SCALABLE HIFI SEQUENCING WITH *TWIST BIOSCIENCE LONG READ* ALLIANCE PANELS

Quick start your HiFi sequencing projects with pre-designed panels curated by leading researchers

Access many benefits of PacBio® HiFi long-read sequencing at a fraction of the cost with Twist Bioscience long-read sequencing panels. Twist Bioscience's target enrichment technology provides high capture efficiency and exceptional uniformity across target regions. Combined with HiFi sequencing, this enables highly accurate variant calling, including structural variants (SVs) and complex variants, as well as direct haplotype phasing, for your regions of interest. Develop your own fully customizable and scalable panel now, or get started quickly with pre-designed Twist Alliance panels that have been developed by researchers at leading institutions for specific applications. Learn more at **twistbioscience.com/products/ngs/Long-Read-Sequencing-Panels**.

Long-read sequencing at scale:

- Probes optimized for high uniformity and sequencing efficiency
- High-accuracy variant calling of SNPs, SVs, and indels
- Unambiguous haplotype phasing without the need for trios
- Cost-effective and high-throughput, compatible with PacBio Sequel® IIe and Revio™ systems

Twist Alliance *Dark Genes* panel

Several medically-relevant genes lie in so called next-generation sequencing (NGS) "dark regions" of the genome; that is, areas of the genome that are difficult or impossible to sequence or map with short-read sequencing. Due to high sequence similarity with pseudogenes or paralogs, or the presence of repeat elements and segmental duplications, short-read sequencing may result in poor mapping, read depth, and sequencing yield. Discover what you've been missing with the Twist Alliance *Dark Genes* panel, a comprehensive 22 Mb panel of 389 medically-relevant dark genes, including *CYP2D6*, *GBA*, *SMN1/2*, and *PMS2* (figure 1). This panel includes genes missed by short-read NGS that might typically require several platforms to adequately capture, allowing for assay consolidation and cost-effectiveness. **A full list of genes can be found on the Twist Long Read Sequencing Panels website.**

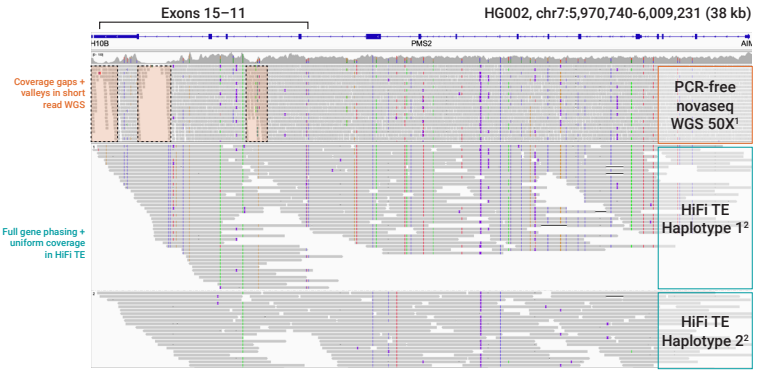

Figure 1. Full-gene phasing and no coverage gaps at *PMS2*. (1) Baid et al (2020) An extensive sequence dataset of gold-standard samples for benchmarking and development. bioRXiv doi: **https://doi.org/10.1101/2020.12.11.422022.** (2) **https://downloads.pacbcloud.com/public/dataset/HiFiTE_Revio/Nov_2022/TwistAllianceDarkGene/**

"The Dark Genes panel enables the accurate detection of genetic diversity in a large and unique set of medically relevant genes. I am confident this workflow will generate significant and novel insights in disease genomics and population-wide genetic studies."

– Fritz Sedlazeck, PhD
Associate Professor Human Genome Sequencing Center, Baylor College of Medicine

Twist Alliance *Long-Read Pharmacogenomics* panel

Pharmacogenomic (PGx) profiles have an impact on medication safety and efficacy. With the Twist Alliance *Long-Read Pharmacogenomics* panel, you can leverage the benefits of HiFi sequencing in a robust, scalable, and cost-effective PGx assay. Stuart Scott, PhD, FACMG, Director of the Stanford Medicine Clinical Genomics Laboratory, highlighted the importance of phasing enabled by the panel, noting "really clean characterization of haplotypes and complete star allele architecture." This is a comprehensive 2 Mb panel that includes 49 genes, the majority of which are captured full-length (introns and exons) (table 1). This panel was designed to capture all 20 current genes with Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines, as well as FDA PGx genes and genes of PGx research interest.

