

The PacBio logo is displayed in a bold, pink, sans-serif font. A single, large pink droplet is positioned at the end of the word 'Bio', appearing to be suspended in mid-air as if falling from a pipette. The background of the slide is a blurred laboratory setting with a rack of microcentrifuge tubes containing pink liquid.

PacBio

**Technical overview**

# **Revio system ICS v13 + SMRT Link v13**

Instrument control software and SMRT Link software updates for Revio and Sequel II systems

# Revio system ICS v13 + SMRT Link v13 release technical overview

## Technical overview

### Revio system ICS v13 + applications

- Revio system ICS v12 features recap
- Revio system ICS v13 key features and benefits overview
- Revio system ICS v13 user experience improvements
- Revio system ICS v13 applications updates

### SMRT Link v13

- SMRT Link v13 Instruments module updates
- SMRT Link v13 Sample Setup module updates
- SMRT Link v13 Runs module updates
- SMRT Link v13 Data Management module updates
- SMRT Link v13 SMRT Analysis module updates
- SMRT Link Lite

### Technical documentation & applications support resources

#### Appendix

- Example Revio system ICS v13 instrument robotic workflow schedules
- SMRT Link v13 installation & configuration settings
- SMRT Link v13 API documentation
- Revio system ICS v13 / SMRT Link v13 fixed & known issues



# Revio system ICS v13 key features and benefits

# What's staying the same in Revio system ICS v13?



## Workflow

SMRTbell prep kit 3.0



## Chemistry

Revio pol kit + Revio sequencing plate



## File formats

hifi\_reads.bam + fail\_reads.bam





# What is new in Revio system ICS v13?

*More of HiFi sequencing at scale*



## More applications

AAV, < 3kb amplicons, full-length RNA-seq, targeted panels, Kinnex



## More flexibility

Multiple movie time options, rich HiFi reads



## More streamlined

Improved software user interface experience for easier run set up and run monitoring



## More reliability

SMRT Link and Revio instrument software bug fixes



## More accuracy

Improved read quality, more consistent indel performance

# Revio system v13 key feature updates










**ICS v13**

**Revio system only**








**SMRT Link v13**

**Sequel II/IIe systems & Revio system**

Revio workflow step	What's New	What stays the same
<b>SMRTbell library prep protocols &amp; consumables</b>	 <ul style="list-style-type: none"> <li>N/A</li> </ul>	<ul style="list-style-type: none"> <li>Existing SMRTbell prep kit 3.0 &amp; protocols</li> <li>Existing Kinnex kits &amp; protocols</li> </ul>
<b>Supported applications</b>	 <ul style="list-style-type: none"> <li>Full-length RNA (Iso-Seq)</li> <li>&lt;3 kb Amplicon</li> <li>Adeno-associated virus (AAV)</li> <li>Kinnex single-cell RNA</li> <li>Kinnex full-length RNA</li> <li>Kinnex 16S RNA</li> </ul>	<ul style="list-style-type: none"> <li>WGS (plant/animal / microbial / shotgun metagenomics)</li> <li>MAS-seq single cell</li> <li>3+ kb Amplicons</li> </ul>
<b>Sample setup (ABC)</b>	 <ul style="list-style-type: none"> <li>Kinnex sequencing primer (in Revio polymerase kit)</li> <li>SMRT Link Sample Setup support for Kinnex libraries</li> <li>SMRT Link Sample Setup GUI usability improvements</li> </ul>	<ul style="list-style-type: none"> <li>Overall sample setup workflow</li> <li>Existing Revio polymerase kit core components &amp; reagent formulations</li> </ul>
<b>Run design</b>	 <ul style="list-style-type: none"> <li>12 / 24 / 30-hour movie time options</li> <li>Updated SMRT Link Run Design barcoding / auto-analysis / other options</li> <li>Updated run design CSV format for improved usability</li> </ul>	<ul style="list-style-type: none"> <li>Overall run design workflow</li> </ul>
<b>Sequencing</b>	 <ul style="list-style-type: none"> <li>Adaptive loading</li> <li>Revio SMRT Cell single-use tray (1 SMRT Cell)</li> <li>Revio sequencing plate – 1 rxn</li> <li>Instrument reliability &amp; serviceability improvements</li> </ul>	<ul style="list-style-type: none"> <li>Overall run setup workflow</li> <li>Existing Revio sequencing plate reagent formulations</li> <li>Existing Revio on-instrument analysis features</li> </ul>
<b>Run QC</b>	 <ul style="list-style-type: none"> <li>SMRT Link <b>Run Preview</b> feature provides early feedback on performance</li> </ul>	<ul style="list-style-type: none"> <li>Existing SMRT Link Run Details reports</li> </ul>
<b>Data analysis</b>	 <ul style="list-style-type: none"> <li>SMRT Analysis <b>HiFi Target Enrichment, Read Segmentation &amp; Iso-Seq</b> applications</li> <li><b>SMRT Link Lite</b> (omits SMRT Analysis module)</li> </ul>	<ul style="list-style-type: none"> <li>Existing SMRT Analysis applications</li> </ul>

# Revio system v13 key benefits

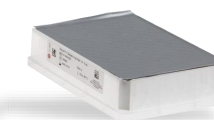
New Revio system release enables more applications, more sample types and more flexibility

	Whole genome SPK 3.0	●	1,300
	Metagenome – Profiling & assembly SPK 3.0	●	15,600
	Metagenome – Full-length 16S RNA Kinnex	+	1 M+
	Single-cell RNA Kinnex	+	1,300
	Full-length RNA Kinnex	+	5,200
	Full-length RNA SPK 3.0	+	2,600
	Target enrichment (5+ kb fragment) SPK 3.0	●	15,600 20 Mb panel
	Amplicon 3+ kb SPK 3.0	●	1.3 M+
	Amplicon <3 kb SPK 3.0	+	2.6 M+
	Adeno-associated virus (AAV) SPK 3.0	+	31,200

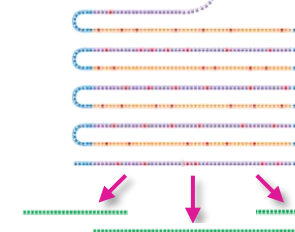
Samples / yr<sup>1</sup>

## Revio system v13

- Increases instrument reliability
- Supports all insert sizes
- Enables more movie time options



All insert sizes now supported with existing Revio polymerase kit (102-817-600) and Revio sequencing plate (102-587-400)



More movie time options to suit different insert sizes (12 / 24 / 30 hrs)



Increased Revio system instrument reliability

# Revio system v13 key benefits (cont.)

New ICS and SMRT Link software enables more robust sequencing performance and increased flexibility

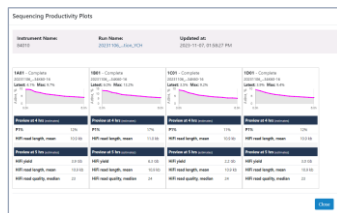
## Updated software for Revio system

### ICS v13



- ICS v13 is available for Revio systems only

### SMRT Link v13



- SMRT Link v13 requires Revio systems to be upgraded to ICS v13<sup>1</sup>

### SMRT Link v13 key feature updates



#### Instruments

- **New Run Preview<sup>2</sup>** feature provides early feedback on summary metrics (P1 loading, HiFi read length performance and/or HiFi yield) [Revio system only]



#### Sample Setup

- **New support for Kinnex libraries**
- **General usability and user experience improvements** to make annealing / binding / cleanup (ABC) steps clearer and easier to follow



#### Runs

- **Updated sample barcoding and run / data / auto-analysis options<sup>2</sup>**
- **Updated run design CSV<sup>2</sup>** format for improved usability
- **New Run Preview and Barcode Preview<sup>2</sup>** feature provides early feedback on summary metrics and sample demultiplexing performance



#### Data Management

- **New Run Preview and Barcode Preview<sup>2</sup>** feature provides early feedback on summary metrics and sample demultiplexing performance



#### SMRT Analysis

- **New HiFi Target Enrichment** application for analysis of long-read panels
- **New Read Segmentation & Iso-Seq** application for analysis of Kinnex full-length RNA samples
- **SMRT Link Lite** (omits SMRT Analysis module) for run setup & monitoring



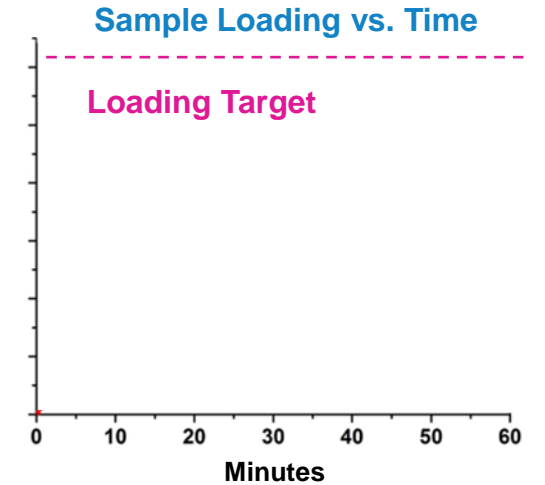
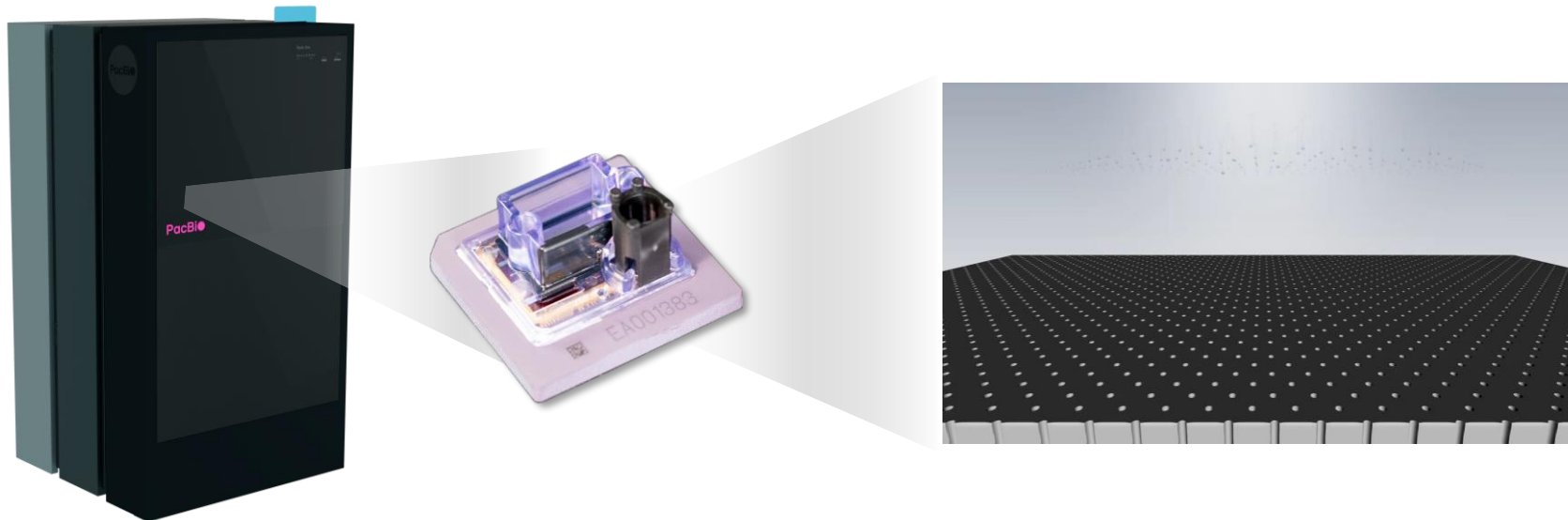


# Revio system v13 user experience improvements

# Revio system v13 adaptive loading feature

## Adaptive loading reduces sample overloading on SMRT Cells

- Adaptive loading technology actively monitors polymerase complex binding to ZMWs during sample immobilization step
- Revio system terminates the immobilization reaction when the desired loading target has been achieved
  - Reduces sample overloading and run-to-run yield variability



# Revio system v13 adaptive loading feature (cont.)

To enable adaptive loading, go to Run Options in Run Design page and select 'YES'

The screenshot shows the 'New Run Design' page in the PacBio software. The 'Run Information' section on the left includes fields for Instrument Type (Sequel II or Revio), Run Name, Plate 1 and 2 information, Run Comments, and Transfer Subdirectory. The 'Sample Information' section on the right includes Polymerase Kit, Movie Acquisition Time, Application, Samples, and Run Options. The 'Run Options' section is highlighted with a pink box and contains the following fields:

- Library Concentration (pM) Required: 0
- Use Adaptive Loading:  YES  NO

**Note:** Adaptive Loading setting must be the same for all SMRT Cells in a run.<sup>1</sup>

**Run Options**

Library Concentration (pM) Required: 0

Use Adaptive Loading  YES  NO

For Revio system v13, **adaptive loading target** and **maximum loading time** settings are **not** configurable by users in Run Design)

<sup>1</sup> In SMRT Link v13 GUI, Adaptive Loading setting (YES or NO) is configurable per sample but will switch to be configurable per run in SMRT Link v13.1. If different Adaptive Loading settings are specified for different samples within the same run design, all settings will be overwritten by the last chosen one. Furthermore, importing a Run Design CSV with multiple values for Adaptive Loading field results in an error.

# Revio system v13 movie time options

In SMRT Link v13 Run Design, movie acquisition time can be optionally set to 12, 24 or 30 hours for increased flexibility when running different library insert sizes



Movie Acquisition Time (hours)	24
Application	24

Can specify different movie times for different SMRT Cells within the same Revio run design

Shorter

12 hrs

~1 – 5 kb

Short fragments<sup>1</sup>



Longer

24 hrs

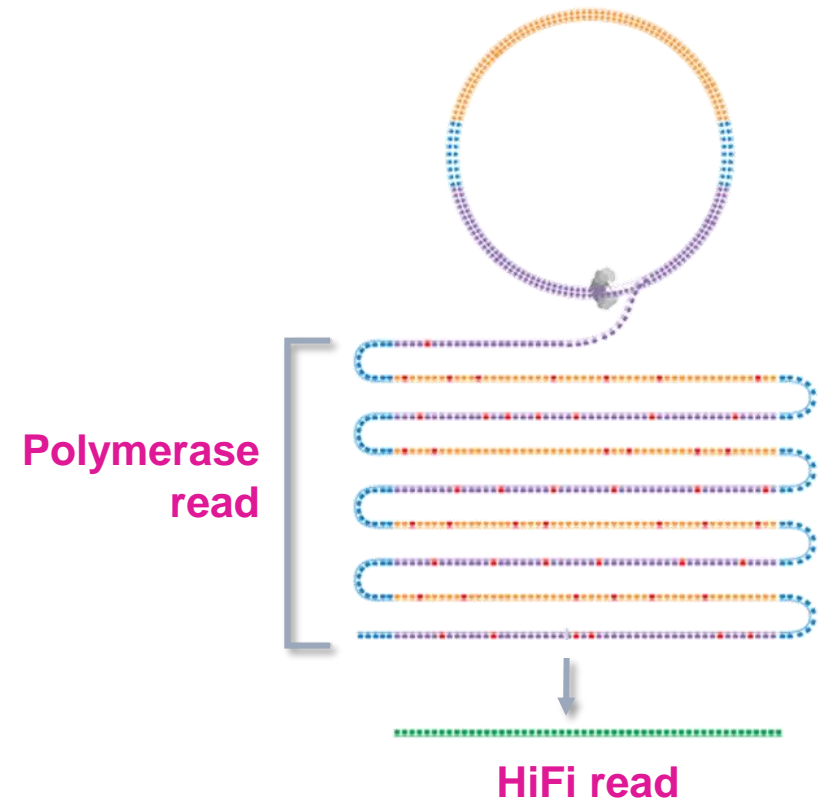
~5 – 20 kb

Most applications

30 hrs

~20 – 25 kb

Longer fragments





# Revio system v13 movie time options (cont.)

Example human 20 kb WGS library sequenced with 24 hrs vs. 30 hrs movie times<sup>1</sup>



24 hrs

110 Gb

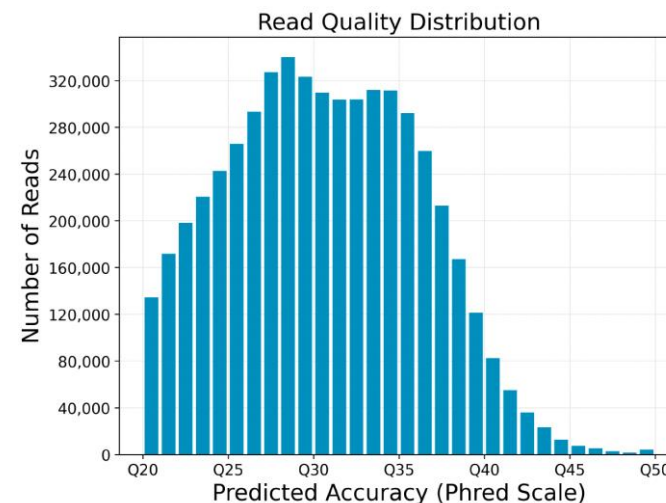
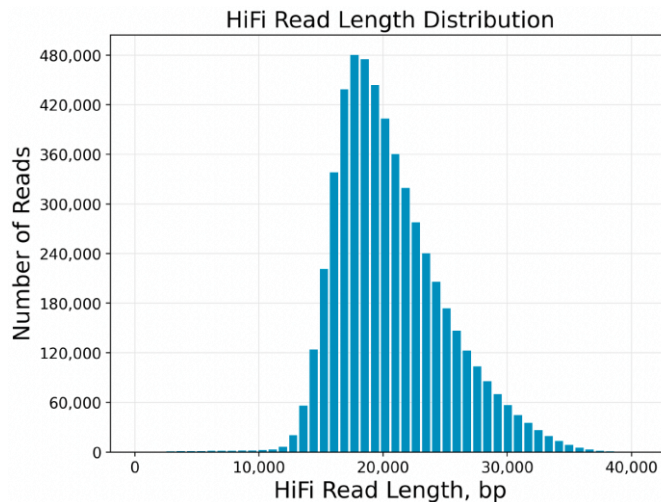
HiFi yield

Q30

HiFi read quality

20.6 kb

HiFi read length



Can improve read quality  
for 20 – 25 kb libraries



30 hrs

113 Gb

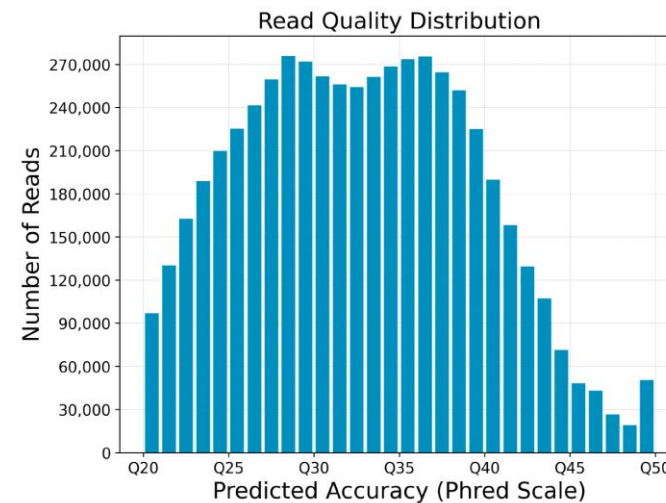
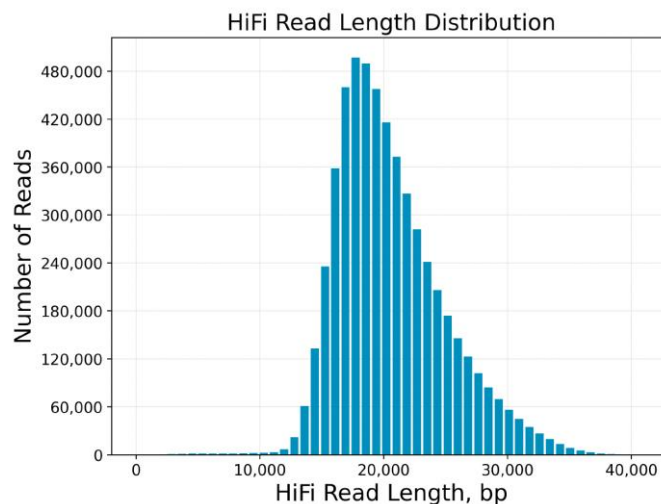
HiFi yield

Q33

HiFi read quality

20.7 kb

HiFi read length



# Revio system v13 movie time options (cont.)

Example weekly instrument throughput and run schedules for 12 / 24 /30-hour movie times

Example Revio system instrument throughput  
(Maximum number of SMRT Cells per week)

Movie acquisition time	No adaptive loading	Adaptive loading
12 hrs	56	32
24 hrs	28	24
30 hrs	22 <sup>1</sup>	20

Example Revio system instrument run schedule with adaptive loading<sup>2</sup>

Movie acquisition time (with adaptive loading)	Week	Number of Revio SMRT Cells				
		Monday	Tuesday	Wednesday	Thursday	Friday
12 hrs	Week 1	8	4	8	4	8
24 hrs	Week 1	8	0	8	0	8
30 hrs	Week 1	8	0	4	0	8
	Week 2	8	0	0	4	8

Ideally load 8-cell run on Friday to let instrument run over weekend

<sup>1</sup> For Revio runs using a 30 hrs movie time without adaptive loading, the maximum allowable number of SMRT Cells run per week will alternate between 24 cells for one week and 20 cells for the following week due to shifts in the run schedule.

<sup>2</sup> **Note:** Adaptive loading feature adds ~4 hours to time required to process one Revio SMRT Cell due to non-parallelization of cell prep and data acquisition steps → Revio system work deck will initially become available for pre-loading ~10 hours after starting a 4-SMRT Cell run.

# Revio system v13 support for all library insert sizes

Revio system v13 supports all insert sizes<sup>1</sup> through optimized instrument workflows without any change in sequencing reagent formulations

Runs / Create New  
**New Run Design**

Run Information

Instrument Type

Sequel II  Sequel IIe  Revio

Run Name  
Run 11.23.2023 14:25

Plate 1 Required ⓘ

Lot Serial Expiry

Plate 2 ⓘ

Lot Serial Expiry

Sample Information

▼ Plate 1, Well A01: Copy Delete

Plate Well ⓘ Required Plate 1, Well A01

Well Name ⓘ Required

Well Comment

Library Type Required Standard

**Insert Size (bp) Required**

Polymerase Kit Required Revio polymerase kit

Movie Acquisition Time (hours) 24

**Library insert size information is required in Revio system v13 run designs to enable correct instrument workflow**



All insert sizes now supported with existing Revio polymerase kit (102-817-600) and Revio sequencing plate (102-587-400)

# Revio system v13 run design CSV

Updated run design CSV format<sup>1</sup> has a simplified, more compact format and improves readability



## Simplified format

Editable in text editor or spreadsheet software



## Organized into three sections

1. Run Settings
2. SMRT Cell Settings
3. Samples



## Supports advanced settings

Access rich HiFi tags and full-resolution base quality values

[Run Settings]		
Instrument Type	Revio	
Run Name	Example Run	
Run Comments	Example Run comment	
Plate 1	102118800	
Plate 2	102118800	
Transfer Subdirectory	Example_Transfer_Subdirectory	
CSV Version	1	
[SMRT Cell Settings]		
	1_A01	1_B01
Well Name	Sample1_CCS	Sample2_CCS_BC
Well Comment	Sample 1 comment	Sample 2 comment
Application	HiFi Reads	HiFi Reads
Library Type	Standard	Standard
Movie Acquisition Time (hours)	24	24
Insert Size (bp)	2000	2000
Assign Data To Project	1	1
Library Concentration (pM)	7	7
Include Base Kinetics	FALSE	FALSE
Polymerase Kit	Lxxxxx102739100123199	Lxxxxx102739100123199
Sample is indexed	TRUE	TRUE
Use Adaptive Loading	TRUE	TRUE
Consensus Mode	molecule	molecule
[Samples]		
Bio Sample Name	Plate Well	Adapter
Lambda1	1_A01	bc2001
Lambda1	1_B01	lbc1
Lambda2	1_B01	lbc2
Lambda3	1_B01	lbc3

**Note:** Old SMRT Link v12 run design CSVs will be supported until SMRT Link v13.1 release  
(No change for Sequel II/Ile systems)

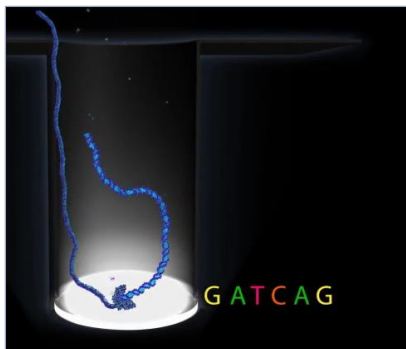
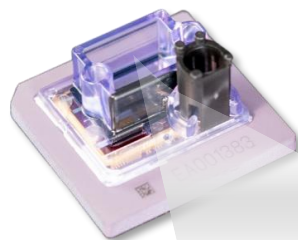


# Revio system v13 Run Preview feature

Run Preview<sup>1</sup> provides early feedback on sequencing performance 4 hrs after movie starts and 1 hour before movie ends



## Subsample across SMRT Cell

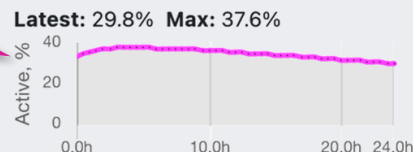


Run Preview metrics are calculated based on **subsampling** ~0.1% of total ZMWs on a Revio SMRT Cell



## Run Preview – Primary sequencing metrics

For Sequel II/Ile systems, Run Preview<sup>1</sup> only shows % Active ZMWs plot



### Preview at 4 hrs (estimates)

P1%	63%
HiFi read length, mean	13.0 kb

### Preview at 23 hrs (estimates)

HiFi yield	91.3 Gb
HiFi read length, mean	17.3 kb
HiFi read quality, median	32

### Available in:

- SMRT Link Instruments page
- SMRT Link Run Details report
- SMRT Link Data Management report



## Run Preview – Barcode demultiplexing metrics

Barcode	HiFi reads ↓	HiFi read length (mean, bp)
bc2001--bc2001	25.2%	17,255
bc2002--bc2002	25.2%	17,799
bc2003--bc2003	24.5%	17,186
bc2004--bc2004	23.9%	17,380

### Available in:

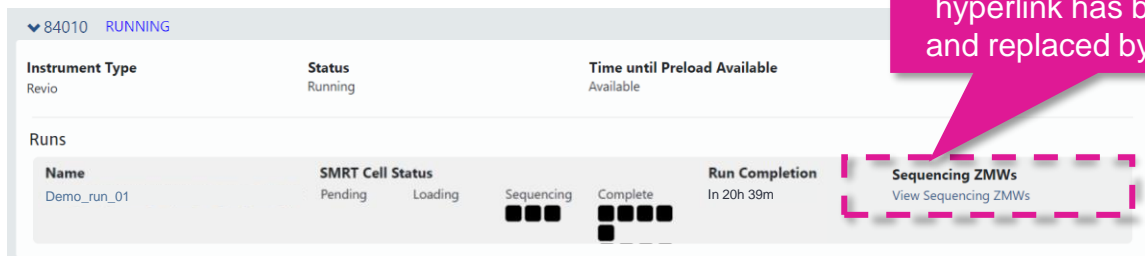
- SMRT Link Run Details report
- SMRT Link Data Management report

Run Preview info is **approximate** and intended to guide future runs by providing early information on loading, library fragment size, and representation of barcodes in a pool

# Revio system v13 Run Preview feature (cont.)

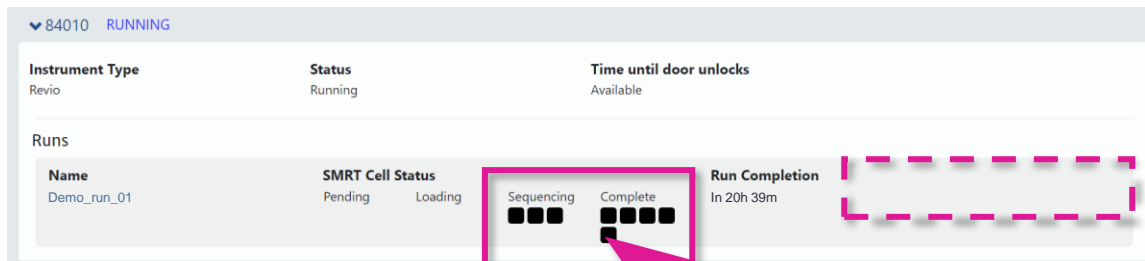
How to access Run Preview info from SMRT Link Instruments page

## SMRT Link v12.0

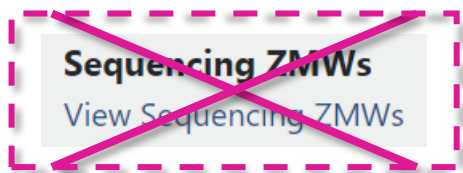


'View Sequencing ZMWs' hyperlink has been removed and replaced by Run Preview

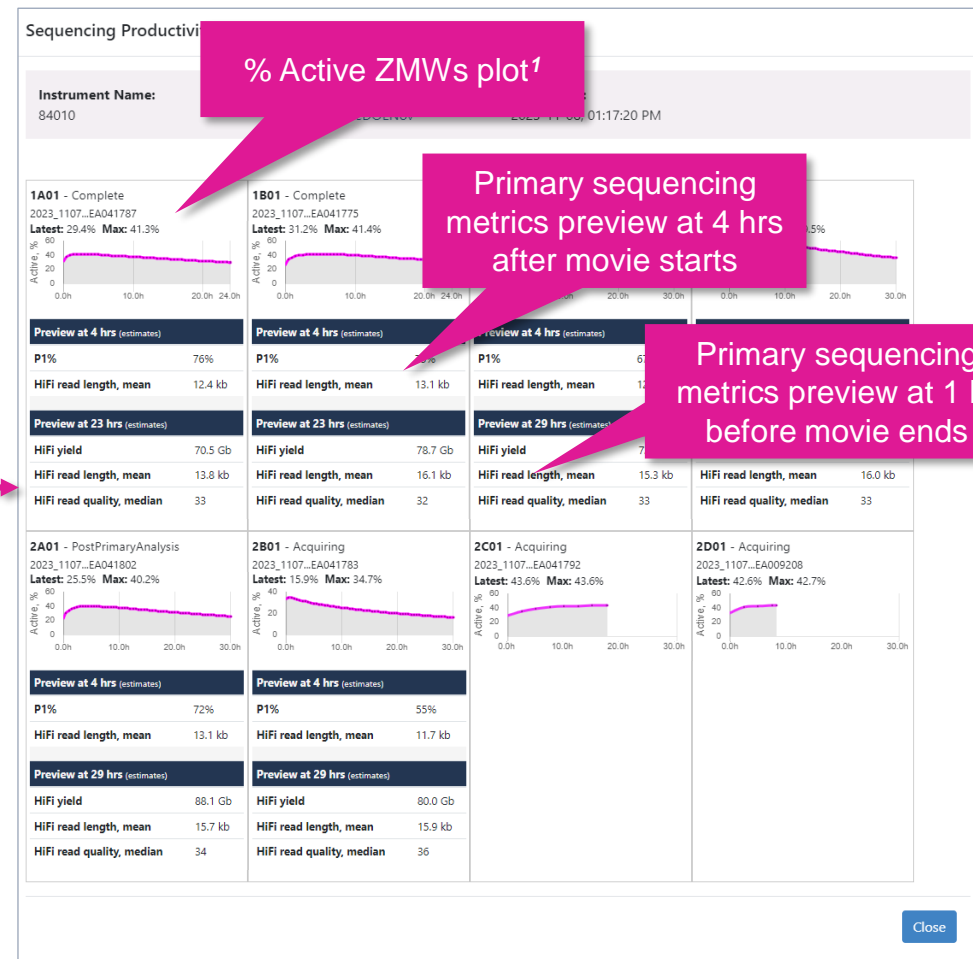
## SMRT Link v13



Access run preview by clicking on any SMRT Cell icon in 'Sequencing' or 'Completed' status → Run Preview metrics will be displayed for ALL cells in the run

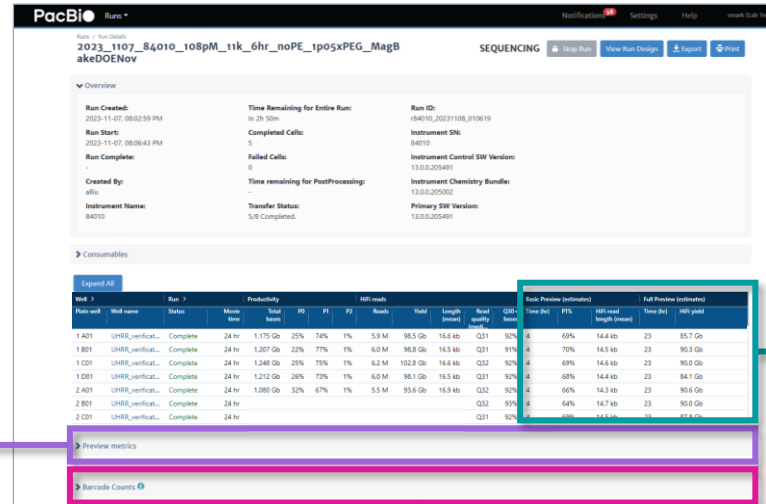


## Run Preview



# Revio system v13 Run Preview feature (cont.)

How to view Run Preview info in SMRT Link Run Details report



Preview metrics + % active ZMWs plots are displayed below run QC metrics table only while run is still in progress

Preview metrics are displayed in run QC metrics table while run is still in progress and after run ends

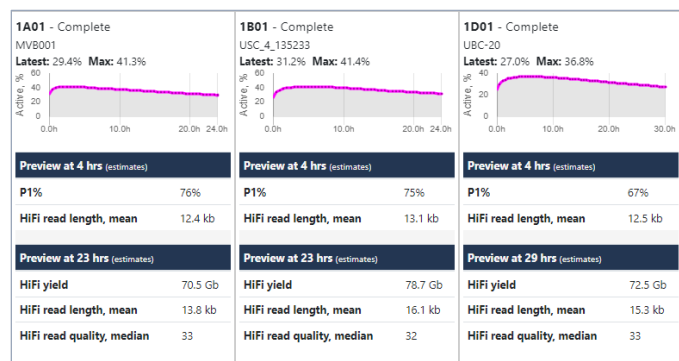
Preview metrics

Barcode Counts

Basic Preview (estimates)

Full Preview (estimates)

Barcode Counts preview metrics are available while run is still in progress and after run ends



Well name: UHRR\_verification\_DL\_4 | Time point: 4 hr

Barcode ID	HiFi reads %	HiFi read length (mean, bp)
bcM0001--bcM0001	30.2%	14209
bcM0002--bcM0002	23.2%	15142
bcM0003--bcM0003	20.0%	14110
bcM0004--bcM0004	20.0%	14383
Not Barcoded	6.7%	13844

Time (hr)	P1%	HiFi read length (mean)
4	69%	14.4 kb
4	70%	14.5 kb
4	69%	14.6 kb
4	68%	14.4 kb
4	66%	14.3 kb
4	64%	14.7 kb
4	69%	14.5 kb
4	67%	14.6 kb

Time (hr)	HiFi yield	HiFi read length (mean)	HiFi read quality (median)
23	85.7 Gb	16.5 kb	Q31
23	90.3 Gb	16.4 kb	Q30
23	90.0 Gb	16.6 kb	Q31
23	84.1 Gb	16.4 kb	Q30
23	90.6 Gb	16.9 kb	Q31
23	90.0 Gb	17.5 kb	Q32
23	87.8 Gb	16.9 kb	Q31
23	81.2 Gb	16.8 kb	Q31

Run Preview primary sequencing metrics + % Active ZMWs plots

Run Preview barcode demultiplexing metrics<sup>1</sup>

Run Preview primary sequencing metrics



<sup>1</sup> Note: The barcode demultiplexing metrics displayed in Run Preview may overestimate the actual number of unbarcoded reads. In addition, all estimates may be less accurate for barcodes at low frequency (<10%) due to sample size. Any barcodes below a 1% frequency are not displayed, and are grouped into the "Other" category.

