Oxford Nanopore Technologies

Transcriptomics with Oxford Nanopore

Introduction

Unlock high-definition transcriptomics with Oxford Nanopore sequencing.

A deeper understanding of the diversity of RNA within a transcriptome is essential for the better study of biological mechanisms and cellular processes. RNA diversity arises in part from alternative splicing, which is the mechanism that generates different isoforms from a single gene, and, in humans, up to 95% of multi-exon genes undergo this process[1].

Alternative transcript usage is a key source of variation between healthy and diseased tissues, and approximately 15% of hereditary diseases and cancers are associated with alternative splicing[1], highlighting the significance of isoform-level expression analysis.

Despite the importance of isoform-level information, legacy short-read sequencing techniques often cannot generate reads that span entire transcripts (**Figure 1**). As a result, novel transcripts can be missed[2], which impacts precise isoform identification and quantification.

Oxford Nanopore technology uniquely delivers high outputs of reads that span full-length transcripts, resulting in unambiguous isoform detection and comprehensive transcriptome characterisation. Oxford Nanopore reads enable differential isoform expression analysis, as well as allele-specific expression analysis. Additionally, nanopore sequencing can identify fusion transcripts that cannot be captured in full with short-read approaches.

With the latest Oxford Nanopore technology, it is possible to obtain isoform-level expression even at single-cell resolution — find out more in the single-cell transcriptomics with Oxford Nanopore getting started guide[3].

For bulk transcriptomics, Oxford Nanopore Technologies offers streamlined, end-to-end workflows based on two different sequencing methods, and your experimental aims determine which approach to use.

From the two methods, cDNA nanopore sequencing generates the highest outputs of reads from total RNA or poly-A-enriched RNA, from inputs as low as 10 ng. The cDNA sequencing workflow is ideally suited for measuring gene expression, and detecting and quantifying full-length isoforms, splice variants, fusion transcripts, and poly-A tail lengths. The workflow also includes steps to significantly reduce internal poly-T priming artefacts, making it a precise tool for transcriptomics.

Direct RNA nanopore sequencing is the only available technique that directly reads single, native RNA molecules. This method is amplification free, eliminating PCR bias and enabling the direct detection of RNA base modifications (such as methylation) that are usually erased during cDNA preparation. Therefore, this workflow not only provides resolution of full-length isoforms but also detects m^6A, pseU, inosine, m^5C, and 2'OMe modifications.

All Oxford Nanopore sequencing devices are compatible with both the cDNA and direct RNA workflows. The sequencers are built around independent flow cells that can typically accommodate one to several cDNA or RNA libraries, removing the need to batch a high number of samples and providing quick turnaround times and fast results.

This guide introduces cDNA and direct RNA Oxford Nanopore sequencing, for ultra-rich transcriptomic data without compromise.

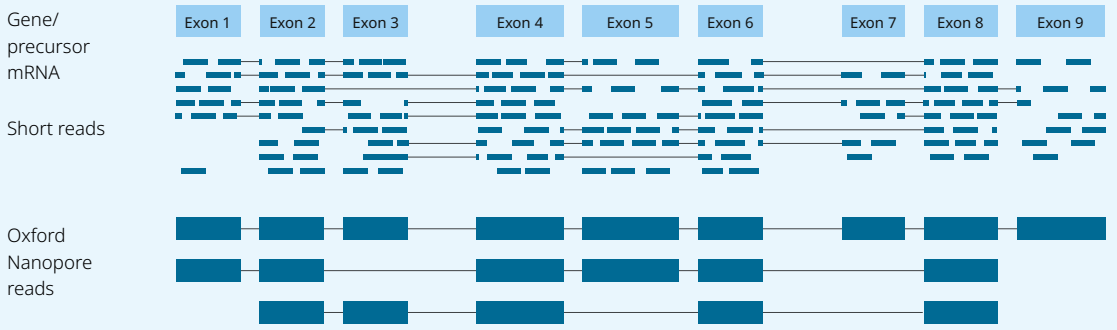


Figure 1. The short reads generated by legacy sequencing techniques often cannot span full-length transcripts, making the correct assembly of isoforms challenging. Oxford Nanopore reads can span full-length transcripts, simplifying isoform detection and quantification.

