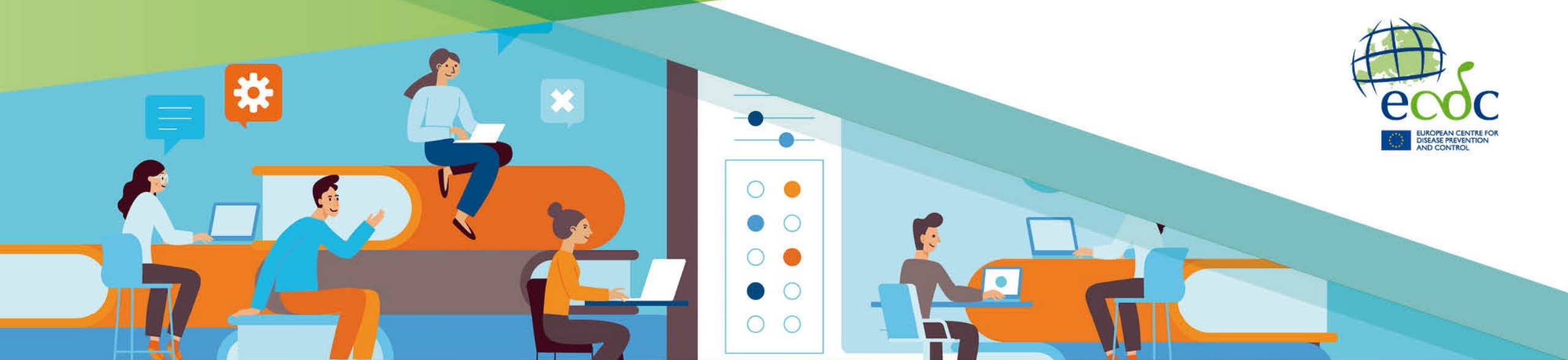




Genomics and Epidemiology of Polioviruses

Epidemiology and Genome Surveillance



MRC Centre for
Global Infectious
Disease Analysis

IMPERIAL



Polio Sequencing
Consortium

17th July 2026

Dr Alex Shaw, Advanced Research Fellow, Imperial College London

Aim and learning objectives



Aim

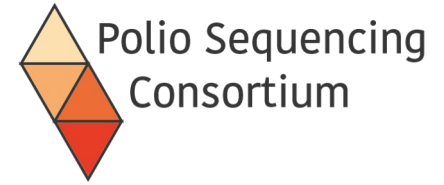
To support the adoption and effective use of Direct Molecular Detection and Nanopore Sequencing (DDNS) and PIRANHA analysis workflows for timely poliovirus detection

Specific learning objectives

At the end of this session, you will be able to:

1. Describe how DDNS is performed
2. Use PIRANHA software to analyse DDNS data
3. Identify resources for further learning and troubleshooting

Outline



1. Introduction to the DDNS protocol
2. Demonstration of PIRANHA for analysing DDNS data

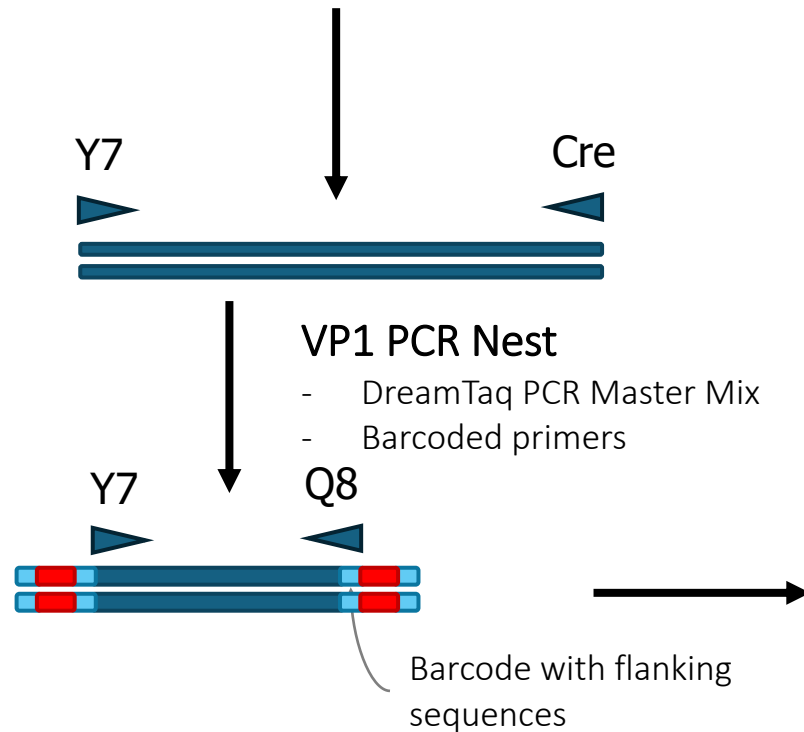
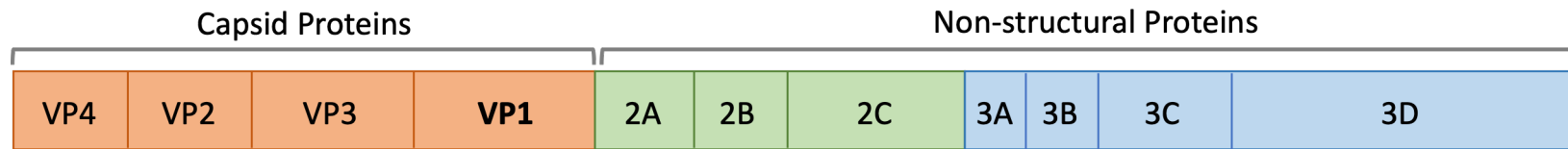
Why use molecular methods for poliovirus?

- Shorten time to detection compared to viral culture
- Avoid the bottleneck for diversity due to competitive culture
- Support containment goals
- NGS methods can resolve viral mixtures
- Can pivot more easily to surveillance other organisms



Summary of DDNS

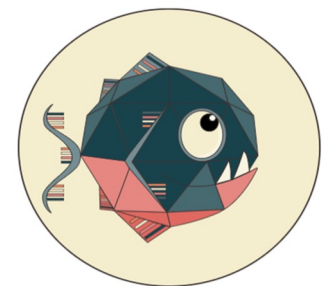
(Direct molecular Detection and Nanopore Sequencing)



Half capsid RT-PCR

- SuperScript III One-Step rtPCR System
- Primers: Y7 and Cre +nOPV-MM-R)
- 2kb product, ~4-5 hrs program

PIRANHA



Poliovirus direct detection by nanopore sequencing

- Fast (2-3 days)
- Low cost (~\$15 per sample)
- Scalable (simple protocol, few capital costs)
- Multiplex (barcoding)
- Can sequence mixtures of polioviruses, enteroviruses in same sample



Protocol availability

Updated protocols maintained on protocols.io – can be reached through

<http://polionanopore.org>

- Forums on the protocols.io site to raise any queries you have about the protocol
- New version of the protocols will be published here.



POLIOVIRUS SEQUENCING CONSORTIUM

Free, open source protocols and software for poliovirus detection and sequencing using nanopore.

