Rational Design of Attenuated FMDV Vaccines By Elevation of –CpG- and –UpA- Dinucleotide Frequencies

Prof. Martin D. Ryan, Dr. Fiona Tulloch, Dr. Garry A. Luke & Dr. Sean Wilson

Biomedical Sciences Research Complex, School of Biology, University of St. Andrews, North Haugh, St. Andrews KY16 9ST, UK.

Dr. Toby Tuthill, Dr. Don King, Dr. Sarah Gold, Dr. Lidia Laseka-Dykes & Dr. Caroline Wright

The Pibright Institute, Ash Rd., Pirbright, Surrey GU24 0NF, UK.

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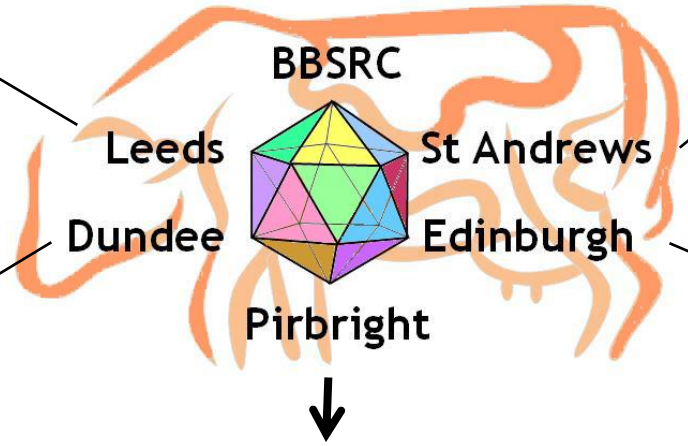
Prof. Dave Rowlands
Prof. Nic Stonehouse
Dr. Morgan Herod
Mr. Joseph Ward
Ms. Eleni-Anna Loundras
Mr. Oluwapelumi Adeyemi

Prof. Angus Lamond



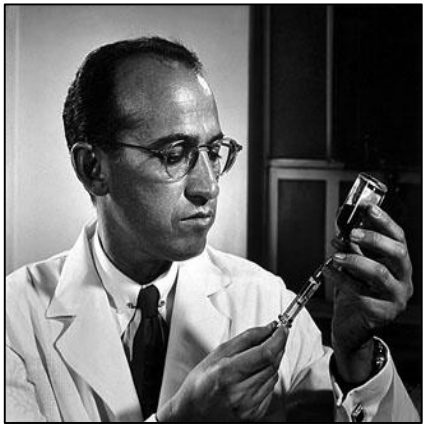
Dr. Fiona Tulloch
Dr. Garry Luke
Dr. Sean Wilson

Prof. Juergen Haas
Dr. Sam Griffiths
Dr. Michelle McNab

Dr. Toby Tuthill (Dr. Terry Jackson)
Dr. Don King
Dr. Sarah Gold
Dr. Lidia Laseka-Dykes
Dr. Caroline Wright

Prof. Peter Simmonds – University of Oxford

Killed *versus* Live, Attenuated, Vaccines: Poliovirus

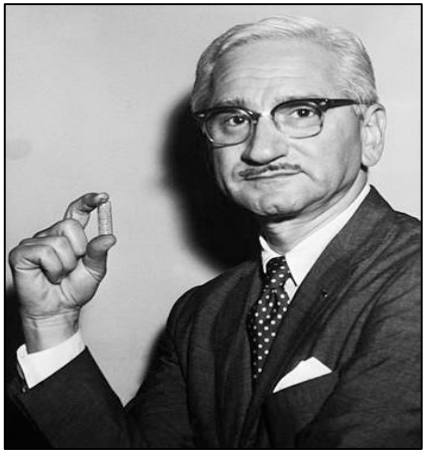


Jonas Salk: 1955

The 'Cutter Incident': April 1955

Incomplete formaldehyde inactivation of poliovirus;

