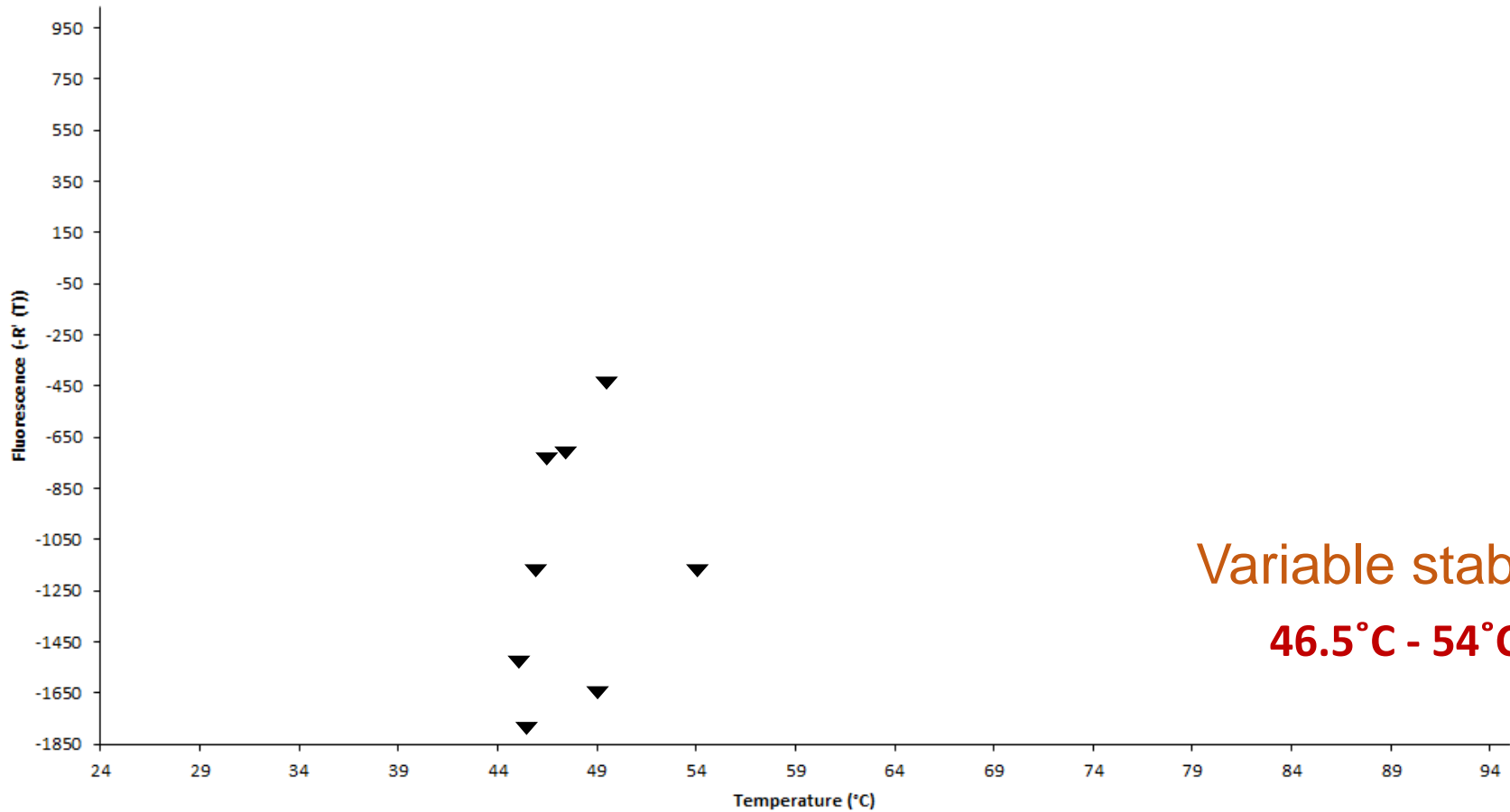
Variable stabilities

43°C – 50.5°C



# Candidate selection - Stability analysis

## SAT2 serotype strains



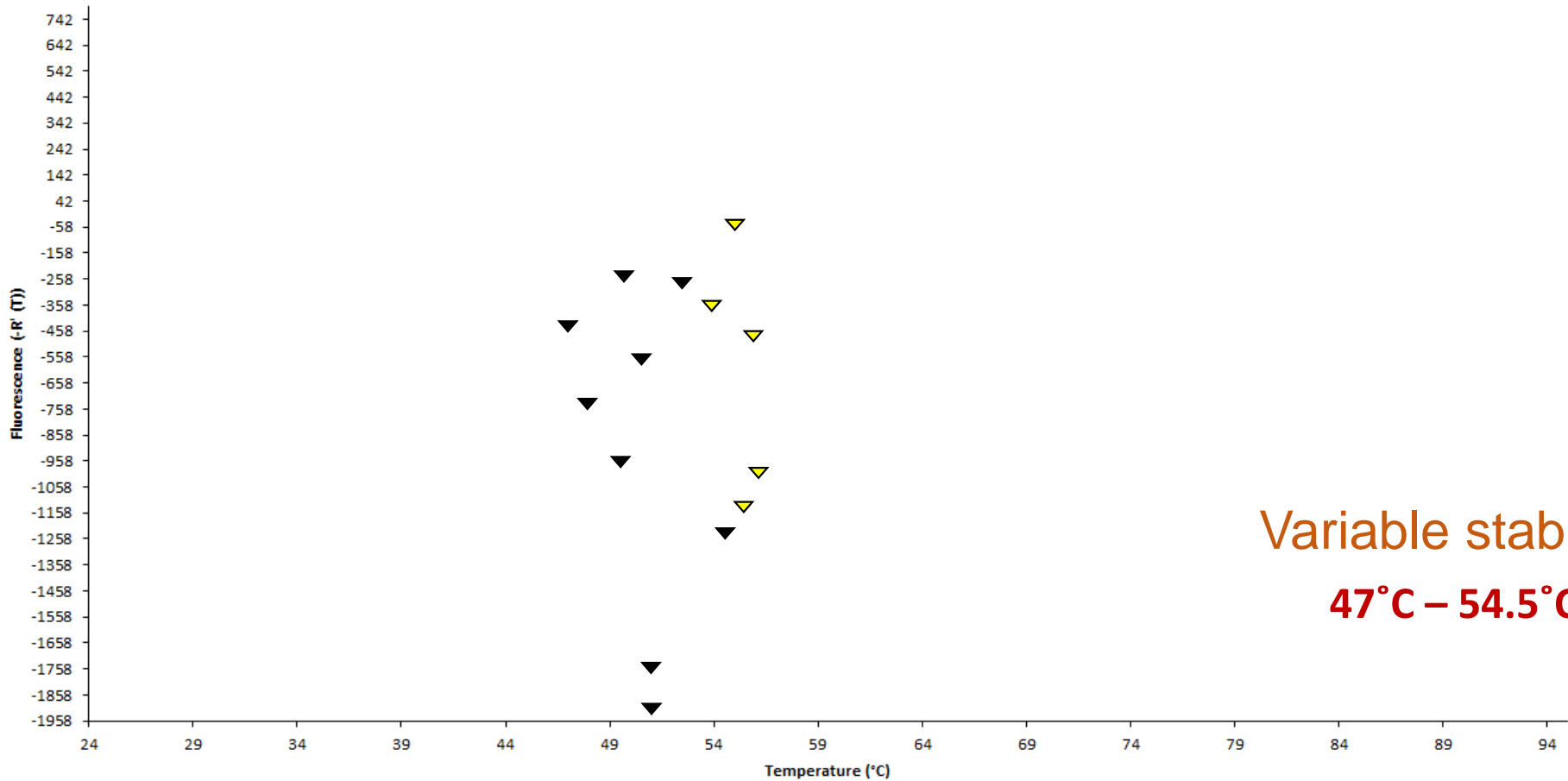
