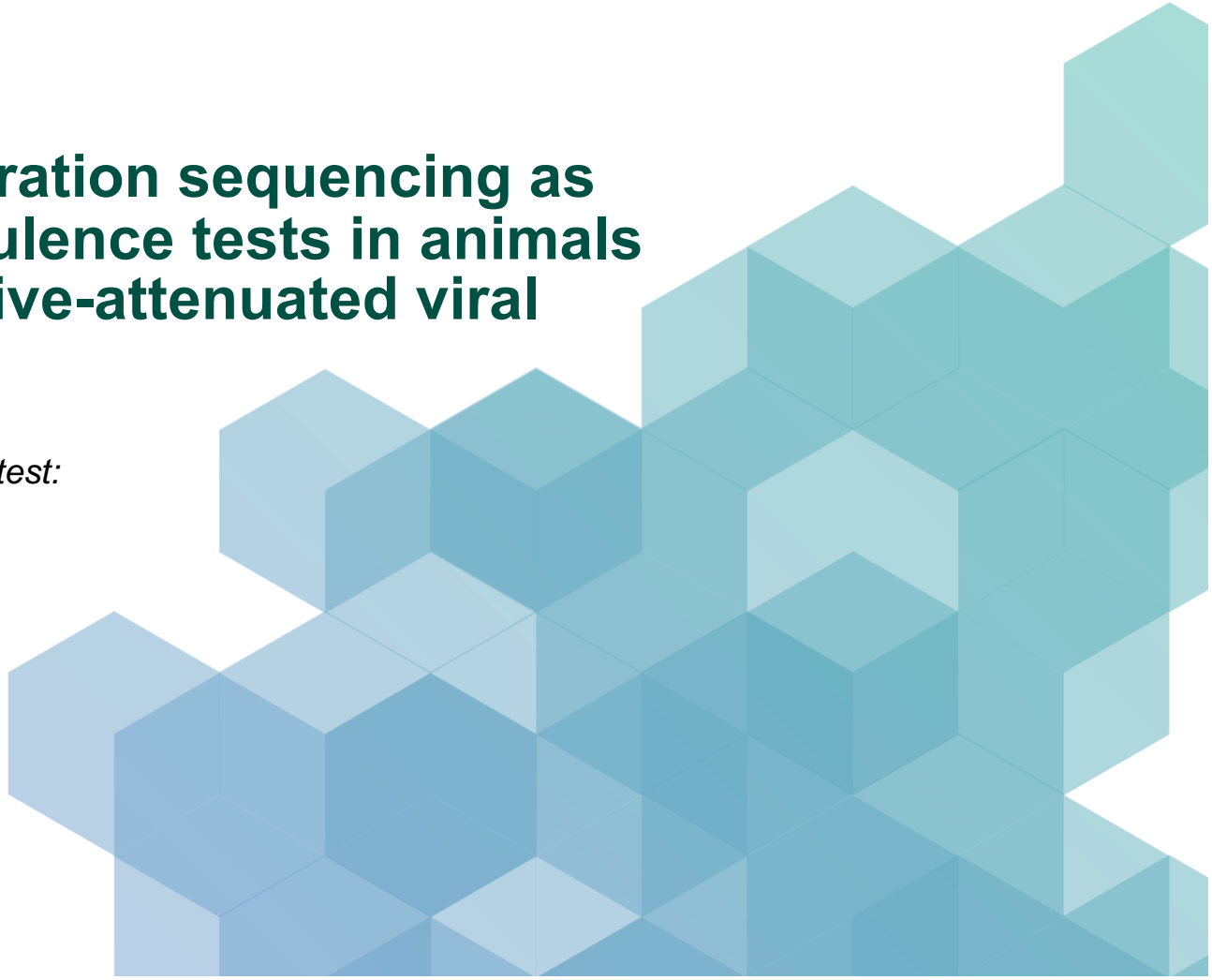
Medicines & Healthcare products Regulatory Agency

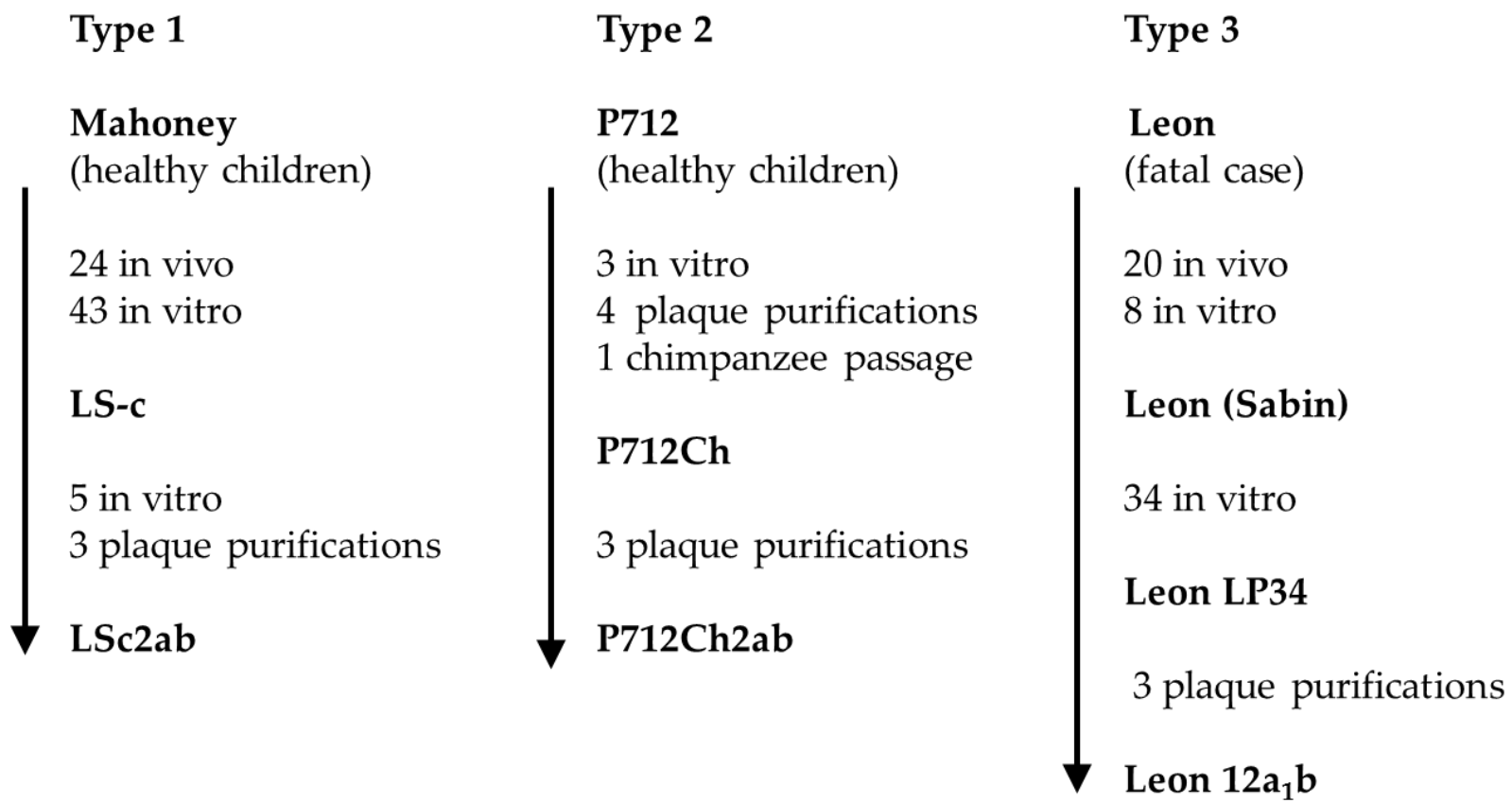
Whole-genome next generation sequencing as an alternative to neurovirulence tests in animals for the quality control of live-attenuated viral vaccines