# Revio system v13 Run Preview feature (cont.)

## How to view Run Preview info in SMRT Link Data Management report

▼ 4hr Run Preview

Summary Metrics

Barcode preview

PacBio Data Management

MasBulk\_pool\_op2-Cells (all samples)

Dataset Overview

Status

4hr Run Preview

23hr Run Preview

▼ 23hr Run Preview

Summary Metrics

Barcode preview

Estimated primary sequencing metrics displayed in table are the same as those shown in Run Details report and Instruments page

Estimated barcode demultiplexing metrics displayed in table are the same as those shown in Run Details report

Value	Analysis Metric
undefined	Estimated HiFi yield
undefined	HiFi read quality, median
15881	HiFi read length, mean
63.5	P1%
4	Time (Hrs)
Basic	Workflow
m84034_231103_151641_s1_basic_preview_4hr	Id
m84034_231103_151641_s1	Movie Id

Value	Analysis Metric
113987288120	Estimated HiFi yield
32	HiFi read quality, median
16532	HiFi read length, mean
undefined	P1%
23	Time (Hrs)
Full	Workflow
m84034_231103_151641_s1_full_preview_23hr	Id
m84034_231103_151641_s1	Movie Id

Barcode ↓↑	HiFi reads ↓↑	HiFi read length (mean, bp) ↓↑
bcM0001--bcM0001	54.2%	15740
bcM0002--bcM0002	39.5%	16153
Other	0.6%	15556
Not Barcoded	5.8%	15176

Barcode ↓↑	HiFi reads ↓↑	HiFi read length (mean, bp) ↓↑
bcM0001--bcM0001	55.2%	16234
bcM0002--bcM0002	42.8%	16988
Not Barcoded	2.0%	14412



# Revio system v13 Run Preview feature (cont.)

Example comparison of preview metrics *versus* final metrics displayed in SMRT Link v13 Run Details report

**Final metrics**

Well >		Run >		Productivity			HiFi reads				Polymerase reads >		Control reads >		
Plate well	Well name	Status	Movie time	Total bases	P0	P1	P2	Reads	Yield	Length (mean)	Read quality (median, QV)	Q30+ bases	Pol. read length (mean)	Reads	Read length (mean)
1 A01	Sample 01	Complete	24 hr	1,175 Gb	25%	74%	1%	5.9 M	98.5 Gb	16.6 kb	Q31	92%	63.2 kb	3,351	47.1 kb
1 B01	Sample 02	Complete	24 hr	1,207 Gb	22%	77%	1%	6.0 M	98.8 Gb	16.5 kb	Q31	91%	61.9 kb	3,466	47.1 kb
1 C01	Sample 03	Complete	24 hr	1,248 Gb	25%	75%	1%	6.2 M	102.8 Gb	16.6 kb	Q32	92%	66.5 kb	3,307	50.4 kb
1 D01	Sample 04	Complete	24 hr	1,212 Gb	26%	73%	1%	6.0 M	98.1 Gb	16.5 kb	Q31	92%	66.1 kb	3,520	51.2 kb
2 A01	Sample 05	Complete	24 hr	1,080 Gb	32%	67%	1%	5.5 M	93.6 Gb	16.9 kb	Q32	92%	63.7 kb	3,462	49.9 kb
2 B01	Sample 06	Complete	24 hr	1,095 Gb	34%	65%	1%	5.4 M	91.9 Gb	17.0 kb	Q32	93%	66.8 kb	3,703	51.8 kb
2 C01	Sample 07	Complete	24 hr	1,146 Gb	28%	72%	1%	5.7 M	96.2 Gb	16.9 kb	Q31	92%	63.4 kb	3,552	49.9 kb
2 D01	Sample 08	Complete	24 hr	1,109 Gb	31%	69%	1%	5.5 M	92.8 Gb	16.9 kb	Q32	92%	64.1 kb	3,572	50.1 kb

**Preview metrics**

Well >		Run >		Basic Preview (estimates)			Full Preview (estimates)			
Plate well	Well name	Status	Movie time	Time (hr)	P1%	HiFi read length (mean)	Time (hr)	HiFi yield	HiFi read length (mean)	HiFi read quality (median)
1 A01	Sample 01	Complete	24 hr	4	69%	14.4 kb	23	85.7 Gb	16.5 kb	Q31
1 B01	Sample 02	Complete	24 hr	4	70%	14.5 kb	23	90.3 Gb	16.4 kb	Q30
1 C01	Sample 03	Complete	24 hr	4	69%	14.6 kb	23	90.0 Gb	16.6 kb	Q31
1 D01	Sample 04	Complete	24 hr	4	68%	14.4 kb	23	84.1 Gb	16.4 kb	Q30
2 A01	Sample 05	Complete	24 hr	4	66%	14.3 kb	23	90.6 Gb	16.9 kb	Q31
2 B01	Sample 06	Complete	24 hr	4	64%	14.7 kb	23	90.0 Gb	17.5 kb	Q32
2 C01	Sample 07	Complete	24 hr	4	69%	14.5 kb	23	87.8 Gb	16.9 kb	Q31
2 D01	Sample 08	Complete	24 hr	4	67%	14.6 kb	23	81.2 Gb	16.8 kb	Q31

Basic Preview metrics tend to be under-estimates of final values

Full Preview metrics tend to be closer to final values

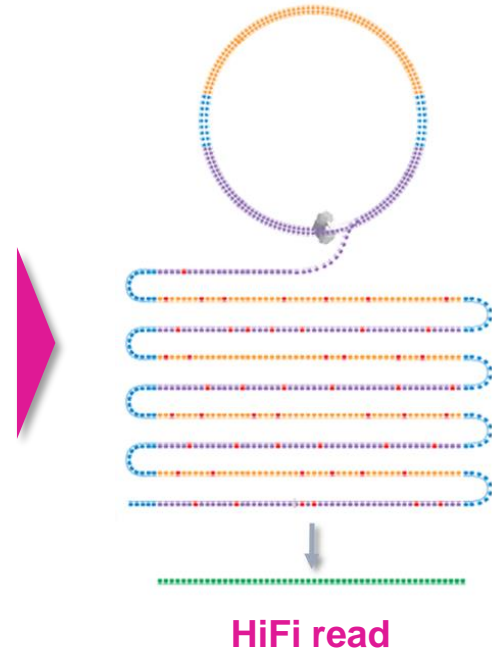
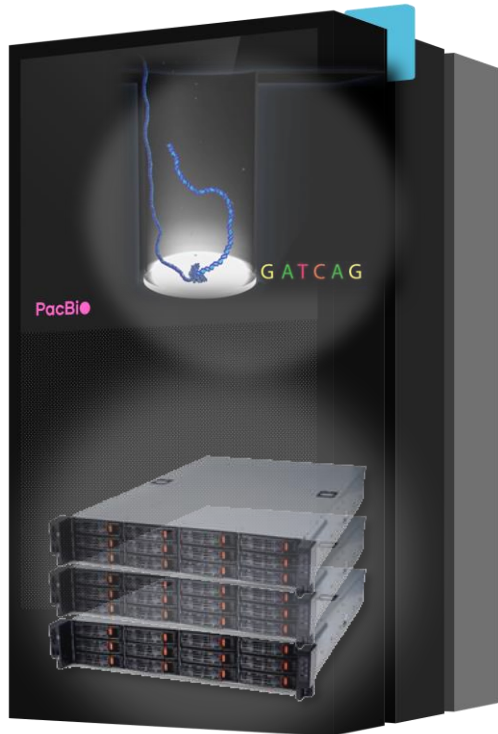
Run Preview info is **approximate** and intended to guide future runs by providing early information on primary sequencing metrics

# Revio system v13 on-instrument analysis updates

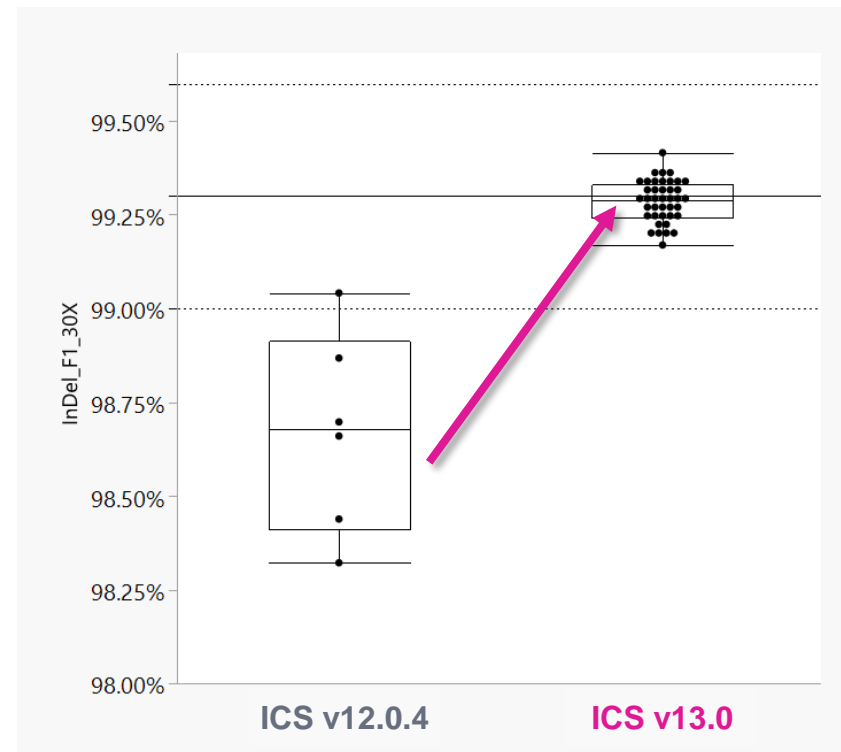
Updated basecaller and circular consensus sequencing (CCS) read calling models to improve accuracy

## Revio system v13 basecaller and CCS analysis updates

- Implemented **improvements to primary analysis basecalling, CCS analysis and DeepConsensus** to improve HiFi accuracy performance
- Inputs, outputs, and model architecture are **unchanged** from the previous release.
  - Can combine Revio system v13 datasets with existing v12.0 datasets



## Indel F1 score @ 30x<sup>1</sup>



# Revio system v13 on-instrument analysis updates (cont.)

Updated 5mC methylation-calling software to include training for Revio system chemistry

## Revio ICS v12.0 / SMRT Link v12.0

### primrose 5mC calling software

- `primrose` software only includes training for Sequel II/IIe system chemistry



## Revio ICS v12.0 / SMRT Link v13

### jasmine 5mC calling software

- `jasmine` software includes training for Revio system chemistry and uses a new model for 5mC methylation calling
  - `jasmine` software replaces the `primrose` software used in previous SMRT Link releases)
- Inputs, outputs, and model architecture are unchanged from the previous release
  - It is not necessary to reprocess existing methylation datasets already called with `primrose`

## Example 5mC methylation calling performance<sup>1</sup>

N = 8	Sensitivity	Specificity
<code>primrose</code>	83%	83%
<code>jasmine</code>	81%	84%

## Correlation of 5mC methylation calling with bisulfite sequencing<sup>2</sup>

	HG002
<code>primrose</code>	0.94
<code>jasmine</code>	0.94

<sup>1</sup> Methylation analysis was performed for each human WGS sample on single CpG sites within single reads (8 HG002 replicates). True negatives = HG002 + whole-genome amplification; True positives = HG002 + WGA+ M.SssI treatment (to generate CpG methylated DNA.)

<sup>2</sup> Analysis was performed using [pb-CpG-tools](#).

# Revio system v13 on-instrument analysis updates (cont.)

## Updated run design parameter settings for non-multiplexed samples

### SMRT Link v12.0 Run Design setup procedure for non-multiplexed samples

**Samples**

Adapters / Barcodes Required: Revio SMRTbell adapters + barcodes

Sample Names Required: Interactively



For non-multiplexed samples, select 'Revio SMRTbell adapters + barcodes'

**Barcode Selector and Sample Name Editor**

Available Barcodes: Filter...

Barcode ID
bc2001--bc2001
bc2002--bc2002
bc2003--bc2003
bc2004--bc2004
bc2005--bc2005
bc2006--bc2006
bc2007--bc2007
bc2008--bc2008

Included Barcodes: Filter...

Barcode ID	Bio Sample ID
default--default	Non-Barcoded_Sample

For non-multiplexed samples, select 'default--default' barcode and enter in a Bio Sample name

### SMRT Link v13 Run Design setup procedure for non-multiplexed samples

**Samples**

Sample is indexed: YES NO

Bio Sample Name Required: [Empty field]



### For non-multiplexed samples:

→ Select 'Sample is indexed' = NO and directly enter a Bio Sample Name

- No longer need to specify "default--default" barcode in Run Design Bio Sample Name sheet for non-multiplexed samples

# Revio system v13 on-instrument analysis updates (cont.)

Updated run design parameter settings for multiplexed samples

## SMRT Link v12.0 Run Design setup procedure for multiplexed samples

For multiplexed samples, select 'Revio SMRTbell adapters + barcodes'

Adapters / Barcodes Required: Revio SMRTbell adapters + barcodes

Sample Names Required: Interactively From a File

Multiplexed library sample containing barcoded SMRTbell adaptors

Barcode ID	Bio Sample ID
<input type="checkbox"/> bc2001--bc2001	Adapter-barcoded_sample_1
<input type="checkbox"/> bc2002--bc2002	Adapter-barcoded_sample_2
<input type="checkbox"/> bc2003--bc2003	Adapter-barcoded_sample_3
<input type="checkbox"/> bc2004--bc2004	Adapter-barcoded_sample_4

Select desired barcodes and enter in Bio Sample names  
→ Barcode demultiplexing automatically performed on-instrument

Multiplexed library sample containing barcoded PCR primers

Barcode ID	Bio Sample ID
<input type="checkbox"/> default--default	Primer-barcoded sample

Select 'default--default' barcode and enter in a Bio Sample name  
→ Manually perform barcode demultiplexing in SMRT Link using the appropriate barcode set FASTA file

## SMRT Link v13 Run Design setup procedure for multiplexed samples

For multiplexed samples, select Sample is indexed = YES

Sample is indexed:  YES  NO

Indexes Required: SMRTbell adapter indexes

Select appropriate index set FASTA<sup>1</sup>

Same Barcodes on Both Ends of Sequence:  YES  NO

Specify YES or NO

Biosample names Required: Interactively From a File

Formerly called 'Adapters / Barcodes'

Barcode Selector and Sample Name Editor

Available Barcodes

Barcode ID	Bio Sample ID
<input type="checkbox"/> bc2003--bc2003	
<input type="checkbox"/> bc2004--bc2004	
<input type="checkbox"/> bc2005--bc2005	
<input type="checkbox"/> bc2006--bc2006	
<input type="checkbox"/> bc2007--bc2007	

Included Barcodes

Barcode ID	Bio Sample ID
<input checked="" type="checkbox"/> bc2001--bc2001	Human_WGS_Sample_1
<input checked="" type="checkbox"/> bc2002--bc2002	Human_WGS_Sample_2

Select desired barcodes and enter in Bio Sample names  
→ Barcode demultiplexing automatically performed on-instrument

# Revio system v13 output files

Output BAM files are more efficiently compressed and updated BAM file header includes additional run information

## Revio system data output file structure

r84001\_20220722\_134701

```
├── 1_A01
│   ├── fail_reads
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2001.bam
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2001.bam.pbi
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2002.bam
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2002.bam.pbi
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2003.bam
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2003.bam.pbi
│   │   ├── m84001_221201_123456_s1.fail_reads.unassigned.bam
│   │   └── m84001_221201_123456_s1.fail_reads.unassigned.bam.pbi
│   ├── hifi_reads
│   │   ├── m84001_221201_123456_s1.hifi_reads.bc2001.bam
│   │   ├── m84001_221201_123456_s1.hifi_reads.bc2001.bam.pbi
│   │   ├── m84001_221201_123456_s1.hifi_reads.bc2002.bam
│   │   ├── m84001_221201_123456_s1.hifi_reads.bc2002.bam.pbi
│   │   ├── m84001_221201_123456_s1.hifi_reads.bc2003.bam
│   │   ├── m84001_221201_123456_s1.hifi_reads.bc2003.bam.pbi
│   │   ├── m84001_221201_123456_s1.hifi_reads.unassigned.bam
│   │   └── m84001_221201_123456_s1.hifi_reads.unassigned.bam.pbi
│   ├── metadata
│   ├── pb_formats
│   └── statistics
```

## Revio system v13 BAM files

- More efficient compression of output BAM files by sorting records **reduces file sizes by approximately 10%**
- Updated BAM file header includes:
  - SMRTCELLKIT – Part number for SMRT Cell
  - SMRTCELLID – Serial number for SMRT Cell
  - RUNID – Run identifier
  - ICSVERSION – Instrument software version
  - MOVIELENGTH – Movie length in minutes

## Example Revio system v13 BAM file header

```
$ samtools view -H m84200_231109_020206_s3.hifi_reads.bam | grep '^@RG' | tr '\t' '\n'
@RG
ID:39b34537
PL:PACBIO
DS:READTYPE=CCS;Ipd:CodecV1=ip;PulseWidth:CodecV1=pw;BINDINGKIT=102-739-100;
SEQUENCINGKIT=102-412-400;BASECALLERVERSION=5.0;SMRTCELLKIT=102-202-300;
SMRTCELLID=EA051064;RUNID=r84200_20231109_015443;ICSVERSION=13.0.0.206556;
MOVIELENGTH=1440.0;FRAMERATEHZ=100.000000
LB:HG002-17kb_Acq29
PU:m84200_231109_020206_s3
SM:HG002-17kb
PM:REVIO
CM:R/P1-C1/5.0-25M
```



# Revio system v13 output files (cont.)

Added analysis support for “rich HiFi” tags that provide a summary of subread-to-consensus read alignment

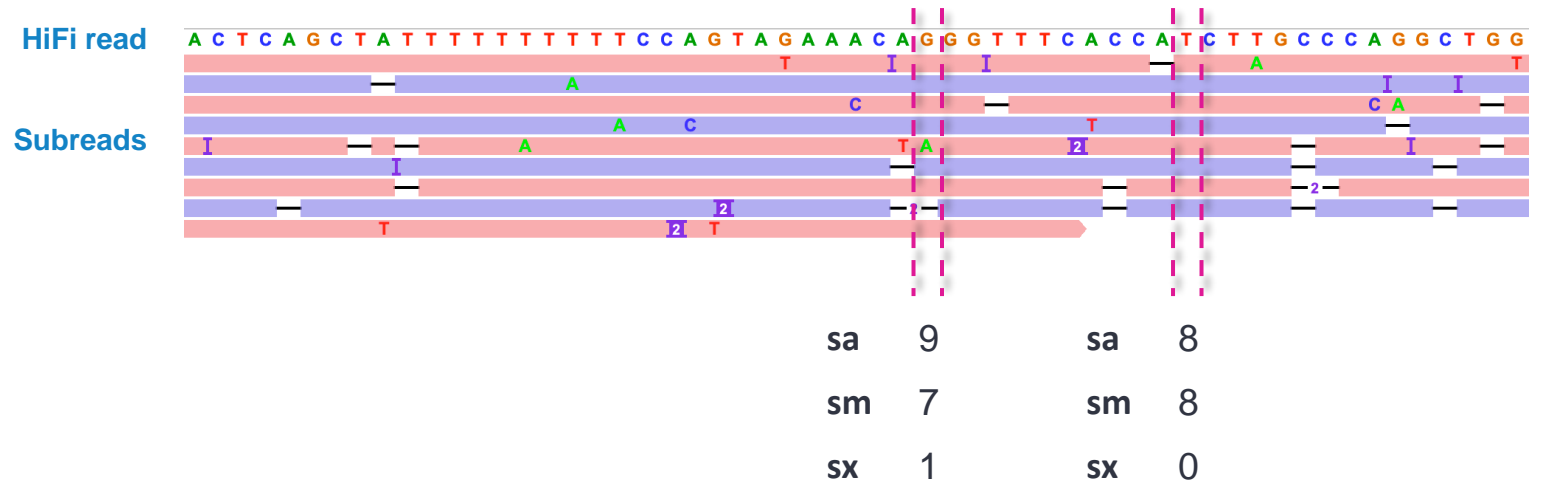
## Revio system data output file structure

```

r84001_20220722_134701
├── 1_A01
│   ├── fail_reads
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2001.bam
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2001.bam.pbi
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2002.bam
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2002.bam.pbi
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2003.bam
│   │   ├── m84001_221201_123456_s1.fail_reads.bc2003.bam.pbi
│   │   ├── m84001_221201_123456_s1.fail_reads.unassigned.bam
│   │   └── m84001_221201_123456_s1.fail_reads.unassigned.bam.pbi
│   └── hifi_reads
│       ├── m84001_221201_123456_s1.hifi_reads.bc2001.bam
│       ├── m84001_221201_123456_s1.hifi_reads.bc2001.bam.pbi
│       ├── m84001_221201_123456_s1.hifi_reads.bc2002.bam
│       ├── m84001_221201_123456_s1.hifi_reads.bc2002.bam.pbi
│       ├── m84001_221201_123456_s1.hifi_reads.bc2003.bam
│       ├── m84001_221201_123456_s1.hifi_reads.bc2003.bam.pbi
│       ├── m84001_221201_123456_s1.hifi_reads.unassigned.bam
│       └── m84001_221201_123456_s1.hifi_reads.unassigned.bam.pbi
│   ├── metadata
│   ├── pb_formats
│   └── statistics
    
```

hifi\_reads.bam now contains **optional “rich HiFi” tags** that summarize the subreads that produced a HiFi read to support downstream analysis applications

→ The rich HiFi tags are accessible using the **Subread to HiFi Pileup** option when creating new run designs by importing a run design CSV file<sup>1</sup>



- sa** Subread alignments that span each consensus position
- sm** Subreads that match the consensus base at each position
- sx** Subreads that mismatch the consensus base at each position

<sup>1</sup> The option to include rich HiFi tags in hifi\_reads.bam file is not available in the SMRT Link Run Design graphical user interface – it can only be specified by creating a new Run Design CSV and importing it into SMRT Link.

# Revio system v13 output files (cont.)

Updated fail\_reads.bam file contents to include more useful data

## Revio system data output file structure

r84001\_20220722\_134701

└─ 1\_A01

```
└─ fail_reads
  └─ m84001_221201_123456_s1.fail_reads.bc2001.bam
  └─ m84001_221201_123456_s1.fail_reads.bc2001.bam.pbi
  └─ m84001_221201_123456_s1.fail_reads.bc2002.bam
  └─ m84001_221201_123456_s1.fail_reads.bc2002.bam.pbi
  └─ m84001_221201_123456_s1.fail_reads.bc2003.bam
  └─ m84001_221201_123456_s1.fail_reads.bc2003.bam.pbi
  └─ m84001_221201_123456_s1.fail_reads.unassigned.bam
  └─ m84001_221201_123456_s1.fail_reads.unassigned.bam.pbi
```

```
└─ hifi_reads
  └─ m84001_221201_123456_s1.hifi_reads.bc2001.bam
  └─ m84001_221201_123456_s1.hifi_reads.bc2001.bam.pbi
  └─ m84001_221201_123456_s1.hifi_reads.bc2002.bam
  └─ m84001_221201_123456_s1.hifi_reads.bc2002.bam.pbi
  └─ m84001_221201_123456_s1.hifi_reads.bc2003.bam
  └─ m84001_221201_123456_s1.hifi_reads.bc2003.bam.pbi
  └─ m84001_221201_123456_s1.hifi_reads.unassigned.bam
  └─ m84001_221201_123456_s1.hifi_reads.unassigned.bam.pbi
```

```
└─ metadata
└─ pb_formats
└─ statistics
```

Many expanded repeat loci, especially in GA repeats and heavily methylated targets, generate single-pass reads

- **fail\_reads.bam contents now expanded to include single-pass subreads<sup>1</sup>**
- This allows retaining coverage information from difficult-to-sequence regions like GA-rich loci and some long tandem repeat regions

## Additional information included in Revio system v13 fail\_reads.bam file:

- **Subreads that have at least one full pass but do not produce a consensus read<sup>1</sup>**
  - Note: Included subreads do not contain kinetics information
- **Control reads** – reads that match the known (spike-in) DNA internal control sequence (previously, sequencing control reads were not included in output BAM files.)
  - Enables examination of which specific reads were classified as control reads



# Revio system v13 output files (cont.)

Updated fail\_reads.bam file contents to include ff ("fail flag") bitwise BAM tag

## Revio system data output file structure

r84001\_20220722\_134701

└─ 1 A01

```
└─ fail_reads
  ├── m84001_221201_123456_s1.fail_reads.bc2001.bam
  ├── m84001_221201_123456_s1.fail_reads.bc2001.bam.pbi
  ├── m84001_221201_123456_s1.fail_reads.bc2002.bam
  ├── m84001_221201_123456_s1.fail_reads.bc2002.bam.pbi
  ├── m84001_221201_123456_s1.fail_reads.bc2003.bam
  ├── m84001_221201_123456_s1.fail_reads.bc2003.bam.pbi
  ├── m84001_221201_123456_s1.fail_reads.unassigned.bam
  └─ m84001_221201_123456_s1.fail_reads.unassigned.bam.pbi
```

```
└─ hifi_reads
  ├── m84001_221201_123456_s1.hifi_reads.bc2001.bam
  ├── m84001_221201_123456_s1.hifi_reads.bc2001.bam.pbi
  ├── m84001_221201_123456_s1.hifi_reads.bc2002.bam
  ├── m84001_221201_123456_s1.hifi_reads.bc2002.bam.pbi
  ├── m84001_221201_123456_s1.hifi_reads.bc2003.bam
  ├── m84001_221201_123456_s1.hifi_reads.bc2003.bam.pbi
  ├── m84001_221201_123456_s1.hifi_reads.unassigned.bam
  └─ m84001_221201_123456_s1.hifi_reads.unassigned.bam.pbi
```

```
└─ metadata
└─ pb_formats
└─ statistics
```

For increased clarity, a bitwise BAM tag (**ff for "fail flag"**) is now added to each read in the fail\_reads.bam file to indicate the reasons why it was filtered

Fail reason	Bit
For CCS reads with predicted accuracy below QV 20	0x1
For control CCS reads	0x2
For single-stranded CCS reads	0x4
For median full-length subread from molecules that do not produce a CCS read but have at least one full pass	0x8
For CCS reads which are a concatenation of the adapter, with possible short non-adapter sequences in between	0x10
For CCS reads with miscalled adapter which is enclosed by a sequence and its reverse complement, either spanning to the end	0x20
For CCS reads that have one or more adapters close to either end	0x40

# Revio system v13 instrument reliability & serviceability improvements

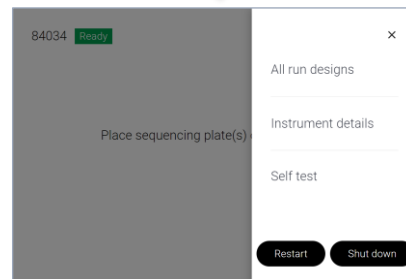
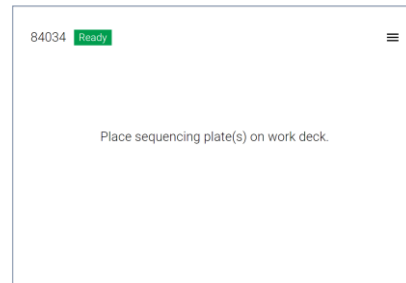
Updated Revio system v13 ICS enables improved instrument reliability and facilitates remote troubleshooting

Work deck camera

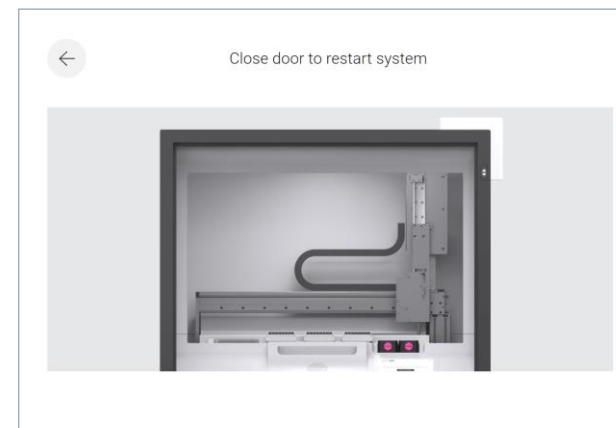


## Revio instrument control software v13 reliability updates

- Updated firmware on the Revio system for improved instrument robustness
- Enabled Revio work deck camera for remote troubleshooting with PacBio Technical Support
- Added Shut down and Restart options to work deck touchscreen user interface to enable users to perform a graceful instrument shutdown or re-boot
- Collect additional instrument and consumable performance metrics and logs to increase efficiency of instrument support.
  - New metrics cover SMRT Cell loading and compute utilization (GPU, CPU, RAM); new log files cover primary and post-primary analysis.