Variable stabilities

46.5°C - 54°C



# Candidate selection - Stability analysis

## A serotype strains



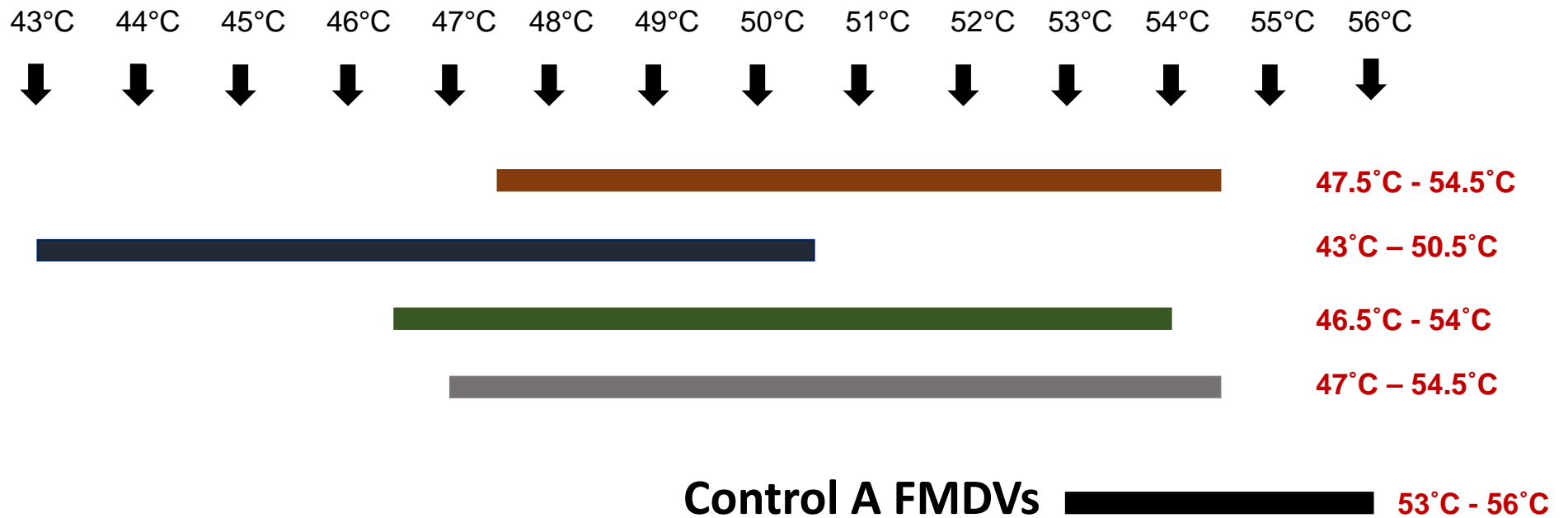
Variable stabilities

