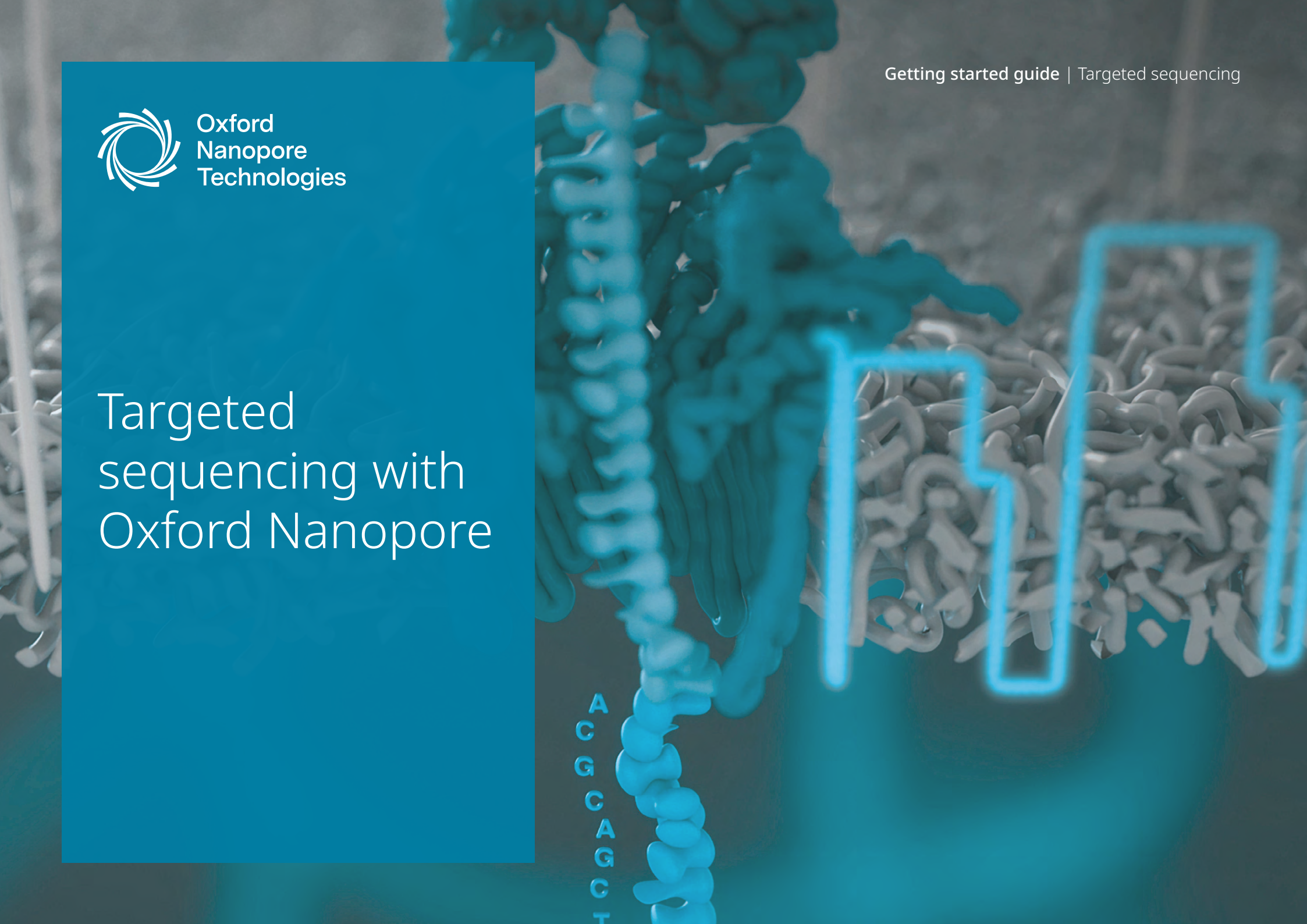
Oxford Nanopore Technologies

Targeted sequencing with Oxford Nanopore



Introduction

Targeted sequencing — the enrichment of target DNA/RNA molecules or depletion of unwanted molecules — is a valuable method of generating sufficient depth of coverage for regions of interest, enabling informative and cost-effective analysis.

In many sequencing applications, the focus of study — a single gene, or a selection of genomic regions — comprises only a tiny fraction of a genome. In these cases, characterisation through whole-genome sequencing is inefficient, costly, and time-consuming. For example, in cancer research, rapid analysis of an oncogenic fusion is impractical through whole-genome sequencing, while in metagenomics, it may be difficult to enrich for a microbe of interest where it is poorly represented in a mixed sample.

Target enrichment is an effective strategy to address these difficulties: by dedicating more sequencing time to regions of interest, their depth of coverage can be greatly increased. This can significantly reduce the number of sequencing libraries and runs required and the data analysis burden for a quicker and more cost-efficient workflow. Target enrichment is commonly achieved through PCR-based amplification of selected sequences, which can provide a simple and inexpensive method of sequencing regions to a high depth of coverage. PCR-based enrichment is often utilised in clinical applications, such as pharmacogenomics to enrich gene panels, such as genes affecting drug metabolism[1].

