Technical overview

Revio system v13.5 + SPRQ-Nx chemistry and SMRT Link v26.1

Revio system ICS v13.5
SMRT Link v26.1

PN 103-849-300 Rev 01 | May 2026

Technical overview

Revio system ICS v13.5 + SPRQ-Nx chemistry and SMRT Link v26.1

§ Part 1: Revio system v13.5 + SPRQ-Nx chemistry

1. Revio system v13.5 key features & benefits
2. New Revio SPRQ-Nx consumables and instrument control software
3. Revio multi-use SMRT Cell workflow overview
4. Revio system v13.5 user experience improvements
5. Revio system v13.5 example sequencing performance

§ Part 2: SMRT Link v26.1 + SMRT Link Cloud v26.1

1. SMRT Link v26.1 key features & benefits
2. New SPRQ-Nx chemistry support
3. New SMRT Link v26.1 audit trail support
4. SMRT Link v26.1 user experience improvements
    - Improved asymmetric barcode demultiplexing support
    - Improved Ampli-Fi run design support
    - Improved methylation reports

§ Part 3: Technical documentation & applications support resources

§ Part 4: Appendix

§ Part 1:

Revio system v13.5 + SPRQ-Nx

1. Revio system v13.5 key features & benefits
2. New Revio SPRQ-Nx consumables & ICS
3. Multi-use SMRT Cell workflow overview
4. Revio system v13.5 user experience improvements
5. Revio system v13.5 example sequencing performance

Revio system v13.5 key features and benefits

What is staying the same in Revio system v13.5?

No major changes to core HiFi library prep kits / sequencing prep workflow and Revio instrument hardware / data file formats

HiFi library prep workflow

Continue to use existing SMRTbell prep kit 3.0 / HiFi prep kit 96 / HiFi plex prep kit 96 / Kinnex / PureTarget 2.0 products

Sequencing prep workflow

Continue to follow existing sample setup ABC instructions provided in HiFi library prep protocols

Revio instrument hardware and run setup

No changes to sequencing stage design and work deck loading steps

Revio data file formats

No changes to hifi_reads.bam + fail_reads.bam file structures

What is new in Revio system v13.5?

New Revio system v13.5 with SPRQ-Nx chemistry makes HiFi sequencing easier and more cost-effective

Lower-cost HiFi genomes

New multi-use SMRT Cells enable cost-effective HiFi sequencing projects at scale

Richer multiomic data

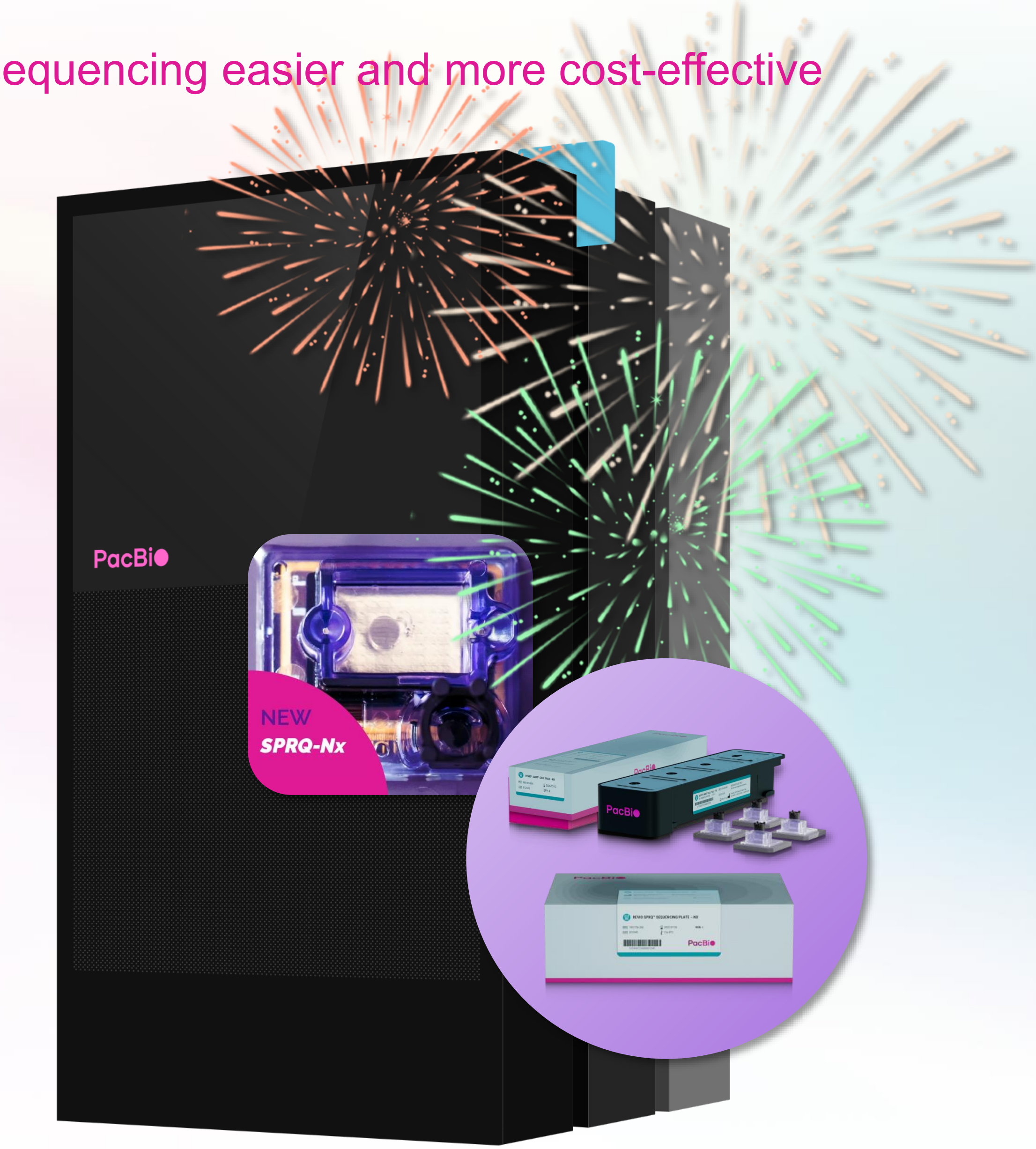
New 5hmC caller expands Revio system on-instrument methylation detection capabilities beyond 5mC and 6mA

More accuracy

Revio system v13.5 improves HiFi sequencing accuracy and 5mC calling performance in CpG contexts

Stronger compliance support

New Revio system ICS v13.5 + SMRT Link v26.1 software enables detailed audit logging of user actions[1]



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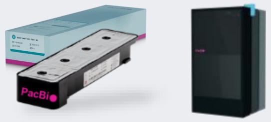
[1] New audit logging feature is available for both Revio system v13.5 and Vega system v1.1 with SMRT Link v26.1.

Revio system v13.5 key workflow updates

Revio workflow step	What's new		What stays the same
DNA sample extraction		N/A	• Existing Nanobind HMW DNA extraction workflows • Existing Nanobind kits
SMRTbell library preparation		N/A	• Core SMRTbell library construction workflow • Existing HiFi library preparation kits • Existing applications support
Sample setup (ABC)		N/A	• Core Revio sample setup ABC workflow • Existing applications support • Existing Revio SPRQ polymerase kit
Run design		• SMRT Link v26.1 run design adds support for new Revio SPRQ sequencing plate-Nx	• Overall Revio run design workflow • Existing applications support
Sequencing		• Revio ICS v13.5 adds support for new Revio SPRQ sequencing plate-Nx chemistry and multi-use Revio SMRT Cell tray-Nx • Revio ICS v13.5 adds support for new on-instrument 5hmC calling	• Overall Revio run setup workflow • Existing on-instrument analysis workflow

[1] ABC = Primer annealing + polymerase binding + complex cleanup steps in Revio sample setup workflow.

Revio system v13.5 key software updates

New ICS and SMRT Link software enable support for Revio SPRQ-Nx chemistry

Revio system supported software

Revio ICS v13.5[1]		SMRT Link v26.1[2]		
Instrument control	- Minor ICS software bug fixes - Updated robotic workflow to support multi-use SMRT Cells	Instruments	- User interface improvements to include multi-use SMRT Cell info	Audit logging support
Primary analysis	- Added support for new Revio SPRQ-Nx sequencing chemistry[3]	Sample Setup	No major changes	
On-instrument CCS analysis	- Updated consensus basecalling model (DeepConsensus) to support new Revio SPRQ-Nx sequencing chemistry[3]	Runs	- Run design support for new Revio SPRQ sequencing plate – Nx[3] - User interface improvements to include multi-use SMRT Cell info	
On-instrument methylation calling	- Improved accuracy and increased confidence of 5mCpG calling - Added on-instrument 5hmC caller	Data Management	No major changes	
On-instrument barcode demux	No major changes	SMRT Analysis	- Improved asymmetric barcode demultiplexing support - Improved methylation reports	

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[1] See Revio system v13.5 release notes (103-854-800) for latest ICS software update details.

[2] SMRT Link v26.1 supports Revio system ICS v13.5 and Vega system ICS v1.1. SMRT Link v26.1 does not support Sequel II/IIe systems.

[3] Revio system ICS v13.5 retains support for original Revio sequencing chemistry (‘v1’) and Revio SPRQ chemistry.

New Revio SPRQ-Nx consumables & instrument control software

Revio system v13.5 release includes new SPRQ-Nx consumables & ICS

New consumables and instrument control software enable an improved user experience for HiFi sequencing

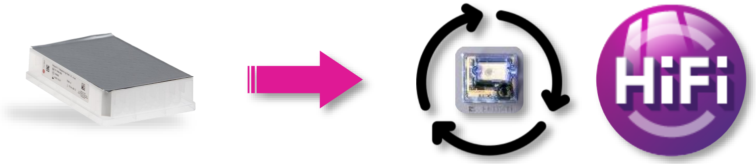
Revio SPRQ sequencing plate – Nx

PN 103-726-200 (4-rxn)

SPRQ chemistry upgrade

multi-use SMRT Cell support

New SPRQ-Nx chemistry supports multi-use SMRT Cells and provides excellent HiFi sequencing performance[1]



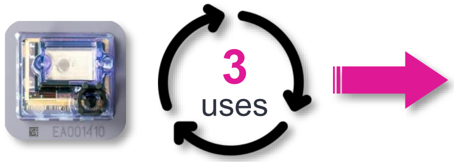
Revio SMRT Cell tray – Nx

PN 103-483-800 (4 SMRT Cells)

Multi-use SMRT Cells

lower \$/genome + simpler workflow

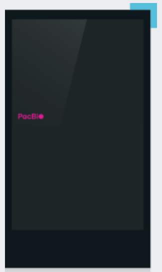
New multi-use SMRT Cells can be used for up to 3 acquisitions to enable lower cost per genome[2]



~\$690 per acquisition

(~\$345 / genome)

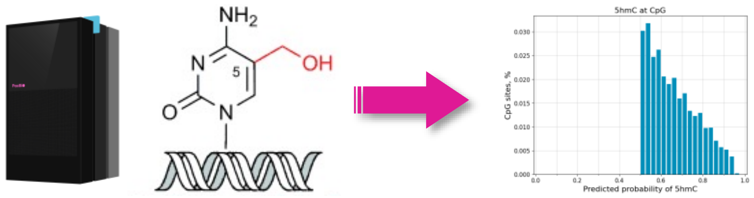
Revio ICS v13.5



Richer multiomic data

5hmC along with 5mC and 6mA

Revio ICS v13.5 improves 5mC + 6mA calling accuracy and features new on-instrument 5hmC calling[3]



5-hydroxymethylcytosine
(5hmC)

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[1] Revio SPRQ-Nx sequencing plate chemistry supports all existing HiFi applications.

[2] \$345 / genome cost shown is calculated based on sequencing 2 human genomes per acquisition to achieve 20X HiFi coverage per genome.

[3] 5-hydroxymethylcytosine (5hmC) is enriched in neuronal and developmental tissues, where it contributes to cell-type-specific gene expression patterns.

New Revio SPRQ-Nx consumables packaging and kit configurations

New Revio SPRQ-Nx consumables product packaging features white-colored boxes with magenta trim to easily distinguish them from old “single-use” Revio SPRQ consumables

	Old “single-use” Revio SPRQ consumables[1]		***NEW* Revio SPRQ-Nx consumables**	
Polymerase kit		Revio SPRQ polymerase kit 103-520-100		Revio SPRQ polymerase kit 103-520-100
Sequencing plate		Revio SPRQ sequencing plate 103-504-900 (4-rxn)		**Revio SPRQ sequencing plate – Nx[2]** 103-726-200 (4-rxn)
SMRT Cell tray		Revio SMRT Cell tray 102-202-200 (4 single-use SMRT Cells)		**Revio SMRT Cell tray – Nx** 103-483-800 (4 multi-use SMRT Cells)
24-pack reagent kit		Revio SPRQ reagent kit, 24-pack 103-520-200 (24 acquisitions) Contains: (1) Revio SPRQ polymerase kit, (6) Revio SPRQ sequencing plate (6) Revio SMRT Cell tray (1) SMRTbell cleanup beads (10 mL)		**Revio SPRQ-Nx reagent kit, 24-pack** 103-748-700 (24 acquisitions) Contains: (1) Revio SPRQ polymerase kit, (6) Revio SPRQ sequencing plate – Nx, (2) Revio SMRT Cell tray – Nx (1) SMRTbell cleanup beads (10 mL)

SPRQ-Nx packaging is **white-colored with magenta trim**

Continue to use **existing** SPRQ polymerase kit

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[1] Old “Single-use” Revio SPRQ consumables will remain available for purchase.

[2] Revio SPRQ-Nx sequencing reagent plates can be stored at 4°C for up to two weeks after first use (each reagent plate consumable can only be used twice).

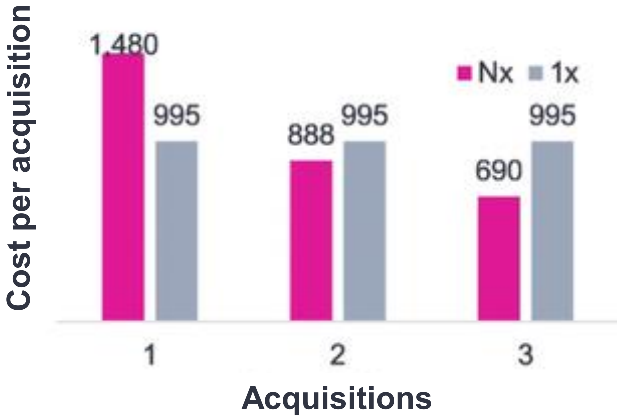
Enhanced economics of HiFi long-read sequencing with SPRQ-Nx (cont.)

With SPRQ-Nx chemistry, multi-use SMRT Cells on the Revio system can lower your sequencing cost by ~30% versus single-use SMRT Cells

SPRQ-Nx pricing benefits increase with the number of uses

- SPRQ-Nx is more expensive for 1st use
- SPRQ-Nx is less expensive at 2 uses
- SPRQ-Nx is least expensive at 3 uses

~$690 per acquisition
(~$345 / genome)



SPRQ-Nx makes large-scale HiFi projects more financially accessible than ever before

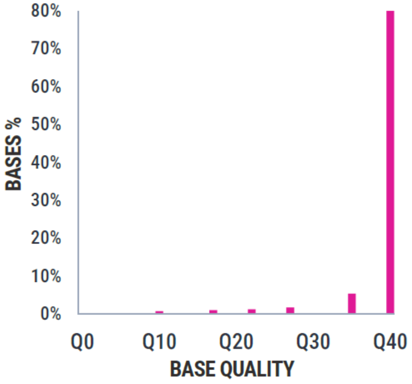
Example HiFi sequencing costs[1] for a hypothetical 10,000-sample human whole genome study at 20× coverage.

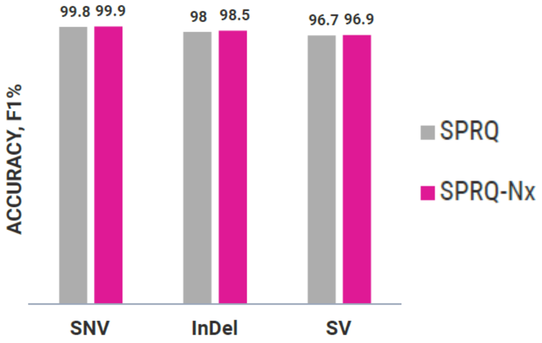
	Revio SPRQ (single-use)	Revio SPRQ-Nx (multi-use)	Cost benefit at scale[1]
Cost per human genome (20×)	~$500	~$345	**~30% savings in reagent cost** → ~$1.55 M in cost savings for this example project
Total reagent cost	~$5 M	~$3.45 M	

Study design, sample type, and level of multiplexing may affect the number of SMRT Cells required. All prices are listed in USD and cost may vary by region. Pricing includes sequencing reagents run on your system and does not include instrument amortization or other reagents. Talk to your local PacBio representative for your local pricing.

SPRQ-Nx provides low HiFi sequencing cost without compromising accuracy

Data shown is for a single Revio SMRT Cell acquisition for HG002/GM24385 sequenced with SPRQ or SPRQ-Nx chemistry





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[1] Cost savings and benefits are calculated based on 3 acquisitions (uses) per multi-use Revio SMRT Cell using Revio SPRQ-Nx chemistry and pooling two human genomes per acquisition to achieve 20X coverage per genome. See ***Application brief – Head-to-head performance and economics of SPRQ-Nx*** (102-326-664)

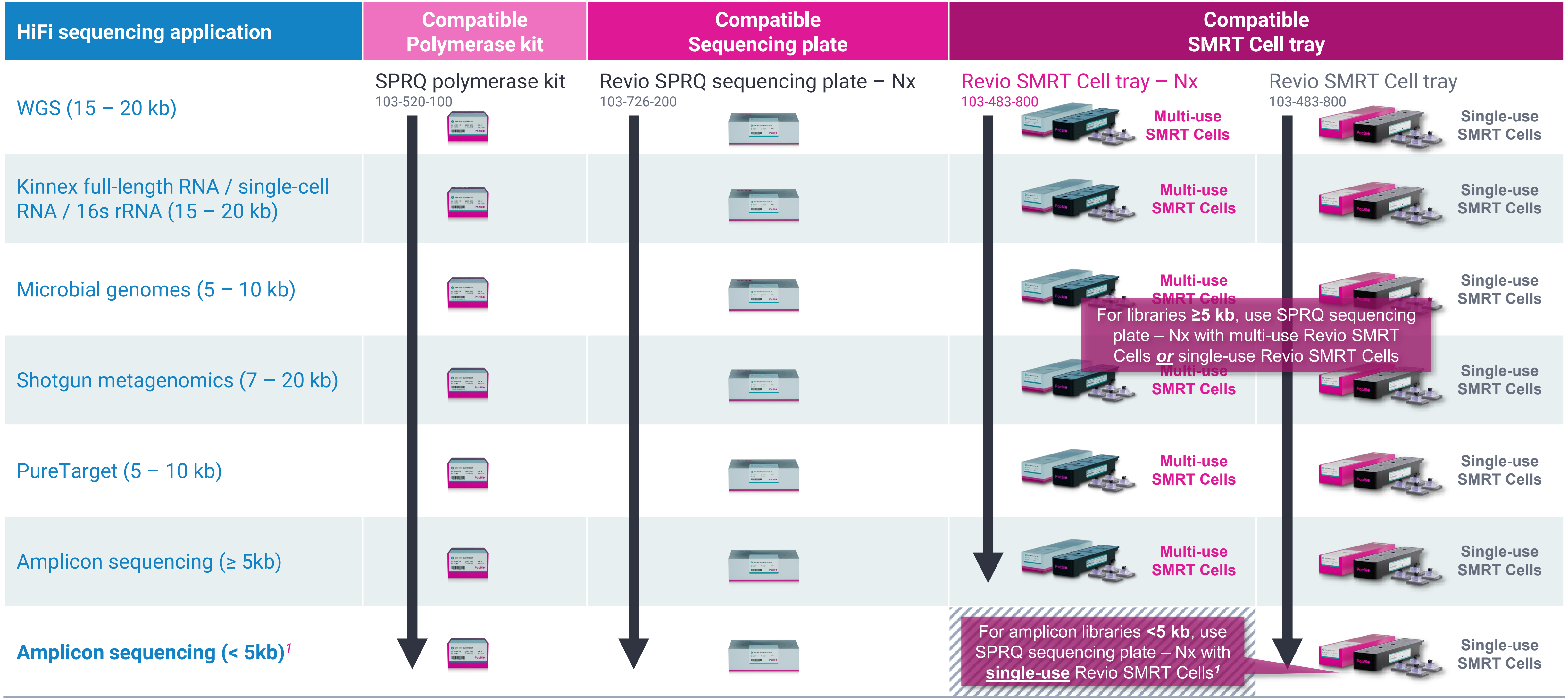
Revio SPRQ *versus* SPRQ-Nx consumable cross-compatibility

Revio SPRQ-Nx sequencing plate chemistry is compatible with both single-use Revio SMRT Cells and multi-use Revio SMRT Cells

	Revio SPRQ sequencing plate **103-504-900** 	**Revio SPRQ sequencing plate – Nx** **103-726-200** 
Revio SMRT Cell tray **(single-use SMRT Cells)** **102-202-200** 	✓	✓
Revio SMRT Cell tray – Nx **(multi-use SMRT Cells)** **103-483-800** 	✗	✓

Revio SPRQ-Nx sequencing plate chemistry supports all existing applications

Note: For libraries **<5 kb**, use Revio SPRQ sequencing plate – Nx with single-use Revio SMRT Cells[1]



HiFi sequencing application	Compatible Polymerase kit	Compatible Sequencing plate	Compatible SMRT Cell tray	
	SPRQ polymerase kit 103-520-100	Revio SPRQ sequencing plate – Nx 103-726-200	Revio SMRT Cell tray – Nx 103-483-800	Revio SMRT Cell tray 103-483-800
WGS (15 – 20 kb)			**Multi-use SMRT Cells**	**Single-use SMRT Cells**
Kinnex full-length RNA / single-cell RNA / 16s rRNA (15 – 20 kb)			**Multi-use SMRT Cells**	**Single-use SMRT Cells**
Microbial genomes (5 – 10 kb)			**Multi-use SMRT Cells**	**Single-use SMRT Cells**
Shotgun metagenomics (7 – 20 kb)			**Multi-use SMRT Cells**	**Single-use SMRT Cells**
PureTarget (5 – 10 kb)			**Multi-use SMRT Cells**	**Single-use SMRT Cells**
Amplicon sequencing (≥ 5kb)			**Multi-use SMRT Cells**	**Single-use SMRT Cells**
Amplicon sequencing (< 5kb)[1]			For amplicon libraries **<5 kb**, use SPRQ sequencing plate – Nx with **single-use** Revio SMRT Cells[1]	**Single-use SMRT Cells**

For libraries **≥5 kb**, use SPRQ sequencing plate – Nx with multi-use Revio SMRT Cells ***or*** single-use Revio SMRT Cells

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[1] For amplicon libraries <5 kb, using Revio SPRQ sequencing plate with multi-use Revio SMRT Cells may result in reduced P1/.HiFi yield performance for Use 2 and/or Use 3.

Revio system v13.5 DeepConsensus improves HiFi sequencing accuracy

Improved DeepConsensus model[1] results in more usable high-quality reads per run, more reliable quality values, and faster time to results

Updated DeepConsensus model delivers:

- Increased percentage of reads that achieve empirical Q30 accuracy from 47.9% to 53.2% (+5.3% of reads)
- Improved quality calibration, particularly near the Q20 HiFi threshold

Additional software improvements enable faster data processing times and increased HiFi data throughput to ~18 Gb/hour through:

- Model optimization to reduce computational overhead
- Floating point quantization to accelerate inference while maintaining accuracy
- Adoption of updated inference frameworks such as ONNX (Open Neural Network Exchange) for improved hardware utilization

PacBio | Google Research

HiFi yield distribution by empirical quality binning for Revio system data generated with SPRQ – Nx chemistry

- Revio v13.5 DeepConsensus model is trained on both SPRQ and SPRQ-Nx data
- Both SPRQ and SPRQ-Nx chemistry perform similarly with Revio 13.5 with no major change in error modes from Revio 13.3

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[1] See ***Improving HiFi sequencing accuracy with Google DeepConsensus and AlphaEvolve*** for further details about DeepConsensus model improvements enabled by Google's AlphaEvolve AI coding agent (PacBio BLOG, April 30, 2026).

[2] RevRevio v13.3 DeepConsensus model is trained with SPRQ chemistry. RevRevio v13.5 DeepConsensus model is trained with SPRQ chemistry and SPRQ-Nx chemistry.

Revio system v13.5 improves methylation detection capabilities

Updated methylation analysis software supports 5hmC calling

Revio on-instrument methylation analysis

Jasmine v26.1.3

Available now in GitHub

https://github.com/PacificBiosciences/jasmine

- New models for Revio SPRQ-Nx chemistry
- 5-methylcytosine (5mC in CpG) calls more strongly match bisulfite data
- Improved 6mA strand calling annotation[1]
- New 5-Hydroxymethylcytosine (5hmC in CpG) caller

Off-instrument methylation analysis

MethBat v1.0.0

Available now in GitHub

https://github.com/PacificBiosciences/methbat

- MethBat secondary analysis tool aggregates and analyzes CpG methylation calls made from PacBio HiFi datasets[2]
  - Now includes 5mC, 6mA, and 5hmC pileup functionality
- **Note:** pb-CpG-tools will not undergo further development and will be **deprecated in May 2026**[3]
- If using Jasmine version ≥26.x
  - → Recommend to use **MethBat tool** since it supports analysis of all methylation types
- If using Jasmine version <26.x
  - → Can continue to use **pb-CpG-tools**

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[1] Balanced calling of 6mA on both strands. The prior caller had slightly higher recall for A in forward strand compared to T in reverse strand..

[2] See MethBat GitHub page for more details about methylation secondary analysis features, including: Creation of a methylation profile for a collection of genomic regions (e.g., CpG islands) Creation of cohort / background profiles from a collection of methylation profiles.

[3] pb-CpG-tools will remain available for at least the remainder of 2026.

PacBio

Revio system v13.5 performance specifications remain unchanged with SPRQ-Nx chemistry

Revio system v13.5 data throughput

Library	Run time[1]	Q30+ bases	HiFi yield per acquisition[2]	Methylation
0.5–5 kb	12 hours	95%	6 – 8 Million reads	5mC + 5hmC at CpG sites and 6mA[3] for native DNA
5–10 kb	24 hours	90%	35 – 70 Gb	
10–15 kb			70 – 100 Gb	
15–20 kb			100 – 120 Gb	
20–25 kb	30 hours	85%	100 – 120 Gb	

[1] Run time refers to the data collection step, which determines the time between processing SMRT Cell acquisitions.

[2] HiFi yield is dependent on library quality and sequencing preparation procedures. Specified yield is based on high-quality samples prepared following best practices.

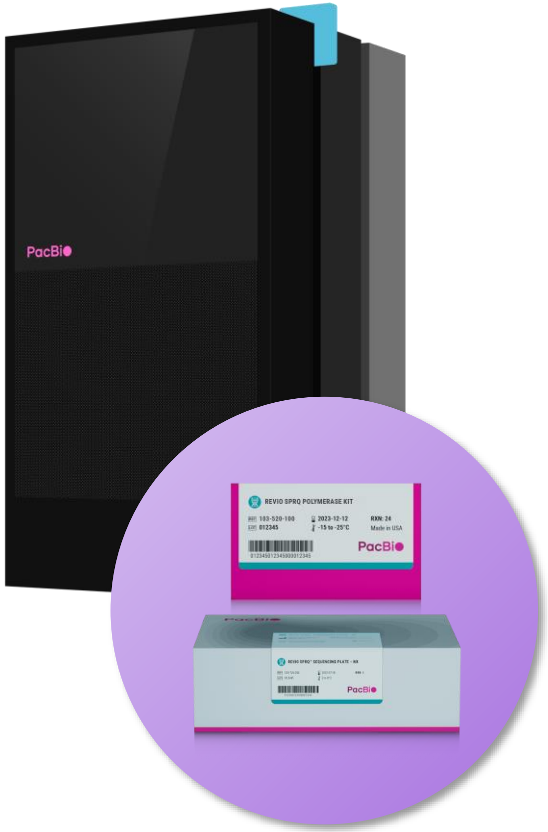
[3] The 6mA caller is designed to detect methylation in the context of the Fiber-seq chromatin assay.

Revio system v13.5 key applications and sample throughput

Library	Sample	Expected coverage[4]	Samples per acquisition	Samples per year[5]
0.5–5 kb	Amplicon sequencing	50×	>1,000	>2.5M
5–10 kb	Microbial genome	50×	384	480,000
5–10 kb	PureTarget repeat expansion panel	200×	48	60,000
5–10 kb	Ampli-Fi human genome	20×	1	1,250
15–20 kb	Human genome	20×	2	2,500
15–20 kb	Human methylation profiling	5×	8	10,000
15–20 kb	Transcriptome with Kinnex full-length RNA kit	10M reads	6	7,500

[4] Expected coverages are estimates.

[5] Annual throughput is estimated and based on 2,500 Revio SMRT Cell acquisitions for 12 hour runs; 1,250 Revio SMRT Cell acquisitions for 24 hr runs; and 1,050 SMRT Cell acquisitions for 30 hour runs.



Revio system v13.5
+
SPRQ-Nx chemistry

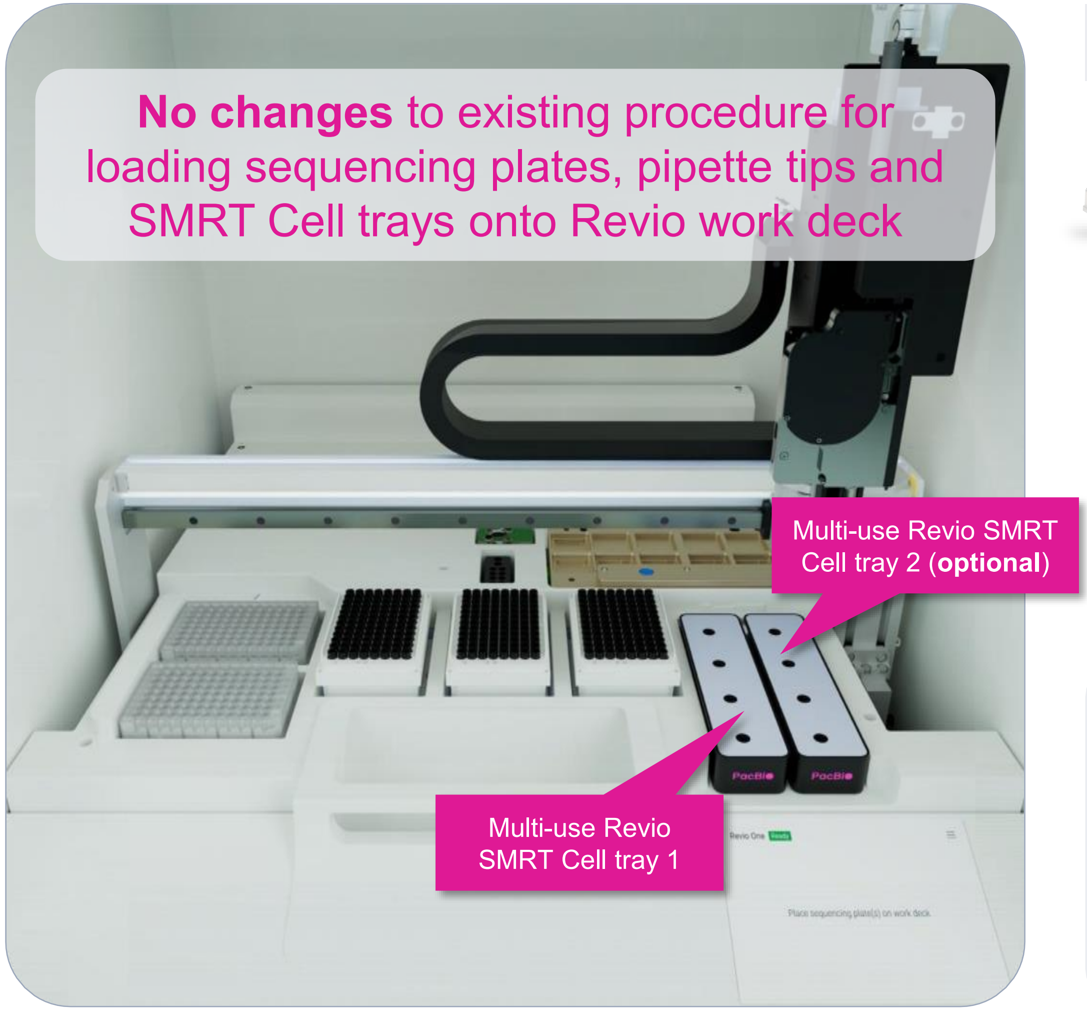
PacBio

See ***Revio system specification sheet*** (102-326-552) for the latest Revio system performance specifications.

