# TARGETED SEQUENCING FOR AMPLICONS – BEST PRACTICES

With PacBio long reads you can easily and cost-effectively sequence full length amplicons that target genes or regions of interest, from several hundred base pairs to kilobase scale. Highly accurate long reads allow you to target all types of variation, from single nucleotide variants and indels, to structural variants. HiFi reads can reveal biology that other technologies miss, like low complexity regions and repeat expansions, GC-rich promoter regions, and insertion sites of transposable elements.

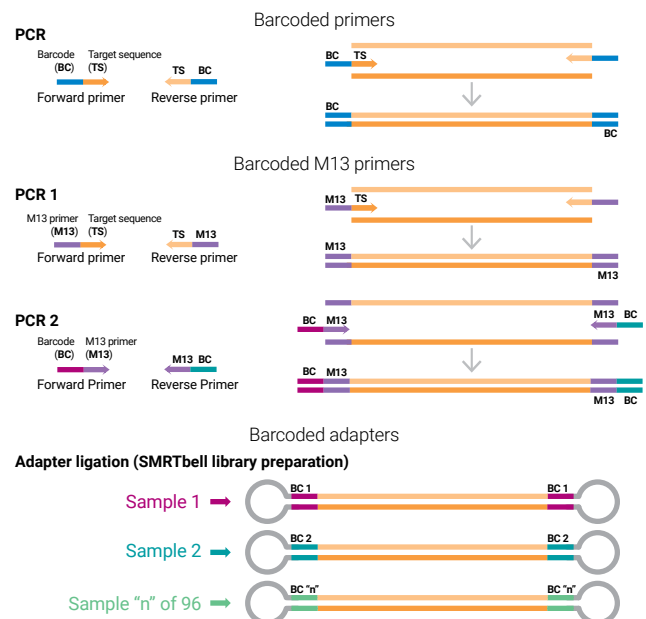## From DNA to target quickly and efficiently
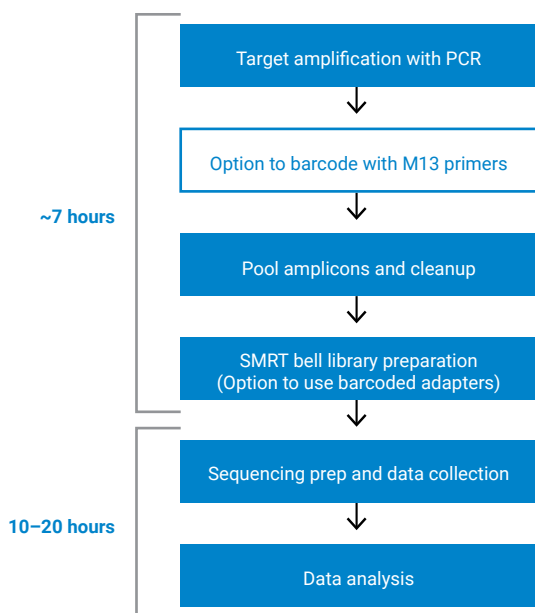
Prepare your DNA for sequencing in less than 1 day with a simple workflow and flexible barcoding options that support up to 384 unique barcodes.

Flexible multiplexing options

Multiplexing is supported with three barcoding options, providing flexibility to incorporate unique sample identifiers during target amplification or library preparation.

Sample preparation recommendations

- Start with high-quality DNA or RNA, as low as 250 ng for small amplicons

- Create SMRTbell® templates from amplicons as small as 250 bp to kilobase scale

- Long and accurate HiFi reads
  - Up to 4M reads on the Sequel® systems[1]
  - Median read quality of Q30

- Sequence to desired coverage based on project needs
  - Target 50-fold coverage for variant detection
  - Increase coverage for minor variant detection (~6000-fold coverage for 1% sensitivity)

- Optimize throughput with flexible barcoding options
  - Amplify targets with one round of PCR using target-specific primers with incorporated barcodes[2]
  - Add barcodes during PCR using a two round design with PacBio's Barcoded M13 primer plate[3]
  - Add barcodes during SMRTbell library prep with PacBio's barcoded SMRTbell adapter plate[4]

- Fast turnaround time
  - Less than one day for sample prep
  - 10–20 hour movies depending on amplicon size[5]

Products to support sample indexing[6]