Targeted sequencing can be achieved via legacy short-read technology, but the length of targets that can be analysed is limited, precluding the interrogation of larger regions such as those associated with structural variants (SVs). Instead, large regions must be reassembled from shorter segments; this can lead to errors, especially where regions are highly repetitive or poorly represented in reference genomes. Oxford Nanopore sequencing overcomes this challenge by producing reads of unrestricted length, from short to ultra long, making it possible to span large target regions. Furthermore, with scalable technology and sample multiplexing options, Oxford Nanopore provides fast, flexible, and cost-effective solutions for sequencing at scale, overcoming the limitations of legacy technologies, such as slow and expensive sequential Sanger sequencing[2].

Nonetheless, not all sequences are amenable to PCR: those that are GC rich or highly repetitive can be difficult or even impossible to amplify. PCR also removes base modifications, preventing their analysis. To address this limitation of PCR-based targeted sequencing, Oxford Nanopore Technologies offers digital panels, delivering bioinformatics-based target enrichment with no need for additional library prep. Digital panels are powered by Adaptive Sampling: a PCR-free enrichment and depletion technique to target regions of interest during sequencing (page 4).

Nanopore technology provides significant advantages over conventional targeted sequencing methodologies, providing both PCR-based and PCR-free target enrichment options combined with real-time sequencing and analysis (Table 1).

In this guide, we introduce the different approaches and benefits of targeted nanopore sequencing.

Table 1. Advantages of Oxford Nanopore technology for targeted sequencing

Enrich regions inaccessible to legacy technologies
Target repetitive or GC-rich regions with PCR-free enrichment and access large, complex regions with long nanopore reads for both PCR and PCR-free methods

Characterise regions of interest without restrictions on read length
Enrich and sequence targets of any read length, from 20 bp to over 4 Mb

Expand your targeted sequencing assays
Detect single nucleotide variants (SNVs), SVs, repeat expansions, and modified bases in targets in a single sequencing run

Sequence when you need to, tailored to your application
Sequence to suit your needs — from portable devices for in-field sequencing to high-throughput benchtop systems

Gain rapid access to results
Reduce turnaround times to hours or less with rapid enrichment and library prep methods, plus real-time targeted sequencing and analysis

Sequence in your lab, with minimal start-up costs
Get started with nanopore sequencing at the scale you need, and sequence targets in multiplex to increase cost efficiency

Perform targeted sequencing without lab-based enrichment
Enrich chosen regions during real-time nanopore sequencing with our digital panels

Deploy simple, end-to-end workflows
Get set up for sequencing and analysis quickly and effectively, with sample-to-answer guidance

1. Verma, R. et al. *Am. J. Respir. Crit. Care Med*. 209(12): 1486–1496 (2024). DOI: https://doi.org/10.1164/rccm.202309-1583OC

2. CD Genomics. Sanger sequencing: introduction, principle, and protocol. https://www.cd-genomics.com/blog/sanger-sequencing-introduction%20principle-and-protocol/ (2020) [Accessed 12 January 2026]

PCR-based targeted sequencing

Oxford Nanopore sequencing is built on highly scalable chemistry, available across a range of devices to suit your application. The portable MinION™ can sequence anywhere, whereas the benchtop GridION™ and high-throughput PromethION™ devices provide the flexibility to sequence multiple libraries in parallel. Plus, flow cells operate independently, so you can sequence across one or multiple flow cells without the need to batch your samples. Sequencing experiments can also be run in multiplex with the barcoding kits, reducing turnaround time and cost.

With nanopore sequencing, data is streamed in real time, meaning basecalling and analysis can begin as soon as sequencing starts. Combined with rapid library preparation kits, the entire sample-to-answer workflow can be performed in just hours.

Nanopore technology sequences fragments of any length, making it suitable for PCR-based targeted sequencing approaches. Combined with long-range PCR, targets spanning several kilobases can be sequenced to a high depth of coverage, even across complex genomic regions.

PCR-based nanopore sequencing approaches include full-length 16S ribosomal RNA (rRNA) gene sequencing for bacterial species identification[3] and internal transcribed spacer (ITS) sequencing for fungal profiling[4]. Both of which can now be accomplished with our Microbial Amplicon Barcoding Kit. Gene panels can also be targeted with PCR-based methods, for example, to detect antimicrobial resistance (AMR) in *Mycobacterium tuberculosis*[5] or to screen carriers for genetic conditions. To support carrier screening research applications, we have collaborated with Asuragen, a Bio-Techne brand, to develop a PCR-based enrichment workflow for carrier screening using nanopore sequencing[6].

Oxford Nanopore sequencing also supports tiled amplicon sequencing for viral genome analysis. This has been commonly applied to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[7] and has been adopted for other viruses, including Zika and avian influenza viruses[8]. This method has been deployed in public health emergencies to provide rapid, high-quality data to inform outbreak responses and surveillance.

Other targeted methods suitable for nanopore sequencing include hybridisation capture to enrich regions of interest before sequencing. For example, the Agilent SureSelect XT HS2 target enrichment system generates libraries with fragment lengths of approximately 5 kb for nanopore sequencing, resulting in high coverage across targeted regions[9]. We have also partnered with 4bases to support the sequencing of hereditary and somatic mutations in *BRCA*-related cancers[10,11].

Beyond the flexible PCR-based options, targeted nanopore sequencing can also be performed without amplification by using digital panels for on-device target enrichment via Adaptive Sampling, an innovative PCR-free targeted sequencing method unique to Oxford Nanopore (**page 4**).

