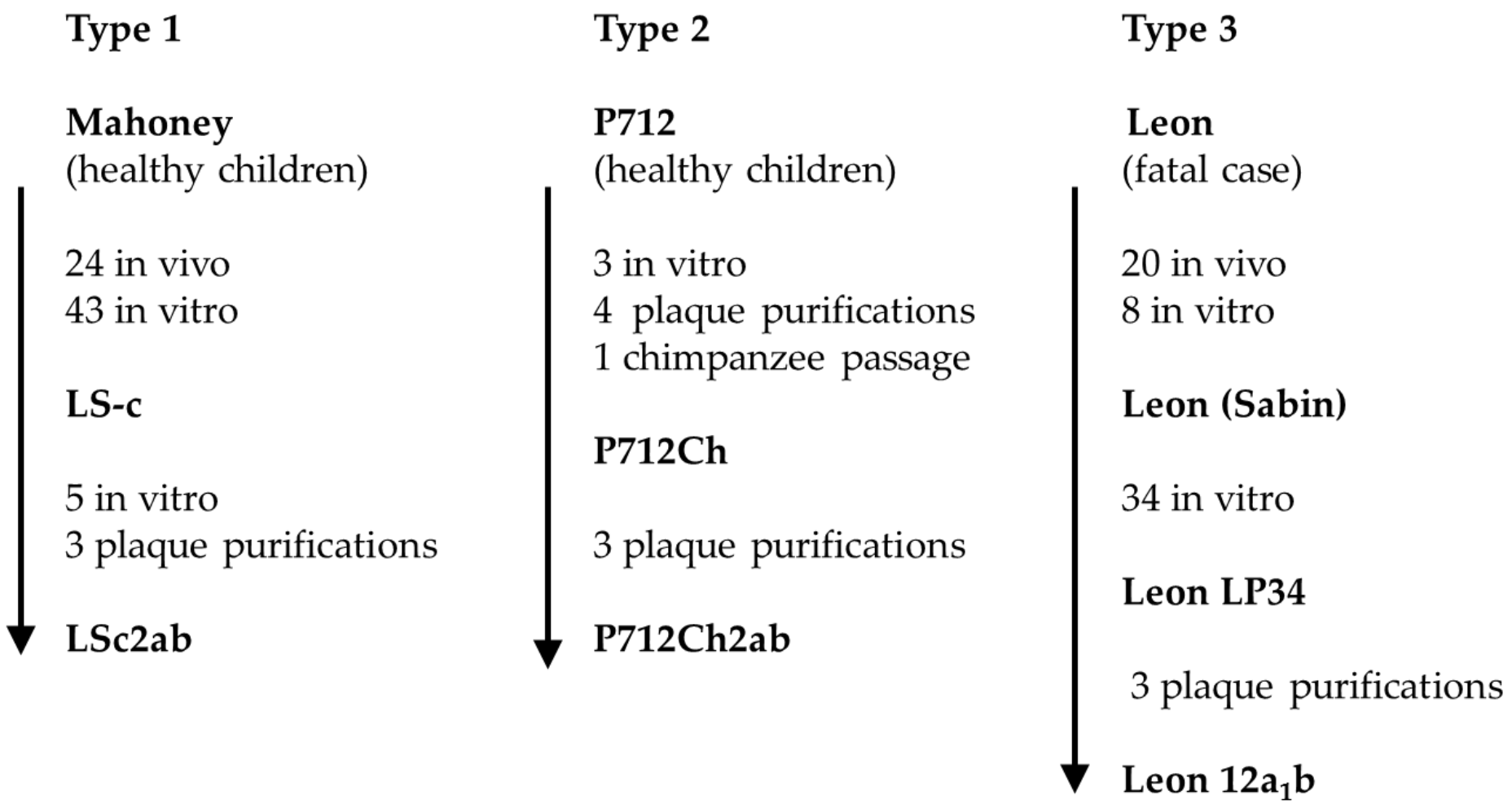
Medicines & Healthcare products Regulatory Agency

Next generation sequencing as an alternative to neurovirulence tests in animals for the quality control of live-attenuated viral vaccines

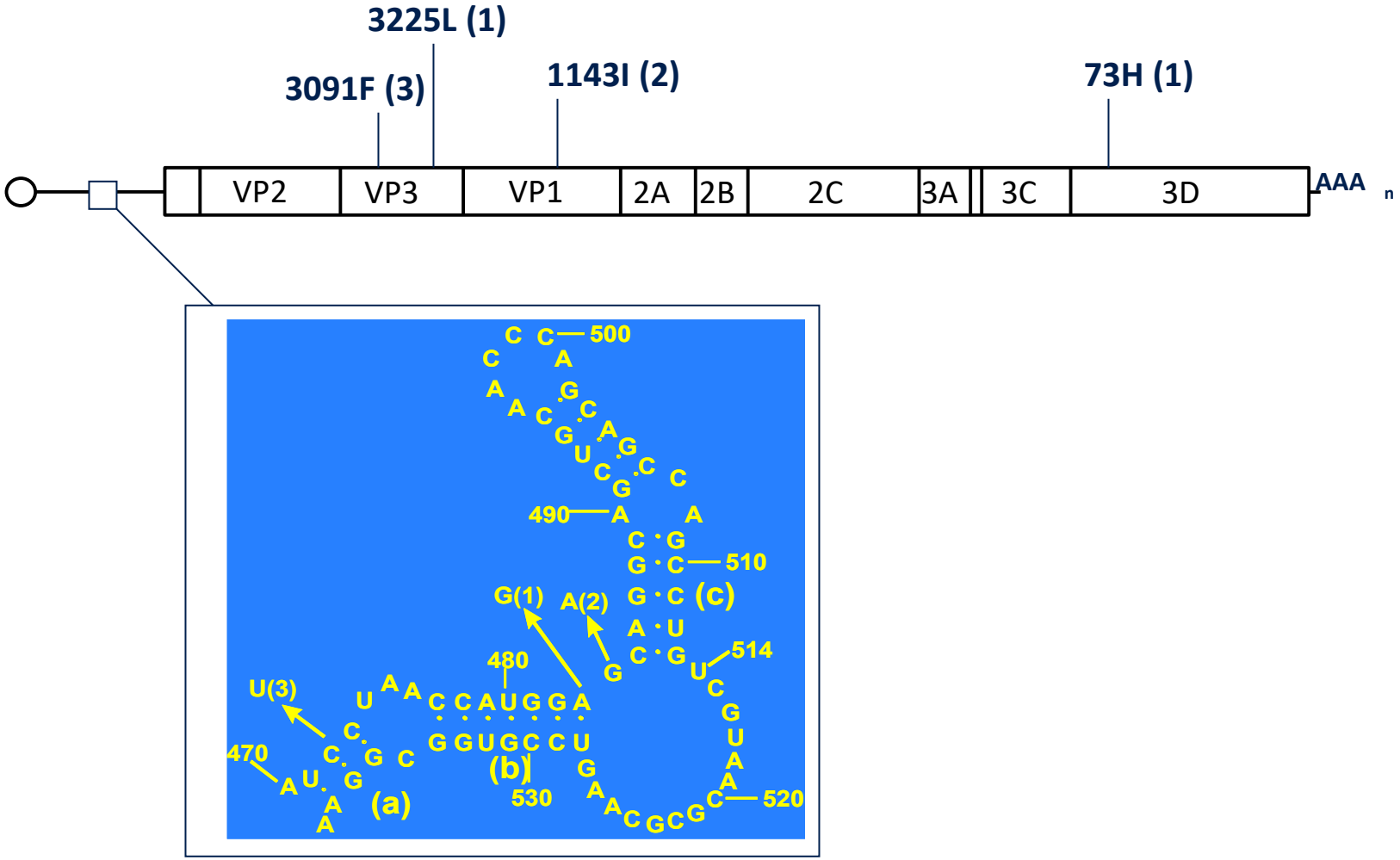
Animal testing replacement for vaccines. A One Health View: global outlook and future strategy.
An Animal-Free Safety Assessment (AFSA) Collaboration conference co-organized by
Humane World for Animals and IABS
Bangkok, Thailand. 2-4 December 2025

