Real-time enrichment of a comprehensive pharmacogenomic panel with Oxford Nanopore sequencing

Accurate, rapid identification of pharmacogenomic (PGx) variants can provide information critical to understanding an individual's response to pharmaceutical compounds, holding the potential for personalised medicine. However, the limited resolution of legacy microarray and short-read sequencing methods necessitates either analysis of a small subset of PGx targets — potentially missing important variants — or a complex cascade of assays, adding to turnaround times and costs.

Oxford Nanopore sequencing delivers accurate, comprehensive analysis of PGx targets from a single sample-to-star-allele workflow. Utilising adaptive sampling[1–4] — a real-time, bioinformatics-based target enrichment technique — it is possible to enrich a comprehensive panel of 375 genes, with no need for additional library preparation steps. Combined with PCR-free sequencing of any-length fragments, this streamlined workflow provides unambiguous allele and variant calls, phased haplotypes, and full resolution of *CYP2D6*, for confident PGx analysis without compromise.

Here we present an end-to-end workflow for the rapid characterisation of PGx targets from a blood research sample, using adaptive sampling for real-time target enrichment on a PromethION™.

Extraction: obtaining high molecular-weight DNA

View documentation, including protocols and best-practice guidance: nanoporetech.com/documentation

When starting from human blood research samples, we recommend using the **QIAGEN Puregene Blood Kit** to extract high molecular-weight DNA, then using a **Covaris g-TUBE** to shear the DNA to an average fragment length of 10 kb.

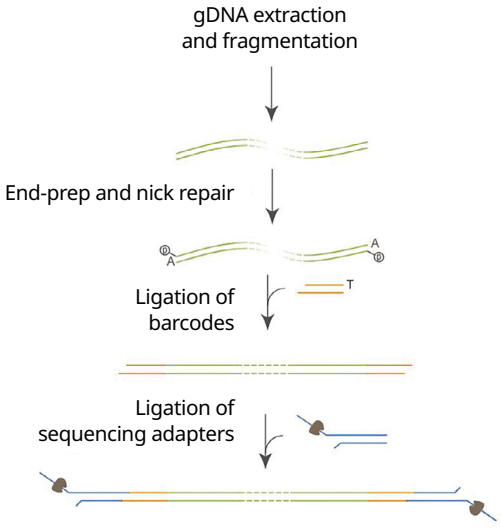
The sample-to-answer protocol also features verified extraction methods for human cell samples and saliva, as well as guidance on how to quality check your samples for best results.

Library preparation: preparing whole native DNA samples for multiplexed sequencing

Learn more about Oxford Nanopore library prep: nanoporetech.com/prepare

No special enrichment or depletion steps are required to prepare your library, thanks to the use of adaptive sampling for target enrichment — instead, the whole genomic DNA sample is prepared in a fast, simple workflow.

To prepare samples for multiplexed sequencing, use the **Native Barcoding Kit** (24 or 96). This PCR-free method features a ligation-based approach to attach a unique barcode to each sample, followed by pooling of the samples to be run on the same flow cell, then rapid attachment of sequencing adapters. Taking only around 145 minutes, the technique preserves long fragments of DNA for targeted sequencing. You can multiplex up to four samples on a single **PromethION Flow Cell**.



Real-time targeted sequencing:
enriching for PGx targets with adaptive sampling

Learn more about PromethION devices:
nanoporetech.com/promethion

For high depth of coverage across the PGx targets, sequence your library on one **PromethION Flow Cell** on any device in the PromethION range. The **PromethION 24** enables sequencing on up to 24 independent flow cells, while the **PromethION 2** devices provide sequencing on up to two flow cells, offering scalable options for the throughput you require.

Adaptive sampling is set up using **MinKNOW™**, the intuitive software that controls Oxford Nanopore sequencing devices. Everything you need to set this up will be supplied in a data bundle, which you will receive after registering. In MinKNOW, simply select 'enrich' mode, then input the provided BED file containing the target coordinates, the reference genome, and, optionally, a sample sheet. During sequencing, the software will then selectively sequence on-target fragments from the PGx panel while rejecting off-target fragments.

To maximise output, we recommend washing and reloading the flow cell every 24 hours, for a total of three sequencing runs, using the **Flow Cell Wash Kit**. You will have sufficient library for all three runs from the previous step.

Learn more about adaptive sampling: nanoporetech.com/blog-adaptive-sampling

Analysis:
calling and phasing PGx variants with EPI2ME™

Discover streamlined data analysis with EPI2ME:
nanoporetech.com/epi2me

Your targeted sequencing data is analysed using the **EPI2ME** platform, which features data analysis workflows for all levels of expertise. The EPI2ME PGx workflow, **wf-pgx**, delivers all-in-one analysis of 375 PGx targets, including *CYP2D6*, in a single workflow. After registering, you will receive a link to download the 2ME file from which you can install the workflow.

From an input BAM file, the pipeline provides single nucleotide variant (SNV) and insertion/deletion (indel) calling across the targets, while the tool **PharmCAT**[5] generates star allele calls. The Oxford Nanopore tool **Chinook**[6] provides comprehensive variant calling for *CYP2D6*, encompassing SNVs, indels, copy number variants, and structural variants, plus identification of hybrid genes and whole-gene deletions.

Register your interest in the PGx workflow: nanoporetech.com/pgx-ryi

View the end-to-end protocol: nanoporetech.com/pgx-adaptive-sampling-protocol

References:

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