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# Sequel II System v8.0 & SMRT Link v8.0 Technical Overview

*Sequel II System v8.0 / Sequel II Chemistry 2.0 / SMRT Link v8.0*

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# Sequel II System v8.0 & SMRT Link v8.0 Technical Overview

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# SEQUEL II SYSTEM V8.0 & SMRT LINK V8.0 OVERVIEW & FEATURES

## Complete Larger Sequencing Projects by Reducing Cost and Time-to-Results

### New Sequel II System Chemistry, Software & Protocols

- **New Sequel II Chemistry 2.0** (Sequel II Sequencing Kit 2.0 & Sequel II Binding Kits 2.0 / 2.1) improves average read lengths up to ~50%
- **New Sequel II System Instrument Control Software v8.0** supports new chemistry
- **New SMRT Link v8.0 Software** version includes several highly beneficial updates and features for improved data analysis support
- **New HiFi long-insert sample preparation workflow** using SMRTbell Express Template Prep Kit 2.0 provides faster library construction times and enables automation

### Increased Sequencing Performance

- **Longer average read lengths** achievable with Sequel II Chemistry 2.0 enable:
  - Higher yields of >Q20 and Q30 HiFi reads per SMRT Cell 8M
  - Sequencing of larger HiFi inserts (*e.g.*, 15-20 kb) to support *de novo* assembly applications with HiFi data
  - High precision and recall for variant detection with fewer SMRT Cells per sample by using ~15 kb HiFi reads
  - Characterization of a whole transcriptome in a single SMRT Cell 8M and increased detection of long isoforms

# SEQUEL II SYSTEM V8.0 & SMRT LINK V8.0 OVERVIEW & FEATURES (CONT.)

## Improved Data Analysis Support

- **New workflow engine for SMRT Link v8.0** – Cromwell, replaces pbsmrtpipe and provides improved scalability
- **Faster CCS analysis time-to-results** (4- to 6-fold improvement over previous CCS analysis algorithm)
- **New Microbial Assembly-specific application** provides end-to-end analysis including assembly, circularization, and plasmid identification
- **New-style, refreshed SMRT Link Graphical User Interface** retains the same core functionality with improved data visualization features and other general usability improvements

# SEQUEL II SYSTEM V8.0 KEY APPLICATIONS IMPROVEMENTS



## Variant Detection

- Faster and easier HiFi Library construction with SMRTbell Express TPK 2.0-based protocol
- Rapid CCS data analysis time (4.5 hours) *via* algorithm improvements
- Achieve high precision and recall for comprehensive variant detection (SNVs, InDels, SVs, CNVs) with fewer SMRT Cells per sample by using longer HiFi Reads (~15 – 25 kb)
- Multiplex two samples per SMRT Cell 8M for structural variant detection using Long Reads (CLR sequencing mode)



## De novo Assembly

- Construct large HiFi library inserts (~15-20 kb) for *de novo* assembly applications
  - Long HiFi reads enable higher accuracy assemblies, better phasing, and faster data analysis times
- Multiplex up to 48 microbial samples per SMRT Cell 8M for high quality assemblies
  - New end-to-end microbial assembly workflow including circularization and plasmid identification through SMRT Link GUI



## Long-Read RNA Sequencing (Iso-Seq Analysis)

- Characterize a whole transcriptome in a single SMRT Cell 8M and detect even more longer isoforms

# EFFICIENT SMRT SEQUENCING WORKFLOWS FOR THE SEQUEL II SYSTEM

1 Day



**LIBRARY PREP**

- Complete template preparation in ~6 hours
- Accepts a variety of sample types and insert sizes
- Size-selection options to enrich for longest inserts
- Multiplexing and barcoding solutions available

1 Day



**SMRT  
SEQUENCING**

- Rapid sequence time (0.5 to 30 hours)
- Serially process up to 8 SMRT Cells
- Walk away time up to four days

1 Day







**DATA ANALYSIS**

- Variety of analysis methods available through SMRT Analysis and PacBio Analytical Portfolio
- DevNet and Analytical Partners applications
- Advanced data visualization and mining

# WHAT CAN YOU DO WITH ONE SEQUEL II SYSTEM SMRT CELL 8M?

With PacBio Single Molecule, Real-Time Sequencing on the Sequel II System you can characterize whole genomes and transcriptomes with just one SMRT Cell 8M

	SMRT Sequencing Applications	Number of SMRT Cells 8M*	PacBio Consumable Estimated Costs (US List Price) <sup>†</sup>
 <b>WHOLE GENOME SEQUENCING</b>	<b>De Novo Assembly:</b> Produce reference-quality assemblies for genomes up to 2 Gb	<b>1</b>	\$1300/sample
	<b>Microbial De Novo Assembly:</b> Generate reference-quality assemblies for up to 48 microbial isolates	<b>1</b>	\$70/sample
	<b>Variant Detection:</b> Call single nucleotide, indel, and structural variants in a ~3 Gb genome	<b>2</b>	\$2600/sample
	<b>Structural Variant Detection:</b> Call structural variants for up to 2 samples with ~3 Gb genomes	<b>1</b>	\$670/sample
 <b>RNA SEQUENCING</b>	<b>Whole Transcriptome:</b> Characterize alternative splicing with full-length transcripts	<b>1</b>	\$1300/sample
	<b>Genome Annotation:</b> Sequence full-length transcripts and multiplex up to 8 tissues	<b>1</b>	\$185/tissue
 <b>TARGETED SEQUENCING</b>	<b>Amplicon Sequencing:</b> Detect variation in specific regions by multiplexing	<b>1</b>	\$1-4/sample
	<b>No-Amp Sequencing:</b> Enrich hard-to-amplify targets and multiplex up to 10 samples	<b>1</b>	\$120/sample
 <b>COMPLEX POPULATIONS</b>	<b>Full-length 16S:</b> Gain strain-level resolution by multiplexing up to 96 samples	<b>1</b>	\$30/sample
	<b>Metagenomic Functional Profiling:</b> Examine up to 3 low-complexity samples with multiplexing	<b>1</b>	\$450/sample
	<b>Shotgun Metagenomic Assembly:</b> Generate near-complete assemblies of high-complexity samples (e.g. gut microbiome)	<b>1</b>	\$1300/sample

\*Study design, sample type, and level of multiplexing may affect the number of SMRT Cells 8M required. †All prices are listed in USD and cost may vary by region. Pricing includes library and sequencing reagents run on your Sequel II System and does not include instrument amortization or other reagents.



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# Sequel II System v8.0 Overview

*Sequel II System v8.0 / Sequel II Chemistry 2.0*



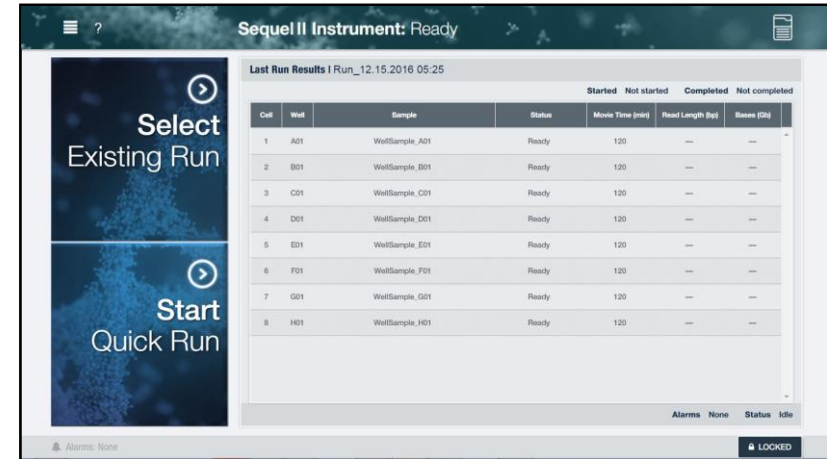


# **Sequel II System v8.0 Instrument Software & Consumables Products Updates**

# SEQUEL II SYSTEM INSTRUMENT CONTROL SOFTWARE (ICS)

## Sequel II System ICS v8.0 Features

- Sequel II System ICS v8.0 supports new Chemistry 2.0 reagents and implements an updated on-deck robotic workflow for sample immobilization using Sequel II Sequencing Kit 2.0
- No substantial changes to GUI from Sequel II System ICS v7.0
  - Selectable run designs from SMRT Link
  - Guided work deck loading
  - Real-time run feedback and run metrics
  - Directed post-run instrument clean-up



# SEQUEL II SYSTEM REAGENTS & CONSUMABLES

## SMRTbell Template Preparation

- SMRTbell Template Prep Kit 1.0
- SMRTbell Express Template Prep Kit 2.0
- SMRTbell Enzyme Clean Up Kit *(NEW)*



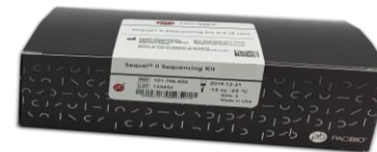
## Sequel II Binding Kits

- Sequel II DNA Polymerase 2.0 *(NEW)*
- Sequel II DNA Polymerase 2.1 *(NEW)*
- Sequel II DNA Internal Control 1.0



## Sequel II Sequencing Kit

- Sequel II Sequencing Kit 2.0 (4 rxn) *(NEW)*



## SMRT Cell 8M

- Sequel II SMRT Cell 8M (Supports movie times up to 30 hours)



# NEW SEQUEL II SYSTEM CONSUMABLES KITS & BUNDLES

Part Number	Description
101-820-200	Sequel II Sequencing 2.0 Kit (4 rxn)
101-849-000	Sequel II Sequencing Kit 2.0 Bundle (5-pk) (Five Plates plus Tube Septa and SMRT Cell Oil)
101-842-900	Sequel II Binding Kit 2.0 and Internal Control 1.0 Kit
101-843-000	Sequel II Binding Kit 2.1 and Internal Control 1.0 Kit
101-789-500	Sequel II Binding 2.0 Kit
101-820-500	Sequel II Binding 2.1 Kit
101-746-400	SMRTbell Enzyme Clean Up Kit (Provides similar capabilities as Exo III / VII for library cleanup)
101-843-100	SMRTbell Express TPK 2.0 and Enzyme Clean Up Kit + Sequencing Primer v2 (Bundle for generating HiFi Libraries with Express TPK 2.0)
101-753-300	Sequel II Consumables Starter Kit 2.0 (Updated to Binding Kit 2.0 and Sequel II Sequencing Kit 2.0)
101-847-900	Sequencing Primer v2 (Now a stand-alone product offering; also still included in TPK 1.0)
101-719-600	Sequel II System Training Kit 2.0 (Updated to Binding Kit 2.0 and Sequel II Sequencing Kit 2.0)

# SEQUEL II BINDING KIT, SEQUENCING PLATE AND DNA INTERNAL CONTROL COMPATIBILITY MATRIX

Binding Kit	Sequencing Plate	Internal Control
Sequel II Binding Kit 1.0	Sequel II Sequencing Plate 1.0	Sequel II DNA Internal Control 1.0
Sequel II Binding Kit 2.0 <sup>1</sup>	Sequel II Sequencing Plate 2.0	Sequel II DNA Internal Control 1.0
	Sequel II Sequencing Plate 2.0EA <sup>2</sup>	Sequel II DNA Internal Control 1.0
Sequel II Binding Kit 2.1 <sup>1</sup>	Sequel II Sequencing Plate 2.0	Sequel II DNA Internal Control 1.0
	Sequel II Sequencing Plate 2.0EA <sup>2</sup>	Sequel II DNA Internal Control 1.0

<sup>1</sup> Sequel II Binding Kit 2.1 is recommended for Amplicons <3 kb and Standard Iso-Seq Samples. For all other library types (including Amplicons ≥3 kb and Iso-Seq samples with a focus on long transcripts), Sequel II Binding Kit 2.0 is recommended.

<sup>2</sup> Not commercially available



# Sequel II Sample Library Preparation Protocol & Workflow Updates

# NEW AND UPDATED SEQUEL II SYSTEM SMRTBELL LIBRARY PREPARATION PROTOCOLS

SMRTBELL LIBRARY TYPE OR PROTOCOL	SEQUENCING MODE	SMRTBELL EXPRESS TPK 2.0	PROCEDURE & CHECKLIST
HiFi Express TPK 2.0 Library	CCS	✓	<a href="#">Link</a>
Large Insert gDNA Library	CLR	✓	<a href="#">Link</a>
Multiplexed SV Library	CLR	✓	<a href="#">Link</a>
Low Input gDNA Library	CLR / CCS	✓	<a href="#">Link</a>
Microbial Multiplexing Library	CLR	✓	<a href="#">Link</a>
Metagenomics Shotgun Library	CCS	✓	<a href="#">Link</a>
16S Amplicon Library	CCS	✓	<a href="#">Link</a>
Iso-Seq Express Library	CCS	✓	<a href="#">Link</a>
Multiplexed BOA Amplicon Library	CCS	✓	<a href="#">Link</a>
Multiplexed BUP Amplicon Library	CCS	✓	<a href="#">Link</a>
AMPure PB Size Selection	N/A	✓	<a href="#">Link</a>

# SMRTBELL LIBRARY CONSTRUCTION PROTOCOL UPDATES

## New HiFi SMRTbell Express 2.0 Library Construction Protocol for Comprehensive Variant Detection and *De Novo* Assembly Applications

- Protocol (PN 101-853-100) describes the construction of HiFi libraries with insert sizes ~11 – 20 kb (or larger) using **SMRTbell Express TPK 2.0** and **SMRTbell Enzyme Clean Up Kit**
- Rapid single-tube, addition-only workflow (with 1-hour ligation time) that is amenable to automation
- Eliminates multiple AMPure purification steps and thus minimizes handling-induced DNA damage compared to previous SMRTbell TPK 1.0 protocol
- Recommended size selection method for HiFi Variant Detection and *De Novo* Assembly applications is SageELF (“gold standard”) or BluePippin
- Includes reference to (optional) alternative AMPure PB Size Selection method for HiFi *De Novo* Assembly applications



### Procedure & Checklist - Preparing HiFi SMRTbell® Libraries using SMRTbell Express Template Prep Kit 2.0

This document describes a method for constructing HiFi SMRTbell libraries for generating high-accuracy long reads on the Sequel II System using PacBio's SMRTbell Express Template Prep Kit 2.0.

High quality genomic DNA (gDNA) can be sheared using a Megaruptor instrument (Diagenode) or g-TUBEs (Covaris). Depending on your project requirements, SMRTbell libraries are size-selected using a SageELF system (Sage Science), BluePippin system (Sage Science) or AMPure PB Beads (PacBio). Table 1 is a summary of recommendations for constructing HiFi long reads for specific applications.

Application	Size-Selection Method	Number of Collected Fractions	Note
HiFi for Variant Detection	SageELF	5 (~11 kb, ~13 kb, ~15 kb, ~17 kb, >19 kb)	Reads may also be used for <i>de novo</i> assembly
	BluePippin	2 (11-13 kb, 13-20 kb)	Reads may also be used for <i>de novo</i> assembly
HiFi for <i>de novo</i> Assembly	AMPure PB Beads	1 (5-20 kb, depending on shear distribution)	Reads are not suitable for variant detection. Removes <5 kb and reduces <10 kb SMRTbells from final library.

Table 1: Library construction recommendations for applications requiring HiFi long reads

This procedure describes construction of HiFi libraries from sheared gDNA with a mode size of 15 kb or larger. Table 2 summarizes DNA input, quality and DNA shear mode requirements for specific size-selection options. The final SMRTbell library yield (%) of the collected and purified HiFi fractions depends on the quality of the starting genomic DNA and distribution of the DNA shear.

To increase the recovery yield of larger fraction sizes (>20 kb), the target shear size distribution must be adjusted so that the mode is 20 kb. Always perform test shears prior to starting SMRTbell library construction.

Size-Selection Method	Required Input gDNA Amount	Required Input gDNA Quality (Mode Size)	Target Sheared Fragment Size Distribution Mode	Shearing Method
SageELF	15 µg	>40 kb	>15-20 kb	g-TUBE or Megaruptor
BluePippin	15 µg	>40 kb	>15-20 kb	g-TUBE or Megaruptor
AMPure PB	15 µg	>40 kb	>20 kb	g-TUBE or Megaruptor


Table 2: DNA requirements and recommended shearing methods for constructing HiFi libraries.



# SMRTBELL LIBRARY CONSTRUCTION PROTOCOL UPDATES (CONT.)

## New Optional AMPure PB Size Selection Protocol for Use with HiFi *De Novo* Assembly Applications

- New Procedure & Checklist (PN 101-854-900) provides instructions for using AMPure PB beads for size-selection of SMRTbell libraries
- AMPure PB size selection method is compatible with both HiFi TPK 1.0 and HiFi Express 2.0 library construction procedures
- AMPure PB beads are diluted with PacBio Elution Buffer to achieve a specified volumetric ratio and then used for size-selection
- SMRTbell library recovery yields with AMPure PB size-selection are typically >40%
- Protocol document contains:
  1. List of required materials
  2. Procedure for diluting AMPure PB Beads in Elution Buffer to achieve a specified volumetric concentration
  3. Procedure for performing size selection to remove <3 kb SMRTbell templates or <5 kb SMRTbell templates using a specified volumetric ratio of diluted AMPure PB Beads:Sample



### Procedure & Checklist – Using AMPure® PB Beads for Size-Selection

This procedure provides instructions for using AMPure PB beads for size-selection of SMRTbell libraries. AMPure PB beads are diluted with PacBio Elution Buffer to 35% (volume by volume) and then used for size-selection.

**Important:** To efficiently remove SMRTbell templates <3 kb or <5 kb, be sure to use this procedure **after** the first AMPure PB bead purification step (post-adaptor ligation in the SMRTbell library construction workflow).

#### Required Materials

Item	Vendor	Part Number
AMPure PB Beads	Pacific Biosciences	100-265-900
Elution Buffer	Pacific Biosciences	101-633-500
15 mL High-Clarity Polypropylene Conical Tube	Corning	352096
Wide Orifice Tips (RT-LTS-A-200 µL-/F/LW-960/10)	Rainin	30389241
1X dsDNA HS Assay kit and Qubit 2.0, 3.0 or 4.0 fluorometer	Thermo Fisher	Q33230

#### Dilute AMPure PB Beads with Elution Buffer

The final AMPure PB bead concentration is critical to the success of this procedure. Therefore, accurate pipetting is of utmost importance to achieve a final 35% (v/v) AMPure PB bead solution in Elution Buffer.

Reagent	Volume	✓	Notes
Elution Buffer	6.5 mL		
AMPure PB Beads (stock reagent)	3.5 mL		
<b>Total Volume</b>	<b>10.0 mL</b>		


1. Bring the AMPure PB bead stock to room temperature.
2. Vortex the stock solution for 30 seconds to mix well.
3. Using a P1000 pipette, transfer 6.5 mL of Elution Buffer into a 15 mL conical tube.
4. Add 3.5 mL of the stock AMPure PB beads to the Elution Buffer. When pipetting the viscous AMPure PB bead solution, pipette slowly to ensure that the volume aspirated is as precise as possible. Large residual AMPure PB beads adhering outside of the tip should be removed prior to adding to the Elution Buffer.
5. Vortex the diluted AMPure PB beads solution for 30 seconds to mix well before use. This solution may be stored at 4°C for 2 months for future use.

Page 1 Part Number 101-854-900 Version 02 (January 2020)

# SMRTBELL LIBRARY CONSTRUCTION PROTOCOL UPDATES (CONT.)

## Updated Low DNA Input Protocol Now Supports Preparation of Multiplexed SMRTbell Libraries for Sequencing on the Sequel II System

- This Procedure & Checklist (PN 101-730-400) describes how to prepare SMRTbell libraries for a **single sample** using Express Template Prep Kit 2.0 with low DNA input for sequencing on the Sequel and Sequel II Systems
- The procedure also describes preparing **multiplexed** SMRTbell libraries with a maximum of 2 pooled low DNA input samples (up to 600 Mb per genome) for sequencing on the Sequel II System
- Protocol document contains:
  1. General laboratory best practices recommendations
  2. Recommendations for gDNA QC and quantification
  3. Guidelines for evaluation of gDNA samples for low-Input library construction
  4. Enzymatic steps for preparation of non-multiplexed and multiplexed low DNA input SMRTbell libraries
  5. Instructions for size-selection of low DNA input libraries using the AMPure BP Size Selection method
  6. Sample setup guidance for preparing low DNA input libraries for sequencing on the Sequel or Sequel II Systems



### Procedure & Checklist - Preparing SMRTbell® Libraries Using Express Template Prep Kit 2.0 With Low DNA Input

This document describes preparing SMRTbell libraries from >150 ng of input genomic DNA (gDNA) for the Sequel System and from >400 ng of input gDNA for the Sequel II System using SMRTbell Express Template Prep Kit 2.0. This low DNA input procedure also enables multiplexing a maximum of 2 small genomes (up to 600 Mb/genome) on the Sequel II System, from >300 ng of genomic DNA per genome. The two samples are pooled (Figure 2) after ligation and nuclease-treated.

Table 1 below lists the three main types of low DNA input SMRTbell libraries supported in this document and summarizes the DNA quality and quantity requirements for each.

SMRTbell Library Type	Required Minimum gDNA	Required Quality of Input gDNA	gDNA Shearing Method	Required Distribution
Low DNA input for the Sequel System (1 sample)	>150 ng	Majority of gDNA >20 kb	Not Required	Mean fragment length of 50-60 kb is optimal
Low DNA input for the Sequel II System (1 sample)	>400 ng	Majority of gDNA >20 kb	Not Required	Mean fragment length of 50-60 kb is optimal
Multiplexed Low DNA input for the Sequel II System (2 samples up to 600 Mb per genome)	>300 ng per sample	Majority of gDNA >30 kb	Required using Megaruptor System	12-18 kb sheared DNA is optimal

Table 1: Recommended gDNA quality and quantity requirements for the three supported low DNA input library types.

PacBio recommends using the Femto Pulse system for assessing the integrity of the starting gDNA material. The Femto Pulse system requires significantly lower sample amounts (200-500 picograms) compared to other systems that require >50 ng of DNA for sizing.

When working with low amounts of gDNA, accurate quantification is required. The Qubit High Sensitivity (HS) assay system can be used to obtain accurate dsDNA concentration measurements for low DNA input samples.