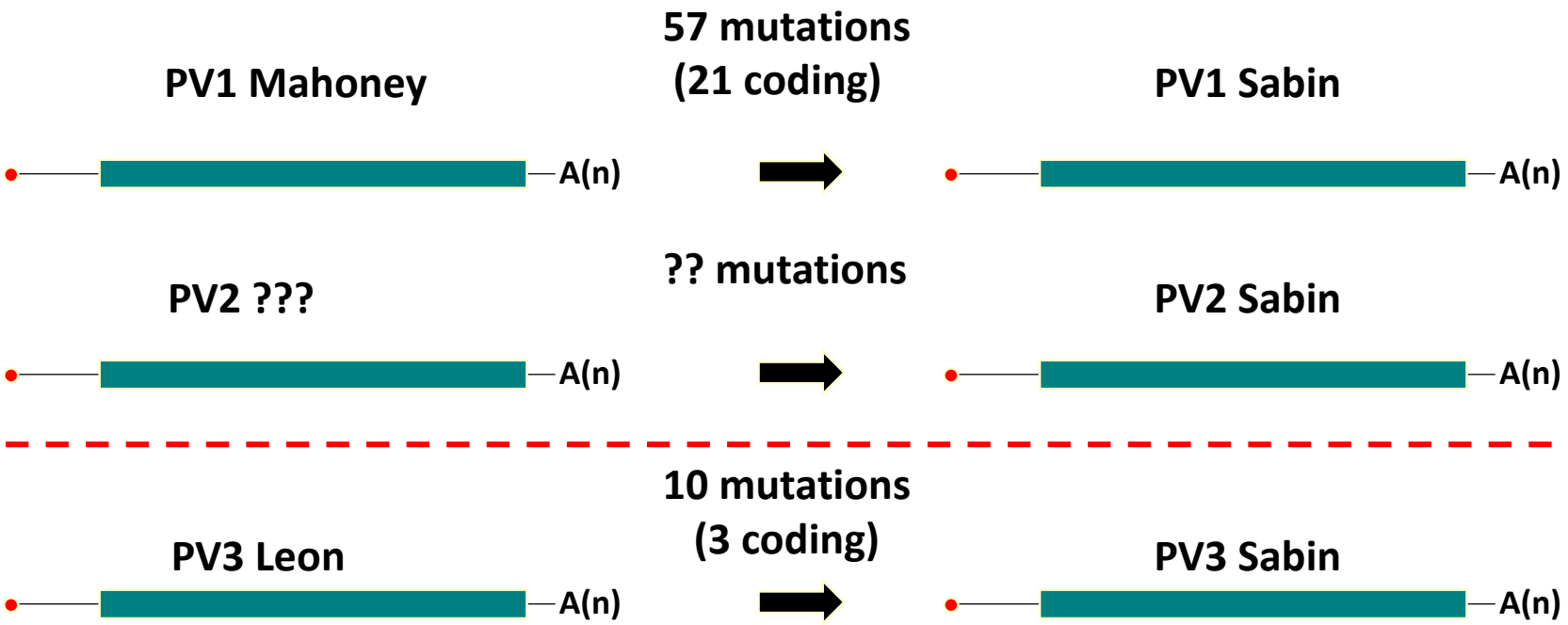
- 40,000 'abortive' poliomyelitis cases (no CNS involvement)
- 113 people paralyzed – mainly children
- 5 children died



Albert Sabin: 1956

Live, attenuated, poliovirus types 1,2 and 3

Live, Attenuated, Vaccines: Reversion to Virulence



PV1(S): the P1 domain harbours 22 nucleotide mutations resulting in 12 amino acid changes.
PV3(S): the P1 domain harbours only 2 nucleotide changes resulting in only 2 amino acid changes.

Mutations in the P1 domain of both viruses confer a *ts* phenotype to the vaccine viruses and they are now considered the most important determinants for attenuation.

Live, Attenuated, FMDV Vaccines…..?

Mowat, G.N. (1961). **Multiplication in vivo of modified foot and mouth disease virus**. *Res. Vet. Sci*. 2:153-161.

Brooksby, J.B., Thorp, A.C.P., Davie, J., Mowat, G.N. & O'Reilly K.J. (1962). **Experiments with modified strains of the virus of foot and mouth disease**. *Res. Vet. Sci.* 3:315-325.

Mowat, G.N. & Prydie, J. (1962*).* **Observation in East African cattle of the innocuity and immunogenicity of a modified strain of foot and mouth disease virus type SAT 2.** Res. Vet. Sci. 3:368-381.

Mowat, G.N., Brooksby, J.B. & Pay, T.W. (1962). **Use of BHK 21 cells in the preparation of mouse attenuated live foot-and-mouth disease vaccines for the immunization of cattle.** *Nature* 196:655–656.

Mowat, G.N. (1964). **Selection of attenuated strains of foot and mouth disease virus by cloning in tissue culture.** *Bull Off int Epiz*, 61:639-649.

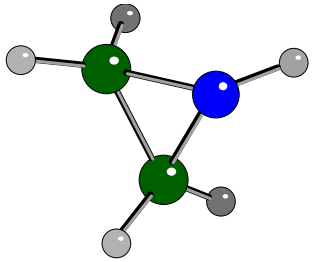
Martin, W.B. & Edwards, L.T. (1965). **A field trial in South Africa of an attenuated vaccine against foot-and-mouth disease**. *Res. Vet. Sci.* 6:196–201.

Zhidkov, S.A. & Sergeev, V.A. (1969). **A study of the properties of attenuated cold variant of type O foot-and-mouth disease virus**. *Veterinariia* 10:29–31.

Mowat, G.N., Barr, D.A. & Bennett, J.H. (1969). **The development of an attenuated foot-and-mouth disease virus vaccine by modification and cloning in tissue cultures of BHK21 cells**. *Archiv fur die gesamte Virusforschung* 26:341–54.