Webinar: *Replacing the monkey neurovirulence test: challenges and opportunities for the future.*
22 October 2025

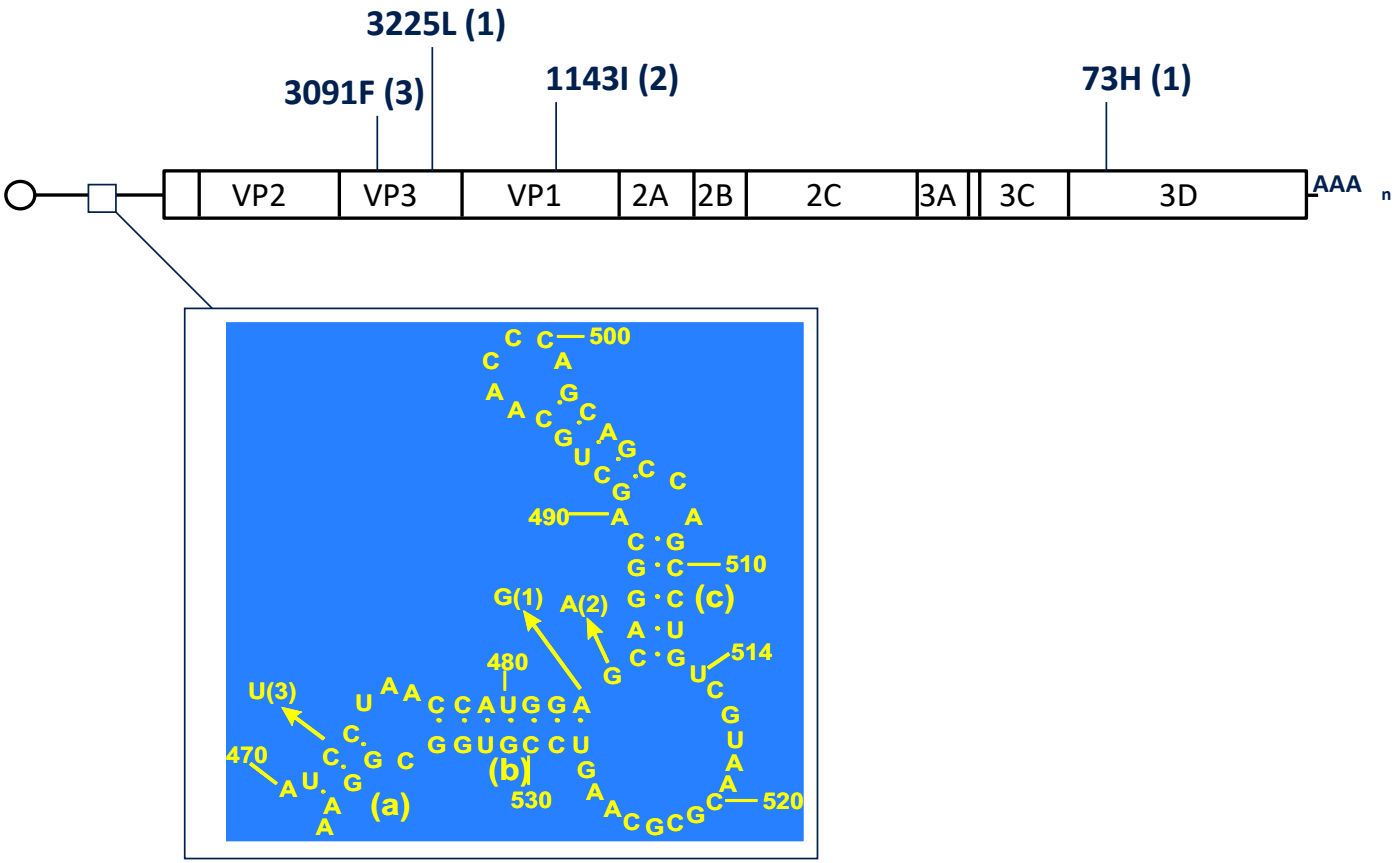
Javier Martin



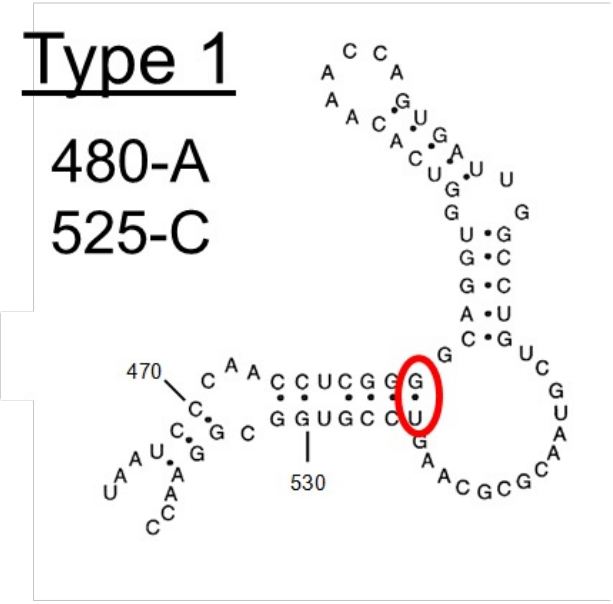
Development of Sabin live-attenuated OPV strains

