Rapid plasmid analysis with nanopore sequencing and EPI2ME™ workflows

At the core of a researcher's ability to synthesise novel proteins is the plasmid construct, containing genes coding for proteins of interest, as well as accessory components offering control of expression or genetic features for selective pressure options. These genetic toolboxes enable exquisite control, but it is vital that all features are validated as present and correct for experimental success.

Nanopore sequencing enables the highly accurate, flexible, and secure characterisation of full-length plasmids in-house, 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 accuracy of your constructs is also no longer required.

EPI2ME

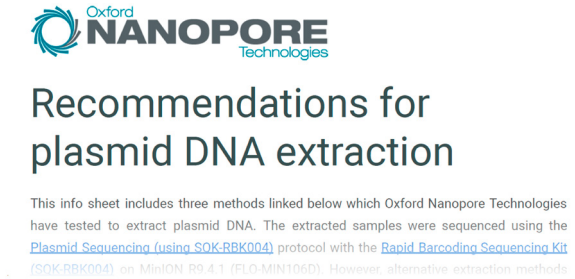
Here we present a flexible, rapid workflow for sequence assembly of plasmid constructs using MinION™ Flow Cells on MinION or GridION™ sequencing devices and the EPI2ME analysis platform.

EXTRACTION:
obtaining high molecular-weight DNA

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 mini prep kit, such as the VWR **Plasmid Miniprep Kit II**, which enables extraction of reliable quantities of high-purity DNA from overnight cultures for up to 200 samples. For each sample, 400 ng of plasmid DNA is then taken forwards into sample preparation. We recommend the **Qubit fluorometer** for accurate DNA quantification.

Find more guidance and recommendations for plasmid extraction in our extraction protocols library:
nanoporetech.com/docs/prepare/extraction_protocols


Oxford NANOPORE Technologies

Recommendations for plasmid DNA extraction

This info sheet includes three methods linked below which Oxford Nanopore Technologies have tested to extract plasmid DNA. The extracted samples were sequenced using the Plasmid Sequencing (using SQK-RBK004) protocol with the Rapid Barcoding Sequencing Kit (SQK-RBK004) on MinION R9.4.1 (FLO-MIN106D). However, alternative extraction methods

LIBRARY PREPARATION:
sample multiplexing

To prepare your library for sequencing and downstream analysis, you can choose from either the 12- or 96-plex **Rapid Barcoding Kits**. These PCR-free kits use a transposase to fragment and attach barcodes to your plasmid DNA before adding a sequencing adapter.

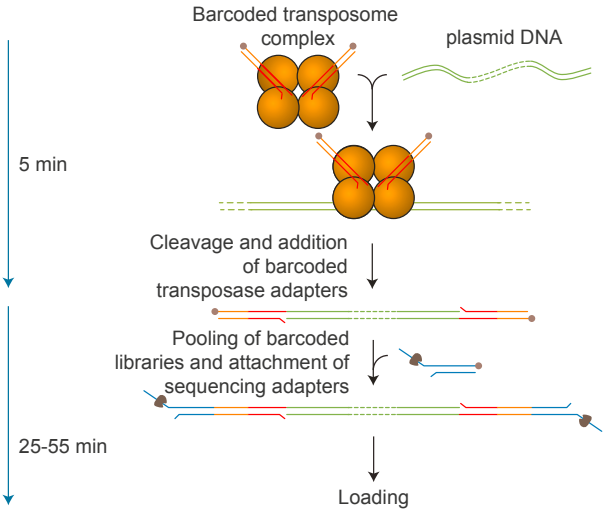
For improved sequencing performance, a bead-based clean up of your library can also be performed with solutions such as **Agencourt AMPure XP**. Whilst not a requirement, a clean up will improve the efficiency of sequencing, delivering more data in a shorter time frame.

Through multiplexing a number of samples on a single MinION Flow Cell, the cost per sample can be considerably reduced; when utilising all 96 barcodes, data can be generated for as little as $13 per sample.

No. samples	12 plasmids	96 plasmids
Price per sample	$84	$13

Find out more about our 96-plex rapid barcoding kit:
store.nanoporetech.com/rapid-barcoding-kit-1

Rapid Barcoding Kit workflow



SEQUENCING: running until the necessary coverage is achieved

Find out more about MinION:
nanoporetech.com/products/minion

We recommend sequencing your plasmid libraries on MinION Flow Cells, which can be run on the portable **MinION** and **MinION Mk1C** devices for easily accessible, routine sequencing. For consistently running higher sample numbers, the benchtop **GridION** device enables on-demand sequencing of up to five flow cells at one time.

The clone validation analysis workflow can generate a plasmid consensus sequence from fast, high-accuracy, or super-accuracy basecalling models. For experiments requiring very high per-base accuracy, we advise the use of the super-accuracy basecalling model and the GridION device.

Run time will depend on sample numbers and plasmid length, but only ~2,500 reads are required per plasmid for highly confident results; for a 96-plex run of ~5 kb plasmids, this could be generated in less than 2 hours. Flow cells can be re-used following removal of your library using the **Flow Cell Wash Kit**, further reducing costs.

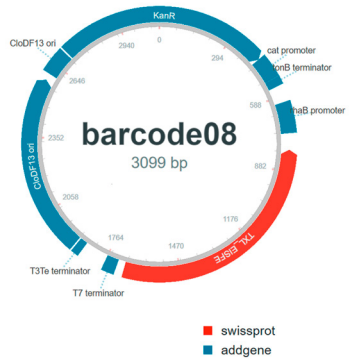
Find out more and compare nanopore sequencing platforms: nanoporetech.com/products/comparison

ANALYSIS: using the EPI2ME Clone Validation workflow

Read more about EPI2ME Labs tutorials:
labs.epi2me.io/nbindex

Assembly and annotation of your plasmids is achieved using the **Clone Validation workflow**, either in **EPI2ME**, our secure cloud-based analysis platform, or **EPI2ME Labs**, a tutorial-based analysis platform that runs on your local device. The Clone Validation workflow integrates a number of best practice tools for plasmid assembly and annotation into an easy-to-use analysis pipeline, including Canu[1] (for plasmid assembly), Trycycler[2] (for cirularising and refining the assembly), Medaka[3] (for sequence polishing), and pLannotate[4] (for annotation, which uses entries from databases such as Swiss-Prot[5] and Addgene[6]).

The report generated by this workflow presents you with identified promoters, operators, protein-coding genes and more, colour coded by the database from which they were identified.



Additional information about each feature can be obtained through links to external sources. The complete FASTA sequence for each plasmid can also be downloaded for use in any further downstream processes.

Find all your nanopore sequencing products: **store.nanoporetech.com**

Oxford NANOPORE Technologies

Twitter: @nanopore
www.nanoporetech.com

References:
1. Koren, S. et al. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Research 27, 722-736 (2017).
2. Wick, Ryan R. et al. Trycycler: consensus long-read assemblies for bacterial genomes. Genome Biol. 22, 266 (2021).
3. Oxford Nanopore Technologies. Medaka. Software. Available at: https://nanoporetech.github.io/medaka [Accessed: 14 January 2022]
4. McGuffie, M. & Barrick, J. pLannotate: engineered plasmid annotation. Nucleic Acids Research 49, W516-W522 (2021)
5. UniProtKB/Swiss-Prot - SIB Swiss Institute of Bioinformatics | Expasy. Expasy.org (2022). at https://www.expasy.org/resources/uniprotkb-swiss-prot
6. Kamens, J. The Addgene repository: an international nonprofit plasmid and data resource. Nucleic Acids Research 43, D1152-D1157 (2014).