1. Jiang, W. and Chen, L. Alternative splicing: human disease and quantitative analysis from high-throughput sequencing. *Comput. Struct. Biotechnol. J.* 19:183–195 (2021). DOI: https://doi.org/10.1016/j.csbj.2020.12.009

2. Lee, J. et al. Long-read RNA sequencing of archival tissues reveals novel genes and transcripts associated with clear cell renal cell carcinoma recurrence and immune evasion. *Genome Res.* gr.278801.123 (2024). DOI: https://doi.org/10.1101/gr.278801.123

3. Oxford Nanopore Technologies. Single-cell transcriptomics with Oxford Nanopore (2025). Available at: https://nanoporetech.com/resource-centre/getting-started-guide-single-cell-transcriptomics [Accessed 25 July 2025]

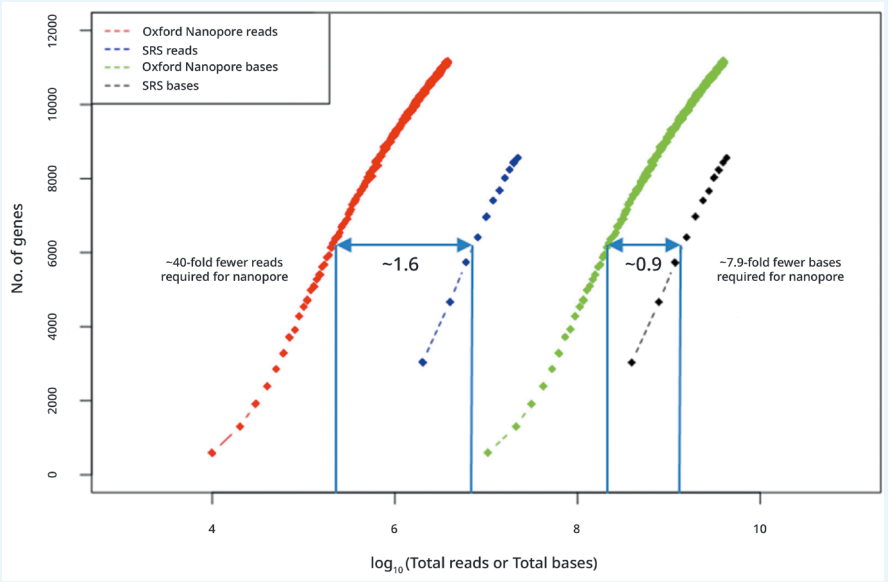
At the same time as capturing full-length isoforms in single reads, Oxford Nanopore sequencing delivers robust gene expression profiles that are comparable to those of short-read sequencing technologies[4,5]. Therefore, nanopore technology enables accurate quantification of gene expression levels while retaining the capability to characterise splice variants and RNA modifications in real time, giving you greater flexibility in the generation of high-quality gene expression data.

Additionally, less data is required to cover the same number of genes with Oxford Nanopore reads than with short reads (**Figure 2**)[6]. Due to their long length, only a single nanopore read is needed to span the whole length of a transcript, while multiple short reads are required to tile across the RNA molecule. Short-read data also results in uneven coverage across a transcript, meaning more bases are required to obtain a minimum level of coverage across the full transcript length. For example, approximately eight-fold fewer Oxford Nanopore bases will cover the same number of genes at 95% versus a short-read sequencing technology[6].

The improved gene coverage efficiency of nanopore sequencing compared with legacy technology, combined with flexible sample batching, means that Oxford Nanopore technology is a cost-effective solution for generating ultra-rich transcriptomic data. For example, with the PromethION™ range of devices, you may be able to multiplex up to five human whole-transcriptome sequencing samples per flow cell (see **page 6** for more on PromethION Flow Cells).

Figure 2. Less data is required to cover the same number of genes with Oxford Nanopore reads than with those from short-read sequencing (SRS).