Revio multi-use SMRT Cell workflow overview

Revio system v13.5 multi-use SMRT Cell workflow key features

Revio system core run setup procedure for multi-use SMRT Cells remains the same as for single-use SMRT Cells

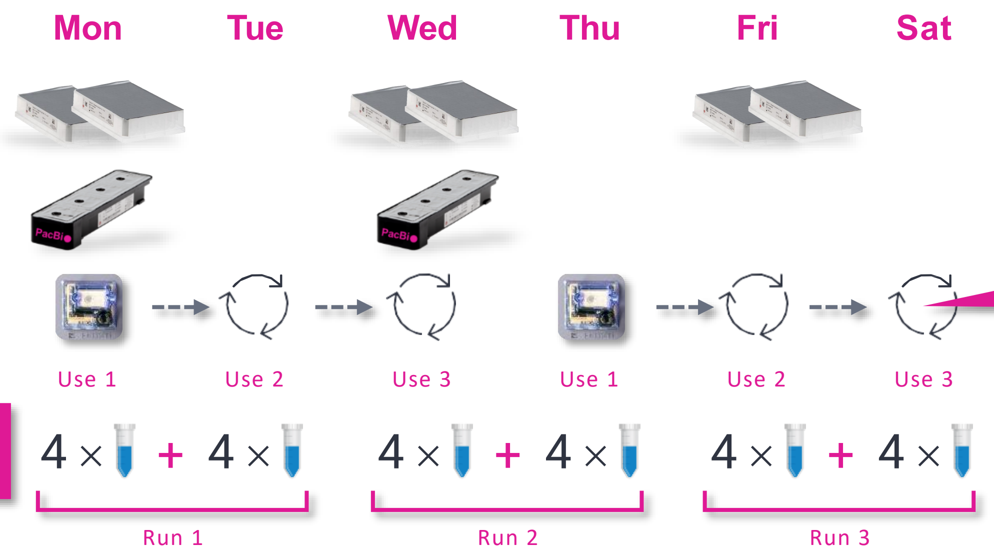


No changes to existing procedure for loading sequencing plates, pipette tips and SMRT Cell trays onto Revio work deck

Multi-use Revio SMRT Cell tray 2 (**optional**)

Multi-use Revio SMRT Cell tray 1

Run multiple acquisitions per Revio SMRT Cell with SPRQ-Nx chemistry[1]



Example run schedule:
24 samples / 3 runs

6 SPRQ-Nx sequencing plates

2 Multi-use SMRT Cell trays

Each SMRT Cell supports up to **3** acquisitions

24 samples
(= 24 acquisitions)

Different samples each acquisition

Minimal carryover[1]

Automatic washing

No manual steps

SMRT Cells stay in instrument

Software managed

Tracking of SPRQ – Nx consumables is **fully automated** *via* updated SMRT Link and Revio ICS software

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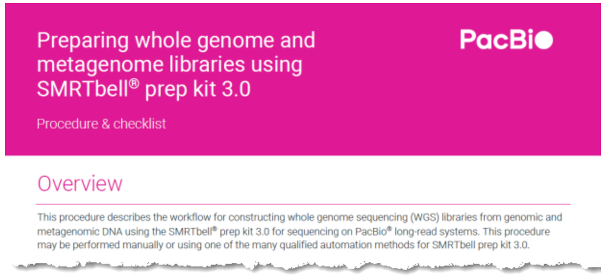
[1] Revio SPRQ-Nx use-to-use barcode carryover level is typically <0.1%

HiFi sample prep, HiFi library prep and Revio system sequencing prep core workflows remain the same for SPRQ-Nx chemistry

No changes to DNA input and DNA QC requirements for HiFi sequencing[1]

- SAMPLE PREPARATION
- LIBRARY PREPARATION
- SAMPLE SETUP (ABC)
- RUN DESIGN
- INSTRUMENT SETUP & RUN START
- ON-INSTRUMENT DATA ANALYSIS
- OFF-INSTRUMENT DATA ANALYSIS

Follow **existing** HiFi sample prep + library prep procedures



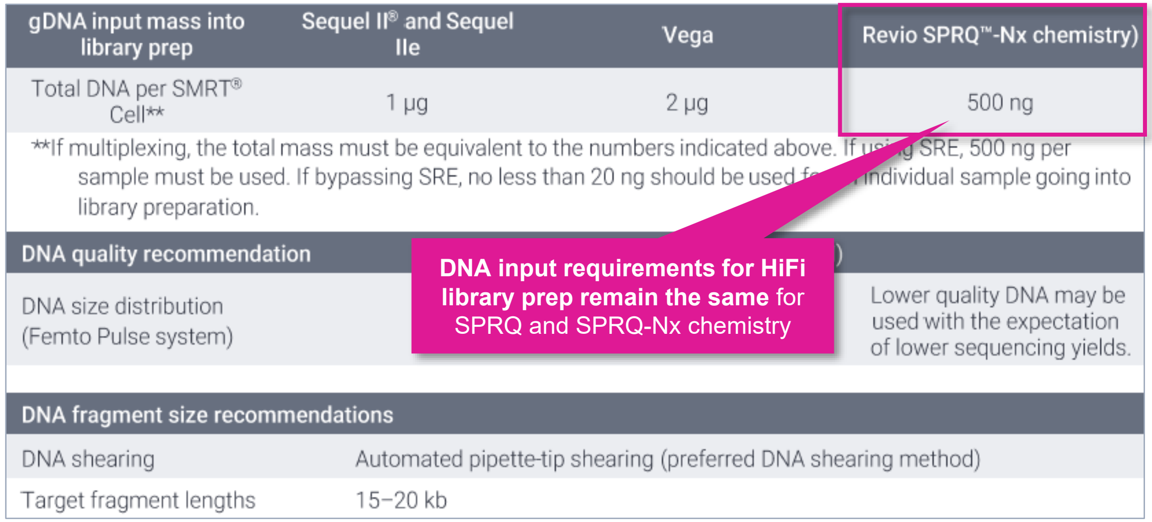
Preparing whole genome and metagenome libraries using SMRTbell® prep kit 3.0

Procedure & checklist

Overview

This procedure describes the workflow for constructing whole genome sequencing (WGS) libraries from genomic and metagenomic DNA using the SMRTbell® prep kit 3.0 for sequencing on PacBio® long-read systems. This procedure may be performed manually or using one of the many qualified automation methods for SMRTbell prep kit 3.0.

Input gDNA QC requirements for HiFi library prep remain the same for SPRQ and SPRQ-Nx chemistry



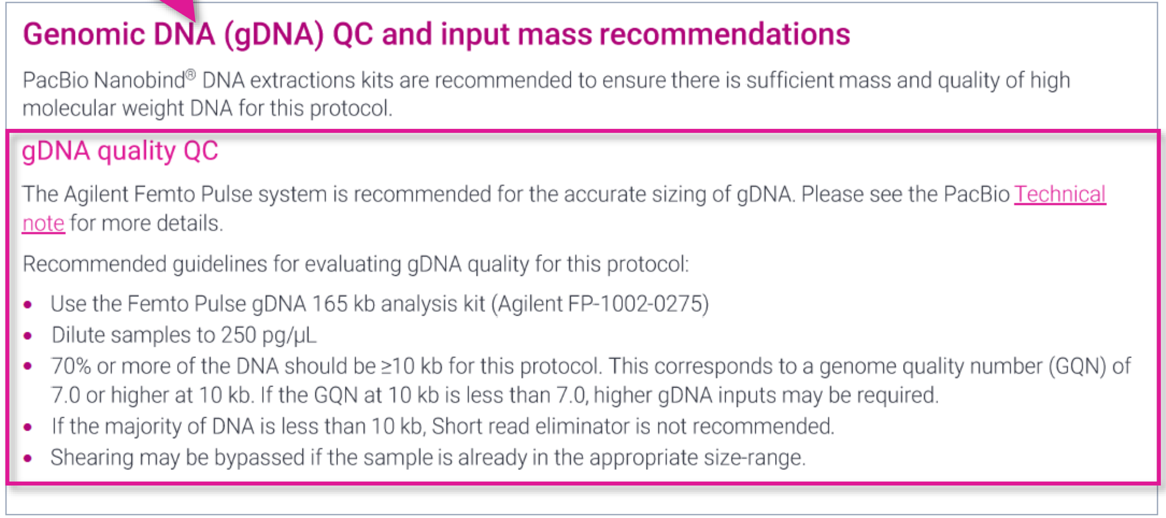
gDNA input mass into library prep	Sequel II® and Sequel IIe	Vega	Revio SPRQ™-Nx chemistry)
Total DNA per SMRT® Cell**	1 µg	2 µg	500 ng

**If multiplexing, the total mass must be equivalent to the numbers indicated above. If us[illegible]g SRE, 500 ng per sample must be used. If bypassing SRE, no less than 20 ng should be used f[illegible] individual sample going into library preparation.

DNA quality recommendation		)
DNA size distribution (Femto Pulse system)	[illegible]	Lower quality DNA may be used with the expectation of lower sequencing yields.

DNA fragment size recommendations	
DNA shearing	Automated pipette-tip shearing (preferred DNA shearing method)
Target fragment lengths	15–20 kb

DNA input requirements for HiFi library prep remain the same for SPRQ and SPRQ-Nx chemistry



Genomic DNA (gDNA) QC and input mass recommendations

PacBio Nanobind® DNA extractions kits are recommended to ensure there is sufficient mass and quality of high molecular weight DNA for this protocol.

gDNA quality QC

The Agilent Femto Pulse system is recommended for the accurate sizing of gDNA. Please see the PacBio Technical note for more details.

Recommended guidelines for evaluating gDNA quality for this protocol:

- Use the Femto Pulse gDNA 165 kb analysis kit (Agilent FP-1002-0275)
- Dilute samples to 250 pg/µL
- 70% or more of the DNA should be ≥10 kb for this protocol. This corresponds to a genome quality number (GQN) of 7.0 or higher at 10 kb. If the GQN at 10 kb is less than 7.0, higher gDNA inputs may be required.
- If the majority of DNA is less than 10 kb, Short read eliminator is not recommended.
- Shearing may be bypassed if the sample is already in the appropriate size-range.

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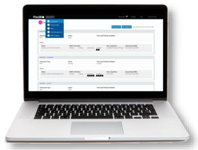
[1] Refer to latest PacBio Procedure & checklist protocol documentation for up-to-date guidance on application-specific DNA input amount requirements.

HiFi sample prep, HiFi library prep and Revio system sequencing prep core workflows remain the same for SPRQ-Nx chemistry (cont.)

No changes to Revio run setup and on-instrument/off-instrument data analysis procedures

SAMPLE PREPARATION → LIBRARY PREPARATION → SAMPLE SETUP (ABC) → **RUN DESIGN** → **INSTRUMENT SETUP & RUN START** → **ON-INSTRUMENT DATA ANALYSIS** → **OFF-INSTRUMENT DATA ANALYSIS**

Follow **existing** Revio run setup and HiFi data analysis procedures

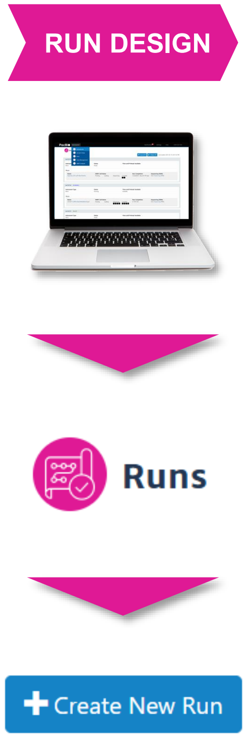


Runs

➕ Create New Run

SMRT Link v26.1 Revio Run Design user interface supports new SPRQ sequencing plate – Nx

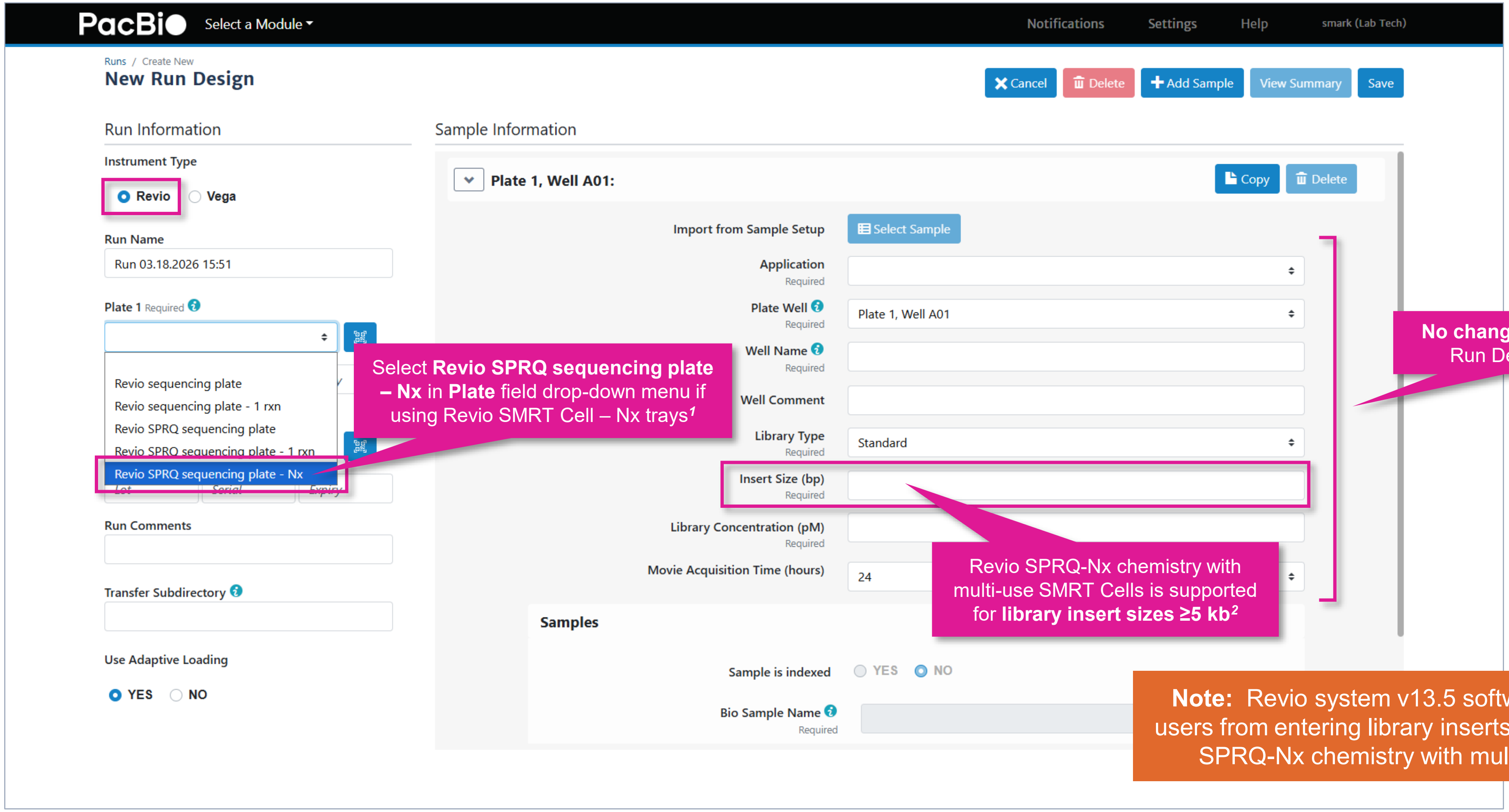
Run design core workflow remains the same when setting up a new Revio run with multi-use SMRT Cells



RUN DESIGN

Runs

+ Create New Run



Runs / Create New

New Run Design

Run Information

Instrument Type: ● Revio ○ Vega

Run Name: Run 03.18.2026 15:51

Plate 1 Required

- Revio sequencing plate
- Revio sequencing plate - 1 rxn
- Revio SPRQ sequencing plate
- Revio SPRQ sequencing plate - 1 rxn
- Revio SPRQ sequencing plate - Nx

Lot / Serial / Expiry

Run Comments

Transfer Subdirectory

Use Adaptive Loading: ● YES ○ NO

Sample Information

Plate 1, Well A01:

Import from Sample Setup: Select Sample

Application Required

Plate Well Required: Plate 1, Well A01

Well Name Required

Well Comment

Library Type Required: Standard

Insert Size (bp) Required

Library Concentration (pM) Required

Movie Acquisition Time (hours): 24

Samples

Sample is indexed: ○ YES ● NO

Bio Sample Name Required

Select **Revio SPRQ sequencing plate – Nx** in **Plate** field drop-down menu if using Revio SMRT Cell – Nx trays[1]

No changes to required Run Design fields

Revio SPRQ-Nx chemistry with multi-use SMRT Cells is supported for **library insert sizes ≥5 kb**[2]

Note: Revio system v13.5 software **does not block** users from entering library inserts sizes **<5 kb** if running SPRQ-Nx chemistry with multi-use SMRT Cells

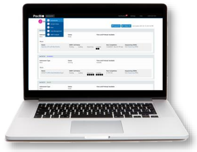
[1] **Note:** Revio SPRQ sequencing plate – Nx is compatible with both single-use Revio SMRT Cells and multi-use Revio SMRT Cells. Revio SPRQ sequencing plate is **not** compatible with multi-use Revio SMRT Cells.

[2] For amplicon libraries <5 kb, **do not** use Revio SPRQ sequencing plate – Nx with multi-use Revio SMRT Cell tray – Nx. For amplicon libraries <5 kb, can use Revio SPRQ sequencing plate – Nx (or Revio SPRQ sequencing plate) with single-use Revio SMRT Cells.

SMRT Link v26.1 Revio Run Design user interface supports new SPRQ sequencing plate – Nx (cont.)

Run design CSV supports new SPRQ-Nx sequencing plate part number

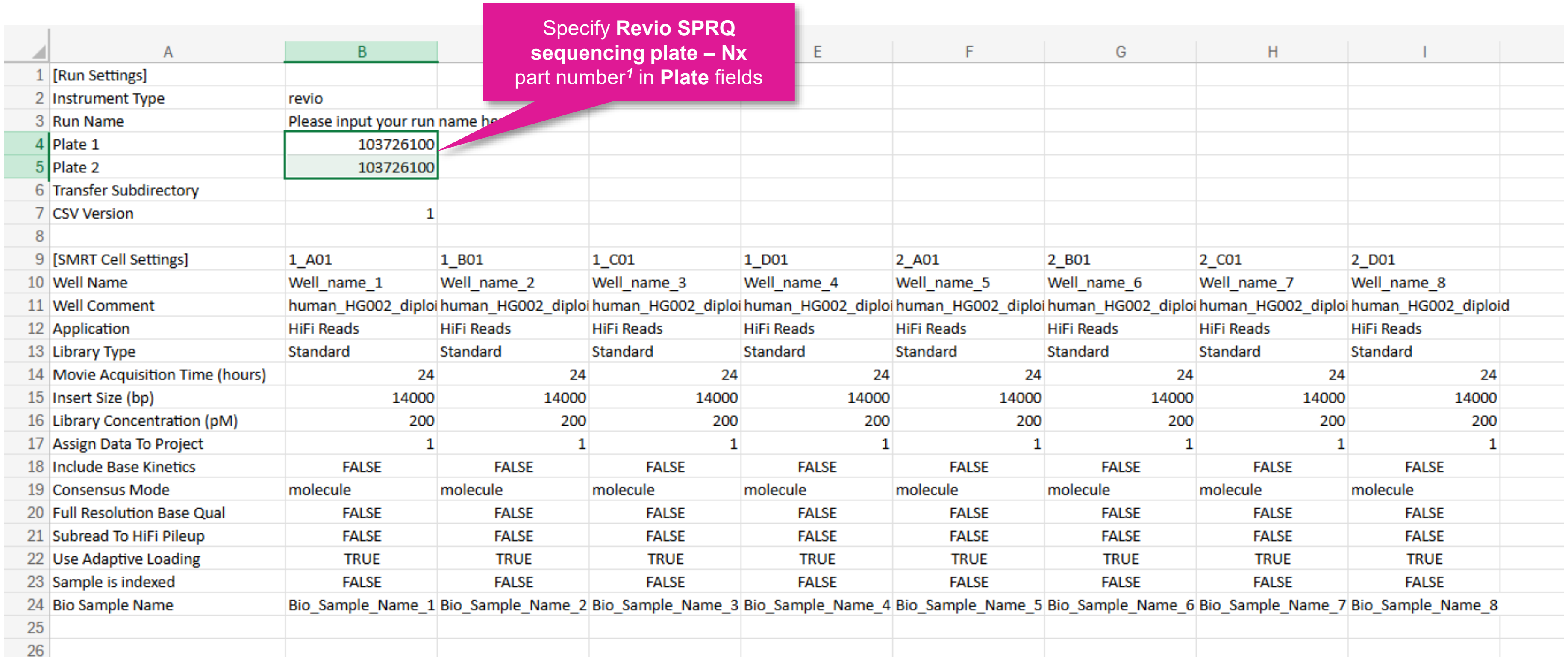
RUN DESIGN



Runs

Import Run

Specify **Revio SPRQ sequencing plate – Nx** part number[1] in **Plate** fields



	A	B			E	F	G	H	I
1	[Run Settings]								
2	Instrument Type	revio							
3	Run Name	Please input your run name he							
4	Plate 1	103726100							
5	Plate 2	103726100							
6	Transfer Subdirectory								
7	CSV Version	1							
8									
9	[SMRT Cell Settings]	1_A01	1_B01	1_C01	1_D01	2_A01	2_B01	2_C01	2_D01
10	Well Name	Well_name_1	Well_name_2	Well_name_3	Well_name_4	Well_name_5	Well_name_6	Well_name_7	Well_name_8
11	Well Comment	human_HG002_diploi	human_HG002_diploi	human_HG002_diploi	human_HG002_diploi	human_HG002_diploi	human_HG002_diploi	human_HG002_diploi	human_HG002_diploid
12	Application	HiFi Reads	HiFi Reads	HiFi Reads	HiFi Reads	HiFi Reads	HiFi Reads	HiFi Reads	HiFi Reads
13	Library Type	Standard	Standard	Standard	Standard	Standard	Standard	Standard	Standard
14	Movie Acquisition Time (hours)	24	24	24	24	24	24	24	24
15	Insert Size (bp)	14000	14000	14000	14000	14000	14000	14000	14000
16	Library Concentration (pM)	200	200	200	200	200	200	200	200
17	Assign Data To Project	1	1	1	1	1	1	1	1
18	Include Base Kinetics	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
19	Consensus Mode	molecule	molecule	molecule	molecule	molecule	molecule	molecule	molecule
20	Full Resolution Base Qual	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
21	Subread To HiFi Pileup	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
22	Use Adaptive Loading	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
23	Sample is indexed	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
24	Bio Sample Name	Bio_Sample_Name_1	Bio_Sample_Name_2	Bio_Sample_Name_3	Bio_Sample_Name_4	Bio_Sample_Name_5	Bio_Sample_Name_6	Bio_Sample_Name_7	Bio_Sample_Name_8
25									
26									

---

[1] **Note:** Individual Revio SPRQ sequencing plate – Nx part number to enter into run design CSV is 103-726-100. (When purchasing the Revio SPRQ sequencing plate – Nx consumable kit product, the orderable part number is 103-726-200.)

Revio system v13.5 run setup and instrument workflow for multi-use SMRT Cell trays

Revio instrument control software automatically tracks SMRT Cell uses and prioritizes allocation of samples to used cells before new cells[1,2]

Example run schedule: Run 1 = 8 samples and **Run 2 = 4 samples (total # acquisitions = 12)**

→ Only **one** multi-use SMRT Cell tray (= 4 SMRT Cells) is needed to sequence all 12 samples since each SMRT Cell can support up to **3** acquisitions (uses)

Multi-use SMRT Cell tray 1 is used for Run 1 **and** Run 2

Multi-use SMRT Cell tray 2 is **not used** since instrument scheduler will allocate Samples 5 – 12 to used cells from tray 1 *before* using new (unused) cells in tray 2

→ **There is no need to load SMRT Cell tray 2**

With SPRQ-Nx, 1 multi-use Revio SMRT Cell tray (= 4 SMRT Cells) can support **12** acquisitions

Run	Samples	Sequencing plates	SMRT Cell trays	Use
Run 1 (8 samples)	Samples 1 – 4 [Plate 1_A01-D01]	SPRQ-Nx sequencing plate 1	SPRQ-Nx multi-use SMRT Cell tray **1**	Use **1** (Cells 1 – 4)
	Samples 5 – 8 [Plate 2_A01-D01]	SPRQ-Nx sequencing plate 2	SPRQ-Nx multi-use SMRT Cell tray **1**	Use **2** (Cells 1 – 4)
Run 2 (4 samples)	Samples 9 – 12 [Plate 1_A01-D01]	SPRQ-Nx sequencing plate 1	SPRQ-Nx multi-use SMRT Cell tray **1**	Use **3** (Cells 1 – 4)

---

[1] **Definitions: New cells** – unused cells on the instrument work deck that are still inside their SMRT Cell tray(s). **Reuse cells** – used cells sitting on the instrument stage(s) that have remaining uses.

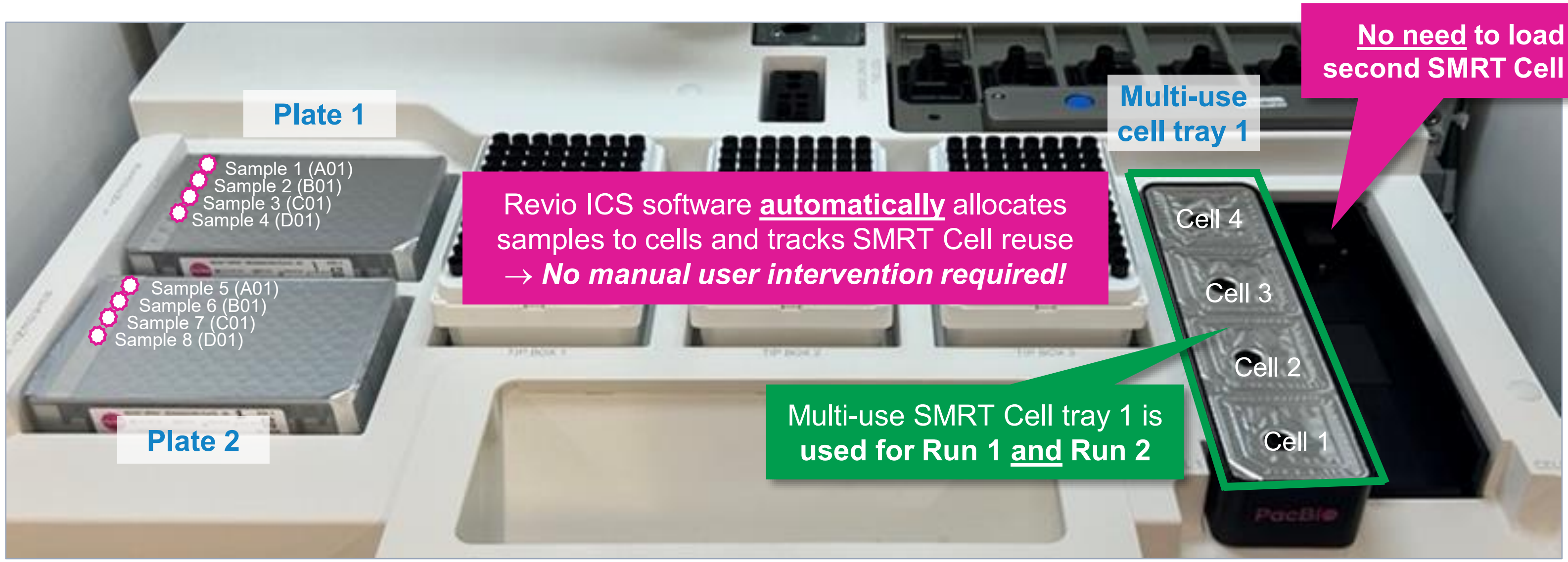
[2] See **Appendix** for general rules for SMRT cell multi-use (Revio system ICS v13.5).

Revio system v13.5 run setup and instrument workflow for multi-use SMRT Cell trays (cont.)

Revio instrument control software automatically tracks SMRT Cell uses and prioritizes allocation of samples to used cells before new cells

Example run schedule: **Run 1 = 8 samples** and **Run 2 = 4 samples (total # acquisitions = 12)**

→ Only **one** multi-use SMRT Cell tray (= 4 SMRT Cells) is needed to sequence all 12 samples since each SMRT Cell can support up to **3** acquisitions (uses)



No need to load a second SMRT Cell tray

Revio ICS software **automatically** allocates samples to cells and tracks SMRT Cell reuse
→ ***No manual user intervention required!***

Multi-use SMRT Cell tray 1 is **used for Run 1 and Run 2**

SPRQ-Nx multi-use SMRT Cell usage

- Each multi-use SMRT Cell supports up to **3 acquisitions (uses)**
- Once removed from cell tray for 1st use, the 3rd use (Use 3) must **start** within **108 hours (= 4.5 days)**[1]
- **Used SMRT Cells stay mounted on the instrument stages** until there are no remaining uses available (or the cell expires)[2]
- **Note:** Expired SMRT Cells and SMRT Cells with no remaining uses are **automatically discarded** into the instrument waste bin → **NO option** to use expired multi-use SMRT Cells for a sequencing run

Run	Samples	Sequencing plates	SMRT Cell trays	Use
Run 1 (8 samples)	Samples 1 – 4 [Plate 1_A01-D01]	SPRQ-Nx sequencing plate 1	SPRQ-Nx multi-use SMRT Cell tray **1**	Use **1** (Cells 1 – 4)
	Samples 5 – 8 [Plate 2_A01-D01]	SPRQ-Nx sequencing plate 2	SPRQ-Nx multi-use SMRT Cell tray **1**	Use **2** (Cells 1 – 4)
Run 2 (4 samples)	Samples 9 – 12 [Plate 1_A01-D01]	SPRQ-Nx sequencing plate 1	SPRQ-Nx multi-use SMRT Cell tray **1**	Use **3** (Cells 1 – 4)

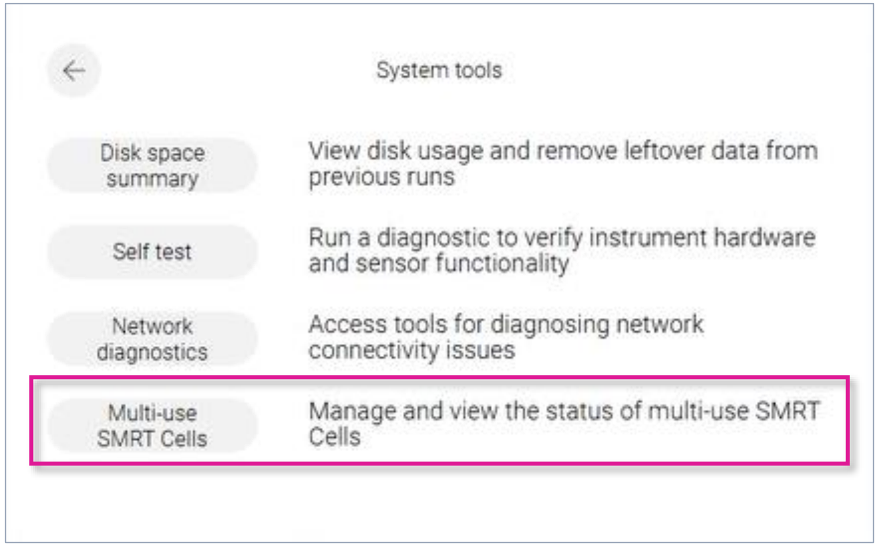
---

[1] If a SMRT Cell has not started another use within 108 hours, then it will be expired and automatically discarded into the waste bin by the instrument control software.