Overall, SMRTbell library yields in this low-input workflow are typically 50% (from input into the first enzymatic reaction) for the single-sample workflow described in Figure 1 and 30% (from sheared DNA) for the multiplexing workflow described in Figure 2. Depending on the final size of the library, sufficient amounts of SMRTbell template material (to run approximately 4 or more Sequel SMRT® Cells 1M) can be generated for the Sequel System. The Sequel II System requires higher on-plate loading concentrations and, as a result, the amount of SMRTbell library material generated in this procedure is enough for only one SMRT Cell 8M.

For large genomes that require multiple SMRT Cells and where DNA can be extracted in abundant quantities from a single individual sample, we recommend constructing a library starting with >3 µg gDNA using the standard workflow located [here](#).

Page 1      Part Number 101-730-400 Version 04 (November 2019)



# SMRT Link Sample Setup, Run Design & Run QC Updates

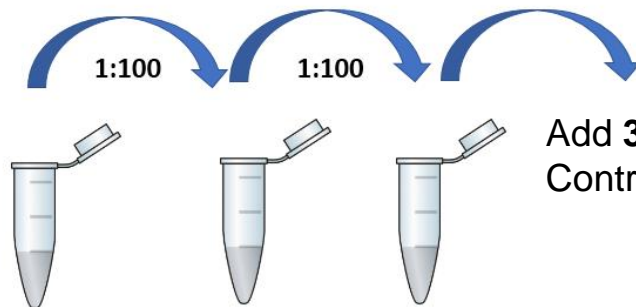
# SMRTBELL LIBRARY ANNEALING, BINDING & CLEANUP WORKFLOW AND DNA INTERNAL CONTROL (SEQUEL II CHEMISTRY 2.0)

## Sequel II SMRTbell Library A/B/C Requirements

- Follow instructions outlined in **SMRT Link v8.0 Sample Setup** for annealing / binding / complex cleanup unless specified otherwise in the relevant Procedure & Checklist
- Recommended method for complex cleanup for most library types (except Iso-Seq Express samples) is **AMPure PB Bead purification**
  - For **Iso-Seq Express** libraries, used **ProNex Beads (Promega)** for complex cleanup
- Final on-plate sample volume per SMRT Cell 8M = **115  $\mu$ l** (robot aspirates 105  $\mu$ l from sample plate; adds 105  $\mu$ l of Dilution Buffer; and then dispenses 200  $\mu$ l into a SMRT Cell)

## Sequel II DNA Internal Control

- No change to DNA Internal Control spike-in procedure:



Add **3.8  $\mu$ l** (per SMRT Cell 8M) of serially diluted DNA Internal Control to final on-plate sample dilution

- Expect ~1000 internal control reads per SMRT Cell 8M; ~50 – 60 kb Control Pol RL for 30-h movies with 2- to 4-h pre-extension time

# QUICK REFERENCE CARD UPDATES FOR SEQUEL II SYSTEM

## New and Updated Sample Setup QRC Info Fields & Parameter Values for Sequel II System Chemistry 2.0

### - New Sample Setup QRC Fields

- Sequencing Mode
- Binding Kit
- Sequencing Primer
- Polymerase Binding Time
- Complex Cleanup Method

### - Refined list of supported applications now includes:

- Low DNA Input
- Larger HiFi insert size range
- Amplicons “<3 kb” & “≥3 kb”
- Iso-Seq “Standard” vs. “Focus on Long Transcripts” [Was previously “Iso-Seq Libraries”]

### - Use Sequel II Sequencing Kit 2.0 for all listed applications

- Target P1 is 50% – 70% for all listed applications

Refer to the table below for loading recommendations for the Sequel II System. Note that the sample quality, size, and binding efficiency may affect loading concentrations, which may result in optimum loading concentrations as low as 30 pM or as high as 100 pM. **Use Sequel II Sequencing Plate 2.0 for all application types.**

Applications	Sequencing Mode	Library Prep Kit	Binding Kit	Sequencing Primer	Pol Binding Time (hr)	Complex Cleanup	Loading Concentration Range (pM)
Large insert (>15 kb size- selection cutoff)	CLR	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X AMPure PB Beads	30 - 70
Microbial Multiplex (10 kb)	CLR	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X AMPure PB Beads	60 - 100
Low DNA Input (>10 kb, AMPure PB Bead size-selection)	CLR	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X AMPure PB Beads	40 - 100
HiFi (10 kb – 25 kb)	CCS	Express Prep 2.0	Binding Kit 2.0	v2	4	1.2X AMPure PB Beads	30 - 70
Shotgun Metagenomics (10 kb, AMPure PB Bead size-selection)	CCS	Template Prep Kit 1.0	Binding Kit 2.0	v2	4	1.2X AMPure PB Beads	30 - 70
Amplicons (≥3 kb)	CCS	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X AMPure PB Beads	30 - 70
Amplicons (< 3 kb)	CCS	Express Prep 2.0	Binding Kit 2.1	v4	1	1.2X AMPure PB Beads	40 - 150
16S (1.6 - 2.5 kb)	CCS	Express Prep 2.0	Binding Kit 2.1	v4	1	1.2X AMPure PB Beads	40 - 100
Iso-Seq (standard samples)	CCS	Express Prep 2.0	Binding Kit 2.1	v4	1	1.2X ProNex	40 - 80
Iso-Seq (focus on long transcripts)	CCS	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X ProNex	50 - 100

**Target % P1 is 50 to 70.** Recommended for optimal yield per SMRT Cell (defined as maximized raw yield for long insert reads, and unique molecular yield for HiFi Reads.) Indications for overloaded libraries can be gauged by P0 values. **Note:** If P0 values are <10% then the SMRT Cell is overloaded.

# QUICK REFERENCE CARD UPDATES FOR SEQUEL II SYSTEM (CONT.)

## New and Updated Pre-Extension & Movie Time QRC Info Fields & Parameter Values for Sequel II System Chemistry 2.0

- New Movie Collection Time QRC Field
- Refined list of supported applications now includes:
  - Microbial Multiplex
  - Low DNA Input
  - Larger HiFi insert size range
  - Shotgun Metagenomics
  - Amplicons “<3 kb” & “≥3 kb”
  - Iso-Seq “Standard” vs. “Focus on Long Transcripts”

Pre-extension is a Software feature that allows SMRTbell template molecules to reach rolling circle replication (when the polymerase is most stable) before movie collection is initiated. Generalized pre-extension guidelines by mean insert size and applications are summarized in the table below. Further optimization of pre-extension time is recommended for specific applications to maximize read length and yield.

Applications	Pre-Extension Time (hr)	Movie Collection Time (hr)
Large insert (>15 kb size-selection cutoff)	0	15
Microbial Multiplex (10 kb)	2	15
Low DNA Input (>10 kb, AMPure PB bead size-selection)	2	30
HiFi (10 kb – 25 kb)	2 hrs (≤20 kb), 4 hrs (>20 kb)	30
Shotgun Metagenomics (10 kb, AMPure PB bead size-selection)	2	30
Amplicons (≥3 kb)	Use default values in Run Design	10 - 30
Amplicons (< 3 kb)	Use default values in Run Design	10
16S (1.6 - 2.5 kb)	1	10
Iso-Seq (standard samples)	2	24
Iso-Seq (focus on long transcripts)	2	24

# GENERAL RUN SETUP WORKFLOW FOR LOADING SMRTBELL LIBRARIES FOR SEQUENCING ON THE SEQUEL II SYSTEM (CHEMISTRY 2.0 / SMRT LINK V8.0)

Perform final sample dilution per instructions in **SMRT Link v8.0 Sample Setup** (unless stated otherwise in the Procedure & Checklist) and transfer sample to 96-well sample plate



Complete all required sample information fields in **SMRT Link v8.0 Run Design** (*do not* modify any parameters under Advanced Options unless instructed to do so in the Procedure & Checklist)

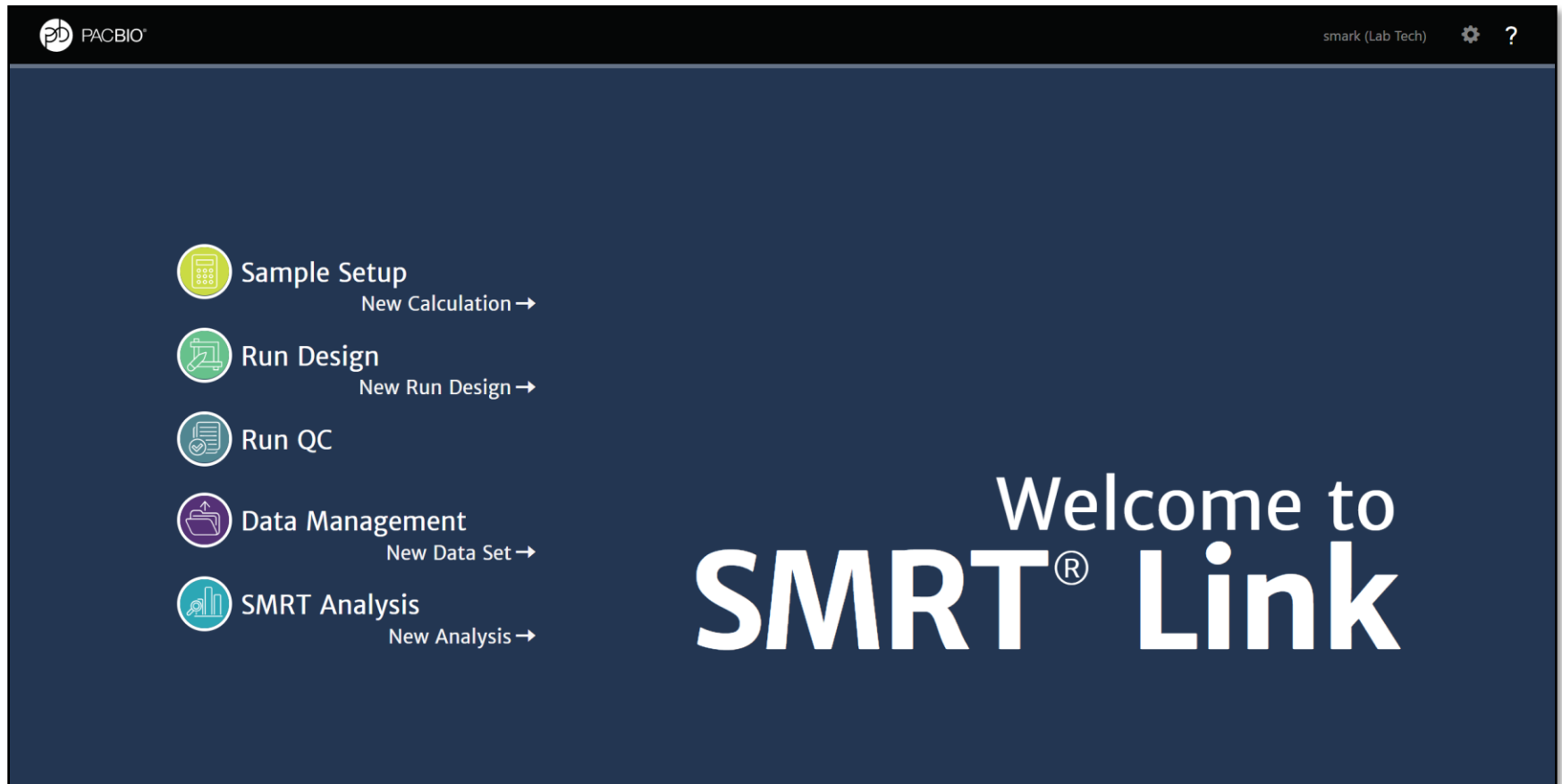


Load sample plate and all other required reagents & consumables into the Sequel II System instrument and **START** run

- Refer to application-specific **Procedure & Checklist** document and **Diffusion Loading and Pre-Extension Recommendations for the Sequel II System [Quick Reference Card](#)** for the latest sample loading and run setup recommendations

# NEW UPDATED LOOK FOR SMRT LINK V8.0 GRAPHICAL USER INTERFACE

New-style GUI retains the same basic functionality as previous SMRT Link versions



- SMRT Link v8.0 features a newly re-designed, more intuitive GUI that is easy to use
- Offers better navigation and improved data visualization features



# SMRT LINK V8.0 SAMPLE SETUP HOMEPAGE

New-style GUI retains the same basic functionality as previous SMRT Link Sample Setup modules

Sample Setup

+ New Calculation Edit/Print Delete

Sequel Sequel II

<input type="checkbox"/>	Name	Date Created ↓	Created By	Sample Concentration (ng/uL)	Locked	UUID
<input type="checkbox"/>	SMRT Link v8.0 Sample Setup Demo 4	2019-09-15, 07:02:28 PM	smark	100	false	2b22635b-5531-bc65-3538-7d6cea5d65a2
<input type="checkbox"/>	SMRT Link v8.0 Sample Setup Demo 3	2019-09-15, 07:02:23 PM	smark	100	false	75d1148b-68ea-3255-2eb1-3b29374d7674
<input type="checkbox"/>	SMRT Link v8.0 Sample Setup Demo 2	2019-09-15, 07:02:16 PM	smark	100	false	09ab0564-4c87-073d-7ebe-31a6765a23b9
<input type="checkbox"/>	SMRT Link v8.0 Sample Setup Demo 1	2019-09-15, 06:59:32 PM	smark	100	false	36fd8e75-ba36-774d-feb9-118e640b9a0a

v8.0 Sample Setup Demo

Displaying rows 1 to 4 out of 4

1. Access SMRT Link using the Chrome web browser.
2. Select **Sample Setup**.
3. Specify if this calculation is to be used with a **Sequel** or **Sequel II System**. This affects the initial default values.
4. Click **+ New Calculation**.

# SMRT LINK V8.0 SAMPLE SETUP FIELDS

## No Major Changes to SMRT Link Sample Setup GUI Workflow

- Replaced 'Chip Type' field with 'Instrument Type' field
- **New field** under 'Advanced Options' menu: **Annealing to Binding Volume Margin**
  - Specifies the amount of overage volume to use for setting up the Annealing Primer reaction in order to account for potential pipetting errors, lost droplets, etc., so that there is always enough volume to carry into the Polymerase Binding reaction
  - Default value is 10% (e.g., if the Binding reaction volume is 20  $\mu$ L, then the target Annealing reaction volume =  $20 * 1.10 = 22.0 \mu$ L.)
- No changes to 'Lock' feature
  - Locks the calculation to prevent further changes. This is **required** before samples can be imported into the Run Design module, and also sends a copy of the instructions to the server for use in Data Set reports.
    - Locking ensures that calculations are always synchronized with their run time state if a report is generated at a later date.

Sample 1	
Sample Name	SMRT Link v8.0 Sample Setup Demo 1
Instrument Type	Sequel II
Available Volume	10 $\mu$ L
Concentration	100 ng/ $\mu$ L
Insert Size	30000 bp
Sequencing Primer	Sequencing Primer v4
Binding Kit	Sequel® II Binding Kit 2.0
Sequencing Mode	CLR
Iso-Seq experiment	No
Iso-Seq Version	None
Internal Control	Sequel® II DNA Internal Control
Use Cleanup Step	Yes
Cleanup Anticipated Yield	50 %
Cells to Bind	1 cells
Specify Concentration on Plate	50 pM
Number of SMRT Cells possible	4
▼ Advanced Options	
Use Predictive Loading (Beta)	No
Minimum Pipetting Volume	1 $\mu$ L
Annealing to Binding Volume Margin	10 %
Warnings	
Actions	Copy Remove Lock

# SMRT LINK V8.0 SAMPLE SETUP FIELDS (CONT.)

- **New field** under 'Final Loading Dilution' section: **Show values for a different number of cells**

- This **optional** field allows users to specify an alternate number of SMRT Cells to load for a given amount of prepared complex
- This is useful in case, for example, a user wants Sample Setup instructions for preparing a large amount of polymerase-bound complex, but only wants to load a subset of the sample at any given time (e.g., prepare enough purified bound complex for 10 SMRT Cells, but only load 2 cells that day; or, e.g., the complex cleanup yield is better than expected and thus the desired number of actual cells to run can be revised upwards.)

## Final Loading Dilution

Reagent	SL v8.0 Sample Setup Demo 01B - SQII - CLR Large Insert - UNLOCKED	✓	
Sequel® Complex Dilution Buffer	132.9 uL		
Prepared sample	64.1 uL		
Diluted Internal Control (Dilution 2)	7.7 uL		
DTT	23.0 uL		
Sequel Additive	2.3 uL		
Total Volume	230.0 uL		
# of SMRTCells requested	2		
Show values for a different number of cells	2		



## Final Loading Dilution

Reagent	SL v8.0 Sample Setup Demo 01B - SQII - CLR Large Insert - UNLOCKED	✓	
Sequel® Complex Dilution Buffer	66.5 uL		
Prepared sample	32.0 uL		
Diluted Internal Control (Dilution 2)	3.8 uL		
DTT	11.5 uL		
Sequel Additive	1.2 uL		
Total Volume	115.0 uL		
# of SMRTCells requested	2		
Show values for a different number of cells	1		

Load 115 uL of sample per well and store at 4C for up to 24 hours before use.

Load 115 uL of sample per well and store at 4C for up to 24 hours before use.

## NEW AND UPDATED SAMPLE SETUP SELECTION OPTIONS

Sample Setup Field	SQII Chem 1.0	SQII Chem 2.0
Sequencing Primer	Sequencing Primer v3	<a href="#">Sequencing Primer v2</a>
	Sequencing Primer v4	Sequencing Primer v4
Binding Kit	Sequel II Binding Kit 1.0	Sequel II Binding Kit 1.0
		<a href="#">Sequel II Binding Kit 2.0*</a>
		<a href="#">Sequel II Binding Kit 2.1*</a>
Internal Control	Sequel II DNA Internal Control 1.0	Sequel II DNA Internal Control 1.0

\* **Sequel II Binding Kit 2.1** is recommended for Amplicons <3 kb and Standard Iso-Seq Samples. For all other library types (including Amplicons ≥3 kb and Iso-Seq samples with a focus on long transcripts), **Sequel II Binding Kit 2.0** is recommended.

# SMRT LINK V8.0 SAMPLE SETUP – APPLICATION-SPECIFIC NOTES

## Low DNA Input Libraries (SMRTbell Express TPK 2.0)

- To help drive the Annealing and Binding reaction kinetics forward, Sample Setup no longer prioritizes holding the Primer:Template and Pol:Template ratios constant for this use case
- Instead, Sample Setup aims to hold the nominal primer concentration (20 nM) and polymerase concentration (15 nM) constant and allows the DNA template concentration to fall if the sample is not concentrated enough to attain the normal target annealing and binding values (i.e., 1 nM and 0.5 nM, respectively, for all library types)

## HiFi Libraries (SMRTbell TPK 1.0 or SMRTbell Express TPK 2.0)

- For HiFi Libraries, Sequencing Primer v2 is currently recommended but Sample Setup does not prohibit Sequencing Primer v4 to be specified
  - Refer to application-specific **Procedure & Checklist** document and **Diffusion Loading and Pre-Extension Recommendations for the Sequel II System** [Quick Reference Card](#) for the latest HiFi sample loading and run setup recommendations

# SMRT LINK V8.0 RUN DESIGN HOMEPAGE

New-style GUI retains the same basic functionality as previous SMRT Link Run Design modules

PACBIO Run Design smark (Lab Tech) ?

Run Design

+ Create New Design Import Run Design

Sequel Sequel II

Name	Summary	Date Created ↓	Created By	Reserved	UUID
Sequel II Demo 1		2019-09-15, 08:48:04 PM	smark	false	6f480126-917d-4740-9c98-97b56ca899dd

SMRT Link v8.0 Demo x

Displaying rows 1 to 1 out of 1

1. Access SMRT Link using the Chrome web browser.
2. Select **Run Design**.
3. Specify if this Run Design is to be used with a Sequel or Sequel II System. This affects the initial default values.
4. To initiate a new Run Design, click **+ Create New Design**.

# SMRT LINK V8.0 RUN DESIGN FIELDS

## No Major Changes to SMRT Link Run Design GUI Workflow

The screenshot displays the 'Edit Run Design' interface in the PacBio smark (Lab Tech) application. The interface is divided into several sections:

- Run Information:** Includes 'System Type' (radio buttons for SEQUEL and SEQUEL II), 'Run Name' (text input: SMRT Link v8.0 Run Design Demo 1), 'Run Comments' (text area), 'Experiment Name' (text input: Demo Experiment Name), and 'Experiment ID' (text input: 1234). An 'Estimated Run Duration (hours): 18' is also shown.
- Run Reagents / Consumables:** Lists required items: 1 SMRT Cell, 1 sequencing reagent plate, 1 mineral oil tube, 3 boxes of tips, 1 mixing plate, and 1 sample plate.
- Sample Information:** A scrollable panel for 'SAMPLE 1: Demo Sample 1, A01, 15 hour movie, 30000 bp insert'. It includes:
  - 'Import from Sample Setup' with a 'Select Sample' button.
  - 'Sample Name' (Required): Demo Sample 1
  - 'Sample Comment' (Text area)
  - 'Sample Well': A01
  - 'Template Prep Kit' (Required): SMRTbell® Express Template Prep Kit 2.0
  - 'Binding Kit' (Required): Sequel® II Binding Kit 2.0
  - 'Sequencing Kit' (Required): Sequel® II Sequencing Plate 2.0 (4 rxn)
  - 'DNA Control Complex': Sequel® II DNA Internal Control 1.0
  - 'Sequencing Mode': Radio buttons for CONTINUOUS LONG READS (selected) and CCS READS.
  - 'Insert Size (bp)' (Required): 30000
  - 'On-Plate Loading Concentration (pM)' (Text input)

Navigation buttons at the top right include Delete, Cancel, View Summary, Auto Analysis, and Save.

# SMRT LINK V8.0 RUN DESIGN FIELDS (CONT.)

The screenshot displays the 'Edit Run Design' interface in the PacBio software. The top navigation bar includes the PacBio logo, 'Run Design', and user information 'smark (Lab Tech)'. The main content area is divided into two columns: 'Run Information' on the left and 'Sample Information' on the right. The 'Run Information' column contains fields for System Type (radio buttons for SEQUEL and SEQUEL II), Run Name (text input), Run Comments (text area), Experiment Name (text input), and Experiment ID (text input). The 'Sample Information' column contains a dropdown for 'Movie Time per SMRT Cell (hours)' (set to 15), a radio button for 'Use Pre-Extension' (set to NO), a dropdown for 'Immobilization Time (hrs)' (set to 2), and two expandable sections: 'Advanced Options' and 'Barcoded Sample Options'. The 'Advanced Options' section includes a radio button for 'Use Predictive Loading (Beta)' (set to NO). The 'Barcoded Sample Options' section includes a radio button for 'Sample Is Barcoded' (set to NO). At the bottom of the 'Sample Information' column, there are 'Copy' and 'Delete' buttons, and an 'Add Sample' button. The top right of the interface features buttons for 'Delete', 'Cancel', 'View Summary', 'Auto Analysis', and 'Save'.

