Oxford Nanopore sequencing of pharmacogenomic (PGx) loci delivers unambiguous variant calling in a single assay

Featuring highly complex structural variants (SVs), pseudogene homology, and copy number variants (CNVs), PGx loci are incredibly challenging to resolve using conventional microarrays or short-read sequencing. Oxford Nanopore sequencing provides the solution to resolve these complexities, by providing any-length reads for calling all variants in a single assay, via an accessible, scalable platform.

The following highlighted publications showcase how researchers are taking advantage of this technology, focusing on targeted analysis through either sample prep-based enrichment, or adaptive sampling — a fast and flexible method unique to Oxford Nanopore devices that involves software-controlled enrichment.

Sample prep-based targeted enrichment

Featured publication	Workflow	Research summary
Characterisation of complex structural variation in the *CYP2D6-CYP2D7-CYP2D8* gene loci using single-molecule long-read sequencing Turner, A.J. et al. *Front. Pharmacol.* 14:1195778 (2023).	**Enrichment method:** CRISPR-Cas9 (PCR-free) **Device:** MinION™	**Aim:** develop an improved method to characterise complex SVs and CNVs in the *CYP2D6-CYP2D7-CYP2D8* loci, in a single assay. **Results:** the researchers achieved full haplotype phasing and precise characterisation of *CYP2D* SVs. Novel *CYP2D6* suballeles were identified, and complex diplotypes were resolved in multiple samples. The team uncovered 17 *CYP2D7* and 18 *CYP2D8* unique haplotypes. They also determined the haplotype structure of the entire region for all samples.
Approach for phased sequence-based genotyping of the critical pharmacogene dihydropyrimidine dehydrogenase *(DPYD)* Ambrodji, A. et al. *Int. J. Mol. Sci.* 25(14):7599 (2024).	**Enrichment method:** PCR **Device:** GridION™	**Aim:** develop a cost-effective method to genotype the complex *DPYD* gene. **Results:** all *DPYD* variants detected *a priori* were identified. Fully phased genotypes were obtained for all samples, overcoming the limitations of conventional genotyping methods. An additional 11 heterozygous genotypes — previously missed by standard methods — were detected, then validated by orthogonal methods. As few as 619 high-quality, full-length reads were needed to reliably call and phase variants.

Adaptive sampling-based targeted enrichment

Featured publication	Workflow	Research summary
Targeted haplotyping in pharmacogenomics using Oxford Nanopore Technologies' adaptive sampling Deserranno, K. et al. *Front. Pharmacol*. 14:1286764 (2023).	**Enrichment method:** Adaptive sampling **Device:** PromethION™ 24	**Aim:** assess the potential of adaptive sampling on an extended panel of 1,036 genes associated with drug response, to determine its feasibility for cost-effective PGx gene analysis. **Results:** calling and phasing of single nucleotide variants (SNVs), indels, and SVs was performed successfully. Even with three samples multiplexed on a single flow cell, high recall (99.35%) and precision (99.84%) were obtained for calling of targetedPGx variants.
Cas9 targeted nanopore sequencing with enhanced variant calling improves *CYP2D6-CYP2D7* hybrid allele genotyping Rubben, K. et al. *PLoS Genet*. 18(9):e1010176 (2022).	**Enrichment method:** Adaptive sampling + CRISPR-Cas9 **Device:** GridION	**Aim:** develop a more accurate, complete method, versus current approaches, for genotyping complex PGx regions such as the highly polymorphic *CYP2D6* gene. **Results:** *CYP2D6-CYP2D7* hybrid alleles were accurately identified via an optimised Oxford Nanopore sequencing-based assay and tailored analysis pipeline for simultaneous SNV and SV detection. Novel SVs and SNVs were also detected that were missed by other genotyping methods.

'We conclude that PGx based on targeted [long-read sequencing] is a valuable tool to advance the implementation of personalized medicine.' Deserrano *et al.* 2023

Find out more about pharmacogenomic sequencing with Oxford Nanopore: nanoporetech.com/pgx

Additional references

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