[2] Once removed from tray, a SMRT Cell is never returned to the tray. **Note:** Expired SMRT Cells and SMRT Cells with no remaining uses are **automatically discarded** into the instrument waste bin. There is **no option** to use expired multi-use SMRT Cells for a sequencing run. Used cells can also be manually discarded by specifying to clear all stages through the instrument touchscreen display.

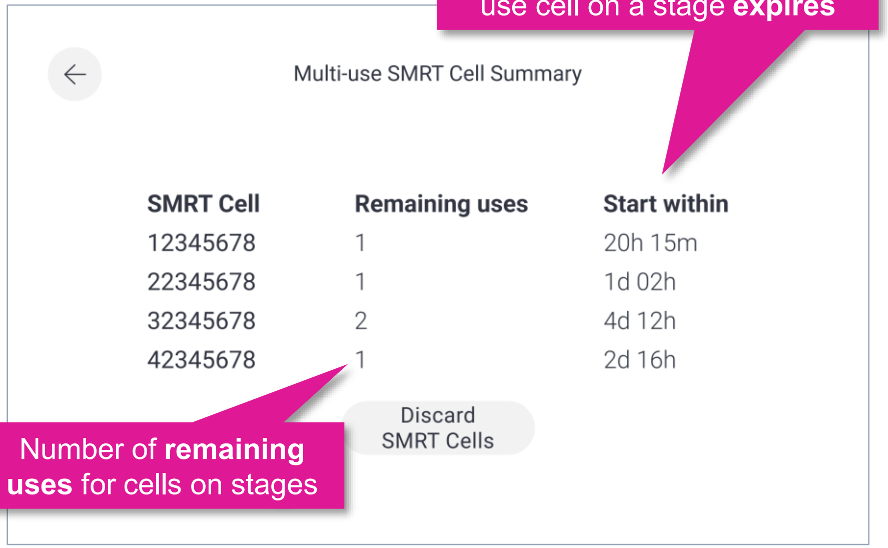
Revio work deck touchscreen displays SMRT Cell multi-use information

Revio work deck touchscreen shows remaining SMRT Cell uses and remaining time until a multi-use cell on an instrument stage expires



System tools

Disk space summary	View disk usage and remove leftover data from previous runs
Self test	Run a diagnostic to verify instrument hardware and sensor functionality
Network diagnostics	Access tools for diagnosing network connectivity issues
Multi-use SMRT Cells	Manage and view the status of multi-use SMRT Cells



Multi-use SMRT Cell Summary

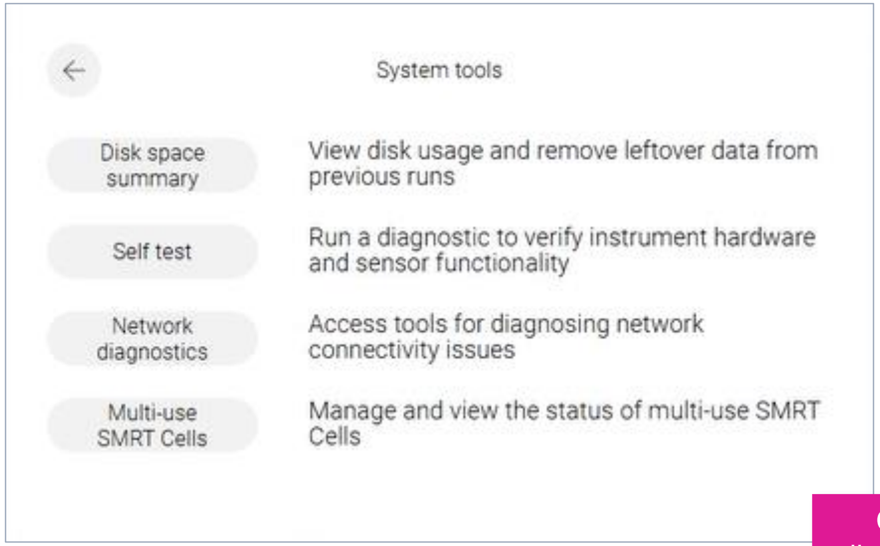
SMRT Cell	Remaining uses	Start within
12345678	1	20h 15m
22345678	1	1d 02h
32345678	2	4d 12h
42345678	1	2d 16h

Remaining time until the multi-use cell on a stage **expires**

Number of **remaining uses** for cells on stages

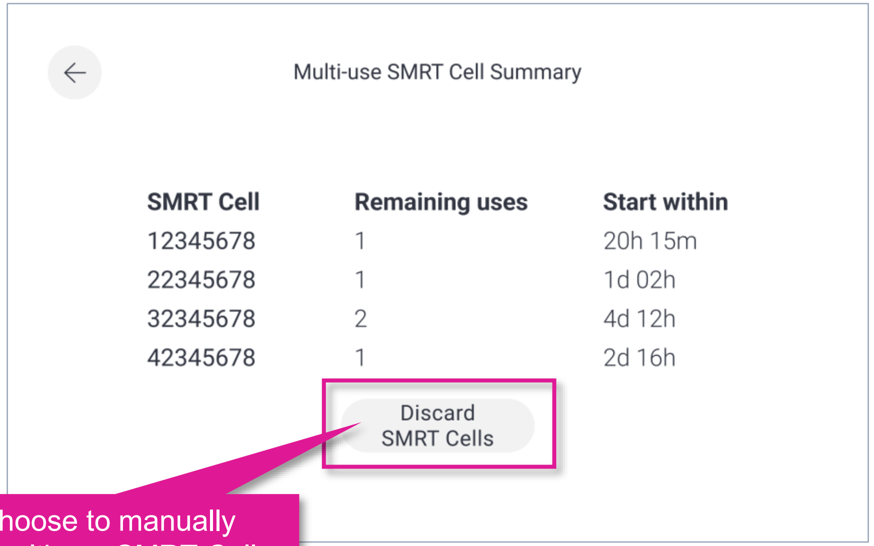
Revio work deck touchscreen displays SMRT Cell multi-use information (cont.)

Users can manually specify to discard multi-use SMRT Cells *via* work deck touchscreen



System tools

Disk space summary	View disk usage and remove leftover data from previous runs
Self test	Run a diagnostic to verify instrument hardware and sensor functionality
Network diagnostics	Access tools for diagnosing network connectivity issues
Multi-use SMRT Cells	Manage and view the status of multi-use SMRT Cells



Multi-use SMRT Cell Summary

SMRT Cell	Remaining uses	Start within
12345678	1	20h 15m
22345678	1	1d 02h
32345678	2	4d 12h
42345678	1	2d 16h

Can choose to manually discard multi-use SMRT Cells

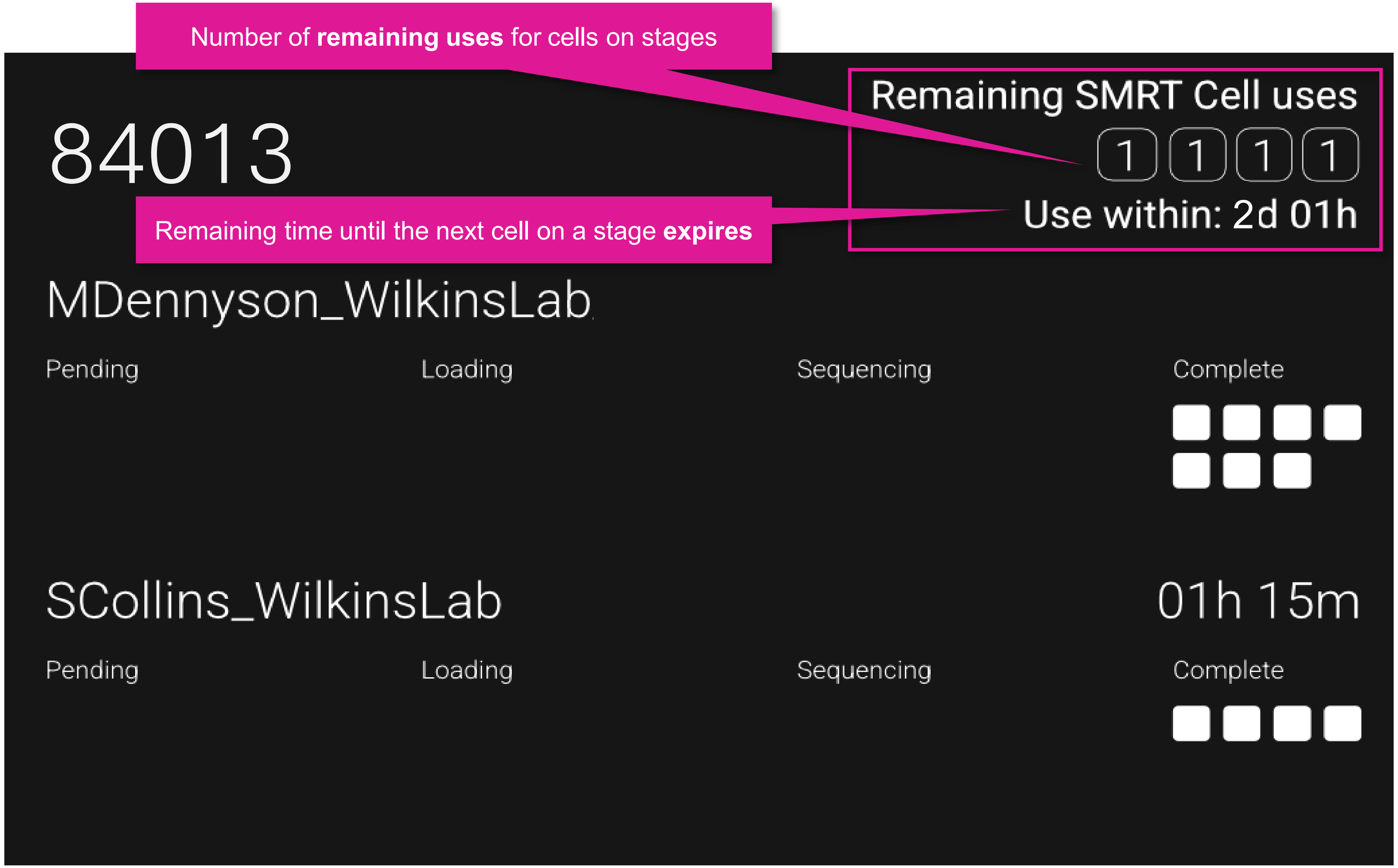
NOTE: You cannot select individual SMRT Cells to discard
→ Selecting **Discard SMRT Cells** will discard **ALL** multi-use cells currently on instrument stages regardless of the remaining number of uses for each cell[1]

---

[1] After clicking **Discard SMRT Cells**, a follow-up message will then appear to ask the user to confirm the selected action.

Revio system front panel display shows SMRT Cell multi-use information

Revio front panel display shows remaining SMRT Cell uses and remaining time until next cell expires[1]



Number of **remaining uses** for cells on stages

Remaining time until the next cell on a stage **expires**

Remaining SMRT Cell uses

1 1 1 1

Use within: 2d 01h

84013

MDennyson_WilkinsLab

Pending	Loading	Sequencing	Complete

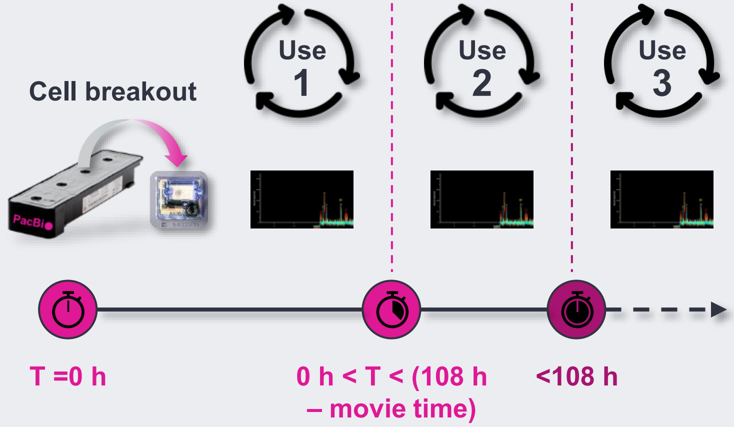
SCollins_WilkinsLab

01h 15m

Pending	Loading	Sequencing	Complete

Multi-use SMRT Cell expiration time

- Expiration countdown timer starts when SMRT Cell is first removed from the cell tray for 1st use (Use 1)
- ***IMPORTANT!*** – Once removed from cell tray for Use 1, the 3rd use (Use 3) must **start** within **108 hours (= 4.5 days)**[1]



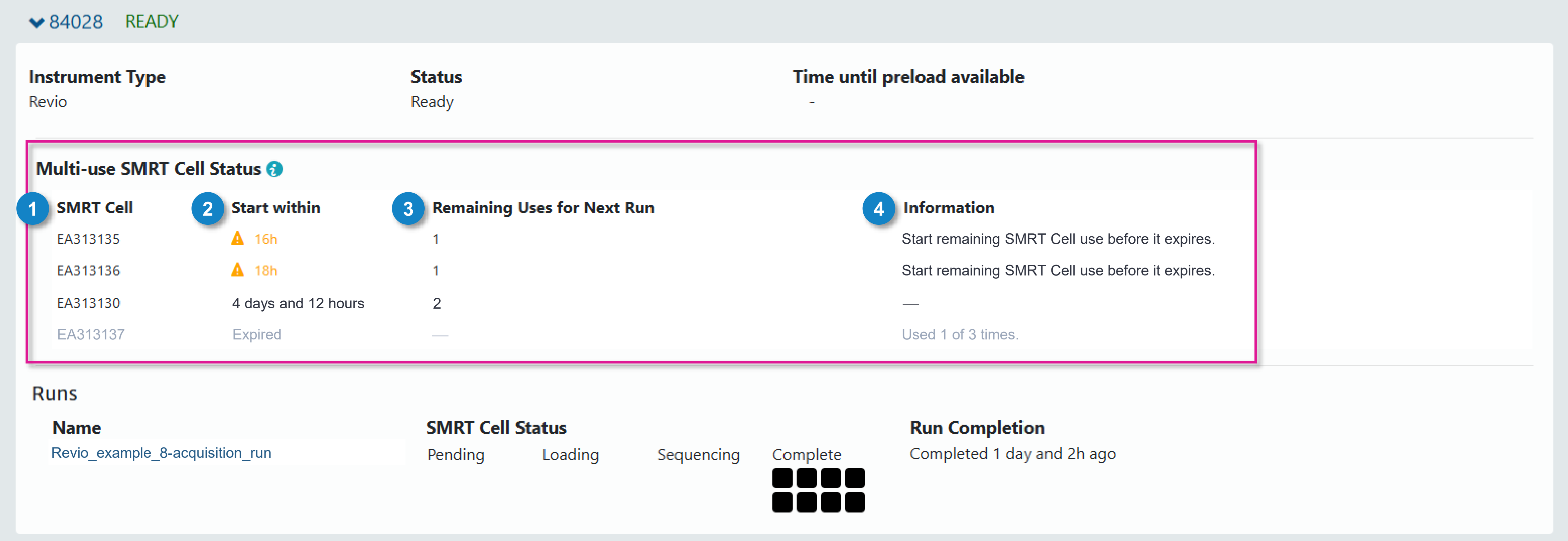
- **Note:** Expired SMRT Cells and SMRT Cells with no remaining uses are **automatically discarded** into the instrument waste bin → **NO option** to use expired multi-use SMRT Cells for a sequencing run

---

[1] See SMRT Link Instruments module for more detailed SMRT Cell status and expiration time information.

SMRT Link Instruments module is updated to display SMRT Cell multi-use information

SMRT Link Instruments module shows more detailed multi-use SMRT Cell status and expiration time information



84028 READY

Instrument Type	Status	Time until preload available
Revio	Ready	-

Multi-use SMRT Cell Status

1 SMRT Cell	2 Start within	3 Remaining Uses for Next Run	4 Information
EA313135	⚠ 16h	1	Start remaining SMRT Cell use before it expires.
EA313136	⚠ 18h	1	Start remaining SMRT Cell use before it expires.
EA313130	4 days and 12 hours	2	—
EA313137	Expired	—	Used 1 of 3 times.

Runs

Name	SMRT Cell Status	Run Completion
Revio_example_8-acquisition_run	Pending Loading Sequencing Complete ■■■■ ■■■■	Completed 1 day and 2h ago

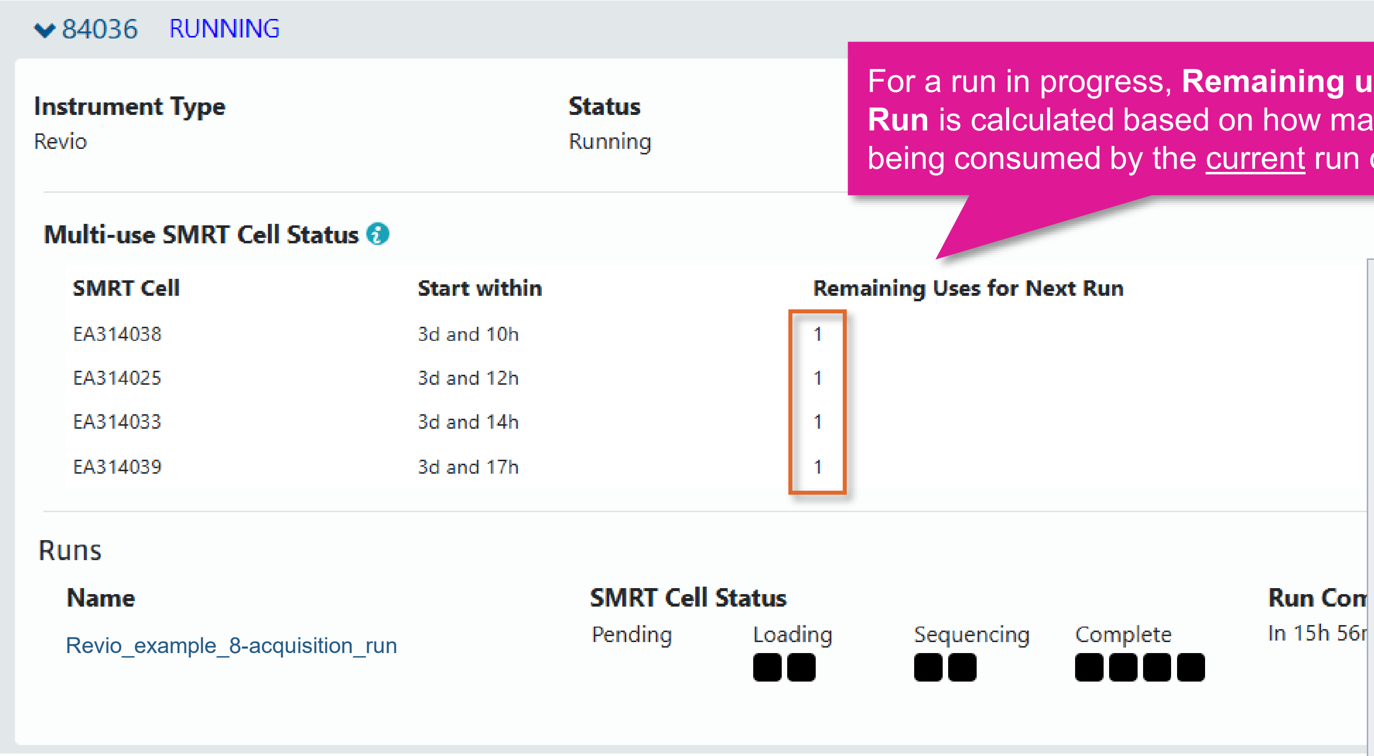
1. **SMRT Cell**
   - SMRT Cell ID serial number
2. **Start within**
   - Remaining time left before a multi-use SMRT Cell expires and becomes unusable[1]
3. **Remaining uses for Next Run**[1,2]
   - Number of uses remaining for a specific multi-use SMRT Cell
4. **Information**
   - Other status information or warning messages for a specific SMRT Cell

---

[1] For a run in progress, **Remaining uses for Next Run** is calculated based on how many uses are being consumed by the current run design.

[2] **Note:** Expired SMRT Cells and SMRT Cells with no remaining uses are automatically discarded into the instrument waste bin. There is no option to use expired multi-use SMRT Cells for a sequencing run.

SMRT Link Instruments module is updated to display SMRT Cell multi-use information (cont.)



84036 RUNNING

Instrument Type
Revio

Status
Running

Multi-use SMRT Cell Status

SMRT Cell	Start within	Remaining Uses for Next Run
EA314038	3d and 10h	1
EA314025	3d and 12h	1
EA314033	3d and 14h	1
EA314039	3d and 17h	1

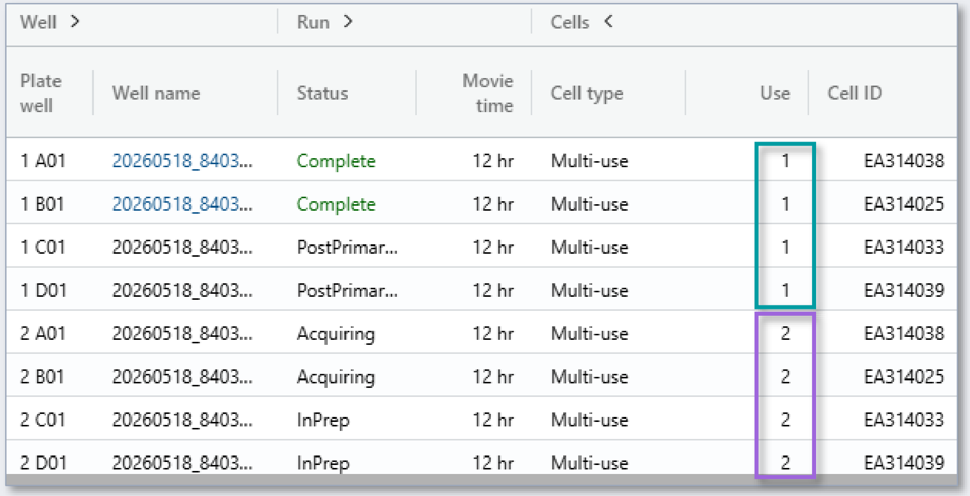
Runs

Name	SMRT Cell Status				Run Com
	Pending	Loading	Sequencing	Complete	
Revio_example_8-acquisition_run		■■	■■	■■■■	In 15h 56

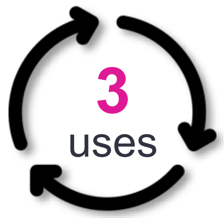
For a run in progress, **Remaining uses for Next Run** is calculated based on how many uses are being consumed by the current run design

Example: For each multi-use SMRT Cell in this 8-acquisition run design using a new SMRT Cell tray – Nx (= 4 new Nx cells):

- First 4 acquisitions were completed on **Use 1** (or are in post-primary analysis)
- Second 4 acquisitions are in progress on **Use 2** (or in cell prep for Use 2)
- Remaining uses for next run = **1 for each SMRT Cell**
- Total # acquisitions remaining **= 4**



Well >		Run >		Cells <		
Plate well	Well name	Status	Movie time	Cell type	Use	Cell ID
1 A01	20260518_8403...	Complete	12 hr	Multi-use	1	EA314038
1 B01	20260518_8403...	Complete	12 hr	Multi-use	1	EA314025
1 C01	20260518_8403...	PostPrimar...	12 hr	Multi-use	1	EA314033
1 D01	20260518_8403...	PostPrimar...	12 hr	Multi-use	1	EA314039
2 A01	20260518_8403...	Acquiring	12 hr	Multi-use	2	EA314038
2 B01	20260518_8403...	Acquiring	12 hr	Multi-use	2	EA314025
2 C01	20260518_8403...	InPrep	12 hr	Multi-use	2	EA314033
2 D01	20260518_8403...	InPrep	12 hr	Multi-use	2	EA314039



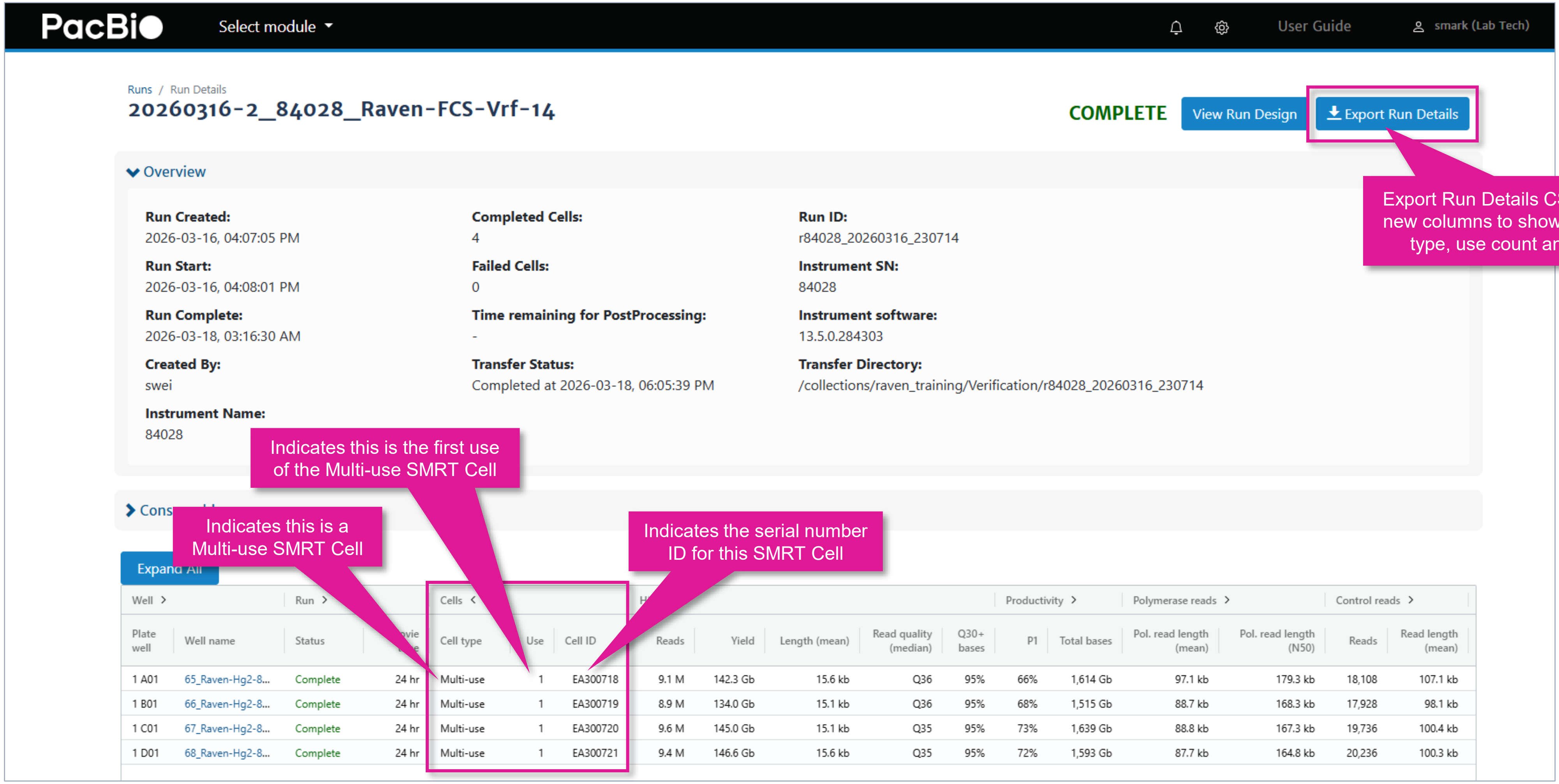
SMRT Link Run Details report page is updated to display SMRT Cell multi-use information

Run Details report page contains new columns to show SMRT Cell type, use count and cell ID[1]

RUN QC

Runs

Run Details



Runs / Run Details

20260316-2_84028_Raven-FCS-Vrf-14

COMPLETE

Overview

Run Created:
2026-03-16, 04:07:05 PM

Run Start:
2026-03-16, 04:08:01 PM

Run Complete:
2026-03-18, 03:16:30 AM

Created By:
swei

Instrument Name:
84028

Completed Cells:
4

Failed Cells:
0

Time remaining for PostProcessing:
-

Transfer Status:
Completed at 2026-03-18, 06:05:39 PM

Run ID:
r84028_20260316_230714

Instrument SN:
84028

Instrument software:
13.5.0.284303

Transfer Directory:
/collections/raven_training/Verification/r84028_20260316_230714

Cons

Well		Run		Cells			H					Productivity		Polymerase reads		Control reads	
Plate well	Well name	Status	vie e	Cell type	Use	Cell ID	Reads	Yield	Length (mean)	Read quality (median)	Q30+ bases	P1	Total bases	Pol. read length (mean)	Pol. read length (N50)	Reads	Read length (mean)
1 A01	65_Raven-Hg2-8...	Complete	24 hr	Multi-use	1	EA300718	9.1 M	142.3 Gb	15.6 kb	Q36	95%	66%	1,614 Gb	97.1 kb	179.3 kb	18,108	107.1 kb
1 B01	66_Raven-Hg2-8...	Complete	24 hr	Multi-use	1	EA300719	8.9 M	134.0 Gb	15.1 kb	Q36	95%	68%	1,515 Gb	88.7 kb	168.3 kb	17,928	98.1 kb
1 C01	67_Raven-Hg2-8...	Complete	24 hr	Multi-use	1	EA300720	9.6 M	145.0 Gb	15.1 kb	Q35	95%	73%	1,639 Gb	88.8 kb	167.3 kb	19,736	100.4 kb
1 D01	68_Raven-Hg2-8...	Complete	24 hr	Multi-use	1	EA300721	9.4 M	146.6 Gb	15.6 kb	Q35	95%	72%	1,593 Gb	87.7 kb	164.8 kb	20,236	100.3 kb

Export Run Details CSV contains new columns to show SMRT Cell type, use count and cell ID

Indicates this is the first use of the Multi-use SMRT Cell

Indicates this is a Multi-use SMRT Cell

Indicates the serial number ID for this SMRT Cell

---

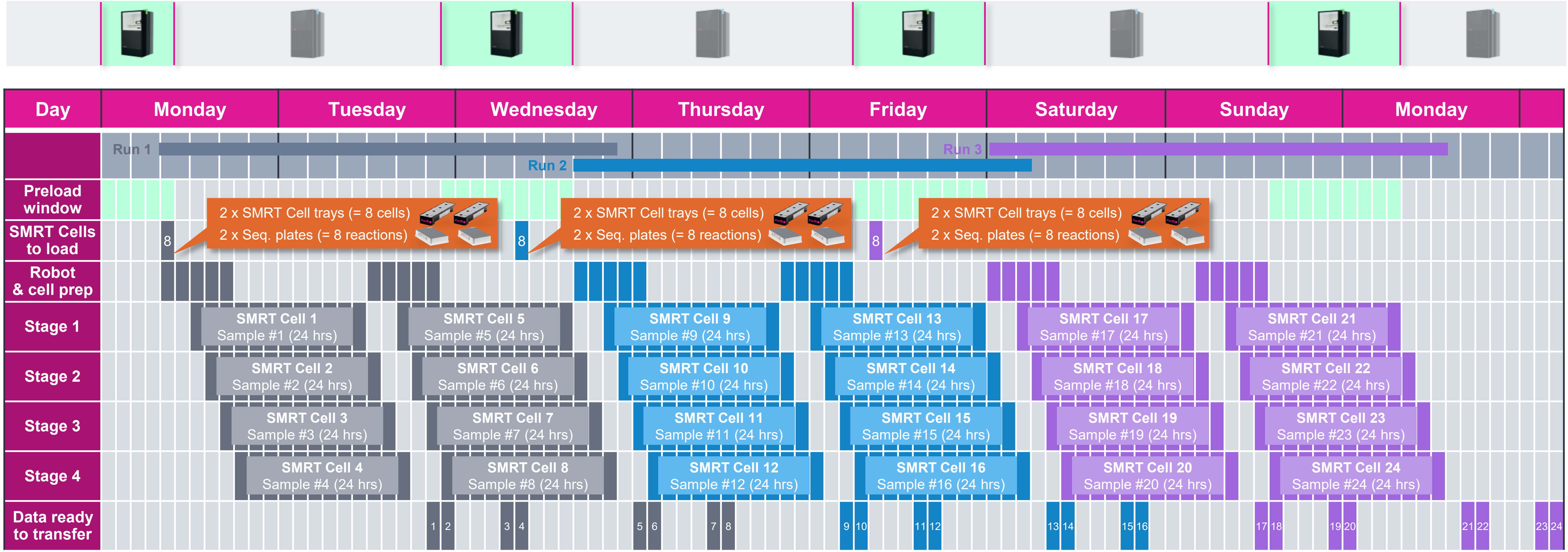
[1] **Note:** 'Cells' section is displayed in the Run Details table only when at least one sample/collection in the run used a multi-use SMRT Cell.