- No changes to **'Advanced Options'**
- No changes to **'Barcoded Sample Options'**



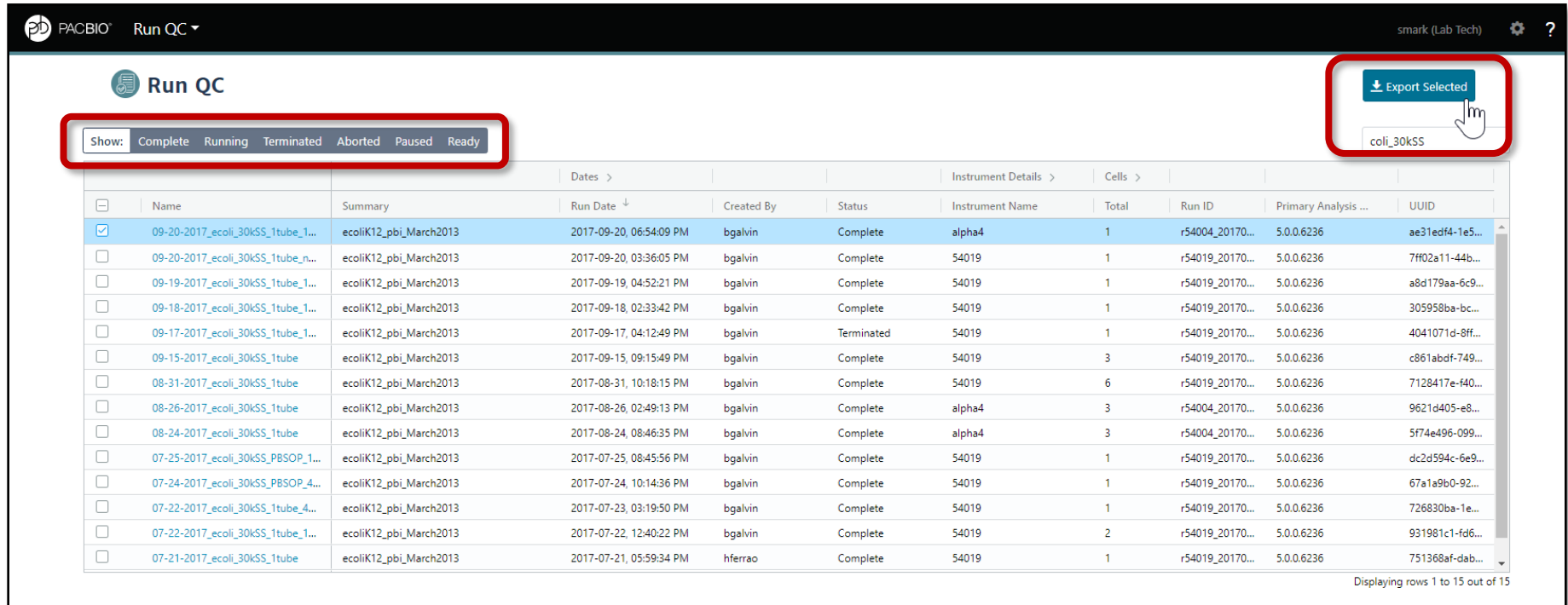
## NEW AND UPDATED RUN DESIGN SELECTION OPTIONS

Run Design Field	SQII Chem v1.0	SQII Chem 2.0
Template Prep Kit	SMRTbell TPK 1.0	SMRTbell TPK 1.0
	<b>SMRTbell Express TPK*</b>	SMRTbell Express TPK 2.0
	SMRTbell Express TPK 2.0	
Binding Kit	Sequel II Binding Kit 1.0	Sequel II Binding Kit 1.0
		Sequel II Binding Kit 2.0
		Sequel II Binding Kit 2.1
Sequencing Kit	Sequel II Sequencing Kit 1.0	Sequel II Sequencing Kit 1.0
		Sequel II Sequencing Kit 2.0
DNA Control Complex	Sequel II DNA Internal Control 1.0	Sequel II DNA Internal Control 1.0
Movie Time per SMRT Cell	Up to 30 hours	Up to 30 hours

\* **SMRTbell Express TPK** is **obsoleted** and has been discontinued

# SMRT LINK V8.0 RUN QC HOMEPAGE

New-style GUI retains the same basic functionality as previous SMRT Link Run QC modules



The screenshot displays the SMRT Link Run QC homepage. At the top left, the PacBio logo and 'Run QC' are visible. The top right shows the user 'smark (Lab Tech)' and a settings icon. Below the header, there is a 'Run QC' section with a 'Show:' filter menu containing options: Complete, Running, Terminated, Aborted, Paused, and Ready. A red box highlights this filter menu. To the right, there is an 'Export Selected' button with a download icon, also highlighted with a red box. Below the filter and export buttons is a table of runs. The table has columns for Name, Summary, Dates, Created By, Status, Instrument Name, Total, Run ID, Primary Analysis, and UUID. The first row is selected, and its details are visible in the table below.

	Name	Summary	Dates >	Created By	Status	Instrument Details >	Cells >				
<input type="checkbox"/>			Run Date ↓			Instrument Name	Total	Run ID	Primary Analysis ...	UUID	
<input checked="" type="checkbox"/>	09-20-2017_ecoli_30kSS_1tube_1...	ecoliK12_pbi_March2013	2017-09-20, 06:54:09 PM	bgalvin	Complete	alpha4	1	r54004_20170...	5.0.0.6236	ae31edf4-1e5...	
<input type="checkbox"/>	09-20-2017_ecoli_30kSS_1tube_n...	ecoliK12_pbi_March2013	2017-09-20, 03:36:05 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	7ff02a11-44b...	
<input type="checkbox"/>	09-19-2017_ecoli_30kSS_1tube_1...	ecoliK12_pbi_March2013	2017-09-19, 04:52:21 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	a8d179aa-6c9...	
<input type="checkbox"/>	09-18-2017_ecoli_30kSS_1tube_1...	ecoliK12_pbi_March2013	2017-09-18, 02:33:42 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	305958ba-bc...	
<input type="checkbox"/>	09-17-2017_ecoli_30kSS_1tube_1...	ecoliK12_pbi_March2013	2017-09-17, 04:12:49 PM	bgalvin	Terminated	54019	1	r54019_20170...	5.0.0.6236	4041071d-8ff...	
<input type="checkbox"/>	09-15-2017_ecoli_30kSS_1tube	ecoliK12_pbi_March2013	2017-09-15, 09:15:49 PM	bgalvin	Complete	54019	3	r54019_20170...	5.0.0.6236	c861abdf-749...	
<input type="checkbox"/>	08-31-2017_ecoli_30kSS_1tube	ecoliK12_pbi_March2013	2017-08-31, 10:18:15 PM	bgalvin	Complete	54019	6	r54019_20170...	5.0.0.6236	7128417e-f40...	
<input type="checkbox"/>	08-26-2017_ecoli_30kSS_1tube	ecoliK12_pbi_March2013	2017-08-26, 02:49:13 PM	bgalvin	Complete	alpha4	3	r54004_20170...	5.0.0.6236	9621d405-e8...	
<input type="checkbox"/>	08-24-2017_ecoli_30kSS_1tube	ecoliK12_pbi_March2013	2017-08-24, 08:46:35 PM	bgalvin	Complete	alpha4	3	r54004_20170...	5.0.0.6236	5f74e496-099...	
<input type="checkbox"/>	07-25-2017_ecoli_30kSS_PBSOP_1...	ecoliK12_pbi_March2013	2017-07-25, 08:45:56 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	dc2d594c-6e9...	
<input type="checkbox"/>	07-24-2017_ecoli_30kSS_PBSOP_4...	ecoliK12_pbi_March2013	2017-07-24, 10:14:36 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	67a1a9b0-92...	
<input type="checkbox"/>	07-22-2017_ecoli_30kSS_1tube_4...	ecoliK12_pbi_March2013	2017-07-23, 03:19:50 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	726830ba-1e...	
<input type="checkbox"/>	07-22-2017_ecoli_30kSS_1tube_1...	ecoliK12_pbi_March2013	2017-07-22, 12:40:22 PM	bgalvin	Complete	54019	2	r54019_20170...	5.0.0.6236	931981c1-fd6...	
<input type="checkbox"/>	07-21-2017_ecoli_30kSS_1tube	ecoliK12_pbi_March2013	2017-07-21, 05:59:34 PM	hferrao	Complete	54019	1	r54019_20170...	5.0.0.6236	751368af-dab...	

Displaying rows 1 to 15 out of 15

1. Access SMRT Link using the Chrome web browser.
2. Select **Run QC**.
3. To filter the list of runs to display, click one or more of the following buttons: Complete, Running, Terminated, Aborted, Paused, and/or Ready. (Click Show to remove or select all the filters.)
4. To export Run QC data in CSV format: Select one or more runs in the table, then click Export Selected

# SMRT LINK V8.0 RUN QC HOMEPAGE (CONT.)

The screenshot displays the 'Run QC' interface. At the top left, the PacBio logo and 'Run QC' are visible. On the top right, there is a user profile 'smark (Lab Tech)' and a settings icon. Below the header, there is a 'Show:' filter menu with options: Complete, Running, Terminated, Aborted, Paused, Ready. To the right of the filter is an 'Export Selected' button and a search box containing 'coli\_30kSS'. The main area is a table with 15 rows. The first row is selected. Three column headers are highlighted with red boxes: 'Dates >', 'Instrument Details >', and 'Cells >'. The table columns are: Name, Summary, Run Date, Created By, Status, Instrument Name, Total, Run ID, Primary Analysis, and UUID. The bottom right corner of the table area says 'Displaying rows 1 to 15 out of 15'.

	Name	Summary	Dates >	Created By	Status	Instrument Details >	Cells >	Run ID	Primary Analysis ...	UUID
<input checked="" type="checkbox"/>	09-20-2017_ecoli_30kSS_1tube_1...	ecoliK12_pbi_March2013	2017-09-20, 06:54:09 PM	bgalvin	Complete	alpha4	1	r54004_20170...	5.0.0.6236	ae31edf4-1e5...
<input type="checkbox"/>	09-20-2017_ecoli_30kSS_1tube_n...	ecoliK12_pbi_March2013	2017-09-20, 03:36:05 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	7f02a11-44b...
<input type="checkbox"/>	09-19-2017_ecoli_30kSS_1tube_1...	ecoliK12_pbi_March2013	2017-09-19, 04:52:21 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	a8d179aa-6c9...
<input type="checkbox"/>	09-18-2017_ecoli_30kSS_1tube_1...	ecoliK12_pbi_March2013	2017-09-18, 02:33:42 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	305958ba-bc...
<input type="checkbox"/>	09-17-2017_ecoli_30kSS_1tube_1...	ecoliK12_pbi_March2013	2017-09-17, 04:12:49 PM	bgalvin	Terminated	54019	1	r54019_20170...	5.0.0.6236	4041071d-8ff...
<input type="checkbox"/>	09-15-2017_ecoli_30kSS_1tube	ecoliK12_pbi_March2013	2017-09-15, 09:15:49 PM	bgalvin	Complete	54019	3	r54019_20170...	5.0.0.6236	c861abdf-749...
<input type="checkbox"/>	08-31-2017_ecoli_30kSS_1tube	ecoliK12_pbi_March2013	2017-08-31, 10:18:15 PM	bgalvin	Complete	54019	6	r54019_20170...	5.0.0.6236	7128417e-f40...
<input type="checkbox"/>	08-26-2017_ecoli_30kSS_1tube	ecoliK12_pbi_March2013	2017-08-26, 02:49:13 PM	bgalvin	Complete	alpha4	3	r54004_20170...	5.0.0.6236	9621d405-e8...
<input type="checkbox"/>	08-24-2017_ecoli_30kSS_1tube	ecoliK12_pbi_March2013	2017-08-24, 08:46:35 PM	bgalvin	Complete	alpha4	3	r54004_20170...	5.0.0.6236	5f74e496-099...
<input type="checkbox"/>	07-25-2017_ecoli_30kSS_PBSOP_1...	ecoliK12_pbi_March2013	2017-07-25, 08:45:56 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	dc2d594c-6e9...
<input type="checkbox"/>	07-24-2017_ecoli_30kSS_PBSOP_4...	ecoliK12_pbi_March2013	2017-07-24, 10:14:36 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	67a1a9b0-92...
<input type="checkbox"/>	07-22-2017_ecoli_30kSS_1tube_4...	ecoliK12_pbi_March2013	2017-07-23, 03:19:50 PM	bgalvin	Complete	54019	1	r54019_20170...	5.0.0.6236	726830ba-1e...
<input type="checkbox"/>	07-22-2017_ecoli_30kSS_1tube_1...	ecoliK12_pbi_March2013	2017-07-22, 12:40:22 PM	bgalvin	Complete	54019	2	r54019_20170...	5.0.0.6236	931981c1-fd6...
<input type="checkbox"/>	07-21-2017_ecoli_30kSS_1tube	ecoliK12_pbi_March2013	2017-07-21, 05:59:34 PM	hferao	Complete	54019	1	r54019_20170...	5.0.0.6236	751368af-dab...

## - New collapsible column headers:

- **Dates >** Run Date, Completion Date, Transferred Date
- **Instrument Details >** Instrument Name, Instrument SN, Instrument SW
- **Cells >** Total, Completed, Failed

# SMRT LINK V8.0 RUN QC PRIMARY ANALYSIS METRICS SUMMARY TABLE

Run QC / Run Data  
Appslab\_E.coli\_CLR\_SC

COMPLETE [Export](#) [Print](#)

Overview

Run Start: 2019-09-09, 05:22:02 PM  
Run Complete: 2019-09-11, 03:27:40 AM  
Transfer Complete: 2019-09-11, 06:44:14 AM

Run Id: r64001\_20190909\_210949  
Description:  
Instrument: 64001

Instrument SN: 64001  
Instrument Control SW Version: 8.0.0.78320  
Instrument Chemistry Bundle: 8.0.0.77154

Primary SW Version: 8.0.0.78320

Consumables

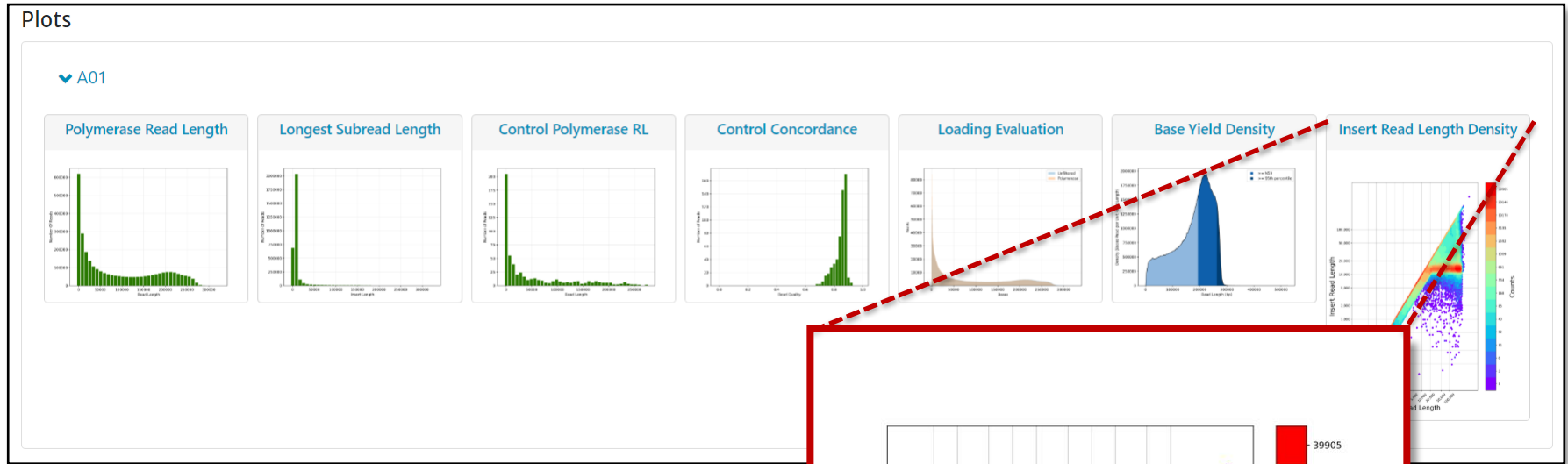
Well	Name	Movie Time (hrs)	Status	Total Bases (Gb)	Unique Molecular Yield (Gb)	Productivity (%)			Read Length (bp)				Local Base Rate	Adapter Dimer
						P0	P1	P2	Polymerase		Longest Subread			
									Mean	N50	Mean	N50	Poly RL Mean (bp)	
A01	E.coli CLR_V2	15	Complete	153.37	131.52	47.1	52.1	0.8	36735	54321	31509	44419	2.30	0
B01	E.coli CLR_V4	15	Complete	182.59	160.80	33.8	65.0	1.2	35043	52456	30869	44533	2.28	0

1 to 2 of 2 | Page 1 of 1

## - New collapsible column headers:

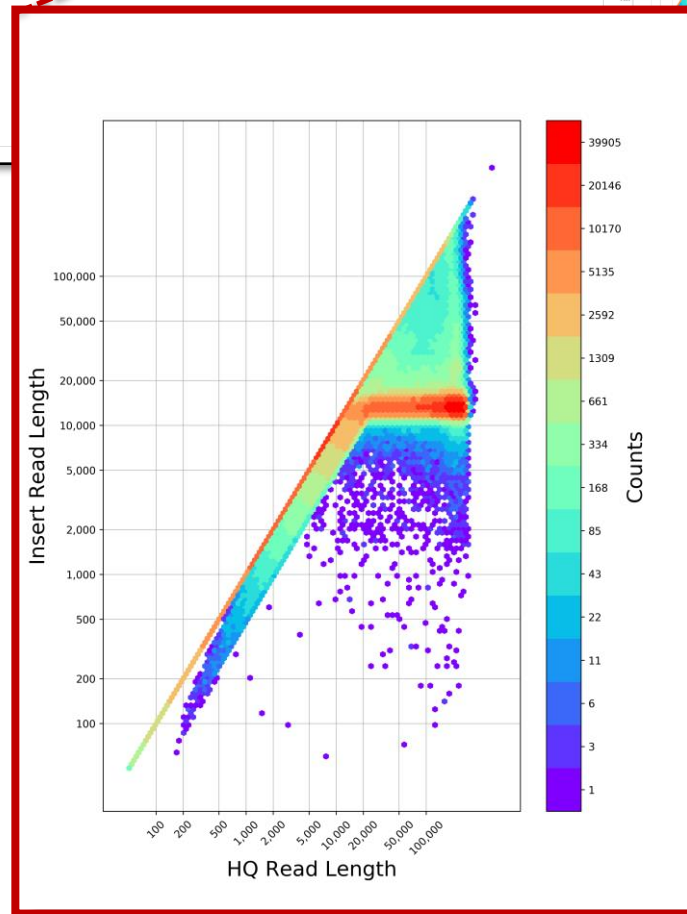
- **Run Settings** > Movie Time (hrs), Pre-extension Time (hrs), Workflow
- **Control** > Poly RL Mean (bp) Total Reads, Concordance Mean, Concordance Mode
- **Template** > Adapter Dimer, Short Insert

# SMRT LINK V8.0 RUN QC PLOTS



## - No major changes to Run QC Plots

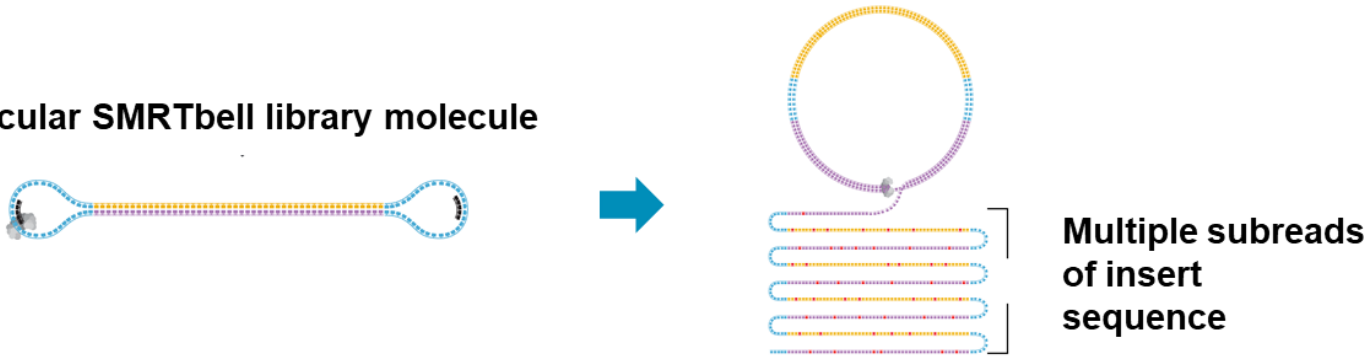
- Polymerase Read Length
  - Longest Subread Length
  - Control Polymerase RL
  - Control Concordance
  - Loading Evaluation
  - Base Yield Density
  - Insert Read Length Density
- **Note:** 'Distribution of HQ Start Positions' plot has been **removed** in SMRT Link v8.0



# RUN QC PRIMARY ANALYSIS METRICS SUMMARY TABLE: TOTAL BASES VS. UNIQUE MOLECULAR YIELD (UMY)

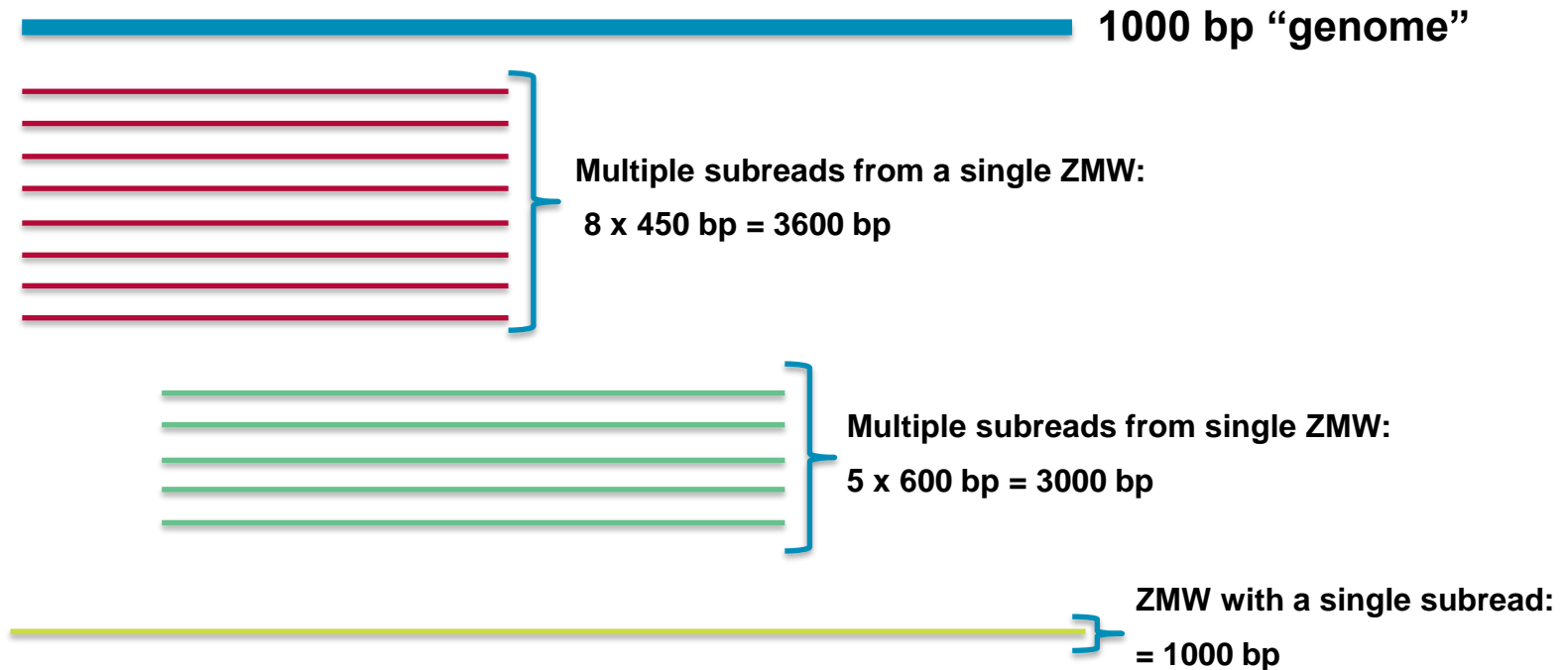
- **Total Bases (GB):** Calculated by multiplying the number of Productive (P1) ZMWs by the mean Polymerase Read Length; displayed in Gigabases.
- **Unique Molecular Yield (UMY):** The sum total length of **unique** single molecules that were sequenced. It is calculated as the sum of per-ZMW median subread lengths.

