



Comprehensive pharmacogenomics profiling using Oxford Nanopore digital panels or hybridisation capture

Both digital panels and hybridisation capture workflows enable comprehensive pharmacogenomic approaches including improved *CYP2D6* resolution, pharmacogene star allele annotation, scalability, and native methylation signatures

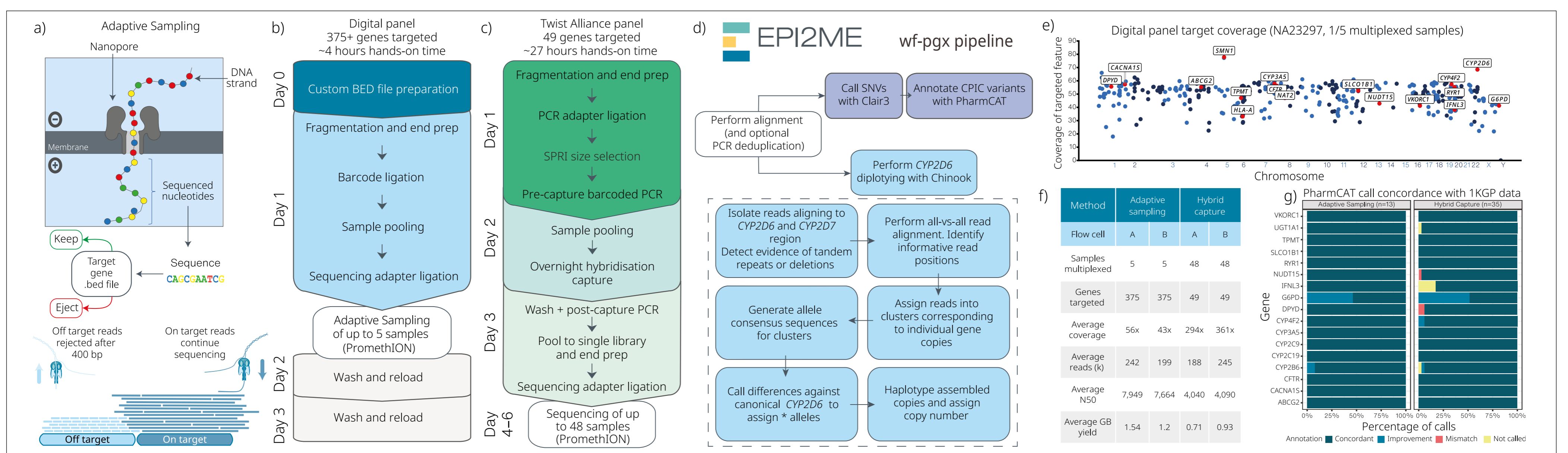


Fig. 1 a) Adaptive Sampling schematic, b) digital panel workflow, c) Twist Alliance Long-Read workflow, d) EPI2ME™ wf-pgx pipeline, e) per-gene coverage, f) run statistics, and g) PharmCAT star allele concordance.

Oxford Nanopore digital panels and the Twist Alliance Long-Read PGx panel both offer scalable library preparation and sequencing approaches, and are compatible with streamlined pharmacogenomic analysis using EPI2ME wf-pgx

Pharmacogenomics (PGx) is the study of how genetic variation affects drug metabolism; pharmacogene variants can affect treatment decisions and patient outcomes. Oxford Nanopore offers multiple methods for targeted enrichment and sequencing of pharmacogenes. Digital panels are a rapid, flexible approach, powered by Adaptive Sampling, to enrich regions of interest during sequencing itself, with no upfront sample manipulation. During Adaptive Sampling (AS), the first ~400 bp of each strand are basecalled and mapped to a reference genome in real time. Off-target strands are ejected, while on-target strands sequence to completion (Fig. 1a). The PGx digital panel targets 375+ genes specified in an easily configurable BED file, and allows multiplexing of up to five samples (Fig. 1b). Alternatively, hybridisation capture using the Twist Alliance Long-Read PGx panel allows highly scalable sequencing of 49 clinically implicated genes in up to 48 samples (Fig. 1c). The EPI2ME wf-pgx pipeline, compatible with both methods, calls star (*) alleles in *CYP2D6* and additional pharmacogene variants with PharmCAT (Fig. 1d). Long reads and sufficient coverage are needed to distinguish between *CYP2D6* homologs; digital panels and hybridisation capture can both produce the >40x depth of coverage required for comprehensive analysis (Fig. 1e, f), with read length N50s of ~8 kb from digital panels, and ~4 kb from hybridisation capture (Fig. 1f). Star allele calling with PharmCAT on Oxford Nanopore wf-pgx VCFs vs. Illumina 1000 Genomes Project (1KGP) VCFs showed 99% agreement or improvement via better phasing (Fig. 1g). Low coverage resulted in some uncalled targets.

