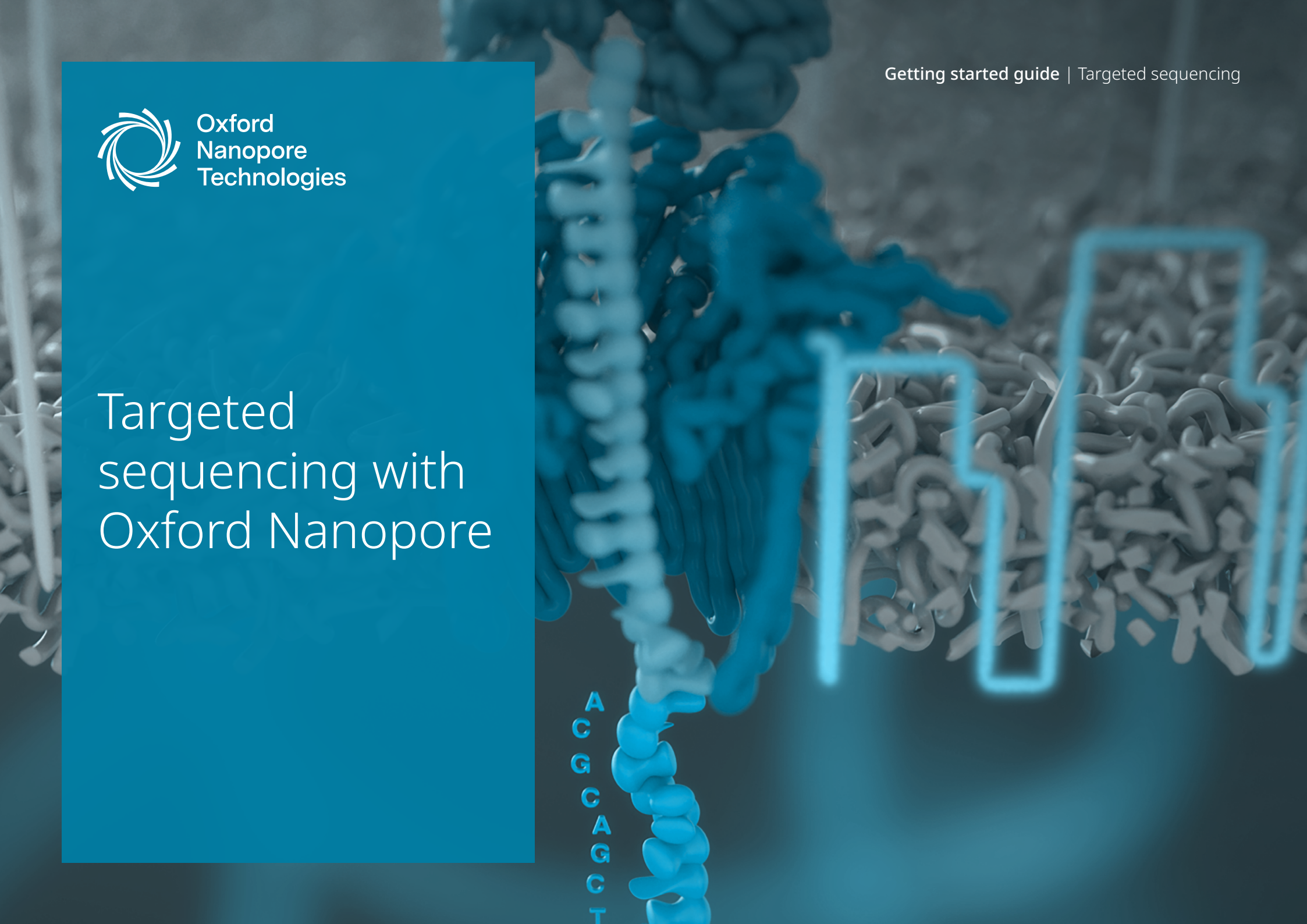
Oxford Nanopore Technologies

Targeted sequencing with Oxford Nanopore



Introduction

Targeted sequencing — by 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.

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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 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, larger regions must be reassembled from shorter segments; this can lead to errors, especially where regions are highly repetitive or poorly represented in the sequence data. 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 adaptive sampling: a software-based enrichment and depletion method to target regions of interest during sequencing — with no additional sample prep (page 4).