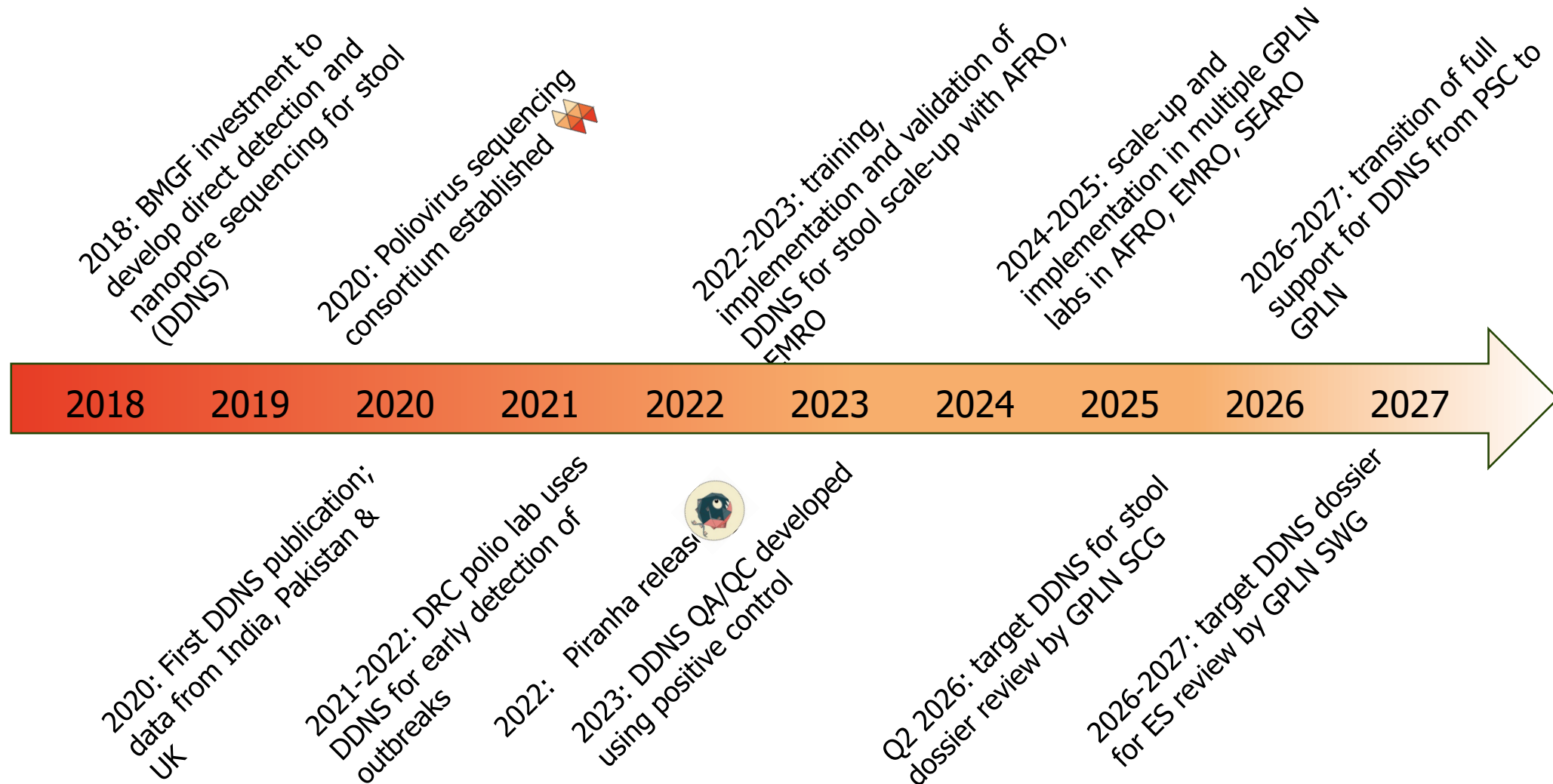
You can find all you need here for direct detection and nanopore sequencing (DDNS) of poliovirus from stool and environmental samples. Helping you detect and respond to poliovirus outbreaks faster. Funded by the Bill and Melinda Gates Foundation.



DDNS timeline



Polio Sequencing
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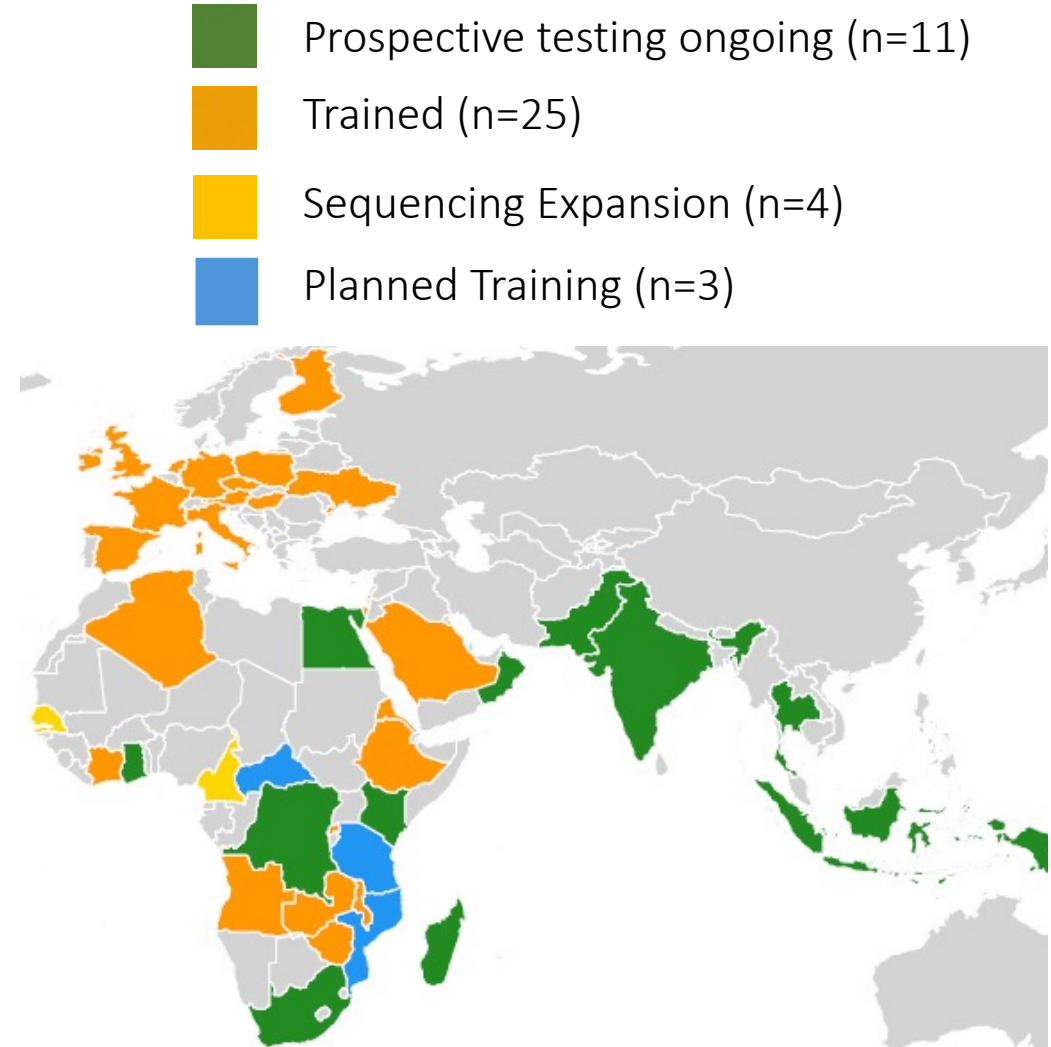


<https://polionanopore.org>

DDNS Pilot

- DDNS pilot being performed within the GPLN generating data to support acceptance as a method
- 35 labs now trained to perform DDNS for parallel testing
- 11 labs started parallel testing of stool samples by DDNS and routine method (culture)
- Ongoing proficiency testing and continuous training as needed
- We have also supported sequencing expansion in 4 labs (nanopore sequencing of poliovirus isolates)