Hybrid capture — agree					
Sample	Chinook	Sample	Chinook	Sample	Chinook
HG00156	*5/*5	NA17039	*2/*17	NA18980	*2/*36+*10
HG00276	*4/*5	NA17102	*1/*40	NA18992	*1/*5
HG00373	*2/*2	NA17113	*17x2/*45	NA19109	*2x2/*29
HG00421	*2/*10x2	NA17114	*1/*5	NA19122	*2/*17
HG00423	*10/*10x2	NA17115	*1/*2	NA19143	*10/*45
HG00436	*2x2/*71	NA17204	*1/*35	NA19174	*4/*40
HG00589	*1/*21	NA17227	*1/*9	NA19178	*1/*1
HG01086	*1/*31	NA17230	*4/*41	NA19207	*2x2/*10
HG01190	*68+*4/*5	NA17234	*1/*41	NA19226	*2/*2x2
HG02373	*36+*10/*14	NA17237	*1/*4	NA19239	*15/*17
HG03225	*5/*56	NA17240	*1/*10	NA19785	*1/*13+*2
NA02016	*2x2/*17	NA17244	*2x2/*4x2	NA19819	*2/*4x2
NA06993	*4/*35	NA17247	*1/*2	NA19920	*1/*4x2
NA07348	*1/*6	NA17269	*2/*41	NA21781	*2x2/*68+*4
NA07439	*4x2/*41	NA17287	*1/*83	NA23093	*1/*36+*10
NA10005	*17/*29	NA17288	*1/*1	NA23296	*2x2/*4
NA10847	*1/*41	NA17290	*1/*41	NA23297	*10x2/*17
NA10860	*1/*4N+*4	NA17454	*1x2/*2x2	NA24027	*2x2/*6
NA11832	*1/*68+*4	NA18518	*17/*29	NA24217	*2/*41x3
NA12154	*68+*4/*33	NA18540	*36+*10/*41		
NA12244	*35/*41	NA18552	*1/*14		

Adaptive Sampling — agree			
Sample	Chinook	Sample	Chinook
HG00276	*4/*5		
HG01190	*68+*4/*5		
NA11832	*1/*68+*4		
NA18518	*17/*29		
NA19174	*4/*40		
NA19207	*2/*2x2		
NA19226	*2/*2x2		

Hybridisation capture — improve GeT-RM			
Sample	Chinook	GeT-RM	GeT-RM
NA02016	*2x2/*17	*2xN/*17	
NA16688	*2/*36+*10	*2/*10	
NA17019	*1/*36+*10	*1/*10	
NA17058	*10/*36+*10	*10/*10	
NA17084	*1/*36+*10	*1/*10	
NA17203	*68+*4/*35	*4/*35	
NA17226	*4/*4N+*4	*4/*4	
NA17245	*68+*4/*35	*2/*4	
NA17264	*1/*68+*4	*1/*4	
NA23873	*1/*68+*4	*1/*4	
NA23874	*4/*68+*4	*4/*4	
NA24008	*1/*68+*4	*1/*4	

Hybrid capture — mismatch GeT-RM			
Sample	Chinook	GeT-RM	GeT-RM
HG00463	*10/*36+*10	*36+*10/*36+*10	
NA17232	*2/*35	*2/*2xN	
NA18526	*1/*36+*10	*1/*36x2+*10	
NA23246	*10x2/*36x3	*10x2/*36+*10	

Fig. 2 Agreement between Chinook results and GeT-RM for a) hybridisation capture and b) digital panel with Adaptive Sampling, and c) genotyping disagreements.

Accurate genotyping of complex *CYP2D6* structural variants using targeted assembly

The *CYP2D6* gene, a key metaboliser of numerous medications, is among the most technically challenging pharmacogenes to sequence and analyse. Its proximity to the highly similar pseudogene *CYP2D7* leads to high structural polymorphism, including deletions, tandem duplications, and hybridisation; standard alignment-based variant-calling tools often struggle with this complex structural variation. Our tool, Chinook, uses an assembly-based approach to directly reconstruct individual gene copies, distinguish between *CYP2D6* and *CYP2D7*, and call hybrid alleles. Compared to 77 samples with genotypes published by the Genetic Testing Reference Materials Program (GeT-RM), Chinook agreed in 94% of cases with both hybridisation capture (Fig. 2a) and Adaptive Sampling data (Fig. 2b). Importantly, following manual investigation, we believe that Chinook calls improve upon GeT-RM genotypes for 12 samples, with most cases involving hybrid alleles or CNVs such as *68, *36, and *4N (Fig. 2c).