Nanopore technology provides significant advantages over conventional targeted sequencing methodologies, providing both PCR-based and PCR-free 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

Perform on-demand sequencing, tailored to your application
Sequence to suit your needs — from portable devices for in-field sequencing to high-throughput, on-demand 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 for the cost of consumables only; sequence targets in multiplex to further increase cost efficiency

Perform targeted sequencing without lab-based enrichment
Enrich or deplete chosen regions during real-time nanopore sequencing with adaptive sampling

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. A nanopore sequencing-based pharmacogenomic panel to personalize tuberculosis drug dosing. *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 principle-and-protocol/ (2020) [Accessed: 15 November 2024]

PCR-based targeted sequencing

The chemistry underpinning Oxford Nanopore sequencing is highly scalable and is deployed in a wide range of devices to suit different applications. The portable MinION™ enables sequencing in any location. Meanwhile, the flexible GridION™ and high-throughput PromethION™ benchtop devices provide the capacity to sequence many targets in parallel.

Additionally, the flow cells are individually addressable, meaning there is no need to wait to batch samples — sequencing can be accessed on demand across one or many experiments. With nanopore sequencing, data is streamed in real time, meaning that basecalling and downstream data analysis can begin as soon as sequencing starts. When combined with rapid enrichment and library preparation options, the entire sample-to-answer workflow can be performed in a matter of hours.

Nanopore technology can sequence any length of fragment, and there is a range of flexible approaches for PCR-based targeted nanopore sequencing available. Therefore, in combination, long-range PCR can be used to enrich and sequence targets spanning several kilobases to a high depth of coverage — even across complex genomic regions. Sequencing experiments can also be run in multiplex, with barcoding kits available to reduce turnaround time and costs.

Multiple PCR-based techniques have been used to perform targeted nanopore sequencing. This includes full-length 16S ribosomal RNA (rRNA) gene sequencing for bacterial species identification[3] and internal transcribed spacer (ITS) sequencing for rRNA for fungal identification[4] — both of which can be used for food safety and environmental monitoring applications. Gene panels can also be targeted using nanopore sequencing — for example, to detect and characterise antimicrobial resistance (AMR) in *Mycobacterium tuberculosis*[5].

Oxford Nanopore technology can also be used to perform tiled amplicon sequencing of viral genomes. This method is commonly used to sequence severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[6] and has been adopted for other viruses, including Zika and avian influenza viruses[7], providing rapid and insightful results for fast responses during outbreaks.

Furthermore, libraries prepared using hybridisation capture to enrich regions of interest can also be sequenced using nanopore technology. This includes the Agilent SureSelect XT HS2 target enrichment system, which can generate target-enriched libraries with an average fragment length of ~5 kb for nanopore sequencing with high on-target percentage and coverage[8].

Beyond the flexible PCR-based options, targeted nanopore sequencing can also be performed without amplification with adaptive sampling, an innovative PCR-free targeted sequencing method unique to Oxford Nanopore Technologies (**pages 4**).

3. Kai, S. et al. Rapid bacterial identification by direct PCR amplification of 16S rRNA genes using the MinION nanopore sequencer. *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. Clinical performance of nanopore targeted sequencing for diagnosing infectious diseases. *Microbiol. Spectr*. 10(2):e00270-22 (2022). DOI: https://doi.org/10.1128/spectrum.00270-22

5. Zhao, K. et al. Rapid identification of drug-resistant tuberculosis genes using direct PCR amplification and Oxford Nanopore technology sequencing. *Can. J. Infect. Dis. Med. Microbiol*. 28:2022:7588033 (2022). DOI: https://doi.org/10.1155/2022/7588033

6. Kalendar, R. et al. Universal whole-genome Oxford Nanopore sequencing of SARS-CoV-2 using tiled amplicons. *Sci. Rep*. 13(1):10334 (2023). DOI: https://doi.org/10.1038/s41598-023-37588-x

7. Ratcliff, J. et al. Improved resolution of avian influenza virus using Oxford Nanopore R10 sequencing chemistry. *Microbiol. Spectr*. 12(12):e01880-24 (2024). DOI: https://doi.org/10.1128/spectrum.01880-24

8. 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: 09 January 2025]

Adaptive sampling

Adaptive sampling is a software-based enrichment and depletion method that targets regions of interest during sequencing with no additional library preparation. It is a PCR-free method and enables targeted sequencing of large regions that could previously only be characterised through high-depth, whole-genome nanopore sequencing. Examples include regions encompassing low-complexity and GC-rich sequences, large SVs, and repeat expansions.