Coverage equivalence between SRS and Oxford Nanopore sequencing. Number of cDNA reads and total number of bases required to detect the same number of genes (at 95% gene length coverage) with either SRS or Oxford Nanopore sequencing. The x-axis and the indicated numbers on the plot are in log10 scale. The data used to derive the plot are from Bayega *et al.*[7]. Figure from Oikonomopoulos *et al.*[6] and available under Creative Commons license (creativecommons.org/licenses/by/4.0).



4. Glinos, D.A. et al. Transcriptome variation in human tissues revealed by long-read sequencing. *Nature* 608(7922):353–359 (2022). DOI: https://doi.org/10.1038/s41586-022-05035-y

5. Chen, Y. et al. A systematic benchmark of nanopore long read RNA sequencing for transcript level analysis in human cell lines. *bioRxiv* 440736 (2021). DOI: https://doi.org/10.1101/2021.04.21.440736

6. Oikonomopoulos, S. et al. Methodologies for transcript profiling using long-read technologies. *Front. Genet.* 11:606 (2020). DOI: https://doi.org/10.3389/fgene.2020.00606

7. Bayega, A. et al. Nanopore long-read RNA-seq and absolute quantification delineate transcription dynamics in early embryo development of an insect pest. *Sci. Rep.* 11(1):7878 (2021). DOI: https://doi.org/10.1038/s41598-021-86753-7

Which approach do I choose?

Every transcriptomics research project is unique. Find out which library preparation kit is right for you.

	cDNA sequencing	Direct RNA sequencing
Library prep kit	cDNA-PCR Sequencing Kit	Direct RNA Sequencing Kit
Preparation time	~225 mins + PCR	135 mins
Input recommendation	10 ng poly(A)+ RNA or 500 ng total RNA	300 ng poly(A)+ RNA or 1 µg total RNA
Reverse transcription	Yes	Optional
Amplification	Yes	No
Barcode options	cDNA-PCR Barcoding Kit (24 plex)	*In development*
Typical output	>100M reads	20M reads
Methylation included	N/A	Yes

Find out more about Oxford Nanopore library preparation solutions:
nanoporetech.com/prepare

From sample to answer

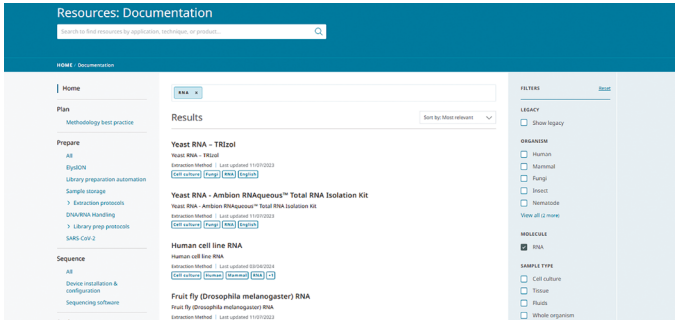
Extraction

How do I extract high-quality RNA from my samples?

Either poly-A-enriched, ribodepleted, or total RNA can be used as input for library preparation for cDNA or direct RNA nanopore sequencing. 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 you are starting with human blood research samples, we recommend using the QIAGEN Paxgene Blood RNA Kit. For these protocols and those covering the extraction of RNA from other organisms, view all our RNA extraction protocols on the Documentation section on our website.

After extraction, poly-A enrichment or ribodepletion can be performed on the total RNA using the Invitrogen Dynabeads mRNA Purification Kit or Invitrogen RiboMinus Eukaryote Kit v2, respectively. For some transcriptomics research projects, you may want to add poly-A tails to non-polyadenylated RNA transcripts (e.g. prokaryotic protein-coding mRNA), and this can be done using NEB *E. coli* Poly(A) Polymerase.