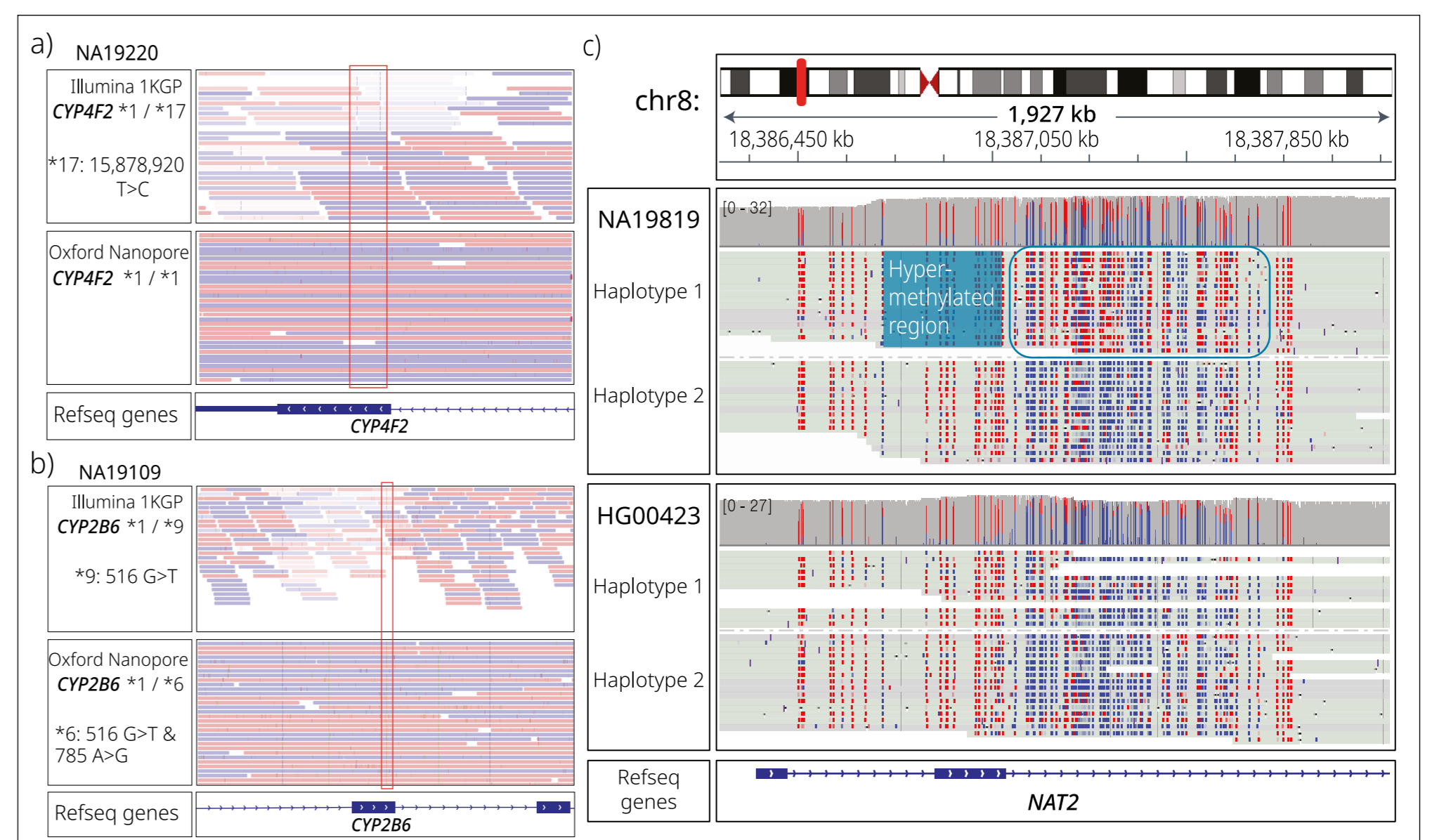


Fig. 3 Short-read mismatching in a) NA19220 of *CYP4F2* reads; darker = higher MAPQ, and b) NA19109 of *CYP2B6* reads, and c) epigenetic variation in *NAT2* reads; red = methylated CpG, blue = unmethylated.

Long reads resolve short-read mapping errors and reveal allele-specific epigenetic variation

From 1KGP short-read sequencing data, two star alleles were miscalled due to low mappability. *CYP4F2* 18629A>G was falsely called due to poorly mapping short reads, shown as pale (low MAPQ) alignments (Fig. 3a). However, stricter filtering introduces errors to *CYP2B6*, where 785A>G falls in a region with high pseudogene homology, again causing short-read mismatching (Fig. 3b). In both cases, long Oxford Nanopore reads were mapped and phased confidently, matching GeT-RM. Additionally, variability in drug responses are only partially explained by genetic sequence variants alone. Digital panels retain per-base methylation information, enabling joint analysis of sequence and epigenetic variation. Haplotype-specific methylation analysis with modkit reveals differentially methylated regions, including *NAT2* promoter hypermethylation in NA19819, which has been linked to anti-tuberculosis drug-induced liver injury (Fig. 3c).