Javier Martin

Development of Sabin live-attenuated OPV strains



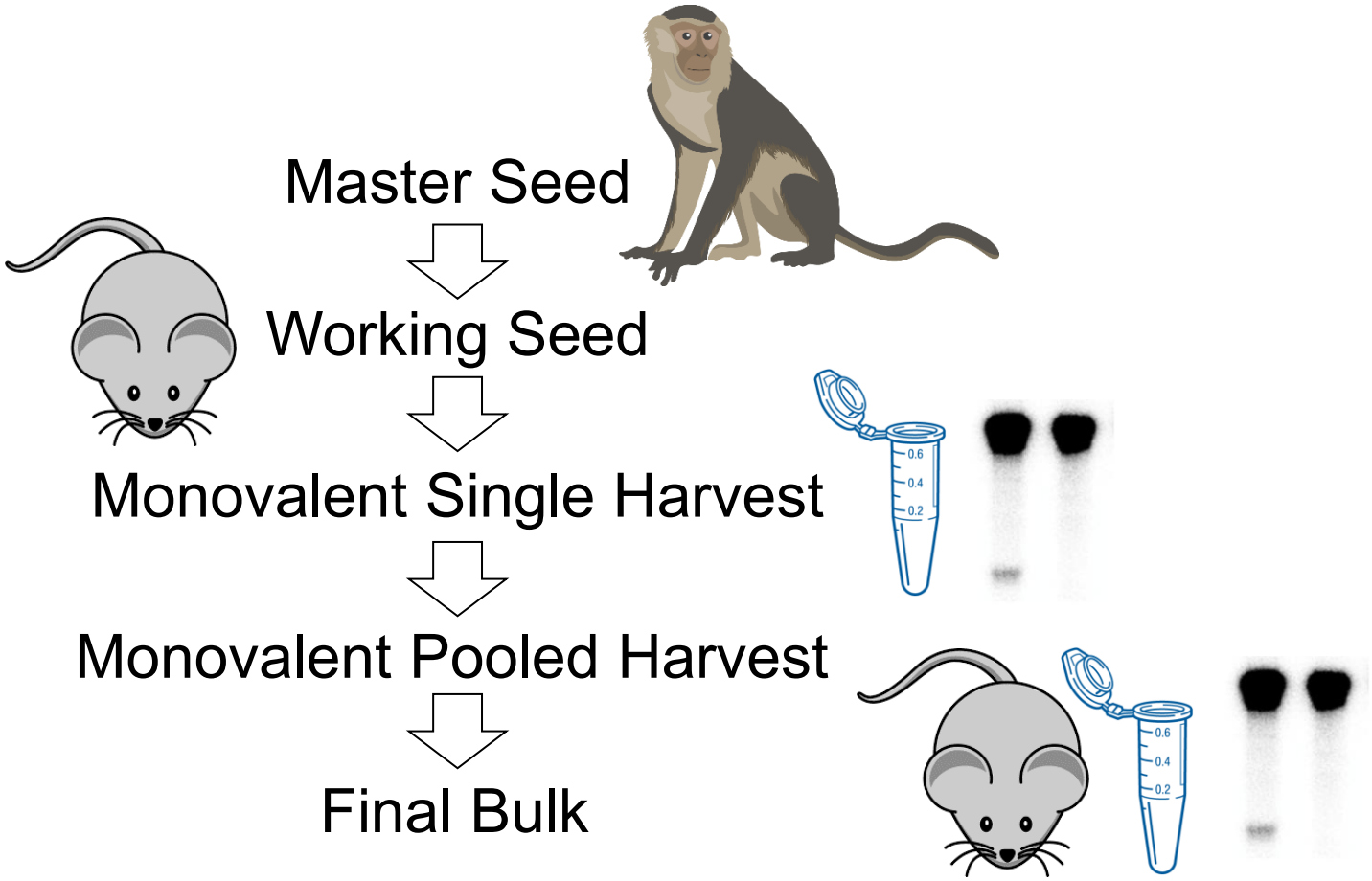
Determinants of attenuation in Sabin poliovirus OPV strains



Quality control of OPV

- OPV prepared by serial passage of wild poliovirus
- Virus weakened by incorporation of mutations
- Low risk of disease 1:10^6 doses, VAPP
- Potency: titration in cell culture
- Safety: reversion to virulence

OPV safety testing



High Throughput (Next Generation, or Deep) Sequencing

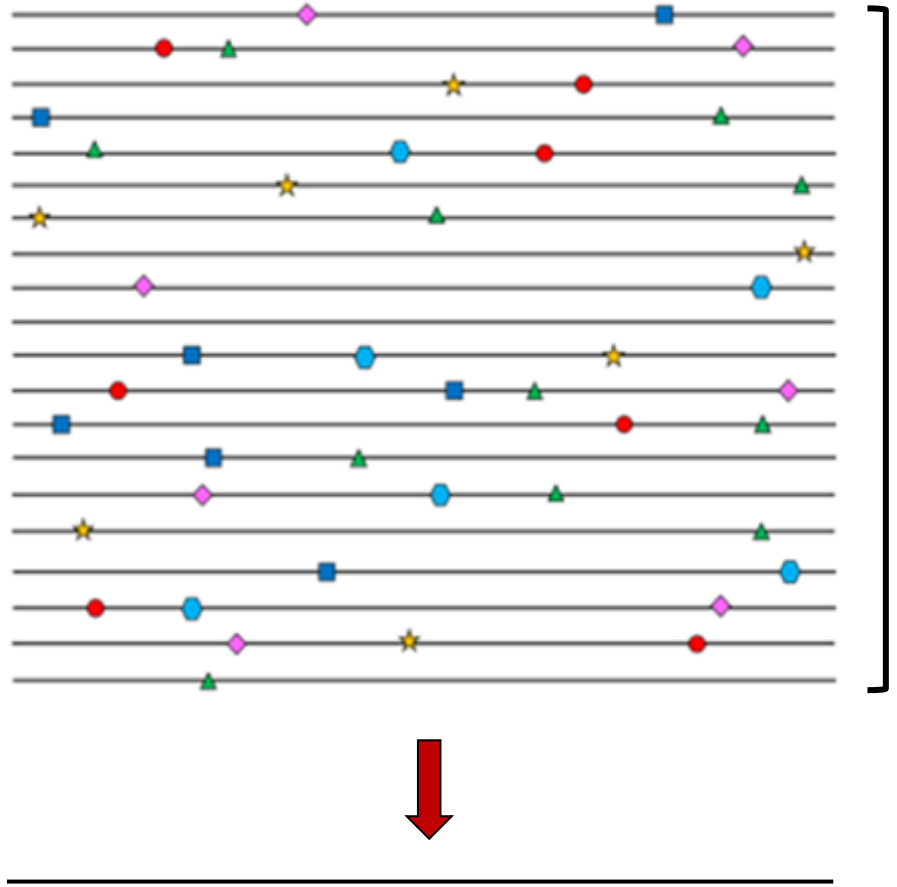
- High throughput allowing multiplexing
- Allows sequencing of individual molecules, accurately measuring variant mutations – Single Nucleotide Polymorphism (SNP)
- Decreasing cost per sample for large amounts of data
- High efficiency
- Technically simpler and more robust than MAREC

Early research evidence of potential of NGS for OPV

Neverov A and Chumakov K. **Massively parallel sequencing for monitoring genetic consistency and quality control of live viral vaccines**. PNAS, 2010

Sarcey E *et al*., **Quantifying low-frequency revertants in oral poliovirus vaccine using next generation sequencing.** J Virol Methods, 2017.

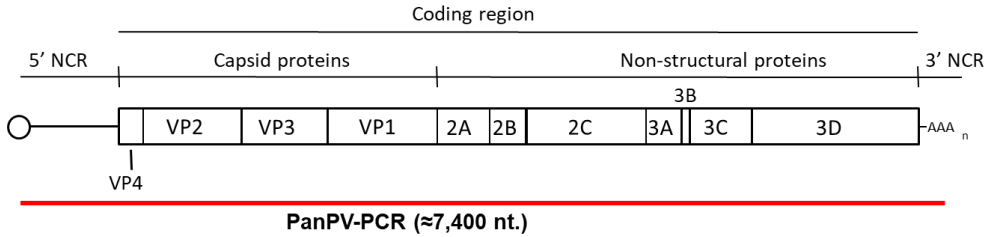
Nucleotide sequence analysis of viral genomes



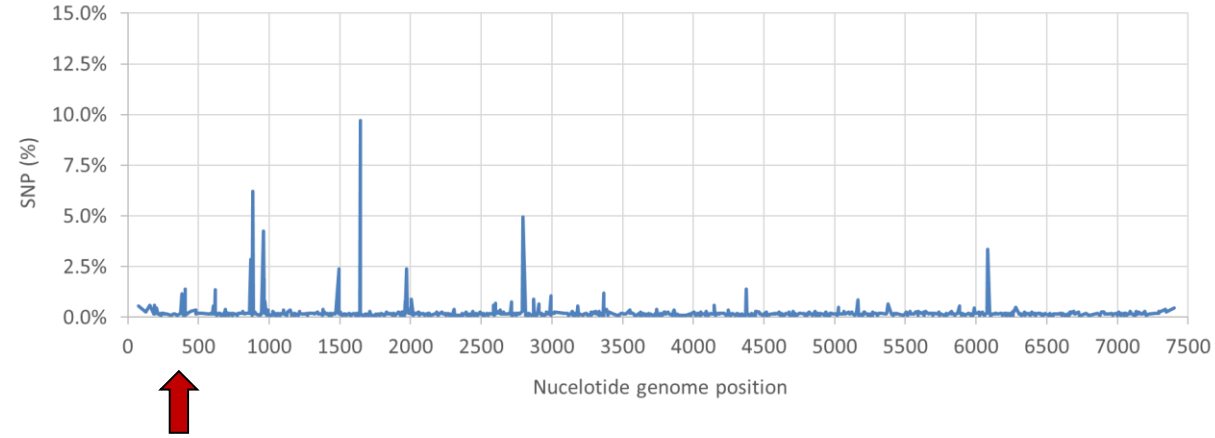
High Throughput (Next Generation, or Deep) Sequencing allows sequencing of individual molecules, accurately measuring variant mutations – Single Nucleotide Polymorphism (SNP) at each genome nucleotide position, creating a unique SNP profile (*molecular fingerprint*) for any given virus preparation that can be compared to that of other related (e.g. master seed vs working seed vs bulk produced from the same seed virus) or unrelated virus preparations (e.g. vaccine bulks from different seeds/manufacturers) .