Documentation



View RNA extraction protocols:
nanoporetech.com/extraction-methods

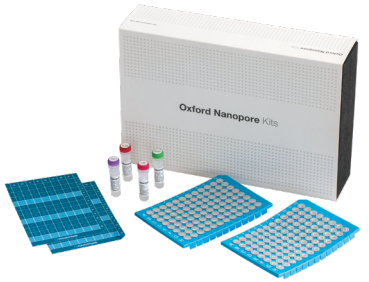
Library prep

How do I prepare my samples for nanopore sequencing?

For the generation of high outputs of reads that span full-length transcripts, prepare your extracted RNA for cDNA sequencing using the cDNA-PCR Sequencing Kit. If you would like to sequence multiple samples in one sequencing experiment, use the cDNA-PCR Barcoding Kit to multiplex up to 24 samples.

For the preparation of native RNA transcripts for direct sequencing, use the Direct RNA Sequencing Kit. This method does not require PCR, preserves RNA base modifications, and only takes approximately two hours to prepare a sequencing-ready library. To perform targeted direct RNA sequencing of transcripts with defined (non-polyadenylated) terminal 3' sequences, see our sequence-specific protocol on the Documentation section.

Library preparation kit



Find out more about nanopore library preparation kits:
nanoporetech.com/prepare

From sample to answer

Sequencing

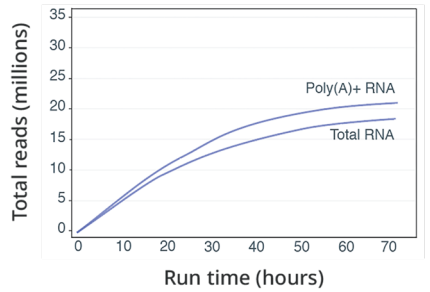
Which device should I choose?

For both cDNA and direct RNA nanopore sequencing, we recommend using the PromethION range of sequencing devices — offering flexible, on-demand access to (up to) terabases of data. The DNA PromethION Flow Cells can deliver >100 million reads per flow cell for cDNA sequencing, and for direct RNA sequencing, the RNA PromethION Flow Cells can generate up to 20 million reads per flow cell.

For research labs requiring high-throughput sequencing on up to 24 independent flow cells and powerful onboard compute, we recommend the PromethION 24. For research projects with lower sample throughput needs, the PromethION 2 range is ideal, delivering sequencing on up to two flow cells — with the same per-flow cell output as the PromethION 24 — and compact device designs. The PromethION 2 Solo can be connected to a GridION™ device or other existing compute infrastructure, while the PromethION 2 Integrated is a self-contained sequencer with onboard compute and a high-resolution touchscreen.

All Oxford Nanopore sequencing devices can run RNA and DNA flow cells, offering you complete flexibility of output to suit your experimental needs.

Median number of reads generated by RNA PromethION Flow Cells (RNA004)



Find out more about PromethION:
nanoporetech.com/promethion

Data analysis

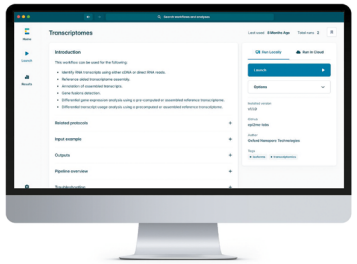
How can I analyse my data?

On-demand, intuitive data analysis begins with MinKNOW™ — the software that controls Oxford Nanopore sequencing devices — performing raw data acquisition and basecalling in real time. Next, transcriptomic data generated by nanopore technology can be easily analysed using the EPI2ME™ workflow wf-transcriptomes. The EPI2ME platform provides data analysis solutions for all levels of experience and can be accessed via an intuitive interface or through the command line. EPI2ME workflows can be run locally or in the cloud.

From cDNA or direct RNA reads, wf-transcriptomes identifies RNA transcripts and isoforms, assembles and annotates transcriptomes, detects gene fusions, and enables differential gene and isoform expression analysis. The pipeline outputs an HTML report detailing the primary findings of the workflow.

Additionally, from direct RNA reads, m^6A, pseU, inosine, m^5C, and 2'OMe modifications can be basecalled alongside the nucleotide sequence.