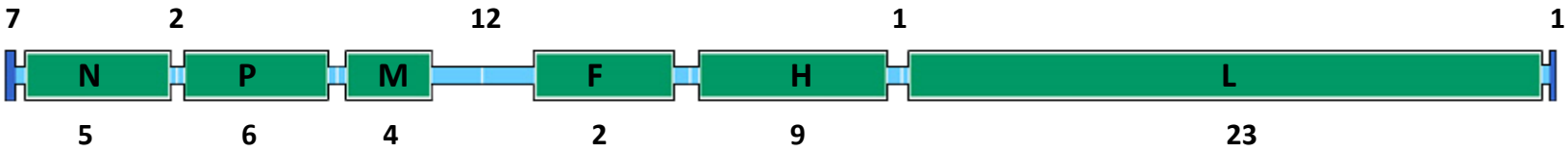
FMDV Chemically Inactivated Vaccine

- Vaccine developed in 1950s – Chemically inactivated (killed) virus preparation
- Bulk growth (BHK-21 suspension cells) of pathogenic virus – *biosecurity + cost*
- Effective inactivation – Aziridine
  (Azacyclopropane, Dimethylenimine, Ethyleneimine, Ethylenimine)



- Does not replicate within the vaccine recipient
- Immune responses against structural proteins *only*
- Does not address the strategic problem of wild-animal reservoirs of virus

Live, Attenuated, Rinderpest Virus – Genetically Stable



23

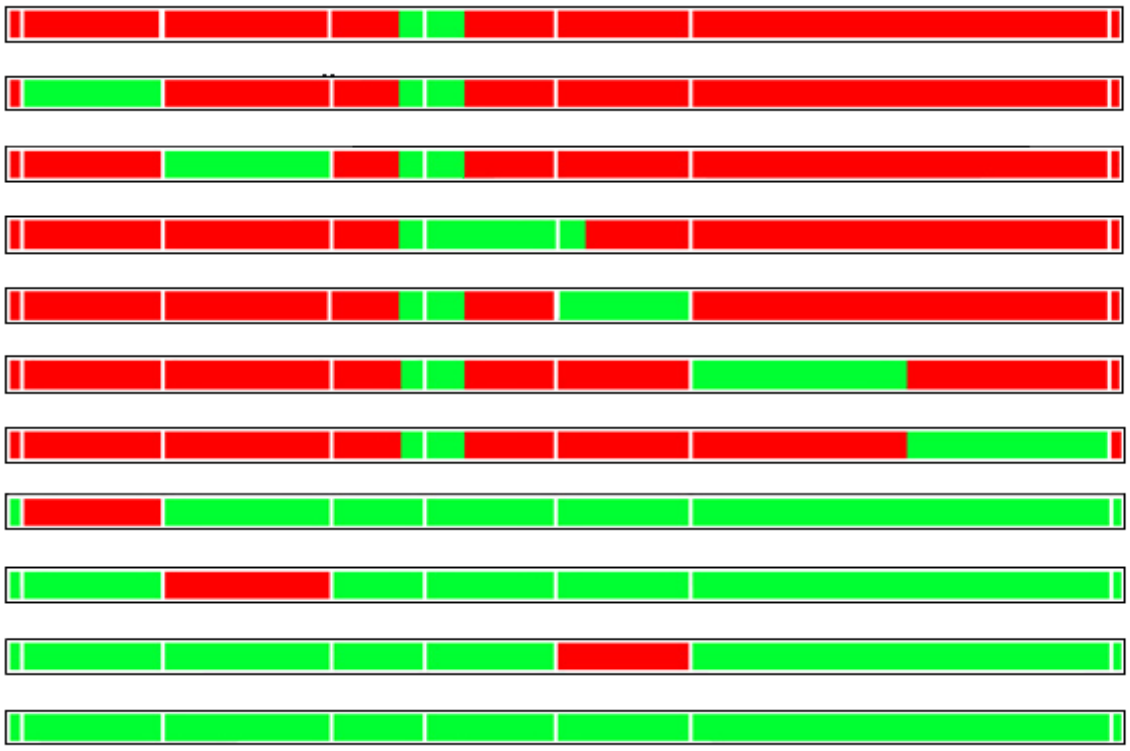
49

Recombinant Genomes:

= 72

Rinderpest (Kabete 'O' strain) – Plowright attenuated strain (RBOK)

Pathogenesis



+++	
+	
+	
+	
++	
+	
++	
-	67
-	66
-	70
-	72

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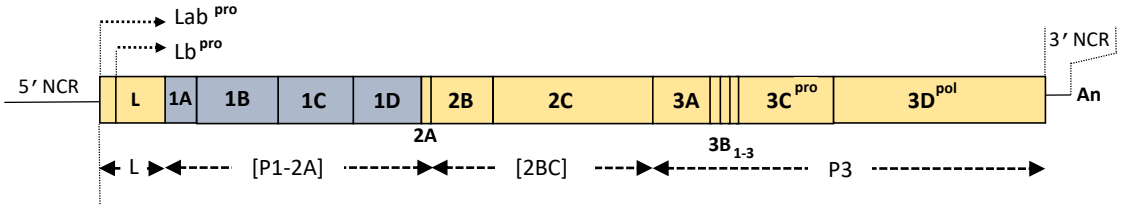
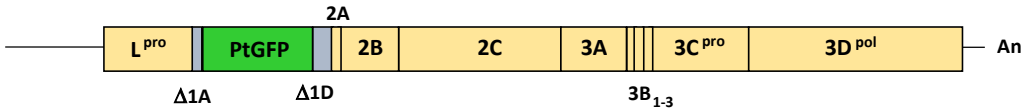
FMDV Genome
(infectious copy)

