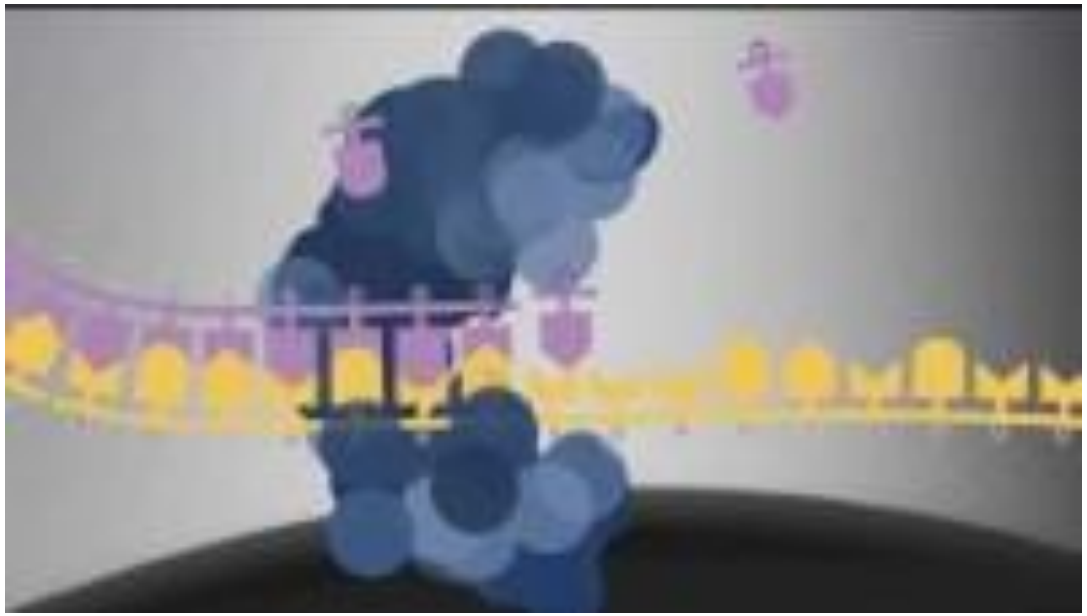
PACIFIC BIOSCIENCES®

Back to the lab package

Contents

Getting started!

PACBIO SEQUENCING 101 SERIES



PacBio Sequencing - How it works

PacBio sequencing instruments are powered by Single Molecule, Real-Time (SMRT) Sequencing technology. Learn how to go from DNA to discovery and explore the versatility of SMRT Sequencing across a broad range of applications, including whole genome sequencing, variant detection, RNA sequencing, to resolve complex populations, targeted sequencing, and epigenetics.

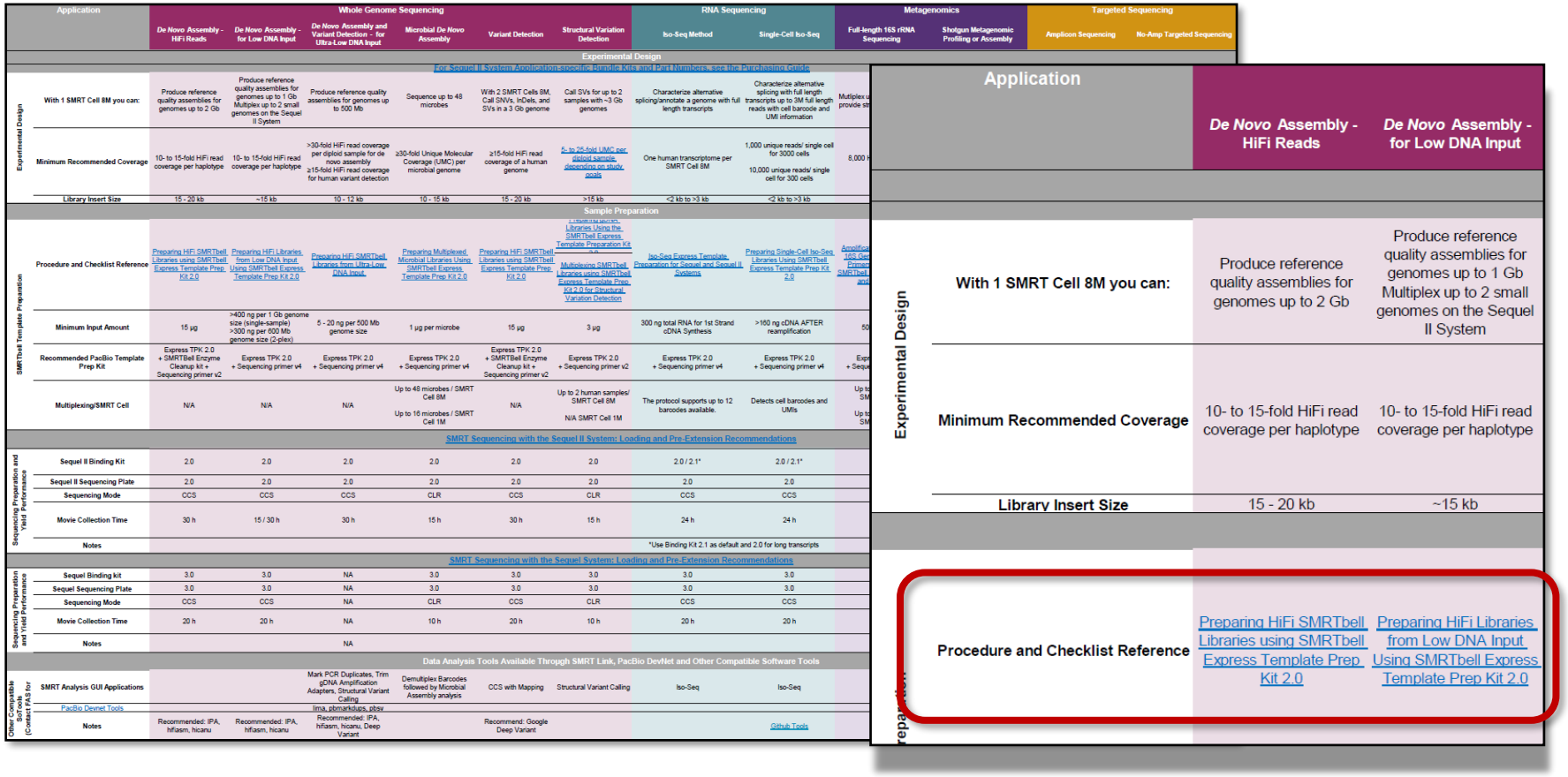
https://www.youtube.com/watch?v=_lD8JyAbwEo

- *PacBio's sequencing 101 series provides information on SMRT sequencing technology, genomics and much more!*

Explore other posts in the sequencing 101 series

OVERVIEW – SEQUEL SYSTEMS APPLICATION OPTIONS AND SEQUENCING RECOMMENDATIONS

Document provides high-level application workflow guidance and links to protocols for preparing samples for sequencing on the Sequel Systems and analysis.