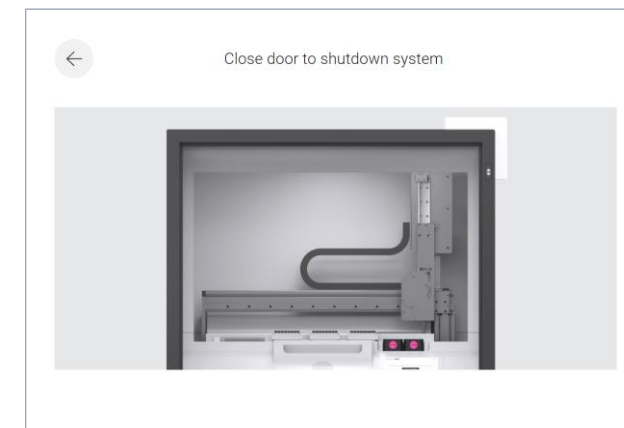


### Instrument restart



Restart + bootup takes ~10 min

### Instrument shutdown

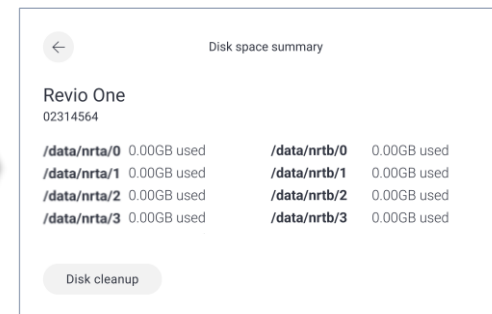
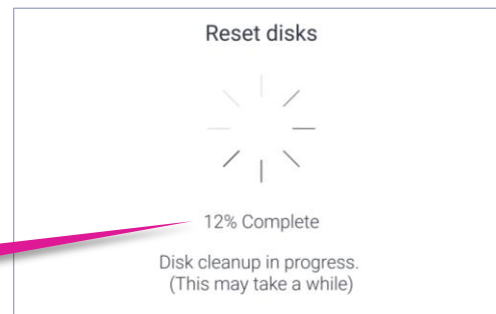
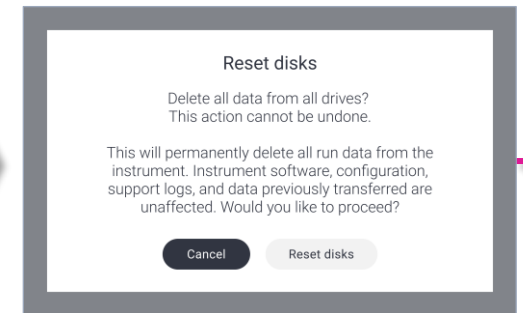
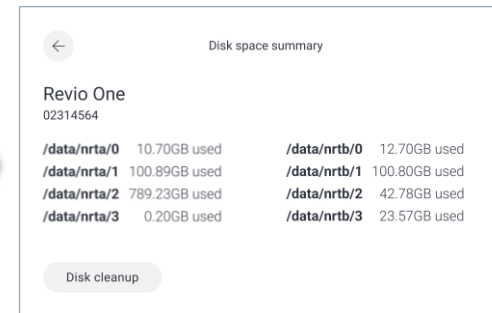
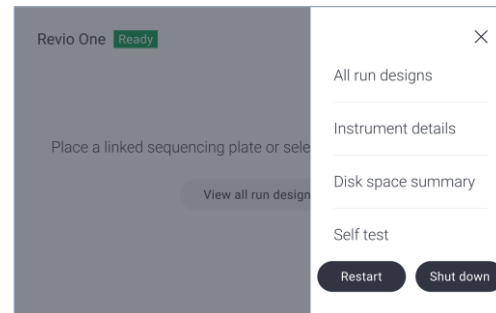


Shutdown takes ~1 min

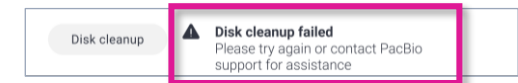
# Revio system v13 instrument reliability & serviceability improvements (cont.)

Added work deck touchscreen feature to enable users to manually clear instrument disk space

- Users can optionally use this touchscreen feature to **clear disk partitions to restore an instrument** if unwanted data files are left behind from a previous failed run
- We recommend that users contact PacBio Technical Support to review data **before** clearing as **deleting files cannot be undone**



OR



Disk cleanup process takes <1 minute



# Revio system v13 applications updates



# Revio system v13 supports short-insert library sequencing

Updated Revio ICS supports short amplicons and fragmented DNA libraries prepared with SMRTbell prep kit 3.0



SMRT Link v13

- Sample Setup
- Runs

Application type

- Targeted Sequencing
- HiFi Target Enrichment
- <3kb Amplicons**
- >=3kb Amplicons

Recommended target P1 loading range for Revio system is ~50 – 70%

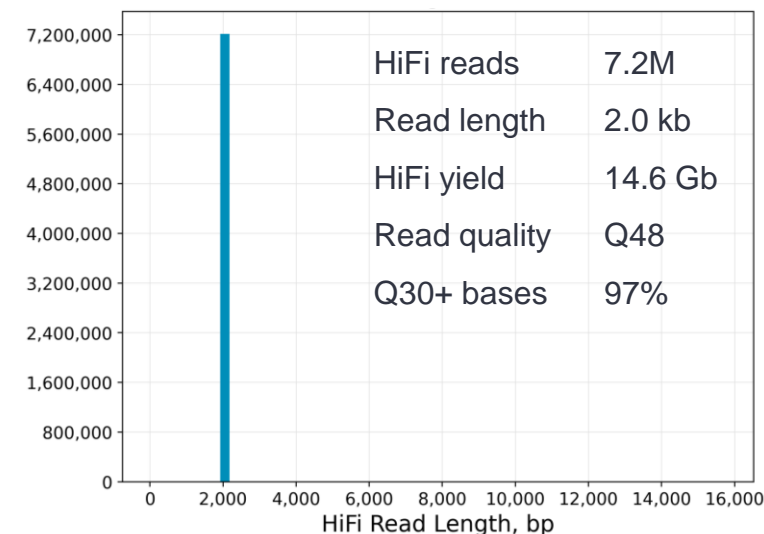
Run design sample information

Library Type <small>Required</small>	Standard
Insert Size (bp) <small>Required</small>	2000
Polymerase Kit <small>Required</small>	Revio polymerase kit
Movie Acquisition Time (hours)	12
Application	<3kb Amplicons

**IMPORTANT:** Specification of **Insert Size** is **required** for Revio run designs in SMRT Link v13  
 → For all applications, Revio system performs optimized instrument workflows based on entered library insert size value



2 kb amplicon library (SPK 3.0)



SMRT Link module	Key setup parameters for <3 kb Amplicons	Sequel II/Ile system recommended settings for <3 kb amplicon libraries	Revio system recommended settings for <3 kb amplicon libraries
Sample setup	Library type	Standard	
	Primer	Standard sequencing primer	
	Binding/Polymerase kit <sup>1</sup>	Sequel II binding kit 3.1	Revio polymerase kit
	Concentration on plate	40 – 60 pM	200 – 400 pM
Runs → Run design	Adapter / Library type	SMRTbell Adapter Design = Overhang – SMRTbell Prep Kit 3.0	Library type = Standard
	Movie collection time	10 hrs	12 hrs
	Use adaptive loading	NO	YES
	On-instrument CCS	Detect and Resolve Heteroduplex Reads = YES	Consensus Mode = MOLECULE

# Revio system v13 supports adeno-associated virus (AAV) sequencing

Full workflow support for Adeno-associated Virus (AAV) libraries prepared using SMRTbell prep kit 3.0

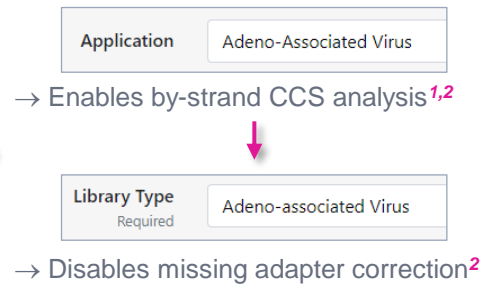


## New 'AAV' Application & Library Type options in Revio Run Design

Specifies primary analysis settings appropriate for AAV samples

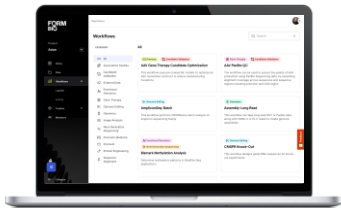


SMRT Link v13

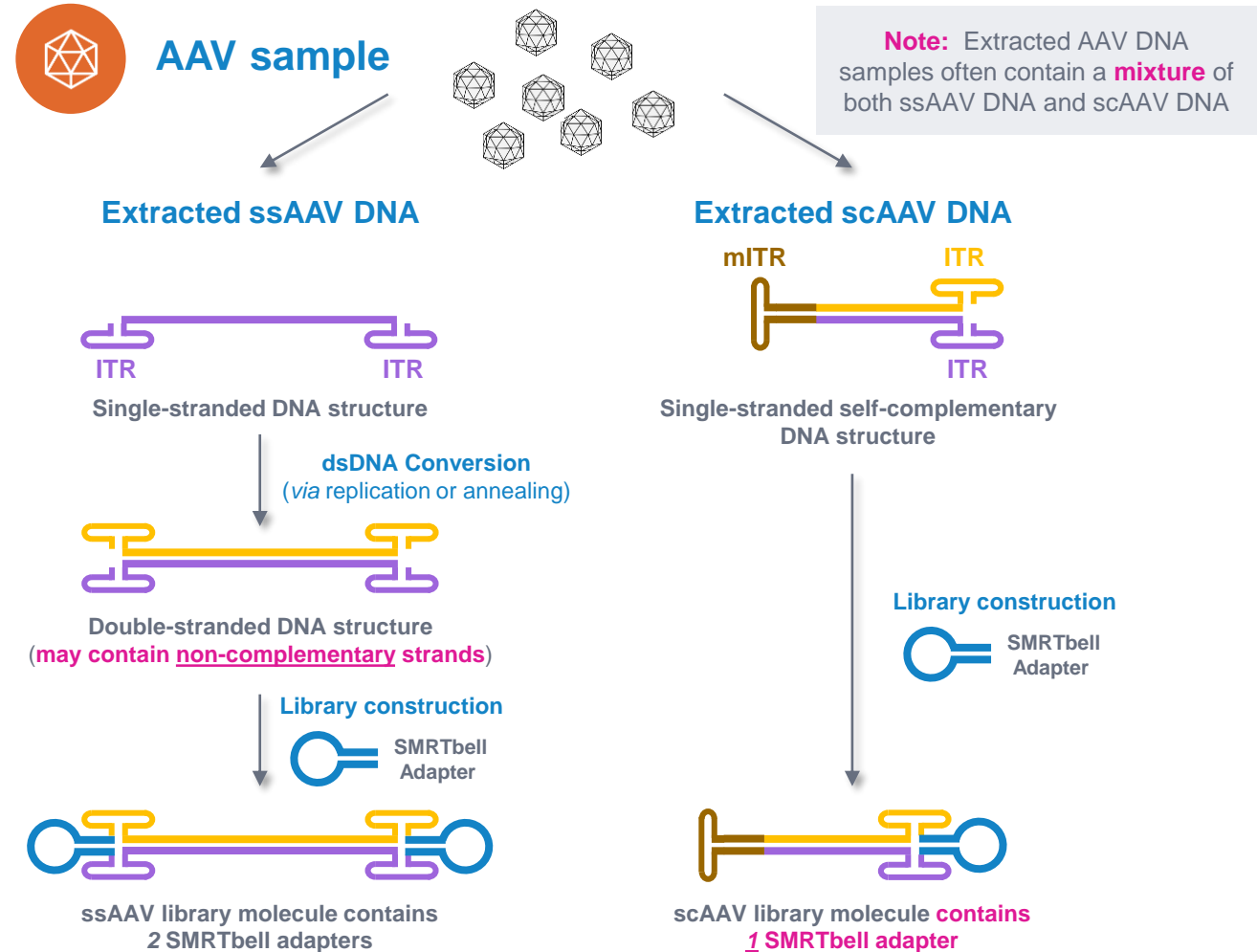


## Form Bio provides an all-in-one AAV secondary analysis solution

Platform + expertise to generate a report on the mix of full and partial ssAAV and scAAV molecules in a sample



FORM BIO



<sup>1</sup> On Sequel II/IIe systems, post-primary analysis pipeline for AAV samples uses heteroduplex calling (where sometimes one CCS read is output per molecule and sometimes one CCS read is output per strand). On Revio system, AAV post-primary analysis pipeline uses explicit by-strand CCS analysis (i.e., always get one CCS read per strand).

<sup>2</sup> **IMPORTANT:** For AAV samples, specify Application Type = AAV and Library Type = AAV in SMRT Link run designs. Modified adapter calling during post-primary analysis is needed to enable correct annotation of full-pass subreads within library molecules containing only 1 SMRTbell adapter → Required for analysis of scAAV libraries.

# Revio system v13 supports adeno-associated virus (AAV) sequencing (cont.)

## Sample Setup & Run Design recommendations for AAV libraries



SMRT Link v13

Sample Setup

Runs

Application type

Viral Sequencing

Adeno-Associated Virus

Recommended target P1 loading range for Revio system is ~50 – 70%

Run design sample information

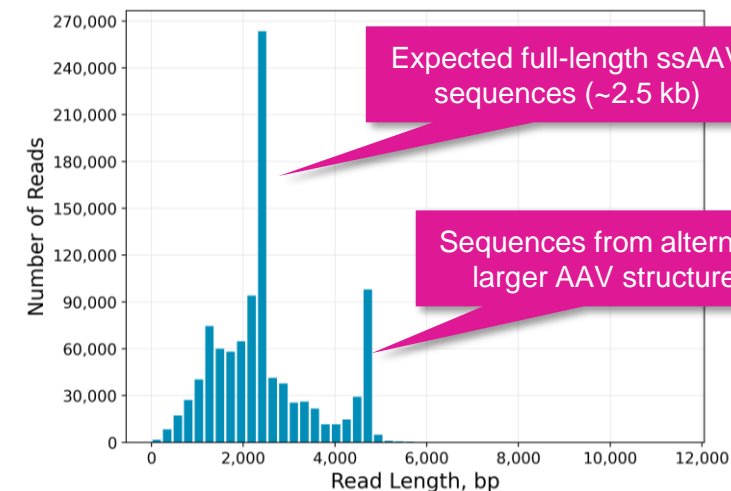
Library Type <small>Required</small>	Adeno-associated Virus
Insert Size (bp) <small>Required</small>	
Polymerase Kit <small>Required</small>	Revio polymerase kit
Movie Acquisition Time (hours)	24
Application	Adeno-Associated Virus

**IMPORTANT:** For AAV samples, specify **Application Type = AAV** and **Library Type = AAV** in Revio run designs in SMRT Link v13

- Enables modified adapter calling required for correct annotation of AAV libraries
- Enables correct on-instrument CCS analysis mode required for AAV



ssAAV Library  
(SPK 3.0)



<sup>1</sup> See *Application brochure — AAV Sequencing with HiFi Reads (102-326-502)*.

SMRT Link module	Key setup parameters for AAV	Sequel II/Ile system recommended settings For AAV libraries	Revio system recommended settings for AAV libraries
Sample setup	Library type	Standard <sup>1</sup>	
	Primer	Standard sequencing primer	
	Binding/Polymerase kit <sup>1</sup>	Sequel II binding kit 3.1	Revio polymerase kit
	Concentration on plate	40 – 150 pM	200 – 400 pM
Runs → Run design	Adapter / Library type	SMRTbell Adapter Design = Overhang – SMRTbell Prep Kit 3.0	Library type = Adeno-Associated Virus
	Movie collection time	24 hrs	
	Use adaptive loading	NO	YES
	On-instrument CCS	Detect and Resolve Heteroduplex Reads = YES	Consensus Mode = STRAND

<sup>1</sup> **Note:** No 'AAV' library type is available in SMRT Link Sample Setup → Specify 'Standard' library type for AAV samples in SMRT Link Sample Setup.

# Revio system v13 supports full-length RNA sequencing (non-Kinnex libraries)

Full workflow support for full-length RNA libraries prepared using SMRTbell prep kit 3.0



SMRT Link v13

- Sample Setup
- Runs



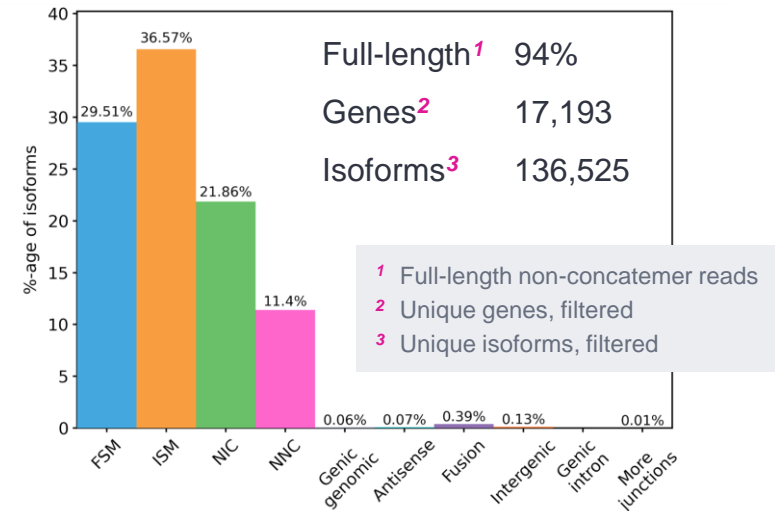
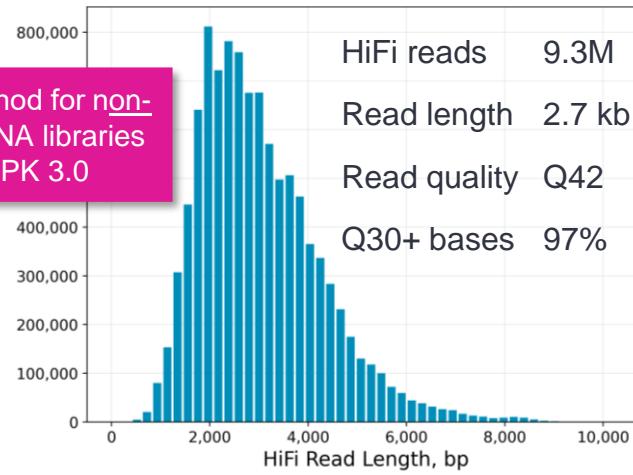
Example sequencing & analysis results<sup>1</sup> for (non-Kinnex) universal human reference RNA (UHRR) full-length RNA library prepared with SPK 3.0

Application type

- RNA Sequencing
  - Iso-Seq Method**
  - MAS-Seq single cell
  - Kinnex single-cell RNA
  - Kinnex full-length RNA

Select 'Iso-Seq' method for non-Kinnex full-length RNA libraries prepared with SPK 3.0

Recommended target P1 loading range for Revio system is **-50 – 70%**



SMRT Link module	Key setup parameters for Iso-Seq Method	Sequel II/Ile system recommended settings for (non-Kinnex) full-length RNA libraries	Revio system recommended settings for (non-Kinnex) full-length RNA libraries
Sample setup	Library type	Standard	
	Primer	Standard sequencing primer	
	Binding/Polymerase kit <sup>1</sup>	Sequel II binding kit 3.1	Revio polymerase kit
	Concentration on plate	40 – 80 pM	200 – 400 pM
Runs → Run design	Adapter / Library type	SMRTbell Adapter Design = Overhang – SMRTbell Prep Kit 3.0	Library type = Standard
	Movie collection time	24 hrs	
	Use adaptive loading	NO	YES
	On-instrument CCS	CCS Analysis Output - Include Low Quality Reads = NO CCS Analysis Output - Include Kinetics Information = NO	Consensus Mode = MOLECULE

# Revio system v13 supports Kinnex full-length RNA, single-cell RNA, and 16S rRNA sequencing

Full workflow support for Kinnex libraries prepared using Kinnex library prep kits



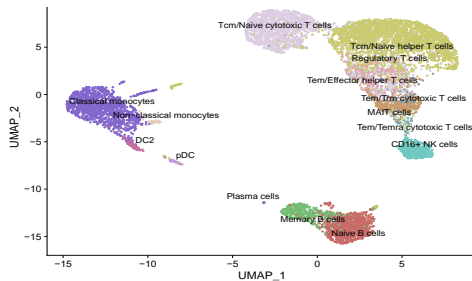
## Kinnex single-cell RNA kit



Single-cell RNA sequencing  
[(10x Chromium Single Cell 3' or 5' cDNA input)]

Up to 4-plex Kinnex library  
[16-fold concatenation]

80M reads (Revio SMRT Cell)  
[40M reads (Sequel II/Ile SMRT Cell 8M)]



Identify cell type-specific isoform expression



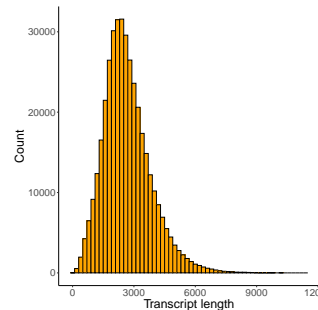
## Kinnex full-length RNA kit



Full-length RNA sequencing

Up to 48-plex Kinnex library  
[8-fold concatenation]

40M reads (Revio SMRT Cell)  
[15M reads (Sequel II/Ile SMRT Cell)]



Full-length transcripts from 1–10 kb for bulk RNA samples



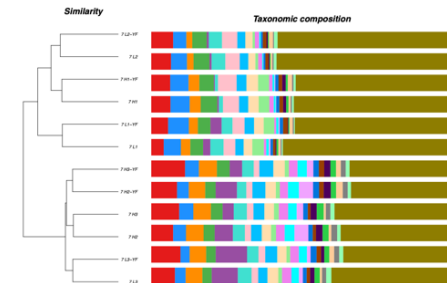
## Kinnex 16S rRNA kit



Full-length 16S rRNA for species identification

Up to 1,536-plex Kinnex library  
[12-fold concatenation]

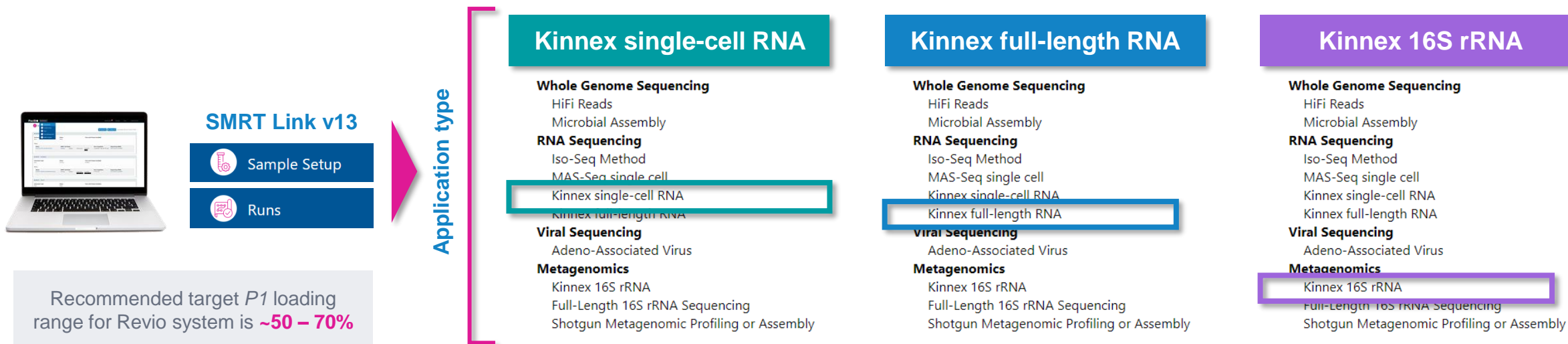
60M (Revio SMRT Cell)  
[25M reads (Sequel II/Ile SMRT Cell)]



Taxonomic profiling for human, animal, and environmental samples

# Revio system v13 supports Kinnex full-length RNA, single-cell RNA, and 16S rRNA sequencing (cont.)

## Sample Setup & Run Design recommendations for Kinnex libraries



SMRT Link module	Key setup parameters For Kinnex libraries	Sequel II/IIe system recommended settings for Kinnex libraries	Revio system recommended settings for Kinnex libraries
Sample setup	Library type	Kinnex	
	Primer	Kinnex sequencing primer	
	Binding/Polymerase kit <sup>1</sup>	Sequel II binding kit 3.2 (includes Kinnex sequencing primer)	Revio polymerase kit (includes Kinnex sequencing primer)
	Concentration on plate	40 – 60 pM	100 – 150 pM
Runs → Run design	Adapter / Library type	SMRTbell Adapter Design = SMRTbell Kinnex Prep Kit	Library type = Kinnex
	Movie collection time	30 hrs	24 hrs
	Use adaptive loading	YES	
	On-instrument CCS	CCS Analysis Output - Include Low Quality Reads = NO CCS Analysis Output - Include Kinetics Information = NO	Consensus Mode = MOLECULE



# New HiFi Target Enrichment application option in SMRT Link v13

Sample Setup recommendations for HiFi target enrichment libraries – Sequel II/Ile and Revio systems

Select desired application type in SMRT Link Sample Setup module



Sample Setup

- Whole Genome Sequencing
  - HiFi Reads
  - Microbial Assembly
- RNA Sequencing
  - Iso-Seq Method
  - MAS-Seq single cell
  - Kinnex single-cell RNA
  - Kinnex full-length RNA
- Viral Sequencing
  - Adeno-Associated Virus
- Metagenomics
  - Kinnex 16S rRNA
  - Full-Length 16S rRNA Sequencing
  - Shotgun Metagenomic Profiling or Assembly
- Targeted Sequencing
  - <3kb Amplicons
  - >=3kb Amplicons
  - HiFi Target Enrichment
- Other

## Sequel II/Ile systems

< Sample group >	
Name	HiFi_Targ_Enrichment_Lib_Demo
Application	HiFi Target Enrichment
Library type	Standard
Polymerase / Binding kit	Sequel II Binding Kit 3.2
Number of samples	1 samples
SMRT Cells per sample	1 cells
Available volume per sample	15 uL
Insert size	5000 bp
Sample concentration	7.5 ng/uL
Cleanup anticipated yield	75 %
Concentration on plate	90 pM Recommended: 85-100 pM
Minimum pipetting volume	1 uL

## Revio system

< Sample group >	
Name	HiFi_Targ_Enrichment_Lib_Demo
Application	HiFi Target Enrichment
Library type	Standard
Polymerase / Binding kit	Revio polymerase kit
Number of samples	1 samples
SMRT Cells per sample	1 cells
Available volume per sample	15 uL
Insert size	5000 bp
Sample concentration	7.5 ng/uL
Cleanup anticipated yield	75 %
Concentration on plate	250 pM Recommended: 200-300 pM
Minimum pipetting volume	1 uL

After specifying your application type, SMRT Link auto-fills selected Sample Setup & Run Design parameter fields with default recommended values

SMRT Link module	Key setup parameters for HiFi Targ. Enrich.	Sequel II/Ile system recommended settings for HiFi Target Enrichment libraries	Revio system recommended settings for HiFi Target Enrichment libraries
Sample setup	Library type	Standard	
	Primer	Standard sequencing primer	
	Binding/Polymerase kit <sup>1</sup>	Sequel II binding kit 3.2	Revio polymerase kit
	Concentration on plate	85 – 100 pM	200 – 300 pM

# New HiFi Target Enrichment application type option SMRT Link v13 (cont.)

Run Design recommendations for HiFi target enrichment libraries – Revio system

Select desired application type in SMRT Link Run Design page



Runs

## Whole Genome Sequencing

- HiFi Reads
- Microbial Assembly

## RNA Sequencing

- Iso-Seq Method
- MAS-Seq single cell
- Kinnex single-cell RNA
- Kinnex full-length RNA

## Viral Sequencing

- Adeno-Associated Virus

## Metagenomics

- Kinnex 16S rRNA
- Full-Length 16S rRNA Sequencing
- Shotgun Metagenomic Profiling or Assembly

## Targeted Sequencing

- <3kb Amplicons
- >=3kb Amplicons

- HiFi Target Enrichment

## Other

After specifying your application type, **SMRT Link auto-fills** selected Sample Setup & Run Design parameter fields with default recommended values

## Sample and run information

Plate Well Required Plate 1, Well A01

Well Name Required HiFi\_Targ\_Enrichment\_Lib\_Demo

Well Comment

Library Type Required Standard

Insert Size (bp) Required 5000

Polymerase Kit Required Revio polymerase kit

Movie Acquisition Time (hours) 24

Application HiFi Target Enrichment

## Indexing (barcoding) information

Sample is indexed  YES  NO

Indexes Required Twist Universal Adapters with UDI

Same Barcodes on Both Ends of Sequence  YES  NO

Biosample names Required Interactively From a File

## Run options and data options

Library Concentration (pM) Required 250

Use Adaptive Loading  YES  NO

Include Base Kinetics  YES  NO

Consensus Mode  MOLECULE  STRAND

Assign Data To Project Required General Project

## Analysis options

Add Analysis  YES  NO

Analysis Name Required Demo\_Analysis\_Job\_Name

Select Analysis Workflow Required HiFi Target Enrichment

Reference Set Required Human Genome hg38, with Gencode v39 annotations

Target BED file Required

**Library Type** identifies structure of molecules to be sequenced, which determines how instrument performs adapter calling and consensus read generation

SMRT Link module	Key setup parameters for HiFi Targ. Enrich	Revio system recommended settings for HiFi Target Enrichment libraries
Runs → Run design	Library type	Standard
	Movie collection time	24 hrs
	Use adaptive loading	YES
	On-instrument CCS	Consensus Mode = MOLECULE

# New HiFi Target Enrichment application type option SMRT Link v13 (cont.)

Run Design recommendations for HiFi target enrichment libraries – Sequel II and IIS systems

Select desired application type in SMRT Link Run Design page



Runs

- Whole Genome Sequencing
  - HiFi Reads
  - Microbial Assembly
- RNA Sequencing
  - Iso-Seq Method
  - MAS-Seq single cell
  - Kinnex single-cell RNA
  - Kinnex full-length RNA
- Viral Sequencing
  - Adeno-Associated Virus
- Metagenomics
  - Kinnex 16S rRNA
  - Full-Length 16S rRNA Sequencing
  - Shotgun Metagenomic Profiling or Assembly
- Targeted Sequencing
  - <3kb Amplicons
  - >=3kb Amplicons
  - HiFi Target Enrichment
- Other

After specifying your application type, SMRT Link auto-fills selected Sample Setup & Run Design parameter fields with default recommended values

## Sample and run information

Application: HiFi Target Enrichment

Well Sample Name: HiFi\_Targ\_Enrichment\_Lib\_Demo

Sample Well: A01

SMRTbell Adapter Design: Overhang - SMRTbell Prep Kit 3.0

Sequencing Kit: Sequel II Sequencing Plate 2.0 (4 rxn)

Sequencing Kit: Sequel II Binding Kit 3.2

DNA Control Complex: Sequel II DNA Internal Control Complex 3.2

Insert Size (bp):

Recommended Concentration on Plate (pM): 85-100 pM

On-Plate Loading Concentration (pM): 90

Movie Time per SMRT Cell (hours): 24

Use Pre-Extension: YES

Pre-Extension Time (hours): 2

Detect and Resolve Heteroduplex Reads: YES

SMRTbell Adapter Design determines which adapter finding algorithm is used during post-primary analysis