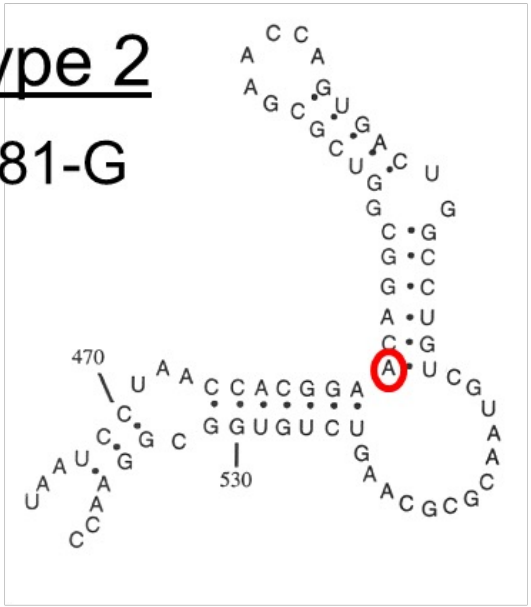


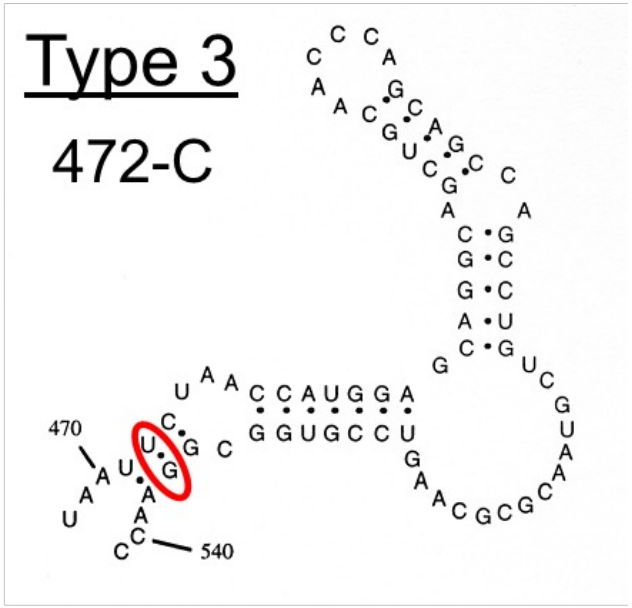
Determinants of attenuation in Sabin poliovirus OPV strains