Application	Whole Genome Sequencing						RNA Sequencing		Metagenomics		Targeted Sequencing	
	De Novo Assembly - HiFi Reads	*De Novo* Assembly - for Low DNA Input	*De Novo* Assembly and Variant Detection - for Ultra-Low DNA Input	Microbial *De Novo* Assembly	Variant Detection	Structural Variation Detection	Iso-Seq Method	Single-Cell Iso-Seq	Full-length 16S rRNA Sequencing	Shotgun Metagenomic Profiling or Assembly	Amplicon Sequencing	No-Amp Targeted Sequencing
Experimental Design												
For Sequel II System Application-specific Bundle Kits and Part Numbers, see The Purchasing Guide												
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 1 Gb Multiplex up to 2 small genomes on the Sequel II System	Produce reference quality assemblies for genomes up to 500 Mb	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	Characterize alternative splicing with full length transcripts up to 3M full length reads with cell barcode and UMI information	Multiplex up to 2 small genomes on the Sequel II System			
Minimum Recommended Coverage	10- to 15-fold HiFi read coverage per haplotype	10- to 15-fold HiFi read coverage per haplotype	>30-fold HiFi read coverage per diploid sample for de novo assembly ≥15-fold HiFi read coverage for human variant detection	≥30-fold Unique Molecular Coverage (UMC) per microbial genome	≥15-fold HiFi read coverage of a human genome	5- to 25-fold UMC per diploid sample, depending on study goals	One human transcriptome per SMRT Cell 8M	1,000 unique reads/ single cell for 3000 cells 10,000 unique reads/ single cell for 300 cells	8,000 HiFi reads per sample			
Library Insert Size	15 - 20 kb	~15 kb	10 - 12 kb	10 - 15 kb	15 - 20 kb	>15 kb	<2 kb to >3 kb	<2 kb to >3 kb				
Sample Preparation												
Procedure and Checklist Reference	Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi Libraries from Low DNA Input Using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi SMRTbell Libraries from Ultra-Low DNA Input	Preparing Multiplexed Microbial Libraries Using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0	Preparing Multiplexed Microbial Libraries Using the SMRTbell Express Template Preparation Kit 2.0 Multiplexing SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0 for Structural Variation Detection	Iso-Seq Express Template Preparation for Sequel and Sequel II Systems	Preparing Single-Cell Iso-Seq Libraries Using SMRTbell Express Template Prep Kit 2.0	Amplification of 16S Gene Primers for SMRTbell Libraries			
Minimum Input Amount	15 µg	>400 ng per 1 Gb genome size (single-sample) >300 ng per 500 Mb genome size (2-plex)	5 - 20 ng per 500 Mb genome size	1 µg per microbe	15 µg	3 µg	300 ng total RNA for 1st Strand cDNA Synthesis	>160 ng cDNA AFTER reamplification	50 ng gDNA			
Recommended PacBio Template Prep Kit	Express TPK 2.0 + SMRTbell Enzyme Cleanup kit + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + SMRTbell Enzyme Cleanup kit + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v2	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4	Express TPK 2.0 + Sequencing primer v4			
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.	Detects cell barcodes and UMIs	Up to 96 samples/ SMRT Cell 8M Up to 24 samples/ SMRT Cell 1M			
SMRT Sequencing with the Sequel II System: Loading and Pre-Extension Recommendations												
Sequel II Binding Kit	2.0	2.0	2.0	2.0	2.0	2.0	2.0 / 2.1*	2.0 / 2.1*				
Sequel II Sequencing Plate	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0				
Sequencing Mode	CCS	CCS	CCS	CLR	CCS	CLR	CCS	CCS				
Movie Collection Time	30 h	15 / 30 h	30 h	15 h	30 h	15 h	24 h	24 h				
Notes							*Use Binding Kit 2.1 as default and 2.0 for long transcripts					
SMRT Sequencing with the Sequel System: Loading and Pre-Extension Recommendations												
Sequel Binding kit	3.0	3.0	NA	3.0	3.0	3.0	3.0	3.0				
Sequel Sequencing Plate	3.0	3.0	NA	3.0	3.0	3.0	3.0	3.0				
Sequencing Mode	CCS	CCS	NA	CLR	CCS	CLR	CCS	CCS				
Movie Collection Time	20 h	20 h	NA	10 h	20 h	10 h	20 h	20 h				
Notes			NA									
Data Analysis Tools Available Through SMRT Link, PacBio DevNet and Other Compatible Software Tools												
SMRT Analysis GUI Applications			Mark PCR Duplicates, Trim gDNA Amplification Adapters, Structural Variant Calling	Demultiplex Barcodes followed by Microbial Assembly analysis	CCS with Mapping	Structural Variant Calling	Iso-Seq	Iso-Seq				
PacBio DevNet Tools			lima, pbmarkdups, pbsv									
Notes	Recommended: IPA, hifiasm, hicanu	Recommended: IPA, hifiasm, hicanu	Recommended: IPA, hifiasm, hicanu, Deep Variant		Recommend: Google Deep Variant			Github Tools				

Application	*De Novo* Assembly - HiFi Reads	*De Novo* Assembly - for Low DNA Input
Experimental Design		
With 1 SMRT Cell 8M you can:	Produce reference quality assemblies for genomes up to 2 Gb	Produce reference quality assemblies for genomes up to 1 Gb Multiplex up to 2 small genomes on the Sequel II System
Minimum Recommended Coverage	10- to 15-fold HiFi read coverage per haplotype	10- to 15-fold HiFi read coverage per haplotype
Library Insert Size	15 - 20 kb	~15 kb
Sample Preparation		
Procedure and Checklist Reference	Preparing HiFi SMRTbell Libraries using SMRTbell Express Template Prep Kit 2.0	Preparing HiFi Libraries from Low DNA Input Using SMRTbell Express Template Prep Kit 2.0

https://www.pacb.com/wp-content/uploads/Overview-Sequel-Systems-Application-Options-and-Sequencing-Recommendations.pdf

LIBRARY CONSTRUCTION

PRIMER ANNEALING AND POLY BINDING

Libraries can be stored @4C for 1 week (elution buffer, 101-633-500)

Libraries can be stored @-20C for ~6 months

Libraries can be stored @4C for 1 week

Application-specific Accessory Kits

- For Ultra-Low input: SMRTbell gDNA Sample Amplification (101-980-000) 1
  8 standard libraries
- For Iso-Seq Method: Iso-Seq Express Oligo Kit (101-737-500) 24 reactions
- For No-Amp: No-Amp Accessory Kit (101-788-900)

+

Template Preparation Kits

- SMRTbell Express Template 2.0 (100-938-900)
  18 standard libraries
  48 microbial genomes (12kb)
  96 amplicon samples
- Protocol specific:
  SMRTbell Enzyme Clean Up (101-746-400)
  18 reactions
  SMRTbell Enzyme Clean Up 2.0 (101-932-600) NEW
  18 reactions