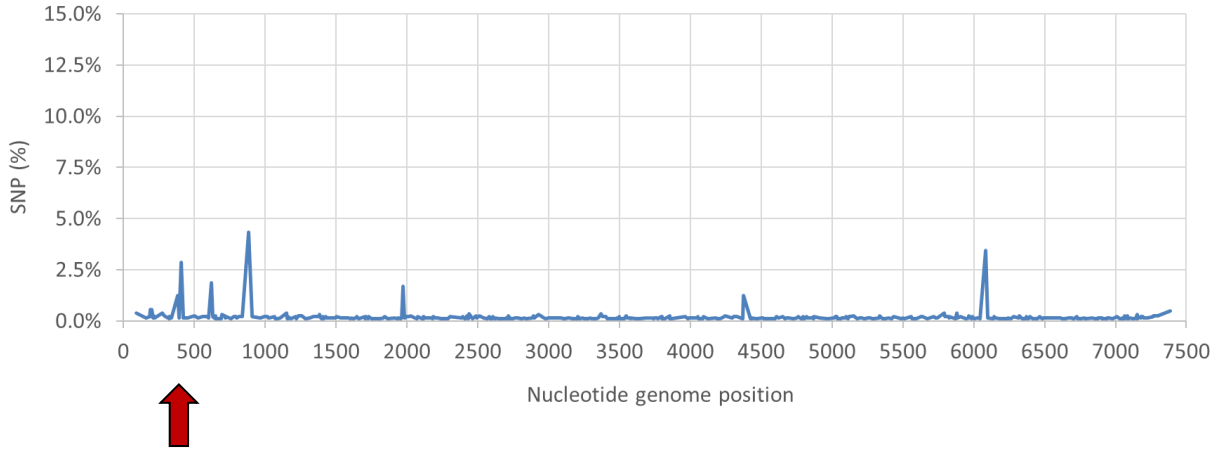
Consensus sequence by **Sanger sequence analysis**

NGS analysis of OPV strains – SNP analysis



Samples analyzed	OPV sample
RNA extraction	High Pure Viral RNA kit (Roche)
cDNA-PCR synthesis	Whole-genome Pan-PV PCR
Library preparation	Nextera XT reagents
NGS	MiSeq Nextera™ XT kit v2
Data analysis	**Geneious R10** *Mapping to Sabin reference* *SNP calling*





MAPREC mutation

NGS of OPV for the assessment of molecular consistency

- Phase 1:
  - Can NGS be used as a replacement for MAPREC to quantify 5’-NTR mutations?
- Phase 2:
  - Can whole-genome sequence SNP profiles be used as a replacement for NVT eventually removing the need for animal testing?
  - This would be useful for both OPV and OPV seeds used for Sabin-IPV production, to monitor not only the preservation of attenuation mutations but of any other mutations that might affect antigenicity/immunogenicity

NGS for OPV3 - International Collaborative Study

The Journal of Infectious Diseases

MAJOR ARTICLE

The Use of Next-Generation Sequencing for the Quality Control of Live-Attenuated Polio Vaccines

Bethany Charlton,[1] Jason Hockley,[2] Majid Laassri,[3] Thomas Wilton,[1] Laura Crawt,[1] Mark Preston,[4] NGS Study Group, Peter Rigsby,[2] Konstantin Chumakov,[3] and Javier Martin[1]

[1]Division of Virology, National Institute for Biological Standards and Control, Potters Bar, United Kingdom, [2]Division of Biostatistics, National Institute for Biological Standards and Control, Potters Bar, United Kingdom, [3]US Food and Drug Administration, Silver Spring, Maryland, USA, and [4]Division of Bioinformatics, National Institute for Biological Standards and Control, Potters Bar, United Kingdom

World Health Organization

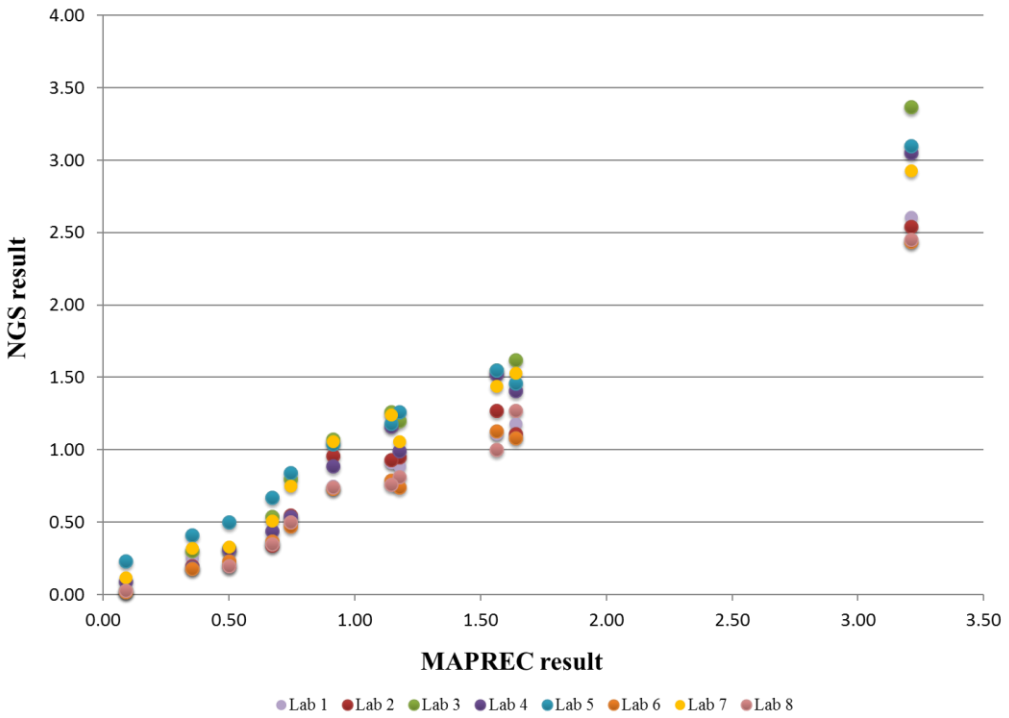
WHO/BS/2019.2359
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 21 to 25 October 2019

Report on the WHO collaborative study to investigate the utility of next generation sequencing (NGS) as a molecular test of virus stocks used in the manufacture of Poliovirus vaccine (Oral)

Javier Martin[1,5], Kostya Chumakov[4,5], Jason Hockley[2], Thomas Wilton[1], Laura Crawt[1], NGS Study Group (see Appendix 2), Mark Preston[3], Peter Rigsby[2] and Bethany Charlton[1]

Division of Virology[1], Biostatistics[2] and Bioinformatics[3]
National Institute for Biological Standards and Control (NIBSC),
South Mimms, Potters Bar, Herts, EN6 3QG, UK
US Food and Drug Administration[4], Silver Spring, MD 20993, USA.
[5]Study Coordinators
E-mail: Javier.Martin@nibsc.org; konstantin.chumakov@fda.hhs.gov