Why pharmacogenomics with PacBio?

- Comprehensive coverage includes SNVs, indels, and SVs
- Direct phasing for unambiguous star allele assignment
- Ancestry-agnostic coverage of actionable PGx genes

CYP genes	HLA	Others			
CYP1A2	***HLA-A***	***ABCB1***	***COMT***	*HTR2C*	***OPRM1***
CYP2B6	***HLA-B***	***ABCG2***	***CTBP2P2***	***IFNL3***	*POLG*
CYP2C19	***HLA-DQA1***	*ADD1*	*DPYD*	***MT-RNR1****	***RYR1***
CYP2C8	***HLA-DRB1***	***ADRA2A***	***DRD2***	***MTHFR***	***SLC6A4***
CYP2C9		***ANKK1***	*F2*	***NAGS***	***SLCO1B1***
CYP2D6		***APOL1***	*F5*	***NAT2***	***TPMT***
CYP3A4		***BCHE***	***G6PD***	***NUDT15***	***UGT1A1***
CYP3A5		*CACNA1S*	***GBA***	***OPRD1***	***UGT2B15***
CYP4F2		*CFTR*	*GRIK4*	***OPRK1***	***VKORC1***
					YEATS4

Table 1: Genes covered in the Twist Alliance Long-Read PGx panel. ***Bold*** denotes full-gene coverage. Underline denotes inclusion in a CPIC Level A guideline. **Full length mtDNA coverage is available for spike-in separately through Twist.

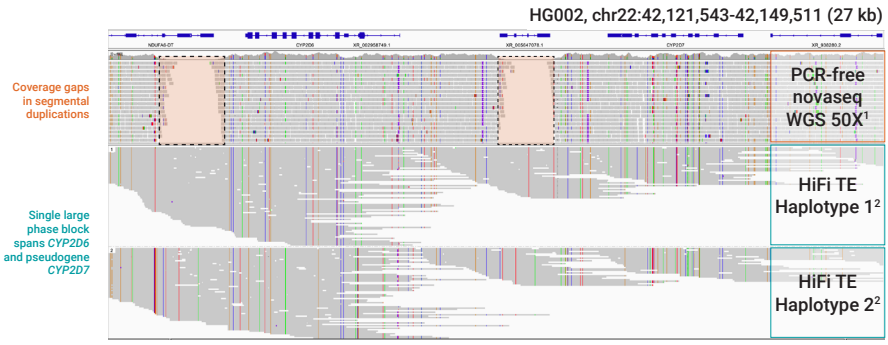

Figure 2. *CYP2D6* region captured by HiFi target enrichment, compared to 50× short-read whole genome. Large phase blocks formed by HiFi reads span the *CYP2D6-CYP2D7* region, allowing for phased pseudogene disambiguation with the highly homologous *CYP2D7* gene, avoiding off-target read mapping as seen with short reads. HiFi reads also sequence through a segmental duplication region between the pseudogenes that is inaccessible to short-read NGS, as seen by the gap in coverage in the orange boxes. (1) Baid et al (2020) An extensive sequence dataset of gold-standard samples for benchmarking and development. bioRXiv doi: **https://doi.org/10.1101/2020.12.11.422022.** (2) **https://downloads.pacbcloud.com/public/dataset/HiFiTE_SqIIe/Oct_2022/TwistAllianceLongReadPGx/**

Panels at a glance

Twist Alliance panel	Number of genes	Samples per Sequel IIe SMRT® Cell 8M	Samples per Revio SMRT Cell	Mean target coverage	Target bases ≥10-fold read depth
Long-read PGx	49	24	72	190-fold	99%
Dark Genes	389	4	12	75-fold	93%

RESOURCES

1. Sample data for both panels can be found at **https://www.pacb.com/connect/datasets/#targeted-datasets**
2. Twist long read resources **https://www.twistbioscience.com/products/ngs/Long-Read-Sequencing-Panels**
3. Application brief **https://www.pacb.com/wp-content/uploads/Application-Brief-HiFi-Target-Enrichment-Best-Practices.pdf**

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