3. Kai, S. et al. *FEBS Open Bio*. 9(3):548–557 (2019). DOI: https://doi.org/10.1002/2211-5463.12590
4. Fu, Y. and Chen, Q. et al. *Microbiol. Spectr*. 10(2): e00270-22 (2022). DOI: https://doi.org/10.1128/spectrum.00270-22
5. Zhao, K. et al. *Can. J. Infect. Dis. Med. Microbiol*. 28:2022:7588033 (2022). DOI: https://doi.org/10.1155/2022/7588033
6. Oxford Nanopore Technologies. Asuragen. https://nanoporetech.com/about/partners/nanopore-compatible-products-programme/asuragen (2025) [Accessed 18 December 2025]
7. Kalendar, R. et al. *Sci. Rep*. 13(1):10334 (2023). DOI: https://doi.org/10.1038/s41598-023-37588-x
8. Ratcliff, J. et al. *Microbiol. Spectr*. 12(12):e01880-24 (2024). DOI: https://doi.org/10.1128/spectrum.01880-24
9. Agilent. Target capture long-read sequencing using the Agilent SureSelect XT HS2 Target Enrichment System application note (2024). Available at: https://www.agilent.com/en/product/next-generation-sequencing/ngs-library-prep-target-enrichment-reagents/dna-seq-reagents/sureselect-xt-hs2-dna-reagent-kit-4252207 [Accessed 12 January 2026]
10. Oxford Nanopore Technologies. 4bases. https://nanoporetech.com/about/partners/nanopore-compatible-products-programme/4bases (2025) [Accessed 27 January 2026]
11. 4bases. Oncological kits: BRaCA panel. https://4bases.ch/oncological-kits/ (2025) [Accessed 27 January 2026]

Digital panels for targeted sequencing

Digital panels are powered by Adaptive Sampling, a software-based enrichment and depletion method that targets regions of interest during sequencing with no additional library preparation or PCR steps. With this PCR-free method, you can create digital panels targeting genes in difficult-to-sequence regions that could previously only be characterised through high-depth, whole-genome sequencing. Examples include regions encompassing low-complexity, GC-rich sequences, large SVs, and repeat expansions.

By sequencing PCR-free, native DNA, modifications are preserved in targets and can be analysed alongside the nucleotide sequence. Adaptive Sampling, an innovation developed by Oxford Nanopore Technologies with support from the Nanopore Community[12–15], negates the need for any lab-based enrichment steps. Instead, the enrichment is performed in real time, during the sequencing itself. The method is fully incorporated into MinKNOW™, the operating software that drives all nanopore sequencing devices.

Firstly, the whole DNA library is prepared for sequencing without any amplification or enrichment steps. The sample is then added to the flow cell, and the sequencing run is set up in MinKNOW. Here, Adaptive Sampling is selected and a reference file for alignment in FASTA format, along with a digital panel as a BED file — containing the coordinates for enrichment — are uploaded. Adaptive Sampling can also be used for targeted depletion of the sequences in the BED file. The run is then started as normal, and targeted sequencing begins (**Figure 1**).

With real-time nanopore sequencing and Adaptive Sampling, the first section of the sequenced DNA strand is aligned to the provided reference file as it passes through a nanopore. If the sequence lies within a target to be enriched — or is not a sequence to be depleted — the strand is allowed by MinKNOW to continue through the nanopore. If the sequence is not a target — or is to be depleted — the strand is selectively ejected from that nanopore, preventing further sequencing and freeing up pore occupancy time for other DNA strands containing regions of interest. Target from one gene, up to <10% of the genome. In this way, even very large panels, or entire genomes, can be enriched or depleted during sequencing.

Adaptive Sampling is available on all devices.

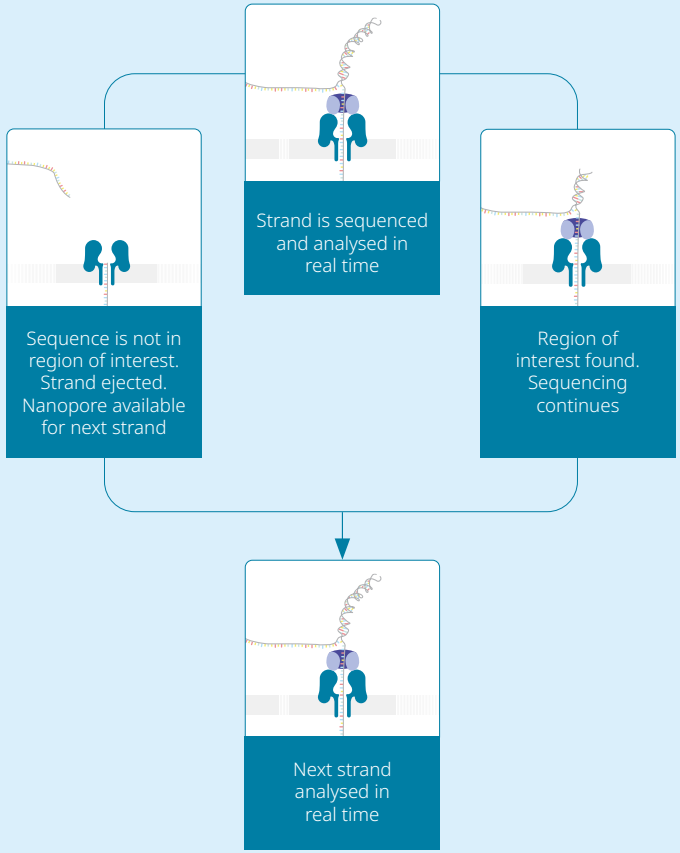


Figure 1. Enrichment/depletion of DNA strands during real-time sequencing with Adaptive Sampling