By sequencing native DNA, modifications are preserved in targets and can be analysed alongside the nucleotide sequence. Adaptive sampling, an innovation from the Nanopore Community[9–12], negates the need for any lab-based enrichment steps and instead, the enrichment is performed in real time, during the sequencing itself. The method is 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 and an optional BED file — containing the coordinates for enrichment or depletion — are uploaded (**Figure 1**). The run is then started as normal and targeted sequencing begins.

With real-time nanopore sequencing, it is possible for a sequence that represents a region of interest to be identified as the DNA strand passes through a nanopore. This is achieved through the rapid mapping of the beginning of the sequenced strand to the provided reference file. If the sequence lies within a target to be enriched — or is not a sequence to be depleted — it is allowed by MinKNOW to continue sequencing. 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 regions of interest. There is no limit to read length or target number. In this way, even very large panels, or entire genomes, can be enriched or depleted entirely during sequencing.

Adaptive sampling is available on all devices.

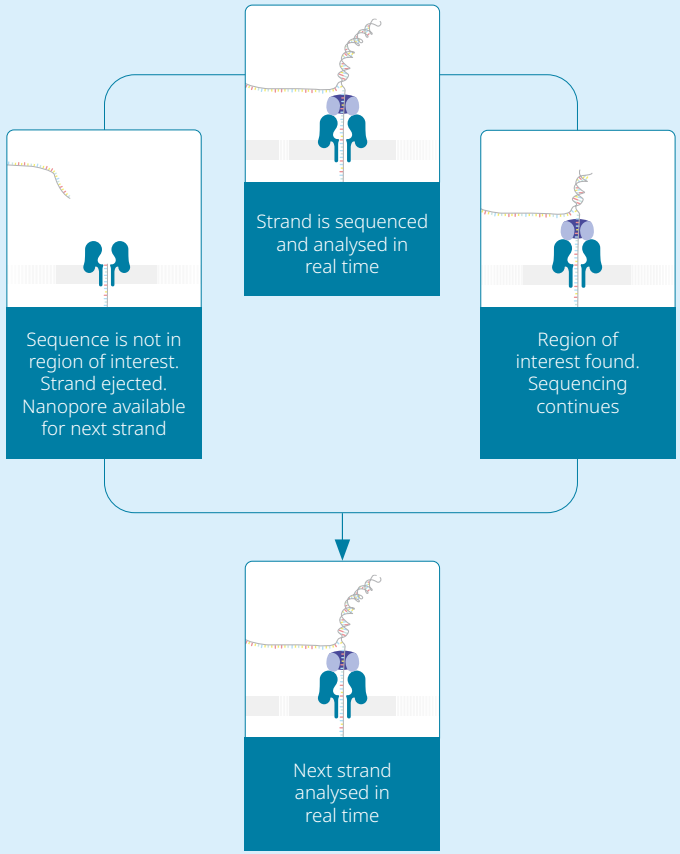


Figure 1. Enrichment/depletion of DNA strands during real-time sequencing with adaptive sampling

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

9. Loose, M., Malla, S., and Stout, M. Real-time selective sequencing using nanopore technology. *Nat. Methods* 13(9):751–754 (2016). DOI: https://doi.org/10.1038/nmeth.3930
10. Payne, A. et al. Readfish enables targeted nanopore sequencing of gigabase-sized genomes. *Nat. Biotechnol*. 39(4):442–450 (2021). DOI: https://doi.org/10.1038/s41587-020-00746-x
11. Kovaka, S., Fan, Y., Ni, B., Timp, W. and Schatz, M. Targeted nanopore sequencing by real-time mapping of raw electrical signal with UNCALLED. *Nat. Biotechnol*. 39(4):431–441 (2021). DOI: https://doi.org/10.1038/s41587-020-0731-9
12. Weilguny, L. et al. Dynamic, adaptive sampling during nanopore sequencing using Bayesian experimental design. *Nat. Biotechnol*. 41(7):1018–1025 (2023). DOI: https://doi.org/10.1038/s41587-022-01580-z

Adaptive sampling applications

Adaptive sampling can be used in many applications to perform targeted sequencing for many applications, for example, to identify and quantify genome-wide DNA methylation with reduced representation methylation sequencing (RRMS).