Pearson correlation coefficient

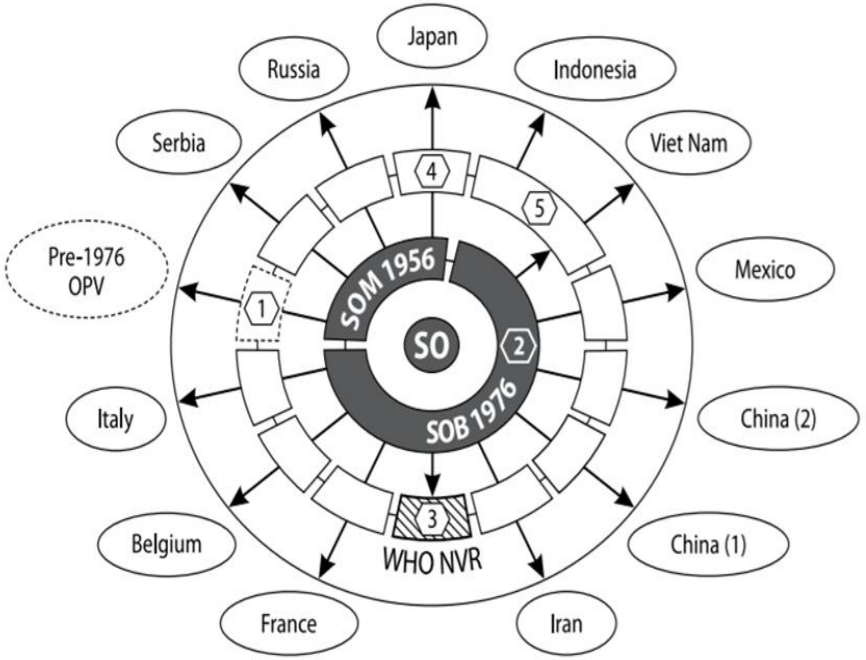
Analysis	Lab								
	1	2	3	4	5	6	7	8	Overall
In-House	0.997	0.979	0.994	0.994	0.990	0.995	0.995	0.992	0.996
NIBSC	0.996	0.984	0.995	0.993	0.996	0.996	0.991	0.993	0.996

NGS of OPV for the assessment of molecular consistency

- Phase 1:
  - Can NGS be used as a replacement for MAPREC to quantify 5’-NTR mutations?
- Phase 2:
  - Can whole-genome sequence SNP profiles be used as a replacement for NVT eventually removing the need for animal testing?
  - This would be useful for both OPV and OPV seeds used for Sabin-IPV production, to monitor not only the preservation of attenuation mutations but of any other mutations that might affect antigenicity/immunogenicity

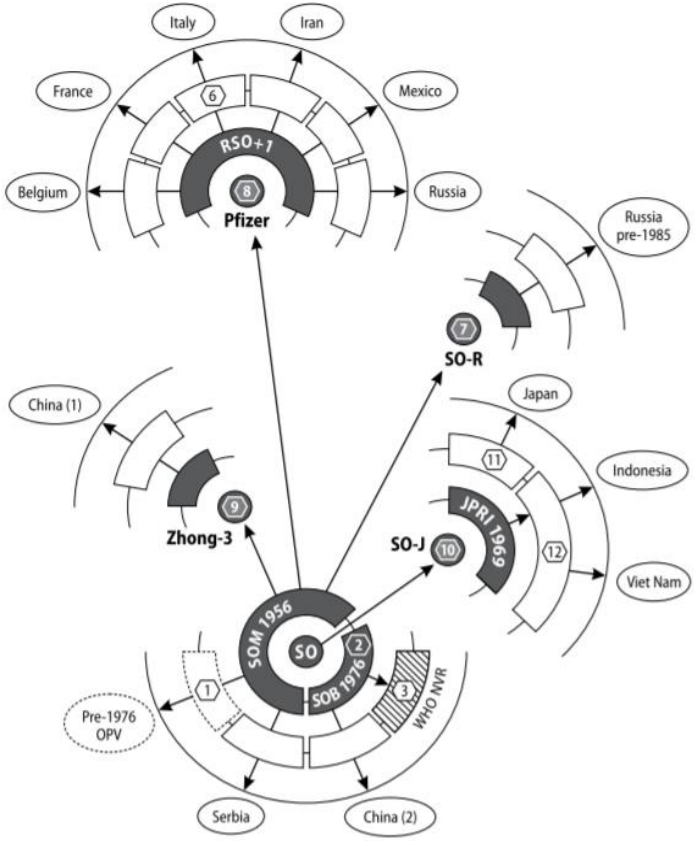
Passage history of OPV seeds

Figure 2.1
History of seed virus and reference materials used to produce type 1 and type 2 OPV from Sabin 1 and Sabin 2



WHO Technical Report Series No. 980, 2014

Figure 2.2
History of seed virus and reference materials used to produce type 3 OPV from Sabin 3