47°C – 54.5°C



# Stability analysis – Serotype variability

## Summary:



# Cell adaptation

3 candidate strains per serotype



Passaged in suspension BHK Cells

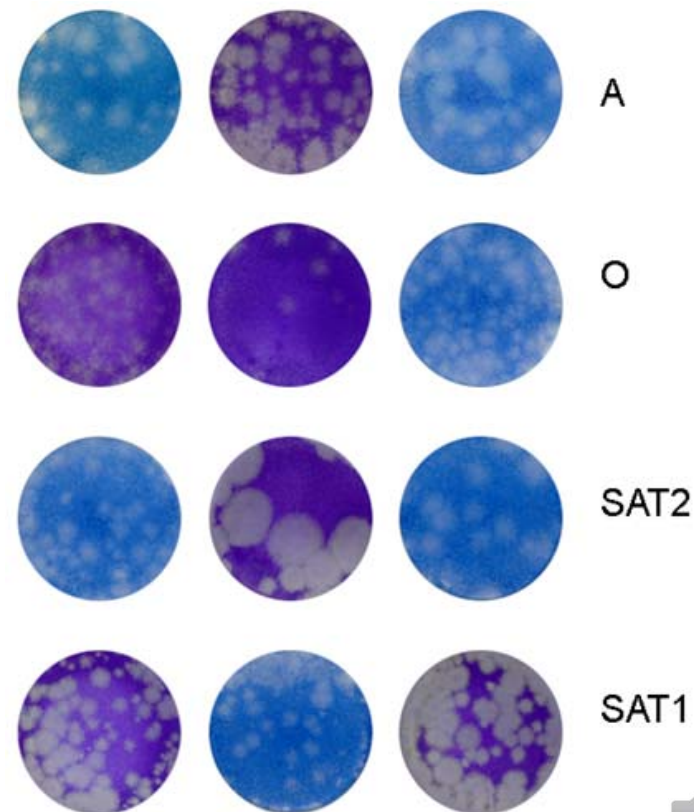


CPE confirmed in adherent BHK cells

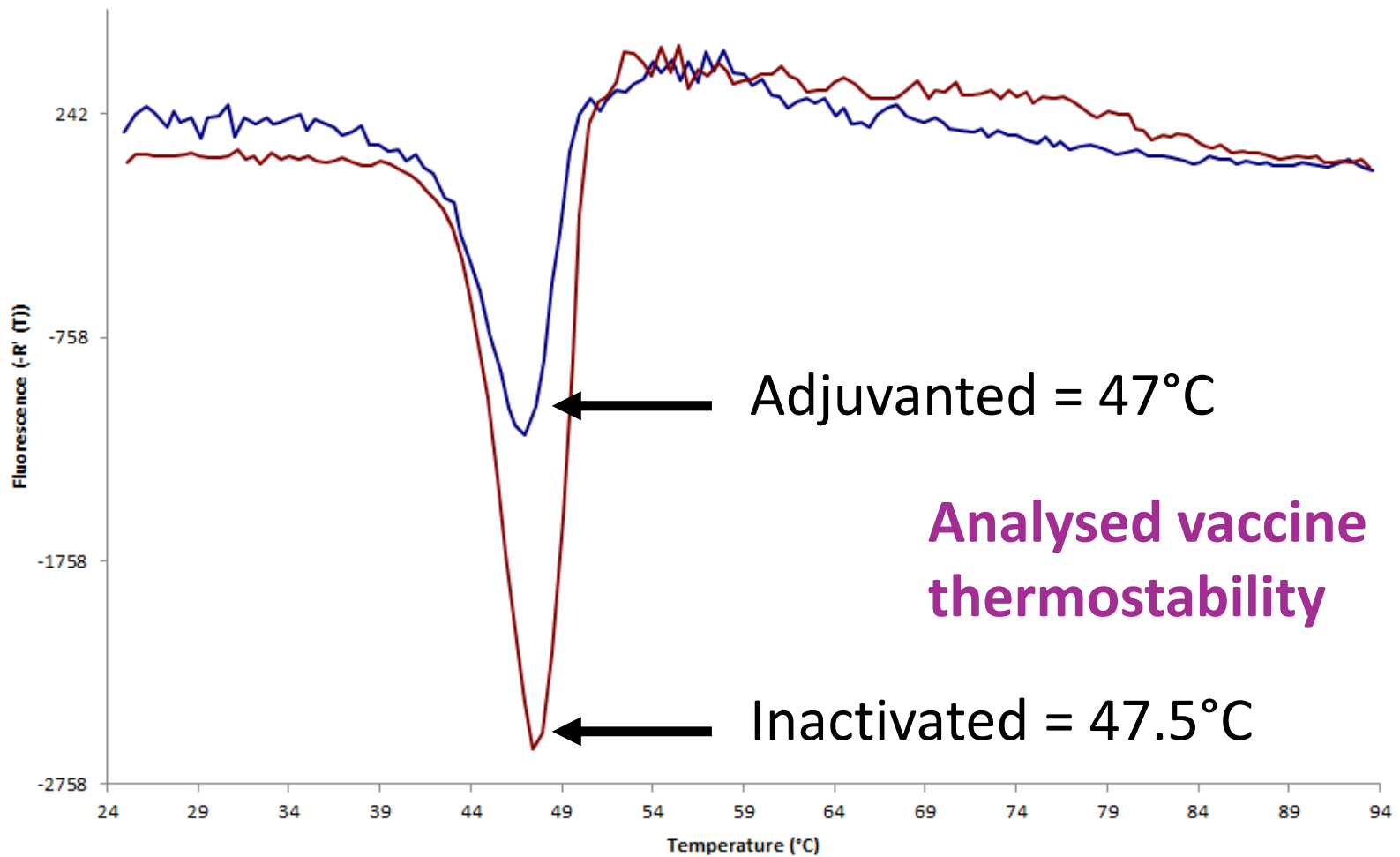
Currently analysing growth of candidates in different suspension cell lines