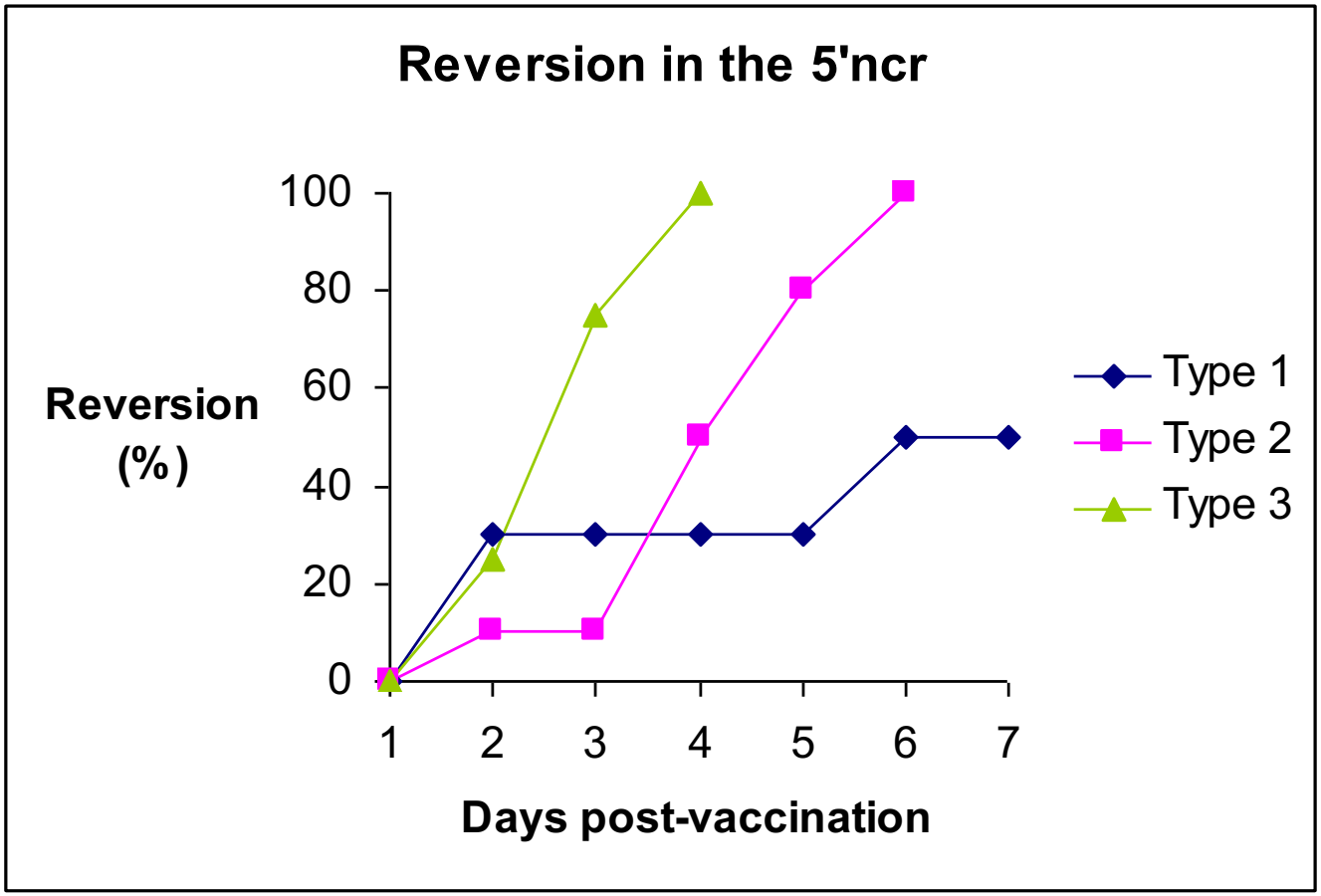
Poliovirus 5’NTR Domain V Mutations







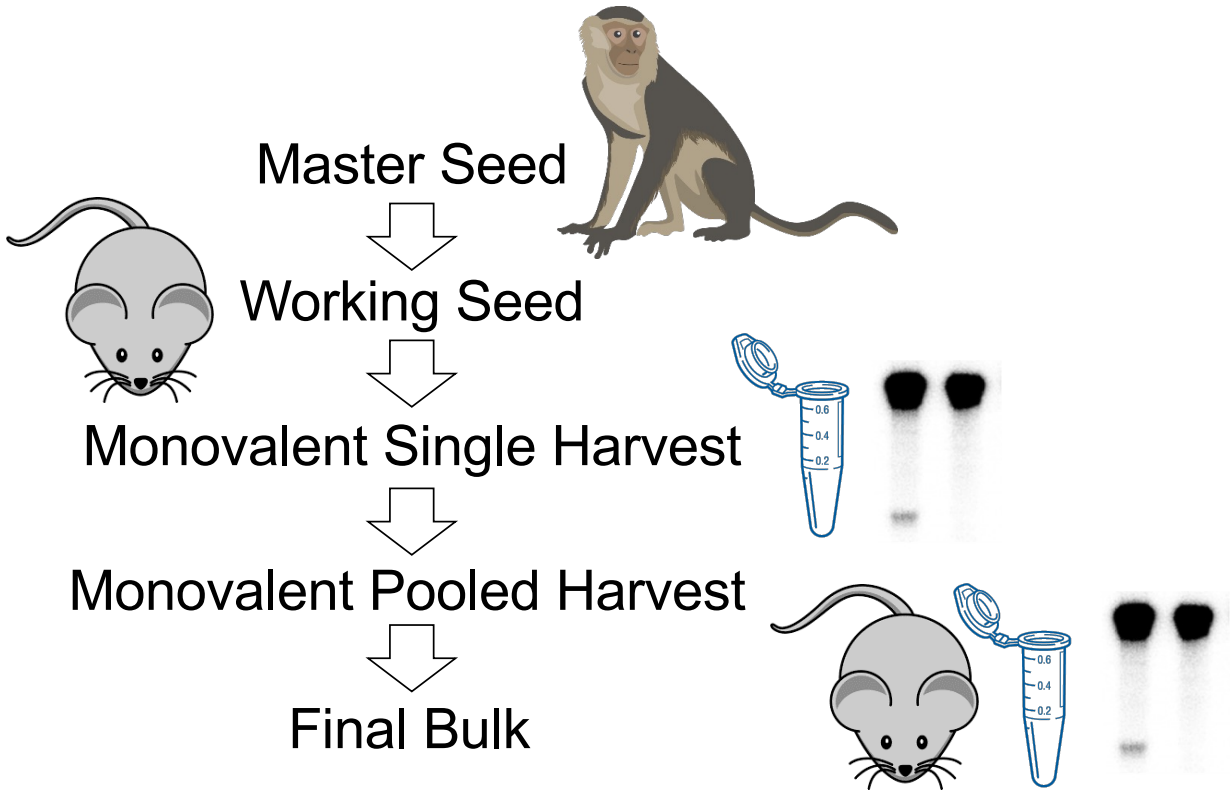
Genetic stability of OPV strains



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



OPV safety testing through time

OPV Production	**Testing**		
	Until 2002	*2002-2013*	*2013 onwards*
Master Seed	MNVT	MNVT	MNVT
↓	↓	↓	↓
Working Seed	MNVT	MNVT	TgmNVT
↓	↓	↓	↓
Monovalent Single Harvest	-	MAPREC	MAPREC
↓	↓	↓	↓
Monovalent Pooled Harvest	MNVT	TgmNVT MAPREC	TgmNVT MAPREC
↓			
Final Bulk Vaccine (mOPV, bOPV, tOPV)			

OPV safety testing through time

OPV Production	**Testing**			
	Until 2002	*2002-2013*	*2013 onwards*	*202X onwards*
Master Seed	MNVT	MNVT	MNVT	MNVT
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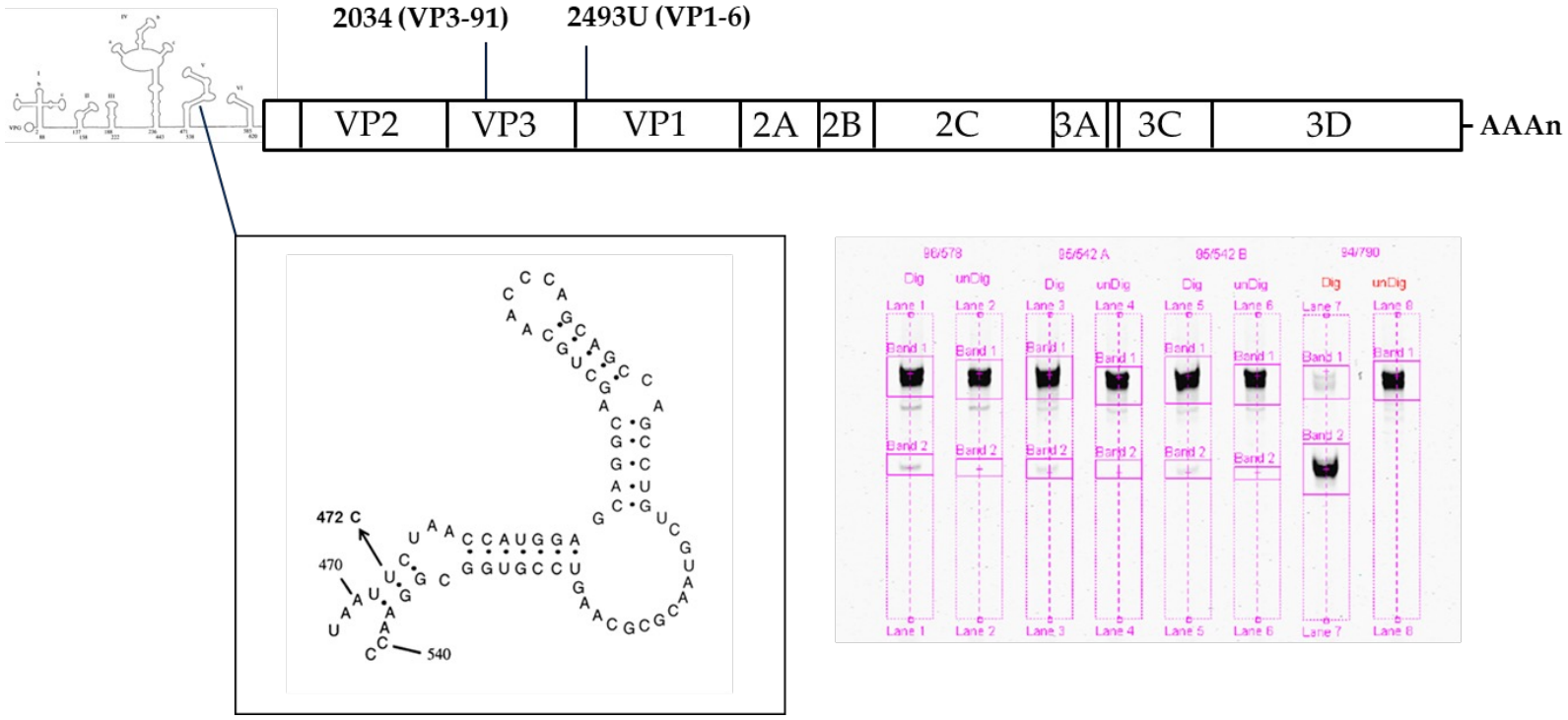
Monovalent Single Harvest	-	MAPREC	MAPREC	MAPREC
↓	↓	↓	↓	↓
Monovalent Pooled Harvest	MNVT	TgmNVT MAPREC	TgmNVT MAPREC	TgmNVT MAPREC
↓				
Final Bulk Vaccine (mOPV, bOPV, tOPV)				

NGS

MNVT and TgmNVT for OPV

Feature	MNVT	TgmNVT
Model	Old-world macaques	Transgenic mice; modified to express the human poliovirus receptor
Inoculation	Intraspinal	Intraspinal
Number of Doses	Single dose. All serotypes at the one dose.	Two doses. Doses are different for each serotype
Scoring the Test	Histological analysis of CNS tissues.	Clinical scoring of mice. Eventually reduced to +/- for paralysis.
Independent Assessment	Scoring of the slides.	Paper-trail only of randomization and clinical scores.
Time to complete (to end of analysis)	Minimum of 8 weeks – usually closer to 12 weeks.	Minimum of 3 weeks – usually around 6 weeks.
Demonstration of inoculator competence	In-line demonstration with each test with animal validity scored for each animal	External indicators e.g. surrogate tests (Inert dye inoculation). Can be assessed in-line with histology.