Typically, bisulfite conversion and sequencing are used for this application; however, whole-genome bisulfite conversion is expensive, labour-intensive, and introduces PCR bias resulting in poor representation of targets that are difficult to amplify. As a result, only about 75% of the CpGs in the human genome are accessed with bisulfite sequencing to 50x depth of coverage[13]. Meanwhile, reduced representation bisulfite sequencing (RRBS), though more cost effective, only captures 10–15% of CpGs in a mammalian genome.

Through Oxford Nanopore sequencing of native DNA with adaptive sampling, RRMS enables direct detection of methylated cytosines (e.g. at CpG sites) without the need for bisulfite conversion or PCR. The method targets 310 Mb of the human genome, including regions that are highly enriched for CpGs to enable direct, targeted analysis of epigenetic modifications, such as 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC).

Utilising adaptive sampling for flexible, real-time enrichment during sequencing, RRMS requires no special library prep, while SVs, SNVs, and methylation can all be called from a single dataset.

Adaptive sampling has also been used in applications such as pharmacogenomics (PGx) to target relevant PGx genes for haplotype phasing[14] and to identify novel pathogenic variants associated with hereditary cancer[15]. Adaptive sampling can also provide rapid strain-level analysis of pathogenic microbes for fast responses to outbreaks such as foodborne contaminants[16] and efficient microbial characterisation in samples with a high background of host DNA by providing host depletion[17].

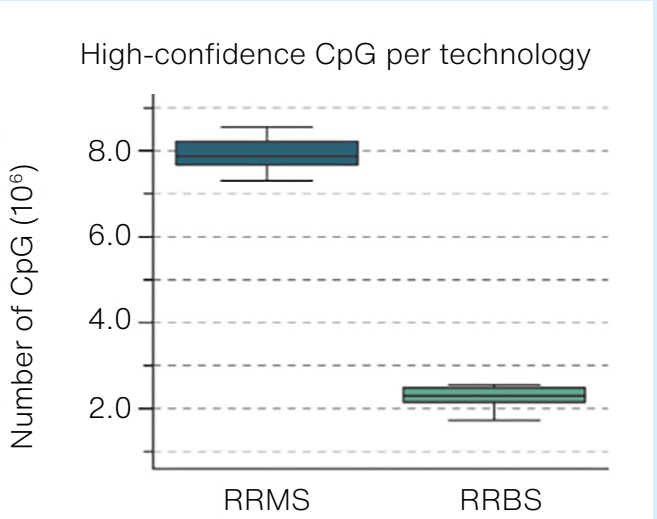


Figure 2. RRMS covers a greater number of CpGs than RRBS

For more information on RRMS, view the methylation getting started guide:

nanoporetech.com/resource-centre/investigating-methylation-in-the-human-genome

13. Oxford Nanopore Technologies. Introduction to reduced representation methylation sequencing (RRMS). Introduction (2024). Available at: https://nanoporetech.com/document/ligation-sequencing-gdna-lsk114-rrms [Accessed: 23 January 2025]
14. Deserranno, K., Tilleman, L., Rubben, K., Deforce, D. and Nieuwerburgh, F. Targeted haplotyping in pharmacogenomics using Oxford Nanopore Technologies' adaptive sampling. *Front. Pharmacol.* 13:14:1286764 (2023). DOI: https://doi.org/10.3389/fphar.2023.1286764
15. Nakamura, W. and Hirata, M. et al. Assessing the efficacy of target adaptive sampling long-read sequencing through hereditary cancer patient genomes. *NPJ Genom. Med.* 9(1):11 (2024) DOI: https://doi.org/10.1038/s41525-024-00394-z
16. Buytaers, F. et al. Strain-level characterisation of foodborne pathogens without culture enrichment for outbreak investigation using shotgun metagenomics facilitated with nanopore adaptive sampling. *Front. Microbiol.* 1:15:1330814 (2024). DOI: https://doi.org/10.3389/fmicb.2024.1330814
17. Kipp, E. and Lindsey, L. et al. Nanopore adaptive sampling for targeted mitochondrial genome sequencing and bloodmeal identification in haematophagous insects. *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 bacteria or archaea for identification

