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

View the protocol

Influenza is a global health concern due to annual epidemics of the disease and its potential to cause a pandemic because of 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 Oxford 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 Perform RT-PCR

Perform RT-PCR amplification with SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (ThermoFisher) for influenza A and influenza B virus genomes

Purify amplicons using 1x AMPure XP Beads (Beckman Coulter)

Quantify 1 µl of each sample using a Qubit fluorometer

2 Prepare samples

Add 200 fmol of DNA per sample to a 96-well plate and perform end repair using the NEBNext Ultra II End Repair/dA-Tailing Module (NEB)

3 Ligate native barcodes

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

Pool up to 96 barcoded samples and purify using 0.4x AMPure XP Beads, washing twice with Short Fragment Buffer (SFB)

Quantify 1 µl of purified sample using a Qubit fluorometer

4 Ligate sequencing adapter

Ligate the Native Adapter (NA) using the NEBNext Quick Ligation Module (NEB), purify using 0.4x AMPure XP Beads, washing twice with SFB, and quantify 1 µl using a Qubit fluorometer

5 Sequence

Prepare the library for loading by combining 12 µl of the library with 37.5 µl of Sequencing Buffer (SB) and 25.5 µl of Library Beads (LIB)

Load 75 µl of the prepared library dropwise on to a MinION™ Flow Cell, set up the sequencing run on MinKNOW™ using the high accuracy (HAC) basecaller, and sequence on a MinION or GridION™ device

6 Analyse

Use the wf-flu workflow[3] from EPI2ME™ to analyse basecalled and demultiplexed data generated by MinKNOW

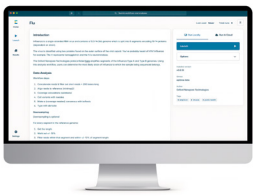
The workflow performs influenza strain typing and outputs an interactive HTML report, as well as typing results in a CSV file

For users preferring an easy-to-use graphical interface, this preconfigured workflow is free to access from the EPI2ME Desktop Application

For users with advanced bioinformatics experience, the workflow is simple to run in the command line

Both options can be run on local compute or in the cloud

Kits, devices, and software



Library preparation
Native Barcoding Kit V14

Sequencing
MinION Flow Cells on MinION or GridION devices

Analysis
EPI2ME wf-flu

View the protocol:
nanoporetech.com/influenza-sequencing-protocol

References:

1. Zhou, B. et al. *J. Virol.* 83(19):10309–13 (2009). DOI: https://doi.org/10.1128/jvi.01109-09
2. Zhou, B. et al. *J. Clin.* Microbiol. 52(5):1330–7 (2014). DOI: https://doi.org/10.1128/jcm.03265-13
3. GitHub. wf-flu. Available at: https://github.com/epi2me-labs/wf-flu [Accessed 21 August 2025]

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