View an animation of Adaptive Sampling:
nanoporetech.com/adaptive-sampling-animation

12. Loose, M., Malla, S., and Stout, M. *Nat. Methods* 13(9):751–754 (2016). DOI: https://doi.org/10.1038/nmeth.3930
13. Payne, A. et al. *Nat. Biotechnol*. 39(4):442–450 (2021). DOI: https://doi.org/10.1038/s41587-020-00746-x
14. Kovaka, S., Fan, Y., Ni, B., Timp, W., and Schatz, M. *Nat. Biotechnol*. 39(4):431–441 (2021). DOI: https://doi.org/10.1038/s41587-020-0731-9
15. Weilguny, L. et al. *Nat. Biotechnol*. 41(7):1018–1025 (2023). DOI: https://doi.org/10.1038/s41587-022-01580-z

Digital panel applications

Digital panels can be used in many applications to perform targeted sequencing. We have developed the Hereditary Cancer Panel, an end-to-end sequencing solution with a pre-designed digital panel that targets 258 key genes implicated in cancer predisposition, with the potential to advance precision oncology in the future.

Hereditary cancers are caused by inherited gene abnormalities that disrupt cellular pathways, such as DNA repair and cell cycle regulation[16]. Current germline genetic testing typically investigates a single or a few high-risk genes, for example, *BRCA* or *TP53*. These limited targets are often selected through clinical criteria such as tumour type and family history[17]. If results are inconclusive, large panels or whole-genome sequencing may be used[18]. However, these methods currently rely on short-read sequencing, making complex structural variants, intronic regions, and epigenetic modifications challenging to analyse, limiting diagnostic yield.

Through Oxford Nanopore sequencing of native DNA with Adaptive Sampling, the Hereditary Cancer Panel captures both genetic and epigenetic modifications to provide a comprehensive view of the genome in one workflow. Following the Hereditary Cancer Panel end-to-end solution, we have demonstrated that over 45 Gb of total data per flow cell can be generated, guaranteeing a mean depth of coverage of >30x across all target genes alongside low-pass, whole-genome sequencing of 3–5x (**Figure 2**)[19].

Digital panels provide flexible, real-time enrichment during sequencing. This means the Hereditary Cancer Panel requires no separate library preparation, whilst enhancing detection of SNVs, insertions and deletions (indels), SVs, pseudogenes, methylation, and repetitive regions, alongside enabling copy number analysis with low-pass whole-genome coverage.

Digital panels have also been used in applications, including pharmacogenomics (PGx)[20], where pharmacogene variants have been shown in a research setting to provide critical information about an individual's potential response to pharmaceutical compounds. We have developed an end-to-end PGx workflow with a pre-designed digital panel to target pharmacogene-associated genes, providing unambiguous allele and variant calls, phased haplotypes, and full resolution of *CYP2D6*.

Adaptive Sampling, our unique targeted sequencing method behind digital panels, can also provide rapid strain-level analysis of pathogenic microbes. This allows for fast responses to outbreaks such as foodborne contaminants[21] and efficient microbial characterisation in samples with a high background of host DNA by providing host depletion[22].

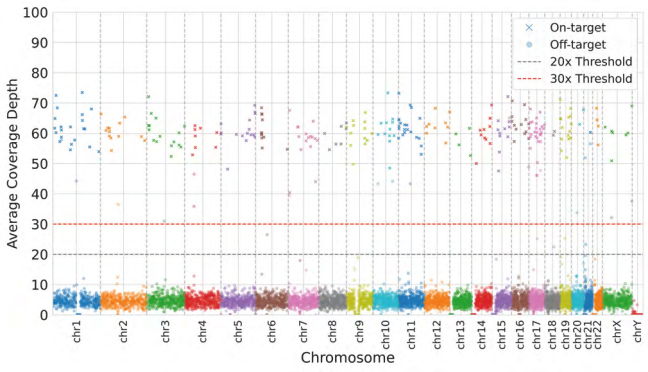


Figure 2. Gene coverage profile from a male research sample demonstrating on-target coverage depth significantly above 30x, with uniform low-pass whole-genome (off-target) coverage across all chromosomes.