Circular SMRTbell library molecule



Run Settings >				Productivity (%)			Read Length (bp)					
Well	Name	Movie Time (hrs)	Status	Total Bases (Gb)	Unique Molecular Yield (Gb)	P0	P1	P2	Polymerase		Longest Subread	
									Mean	N50	Mean	N50
A01	CLR Sample 01	15	Complete	153.37	131.52	47.1	52.1	0.8	36735	54321	31509	44419
B01	CLR Sample 02	15	Complete	182.59	160.80	33.8	65.0	1.2	35043	52456	30869	44533

# UNIQUE MOLECULAR YIELD (UMY) CAN BE USED TO CALCULATE UNIQUE MOLECULAR COVERAGE (UMC)



**Raw Coverage = Total Bases / Genome Size**

$$\rightarrow (3600 + 3000 + 1000) / 1000 = 7600 / 1000 = \mathbf{7.60\text{-fold}}$$

**Unique Molecular Coverage (UMC) = Unique Molecular Yield (UMY) / Genome Size**

$$\rightarrow (450 + 600 + 1000) / 1000 = 2050 / 1000 = \mathbf{2.05\text{-fold}}$$

# TOTAL BASES VS. UNIQUE MOLECULAR YIELD EXAMPLES

>35 kb CLR Library (15-h movie; No Pre-Extension Time)

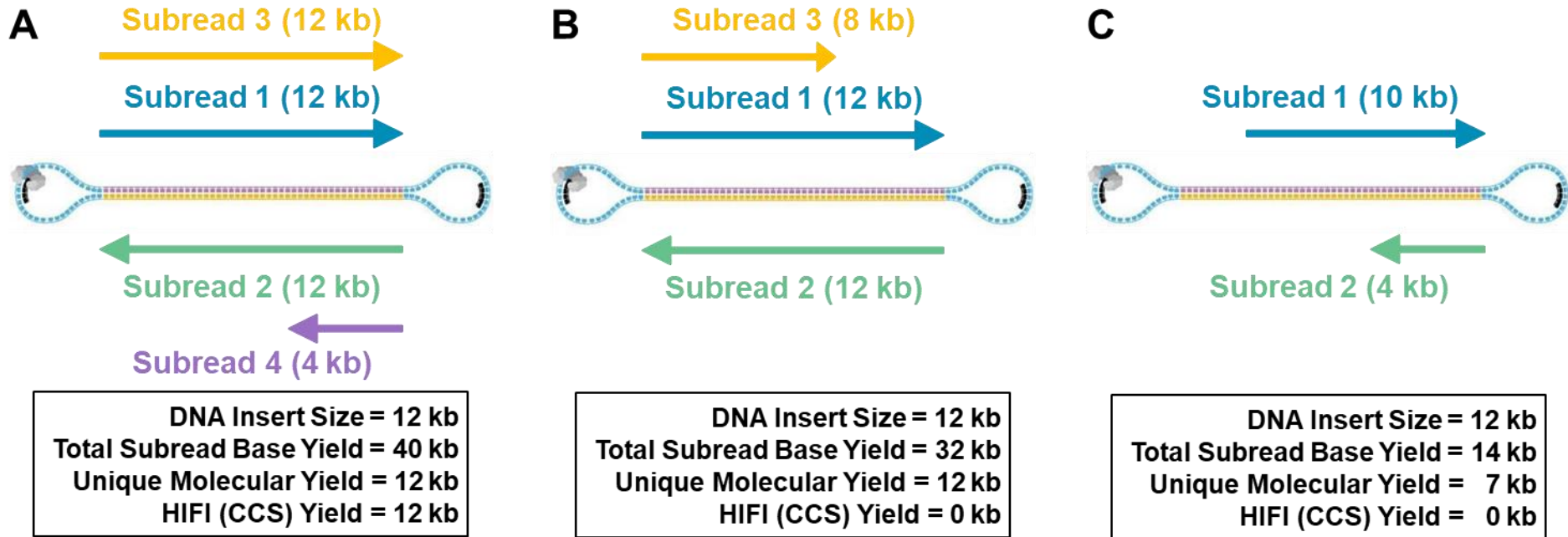
Run Settings >					Productivity (%)	Read Length (bp)
Well	Name	Movie Time (hrs)	Status	Total Bases (Gb)	Total Bases (Gb)	Unique Molecular Yield (Gb)
A01	CLR Sample 01	15	Complete	153.37	153.37	131.52
B01	CLR Sample 02	15	Complete	182.59	182.59	160.80

15 kb CCS (HiFi) Library (30-h movie; 2-h Pre-Extension Time)

Run Settings >					Productivity (%)	Read Length (bp)
					Polymerase	Longest Subread
Well	Name	Movie Time (hrs)	Status	Total Bases (Gb)	Total Bases (Gb)	Unique Molecular Yield (Gb)
A01	HiFi Sample 01	30	Complete	395.31	395.31	56.20
B01	HiFi Sample 02	30	Complete	358.11	358.11	54.21
C01	HiFi Sample 03	30	Complete	382.96	382.96	63.26



# TOTAL SUBREAD BASE YIELD VS. UNIQUE MOLECULAR YIELD (UMY)\* VS. HIFI (CCS) YIELD\*\*



\* **UMY:** The sum total length of unique single molecules that were sequenced. It is calculated as the sum of per-ZMW median subread lengths

\*\* **HiFi Reads:** Long reads with  $\geq Q20$  (99%) single-molecule accuracy generated using the circular consensus sequencing (CCS) analysis method. Note: In SMRT Link v8.0, the minimum number of **full-length subreads** required to generate a HiFi Read for a ZMW = 3



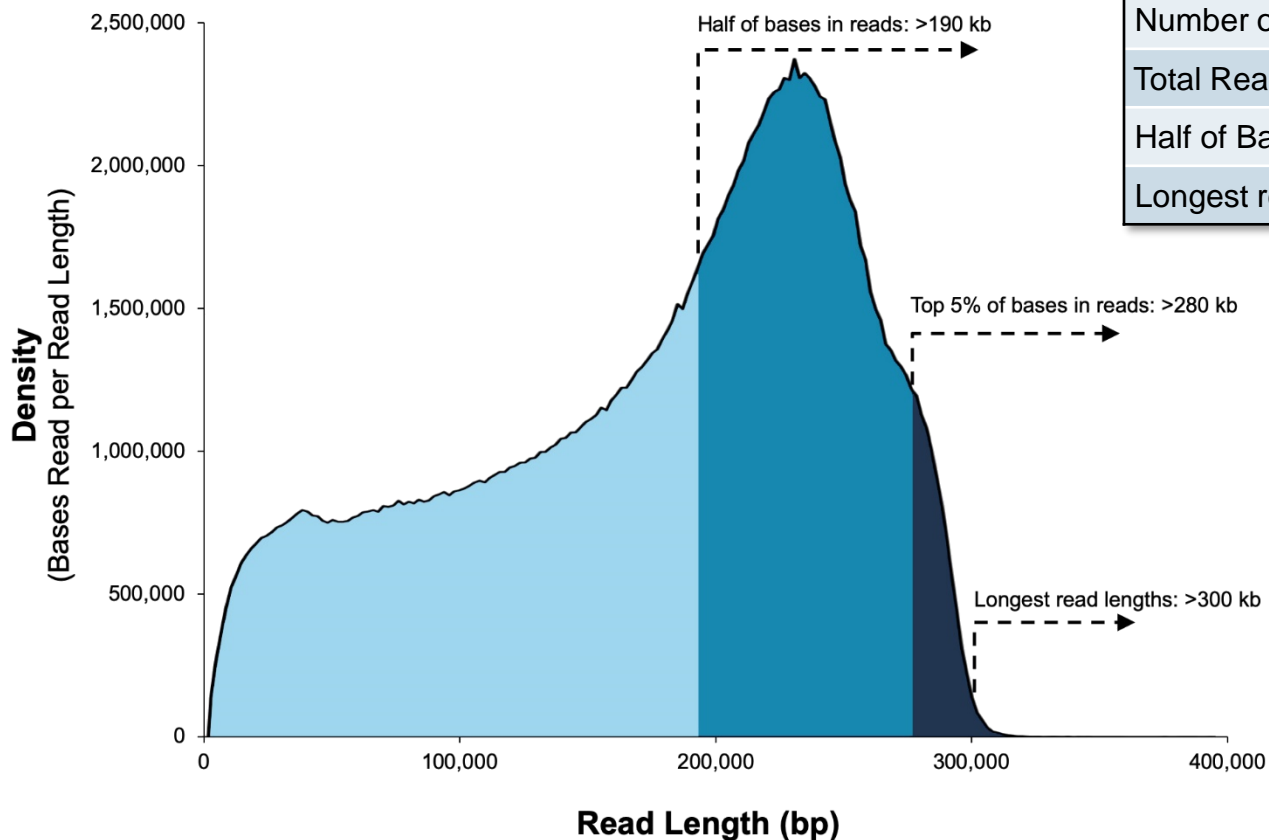
# Example Sequel II System Performance Data



# HIFI SEQUENCING (20 KB INSERT): RAW DATA

Up to 500 Gb  
Customer average: 325 Gb

Metrics	
Insert Size	20 kb
Number of Raw Bases (Gb)	377
Total Reads	4,266,403
Half of Bases in Reads	>193,403
Longest read lengths	>300,000

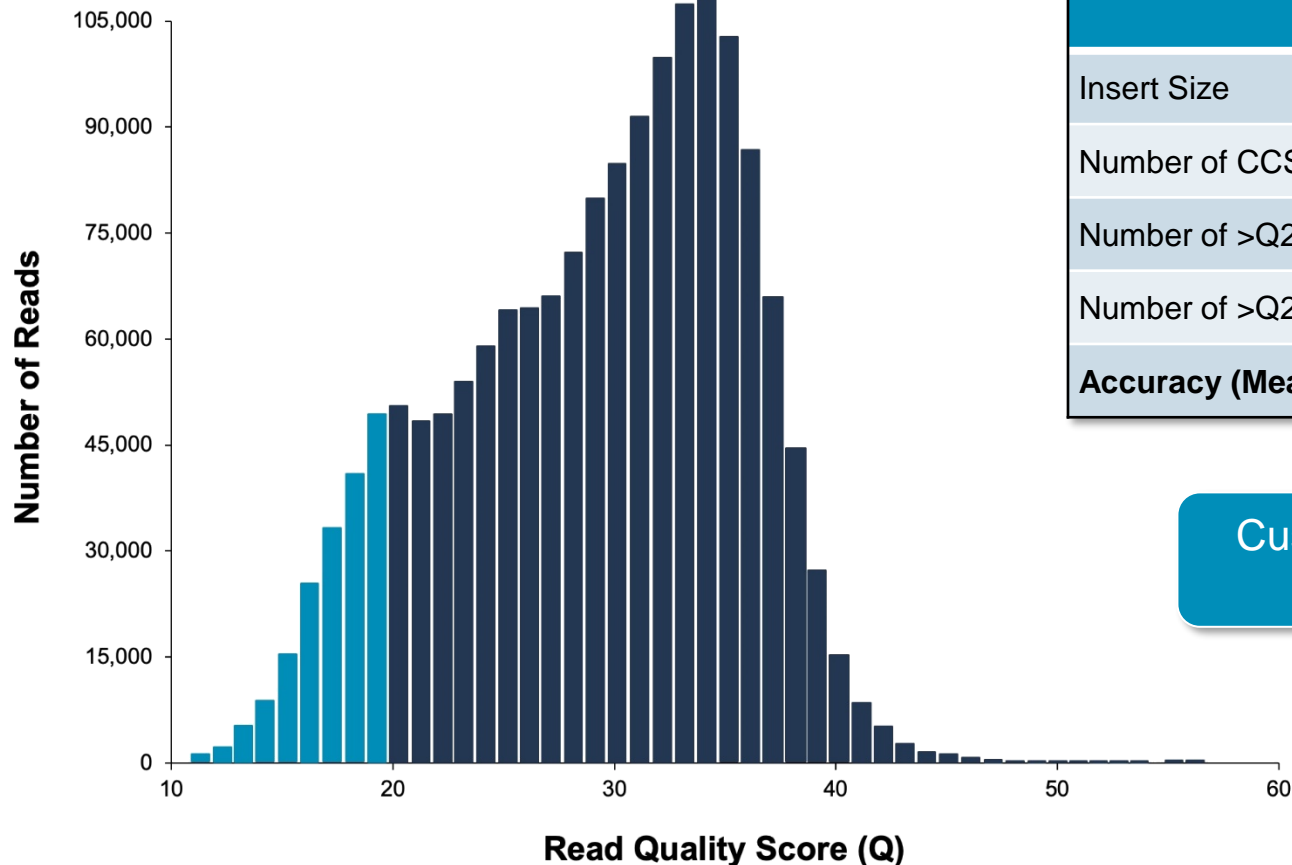


Data shown above from a 20 kb size-selected human library using the SMRTbell Template Prep Kit on a Sequel II System (2.0 Chemistry, Sequel II System Software v8.0, 30-hour movie). Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.



# HIFI SEQUENCING (20 KB INSERT): POST-CCS PROCESSING

Q20 (99%) HiFi accuracy



Metrics	
Insert Size	20 kb
Number of CCS Bases (Gb)	30 Gb
Number of >Q20 Bases	26 Gb
Number of >Q20 Reads	1,423,277
<b>Accuracy (Mean)</b>	<b>99.92%</b>

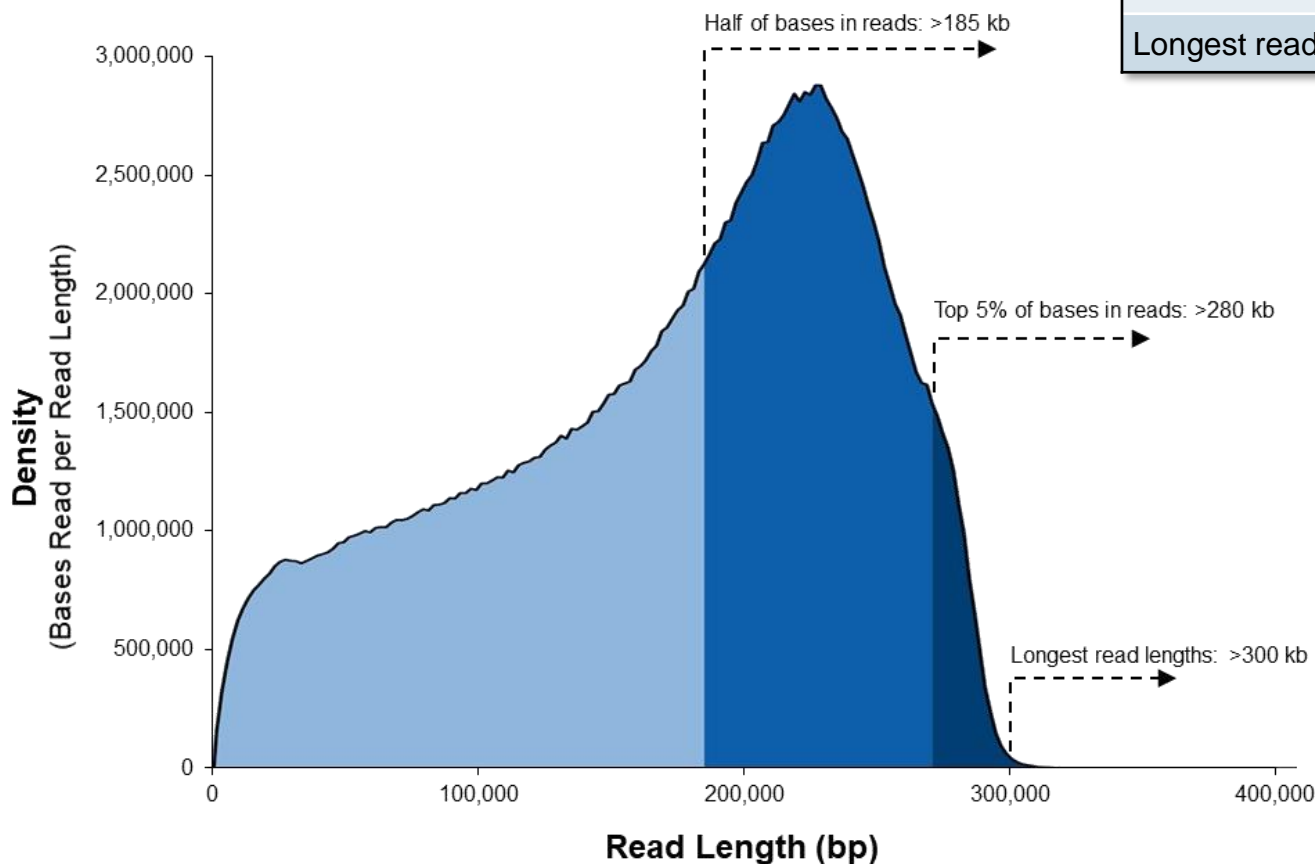
Customer average:  
25-30 Gb

Data shown above from a 20 kb size-selected human library using the SMRTbell Template Prep Kit on a Sequel II System (2.0 Chemistry, Sequel II System Software v8.0, 30-hour movie). Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.



# EXAMPLE: VARIANT CALLING WITH HIFI READS (RAW DATA)

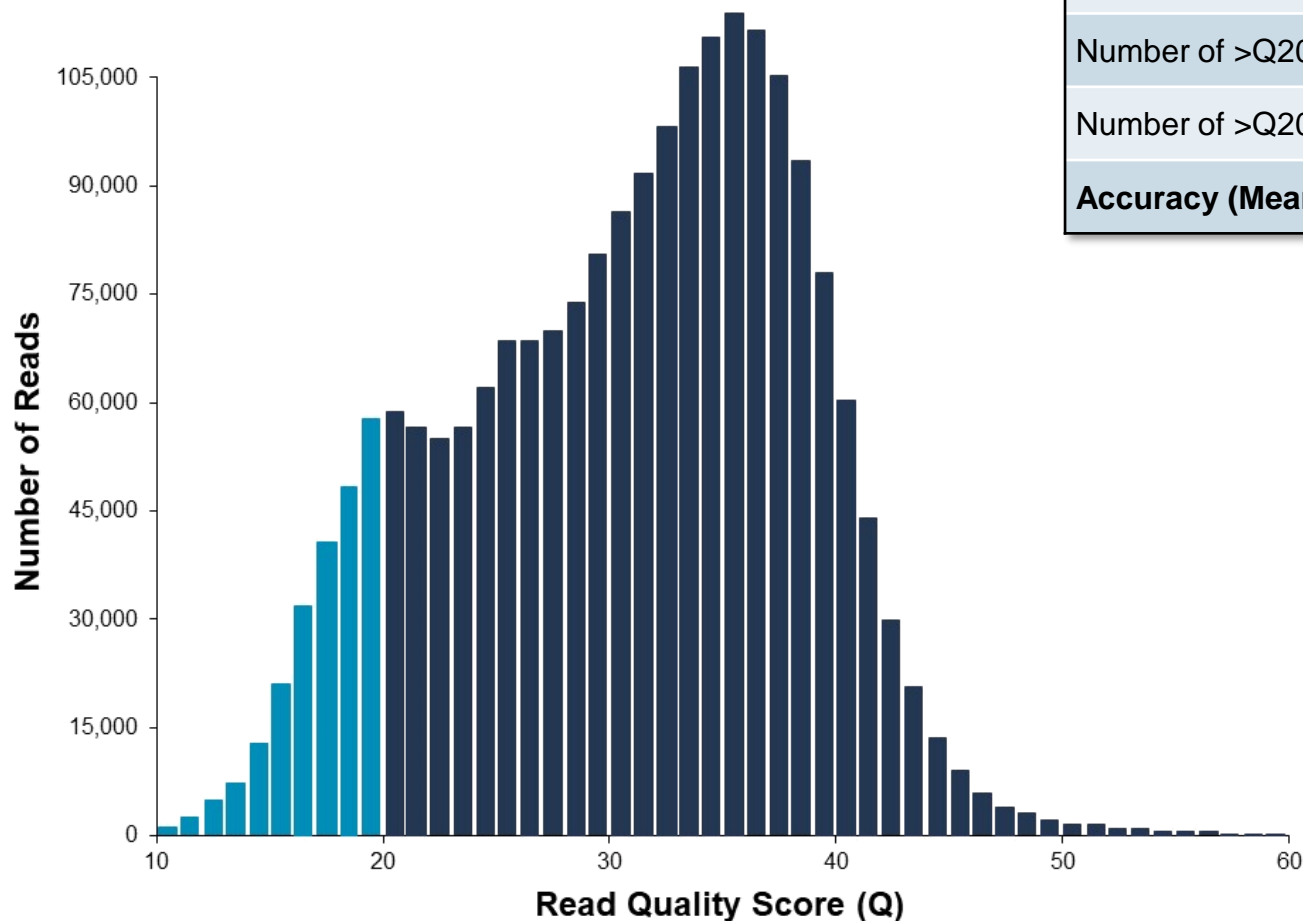
Metrics	
Insert Size	15 kb
Number of Raw Bases (Gb)	449
Total Reads	4,976,882
Half of Bases in Reads	>186,554
Longest read lengths	>300,000



Data shown above from a 15 kb size-selected human library using the SMRTbell Template Prep Kit on a Sequel II System (2.0 Chemistry, Sequel II System Software v8.0, 30-hour movie). Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.