## 2 Litre Bioreactor

- Control for temperature, dissolved oxygen, pH and level/foam.



# *In vitro* cross protection analysis

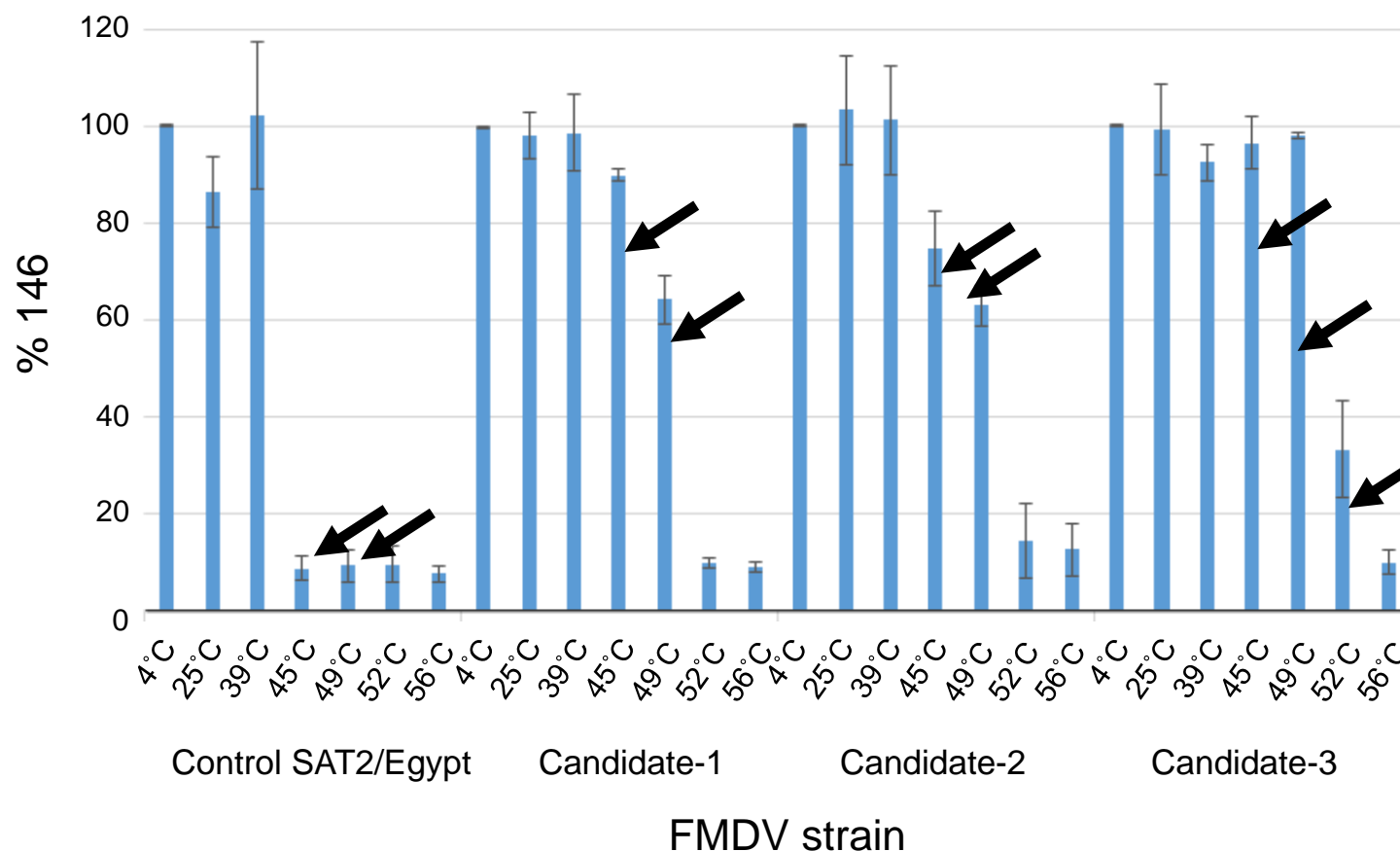


Analysed vaccine  
thermostability



# Stability analysis by ELISA

Accelerated thermostability studies: Inactivated FMDV heated for 20 minutes



