Rapid plasmid characterisation with nanopore sequencing and EPI2ME™ analysis

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.

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

EPI2ME

EXTRACTION:
obtaining high molecular-weight DNA

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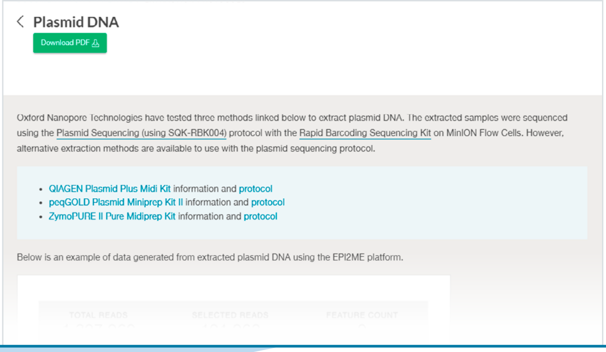
Selecting an extraction method that will effectively remove contaminants — such as detergents, denaturants, chelating agents, or high salt concentrations — will ensure clean, high-quality DNA samples are taken forward to library preparation.

We recommend the use of a plasmid mini prep kit, such as the **ZymoPURE II Midiprep Kit**, which enables extraction of reliable quantities of high-purity DNA from overnight cultures for up to 200 samples. For each sample, 50 ng of plasmid DNA is then taken forward into sample preparation. We recommend the **Qubit fluorometer** for accurate DNA quantification.



Plasmid DNA

Oxford Nanopore Technologies have tested three methods linked below to extract plasmid DNA. The extracted samples were sequenced using the Plasmid Sequencing (using SQK-RBK004) protocol with the Rapid Barcoding Sequencing Kit on MinION Flow Cells. However, alternative extraction methods are available to use with the plasmid sequencing protocol.

- QIAGEN Plasmid Plus Midi Kit information and protocol
- peqGOLD Plasmid Miniprep Kit II information and protocol
- ZymoPURE II Pure Midiprep Kit information and protocol

Below is an example of data generated from extracted plasmid DNA using the EPI2ME platform.

LIBRARY PREPARATION:
sample multiplexing

Find out more about the Rapid Barcoding Kit 96:
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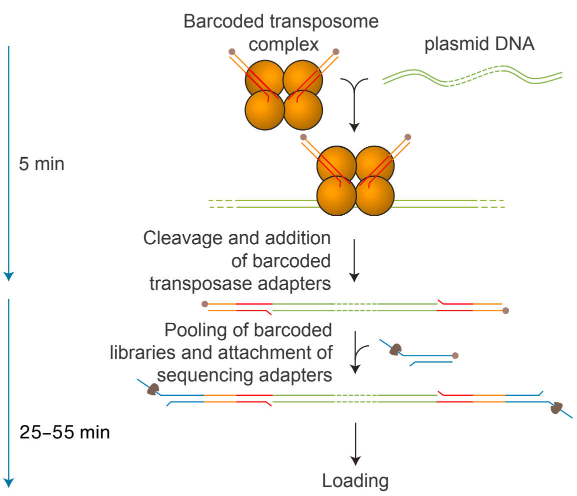
To prepare your library for sequencing and downstream analysis, you can choose from either the 24- or 96-plex **Rapid Barcoding Kits**. These PCR-free kits use a transposase to fragment and attach barcodes to your plasmid DNA before adding a sequencing adapter.

For improved sequencing performance, a bead-based clean up of your library can also be performed with solutions such as **Mag-Bind TotalPure NGS** or **sparQ PureMag Beads** (available at **vwr.com**). 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; this makes nanopore sequencing of plasmids cost-competitive with existing technologies. Flow cells can be also be washed and reused, reducing cost per sample even further. The **Flow Cell Wash Kit** provides a cost-effective method to wash and re-run a flow cell multiple times.

Rapid Barcoding Kit workflow



SEQUENCING: running until the necessary coverage is achieved

We recommend sequencing your plasmid libraries on MinION Flow Cells, which can be run on the portable **MinION** devices for easily accessible, routine sequencing.

The clone validation analysis workflow can generate a plasmid consensus sequence from fast, high-accuracy, or super-accuracy basecalling models.

Find out more about MinION:
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Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
X5	FAQ17853	LAMPore_Test		1 h / 2 h	Active	57.69 k	31.66 Mb basecalled 33.44 Mb estimated	100%

☐ Display failed ☐ Display unclassified ☑ Hide zero values

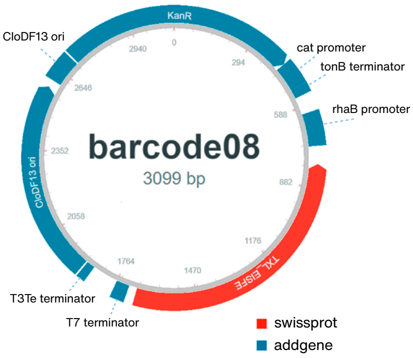
Run time will depend on sample numbers and plasmid length, but only ~2,500 reads are required per plasmid for highly confident results. Flow cells can be reused following removal of your library using the **Flow Cell Wash Kit**.

ANALYSIS: using the EPI2ME Clone Validation workflow

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 plasmid assembly and annotation into an easy-to-use analysis pipeline, including Flye[1] for plasmid assembly, Trycycler[2] for cirularising and refining the assembly, Medaka[3] for sequence polishing, and pLannotate[4] for annotation.

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.

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



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.

Discover more about nanopore sequencing and order today at **vwr.com**

Oxford NANOPORE Technologies

References:

1. GitHub. Flye. Available at: https://github.com/fenderglass/Flye. [Accessed: 02 Aug 2023]
2. Wick, R.R. et al. Trycycler: consensus long-read assemblies for bacterial genomes. *Genome Biol*. 22, 266 (2021).
3. GitHub. Medaka. Available at: https://github.com/nanoporetech/medaka. [Accessed: 02 Aug 2023]
4. McGuffie, M. & Barrick, J. pLannotate: engineered plasmid annotation. *Nucleic Acids Res.* 49, W516-W522 (2021).

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