Genomic and epidemiological monitoring of yellow fever virus transmission potential

Yellow fever (YF) is an acute viral haemorrhagic disease responsible for up to 60,000 human deaths annually. The disease is caused by a member of the *Flaviviridae* family with transmission primarily through mosquito species that feed on non-human primates. The MinION™ was used in this investigation to characterise genetic diversity and monitor transmission of the virus in real-time, allowing more effective disease surveillance and outbreak control strategies to be implemented[1].

This workflow presented here, as described by Faria *et al*[1] for yellow fever virus (YFV), has been applied to other viruses including Ebola and Zika. More detailed protocol information can be found in Quick *et al.*[2]

Sample preparation

Sample preparation ~ 9 hours

Viral RNA extracted from 200 µl clinical sample (tissue/serum/plasma) using the QIAamp Viral Mini Kit with minor protocol modifications.

cDNA SYNTHESIS

cDNA synthesised from viral RNA using Protoscript II First Strand Sequencing Kit (NEB) with random hexamers.

PRIMER DESIGN

Primal Scheme used to generate overlapping 500 bp primers covering complete yellow fever virus (YFV) genome.

MULTIPLEX PCR

Multiplex PCR using Q5 High Fidelity Hot-Start DNA Polymerase (NEB) and 500 bp YFV primers.

CLEAN-UP

PCR products purified using 1X AMPure XP beads.

QUANTIFICATION

DNA concentration measured using Qubit.

The Primal Scheme whole genome primer design tool and additional supporting information is available at http://primal.zibraproject.org.

Library preparation

As per Ligation Sequencing 1D (SQK-LSK108) and Native Barcoding (EXP-NBD103) for the MinION with noted amendments*.

Library preparation ~ 1.5 hours

20 µl input DNA per sample, standardised to give 100-200 ng total DNA across all samples (as recommended by Quick *et al.*[2])

END-REPAIR, dA-TAILING

End-repair and dA-tailing using 20 µl input DNA, 2.8 µl reaction buffer, 1.2 µl enzyme mix. Clean-up using 0.8-1X AMPure XP beads. Elute in 10 µl nuclease-free water.

BARCODING

Barcoding using Native Barcoding Kit (EXP-NBD103) with 10 µl dA-tailed amplicons, 2.5 µl native barcode, 12.5 µl blunt/TA ligase master mix. Incubate 10 min at RT then denature at 65ºC for 10 min.

CLEAN-UP

Pool barcoded amplicons. Clean-up using 0.8-1X AMPure XP beads. Elute in 30 µl nuclease-free water.

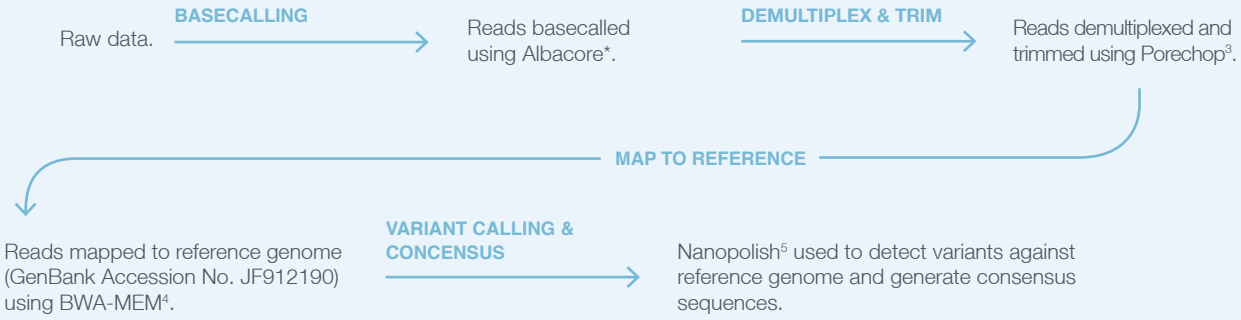
ADAPTER LIGATION

Barcoding adapter ligation using 50 µl Blunt/TA Ligase, 30 µl pooled barcoded samples and 20 µl Barcode Adapter Mix. Clean-up using 0.8-1X AMPure XP beads. 14.5 µl DNA library and no water used in library loading mix.

SEQUENCING

*Please note these kits are no longer available. The Ligation Sequencing Kit 1D SQK-LSK108 has since been superseded by kit SQK-LSK109. Two Native Barcoding Expansion kits are now available, providing a total of 24 barcodes (EXP-NBD104 and EXP-NBD114).

Data analysis



In addition to the analysis tools used by Faria *et al*[1], the cloud-based EPI2ME data analysis platform offers a number of real-time analysis pipelines, including species identification and antimicrobial resistance profiling. Find out more at www.nanoporetech.com/analyse.

References

1. Faria, N. R. et al (2018) Genomic and epidemiological monitoring of yellow fever virus transmission potential. Science. 361(6405):894-899
2. Quick, J. et al (2017) Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. Nat Protoc. 12(6):1261-1276.
3. GitHub. PoreChop. Available at: <https://github.com/rrwick/Porechop> [Accessed: 3 May 2018].
4. Li. H. (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv. 1303.3997v1 [q-bio.GN].
5. Loman, N.J. et al (2015). A complete bacterial genome assembled *de novo* using only nanopore sequencing data. Nature methods. 12(8):733-5.

Find out more about portable, real-time analysis of viral genomes at www.nanoporetech.com.

*Guppy has superseded Albacore as the production basecaller

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