# EXAMPLE: VARIANT CALLING WITH HIFI READS (POST-CCS PROCESSING)



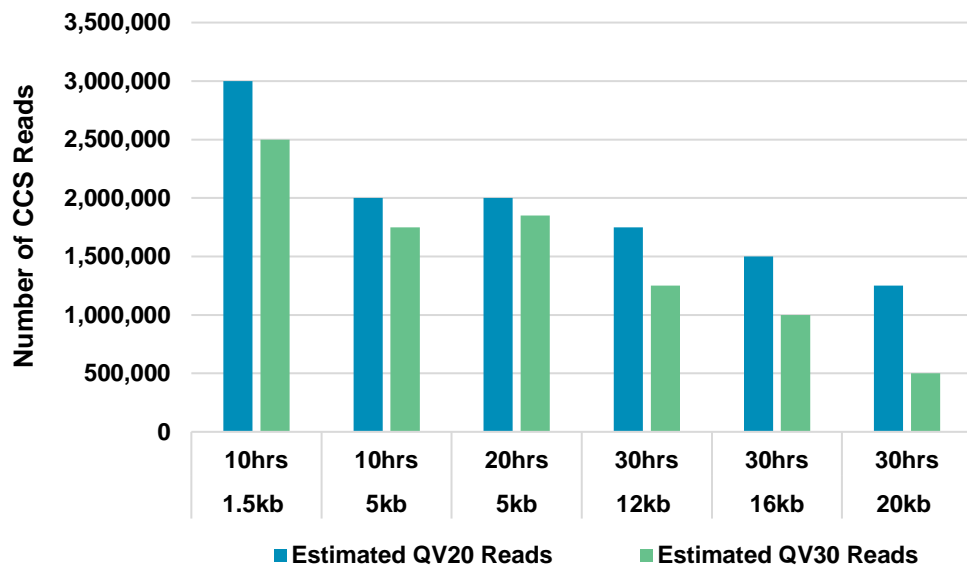
Metrics	
Insert Size	15 kb
Number of CCS Bases (Gb)	30.4 Gb
Number of >Q20 Bases	27.4 Gb
Number of >Q20 Reads	2,099,017
<b>Accuracy (Mean)</b>	<b>99.96%</b>

Data shown above from a 15 kb size-selected human library using the SMRTbell Template Prep Kit on a Sequel II System (2.0 Chemistry, Sequel II System Software v8.0, 30-hour movie). Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.



# HIFI (Q20, Q30) READ YIELD PERFORMANCE FOR DIFFERENT AMPLICON LIBRARY SIZES (SEQUEL II CHEMISTRY 2.0)

**CCS Amplicon Yield\***

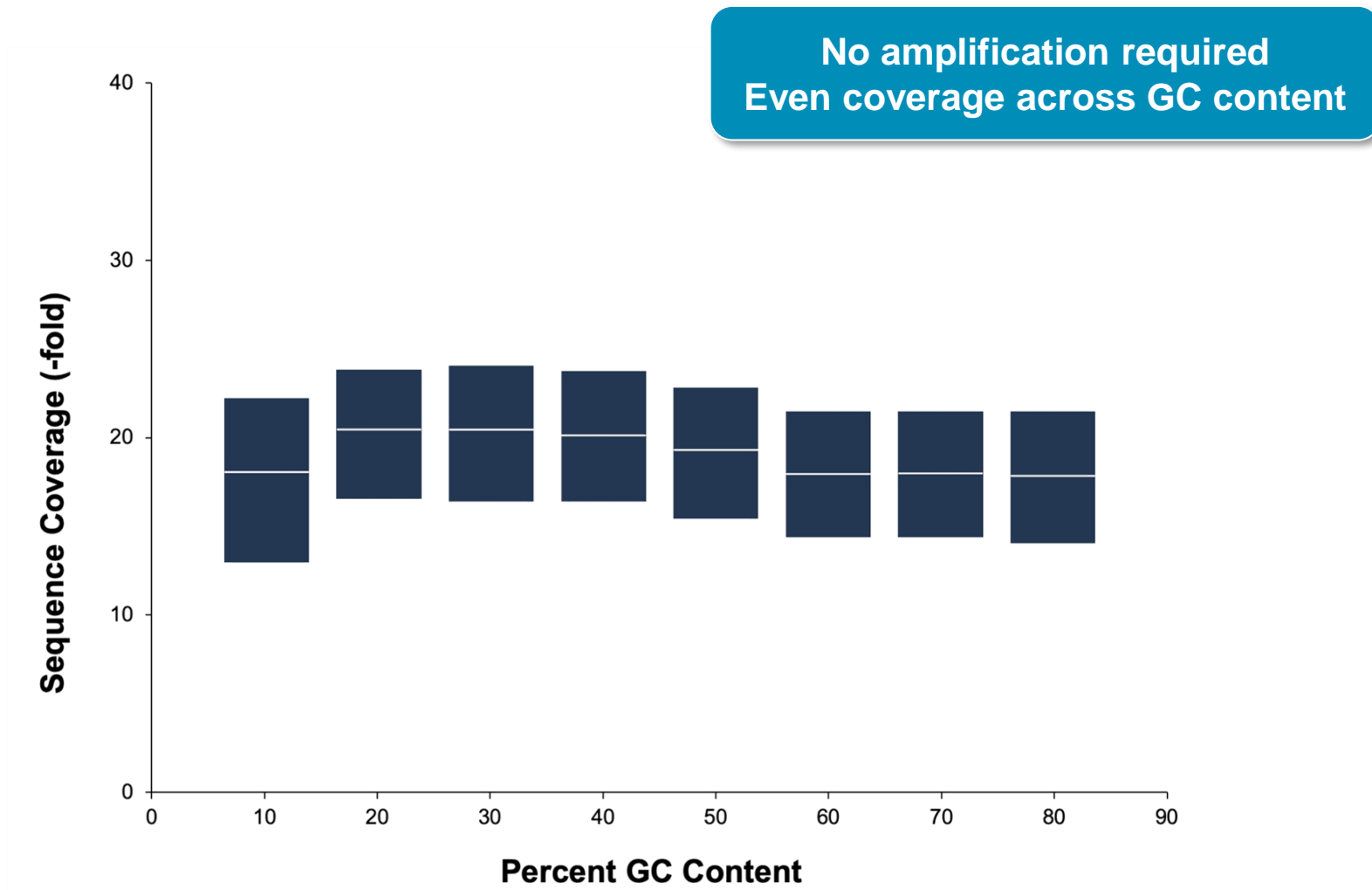


INSERT SIZE	MOVIE LENGTH	ESTIMATED Q20 READS	ESTIMATED Q30 READS
1.5 kb	10 hrs	3,000,000	2,500,000
5 kb	10 hrs	2,000,000	1,750,000
5 kb	20 hrs	2,000,000	1,850,000
12 kb	30 hrs	1,750,000	1,250,000
16 kb	30 hrs	1,500,000	1,000,000
20 kb	30 hrs	1,250,000	500,000

\* 50% P1 Loading

Data shown above are from amplicon libraries constructed using the SMRTbell Express Template Prep Kit 2.0 and sequenced on a Sequel II System (2.0 Chemistry, Sequel II System Software v8.0) using different movie collection times. Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.

# UNIFORM COVERAGE

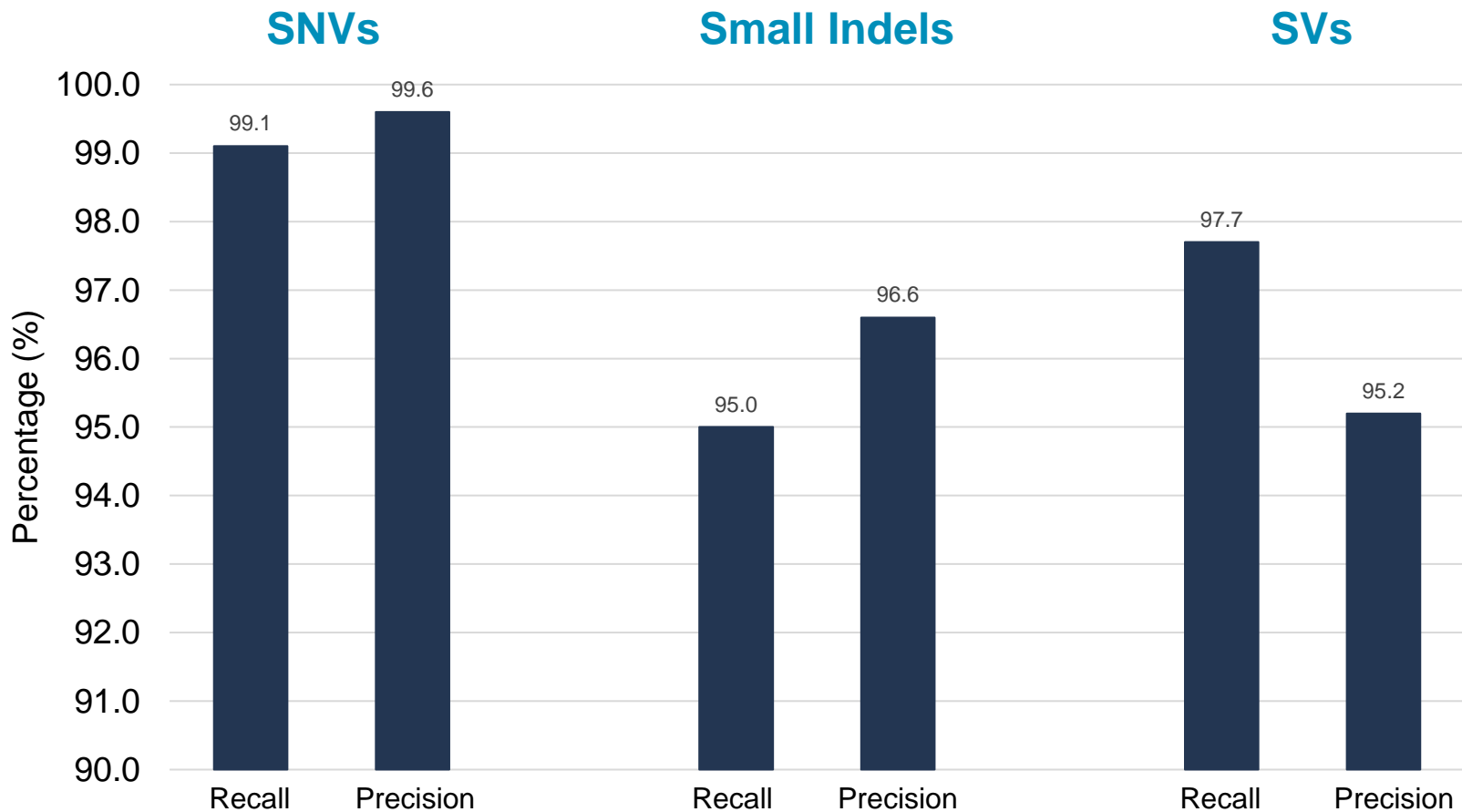


Mean coverage per GC window across a human sample. Data generated with a 15 kb human HiFi library on a Sequel II System using 2.0 Chemistry and Sequel II System Software v8.0





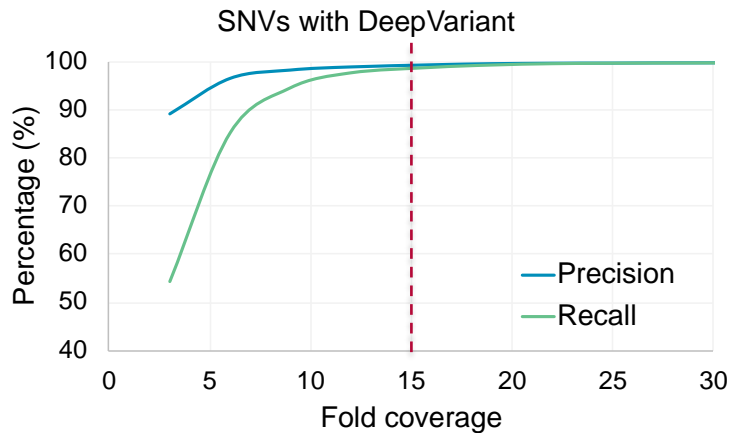
# EXAMPLE: VARIANT CALLING WITH HIFI READS



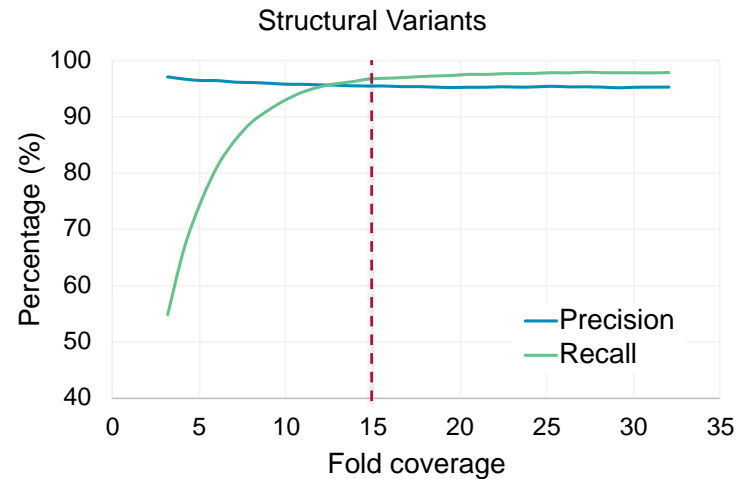
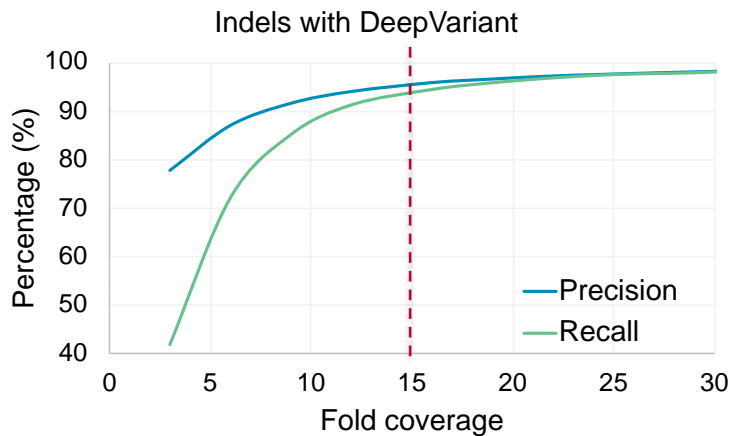
Variant calls from ~15-fold HiFi read coverage of a human genome (HG002) were measured against the Genome in a Bottle small variant benchmark (v3.3.2) for SNVs and indels using Deep Variant and SMRT Link 8.0 for SVs. Libraries were generated using a 15 kb insert and sequenced using Chemistry 2.0.



# VARIANT DETECTION COVERAGE RECOMMENDATION



**15-fold HiFi ( $\geq Q20$ ) Coverage [2 SMRT Cells 8M] provides a good trade-off between cost and results**

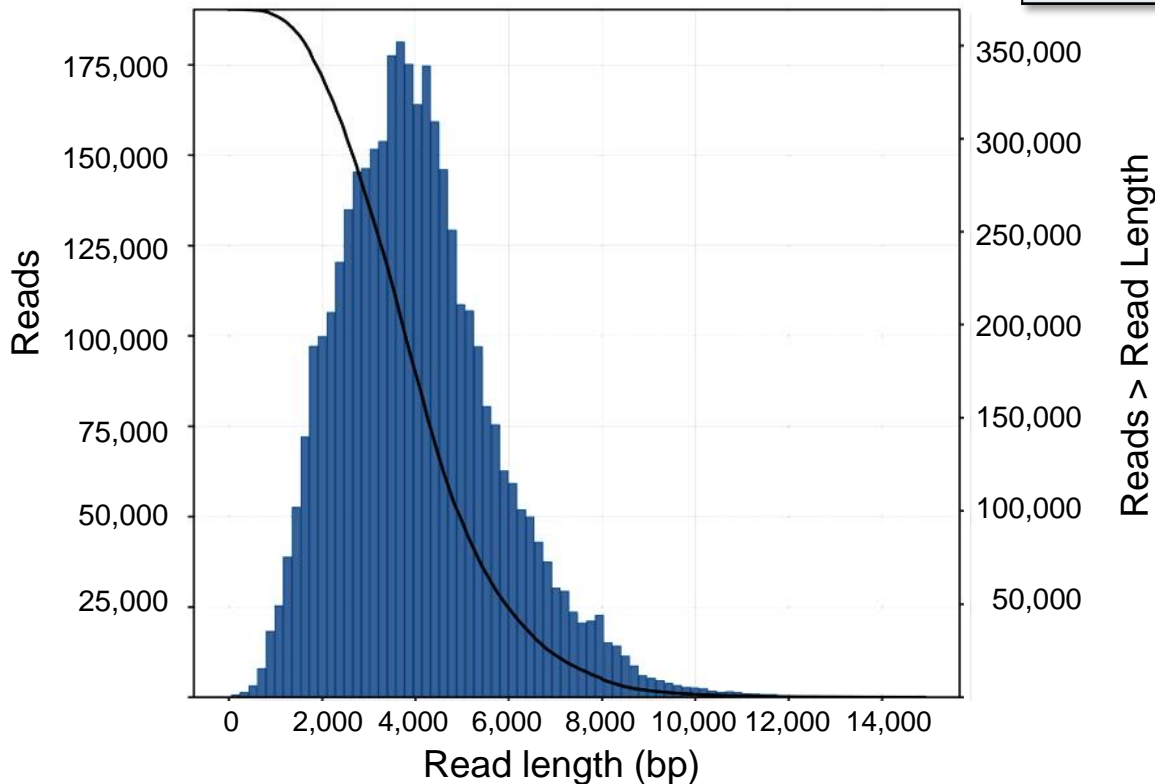




# ISO-SEQ PERFORMANCE

- Iso-Seq: Universal Human Reference RNA

Metrics	
Number of Raw Bases (Gb)	502
Total Reads	5,433,706
Full Length Non-chimeric Reads	3,693,801 (86%)
CCS Passes (Mean)	8

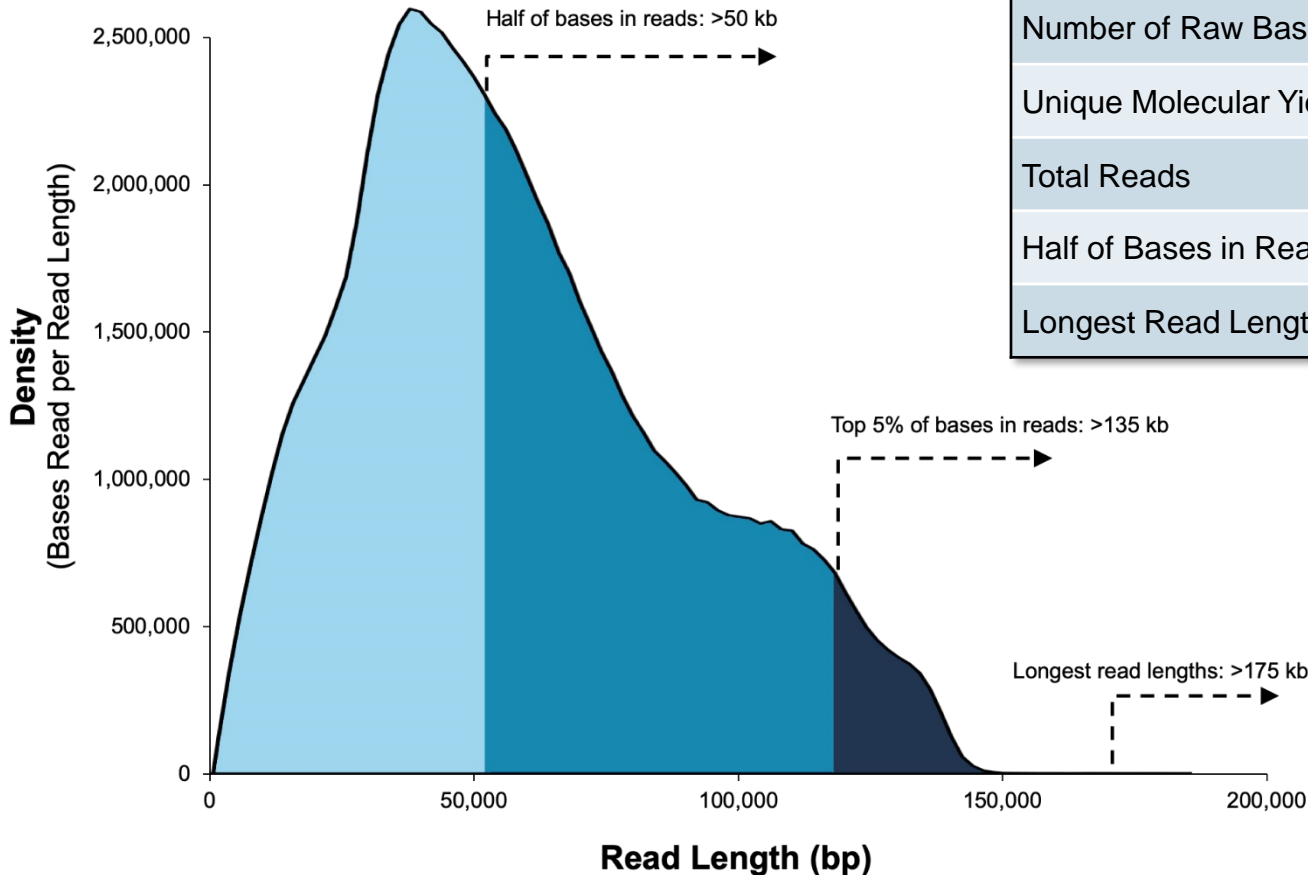


Data shown above from a Universal Human Reference RNA (human) and Lexogen SIRV spike-in controls. The library was constructed using the Iso-Seq Express workflow including the SMRTbell Express Template Prep Kit 2.0 on a Sequel II System (Sequel II Sequencing kit 2.0, Sequel II Binding Kit 2.0, Sequel II System Software v8.0, 24-hour movie). Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.