Mutation Analysis by PCR and Restriction Enzyme Cleavage



High Throughput (Next Generation, or Deep) Sequencing

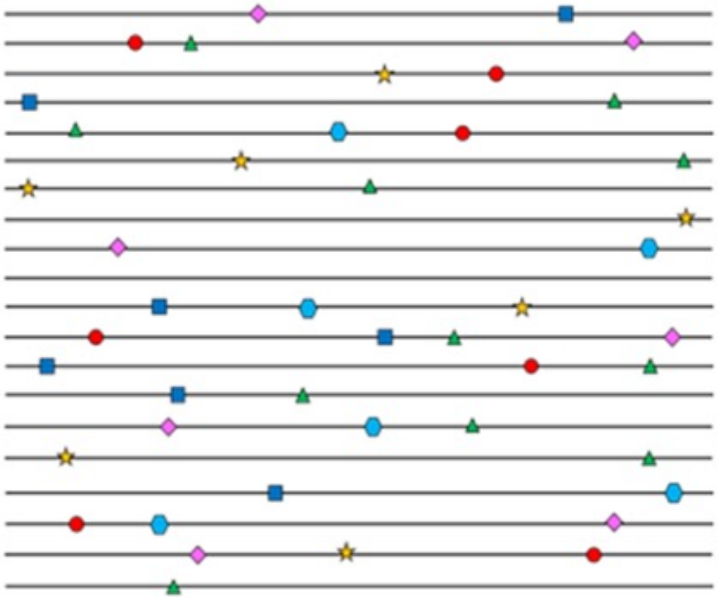
- High throughput allowing multiplexing
- Allows sequencing of individual molecules, accurately measuring variant mutations – Single Nucleotide Polymorphism (SNP)
- Decreasing cost per sample for large amounts of data
- High efficiency
- Technically simpler and more robust than MAREC

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

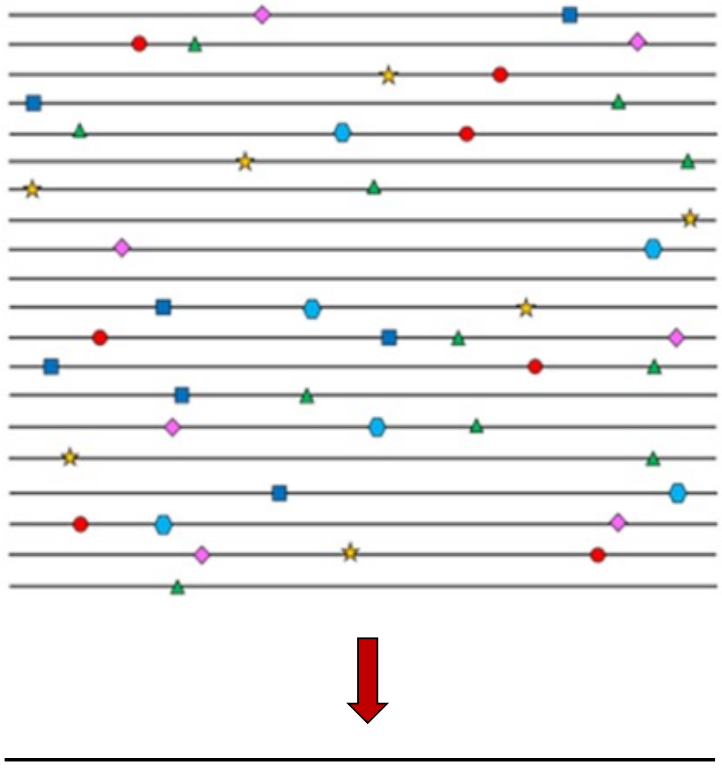
Sarcey E *et al*., **Quantifying low-frequency revertants in oral poliovirus vaccine using next generation sequencing.** J Virol Methods, 2017.

Viral quasispecies



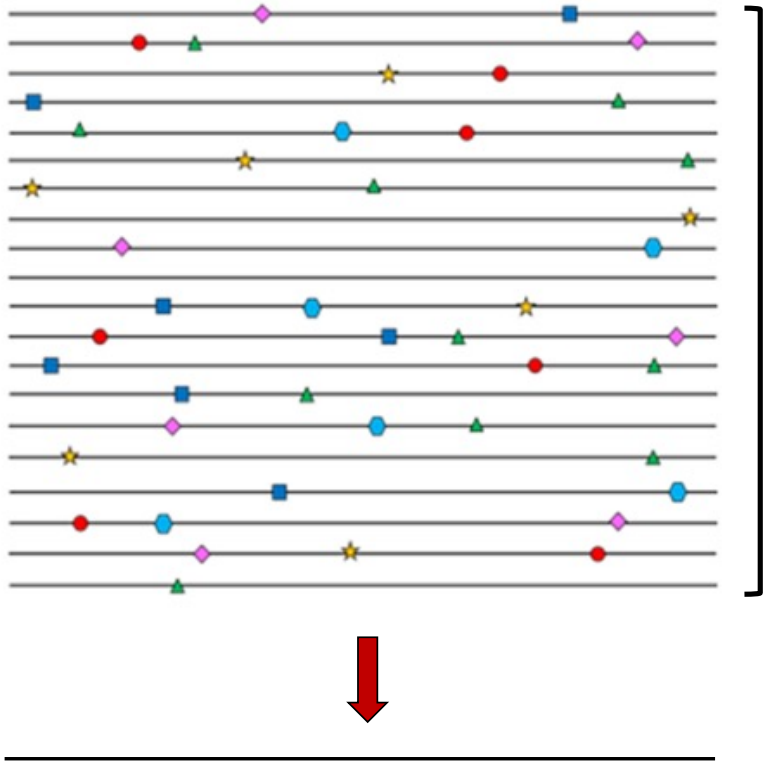
RNA viruses exist as **viral quasispecies**, which refers to a population structure that consists of large numbers of variant genomes, termed mutant spectra, mutant swarms or mutant clouds. Fuelled by high mutation rates, mutants arise continually, and they change in relative frequency as viral replication and selection proceeds.

Nucleotide sequence analysis of viral genomes



Consensus sequence by **Sanger sequence analysis**

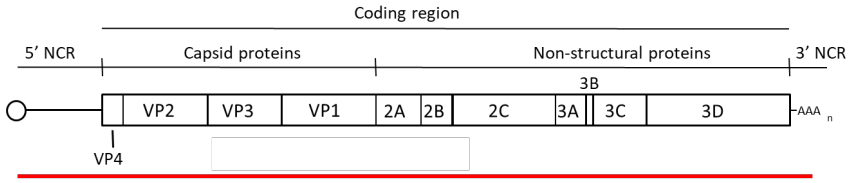
Nucleotide sequence analysis of viral genomes



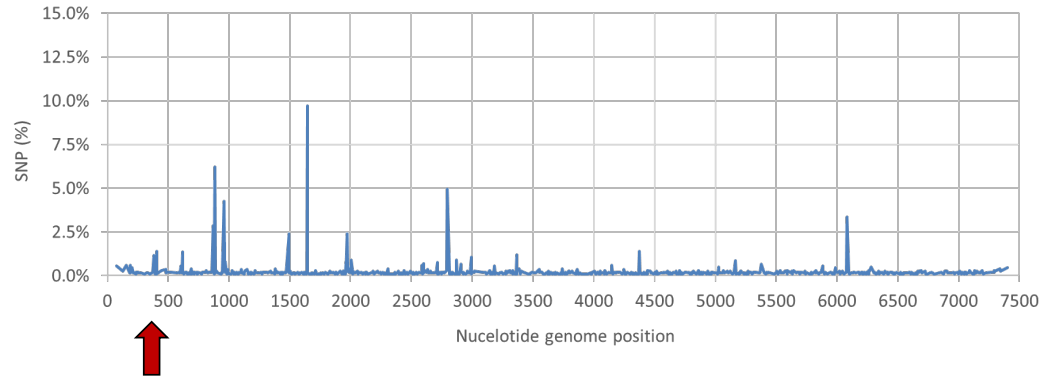
High Throughput (Next Generation, or Deep) Sequencing allows sequencing of individual molecules, accurately measuring variant mutations – Single Nucleotide Polymorphism (SNP) at each genome nucleotide position, creating a unique SNP profile (*molecular fingerprint*) for any given virus preparation that 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) .

