

Application		Whole Genome Sequencing					RNA Sequencing	Metagenomics		Targeted Sequencing			
		<i>De Novo</i> Assembly - HiFi Reads	<i>De Novo</i> Assembly - Long Reads	<i>De Novo</i> Assembly - for Low DNA Input	Microbial <i>De Novo</i> Assembly	Variation Detection	Structural Variation Detection	Iso-Seq Method	Full-length 16S rRNA Sequencing	Shotgun Metagenomic Profiling or Assembly	Amplicon Sequencing	No-Amp Targeted Sequencing	
Experimental Design													
Experimental Design	<b>With 1 SMRT Cell 8M you can:</b>	Produce reference quality assemblies for genomes up to 2 Gb	Produce reference quality assemblies for genomes up to 3 Gb	Produce reference quality assemblies for genomes up to 1 Gb	Sequence up to 48 microbes	With 2 SMRT Cells 8M, Call SNVs, InDels, and SVs in a 3 Gb genome	Call SVs for up to 2 samples with ~3 Gb genomes	Characterize alternative splicing/annotate a genome with full length transcripts	Multiplex up to 96 samples to provide strain level resolution	Generate near-complete assemblies of high-complexity sample(s) (e.g. gut microbiome)	Sequence 384 barcoded amplicons	Sequence 5 targeted regions in a multiplex of 10 samples	
	<b>Minimum Recommended Coverage</b>	>15-fold HiFi read coverage	≥30-fold Unique Molecular Coverage (UMC) per haplotype	≥30-fold UMC per haplotype	≥30-fold UMC coverage per microbial genome	>15-fold HiFi of a human genome	5-25-fold Continuous Long Read (CLR) coverage	One human transcriptome per SMRT Cell 8M	8,000 reads/sample	See Best practices guide	30-fold ≥Q20 CCS read coverage for variant detection 6,000-fold ≥Q20 CCS read coverage for minor variant detection (1% sensitivity)	≥100-fold ≥Q20 CCS read coverage per target locus	
	<b>Library Insert Size</b>	15 - 20 kb	>30 kb	~20 kb	10 - 15 kb	15 - 20 kb	>15 kb	<2 kb to >3 kb	1 - 2 kb	10 kb	500 bp - 15 kb	4-6 kb or larger	
Sample Preparation													
SMRTbell Template Preparation	<b>Procedure and Checklist Reference</b>	<a href="#">Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0</a>	<a href="#">Preparing gDNA Libraries Using the SMRTbell Express Template Preparation Kit 2.0</a>	<a href="#">Preparing SMRTbell Libraries Using Express Template Prep Kit 2.0 With Low DNA Input</a>	<a href="#">Preparing Multiplexed Microbial Libraries Using SMRTbell Express Template Prep Kit 2.0</a>	<a href="#">Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0</a>	<a href="#">Preparing gDNA Libraries Using the SMRTbell Express Template Preparation Kit 2.0</a>	<a href="#">Iso-Seq Express Template Preparation for Sequel and Sequel II Systems</a>	<a href="#">Full-Length 16S Amplification, SMRTbell Library Preparation and Sequencing</a>	<a href="#">Preparing 10 kb SMRTbell Library for Metagenomic Shotgun Sequencing</a>	<a href="#">Preparing SMRTbell Libraries using PacBio Barcoded Overhang Adapters for Multiplex SMRT Sequencing</a>	<a href="#">No-Amp-Targeted-Sequencing-Utilizing-the-CRISPR-Cas9-System</a>	
	<b>Minimum Input Amount</b>	15 µg	≥1 µg for 10 kb ≥3 µg for >15 kb ≥5 µg for >30 kb	150 ng per 300 Mb genome size	1 µg per microbe	15 µg	3 µg	300 ng total RNA for 1st Strand cDNA Synthesis	500 ng - 1 µg	1.5 µg	250-500 ng for 250-1000 bp 500-1000ng for 1-3 kb bp 1000-2000 ng for 3-10 kb 1500-3000 ng for 15kb	5 to 10 µg (represented by either a single sample or the total of multiple samples that will be multiplexed)	
	<b>Recommended PacBio Template Prep Kit</b>	Express TPK 2.0 + SMRTbell Enzyme Cleanup kit + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + SMRTbell Enzyme Cleanup kit + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	SMRTbell TPK 1.0 + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v4	No-Amp Accessory Kit
	<b>Multiplexing/SMRT Cell</b>	N/A	N/A	N/A	Up to 48 microbes / SMRT Cell 8M Up to 16 microbes / SMRT Cell 1M	N/A	Up to 2 human samples/ SMRT Cell 8M N/A SMRT Cell 1M	The protocol supports up to 12 barcodes available.	Up to 96 samples/ SMRT Cell 8M Up to 12 samples/ SMRT Cell 1M	Profile up to 8 communities/ SMRT Cell 8M Profile one community/ SMRT Cell 1M	Up to 1,000+ samples/ SMRT Cell 8M or SMRT Cell 1M	Up to 10 samples/SMRT Cell	
<b>SMRT Sequencing with the Sequel II System: Loading and Pre-Extension Recommendations</b>													
Sequencing Preparation and Yield Performance	<b>Sequel II Binding Kit</b>	2.0	2.0	2.0	2.0	2.0	2.0	2.0 / 2.1*	2.1	2.0	2.1: 500bp - 3,000 bp 2.0: ≥3,000 bp	2.0	
	<b>Sequel II Sequencing Plate</b>	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	
	<b>Sequencing Mode</b>	CCS	CLR	CLR / CCS	CLR	CCS	CLR	CCS	CCS	CCS	CCS	CCS	CCS
	<b>Movie Collection Time</b>	30 h	15 h	15 / 30 h	15 h	30 h	15 h	24 h	10 h	30 h	Insert Size-Dependent	≥10 h (20 h for repeat expansion targets)	
	<b>Notes</b>												
<b>SMRT Sequencing with the Sequel System</b>													
Sequencing Preparation and Yield Performance	<b>Sequel Binding kit</b>	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
	<b>Sequel Sequencing Plate</b>	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
	<b>Sequencing Mode</b>	CCS	CLR	CLR	CLR	CCS	CLR	CCS	CCS	CCS	CCS	CCS	
	<b>Movie Collection Time</b>	20 h	10 h	10 h	10 h	20 h	10 h	20 h	10 h	10 h	Insert Size-Dependent	≥10 h (20 h for repeat expansion targets)	
	<b>Notes</b>												
<b>Data Analysis Tools Available Through SMRT Link, PacBio DevNet and Other Compatible Software Tools</b>													
Other Compatible So Tools (Contact FAS for	<b>SMRT Analysis GUI Applications</b>	CCS	Assembly (HGAP 4)	Assembly (HGAP 4)	Demultiplex Barcodes followed by Microbial Assembly analysis	CCS with Mapping	Structural Variant Calling	Iso-Seq	CCS	CCS	CCS or Long Amplicon Analysis	Demultiplex Barcodes followed by CCS with Mapping analysis	
	<a href="#">PacBio Devnet Tools</a>	Falcon, Falcon Unzip, Falcon Phase	Falcon, Falcon Unzip, Falcon Phase									Repeat Analysis Tools	
	<b>Notes</b>					Recommend: GATK, Google Deep Variant							

Read lengths, reads/data per SMRT Cell and other sequencing performance results vary based on sample quality/type and insert size

[Pac Bio Glossary of Terms](#)

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