

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	With 1 SMRT Cell 8M you can:	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	
	Minimum Recommended Coverage	>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	
	Library Insert Size	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	Procedure and Checklist Reference	Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0	Preparing gDNA Libraries Using the SMRTbell Express Template Preparation Kit 2.0	Preparing SMRTbell Libraries Using Express Template Prep Kit 2.0 With Low DNA Input	Preparing Multiplexed Microbial Libraries Using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0	Preparing gDNA Libraries Using the SMRTbell Express Template Preparation Kit 2.0	Iso-Seq Express Template Preparation for Sequel and Sequel II Systems	Full-Length 16S Amplification, SMRTbell Library Preparation and Sequencing	Preparing 10 kb SMRTbell Library for Metagenomic Shotgun Sequencing	Preparing SMRTbell Libraries using PacBio Barcoded Overhang Adapters for Multiplex SMRT Sequencing	No-Amp-Targeted-Sequencing-Utilizing-the-CRISPR-Cas9-System	
	Minimum Input Amount	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)	
	Recommended PacBio Template Prep Kit	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
	Multiplexing/SMRT Cell	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	
SMRT Sequencing with the Sequel II System: Loading and Pre-Extension Recommendations													
Sequencing Preparation and Yield Performance	Sequel II Binding Kit	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	
	Sequel II Sequencing Plate	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	
	Sequencing Mode	CCS	CLR	CLR / CCS	CLR	CCS	CLR	CCS	CCS	CCS	CCS	CCS	CCS
	Movie Collection Time	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)	
	Notes												
SMRT Sequencing with the Sequel System													
Sequencing Preparation and Yield Performance	Sequel Binding kit	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
	Sequel Sequencing Plate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
	Sequencing Mode	CCS	CLR	CLR	CLR	CCS	CLR	CCS	CCS	CCS	CCS	CCS	
	Movie Collection Time	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)	
	Notes												
Data Analysis Tools Available Through SMRT Link, PacBio DevNet and Other Compatible Software Tools													
Other Compatible So Tools (Contact FAS for	SMRT Analysis GUI Applications	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	
	PacBio Devnet Tools	Falcon, Falcon Unzip, Falcon Phase	Falcon, Falcon Unzip, Falcon Phase									Repeat Analysis Tools	
	Notes					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

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