Sequencing full-length transcripts for isoform-level expression analysis

Alternative splicing is the biological mechanism that generates different isoforms from a single gene, contributing to the regulation of cell differentiation and function[1]. Global changes in differential isoform expression have been associated with psychiatric conditions[2,3] and aberrant splicing has been implicated in many diseases, including neurological and autoimmune diseases[1]. Taken together, these findings highlight the importance of accurately quantifying and characterising isoform expression to investigate the mechanisms of diseases[2].

Despite the significance of isoform-level information, legacy short-read sequencing techniques are limited in resolving and quantifying full-length transcripts as short reads often cannot span the entire transcript. Therefore, reference genomes are required to reconstruct full-length transcripts — often leading to missed or discarded novel transcripts[4].

Conversely, Oxford Nanopore sequencing can span full-length transcripts in single reads, enabling comprehensive transcriptome characterisation at the isoform level[2]. Nanopore reads can be any length (from short to ultra long) meaning the full transcript from the input RNA is reflected. This simplifies transcriptome annotation, revealing novel transcripts and, in turn, novel isoform expression.

As well as characterising full-length transcripts, nanopore reads of unrestricted length reveal more isoforms than the same number of reads generated by short-read sequencing[5], significantly improving isoform detection compared with legacy technology[6]. Oxford Nanopore reads also have the added benefit of reduced multimapping, which provides more precise insights into isoform-level expression[7]. Furthermore, nanopore sequencing is cost effective and does not require sample batching due to the scalable solutions available.

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 cell line research samples, we recommend extracting total RNA using the TRIzol-based method, as described in the human cell line RNA extraction protocol. If starting with human blood research samples, we recommend using the **QIAGEN PAXgene Blood RNA Kit**.

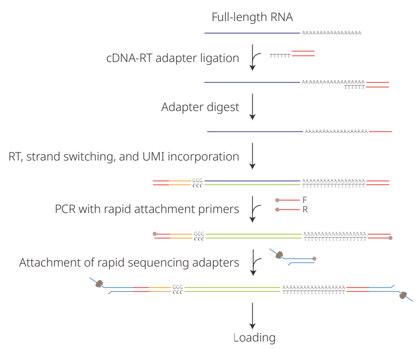
After extraction, polyadenylation enrichment can be performed on total RNA, either using the **Invitrogen Dynabeads mRNA Purification Kit** to isolate poly-A-tailed RNA or the **Invitrogen RiboMinus Eukaryote Kit v2** to selectively deplete ribosomal RNA.

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 and for isoform identification and quantification. With the cDNA-PCR Barcoding Kit, up to 24 samples can be run in parallel, reducing cost and input requirements per sample.

During library preparation, the RNA undergoes reverse transcription and strand switching to prepare full-length cDNA. Full-length cDNA molecules are then selected for and amplified using PCR before the cDNA library is sequenced.



Sequencing:
generating high output on PromethION

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

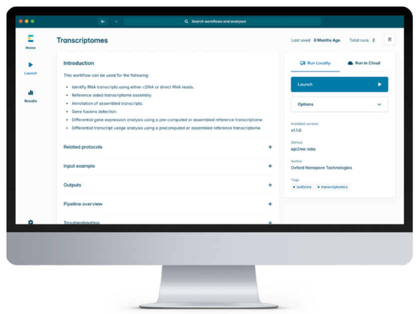
We recommend sequencing using the PromethION device range, which can deliver >100 million reads per flow cell, ideal for transcriptome-wide analysis of isoform-level expression.

The PromethION range includes the **PromethION 24** sequencing device, with the capacity to sequence on up to 24 independent PromethION Flow Cells. For lower sample processing requirements, the **PromethION 2** Integrated and PromethION 2 Solo devices, with capacity for up to two PromethION Flow Cells, are available and maintain the benefits of high-output Oxford Nanopore sequencing.

Analysis:
analysing full-length transcripts

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

The Oxford Nanopore **EPI2ME** workflow, **wf-transcriptomes**[8], provides transcriptome analysis through the assembly and annotation of cDNA data to identify RNA transcripts, isoforms, and gene fusions. The workflow uses raw nanopore read (FASTQ) files generated by the sequencing device to produce an HTML report detailing the primary findings, including assembled transcriptomes and differential gene expression.



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

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