## Barcoded sample options

Sample Is Barcoded: YES

Barcode Set: Twist Universal Adapters with UDI

Same Barcodes on Both Ends of Sequence: NO

Assign Bio Sample Names to Barcodes: Interactively

Demultiplex Barcodes: ON INSTRUMENT

## Advanced options

Use Adaptive Loading: YES

Loading Target (P1 + P2): 0.85

Maximum Loading Time (hours): 2

CCS Analysis Output - Include Low Quality Reads: NO

CCS Analysis Output - Include Kinetics Information: NO

Add Data to Project: General Project

## Analysis options

Add Analysis: YES

Analysis Name: Demo\_Analysis\_Job\_Name

Select Analysis Workflow: HiFi Target Enrichment

Reference Set: Human Genome hg38, with Gencode v39 annotations

Target BED file:

SMRT Link module	Key setup parameters For HiFi Targ. Enrich.	Sequel II/IIS system recommended settings for HiFi Target Enrichment libraries
	SMRTbell adapter design	Overhang – SMRTbell Prep Kit 3.0
	Movie collection time	24 hrs
	Use adaptive loading	YES
Runs → Run design	On-instrument CCS	CCS Analysis Output - Include Low Quality Reads = NO CCS Analysis Output - Include Kinetics Information = NO



# Revio system v13 summary

# Revio v13 extends the core capabilities of the Revio system

## Revio system specifications

Library	Run time <sup>1</sup>	Q30+ bases	HiFi yield per SMRT Cell <sup>2</sup>	Methylation
1-5 kb	12 hours	95%	6M reads	5mC at CpG sites for native DNA
5-10 kb			30 Gb	
10-15 kb	24 hours	90%	60 Gb	
15-20 kb			90 Gb	
20-25 kb	30 hours	85%	90 Gb	

## Common applications and sample throughput

Library	Sample	Per Revio SMRT Cell	Per year	Expected coverage
1-5 kb	Amplicon	>1,000	>2.6 million	50X
5-10 kb	Microbial genome	96	124,800	50X
15-20 kb	Human genome	1	1,300	30X
15-20 kb	Transcriptome w/ Kinnex	4	5,200	10M reads





# SMRT Link v13 Instruments module updates



# SMRT Link v13 Instruments module user interface improvements

Instruments module user interface is updated for increased clarity of instrument & run status information

## SMRT Link v12.0

84034 RUNNING

<b>Instrument Type</b> Revio	<b>Status</b> Running	<b>Time until Preload Available</b> 5h 23m
---------------------------------	--------------------------	---

---

**Runs**

Name	SMRT Cell Status				Run Completion	Sequencing ZMWs
20221229_84034_24hPE_11K-unclean	Pending ■ ■	Loading ■ ■	Sequencing	Complete	In 1 day and 6h	<a href="#">View Sequencing ZMWs</a>

'Time until preload is available' is updated to show 'Time until door unlocks' to more accurately reflect the information relayed by the countdown timer<sup>1</sup>

## SMRT Link v13

84036 RUNNING

<b>Instrument Type</b> Revio	<b>Status</b> Running	<b>Time until door unlocks</b> 4h 40m
---------------------------------	--------------------------	--

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**Runs**

Name	SMRT Cell Status				Run Completion	
20231113_5pHG2_InputTitr	Pending ■	Loading ■ ■	Sequencing	Complete	In 1 day and 5h	

'View Sequencing ZMWs' hyperlink has been **deprecated** and replaced by new **Run Preview** feature

# SMRT Link v13 Instruments module user interface improvements (cont.)

New Run Preview feature provides early information on sample loading performance

## SMRT Link v12.0

84032 READY

Instrument Type	Status	Time until Preload Available
Revo	Ready	-

Runs

Name	SMRT Cell Status	Run Completion	Sequencing ZMWs
20230922_UCI-91-92-93-94-95-96-97-98	Pending Loading Sequencing Complete	-	View Sequencing ZMWs

## SMRT Link v13

84010 RUNNING

Instrument Type	Status	Time until door unlocks
Revo	Running	Available

Runs

Name	SMRT Cell Status	Run Completion
2023_1107_84010_108pM_11k_6hr_noPE_1pC	Pending Loading Sequencing Complete	In 4h 39m

Access run preview by clicking on any SMRT Cell icon in 'Sequencing' or 'Completed' status → Run Preview metrics will be displayed for ALL cells in the run



## Run Preview – Primary sequencing metrics

Latest: 29.8% Max: 37.6%



### Preview at 4 hrs (estimates)

P1%	63%
HiFi read length, mean	13.0 kb

### Preview at 23 hrs (estimates)

HiFi yield	91.3 Gb
HiFi read length, mean	17.3 kb
HiFi read quality, median	32

Run Preview info is **approximate** and intended to guide future runs by providing early information on sample loading and library size



# SMRT Link v13 Sample Setup module updates

# SMRT Link v13 Sample Setup user interface updates

Updated Sample Setup user interface is simplified and enables a more streamlined calculation procedure

## SMRT Link v12.0

Sample Setup

Version:  Classic  High-Throughput

+ Add Calculation Edit/Print Import Export Delete

<input type="checkbox"/>	Name <i>IT</i>	Date Created <i>↓</i>	Number of S... <i>IT</i>	SMRT Cells per ... <i>IT</i>	Created By <i>IT</i>	Lo... <i>IT</i>	Comment
<input type="checkbox"/>	My Batch of Samples	2023-11-13, 01:32:55 PM	N/A		skalla	false	
<input type="checkbox"/>	My Batch of Samples	2023-11-13, 11:36:17 AM	1	1	skalla	false	
<input type="checkbox"/>	My Batch of Samples	2023-11-08, 12:04:03 AM	1	1	smark	false	
<input type="checkbox"/>	UNAM-4146	2023-11-07, 04:50:46 PM	1	1	anpham	false	
<input type="checkbox"/>	BCH-62	2023-11-06, 01:48:24 PM	1	3	anpham		

In Sample Setup v13, click Add Calculation to specify binding kit type:

- Sequel II binding kit 2.1/2.2 = "Classic mode"
- Sequel II binding kit 3.1/3.2, Revio pol kit = "High Throughput Mode"

## SMRT Link v13

Sample Setup

+ Add Calculation *▼* Edit Import Export

Sequel II binding kit 2.1/2.2  
✓ Sequel II binding kit 3.1/3.2, Revio polymerase kit

<input type="checkbox"/>	Sample name <i>IT</i>	Date created <i>↓</i>	Created by <i>IT</i>	Polymerase / Binding kit <i>IT</i>	Comment <i>IT</i>
<input type="checkbox"/>	My Batch of Samples	2023-11-11, 11:31:20 PM	awenger	Revio polymerase kit	
<input type="checkbox"/>	My Batch of Samples	2023-11-11, 11:30:23 PM	awenger	Revio polymerase kit	
<input type="checkbox"/>	Sample 1	2023-11-08, 04:34:52 PM	jliou	Sequel® II Binding Kit 2.2	
<input type="checkbox"/>	ExptFQdev_004	2023-11-08, 03:39:40 PM	labtech	Sequel® II Binding Kit 3.2	
<input type="checkbox"/>	My Batch of Samples	2023-11-07, 04:27:09 PM	awenger	Revio polymerase kit	

Deprecated Sample Setup Version field and enabled selection of calculation type in 'Add Calculation' button

- Simplified naming to use consumable type rather than "Classic" and "High Throughput"

All Sample Setup calculations are displayed in a single table instead of displaying "Classic" and "High-throughput" calculations in separate tables

# SMRT Link v13 Sample Setup user interface updates (cont.)

Updated Sample Setup user interface is simplified and enables a more streamlined calculation procedure

The image displays three sequential screenshots of the 'Sample group' setup interface. The first screenshot shows the 'Name' field filled with 'My Batch of Samples' and the 'Application\*' field as an empty dropdown menu. The second screenshot shows the 'Application\*' dropdown menu open, displaying a list of sequencing options such as 'Whole Genome Sequencing', 'RNA Sequencing', and 'Viral Sequencing'. The third screenshot shows the 'Application' field set to 'HiFi Reads', which has triggered the appearance of additional fields: 'Library type' (set to 'Standard'), 'Polymerase / Binding kit' (set to 'Revio polymerase kit'), 'Number of samples\*' (input field), 'SMRT Cells per sample\*' (input field), 'Available volume per sample\*' (input field), 'Insert size\*' (input field), 'Sample concentration\*' (input field), 'Cleanup anticipated yield' (set to 75%), 'Concentration on plate\*' (input field), and 'Minimum pipetting volume' (input field). A 'Comment' field is also visible at the bottom.

**Only Name, Application, and Polymerase / Binding kit fields appear at first**  
→ Other Sample Setup fields will appear once Application type is selected from drop-down menu

**New Library Type field specifies the structure of the SMRTbell library molecule**

# SMRT Link v13 Sample Setup user interface updates (cont.)

New Sample Setup Library Type field determines which type of sequencing primer is used for annealing

< Sample group >	
	Copy Remove Lock Download CSV
Name	My Batch of Samples
Application	HiFi Reads
Library type	Standard
Polymerase / Binding kit	Revo polymerase kit
Number of samples*	samples

**IMPORTANT:** Sample Setup **Library type** field specifies structure of SMRTbell library and determines **sequencing primer type** to use for annealing step<sup>1</sup>

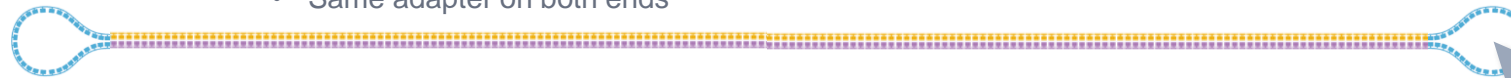
Library type	Standard
Polymerase / Binding kit	Kinnex

**For non-Kinnex libraries:**  
→ Specify Library type = Standard

**For Kinnex libraries:**  
→ Specify Library type = Kinnex

## Standard SMRTbell library type

- Contains standard SPK3 (barcoded or non-barcoded) terminal adapters
- Standard sequencing primer
- Same adapter on both ends



Forward and reverse Kinnex terminal SMRTbell adapters have **different structures** and require use of **Kinnex sequencing primer** for primer annealing step

## Kinnex SMRTbell library type

- Contains Kinnex terminal adapters
- Kinnex sequencing primer
- Different adapters on each end

Forward and reverse standard terminal SMRTbell adapters have **the same structure** and require use of **Standard sequencing primer** for primer annealing step





# SMRT Link v13 Sample Setup user interface updates (cont.)

Sample Setup instructions now display in a format that improves overall clarity and readability when performing annealing / binding / complex cleanup (ABC) steps

### Sample Setup Instructions for Loading

SMRT Link: 13.0.0.206978; Chemistry Bundle: 13.0.0.205983; Params: 13.0.0

#### General best practices

Eppendorf Lo-bind tubes (Eppendorf 022431021) are recommended for this protocol, but PCR tube strips (USA Scientific TempAssure 1402-4708) or 0.2 mL 96-well PCR plates are also acceptable. No difference in performance is expected across Lo-bind tubes, PCR tube strips, or plates.

To pipette-mix, gently pipette up and down until the solution appears homogenous, typically 10 times.

To prepare for the steps below, thaw the **Loading buffer** at **room temperature** and equilibrate the **SMRTbell cleanup beads** to **room temperature**. Store all other reagents on ice unless otherwise noted.

Mix reagent buffers with a brief vortex prior to use. Do not vortex enzymes.

Quick-spin all reagents in a microcentrifuge to collect liquid at bottom prior to use.

If multiplexing, it is recommended to pool SMRTbell libraries prior to Sample Setup. If pooling after Sample Setup, pool samples in equimolar ratios. Only pool samples that were bound with the same sequencing polymerase.

#### 1. Annealing Sequencing primer

✓	Step	Instructions																				
		Add the following components in the order and volume listed to a new Lo-bind tube.																				
		<table border="1"><thead><tr><th>✓</th><th>Tube</th><th># of Samples in Batch</th><th>My Batch of Samples</th></tr></thead><tbody><tr><td>1.1</td><td></td><td>Sample</td><td>8.5 uL</td></tr><tr><td></td><td>light blue</td><td>Annealing buffer</td><td>4.3 uL</td></tr><tr><td></td><td>light green</td><td>Standard sequencing primer</td><td>4.3 uL</td></tr><tr><td></td><td></td><td><b>Total volume</b></td><td><b>17.0 uL</b></td></tr></tbody></table>	✓	Tube	# of Samples in Batch	My Batch of Samples	1.1		Sample	8.5 uL		light blue	Annealing buffer	4.3 uL		light green	Standard sequencing primer	4.3 uL			<b>Total volume</b>	<b>17.0 uL</b>
✓	Tube	# of Samples in Batch	My Batch of Samples																			
1.1		Sample	8.5 uL																			
	light blue	Annealing buffer	4.3 uL																			
	light green	Standard sequencing primer	4.3 uL																			
		<b>Total volume</b>	<b>17.0 uL</b>																			
	1.2	Pipette-mix each sample.																				
	1.3	Incubate at <b>room temperature</b> for <b>15</b> minutes.																				
	1.4	Proceed to the next step of the protocol.																				

## User interface improvements

- Added **General best practices** section
- Generated ABC protocol Protocols now display in a format that **matches PacBio library preparation protocols**
- Shows **numbered steps**
- Shows **tube cap colors**
- Improved wording consistency and removed notes section

## ABC calculation updates

- Increased resolution of required Sample volume value to nearest 0.5  $\mu\text{L}$  instead of 1  $\mu\text{L}$
- Enables finer control over final on-plate loading concentration (OPLC)



# SMRT Link v13 Runs module updates

# SMRT Link v13 Run Design user interface updates – Run information panel

Updated Run Information panel<sup>1</sup> for Revio run designs supports new Revio sequencing plate – 1 rxn product

Run Information

Instrument Type

Sequel II  
 Sequel IIe  Revio

Run Name

Run 11.15.2023 09:07

Plate 1 Required ⓘ

Revio sequencing plate  
Revio sequencing plate - 1 rxn

Plate 2 ⓘ

Lot Serial Expiry

Run Comments

Transfer Subdirectory ⓘ

**Revio sequencing plate – 1 rxn (102-587-500)** contains sufficient reagents to support 1 Revio SMRT Cell

→ Main intended use is to enable more flexible customer FOC reimbursement options when only 1 SMRT Cell needs to be reimbursed instead of 4

Select **Revio sequencing plate – 1 rxn** in Plate field drop-down menu



**Revio sequencing plate – 1 rxn (102-587-500)**

- Contains reagents for sequencing 1 Revio SMRT Cell on the Revio system
- Includes foil seals to prevent sample evaporation

**Run Comments** entered into run designs are now searchable in the Runs module main table

**Transfer Subdirectory** formerly called "Experiment ID"

- Specifies a subdirectory within the transfer location<sup>2</sup>

PacBio Runs

Notification 49 Settings Help smark (Lab Tech)

Runs

Import Run Create New Run Export Selected

Displaying rows 1 to 13 out of 241 Search ...

Run Name II	Run St... II	Instrument ... II	Instrument Type II	Create... II	Comments II	Date Created II	Date Completed II	Samples II
Q20231121_5pHG2...	Running	84198	Revio	jzhou		2023-11-21, 02:48:38 PM		8
Q20231121_Sharan...	Running	84198	Revio	jzhou	Aug input	2023-11-21, 02:47:50 PM		8
Q20231120_5pHG2...	Complete	84198	Revio	jzhou		2023-11-20, 05:54:31 PM	2023-11-22, 06:10:54 AM	16
Q20231120_AS_840...	Complete	84036	Revio	asoupe		2023-11-20, 04:46:31 PM	2023-11-22, 05:19:51 AM	8

<sup>1</sup> Note: **Experiment Name** field has been **removed** from Run Design GUI and CSV → will be ignored if included in Run Design CSV (no error thrown and won't be used anywhere).

<sup>2</sup> Run files are transferred to <TransferRoot>/<ExperimentID>/<RunDirectory> instead of <TransferRoot>/<RunDirectory>.

# SMRT Link v13 Run Design user interface updates – Sample & run info (cont.)

Updated sample & run info fields to enable support for additional insert size, movie time, and application type options for **Revio system**

▼ Plate 1, Well A01: Demo\_Library\_Sample\_1 Copy Delete

Plate Well Required Plate 1, Well A01

Well Name Required Demo\_Library\_Sample\_1

Well Comment

Library Type Required Standard

Insert Size (bp) Required 18000

Polymerase Kit Required Revio polymerase kit

Movie Acquisition Time (hours) 24

Application Unspecified

- 24
- 12
- 24
- 30

**Movie acquisition time**

- Can be optionally set to **12, 24 or 30 hrs<sup>1</sup>** for increased flexibility when running different library insert sizes

Selecting **Kinnex** application type enables support for Kinnex library prep and sequencing reagents

- Other
- Unspecified
- Whole Genome Sequencing**
- HiFi Reads
- Microbial Assembly
- RNA Sequencing**
- Iso-Seq Method
- MAS-Seq single cell
- Kinnex single-cell RNA
- Kinnex full-length RNA
- Viral Sequencing**
- Adeno-Associated Virus
- Metagenomics**
- Kinnex 16S rRNA
- Full-Length 16S rRNA Sequencing
- Shotgun Metagenomic Profiling or Assembly
- Targeted Sequencing**
- HiFi Target Enrichment
- <3kb Amplicons
- >=3kb Amplicons
- Unspecified

**Application**

- Added **Kinnex, AAV, and HiFi Target Enrichment** application types
- After specifying application type, SMRT Link **auto-fills** selected run design parameter fields with default recommended values

**IMPORTANT:** Selecting **AAV** application type automatically sets correct default CCS analysis mode<sup>2</sup>

Selecting **HiFi Target Enrichment** application type automatically enables auto-analysis to be performed using SMRT Link HiFi Target Enrichment analysis workflow

**Insert Size**

- IMPORTANT:** Specification of **Insert Size** is **required** for Revio run designs in SMRT Link v13
- For all applications, Revio system performs optimized instrument workflows based on entered library insert size value

<sup>1</sup> Custom movie times are **unsupported** in SMRT Link v13 – an error will be displayed if a user attempts to upload a run design CSV specifying a movie time that is not 12, 24, or 30 hrs.  
<sup>2</sup> **Note:** AAV analysis requires run design to specify **Consensus Mode = Strand** to enable proper analysis of AAV data. If inadvertently not specified, the AAV sample must be re-run.

# SMRT Link v13 Run Design user interface updates – Sample & run info (cont.)

Updated sample & run info fields to enable support for additional application type options for Sequel II/IIe systems

SAMPLE 1: , A01, 30 hour movie

Copy Delete

Import from Sample Setup Select Sample

Application Required

Well Sample Name Required

Bio Sample Name Required

Sample Comment

Sample Well

SMRTbell Adapter Design Required

**Whole Genome Sequencing**  
HiFi Reads  
Microbial Assembly

**RNA Sequencing**  
Iso-Seq Method  
MAS-Seq single cell  
Kinnex single-cell RNA  
Kinnex full-length RNA

**Viral Sequencing**  
HiFiViral SARS-CoV-2  
Adeno-Associated Virus

**Meta-genomics**  
Kinnex 16S rRNA  
Full-Length 16S rRNA Sequencing  
Shotgun Metagenomic Profiling or Assembly

**Targeted Sequencing**  
HiFi Target Enrichment  
<3kb Amplicons  
>=3kb Amplicons

Selecting **Kinnex** application type enables support for Kinnex library prep and sequencing reagents

## Application

- Added **Kinnex** and **HiFi Target Enrichment** application types
- After specifying application type, SMRT Link **auto-fills** selected run design parameter fields with default recommended values

Selecting **HiFi Target Enrichment** application type automatically enables auto-analysis to be performed using SMRT Link HiFi Target Enrichment analysis workflow

**IMPORTANT:** AAV application type specification in Run Design is required for proper analysis of AAV libraries<sup>1</sup>

→ If inadvertently not specified, the AAV sample must be re-run

<sup>1</sup> **IMPORTANT:** For AAV libraries, select **Adeno-Associated Virus** from the **Application** field drop-down menu in SMRT Link Run Design. Modified adapter calling + heteroduplex detection (HD) are automatically enabled by default when AAV application type is selected. In addition, **YES** for '**Detect and Resolve Heteroduplex Reads**' regardless of whether you sample contains ssAAV, scAAV, or a mixture of ssAAV and scAAV DNA (if inadvertently not specified in Run Design, the AAV sample must be re-run).

# SMRT Link v13 Run Design user interface updates – Sample info

Updated sample & run info fields to enable support for sequencing Kinnex and AAV libraries on Revio system

▼ Plate 1, Well A01: Demo\_Library\_Sample\_1 Copy Delete

Plate Well Required

Well Name Required

Well Comment

**Library Type** Required

**Library Type**

- Specifies structure of SMRTbell library and determines **adapter annotation algorithm** to use for post-primary analysis

Standard

**Standard**

Kinnex

Adeno-associated Virus

## Standard

- Library molecule contains **same adapter on both ends**



→ Specifies a single sequence for adapter calling

## Kinnex

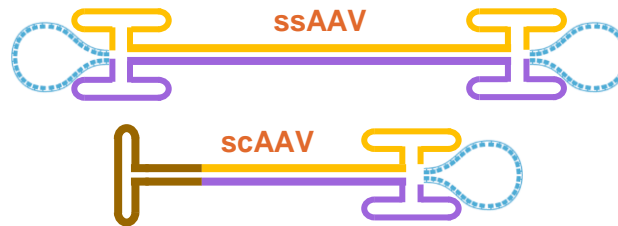
- Library molecule contains a **different adapter on each end**



→ Specifies two sequences for adapter calling<sup>2</sup>

## AAV

- Sample contains **ssAAV** library molecules, **scAAV** library molecules, or a mixture of both<sup>1</sup>
  - **ssAAV** library molecule contains **same adapter on each end** and **non-complementary forward/reverse (F/R) strands**
  - **scAAV** library molecule contains **an adapter at only one end**



→ **Disables adapter correction** (i.e., disables splitting molecules with an adapter on only one end)

- IMPORTANT: Specify Library Type = AAV to enable proper analysis of AAV samples.** If inadvertently not specified, the AAV sample must be re-run.

<sup>1</sup> ssAAV = Single-stranded Adeno-associated virus; scAAV = self-complementary Adeno-associated virus.

<sup>2</sup> When sequencing a Kinnex library sample, if 'Standard' library type is mistakenly selected instead of 'Kinnex' then a higher missing adapter rate (> 95%) and a slight degradation in barcode demultiplexing performance (~93-96% barcoded HiFi read yield) will be observed.



# SMRT Link v13 Run Design user interface updates – Sample & run info (cont.)

Updated sample info panel to enable support for sequencing Kinnex libraries on Sequel II/Ile systems

Application Required: Kinnex full-length RNA

Well Sample Name Required: Kinnex\_Full-length\_RNA\_Demo\_Library\_Sample\_01

Bio Sample Name ?

Sample Comment

Sample Well: A01

SMRTbell Adapter Design Required: SMRTbell® Kinnex Prep Kit

## SMRTbell Adapter Design

- Specifies structure of SMRTbell library and determines **adapter annotation algorithm** to use for post-primary analysis

SMRTbell® Kinnex Prep Kit

Blunt - SMRTbell® Template Prep Kit 1.0

Overhang - SMRTbell® Express Template Prep Kit 2.0

Overhang - SMRTbell® Prep Kit 3.0

SMRTbell® Kinnex Prep Kit

Blunt – SMRTbell TPK 1.0  
Overhang – SMRTbell Express TPK 2.0  
Overhang – SMRTbell Prep Kit 3.0

Library molecule contains same adapter on both ends<sup>1</sup>



→ Specifies a single sequence for adapter calling

SMRTbell Kinnex Prep Kit

Library molecule contains a different adapter on each end



Specifies two sequences for adapter calling<sup>2</sup>

<sup>1</sup> For AAV libraries constructed with SMRTbell Prep Kit 3.0, specify SMRTbell Adapter Design = Overhang – SMRTbell prep Kit 3.0.

<sup>2</sup> When sequencing a Kinnex library sample, if a different SMRTbell adapter design is selected instead of 'SMRTbell Kinnex Prep Kit' then a higher missing adapter rate (> 95%) and a slight degradation in barcode demultiplexing performance (~93-96% barcoded HiFi read yield) will be observed.

# SMRT Link v13 Run Design user interface updates – Sample indexing options

## Updated sample indexing parameter fields for sequencing multiplexed & non-multiplexed samples on Revio system

1. Click **YES** to specify indexed (barcoded) samples.
  - For non-multiplexed samples, can specify 'No' (No longer need to specify "default—default" barcode in Run Design Bio Sample Name)
2. Specify **Index (barcode) set** that contains index sequences of interest by selecting appropriate index set FASTA file<sup>1</sup>.
3. Click on **YES** or **NO** to specify if sample contains the same barcode sequence on both ends of the insert.
  - **YES** – Assumes symmetric barcode designs → **Demultiplexing performed on-instrument**
  - **NO** – Assumes asymmetric designs where barcodes are different on each end of the insert sequence → **Demultiplexing performed in SMRT Link<sup>2</sup>**
4. Assign a Biosample Name to each barcoded sample using one of two ways: **Interactively** or **From a (CSV) File**.

**Samples**

1 Sample is indexed  YES  NO

2 Indexes Required

3 Same Barcodes on Both Ends of Sequence  YES  NO

4 Biosample names Required

<sup>1</sup> Refer to PacBio's [Multiplexing](#) website for the recommended Barcode Set FASTA file to use for demultiplexing samples for a specific application.

<sup>2</sup> Demultiplexed data for asymmetrically indexed samples are output to a location within network file system (NFS). Note: Automated demultiplexing of asymmetrically indexed samples is not currently available in SMRT Link Lite v13.

# SMRT Link v13 Run Design user interface updates – Run options

## Updated Run Options panel to enable adaptive loading feature on Revio system

Run Options

Library Concentration (pM)   
Required

Use Adaptive Loading  YES  NO

Well >	Run <						
Plate well	Well name	Status	Movie time	Loading concentration	Pre-extension time	Workflow	Loading time
1 A01	2023_1116_H...	Complete	24 hr	225 pM	1.4 hr	Adaptive Loading	60 min
1 B01	2023_1116_H...	Complete	24 hr	225 pM	1.4 hr	Adaptive Loading	65 min
1 C01	2023_1116_H...	Complete	24 hr	225 pM	1.4 hr	Adaptive Loading	62 min
1 D01	2023_1116_H...	Complete	24 hr	225 pM	1.4 hr	Adaptive Loading	65 min

### Library Concentration

- **Required** field to facilitate sequencing performance troubleshooting
- Entered library concentration value appears in SMRT Link Run Details report

### Adaptive Loading

- By default, **Use Adaptive Loading = YES** for all applications and library insert sizes
- No need to specify adaptive loading target and maximum loading time<sup>1</sup>
- Note: Specified **Adaptive Loading setting must be the same for all SMRT Cells** in a run<sup>2</sup>  
→ Importing a Run Design CSV with multiple values for Adaptive Loading field results in an error.

One or more errors in this run design: Multiple values found for adaptive loading. Adaptive loading must be set to the same value for all SMRT Cells.

- Actual loading time appears in SMRT Link Run Details report

<sup>1</sup> In SMRT Link v13, default adaptive loading target ( $P1 + P2$ ) = 0.82 and default maximum loading time = 65 min (these settings are **not configurable** by users in Run Design).

<sup>2</sup> In SMRT Link v13 GUI, Adaptive Loading setting (YES or NO) is configurable per sample but will switch to be configurable per run in SMRT Link v13.1. If different Adaptive Loading settings are specified for different samples within the same run design, all settings will be overwritten by the last chosen one.

# SMRT Link v13 Run Design user interface updates – Data Options

Updated Data Options panel to support alternative CCS analysis modes for **Revio system**

**Data Options**

Include Base Kinetics  YES  NO

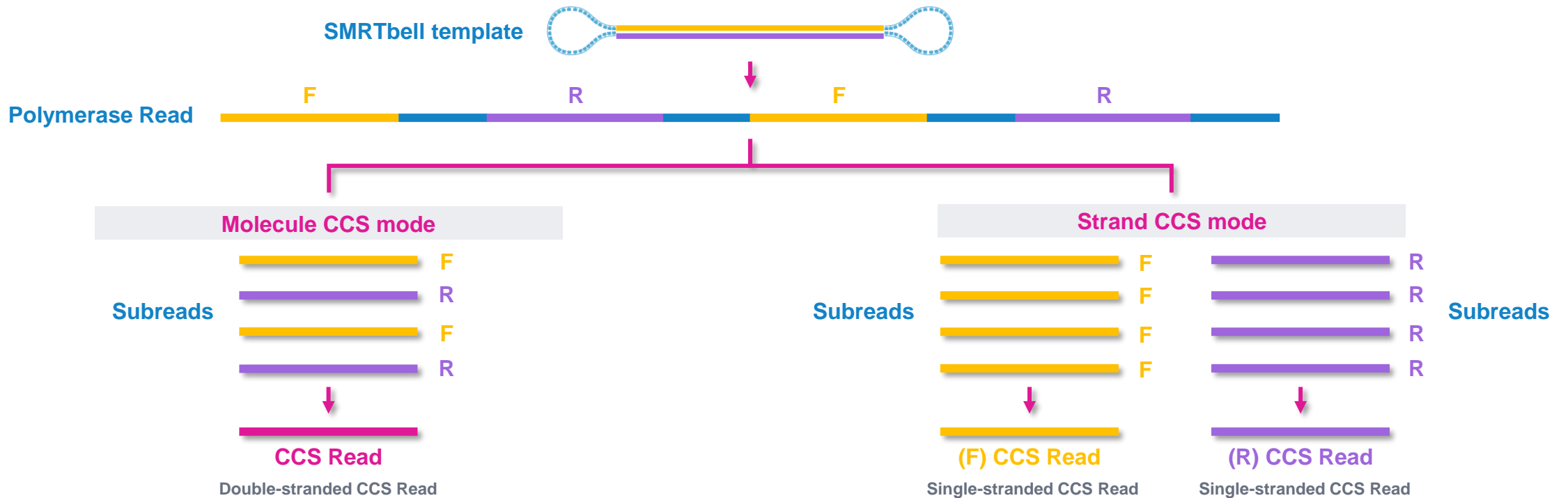
Consensus Mode  MOLECULE  STRAND

Assign Data To Project General Project

**IMPORTANT:** AAV analysis requires run design to specify **Consensus Mode = Strand**<sup>1</sup>

## Consensus Mode

- **Molecule = Standard CCS** (one consensus read per molecule)
  - **Strand = CCS --by-strand** (two consensus reads per molecule, one for forward strand and one for reverse)
- Useful for analysis of SMRTbell library templates containing non-complementary forward & reverse strands



# SMRT Link v13 Run Design user interface updates – Analysis Options

Updated Analysis options panel to support analysis of HiFi target enrichment and Kinnex full-length RNA samples

**Analysis Options**

Add Analysis  YES  NO

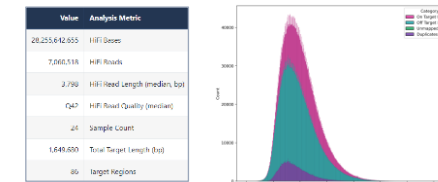
Analysis Name   
Required

Select Analysis Workflow   
Required

- Genome Assembly
- HiFi Mapping
- HiFi Target Enrichment**
- Iso-Seq Analysis
- Microbial Genome Analysis
- Read Segmentation
- Read Segmentation and Iso-Seq**
- Read Segmentation and Single-Cell Iso-Seq
- Structural Variant Calling
- Variant Calling

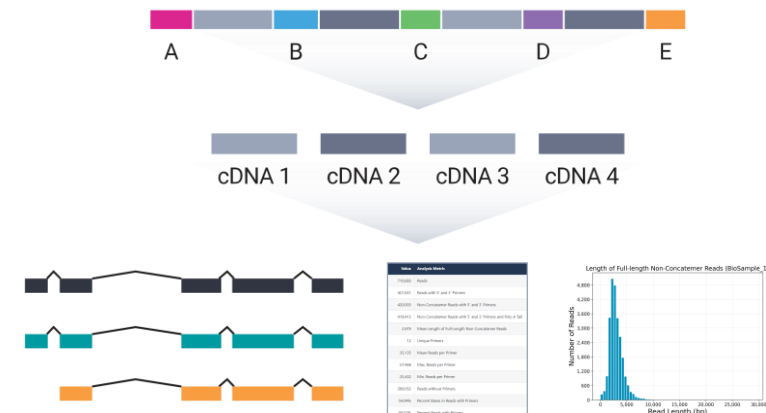
## HiFi target enrichment analysis

Obtain summary metrics, sample / target coverage stats & plots, variant call sets for samples generated with **hybrid capture workflows**



## Read segmentation and Iso-Seq analysis

HiFi read splitting (de-concatenation) and Isoform-classification analysis of **Kinnex full-length RNA** samples to identify novel genes & isoforms with abundance info



# SMRT Link v13 Run Design user interface updates – Run Design Summary

Updated Run Design Summary page is more streamlined and easier to read

## New Duplicate feature

- Click on **Duplicate** to open a new Run Design filled out with all the information from the Run Design you are copying

The image shows two screenshots of the PacBio Run Design Summary page. The top screenshot is for SMRT Link v12.0, and the bottom screenshot is for SMRT Link v13.0. The v13.0 version is more streamlined, with a 'Duplicate' button added to the top right. The v12.0 version has a 'Run Information' section on the left and a 'Sample Information' table on the right. The v13.0 version has a 'Sample Information' section on the left and a 'Sample Information' table on the right. The v13.0 table has columns for Plate Number, #, Well Name, Sample Well, Bio Sample Name, Library Type, Library Concentration, Movie Time, BarcodeSet Name, and Barcode ID.