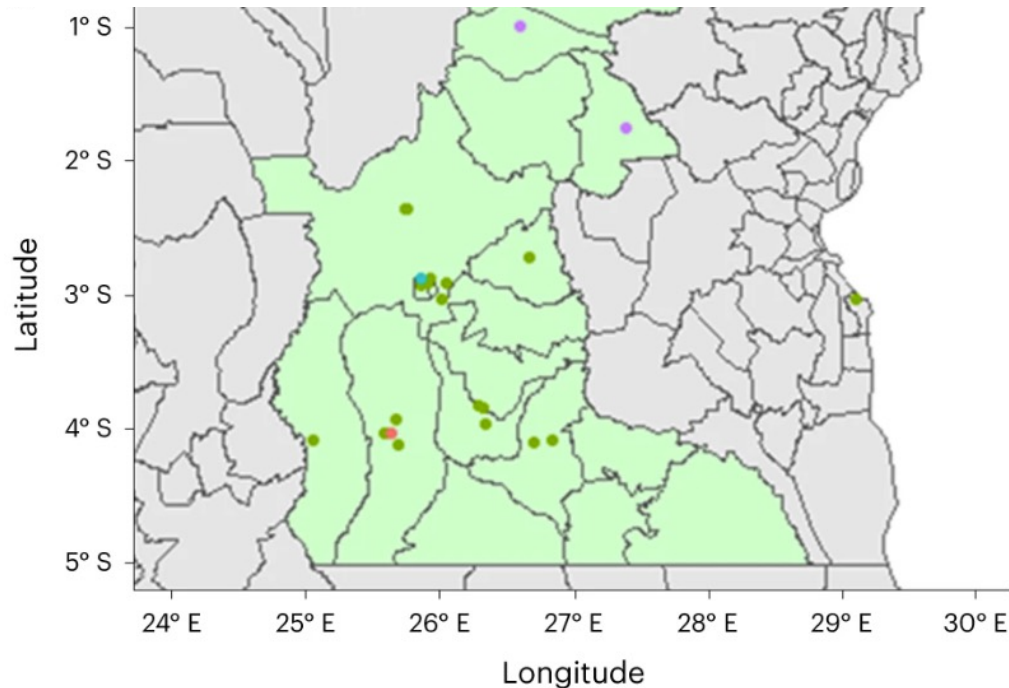
* note 2 labs with routine testing (green) are also doing isolate sequencing as part of sequencing expansion



Outbreak surveillance in DRC



Polio Sequencing
Consortium

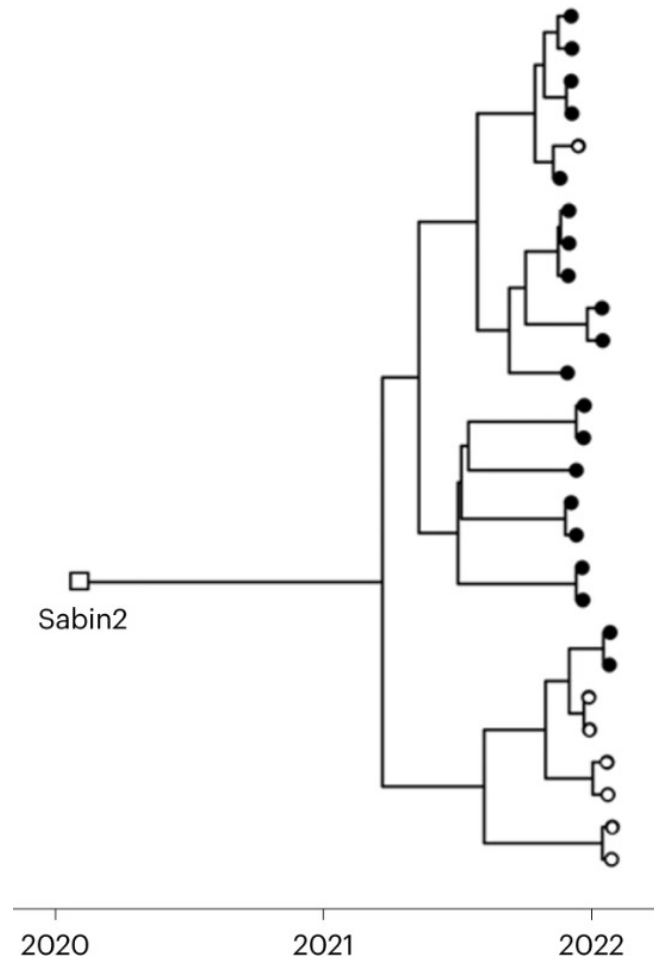


Emergence group

- RDC-MAN-2
- RDC-MAN-3
- RDC-MAN-4
- RDC-MAN-5

Outbreaks of the cVDPV2 in Maniema, DRC

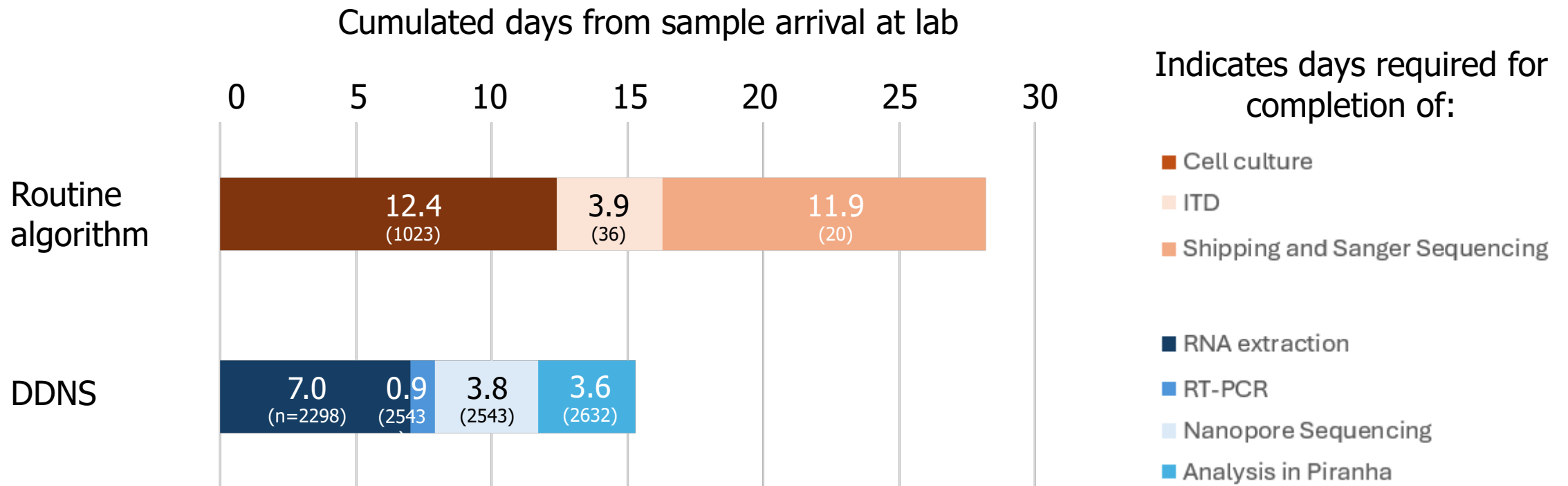
Shaw et al, 2023, Nature Microbiology, s41564-023-01453-4



Phylogenetic tree of the RDC-Man-3 lineage (solid tips indicate that the DDNS detection was matched by a cell-culture-based detection of a cVDPV2 from the same sample)

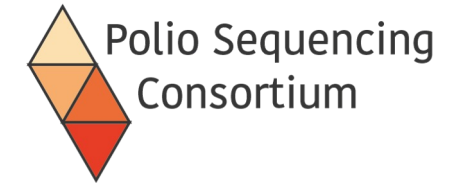
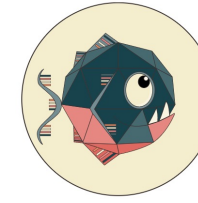
- 2339 stools from DRC tested over a 141 day case study period
- Four VDPV2 lineages detected in Maniema
- Outbreaks detected a mean of 23 days quicker

Time required for detections



Result obtained from 41 sequencing runs by INRB, DRC (2025-04-11 to 2025-09-17)