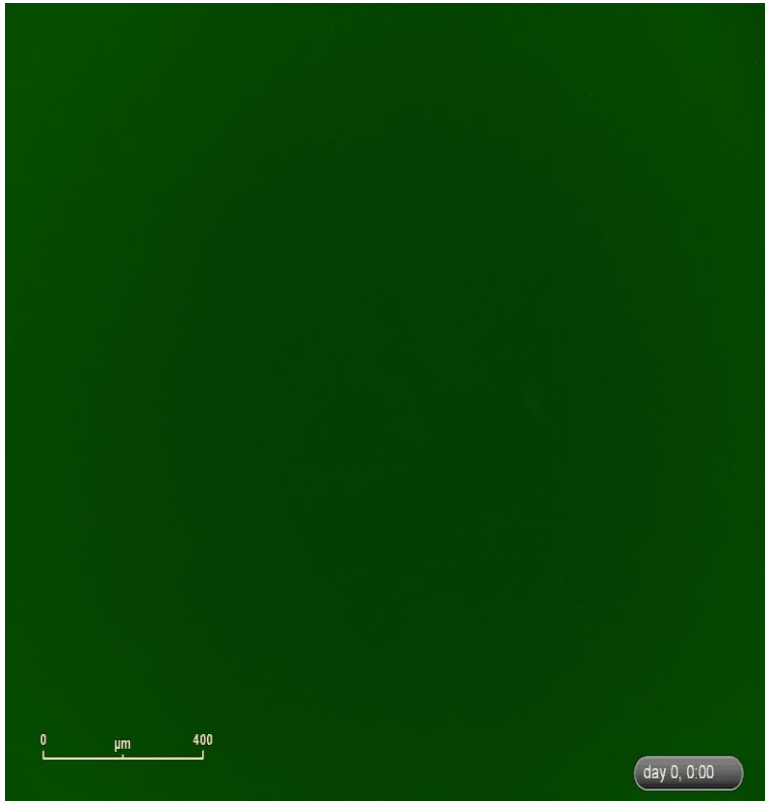


PtGFP FMDV Replicon



Cells <u>cannot</u> make virus particles
- cells turn green!

- **Biosecure**
- **Can quantify genome replication**
- **Can assess – screen - degree of attenuation**
- **Convertion into Infectious copies**
  - **remove GFP from genome,**
  - **re-insert capsid proteins**
  - **corresponding infectious copy**
  - **attenuated viruses?**



SAVE – **S**ynthetic **A**ttenuated **V**irus **E**ngineering (SAVE)

Use **synthetic biology** to alter how the virus encodes it's proteins

Altered codon usage: all **synonymous** mutations: ***protein sequence exactly the same***

Initially attributed to changes in **codon-pair bias**; Wimmer group, Stoney Brook USA
- Codegenix (patent????)

Our research showed attenuation was to due to **increased levels of UpA / CpG dinucleotide frequencies** – **<u>not</u> codon-pair bias**

Tulloch,F., Atkinson, N.J., Evans, D.J., Ryan, M.D. & Simmonds, P. ***The attenuated replication phenotype of codon pair de-optimised RNA viruses is an artefact of increasing CpG and UpA dinucleotiude frequencies - implications for the design and safety evaluation of future live attenuated vaccines.*** (2014). *eLIFE* Dec 9;3. doi: 10.7554/eLife.04531.

SAVE – **S**ynthetic **A**ttenuated **V**irus **E**ngineering (SAVE)

Pro Gly	Pro Met
-CCU-GGG-	-CCC-AUG-
↓	↓
Pro Gly	Pro Met
-CCC-GGG-	-CCU-AUG-

‘Natural’ Sequence

↓

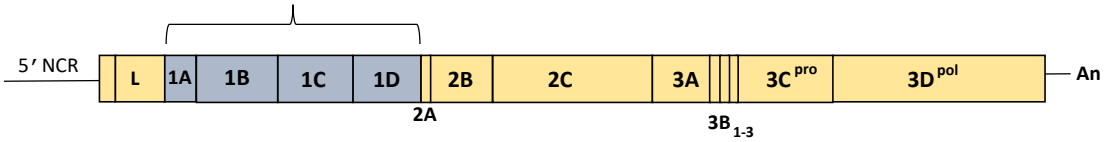
Synthetic Biology:
increase of –CpG- or –UpA-dinucleotide frequencies

(RNA secondary structures??)

Every virus protein has <u>exactly</u> the same amino acid sequence

Synonymous codon pair de-optimization (Plum Island);

FMDV A12 P1 deopt



all nucleotide changes introduced in the P1 region were maintained after 7 passages in BHK-21 cells

- genetically stable

small plaque phenotype as compared to WT in all cell lines analyzed BHK-21, IBRS-2 or LF-PK

no plaques were detected in primary porcine kidney cells (PK)

observed/ expected –CpG- ratio in FMDV A12-WT P1 region = 0.83
observed/ expected –CpG- ratio in FMDV A12-deopt P1 region = 1.35

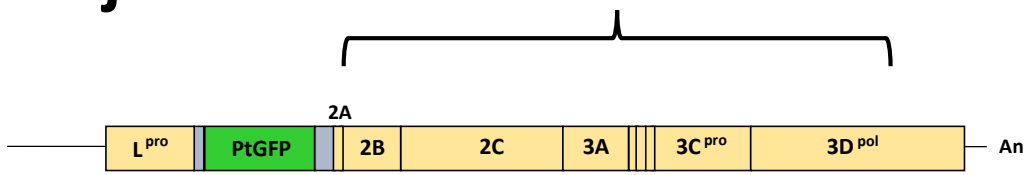
FMDV A12-P1 deopt is attenuated *in vivo* in mice at doses 10,000 times higher than WT
FMDV A12-P1 deopt does not cause death or clinical signs, although virus can be detected in serum

