Obtaining full-length isoforms from single cells with long nanopore sequencing reads

Sequencing cDNA at single-cell resolution can reveal transcriptomic differences between individual cells. The ability to characterise single-cell gene expression has provided an insight into how the different tissues and cell types in an organism utilise the genome for specialised functions, and has elucidated mechanisms of disease. For example, single-cell sequencing has identified aberrant gene expression associated with human neurological diseases[1] and in cancer[2].

RNA from single cells can be prepared using the 10x Genomics microfluidics-based Chromium platforms, which produce barcoded, full-length cDNA from individual cells. However, 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 or isoform switching, such as during development. In addition, long nanopore reads enable the detection of both single nucleotide polymorphisms (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' gene expression or Visium spatial library — sufficient for many gene and isoform analyses of 5,000 or more cells.

Here, we present a complete workflow for single-cell transcriptome analysis from 10x Genomics cDNA libraries, using the PromethION platform.

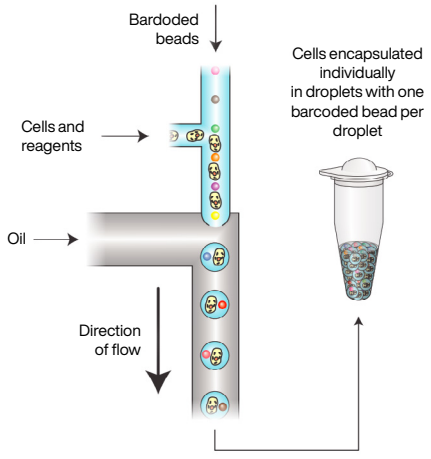
THE SINGLE-CELL WORKFLOW: generating full-length barcoded cDNA and preparing libraries for sequencing

The starting material consists of cell suspensions, which should be viable and free of debris prior to 10x Genomics single-cell preparation. For information on the optimal cell preparation protocol for your experiment, consult the 10x Genomics Support Cell Preparation Guide[4].

Full-length, barcoded cDNA is generated using the protocol for the **10x Genomics Next GEM Single Cell 3'**, **5' gene expression** or **Visium Spatial Kits**. The 10x Genomics barcoded cDNA sample is PCR amplified with biotinylated primers. Then, truncated cDNA molecules lacking cell barcodes are depleted 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 Oxford Nanopore **cDNA-PCR Sequencing Kit**.

Find out more about Oxford Nanopore sequencing kits:
store.nanoporetech.com/sample-prep.html

Overview of 10x Genomics single-cell barcoding process



For detailed protocols on single-cell sequencing using our platforms, visit: **nanoporetech.com/protocols_singlecell**

SEQUENCING:
generating high yields on PromethION

Find out more about PromethION sequencing devices:
nanoporetech.com/products/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 (~150 M total reads before 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 and PromethION 2 Solo devices maintain the benefits of high-yield nanopore sequencing for users with lower sample processing requirements.

ANALYSIS:
using the single-cell analysis pipeline

View the wf-single-cell analysis pipeline at:
github.com/epi2me-labs/wf-single-cell

The Oxford Nanopore **EPI2ME™ Labs** workflow, **wf-single-cell**[5] (previously Sockeye), enables the direct demultiplexing of single-cell barcoded reads without the need to add paired short-read data. This workflow, which is implemented in Nextflow, enables isoform-based analysis. For SNP and fusion detection, we recommend the third-party tool, **JAFFAL**[6].

The workflow takes input files of raw nanopore reads (FASTQ) generated by the sequencing device, and reference files. The standard outputs of the workflow are gene and isoform count matrices, UMAP cell clustering plots, and a BAM file of aligned reads tagged with cell barcode and UMI.

Inputs:
FASTQ file
Reference genome FASTA
Gene annotation GTF

wf-single-cell pipeline

Outputs:
Isoform count matrix
Gene count matrix
UMAP Plots
Aligned BAM with cell barcode/UMI/ gene annotations as tags

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

References:

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