Oxford Nanopore Technologies

Sequencing full-length transcripts for isoform-level expression analysis

Alternative splicing is the biological mechanism that generates multiple transcript isoforms from a single gene, increasing protein diversity and contributing to the regulation of cell differentiation and function[1]. Aberrant splicing has been implicated in many diseases, including cancer and rare diseases[1], highlighting the importance of accurately quantifying and characterising isoform expression to investigate disease mechanisms[2].

However, resolving transcript isoforms is challenging. Transcripts from the same gene are highly similar, often differing by a small number of exons. Short-read sequencing technology typically cannot capture full-length transcripts, so as a result, transcripts must be inferred, which can lead to the improper detection of isoforms that are not expressed in the sample or the failure to detect novel isoforms[3,4].

Oxford Nanopore sequencing overcomes these limitations by capturing full-length transcripts in single reads, spanning all splice junctions. This enables isoform-level characterisation, even without a reference genome. Nanopore reads of unrestricted length reflect complete RNA molecules, simplifying genome annotation and revealing novel transcripts. Additionally, with our scalable devices and barcoding options, nanopore sequencing is cost-effective and flexible, allowing you to sequence as you need to for comprehensive bulk transcriptomic research.

Here we present a simple workflow for characterising full-length isoforms from human blood and cell line research samples, using PromethION™ sequencing devices and the EPI2ME™ analysis platform.

Extraction:
obtaining high-quality RNA

Find extraction protocols and guidance on RNA handling:
nanoporetech.com/extraction-methods

Either poly(A)-enriched, ribodepleted, or total RNA can be used as input for Oxford Nanopore cDNA library preparation, depending on your experimental aims.

For human blood samples, we recommend using the **QIAGEN PAXgene Blood RNA Kit**, followed by globin depletion using the **Invitrogen GLOBINclear-Human Kit** to improve sequencing quality. If you are starting with human cell line samples, we recommend extracting total RNA using the **Invitrogen TRIzol RNA Isolation Reagent.**

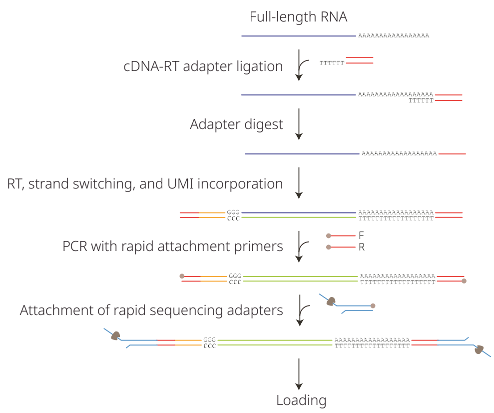
After extracting total RNA, you can maximise sequencing output by using the **NEBNext High Input Poly(A) mRNA Isolation Module** to enrich for poly(A)-tailed RNA or the **Invitrogen RiboMinus Eukaryote Kit v2** to selectively deplete ribosomal RNA. For non-polyadenylated transcripts, we recommend using **NEB *E. coli* Poly(A) Polymerase** reagent to add poly(A) tails for library preparation compatibility.

Library preparation:
preparing full-length transcripts

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

To prepare your extracted RNA for sequencing, we recommend the **cDNA-PCR Sequencing Kit**, which is optimised for generating high outputs of full-length transcripts from inputs as low as 10 ng poly(A)+ RNA sample. To increase cost efficiency and reduce hands-on time, you can use the **cDNA-PCR Barcoding Kit** to sequence up to 24 samples in parallel.

During library preparation, the reverse transcription adapter is ligated to the end of the poly(A) tail and the bottom strand of the adapter is digested to prevent internally primed reads. The RNA is then reverse transcribed, and the resulting cDNA is amplified by PCR. Then, sequencing adapters are added via ligation for nanopore sequencing.



Sequencing:
generating high output on PromethION

Find out more about PromethION sequencing devices: **nanoporetech.com/promethion**

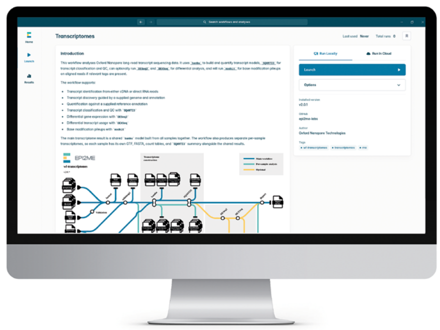
We recommend sequencing using a PromethION device, which can deliver 60–80 million reads per flow cell, ideal for transcriptome-wide analysis of isoform-level expression.

The **PromethION 24** sequencing device has the capacity to sequence up to 24 independent PromethION Flow Cells. For lower sample processing requirements, the **PromethION 2 Integrated** device has the capacity for up to two PromethION Flow Cells whilst maintaining the benefits of high-output Oxford Nanopore sequencing.

Analysis:
analysing full-length transcripts

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

EPI2ME workflows enable Oxford Nanopore data analysis for all levels of expertise. The **wf-transcriptomes**[5], is the analysis pipeline for cDNA-PCR sequencing data. The workflow uses nanopore read files (FASTQ or BAM) generated by the sequencing device to produce output files and an HTML report detailing the findings, including QC, assembled transcriptomes, transcript classification, and transcript and gene counts, including differential expression and differential transcript usage.



View the cDNA-PCR Sequencing Kit protocol: **nanoporetech.com/cdna-pcr-sequencing-protocol**

References:

1. Tao, Y. and Zhang, Q. et al. *Signal Transduct. Target. Ther*. 9(1):26 (2024). DOI: https://doi.org/10.1038/s41392-024-01734-2
2. Wright, D.J. et al. *BMC Genomics* 23(1):79 (2022). DOI: https://doi.org/10.1186/s12864-022-08318-w
3. Lee, J. et al. *Genome Res*. 34(11):1849–1864 (2024). DOI: https://doi.org/10.1101/gr.278801.123
4. You, Y., Solano, A.N., Lancaster, J., David, M., and Wang, C. et al. *bioRxiv* 675724 (2025). DOI: https://doi.org/10.1101/2025.09.11.675724
5. GitHub. wf-transcriptomes. Available at: https://github.com/epi2me-labs/wf-transcriptomes [Accessed 05 June 2026]

Oxford Nanopore Technologies

phone +44 (0)845 034 7900

email support@nanoporetech.com

oxford-nanopore-technologies

@nanopore

@nanoporetech.com

www.nanoporetech.com

WF_1271(EN)_V3_08Jul2026