FMDV A12-P1 deopt caused no clinical signs in swine inoculated with 10^3 or 10^5 pfu throughout the entire experiment

FMDV A12 P1 deopt elicits strong adaptive immune response in swine

Diaz-San Segundo ,F., Medina, G.N., Ramirez-Medina, E., Velazquez-Salinas, L., Koster ,M., Grubman M.J. & de los Santos, T. (2015). **Synonymous Deoptimization of Foot-and-Mouth Disease Virus Causes Attenuation In Vivo while Inducing a Strong Neutralizing Antibody Response**. J Virol. 90:1298-310.

BBSRC SLoLa Project



Project Strategy:

- create an attenuated replication 'backbone' into which (any) P1 regions can be inserted
- FMDV O1K, A12 and Asia1 replicon systems
- Replicon RNA replicates as quickly as vRNA
- Convert replicon constructs into corresponding infectious copies (Pirbright)
- Analyses of virus growth in tissue-cultured cells / animals
- Morbidity / immune responses / protection studies

Utility of Live, Attenuated, FMDV Strains

(i) Enhanced Biosecurity during Killed Vaccine Production

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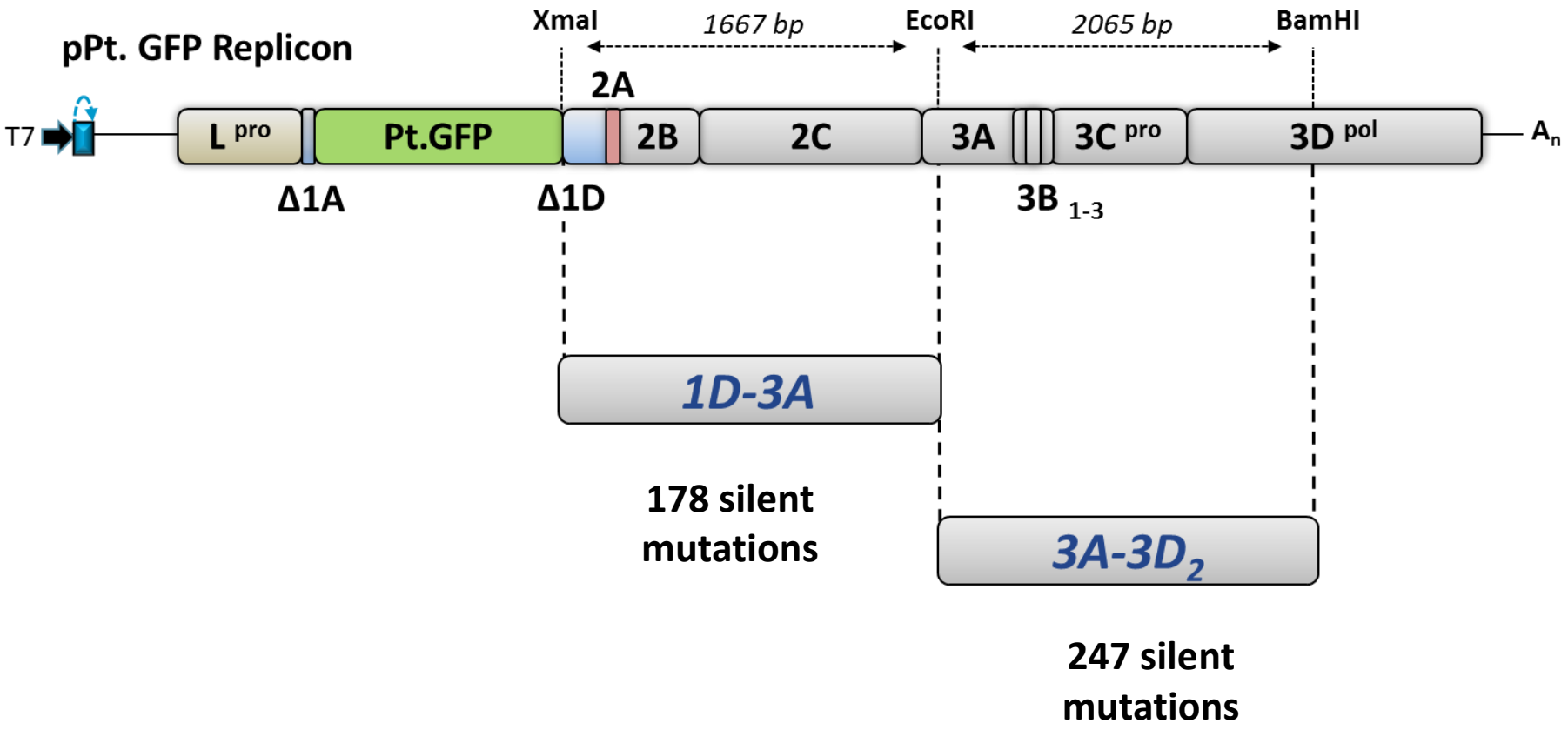
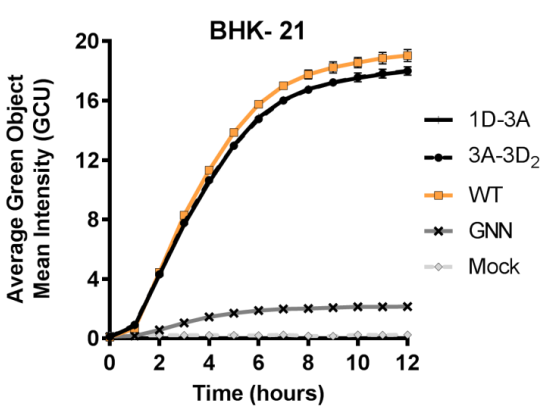
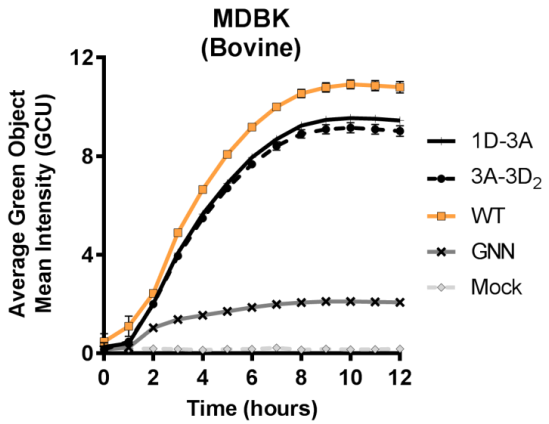
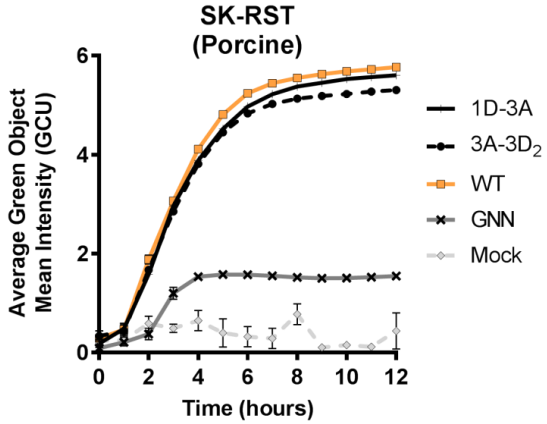

