10x GENOMICS | Compatible Products

Obtaining full-length isoforms from single cells with 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 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. Traditional 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]. 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
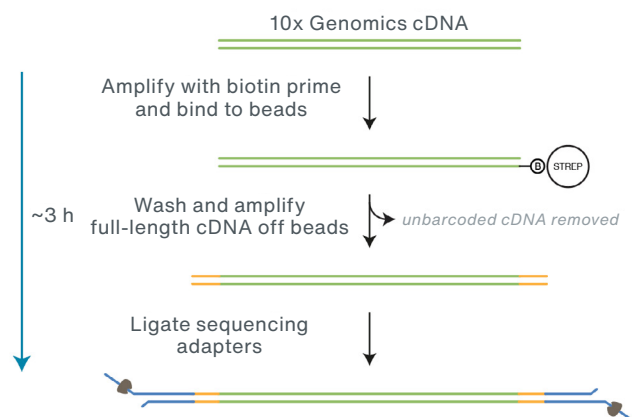
The starting material for nanopore library preparation is full-length, barcoded cDNA generated using the standard protocol for the **10x Genomics Next GEM Single Cell 3' Gene Expression, Single Cell 5' Gene Expression,** or **Visium Spatial Gene Expression kits**.

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.

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

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

10x Genomics cDNA

Amplify with biotin prime and bind to beads

~3 h   Wash and amplify full-length cDNA off beads — *unbarcoded cDNA removed*

Ligate sequencing adapters

For a detailed protocol on single-cell sequencing using the PromethION platform, visit: **community.nanoporetech.com/docs**

SEQUENCING:
generating high outputs on PromethION

For typical single-cell experiments with thousands of cells, we recommend sequencing using the PromethION platform 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 Next GEM Chip**. The typical output expected from a PromethION Flow Cell is ~80 M cell-assigned reads after filtering.

The PromethION 24 and PromethION 48 platforms, with 24 or 48 independently addressable flow cells, respectively, provide you with the flexibility to scale from low to high throughput as needed. 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:
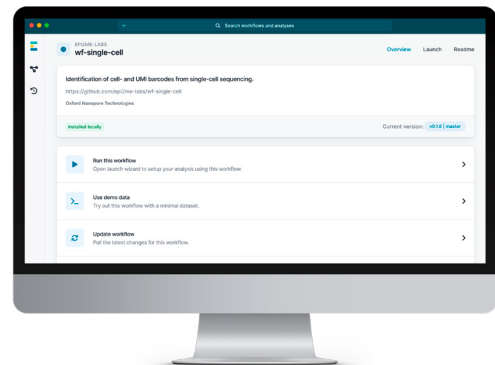### using the single-cell analysis pipeline

EPI2ME™ workflows enable 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 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-transcriptomics**

References:
1. Winkler, E.A. et al. *Science* 375(6584). DOI: https://doi.org/10.1126/science.abi7377 (2022).
2. Chung, W. et al. *Nat. Commun.* 8(15081). DOI: https://doi.org/10.1038/ncomms15081 (2017).
3. Ebrahimi, G. et al. *iScience* 25(104530). DOI: https://doi.org/10.1016/j.isci.2022.104530 (2022).
4. EPI2ME wf-single-cell. Available at: https://labs.epi2me.io/workflows/wf-single-cell [Accessed: 13 February 2024].

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