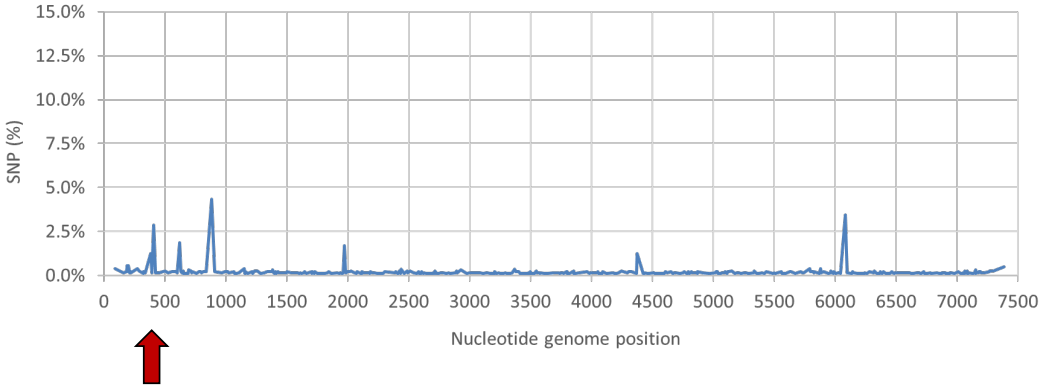
Consensus sequence by **Sanger sequence analysis**

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

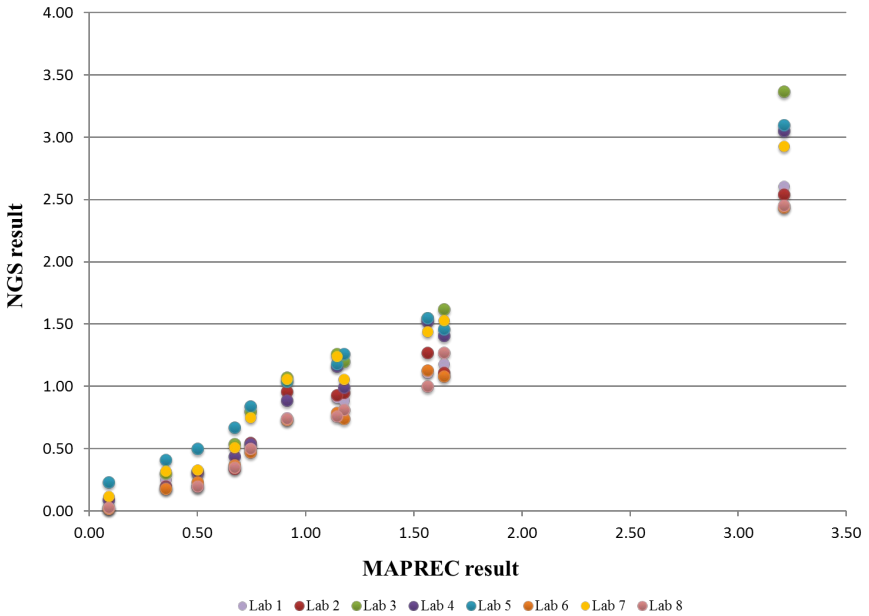
WHO/BS/2019.2359
ENGLISH ONLY

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?
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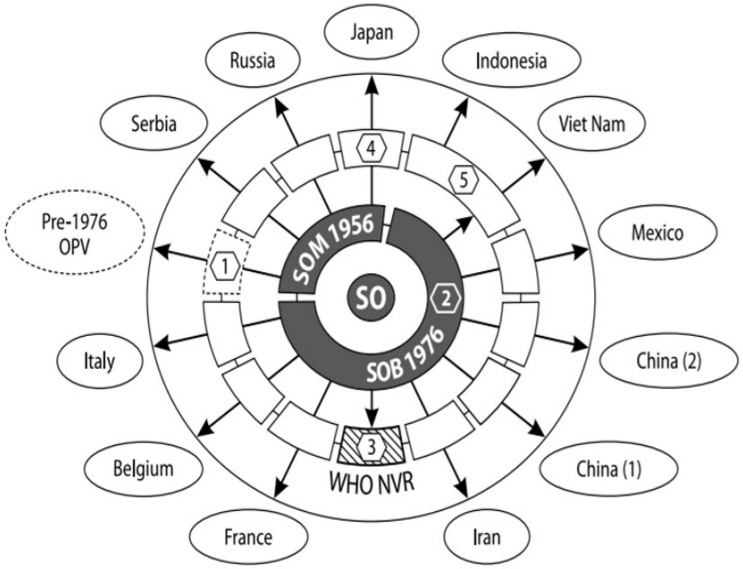
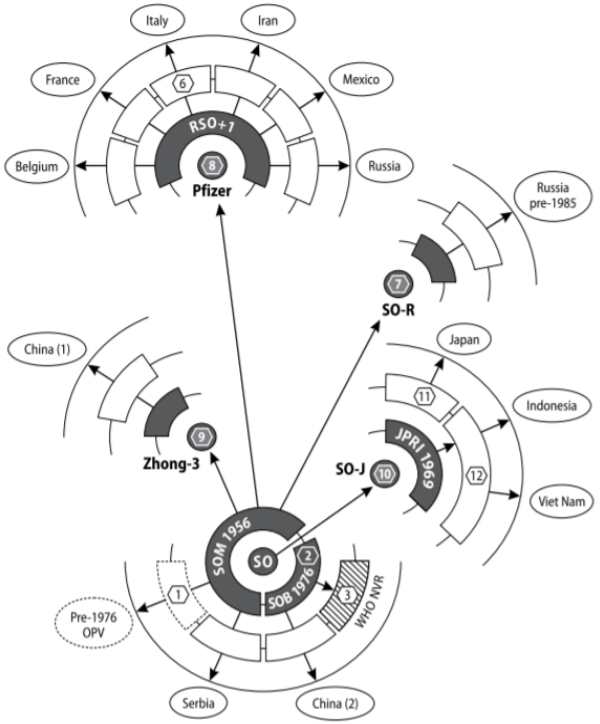
Passage history of OPV seeds