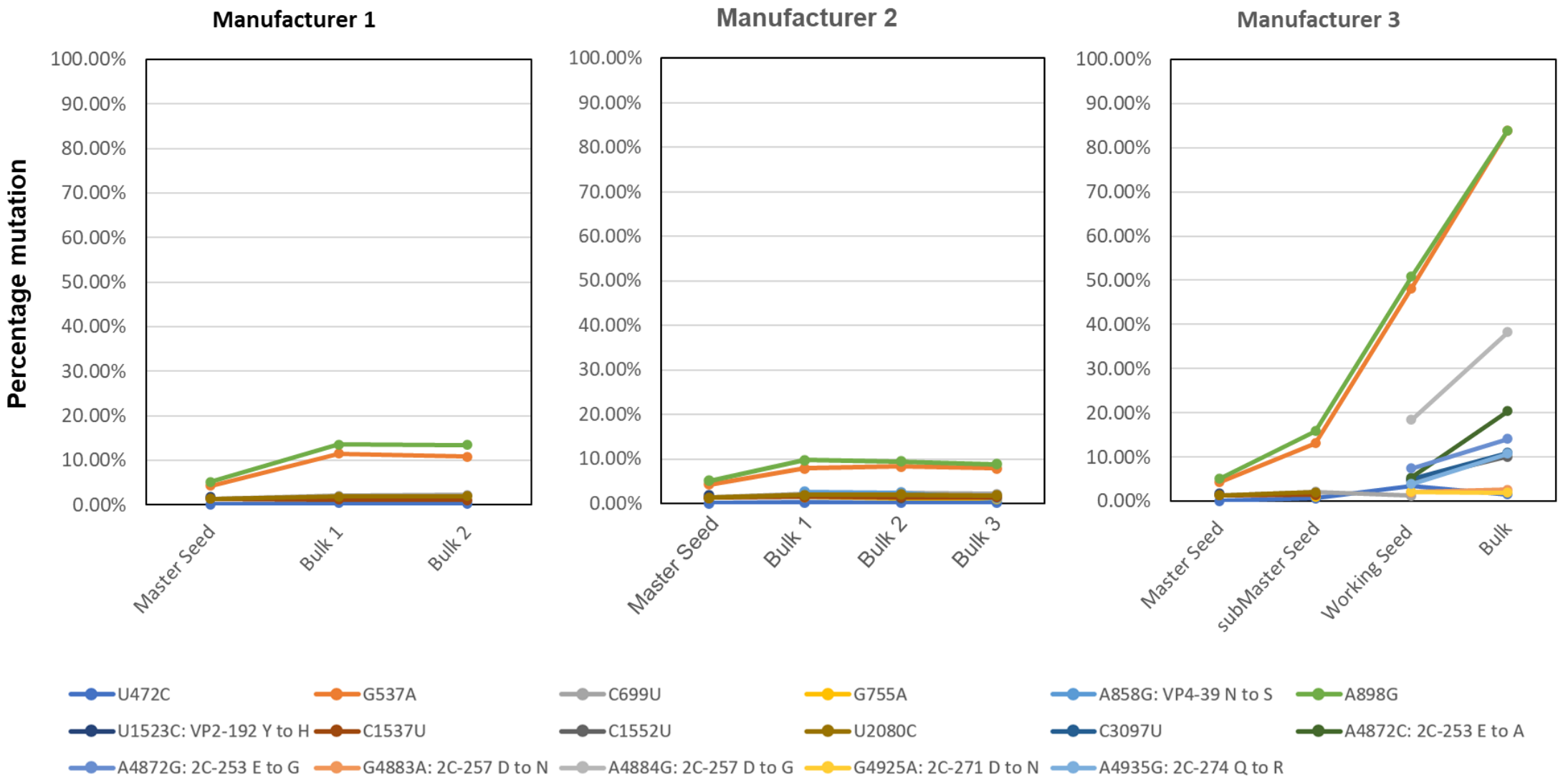
NGS analysis of OPV3 strains

	G537A	A898G	A2440U	C2493U: VP1-6 T to I	A2696G: VP1-74 T to A	G2702C: VP1-76 E to Q	A2703G: VP1-76 E to G	C3262U	C3640U	A3723G: 2A-116 Q to R	G4054A	A4171G	A4872C: 2C-253 E to A	A4890G: 2C-259 K to R	U5473C	U5476C	A6001G	C6421U	A6760U: 3D-261 R to S
SO+1				30.1							1.4			4.7	2.4	2.0		1.1	
05/146				71.0							3.3			2.2	5.7	5.1	1.8	2.7	
M1-mOPV3				72.2							3.1			3.2	5.9	5.5	1.8	2.9	
M2-mOPV3				82.6							3.7			1.3	6.8	5.4	1.9	2.5	
M3-mOPV3				80.9							3.6			1.4	6.6	5.9	1.9	2.7	
M4-mOPV3				92.8							4.8			1.0	7.4	5.8	2.2	3.1	
M5-mOPV3				94.6							5.0			4.7	7.4	5.9	2.2	3.1	
RSO					1.3		2.8	2.3	3.8	2.4		4.9							
M6a-mOPV3					7.0	2.5	4.7	2.2	3.4	7.4		4.2							
M6b-mOPV3					7.8	1.3	6.8	2.2	3.3	7.9		4.3							
M7-mOPV3				1.7	5.8	1.9	3.1	1.9	3.5	6.2		4.3							
M8-mOPV3								1.9	3.1	1.3		4.4							
M9a-mOPV3				18.9				1.5	2.9	1.1		3.3	3.7						
M9b-mOPV3				13.0				2.0	3.0	1.3		4.0	5.3						
SOJ-seed	4.3	5.1	99.8	100															99.7
M10a-mOPV3	8.6	9.3	99.8	99.9															99.7
M10b-mOPV3	10.2	9.9	99.4	99.7															99.2
M11-tOPV	7.8	9.2	99.8	99.8															99.1
M12-tOPV	7.5	7.6	99.7	99.8															99.2

Whole-genome NGS analysis of OPV

- Whole-genome NGS analysis demonstrated high consistency in vaccine production of most OPV products by different manufacturers.
- Whole-genome SNP profiles were highly consistent between vaccine products from the same manufacturer.
- Different vaccine seeds and associated products were found to contain unique SNP profiles.
- Vaccine products from manufacturers using the same vaccine seed were found to contain common SNPs unique to that seed but also manufacturer-specific SNPs often associated with the cell substrate used for vaccine production (e.g. monkey kidney vs vero vs human diploid cells).
- These results suggest that whole-genome NGS analysis has a great potential as a QC test for OPV measuring vaccine production consistency and potentially replacing neurovirulence testing using animals

Mutations in OPV3 lots made from Japanese seed

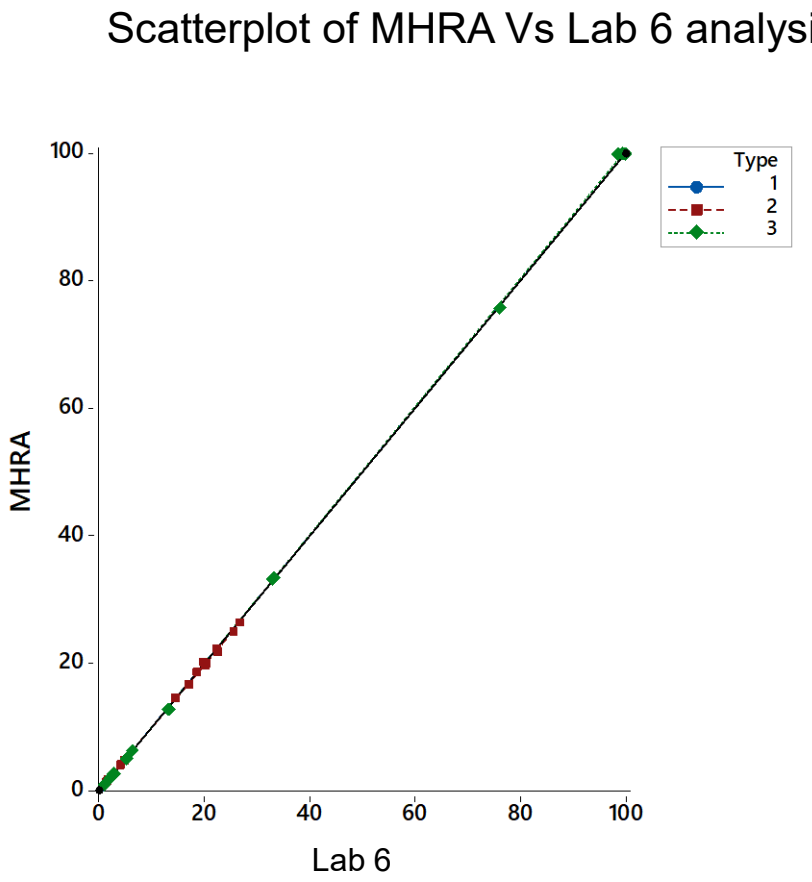


Collaborative study to support Phase 2

Aim of the Study:

- The primary aim of this study is to establish reference reagents to be used in NGS methods to monitor the consistency of production of OPV and the characterization of virus bulks used for the manufacture of sIPV prior to virus inactivation.
- The objective is to establish reference reagents suitable for measuring MAPREC-specific single mutations and/or whole genome sequence analysis.
- The study will also focus on providing appropriate test formats and bioinformatics analytical processes for establishing assay validity criteria.
- Overall, the study will provide further scientific assessment of NGS as a replacement test of animal NVTs for vaccine lot release.

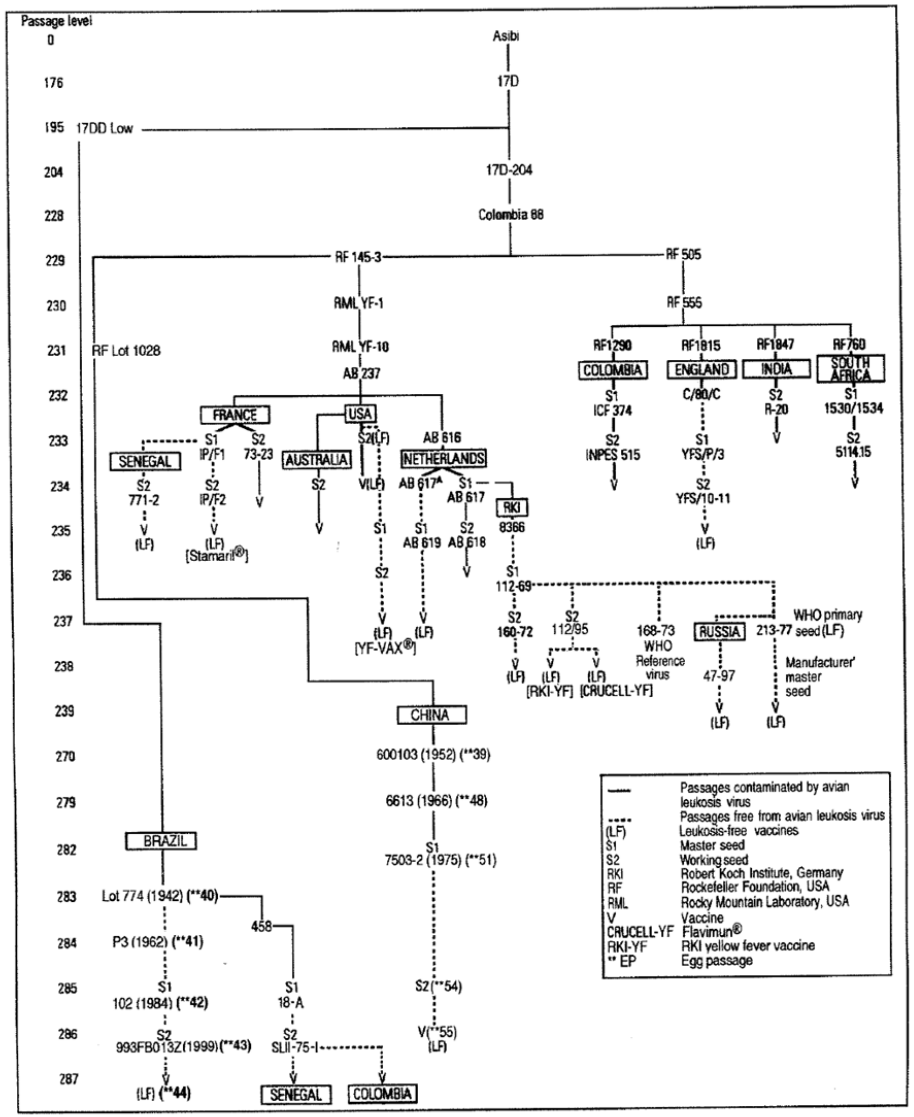
Concordance between SNP profiles generated by MHRA and Lab 6