# LONG READ SEQUENCING EXAMPLE: >35 KB SAMPLE – RAW DATA

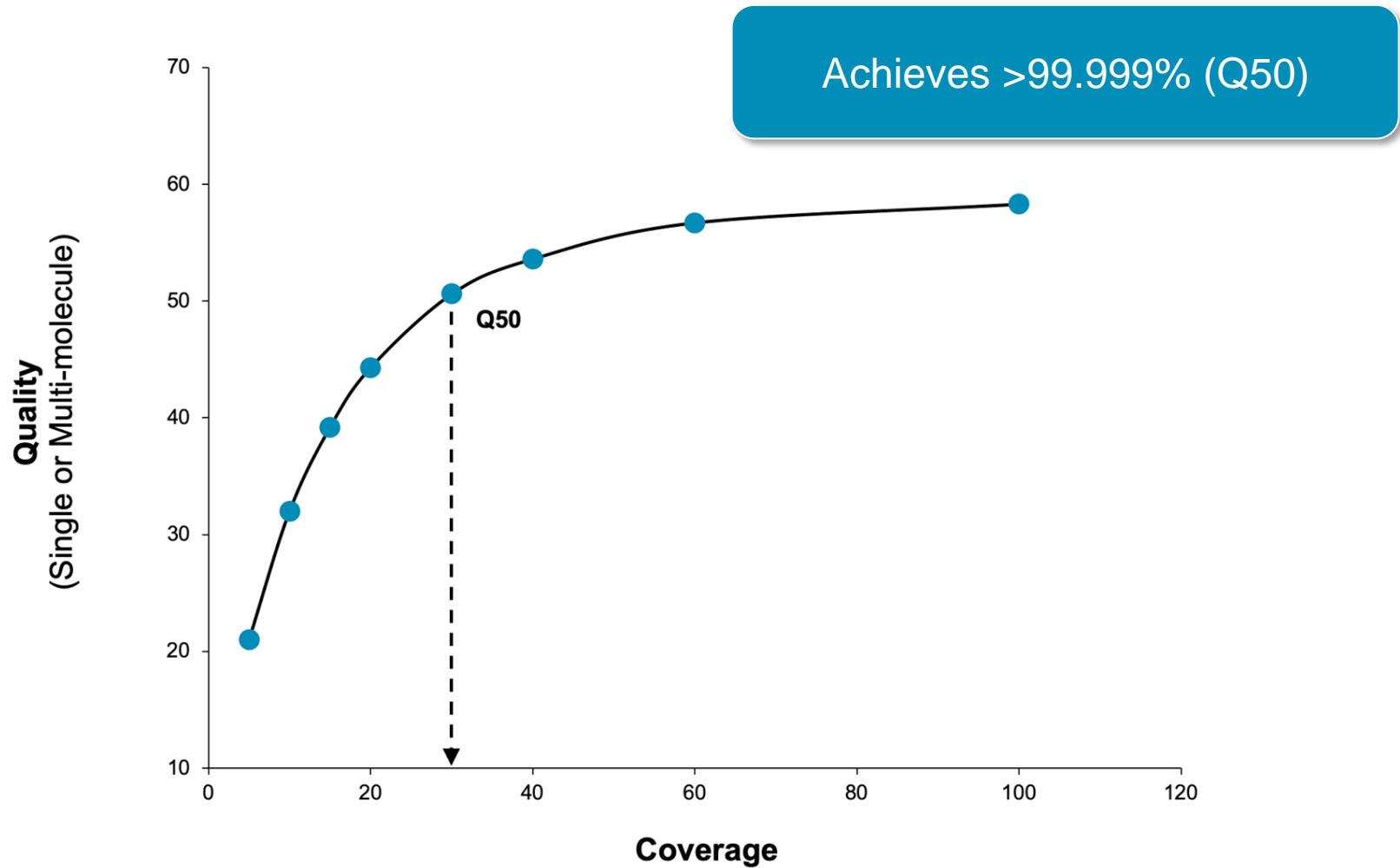
Up to 160 Gb  
Average: 100 – 120 Gb



Metrics	
Number of Raw Bases (Gb)	182
Unique Molecular Yield (Gb)	161
Total Reads	5,210,363
Half of Bases in Reads	>52,456
Longest Read Lengths	>175,000

Data shown above from a 35 kb size-selected E. coli library using the SMRTbell Template Prep Kit on a Sequel II System (2.0 Chemistry, Sequel II System Software v8.0, 15-hour movie). Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.

# HIGH CONSENSUS ACCURACY



Consensus accuracy is a function of coverage and chemistry. The data above is based on a haploid bacterial genome run on the Sequel II System (2.0 Chemistry, Sequel II System Software v8.0). Single-molecule accuracy has similar coverage requirements.



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# SMRT Link v8.0 Overview

*SMRT Link v8.0*

# SMRT LINK V8.0: SUMMARY OVERVIEW OF KEY IMPROVEMENTS

## New Workflow Engine - Cromwell

- Scalable, widely adopted in scientific environments, developed by Broad

## Further Enhancements for CCS Analysis

- Faster CCS analysis (4-6 times faster time-to-results)

## Analysis Applications

- **NEW:** Microbial Assembly application
- *de novo* assembly for CCS data – Falcon Unzip (Bioconda)

## UI and Usability improvements

- New style GUI – same functionality
- Support for IGV visualization
- Analysis restart from the point of failure



# COMPATIBILITY AND SUPPORT

## SMRT Link v8.0 Supports Sequel and Sequel II Systems only

- Use SMRT Link v7.0 or earlier for PacBio RS II data

## SMRT Link v8.0 Upgrades

- **Sequel II Customers** – Upgrade from SMRT Link v7.0 or SMRT Link v7.1
- **Sequel Customers** – Upgrade from SMRT Link v7.0 or SMRT Link v6.0

## Data Visualization

- IGV compatibility - URL links to alignment results (BAM and BAI)
- SMRT View is no longer included with SMRT Link

## SMRT Link v8.0 Migration Quick Reference Card

- [QRC](#) document summarizes the changes in SMRT Link v8.0 in relation to prior SMRT Link versions and provides useful information for planning an upgrade to SMRT Link v8.0.
- This is a major release – requires more efforts for customers to upgrade
  - Relatively minimal impact on GUI users; cmd and APIs users are more impacted



# SMRT LINK V8.0 MIGRATION QUICK REFERENCE CARD

[QRC](#) document summarizes the changes in SMRT Link v8.0 in relation to prior SMRT Link versions and provides useful information for planning an upgrade to SMRT Link v8.0

PacBio is pleased to announce the upcoming release of SMRT Link v8.0 as part of a launch of new consumables and ICS software for the Sequel II System. SMRT Link v8.0 will be released in early Q4 2019 and will support both Sequel and Sequel II Systems. It includes several highly beneficial updates and features:

- A new workflow engine, [Cromwell](#), replaces pb
- 4-6-times faster Circular Consensus Sequencing
- A new analysis application, Microbial Assembly, assembly, circularization, and plasmid identification
- A refreshed SMRT Link Graphical User Interface with improved appearance

## New Capabilities

The most impactful changes for users of SMRT Link v7.0 and earlier versions are related to implementation of the [Cromwell scientific workflow engine](#) which was developed by the Broad Institute and designed for simplicity and scalability.

Using Cromwell in SMRT Link provides the following new capabilities:

- One-click retry in case of analysis failure
- Better parallelization of analysis tasks, resulting in faster time to results
- Enables consolidation of analysis pipelines

We've minimized functional changes for SMRT Link GUI users. SMRT Analysis applications remain the same, with a new back-end implementation.

Details related to upgrading to SMRT Link v8.0 for users utilizing the GUI, command line, APIs and for SMRT Link administrators are included in the [SMRT Link v8.0 Migration Quick Reference Card](#).

We encourage you to review this document and discuss upgrade and migration with your dedicated Bioinformatics FAS.

Please let us know if you have any questions.



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# SMRT Analysis Workflow Engine Management System Updates

# NEW SMRT ANALYSIS WORKFLOW MANAGEMENT ENGINE

`pbsmrtpipe` Replaced By [Cromwell](#) – A Scientific workflow engine designed for simplicity and scalability by the Broad Institute

## Advantages

- Industry tool used in many scientific environments
- Fault tolerance, call caching, efficient multithreading, lower memory overhead
- Support for 3<sup>rd</sup>-party tools and Cloud environment

## Changes

- Directory structure, logs, and primary outputs
- Workflow IDs and task options
- `pbcromwell` replaces `pbsmrtpipe` for CLI users
- APIs – *analysis* job type replaces *pbsmrtpipe* job type

## Documentation

- [Quick Reference Card \(QRC\) SMRT Link v8.0 Migration Guide](#)
- SMRT Link User Guide and SMRT Tools Reference Guide



# SMRT Analysis Applications Updates

# SMRT ANALYSIS APPLICATIONS UPDATES



## Microbial Assembly (*NEW Application*)

- *de novo* assembly for small genomes between 1.9 – 10 Mb
- Circularization
- Plasmid assembly (2 – 220 kb)



## CCS Analysis

- Time-to-results 6.5 hours.
  - For a standard CCS sequencing collection (415 GB sequencing data, 25 GB Unique Molecular Yield, 15 kb insert size, 30-hours movie on PacBio required HPC\*)
  - SNP precision and recall identical with SMRT Link v7.0; Indels – 0.4% and 0.3% decrease in precision and recall compared to SMRT Link v7.0



## Iso-Seq Analysis

- Analysis from CCS data



## *De novo* Assembly With HiFi Reads - Bioconda

- Direct CCS (FASTA+FASTQ) input to FALCON and FALCON-Unzip
- Significantly faster assemblies using HiFi reads

\* See [SMRT Link Installation instructions document](#) for HPC specifications for the Sequel II System

# MICROBIAL ASSEMBLY APPLICATION

SMRT Analysis / Create New Analysis

Analysis Application Required

- Assembly (HGAP4)
- Base Modification Analysis
- CCS with Mapping
- Circular Consensus Sequencing (CCS)
- Convert BAM to FASTX
- Demultiplex Barcodes
- Iso-Seq
- Long Amplicon Analysis (LAA)
- Microbial Assembly**
- Resequencing
- Site Acceptance Test (SAT)
- Structural Variant Calling

Analysis Name: test

Analysis Datasets

ID	Name
1019...	3260356_INDOs-dT_HO808-133_LP1p1_all4mer_10min_3uM_SSB_SP2p1_RecycledCustomerChip-DA015396-Cell1

Displaying rows 1 to 1 out of 1

- *de novo* assembly for small genomes between 1.9-10 Mb
- Circularization
- Plasmid assembly (2 – 220 kb)

- Same report as HGAP4, but without Preassembly section

SMRT Analysis / Analysis Results

assembly\_microbial-d09-escherichia\_coli\_strain\_w\_atcc\_9637

7

SUCCESSFUL

Analysis Overview

- Status
- Thumbnails
- Display All
- Polished Assembly
- Coverage
- Alignment to Draft Assembly
- Data

Status

Analysis: assembly\_microbial-d09-escherichia\_coli\_strain\_w\_atcc\_9637

Analysis ID: 481

Status: SUCCESSFUL: 250 tasks finished

Created By: isovic

Date Created: 2019-08-29, 11:45:28 AM

Date Updated: 2019-08-29, 01:07:10 PM

Application: Microbial Assembly

SMRT Link Version: 8.0.0.SNAPSHOT77325

Inputs: Sequel Data: [microasm-d09-escherichia\\_coli\\_strain\\_w\\_atcc\\_9637](#)

Path: /jbi/dept/secondary/sv/smrlink/smrlink-alpha-nightly/smrtsuite\_8.0.0.SNAPSHOT77325/userdata/jobs\_root/0000/0000000/0000000481

Analysis Parameters

# MICROBIAL ASSEMBLY APPLICATION - METRICS

The top screenshot displays the 'Polished Assembly' section with the following summary metrics table:

Value	Analysis Metric
4	Polished Contigs
4,898,391	Maximum Contig Length
4,898,391	N50 Contig Length
5,042,832	Sum of Contig Lengths
4,760,443	E-size (sum of squares / sum)

The bottom screenshot displays the 'Polished Contigs' table with the following data:

Contig	Length (bases)	Circular
ctg.s1.000000f arrow	4,898,391	yes
ctg.s2.29 arrow	102,535	yes
ctg.s2.000000f arrow	36,546	no
ctg.s2.31 arrow	5,360	yes

- Additional Summary Metrics – contig length and circularization identification



# SMRT Link General Usability Improvements



## USER INTERFACE AND USABILITY IMPROVEMENTS

### New-Style SMRT Link v8.0 Graphical User Interface (GUI) with the Same Functionality

#### **Re-designed, intuitive GUI**

- Better navigation and data display
- Contemporary “look and feel”

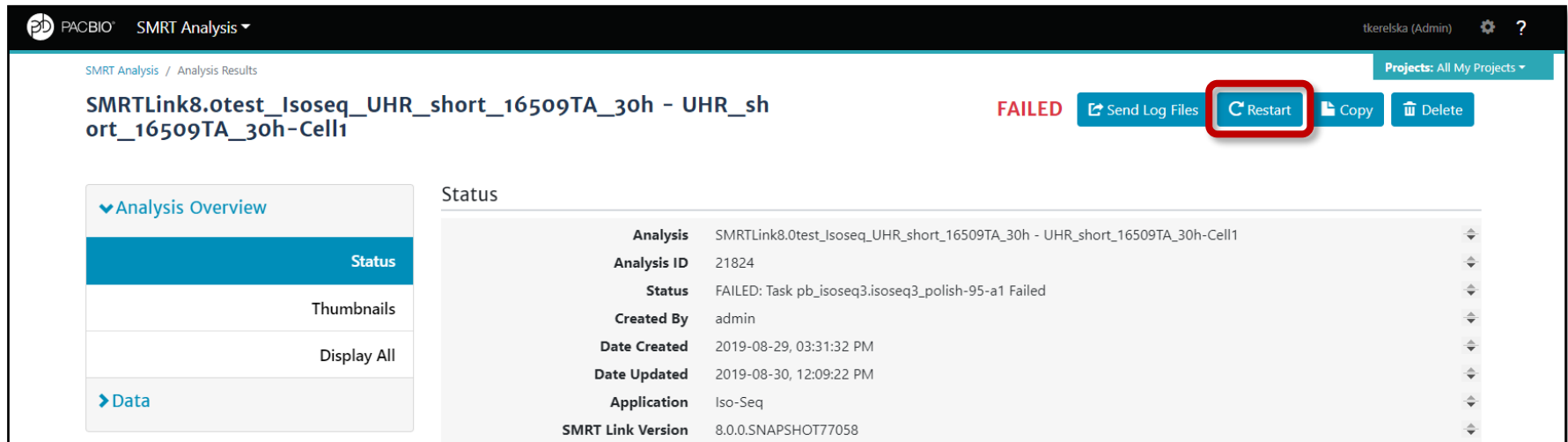
#### **Support for IGV visualization**

- URL links to alignment results (BAM and BAI) for input in IGV

#### **Restart from the point of failure for failed SMRT Analysis applications**

- UI button enables this feature

# RESTART FAILED ANALYSES FROM THE POINT OF FAILURE



The screenshot shows the PACBIO SMRT Analysis interface. At the top, the user is logged in as 'tkerejska (Admin)'. The main heading is 'SMRTLink8.0test\_Isoseq\_UHR\_short\_16509TA\_30h - UHR\_short\_16509TA\_30h-Cell1', which is marked as 'FAILED'. To the right of the heading are buttons for 'Send Log Files', 'Restart' (highlighted with a red box), 'Copy', and 'Delete'. Below the heading is a sidebar with 'Analysis Overview' and 'Data' sections. The main content area shows the 'Status' of the analysis, including details like 'Analysis ID', 'Status', 'Created By', 'Date Created', 'Date Updated', 'Application', and 'SMRT Link Version'.

Status	
<b>Analysis</b>	SMRTLink8.0test_Isoseq_UHR_short_16509TA_30h - UHR_short_16509TA_30h-Cell1
<b>Analysis ID</b>	21824
<b>Status</b>	FAILED: Task pb_isoseq3.isoseq3_polish-95-a1 Failed
<b>Created By</b>	admin
<b>Date Created</b>	2019-08-29, 03:31:32 PM
<b>Date Updated</b>	2019-08-30, 12:09:22 PM
<b>Application</b>	Iso-Seq
<b>SMRT Link Version</b>	8.0.0.SNAPSHOT77058

- Restarted analysis briefly checks completed analysis steps and continues with the failed one/s
- **Note:** Results from the failed analysis should not be deleted before the restarted analysis job completes

# IGV VISUALIZATION

SMRT Analysis / Analysis Results

**Bauhaus2\_Job\_3280305-0007\_1442** SUCCESSFUL Copy Delete

Projects: All My Projects

- ▶ Analysis Overview
- ▶ Coverage
- ▶ Mapping Report
- ▼ Data

File Downloads

IGV Visualization Files

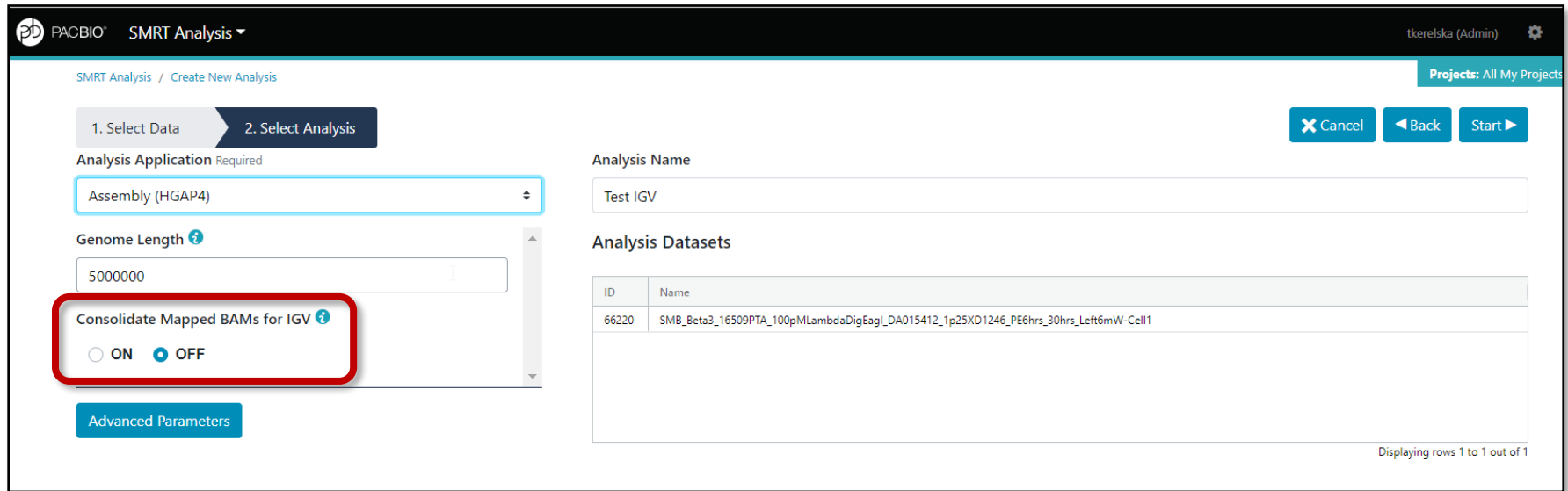
SMRT Link Log

### IGV Visualization Files

File	Path	Size	Type
Mapped BAM Index	<a href="http://smrtlink-sms.nanofluidics.com:8080/job-data/sl_consolidate_alignments/9ca473a2-e039-486b-a809-48eef5428694/call-auto_consolidate_alignments/execution/mapped.bam.bai">http://smrtlink-sms.nanofluidics.com:8080/job-data/sl_consolidate_alignments/9ca473a2-e039-486b-a809-48eef5428694/call-auto_consolidate_alignments/execution/mapped.bam.bai</a>	4 MB	bam_bai
Mapped BAM	<a href="http://smrtlink-sms.nanofluidics.com:8080/job-data/sl_consolidate_alignments/9ca473a2-e039-486b-a809-48eef5428694/call-auto_consolidate_alignments/execution/mapped.bam">http://smrtlink-sms.nanofluidics.com:8080/job-data/sl_consolidate_alignments/9ca473a2-e039-486b-a809-48eef5428694/call-auto_consolidate_alignments/execution/mapped.bam</a>	31 GB	bam

- IGV Visualization files available in SMRT Analysis results
  - URL links for BAM and BAM index files to copy/paste in IGV
  - Reference sequence should be provided in IGV by the user