**SMRT Link v12.0**

Run Information

Run Name  
20231030\_84006\_11k\_v2p2\_Open\_Revio\_v01\_tes

Run Comments

Experiment Name

Experiment ID  
357

Sample Information

#	Name
1	202310
2	202310

**SMRT Link v13**

Sample Information

Plate Number	#	Well Name	Sample Well	Bio Sample Name	Library Type	Library Concentration	Movie Time (hrs)	BarcodeSet Name	Barcode ID
1	1	2023_1107_8401...	A01	11K-clean_108pM	Standard	108	6	-	-
1	2	2023_1107_8401...	B01	11K-clean_108pM	Standard	108	6	-	-
1	3	2023_1107_8401...	C01	11K-clean_108pM	Standard	108	6	-	-
1	4	2023_1107_8401...	D01	11K-clean_108pM	Standard	108	6	-	-
2	5	2023_1107_8401...	A01	11K-clean_108pM	Standard	108	6	-	-
2	6	2023_1107_8401...	B01	11K-clean_108pM	Standard	108	6	-	-
2	7	2023_1107_8401...	C01	11K-clean_108pM	Standard	108	6	-	-
2	8	2023_1107_8401...	D01	11K-clean_108pM	Standard	108	6	-	-

## Run Information updates

- Run Information** has been **moved** to bottom of Sample Information table
- Experiment Name** field is **removed**
- “Experiment ID” field is now **renamed Transfer Subdirectory**

Run Information

Run Name  
2023\_1107\_84010\_108pM\_11k\_6hr\_noPE\_1p05xPEG

Run Comments

Transfer Subdirectory  
362



# SMRT Link v13 Run Design CSV updates (Revio system only)

Added analysis support for full-resolution (non-binned) base quality values

**Full-resolution base quality values** are accessible using the **Full Resolution Base Qual** option field when creating new run designs by importing a run design CSV file<sup>1</sup> (**Default setting = FALSE**)

[Run Settings]	
Instrument Type	Revio
Run Name	20231110_5pHG2_verif_4plex_5pHG2_ILNA
Plate 1	102118800
Transfer Subdirectory	358
CSV Version	1
[SMRT Cell Settings]	
Well Name	1_A01
Well Name	5pHG2_verif_4plex_DL_rep1
Application	Kinnex single-cell RNA
Library Type	Kinnex
Movie Acquisition Time (hours)	24
Insert Size (bp)	16000
Assign Data To Project	1
Library Concentration (pM)	130
Include Base Kinetics	FALSE
Polymerase Kit	Lxxxxx102739100123199
Indexes	cf5d7a6e-d95b-3360-cde5-d579f9abf06b
Minimum Barcode Score	80
Sample is indexed	TRUE
Use Adaptive Loading	FALSE
Consensus Mode	molecule
Full Resolution Base Qual	FALSE
Subread To HiFi Pileup	FALSE
Same Barcodes on Both Ends of Sequence	TRUE
[Samples]	
Bio Sample Name	Plate Well
Bio Sample 1	1_A01
Bio Sample 2	1_A01

## Full resolution base qual = FALSE (default)

- By default, base QV scores are grouped into seven discrete bins
  - Revio HiFi data sets are lighter and easier to work with.

## Full resolution base qual = TRUE

- **Not recommended** as the default setting since HiFi data set file size increases by ~50%.
  - Intended use case is for advanced users with non-standard data analysis needs

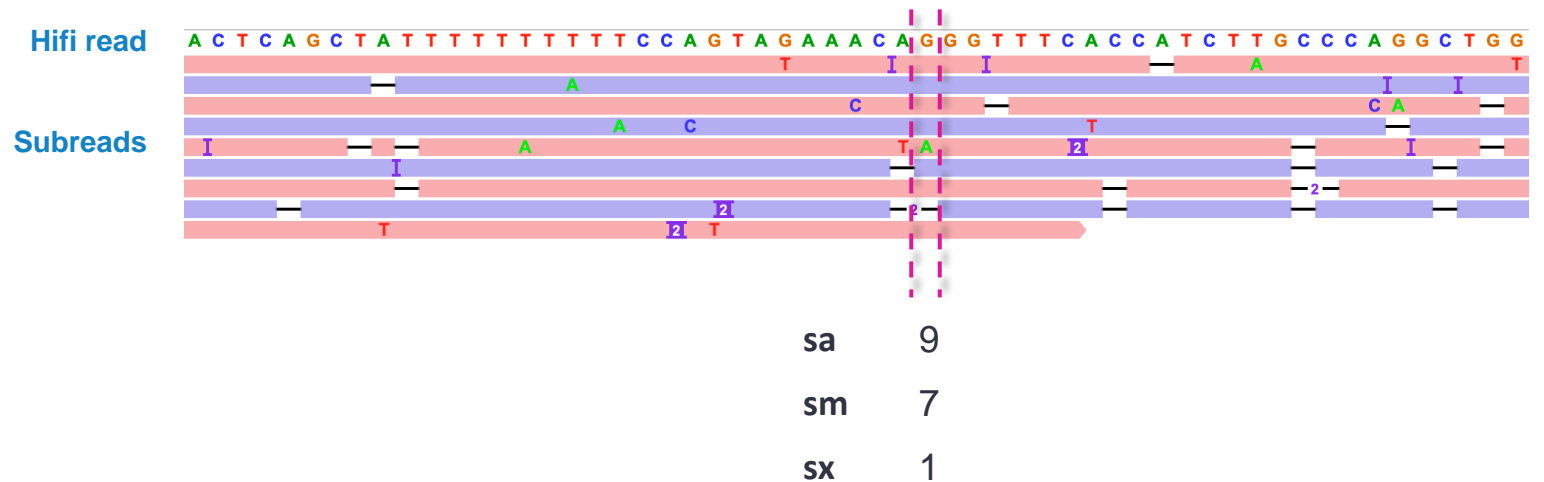
# SMRT Link v13 Run Design CSV updates (Revio system only) (cont.)

Added analysis support for “rich HiFi” tags that provide a summary of subread-to-consensus read alignment

hifi\_reads.bam now contains **optional “rich HiFi” tags** that summarize a HiFi read to support downstream variant calling analysis applications

→ Rich HiFi tags are accessible using the **Subread to HiFi Pileup** option when creating new run designs by importing a run design CSV file<sup>1</sup> (**Default setting = FALSE**)

[Run Settings]	
Instrument Type	Revio
Run Name	20231110_5pHG2_verif_4plex_5pHG2_ILNA
Plate 1	102118800
Transfer Subdirectory	358
CSV Version	1
[SMRT Cell Settings]	
Well Name	1_A01
Well Name	5pHG2_verif_4plex_DL_rep1
Application	Kinnex single-cell RNA
Library Type	Kinnex
Movie Acquisition Time (hours)	24
Insert Size (bp)	16000
Assign Data To Project	1
Library Concentration (pM)	130
Include Base Kinetics	FALSE
Polymerase Kit	Lxxxxx102739100123199
Indexes	cf5d7a6e-d95b-3360-cde5-d579f9abf06b
Minimum Barcode Score	80
Sample is indexed	TRUE
Use Adaptive Loading	FALSE
Consensus Mode	molecule
Full Resolution Base Qual	FALSE
<b>Subread To HiFi Pileup</b>	<b>FALSE</b>
Same Barcodes on Both Ends of Sequence	TRUE
[Samples]	
Bio Sample Name	Plate Well
Bio Sample 1	1_A01
Bio Sample 2	1_A01



**sa** Subread alignments that span each consensus position

**sm** Subreads that match the consensus base at each position

**sx** Subreads that mismatch the consensus base at each position

# SMRT Link v13 Run Details report updates – Run QC metrics table

Updated Run QC metrics table is more streamlined and includes Run Preview information for **Revio** systems

## SMRT Link v12.0

Sample Information >			Run Settings >		Productivity (%)			Reads >				Control >		File Transfer	
								HiFi Reads							
Plate Number ¶	Well ¶	Name ¶	Movie Time (hrs) ¶	Total Bases (Gb) ¶	P0 ¶	P1 ¶	P2 ¶	≥Q20 Reads ¶	Yield ¶	Mean Length ¶	Median QV ¶	Poly RL Mean (bp) ¶	Local Base Rate ¶	File Transfer Status ¶	File Transfer Status ¶
1	A01	HiFi WGS Sample 01	24	1365.69	32.1	66.5	1.4	6396491	124.64 Gb	19485	Q30	69991	2.55	Complete	<a href="#">Retry File Transfer</a>
1	B01	HiFi WGS Sample 02	24	1310.57	28.8	69.4	1.8	5656160	107.84 Gb	19065	Q29	63761	2.43	Complete	<a href="#">Retry File Transfer</a>
1	C01	HiFi WGS Sample 03	24	1219.80	28.8	69.3	2.0	5644181	107.00 Gb	18956	Q30	61246	2.18	Complete	<a href="#">Retry File Transfer</a>
1	D01	HiFi WGS Sample 04	24	1267.79	31.6	66.8	1.6	6064580	114.87 Gb	18941	Q30	64203	2.31	Complete	<a href="#">Retry File Transfer</a>

Column headers and run QC metrics are formatted for easier viewing

## SMRT Link v13

Well >		Run >		Productivity			HiFi reads				Polymerase reads >		Control reads >		Library	File Transfer		
Plate well	Well name	Status	Movie time	Total bases	P0	P1	P2	Reads	Yield	Length (mean)	Read quality (medi...)	Q30+ bases	Pol. read length (mean)	Reads	Read length (mean)	Missing adapter	Status	Action
1 D01	HiFi WGS Sample	Complete	24 hr	1,163 Gb	33%	67%	1%	6.4 M	104.1 Gb	16.4 kb	Q28	93%	69.0 kb	2,479	51.3 kb	7.0%	Complete	<a href="#">Retry File Transfer</a>

Basic Preview (estimates)			Full Preview (estimates)			
Time (hr)	P1%	HiFi read length (mean)	Time (hr)	HiFi yield	HiFi read length (mean)	HiFi read quality (median)
4	64%	14.4 kb	23	97.1 Gb	16.1 kb	Q28

Added Run Preview information to run QC metrics table

# SMRT Link v13 Run Details report updates – Barcode Counts preview

Updated Run Details report page includes Barcode Counts preview for **Revo** systems

## SMRT Link v13

Well >		Run >		Productivity			HiFi reads				Basic Preview (estimates)			Full Preview (estimates)				
Plate well	Well name	Status	Movie time	Total bases	P0	P1	P2	Reads	Yield	Length (mean)	Read quality (median)	Time (hr)	P1%	HiFi read length (mean)	Time (hr)	HiFi yield	HiFi read length (mean)	HiFi read quality (median)
1 D01	HiFi WGS Sample	Complete	24 hr	1,163 Gb	33%	67%	1%	6.4 M	104.1 Gb	16.4 kb	Q28		64%	14.4 kb	23	97.1 Gb	16.1 kb	Q28

Barcode Counts ⓘ

Barcode Counts ⓘ

Well name: HiFi WGS Sample

Time point: 4 hr

Barcode ⚡	HiFi reads ⚡	HiFi read length (mean, bp) ⚡
bcM0004--bcM0004	93.8%	14514
Other	1.1%	12521
Not Barcoded	5.2%	12864

Barcode Counts ⓘ

Well name: HiFi WGS Sample

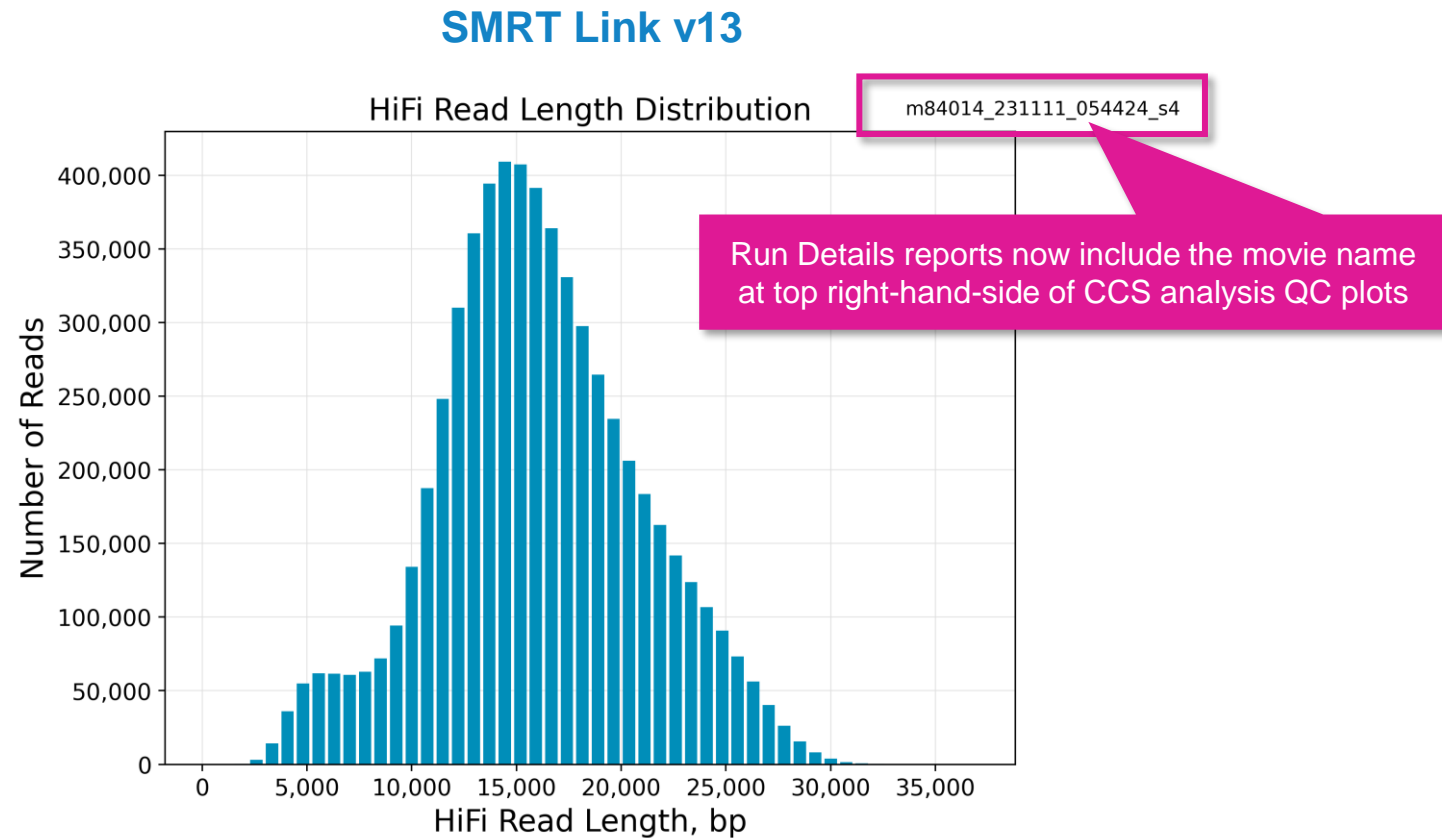
Time point: 23 hr

Barcode ⚡	HiFi reads ⚡	HiFi read length (mean, bp) ⚡
bcM0004--bcM0004	99.8%	16132
Not Barcoded	0.2%	4728

Can select to view Barcode Counts preview for two time points (4 hrs after movie starts or 1 hour before movie ends)

# SMRT Link v13 Run Details report updates – Plots

Run Details report plots now contain the movie name for easier reference when evaluating run performance

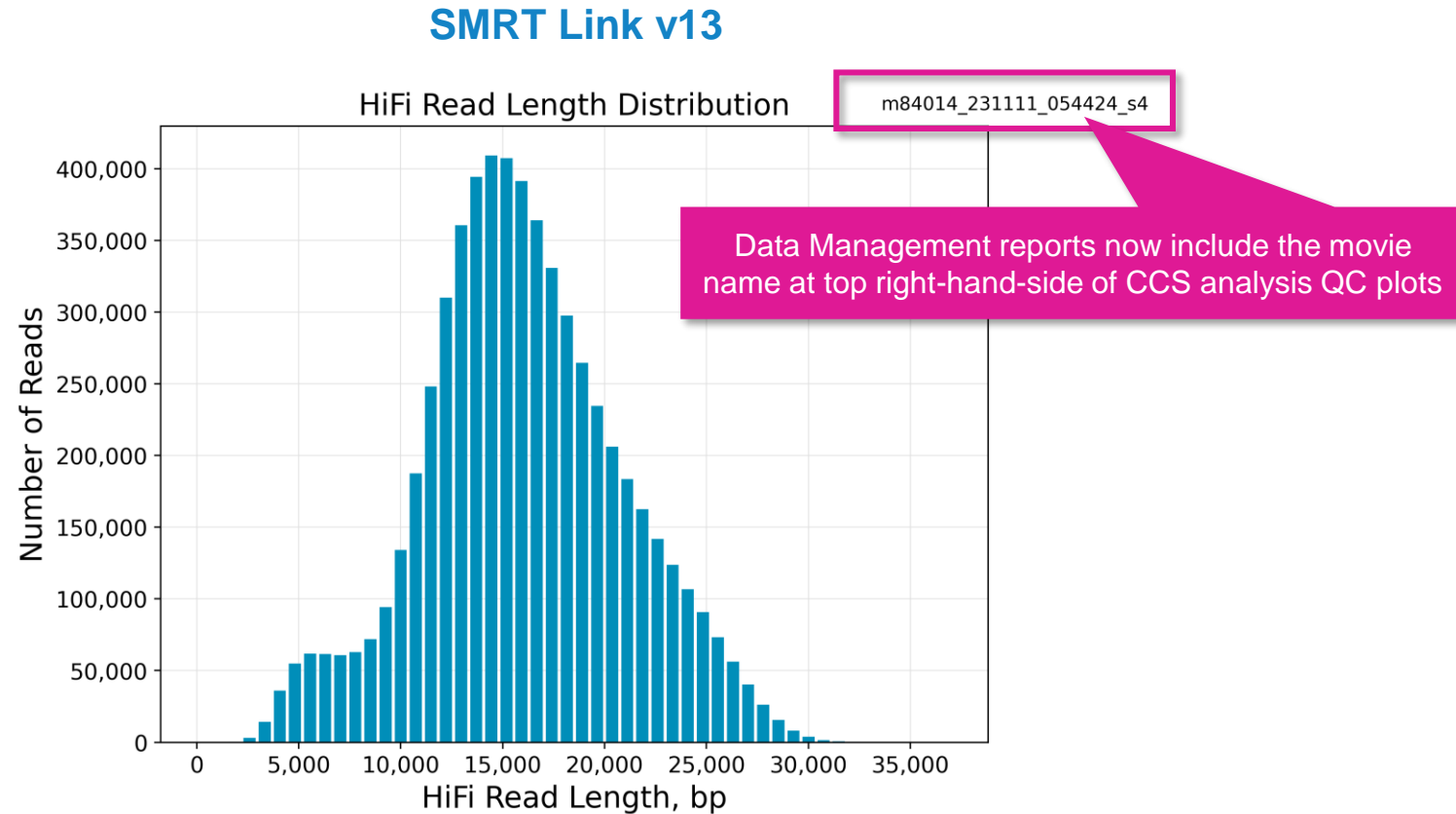




# SMRT Link v13 Data Management module updates

# SMRT Link v13 Data Management module user interface improvements

Data management report plots now contain the movie name for easier reference when evaluating run performance





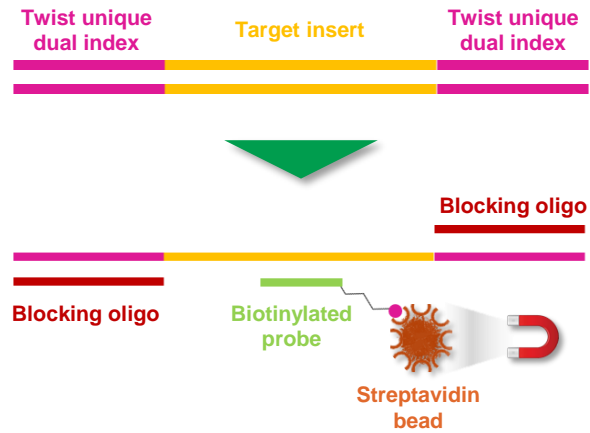


# SMRT Link v13 SMRT Analysis updates

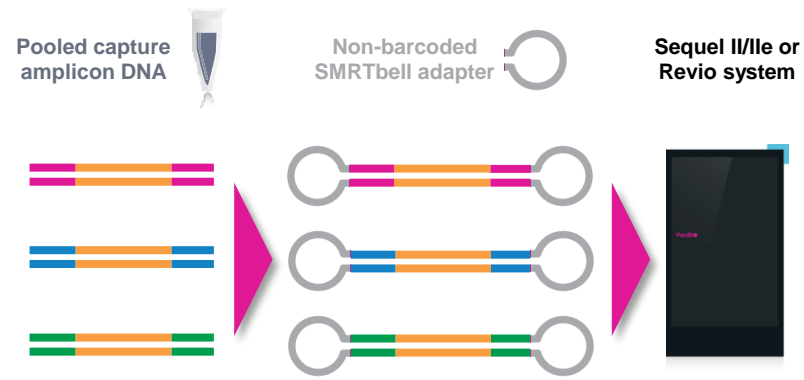
# New SMRT Link v13 HiFi Target Enrichment analysis application

New HiFi Target Enrichment pipeline analyzes multiplexed samples prepared with a target enrichment workflow

## Target enrichment using Twist hybrid capture

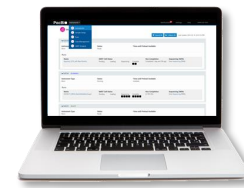


## SMRTbell library prep & PacBio long-read sequencing



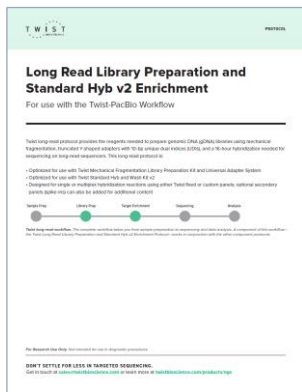
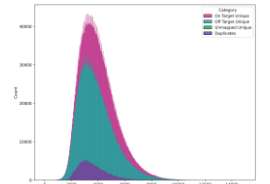
## SMRT Link HiFi Target Enrichment analysis

HiFi Target Enrichment analysis application

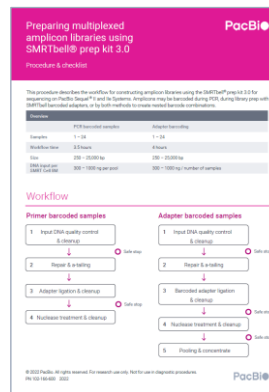


Summary metrics  
Sample / target coverage stats & plots  
Variant call sets

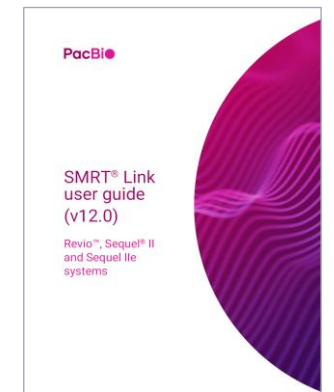
Value	Analysis Metric
28,255,642,955	HiFi Reads
7,660,518	HiFi Reads
2,799	HiFi Read Length (median, bp)
92.7	HiFi Read Quality (median)
24	Sample Count
1,640,820	Total Target Length (bp)
86	Target Regions



Long read library preparation and standard hyb v2 enrichment ([DOC-001320](#))



Procedure & checklist – Preparing multiplexed amplicon libraries using PacBio barcoded M13 primers and SMRTbell prep kit 3.0 ([101-921-300](#))



SMRT Link User Guide v13 ([103-008-800](#))

# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

## HiFi Target Enrichment analysis application supported use cases and workflow input requirements

### Sequencing platform

- Sequel II and Ile systems
- Revio system

### Library preparation

- Target enrichment libraries generated from Twist Biosciences long-read hybrid capture procedure<sup>1</sup> and PacBio SMRTbell library prep procedure
  - Twist protocol – Long read library preparation and standard hyb v2 enrichment ([DOC-001320](#))
  - PacBio Procedure & checklist – Preparing multiplexed amplicon libraries using SMRTbell prep kit 3.0 ([102-359-000](#))

### Data preparation for HiFi target enrichment analysis

- The application accepts HiFi reads (BAM format) as input from one or more datasets.
- Demultiplexing must be performed **before** the HiFi Target Enrichment workflow
  - Run the SMRT Link **Demultiplex Barcodes** data utility (see “Demultiplex Barcodes” in [SMRT Link user guide](#))
  - Recommended barcode set is **Twist Universal Adapter with UDI**<sup>2</sup>

### SMRT Analysis workflow inputs

- Demultiplexed HiFi reads
  - Default barcodes = Twist Universal Adapters with UDI<sup>2</sup>
- Reference genome
  - Default = Human hg38
- Target file
  - User supplied BED file containing target regions (not probes)

#### Example BED file

chr1	97721667	97721668	DPYD_rs1212037891
chr1	97740410	97740415	DPYD_rs72549309
chr1	97828264	97828265	DPYD_rs115632870
chr1	97883328	97883329	DPYD_rs1801265
chr1	155234451	155244670	GBA_fullgene
chr1	169549801	169550015	F5
chr1	201060658	201060816	CACNA1S_1
chr1	201091972	201092114	CACNA1S_2
chr10	94646920	94648182	CTBP2P2
chr10	94762681	94855547	CYP2C19
chr10	94938658	94990091	CYP2C9
chr10	95036772	95069497	CYP2C8
chr10	111077029	111080907	ADRA2A
chr11	46739265	46739506	F2
chr11	113393481	113402454	ANKK1
chr11	113409605	113475398	DRD2
chr11	120792404	120792905	GRIK4_1
chr11	120965795	120966296	GRIK4_2

<sup>1</sup> HiFi target enrichment analysis workflow is untested on other target enrichment libraries but theoretically should work.

<sup>2</sup> When specifying sample indexing options in SMRT Link Run Design or SMRT Analysis, select ‘No’ for Same Barcodes on Both Ends. You may optionally specify a different barcode set for demultiplexing.

# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

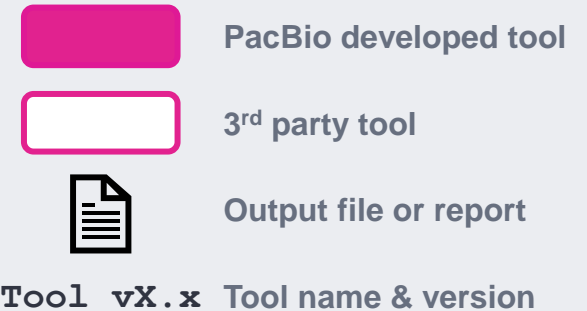
## HiFi Target Enrichment data analysis workflow

- This application accepts **HiFi reads** (BAM format) as input from one or more datasets
- **Note: Demultiplexing** must be performed **before** the HiFi Target Enrichment workflow

### Read processing

Mark PCR dups  
pbmarkdups v1.03

Map to reference  
pbmm2 v1.17 (?)



Target capture stats

hsmetrics v1.3.1 (picard)



Sample Summary

Target enrichment metrics calculation

SV calling

pbsv v2.8



VCF

Variant calling and phasing

Small variant calling

Deep Variant v1.4



VCF

Haplotype phasing

Whatshap v1.1



Haplotagged BAM

# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

Sample Setup recommendations for HiFi target enrichment libraries – Sequel II/Ile and Revio systems

Select desired application type in SMRT Link Sample Setup module



Sample Setup

- Whole Genome Sequencing
  - HiFi Reads
  - Microbial Assembly
- RNA Sequencing
  - Iso-Seq Method
  - MAS-Seq single cell
  - Kinnex single-cell RNA
  - Kinnex full-length RNA
- Viral Sequencing
  - Adeno-Associated Virus
- Metagenomics
  - Kinnex 16S rRNA
  - Full-Length 16S rRNA Sequencing
  - Shotgun Metagenomic Profiling or Assembly
- Targeted Sequencing
  - <3kb Amplicons
  - >=3kb Amplicons
  - HiFi Target Enrichment
- Other

After specifying your application type, SMRT Link auto-fills selected Sample Setup & Run Design parameter fields with default recommended values

## Sequel II/Ile systems

< Sample group >	
<input type="button" value="Copy"/> <input type="button" value="Remove"/> <input type="button" value="Lock"/> <input type="button" value="Download CSV"/>	
Name	HiFi_Targ_Enrichment_Lib_Demo
Application	HiFi Target Enrichment
Library type	Standard
Polymerase / Binding kit	Sequel II Binding Kit 3.2
Number of samples	1 samples
SMRT Cells per sample	1 cells
Available volume per sample	15 uL
Insert size	5000 bp
Sample concentration	7.5 ng/uL
Cleanup anticipated yield	75 %
Concentration on plate	90 pM Recommended: 85-100 pM
Minimum pipetting volume	1 uL

## Revio system

< Sample group >	
<input type="button" value="Copy"/> <input type="button" value="Remove"/> <input type="button" value="Lock"/> <input type="button" value="Download CSV"/>	
Name	HiFi_Targ_Enrichment_Lib_Demo
Application	HiFi Target Enrichment
Library type	Standard
Polymerase / Binding kit	Revio polymerase kit
Number of samples	1 samples
SMRT Cells per sample	1 cells
Available volume per sample	15 uL
Insert size	5000 bp
Sample concentration	7.5 ng/uL
Cleanup anticipated yield	75 %
Concentration on plate	250 pM Recommended: 200-300 pM
Minimum pipetting volume	1 uL

SMRT Link module	Key setup parameters for HiFi Targ. Enrich.	Sequel II/Ile system recommended settings for HiFi Target Enrichment libraries	Revio system recommended settings for HiFi Target Enrichment libraries
Sample setup	Library type	Standard	
	Primer	Standard sequencing primer	
	Binding/Polymerase kit <sup>1</sup>	Sequel II binding kit 3.2	Revio polymerase kit
	Concentration on plate	85 – 100 pM	200 – 300 pM

# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

## Run Design recommendations for HiFi target enrichment libraries – Revio system

Select desired application type in SMRT Link Run Design page



Runs

### Whole Genome Sequencing

- HiFi Reads
- Microbial Assembly

### RNA Sequencing

- Iso-Seq Method
- MAS-Seq single cell
- Kinnex single-cell RNA
- Kinnex full-length RNA

### Viral Sequencing

- Adeno-Associated Virus

### Metagenomics

- Kinnex 16S rRNA
- Full-Length 16S rRNA Sequencing
- Shotgun Metagenomic Profiling or Assembly

### Targeted Sequencing

- <3kb Amplicons
- >=3kb Amplicons

HiFi Target Enrichment

### Other

After specifying your application type, **SMRT Link auto-fills** selected Sample Setup & Run Design parameter fields with default recommended values

## Sample and run information

Plate Well <small>Required</small>	Plate 1, Well A01
Well Name <small>Required</small>	HiFi_Targ_Enrichment_Lib_Demo
Well Comment	
Library Type <small>Required</small>	Standard
Insert Size (bp) <small>Required</small>	5000
Polymerase Kit <small>Required</small>	Revio polymerase kit
Movie Acquisition Time (hours)	24
Application	HiFi Target Enrichment

## Indexing (barcoding) information

Sample is indexed	<input checked="" type="radio"/> YES <input type="radio"/> NO
Indexes <small>Required</small>	Twist Universal Adapters with UDI
Same Barcodes on Both Ends of Sequence	<input type="radio"/> YES <input checked="" type="radio"/> NO
Biosample names <small>Required</small>	Interactively <input type="checkbox"/> From a File <input type="checkbox"/>

## Run options and data options

Library Concentration (pM) <small>Required</small>	250
Use Adaptive Loading	<input checked="" type="radio"/> YES <input type="radio"/> NO
Include Base Kinetics	<input type="radio"/> YES <input checked="" type="radio"/> NO
Consensus Mode	<input checked="" type="radio"/> MOLECULE <input type="radio"/> STRAND
Assign Data To Project	General Project

## Analysis options

Add Analysis	<input checked="" type="radio"/> YES <input type="radio"/> NO
Analysis Name <small>Required</small>	Demo_Analysis_Job_Name
Select Analysis Workflow <small>Required</small>	HiFi Target Enrichment
Reference Set <small>Required</small>	Human Genome hg38, with Gencode v39 annotations
Target BED file <small>Required</small>	

**Library Type** identifies structure of molecules to be sequenced, which determines how instrument performs adapter calling and consensus read generation

SMRT Link module	Key setup parameters for HiFi Targ. Enrich	Revio system recommended settings for HiFi Target Enrichment libraries
Runs → Run design	Library type	Standard
	Movie collection time	24 hrs
	Use adaptive loading	YES
	On-instrument CCS	Consensus Mode = MOLECULE

# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

Run Design recommendations for HiFi target enrichment libraries – Sequel II and Ie systems

Select desired application type in SMRT Link Run Design page



Runs

- Whole Genome Sequencing
  - HiFi Reads
  - Microbial Assembly
- RNA Sequencing
  - Iso-Seq Method
  - MAS-Seq single cell
  - Kinnex single-cell RNA
  - Kinnex full-length RNA
- Viral Sequencing
  - Adeno-Associated Virus
- Metagenomics
  - Kinnex 16S rRNA
  - Full-Length 16S rRNA Sequencing
  - Shotgun Metagenomic Profiling or Assembly
- Targeted Sequencing
  - <3kb Amplicons
  - >=3kb Amplicons
  - HiFi Target Enrichment
- Other

After specifying your application type, **SMRT Link auto-fills** selected Sample Setup & Run Design parameter fields with default recommended values

## Sample and run information

**SMRTbell Adapter Design** determines which adapter finding algorithm is used during post-primary analysis

## Barcoded sample options

## Advanced options

## Analysis options

SMRT Link module	Key setup parameters For HiFi Targ. Enrich.	Sequel II/Ie system recommended settings for HiFi Target Enrichment libraries
	SMRTbell adapter design	Overhang – SMRTbell Prep Kit 3.0
	Movie collection time	24 hrs
	Use adaptive loading	YES
Runs → Run design	On-instrument CCS	CCS Analysis Output - Include Low Quality Reads = NO CCS Analysis Output - Include Kinetics Information = NO



# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

## Input data set selection

SMRT Analysis / Create New Analysis

Projects: All My Projects

1. Select Data 2. Select Analysis

Copy From... Next

Job Name Required

test HiFi TE run

Workflow Type

ANALYSIS  DATA UTILITY

Analysis of Multiple Data Sets

One Analysis for All Data Sets

Choose an option when multiples Data Sets are selected.