With new multi-use SMRT Cell trays, Revio system can be pre-loaded with fewer consumables to enable continuous instrument operation

Pre-load procedure is generally the same for Revio system v13.3 and v13.5

- After run is started, Revio door is **locked** and the work deck becomes **unavailable** while reagent prep, cell immobilization and pre-extension (PE) take place
- Door becomes **unlocked** after cell prep is completed for all SMRT Cells in the 1st run to allow pre-loading of consumables needed for a 2nd run while 1st run is still in progress[1]

Example Revio run schedule to process 24 samples per week with single-use SMRT Cells (24-hour movies)



Day | Monday | Tuesday | Wednesday | Thursday | Friday | Saturday | Sunday | Monday

Run 1

Run 2

Run 3

Preload window

SMRT Cells to load

Robot & cell prep

Stage 1

Stage 2

Stage 3

Stage 4

Data ready to transfer

8

2 x SMRT Cell trays (= 8 cells)
2 x Seq. plates (= 8 reactions)

8

2 x SMRT Cell trays (= 8 cells)
2 x Seq. plates (= 8 reactions)

8

2 x SMRT Cell trays (= 8 cells)
2 x Seq. plates (= 8 reactions)

Stage 1	SMRT Cell 1 Sample #1 (24 hrs)	SMRT Cell 5 Sample #5 (24 hrs)	SMRT Cell 9 Sample #9 (24 hrs)	SMRT Cell 13 Sample #13 (24 hrs)	SMRT Cell 17 Sample #17 (24 hrs)	SMRT Cell 21 Sample #21 (24 hrs)
Stage 2	SMRT Cell 2 Sample #2 (24 hrs)	SMRT Cell 6 Sample #6 (24 hrs)	SMRT Cell 10 Sample #10 (24 hrs)	SMRT Cell 14 Sample #14 (24 hrs)	SMRT Cell 18 Sample #18 (24 hrs)	SMRT Cell 22 Sample #22 (24 hrs)
Stage 3	SMRT Cell 3 Sample #3 (24 hrs)	SMRT Cell 7 Sample #7 (24 hrs)	SMRT Cell 11 Sample #11 (24 hrs)	SMRT Cell 15 Sample #15 (24 hrs)	SMRT Cell 19 Sample #19 (24 hrs)	SMRT Cell 23 Sample #23 (24 hrs)
Stage 4	SMRT Cell 4 Sample #4 (24 hrs)	SMRT Cell 8 Sample #8 (24 hrs)	SMRT Cell 12 Sample #12 (24 hrs)	SMRT Cell 16 Sample #16 (24 hrs)	SMRT Cell 20 Sample #20 (24 hrs)	SMRT Cell 24 Sample #24 (24 hrs)

Data ready to transfer: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

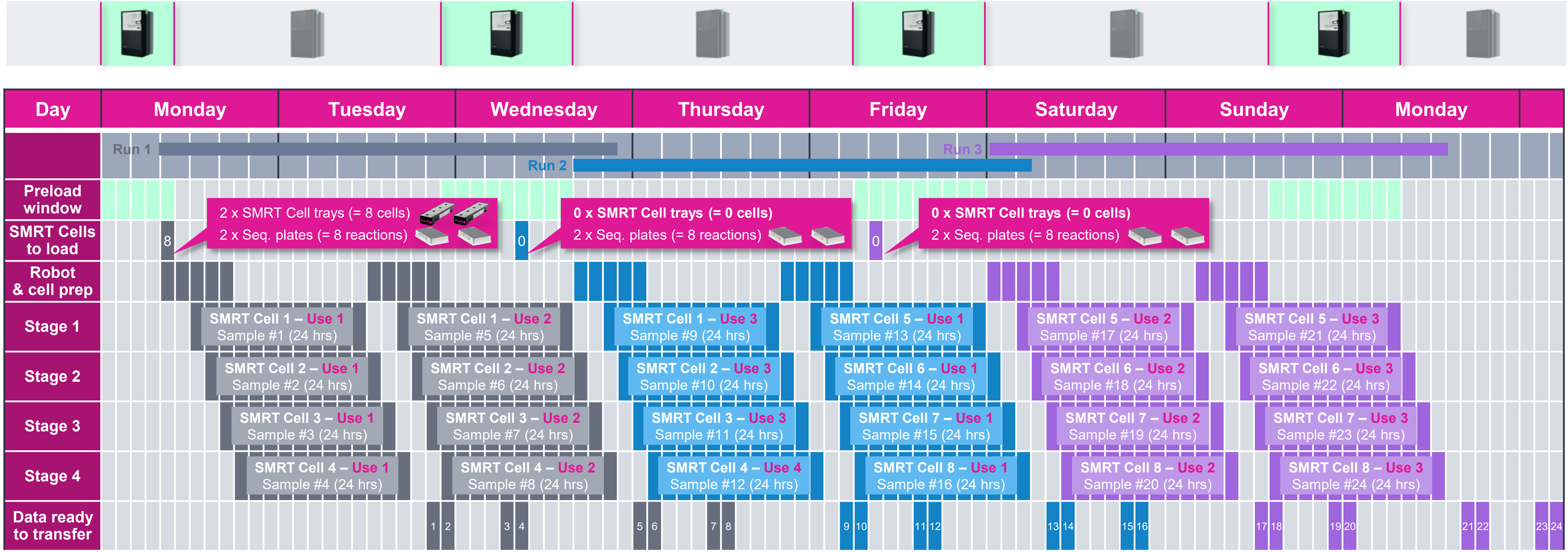
---

[1] For 8-sample (= 8 acquisition) run designs with adaptive loading, door becomes unlocked ~40 hours after first run is initiated. For 4-sample (= 4 acquisition) run designs with adaptive loading, door becomes unlocked ~10 hours after first run is initiated.

With new multi-use SMRT Cell trays, Revio system can be pre-loaded with fewer consumables to enable continuous instrument operation (cont.)

- After run is started, Revio door is **locked** and the work deck becomes **unavailable** while reagent prep, cell immobilization and pre-extension (PE) take place
- Door becomes **unlocked** after cell prep is completed for all SMRT Cells in the 1st run to allow pre-loading of consumables needed for a 2nd run while 1st run is still in progress[1]

Example Revio run schedule to process 24 samples per week with multi-use SMRT Cells (24-hour movies)[2]



Day | **Monday** | **Tuesday** | **Wednesday** | **Thursday** | **Friday** | **Saturday** | **Sunday** | **Monday**

Run 1
Run 2
Run 3

Preload window

SMRT Cells to load

8
2 x SMRT Cell trays (= 8 cells)
2 x Seq. plates (= 8 reactions)

0
0 x SMRT Cell trays (= 0 cells)
2 x Seq. plates (= 8 reactions)

0
0 x SMRT Cell trays (= 0 cells)
2 x Seq. plates (= 8 reactions)

Robot & cell prep

Stage 1
SMRT Cell 1 – Use 1 Sample #1 (24 hrs)
SMRT Cell 1 – Use 2 Sample #5 (24 hrs)
SMRT Cell 1 – Use 3 Sample #9 (24 hrs)
SMRT Cell 5 – Use 1 Sample #13 (24 hrs)
SMRT Cell 5 – Use 2 Sample #17 (24 hrs)
SMRT Cell 5 – Use 3 Sample #21 (24 hrs)

Stage 2
SMRT Cell 2 – Use 1 Sample #2 (24 hrs)
SMRT Cell 2 – Use 2 Sample #6 (24 hrs)
SMRT Cell 2 – Use 3 Sample #10 (24 hrs)
SMRT Cell 6 – Use 1 Sample #14 (24 hrs)
SMRT Cell 6 – Use 2 Sample #18 (24 hrs)
SMRT Cell 6 – Use 3 Sample #22 (24 hrs)

Stage 3
SMRT Cell 3 – Use 1 Sample #3 (24 hrs)
SMRT Cell 3 – Use 2 Sample #7 (24 hrs)
SMRT Cell 3 – Use 3 Sample #11 (24 hrs)
SMRT Cell 7 – Use 1 Sample #15 (24 hrs)
SMRT Cell 7 – Use 2 Sample #19 (24 hrs)
SMRT Cell 7 – Use 3 Sample #23 (24 hrs)

Stage 4
SMRT Cell 4 – Use 1 Sample #4 (24 hrs)
SMRT Cell 4 – Use 2 Sample #8 (24 hrs)
SMRT Cell 4 – Use 4 Sample #12 (24 hrs)
SMRT Cell 8 – Use 1 Sample #16 (24 hrs)
SMRT Cell 8 – Use 2 Sample #20 (24 hrs)
SMRT Cell 8 – Use 3 Sample #24 (24 hrs)

Data ready to transfer
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

---

[1] For 8-sample (= 8 acquisition) run designs with adaptive loading, door becomes unlocked ~40 hours after first run is initiated. For 4-sample (= 4 acquisition) run designs with adaptive loading, door becomes unlocked ~10 hours after first run is initiated.

[2] **Note:** When running multi-use SMRT Cell trays, Revio system v13.5 workflow adds ~45 min to cell prep time for Use 2 and Use 3 of each SMRT Cell compared to cell prep time for single-use SMRT Cells.

PacBio

Revio system v13.5 example sequencing performance

Revio system v13.5 + SPRQ-Nx chemistry example performance – WGS

SPRQ-Nx chemistry shows excellent HiFi sequencing performance across multiple SMRT Cell uses that meets or exceeds Revio system specifications for every use[1,2,3]

Application									
Whole genome sequencing[2]	Use	HiFi yield (Gb)	Reads (M)	Length (kb)	Read QV	*P1*	SNV F1	INDEL F1	SV F1
HG002 SMRT Cell 1	1	143.9	9.3	15.5	36	68.3	0.9987	0.9895	0.9752
	2	144.2	8.9	16.3	35	68.7	0.9988	0.9897	0.9747
	3	119.9	8.0	15.0	36	64.1	0.9989	0.9898	0.9729
HG002 SMRT Cell 2	1	147.0	9.2	16.0	35	68.7	0.9988	0.9892	0.9731
	2	148.6	9.1	16.3	35	73.0	0.9987	0.9867	0.9754
	3	124.7	6.9	17.9	35	53.9	0.9988	0.9877	0.9751
HG002 SMRT Cell 3	1	148.9	9.7	15.4	35	70.1	0.9987	0.9843	0.9740
	2	154.5	9.6	16.1	35	75.7	0.9988	0.9889	0.9739
	3	136.7	8.6	15.9	36	64.9	0.9988	0.9896	0.9754

---

[1] See ***Revio specifications sheet*** (102-326-552) for expected HiFi yield performance range for different library insert sizes and applications. For 15 – 20 kb WGS libraries, the specified HiFi yield range is 100 – 120 Gb of HiFi data per acquisition when using Revio SPRQ chemistry or Revio SPRQ-Nx chemistry. **Note:** HiFi yield is dependent on library quality and sequencing preparation procedures. Specified yields are based on high-quality samples prepared following best practices.

[2] Reduced HiFi yield on Use 2 and/or Use 3 is typically caused by lower *P1* loading efficiency.

[3] Example data shown are for HG002 17 kb WGS library run on a Revio system with SPRQ-Nx chemistry (24-hour acquisition time; 250 pM on-plate loading concentration). Variant calling performed with PacBio WGS variant pipeline; GIAB v4.2.1 small variant benchmark; GIAB T2TQ100 v1.1 SV benchmark.

Revio system v13.5 + SPRQ-Nx chemistry example performance

SPRQ-Nx chemistry shows excellent HiFi sequencing performance across multiple SMRT Cell uses that meets or exceeds Revio system specifications for every use[1]

Application								
RNA sequencing	**Use**	**HiFi yield (Gb)**	**HiFi reads (M)**	**HiFi length (kb)**	**HiFi read QV**	**P1**	**Transcript reads per acquisition**	**Product specifications[5]**
Kinnex full-length RNA, UHRR[2]	1	137.1	9.5	14.4	27	84.4	72.1 M	**50 – 60 M** **transcript reads per acquisition**
	2	135.2	9.3	14.5	29	68.8	71.0 M	
	3	116.2	7.9	14.6	29	60.2	61.2 M	
16S sequencing	**Use**	**HiFi yield (Gb)**	**HiFi reads (M)**	**HiFi length (kb)**	**HiFi read QV**	**P1**	**16S reads per acquisition**	**Product specifications[5]**
Kinnex full-length 16S rRNA, 384-plex ATCC MSA1002[3]	1	170.5	8.8	19.3	33	78.8	103.4 M	**70 – 80 M** **16S reads per acquisition**
	2	168.2	8.7	19.4	35	65.4	102.1 M	
	3	139.8	7.2	19.5	36	50.3	84.9 M	
PureTarget sequencing	**Use**	**HiFi yield (Gb)**	**HiFi reads (M)**	**HiFi length (kb)**	**HiFi read QV**	**P1**	**Mean target coverage**	**Product specifications[5]**
PureTarget 2.0 repeat expansion panel 2.0, 24-plex whole blood / Coriell cell line[4]	1	12.6	1.84	6.7	44	29.5	1064	**Mean target coverage** 100-fold or greater per 1 ug DNA per sample **Min. target coverage** 20-fold per sample
	2	6.5	0.93	6.9	44	17.7	523	
	3	7.1	0.82	8.6	39	13.8	350	

[1] Reduced HiFi yield on Use 2 and/or Use 3 is typically caused by lower P1 loading efficiency.

[2] Kinnex full-length RNA UHRR libraries were run on a Revio system with SPRQ-Nx chemistry (24-hour acquisition time; 140 pM on-plate loading concentration).

[3] Kinnex full-length 16S rRNA, ATCC MSA1002 libraries were run on a Revio system with SPRQ-Nx chemistry (24-hour acquisition time; 120 pM on-plate loading concentration).

[4] PureTarget 2.0 repeat expansion panel 24-plex whole blood/Coriell cell line libraries (2 µg of input DNA per sample) were run on a Revio system with SPRQ-Nx chemistry (24-hour acquisition time).

[5] See ***Revio specifications sheet*** (102-326-552); ***Application note – Kinnex full-length RNA kit for isoform sequencing*** (102-326-591); ***Application note - Kinnex 16S rRNA kit for full-length 16S sequencing*** (102-326-601); and ***Application note – Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing*** (102-326-614). **Note:** HiFi yield is dependent on library quality and sequencing preparation procedures. Specified yields are based on high-quality samples prepared following best practices.

Revio system v13.5 + SPRQ-Nx chemistry example datasets

Example Revio SPRQ-Nx datasets are available for whole genome sequencing and epigenetics

Application	Dataset	Data type	PacBio system
Whole genome sequencing			
Whole genome sequencing	Homo sapiens – HG002	HiFi long read	Revio system – SPRQ-Nx chemistry
Whole genome sequencing	Various plants + animals	HiFi long read	Revio system – SPRQ-Nx chemistry
Whole genome sequencing – Variant detection, assembly, epigenetics	Coming soon	HiFi long read	Revio system – SPRQ-Nx chemistry

Revio system v13.5 summary

New SPRQ-Nx extends Revio SPRQ chemistry with new 5hmC methylation caller and multi-use SMRT Cells, enabling a richer and cost-effective HiFi workflow

2026

SPRQ-Nx

Multi-use SMRT Cells

Up to 4 simultaneous acquisitions

480 Gb per day

$599k system

$345 per 20× genome (30% lower)

500 ng DNA input

2,500 genomes per year

5mC, 5hmC, and 6mA methylation caller

30 – 40% lower cost

Most affordable long-read genome

5 hmC in every run

Built-in multiomic insights

No change in library prep or analysis

Same HiFi quality, same workflow, less waste

Revio system v13.5 + SPRQ-Nx key specifications

Revio system v13.5 data throughput

Library	Run time[1]	Q30+ bases	HiFi yield per acquisition[2]	Methylation
0.5–5 kb	12 hours	95%	6 – 8 Million reads	5mC + 5hmC at CpG sites and 6mA[3] for native DNA
5–10 kb	24 hours	90%	35 – 70 Gb	
10–15 kb			70 – 100 Gb	
15–20 kb			100 – 120 Gb	
20–25 kb	30 hours	85%	100 – 120 Gb	

[1] Run time refers to the data collection step, which determines the time between processing SMRT Cell acquisitions.

[2] HiFi yield is dependent on library quality and sequencing preparation procedures. Specified yield is based on high-quality samples prepared following best practices.

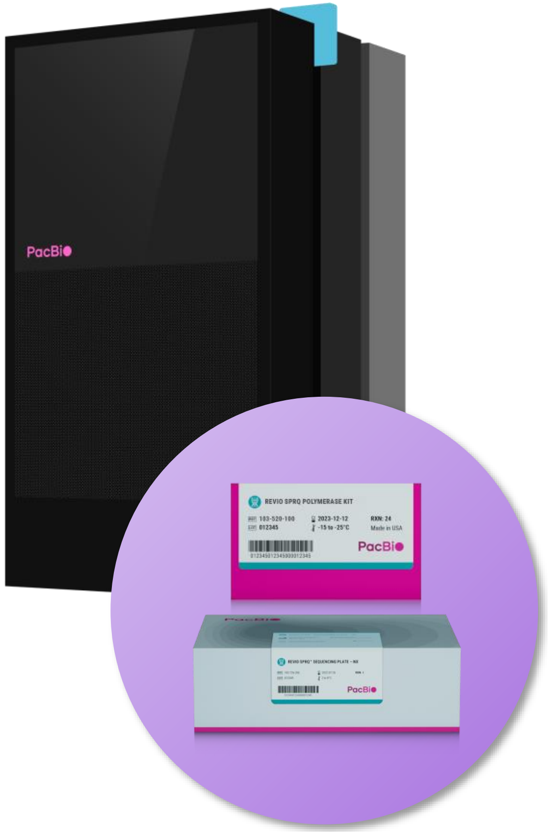
[3] The 6mA caller is designed to detect methylation in the context of the Fiber-seq chromatin assay.

Revio system v13.5 key applications and sample throughput

Library	Sample	Expected coverage[4]	Samples per acquisition	Samples per year[5]
0.5–5 kb	Amplicon sequencing	50×	>1,000	>2.5M
5–10 kb	Microbial genome	50×	384	480,000
5–10 kb	PureTarget repeat expansion panel	200×	48	60,000
5–10 kb	Ampli-Fi human genome	20×	1	1,250
15–20 kb	Human genome	20×	2	2,500
15–20 kb	Human methylation profiling	5×	8	10,000
15–20 kb	Transcriptome with Kinnex full-length RNA kit	10M reads	6	7,500

[4] Expected coverages are estimates.

[5] Annual throughput is estimated and based on 2,500 Revio SMRT Cell acquisitions for 12 hour runs; 1,250 Revio SMRT Cell acquisitions for 24 hr runs; and 1,050 SMRT Cell acquisitions for 30 hour runs.



Revio system v13.5
+
SPRQ-Nx chemistry

PacBio

See ***Revio system specification sheet*** (102-326-552) for the latest Revio system performance specifications.

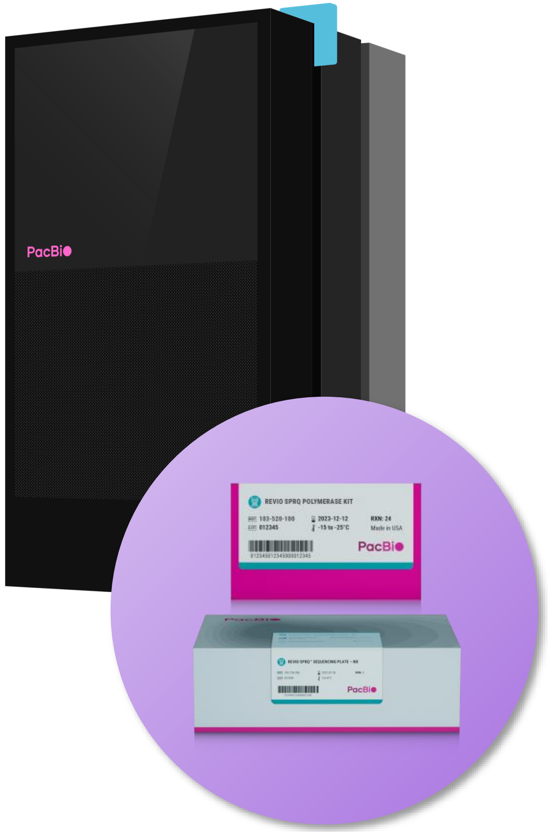
Revio system v13.5 with SPRQ-Nx chemistry enables HiFi sequencing at scale

See ***What can you do with one HiFi run*** (102-326-578)

Revio system with SPRQ-Nx chemistry

Application	Samples per run	
	1 acquisition	4 acquisitions
Whole genome sequencing		
Human genome (20× coverage)	2	8
Human methylation profiling (5× coverage)	8	32
De novo assembly (1 Gb genome)	4	16
Microbial de novo assembly (1 Gb total sum of genomes)	384	1,536
Targeted panels		
Amplicon sequencing	>1,000	>4,000
Hybrid capture sequencing		
20 Mb panel	16	64
2 Mb panel	96	384
100 kb panel	384	1,536
PureTarget panel sequencing	96	384
RNA sequencing		
Kinnex single-cell RNA sequencing	1 (6,000 – 10,000 cells)	4 (6,000 – 10,000 cells)
Kinnex full-length RNA sequencing		
5M reads	12	48
10M reads	6	24
Microbial		
Shotgun metagenomic profiling	128 communities	512 communities
Shotgun metagenomic assembly	16 communities	64 communities
Kinnex 16S rRNA	1,536 communities	6,144 communities

All sample throughputs are estimates for either the Vega system with 1 acquisition or the Revio system using SPRQ or SPRQ-Nx chemistry with both 1 or 4 acquisitions. Coverage may vary based on sample quality, library quality, and fragment lengths. Currently available SMRTbell® adapter index plates 96A-96D contain a total of 384 SMRTbell barcoded adapters. Microbial de novo assembly assumes microbes with 2 Gb of total genome size at 30x per sample. Single-cell transcriptomics assumes ≥80 million reads per library on the Revio system and ~50-60 million reads per library on the Vega system. Full-length RNA sequencing assumes a total of 60M reads for Revio SPRQ and 30M reads for Vega, regardless of plexity. Amplicon sequencing assumes a 12-hour movie time for 1–5 kb, 24-hour movie time for 5+ kb, and >50× per sample. PureTarget panel assumes >100× mean per target and Revio throughput assumes automated library prep.

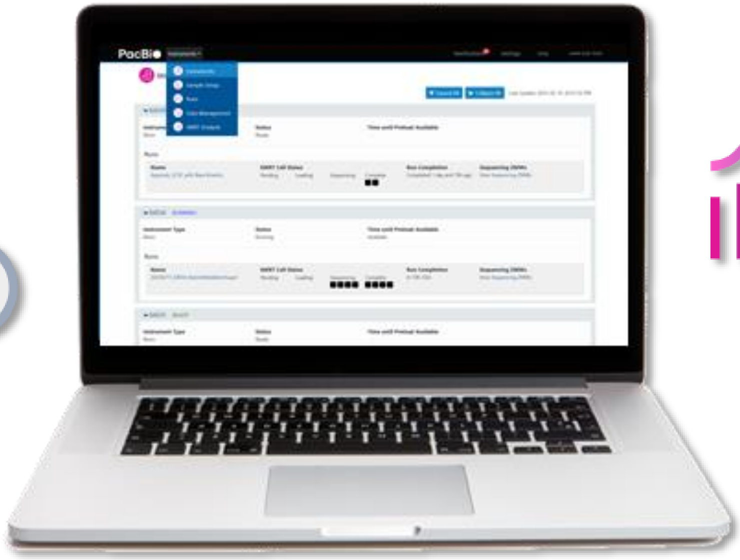


Revio system v13.5
+
SPRQ-Nx chemistry

§ Part 2:

SMRT Link v26.1
+ SMRT Link Cloud v26.1

1. SMRT Link v26.1 key features & benefits
2. New SPRQ-Nx chemistry support
3. New SMRT Link v26.1 audit logging support
4. SMRT Link v26.1 user experience improvements
    - Improved targeted sequencing analysis (asymmetric barcode demultiplexing + export FASTQ) support
    - Improved Ampli-Fi run design support
    - Improved methylation reports



SMRT Link v26.1 key features & benefits

SMRT Link v26.1 key features and updates

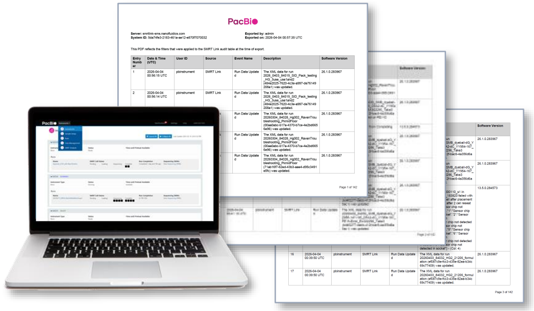
SMRT Link v26.1 makes HiFi sequencing easier with Revio SPRQ-Nx chemistry support, stronger compliance support and data analysis workflow usability improvements

SPRQ-Nx chemistry support

- Support for new Revio SPRQ-Nx consumables and multi-use SMRT Cells

Audit logging support

- New audit trail feature provides 21 CFR Part 11-aligned, tamper-resistant logging with centralized visibility and exportable records



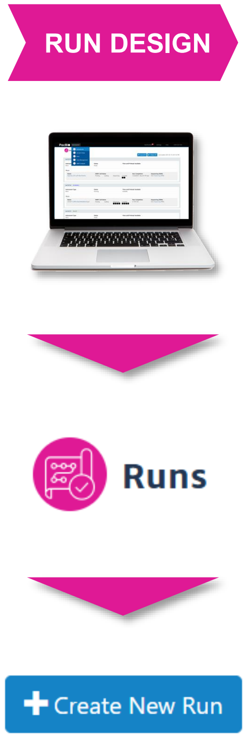
Data analysis workflow usability improvements

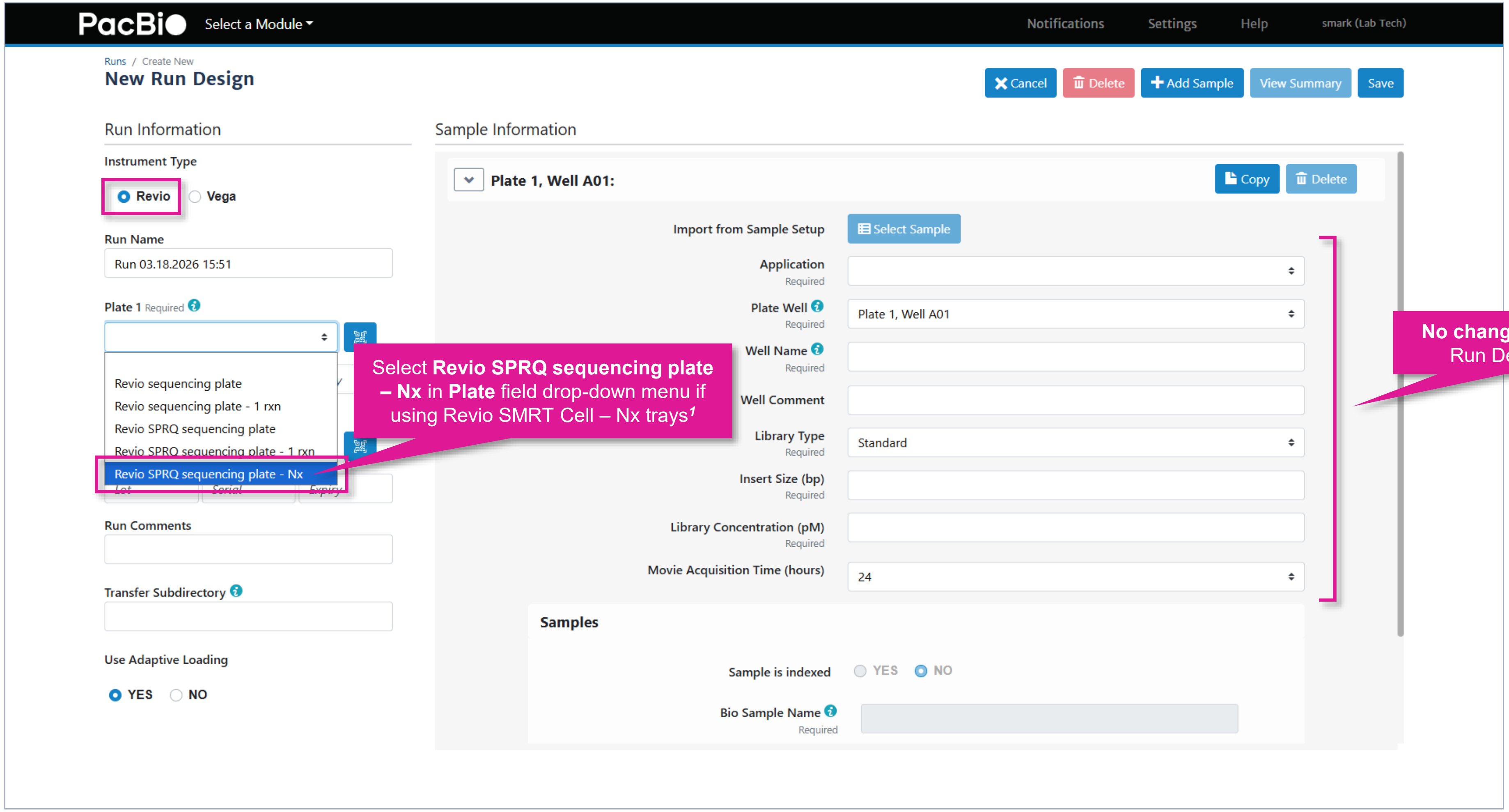
- Improved asymmetric barcode demultiplexing support to enable automatic demultiplexing (in-SMRT Link), predefined barcode pairing rules, and built-in GUI validation
- Improved Ampli-Fi run design support to enable use of preloaded, protocol-specific barcodes and automatic demultiplexing (in-SMRT Link)
- Improved methylation reports contain richer multiomic data (5hmC + 5mC + 6mA) and expanded visual plots

New SMRT Link v26.1
SPRQ-Nx chemistry support

SMRT Link Run Design page is updated for Revio system to support SPRQ-Nx consumables