# IGV VISUALIZATION – CONSOLIDATING BAM FILES

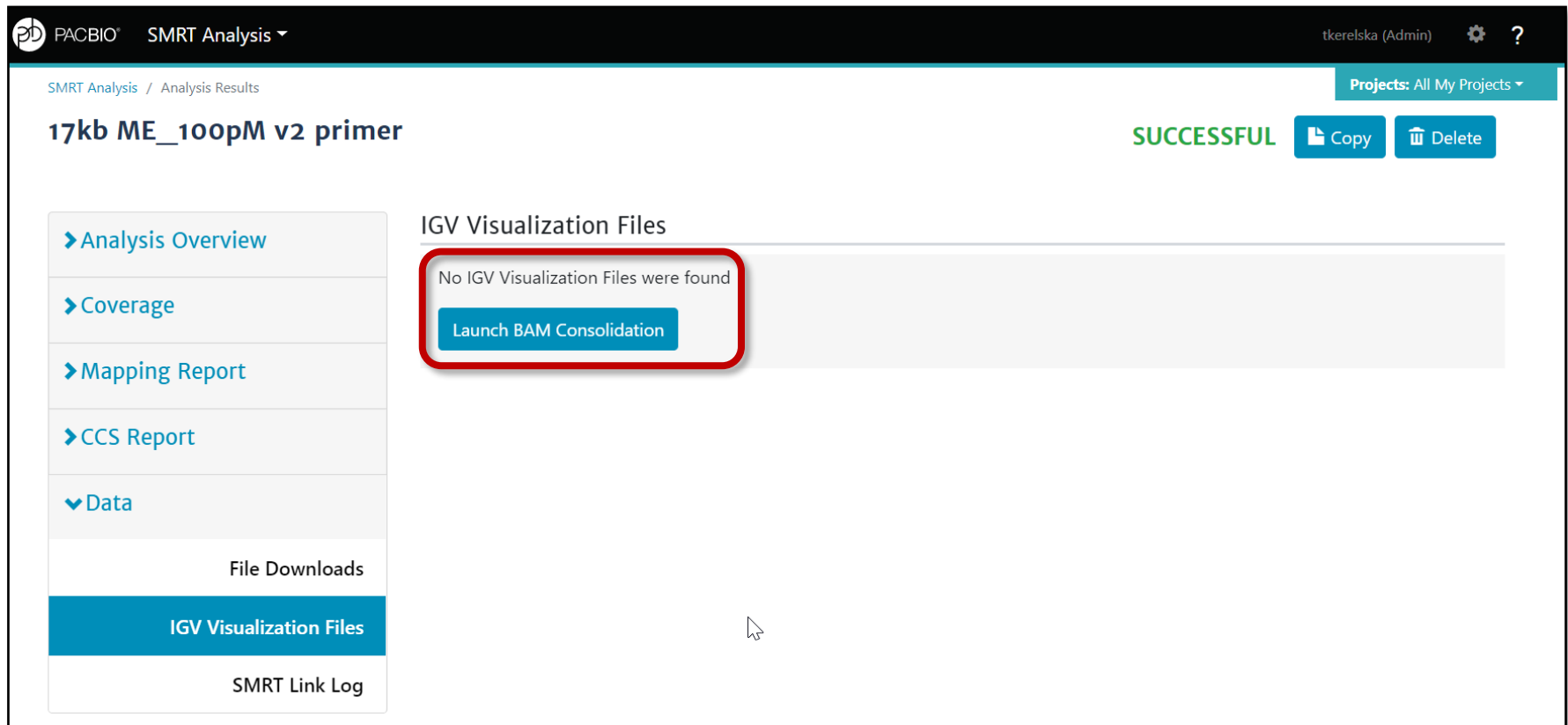


The screenshot shows the 'SMRT Analysis' web interface. The 'Analysis Application' is set to 'Assembly (HGAP4)'. The 'Genome Length' is set to '5000000'. The 'Consolidate Mapped BAMs for IGV' option is highlighted with a red box and is currently set to 'OFF'. The 'Analysis Name' is 'Test IGV'. The 'Analysis Datasets' table shows one dataset with ID 66220 and Name 'SMB\_Beta2\_16509PTA\_100pMLambdaDigEagL\_DA015412\_1p25XD1246\_PE6hrs\_30hrs\_Left6mW-Cell1'.

ID	Name
66220	SMB_Beta2_16509PTA_100pMLambdaDigEagL_DA015412_1p25XD1246_PE6hrs_30hrs_Left6mW-Cell1

- When analyzing large genomes, output may be in multiple BAM file “chunks”
  - SMRT Link **defaults** to combining chunked alignment BAM files if the combined file sizes are **10 GB** or less
  - Combined BAM file size is not known in advance
- IGV can only use consolidated BAM file for visualization
- Consolidate Mapped BAM files is off by default because consolidation results in doubling the space used for BAM files

# IGV VISUALIZATION – CONSOLIDATING BAM FILES (CONT.)



The screenshot shows the PACBIO SMRT Analysis interface. The top navigation bar includes the PACBIO logo, 'SMRT Analysis', and user information 'tkerelska (Admin)'. The main content area is titled '17kb ME\_100pM v2 primer' and shows a 'SUCCESSFUL' status with 'Copy' and 'Delete' buttons. On the left, a sidebar menu lists 'Analysis Overview', 'Coverage', 'Mapping Report', 'CCS Report', 'Data', 'File Downloads', 'IGV Visualization Files' (highlighted), and 'SMRT Link Log'. The main content area under 'IGV Visualization Files' displays the message 'No IGV Visualization Files were found' and a blue button labeled 'Launch BAM Consolidation' which is circled in red.

- If BAM file is greater than 10 GB and “Consolidate BAM files” was not turned ON, IGV Visualization files will be missing
- BAM consolidation can be run after analysis



# SMRT Link Known Issues

## SMRT LINK V8.0 KNOWN ISSUES

See the latest SMRT Link [Release Notes](#) for an updated list of known issues

### SMRT Link

- Date sorting does not work correctly for entries created **on the same day**

### Run Design

- Run Design CSV does not allow uploading of two samples with identical names and identical barcodes in the same run. The workaround is to set up the run using the GUI.
- Successful upload of a Bio Sample Name file in the Run Design editor does not display a confirmation message.
- Insert sizes must be integer values. Entering decimal value results in error.
- CLR Run Designs created prior to release of SMRT Link v7.0 may appear incorrectly as CCS runs when viewed or edited in SMRT Link v8.0.
- Creating or importing Run Designs that employ more than two chemistries causes an error, since only two different chemistries may be loaded on to the instrument.

## SMRT LINK V8.0 KNOWN ISSUES (CONT.)

### SMRT Analysis

- If a **Bio Sample Name file is not provided** during run design **and only a subset of barcodes from the Barcode Set is used**, the Demultiplex Barcodes analysis will output all barcodes in the Barcode Set. When designing a run, provide a Bio Sample Name file with rows containing Bio Sample Names for the barcodes used only. Alternatively, rerun the Demultiplex Barcodes application with the advanced parameter Ignore BioSamples records from Run Design set to On.
- If a **wrong or incomplete list of barcode Bio Sample names** is included in Run Design, or if a **wrong Barcode Set** is used during Run Design, the demultiplexed output will contain wrong number of barcodes. To obtain correct demultiplexing results, rerun the Demultiplex Barcodes application with the advanced parameter Ignore BioSamples records from Run Design set to On.
- Analysis applications Resequencing, CCS with Mapping, HGAP 4, Structural Variant Calling, and Base Modification Analysis fail when launched **on extremely large genomes** containing chromosomes >500 Mb.
- Structural Variant Calling fails when launched with demultiplexed data sets. The workaround is to run pbmm2 and pbsv at the command line, using "--sample" to pbmm2 to indicate the Bio Sample Name for a demultiplexed data set.
- The sections of an analysis results page are sometimes reordered upon relaunch. All content remains correct.





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# Sequel II System Applications Support Resources

*Sequel II System v8.0 / Sequel II Chemistry 2.0 / SMRT Link v8.0*



# **Sequel II System Applications Overview Guide & Quick Reference Card**

# SEQUEL II SYSTEM APPLICATIONS OVERVIEW GUIDE

New **Overview – Sequel Systems Application Options and Sequencing Recommendations** document summarizes recommended Experimental Design, Sample Preparation, Sequencing Setup, and Data Analysis Tools for all supported SMRT Sequencing Applications in a single reference guide

## WHOLE GENOME SEQUENCING

## VARIANT DETECTION

## COMPLEX POPULATIONS

Application	Whole Genome Sequencing					RNA Sequencing	Metagenomics		Targeted Sequencing				
	De Novo Assembly - HFI Reads	De Novo Assembly - Long Reads	De Novo Assembly - for Low DNA Input	Microbial De Novo Assembly	Variant Detection	Structural Variation Detection	Iso-Seq Method	Full length 16S rRNA Sequencing	Shotgun Metagenomics Profiling or Assembly	Amplicon Sequencing	No-Amp Targeted Sequencing		
<b>Experimental Design</b>													
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	Multiplies up to 96 samples to provide strain level resolution	Generate near-complete assemblies of high-complexity samples (e.g. gut microbiome)	Sequence 384 barcoded amplicons	Sequence 5 targeted regions in a multiplex of 10 samples		
Minimum Recommended Coverage	>15-fold HFI read coverage	≥30-fold Unique Molecular Coverage (UMC) per haplotype	≥30-fold UMC per haplotype	≥30-fold UMC coverage per microbial genome	>15-fold HFI 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 ≥200 CCS read coverage for variant detection 6,000-fold ≥200 CCS read coverage for minor variant detection (1% sensitivity) 500 bp - 15 kb	≥100-fold ≥200 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		4-6 kb or larger		
<b>Sample Preparation</b>													
Procedure and Checklist Reference	<a href="#">Preparing HFI SMRTbell Libraries Using the SMRTbell Express Template Prep Kit 2.0</a>	<a href="#">Preparing cDNA 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 HFI SMRTbell Libraries Using the SMRTbell Express Template Prep Kit 2.0</a>	<a href="#">Preparing cDNA Libraries Using the SMRTbell Express Template Prep 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 Libraries for Metagenomic Shotgun Sequencing</a>	<a href="#">Preparing SMRTbell Libraries Using Paired Barcoded Overhang Adapters for Multiplexed SMRT Sequencing</a>	<a href="#">No-Amp Targeted Sequencing Using the CCS/PPS-Case-System</a>		
Minimum Input Amount	≥1 µg for 10 kb ≥3 µg for >15 kb ≥5 µg for >30 kb	≥1 µg for 10 kb ≥3 µg for >15 kb ≥5 µg for >30 kb	100 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 200-1000 bp 500-1000 ng 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 + 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	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		
<b>SMRT Sequencing with the Sequel II System: Loading and Pre-Extension Recommendations</b>													
Sequencing Preparation and Yield Performance	Sequel II Binding Kit	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		
	Sequencing Mode	CCS	CLR	CLR / CCS	CLR	CCS	CLR	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													
<b>SMRT Sequencing with the Sequel System</b>													
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		
	Sequel Sequencing Plate	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		
	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													
<b>Data Analysis Tools Available Through SMRT Link, PacBio DevNet and Other Compatible Software Tools</b>													
Other Compatible Software Tools (Contact PacBio for details)	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
	<a href="#">PacBio DevNet Tools</a>	Falcon, Falcon Unzip, Falcon Phase	Falcon, Falcon Unzip, Falcon Phase	Falcon, Falcon Unzip, Falcon Phase									Repeat Analysis Tools
Notes	Recommend: GATK, Google Deep Variant												

## RNA SEQUENCING

## TARGETED SEQUENCING

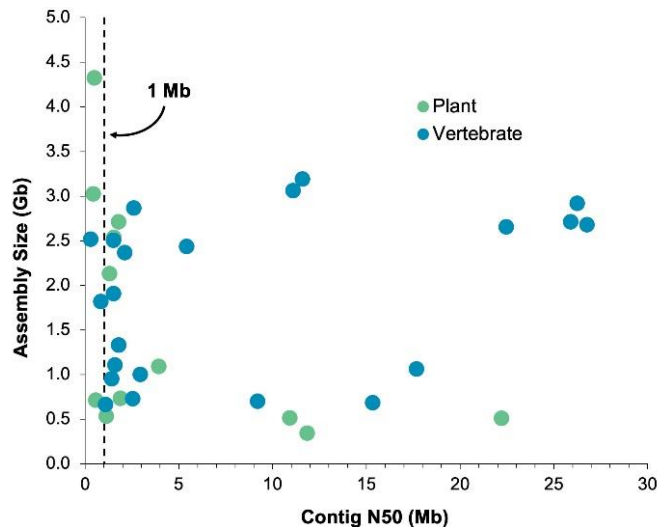
# WHAT COULD YOU DO WITH A SEQUEL II SYSTEM?

## With a single SMRT Cell 8M:

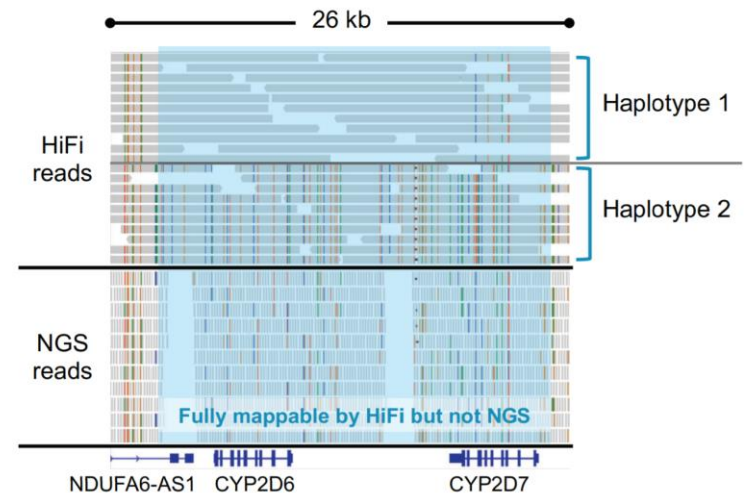
- Generate a 2 Gb genome assembly
- Call structural variants in a human genome
- Sequence a whole transcriptome
- Determine the composition of >90 gut microbiome samples

## With 2-3 SMRT Cells 8M:

- Detect all types of variants in a human genome
- Phase a diploid assembly of a human genome



Plant and vertebrate genome assemblies generated with SMRT Sequencing data from the genome database on NCBI showing PacBio assemblies readily achieve contig N50s  $\geq 1$  Mb.



Coverage of the Cytochrome P450 2D6 (CYP2D6) and CYP2D7 genes with HiFi reads and NGS reads visualized in IGV. CYP2D6 is responsible for the metabolism and elimination of approximately 25% of clinically used drugs.

# SEQUEL II SYSTEM QUICK REFERENCE CARD – DIFFUSION LOADING AND PRE-EXTENSION RECOMMENDATIONS

Refer to QRC for the Latest Sample Setup & Run Design Recommendations



## Loading and Pre-Extension Recommendations for the Sequel® II System Quick Reference Card

Refer to the table below for loading recommendations for the Sequel II System. Note that the sample quality, size, and binding efficiency may affect loading concentrations. This may result in optimum loading concentrations as low as 30 pM or as high as 100 pM. Use Sequel II Sequencing Plate 2.0 for all application types.

Applications	Sequencing Mode	Library Prep Kit	Binding Kit	Sequencing Primer	Pol Binding Time (hr)	Complex Cleanup	Loading Concentration Range (pM)
Large Insert (>15 kb size-selection cutoff)	CLR	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X AMPure PB Beads	30 - 70
Structural Variant Multiplex	CLR	Express Prep 2.0	Binding Kit 2.0	v2	4	1.2X AMPure PB Beads	70 - 100
Microbial Multiplex (10 kb – 15 kb)	CLR	Express Prep 2.0	Binding Kit 2.0	v4	4	1.2X AMPure PB Beads	70 – 100
Low DNA Input (>10 kb, multiplex)	CLR	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X AMPure PB Beads	40 – 100
HiFi (10 kb – 25 kb)	CCS	Express Prep 2.0	Binding Kit 2.0	v2	4	1.2X AMPure PB Beads	30 - 70
Shotgun Metagenomics (10 kb, Multiplex)	CCS	Express Prep 2.0	Binding Kit 2.0	v2	4	1.2X AMPure PB Beads	50 – 70
Amplicons (≥3 kb)	CCS	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X AMPure PB Beads	30 - 70
Amplicons (< 3 kb)	CCS	Express Prep 2.0	Binding Kit 2.1	v4	1	1.2X AMPure PB Beads	40 - 150
16S (1.6 kb - 2.5 kb)	CCS	Express Prep 2.0	Binding Kit 2.1	v4	1	1.2X AMPure PB Beads	40 - 100
Iso-Seq (standard samples)	CCS	Express Prep 2.0	Binding Kit 2.1	v4	1	1.2X ProNex	40 - 80
Iso-Seq (focus on long transcripts)	CCS	Express Prep 2.0	Binding Kit 2.0	v4	1	1.2X ProNex	50 - 100

Target % P1 is 50 to 70. Recommended for optimal yield per SMRT Cell (defined as maximized raw yield for long insert reads, and unique molecular yield for HiFi Reads.) Indications for overloaded libraries can be gauged by PO values. Note: If PO values are <10% then the SMRT Cell is overloaded.

## Pre-Extension and Movie Time Recommendations

Pre-extension is a feature that allows SMRTbell template molecules to reach rolling circle replication (when the polymerase is most stable) before movie collection is initiated. Generalized pre-extension guidelines by mean insert size and applications are summarized in the table below. Further optimization of pre-extension time is recommended for specific applications to maximize read length and yield.

Applications	Pre-Extension Time (hr)	Movie Collection Time (hr)
Large insert (>15 kb size-selection cutoff)	0	15
Structural Variant Multiplex	2 hrs (≤20 kb), 4 hrs (>20 kb)	15
Microbial Multiplex (10 kb - 15 kb)	2	15
Low DNA Input (>10 kb, Multiplex, AMPure PB bead size-selection)	2	30
HiFi (10 kb – 25 kb)	2 hrs (≤20 kb), 4 hrs (>20 kb)	30
Shotgun Metagenomics (10 kb, Multiplex)	2	30
Amplicons (≥3 kb)	Use default values in Run Design	10 - 30
Amplicons (< 3 kb)	Use default values in Run Design	10
16S (1.6 kb - 2.5 kb)	1	10
Iso-Seq (standard samples)	2	24
Iso-Seq (focus on long transcripts)	2	24

Revision History (Description)	Version	Date
Initial release.	01	April 2019
Added loading recommendations for Iso-Seq and 16S applications.	02	June 2019
Updated recommendations for the new Binding Kit and Sequencing plate	03	September 2019
Updated to add multiplex options for various applications.	04	November 2019

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# Sequel II System Applications Best Practices Guides

# SEQUEL II SYSTEM BEST PRACTICES OVERVIEW GUIDES



**Whole Genome Sequencing for *De novo* Assembly**  
*(Includes NEW recommendations for using HiFi Reads for de novo assembly)*



**Variant Detection Using Whole Genome Sequencing with HiFi Reads**



**Structural Variation Detection**



**No-Amp Targeted Sequencing**



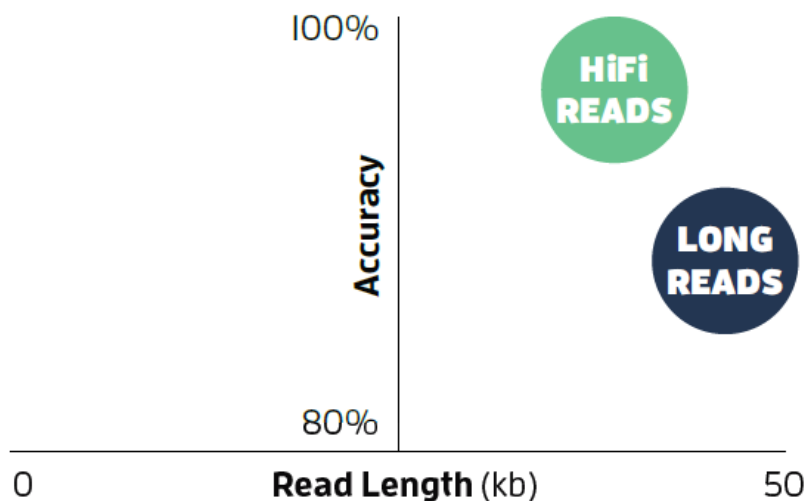
**Long-Read RNA Sequencing**



**Metagenomic Sequencing of Complex Populations**

# **BEST PRACTICES: WHOLE GENOME SEQUENCING (WGS) FOR DE NOVO ASSEMBLY (SEQUEL II CHEMISTRY 2.0)**

## **Choose the Sequencing Mode That's Right for Your Project**



**PacBio is the *only* sequencing technology to offer highly accurate long reads (HiFi reads)**

Sequel II System Best Practices ...

### **HiFi Reads**

- Highly accurate long reads with minimum accuracy of Q20 (99%)
- Small file sizes and fast analysis time
- Assemble up to a 2 Gb genome in a single SMRT Cell 8M for ~\$1,300\* or scale up for larger genomes
- Run up to 200 samples (2 Gb) per year, per system\*




### **Long Reads**

- Longest reads, with half of data >50 kb and maximum read lengths up to 175 kb
- Shorter sequencing run times
- Assemble up to a 3 Gb genome in a single SMRT Cell 8M for ~\$1,300\* or scale up for larger genomes
- Run up to 400 samples (3 Gb) per year, per system\*

\* Read lengths, reads/data per SMRT Cell 8M, and other sequencing performance results vary based on sample quality/type and insert size. Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Sequel II System and does not include instrument amortization or other reagents.