Back

Members of AS\_hg2\_4plex\_SpeedVac-Cell3 (CCS) (demux) (all samples)

Showing rows 1 to 7 out of 7 Search ...

	Data Set Details >		Sample Details				Run Data >		Metadata >	
<input checked="" type="checkbox"/>	Name ↑ ▾	Well Sample Name	Run Name ↑ ▾	Date Created ↑ ▾	Created By ↑ ▾	Bio Sample Name ↑ ▾	Barcode Name ↑ ▾	Total Length of Read	Instrument Name	Version ↑ ▾
<input checked="" type="checkbox"/>	AS_hg2_4plex...	AS_hg2_4plex_...	20231019_AS...	2023-10-28, ...	asoupe	Bio Sample 26	Plate_A_26_B0...	3,937,318,850	Sequel Ile SQ...	3.0.1
<input checked="" type="checkbox"/>	AS_hg2_4plex_...	AS_..._4plex_...	20231019_AS...	2023-10-28, ...	asoupe	Bio Sample 25	Plate_A_25_A0...	3,600,808,389	Sequel Ile SQ...	3.0.1
<input checked="" type="checkbox"/>	AS_hg2_4plex_...	AS_hg2_4plex_...	20231019_AS...	2023-10-28, ...	asoupe	Bio Sample 28	Plate_A_28_D0...	3,202,845,877	Sequel Ile SQ...	3.0.1
<input checked="" type="checkbox"/>	AS_hg2_4plex_...	AS_hg2_4plex_...	20231019_AS...	2023-10-28, ...	asoupe	Bio Sample 27	Plate_A_27_C0...	3,147,642,610	Sequel Ile SQ...	3.0.1

Select One Analysis for All Data Sets

Note: To ensure compatibility with third-party tools and formats, space characters in the Bio Sample Name of input datasets are automatically replaced with underscores by the HiFi Target Enrichment workflow

Select all desired sample data sets (from a previously demultiplexed job) with one click

# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

## HiFi Target Enrichment analysis outputs – Summary Metrics

The screenshot displays the PacBio SMRT Analysis web interface. The top navigation bar includes the PacBio logo, 'SMRT Analysis', and user options like 'Notifications', 'Settings', 'Help', and 'skingan (Lab Tech)'. The main content area shows the analysis ID '20231106\_TE\_test\_YW' and a 'SUCCESSFUL' status with 'Copy' and 'Delete' buttons. A left sidebar contains a menu with 'Analysis Overview', 'Target Enrichment', 'Summary Metrics' (highlighted), 'Sample Summary', 'Read Categories', 'Target Coverage', 'Sample Coverage', and 'Data'. The 'Target Enrichment' section features a table with the following data:

Value	Analysis Metric
28,255,642,655	HiFi Bases
7,060,518	HiFi Reads
3,798	HiFi Read Length (median, bp)
Q42	HiFi Read Quality (median)
24	Sample Count
1,649,680	Total Target Length (bp)
86	Target Regions

Top level summary of HiFi data, samples, targets

# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

## HiFi Target Enrichment analysis outputs – Sample Summary part 1

SMRT Analysis / Analysis Results

20231106\_TE\_test\_YW

SUCCESSFUL [Copy](#) [Delete](#)

Analysis Overview

Target Enrichment

Summary Metrics

Sample Summary

Read Categories

Target Coverage

Sample Coverage

Data

### Sample Summary

Sample ↑	HiFi Reads ↑↓	HiFi Read Length... ↑↓	HiFi Read Qual... ↑↓	Mean Target Cov... ↑↓	Percent Target ≥10-fold Cov... ↑↓	Percent Target ≥20-fold Cove
20230202_HG002a_PGx	282,665	4,108	Q42	219.8	98.96%	97.74%
20230202_HG002b_PGx	282,475	4,108	Q42	219.9	98.80%	97.87%
20230202_HG002c_PGx	251,704	4,131	Q42	197.2	98.81%	97.68%
20230202_HG002d_PGx	256,882	4,169	Q42	203.1	98.95%	97.91%
20230202_HG002e_PGx	268,712	4,162	Q42	212.1	98.75%	98.05%
20230202_HG002f_PGx	246,929	4,183	Q42	196.1	98.91%	97.79%
20230202_HG002g_PGx	254,116	4,216	Q42	203.9	98.68%	97.61%
20230202_HG002h_PGx	234,274	4,159	Q42	184.5	98.67%	97.41%
20230405_HG00118_PGx	231,015	3,677	Q42	138.9	99.22%	97.88%
20230405_HG00130_PGx	179,148	3,960	Q42	139.5	98.60%	97.25%
20230405_HG00185_PGx	314,139	3,673	Q43	162.6	99.12%	97.93%
20230405_HG00246_PGx	410,608	3,486	Q44	160.4	99.14%	98.24%
20230405_HG00276_PGx	296,535	3,621	Q43	139.8	99.07%	97.79%
20230405_HG00332_PGx	330,316	3,833	Q43	177.3	99.32%	98.31%
20230405_HG00437_PGx	234,555	3,821	Q42	159.5	98.72%	97.61%

All sample summary metrics shown in table are based on hs\_metrics from Picard tools

# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

## HiFi Target Enrichment analysis outputs – Sample Summary part 2

PacBio SMRT Analysis ▾ Notifications Settings Help skingan (Lab Tech)

SMRT Analysis / Analysis Results

20231106\_TE\_test\_YW SUCCESSFUL Copy Delete

Analysis Overview

Target Enrichment

Summary Metrics

**Sample Summary**

Read Categories

Target Coverage

Sample Coverage

Data

### Sample Summary

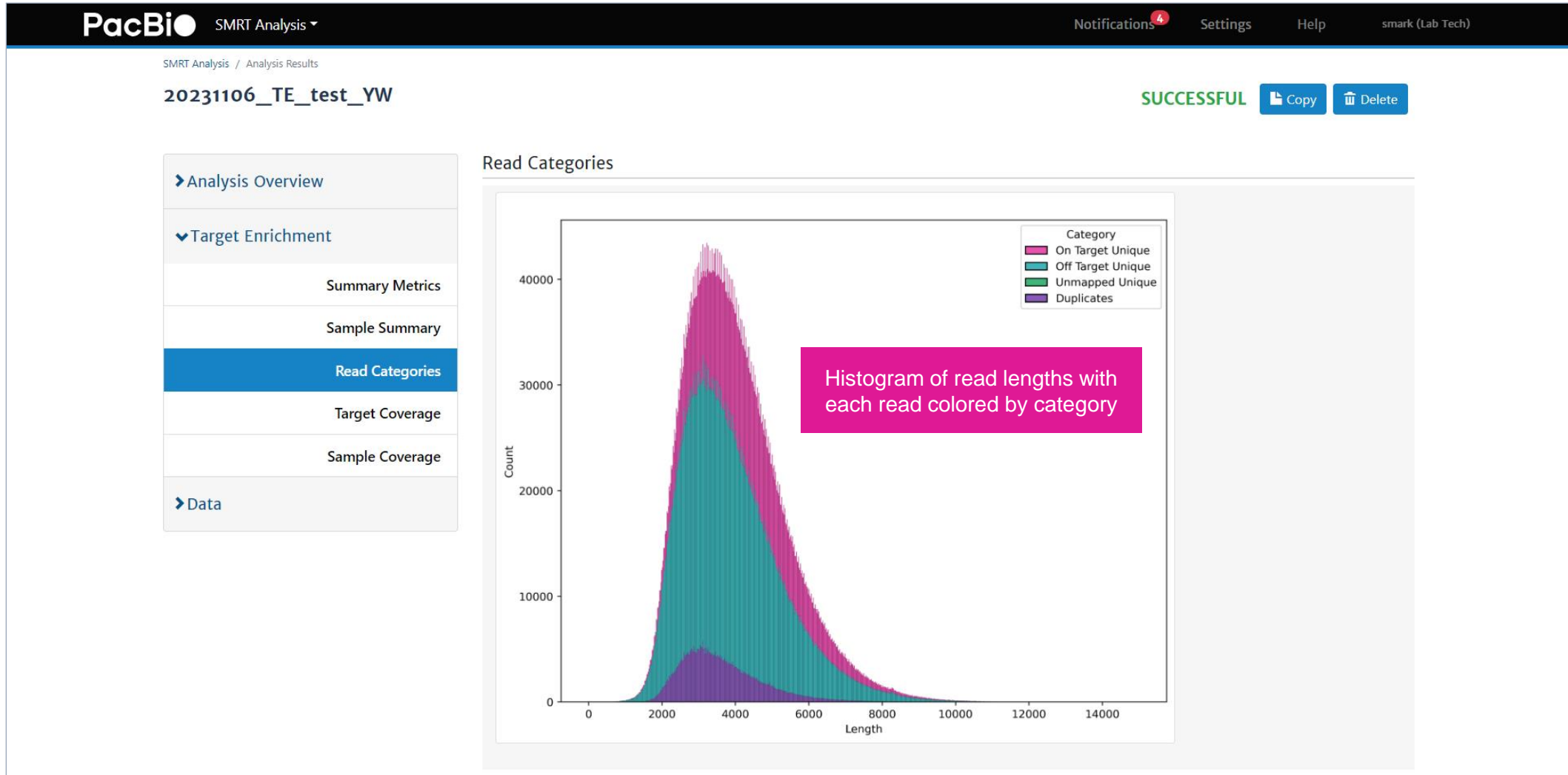
Search ...

Sample ↑	Percent Target ≥30...	Percent Target Zero...	Target Coverage Uniformity	Percent On-Target Bases	Percent Duplicate R
20230202_HG002a_PGx	24,0•2199796.43%	1.16%	2.00	44.07%	7.55%
20230202_HG002b_PGx	24,0•2199796.39%	1.16%	2.00	44.01%	7.37%
20230202_HG002c_PGx	24,0•1999795.88%	1.16%	2.01	43.98%	7.27%
20230202_HG002d_PGx	24,0•2099796.13%	1.16%	2.01	44.04%	7.34%
20230202_HG002e_PGx	24,0•2199996.54%	1.16%	1.98	44.26%	7.65%
20230202_HG002f_PGx	24,0•1999796.00%	1.16%	2.00	44.08%	7.35%
20230202_HG002g_PGx	24,0•2099796.04%	1.16%	1.98	44.12%	7.23%
20230202_HG002h_PGx	24,0•1899795.46%	1.16%	1.98	43.75%	7.00%
20230405_HG00118_PGx	23,0•1399795.65%	1.16%	1.74	38.31%	6.43%
20230405_HG00130_PGx	17,0•1399795.04%	1.16%	1.81	43.83%	4.88%
20230405_HG00185_PGx	38,0•1699796.07%	1.16%	1.83	34.77%	7.62%
20230405_HG00246_PGx	48,0•1699996.55%	1.16%	1.80	32.72%	12.21%
20230405_HG00276_PGx	28,0•1399795.42%	1.16%	1.77	33.22%	8.24%
20230405_HG00332_PGx	33,0•1799996.83%	1.16%	1.72	34.85%	7.60%

All sample summary metrics shown in table are based on hs\_metrics from Picard tools

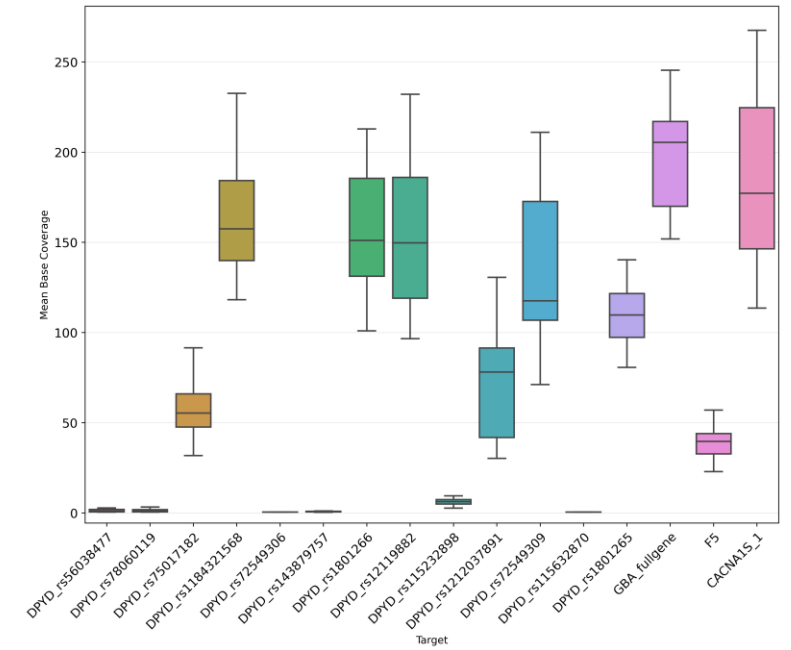
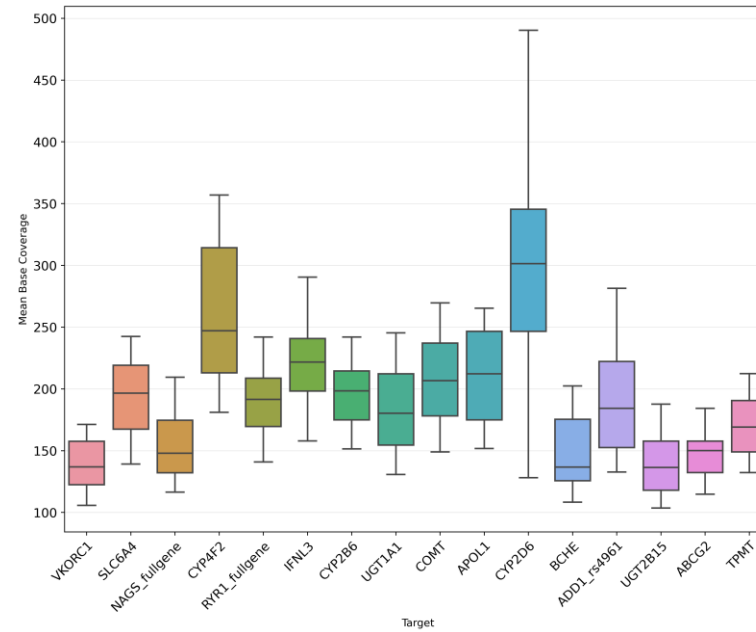
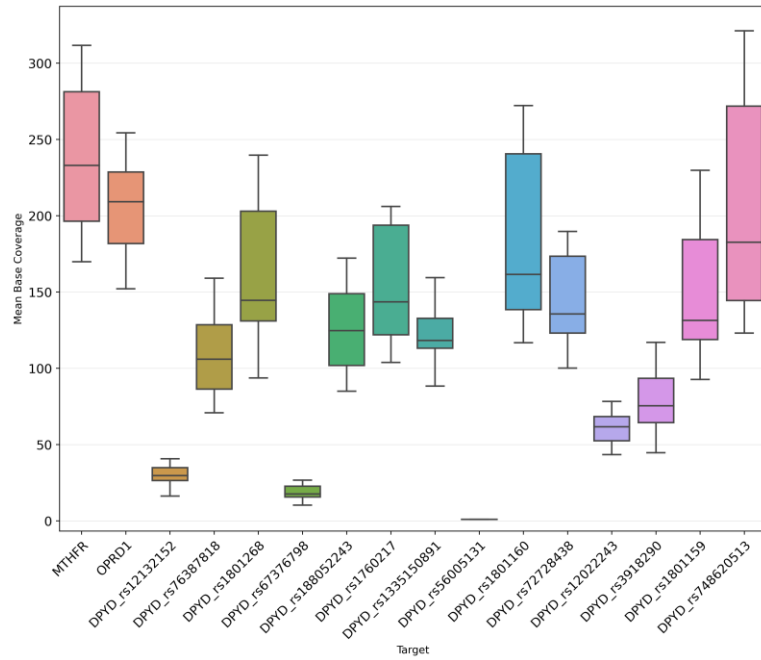
# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

## HiFi Target Enrichment analysis outputs – Read Categories



# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

## HiFi Target Enrichment analysis outputs – Target Coverage

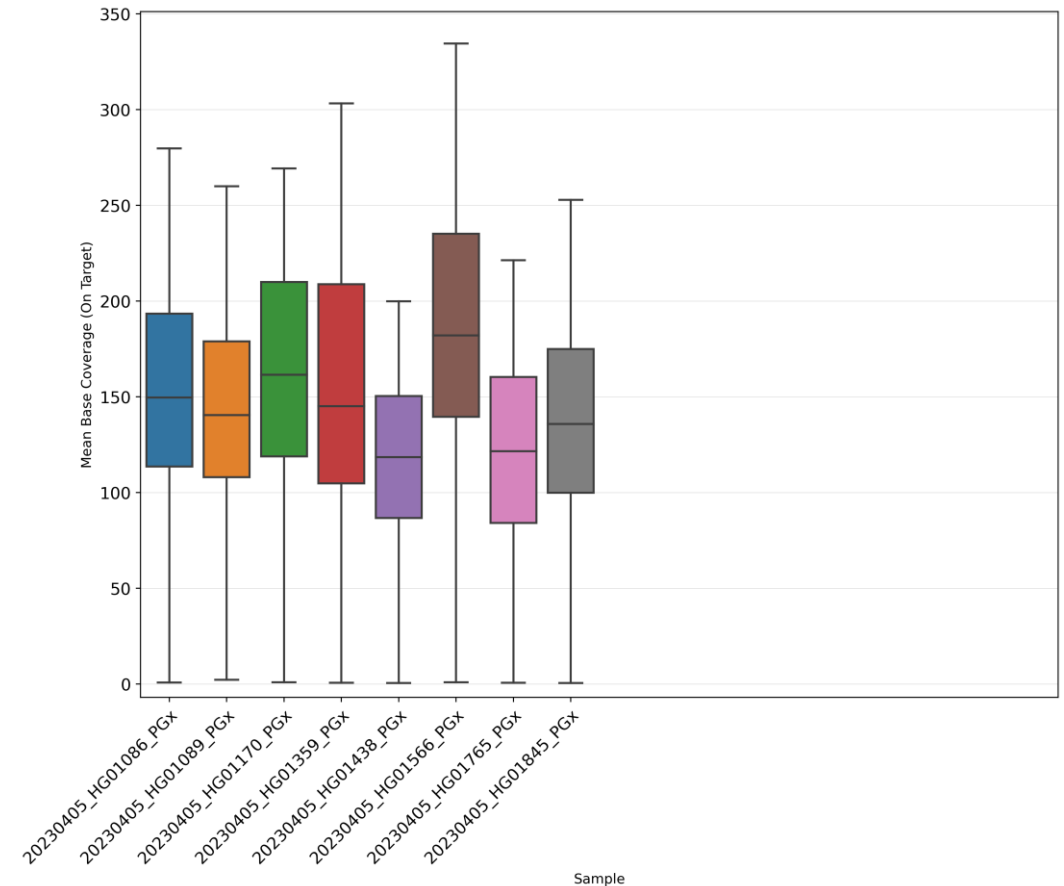
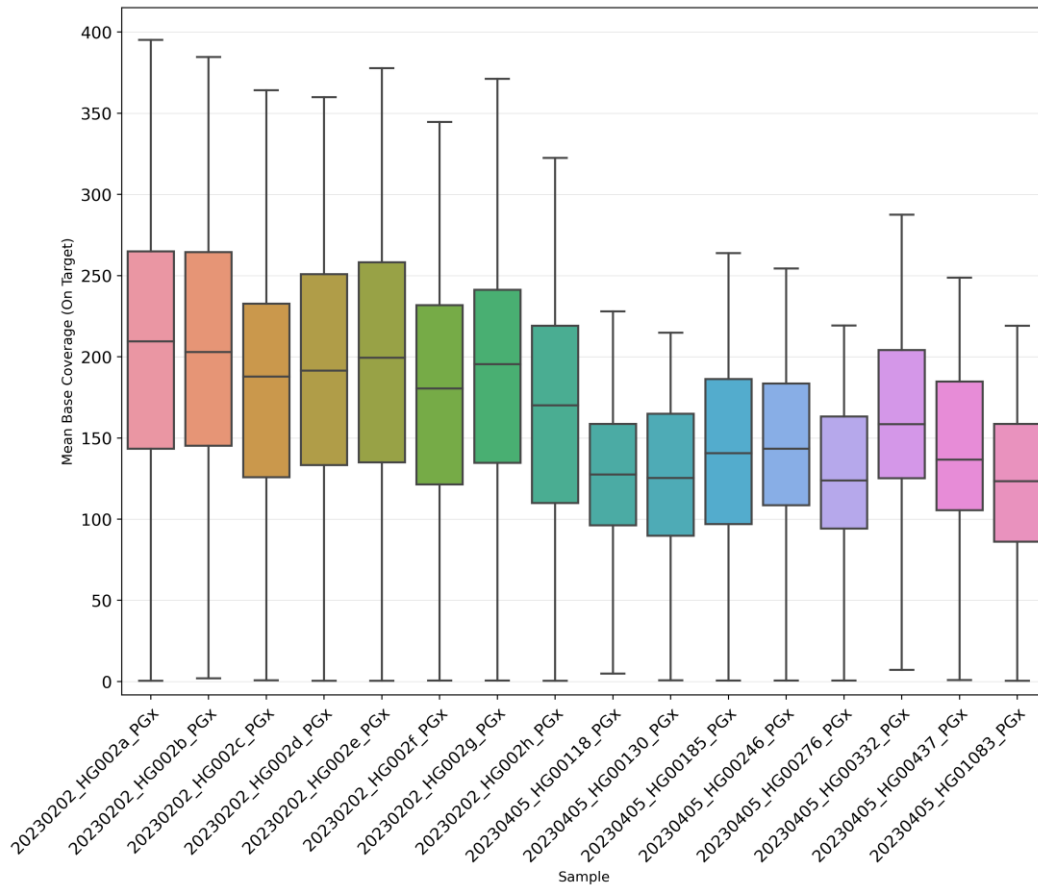


**X-axis:** Targets

**Y-axis:** Boxplot of mean base coverage across samples in target regions

# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

## HiFi Target Enrichment analysis outputs – Sample Coverage



**X-axis:** Samples  
**Y-axis:** Boxplot of mean base coverage across target regions



# New SMRT Link v13 HiFi Target Enrichment analysis application (cont.)

## HiFi Target Enrichment analysis outputs – Data downloads

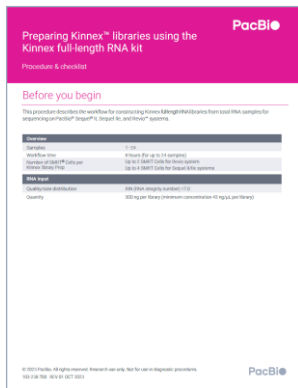
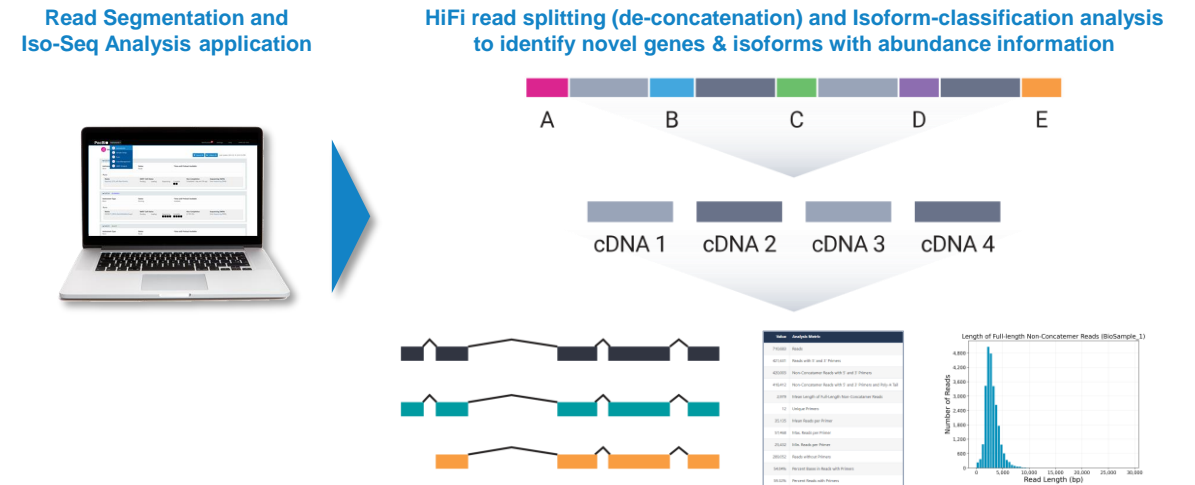
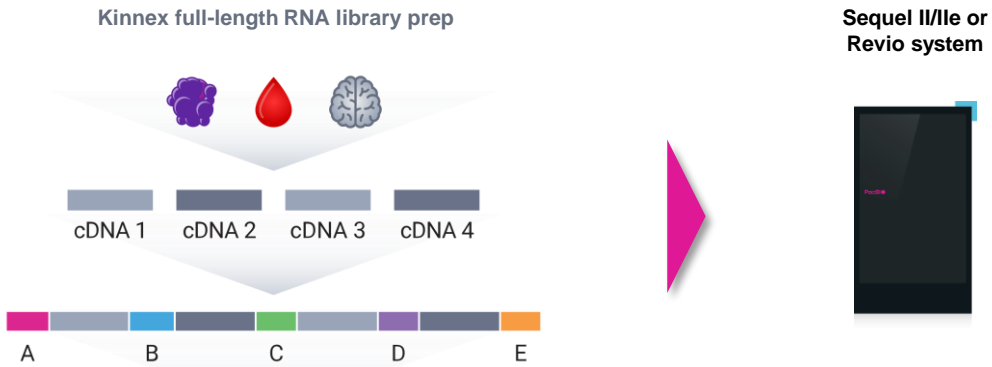
File	Description
<b>Sample Mapped BAMs</b>	Zipped archive of haplotagged, mapped reads in BAM format
<b>Sample VCFs for SVs</b>	Zipped archive of PBSV structural variant calls in VCF format for each sample
<b>Sample VCFs for small variants</b>	Zipped archive of DeepVariant, small variant calls in VCF format for each sample.
<b>Sample Summary CSV</b>	CSV version of the data displayed in the Target Enrichment Sample Summary table.
<b>Target Coverage by Sample CSV</b>	Comma-delimited text file of the matrix of all mean coverage values for each target (rows) and sample (columns). The data in this file is used to generate the Target Coverage and Sample Coverage box plot

# New SMRT Link v13 Read Segmentation and Iso-Seq Analysis pipeline

New Read Segmentation and Iso-Seq Analysis pipeline enables analysis of Kinnex full-length RNA libraries to split arrayed HiFi reads and characterize full-length transcript isoforms

## Kinnex full-length RNA library preparation & PacBio long-read sequencing

## SMRT Link Read Segmentation and Iso-Seq Analysis



**PacBio**

Procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit ([103-238-700](https://www.pacb.com/support/103-238-700))



**PacBio**

SMRT Link User Guide v13 ([103-008-800](https://www.pacb.com/support/103-008-800))

# New SMRT Link v13 Read Segmentation and Iso-Seq Analysis pipeline

## Read Segmentation and Iso-Seq Analysis application supported use cases and workflow input requirements

### Sequencing platform

- Sequel II and IIe systems
- Revio system

### Library preparation

- Kinnex full-length RNA libraries generated using PacBio Kinnex full-length RNA kit and Iso-Seq express 2.0 kit
  - PacBio Procedure & checklist – Preparing Kinnex ([102-359-000](https://www.pacb.com/support/knowledge-base/preparing-kinnex))
- If the library is a regular Iso-Seq library **without** MAS-Seq concatenation, use the SMRT Link **Iso-Seq Analysis** workflow instead

### Data preparation for Read Segmentation and Iso-Seq Analysis

- The application accepts HiFi reads (BAM format) as input from one or more datasets.
- The Read Segmentation and Iso-Seq workflow will **only process barcoded libraries at the cDNA level** (such as using Iso-Seq v2 Barcoded cDNA Primers as part of the Kinnex full-length RNA kit)
  - Demultiplexing of Kinnex barcoded adapters (also part of the Kinnex full-length RNA kit) should first run through the Demultiplexing workflow in SMRT Link.
    - Run the SMRT Link **Demultiplex Barcodes** data utility (see “Demultiplex Barcodes” in SMRT Link user guide)
    - Recommended barcode set is **MAS SMRTbell barcoded adapters (v2)**<sup>1</sup>

### SMRT Analysis workflow inputs

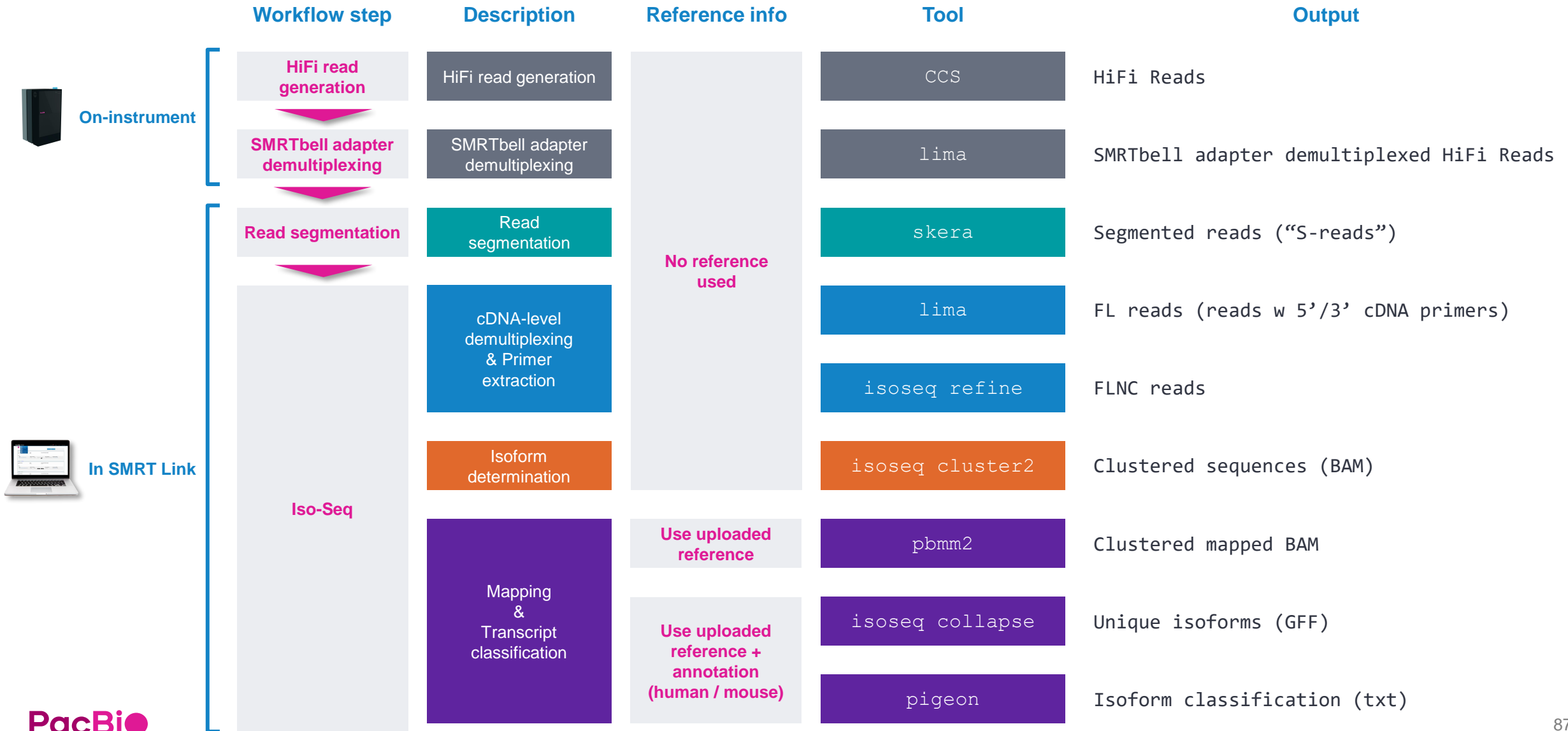
- Demultiplexed HiFi reads
  - Default barcodes = MAS SMRTbell barcoded adapters (v2)
- Segmentation Adapter Set
  - Specify a FASTA file, provided by PacBio, containing segmentation adapters
  - Default = MAS-Seq Adapter v3 (MAS8)
- Primer Set (Required)
  - Specify a primer sequence file in FASTA format to identify cDNA primers for removal (barcode FASTA includes the 5' and 3' cDNA primers)
  - Each primer sequence must be unique
  - (Default = Iso-Seq v2 Barcoded cDNA Primers)
- Reference Set (Required)
  - Specify one of two default reference genome and annotation sets to align high-quality isoforms to, and to collapse isoforms mapped to the same genomic loci
  - Default sets are Human Genome hg38, with Gencode v39 annotations and Mouse Genome mm39, with Gencode vM28 annotations
  - Alternatively, choose other reference genomes (but not with annotations) that were custom-uploaded to SMRT Link
  - If Reference Set field is left blank, the workflow will stop after the isoform clustering step (`isoseq_cluster2`)

<sup>1</sup> When specifying sample indexing options in SMRT Link Run Design or SMRT Analysis, select 'Yes' for Same Barcodes on Both Ends.