Plate field dropdown selection menu includes new SPRQ sequencing plate – Nx (4-rxn)





New Run Design

Run Information

Instrument Type: ● Revio ○ Vega

Run Name: Run 03.18.2026 15:51

Plate 1 Required

- Revio sequencing plate
- Revio sequencing plate - 1 rxn
- Revio SPRQ sequencing plate
- Revio SPRQ sequencing plate - 1 rxn
- Revio SPRQ sequencing plate - Nx

Run Comments

Transfer Subdirectory

Use Adaptive Loading: ● YES ○ NO

Sample Information

Plate 1, Well A01:

Import from Sample Setup

Application Required

Plate Well Required: Plate 1, Well A01

Well Name Required

Well Comment

Library Type Required: Standard

Insert Size (bp) Required

Library Concentration (pM) Required

Movie Acquisition Time (hours): 24

Samples

Sample is indexed: ○ YES ● NO

Bio Sample Name Required

Select **Revio SPRQ sequencing plate – Nx** in **Plate** field drop-down menu if using Revio SMRT Cell – Nx trays[1]

No changes to required Run Design fields

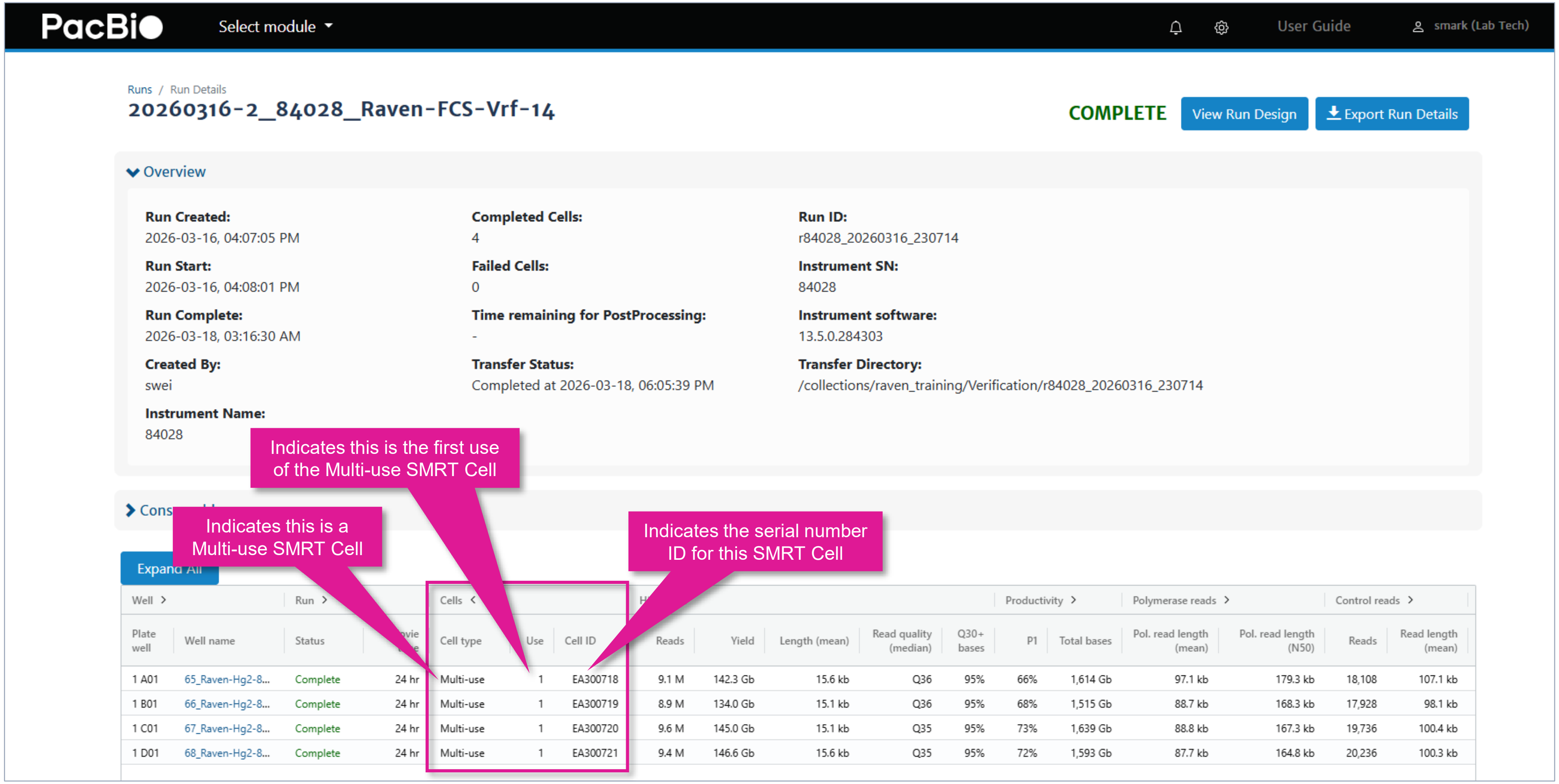
---

[1] **Note:** Revio SPRQ sequencing plate – Nx is compatible with both single-use Revio SMRT Cells and multi-use Revio SMRT Cells. Revio SPRQ sequencing plate is **not** compatible with multi-use Revio SMRT Cells.

[2] For amplicon libraries <5 kb, **do not** use Revio SPRQ sequencing plate – Nx with multi-use Revio SMRT Cell tray – Nx. For amplicon libraries <5 kb, can use Revio SPRQ sequencing plate – Nx (or Revio SPRQ sequencing plate) with single-use Revio SMRT Cells.

SMRT Link Run Details report page is updated to display SMRT Cell multi-use information

Run Details report page contains new columns to show SMRT Cell type, use count and cell ID[1]



Runs / Run Details

20260316-2_84028_Raven-FCS-Vrf-14

Run Created: 2026-03-16, 04:07:05 PM

Run Start: 2026-03-16, 04:08:01 PM

Run Complete: 2026-03-18, 03:16:30 AM

Created By: swei

Instrument Name: 84028

Completed Cells: 4

Failed Cells: 0

Time remaining for PostProcessing: -

Transfer Status: Completed at 2026-03-18, 06:05:39 PM

Run ID: r84028_20260316_230714

Instrument SN: 84028

Instrument software: 13.5.0.284303

Transfer Directory: /collections/raven_training/Verification/r84028_20260316_230714

Well		Run		Cells			H					Productivity		Polymerase reads		Control reads	
Plate well	Well name	Status		Cell type	Use	Cell ID	Reads	Yield	Length (mean)	Read quality (median)	Q30+ bases	P1	Total bases	Pol. read length (mean)	Pol. read length (N50)	Reads	Read length (mean)
1 A01	65_Raven-Hg2-8...	Complete	24 hr	Multi-use	1	EA300718	9.1 M	142.3 Gb	15.6 kb	Q36	95%	66%	1,614 Gb	97.1 kb	179.3 kb	18,108	107.1 kb
1 B01	66_Raven-Hg2-8...	Complete	24 hr	Multi-use	1	EA300719	8.9 M	134.0 Gb	15.1 kb	Q36	95%	68%	1,515 Gb	88.7 kb	168.3 kb	17,928	98.1 kb
1 C01	67_Raven-Hg2-8...	Complete	24 hr	Multi-use	1	EA300720	9.6 M	145.0 Gb	15.1 kb	Q35	95%	73%	1,639 Gb	88.8 kb	167.3 kb	19,736	100.4 kb
1 D01	68_Raven-Hg2-8...	Complete	24 hr	Multi-use	1	EA300721	9.4 M	146.6 Gb	15.6 kb	Q35	95%	72%	1,593 Gb	87.7 kb	164.8 kb	20,236	100.3 kb

Indicates this is the first use of the Multi-use SMRT Cell

Indicates this is a Multi-use SMRT Cell

Indicates the serial number ID for this SMRT Cell

---

[1] **Note:** 'Cells' section is displayed in the Run Details table only when at least one sample/collection in the run used a multi-use SMRT Cell.

New SMRT Link v26.1 Audit logging support

SMRT Link v26.1 provides the first step in 21 CFR part 11 enablement

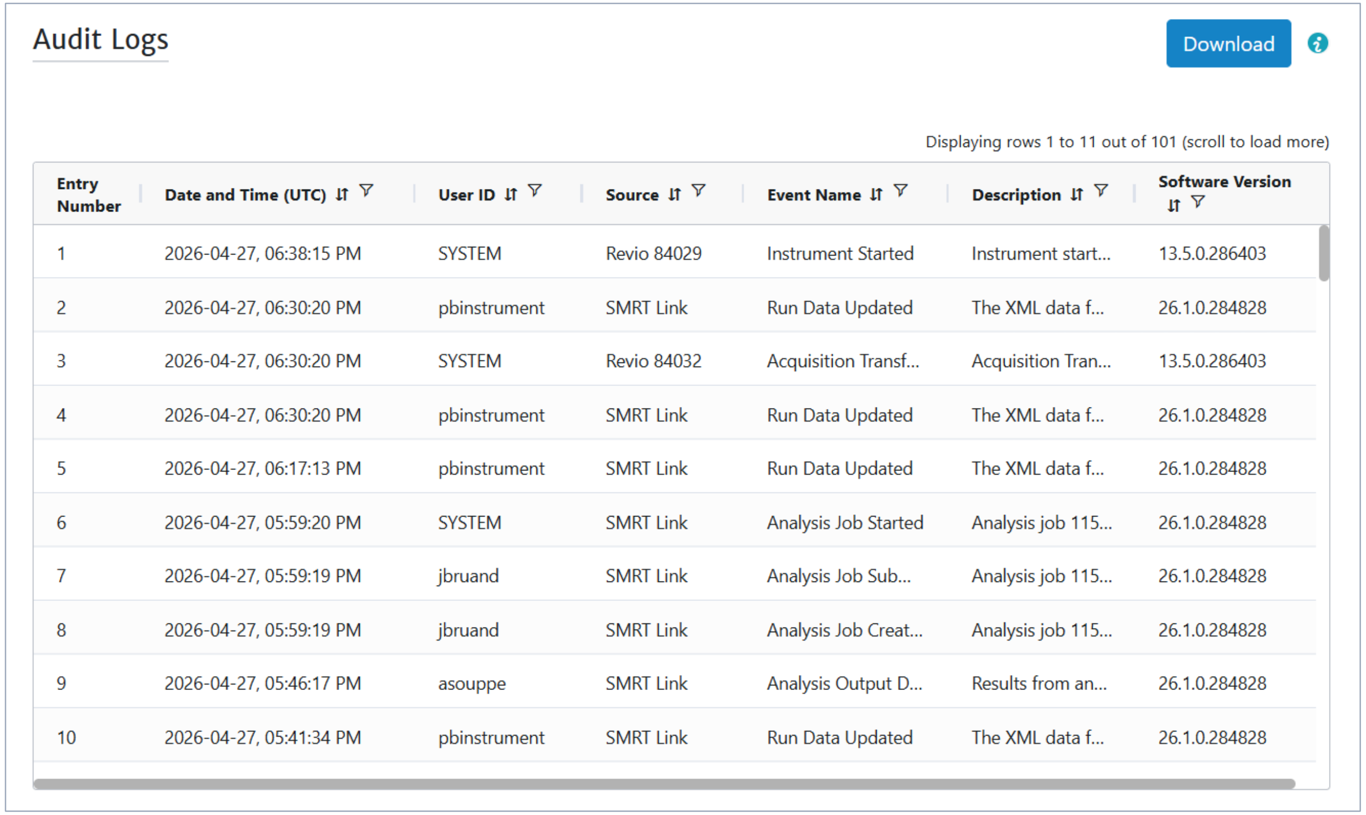
New audit trail logging feature provides 21 CFR Part 11-aligned, tamper-resistant logging with centralized visibility and exportable records for SMRT Link servers connected to Revio and Vega systems

What is 21 CFR part 11?

- 21 CFR part 11 is a U.S. Food and Drug Administration (FDA) regulation outlining how regulated organizations should maintain electronic records
- Covers user access and authentication, audit trails of system activity, data integrity and traceability, and system validation and accountability

How does SMRT Link v26.1 help enable 21 CFR part 11 compliance?

- With SMRT Link v26.1, actions taken in SMRT Link and on Revio and Vega instruments will be traceable *via* new SMRT Link audit log feature that contains a table highlighting *who* performed *what* actions *where* and *when* starting from the SMRT Link v26.1 install date[1]



Displaying rows 1 to 11 out of 101 (scroll to load more)

Entry Number	Date and Time (UTC)	User ID	Source	Event Name	Description	Software Version
1	2026-04-27, 06:38:15 PM	SYSTEM	Revio 84029	Instrument Started	Instrument start...	13.5.0.286403
2	2026-04-27, 06:30:20 PM	pbinstrument	SMRT Link	Run Data Updated	The XML data f...	26.1.0.284828
3	2026-04-27, 06:30:20 PM	SYSTEM	Revio 84032	Acquisition Transf...	Acquisition Tran...	13.5.0.286403
4	2026-04-27, 06:30:20 PM	pbinstrument	SMRT Link	Run Data Updated	The XML data f...	26.1.0.284828
5	2026-04-27, 06:17:13 PM	pbinstrument	SMRT Link	Run Data Updated	The XML data f...	26.1.0.284828
6	2026-04-27, 05:59:20 PM	SYSTEM	SMRT Link	Analysis Job Started	Analysis job 115...	26.1.0.284828
7	2026-04-27, 05:59:19 PM	jbruand	SMRT Link	Analysis Job Sub...	Analysis job 115...	26.1.0.284828
8	2026-04-27, 05:59:19 PM	jbruand	SMRT Link	Analysis Job Creat...	Analysis job 115...	26.1.0.284828
9	2026-04-27, 05:46:17 PM	asouppe	SMRT Link	Analysis Output D...	Results from an...	26.1.0.284828
10	2026-04-27, 05:41:34 PM	pbinstrument	SMRT Link	Run Data Updated	The XML data f...	26.1.0.284828

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[1] **Note:** Audit events start to be logged from the timepoint when the SMRT Link v26.1 software is installed. Events that occurred prior to installation of the SMRT Link v26.1 software cannot be retroactively added to audit logs after the SMRT Link v26.1 software installation is completed.

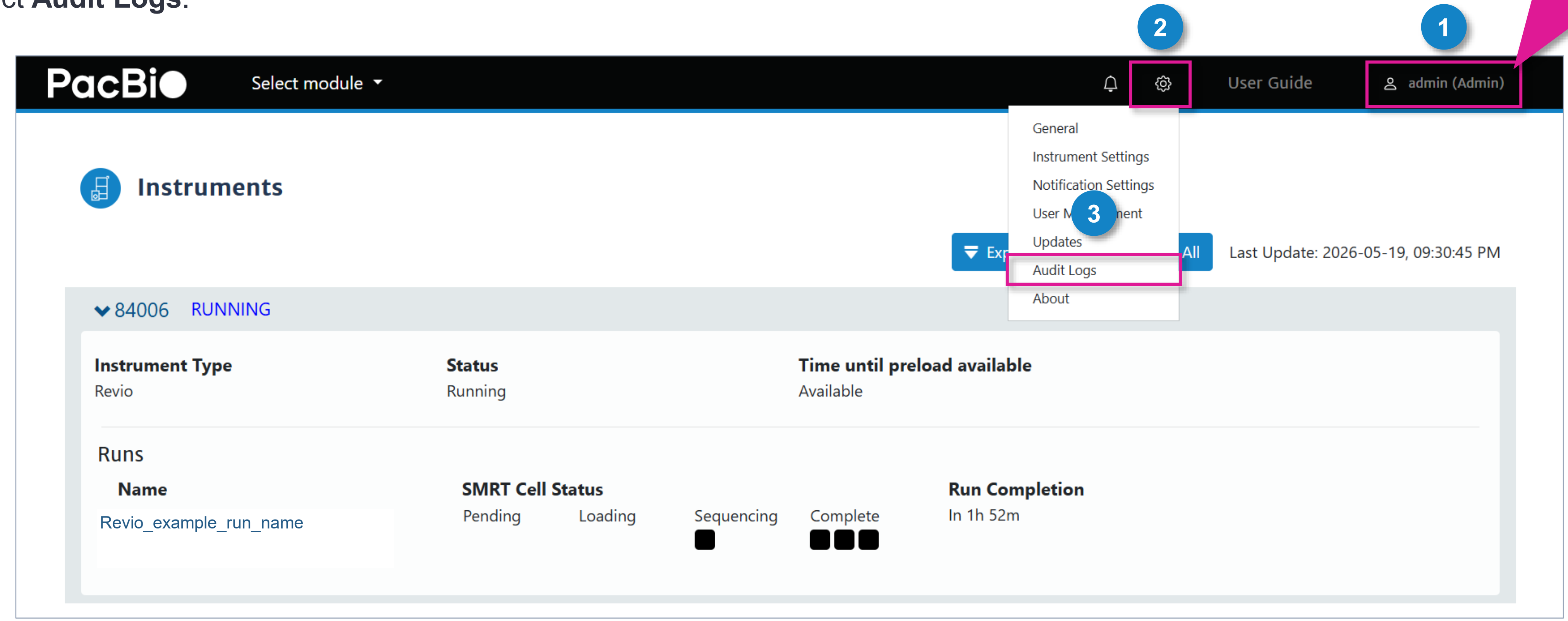
How to access SMRT Link audit logs

Admin users can access audit logs through SMRT Link Settings menu[1]

How to access audit logs

1. Log into SMRT Link using a user profile with Admin privileges.
2. Click **Settings** button.
3. Select **Audit Logs**.

Access to Audit Logs page is **restricted to users with the Admin role**



Last Update: 2026-05-19, 09:30:45 PM

84006 RUNNING

Instrument Type	Status	Time until preload available
Revio	Running	Available

Runs

Name	SMRT Cell Status: Pending	Loading	Sequencing	Complete	Run Completion
Revio_example_run_name			■	■■■	In 1h 52m

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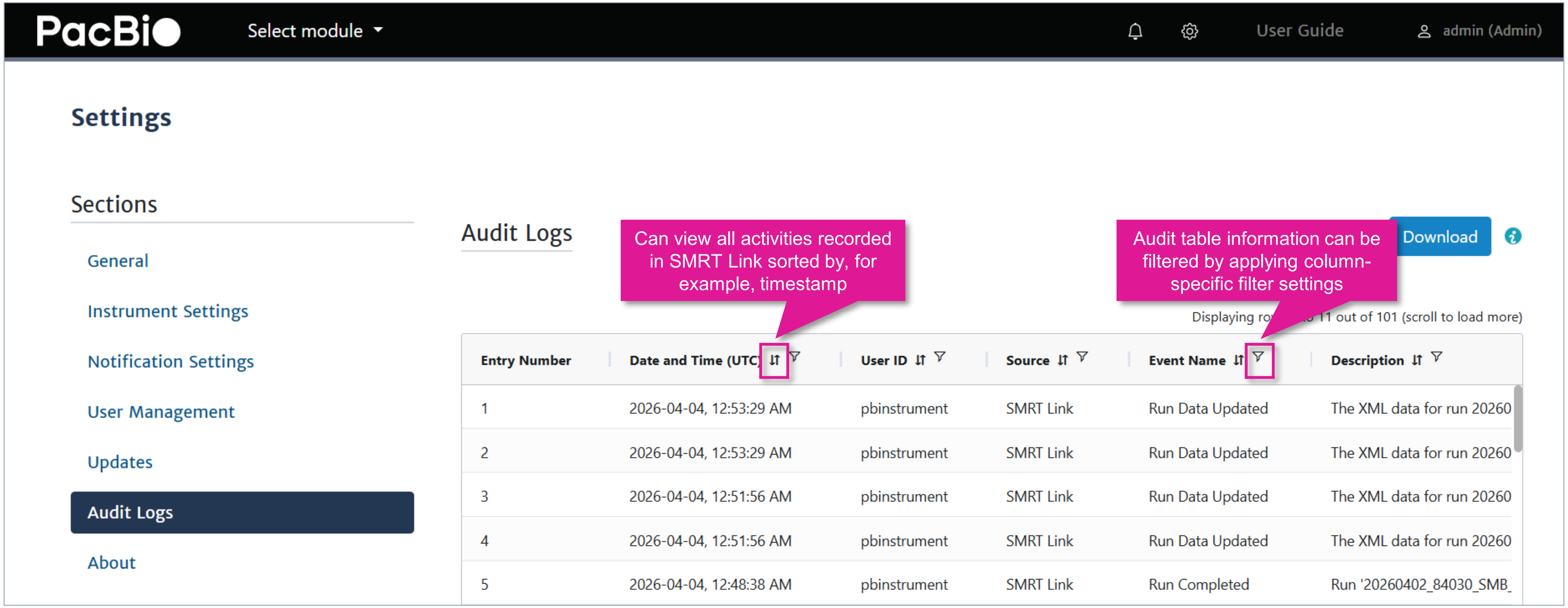
[1] New audit log API endpoints are documented at https://{dnsname}:8243/sl/docs/services/#/default.

Audit Logs page displays a table of recorded activity across all connected instruments

Each entry includes: Entry Number, Date and Time (UTC), User ID, Source, Event Name, Description, and Software Version



Audit Logs

Can view all activities recorded in SMRT Link sorted by, for example, timestamp

Audit table information can be filtered by applying column-specific filter settings

Displaying ro [illegible] 11 out of 101 (scroll to load more)

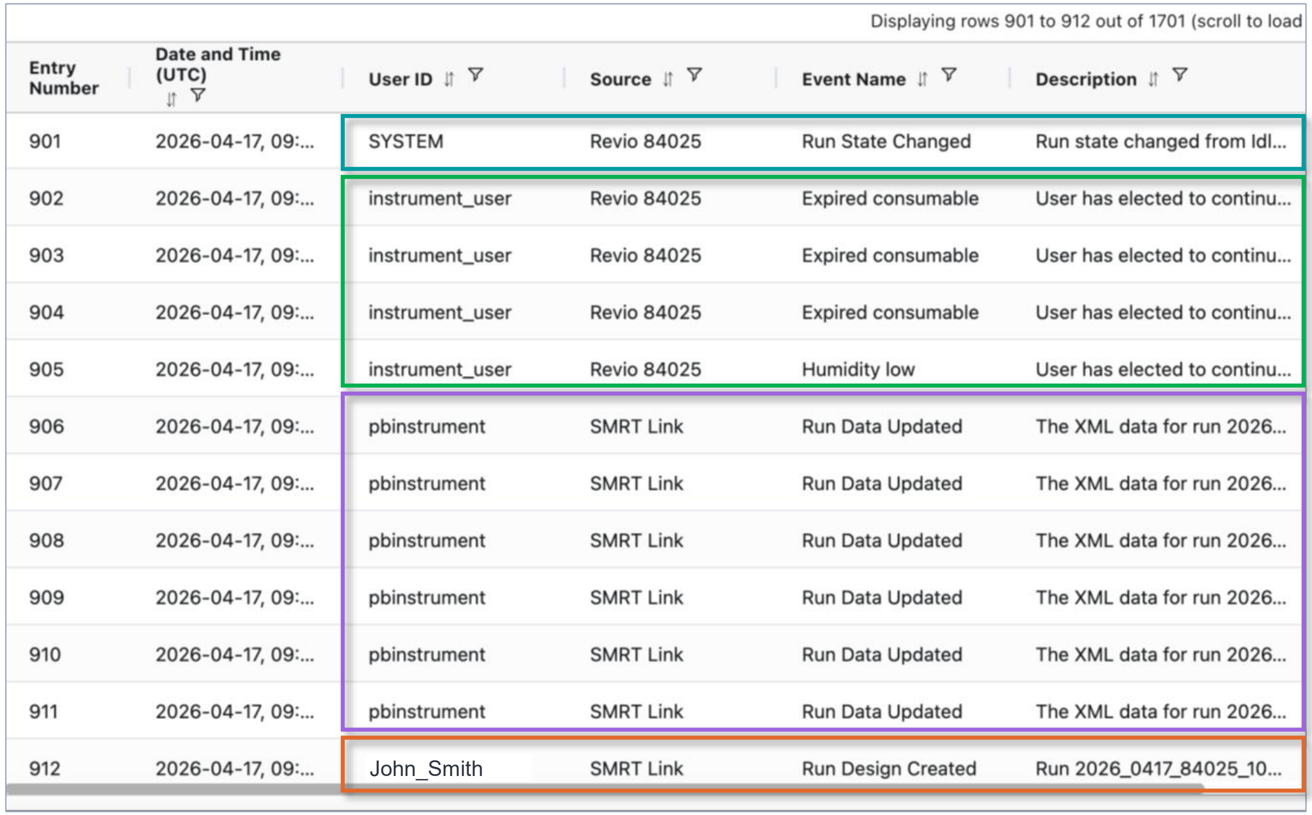
Entry Number	Date and Time (UTC)	User ID	Source	Event Name	Description
1	2026-04-04, 12:53:29 AM	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260
2	2026-04-04, 12:53:29 AM	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260
3	2026-04-04, 12:51:56 AM	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260
4	2026-04-04, 12:51:56 AM	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260
5	2026-04-04, 12:48:38 AM	pbinstrument	SMRT Link	Run Completed	Run '20260402_84030_SMB_

Audit logs capture system and user actions performed in SMRT Link and on connected instruments, including who performed the action, what action was performed, where it occurred, and when it occurred.

Audit log entries include both system-generated and user-performed actions

User ID field identifies whether an action was performed by a PacBio user or by the PacBio system ICS

Action generated by	User ID type	Example event or activity
PacBio system ICS	**SYSTEM**[1]	- **Actions that affect instrument status** - E.g., "run state changed" or "data transfer initiated"
	pbinstrument[1]	- **Actions that affect SMRT Link status** - E.g., ““run data updated”
PacBio (human) end-user	**Instrument user (e.g., “instrument_user”)**[2]	- **On-instrument user actions** - E.g., electing to start a run with expired consumables
	SMRT Link user (e.g., “John_Smith”)	- **In-SMRT Link user actions** - E.g., creating a new run design or a new analysis job



Displaying rows 901 to 912 out of 1701 (scroll to load

Entry Number	Date and Time (UTC)	User ID	Source	Event Name	Description
901	2026-04-17, 09:...	SYSTEM	Revio 84025	Run State Changed	Run state changed from Idl...
902	2026-04-17, 09:...	instrument_user	Revio 84025	Expired consumable	User has elected to continu...
903	2026-04-17, 09:...	instrument_user	Revio 84025	Expired consumable	User has elected to continu...
904	2026-04-17, 09:...	instrument_user	Revio 84025	Expired consumable	User has elected to continu...
905	2026-04-17, 09:...	instrument_user	Revio 84025	Humidity low	User has elected to continu...
906	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
907	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
908	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
909	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
910	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
911	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
912	2026-04-17, 09:...	John_Smith	SMRT Link	Run Design Created	Run 2026_0417_84025_10...

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[1] For actions taken by the PacBio system ICS, the User ID type (“SYSTEM” or “pbinstrument”) is taken from literal strings used in the system logs.

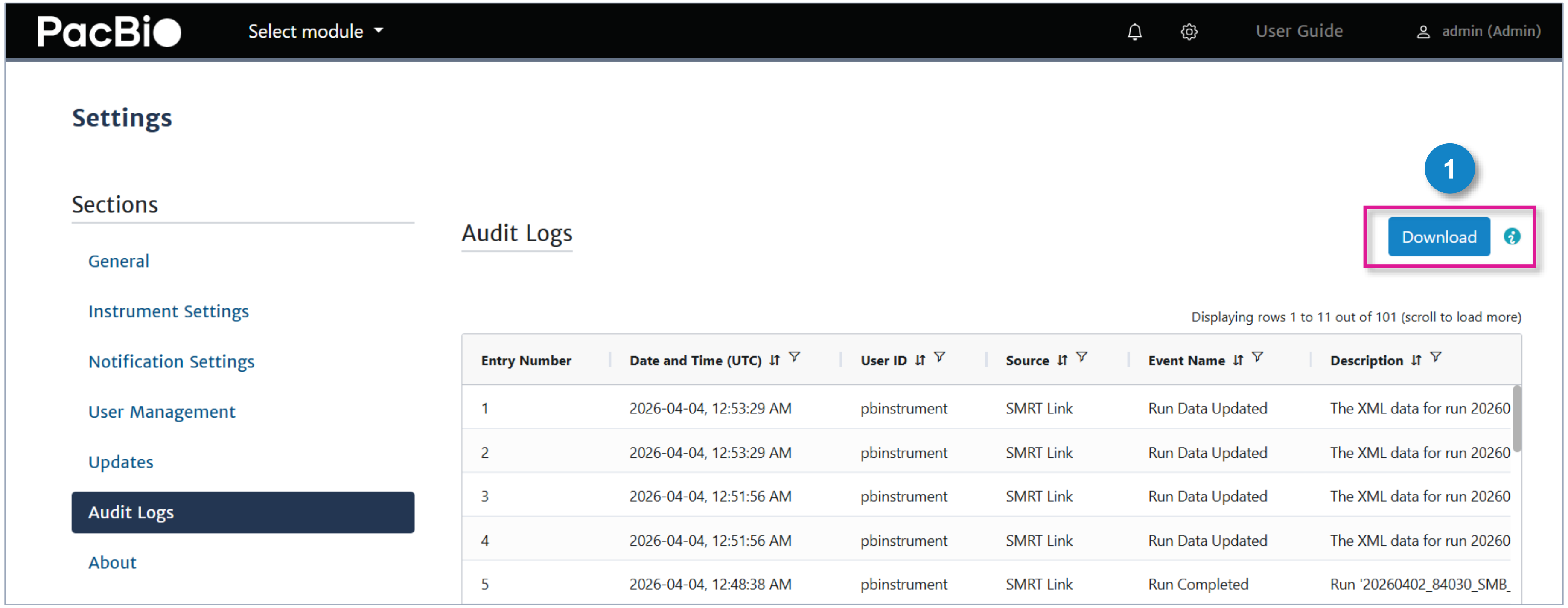
[2] In Revio system ICS v13.5 + SMRT Link v26.1, on-instrument actions taken by a human end-user are recorded in audit logs with User ID = “instrument_user”. In future software versions, the User ID will reflect the (human) end-user’s actual ID username.

How to download SMRT Link audit report

Admin users can download audit report through SMRT Link Audit Logs page

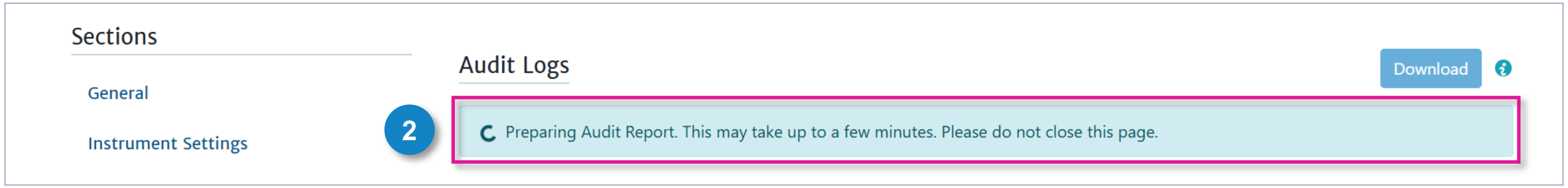
How to download audit report PDF

1. Click **Download** on Audit Logs page and wait for Audit Report PDF to be generated. This may take up to a few minutes.