For more information on the Hereditary Cancer Panel, view the application note:
nanoporetech.com/resource-centre/hcp-app-note

16. Romero-Laorden, N. and Castro, E. *Curr. Probl. Cancer* 41(4):251–264 (2017). DOI: https://doi.org/10.1016/j.currproblcancer.2017.02.009
17. Ceyhan-Birsoy, O. and Jayakumaran, G., et al. *Genome Med*. 14:19 (2022). DOI: https://doi.org/10.1186/s13073-022-01101-2
18. Bilyalov, A., Nikolaev, S., and Shigapova, L., et al. *Biology* 11(10):1461 (2022). DOI: https://doi.org/10.3390/biology11101461
19. Oxford Nanopore Technologies. Application Note (2025) https://nanoporetech.com/resource-centre/application-note-comprehensive-genomic-and-epigenomic-profiling-with-the-oxford-nanopore-hereditary-cancer-panel (2025) [Accessed 18 December 2025]
20. Deserranno, K., Tilleman, L., Rubben, K., Deforce, D. and Nieuwerburgh, F. *Front. Pharmacol*. 13:14:1286764 (2023). DOI: https://doi.org/10.3389/fphar.2023.1286764
21. Buytaers, F. et al. *Front. Microbiol*. 1:15:1330814 (2024). DOI: https://doi.org/10.3389/fmicb.2024.1330814
22. Kipp, E. and Lindsey, L. et al. *Parasit. Vectors* 16(68) (2023). DOI: https://doi.org/10.1186/s13071-023-05679-3

Which PCR-based approach do I choose?

START HERE

What is your experimental aim?

Target PCR products

Singleplex

Amplicon: Ligation Sequencing Kit

Preparation time	~60 minutes + PCR
Input recommendation	100–200 fmol amplicon DNA
Fragmentation	Not required
Read length	Equal to fragment length

Multiplex

Amplicon: Rapid Barcoding Kit

Preparation time	~60 minutes + PCR
Input recommendation	50 ng amplicon DNA per sample
Fragmentation	Not required
Read length	Random distribution, dependenton input fragment length

View amplicon sequencing protocols at:
nanoporetech.com/documentation

Target bacteria, archaea, and fungi for identification

Microbial Amplicon Barcoding Kit

Preparation time	~60 minutes + PCR
Input recommendation	10 ng gDNA per sample
Fragmentation	Not required
Read length	Full-length 16S gene and ITS region

Find out more at
nanoporetech.com/prepare

Target viral genomes using tiled amplicon sequencing

SARS-CoV-2

Rapid Barcoding Kit

End-to-end protocol preparation time	~315 minutes + RT-PCR
Input recommendation	8 µl RNA
Fragmentation	Not required
Read length	Enriched for whole viral genome

End-to-end protocol available:
SARS-CoV-2 virus PCR tiling and sequencing from RNA

Influenza

Native Barcoding Kit

End-to-end protocol preparation time	~160 minutes + RT-PCR
Input recommendation	2 µl RNA
Fragmentation	Not required
Read length	Enriched for whole viral genome

End-to-end protocol available:
Influenza virus sequencing from RNA

View end-to-end protocols on the application workflows page:
nanoporetech.com/documentation/application-workflows

Which PCR-free approach do I choose?

START HERE

What is your experimental aim?

Target genes with pre-designed digital panels

PGx

Native Barcoding Kit

Preparation time	~390 minutes
Input recommendation	5 µg gDNA
Fragmentation	Not required
Read length	Enriched for targeted genes

End-to-end protocol available: pharmacogenomic sequencing workflow with Adaptive Sampling

Hereditary cancer

Native Barcoding Kit

Preparation time	~560 minutes
Input recommendation	1 µg gDNA
Fragmentation	Not required
Read length	Enriched for targeted genes

End-to-end protocol available: Hereditary Cancer Panel

Target regions highly enriched for CpGs in the human genome with a digital panel

Ligation Sequencing Kit

Preparation time	~60 minutes
Input recommendation	2 µg gDNA
Fragmentation	Optional; recommended for inputs of 100–500 ng
Read length	Equal to fragment length

End-to-end protocol available: reduced representation methylation sequencing (RRMS) from cells

Enrich genomic regions of any size with a digital panel

Native Barcoding Kit

Preparation time	~140 minutes
Input recommendation	400–1,000 ng gDNA
Fragmentation	Optional; recommended for inputs of 100–500 ng
Read length	Equal to fragment length

Target sequence-specific transcripts

Direct RNA Sequencing Kit

Preparation time	~135 minutes
Input recommendation	1 µg total RNA or 300 ng poly(A)+ RNA
Fragmentation	Not required
Read length	Equal to RNA length

View end-to-end protocols on the application workflows page:
nanoporetech.com/documentation/application-workflows

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

From sample to answer

Preparation

Should I amplify my samples?

Oxford Nanopore provides both PCR-based and PCR-free target enrichment solutions. We recommend a PCR-based approach to amplify your samples when a low amount of starting input is available and high coverage is required. We also recommend amplification to detect low-frequency variants to ensure your targets are sequenced to a high depth of coverage.

Adaptive Sampling does not require amplification and enables the targeting of very large, GC-rich, and/or low-complexity regions that cannot be accessed through PCR, whilst retaining base modifications. However, this method acts by freeing up sequencing time for target DNA molecules, rather than increasing the number of on-target molecules in a sample prior to sequencing. As a result, input requirements are higher for this method, and low-frequency variants may not be present in sufficient numbers to generate a good depth of coverage for a robust analysis.

Experimental design

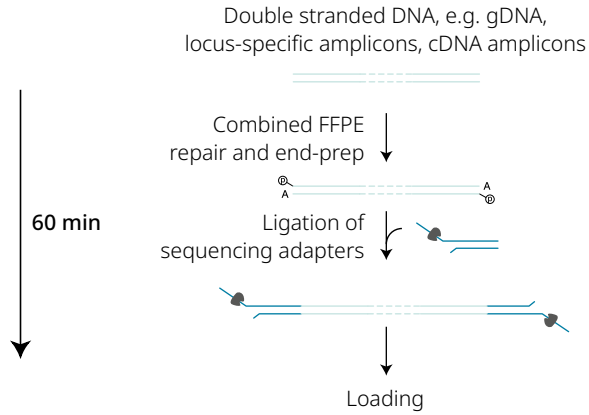
Which PCR-based approach should I choose for custom targets?