<sup>2</sup> If you need a custom segmentation adapter set, click Advanced Parameters and use a custom FASTA file formatted as described in SMRT Link User Guide.

# New SMRT Link v13 Read Segmentation and Iso-Seq Analysis pipeline

## Read Segmentation and Iso-Seq Analysis workflow



# New SMRT Link v13 Read Segmentation and Iso-Seq Analysis pipeline

## Read Segmentation and Iso-Seq Analysis clustering options

### SMRT Link Run Design > Analysis options

Add Analysis  YES  NO

Analysis Name Required

Select Analysis Workflow Required

Segmentation Adapter Set MAS-Seq Adapter v3 (MAS8)

Primer Set Required Iso-Seq v2 Barcoded cDNA Primers

Reference Set Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples

Advanced Parameters

Specify how to perform read clustering for barcoded samples

Pool reads and cluster together

-- select --

Cluster reads separately

Pool reads and cluster together

### SMRT Analysis > Associated inputs

Analysis Application Required

Read Segmentation and Iso-Seq

Import Analysis Settings Export

Associated Inputs

Segmentation Adapter Set MAS-Seq Adapter v3 (MAS8)

Primer Set Required Iso-Seq v2 Barcoded cDNA Primers

Reference Set Human Genome hg38, with Gencode v39 annotations

Cluster of Barcoded Samples

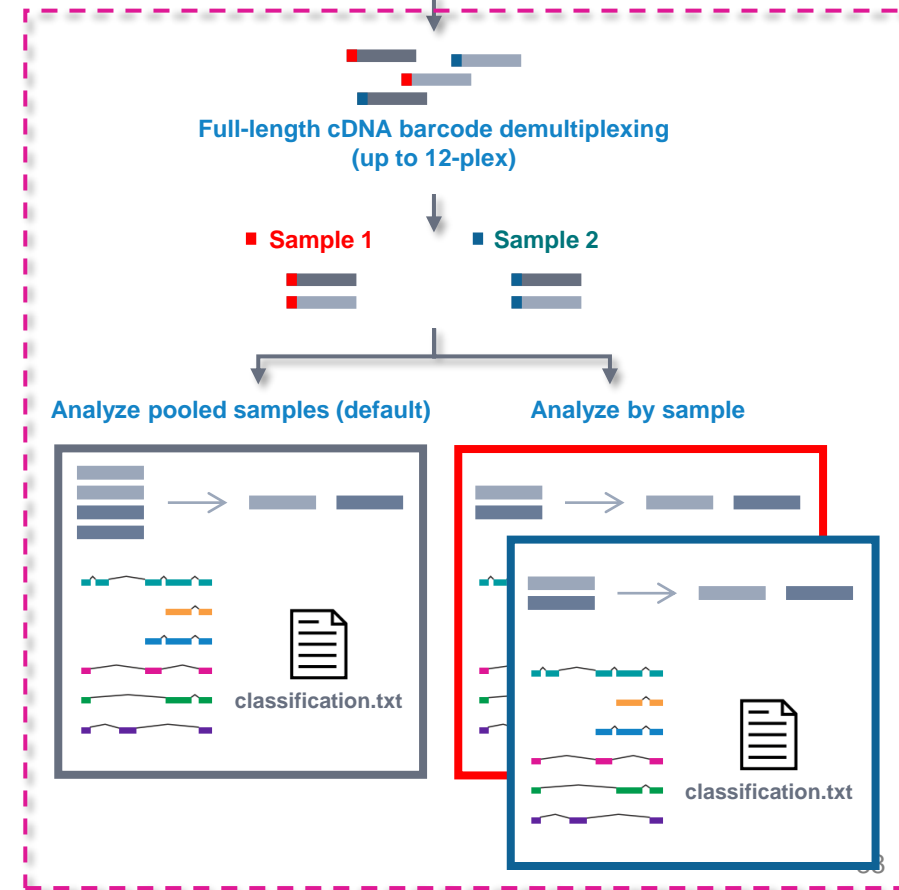
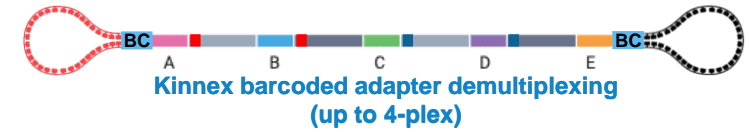
Advanced Parameters

Pool reads and cluster together

-- select --

Cluster reads separately

Pool reads and cluster together



**Cluster of Barcoded Samples** option applies to barcoded samples that were barcoded at the cDNA level, where the (barcoded) cDNA primers are specified in the Primer Set option

→ Setting determines whether all FLNC reads will be pooled for clustering<sup>1</sup> (Does not apply to non-barcoded cDNA samples.)

# Updated Read Segmentation and Single-cell Iso-Seq Analysis pipeline

Updated Read Segmentation and Single-cell Iso-Seq Analysis pipeline supports analysis of Kinnex single-cell RNA libraries generated from 10x 3' or 5' single-cell cDNA generation kit

## Kinnex single-cell RNA library preparation & PacBio long-read sequencing

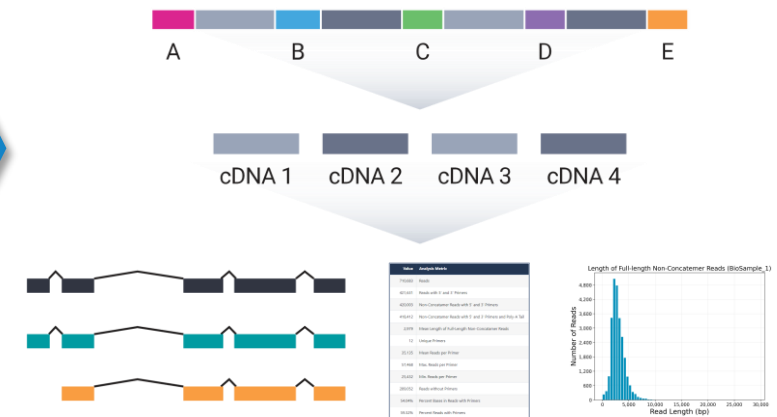
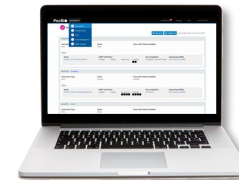
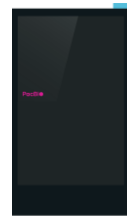
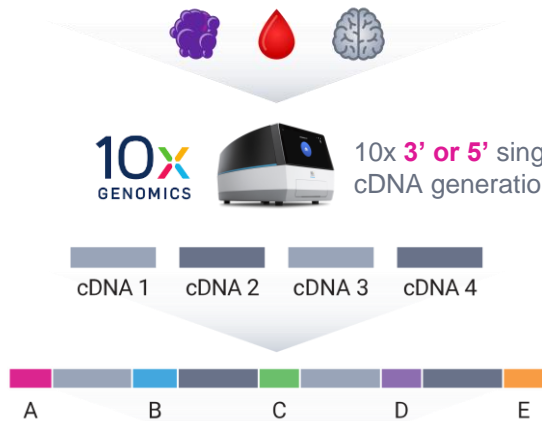
## SMRT Link Read Segmentation and Iso-Seq Analysis

Kinnex single-cell RNA library prep

Sequel II/IIe or Revio system

Read Segmentation and Single-cell Iso-Seq Analysis application

HiFi read splitting (de-concatenation) and Isoform-classification analysis to identify novel genes & isoforms



**PacBio**

Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit ([102-254-300](https://www.pacb.com/support/102-254-300))



**PacBio**

SMRT Link User Guide v13 ([103-008-800](https://www.pacb.com/support/103-008-800))

Updated Read Segmentation and Single-cell Iso-Seq Analysis pipeline supports both 3' and 5' single-cell cDNA samples

# Updated Read Segmentation and Single-cell Iso-Seq Analysis pipeline (cont.)

Read Segmentation and Single-cell Iso-Seq Analysis includes options for 10x 3' and 10x 5' single cell kits

## SMRT Link Run Design > Analysis options

Add Analysis  YES  NO

Analysis Name Required: Kinnex\_Single-Cell\_RNA\_Demo\_Analysis

Select Analysis Workflow Required: Read Segmentation and Single-Cell Iso-Seq

Segmentation Adapter Set: MAS-Seq Adapter v1 (MAS16)

Primer Set Required: 10x Chromium single cell 3' cDNA primers

Reference Set Required: Human Genome hg38, with Gencode v39 annotations

Kit Type  10X 3' KIT  10X 5' KIT

Advanced Parameters

Specify Primer Set used for cDNA amplification

Specify Kit Type used for single-cell cDNA generation

## SMRT Analysis > Associated inputs

Analysis Application Required: Read Segmentation and Single-Cell Iso-Seq

Import Analysis Settings | Export

Associated Inputs

Segmentation Adapter Set: MAS-Seq Adapter v1 (MAS16)

Primer Set Required: 10x Chromium single cell 3' cDNA primers

Reference Set Required: Human Genome hg38, with Gencode v39 annotations

Kit Type  10X 3' KIT  10X 5' KIT

Advanced Parameters

Specify **Primer Set** sequence file in FASTA format to **identify cDNA primers for removal** (include the 5' and 3' cDNA primers)

10x Forward (F) PCR primer: [CBC][UMI] TTTTTTTTTT LIBRARY INSERT CCC TSO 3'

10x Reverse (R) PCR primer: [CBC][UMI] AAAAAAAAAA LIBRARY INSERT GGG TSO 5'

5' [CBC][UMI] TTTTTTTTTT LIBRARY INSERT CCC TSO 3'

3' [CBC][UMI] AAAAAAAAAA LIBRARY INSERT GGG TSO 5'

For Kinnex single-cell 3' RNA analysis, select '10x Chromium single cell 3' cDNA primers'

OR

5' [CBC][UMI] [TSO]GGG LIBRARY INSERT AAAAAAAAAA 3'

3' [CBC][UMI] [TSO]CCC LIBRARY INSERT TTTTTTTTTT 5'

For Kinnex single-cell 5' RNA analysis, select '10x Chromium single cell 5' cDNA primers'

Specification of **Kit Type** (10x 3' Kit or 10x 5' Kit) **determines which set of 10x barcode sequences to use**, and also affects **UMI and single-cell barcode design settings<sup>1</sup>**

5' [CBC][UMI] TTTTTTTTTT LIBRARY INSERT CCC TSO 3'

3' [CBC][UMI] AAAAAAAAAA LIBRARY INSERT GGG TSO 5'

For Kinnex single-cell 3' RNA analysis, select '10x 3' Kit'

OR

5' [CBC][UMI] [TSO]GGG LIBRARY INSERT AAAAAAAAAA 3'

3' [CBC][UMI] [TSO]CCC LIBRARY INSERT TTTTTTTTTT 5'

For Kinnex single-cell 5' RNA analysis, select '10x 5' Kit'



**PacBio**

**SMRT Link Lite**

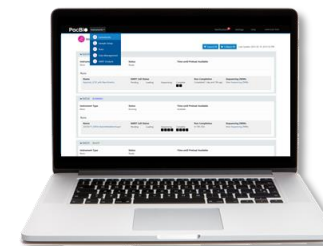
# SMRT Link Lite

New SMRT Link Lite can be installed for users who do not require SMRT Analysis and need more minimal compute requirements.

## SMRT Link Lite feature set

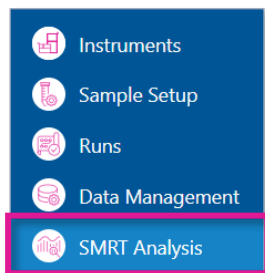
- No secondary analysis in SMRT Link
- Users can run secondary analysis on a separate server via command-line tools
- Sample Setup
- Run Design
- Run QC plots
- Real-time run monitoring (Run previews, run and instrument state updates, instrument error & alarm notifications)
- Settings to control instrument [Revio system only]
- Notifications [Revio system only]

## SMRT Link Lite installation

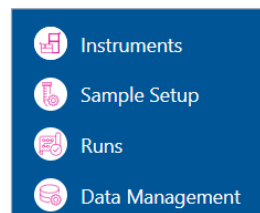


- Same installer as SMRT Link
- Use `--lite true --nworkers 4`
- Note: Only Sequel IIe and Revio systems are supported  
→ SMRT Link Lite does not support Sequel II system

## SMRT Link modules



## SMRT Link Lite modules



## SMRT Link compute requirements<sup>1</sup>

	SMRT Link server	
	SMRT Link	SMRT Link lite
CPU core	16	4
RAM	64 GB	16 GB
Local storage	1 TB SSD	500 GB SSD



# Technical documentation & applications support resources

# Revio system and SMRT Link technical documentation

## Revio system documentation

- Revio system Instrument Control Software release notes ([103-324-700](#))
- Revio system operations guide ([102-962-600](#))
- Revio system specifications sheet ([102-326-522](#))

## SMRT Link & other data analysis documentation

- Brief primer and lexicon for PacBio SMRT sequencing webpage ([v13.0](#))
- PacBio bioinformatics file formats documentation webpage ([v13.0](#))
- SMRT Link v13 Python API reference ([103-285-100](#))
- SMRT Link v13 release notes ([103-083-400](#))
- SMRT Link v13 software installation guide ([103-009-000](#))
- SMRT Link v13 user guide ([103-008-800](#))
- SMRT Link v13 web services API use cases ([103-234-100](#))
- SMRT Tools v13 reference guide ([103-008-900](#))



# Revio system library preparation technical documentation

## SMRTbell library preparation literature

- Overview – HiFi application options ([101-851-300](#))
- Procedure & checklist – Amplification of bacterial full-length 16S rRNA gene with barcoded primers ([101-599-700](#))
- Procedure & checklist – PacBio HiFiViral high-throughput multiplexing for full-viral genome sequencing of SARS-CoV-2 using SMRTbell prep kit 3.0 ([102-396-100](#))
- Procedure & checklist – Preparing Iso-Seq libraries using SMRTbell prep kit 3.0 ([102-396-000](#))
- Procedure & checklist – Preparing Kinnex libraries using Kinnex single-cell RNA kit ([103-254-300](#))
- Procedure & checklist – Preparing Kinnex libraries using Kinnex full-length RNA kit ([103-238-700](#))
- Procedure & checklist – Preparing Kinnex libraries from 16s rRNA amplicons ([103-238-800](#))
- Procedure & checklist – Preparing multiplexed AAV SMRTbell libraries using SMRTbell prep kit 3.0 ([102-126-400](#))
- Procedure & checklist – Preparing multiplexed amplicon libraries using PacBio barcoded M13 primers and SMRTbell prep kit 3.0 ([101-921-300](#))
- Procedure & checklist – Preparing multiplexed amplicon libraries using SMRTbell prep kit 3.0 ([102-359-000](#))
- Procedure & checklist – Preparing whole genome and metagenome sequencing libraries using SMRTbell prep kit 3.0 ([102-166-600](#))
- Twist protocol – Long read library preparation and standard hyb v2 enrichment ([DOC-001320](#))



# Revio system applications support documentation

## Application notes & best practices guides

### Whole genome sequencing applications

- Application brief – Whole genome sequencing for de novo assembly – Best Practices ([102-193-627](#))
- Application brief – Variant detection using whole genome sequencing with HiFi reads – Best Practices ([102-193-604](#))
- Application brief – Microbial whole genome sequencing – Best Practices ([102-193-601](#))

### Viral sequencing applications

- Application brief – AAV sequencing – Best Practices ([102-193-502](#))
- Application brief – HiFiViral SARS-CoV-2 for COVID-19 whole genome sequencing – Best Practices ([102-193-692](#))

### RNA sequencing applications

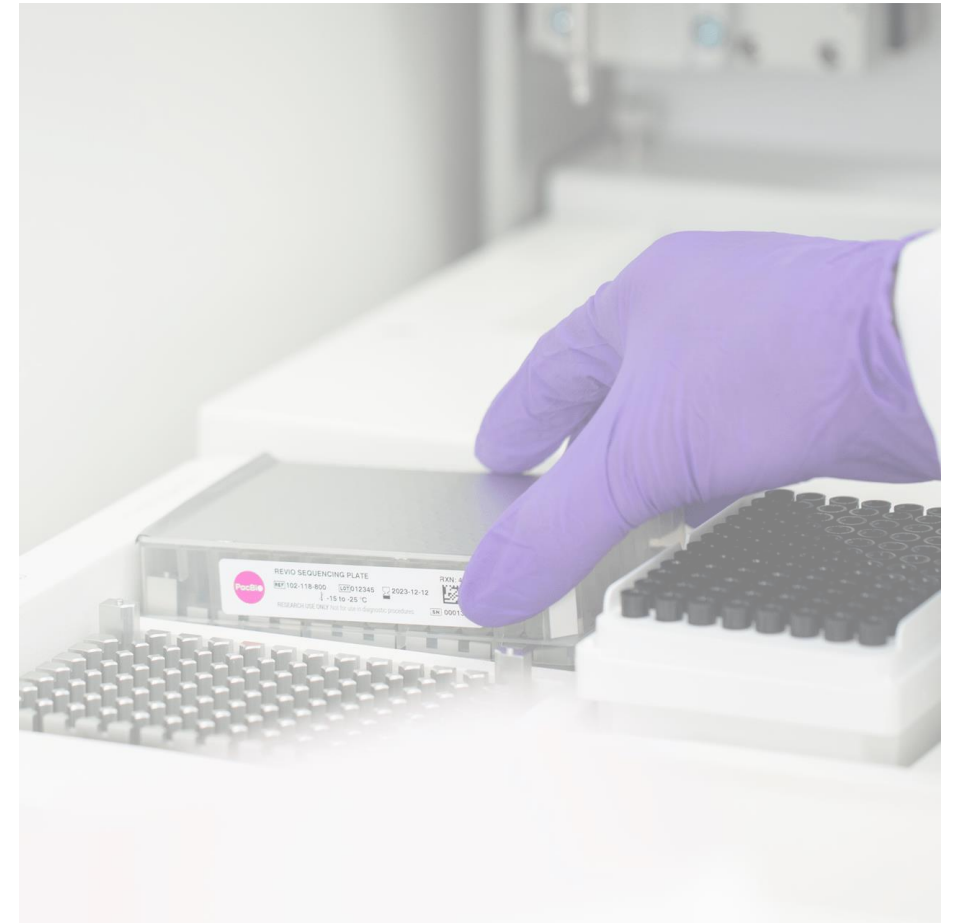
- Application note – Kinnex full-length RNA kit for isoform sequencing ([102-326-591](#))
- Application note – Kinnex single-cell RNA kit for single-cell isoform sequencing ([102-326-549](#))

### Metagenomics applications

- Application brief – Metagenomic sequencing with HiFi reads – Best Practices ([102-193-684](#))
- Application note – Kinnex 16S rRNA kit for full-length 16S sequencing ([102-326-601](#))

### Targeted sequencing applications

- Application brief – HiFi target enrichment – Best practices ([102-326-515](#))
- Application brief – Targeted sequencing for amplicons – Best Practices ([102-193-603](#))





# Revio system applications support documentation (cont.)

## Application technical overviews

- Technical overview – Adeno-associated virus (AAV) library preparation using SMRTbell prep kit 3.0 ([102-390-400](#))
- Technical overview – Iso-Seq library preparation using SMRTbell prep kit 3.0 ([102-393-400](#))
- Technical overview – Kinnex library preparation for 16S rRNA amplicon sequencing ([103-344-800](#))
- Technical overview – Kinnex library preparation using Kinnex full-length RNA kit ([103-344-700](#))
- Technical overview – MAS-Seq library preparation using the MAS-Seq for 10x Single Cell 3' kit ([102-829-300](#))
- Technical overview – Multiplexed amplicon library preparation using SMRTbell prep kit 3.0 ([102-395-900](#))
- Technical overview – Multiplexed SARS-CoV-2 library preparation for full-viral genome sequencing using SMRTbell prep kit 3.0 ([102-399-300](#))
- Technical overview – Nanobind HT kits for automated HMW DNA extraction ([103-020-800](#))
- Technical overview – Whole genome and metagenome library preparation using SMRTbell prep kit 3.0 ([102-390-900](#))





# Revio system applications support documentation (cont.)

## DNA sample extraction literature

- Nanobind HMW DNA extraction [Procedures & checklists](#)
- Nanobind kit [Guides & overviews](#)
- Nanobind HT kit Guide & overview ([103-028-100](#))
- Overview – Nanobind HMW DNA extraction procedures ([102-326-562](#))
- Overview – PacBio Nanobind HT HMW DNA extraction – robotic procedures ([103-032-000](#))
- Technical note – Preparing DNA for PacBio HiFi sequencing – Extraction and quality control ([102-193-651](#))
- Technical note – Sample preparation for PacBio HiFi sequencing from human whole blood ([102-326-500](#))



# PacBio long-read system supported applications & protocols

For Revio system v13, the Revio polymerase kit is optimized for use with all SMRTbell library insert sizes

Sequencing method	Application	Protocol	Sequel II/Ile <sup>1</sup>	Revio <sup>2</sup>
Whole genome sequencing	Whole genome assembly & VC	Preparing whole genome and metagenome libraries using SPK 3.0 [ <a href="#">102-166-600</a> ]	✓ (BK3.2)	✓ (RPK)
	Ultra-Low DNA input	Preparing HiFi SMRTbell Libraries from Ultra-Low DNA Input [ <a href="#">101-987-800</a> ]	✓ (BK3.2)	✓ (RPK)
RNA sequencing	Single-cell transcriptome	Preparing Kinnex libraries using Kinnex single-cell RNA kit [ <a href="#">103-254-300</a> ]	✓ (BK3.2)	✓ (RPK)
		Preparing MAS-Seq libraries using MAS-Seq for 10x 3' concatenation kit [ <a href="#">102-678-600</a> ]	✓ (BK3.2)	✓ (RPK)
	Bulk transcriptome	Preparing Iso-Seq libraries using SPK 3.0 [ <a href="#">102-396-000</a> ]	✓ (BK3.1)	✓ (RPK)
		Preparing Kinnex libraries using Kinnex full-length RNA kit [ <a href="#">103-238-700</a> ]	✓ (BK3.2)	✓ (RPK)
Targeted sequencing	Amplicon ≥3 kb (includes targeted gene panels)	Preparing multiplexed amplicon libraries using SPK 3.0 [ <a href="#">102-359-000</a> ]	✓ (BK3.2)	✓ (RPK)
		Preparing multiplexed amplicon libraries using PacBio barcoded M13 primers and SPK 3.0 [ <a href="#">101-921-300</a> ]	✓ (BK3.2)	✓ (RPK)
	Amplicon <3 kb	Preparing multiplexed amplicon libraries using SPK 3.0 [ <a href="#">102-359-000</a> ]	✓ (BK3.1)	✓ (RPK)
		Preparing multiplexed amplicon libraries using PacBio barcoded M13 primers and SPK 3.0 [ <a href="#">101-921-300</a> ]	✓ (BK3.1)	✓ (RPK)
Viral sequencing	HiFiViral SARS-CoV-2	PacBio HiFiViral high-throughput multiplexing for full-viral genome sequencing of SARS-CoV-2 using SPK 3.0 [ <a href="#">102-396-100</a> ]	✓ (BK3.1)	✓ (RPK)
	Adeno-associated virus (AAV)	Preparing multiplexed AAV SMRTbell libraries using SPK 3.0 [ <a href="#">102-126-400</a> ]	✓ (BK3.1)	✓ (RPK)
Metagenomics	Shotgun metagenome	Preparing whole genome and metagenome libraries using SPK 3.0 [ <a href="#">102-166-600</a> ]	✓ (BK3.2)	✓ (RPK)
	16S	Amplification of bacterial full-length 16S rRNA gene with barcoded primers [ <a href="#">101-599-700</a> ]	✓ (BK3.1)	✓ (RPK)
		Preparing Kinnex libraries from 16s rRNA amplicons [ <a href="#">103-238-800</a> ]	✓ (BK3.2)	✓ (RPK)

<sup>1</sup> For Sequel II and Ile systems, use **BK3.1 = Sequel II binding kit 3.1** or **BK3.2 = Sequel II binding kit 3.2** to perform sample setup (ABC) for all supported applications.

<sup>2</sup> For Revio systems, use **Revio polymerase kit (RPK)** to perform sample setup (ABC) for all supported applications.



# Appendix



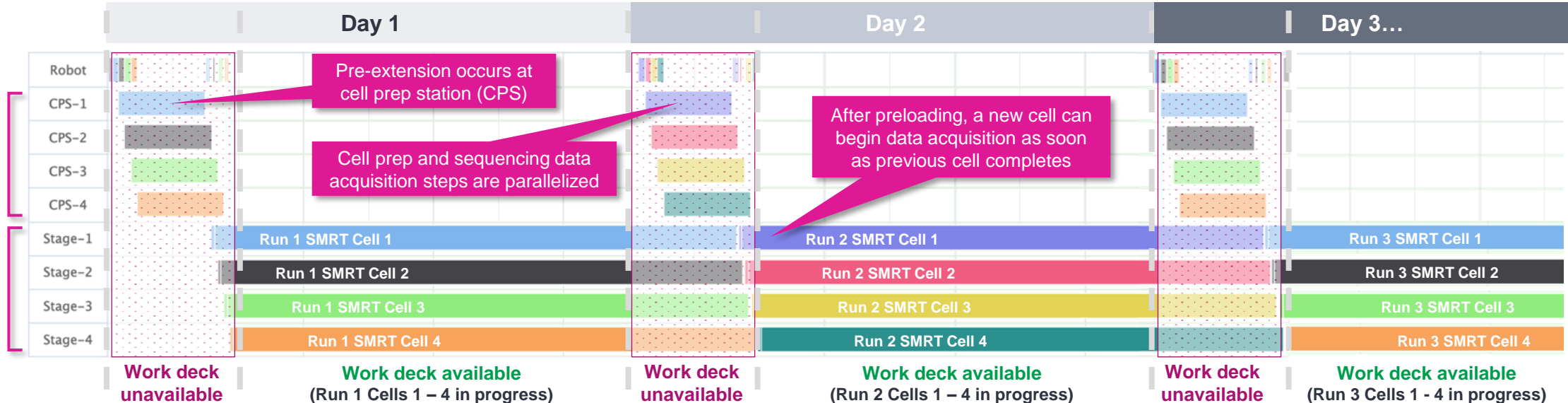
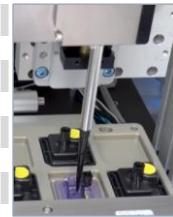
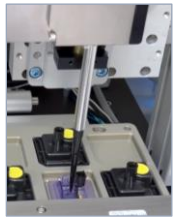
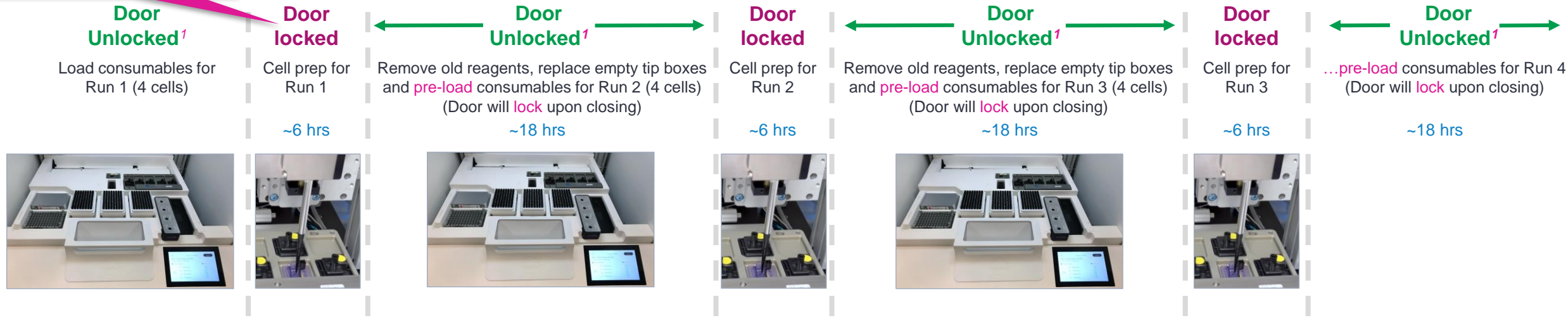
# Example Revio system ICS v13 instrument robotic workflow schedules

# Revio system v13 robotic workflow with adaptive loading (AL) OFF

## Example preloading for 24 hrs movie time with AL OFF: Consecutive 4-SMRT Cell runs

Time to first preload available = ~6 hrs after starting new run

Cell prep and sequencing data acquisition steps are **parallelized** when adaptive loading is turned OFF



<sup>1</sup> Door should always be kept **CLOSED** (even if unlocked) **except** when loading or pre-loading reagents & consumables.  
<sup>2</sup> Door must be closed and locked during any type of robotic activity or when there are unused reagents loaded on work deck.