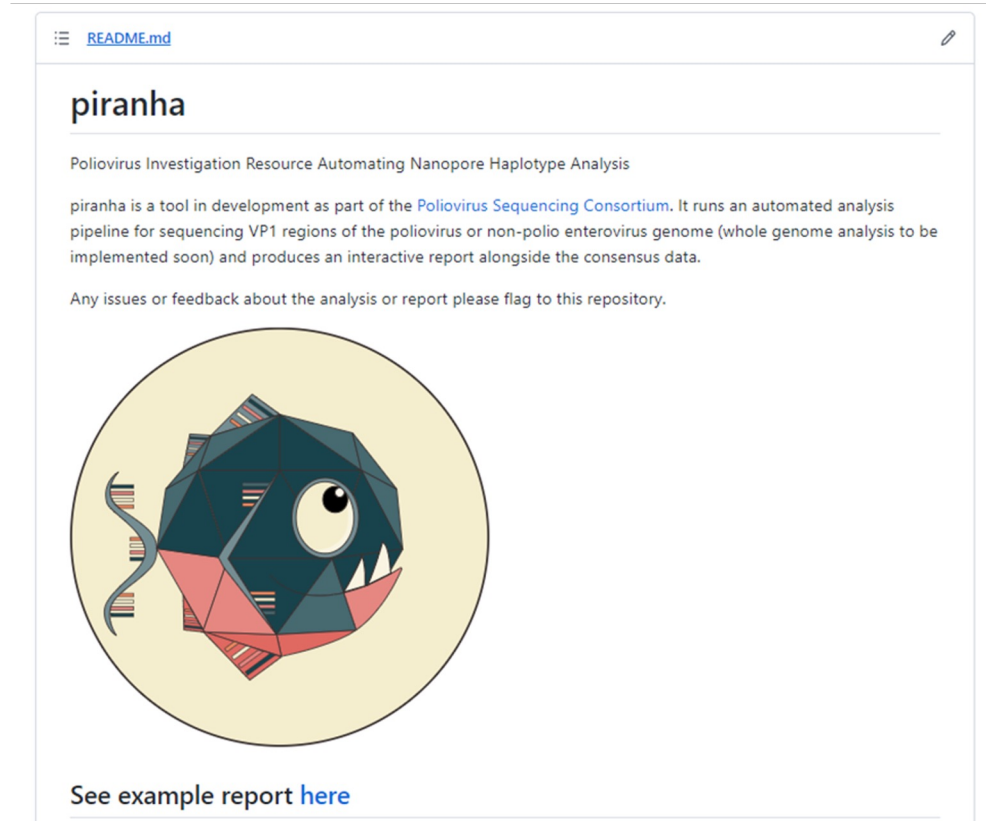
PIRANHA Overview



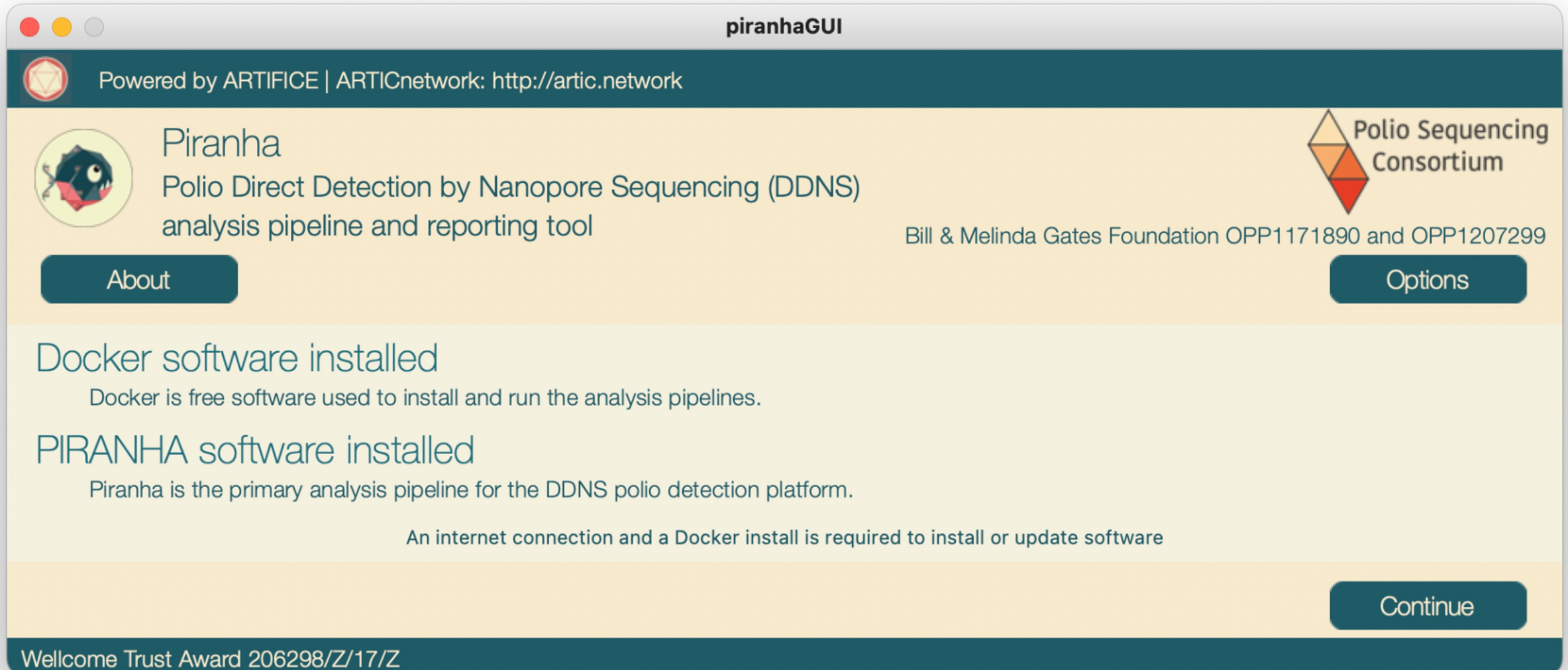
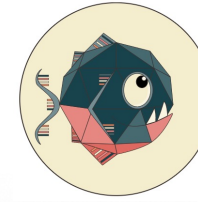
- Converts the fastq file outputs from sequencing into a reportable format
- Requires command line
- Installation instructions and basic usage available on github:

<https://github.com/polio-nanopore/piranha>

O'Toole et al, 2022, Virus Evolution, 10.1093/ve/veae023

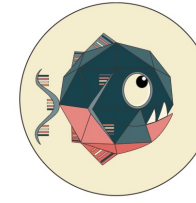


PiranhaGUI



Running PiranhaGUI

- Select your barcode.csv file
- and your demultiplexed sequencing data
- Set your run name
- Select an output folder for your results
- Click “Continue”



Polio Sequencing
Consortium



Piranha

Powered by ARTIFICE | ARTICnetwork: <http://artic.network>

Piranha
Polio Direct Detection by Nanopore Sequencing (DDNS)
analysis pipeline and reporting tool

Polio Sequencing Consortium

Bill & Melinda Gates Foundation OPP1171890 and OPP1207299

Persistent Run Options

Sequencing Run

Select a CSV file containing the IDs and barcodes for each sample:

Samples:

Select the folder containing sequencing reads from MinKnow:

MinKnow Run:

Run Name:

User Name:

Institute:

Notes:

Select a folder for the output of Piranha analysis:

Output Folder:

- Set run options. For DDNS stool testing:

Minimum length - 1000 bp

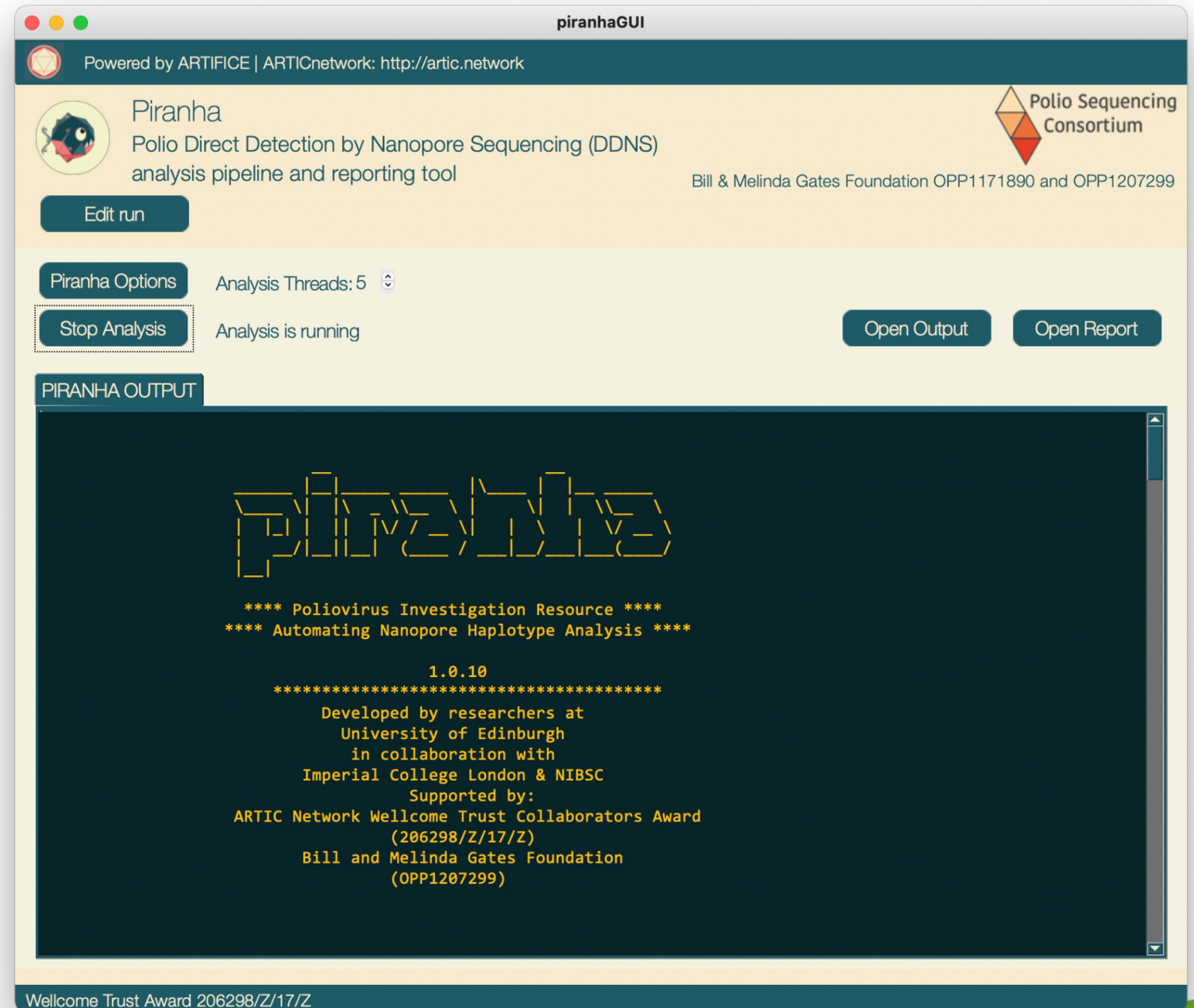
Maximum length - 1300 bp

Minimum read depth – 50

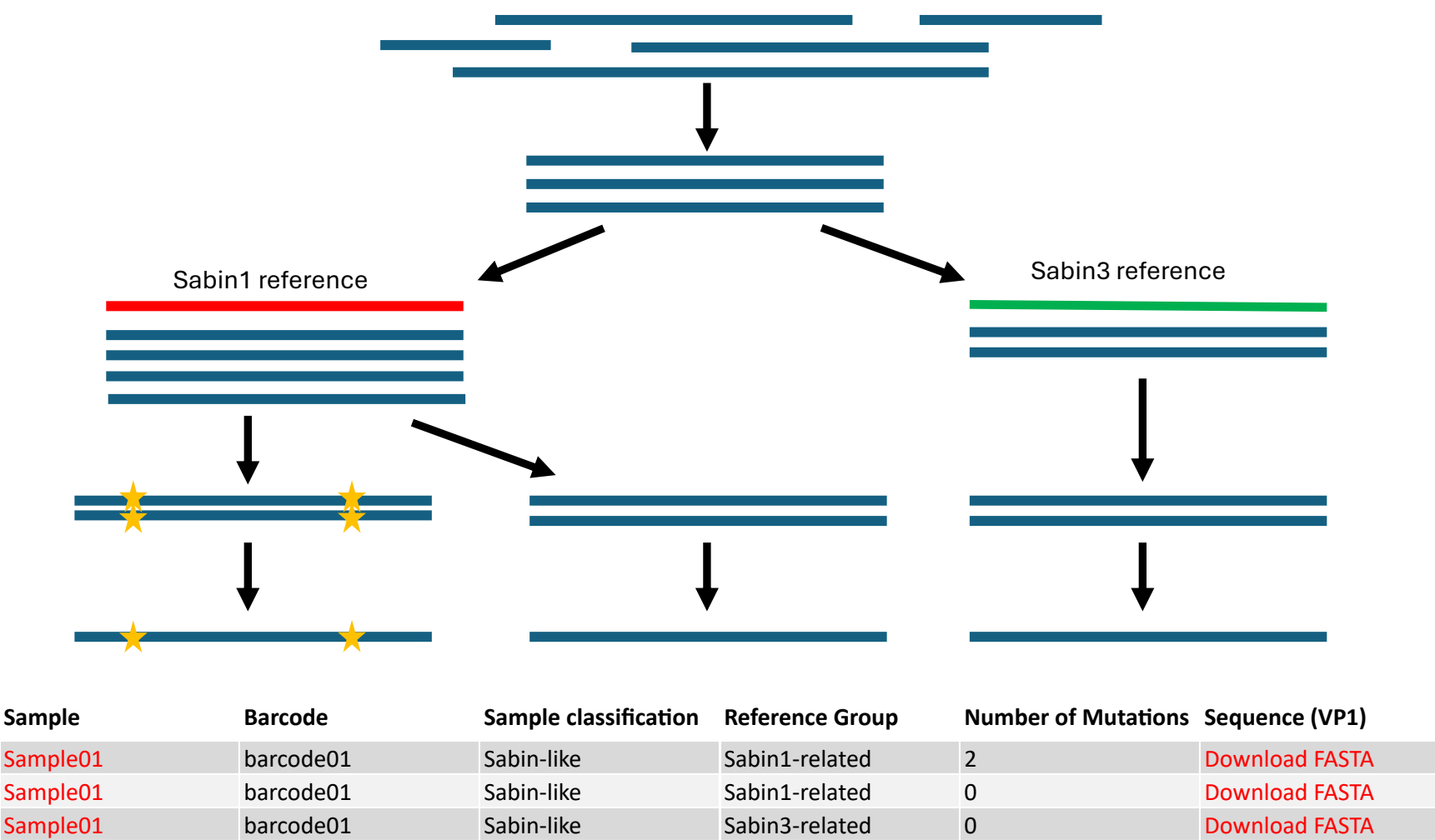
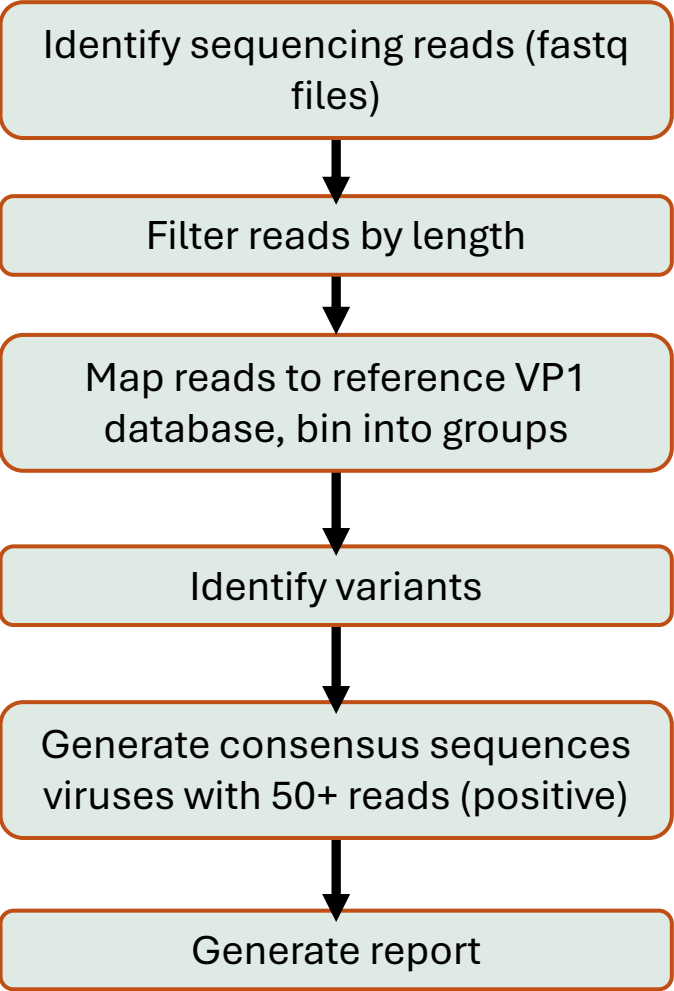
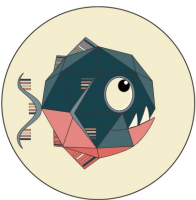
Minimum read percentage – 0