+

Barcoding (optional)

- Barcoded Overhang Adapter 8A (101-628-400)
  6 reactions each barcode
- Barcoded Overhang Adapter 8B (101-628-500)
  6 reactions each barcode
- Bar Univ F/R Primers Plate (101-629-100)
  96 different barcodes
- Ampure PB (100-265-900)
  For library construction and cleanup of polymerase-bound complexes

+

Sequencing Primer

- V4 (included in prep kit)
- V2 (101-847-900)
  10 reactions
- V5 (102-067-400) NEW
  10 reactions

+

Binding Kit *

- 2.0 (101-842-900)
  24 reactions
- 2.1 (101-843-000)
  24 reactions
- 2.2 (102-089-000) NEW
  24 reactions
- MagBead Binding Buffer (101-046-400)
  if run out of Complex Dilution Buffer

* For <3kb fragments, amplicons or transcripts use 2.1

For >3kb fragments, amplicons or transcripts use 2.0/2.2

PREPARATION FOR LOADING ON INSTRUMENT

	# Part Number	# Reactions	# Per Run	
SMRT Cell 8M Tray	101-389-001	4	1	Unused cells stored @RT
Sequel II Seq kit 2.0	101-820-200	4	1	Partially used kit can be stored @4C up to 100 hours
Sequel SMRT Cell Oil	100-621-300	5	1	Mitigates evaporation during runs on the SMRT Cell
Tube septa	100-667-700	10	1	Tube septa for closing reagent tubes on the system
Sample Plate Foil	100-667-400	100	1	Foil seals for covering Sample Plates
Sample Plate	000-448-888	50	1	96-well PCR plates for sample placement on the system
Sequel Mixing Plate	100-667-500	120	1	96-well deep well plates for reagent and sample mixing on the system
Sequel Pipet Tips v2	100-667-601	3840	10-12	Robotic pipet tips compatible for liquid handling on the system

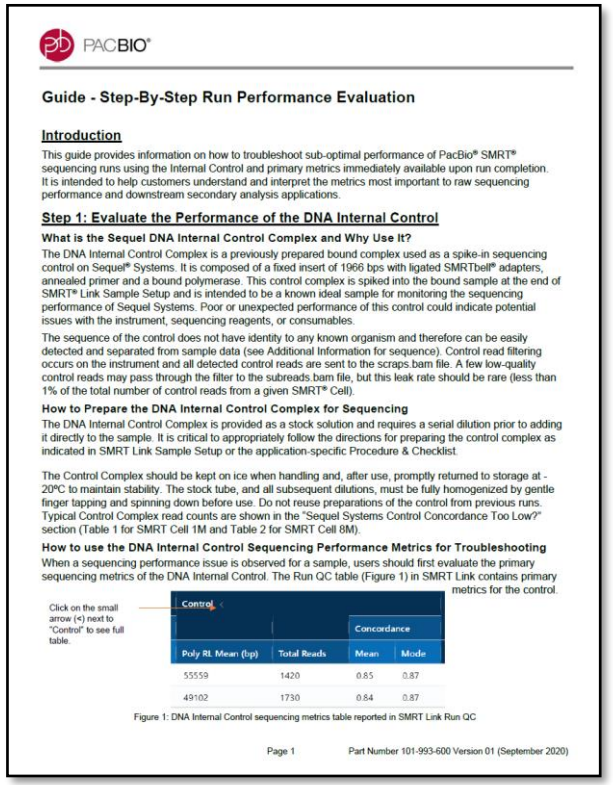
Useful guides and webinars

DNA SAMPLE PREP AND RUN PERFORMANCE GUIDES AND APPLICATION BRIEFS

Sample Preparation Technical Notes

Link

Run Performance Evaluation Guide



PACBIO

Guide - Step-By-Step Run Performance Evaluation

Introduction

This guide provides information on how to troubleshoot sub-optimal performance of PacBio® SMRT® sequencing runs using the Internal Control and primary metrics immediately available upon run completion. It is intended to help customers understand and interpret the metrics most important to raw sequencing performance and downstream secondary analysis applications.

Step 1: Evaluate the Performance of the DNA Internal Control

What is the Sequel DNA Internal Control Complex and Why Use It?

The DNA Internal Control Complex is a previously prepared bound complex used as a spike-in sequencing control on Sequel® Systems. It is composed of a fixed insert of 1966 bps with ligated SMRTbell® adapters, annealed primer and a bound polymerase. This control complex is spiked into the bound sample at the end of SMRT® Link Sample Setup and is intended to be a known ideal sample for monitoring the sequencing performance of Sequel Systems. Poor or unexpected performance of this control could indicate potential issues with the instrument, sequencing reagents, or consumables.

The sequence of the control does not have identity to any known organism and therefore can be easily detected and separated from sample data (see Additional Information for sequence). Control read filtering occurs on the instrument and all detected control reads are sent to the scraps.bam file. A few low-quality control reads may pass through the filter to the subreads.bam file, but this leak rate should be rare (less than 1% of the total number of control reads from a given SMRT® Cell).

How to Prepare the DNA Internal Control Complex for Sequencing

The DNA Internal Control Complex is provided as a stock solution and requires a serial dilution prior to adding it directly to the sample. It is critical to appropriately follow the directions for preparing the control complex as indicated in SMRT Link Sample Setup or the application-specific Procedure & Checklist.

The Control Complex should be kept on ice when handling and, after use, promptly returned to storage at -20ºC to maintain stability. The stock tube, and all subsequent dilutions, must be fully homogenized by gentle finger tapping and spinning down before use. Do not reuse preparations of the control from previous runs. Typical Control Complex read counts are shown in the "Sequel Systems Control Concordance Too Low?" section (Table 1 for SMRT Cell 1M and Table 2 for SMRT Cell 8M).

How to use the DNA Internal Control Sequencing Performance Metrics for Troubleshooting

When a sequencing performance issue is observed for a sample, users should first evaluate the primary sequencing metrics of the DNA Internal Control. The Run QC table (Figure 1) in SMRT Link contains primary metrics for the control.

Click on the small arrow (<) next to "Control" to see full table.

