Oxford NANOPORE Technologies
APPLICATIONS

Targeting and comprehensive characterization of pharmacogenes using Oxford Nanopore Technologies' adaptive sampling

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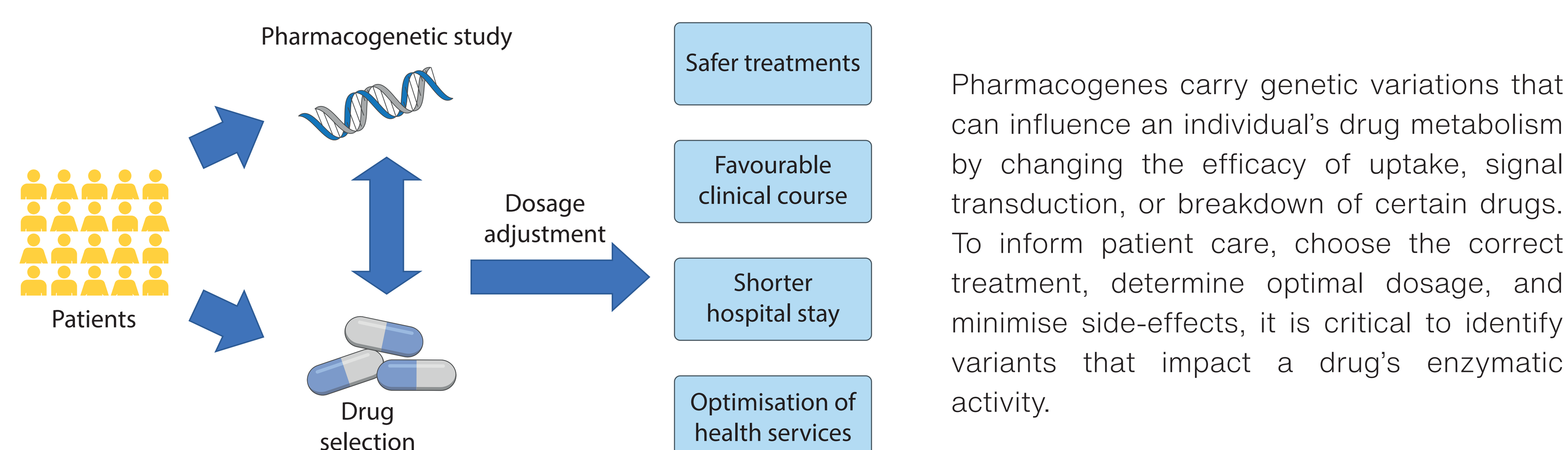
Abstract