Displaying rows 1 to 11 out of 101 (scroll to load more)

Entry Number	Date and Time (UTC)	User ID	Source	Event Name	Description
1	2026-04-04, 12:53:29 AM	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260
2	2026-04-04, 12:53:29 AM	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260
3	2026-04-04, 12:51:56 AM	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260
4	2026-04-04, 12:51:56 AM	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260
5	2026-04-04, 12:48:38 AM	pbinstrument	SMRT Link	Run Completed	Run '20260402_84030_SMB_



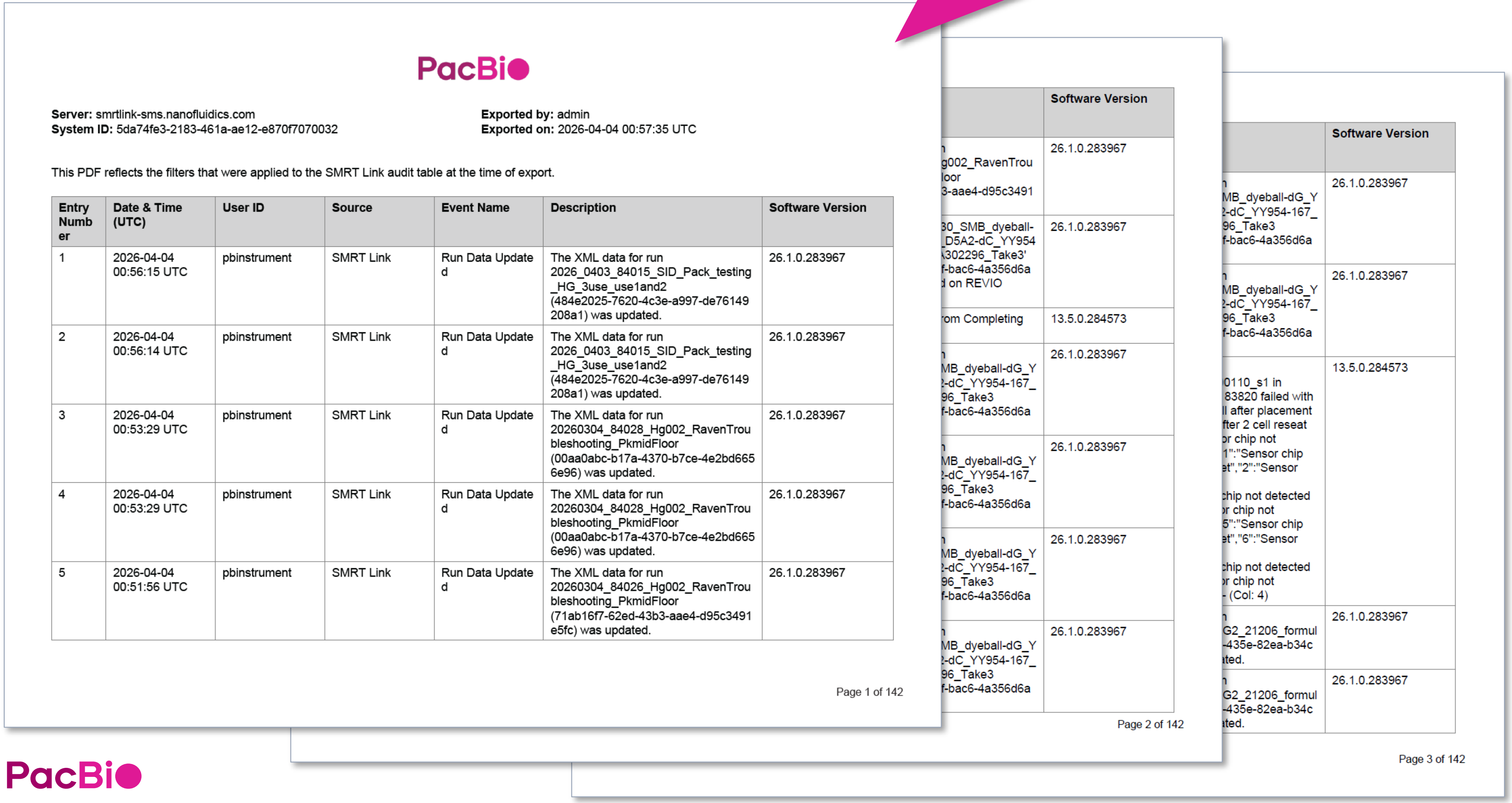
Preparing Audit Report. This may take up to a few minutes. Please do not close this page.

Example Audit Report PDF

Audit report

Example Audit Report

PDF report reflects the filters that were applied to the SMRT Link audit table at the time of export.



Server: smrtlink-sms.nanofluidics.com
System ID: 5da74fe3-2183-461a-ae12-e870f7070032

Exported by: admin
Exported on: 2026-04-04 00:57:35 UTC

This PDF reflects the filters that were applied to the SMRT Link audit table at the time of export.

Entry Number	Date & Time (UTC)	User ID	Source	Event Name	Description	Software Version
1	2026-04-04 00:56:15 UTC	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026_0403_84015_SID_Pack_testing_HG_3use_use1and2 (484e2025-7620-4c3e-a997-de76149208a1) was updated.	26.1.0.283967
2	2026-04-04 00:56:14 UTC	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026_0403_84015_SID_Pack_testing_HG_3use_use1and2 (484e2025-7620-4c3e-a997-de76149208a1) was updated.	26.1.0.283967
3	2026-04-04 00:53:29 UTC	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260304_84028_Hg002_RavenTroubleshooting_PkmidFloor (00aa0abc-b17a-4370-b7ce-4e2bd6656e96) was updated.	26.1.0.283967
4	2026-04-04 00:53:29 UTC	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260304_84028_Hg002_RavenTroubleshooting_PkmidFloor (00aa0abc-b17a-4370-b7ce-4e2bd6656e96) was updated.	26.1.0.283967
5	2026-04-04 00:51:56 UTC	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 20260304_84026_Hg002_RavenTroubleshooting_PkmidFloor (71ab16f7-62ed-43b3-aae4-d95c3491e5fc) was updated.	26.1.0.283967

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	Software Version
n g002_RavenTrou loor 3-aae4-d95c3491	26.1.0.283967
30_SMB_dyeball- _D5A2-dC_YY954 A302296_Take3' f-bac6-4a356d6a d on REVIO	26.1.0.283967
rom Completing	13.5.0.284573
n MB_dyeball-dG_Y 2-dC_YY954-167_ 96_Take3 f-bac6-4a356d6a	26.1.0.283967
n MB_dyeball-dG_Y 2-dC_YY954-167_ 96_Take3 f-bac6-4a356d6a	26.1.0.283967
n MB_dyeball-dG_Y 2-dC_YY954-167_ 96_Take3 f-bac6-4a356d6a	26.1.0.283967
n MB_dyeball-dG_Y 2-dC_YY954-167_ 96_Take3 f-bac6-4a356d6a	26.1.0.283967

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	Software Version
n MB_dyeball-dG_Y 2-dC_YY954-167_ 96_Take3 f-bac6-4a356d6a	26.1.0.283967
n MB_dyeball-dG_Y 2-dC_YY954-167_ 96_Take3 f-bac6-4a356d6a	26.1.0.283967
0110_s1 in 83820 failed with ll after placement fter 2 cell reseat or chip not 1":"Sensor chip et","2":"Sensor chip not detected or chip not 5":"Sensor chip et","6":"Sensor chip not detected or chip not - (Col: 4)	13.5.0.284573
n G2_21206_formul -435e-82ea-b34c ated.	26.1.0.283967
n G2_21206_formul -435e-82ea-b34c ated.	26.1.0.283967

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SMRT Link v26.1 user experience improvements

SMRT Link v26.1 improved asymmetric barcode demultiplexing support

New Barcode Type specification in Data Management enables automatic demultiplexing (in-SMRT Link), predefined barcode pairing rules, and built-in GUI validation for all asymmetrically indexed sample types

SMRT Link v25.3: Run Design only supports symmetric barcoding of samples when using newly imported index sets

- SMRT Link v25.3 **Run Design form does not** enable specification of whether the barcodes at each end are the **same** (symmetric) or **different** (asymmetric) for indexed samples
- SMRT Link v25.3 **Run Design assumes all imported index sets are symmetric**, preventing users from using asymmetric barcode sets that are not already pre-installed with SMRT Link (i.e., Twist UDI barcodes, M13 barcodes)
  - For newly imported index sets, users can **only** select **symmetric index (barcode) pairs** for multiplexed samples
- **Asymmetric index pairing only works for specific SMRT Link-preloaded asymmetric barcode sets** like the Twist UDI barcode set
  - The only workaround for using other asymmetric barcode sets is to set "**Sample is indexed = NO**" in Run Design and then manually run Demultiplex Barcodes utility in SMRT Link **after** the run is finished.

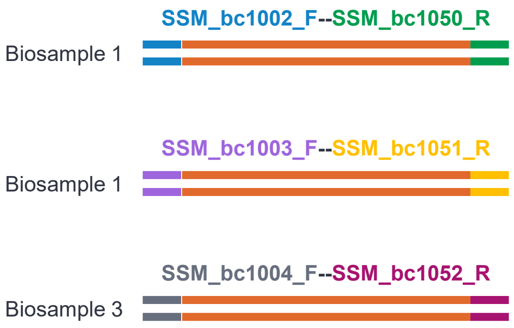
Example:

Newly imported barcode set

>**SSM_bc1002_F**
ACACACAGACTGTGAGGTAAAACGACGGCCAGT
>**SSM_bc1003_F**
ACACATCTCGTGAGAGGTAAAACGACGGCCAGT
>**SSM_bc1004_F**
CACGCACACACGCGCGGTAAAACGACGGCCAGT
>**SSM_bc1050_R**
GATATACGCGAGAGAGCAGGAAACAGCTATGAC
>SSM_bc1051_R
CGTGTCTAGCGCGCGCCAGGAAACAGCTATGAC
>**SSM_bc1052_R**
GTGTGAGATATATATCCAGGAAACAGCTATGAC

User imports a barcode set containing **3 forward (F) indexes + 3 reverse (R) indexes**

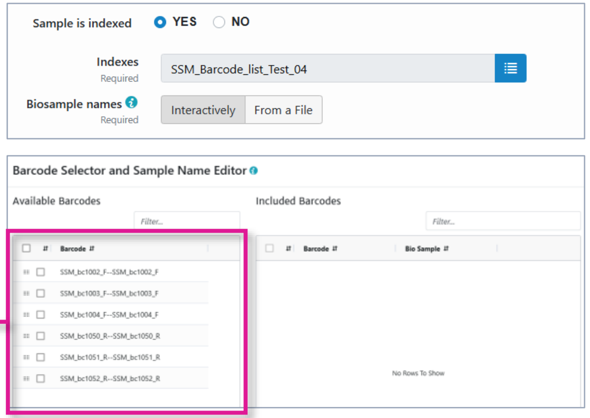
User-desired barcode combinations



User wants to assign **3 asymmetric index pairs** to 3 unique biosamples

SMRT Link v25.3 Run Design only allows symmetric barcode assignments

SSM_bc1002_F--SSM_bc1002_F
SSM_bc1003_F--SSM_bc1003_F
SSM_bc1004_F--SSM_bc1004_F
SSM_bc1050_R--SSM_bc1050_R
SSM_bc1051_R--SSM_bc1051_R
SSM_bc1052_R--SSM_bc1052_R



Sample is indexed ● YES ○ NO

Indexes (Required): SSM_Barcode_list_Test_04

Biosample names (Required): Interactively | From a File

Barcode Selector and Sample Name Editor

Available Barcodes

☐	#	Barcode
	☐	SSM_bc1002_F--SSM_bc1002_F
	☐	SSM_bc1003_F--SSM_bc1003_F
	☐	SSM_bc1004_F--SSM_bc1004_F
	☐	SSM_bc1050_R--SSM_bc1050_R
	☐	SSM_bc1051_R--SSM_bc1051_R
	☐	SSM_bc1052_R--SSM_bc1052_R

Included Barcodes

☐	#	Barcode	Bio Sample

No Rows To Show

User is **unable** to assign **3 asymmetric index pairs** to 3 unique biosamples since **only symmetric barcode combinations are displayed** in SMRT Link v25.3 Run Design interactive barcode selector tool → In this case, user must specify **Sample is indexed = No** and manually perform barcode demultiplexing in SMRT Link v25.3.

SMRT Link v26.1 improved asymmetric barcode demultiplexing support (cont.)

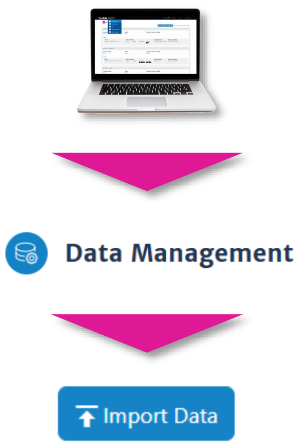
New Barcode Type specification in Data Management enables automatic demultiplexing (in-SMRT Link), predefined barcode pairing rules, and built-in GUI validation for all asymmetrically indexed sample types

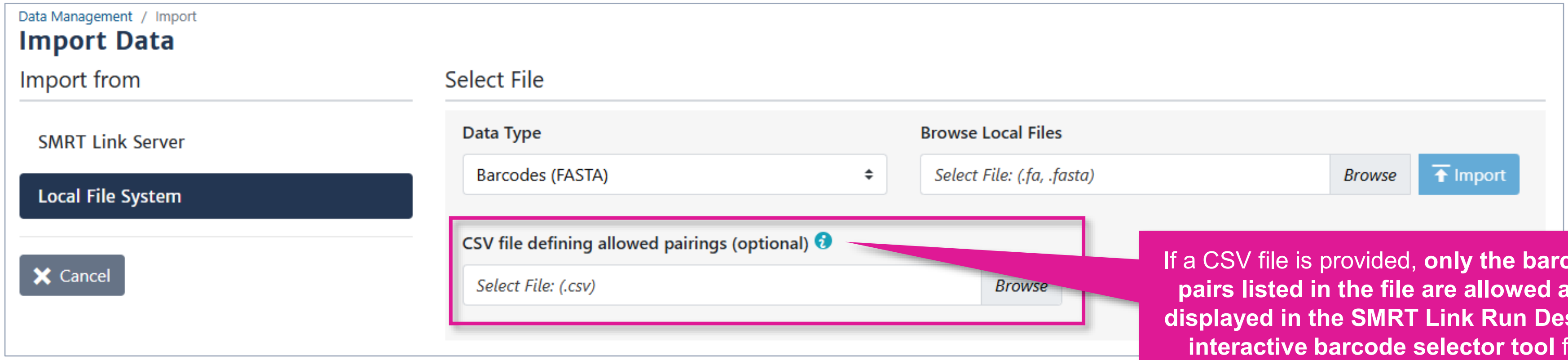
SMRT Link v26.1 (on-premises): Data Management enables option to upload a CSV file defining allowed barcode pairings to support asymmetric indexing of samples[1,2]

- To use specific index sequences for **asymmetric barcoding**, **optionally include a CSV file defining all allowed barcode pairs**, e.g., "bc1001_F,bc1001_R".

To import a barcode set:

1. Navigate to Data Management > Import Data
2. Select a barcode set FASTA file
3. (Optional) Provide a valid pairs CSV file





Data Type: Barcodes (FASTA)

Browse Local Files: Select File: (.fa, .fasta)

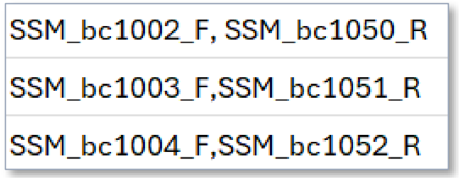
CSV file defining allowed pairings (optional): Select File: (.csv)

If a CSV file is provided, **only the barcode pairs listed in the file are allowed and displayed in the SMRT Link Run Design interactive barcode selector tool** for assigning biosample names

Example barcode set FASTA containing:
3 forward (F) indexes + 3 reverse (R) indexes

```
>SSM_bc1002_F
ACACACAGACTGTGAGGTAAAACGACGGCCAGT
>SSM_bc1003_F
ACACATCTCGTGAGAGGTAAAACGACGGCCAGT
>SSM_bc1004_F
CACGCACACACGCGCGGTAAAACGACGGCCAGT
>SSM_bc1050_R
GATATACGCGAGAGAGCAGGAAACAGCTATGAC
>SSM_bc1051_R
CGTGTCTAGCGCGCGCCAGGAAACAGCTATGAC
>SSM_bc1052_R
GTGTGAGATATATATCCAGGAAACAGCTATGAC
```

Example CSV file defining all allowed barcode pairs (i.e., to enable asymmetric indexing of samples)



```
SSM_bc1002_F, SSM_bc1050_R
SSM_bc1003_F,SSM_bc1051_R
SSM_bc1004_F,SSM_bc1052_R
```

---

[1] **Note:** **If no CSV file is provided**, the barcode set is treated as **symmetric** and each barcode is paired with itself. If a CSV file is provided, a barcode set is treated as asymmetric if at least one defined pair contains different forward and reverse barcodes.

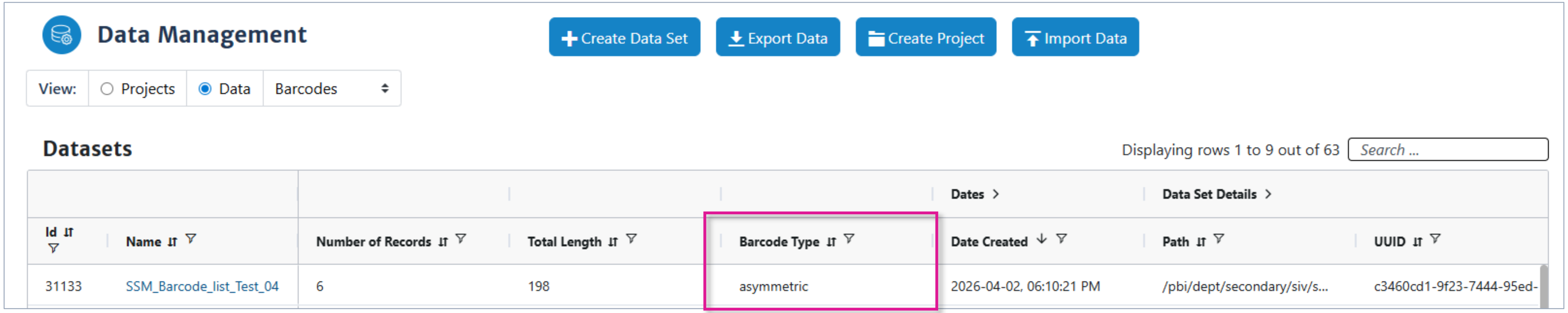
[2] **Note:** To modify allowed barcode pairs for an existing barcode set, you will need to re-import the barcode set in Data Management using a different filename and also upload a new CSV file containing the updated list of valid barcode pairs.

SMRT Link v26.1 improved asymmetric barcode demultiplexing support (cont.)

New Barcode Type specification in Data Management enables automatic demultiplexing (in-SMRT Link), predefined barcode pairing rules, and built-in GUI validation for all asymmetrically indexed sample types

SMRT Link v26.1 (on-premises): Data Managements enables option to upload a CSV file defining allowed barcode pairings to support asymmetric indexing of samples[1,2]

- After importing barcode set FASTA + (optional) CSV file, **Barcode Type** column value is **determined automatically** based on structure of barcode pairings in CSV



Datasets

Displaying rows 1 to 9 out of 63

				Dates		Data Set Details
Id	Name	Number of Records	Total Length	Barcode Type	Date Created	Path	UUID
31133	SSM_Barcode_list_Test_04	6	198	asymmetric	2026-04-02, 06:10:21 PM	/pbi/dept/secondary/siv/s...	c3460cd1-9f23-7444-95ed-

---

[1] Only barcode pairs specified in the CSV will be displayed in SMRT Link Run Design interactive barcode selector tool for assigning biosample names.

[2] To modify allowed barcode pairs for a given barcode set, you will need to re-import the barcode set in Data Management using a different filename and also upload a new CSV file containing the updated list of valid barcode pairs.

SMRT Link v26.1 improved asymmetric barcode demultiplexing support (cont.)

New Barcode Type specification in Data Management enables automatic demultiplexing (in-SMRT Link), predefined barcode pairing rules, and built-in GUI validation for all asymmetrically indexed sample types

SMRT Link v26.1 (on-premises): Data Managements enables option to upload a CSV file defining allowed barcode pairings to support asymmetric indexing of samples[1,2]

- After importing barcode set FASTA + (optional) CSV file, **Barcode Type** column value is **determined automatically** based on structure of barcode pairings in CSV

Barcode type	Description
Symmetric	• Same barcode on both ends of the sequence • No valid pairs CSV required • Demultiplexing is automatically performed on-instrument
Asymmetric	• Different barcodes on each end • Defined using a valid pairs CSV file • Demultiplexing is performed automatically or manually off-instrument (in SMRT Link) after sequencing run completes
Iso-Seq (informational-only)[3]	• Assigned to barcode sets used for Iso-Seq workflows that are pre-loaded into SMRT Link
Other (informational-only)[3]	• Assigned to generic or legacy barcode sets that are pre-loaded into SMRT Link • May not include pairing definitions • May not support asymmetric validation or automated workflows

Demultiplexing behavior depends on barcode type

Symmetric barcode sets

- Demultiplexed automatically on-instrument

Asymmetric barcode sets

- Demultiplexed automatically or manually off-instrument (in SMRT Link) after sequencing run completes

---

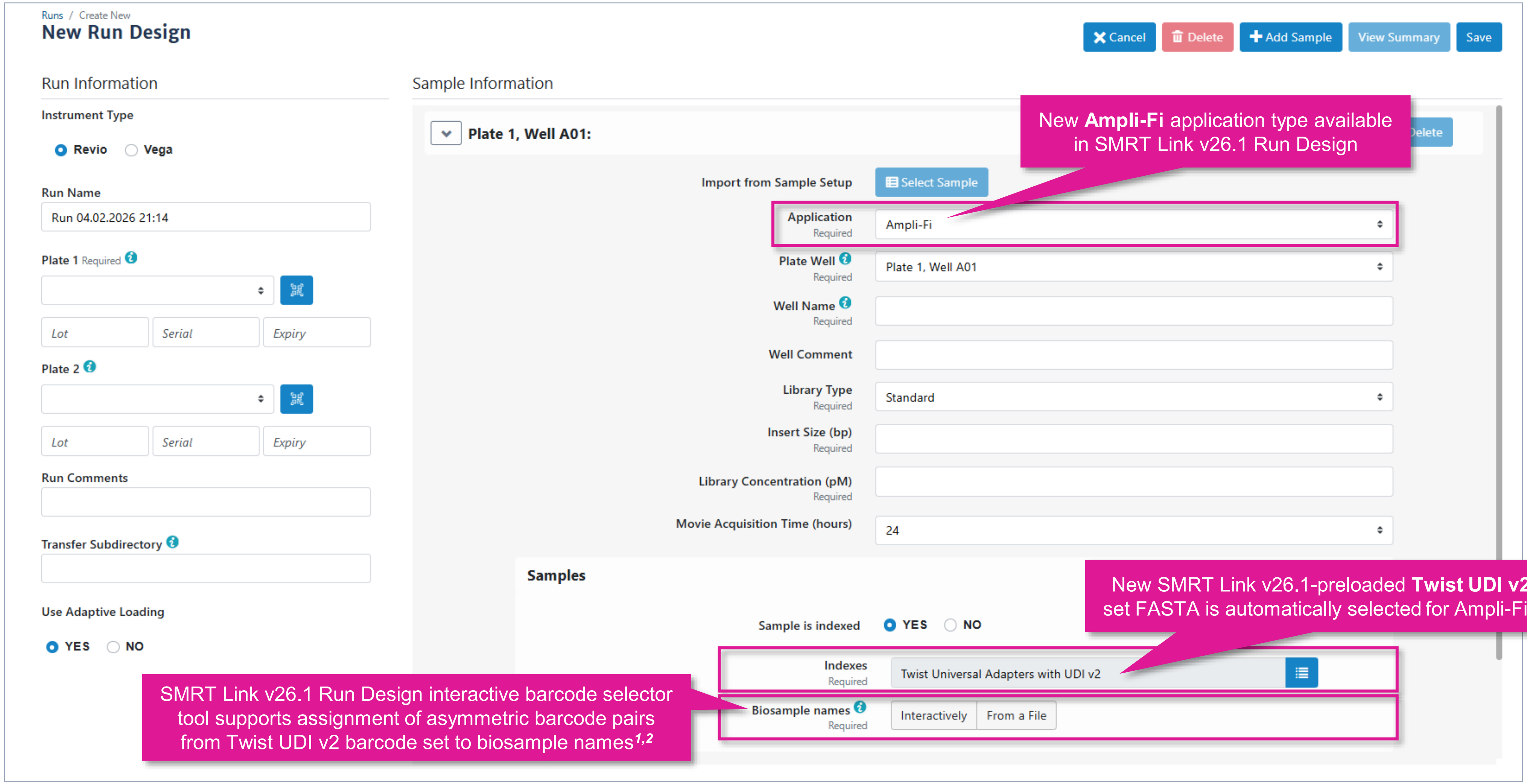
[1] Only barcode pairs specified in the CSV will be displayed in SMRT Link Run Design interactive barcode selector tool for assigning biosample names.

[2] To modify allowed barcode pairs for a given barcode set, you will need to re-import the barcode set in Data Management using a different filename and also upload a new CSV file containing the updated list of valid barcode pairs.

[3] **Note:** **Iso-seq** and **Other** labels are only used for Barcode Sets that are **pre-loaded** with SMRT Link and cannot be assigned or edited by users.

SMRT Link v26.1 improved Ampli-Fi run design support

New ‘Ampli-Fi’ application type option in run design enables use of preloaded, protocol-specific barcodes and automatic demultiplexing (in-SMRT Link) for Ampli-Fi samples



New Run Design

Run Information

Instrument Type: ● Revio ○ Vega

Run Name: Run 04.02.2026 21:14

Plate 1 Required

Lot / Serial / Expiry

Plate 2

Lot / Serial / Expiry

Run Comments

Transfer Subdirectory

Use Adaptive Loading: ● YES ○ NO

Sample Information

Plate 1, Well A01:

Import from Sample Setup

Application (Required): Ampli-Fi

Plate Well (Required): Plate 1, Well A01

Well Name (Required)

Well Comment

Library Type (Required): Standard

Insert Size (bp) (Required)

Library Concentration (pM) (Required)

Movie Acquisition Time (hours): 24

Samples

Sample is indexed: ● YES ○ NO

Indexes (Required): Twist Universal Adapters with UDI v2

Biosample names (Required)

New **Ampli-Fi** application type available in SMRT Link v26.1 Run Design

New SMRT Link v26.1-preloaded **Twist UDI v2** barcode set FASTA is automatically selected for Ampli-Fi samples[1]

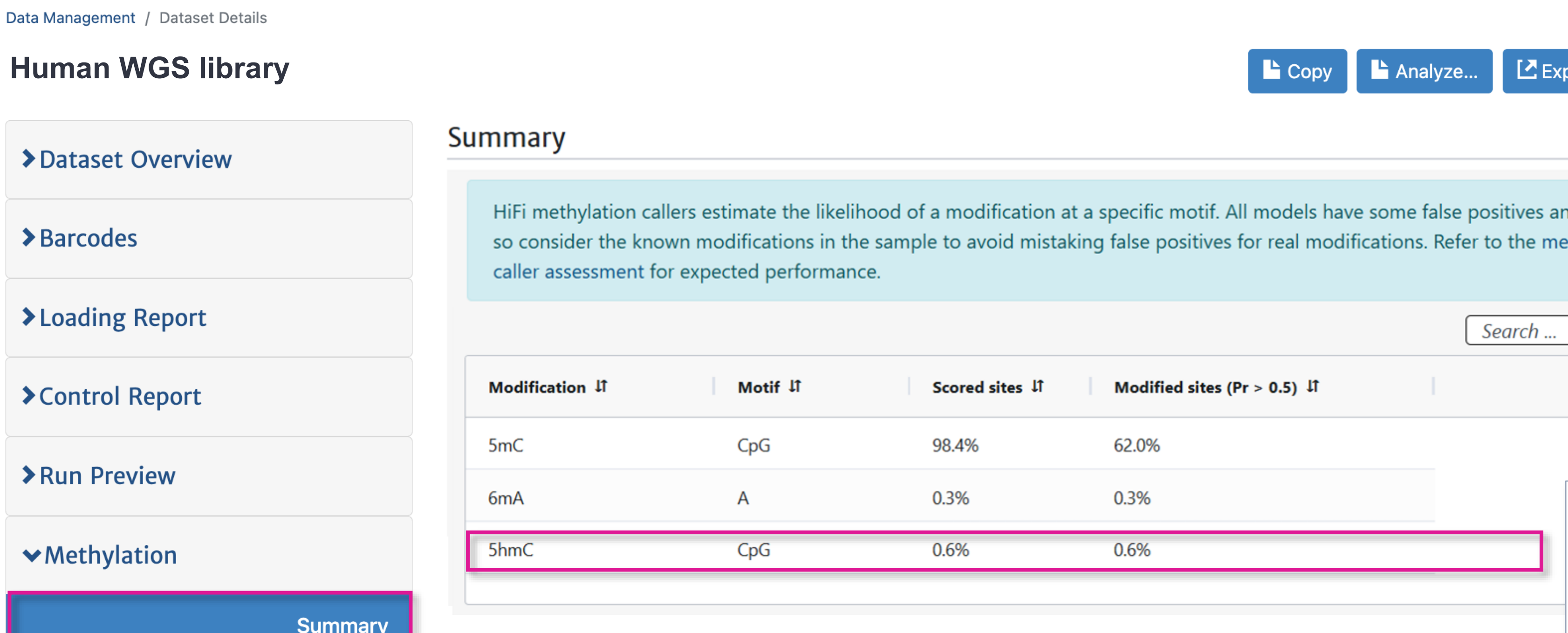
SMRT Link v26.1 Run Design interactive barcode selector tool supports assignment of asymmetric barcode pairs from Twist UDI v2 barcode set to biosample names[1,2]

---

[1] In SMRT Link v25.3, barcode demultiplexing of Ampli-Fi samples requires 1) `Amplifi_TwistUDIadapters_noP7P5` barcode set FASTA file and 2) `Ampli-Fi_Barcoded_Sample_Name_File.csv` template file to be manually downloaded from the PacBio website in order to correctly assign asymmetric Twist barcode pairs to biosample names.

SMRT Link v26.1 improved methylation reports (Revio system only): Data Management Methylation Summary report

Updated Methylation Summary report now includes 5-hydroxymethylcytosine (5hmC)



Data Management / Dataset Details

Human WGS library

- Dataset Overview
- Barcodes
- Loading Report
- Control Report
- Run Preview
- Methylation
  - Summary
  - Score distributions
  - Per-read distributions

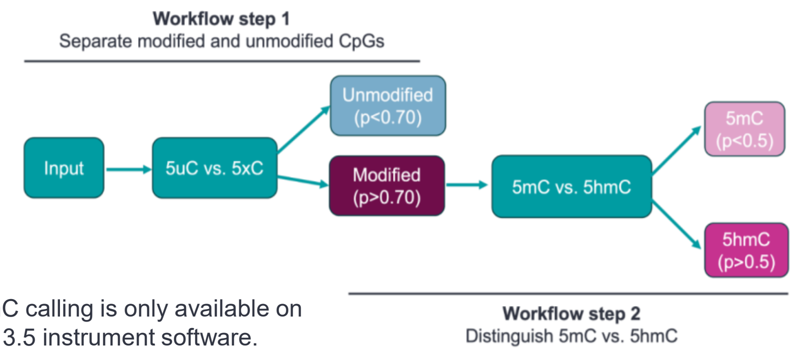
Summary

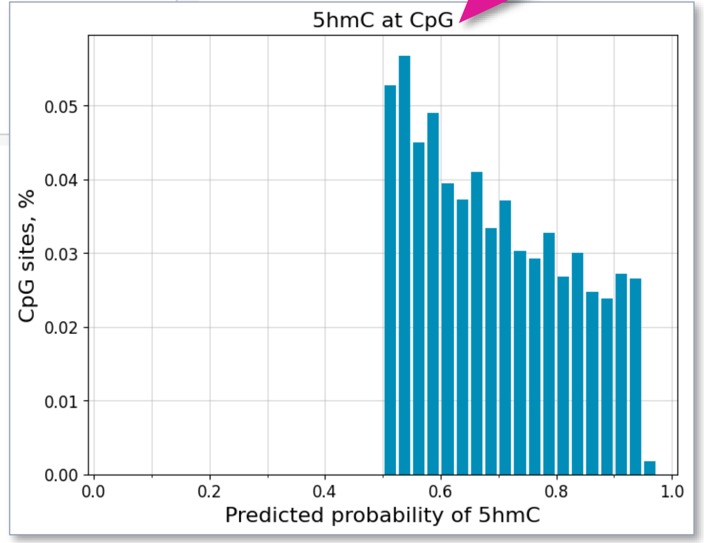
HiFi methylation callers estimate the likelihood of a modification at a specific motif. All models have some false positives and negatives, so consider the known modifications in the sample to avoid mistaking false positives for real modifications. Refer to the methylation caller assessment for expected performance.