pPt. GFP Replicon
XmaI
1667 bp
EcoRI
2065 bp
BamHI
2A
T7
L pro
Pt.GFP
2B
2C
3A
3C pro
3D pol
An
Δ1A
Δ1D
3B 1-3
1D-3A
178 silent mutations
3A-3D2
247 silent mutations

FMDV Replicon Analyses (St. Andrews)





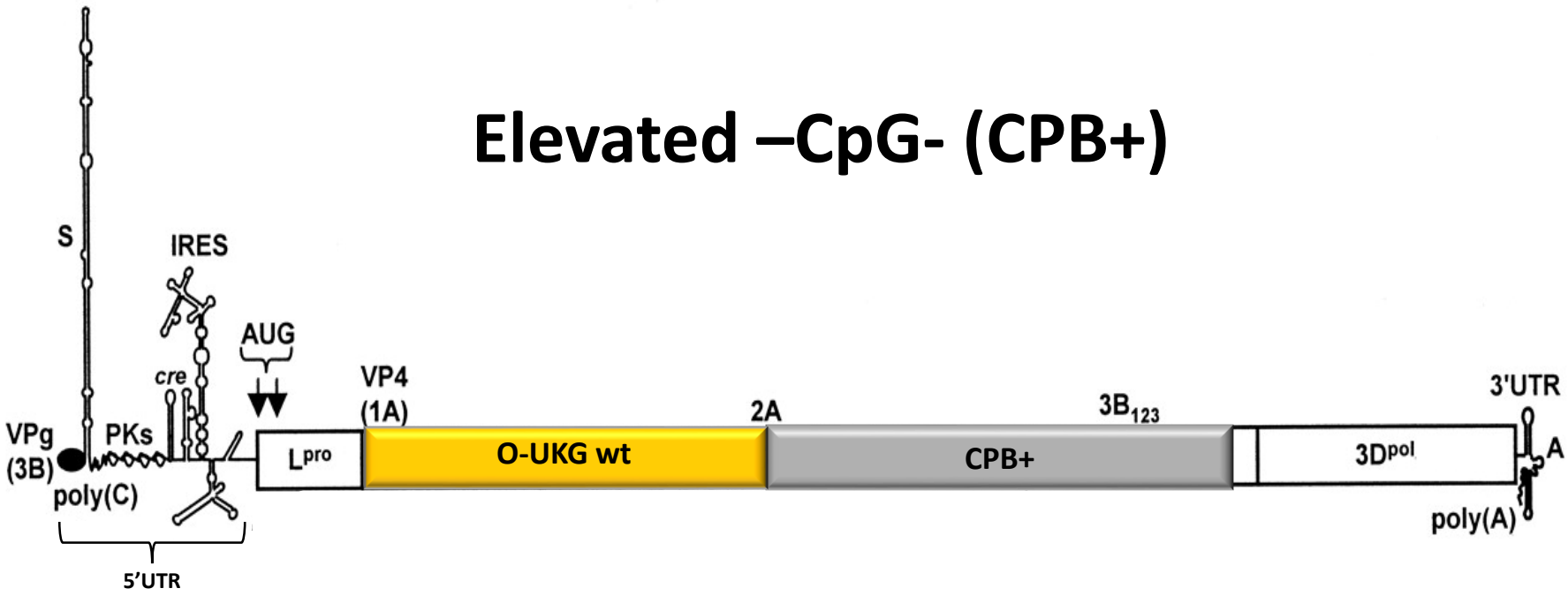


Corresponding Viruses Rescued (Pirbright)