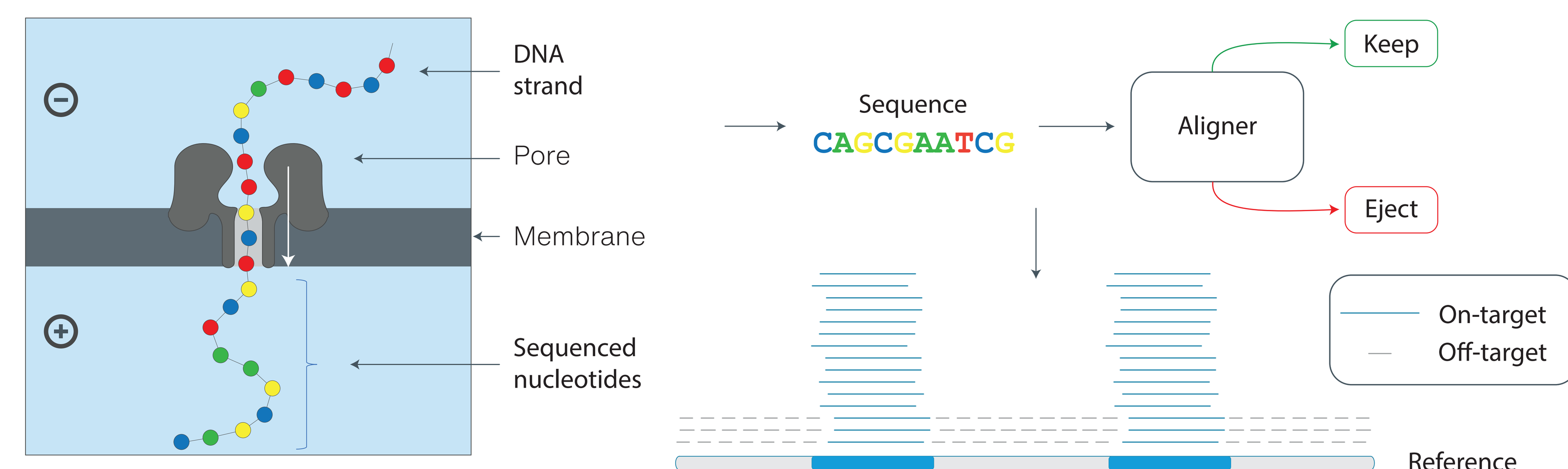
Pharmacogenomics (PGx) is the study of how a person's genome affects drug response. Adaptive sampling (AS) is a fast, flexible, and precise method to enrich for regions of interest by depleting off-target regions during sequencing itself, with no enrichment required during sample preparation. Here, we demonstrate enrichment via adaptive sampling of a PGx gene panel with >278 unique pharmacogenetic targets sourced from the FDA, PharmGKB, and the Clinical Pharmacogenetics Implementation Consortium (CPIC). Samples were analyzed using the wf-human-variation Epi2Me Labs pipeline from Oxford Nanopore Technologies, which called small variants with *Clair3*, structural variants with *Sniffles2*, and phased reads with *whatshap*. We show that coverage is sufficiently high for high accuracy star (*) allele calls on five samples multiplexed on a single flowcell.

1. Background



Pharmacogenes carry genetic variations that can influence an individual's drug metabolism by changing the efficacy of uptake, signal transduction, or breakdown of certain drugs. To inform patient care, choose the correct treatment, determine optimal dosage, and minimise side-effects, it is critical to identify variants that impact a drug's enzymatic activity.

2. Adaptive Sampling



Adaptive sampling (AS) is a software-based approach to enrich regions of interest by uploading a file of target genes and their genomic coordinates. During sequencing, strands are basecalled and mapped to a reference genome in real time. Strands that align outside of the target regions within the first 400 base pairs are ejected as they are sequenced, while strands that are on-target are allowed to sequence completely.

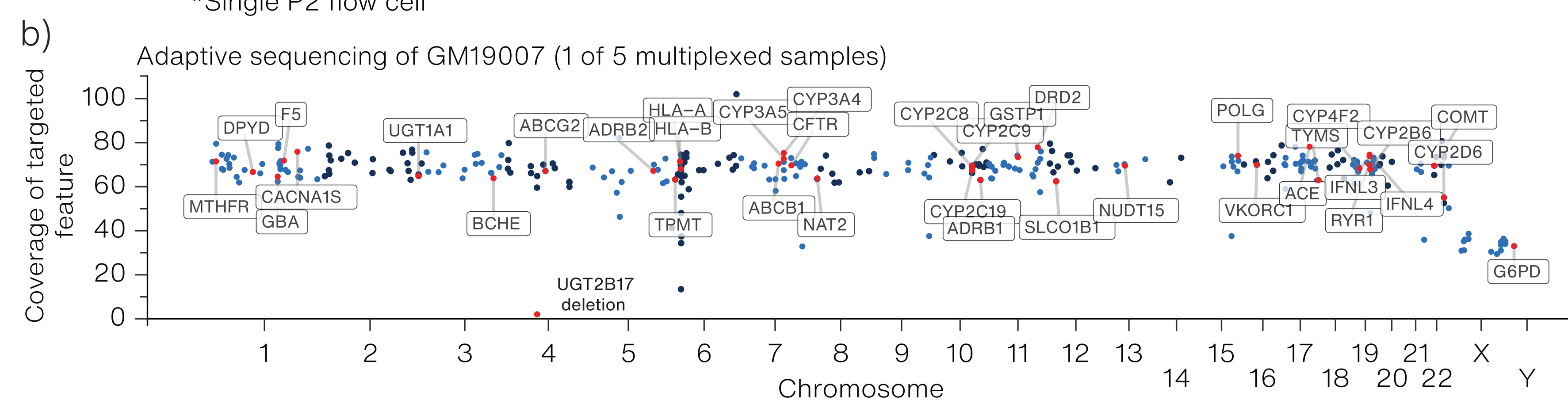
3. Enrichment of PGx targets on PromethION™ 2 solo

a)

