Oxford NANOPORE Technologies

WORKFLOW: MPOX VIRUS SEQUENCING

Rapid viral sequencing for genomic surveillance of mpox using metagenomic or targeted approaches

Mpox is a viral zoonotic disease that can cause painful rashes, swollen lymph nodes, and fever as a result of infection with a virus called *Orthopoxvirus monkeypox* (MPXV)[1], with more severe symptoms often observed for those with comorbidities[2,3]. It is a large (~200 kb), enveloped, linear, double-stranded DNA virus with ~200 genes, part of the *Poxviridae* family, and subclassified into two clades: clade I and II[3]. As well as zoonotic transmission, human-to-human transmission also occurs through close contact with an infected individual, including skin-to-skin contact, and via contaminated objects such as clothing[3].

Mpox is endemic to West and Central African countries, but the world is currently experiencing the largest mpox outbreak, which is also affecting non-endemic countries[2,4]. However, there is limited information about the circulating virus and no specific treatment for mpox[4,5]. Rapid molecular assays are needed to detect MPXV and identify the clade and changes in viral characteristics to understand the epidemiology, prevent further spread, and develop specific treatments[4,5].

Most MPXV sequencing performed early in the current global outbreak used a metagenomic approach, but targeted sequencing has since been developed for MPXV whole-genome analysis[6]. With Oxford Nanopore Technologies, both metagenomic and targeted MPXV sequencing can be performed depending on your experimental needs.

For the generation of complete *de novo* MPXV reference genome assemblies, we recommend metagenomic sequencing. To investigate genomic epidemiology links, we recommend targeted MPXV sequencing of tiled PCR amplicons. Nanopore technology delivers fast and highly accurate sequencing reads of any length, and complex and repeat-rich regions can be sequenced in single nanopore reads, simplifying genome assembly. Furthermore, nanopore sequencing is a cost-effective method with real-time data streaming to provide rapid, actionable results.

Here we provide a simple and rapid end-to-end workflow using either metagenomic or targeted sequencing and the EPI2ME™ analysis platform for the detection and characterisation of MPXV.

EXTRACTION: obtaining viral DNA

Find out more guidance and recommendations for viral DNA extraction in our extraction protocols library: **nanoporetech.com/extraction-methods**

For **metagenomic sequencing**, we recommend extracting viral DNA from either nasopharyngeal or skin lesion swabs using **Roche Diagnostics MagNA Pure Bacteria Lysis Buffer**. In addition, we recommend preparing full-length cDNA from RNA within the sample using **NEB LunaScript RT SuperMix Kit** within the sample using **NEB LunaScript RT SuperMix Kit** and **Applied Biosystems Sequenase Version 2.0 DNA Polymerase**[7] in order to detect potential coinfections from RNA viruses.

For **targeted MPXV sequencing**, we recommend extracting viral DNA from skin lesion swabs using **QIAGEN DNeasy Blood and Tissue Kit**.

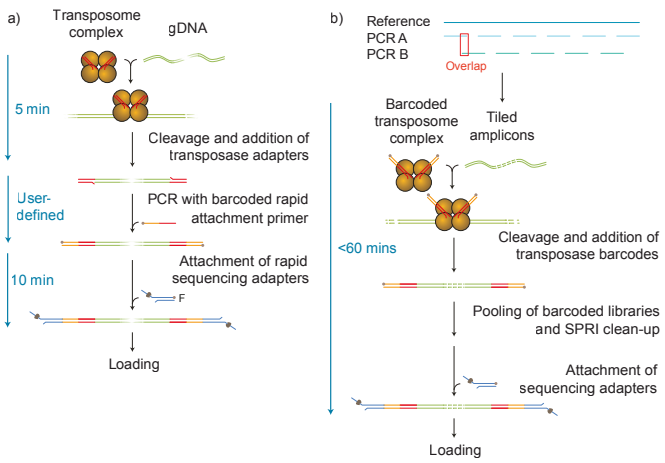
Find out more about the metagenomic sequencing extraction method in the legacy protocol: **nanoporetech.com/document/viral-metagenomics-RNA-DNA**

LIBRARY PREPARATION: preparing DNA for sequencing

Find out more about library preparation: **nanoporetech.com/documentation**

We recommend performing **metagenomic sequencing** to generate high-quality, complete *de novo* MPXV genome assemblies. To prepare your DNA library for sequencing, we recommend using the **Rapid PCR Barcoding Kit 24 V14** and following the standard protocol (panel a), which has been optimised for low quantities of starting input and can sequence MPXV clades I and II.

To perform rapid and cost-effective MPXV whole-genome sequencing for genomic epidemiological analysis, we recommend using the **targeted sequencing** approach where tiled PCR amplicons generate sufficient genome-wide coverage. To prepare your DNA for sequencing, we recommend using the 24- or 96-plex **Rapid Barcoding Kits** and the Midnight PCR tiling of SARS-CoV-2 virus protocol (panel b), replacing the midnight primers with the latest MPXV primer tiling scheme. Two popular tiling schemes have been developed by Welkers *et al.*[8] and Chen, Chaguza, and Gagne *et al.*[6].



SEQUENCING:
generating MPXV sequencing reads

Find out more about the sequencing devices:
nanoporetech.com/products/sequence

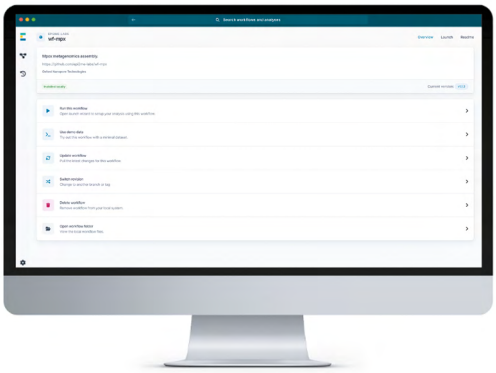
We recommend sequencing viral DNA libraries on **MinION™** Flow Cells. These flow cells are compatible with portable MinION devices for rapid onsite sequencing. To scale up sequencing, up to five independently addressable MinION Flow Cells can be run on **GridION™** devices for large-scale pathogen surveillance.

The sequencing software **MinKNOW™** provides a simple user interface to set up experiments easily. We recommend using the high accuracy (HAC) basecalling model during sequencing for rapid results with real-time data streaming.

ANALYSIS:
assembling the viral genome

View the wf-mpox analysis pipeline at:
labs.epi2me.io/workflows/wf-mpx/

EPI2ME is the data analysis platform, providing a user-friendly bioinformatics experience for all levels of expertise. The **wf-mpx**[9] workflow is a simple data analysis tool that aligns sequencing reads to an MPXV reference genome regardless of the sequencing method used, creating a draft consensus and *de novo* genome assembly. The workflow also assesses coverage, calls variants, and can filter out variants with <20x coverage.



Learn more about data analysis solutions: **nanoporetech.com/products/analyse**

View the protocol for metagenomic sequencing

View the midnight protocol for targeted sequencing

Find out more at: **nanoporetech.com/infectious-disease**

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