A range of methods is available for PCR-based enrichment to suit your experimental aims. When amplifying and sequencing a single target, simply use custom primers to amplify your target, then prepare your sample for sequencing using the Ligation Sequencing Kit or the Rapid Sequencing Kit. Both kits can be used to prepare any pre-amplified samples for sequencing.

To analyse many samples in multiplex without the need for further amplification, a unique barcode can be attached to each amplified sample using the Native Barcoding Kit or Rapid Barcoding Kit, prior to pooling and sequencing on a single flow cell. For low input quantities, the Rapid PCR Barcoding Kit is available.

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

Preparation of amplicon samples for nanopore sequencing using the Ligation Sequencing Kit



From sample to answer

Sequencing

Which sequencing device should I choose?

Oxford Nanopore sequencing is highly scalable and flexible, with devices available to suit a wide range of applications. The portable MinION can be run from a contemporary laptop for sequencing and analysis inside or outside the lab. The MinION is ideal for sequencing single targets or smaller panels. Scaling up, the GridION has the capacity to run up to five independent MinION Flow Cells, providing the flexibility to sequence as and when needed, or for large-scale multiplexing of many targets.

Finally, the powerful PromethION range delivers flexible, large-scale sequencing. The PromethION 2 Integrated enables sequencing on two independent flow cells to provide high-output sequencing for every lab. Meanwhile, the PromethION 24 offers sequencing on up to 24 independent flow cells, providing the capacity to sequence thousands of targets in parallel.

Compare nanopore sequencing devices in detail:
nanoporetech.com/products/sequence

GridION, PromethION 2 Integrated, MinION Mk1D, MinION Flow Cell, and PromethION Flow Cell

Analysis

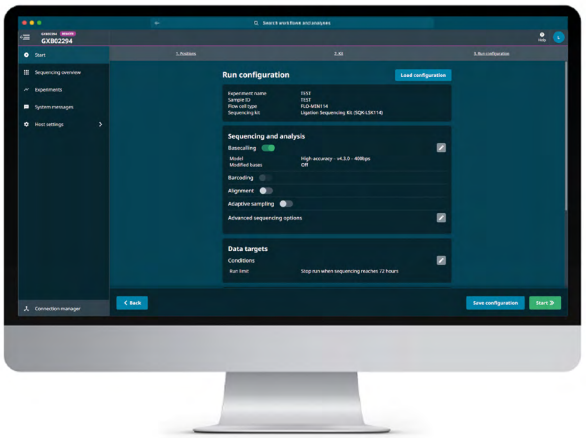
How do I enrich samples during sequencing with digital panels?

Digital panels are powered by Adaptive Sampling, which is integrated into MinKNOW. Simply upload your panel as a BED file to enrich sequences comprising selected regions (e.g. a gene or genes of interest). Adaptive Sampling can also be used to deplete sequences comprising selected regions or whole genomes (e.g. those of abundant organisms to deplete in a metagenomic sample). These targeted sequences are uploaded in the form of a reference file and a BED file that specifies the coordinates of the genomic intervals. If the BED file is not provided, the whole reference will be enriched or depleted.

During an enrichment experiment, all sequences that are not present in the reference or BED file will be rejected, while in a depletion experiment, only sequences that are present in the reference or BED file will be rejected. The fold-enrichment of a run will vary depending on factors, including input read lengths and the proportion of reads you wish to sequence or reject. We recommend that digital panel BED files should target less than 5% of the sample; targeting over 10% will reduce the enrichment values obtained.

View the Adaptive Sampling guide:
nanoporetech.com/document/adaptive-sampling

Setting up Adaptive Sampling via MinKNOW



From sample to answer

Analysis

How can I analyse my data?

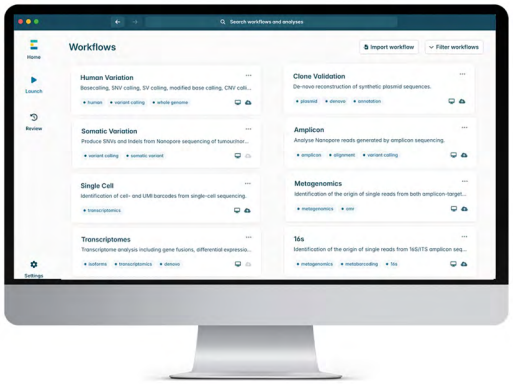
EPI2ME™ is our analysis software, providing workflows available as cloud-based or locally run solutions for sequencing analysis. It has an intuitive user interface that can be used without command-line experience to access bioinformatics workflows for a range of applications. For example, to detect AMR genes in microorganisms, or to characterise SVs, SNVs, and methylation in human genomes.

EPI2ME workflows for the Hereditary Cancer Panel and pharmacogenomics end-to-end solutions are available as part of a comprehensive point-and-click platform with the pre-designed digital panel BED and genome reference files.