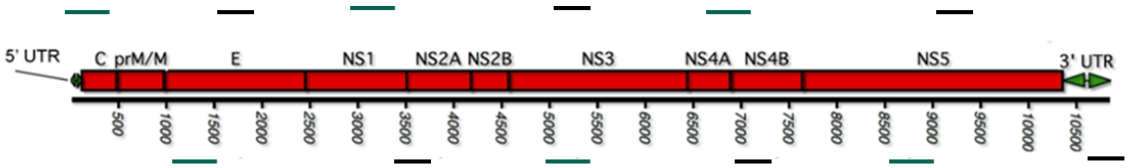


MHRA lower	44.12%
N	68
Wilcoxon p	0.935
Type	**CCC**
1	0.999997
2	0.999518
3	0.999936

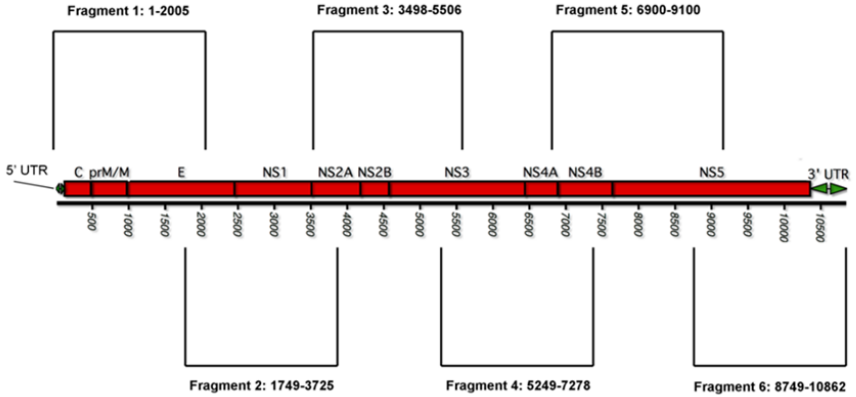
This analysis shows high concordance in reported values for single nucleotide polymorphisms for Type 1, 2 and 3 OPV when values generated by MHRA pipeline and Lab 6 were plotted against each other

Whole-genome analysis of yellow fever vaccines



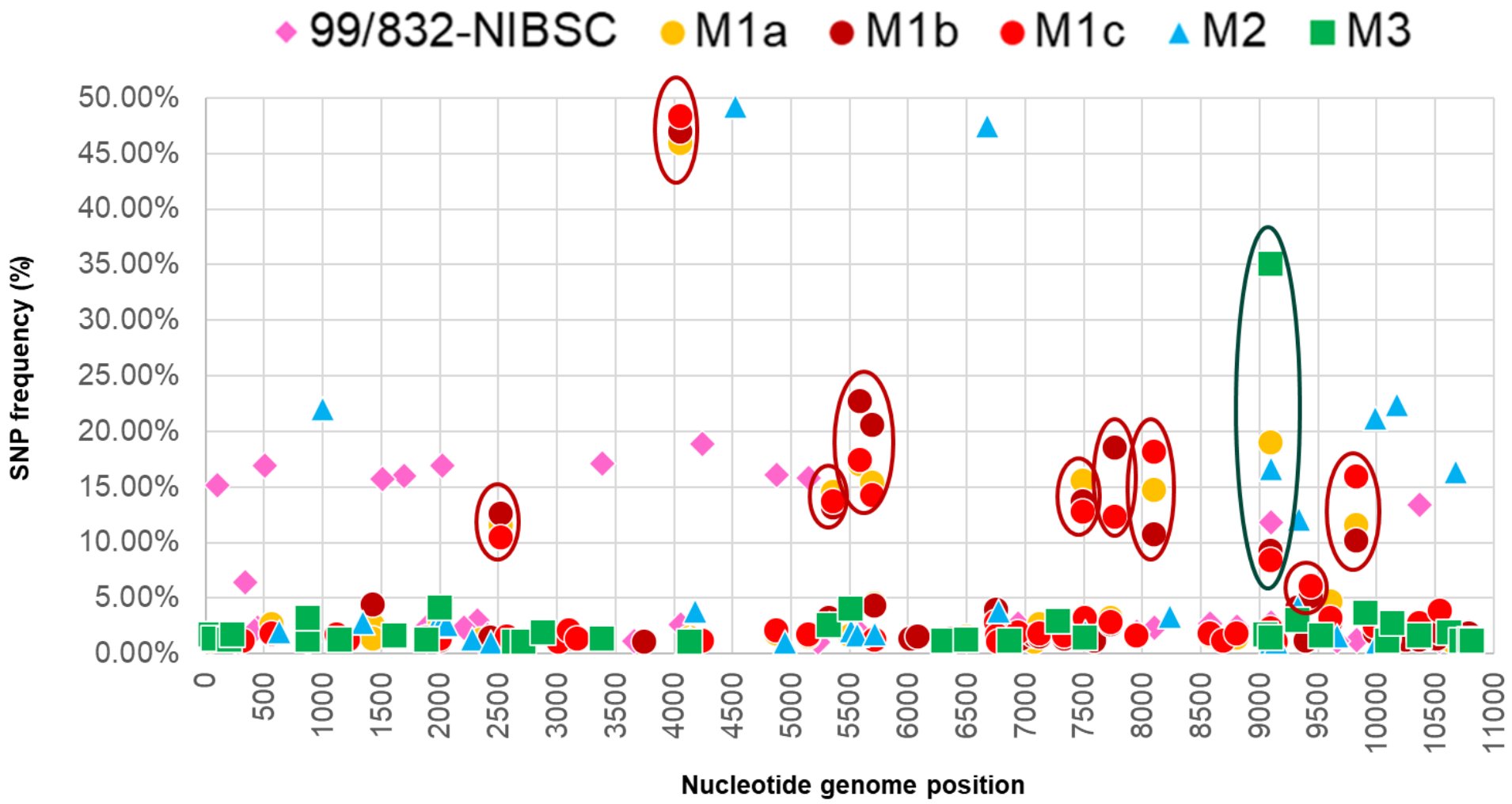


6 different PCR reactions standardized to run on single thermal profile



Approximately equimolar concentration of 6 PCR reactions mixed in a single tube and sent for Miseq