16S Barcoding Kit 1–24

Preparation time	10 minutes + PCR
Input recommendation	<10 ng gDNA
Fragmentation	Not required
Read length	Full length 16S gene (~1.5 kb)

Find out more at **nanoporetech.com/prepare**

Enrich custom regions of interest using prepared locus-specific amplicons

Amplicon: Rapid Barcoding Kit

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

Amplicon: Ligation Sequencing Kit

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

View amplicon protocols at: **nanoporetech.com/documentation**

Target viral genomes using tiled amplicon sequencing

Rapid Barcoding Kit 96 with Midnight RT PCR Expansion

End-to-end protocol preparation time	~280 minutes + RT-PCR
Input recommendation	8 µl RNA
Fragmentation	Not required
Read length	Enriched for whole viral genome

End-to-end protocol available: PCR tiling of SARS-CoV-2 virus

Native Barcoding Kit 24 or 96

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: Ligation sequencing of influenza whole genome

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 regions highly enriched for CpGs in the human genome

Targeted method: reduced representation methylation sequencing (RRMS) via adaptive sampling.

Performed during sequencing; targeted library prep not required.

Ligation Sequencing Kit

Preparation time	~60 minutes
Input recommendation	1 µg gDNA
Fragmentation	Optional; recommended for inputs of 100–500 ng
Read length	Equal to fragment length

Enrich genomic regions of any size — from a single gene to chromosome-scale regions

Targeted method: enrichment/depletion of custom targets via adaptive sampling.

Performed during sequencing; targeted library prep not required.

Native Barcoding Kit 24 or 96

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	~140 minutes
Input recommendation	1 µg total RNA or 300 ng poly(A)+ RNA
Fragmentation	Not required
Read length	Equal to RNA length

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

From sample to answer

Preparation

Should I amplify my samples?

Visit the targeted sequencing page: nanoporetech.com/applications/targeted-sequencing

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

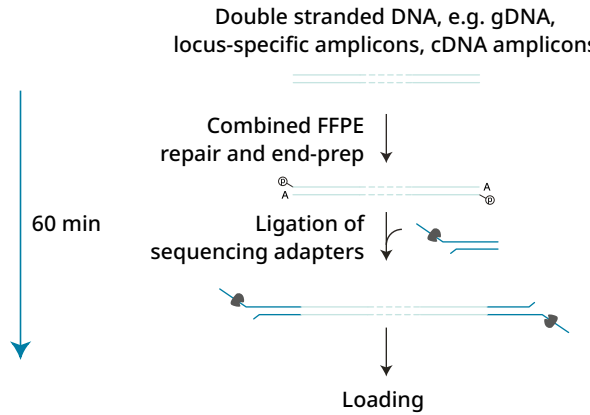
Which PCR-based approach should I choose for custom targets?

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

A range of methods are 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.

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



From sample to answer

Sequencing

Which sequencing device should I choose?

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

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 individually addressable 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 devices enable sequencing on two independently addressable flow cells to provide high-output sequencing for every lab, while the PromethION 24 offers sequencing on up to 24 independently addressable flow cells, providing the capacity to sequence thousands of targets in parallel.

Left to right: PromethION 2 Integrated, PromethION 24, MinION Mk1D, PromethION 2 Solo, and GridION

Analysis

How do I enrich samples during sequencing with adaptive sampling?