- Viruses grow like wild-type in BHK-21 cells (vaccine producer cell-line)
- Viruses are attenuated in BTY (primary bovine thyroid cells)
- The attenuation in BTY cells can be reversed by Ruxolitinib (JAK2/JAK1 inhibitor)

- HIV - ZAP (Zinc-finger antiviral protein)

Elevated –CpG- (CPB+)

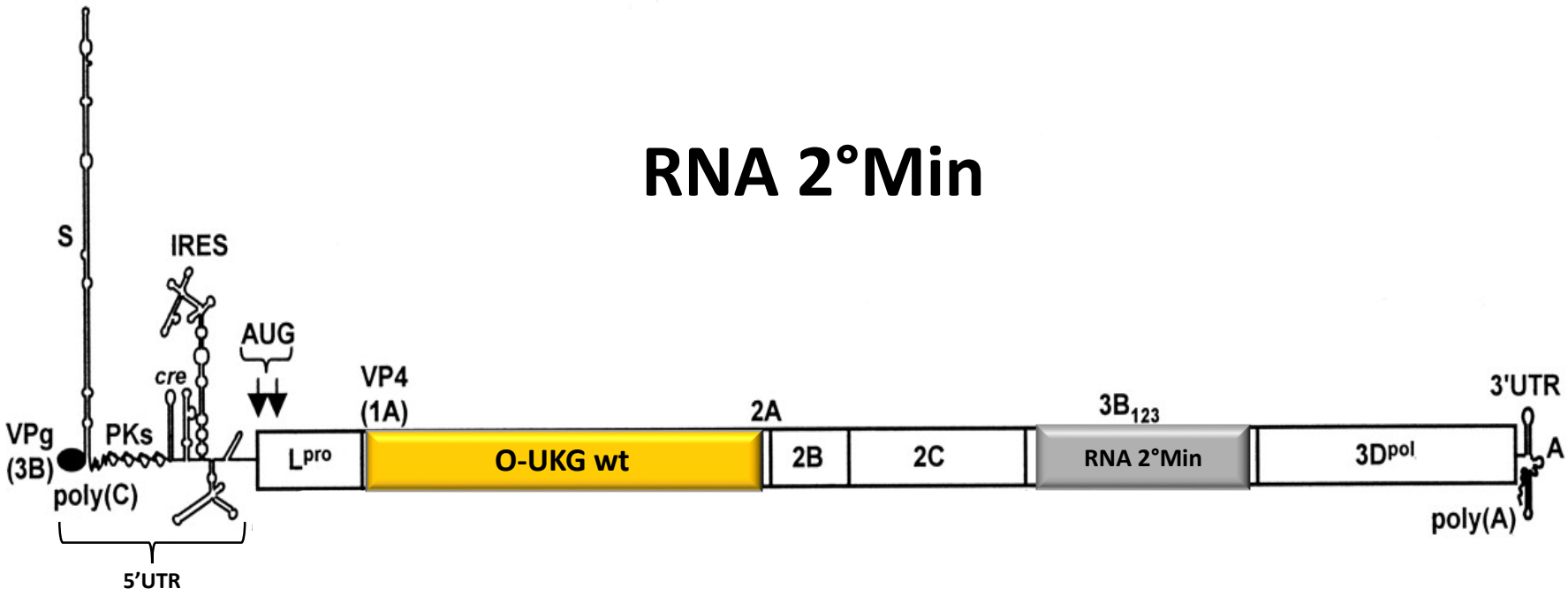


CPB+ designed by Eleanor Cottam and Richard Orton

Replicon work by Lidia Lasecka (not presented)

Initial in vitro work by Sarah Gold (not presented)