Data analysis with wf-transcriptomes



Learn more about EPI2ME:
nanoporetech.com/epi2me

Case studies

Case study 1: full-length RNA isoforms reveal hidden diversity in human health and disease

Using legacy short-read sequencing technologies where RNA must be fragmented, 'researchers have historically been forced to collapse all isoforms into a single gene expression measurement, [which is] a major oversimplification of the underlying biology'[8].

In their recent publication, Aguzzoli Heberle *et al*. used cDNA Oxford Nanopore sequencing to map medically relevant RNA isoforms in the human brain[8]. The team used the cDNA-PCR Sequencing Kit and a PromethION device to perform whole-transcriptome sequencing of 12 post-mortem, aged, frontal cortex brain research samples: six with Alzheimer's disease (AD) and six cognitively unimpaired controls (CT).

The team identified 7,042 genes expressing two or more RNA isoforms, 1,917 of which were determined to be medically relevant. From this, the team reported 428 new isoforms, 53 of which originated from medically relevant genes involved in brain-related diseases.

The team shared that 'the most compelling value' in using Oxford Nanopore reads with unrestricted length is the ability to perform differential isoform expression analyses. Analysis of six AD and six CT samples revealed expression patterns associated with AD that were hidden when performing gene-level analysis. The team reported 176 differentially expressed genes and 105 differentially expressed RNA isoforms. Of the 105 isoforms, 99 came from genes that were not differentially expressed at the gene level.

Understanding disease mechanisms and developing treatments require methods that offer 'substantial improvement over short-read sequencing' approaches. Using nanopore sequencing, Aguzzoli Heberle *et al.* went beyond basic gene expression and found that differential expression analysis of isoforms can dive deeper, revealing insights into which healthy and diseased cell and tissue types express different isoforms.

Read the publication (May 2024):
nanoporetech.com/medically-relevant-rna-isoform

Case study 2: delivering 'unique insights' into infection with direct RNA sequencing

Influenza is a highly contagious respiratory illness caused by influenza viruses. These relatively small RNA viruses (genome size: ~13.5 kb) are exceptionally adaptable and efficient at evading the host immune response, making producing a long-lasting vaccine against them challenging.

Wang *et al*., from the University of Oklahoma, USA, used direct RNA Oxford Nanopore sequencing to explore the changes in viral and host transcripts, and the host immune response, during influenza infection[9]. Using the Direct RNA Sequencing Kit and benchtop MinION™ sequencing device, the team analysed mRNA transcripts and non-coding RNA (both polyadenylated and non-polyadenylated) from influenza-exposed and mock-exposed human bronchial epithelial research samples. In a single assay, they were able to measure base modifications, transcript isoforms, poly-A tail length, and differential expression.

Direct detection of base modifications revealed 502 transcripts with m^{6}A methylation in influenza-exposed samples, and 395 transcripts with m^{6}A methylation in mock-exposed samples; however, the abundance of the majority of transcripts exhibiting differential m^{6}A did not change. The team suggested that, although m^{6}A methylation is an important regulatory mechanism of the transcriptional response to viral infection, it is unlikely to be the main mechanism regulating transcriptional changes in response to influenza in their model system.