(see QC document)

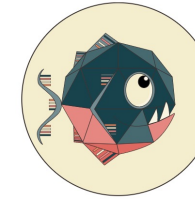
- Start analysis



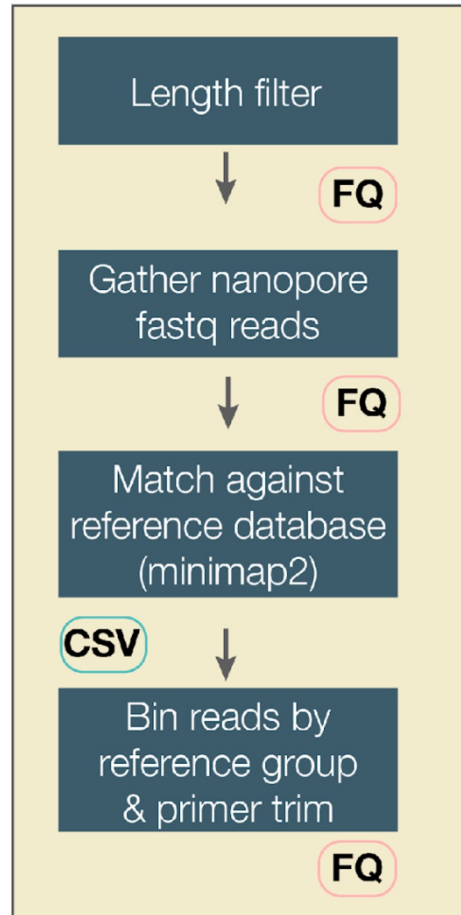
What does PIRANHA do?



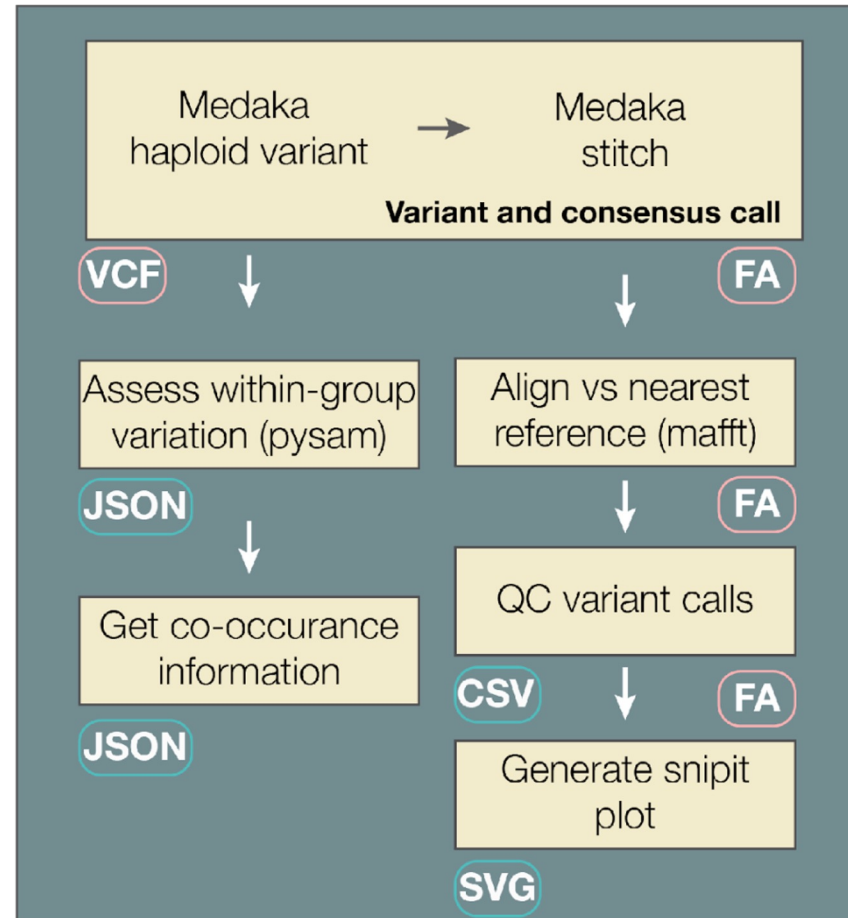
Workflow schema



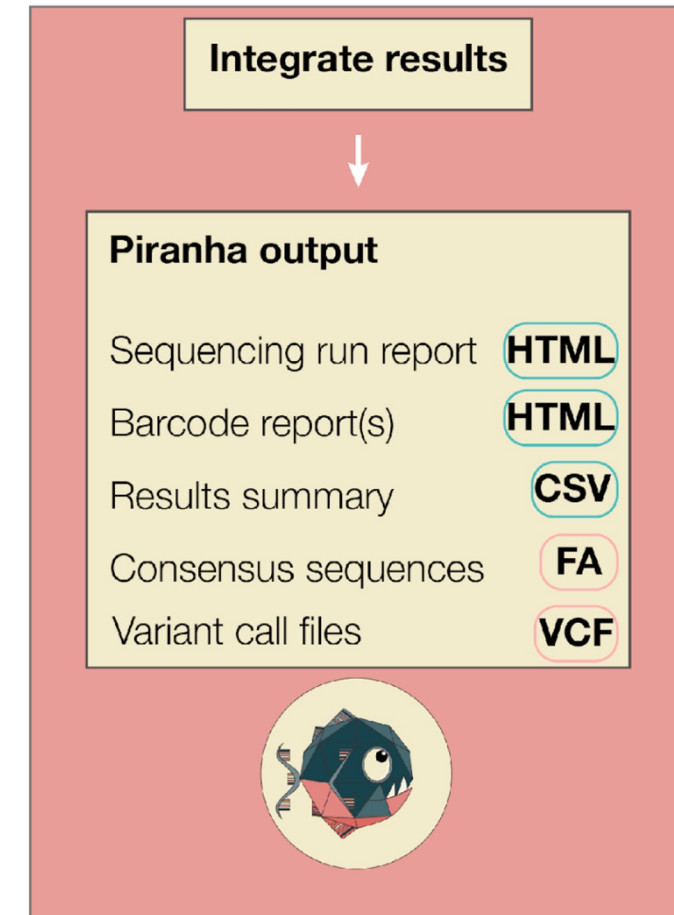
A. Barcode analysis



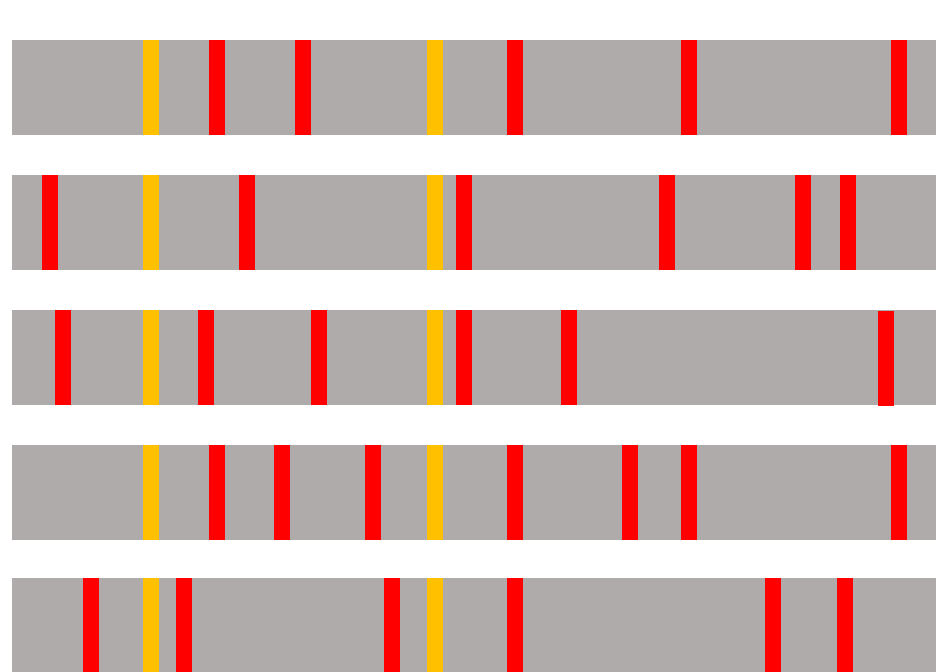
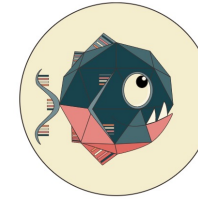
B. Reference group analysis