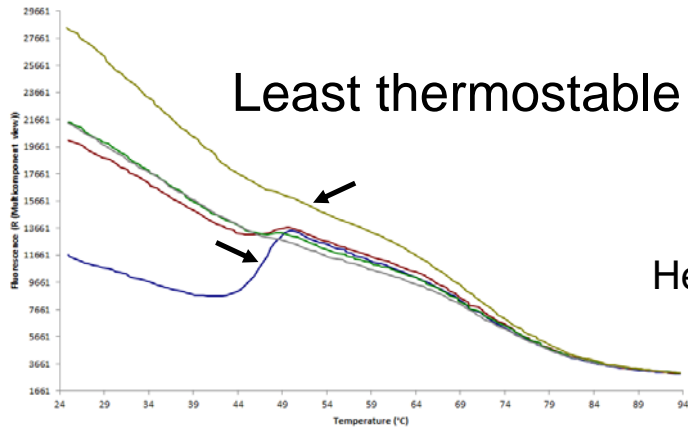
Llama-Ab  
based ELISA





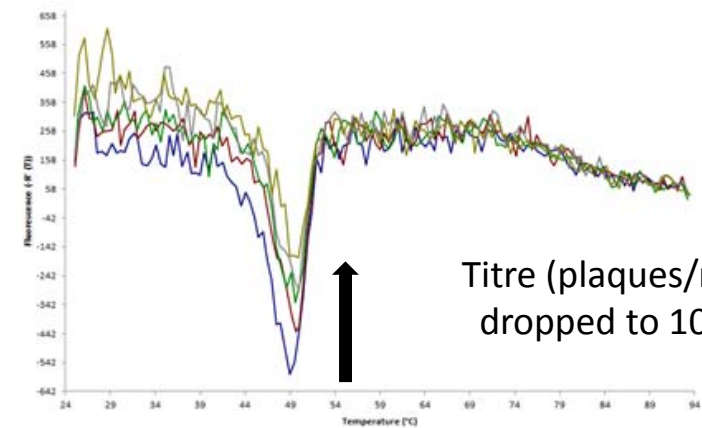
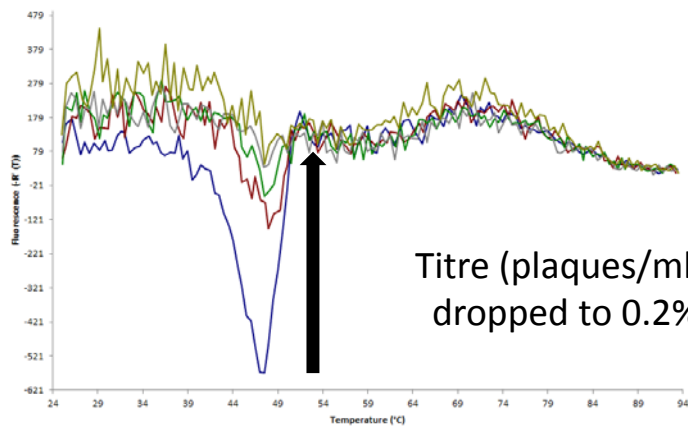
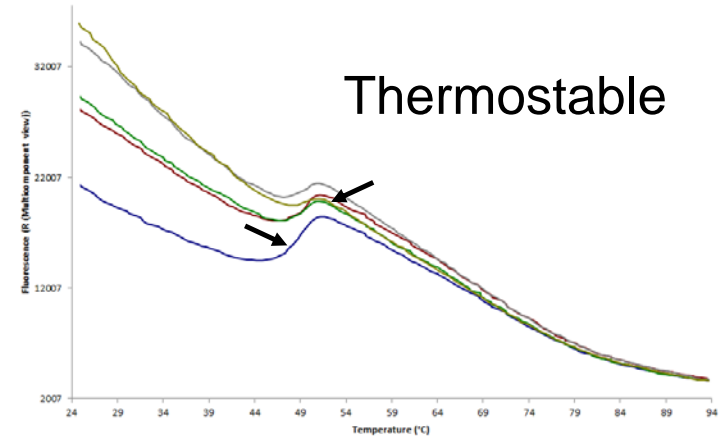
# Stability analysis by thermofluor & plaque assay

