Obtaining full-length isoforms from single cells with Oxford Nanopore sequencing

Characterisation of transcriptomic differences between individual single cells, or spatially across tissues, has provided insight into how the genome is utilised for specialised functions, such as neurological disorders[1] and cancer[2]. However, the majority of these studies recover limited isoform and single nucleotide polymorphism (SNP) content due to the limited read length of short-read sequencing. With the latest Oxford Nanopore sequencing technology, it is now simple to get isoform-level expression from full-length cDNAs at the throughput needed to analyse thousands of cells.

RNA from single cells can be prepared using the 10x Genomics microfluidics-based Chromium platform, which produce barcoded, full-length cDNA from individual cells. Legacy short-read sequencing of these single-cell libraries typically yields only around 90 bp of sequence aligned to one end of each transcript. This limited representation of each transcript makes it difficult to quantify isoform-level expression. Furthermore, as short reads are unlikely to span fusion junctions, fusion transcripts are also challenging to resolve[3]. Oxford Nanopore sequencing is compatible with the 10x Genomics sample preparation approach and can be used to sequence full-length transcripts and splice variants, providing detail on isoform diversity and isoform switching, such as during development. In addition, long nanopore reads enable the detection of both SNPs for RNA-based genotyping and gene fusions that are often associated with cancer.

A single PromethION™ Flow Cell generates ~80 M full-length, cell-assigned reads from a 10x Genomics 3′ or 5′ Chromium Single Cell or Visium Spatial gene expression library — sufficient for many gene and isoform analyses of thousands of cells.

Here, we present a complete workflow for single-cell transcriptome analysis from 10x Genomics cDNA, with library prep in approximately three hours.

The single-cell workflow: preparing full-length 10x Genomics cDNA libraries for sequencing

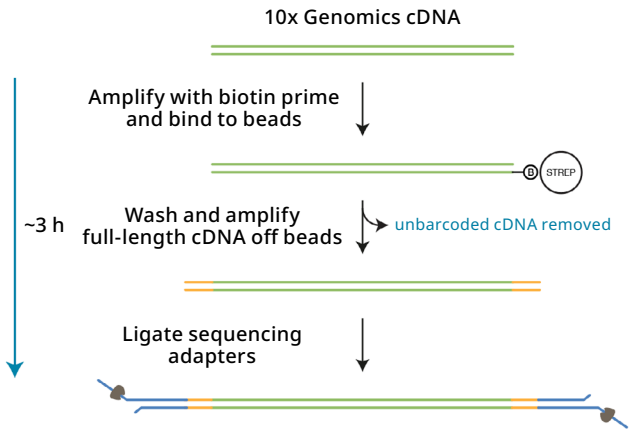
Learn more about nanopore library preparation: nanoporetech.com/prepare

The starting material for nanopore library preparation is full-length, barcoded cDNA generated using the standard protocol for the **10x Genomics GEM-X Universal 3′ Gene Expression**, **Universal 5′ Gene Expression**, or **Visium Spatial Gene Expression** kits.

The 10x Genomics barcoded cDNA sample is PCR amplified with biotinylated primers. cDNA molecules lacking cell barcodes are then removed using a streptavidin pulldown. Depletion of these artefacts is important to maximise sequencing efficiency of full-length cDNAs.

The sequencing library is then prepared using the **Ligation Sequencing Kit**. The entire library preparation workflow, from cDNA to sequencing-ready library, can be completed in approximately three hours.

Oxford Nanopore library preparation with 10x Genomics cell-barcoded cDNA



For a detailed protocol on single-cell sequencing using a PromethION, visit: nanoporetech.com/documentation

Sequencing:
generating high outputs on PromethION

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

For typical single-cell experiments with thousands of cells, we recommend sequencing using the PromethION sequencing device range. PromethION Flow Cells generate the highest number of reads of any Oxford Nanopore flow cell and the output from one flow cell pairs well with one lane from a **10x Genomics Chromium GEM-X Chip**. The typical output expected from a PromethION Flow Cell is ~80 M cell-assigned reads after filtering.

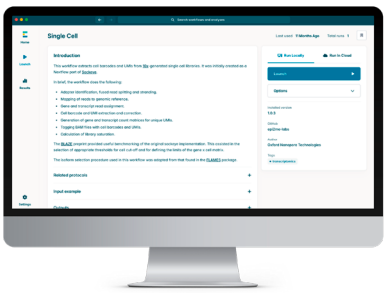
The PromethION 24 device provides you with the flexibility to scale from low to high throughput as needed, with sequencing on up to 24 individually addressable flow cells. The PromethION 2 Integrated and PromethION 2 Solo devices maintain the benefits of high-output nanopore sequencing for users with lower sample processing requirements.

Analysis:
using the single-cell analysis pipeline

View the wf-single-cell analysis pipeline:
labs.epi2me.io/workflows/wf-single-cell

EPI2ME™ workflows enable Oxford Nanopore data analysis for all levels of expertise. The pre-configured analysis packages are free to access from an intuitive interface or the command line, and can be run on local compute or in the cloud.

The dedicated **wf-single-cell** pipeline[4] enables the direct demultiplexing of single-cell barcoded reads without the need to add paired short-read data. The outputs of the workflow are gene, genotype, and isoform count matrices, UMAP plots, and a BAM file of aligned reads.



The **wf-single-cell** pipeline is accessible through the **EPI2ME** application, and simplifies data analysis with an intuitive interface.

Find out more at: nanoporetech.com/single-cell

Oxford Nanopore Technologies

10x GENOMICS Compatible Products

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4. EPI2ME wf-single-cell. Available at: https://labs.epi2me.io/workflows/wf-single-cell [Accessed 17 February 2025]

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