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



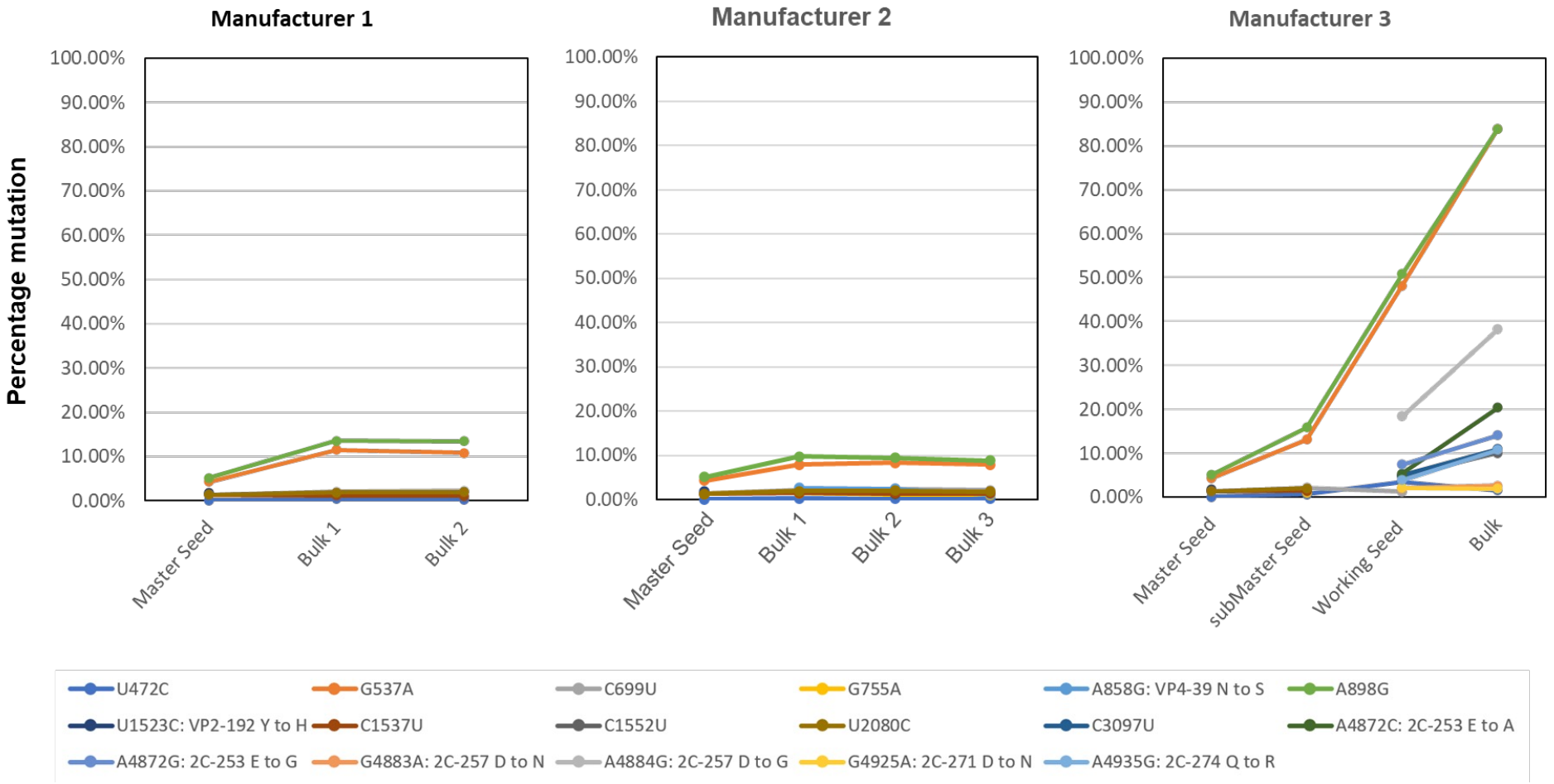
NGS analysis of OPV3 strains

	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



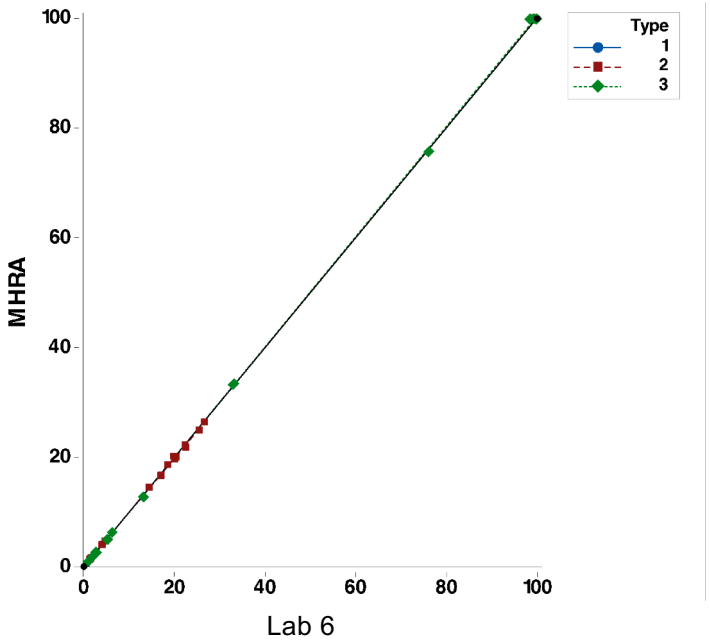
Collaborative study to support Phase 2

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

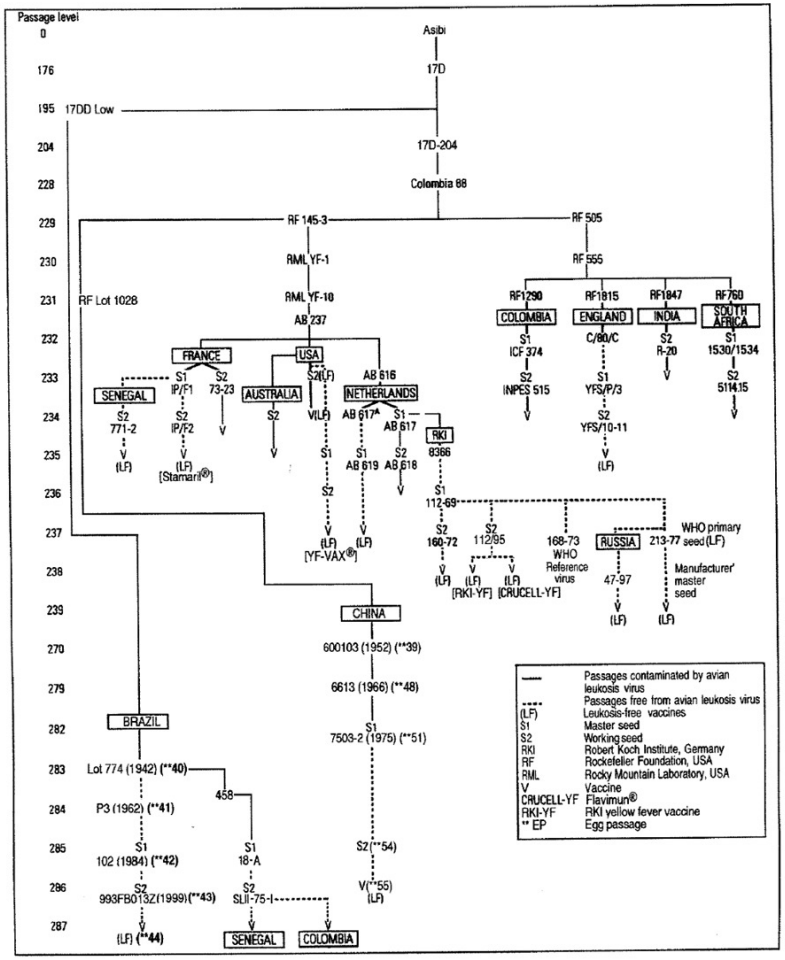
Scatterplot of MHRA Vs Lab 6 analysis

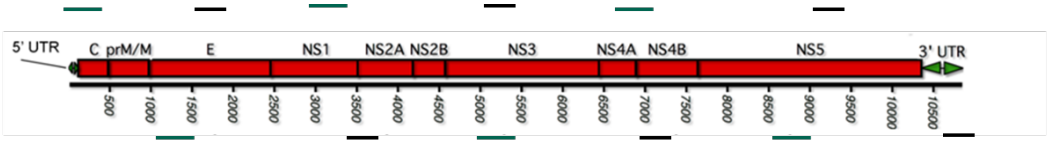


MHRA lower	44.12%
N	68
Wilcoxon p	0.935
Type	**CCC**
1	0.999997
2	0.999518
3	0.999936

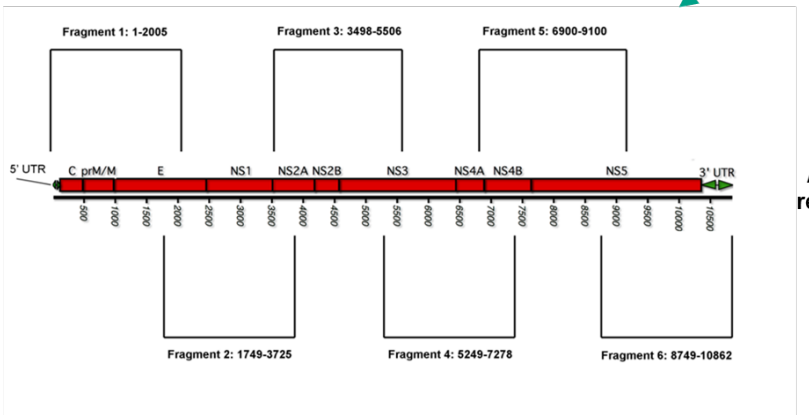
This analysis shows high concordance in reported values for single nucleotide polymorphisms for Type 1, 2 and 3 OPV when values generated by MHRA pipeline and Lab 6 were plotted against each other

