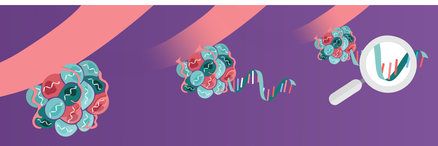
BioTechniques In Focus

Oxford NANOPORE Technologies

# Single-cell analysis with nanopore sequencing



~15% of human hereditary diseases and cancers are associated with alternative RNA splicing[1] while the development of 1 in 6 cancers is driven by gene fusions[2].

Isolating RNA from individual cells is a powerful technique to investigate transcriptomic heterogeneity at the single-cell level.

Using long nanopore sequencing reads, full-length transcripts can be investigated to gain a deeper understanding of how different tissues and cell types utilize the genome during cell development or disease progression.

## HOW IS SINGLE-CELL SEQUENCING PERFORMED?


**1** Single cells are captured using a variety of methods, including droplet- and plate-based solutions.

**2** The mRNA from each single cell is reverse transcribed to cDNA containing a cell-specific barcode. The barcoded cDNA is then used as input into sequencing library construction.

**3** After sequencing, each transcript can be mapped back to its cell of origin using the unique barcode incorporated during reverse transcription.

## LIMITATIONS OF TRADITIONAL SEQUENCING TECHNOLOGY FOR SINGLE-CELL ANALYSES

Short-read sequencing involves fragmentation of the full-length cDNA so that reads typically cover only 90 bp of sequence at either the 5' or 3' end of a transcript.


This limited representation leads to:

Difficulties in quantifying isoform-level expression.

Missed alternative splicing events, since DNA fragments do not typically span entire transcripts.

Challenges in resolving fusion transcripts as short reads are unlikely to span fusion junctions.


## BENEFITS OF LONG NANOPORE SEQUENCING READS

Nanopore sequencing does not require fragmentation and has no read length limitations, enabling full-length transcripts to be sequenced in single reads.


Single-cell analysis with nanopore sequencing can:

Detect gene- and isoform-level expression, plus variants, in a single experiment.

Identify and characterize known and novel isoforms.

Detect structural variants, including gene fusion transcripts, alongside single nucleotide variants.

Reveal alternative splicing and isoform switching events.

To achieve the required depth of coverage for high-output single-cell sequencing, Oxford Nanopore has a range of PromethION sequencing devices to suit your experimental needs, from research lab to core sequencing facilities.

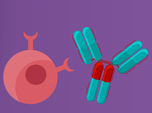
Dedicated data analysis is provided for all levels of expertise, using the wf-single-cell pipeline.

## WHAT WILL YOU DISCOVER?

Detect oncogenic gene fusions and variants invisible to short reads to uncover novel cancer biomarkers.

Reveal isoform diversity to deeply characterize the tumor microenvironment.

Uncover aberrantly spliced transcripts and investigate their role in cancer initiation and progression.



Identify immune-receptor isotypes alongside gene and isoform expression to gain insights into tumor–immune cell interactions.

### References

1. Jiang W, Chen, L. Alternative splicing: Human disease and quantitative analysis from high-throughput sequencing. *Comput. Struct. Biotechnol. J.* 24(19), 183–195 (2020).

2. Gao Q Liang W-W, Foltz SM et al. Driver Fusions and Their Implications in the Development and Treatment of Human Cancers. *Cell Rep.* 23(1), 227–238.e3 (2018).



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