# BEST PRACTICES: WHOLE GENOME SEQUENCING (WGS) FOR DE NOVO ASSEMBLY (SEQUEL II CHEMISTRY 2.0) (CONT.)

	HiFi Reads Workflow <sup>2</sup>	Long Reads Workflow <sup>2</sup>
 <p><b>LIBRARY PREP</b></p>	<ul style="list-style-type: none"> <li>- Start with &gt;15 µg of unamplified genomic DNA</li> <li>- Prepare a library in ~5 hours with the SMRTbell® Template Prep Kit 2.0</li> <li>- Enrich for 15-20 kb inserts with size selection</li> </ul>	<ul style="list-style-type: none"> <li>- Start with &gt;5 µg of unamplified genomic DNA</li> <li>- Prepare a library in ~3 hours with the SMRTbell Express Template Prep Kit 2.0</li> <li>- Enrich for &gt;30 kb inserts with size selection</li> </ul>
 <p><b>SMRT SEQUENCING</b></p>	<ul style="list-style-type: none"> <li>- Sequence using the circular consensus sequencing (CCS) mode</li> <li>- Sequence up to a 2 Gb genome in a single SMRT Cell 8M*</li> <li>- Coverage recommendations:               <ul style="list-style-type: none"> <li>- 15-fold HiFi coverage for typical diploid genomes</li> <li>- &gt;20-fold HiFi coverage for repeat-rich genomes</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- Sequence using the continuous long read (CLR) sequencing mode</li> <li>- Sequence up to a 3 Gb genome in a single SMRT Cell 8M*</li> <li>- Coverage recommendation:               <ul style="list-style-type: none"> <li>- &gt;30-fold long-read coverage per haplotype</li> </ul> </li> </ul>
 <p><b>DATA ANALYSIS</b></p>	<ul style="list-style-type: none"> <li>- Generate HiFi reads with CCS analysis in SMRT Link or at the command line</li> <li>- Use FALCON and FALCON-Unzip3 to assemble and phase HiFi reads</li> </ul>	<ul style="list-style-type: none"> <li>- Utilize the PacBio analytical portfolio, including community developed tools, to assemble and phase long reads</li> </ul>

**HiFi assembly of a ~3 Gb genome in ~31 hours vs ~62 hours with long reads**

**Long read data of a ~3 Gb genome in ~15 hours vs ~60 hours with HiFi**

\* Read lengths, reads/data per SMRT Cell 8M, and other sequencing performance results vary based on sample quality/type and insert size. Prices, listed in USD, are approximate and may vary by region

# **BEST PRACTICES: VARIANT DETECTION USING WHOLE GENOME SEQUENCING WITH HIFI READS (SEQUEL II CHEMISTRY 2.0)**



## **LIBRARY PREP**

### **SMRTbell Template Preparation**

- Start with unamplified genomic DNA input ( $\geq 15 \mu\text{g}$ )
- Prepare a HiFi library for using SMRTbell Express Template Prep Kit 2.0
- Enrich for 15-20 kb inserts with size selection



## **SMRT SEQUENCING**

### **Sequence on the Sequel II System (CCS Sequencing Mode)**

- Maximize output and turn-around-time with adjustable sequencing parameters
- Sequence to desired coverage based on study needs:
  - Recommend 2 SMRT Cells 8M to achieve 15-fold coverage of a human genome for comprehensive variant detection for \$2600<sup>†</sup>



## **DATA ANALYSIS**

### **Data Analysis Solutions with the PacBio Analytical Portfolio**

- Detect all variant types – including SNVs, indels, SVs, and CNVs – with the highest precision and recall using SMRT Analysis, and GATK or Google DeepVariant
- Google DeepVariant achieves higher precision and recall than GATK, particularly for indels
- Expand variant calling into previously inaccessible regions of the genome, including repetitive regions and medically relevant genes that are difficult to map
- Output data in standard file formats – BAM and VCF – for seamless integration with downstream analysis tools
- Phase small variants into phase blocks using WhatsHap
- Confirm variant calls visually with IGV10 and GenomeRibbon

*\* Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.*

*† Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Sequel II System and does not include instrument amortization or other reagents.*

# **BEST PRACTICES: STRUCTURAL VARIANT DETECTION USING WHOLE GENOME SEQUENCING (SEQUEL II CHEMISTRY 2.0)**



## **LIBRARY PREP**

### **SMRTbell Template Preparation**

- Prepare >15 kb library using SMRTbell Express Template Preparation Kit 2.0
- Start with unamplified genomic DNA with a mean fragment size >20 kb (~5.0 µg input)
- Multiplex samples with barcoded SMRTbell adapters
- Enrich for longest inserts with size selection



## **SMRT SEQUENCING**

### **Sequence on the Sequel II System (CLR Sequencing Mode)**

- Select coverage based on study goals\*
  - 5- to 10-fold: Population genetics studies – sensitivity limited per individual, but high for variants shared in the population using joint calling
  - 10- to 20-fold: Rare undiagnosed disease studies – sensitivity high per individual allowing discovery of pathogenic SVs
  - 25-fold: Saturate discovery in an individual, recommended for detection of de novo SVs
- For human population genetics studies, run ~1,000 samples/year by multiplexing 2 samples / SMRT Cell at a cost of ~\$670/sample†



## **DATA ANALYSIS**

### **Data Analysis Solutions with the PacBio Analytical Portfolio**

- Detect the broadest range of SV types including insertions, deletions, duplications, inversions, translocations, and copy number variants
- Discover SVs with the highest precision and recall
- Identify common SVs across multiple samples with joint calling
- Resolve breakpoints to sequence level
- Limit costly validation efforts with a low false discovery rate of <5%
- Output data in standard file formats – BAM and VCF – for seamless integration with downstream analysis tools
- Confirm SV calls visually with IGV and GenomeRibbon

\* Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.

† Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Sequel II System and does not include instrument amortization or other reagents.

# **BEST PRACTICES: NO-AMP TARGETED SEQUENCING (SEQUEL II CHEMISTRY 2.0)**



## **LIBRARY PREP**

### **SMRTbell Template Preparation**

- Start with high-quality genomic DNA (~5-10 µg / SMRT Cell)
- Prepare SMRTbell libraries in 2-days with stream-lined protocol
  - Block 5' & 3' ends to prevent off-target ligation
  - Use custom-design guide RNAs to enrich for regions-of-interest
  - Multiplex samples using barcoded adapters
  - Remove off-target products with trypsin exonuclease digestion



## **SMRT SEQUENCING**

### **Sequence on the Sequel II System (CCS Sequencing Mode)**

- Sequence multiplexed targets and/or samples on the Sequel Systems using the latest chemistry\*
  - Adjust movie time parameters based on repeat expansion size
  - Run up to 10 samples per SMRT Cell at ~\$120/sample on the Sequel II System†



## **DATA ANALYSIS**

### **Data Analysis Solutions with the PacBio Analytical Portfolio**

- Use SMRT Link to recall asymmetric SMRTbell adapters for demultiplexing of samples and perform circular consensus sequencing (CCS) to generate highly accurate long reads (HiFi reads)
- Output data in FASTQ format for results summary reporting on repeat counts and on-target rates
- Visualize results with the IGV and command-line scripts for easy review of repeat count of both alleles, mosaic characterization, identification of interruption sequences and CRISPR / Cas9 off-targets

\* Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.

† Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Sequel II System and does not include instrument amortization or other reagents.

# **BEST PRACTICES: LONG-READ RNA SEQUENCING (ISO-SEQ ANALYSIS) (SEQUEL II CHEMISTRY 2.0)**



## **LIBRARY PREP**

### **Template Preparation with SMRTbell Express Template Prep Kit 2.0**

- Prepare full-length cDNA from 300 ng of total RNA using the NEBNext® Single Cell/Low Input cDNA Synthesis & Amplification Module kit
- Use the SMRTbell Express Template Prep Kit 2.0 to prepare libraries in one day
- Multiplex up to 12 samples with barcoding



## **SMRT SEQUENCING**

### **Sequence on the Sequel or Sequel II System (CCS Sequencing Mode)**

- Maximize output and turn-around-time with adjustable sequencing parameters
  - Sequel II System: Diffusion loading, 24 hour movies with 2 hours pre-extension is recommended
- Use the Sequel II System to generate up to 4 million\* full-length, non-concatemer (FLNC) reads per SMRT Cell 8M
- Scale throughput based on project needs – With a single Sequel II System SMRT Cell 8M you can:
  - Characterize a whole transcriptome
  - Multiplex multiple tissues for genome annotation



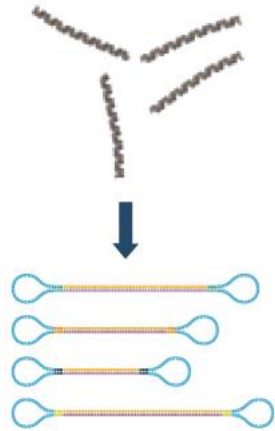
## **DATA ANALYSIS**

### **Data Analysis Solutions with the PacBio Analytical Portfolio**

- Generate highly accurate long reads (HiFi reads), with single-molecule resolution using circular consensus sequencing (CCS) mode
- Use the Iso-Seq analysis in SMRT Link to output high-quality, full-length transcript FASTA sequences, with no assembly required, to characterize transcripts and splice variants
- Run Iso-Seq analysis with or without a reference genome, and annotate the genome using community tools such as [SQANTI2](#), [TAMA](#), and [LoReAn](#)

*\* Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.*

# BEST PRACTICES: METAGENOMIC SEQUENCING (SEQUEL II CHEMISTRY 2.0)



Sequel II System



	Full-length 16S rRNA Sequencing	Long-read Metagenomic Profiling	Shotgun Metagenomic Assemblies
	High-resolution, cost-effective screening for microbial community studies	Unbiased discovery and functional characterization of microbial communities	Generation of complete or near-complete genome assemblies from microbial population
<b>Library Preparation</b>			
<b>Prepare SMRTbell® Template</b>	Full-length 16S rRNA protocol <sup>3</sup> available with recommended full-length primers or use Shoreline Biome <sup>4</sup> kits for DNA extraction and PCR amplification	Create 10 kb libraries	
	Multiplexing and library automation supported		
<b>SMRT Sequencing with the Sequel® II System</b>			
<b>Yield</b>	1-2 kb HiFi reads	10 kb HiFi reads	
	A 10-hour movie collection generates: Q20 (99%): up to 2.7 million reads Q30 (99.9%): up to 2.1 million reads	A 30-hour movie collection generates: Q20 (99%): up to 1.7 million reads	
	Q30 recommended	Q20 recommended	
<b>Scale Throughput*</b>	Resolve up to 96 communities per SMRT Cell 8M	Resolve up to 8 communities per SMRT Cell 8M	Recommend targeting: <ul style="list-style-type: none"> <li>- 5-fold coverage of least abundant species for intact genes and operons</li> <li>- 20-fold coverage for near-complete genome assemblies</li> </ul>

\* Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size. See [Application Brochure: Explore Metagenomes in High Resolution](#) (June 2019)

# BEST PRACTICES: METAGENOMIC SEQUENCING (SEQUEL II CHEMISTRY 2.0) (CONT.)



	<b>Full-length 16S rRNA Sequencing</b> High-resolution, cost-effective screening for microbial community studies	<b>Long-read Metagenomic Profiling</b> Unbiased discovery and functional characterization of microbial communities	<b>Shotgun Metagenomic Assemblies</b> Generation of complete or near-complete genome assemblies from microbial population
<b>Data Analysis Tools Available Through SMRT Analysis or PacBio® Devnet</b>			
<b>SMRT Analysis</b>	Circular consensus sequencing (CCS) analysis produces Hi-Fi reads through multiple observations of single circularized templates		<i>De novo</i> assemble high-quality metagenomes with high consensus accuracies >99.999% using Canu <sup>5</sup>
<b>Tertiary Analysis</b>	Analyze 16S data with either Shoreline Biome SB Analyzer <sup>3</sup> or DADA2 <sup>6</sup>	Study metagenomic profile and functions using public databases and MEGAN <sup>7</sup>	Utilize epigenetic signatures to improve shotgun metagenomic analysis <sup>8</sup>

\*Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.



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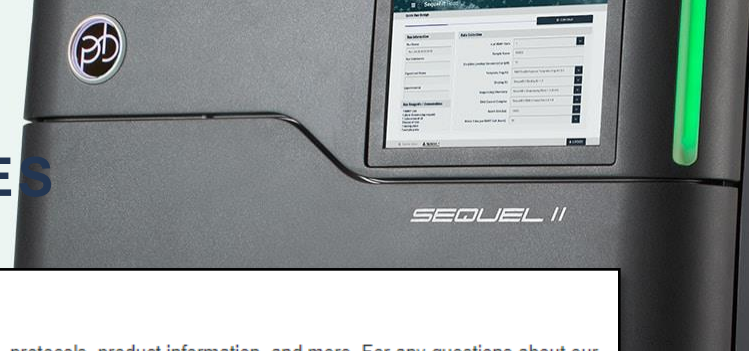


# Technical Documentation & Software Download Resources

*Sequel II System v8.0 / Sequel II Chemistry 2.0 / SMRT Link v8.0*



# PACBIO DOCUMENTATION RESOURCES



## DOCUMENTATION

Welcome to the PacBio Documentation page. The table below allows you to search for and download the latest guides, protocols, product information, and more. For any questions about our documentation, please [contact us](#).

### Search Query



### Asset Type

- SDS (59)  Procedure & Checklist (51)  Package Insert (49)  Guide & Overview (31)  Release Notes (12)  Quick Reference Card (7)  Developer Documentation (5)  Experimental Protocol (5)  
 User Bulletin (2)

### Workflow Step

- Template Preparation (96)  Sequencing (72)  Binding (63)  Analysis (41)  Sample Preparation (5)

### Product Line

- Sequel System (165)  Sequel II System (91)  PacBio RS II (79)

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>>

TOGGLE ALL DESCRIPTIONS



[Sequel SMRT Link Web Services API Use Cases \(v8.0\)](#)

September 27, 2019

**Developer Documentation**  
**Sequel II System, Sequel System**  
**Analysis**

#### Description -

This document describes common tasks performed using the SMRT Link (v8.0) Web Services API and provides "how to" recipes for accomplishing these tasks. To accomplish a task, you usually need to perform several API calls; the document describes the order of these calls.

- PacBio Documentation page allows you to search for and download the latest guides, protocols, product information, and more.

# PACBIO TRAINING RESOURCES

## TRAINING

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[Sequel II System v8.0 & SMRT Link v8.0 Technical Overview](#)

*October 1, 2019*

#### Tutorial

#### Sequel II System

**Analysis, Binding, Sample Preparation, Sequencing, Template Preparation**

#### Description -

This technical training presentation provides an overview of key features & benefits of the Sequel II System v8.0 and SMRT Link v8.0. 'Best Practices' overviews for sample library preparation and sequencing are also presented for whole genome sequencing for de novo assembly, whole genome sequencing for structural variation detection, variant detection using whole genome sequencing with HiFi reads, No-Amp targeted sequencing, long-read RNA sequencing, and metagenomic sequencing of complex populations.

- PacBio Training page allows you to search for and download video tutorials and other training resources

# TECHNICAL DOCUMENTATION & SOFTWARE DOWNLOAD RESOURCES

## Sequel II System and SMRT Link Documentation

- [Sequel II System Operations Guide \(PN 101-774-700\)](#)
- [Sequel II System 8.0 Release Notes \(PN 101-774-400\)](#)
- [Quick Reference Card – Loading and Pre-Extension Recommendations for the Sequel II System \(PN 101-769-100\)](#) [Quick Reference Card – SMRT Link v8.0 Migration \(PN 101-850-100\)](#)
- SMRT Link v8.0 Software Download Site: <https://www.pacb.com/support/software-downloads/>
- [SMRT Link v8.0 Software Installation Instructions \(PN 100-749-900\)](#)
- [SMRT Link v8.0 Release Notes \(PN 100-749-600\)](#)
- [SMRT Link v8.0 User Guide \(PN 101-039-100\)](#)
- [SMRT Tools Reference Guide \(v8.0\) \(PN 100-939-900\)](#)
- [Sequel SMRT Link Web Services API Use Cases \(v8.0\) \(PN 101-430-800\)](#)
- [Pacific Biosciences Glossary of Terms \(PN 000-710-267\)](#)

# TECHNICAL DOCUMENTATION & SOFTWARE DOWNLOAD RESOURCES (CONT.)

## Sample Library Preparation Documentation

- [Overview – Sequel Systems Application Options and Sequencing Recommendations \(PN 101-851-300\)](#)
- [Procedure & Checklist – Using AMPure PB Beads for Size-Selection \(PN 101-854-900\)](#)
- Whole Genome Sequencing Applications
  - *De Novo* Assembly – Long Reads
    - [Procedure & Checklist – Preparing gDNA Libraries Using the SMRTbell Express Template Preparation Kit 2.0 \(PN 101-693-800\)](#)
  - *De Novo* Assembly – HiFi Reads
    - [Procedure & Checklist – Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0 \(PN 101-853-100\)](#)
  - *De Novo* Assembly – Low DNA Input
    - [Procedure & Checklist - Preparing SMRTbell Libraries Using Express Template Prep Kit 2.0 With Low DNA Input \(PN 101-730-400\)](#)
  - Microbial *De Novo* Assembly
    - [Procedure & Checklist – Preparing Multiplexed Microbial Libraries Using SMRTbell Express Template Prep Kit 2.0 \(PN 101-696-100\)](#)
  - Variant Detection
    - [Procedure & Checklist – Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0 \(PN 101-853-100\)](#)
  - Structural Variation Detection
    - [Procedure & Checklist – Multiplexing SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0 for Structural Variation Detection \(PN 101-881-600\)](#)

# TECHNICAL DOCUMENTATION & SOFTWARE DOWNLOAD RESOURCES (CONT.)

## Sample Library Preparation Documentation (Cont.)

### - RNA Sequencing Applications

#### - Iso-Seq Method

- [Procedure & Checklist – Iso-Seq Express Template Preparation for Sequel and Sequel II Systems \(PN 101-763-800\)](#)
- [Procedure & Checklist – Preparing Single-Cell Iso-Seq Libraries Using SMRTbell Express Template Prep Kit 2.0 \(PN 101-892-000\)](#)

### - Metagenomics Applications

#### - Full-length 16S Sequencing

- [Procedure & Checklist – Amplification of Full-Length 16S Gene with Barcoded Primers for Multiplexed SMRTbell Library Preparation and Sequencing \(PN 101-599-700\)](#)

#### - Metagenomics Shotgun Sequencing

- [Procedure & Checklist – Preparing 10 kb Library Using SMRTbell Express Template Prep Kit 2.0 for Metagenomics Shotgun Sequencing \(PN 101-800-800\)](#)

### - Targeted Sequencing Applications

#### - Amplicon Sequencing

- [Procedure & Checklist – Preparing SMRTbell Libraries using PacBio Barcoded Overhang Adapters for Multiplexing Amplicons \(PN 101-791-700\)](#)
- [Procedure & Checklist – Preparing SMRTbell Libraries using PacBio Barcoded Universal Primers for Multiplex SMRT Sequencing \(PN 101-791-800\)](#)

#### - No-Amp Targeted Sequencing

- [Procedure & Checklist – No-Amp Targeted Sequencing Utilizing the CRISPR-Cas9 System \(PN 101-801-500\)](#)

# TECHNICAL DOCUMENTATION & SOFTWARE DOWNLOAD RESOURCES (CONT.)

## Applications Technical Training Documentation

### - Whole Genome Sequencing Applications

- [Technical Overview: HiFi Library Preparation Using SMRTbell Express TPK 2.0 \(PN 101-855-400\)](#)
- [Technical Overview: Low DNA Input Library Preparation Using SMRTbell Express Template Prep Kit 2.0 \(PN 101-781-000\)](#)
- [Technical Overview: Multiplexed Microbial Library Preparation Using SMRTbell Express Template Prep Kit 2.0 \(PN 101-742-600\)](#)
- [Technical Overview: Multiplexed Library Preparation For Structural Variation Detection Using SMRTbell Express Template Prep Kit 2.0 \(PN 101-884-000\)](#)

### - RNA Sequencing Applications

- [Technical Overview: Iso-Seq Express Library Preparation Using SMRTbell Express Template Prep Kit 2.0 \(PN 101-814-400\)](#)

### - Metagenomics Applications

- [Technical Overview: Metagenomics Shotgun Library Preparation Using SMRTbell Express Template Prep Kit 2.0 \(PN 101-894-900\)](#)

### - Targeted Sequencing Applications

- [Technical Overview: Multiplexed Amplicon Library Preparation Using SMRTbell Express Template Prep Kit 2.0 \(PN 101-814-300\)](#)
- [Technical Overview: No-Amp Targeted Sequencing Library Preparation and Data Analysis Technical Overview \(PN 101-840-800\)](#)

# TECHNICAL DOCUMENTATION & SOFTWARE DOWNLOAD RESOURCES (CONT.)

## Data Analysis Documentation

- [Analysis Procedure – Multiplexed Microbial Assembly with SMRT Link v8.0 and SMRTbell Express Template Prep Kit 2.0 \(PN 101-855-300\)](#)
- [Analysis Procedure – No-Amp Data Preparation and Repeat Analysis \(PN 101-801-400\)](#)

# SMRT SEQUENCING RESOURCES

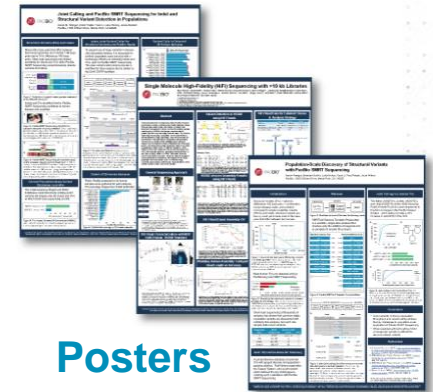
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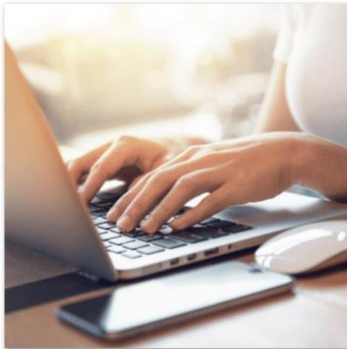
**Scientific Publications**



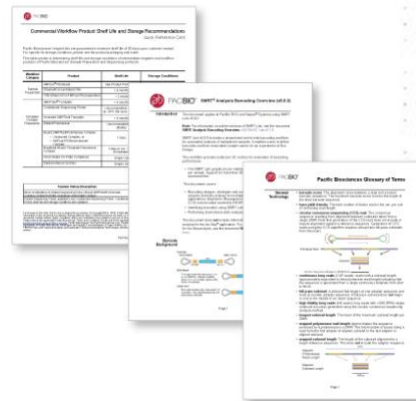
**PacBio Literature**



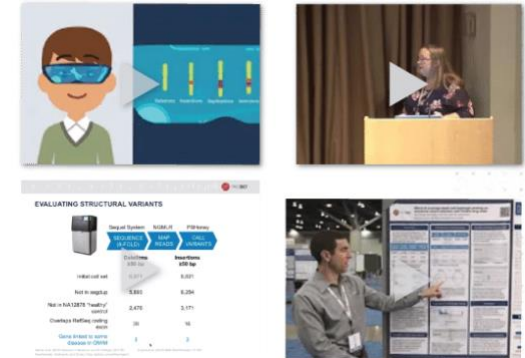
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