Visit the Adaptive Sampling Catalogue (requires Community login):
community.nanoporetech.com/adaptive_sampling_catalogue/

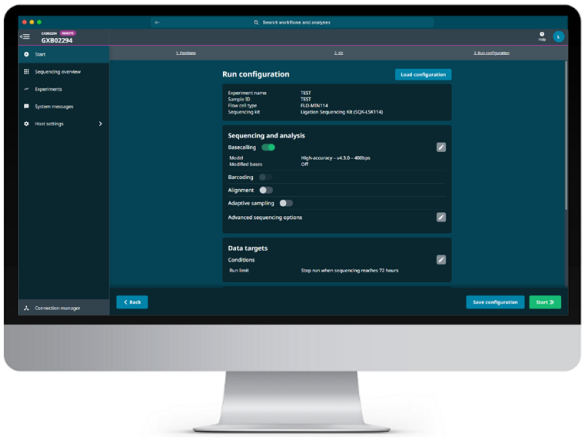
View the adaptive sampling guide:
nanoporetech.com/document/adaptive-sampling

Adaptive sampling is integrated into MinKNOW, where you can select whether you wish to enrich or deplete sequences comprising either selected regions (e.g. a gene or genes of interest) or whole genomes (e.g. those of abundant organisms to deplete in a metagenomic sample). These sequences are uploaded in the form of a reference file and an optional 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, adaptive sampling will reject all sequences that are not present in the reference or BED file, 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 BED files should target less than 5% of the sample; targeting over 10% will reduce the enrichment values obtained.

To browse adaptive sampling BED files submitted by members of the Nanopore Community, or to submit your own, please visit the Adaptive Sampling Catalogue.

Setting up adaptive sampling via MinKNOW



From sample to answer

Analysis

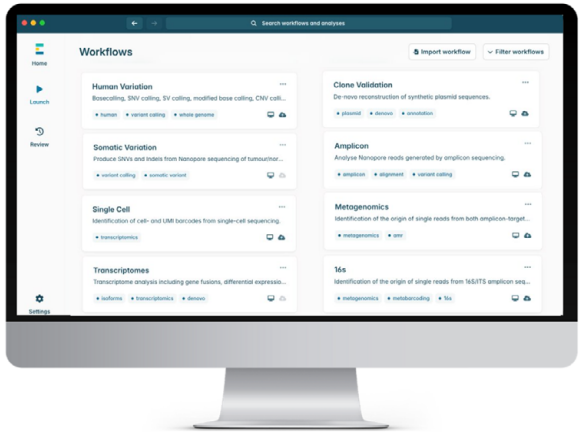
How can I analyse my data?

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

View EPI2ME workflows:
labs.epi2me.io/wfindex/

There are many tools available for the analysis of nanopore sequencing reads, developed both by Oxford Nanopore Technologies and by the Nanopore Community. EPI2ME™ provides analysis workflows that are available as cloud-based or locally run solutions for real-time or post-run analysis. It has an intuitive user interface and can be used without command line experience. This includes bioinformatics workflows for the analysis of microorganisms and AMR genes, and human variants, including SNVs, SVs, and methylation.

Data analysis workflows available on EPI2ME



Analysis

How can I assess methylation in my target regions?

Find out more about RRMS:
labs.epi2me.io/rrms2022.07

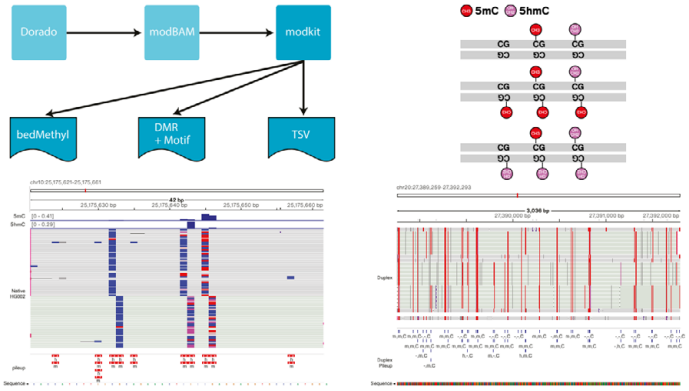
Find Modkit on GitHub:
github.com/nanoporetech/modkit

With adaptive sampling, targets are enriched without PCR, preserving methylation, which 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 Technologies and is integrated into EPI2ME workflows (e.g. wf-human-variant). This software enables downstream methylation analysis, such as methylation aggregation, pattern visualisation, exploration of differentially methylated regions, and automatic identification of DNA methylation motifs.