Single Nucleotide Polymorphism (SNP) analysis of YFV



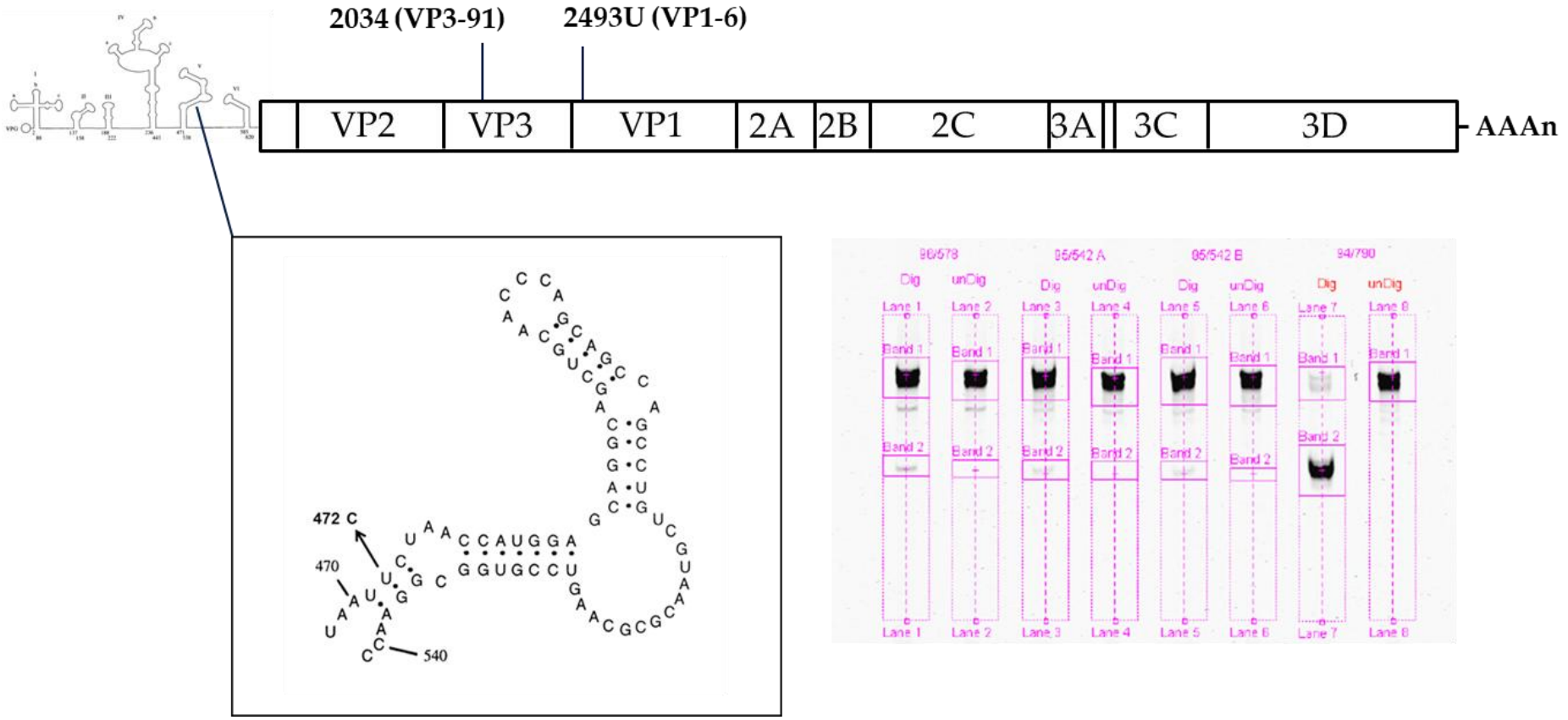
Acknowledgements

- Majid Laassri (FDA)
- Tatiana Zagorodnyaya (FDA)
- Kostya Chumakov (FDA)
- Amy Rosenfeld (FDA)
- Leonid Brodsky (Haifa University)
- Julia Panov (Haifa University)
- Bethany Charlton (MHRA)
- Thomas Wilton(MHRA)
- Laura Stephens (MHRA)
- Edward Mee (MHRA)
- Ryan Mate (MHRA)
- Jason Hockley (MHRA)
- Peter Rigsby (MHRA)
- Dimitra Klapsa (MHRA)
- Manasi Majumdar (MHRA)
- Javier Martin (MHRA)
- Vajra Allan (MHRA)
- Kutub Mahmood (PATH)

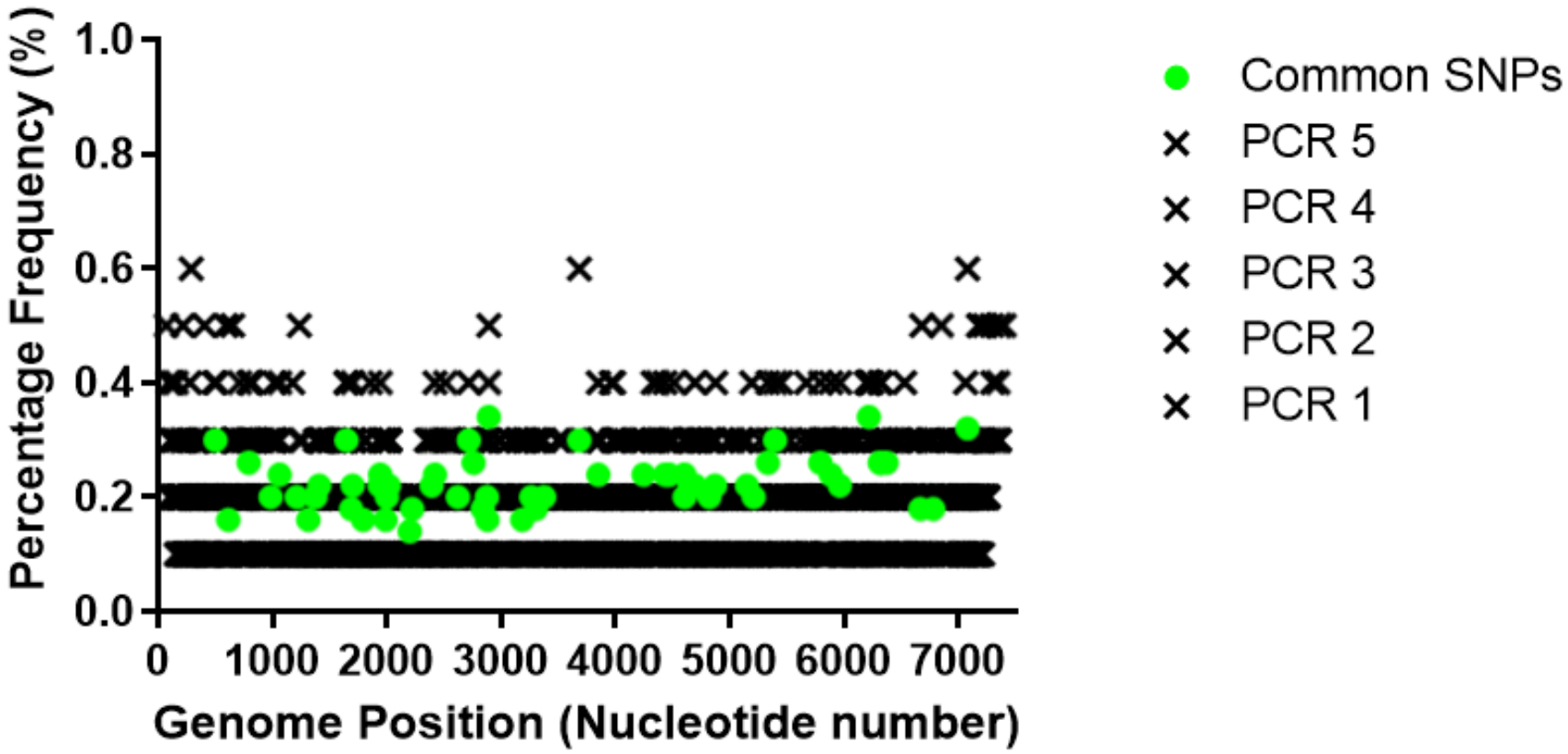
Acknowledgments for the collaborative study

Name	Affiliation
Manasi Majumdar	MHRA,UK
Kafayat Arowolo	MHRA,UK
Marilyn Quinlan	MHRA,UK
Ryan Mate	MHRA,UK
Preni Sinnakandu	MHRA,UK
Peter Rigsby	MHRA,UK
Dionas Maroulis	MHRA,UK
Yemisi Adedeji	MHRA,UK
Edward Mee	MHRA,UK
James Condron	MHRA,UK
Ochiai Susumu	Biken, Japan
Panduranga Pavuluri	BioE, India
Umakantha Madala	BioE, India
Yuvraj Jadhav	BioE, India
Ramurthy Gudla	BioE, India
Saikumar Ale	BioE, India
Jie Song	IMBCAMS, China
Muhammad Luthfi Nugraha	PT Bio Farma, Indonesia
Istianti Nurisa	PT Bio Farma, Indonesia
Vinca Medica	PT Bio Farma, Indonesia
Gemi Utami Pertiwi	PT Bio Farma, Indonesia
Lori A Rowe	Tulane University, USA
Andrew R Hoffmann	Tulane University, USA
Amy Rosenfeld	CBER-FDA, USA
Tatiana Zagorodnyaya	CBER-FDA, USA
Kostya Chumakov	Ex-CBER-FDA, USA
Kutub Mahmood	PATH, USA

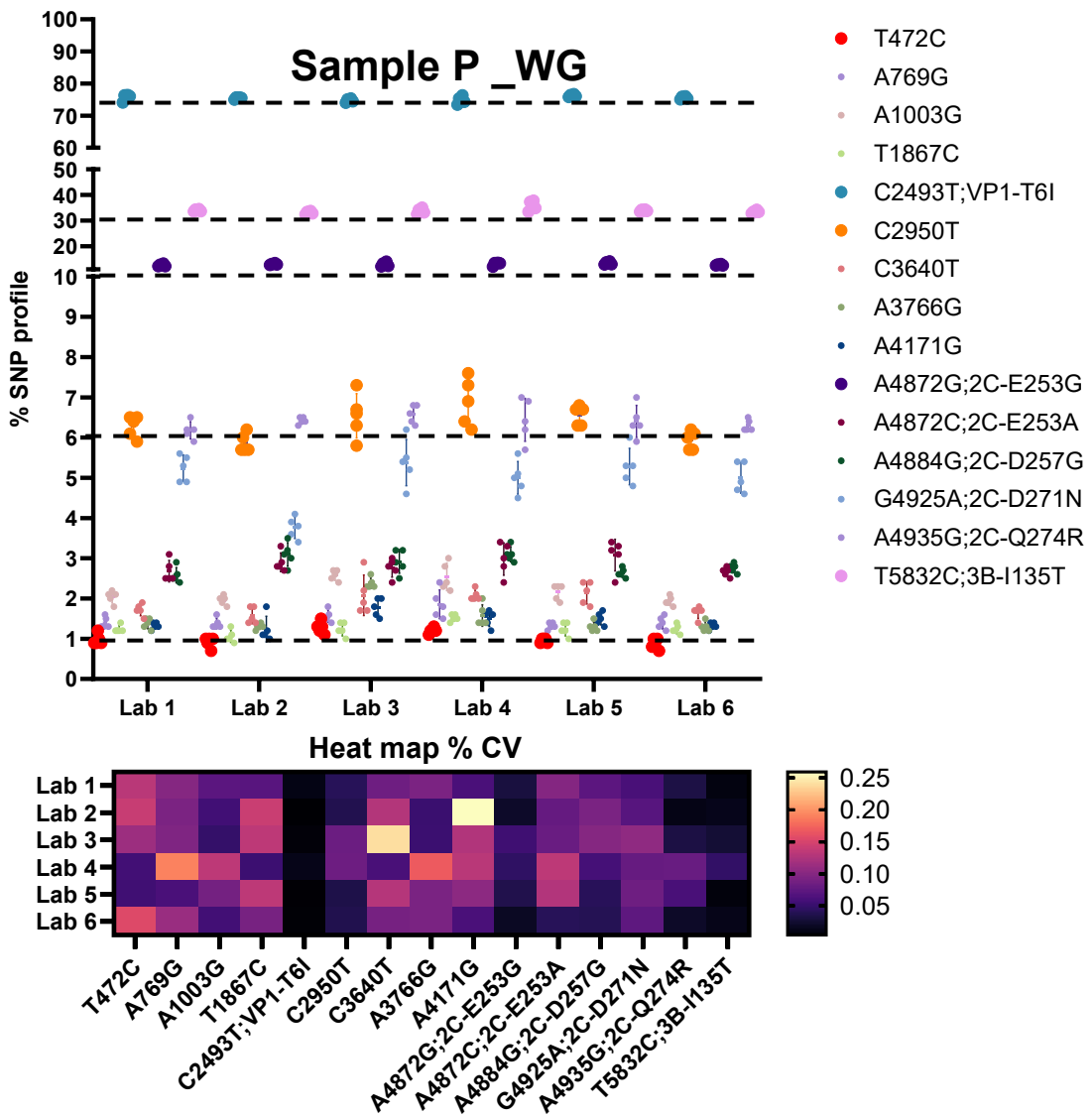
Mutation Analysis by PCR and Restriction Enzyme Cleavage