Strategy	Barcoded primers	Barcoded adapters
Workflow step for barcoding	PCR	SMRTbell library construction
Product name	Barcoded M13 primer plate (102-135-500)	SMRTbell barcoded adapter plate 3.0 (102-009-200)
Unique barcodes	384 dual index	96 single index

Data analysis solutions

- Best-in-class HiFi reads fully characterize genetic complexity — structural variation, rare SNPs, indels, microsatellites, haplotypes, and phasing[7]

- Fast turn around time with on-instrument demultiplexing of HiFi reads on Sequel IIe system[8]

- Output data in standard file formats (BAM and FASTA/Q) for seamless integration with downstream analysis tools

- HiFi reads are compatible with industry-standard analysis tools for variant calling such as Deep Variant and GATK

- Enable reference-free analysis for complex loci like HLA with by pb amplicon analysis[9]

Full length amplicon sequencing of CYP2D6
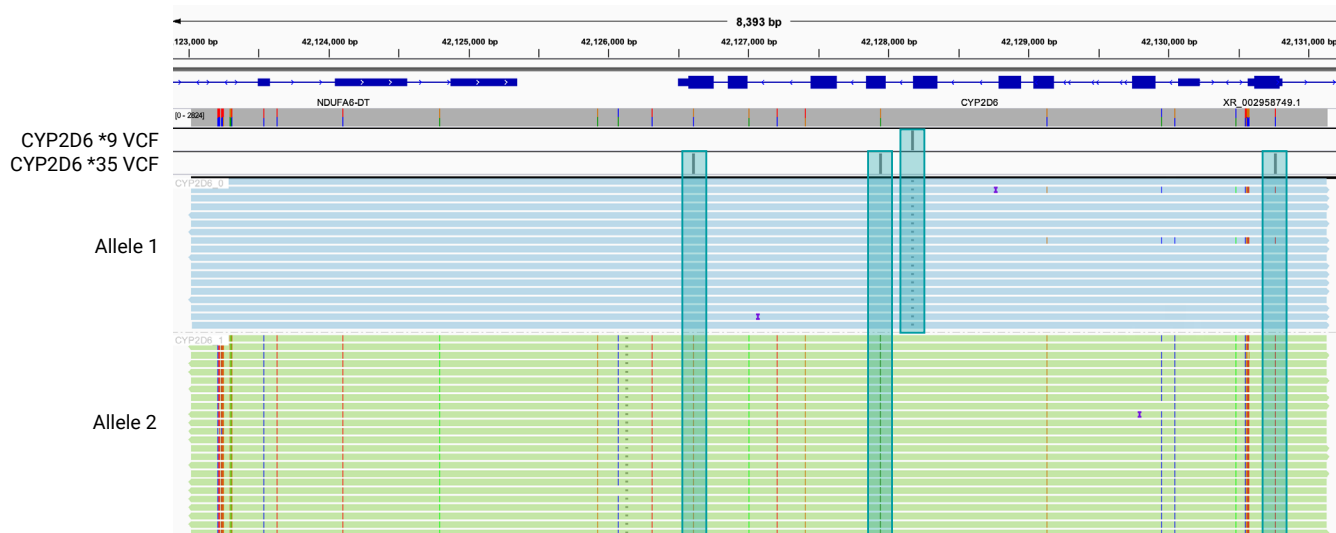
An Integrative Genomics Viewer image highlighting full-length reads across the CYP2D6 region. Over 40 heterozygous variants in an 8.1 kb CYP2D6 amplicon region are fully phased and allow for unambiguous haplotype resolution. Top reads (blue) correspond to the *9 haplotype and bottom reads (green) to *35.

KEY REFERENCES

1. **Sequel system product page** PacBio website
2. **Procedure & checklist – Amplification of full-length 16S gene with barcoded primers for multiplexed SMRTbell® library preparation and sequencing** PacBio literature
3. **Procedure & checklist - Preparing SMRTbell libraries using PacBio barcoded M13 primers for multiplex SMRT sequencing** PacBio literature
4. **Procedure & checklist - Preparing multiplexed amplicon libraries using SMRTbell prep kit 3.0** PacBio literature
5. **Quick reference card** PacBio documentation
6. **PacBio multiplexing products** PacBio website
7. **PacBio blog** PacBio website
8. **SMRT Link user guide (v11.0)** PacBio website
9. **PB amplicon analysis** Github documentation

Learn about targeted sequencing
for amplicons: **pacb.com/target**

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