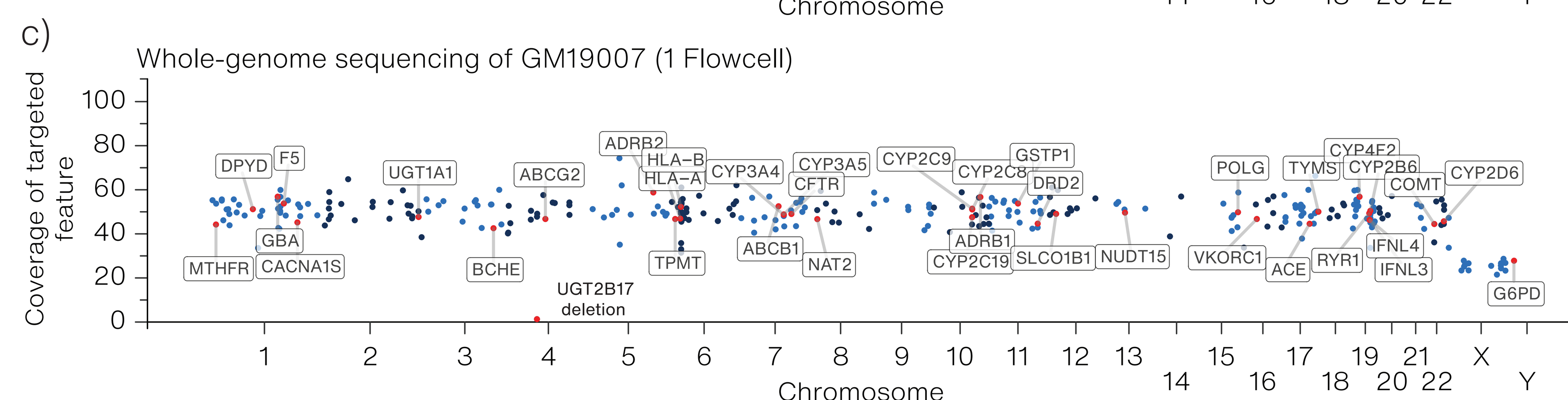
	Sample	Mean on-target coverage*	On-target read N50 (kb)	% on-target reads	% on-target yield	On-target yield (Gb)	Total yield (Gb)	Fold enrichment over WGS
Adaptive sampling (5 barcodes)	GM19785	57x	8.0	1.5%	14.6%	2.7	18.5	6
	GM19007	65x	8.1	1.5%	14.3%	3.0	21.5	7
	GM20296	58x	8.2	1.5%	14.7%	2.7	18.5	6
	HG01190	54x	7.9	1.5%	13.9%	2.6	18.5	6
	HG00276	61x	7.9	1.5%	14.3%	2.9	20	6

*Single P2 flow cell

b)



c)



Five patient-derived cell lines were barcoded and sequenced on a PromethION 2 Solo using the R10 Native Barcoding Sequencing Kit. AS gave 6-fold enrichment over whole-genome sequencing, with on-target read lengths of 8 kb (a). AS enabled all PGx genes from the panel to be enriched to ~59x coverage per barcode for variant calling with no drop-out for any target genes (b). The coverage for each of the five samples is comparable to the coverage from whole genome sequencing on a single sample per flowcell (c).