Find out more about nanopore data analysis of your target regions:
nanoporetech.com/data-analysis

View EPI2ME workflows:
nanoporetech.com/epi2me-workflows

Data analysis workflows available on EPI2ME



Analysis

How can I assess methylation in my target regions?

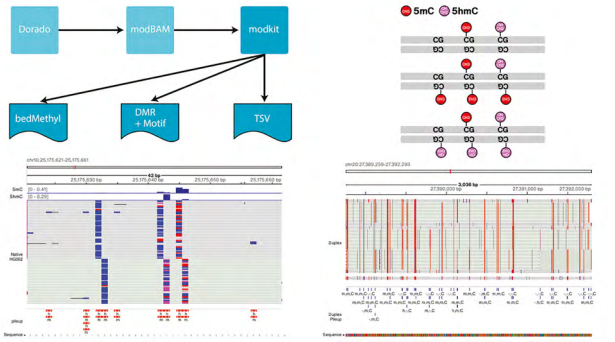
Digital panels allow you to enrich targeted regions without PCR, meaning that methylation is preserved and can be detected without any special library prep steps.

Methylation calling is integrated into both device and standalone software (MinKNOW and Dorado) and is compatible with real-time basecalling. Highly accurate models to detect 5mC, 5hmC, 6mA, and 4mC in all genomic contexts are available. Further analysis of methylation data is supported by Modkit, an open-access tool developed by Oxford Nanopore and is integrated into EPI2ME workflows (e.g. wf-human-variation). This software enables downstream methylation analysis, such as methylation aggregation, pattern visualisation, exploration of differentially methylated regions, and automatic identification of DNA methylation motifs.

To directly detect methylated cytosines using reduced representation methylation multiplex sequencing (RRMS), digital panels for human and mouse genomes are available.

Find Modkit on GitHub:
github.com/nanoporetech/modkit

Downstream methylation analysis from nanopore sequencing data using Modkit



View the Modkit poster:
nanoporetech.com/modkit-poster

Case studies

Case study 1: accurate identification of deep intronic variants in tumour-suppressor genes

Deep intronic variants are genetic mutations located within introns, and some rare variants can alter RNA splicing[23], resulting in exonification of intronic sequences. This, in turn, creates pseudoexons and causes abnormal transcriptional consequences, such as premature stop codons and subsequent loss of gene function[24]. Detrimental deep intronic variants are likely to be understudied and underreported causes of disease because most clinical genetic tests do not sequence full intronic regions due to their repetitive qualities.

To systematically evaluate deep intronic variants, Gulsuner and AbuRayyan *et al*. used targeted nanopore sequencing to analyse DNA and cDNA from research samples from patients and families severely affected by cancers, but with no causal variant identified by conventional methods[25].

The authors carried out two nanopore sequencing workflows in tandem. First, they performed targeted DNA sequencing to identify deep intronic variants in 10 tumour-suppressor genes in which loss-of-function variants are widely thought to cause predisposition to breast and ovarian cancer. Employing a digital panel powered by Adaptive Sampling, they enriched for and sequenced 1 Mb regions around the 10 tumour-suppressor genes, providing high coverage depth across the targets whilst 'simplifying library preparation'. This revealed 92 rare deep intronic variants in 88 (73%) of the families.

Next, the authors prioritised seven deep intronic variants present in eight families for further evaluation. Performing targeted cDNA nanopore sequencing, they achieved >100x depth of sequencing coverage for each sample at the variant of interest. They found that all seven variants led to intron exonification and yielded transcripts with pseudoexons, introducing premature stop codons and ultimately leading to loss of gene function.

Gulsuner and AbuRayyan *et al*. successfully identified rare deep intronic variants contributing to inherited cancer risk in eight of the 120 (6%) previously unsolved families and noted that they would further characterise other variants identified. Nanopore sequencing effectively detected these variants that had been missed by a litany of conventional approaches.

Read the publication (September 2024):
nanoporetech.com/deep-intronic-variants

Case study 2: effectively detecting known and novel zoonotic respiratory viruses with one assay

Zoonotic diseases, such as Ebola and COVID-19, are caused by pathogens originating from animals, which emerge due to rapid mutation and interaction between humans and animal reservoirs[26]. These diseases are a growing public health concern due to factors such as increased urbanisation, causing more human-animal interaction, which increases the risk of zoonotic disease epidemics and pandemics[27].

In recent years, RNA viral zoonotic diseases have been of significant concern[28], causing over three million deaths annually worldwide[29]. Effective surveillance and detection are required to prevent outbreaks. However, typically zoonotic diseases occur in low-resource regions, and current conventional methods are expensive, time-consuming, and require lab expertise, creating a barrier to access. Furthermore, these methods are based on known genomes, which limits the discovery of novel viruses.