C. Results reporting



Building a consensus

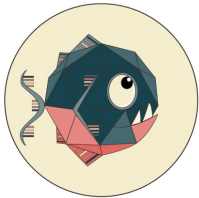


Consensus sequence



 mutation  sequencing error

PIRANHA Outputs- Report



Polio Sequencing Consortium



Search:

Sample	Barcode	Sample Call	Reference Group	Number Of Mutations	VP1 sequence
sample01	barcode25	Sabin-like	Sabin3-related	1	Download FASTA
sample02	barcode26	Sabin-like	Sabin1-related	0	Download FASTA
sample02	barcode26	Sabin-like	Sabin3-related	1	Download FASTA
sample02	barcode26	Sabin-like	Sabin2-related	4	Download FASTA
sample03	barcode27	VDPV	Sabin2-related	106	Download FASTA
sample07	barcode32	Sabin-like	Sabin3-related	2	Download FASTA
sample07	barcode32	Sabin-like	Sabin2-related	0	Download FASTA
sample08	barcode33	Sabin-like	Sabin1-related	1	Download FASTA
sample08	barcode33	Sabin-like	Sabin3-related	1	Download FASTA
sample09	barcode34	Sabin-like	Sabin1-related	0	Download FASTA
sample09	barcode34	Sabin-like	Sabin3-related	1	Download FASTA
sample09	barcode34	Sabin-like	Sabin2-related	2	Download FASTA
sample11	barcode36	VDPV	Sabin2-related	126	Download FASTA
sample12	barcode37	WPV2	WPV2	NA	Download FASTA
sample12	barcode37	NonPolioEV	NonPolioEV	NA	Download FASTA
sample12	barcode37	VDPV	Sabin2-related	135	Download FASTA
sample13	barcode38	WPV2	WPV2	NA	Download FASTA
sample13	barcode38	VDPV	Sabin2-related	142	Download FASTA

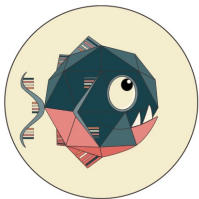
Table 2 | Composition of samples

Export table

Search:

Sample	Barcode	Sabin1-Related	Sabin2-Related	Sabin3-Related	Wpv1	Wpv2	Wpv3	Nonpolioev	Unmapped
neg1	barcode31	0	0	0	0	0	0	0	0
neg2	barcode39	0	0	0	0	0	0	0	0
sample01	barcode25	144	0	2408	3	0	0	1	0
sample02	barcode26	200	499	1003	0	0	0	0	0
sample03	barcode27	0	2803	1	1	221	0	0	1
sample04	barcode28	0	0	0	0	0	0	0	0
sample05	barcode29	0	0	0	0	0	0	0	0
sample06	barcode30	0	0	0	0	0	0	0	0
sample07	barcode32	0	1133	1367	71	0	0	200	1
sample08	barcode33	445	1	2800	0	0	0	7	1
sample09	barcode34	558	436	1696	2	0	0	54	1
sample10	barcode35	0	0	0	0	0	0	0	0
sample11	barcode36	0	68	3	0	0	0	0	1
sample12	barcode37	0	4438	1	0	4400	0	070	1

PIRANHA Outputs- Report



Polio Sequencing Consortium



PoSeCo | Poliovirus Sequencing Consortium

piranha | Poliovirus Investigation Resource Automating Nanopore Haplotype Analysis

sample08 report 2022-09-12

Table 1 | Summary of sample content

Search:

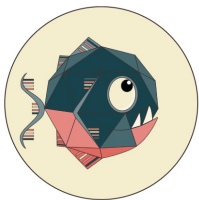
Sample	Barcode	Reference Group
sample08	barcode33	Sabin1-related
sample08	barcode33	Sabin3-related

VP1 sequences

```
>sample08|barcode33|Sabin1-related|Poliovirus1-Sabin_AY184219|1|268:AT
GGGTTAGGTCAGATGCTTGAAAGCATGATTGACACACAGTCCGTGAAACGGTGGGGGCGGCAACGCTAGAGACGCTCTCCAAACACTGAAGCCAGTGGACCAGCACACTCCAAGGAAATTCGGGCACTACCGCAGTGGAAGTGGGGCCAC
AAATCCACTAGTCCCTTCTGATACAGTGCAAACAGACATGTTGTACAACATAGGTCAAGGTCAAGGTCAAGGTCTAGCATAGAGTCTTTCTTCGCGCGGGGTGCATGCGTGCCATTTTAACCGTGGATAACTCAGCTTCCACCAAGAATAAGGATAAGC
TATTTACAGTGTGGAAGATCACTTATAAAGATACTGTCCAGTTACGGAGGAAATTGGAGTTCTTCACCTATTCTAGATTGATATGGAATTTACCTTTGTGGTTACTGCAAATTTCACTGAGACTAACAATGGGCATGCCTTAAATCAAGTGTAC
CAAATTATGTACGTACCACAGGCGCTCCAGTGCCCGAGAAATGGGACGACTACACATGGCAAACCTCATCAAATCCATCAATCTTTTACACCTACGGAACAGCTCCAGCCCGGATCTCGGTACCGTATGTTGGTATTTTGAACGCCTATTACAC
CTTTTACGACGGTTTTTCAAAGTACCCTGAAGGACCAGTCGGCAGCACTAGGTGACTCCCTCTATGGTGACGATCTCTAAATGACTTCGGTATTTTGGCTGTTAGAGTAGTCAATGATCACAACCCGACCAAGGTCACCTCCAAAATCAGAG
```

Download

PIRANHA Outputs- Variants



Sabin1-Related variant report

Table 2 | Sabin1-related

	Information
Reference group	Sabin1-related
Number of mutations	1
Mutations	268:AT