Whole-genome analysis of yellow fever vaccines



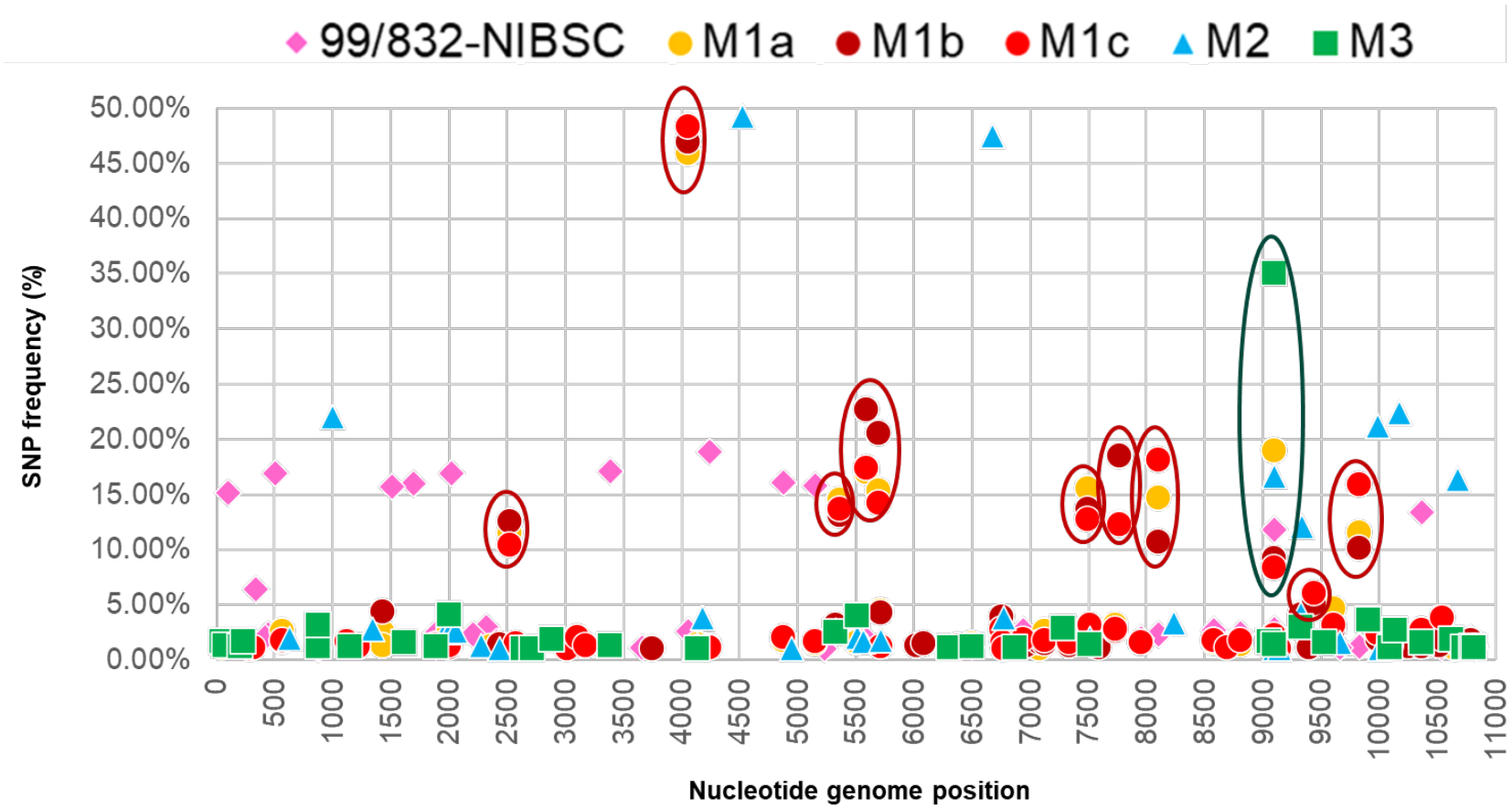


6 different PCR reactions standardized to run on single thermal profile



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

Single Nucleotide Polymorphism (SNP) analysis of YFV



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- Thomas Wilton(MHRA)
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- Jason Hockley (MHRA)
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- Javier Martin (MHRA)
- Vajra Allan (MHRA)
- Kutub Mahmood (PATH)

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