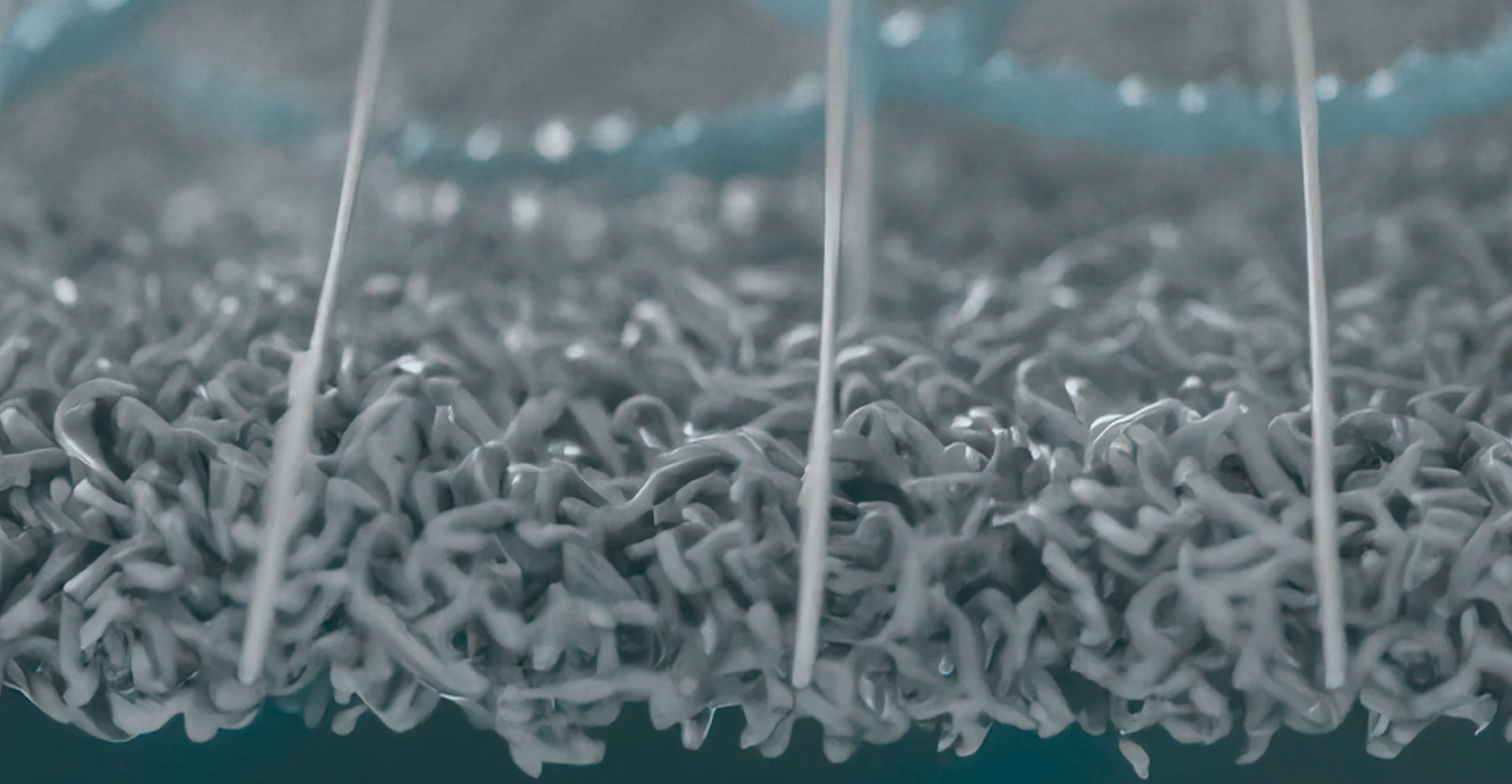
Meki *et al*.[29] developed a multiplex viral family-based PCR method utilising Oxford Nanopore sequencing as an alternative approach to detect multiple respiratory viruses effectively in low-resource settings. The researchers designed a single set of primers based on conserved regions of coronavirus and influenza virus families to broaden virus detection in a single workflow.

Using 78 research samples across a range of birds, beavers, humans, and a pig, the family-based multiplex RT-PCR method detected various known respiratory viruses and, notably, novel viruses, including γ-CoV_IBV. This demonstrates that with a single set of primers, multiple virus families can be targeted, 'expanding the discovery and surveillance of novel viruses' to provide a simple and effective viral detection method.

Furthermore, Oxford Nanopore 'enables mobile sequencing, which is crucial for disease surveillance', alongside easy and scalable library preparation. This workflow offers the potential for rapid detection of viral respiratory diseases to inform public health bodies earlier of potential zoonotic disease outbreaks.

Read the publication (October 2025):
nanoporetech.com/family-wide-PCR

23. Kurosawa, R. et al. *BMC Genomics* 24(1):601 (2023). DOI: https://doi.org/10.1186/s12864-023-09645-2
24. Highsmith, W.E. et al. *N. Engl. J. Med*. 331(15):974–80 (1994). DOI: https://doi.org/10.1056/nejm199410133311503
25. Gulsuner, S. and AbuRayyan, A. et al. *Genome Res*. 34(11):1825–1831 (2024). DOI: https://doi.org/10.1101/gr.279158.124
26. Rahman, M.T., et al. *Microorganisms* 8(9):1405 (2020). DOI: https://doi.org/10.3390/microorganisms8091405
27. Boukli, N. et al. *Front. Med*. 10:1161268 (2023). DOI: https://doi.org/10.3389/fmed.2023.1161268
28. Rosenberg, R. *Cell. Mol. Life Sci*. 72(6):1115–25 (2014). DOI: https://doi.org/10.1007/s00018-014-1785-y
29. Meki, I.K. et al. *Genome Med*. 17(1):123 (2025). DOI: https://doi.org/10.1186/s13073-025-01550-5



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