Oxford Nanopore offers direct detection of methylated cytosines using RRMS by combining methylation calling with adaptive sampling.

Downstream methylation analysis from nanopore sequencing data using Modkit



Case studies

Case study 1: enriching for low-abundance bacterial species in mixed microbial samples using adaptive sampling

Sequencing the genomes of interest from a mixed metagenomic sample can be challenging, especially where some species are not as well represented in the sample as others. This can result in the majority of sequencing data representing abundant genomes, with insufficient data for rarer genomes in the sample to ensure a good depth of coverage. With nanopore sequencing, adaptive sampling can retain or reject reads from specific genomes and regions of interest, dynamic adaptive sampling can, in addition to this, continuously evaluate experimental parameters, such as coverage or genotype uncertainty, and retain or eject the strand based on real-time information from the sequencing run.

BOSS-RUNS (benefit-optimising short-term strategy for read until nanopore sequencing) is a dynamic adaptive sampling tool, developed by EMBL and the University of Nottingham, that allows for real-time assessment of the value of prospective fragments for sequencing[18]. To determine if dynamic adaptive sampling could be used to enrich for rarer genomes in a mixed sample, Weilguny *et al*. used BOSS-RUNS to sequence the Zymobiomics microbial mixture (Zymo Research), comprised of eight differentially abundant species[18]. The most abundant species could be resolved after only a few minutes, and after initially accepting any read from any genome, reads from the most abundant bacteria that had already been resolved were rejected. This resulted in an enrichment for the rarer species during the run, leading to a redistribution in coverage depth from the most abundant to the least abundant species. For example, sequencing yield for *Escherichia coli*, which comprised only 0.1% of the input DNA, was increased by 3.9 times with BOSS-RUNS compared with a control run.

The authors concluded that potentially in the future, 'the resulting reduction in the time-to-answer or increased information gain might be critical in a clinical setting or in pathogen surveillance'.

Read the publication (Jan 2023):
nanoporetech.com/dynamic- adaptive-sampling

Case study 2: solving the genetic cause of a familial DCM case after nearly four decades

Dilated cardiomyopathy (DCM) is a heart muscle disease that causes the heart to enlarge, making pumping inefficient[19]. It is one of the leading causes of heart failure, sudden cardiac death, and arrhythmias[20], with approximately 30% of DCM cases being familial[21] and more than 50 different associated genes, including variants of unknown significance[22].

Sedaghat-Hamedani *et al*. investigated the genetic aetiology of a four-generation family with familial DCM[20]. For nearly four decades, the family underwent genetic testing to identify the pathogenic variant, starting with deep phenotyping, which suggested a laminopathy. However, neither short-read nor Sanger sequencing could identify a pathogenic *LMNA* variant across all affected family members.

High-coverage short-read sequencing revealed a potential splice variant on the *LMNA* allele, but it was of unknown significance. For functional testing, induced pluripotent-stem-cell-derived cardiomyocytes were cultured and RNA was extracted. Short-read sequencing revealed that *LMNA* variant carriers had significantly lower LMNA expression levels. Targeted nanopore sequencing was then utilised, revealing that the variant generated nonsense mRNA decay — therefore causing haploinsufficiency, explaining the low LMNA expression levels.

This study demonstrates the importance of multiomic approaches for accurately identifying complex genetic disease mechanisms and highlights that 'conventional techniques that yield a negative result should not rule out genetic causes ... instead, [they should be] re-evaluated with advanced techniques'. Here, nanopore sequencing unlocked isoform-level information, enabling Sedaghat-Hamedani *et al*. to access the allele-specific expression of the LMNA variant to characterise the mutation and solve the genetic aetiology of this familial DCM case.