Control <

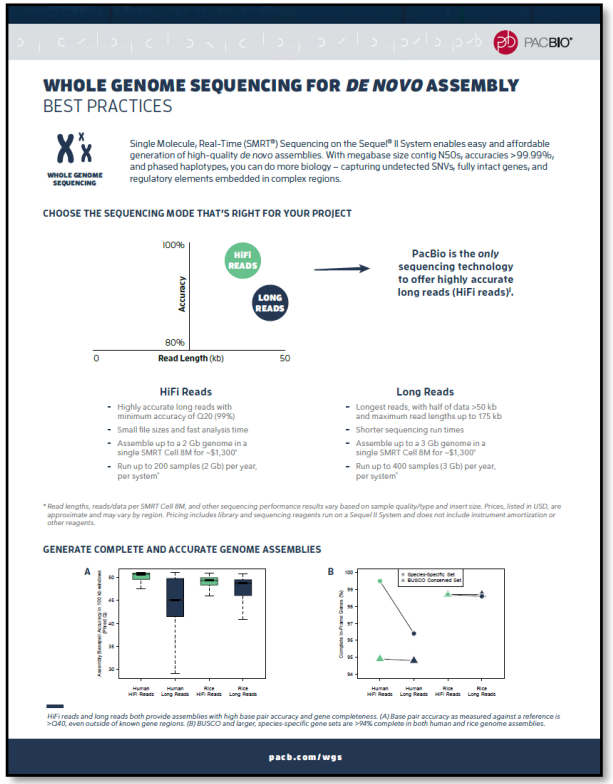
		Concordance	
Poly RL Mean (bp)	Total Reads	Mean	Mode
55559	1420	0.85	0.87
49102	1730	0.84	0.87

Figure 1: DNA Internal Control sequencing metrics table reported in SMRT Link Run QC

Page 1 Part Number 101-993-600 Version 01 (September 2020)

Link

Best Practices Application Briefs



PACBIO

WHOLE GENOME SEQUENCING FOR *DE NOVO* ASSEMBLY
BEST PRACTICES

WHOLE GENOME SEQUENCING

Single Molecule, Real-Time (SMRT®) Sequencing on the Sequel® II System enables easy and affordable generation of high-quality *de novo* assemblies. With megabase size contig N50s, accuracies >99.99%, and phased haplotypes, you can do more biology – capturing undetected SNVs, fully intact genes, and regulatory elements embedded in complex regions.

CHOOSE THE SEQUENCING MODE THAT'S RIGHT FOR YOUR PROJECT

PacBio is the *only* sequencing technology to offer highly accurate long reads (HiFi reads)[1].

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*
- 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*
- 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.*

GENERATE COMPLETE AND ACCURATE GENOME ASSEMBLIES

HiFi reads and long reads both provide assemblies with high base pair accuracy and gene completeness. (A) Base pair accuracy as measured against a reference is >Q40, even outside of known gene regions. (B) BUSCO and larger, species-specific gene sets are >94% complete in both human and rice genome assemblies.

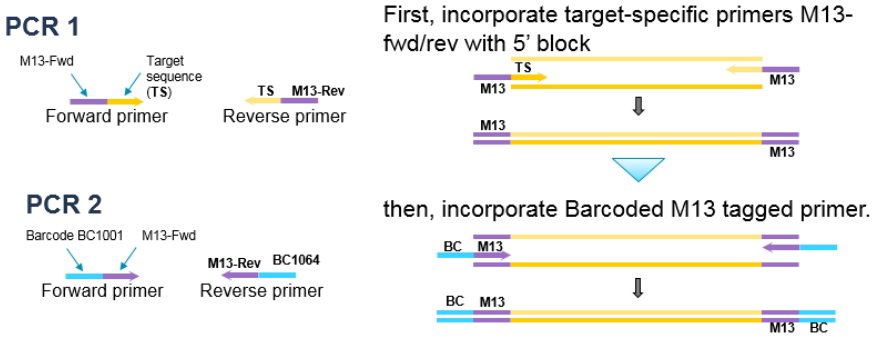
pacb.com/wgs

Link

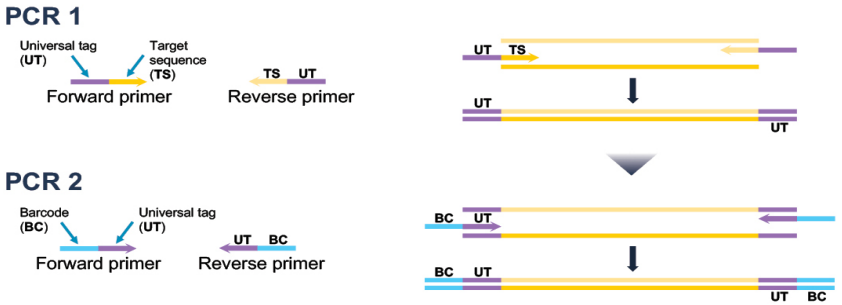
https://www.pacb.com/support/documentation/ and https://www.pacb.com/smrt-science/smrt-resources/pacbio-literature/

FLEXIBLE MULTIPLEXING AND BARCODING SOLUTIONS

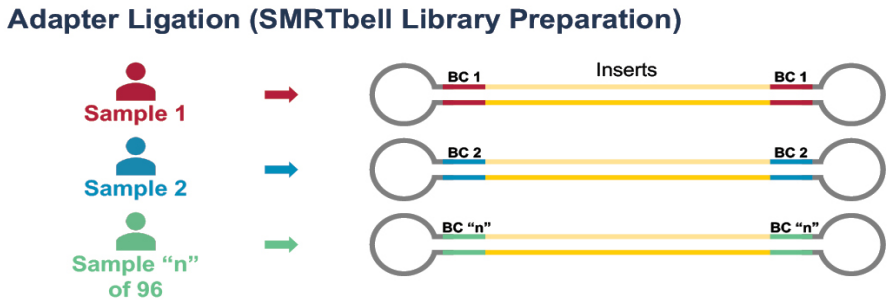
Barcoded M13 Primers



Barcoded Universal Primers



Barcoded Adapters



Barcoded Primers

https://www.pacb.com/wp-content/uploads/Application-Brief-Targeted-sequencing-Best-Practices.pdf

DNA SAMPLE PREPARATION ONLINE RESOURCE

Literature resource for sample collection and DNA extraction protocol references

The listing below is a collection of publications by the scientific community describing extraction protocols for high-molecular weight DNA followed by PacBio sequencing. When possible, the links point directly to the methods section (or supplementary information).

Animals | Plants | Fungi | Protists

If you have protocols you would like to share, or have questions about DNA extraction for PacBio sequencing, contact ExtractDNA@pacb.com.