4. PharmCAT star (*) allele calling of patient-derived samples

Gene	HG00276	NA18518	NA18564	NA19007	NA19109	NA19174	NA19785	NA20296
CYP2B6	*2/*4	*1/*6	*1/*1	*1/*23	*1/*6	*6/*18	*1/*5	*1/*2
CYP2C19	*1/*1	*2/*17	*2/*3	*1/*1	*17/*17	*1/*2	*1/*1	*1/*1
CYP2C9	*1/*2	*1/*1	*1/*1	*1/*1	*1/*1	*1/*1	*1/*1	*1/*1
CYP3A5	*3/*3	*1/*6	*1/*1	*3/*3	*1/*3	*1/*6	*1/*3	*1/*6
CYP4F2	*1/*5	*1/*2	*3/*4	*1/*3	*1/*1	*1/*1	*3/*3	*1/*5
DPYD	*1 / *1	*1/[*9A + *5]	*9A/*5	*1/*1	*9A/c.1218G>A	*1/*1	*1/*1	*1 / *9
SLCO1B1	*1/*15	*1/*37	*1/*37	*37/*37	*15/*37	*1/*27	*1/*37	*27/*37
TPMT	*1/*16	*1/*1	*1/*1	*1/*1	*1/*1	*1/*1	*1/*1	*1/*3C

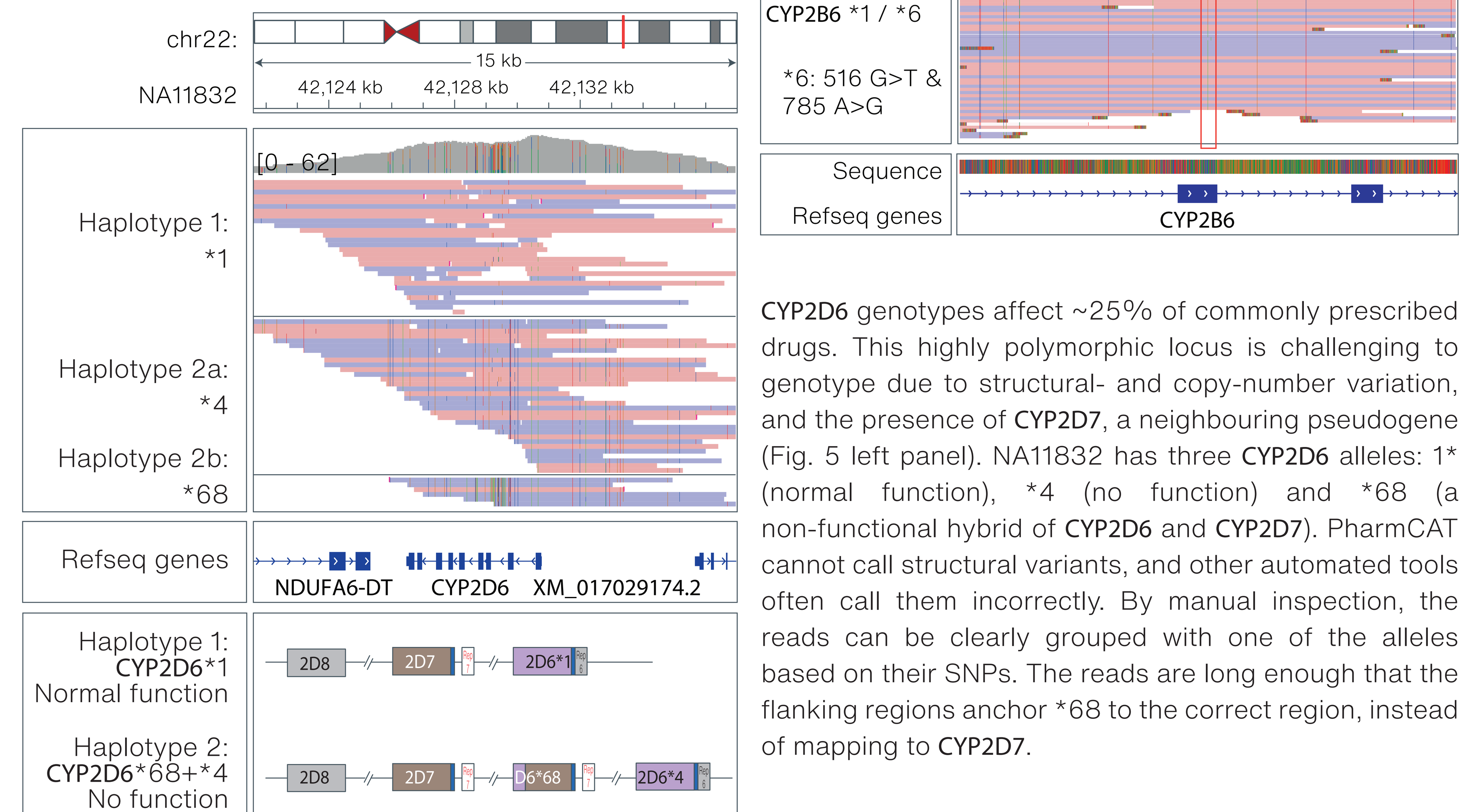
(Column header: Sample)