## Accelerated thermostability studies: SAT2 FMDV



Heated at 43°C for:

- 60 min
- 45 min
- 30 min
- 15 min
- 0 min



## Stability analysis following long-term storage at 4°C

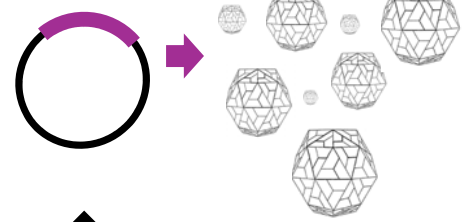
	Day-1)	1 month	2 months	3 months	4 months	5 months	6 months	7 months
O	53.5°C	53°C	53°C	53.5°C	53.5°C	53.5°C	53.5°C	53°C
SAT1	48°C	48°C	48°C	48°C	47.5°C	47.5°C	47.5°C	47°C
SAT2	51°C	51°C	51°C	51°C	51°C	50.5°C	50.5°C	50.5°C
A	53°C	53°C	53°C	52.5°C	52.5°C	52.5°C	52°C	52°C



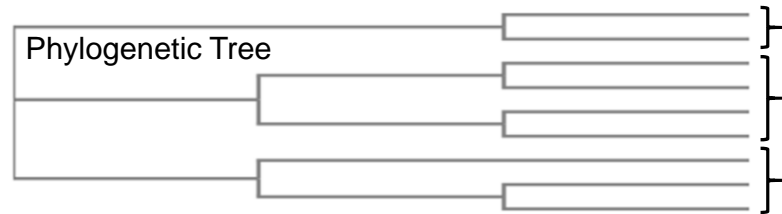
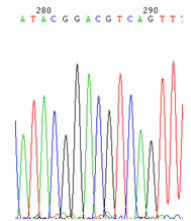
# Sequence analysis



Capsid (VP4,2,3,1)



- Inform genetic basis of capsid stability
- Inform genetic basis of cell adaption



**Most stable** 53.5 - 56°C

**Least stable** 47 - 50°C

**Intermediately stable** 50.5 - 52.5°C



Correlation between phylogeny, region & stability

# Thank you.....

Ben Jackson

Yongie Harvey

Eva Perez

Bryan Charleston

Veronica Carr

Fuquan Zhang

BILL & MELINDA  
GATES *foundation*

Tim Doel

Nick Knowles

Claudia Doel

Byron Moore

Valerie Mioulet

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Jemma Wadsworth

Srijana Rai

Elena Lokhman