Animals

- Invertebrates
  - Panova2016 – DNA extraction protocols for whole-genome sequencing in marine organisms
  - *microinvertebrates*
    - Laumer2020 – protocols for diverse meiofauna species, including *C. elegans* & *Castrella truncata* (SMRT [illegible] presentation)
  - *arthropods*
    - arachnids
      - Guerrero2019 – The Pacific Biosciences de novo assembled genome dataset from a parthenogenetic New Ze[illegible] wild population of the longhorned tick, *Haemaphysalis longicornis* Neumann, 1901
      - Liu2019 – DNA Methylation Patterns in the Social Spider, *Stegodyphus dumicola*
      - Burgess2018 – Draft genome assembly of the sheep scab mite, *Psoroptes ovis*
      - Randall2018 – The draft genome assembly of *Dermatophagoides pteronyssinus* supports identification of novel allergen isoforms in *Dermatophagoides* species
      - Cramaro2017 – Genome scaffolding and annotation for the pathogen vector *Ixodes ricinus* by ultra-long single molecule sequencing
    - crustaceans
      - Liu2019 – Crab muscle extraction & sequencing (Circulomics application note)
      - Baldwin-Brown2018 – A New Standard for Crustacean Genomes: The Highly Contiguous, Annotated Genome Assembly of the Clam Shrimp *Eulimnadia texana* Reveals HOX Gene Order and Identifies the Sex Chromosome
      - Gonçalves Athanasio2016 – Optimisation of DNA extraction from the crustacean *Daphnia*

Methods

Sample collection

A female yellowbelly pufferfish (Fig. 2), reared in the fish breeding centre of Fujian Normal University in Fuzhou City of Fujian Province was used for genome sequencing and assembly. Fresh white muscle, eye, skin, gonad, gut, liver, kidney, blood, gall bladder and air bladder tissues were collected and quickly frozen in liquid nitrogen for one hour. White muscle tissues were used for DNA sequencing for genome assembly, while all tissues were used for transcriptome sequencing.

Fig. 2

A picture of the yellowbelly pufferfish used in the genome sequencing and assembly.

DNA and RNA sequencing

Genomic DNA from white muscle tissue was extracted using the

Plants

- genomic DNA for long-read sequencing of single molecules
- e Genome With Single-Molecule Sequencing Uncovers a Recent Burst of Genes
- ssembly reveals the structure of the *Arabidopsis thaliana* Nd-1 genome and its gene set
- sive, high-throughput plant genomic DNA extraction method suitable for
- consis
- ng and
- ect run
- olly pine mega-genome using long-read single-molecule sequencing
- ce for the Fast-Growing Microalga *Picochlorum celeri*
- *Undaria pinnatifida*: Chromosome-Level Assembly Using PacBio and Hi-C
- e suggests convergent functions of homeobox genes in algae and land plants (algae)
- hole-genome sequencing in marine organisms (algae)

www.ExtractDNAforPacBio.com

Fungi

- Faure2019 – Long-Read Genome Sequence of the Sugar Beet Rhizosphere Mycoparasite *Pythium oligandrum*
- Nagappan2018 – Improved nucleic acid extraction protocols for *Ganoderma boninense*, *G. miniatocinctum* and *G. tornatu*
- Schwessinger2017 – Extraction of high molecular weight DNA from fungal rust spores for long read sequencing
- Solomon2016 – Robust and effective methodologies for cryopreservation and DNA extraction from anaerobic gut fungi
- Sonnenberg2016 – A detailed analysis of the recombination landscape of the button mushroom *Agaricus bisporus var. bisporus*

PacBio does not assume responsibilities/guarantees for these external publications/protocols, but we are happy to help as best as we can to guide / connect. Please contact ExtractDNA@pacb.com for more discussions around your particular species & sequencing project!

DNA AND SAMPLE PREPARATION FOR PACBIO HIFI SEQUENCING – BEST PRACTICES AND FAQ

In this webinar Greg Young covers the points below:

- *Preparing DNA for PacBio HiFi sequencing and selecting the right HiFi library preparation protocol for your project*
- *Interpreting sequencing metrics and troubleshooting*
- *Sharing Frequently Asked Questions*

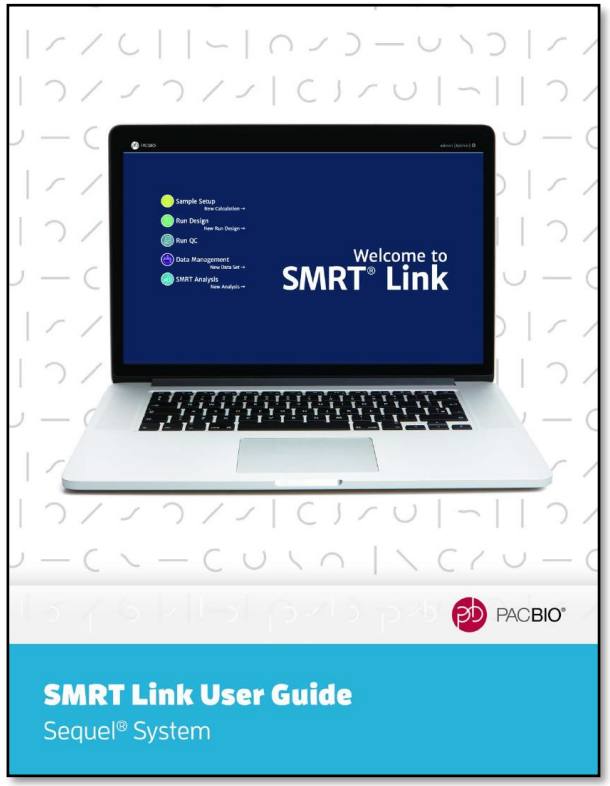
PACBIO DATA DEEPDIVE: A CLOSER LOOK AT HIFI SEQUENCING

In this webinar Dan Browne covers the points below:

- *Exploring the content of PacBio HiFi BAM files (base calls, quality scores and kinetic information)*
- *Providing examples of how HiFi reads can be filtered*
- *Going through recommendations for downstream analysis - for example, how HiFi reads are demultiplexed, assembled and mapped to reference sequences*

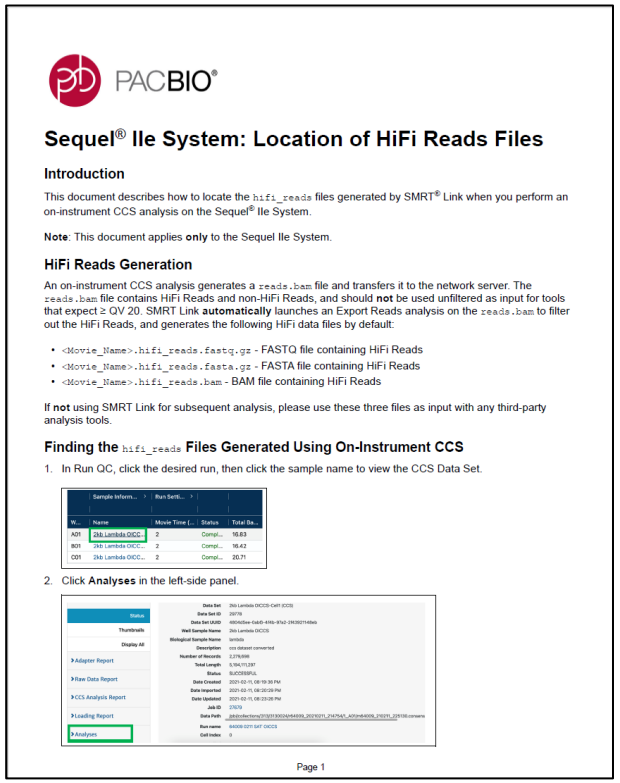
SMRTLINK USER GUIDE

SMRT Link User Guide



Link

Sequel IIe System: Location of HIFI reads files



PACBIO®

Sequel® IIe System: Location of HiFi Reads Files

Introduction

This document describes how to locate the `hifi_reads` files generated by SMRT® Link when you perform an on-instrument CCS analysis on the Sequel® IIe System.