Modification	Motif	Scored sites	Modified sites (Pr > 0.5)
5mC	CpG	98.4%	62.0%
6mA	A	0.3%	0.3%
5hmC	CpG	0.6%	0.6%

For 5hmC, the model only reports 5hmC sites where the probability of modification is **≥ 0.50**

Visual overview of 5hmC analysis workflow. Here, 5xC refers to the combination of 5mC + 5hmC sites.





Note: On-instrument 5hmC calling is only available on Revio systems running v13.5 instrument software.

SMRT Link v26.1 improved methylation reports (Revio system only): Data Management Per-read distribution plots

New per-read methylation distributions provide a view of methylation variability across reads

Native human (HG002) WGS library example

Most reads contain ~50-60% 5mC-modified CpG sites

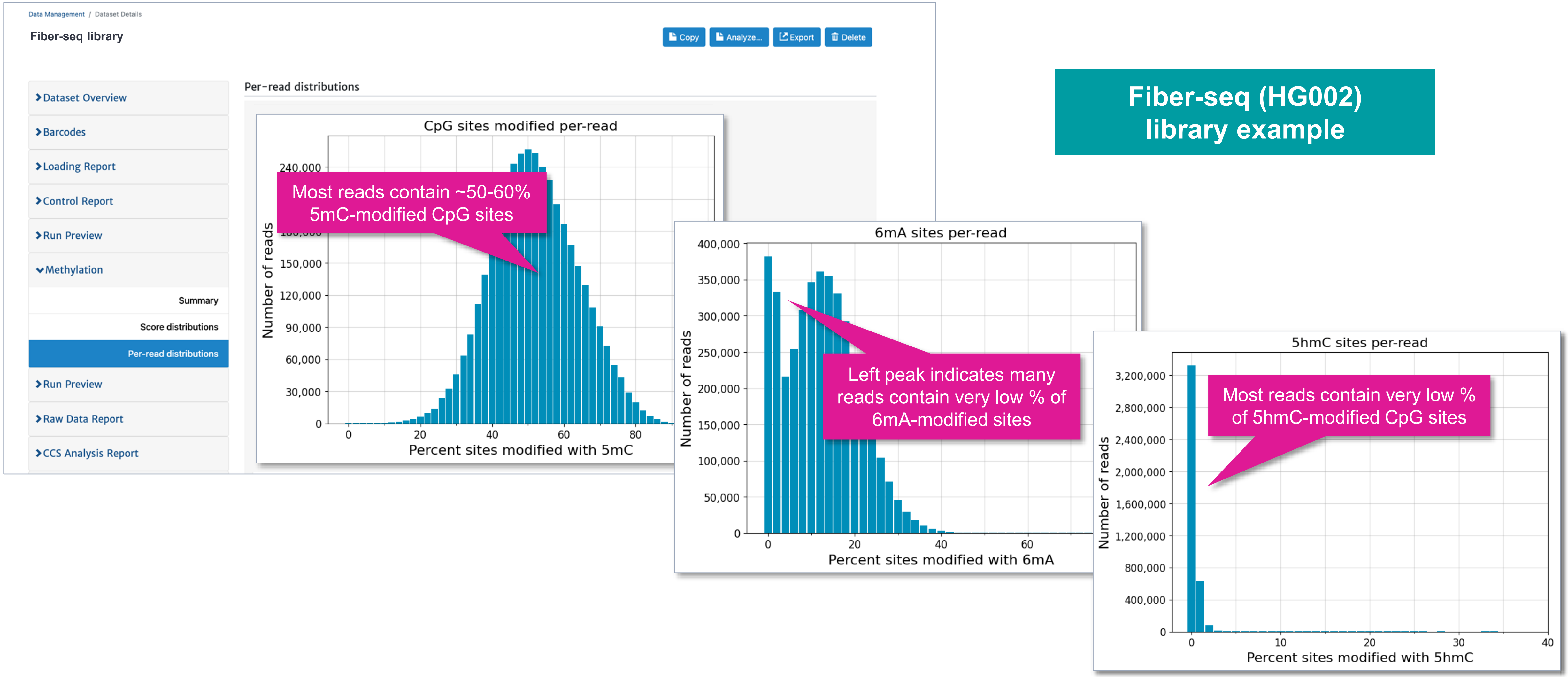
Most reads contain very low % of 6mA-modified sites

Most reads contain very low % of 5hmC-modified CpG sites

[1] Example per-read distributions shown are for a native human (HG002) WGS sample run on a Revio system with SPRQ-Nx chemistry (24-hour acquisition time; 200 pM on-plate loading concentration).

SMRT Link v26.1 improved methylation reports (Revio system only): Data Management Per-read distribution plots (cont.)

New per-read methylation distributions provide a view of methylation variability across reads



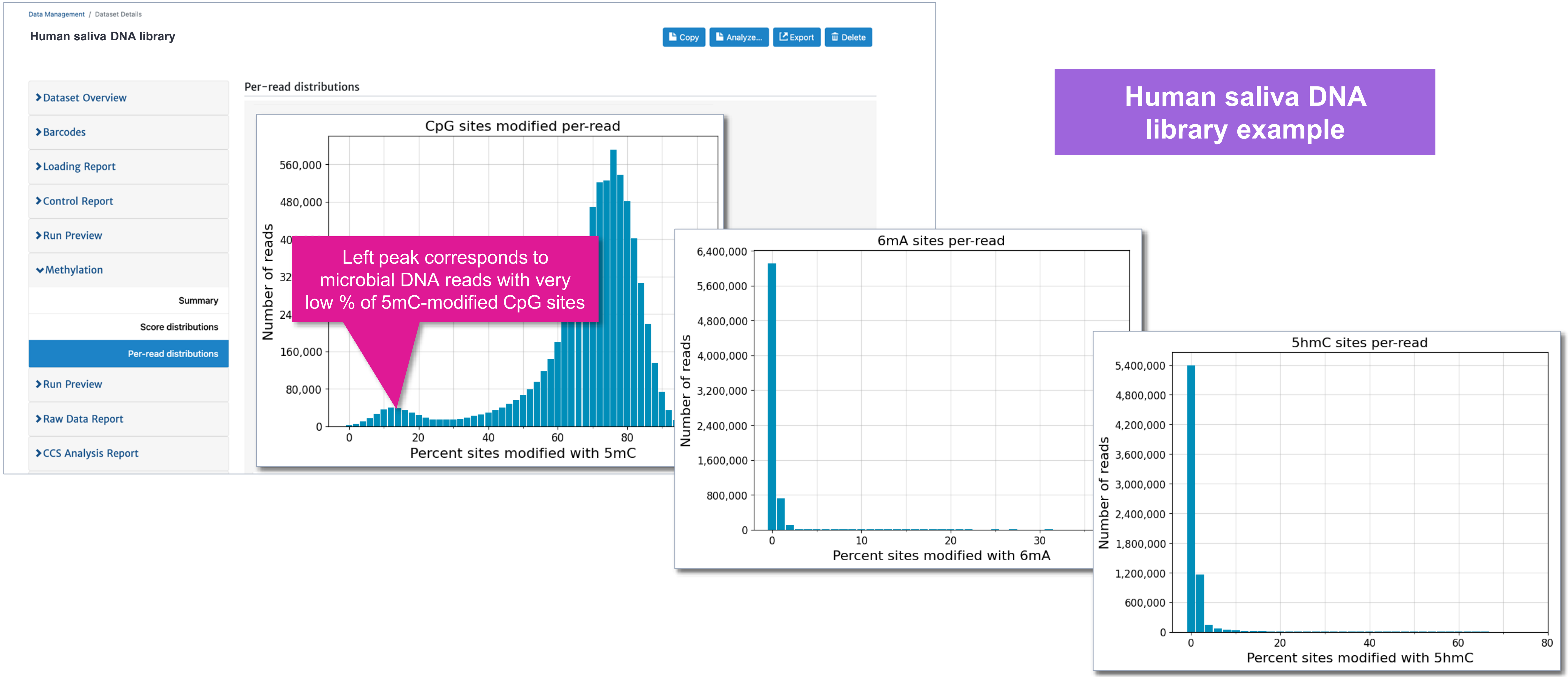
Most reads contain ~50-60% 5mC-modified CpG sites

Left peak indicates many reads contain very low % of 6mA-modified sites

Most reads contain very low % of 5hmC-modified CpG sites

Fiber-seq (HG002) library example

New distributions of modification percentages per read (Revio-only)

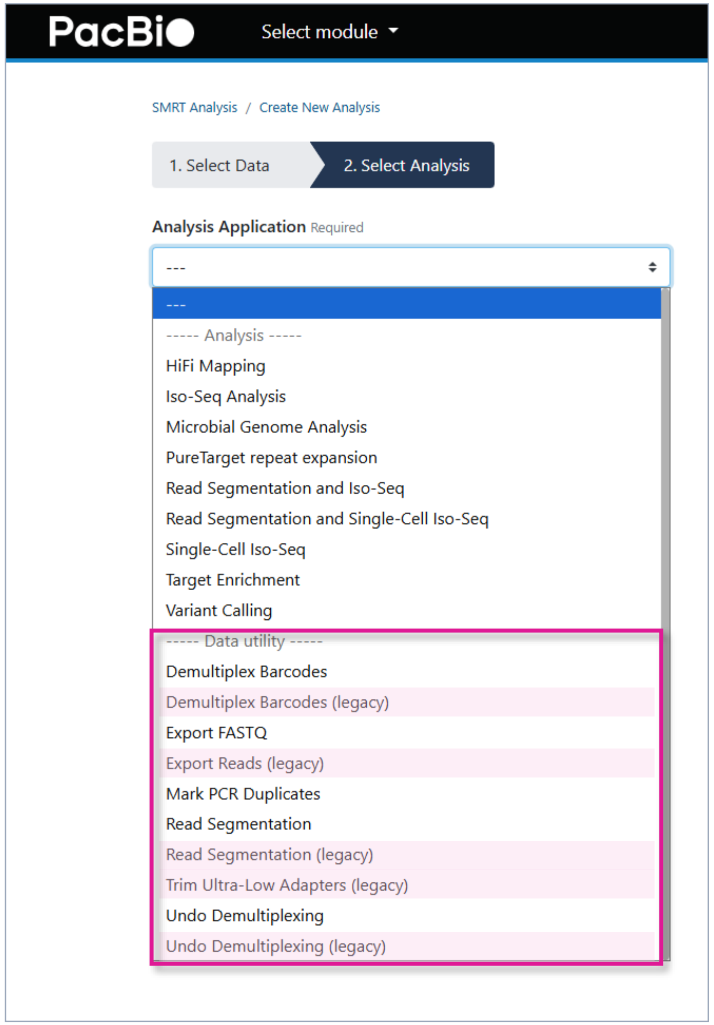


Left peak corresponds to microbial DNA reads with very low % of 5mC-modified CpG sites

Human saliva DNA library example

SMRT Link v26.1 data utilities are updated

Data utility workflows have been updated with a new backend implementation



- Updated workflow names (IDs) (see table below) reflect the new workflow implementations and should be used for all new analyses
- Updated data utility workflow behaviors and outputs remain consistent between versions, enabling a smooth transition[1]
- Previous versions of data utilities are still available as **"(legacy)"** for reference but will not be updated and will be removed in a future SMRT Link release[2]

- Updated analysis workflows are packaged in the wdl-v2.zip
- Direct execution requires cromwell and workflow-specific JSON inputs file

Application or data utility name	Old workflow pipeline ID (SMRT Link v25.4)	New workflow pipeline ID (SMRT Link v26.1)
Demultiplex Barcodes	`cromwell.workflows.pb_demux_ccs`	`pacbio.workflows.pb_demultiplex`
Read Segmentation	`cromwell.workflows.pb_segment_reads`	`pacbio.workflows.pb_read_segmentation`
Undo Demultiplexing	`cromwell.workflows.pb_undo_demux`	`pacbio.workflows.pb_undo_demux_v2`
Export FASTQ (replaces Export Reads)	`cromwell.workflows.pb_export_reads`	`pacbio.workflows.pb_export_fastq`

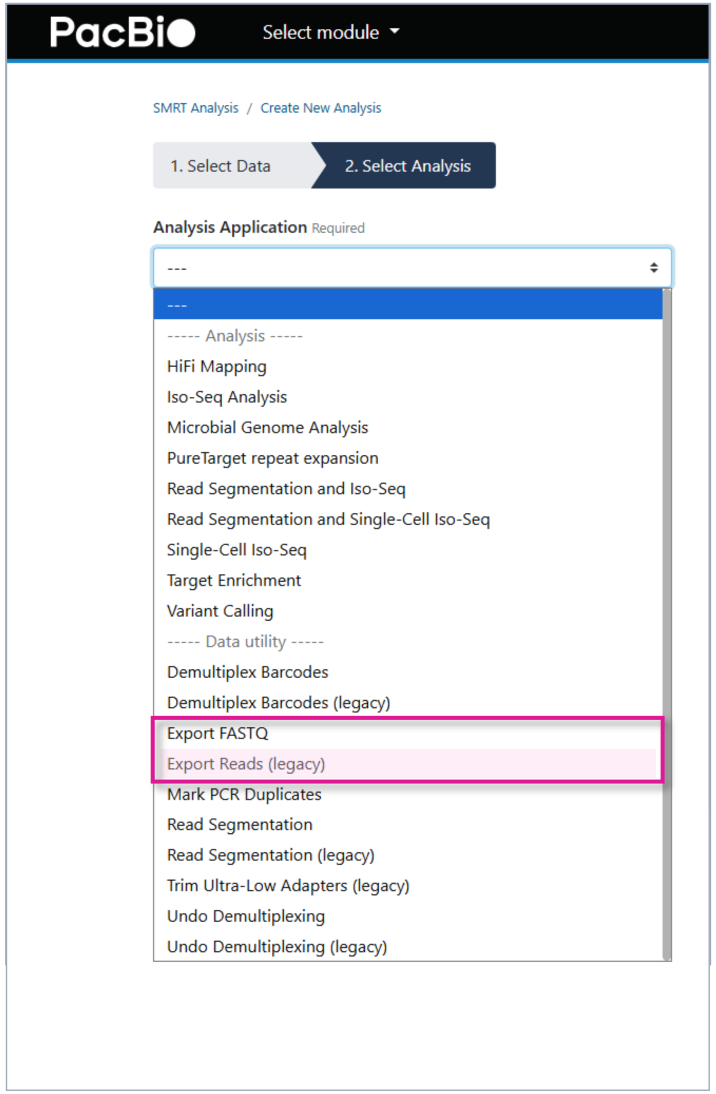
---

[1] **Note: Demultiplex Barcodes** workflow updates change the location of demultiplexed BAM output files, which are now generated in the workflow execution directory rather than the `outputs/` directory; refer to SMRT Link documentation for details.

[2] The "(legacy)" workflow version label is only used for new data utility jobs. Older completed analyses will not be updated to indicate if the legacy version was run.

SMRT Link v26.1 data utilities are updated (cont.)

New Export FASTQ workflow supersedes Export Reads (legacy) and provides richer sequence metadata

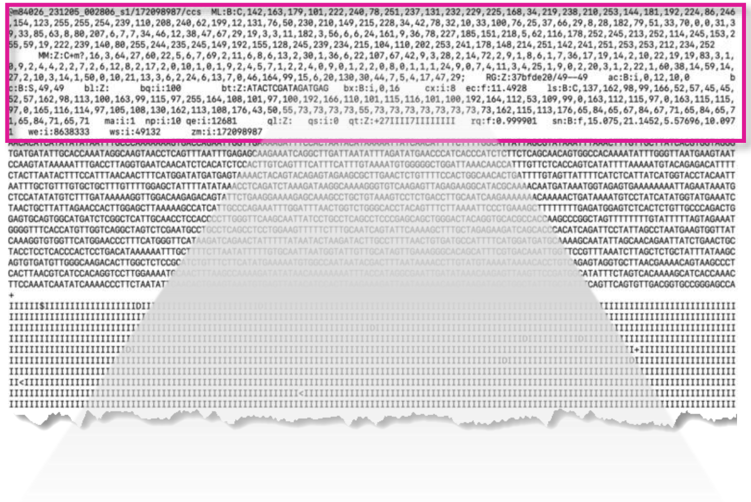


- New **Export FASTQ** workflow supersedes **Export Reads**; unlike the older workflow[1], Export FASTQ **only** outputs FASTQ, and now uses **GZIP/TGZ compression** for all outputs (instead of ZIP)
- New feature in Export FASTQ workflow (and the underlying bam2fastq tool) is that BAM tags are retained in the FASTQ header (line 1), preserving data such as the read quality scores and methylation info

Export Reads (legacy) FASTQ line 1

```
@m84026_231205_002806_s1/172098987/ccs
```

Export FASTQ (new) FASTQ line 1



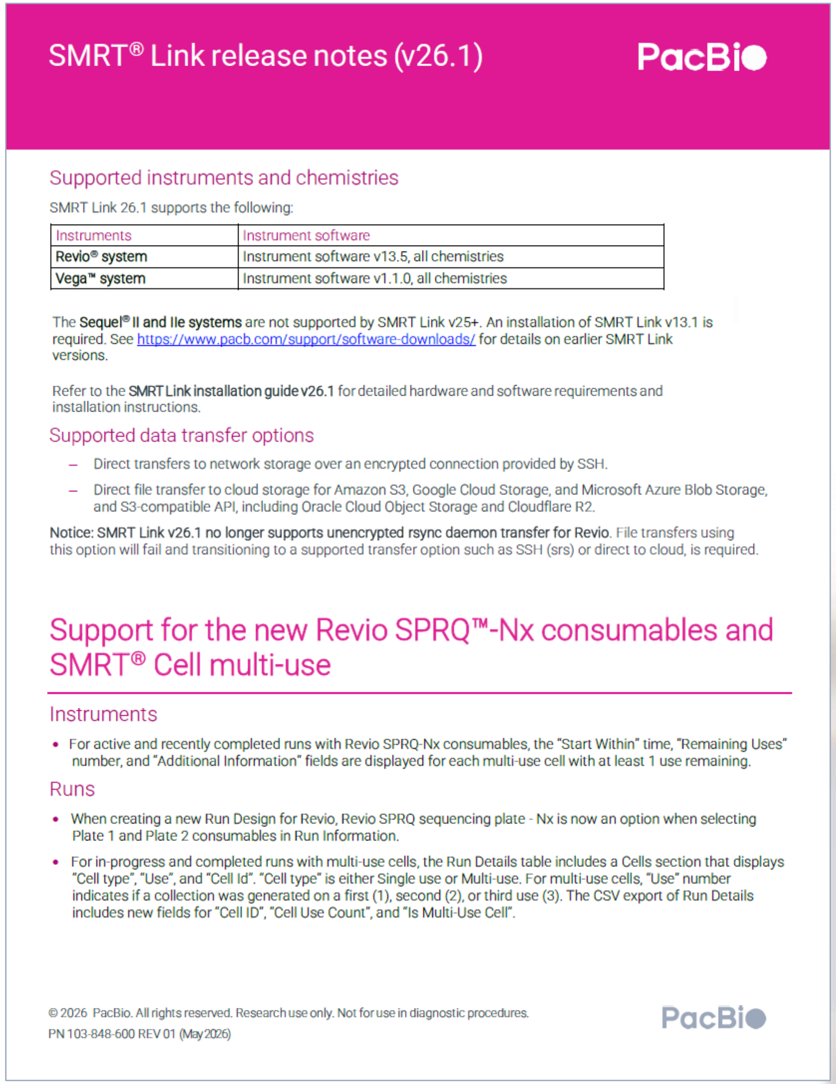
```
@m84026_231205_002806_s1/172098987/ccs
ML:B:C,142,163,179,101,222,240,78,251,237,131...
MM:Z:C+m?,16,3,64,27,60,22,5,6,7,69,1,36;...
RG:Z:37bfde20/49--49  ac:B:i,0,12,10,0  bc:B:S,49,49  bl:Z:
bq:i:100  bt:Z:ATACTCGATAGATGAG  bx:B:i,0,16  cx:i:8
ec:f:11.4928
ls:B:C,137,162,98,99,166,52,57,45,99,115,97,255,... ma:i:1
np:i:10  qe:i:12681  ql:Z:  qs:i:0  qt:Z:+27IIII7IIIIIIII
rq:f:0.999901  sn:B:f,15.075,21.1452,5.57696,10.0971
we:i:8638333  ws:i:49132  zm:i:172098987
```

---

[1] **Note:** Older **Export Reads** workflow outputs FASTQ, FASTA, and BAM file formats and uses ZIP compression.

SMRT Link v26.1 release notes (103-848-600)

Refer to SMRT Link release notes for summary of new features & updates, fixed issues, and known issues[1]



SMRT® Link release notes (v26.1)

PacBio

Supported instruments and chemistries

SMRT Link 26.1 supports the following:

Instruments	Instrument software
Revio® system	Instrument software v13.5, all chemistries
Vega™ system	Instrument software v1.1.0, all chemistries

The **Sequel® II and IIe systems** are not supported by SMRT Link v25+. An installation of SMRT Link v13.1 is required. See https://www.pacb.com/support/software-downloads/ for details on earlier SMRT Link versions.

Refer to the **SMRT Link installation guide v26.1** for detailed hardware and software requirements and installation instructions.

Supported data transfer options

- Direct transfers to network storage over an encrypted connection provided by SSH.
- Direct file transfer to cloud storage for Amazon S3, Google Cloud Storage, and Microsoft Azure Blob Storage, and S3-compatible API, including Oracle Cloud Object Storage and Cloudflare R2.

Notice: SMRT Link v26.1 no longer supports unencrypted rsync daemon transfer for Revio. File transfers using this option will fail and transitioning to a supported transfer option such as SSH (srs) or direct to cloud, is required.

Support for the new Revio SPRQ™-Nx consumables and SMRT® Cell multi-use

Instruments

- For active and recently completed runs with Revio SPRQ-Nx consumables, the "Start Within" time, "Remaining Uses" number, and "Additional Information" fields are displayed for each multi-use cell with at least 1 use remaining.

Runs

- When creating a new Run Design for Revio, Revio SPRQ sequencing plate - Nx is now an option when selecting Plate 1 and Plate 2 consumables in Run Information.
- For in-progress and completed runs with multi-use cells, the Run Details table includes a Cells section that displays "Cell type", "Use", and "Cell Id". "Cell type" is either Single use or Multi-use. For multi-use cells, "Use" number indicates if a collection was generated on a first (1), second (2), or third use (3). The CSV export of Run Details includes new fields for "Cell ID", "Cell Use Count", and "Is Multi-Use Cell".

PN 103-848-600 REV 01 (May 2026)

PacBio

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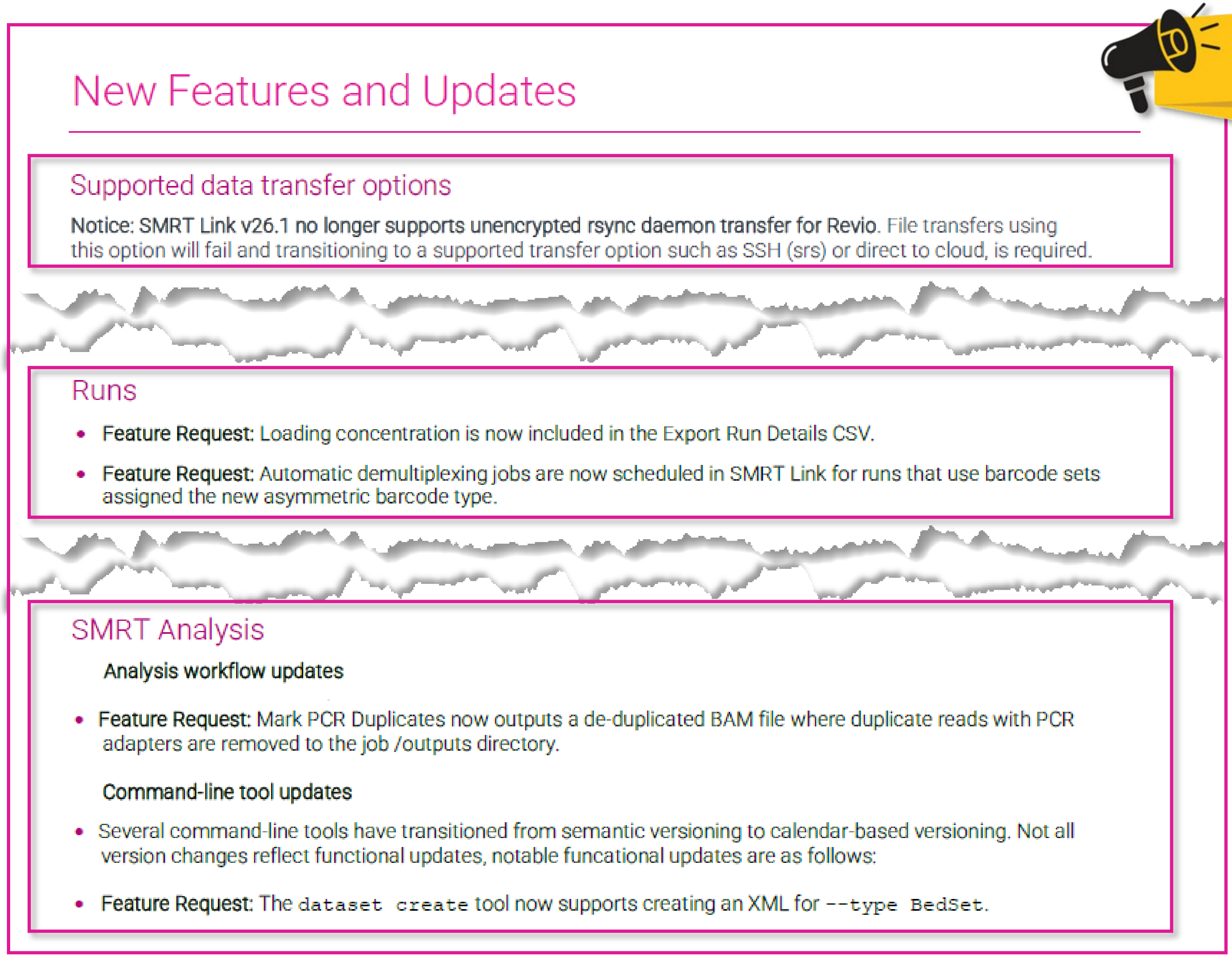
[1] SMRT Link release notes (v26.1) (103-848-600)

SMRT Link v26.1 new features and updates – Examples



New Features and Updates

Supported data transfer options

Notice: SMRT Link v26.1 no longer supports unencrypted rsync daemon transfer for Revio. File transfers using this option will fail and transitioning to a supported transfer option such as SSH (srs) or direct to cloud, is required.

Runs

- **Feature Request:** Loading concentration is now included in the Export Run Details CSV.
- **Feature Request:** Automatic demultiplexing jobs are now scheduled in SMRT Link for runs that use barcode sets assigned the new asymmetric barcode type.

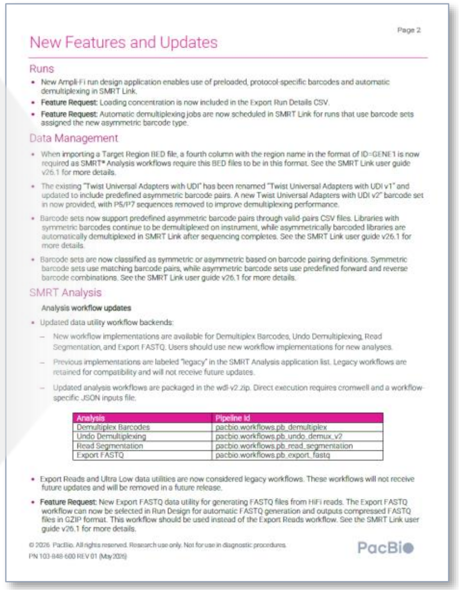
SMRT Analysis

Analysis workflow updates

- **Feature Request:** Mark PCR Duplicates now outputs a de-duplicated BAM file where duplicate reads with PCR adapters are removed to the job /outputs directory.

Command-line tool updates

- Several command-line tools have transitioned from semantic versioning to calendar-based versioning. Not all version changes reflect functional updates, notable funcational updates are as follows:
- **Feature Request:** The `dataset create` tool now supports creating an XML for `--type BedSet`.



Page 2

New Features and Updates

Runs

- New Ampli-Fi run design application enables use of preloaded, protocol-specific barcodes and automatic demultiplexing in SMRT Link.
- **Feature Request:** Loading concentration is now included in the Export Run Details CSV.
- **Feature Request:** Automatic demultiplexing jobs are now scheduled in SMRT Link for runs that use barcode sets assigned the new asymmetric barcode type.

Data Management

- When importing a Target Region BED file, a fourth column with the region name in the format of ID=GENE1 is now required as SMRT® Analysis workflows require this BED files to be in this format. See the SMRT Link user guide v26.1 for more details.
- The existing "Twist Universal Adapters with UDI" has been renamed "Twist Universal Adapters with UDI v1" and updated to include predefined asymmetric barcode pairs. A new Twist Universal Adapters with UDI v2" barcode set in now provided, with P5/P7 sequences removed to improve demultiplexing performance.
- Barcode sets now support predefined asymmetric barcode pairs through valid-pairs CSV files. Libraries with symmetric barcodes continue to be demultiplexed on instrument, while asymmetrically barcoded libraries are automatically demultiplexed in SMRT Link after sequencing completes. See the SMRT Link user guide v26.1 for more details.
- Barcode sets are now classified as symmetric or asymmetric based on barcode pairing definitions. Symmetric barcode sets use matching barcode pairs, while asymmetric barcode sets use predefined forward and reverse barcode combinations. See the SMRT Link user guide v26.1 for more details.

SMRT Analysis

Analysis workflow updates

- Updated data utility workflow backends:
  - New workflow implementations are available for Demultiplex Barcodes, Undo Demultiplexing, Read Segmentation, and Export FASTQ. Users should use new workflow implementations for new analyses.
  - Previous implementations are labeled "legacy" in the SMRT Analysis application list. Legacy workflows are retained for compatibility and will not receive future updates.
  - Updated analysis workflows are packaged in the wdl-v2.zip. Direct execution requires cromwell and a workflow-specific JSON inputs file.

Analysis	Pipeline Id
Demultiplex Barcodes	pacbio.workflows.pb_demultiplex
Undo Demultiplexing	pacbio.workflows.pb_undo_demux_v2
Read Segmentation	pacbio.workflows.pb_read_segmentation
Export FASTQ	pacbio.workflows.pb_export_fastq

- Export Reads and Ultra Low data utilities are now considered legacy workflows. These workflows will not receive future updates and will be removed in a future release.
- **Feature Request:** New Export FASTQ data utility for generating FASTQ files from HiFi reads. The Export FASTQ workflow can now be selected in Run Design for automatic FASTQ generation and outputs compressed FASTQ files in GZIP format. This workflow should be used instead of the Export Reads workflow. See the SMRT Link user guide v26.1 for more details.