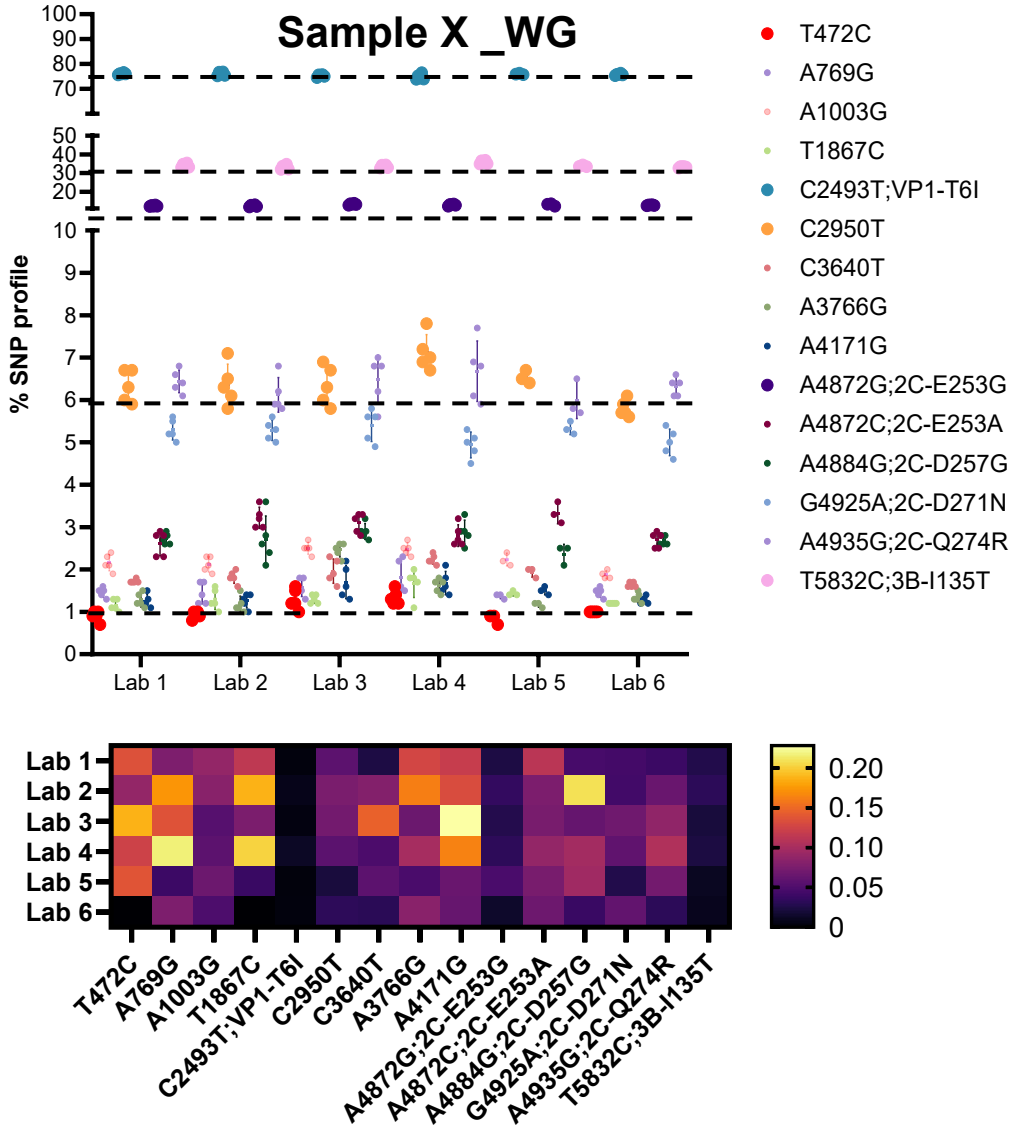


Whole genome background SNP profile – OPV3 clone



Whole Genome profile of selected mutation of Candidate 23/174 (Type 3-OPV)





Final estimates

C2493T	T5832C	A4872G	C2950T	A4935G	G4925A	A4872C	A4884G	A1003G	C3640T	A3766G	A769G	A4171G	T1867C	T472C
75.56	34.00	12.93	6.38	6.36	5.08	2.90	2.78	2.22	1.88	1.52	1.50	1.45	1.30	1.04

Blind Duplicate Type 3 samples P and X

Summary: Whole genome HTS for OPV

- NGS seems to provide a sensitive tool for monitoring consistency of production and identifying outliers
- Close similarity of SNP profiles with historical data is a proof of consistency and suggests that biological properties (neurovirulence) are also very similar
- Caveats:
  - Inconsistency of molecular profiles does not necessarily mean that a vaccine lot is unacceptable
  - It suggests that conditions of virus growth have changes, which is a red flag and may require investigation
- Path forward:
  - Accumulation of SNP profiles of historical vaccine lots that were successfully released and used
  - Development of an algorithm to make pass-fail decisions

ECBS Proposal:

The following proposals will be made to ECBS from this collaborative study:

For Type 1 OPV:

23/160 to be established as WHO RR as background noise control for Type 1 OPV

23/162 to be established as a WHO RR that can be used as an alternative to 00/418 to support Type 1 MAPREC assay in a HTS platform with a mean combined estimate of G480A+U252C as 2.43% (95% CI: 2,33%-2.54%).

For Type 3 OPV:

23/172 to be established as WHO RR as background noise control for Type 3 OPV

23/174 to be established as a WHO RR that can be used as an alternative to 95/542 to support Type 3 MAPREC assay in a HTS platform with a mean estimate of U472C as 1.04% (95% CI: 1.01%-1.08%).

23/174 can also be used for whole genome SNP profiling of OPV vaccines estimates for the position are:

C2493T	T5832C	A4872G	C2950T	A4935G	G4925A	A4872C	A4884G	A1003G	C3640T	A3766G	A769G	A4171G	T1867C	T472C
75.56	34.00	12.93	6.38	6.36	5.08	2.90	2.78	2.22	1.88	1.52	1.50	1.45	1.30	1.04

The collaborative study SOP and trouble shooting will be published as an annex to the proposal to support assay development

The proposal will contain annex describing analytical processes for establishing assay validity criteria. (Kostya's Talk)

A proposed future scheme for routine OPV lot release

- A series of consistency lots of monovalent OPV is tested by WG-HTS analysis to establish the range of variations of SNP profile.
  - Lots are passed with known MNVT, TgmNVT and/or MAPREC results

- **After the consistency of manufacture is established:**

  - Only HTS can be used to test for conformity of molecular composition of each new batch of OPV to the historical profile of mutations
  - If the SNP profile of a new lot falls within pre-defined statistical criteria, it can be released without performing NVT
  - If a new lot falls outside of these criteria, an Investigation is conducted, possibly including performing NVT
  - If the outcome of the investigation is favorable, the historical SNP database can be updated