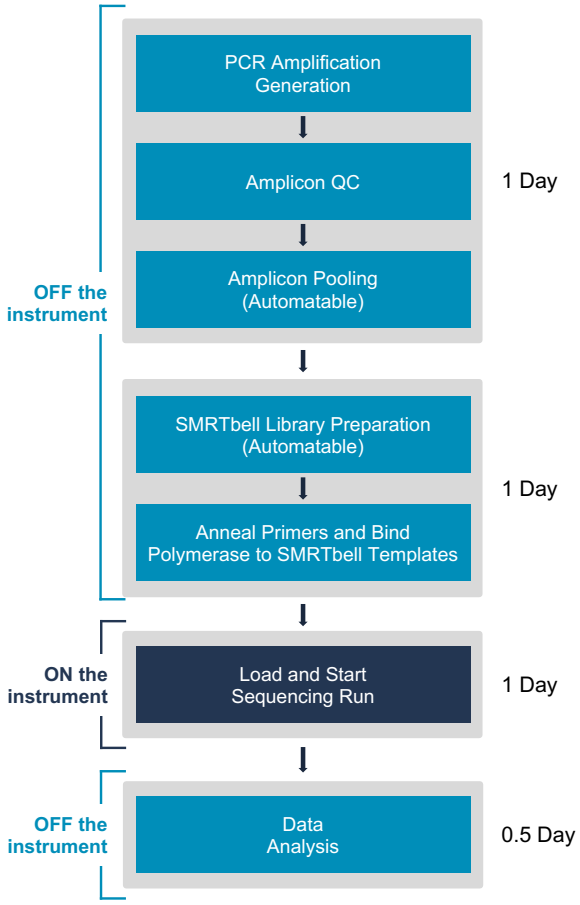
TARGETED SEQUENCING FOR AMPLICONS BEST PRACTICES

PLANT AND ANIMAL SCIENCES

HUMAN BIOMEDICAL RESEARCH

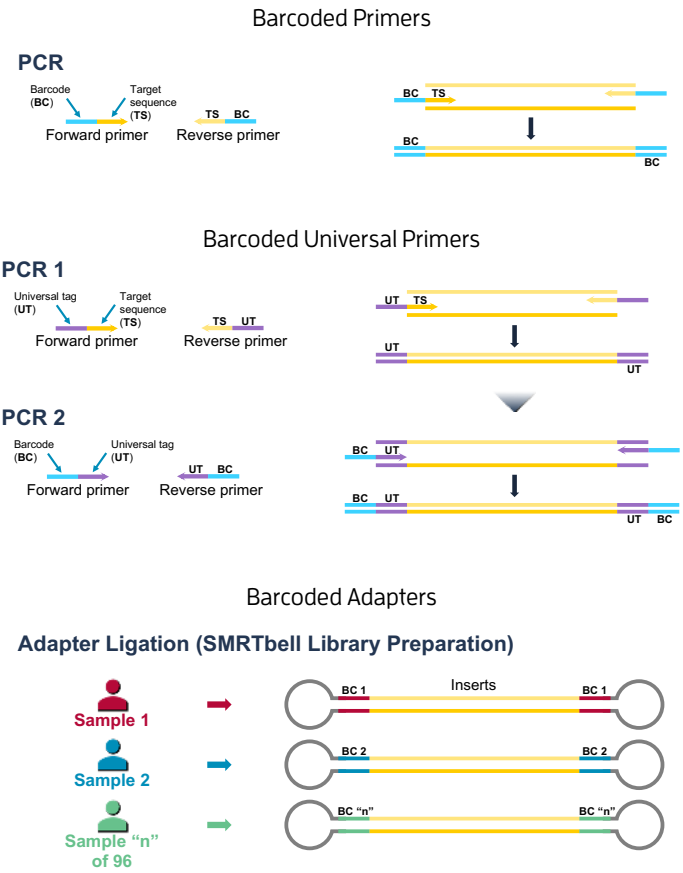
With Single Molecule, Real-Time (SMRT®) Sequencing and the Sequel® System, you can easily and cost effectively generate high-fidelity, long reads (>99% single-molecule read accuracy) from genes or regions of interest ranging in size from several hundred base pairs to 20 kb. Target all types of variation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

FROM DNA TO TARGET QUICKLY AND EFFECIENTLY



From DNA to a complete, high-fidelity answer in just 3.5 days.

FLEXIBLE MULTIPEXING OPTIONS

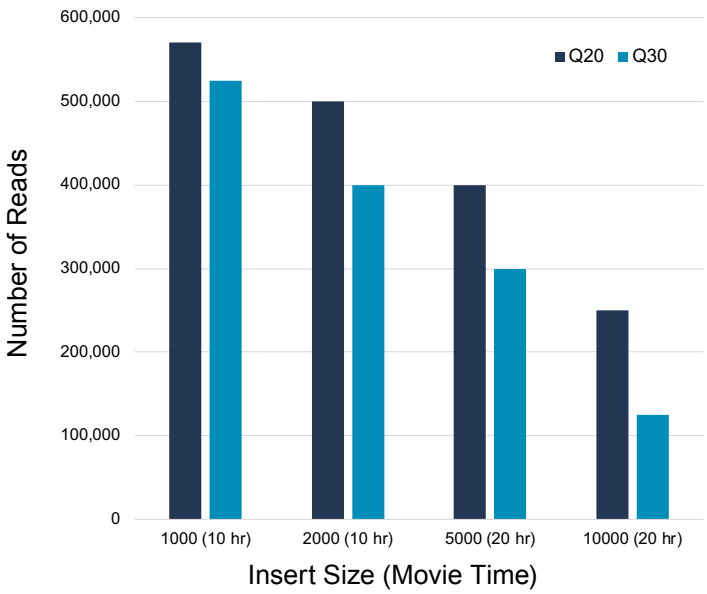


Multiplexing is supported with three barcoding options providing flexibility to incorporate unique sample identifiers during target amplification or library preparation.