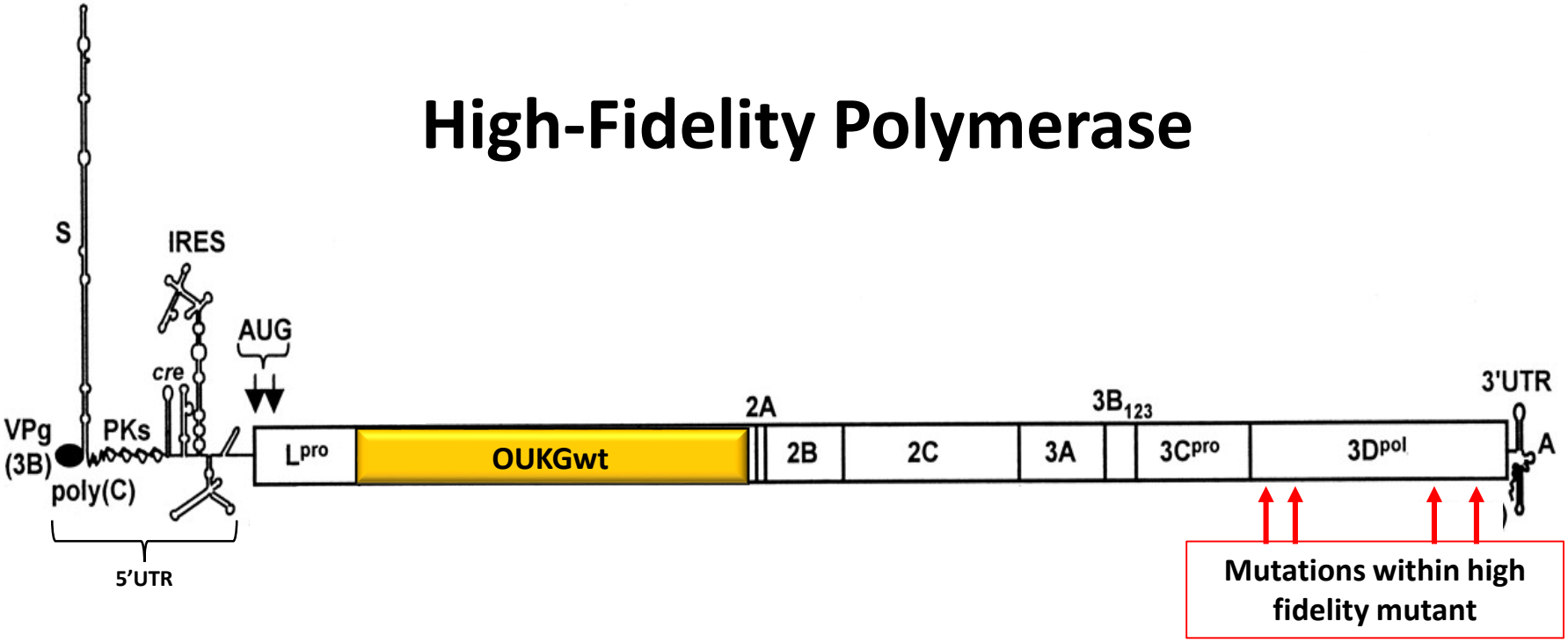
RNA 2°Min



Generated using CDLR algorithm in SSE sequence package (Peter Simmonds)

Replicon work by Fiona Tulloch (not presented)

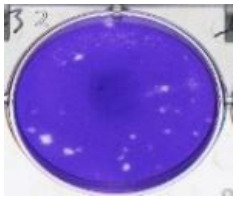
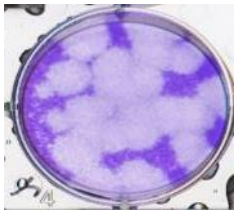
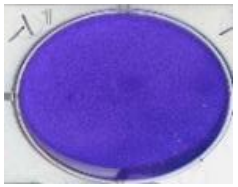
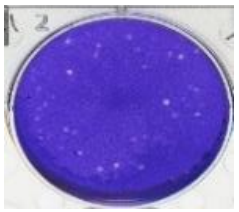
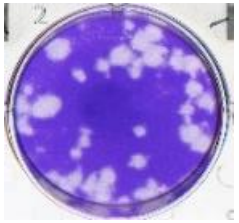
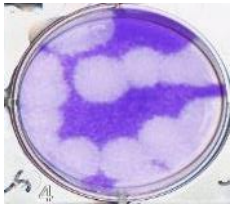
High-Fidelity Polymerase



Viruses rescued, in vitro **characterisation in**

- **primary bovine (BTY)**
- **immortalised goat tongue cells (ZZR)**

Engineered viruses are attenuated in primary bovine cells

	Approx. time to CPE		Plaque phenotype	
	BTY 48hpi	ZZR 16hpi	BTY	ZZR
High fidelity	✗	✓		
RNA 2º Min	✗	✓		
CPB+	✗	✓		
$WT_{OUKGcap}$	✓	✓		

Utility of Live, Attenuated (SAVE) FMDV Strains

(ii) Directly as Vaccines!!

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- Unlike some 'classically' attenuated vaccines with small number of 'key' attenuating mutations, SAVE produces viruses with literally 100s of attenuating mutations - each of which reduces fitness to a small degree, but taken together produce attenuation: ***genetic stability***

- Using synthetic biology, level of attenuation can easily be 'fine-tuned'
  - correct balance between morbidity / immune response / protection

- **Superior immune response** to chemically inactivated viruses

- FMDV serotype O, A and Asia 1 attenuated backbones

- Cohort domesticated animal transmission?

- Transmission to wild-life animal reservoirs?

- Eradication in both domestic and wild-animal reservoirs?

- **AMBITION: ERADICATION**

  not '*living with the enemy*'

- **SAVE (synthetic biology) confers huge versatility**
- **SAVE confers rapid responses to new strains**
- **Applicable to all serotypes**
- **Routine vaccination with live, attenuated, viruses**
- **Biosecurity / cheaper vaccines?**

On 2nd Sep 2014, following a human error, 45 litres of concentrated live polio virus solution were released into the environment in Rixensart city, Belgium