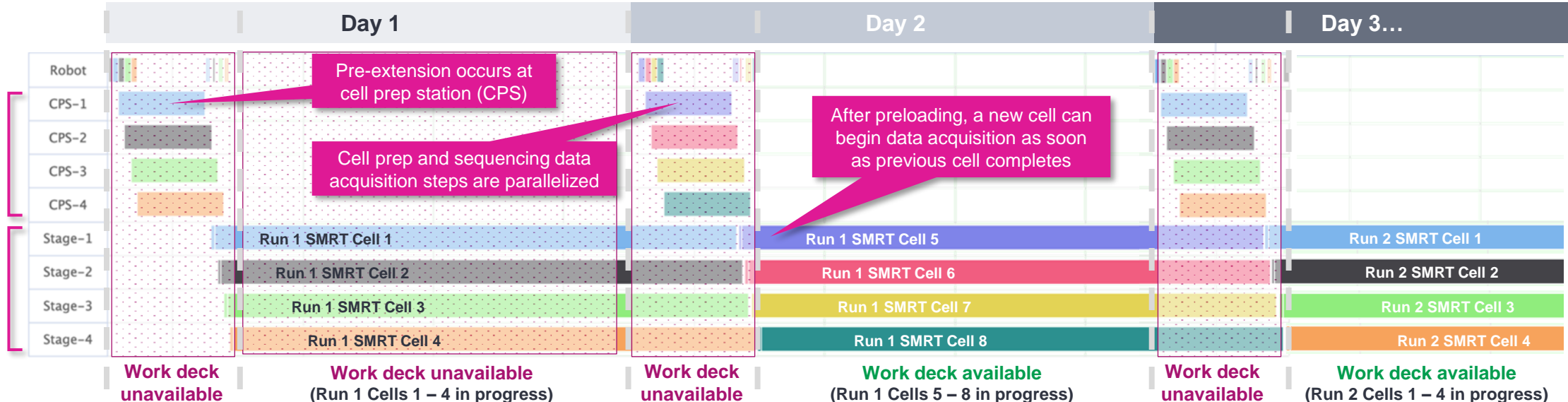
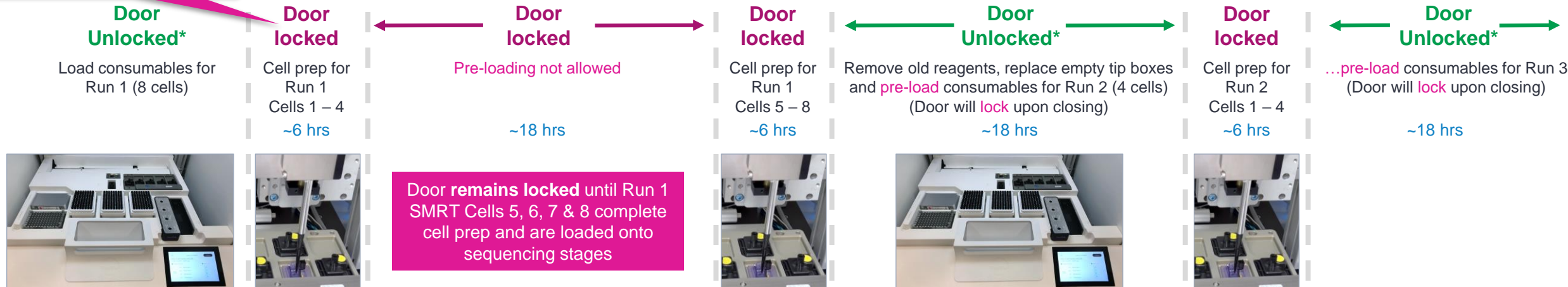


# Revio system v13 robotic workflow with adaptive loading (AL) OFF (cont.)

Example preloading for 24 hrs movie time with AL OFF: 8-SMRT Cell run followed by 4-cell run

Time to first preload available = ~30 hrs after starting new run

Cell prep and sequencing data acquisition steps are **parallelized** when adaptive loading is turned OFF



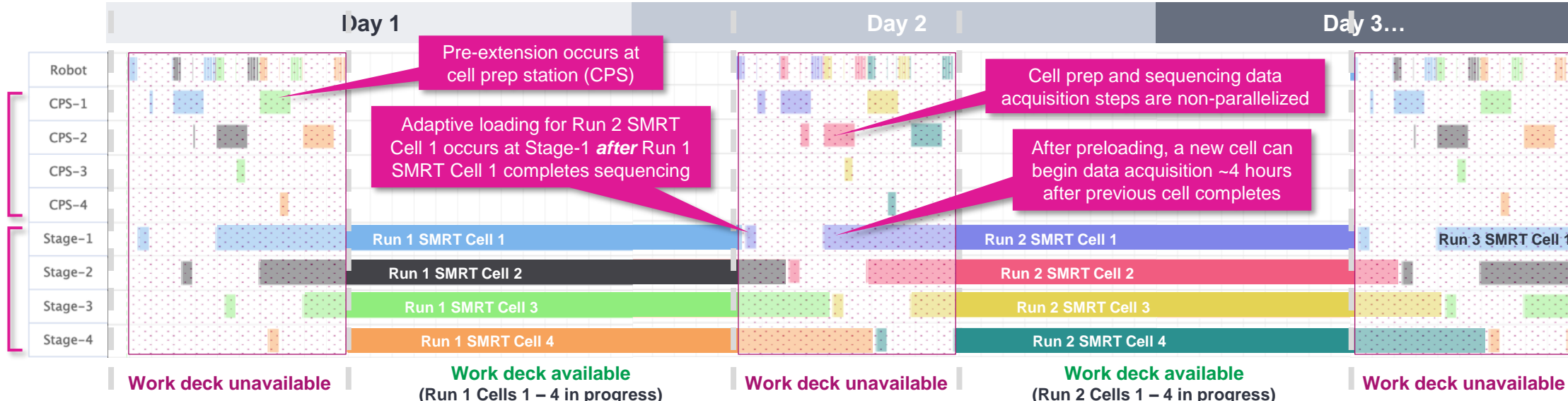
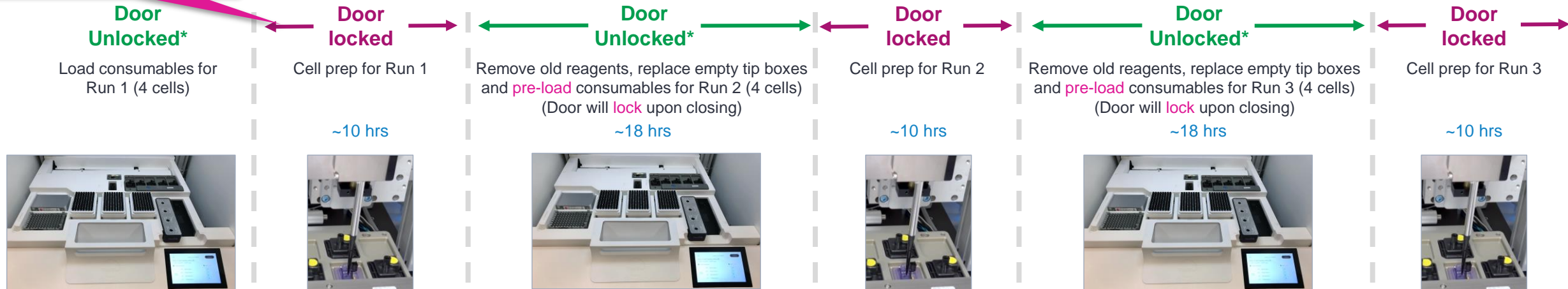
1 Door should always be kept **CLOSED** (even if unlocked) **except** when loading or pre-loading reagents & consumables.  
2 Door must be closed and locked during any type of robotic activity or when there are unused reagents loaded on work deck.

# Revio system v13 robotic workflow with adaptive loading (AL) ON

## Example preloading for 24 hrs movie time with AL ON: Consecutive 4-SMRT Cell runs

Time to first preload available = ~10 hrs after starting new run

Adaptive loading feature adds ~4 hours to time required to process one Revio SMRT Cell due to **non-parallelization** of cell prep and data acquisition steps.



1 Door should always be kept **CLOSED** (even if unlocked) **except** when loading or pre-loading reagents & consumables.  
 2 Door must be closed and locked during any type of robotic activity or when there are unused reagents loaded on work deck.

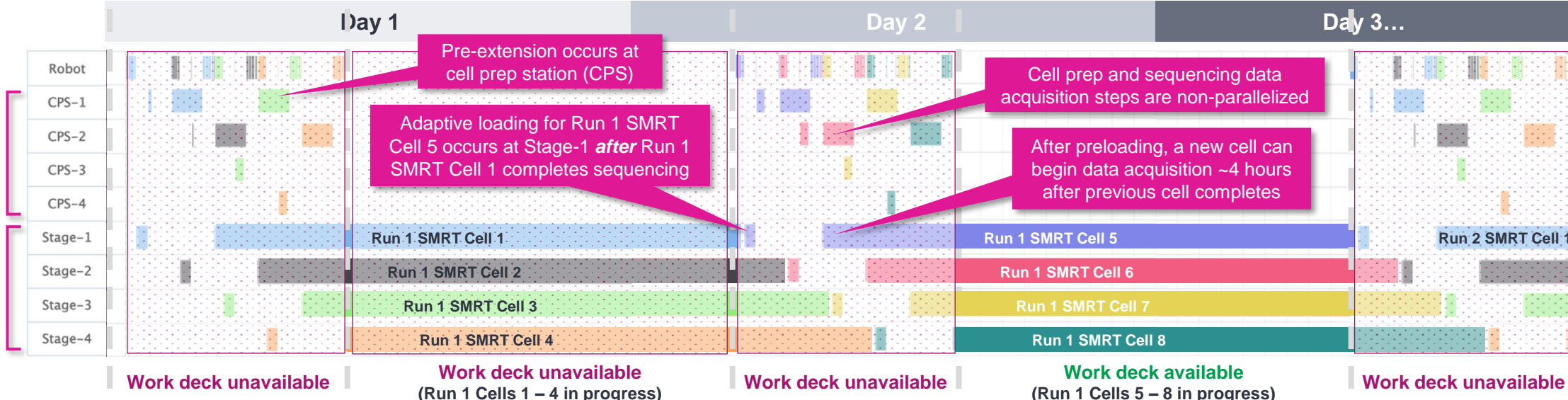
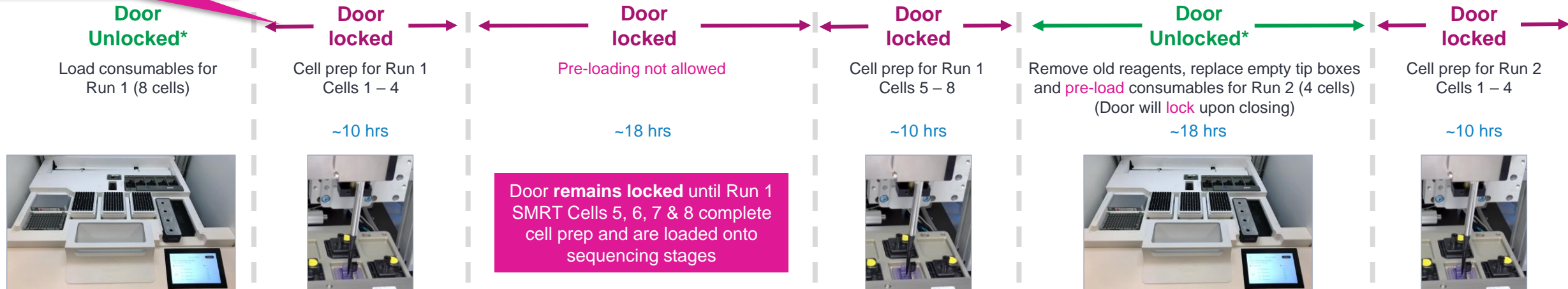


# Revio system v13 robotic workflow with adaptive loading (AL) ON (cont.)

Example preloading for 24 hrs movie time with AL ON: 8-SMRT Cell run followed by 4-cell run

Time to first preload available = ~38 hrs after starting new run

Adaptive loading feature adds ~4 hours to time required to process one Revio SMRT Cell due to **non-parallelization** of cell prep and data acquisition steps.



1 Door should always be kept CLOSED (even if unlocked) except when loading or pre-loading reagents & consumables.  
2 Door must be closed and locked during any type of robotic activity or when there are unused reagents loaded on work deck.



# **SMRT Link v13 installation & configuration settings**

# SMRT Link v13 installation requirements

## Compute requirements for Revio systems and Sequel Ile systems

	Compute requirements	
SMRT Link server	SMRT Link	SMRT Link Lite
<b>CPU cores</b>	32	4
<b>RAM</b>	64 Gb	16 Gb
<b>Local storage</b>	1 TB SSD	500 GB SSD
<b>HPC node (SMRT Link only)</b>	Revio system	Sequel Ile system
<b>Minimum CPU cores</b>	64	
<b>Minimum RAM per core</b>	4 Gb per core (256 GB total)	
<b>Minimum local storage (HDD or SSD)</b>	100 GB SSD or HDD (500 GB preferred as future workflows may use more local disk space.)	
<b>Recommended HPC nodes by application<sup>a</sup> (SMRT Link only)</b>	Per Revio system	Per Sequel Ile system
<b>Human genome</b>	2	1
<b>Single-cell transcriptome</b>	2	1
<b>Large gene panels</b>	1	1
<b>Shared data storage (per year)</b>	Per Revio system	Per Sequel Ile system
<b>Sequencing data<sup>b</sup></b>	72 TB	20 TB
<b>Network connection</b>	Per Revio system	Per Sequel Ile system
<b>Connection speed</b>	1 GbE or 10 GbE copper <sup>c</sup>	

### Revio system data output

- HiFi (without kinetics): ~55 GB compressed hifi\_reads.bam per Revio SMRT Cell
- HiFi + kinetics: ~400 GB per Revio SMRT Cell

- Per Revio system at utilization of 1,300 SMRT Cells per year. Compute is proportional to the number of SMRT Cells run. For example, half the number of nodes is needed at 650 SMRT Cells per year compared to 1,300 SMRT Cells per year. The number of nodes assumes that analysis is performed as data is generated throughout a year.
- Per Revio system. Assumes standard hifi\_reads.bam file without kinetics. Does not include data files produced in data analysis, which approximately doubles the storage requirement.
- For a single Revio SMRT Cell, data transfer takes ~5 – 10 min on a 1 GbE network (~1 minute on a 10 GbE network).

# SMRT Link v13 configuration settings

New Usage Log feature enables administrators to download a log file with information about which user performs each action in SMRT Link



### Usage Log

Download a Log file with information about which user performs each action in SMRT Link

[Download](#)

## Example Usage Log

```
2023-11-02 08:16:13.433UTC [sl-server-akka.actor.default-dispatcher-11] INFO c.p.s.s.app.ServiceLoggingUtils - Logged  
Request:POST:http://localhost:8081/smrt-link/instruments:200 OK:3674 ms (userId=pbinstrument)
```

```
2023-11-02 08:16:13.433UTC [sl-server-akka.actor.default-dispatcher-33] INFO c.p.s.s.app.ServiceLoggingUtils - Logged  
Request:POST:http://localhost:8081/smrt-link/user/preferences:200 OK:34 ms (userId=zali)
```

```
2023-11-02 08:16:13.433UTC [sl-server-akka.actor.default-dispatcher-11] INFO c.p.s.s.app.ServiceLoggingUtils - Logged  
Request:POST:http://localhost:8081/smrt-link/uploader:201 Created:51 ms (userId=zali)
```

```
2023-11-02 08:16:13.433UTC [sl-server-akka.actor.default-dispatcher-7] INFO c.p.s.s.app.ServiceLoggingUtils - Logged  
Request:POST:http://localhost:8081/smrt-link/import-run-design:422 Unprocessable Entity:7 ms (userId=zali)
```

Timestamp

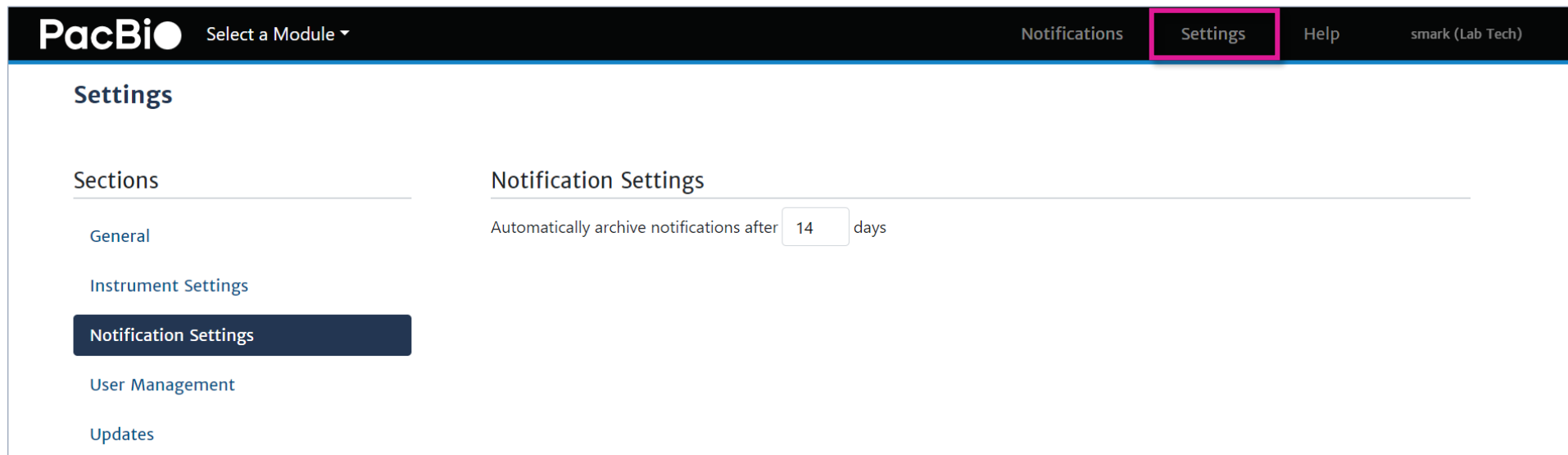
API endpoint

SMRT Link user

# SMRT Link v13 configuration settings (cont.)

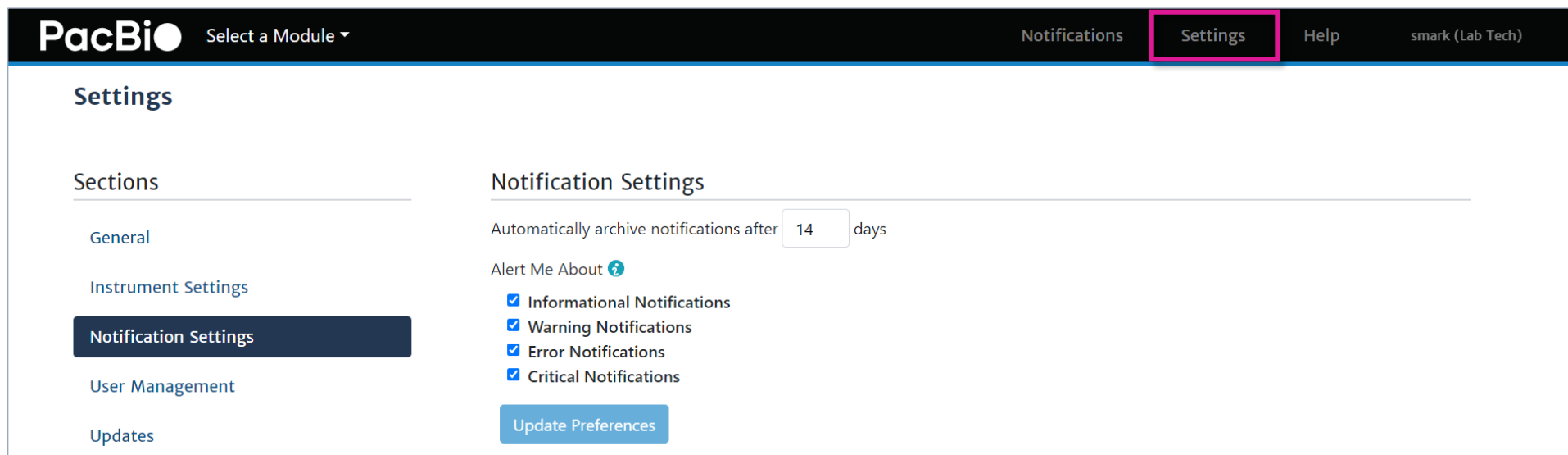
Updated Notification Settings options to enable user preferences

SMRT Link v12.0



The screenshot shows the PacBio Settings page for SMRT Link v12.0. The top navigation bar includes the PacBio logo, a 'Select a Module' dropdown, and links for 'Notifications', 'Settings' (highlighted with a pink box), 'Help', and the user name 'smark (Lab Tech)'. The main content area is titled 'Settings' and features a sidebar with sections: 'General', 'Instrument Settings', 'Notification Settings' (highlighted with a dark blue bar), 'User Management', and 'Updates'. The 'Notification Settings' section is active, showing the option 'Automatically archive notifications after' with a text input field containing '14' and the unit 'days'.

SMRT Link v13



The screenshot shows the PacBio Settings page for SMRT Link v13. The top navigation bar is identical to the v12.0 version, with 'Settings' highlighted in pink. The sidebar is the same, with 'Notification Settings' highlighted in dark blue. The 'Notification Settings' section is active and includes the 'Automatically archive notifications after' field with '14' days. Below this, there is an 'Alert Me About' section with a help icon and four checked checkboxes: 'Informational Notifications', 'Warning Notifications', 'Error Notifications', and 'Critical Notifications'. An 'Update Preferences' button is located at the bottom of this section.



# SMRT Link v13 API documentation

# SMRT Link web services API updates

## SMRT Link v12.0 API → SMRT Link v13 API updates

- New endpoints
  - `GET /smrt-link/runs/{runId}/collections/{collectionId}/reports`  
`GET /smrt-link/runs/{runId}/reports`
- Added a `maxAge` query term to the run, job, and dataset searches, which is internally converted to a search of the corresponding date range
- Allowed user to query using just a date string (e.g., “2020-02-18”) instead of the current UTC string (e.g., “2020-02-18T05:41:43.663Z”)
- Updated the Swagger documentation
- New SMRT Link Python API documentation
  - Python API reference (cookbook) + Python reference client



# SMRT Link web services API updates (cont.)

Added new procedures in SMRT Link v13 web services API use cases documentation (103-234-100)<sup>1</sup>

## SMRT Link v12.0 API



### SMRT® Link web services API use cases (v12.0)

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How to get QC reports for a specific collection .....	15
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How to set up a SMRT Link analysis job for a specific workflow .....	16
How to query job history .....	20
How to copy and rerun a SMRT Link analysis .....	21
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## SMRT Link v13 API



### SMRT® Link web services API use cases (v13.0)

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How to monitor the progress of a SMRT Link run .....	10
How to run jobs using services .....	12
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# SMRT Link Python API resources

SMRT Link Python API reference (v13) (103-285-100)

## Example Python API use cases<sup>1</sup>

### Runs

- Get run(s)
- Get run xml
- Get run collection(s)
- Get run from collection
- Get run collection reports
- Get run collection barcodes
- Get run collection hifi reads
- Get run collection hifi reads barcoded datasets
- Get run reports
- Get run design
- Import run design CSV
- Delete run
- Import run xml
- Update run xml

### Chemistry bundles

- Get active bundle metadata
- Get chemistry bundle metadata
- Get active bundle file
- Get chemistry bundle file

### Jobs

- Get job
- Get job reports
- Download job report resources
- Get job datastore
- Get job entry points
- Get job datasets
- Get job options
- Download job datastore file
- Get analysis jobs
- Get analysis jobs by state
- Get analysis jobs by parent
- Get SMRT Analysis nested jobs
- Create analysis job
- Terminate analysis job
- Get import dataset jobs
- Create import dataset [zip] job
- Create import collection job
- Create merge datasets job
- Get pipeline(s)
- Poll for successful job

### Datasets

- Get consensus read sets
- Get consensus read sets by movie
- Get barcoded child datasets
- Get subread sets
- Get references
- Get barcode sets
- Get consensus read set
- Get subread set
- Get reference set
- Get barcode set
- Get consensus read set reports
- Get barcode set contents
- Get barcode set record names
- Get dataset metadata
- Get dataset jobs
- Get dataset search

# SMRT Link Python API resources (cont.)

API reference “cookbook” example: *How to find a run (or multiple runs) + run details using the SMRT Link API*

## Example request:

```
1 GET /smrt-link/runs
```

Python reference client: `get_runs()` method – gets a list of all PacBio instrument runs, with optional search parameters.

## Python example:

```
1 runs = client.get_runs(name=None, reserved=None, instrumentType=None,  
2                           chipType=None, collectionUuid=None, movieName=None)  
3  
4 '''Example request:  
5 runs = client.get_runs(instrumentType = "Revio)'''
```

## Return value definitions:

`reserved = True` means that the Run Design was used on an instrument

`startedAt` is the time at which user pressed the button to close the instrument door and begin a run.

`completedAt` is recorded once all of the instrument’s movies have completed acquiring and after the cleanup

`uniqueId` is the Run UUID you see in the URL on the Run Details page

## Example return:

```
1 {  
2     "reserved": true,  
3     "numLRCells": 0,  
4     "name": "20230414_84026_225pM_16kbHG002_97Barcodes_24hPE_dT6_snrG4p4_pkmidCeil650",  
5     "completedAt": "2023-04-17T04:19:50.718Z",  
6     "chemistrySwVersion": "12.1.0.SNAPSHOT172286",  
7     "instrumentType": "Revio",  
8     "chipType": "25mChip",  
9     "instrumentName": "84026",  
10    "context": "r84026_20230414_212018",  
11    "instrumentSwVersion": "12.1.0.SNAPSHOT177608",  
12    "numCellsCompleted": 1,  
13    "totalCells": 1,  
14    "primaryAnalysisSwVersion": "12.0.0.1",  
15    "status": "Complete",  
16    "numStandardCells": 1,  
17    "createdAt": "2023-04-14T18:17:23.849Z",  
18    "startedAt": "2023-04-14T21:20:44.057Z",  
19    "createdBy": "admin",  
20    "totalSamples": 97,  
21    "numCellsFailed": 0,  
22    "plate2": "1021188000301580073920231018",  
23    "instrumentSerialNumber": "84026",  
24    "transfersCompletedAt": "2023-04-18T00:13:42.032Z",  
25    "uniqueId": "0f99fea6-5916-4142-9b82-220a7bb04d13",  
26    "ccsExecutionMode": "OnInstrument"  
27 },  
28
```



# Revio system v13 / SMRT Link v13 fixed & known issues

# Revio system v13 fixed & known issues

## Revio system v13 fixed issues

- Modified the behavior of the instrument's Door button so that the button is not lit during startup diagnostics.
- Fixed an issue that sometimes caused the touchscreen to be resized using multitouch gestures.
- Fixed an issue that sometimes caused user-initiated self-tests to fail.
- Fixed an issue that sometimes caused warning messages to persist to a subsequent run.
- Fixed an issue that sometimes caused the incorrect SMRT Cell identifier (e.g. EA010390) to be displayed in SMRT Link.
  - This fix applies only to future runs.
- Improved the reliability of communications between Revio systems and a connected SMRT Link server over DHCP or static IP network connections.
  - SMRT Link will automatically update Revio system IP addresses when they are changed using the instrument network connection settings.
- Added a timeout to the file transfer test performed when loading and launching a run.
- Fixed an issue where run files were sometimes deleted when post-primary analysis failed. Files are now retained to allow recovery. Contact PacBio Technical Support when sequencing completes, but post-primary analysis fails.
- Individual sequencing stage failures no longer prevent sequencing runs from starting, as long as at least one sequencing stage is available.
- Fixed an issue that sometimes prevented users from pre-loading runs in certain conditions.
- Fixed an issue with compute resource utilization in the primary analysis basecaller that contributed to some collections ending early.

# Revio system v13 fixed & known issues (cont.)

## Revio system v13 known issues

- At times the touchscreen may freeze and be unresponsive during initialization.
  - The workaround is to hard reset the instrument to restart initialization by either pressing the power button, or by unplugging the instrument.
  - Contact PacBio Technical Support if the issue persists

# SMRT Link v13 fixed & known issues

## SMRT Link v13 fixed issues

- Fixed an issue that sometimes caused reserved characters to be incorrectly encoded when used in the Edit Output Filename Prefix dialog.
  - This made it impossible for the output files to be downloaded.
- Fixed an issue that sometimes caused the Iso-Seq workflow to request an incorrect amount of memory.
- Fixed an issue that sometimes caused a Microbial Genome Analysis job with Find Modified Base Motifs set to ON to fail.
- Fixed an issue that sometimes caused an edited run design to not be saved.
- Fixed an issue where sometimes clicking Display All Enabled Users caused an error.
- Fixed an issue that sometimes caused the pbservice import-run command to not post a Run Design CSV file.
- Revio, Runs module: Fixed an issue that sometime caused Time Remaining for PostProcessing to not display correctly.
- Revio: Improved the reliability of communications between a Revio system and a connected SMRT Link server over DHCP or static IP network connections. SMRT Link automatically updates Revio system IP addresses when the instrument network connection settings are changed.
- Revio: Added a timeout to the file transfer test performed when loading and launching a run design.
- Instruments module: Time until preload is available was updated to show Time until door unlocks to more accurately reflect the information relayed by the countdown timer. Generally, preloading a subsequent run will be available when the door unlocks, except in cases where there is an instrument issue that requires attention.



# SMRT Link v13 fixed & known issues (cont.)

## SMRT Link v13 known issues

### SMRT Link

- When connecting a new instrument or switching to a different SMRT Link server, it may take a minute or two for update to display in GUI.

### SMRT Analysis module

- Demultiplexing more than one dataset using the One Analysis Per Dataset – Custom parameters option, the Demultiplexed Output Data Set Name may be incorrectly set for datasets 2 and above.
  - The workaround is to manually edit the Output Data Set Name.
- The HiFi Target Enrichment analysis workflow does not progress when the read number is too low.
- To ensure compatibility with third-party tools and formats, the HiFi Target Enrichment workflow replaces space characters in the Bio Sample Name of input datasets with underscores.

### Data Management module

- When merging barcoded Iso-Seq datasets for later downstream analysis, the sample names of the input datasets should be the same so that the summary metrics can display in the output. When sample names do not match, the summary metrics may not display correctly.

### Runs Module

- When a run is specified with Adaptive Loading set to NO, the Loading Time column on the run information page displays as empty.
- When creating a new run design, Adaptive Loading is a run-level setting, but is displayed as a sample level setting in the GUI. If Adaptive Loading is set differently for different samples, only one value is saved.
- The Pre-Extension Time column on the run information page displays as empty for Revio system runs.
- When duplicating a run design, some analysis names and associated workflows sometimes may not be copied correctly.

# SMRT Link v13 fixed & known issues (cont.)

## SMRT Link v13 known issues (cont.)

### Run preview feature

- The 4-hour preview metrics are missing for runs that use a Consensus Mode of Strand. The preview timepoint is also displayed incorrectly as 1-hr before the end of acquisition instead of 4-hrs in the run information table.
- The Latest value in the Active, % plot may be incorrect for some runs.
- Updating the SMRT Link server during an active run may sometimes cause the loss of preview metrics.



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