Note: This document applies **only** to the Sequel IIe System.

HiFi Reads Generation

An on-instrument CCS analysis generates a `reads.bam` file and transfers it to the network server. The `reads.bam` file contains HiFi Reads and non-HiFi Reads, and should **not** be used unfiltered as input for tools that expect ≥ QV 20. SMRT Link **automatically** launches an Export Reads analysis on the `reads.bam` to filter out the HiFi Reads, and generates the following HiFi data files by default:

- `<Movie_Name>.hifi_reads.fastq.gz` - FASTQ file containing HiFi Reads
- `<Movie_Name>.hifi_reads.fasta.gz` - FASTA file containing HiFi Reads
- `<Movie_Name>.hifi_reads.bam` - BAM file containing HiFi Reads

If **not** using SMRT Link for subsequent analysis, please use these three files as input with any third-party analysis tools.

Finding the `hifi_reads` Files Generated Using On-Instrument CCS

1. In Run QC, click the desired run, then click the sample name to view the CCS Data Set.

2. Click **Analyses** in the left-side panel.

Page 1

Link

https://www.pacb.com/support/documentation/

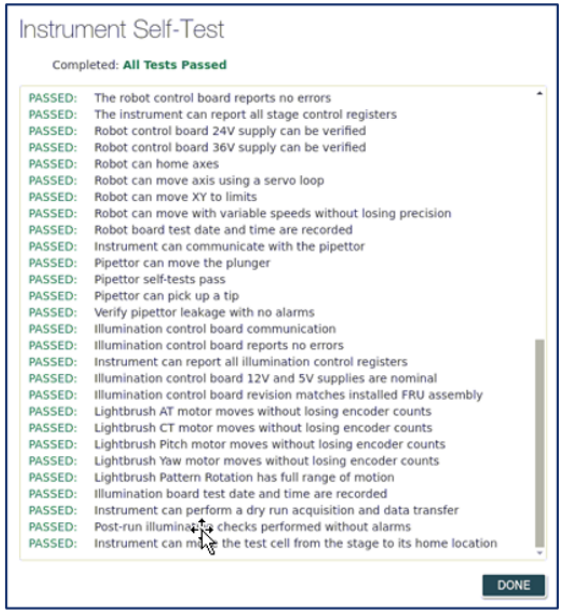
PACBIO®

Instrument start up and technical support

INSTRUMENT SELF-TEST

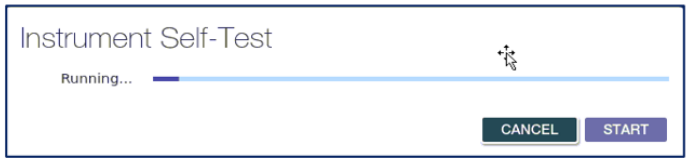
A self-test is recommended for dormant instruments. The robot, optics, chiller and pipettor are tested. In addition, the instrument will perform a work deck clean up and start a 2 minute acquisition with the test cell (found on the workdeck) testing data transfer to data storage location. The self-test takes 45 minutes to complete.

1



2

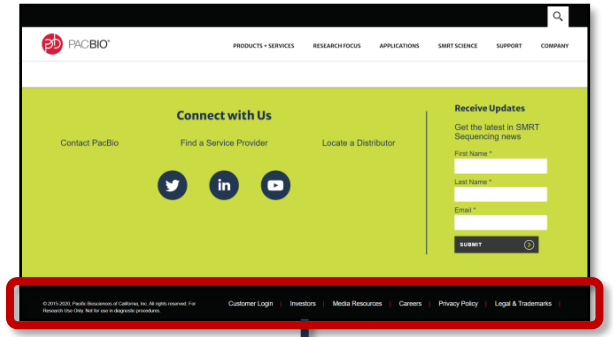
3



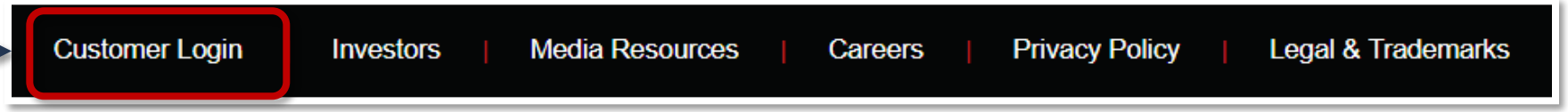
HOW TO CONTACT PACBIO FOR TECHNICAL SUPPORT

We have an experienced team of technical support scientists, engineers, and bioinformaticians to assist you with all of your support needs!

1. Log into the **PacBio Customer Portal** (http://www.pacbioportal.com/) from PacBio's corporate website and create a new case



- **Preferred route** for most customers because PacBio Tech Support and your local field team (FAS and FSE) are immediately notified *via* email of your issue
- Facilitates the exchange of troubleshooting and diagnostic data and maintains a record of all communications between our support team and yourself



2. Email: support@pacb.com

3. Tech Support Toll-free Telephone Number (Mon. – Fri., 7:00 a.m. to 5:00 p.m. PST)

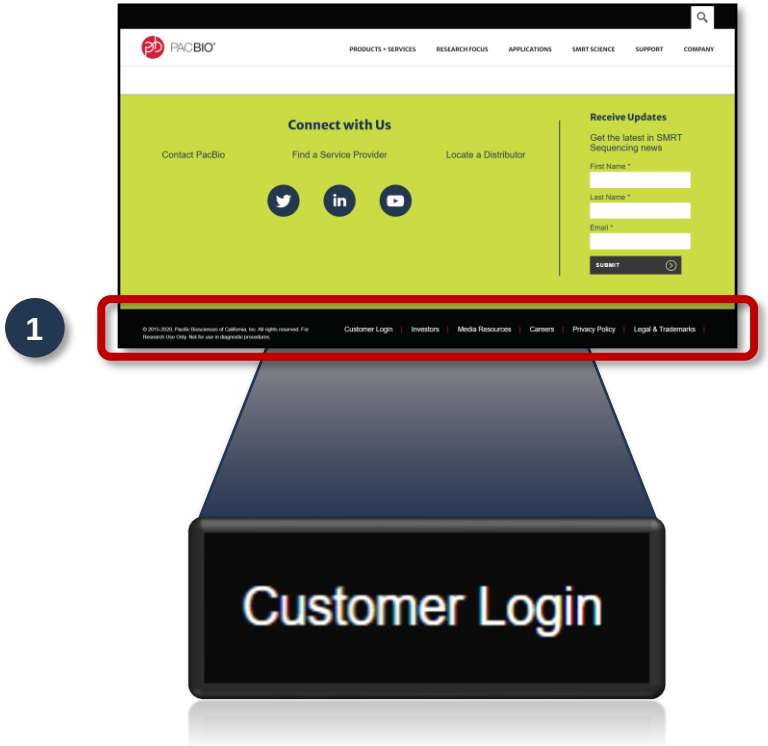
   +1.877.920.PACB [+1 (877) 920-7222]