Legend: Matches GeT-RM and 1KGP; Matches GeT-RM but not 1KGP; Agrees with 1KGB but was unphased by 1KGB or Nanopore; Agrees with 1KGB (GeT-RM allele definition is outdated)

We performed star (*) allele calling with PharmCAT on Illumina VCFs from the 1000 Genomes Project (1KGP) and Nanopore VCFs from wf-human-variation. We compared these calls to those from the Genetic Testing Reference Material Coordination Program (GeT-RM). All variant calls agreed with 1KGP, GeT-RM, or both. In **CYP2B6**, some calls were not concordant with 1KGP because the defining SNP is in a region with lower mappability for short reads.

5. Genotyping heterozygous SNPs and CYP2D6 structural variants

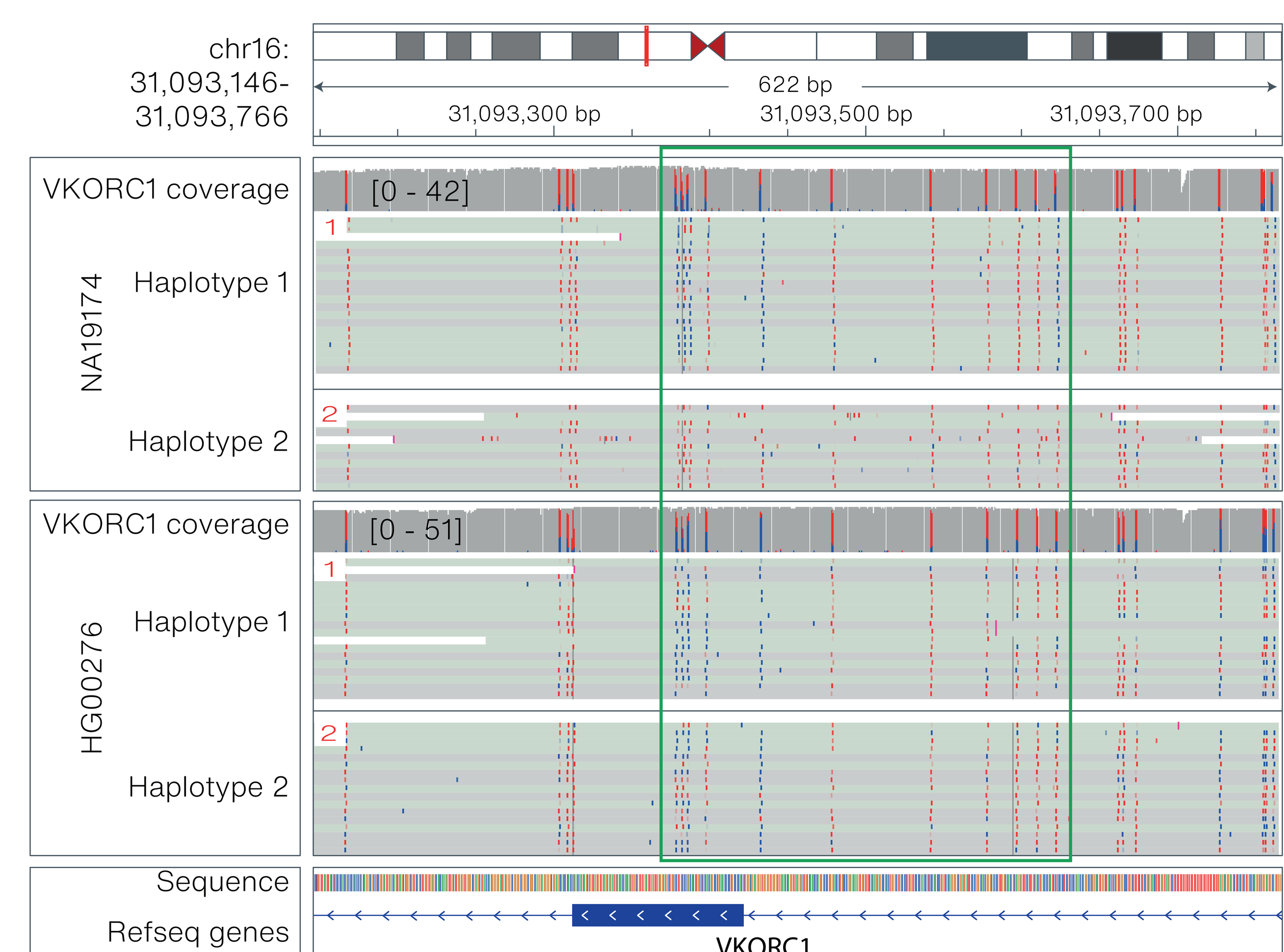
The CYP2B6*6 allele is defined by the presence of two non-synonymous mutations, 516G>T and 785A>G, resulting in decreased function. In short read data, 785A>G falls in a low-mappability region due to the similarity between **CYP2B6** and the pseudogene **CYP2B7** (Fig. 5 right panel). Alignments in this area have decreased MAPQ and some mismapping/clipping; the variant does not pass 1KGP's filters, leading to a miscall of *9. In contrast, Nanopore reads are confidently mapped, phased and match GeT-RM's call of *6.