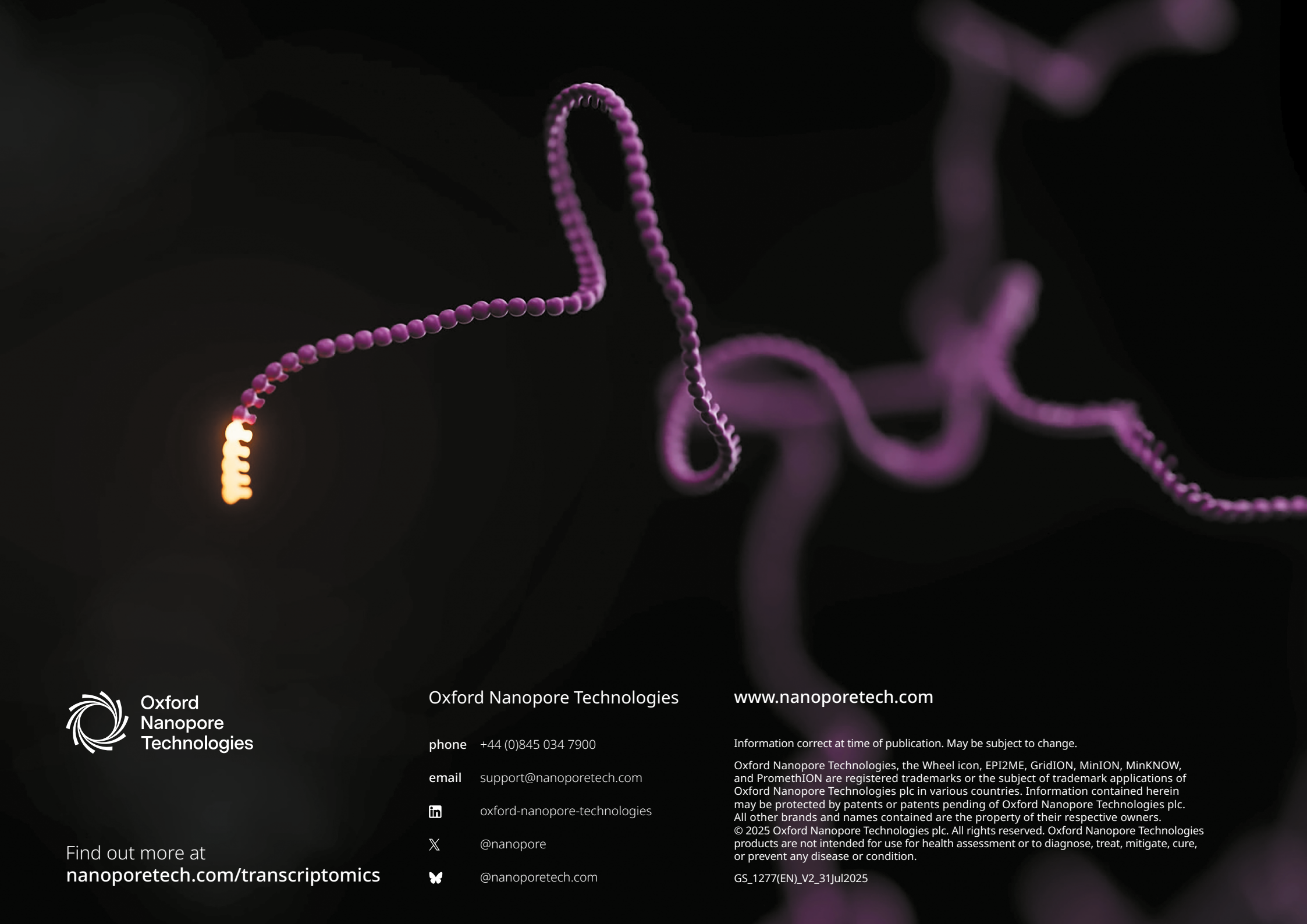
Interestingly, the team observed increased methylation in response to influenza exposure in two long non-coding RNAs (lncRNAs) — CHASERR and LEADR, which were recently discovered to have roles in the immune response.

The team highlighted that further research is necessary to fully understand the mechanisms through which non-coding RNA and RNA modifications fine-tune the host response to influenza infections but that direct RNA sequencing using Oxford Nanopore technology revealed these 'unique insights'.

Read the publication (June 2024):
nanoporetech.com/direct-rna-bronchial-epithelial-cells

8. Aguzzoli Heberle, B. et al. Mapping medically relevant RNA isoform diversity in the aged human frontal cortex with deep long-read RNA-seq. *Nat. Biotechnol.* 10.1038/s41587-024-02245-9 (2024). DOI: https://doi.org/10.1038/s41587-024-02245-9

9. Wang, D. et al. Nanopore direct RNA sequencing reveals virus-induced changes in the transcriptional landscape in human bronchial epithelial cells. *bioRxiv* 600852 (2024). DOI: https://doi.org/10.1101/2024.06.26.600852



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