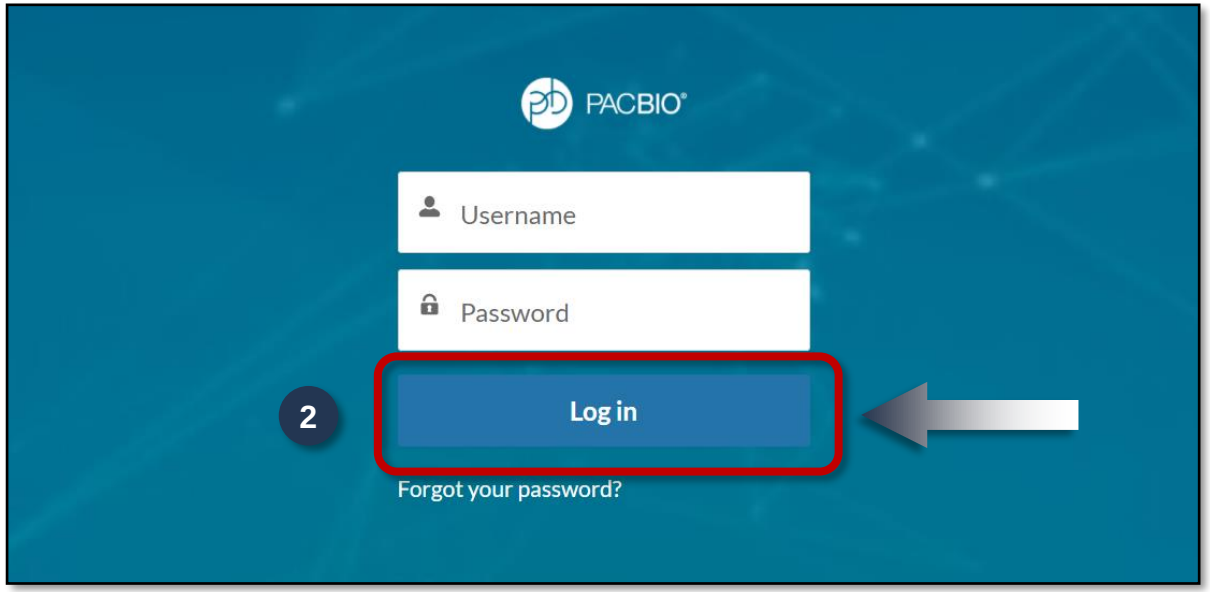
http://www.pacb.com/support/technical-support/

HOW TO LOG INTO THE CUSTOMER PORTAL

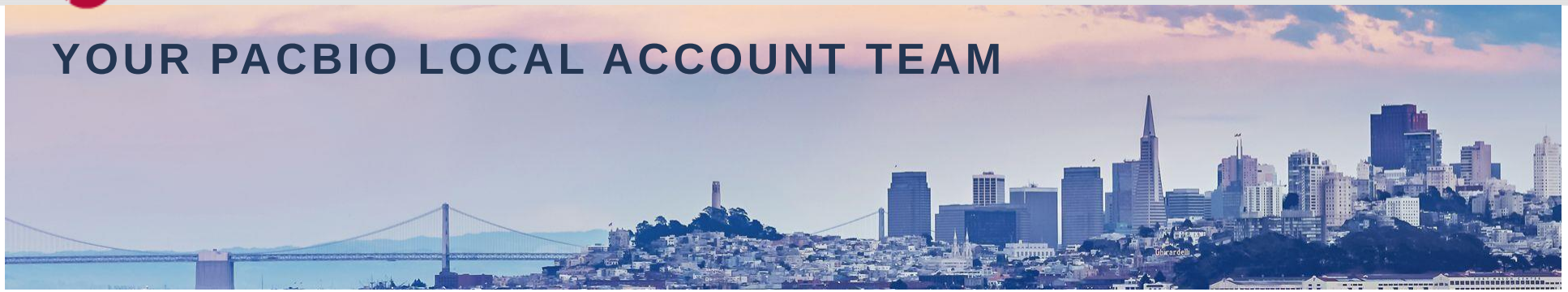
1. Click on the 'Customer Login' link at the bottom of any PacBio webpage
   - Or go to https://mypacbio.force.com/customer/s/login/ directly

2. Enter your Customer Portal login credentials.
   - If you do not remember your password, click on the "**Forgot your password?**" link on the Customer Portal login webpage.
   - For future Users needing accounts, contact your Local FAS to request access or simply click on the "**Request Access**" link on the PacBio Technical Support webpage and the request will be routed to your local PacBio Support Team for activation within 24 hours.





YOUR PACBIO LOCAL ACCOUNT TEAM



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New software and chemistry release

NEW SMRT LINK V10.1 SOFTWARE RELEASE: KEY FEATURES & IMPROVEMENTS

- Supports complete **SMRT Link analysis workflow on Amazon Cloud** (SMRT Link Cloud)
- Features several **new and improved analysis applications** for HiFi de novo assembly, SV calling, multiplexed Iso-Seq analysis and SARS-CoV-2 full-viral genome sequencing
- Reduces HPC requirements to enable **lower-cost data analysis & storage configurations**
- Provides a **simplified user experience** for run setup and includes **usability improvements** to support high-throughput sequencing