CYP2D6 genotypes affect ~25% of commonly prescribed drugs. This highly polymorphic locus is challenging to genotype due to structural- and copy-number variation, and the presence of **CYP2D7**, a neighbouring pseudogene (Fig. 5 left panel). NA11832 has three **CYP2D6** alleles: 1* (normal function), *4 (no function) and *68 (a non-functional hybrid of **CYP2D6** and **CYP2D7**). PharmCAT cannot call structural variants, and other automated tools often call them incorrectly. By manual inspection, the reads can be clearly grouped with one of the alleles based on their SNPs. The reads are long enough that the flanking regions anchor *68 to the correct region, instead of mapping to **CYP2D7**.

6. Haplotyped methylation calling provides additional insight for PGx

Existing automated tools do not incorporate methylation signals in PGx analysis. Because adaptive sampling can enrich without amplification, the reads retain methylation signals. We basecalled reads with *Dorado* to call methylation across VKORC1, an important protein within the vitamin K cycle. NA19174 shows allele-specific methylation within exon 4, and differs overall from the methylation pattern in HG00276. While variants of pharmacogenes and their phenotypes are well established, methylation may play an under-explored role in drug metabolism.



References

1. Sangkuhl K et al. (2020) Clinical Pharmacology and Therapeutics 107(1):203-210
2. Byrska-Bishop M et al. (2022) Cell 185(18):3426-3440.e19
3. Pratt VM et al. (2015) Journal of Molecular Diagnostics 18(1):109-23

Workflow

Conclusion

Overall, we demonstrate targeted nanopore sequencing with AS as a useful tool for the enrichment of PGx gene panels, resulting in high coverage for haplotyped variant calling including structural variations and methylation. Using the variant calls from the Epi2Me Labs wf-human-variation pipeline results in accurate star (*) allele calling with PharmCAT in all samples. The strategy allows for a PGx gene panel that can be easily modified to encompass additional genes of interest as novel pharmacogenomic variants are discovered.

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