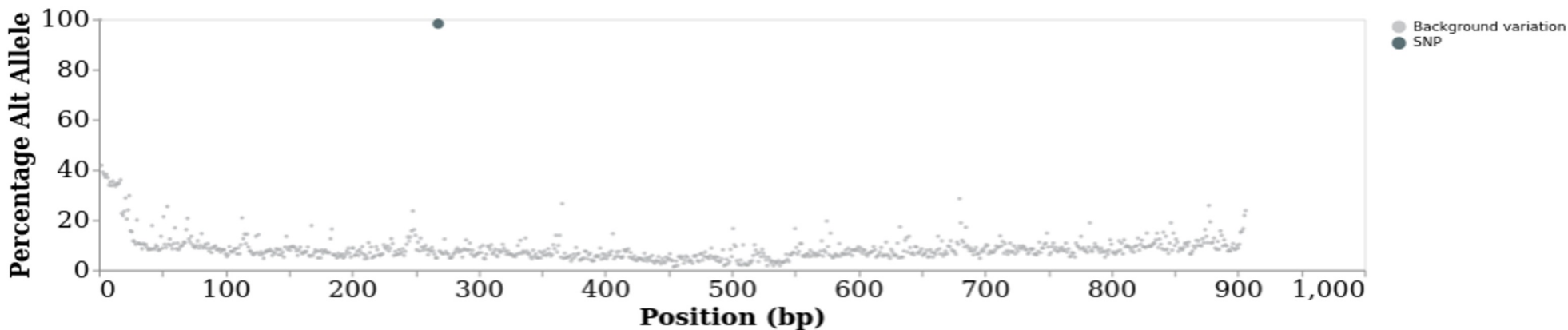
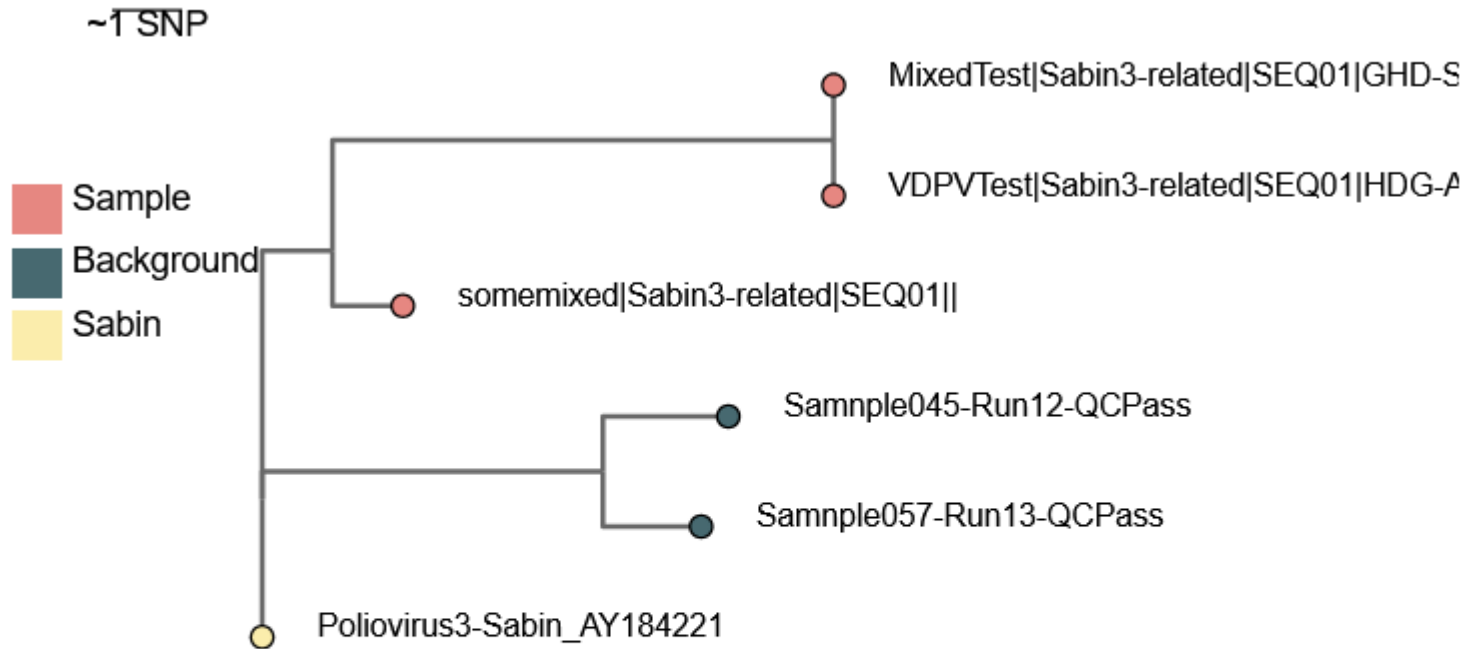
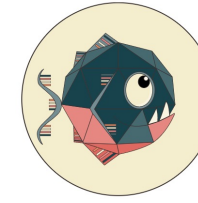


Figure 1 | Variation (errors + mutations) across Sabin1-Related reference in sample08

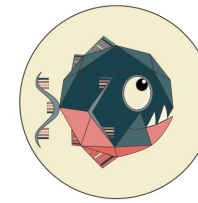
PIRANHA Outputs- Phylogenetic tree



- Trees can be used to identify contamination within the run.
- Historical sequences from the lab (stored in the laboratory sequence QC database) can help identify contamination from experiments.

Figure 3 | Sabin3-related phylogeny

PIRANHA Outputs- Detailed run report



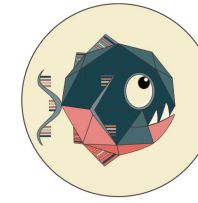
	A	B	C	D	E	F	G
1	sample	barcode	EPID	institute	well	IsQCRetes	IfRetestOri
2	MixedTest	barcode01	GHD-SHD-AKD-02	A01	No		
3	PureTest	barcode02	HDG-AKS-UHE-03	B01	No		
4	WTTest	barcode03	GHD-SHD-AKD-10	C01	No		
5	VDPVTest	barcode04	HDG-AKS-UHE-12	D05	No		
6	negative	barcode05		D06			
7	positively	barcode06		D07			
8	somemixe	barcode07		H12			
9							

...

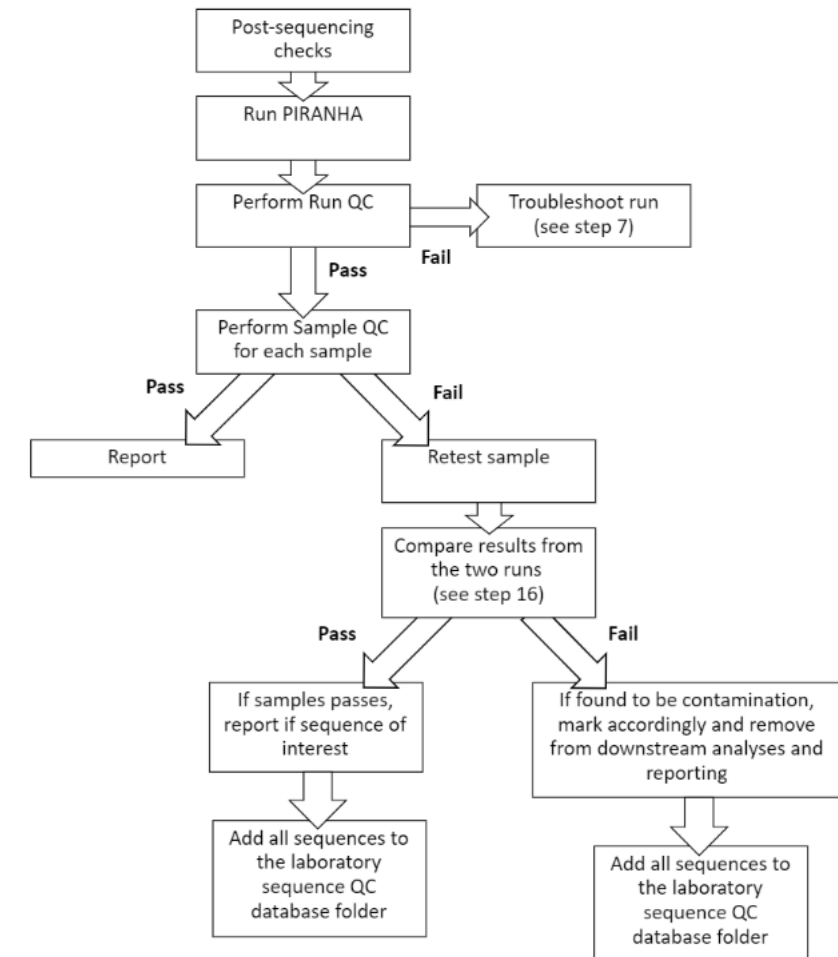
AI	AJ	AK	AL	AM	AN
Sabin1-rel	Sabin2-rel	Sabin2-rel	Sabin2-rel	Sabin2-rel	Sabin2-rel
	Poliovirus2	246	1	99.89	Sabin-like
	Poliovirus2	708	10	98.89	VDPV
		1			
		0			
		0			
		0			
	Poliovirus2	137	0	100	Sabin-like

- Includes data from barcodes.csv with the sequencing results now appended
- Can be annotated during QC process, marking samples to “pass” and report, or those that are “pending” and need further investigation.
- Additional metadata can be added as it becomes available (e.g. ITD results, Sanger results)

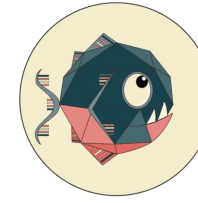
Quality control checks



- SOP available with suggested QC checks:
 - Successful run completion
 - Positive and negative controls
 - Checks for contamination both within and between sequencing runs



In summary



- DDNS is a direct detection method for surveillance of poliovirus currently being evaluated for acceptance by the GPLN
- Protocols and software are supported by the Poliovirus Sequencing Consortium and can be found via <http://polionanopore.org>
- Piranha is an easy-to-use analytical tool for processing data generated by the DDNS protocol

Reference and Recommended material to explore further



<https://polionanopore.org/>

<https://pubmed.ncbi.nlm.nih.gov/32611795/>

- Original DDNS paper

<https://pubmed.ncbi.nlm.nih.gov/38544854/>

- Piranha publication

<https://www.nature.com/articles/s41564-023-01453-4>

- Prospective use of DDNS in DRC

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