SAMPLE PREPARATION RECOMMENDATIONS

- Start with high-quality, double-stranded DNA (1-2 ng per amplicon)
- Create SMRTbell® templates from amplicons[1] between 250 bp to 20 kb
- Optimize throughput with flexible barcoding options[2]
  - Amplify PCR products using target-specific primers with incorporated barcodes[3]
  - Add Barcoded Universal Primers[4] into amplicons via a simple 2-step PCR process
  - Attach Barcoded Adapters[5] during ligation without modifying existing primers
  - Multiplex up to 10,000 samples per SMRT Cell 1M[6]
- Maximize output and turn-around-time with adjustable run parameters[7]
  - For inserts <5 kb, recommend 10-hour movies
  - For inserts >5 kb, recommend 20-hour movies
- Generate high-fidelity, long 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)

HIGH-FIDELITY, LONG READS

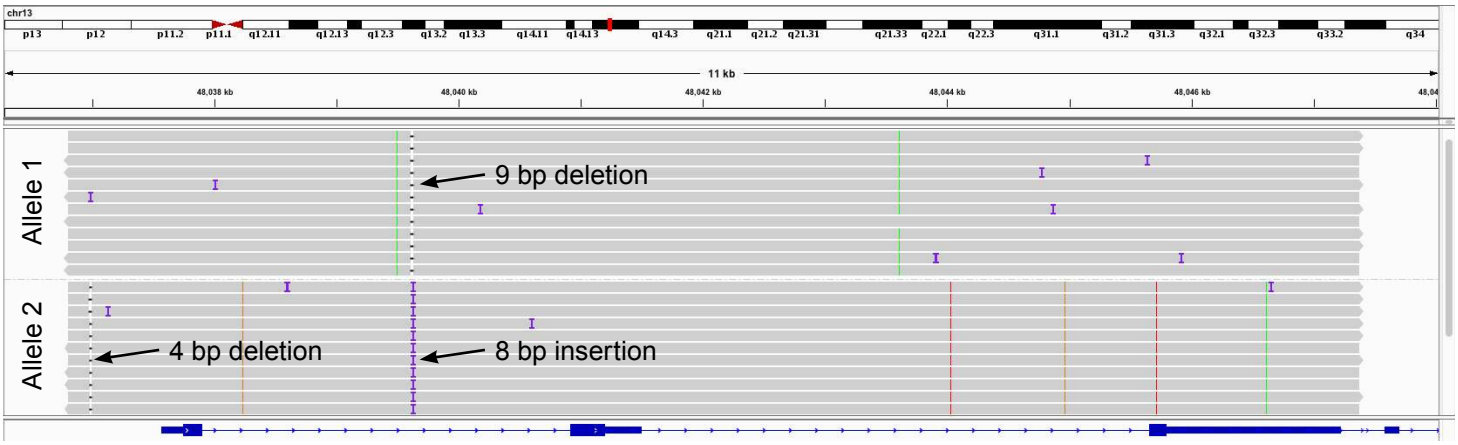


Estimated number of high-fidelity, long reads (Q20 or Q30) per Sequel SMRT Cell 1M (Chemistry 3.0, Sequel System Software v6.0) based on insert size and movie collection time.

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[8]
  - Generate high-fidelity >Q20 single-molecule reads using Circular Consensus Sequencing (CCS)
  - 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[9]
  - 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
- High-fidelity, long reads compatible with standard analysis tools for variant calling such as GATK

VARIANTS DETECTED IN PHASE ACROSS LONG AMPLICON



An Integrative Genomics Viewer image highlighting full-length reads and phased SNVs for a 9.6 kb gene covered by a single 10 kb amplicon. Sequencing results generated with Chemistry 3.0, SMRT Link v6.0 and a 20 hour movie collection time[10].

KEY REFERENCES

1. Procedure & Checklist - Amplicon Template Preparation and Sequencing
2. Product Note: Multiplexing Amplicons Up to 10 kb
3. For recommended barcode primers: https://www.pacb.com/multiplexing
4. Procedure & Checklist – Preparing SMRTbell Libraries using PacBio Barcoded Universal Primers for Multiplex SMRT Sequencing
5. Procedure & Checklist – Preparing Amplicon Libraries using PacBio Barcoded Adapters for Multiplex SMRT Sequencing
6. Herbert, P. et al., (2018) A Sequel to Sanger: Amplicon Sequencing that Scales. *BMC Genomics*. 19:219.
7. Quick Reference Card - Diffusion Loading and Pre-Extension Time Recommendations for the Sequel System
8. SMRT Link User Guide (v6.0)
9. SMRT Analysis Barcoding Overview (v6.0)
10. Collaboration with Stuart A. Scott Laboratory, Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai New York, NY.

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