Rapid and scalable full-length amplicon validation with Oxford Nanopore sequencing

In biopharmaceutical research and development, synthetic biology plays a central role in driving innovation, from engineering cell lines and gene therapy vectors to optimising antibody constructs and CRISPR-based editing tools. These complex and iterative workflows demand precise, rapid validation of sequence integrity to ensure therapeutic potential is not compromised.

Amplicon sequencing is essential at multiple points in this pipeline. This technique enables validation of guide RNA designs and characterisation of CRISPR editing outcomes, high-throughput screening of antibody libraries, construct verification in viral vectors, and clonal stability checks during cell line development.

However, legacy sequencing methods can pose a significant bottleneck in amplicon sequencing workflows. Sanger sequencing is low throughput and limited in read length, making it impractical for multiplexed or long amplicon targets. Short-read sequencing is also limited in read length; the short reads cannot span long genomic targets of interest. Short-read technology often struggles with GC-rich or repetitive regions, meaning that it may fail to fully resolve complex amplicon sequences.

Oxford Nanopore technology delivers rapid and scalable full-length amplicon sequencing, without the need for primers during the sequencing process. The accurate reads generated by Oxford Nanopore sequencing are unrestricted in length and provide the flexibility to handle diverse target lengths and sequence contexts, including repetitive regions. Up to 96 full-length amplicon samples can be multiplexed in a single sequencing run, offering a high-throughput solution that easily integrates into individual or core laboratories. This streamlined workflow enables efficient characterisation of amplicons, reducing turnaround times and bringing confidence to synthetic biology-driven drug discovery.

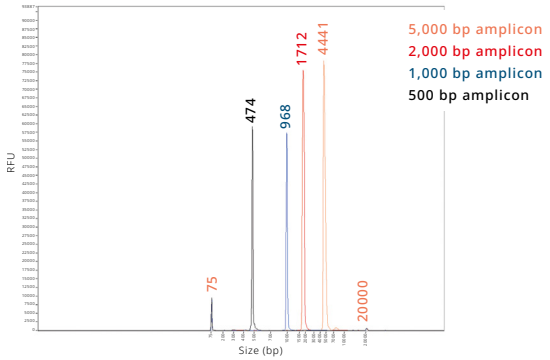
Here we present a fast, simple, and scalable end-to-end workflow for amplicon sequencing using MinION™ or GridION™ devices.

Extraction:
obtaining high-quality DNA and generating amplicon samples

View our sample-specific extraction protocols:
nanoporetech.com/extraction-methods

Selecting a suitable extraction method depends on your sample type. High-quality DNA is required for successful PCR amplification, and we have a range of sample-specific extraction protocols available on the **Documentation** section of our website, alongside guidance on how to check the fragment length, quantity, and purity of your extracted DNA.

To make sure that the whole region of interest is captured, we recommend designing your primers to include an extra 15–20 bp up- and downstream of your target sequence. This protocol has been optimised for amplicons from 500 bp to 5 kb in length.

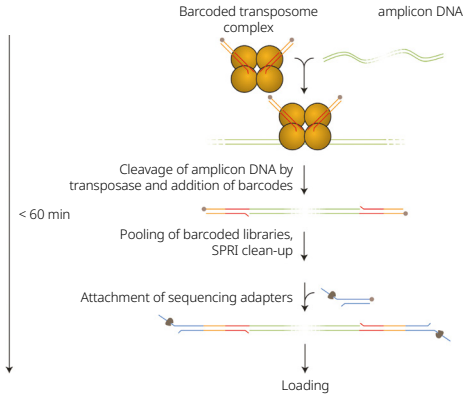


Library preparation:
multiplexing amplicon samples

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

To prepare your amplicon samples 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 amplicon DNA. Barcoded samples are then pooled and sequencing adapters added. We recommend multiplexing up to 96 amplicon libraries per MinION Flow Cell.

Through multiplexing a number of amplicon libraries on a single MinION Flow Cell, the cost per sample can be considerably reduced. Flow cells that are not run to full capacity can be washed and reused, facilitating efficient sample batching while maintaining low cost per amplicon sample. 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 amplicon libraries on **MinION Flow Cells**. These flow cells can be run on the portable **MinION** device for easily accessible, routine sequencing, or on the benchtop **GridION**, which enables on-demand sequencing of up to five independent flow cells at one time for higher throughput needs.

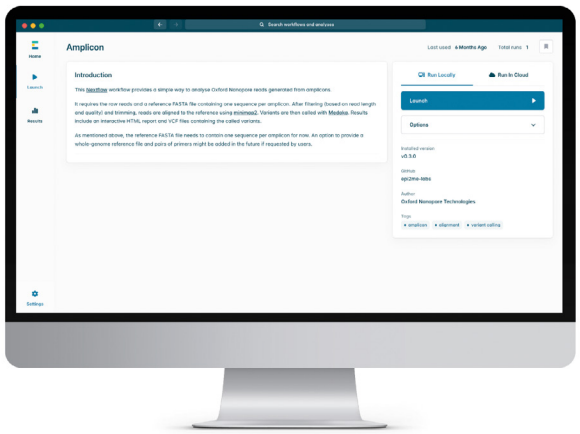
For full-length amplicon sequencing, we recommend basecalling in high accuracy (HAC) mode using the **MinKNOW™** software. If you are new to the method, we recommend sequencing for 12 hours, although a shorter run time may be sufficient. We suggest generating 150x depth of coverage or ~1,500 reads per amplicon library to allow sufficient data for analysis. You can monitor read counts in real time using MinKNOW and stop the run once your desired coverage is achieved.

Analysis:
validating amplicon sequences with EPI2ME™

View the dedicated EPI2ME workflow:
nanoporetech.com/epi2me-wf-amplicon

EPI2ME workflows enable Oxford Nanopore data analysis for all levels of expertise. The pre-configured analysis packages can be accessed from a desktop application with an easy-to-use graphical interface or the command line and can be run on local compute or in the cloud.

The **wf-amplicon** workflow can be run in either variant calling mode or *de novo* consensus mode[1]. In variant calling mode, references with the expected amplicon sequences are supplied, reads are aligned to the references, and variants are called. In *de novo* consensus mode, consensus sequences are generated *de novo* from the reads of each sample and the reads are re-aligned against the draft consensus for polishing. The workflow outputs an interactive HTML report detailing the results alongside the relevant files, such as FASTQ files of *de novo* consensus sequences, BAM files of re-aligned reads, and VCF files of variant calls.



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

References:

1. GitHub. wf-amplicon. Available at: https://github.com/epi2me-labs/wf-amplicon [Accessed 19 August 2025]

Oxford Nanopore Technologies

www.nanoporetech.com

phone +44 (0)845 034 7900

email support@nanoporetech.com

oxford-nanopore-technologies

@nanopore

@nanoporetech.com

WF_1317(EN)_V1_15Sep2025