View the protocol

Characterising influenza type A and B virus genomes through whole-genome nanopore sequencing

Influenza is a global health concern, due to annual epidemics of the disease and its potential to cause a pandemic due to novel strain variants. Whole-genome sequencing enables ongoing surveillance and identification of new emergent strains, and can therefore provide information to inform the development of seasonal vaccines.

There are two main strains of influenza virus, A and B, that are responsible for seasonal epidemics. This overview describes how to generate accurate whole-genome sequences of influenza A and B viruses by PCR amplification and multiplexed nanopore sequencing. This protocol can be used to sequence up to 96 samples in a single sequencing run and provides rapid access to results.

This protocol is based on work by Bin Zhou *et al*., 2009[1] and 2014[2].

Samples: 1 µl influenza RNA

1 Prepare

Extract influenza RNA from up to 96 samples using the RNeasy Mini Kit (QIAGEN) or ZR-96 Viral RNA Kit (Zymo Research)

Check quality of RNA samples using the Agilent 2100 Bioanalyzer to assess the RNA integrity number

2 Perform RT-PCR

Perform RT-PCR amplification with SuperScript III RT/Platinum Taq (ThermoFisher) for influenza A and influenza B virus genomes

Purify amplicons using 1x of AMPure XP beads (Beckman Coulter)

Quantify 1 µl of each sample using a Qubit instrument

3 Prepare sample

Add 200 fmol DNA per sample to a 96-well plate and perform end repair using NEBNext Ultra II End Repair/dA-Tailing module (New England Biolabs)

4 Ligate native barcodes

Use the NEB Blunt/TA Ligase Master Mix (New England Biolabs) to ligate a different native barcode from the Native Barcoding Kit V14 to each end-prepped sample in a 96-well plate

Pool samples and purify using 0.4x AMPure XP Beads, then wash twice with Short Fragment Buffer (SFB)

Quantify 1 µl of each sample using a Qubit instrument

5 Ligate sequencing adapters

To prepare samples for sequencing, ligate sequencing adapters using the NEBNext Quick Ligation Reaction Module (New England Biolabs) according to manufacturer's instructions

Purify samples using 0.4x AMPure XP Beads, washed twice with Short Fragment Buffer

Quantify 1 µl of each sample using a Qubit instrument

6 Sequence

Load 10–20 fmol of the prepared library on to a MinION™ Flow Cell

Sequence up to 96 samples on a single MinION Flow Cell, using a MinION or GridION™ device. The MinION Mk1B and MinION Mk1C sequencing devices are portable, while the benchtop GridION can accommodate up to five independently addressable MinION Flow Cells. Find out more at **nanoporetech.com/MinION** and **nanoporetech.com/GridION**

7 Basecall and demultiplex reads

Perform data acquisition and basecalling in real time with MinKNOW™

8 Analyse

Use **wf-flu** analysis workflow in EPI2ME™ Labs. This workflow performs filtering, sequencing alignment to the CDC influenza reference, and genetic variant calling[3]

The analysis can be run using a fully automated, point-and-click GUI, or using the command line. Visit **nanoporetech.com/data-analysis** for best-practice analysis workflows for all levels of expertise

Kits and devices

Library preparation

Native Barcoding Kit

Sequencing

GridION, MinION, MinION Mk1C, MinION Flow Cell

To find out more, visit:
nanoporetech.com/applications/infectious-disease-sequencing

References

1. Zhou B, Donnelly ME, Scholes DT, St George K, Hatta M, Kawaoka Y, Wentworth DE. (2009) Single-reaction genomic amplification accelerates sequencing and vaccine production for classical and Swine origin human influenza A viruses. *J Virol*. 83(19):10309-13. DOI: 10.1128/JVI.01109-09.
2. Zhou B, Lin X, Wang W, Halpin RA, Bera J, Stockwell TB, Barr IG, Wentworth DE. (2014) Universal influenza B virus genomic amplification facilitates sequencing, diagnostics, and reverse genetics. *J Clin Microbiol*. 52(5):1330-7. DOI: 10.1128/JCM.03265-13.
3. GitHub. wf-flu. Available at: https://github.com/epi2me-labs/wf-flu. [Accessed: 01 Aug 2023]

WF_1203(EN)_V1_10Aug2023.