TARGETED SEQUENCING FOR AMPLICONS
BEST PRACTICES

With Single Molecule, Real-Time (SMRT®) Sequencing and the Sequel® System, you can easily and cost effectively generate highly accurate long reads (HiFi reads, >99% single-molecule accuracy) from genes or regions of interest ranging in size from several hundred base pairs to 20 kb. Target all types of variation across relevant genomic regions, including low complexity regions like repeat expansions, promoters, and flanking regions of transposable elements.

FROM DNA TO TARGET QUICKLY AND EFFICIENTLY

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Amplification Generation</td>
<td>1 Day</td>
</tr>
<tr>
<td>Amplicon QC</td>
<td>1 Day</td>
</tr>
<tr>
<td>Amplicon Pooling (Automatable)</td>
<td>1 Day</td>
</tr>
<tr>
<td>SMRTbell Library Preparation (Automatable)</td>
<td>1 Day</td>
</tr>
<tr>
<td>Anneal Primers and Bind Polymerase to SMRTbell Templates</td>
<td>0.5 Day</td>
</tr>
<tr>
<td>Load and Start Sequencing Run</td>
<td>1 Day</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>0.5 Day</td>
</tr>
</tbody>
</table>

From DNA to a complete, high accuracy answer in just 2.5 days.

SAMPLE PREPARATION RECOMMENDATIONS
- Start with high-quality, nucleic acids, as low as 250 ng for a 250 bp amplicon
- Create SMRTbell® templates from amplicons between 250 bp to 20 kb
- Optimize throughput with flexible barcoding options
  - Amplify PCR products using target-specific primers with incorporated barcodes
  - Add Barcoded Universal Primers into amplicons via a simple 2-step PCR process
  - Attach Barcoded Overhang Adapters during ligation without modifying existing primers
  - Multiplex up to 10,000 samples per SMRT Cell
- Maximize output and turn-around-time with adjustable run parameters
  - For inserts <5 kb, recommend 10-hour movies
  - For inserts >5 kb, recommend 20-hour movies
- Generate HiFi reads
  - Q20 single-molecule accuracy reads
  - Up to 500,000 reads per SMRT Cell 1M
- Sequence to desired coverage based on project needs
  - Target 30-fold coverage for variant detection
  - Increase coverage for minor variant detection (~6,000-fold coverage for 1% sensitivity)

FLEXIBLE MULTIPLEXING OPTIONS

Barcoded Primers

Barcoded Universal Primers

Barcoded Overhang Adapters

Adapter Ligation (SMRTbell Library Preparation)

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

1. For recommended barcode primers: [https://www.pacb.com/multiplexing](https://www.pacb.com/multiplexing)
7. SMRT Analysis Barcoding Overview (v6.0). PacBio Documentation
8. Collaboration with Stuart A. Scott Laboratory, Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai New York, NY.

---

**PACBIO DATA ANALYSIS SOLUTIONS**

- Fully characterize genetic complexity – structural variation, rare SNPs, indels, CNV, microsatellites, haplotypes, and phasing
- Utilize a variety of analysis tools within SMRT Link®
  - Generate HiFi reads (>Q20) using Circular Consensus Sequencing (CCS) mode
  - Perform reference-free analysis of pooled amplicons with Long Amplicon Analysis (LAA)
  - Detect, quantitate, and phase single nucleotide polymorphisms within coding regions using Minor Variants Analysis
- Easily de-multiplex barcodes within SMRT Link®
- SMRT Link GUI supports up to 384 barcodes per sample; command-line supports >384 barcodes
- Output data in standard file formats (BAM and FASTA/Q) for seamless integration with downstream analysis tools
- HiFi reads are compatible with standard analysis tools for variant calling such as GATK