NEW SEQUEL II BINDING KIT 2.2

Part Number	New Products: Name and Description
102-089-000	The Sequel II Binding Kit 2.2 and Internal Control 1.0 contains reagent supplies to bind prepared DNA template libraries to the Sequel II Polymerase 2.2 in preparation for HiFi sequencing. The result is a DNA polymerase template complex ready for use with Adaptive Loading on the Sequel II Systems. Sequel II Polymerase 2.2 should be used only with Sequel II Sequencing Kit 2.0. Reagent quantities support approximately 24 binding reactions, and up to 70 SMRT Cell 8M runs depending on use case, sample size and concentration. Note Sequel II polymerase 2.2 is not recommended for inserts <3 kb on the Sequel II and Sequel IIe Systems. This item also includes the Internal Control 1.0, used as a positive control for loading and sequencing.
101-932-600	The SMRTbell Enzyme Clean Up Kit 2.0 contains 5 enzymes in a single tube and an enzyme reaction buffer. The kit is designed to reduce the template molecules that are not intact to enhance HiFi sequencing. The kit contains sufficient reagents to perform 18 reactions.
102-067-400	Sequencing Primer v5 is a sequencing primer used to anneal to HiFi SMRTbell templates generated with the SMRTbell Express Template Prep Kit 2.0.
102-104-700	Sequel II HiFi Bundle - 18 2.0 contains a bundle of PacBio consumables required to perform the de novo assembly and/or variant detection applications using HiFi reads on the Sequel II and Sequel IIe Systems including template preparation and polymerase binding. The bundle specifically includes the following kits and their associated quantities: SMRTbell Express Template Prep Kit 2.0 (PN: 100-938-900 / Qty: 1), SMRTbell Enzyme Cleanup Kit 2.0 (PN: 101-932-600 / Qty: 1), Sequencing Primer V5 (PN:102-067-400 / Qty: 2), AMPure PB Beads (PN: 100-265-900 / Qty: 1), Sequel II Binding Kit 2.2 and Internal Control 1.0 (PN:102-089-000 / Qty: 1).
102-088-900	The HiFi Express Template Prep Kit 2.0 Bundle contains one each: SMRTbell Express Template Preparation Kit 2.0, SMRTbell Enzyme Clean Up Kit 2.0 and Sequencing Primer v5. Each kit contains sufficient reagents to perform 18 standard reactions.

New binding kit to support HiFi Whole Genome Sequencing applications (*de novo* assembly and variant detection). New chemistry further increases the accuracy of our HiFi reads.

Binding kit 2.2 features:

- Faster polymerase
- Improved version of Enzyme Cleanup kit
- New primer v5

Appendix

Application	Template prep	Sequencing primer	Binding kit
De Novo Assembly HiFi Reads	• SMRTbell Express Template 2.0 (100-938-900) 18 standard libraries • SMRTbell Enzyme Clean Up 2.0 (101-932-600) NEW 18 reactions	• V5 (102-067-400) NEW 10 reactions	• 2.2 (102-089-000) NEW 24 reactions
De Novo Assembly Low Input	• SMRTbell Express Template 2.0 (100-938-900) 18 standard libraries • SMRTbell Enzyme Clean Up (101-746-400) 18 reactions • Barcoded Overhang Adapter 8A (101-628-400) 6 reactions each barcode • Barcoded Overhang Adapter 8B (101-628-500) 6 reactions each barcode	• V4 (included in prep kit)	• 2.0 (101-842-900) 24 reactions
De Novo & Variant Detection Ultra Low Input	• SMRTbell gDNA Sample Amplification (101-980-000) 18 standard libraries • SMRTbell Express Template 2.0 (100-938-900) 18 standard libraries	• V4 (included in prep kit)	• 2.0 (101-842-900) 24 reactions
Microbial *De Novo* Assembly	• SMRTbell Express Template 2.0 (100-938-900) 48 microbial genomes (12kb) • Barcoded Overhang Adapter 8A (101-628-400) 6 reactions each barcode • Barcoded Overhang Adapter 8B (101-628-500) 6 reactions each barcode	• V4 (included in prep kit)	• 2.0 (101-842-900) 24 reactions
Variant Detection	• SMRTbell Express Template 2.0 (100-938-900) 18 standard libraries • SMRTbell Enzyme Clean Up 2.0 (101-932-600) NEW 18 reactions	• V5 (102-067-400) NEW 10 reactions	• 2.2 (102-089-000) NEW 24 reactions

Application	Template prep	Sequencing primer	Binding kit
Structural Variant Detection	• SMRTbell Express Template 2.0 (100-938-900) 18 standard libraries • Barcoded Overhang Adapter 8A (101-628-400) 6 reactions each barcode • Barcoded Overhang Adapter 8B (101-628-500) 6 reactions each barcode	• V2 (101-847-900) 10 reactions	• 2.0 (101-842-900) 24 reactions
Iso-Seq	• Iso-Seq Express Oligo kit (101-737-500) 24 reactions • SMRTbell Express Template 2.0 (100-938-900) 18 standard libraries	• V4 (included in prep kit)	• 2.0 (101-842-900) 24 reactions • 2.1 (101-843-000)* 24 reactions
Full length 16S rRNA Seq	• SMRTbell Express Template 2.0 (100-938-900) 96 samples	• V4 (included in prep kit)	• 2.1 (101-843-000) 24 reactions
Shotgun Metagenomics	• SMRTbell Express Template 2.0 (100-938-900) 18 standard libraries • SMRTbell Enzyme Clean Up (101-746-400) 18 reactions • Barcoded Overhang Adapter 8A (101-628-400) 6 reactions each barcode • Barcoded Overhang Adapter 8B (101-628-500) 6 reactions each barcode	• V2 (101-847-900) 10 reactions	• 2.0 (101-842-900) 24 reactions

* Use Binding kit 2.1 as default and Binding kit 2.0 for transcripts >3kb

	Template prep	Sequencing primer	Binding kit
Amplicon Sequencing	• SMRTbell Express Template 2.0 (100-938-900) 96 amplicon samples • Barcoded Overhang Adapter 8A (101-628-400) 6 reactions each barcode • Barcoded Overhang Adapter 8B (101-628-500) 6 reactions each barcode • Bar Univ F/R Primers 96 Plate (101-629-100) 96 barcodes	• V4 (included in prep kit)	• 2.0 (101-842-900) 24 reactions • 2.1 (101-843-000)** 24 reactions
No-Amp Targeted Sequencing	• No-Amp Accessory kit (101-788-900) 20 reactions • SMRTbell Enzyme Clean Up (101-746-400) 18 reactions	• V4 (included in accessory kit)	• 2.0 (101-842-900) 24 reactions

** Use Binding kit for 500bp-3kb and Binding kit 2.0 for >3kb

Other reagents	
Ampure PB, 5mL (100-265-900)	For library construction and cleanup of polymerase-bound complexes
Elution Buffer, 50mL (101-633-500)	For library prep and barcoding workflows requiring additional clean-up steps
MagBead Binding Buffer (101-046-400)	Used to purify DNA/polymerase complexes after binding of polymerase – functionally equivalent to Complex Dilution Buffer for the Internal Control and final loading dilution steps

Reagents for instrument loading	# Reactions	# Per run	
SMRT Cell 8M Tray (101-389-001)	4	1	Unused cells stored @RT
Sequel II Seq kit 2.0 (101-820-200)	4	1	Partially used kit can be stored @4C up to 100 hours
Sequel SMRT Cell Oil (100-621-300)	5	1	Mitigates evaporation during runs on the SMRT Cell
Tube septa (100-667-700)	10	1	Tube septa for closing reagent tubes on the system
Sample Plate Foil (100-667-400)	100	1	Foil seals for covering Sample Plates
Sample Plate (000-448-888)	50	1	96-well PCR plates for sample placement on the system
Sequel Mixing Plate (100-667-500)	120	1	96-well deep well plates for reagent and sample mixing on the system
Sequel Pipet Tips v2 (100-667-601)	3840	10-12	Robotic pipet tips compatible for liquid handling on the system

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