Rapid and complete plasmid characterisation with Oxford Nanopore sequencing

Plasmids are the backbone of molecular biology, playing a pivotal role in applications such as gene therapy and vaccine development, genetic engineering, industrial biotechnology, and basic scientific research. Therefore, routine verification of plasmid constructs is an essential quality control step. Any errors in plasmids, such as mutations, truncations, or rearrangements, can compromise downstream experimental success.

Oxford Nanopore sequencing enables the highly accurate, flexible, and secure characterisation of full-length plasmid sequences in-house without the need for primers, with results obtained in hours — negating the need to send constructs to third parties for validation. By obtaining full sequence data in a single experiment, the need for multiple techniques to confirm the identity of your constructs is also no longer required.

Here we present a rapid, end-to-end workflow for complete, high-quality whole-plasmid characterisation using a MinION™ or GridION™ sequencing device and the EPI2ME™ analysis platform.

Extraction:
obtaining high molecular-weight DNA

Find more guidance and recommendations for plasmid extraction in our extraction protocols library: nanoporetech.com/extraction-methods

Selecting an extraction method that will effectively remove contaminants — such as detergents, denaturants, chelating agents, or high salt concentrations — will ensure clean, high-quality DNA samples are taken forward to library preparation.

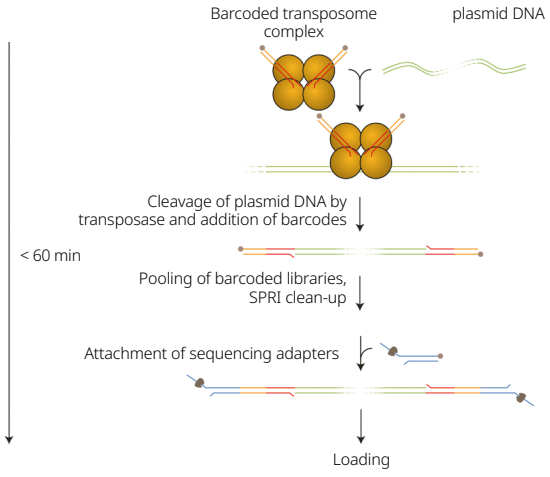
We recommend the use of a plasmid midi prep kit, such as the **QIAGEN Plasmid *Plus* Midi Kit**, which enables extraction of reliable quantities of high-purity DNA from overnight cultures. For each sample, 50 ng of plasmid DNA is then taken forward into library preparation. We recommend the **Qubit fluorometer** for accurate DNA quantification.

Library preparation:
multiplexing samples

Learn more about Oxford Nanopore library preparation: nanoporetech.com/prepare

To prepare your library for sequencing and downstream analysis, you can choose from either the 24- or 96-plex **Rapid Barcoding Kits**. These PCR-free kits use a transposase to cleave and attach barcodes to your plasmid DNA. Barcoded samples are then pooled and sequencing adapters added. We recommend multiplexing up to 96 plasmid libraries per MinION Flow Cell.

Through multiplexing a number of plasmid libraries on a single MinION Flow Cell, the cost per sample can be considerably reduced. Flow cells that are not run at full sample capacity can be washed and reused, facilitating efficient sample batching while maintaining low cost per plasmid. The **Flow Cell Wash Kit** provides a cost-effective method to wash and re-run a flow cell multiple times.



Sequencing:
running until the necessary coverage is achieved

Find out more about Oxford Nanopore sequencing devices:
nanoporetech.com/sequence

We recommend sequencing your plasmid libraries on MinION Flow Cells, which can be run on the portable **MinION** device for easily accessible, routine sequencing. For consistently running higher sample numbers, the benchtop **GridION** device enables on-demand sequencing of up to five independent flow cells at one time.

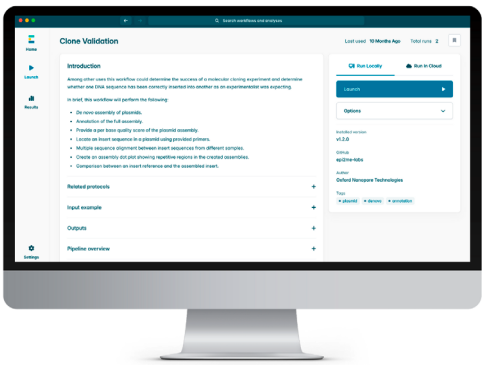
For complete, high-quality whole-plasmid sequencing, we recommend basecalling in high accuracy (HAC) mode using the **MinKNOW**™ software. If you are new to the method, we recommend sequencing for 12 hours, although a shorter run time may be sufficient.

Analysis:
using the EPI2ME wf-clone-validation workflow

View the dedicated EPI2ME workflow:
epi2me.nanoporetech.com/workflows/wf-clone-validation

De novo assembly and annotation of your plasmids is achieved using the **wf-clone-validation** workflow. The wf-clone-validation workflow — an **EPI2ME** solution — integrates a number of best practice tools for complete plasmid assembly and annotation into an easy-to-use analysis pipeline, including **Flye**[1] for plasmid assembly, **Trycycler**[2] for circularising and refining the assembly, **Medaka**[3] for sequence polishing, and **pLannotate**[4] for annotation.

EPI2ME is the user-friendly Oxford Nanopore data analysis platform, suitable for all levels of bioinformatics expertise. The report generated by this EPI2ME workflow presents you with annotated features for each plasmid sequence (e.g. promoters, operators, protein-coding genes), any identified variants in your plasmid sequences compared with a reference, and a multiple sequence alignment between insert sequences from different samples.



Find out more at: nanoporetech.com/microbiology

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

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