Read the publication (Oct 2022):
nanoporetech.com/LMNA-splice-variant/

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19. Medline Plus. Familial dilated cardiomyopathy. https://medlineplus.gov/genetics/condition/familial-dilated-cardiomyopathy/ (2017) [Accessed: 08 January 2025]

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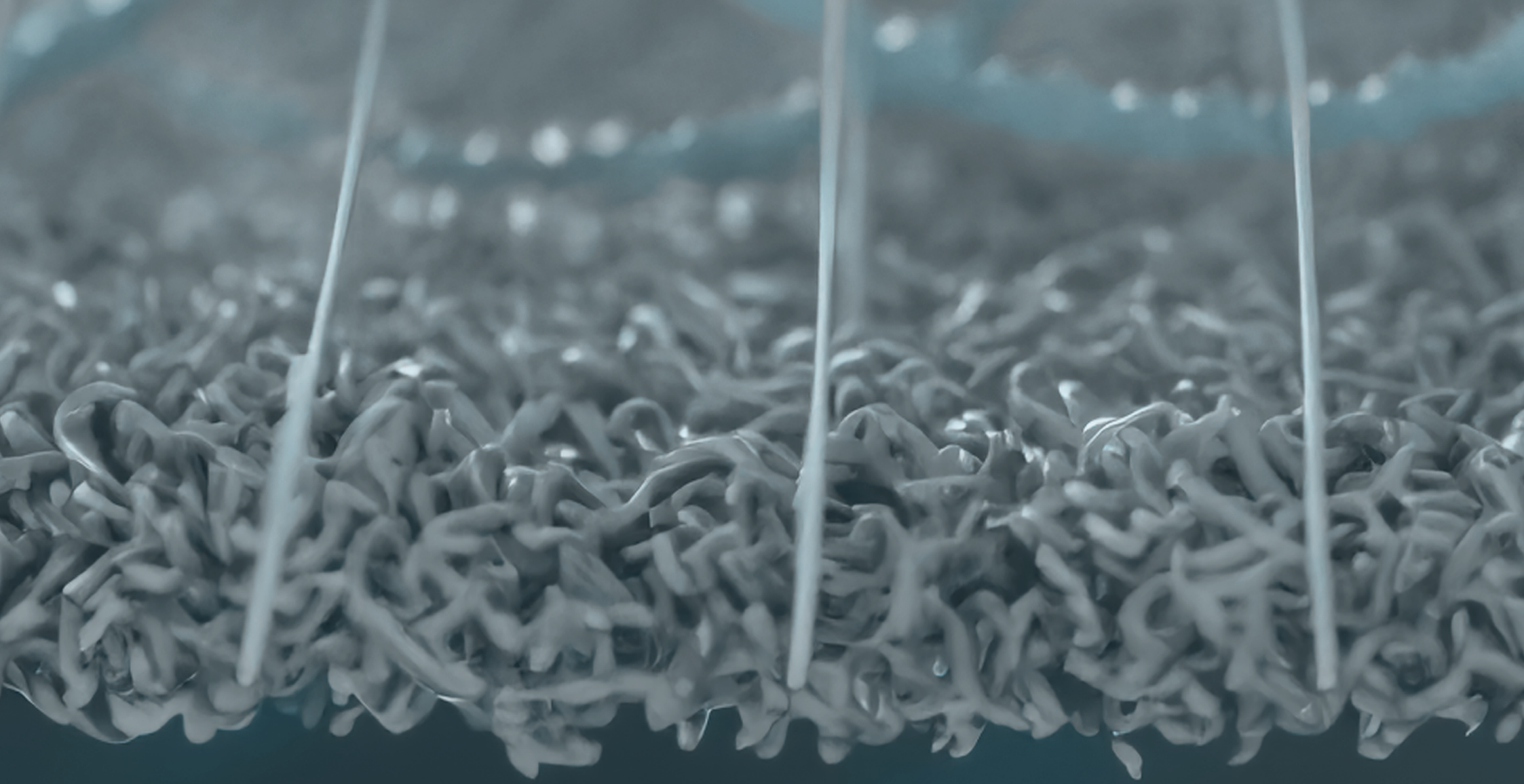
21. Kayvanpour, E. et al. Genotype-phenotype associations in dilated cardiomyopathy: meta-analysis on more than 8000 individuals. *Clin. Res. Cardiol*. 106(2):127–139 (2017). DOI: https://doi.org/10.1007/s00392-016-1033-6

22. Haas, J. et al. Atlas of the clinical genetics of human dilated cardiomyopathy. *Eur. Heart J*. 36(28):1123–35a (2014). DOI: https://doi.org/10.1093/eurheartj/ehu301



Oxford Nanopore Technologies

Find out more at
nanoporetech.com/targeted

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