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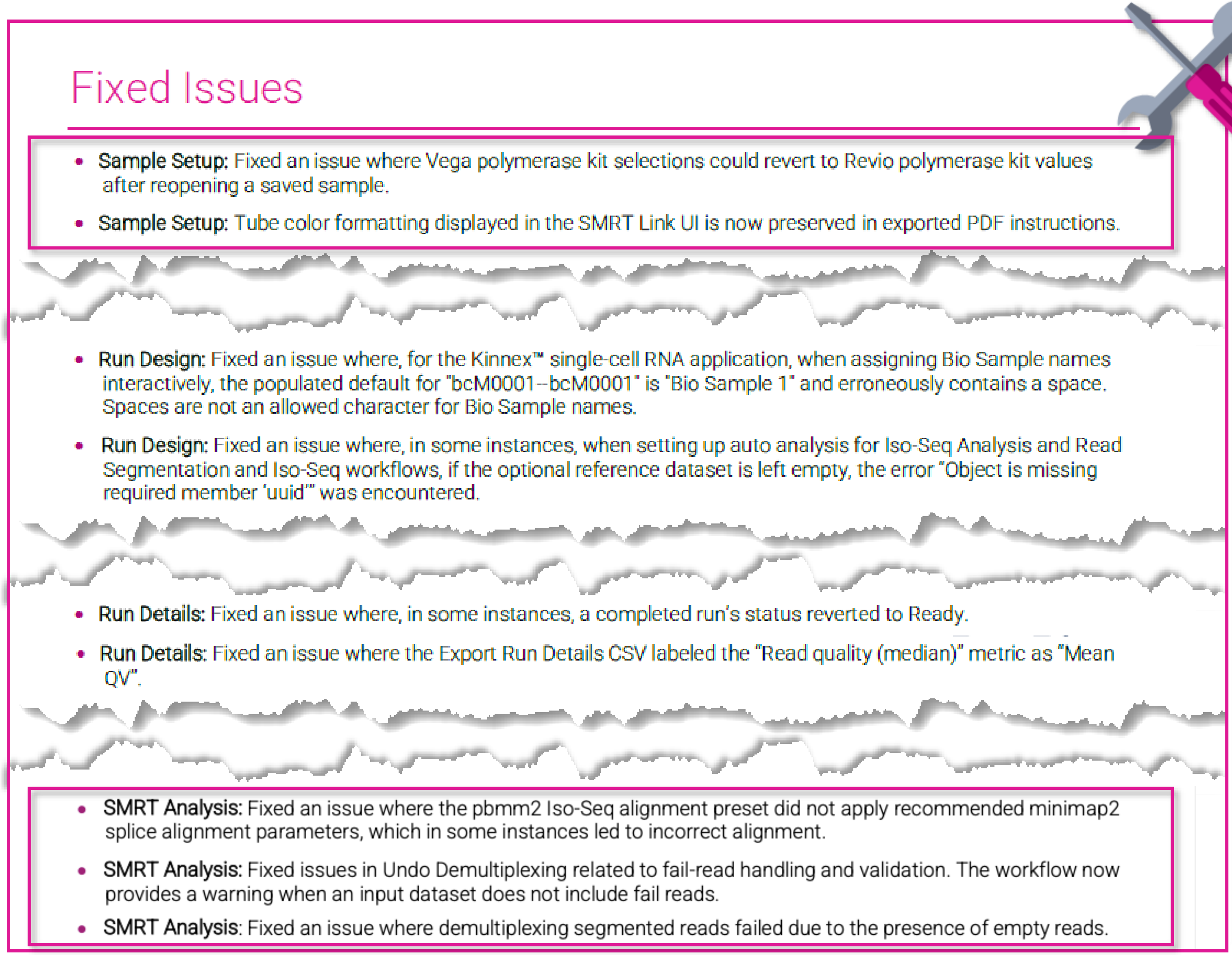
PacBio

SMRT Link v26.1 fixed issues – Examples



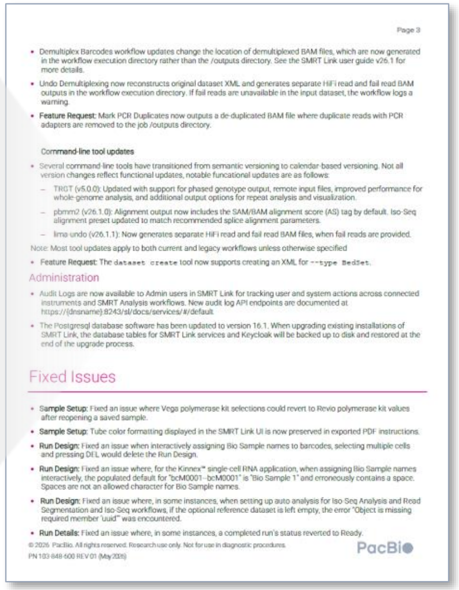
Fixed Issues

- **Sample Setup:** Fixed an issue where Vega polymerase kit selections could revert to Revio polymerase kit values after reopening a saved sample.
- **Sample Setup:** Tube color formatting displayed in the SMRT Link UI is now preserved in exported PDF instructions.

- **Run Design:** Fixed an issue where, for the Kinnex™ single-cell RNA application, when assigning Bio Sample names interactively, the populated default for "bcM0001--bcM0001" is "Bio Sample 1" and erroneously contains a space. Spaces are not an allowed character for Bio Sample names.
- **Run Design:** Fixed an issue where, in some instances, when setting up auto analysis for Iso-Seq Analysis and Read Segmentation and Iso-Seq workflows, if the optional reference dataset is left empty, the error "Object is missing required member 'uuid'" was encountered.

- **Run Details:** Fixed an issue where, in some instances, a completed run's status reverted to Ready.
- **Run Details:** Fixed an issue where the Export Run Details CSV labeled the "Read quality (median)" metric as "Mean QV".

- **SMRT Analysis:** Fixed an issue where the pbmm2 Iso-Seq alignment preset did not apply recommended minimap2 splice alignment parameters, which in some instances led to incorrect alignment.
- **SMRT Analysis:** Fixed issues in Undo Demultiplexing related to fail-read handling and validation. The workflow now provides a warning when an input dataset does not include fail reads.
- **SMRT Analysis:** Fixed an issue where demultiplexing segmented reads failed due to the presence of empty reads.



Page 3

- Demultiplex Barcodes workflow updates change the location of demultiplexed BAM files, which are now generated in the workflow execution directory rather than the /outputs directory. See the SMRT Link user guide v26.1 for more details.
- Undo Demultiplexing now reconstructs original dataset XML and generates separate HiFi read and fail read BAM outputs in the workflow execution directory. If fail reads are unavailable in the input dataset, the workflow logs a warning.
- **Feature Request:** Mark PCR Duplicates now outputs a de-duplicated BAM file where duplicate reads with PCR adapters are removed to the job /outputs directory.

Command-line tool updates

- Several command-line tools have transitioned from semantic versioning to calendar-based versioning. Not all version changes reflect functional updates, notable funcational updates are as follows:
  - TRGT (v5.0.0): Updated with support for phased genotype output, remote input files, improved performance for whole-genome analysis, and additional output options for repeat analysis and visualization.
  - pbmm2 (v26.1.0): Alignment output now includes the SAM/BAM alignment score (AS) tag by default. Iso-Seq alignment preset updated to match recommended splice alignment parameters.
  - lima-undo (v26.1.1): Now generates separate HiFi read and fail read BAM files, when fail reads are provided.

Note: Most tool updates apply to both current and legacy workflows unless otherwise specified

- **Feature Request:** The `dataset create` tool now supports creating an XML for `--type BedSet`.

Administration

- Audit Logs are now available to Admin users in SMRT Link for tracking user and system actions across connected instruments and SMRT Analysis workflows. New audit log API endpoints are documented at https://{dnsname}:8243/sl/docs/services/#/default
- The Postgresql database software has been updated to version 16.1. When upgrading existing installations of SMRT Link, the database tables for SMRT Link services and Keycloak will be backed up to disk and restored at the end of the upgrade process.

Fixed Issues

- **Sample Setup:** Fixed an issue where Vega polymerase kit selections could revert to Revio polymerase kit values after reopening a saved sample.
- **Sample Setup:** Tube color formatting displayed in the SMRT Link UI is now preserved in exported PDF instructions.
- **Run Design:** Fixed an issue when interactively assigning Bio Sample names to barcodes, selecting multiple cells and pressing DEL would delete the Run Design.
- **Run Design:** Fixed an issue where, for the Kinnex™ single-cell RNA application, when assigning Bio Sample names interactively, the populated default for "bcM0001--bcM0001" is "Bio Sample 1" and erroneously contains a space. Spaces are not an allowed character for Bio Sample names.
- **Run Design:** Fixed an issue where, in some instances, when setting up auto analysis for Iso-Seq Analysis and Read Segmentation and Iso-Seq workflows, if the optional reference dataset is left empty, the error "Object is missing required member 'uuid'" was encountered.
- **Run Details:** Fixed an issue where, in some instances, a completed run's status reverted to Ready.

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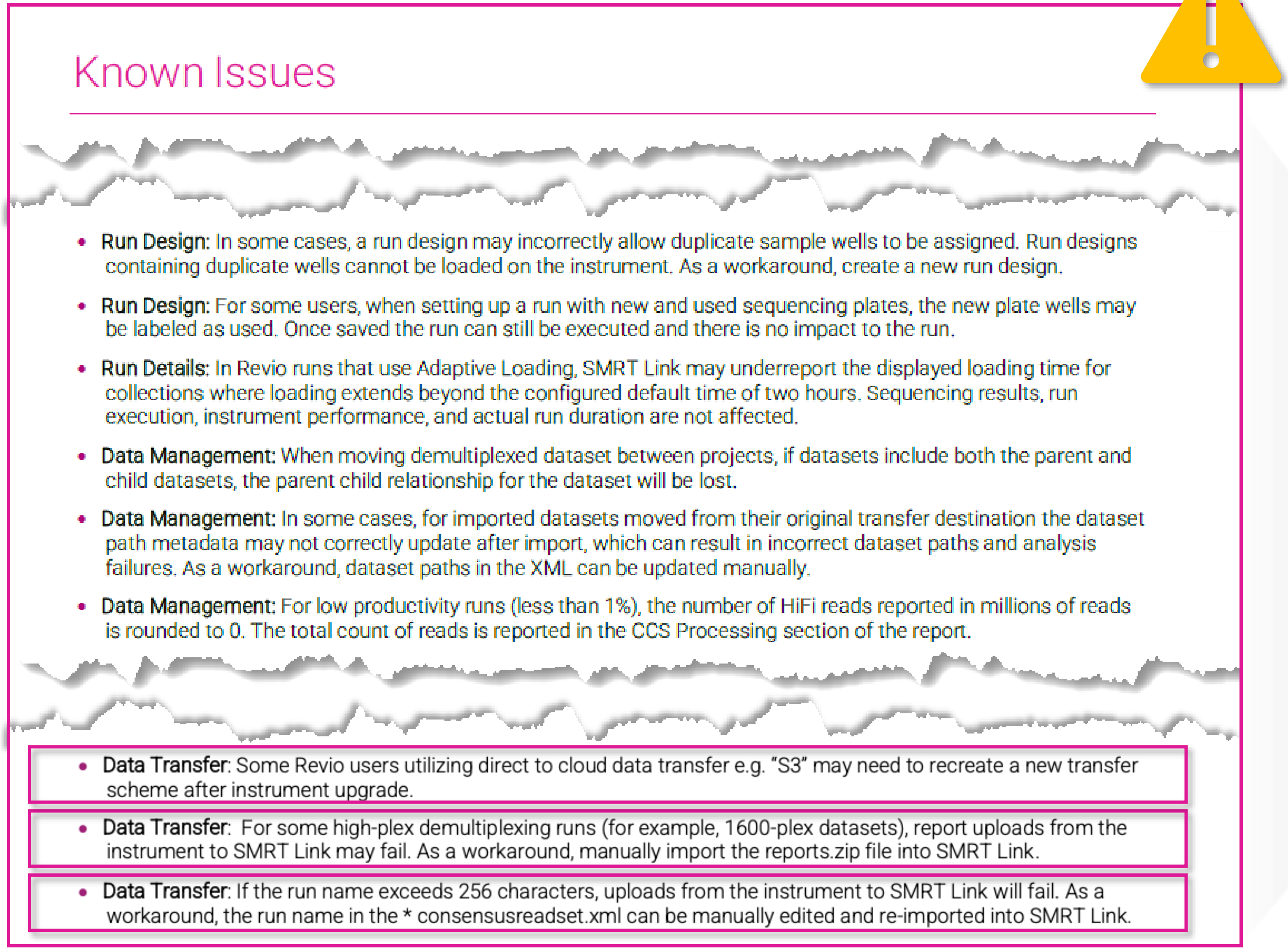
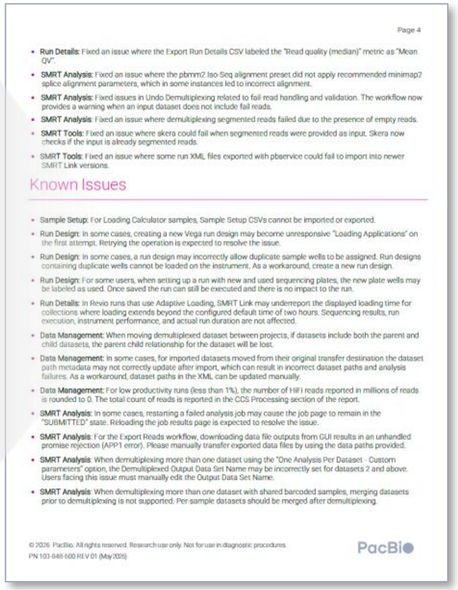
SMRT Link v26.1 known issues – Examples



Known Issues

- **Run Design:** In some cases, a run design may incorrectly allow duplicate sample wells to be assigned. Run designs containing duplicate wells cannot be loaded on the instrument. As a workaround, create a new run design.
- **Run Design:** For some users, when setting up a run with new and used sequencing plates, the new plate wells may be labeled as used. Once saved the run can still be executed and there is no impact to the run.
- **Run Details:** In Revio runs that use Adaptive Loading, SMRT Link may underreport the displayed loading time for collections where loading extends beyond the configured default time of two hours. Sequencing results, run execution, instrument performance, and actual run duration are not affected.
- **Data Management:** When moving demultiplexed dataset between projects, if datasets include both the parent and child datasets, the parent child relationship for the dataset will be lost.
- **Data Management:** In some cases, for imported datasets moved from their original transfer destination the dataset path metadata may not correctly update after import, which can result in incorrect dataset paths and analysis failures. As a workaround, dataset paths in the XML can be updated manually.
- **Data Management:** For low productivity runs (less than 1%), the number of HiFi reads reported in millions of reads is rounded to 0. The total count of reads is reported in the CCS Processing section of the report.

- **Data Transfer**: Some Revio users utilizing direct to cloud data transfer e.g. "S3" may need to recreate a new transfer scheme after instrument upgrade.
- **Data Transfer**: For some high-plex demultiplexing runs (for example, 1600-plex datasets), report uploads from the instrument to SMRT Link may fail. As a workaround, manually import the reports.zip file into SMRT Link.
- **Data Transfer**: If the run name exceeds 256 characters, uploads from the instrument to SMRT Link will fail. As a workaround, the run name in the * consensusreadset.xml can be manually edited and re-imported into SMRT Link.



Page 4

- **Run Details:** Fixed an issue where the Export Run Details CSV labeled the "Read quality (median)" metric as "Mean QV".
- **SMRT Analysis:** Fixed an issue where the pbmm2 Iso-Seq alignment preset did not apply recommended minimap2 splice alignment parameters, which in some instances led to incorrect alignment.
- **SMRT Analysis:** Fixed issues in Undo Demultiplexing related to fail-read handling and validation. The workflow now provides a warning when an input dataset does not include fail reads.
- **SMRT Analysis**: Fixed an issue where demultiplexing segmented reads failed due to the presence of empty reads.
- **SMRT Tools:** Fixed an issue where skera could fail when segmented reads were provided as input. Skera now checks if the input is already segmented reads.
- **SMRT Tools:** Fixed an issue where some run XML files exported with pbservice could fail to import into newer SMRT Link versions.

Known Issues

- **Sample Setup:** For Loading Calculator samples, Sample Setup CSVs cannot be imported or exported.
- **Run Design:** In some cases, creating a new Vega run design may become unresponsive "Loading Applications" on the first attempt. Retrying the operation is expected to resolve the issue.
- **Run Design:** In some cases, a run design may incorrectly allow duplicate sample wells to be assigned. Run designs containing duplicate wells cannot be loaded on the instrument. As a workaround, create a new run design.
- **Run Design:** For some users, when setting up a run with new and used sequencing plates, the new plate wells may be labeled as used. Once saved the run can still be executed and there is no impact to the run.
- **Run Details:** In Revio runs that use Adaptive Loading, SMRT Link may underreport the displayed loading time for collections where loading extends beyond the configured default time of two hours. Sequencing results, run execution, instrument performance, and actual run duration are not affected.
- **Data Management:** When moving demultiplexed dataset between projects, if datasets include both the parent and child datasets, the parent child relationship for the dataset will be lost.
- **Data Management:** In some cases, for imported datasets moved from their original transfer destination the dataset path metadata may not correctly update after import, which can result in incorrect dataset paths and analysis failures. As a workaround, dataset paths in the XML can be updated manually.
- **Data Management:** For low productivity runs (less than 1%), the number of HiFi reads reported in millions of reads is rounded to 0. The total count of reads is reported in the CCS Processing section of the report.
- **SMRT Analysis:** In some cases, restarting a failed analysis job may cause the job page to remain in the "SUBMITTED" state. Reloading the job results page is expected to resolve the issue.
- **SMRT Analysis**: For the Export Reads workflow, downloading data file outputs from GUI results in an unhandled promise rejection (APP1 error). Please manually transfer exported data files by using the data paths provided.
- **SMRT Analysis**: When 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. Users facing this issue must manually edit the Output Data Set Name.
- **SMRT Analysis**: When demultiplexing more than one dataset with shared barcoded samples, merging datasets prior to demultiplexing is not supported. Per sample datasets should be merged after demultiplexing.

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SMRT Link Cloud v26.1
SMRT Analysis support (Beta feature)

SMRT Link Cloud v26.1 SMRT Analysis support (Beta feature)

Revio system customers can optionally connect an existing cloud account (e.g., AWS) to SMRT Link Cloud to enable running core SMRT Analysis workflows in their own cloud computing environment[1,2,3]

- Enables PacBio users to start analysis jobs on a connected AWS account using the SMRT Link Cloud interface
- SMRT Link Cloud v26.1 analysis support is limited to the following data utility workflows:
  - Demultiplex Barcodes
  - Export FASTQ
  - Read segmentation
  - Undo Demultiplexing
- SMRT Link Cloud v26.1 analysis support is enabled for Revio system only

Training & details of the SMRT Analysis beta release features will be provided May 27th

- Instruments
- Sample Setup
- Runs
- Data Management
- SMRT Analysis

- Instruments
- Sample Setup
- Runs
- Data Management (Import run reports)
- SMRT Analysis (Data utilities beta)

Feature	SMRT Link	SMRT Link Cloud
Instrument management	Y	Y
Run setup, run monitoring, run QC	Y	Y
Push-button secondary analysis (SMRT Analysis)	Y	N
Data utilities (e.g., Demultiplex Barcodes)	Y	Beta[2]
3rd party and PacBio Compatible Partner analysis	Y	Y
API access	Y	Y

Beta feature
*(for Revio system **only**)*

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[1] SMRT Link Cloud is a PacBio-hosted and managed private workspace that provides support for instrument management, run setup, run monitoring, and run QC.

[2] SMRT Link Cloud v26.1 software includes the option to enable SMRT Analysis data utility features by configuring a connection to third-party cloud computing resources (BYOC; Bring Your Own Compute). **Note:** This BYOC capability to support data utilities is a **beta feature** in SMRT Link v26.1.

[3] **Note:** SMRT Link Cloud v26.1 Data Management Import Data feature only supports importing Run Reports (*.reports.zip) files and does not support importing of other data types (e.g., HiFi reads, barcodes, references, or target regions (BED).

§ Part 3:
Technical documentation & applications support resources

Revio and Vega system supported applications & protocols

Refer to PacBio Documentation website for the latest application-specific HiFi library preparation procedures

Sequencing method	Application	Protocol or Guide
Whole genome sequencing	Large genome & small (microbial) genome WGS[1]	Preparing whole genome and metagenome libraries using SPK 3.0 [102-166-600]
		Short Read Eliminator (SRE), DNA shearing, and cleanup for the Hamilton Microlab Prep system [103-424-100]
		Preparing whole genome libraries using the HiFi prep kit 96 [103-420-700]
		Automated HiFi prep 96 and HiFi ABC for the Hamilton NGS STAR MOA system [103-425-700]
		Preparing multiplexed whole genome and amplicon libraries using the HiFi plex prep kit 96 [103-418-800]
		Automated HiFi plex prep 96 for the Hamilton NGS STAR MOA system [103-425-800]
Low-DNA input sequencing	Ampli-Fi sequencing	Amplifying genomic DNA for SMRTbell library preparation and HiFi sequencing [**103-648-000**]
RNA sequencing	Kinnex full-length RNA	Preparing Kinnex libraries using Kinnex single-cell RNA kit [103-254-300]
	Kinnex single-cell RNA	Preparing Kinnex libraries using Kinnex full-length RNA kit [103-238-700]
Targeted sequencing	Amplicon sequencing	Preparing multiplexed amplicon libraries using SMRTbell prep kit 3.0 [102-359-000]
		Preparing multiplexed whole genome and amplicon libraries using the HiFi plex prep kit 96 [103-418-800]
	Hybrid capture sequencing	Preparing multiplexed amplicon libraries using SMRTbell prep kit 3.0 [102-359-000]
	PureTarget sequencing	Generating PureTarget libraries with PureTarget kit 24 manual protocol [103-740-700]
		Generating PureTarget libraries with PureTarget kit 96 automation protocol [103-740-800]
		Automated PureTarget kit 96 for the Hamilton NGS STAR MOA system [103-740-900]
Viral sequencing	Adeno-associated virus (AAV)	Preparing multiplexed AAV SMRTbell libraries using SPK 3.0 [102-126-400]
Metagenomics	Shotgun metagenomic assembly	Preparing whole genome and metagenome libraries using SPK 3.0 [102-166-600]
	Shotgun metagenomic profiling	Preparing multiplexed whole genome and amplicon libraries using the HiFi plex prep kit 96 [103-418-800]
	Kinnex full-length 16S	Preparing Kinnex libraries from 16s rRNA amplicons [103-238-800]

[1] Includes human/animal/plant/other WGS, microbial WGS, and shotgun metagenomic profiling or assembly.

PacBio system and SMRT Link documentation

Revio system documentation

- Revio system brochure (102-326-543)
- Revio system instrument control software release notes (103-854-800)
- Revio system operations guide (102-962-600)
- Revio system specifications sheet (102-326-552)

Vega system documentation

- Vega system brochure (102-326-627)
- Vega system instrument control software release notes (103-593-500)
- Vega system operations guide (103-526-600)
- Vega system specifications sheet (102-326-625)

SMRT Link and other data analysis documentation

- Brief primer and lexicon for PacBio SMRT sequencing webpage (v13.1)
- Overview – Analysis options for HiFi data (103-796-000)
- PacBio bioinformatics file formats documentation webpage (v13.1)
- SMRT Link Cloud v26.1 user guide (103-848-800)
- SMRT Link v26.1 software installation guide (103-848-500)
- SMRT Link v26.1 user guide (103-844-300)
- SMRT Link API use cases (103-720-500)

Refer to PacBio Documentation website for the latest PacBio system and SMRT Link technical documentation.

DNA sample extraction documentation

Application, product, and technical notes

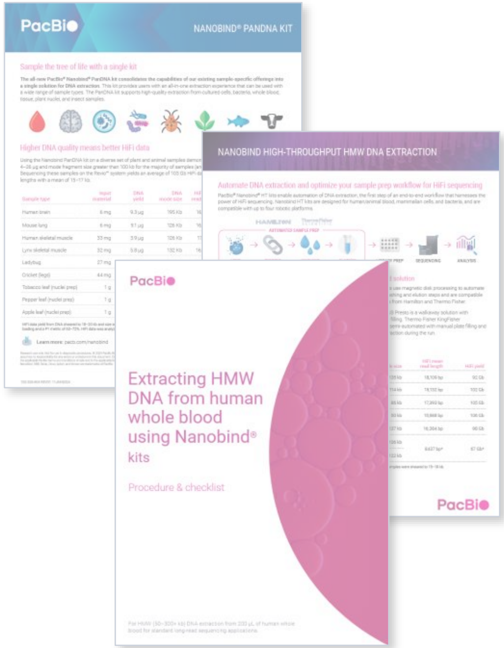
- Application note – HMW DNA extraction from human blood and saliva using Nanobind kits for HiFi long-read sequencing (102-326-656)
- Technical note – Nanobind high-throughput HMW DNA extraction (102-326-565)
- Technical note – Insect DNA extraction (102-326-612)
- Technical note – Preparing DNA for PacBio HiFi sequencing – Extraction and quality control (102-193-651)

Nanobind kit protocols and Guides & overviews

- Guide & overview – Nanobind CBB kit (102-572-200)
- Guide & overview – Nanobind PanDNA kit (103-394-800)
- Nanobind Procedures & checklists – see PacBio Documentation
- Overview – Nanobind CBB HMW DNA extraction protocols (103-515-700)
- Overview – Nanobind HT HMW DNA extraction robotic procedures (103-032-000)
- Overview – Nanobind PanDNA HMW DNA extraction protocols (103-510-000)
- Technical overview – HMW DNA sample preparation for PacBio long-read sequencing using Nanobind PanDNA and SRE kits (103-401-100)

Nanobind high-throughput (HT) automation kit protocols and Guides & overviews

- Guide & overview – Nanobind HT kits (103-028-100)
- Nanobind HT Procedures & checklists – see PacBio Documentation
- Technical overview – Automated high-throughput HMW DNA extraction for PacBio long-read sequencing using Nanobind HT kits (103-401-700)



PacBio

NANOBIND® PANDNA KIT

NANOBIND HIGH-THROUGHPUT HMW DNA EXTRACTION

PacBio

Extracting HMW DNA from human whole blood using Nanobind® kits

Procedure & checklist

PacBio Refer to PacBio Documentation website for the latest Nanobind HMW DNA extraction procedures.

SMRTbell library preparation documentation & other resources

SMRTbell library preparation literature

- Overview – HiFi application options (101-851-300)
- Procedure & checklist – Amplifying genomic DNA for SMRTbell library preparation and HiFi sequencing (103-648-000)
- Procedure & checklist – Generating PureTarget libraries with PureTarget kit 24 – Manual protocol (103-740-700)
- Procedure & checklist – Generating PureTarget libraries with PureTarget kit 96 – Automation protocol (103-740-800)
- 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)

Hybrid capture library preparation literature

- Agilent protocol – Target Capture Long-Read Sequencing Using the Agilent SureSelect XT HS2 Target Enrichment System (5994-7612EN)
- QIAGEN protocol – QIAseq xHYB Long Read Panel Handbook (HB-3695-001)
- Twist protocol – Long read library preparation and standard hyb v2 enrichment (DOC-001320)

Refer to PacBio Documentation website for the latest library preparation protocols and other documentation.

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 – Highly-accurate HiFi reads for AAV-research (102-326-594)

RNA sequencing applications

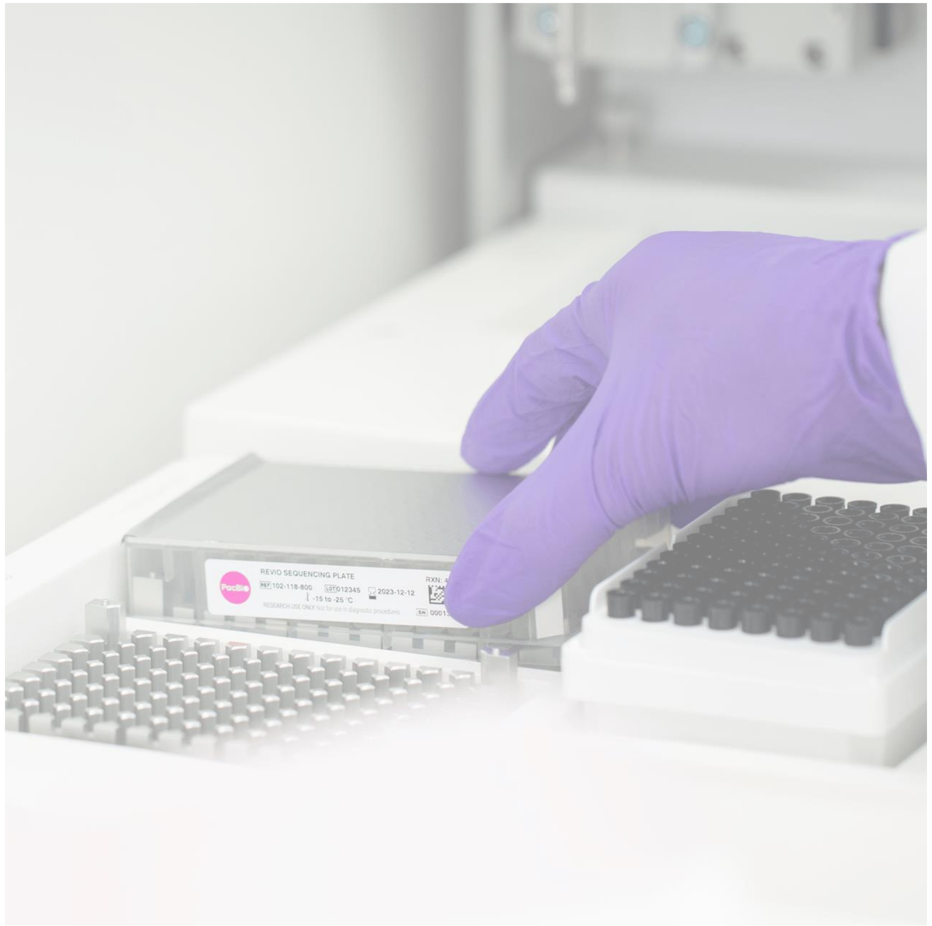
- Application note – Kinnex full-length RNA kit for isoform sequencing (102-326-591)
- Application note – Kinnex single-cell RNA kit for single-cell isoform sequencing (102-326-549)

Metagenomics applications

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

Targeted sequencing applications

- Application brief – Target enrichment using hybrid capture for HiFi sequencing (102-326-515)
- Application brief – Targeted sequencing for amplicons – Best Practices (102-193-603)
- Application note – Comprehensive genotyping with the PureTarget repeat expansion panel and HiFi sequencing (102-326-614)
- Application note – Consolidate challenging genes with PureTarget carrier screen panel



Refer to PacBio Literature website for the latest application notes and other application-specific resources.

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 – Amplifying genomic DNA for SMRTbell library preparation and HiFi sequencing (103-648-000)
- Technical overview – HiFi library preparation using HiFi prep kits for high-throughput sequencing on PacBio long-read systems (103-424-600)
- Technical overview – Kinnex kits for single-cell RNA and full-length RNA and 16S rRNA sequencing (103-343-700)
- Technical overview – Kinnex library preparation for full-length 16S rRNA sequencing (103-344-800)
- Technical overview – Kinnex library preparation using Kinnex full-length RNA kit (103-344-700)
- Technical overview – Kinnex library preparation using Kinnex single-cell RNA kit (103-344-600)
- Technical overview – Multiplexed amplicon and hybrid capture library preparation using SMRTbell prep kit 3.0 (103-705-200)
- Technical overview – PureTarget library preparation using PureTarget kit 2.0 (103-742-600)
- Technical overview – Whole genome and metagenome library preparation using SMRTbell prep kit 3.0 (102-390-900)

Refer to PacBio Training website for the latest application-specific training documentation.

PacBio HiFi sequencing project getting started guides

Equipment and materials checklists

- Get started with HiFi sequencing – Checklist (102-326-639)
- Buyer's guide with automation platforms (102-326-638)
- Getting started with long-read sequencing (102-326-640)

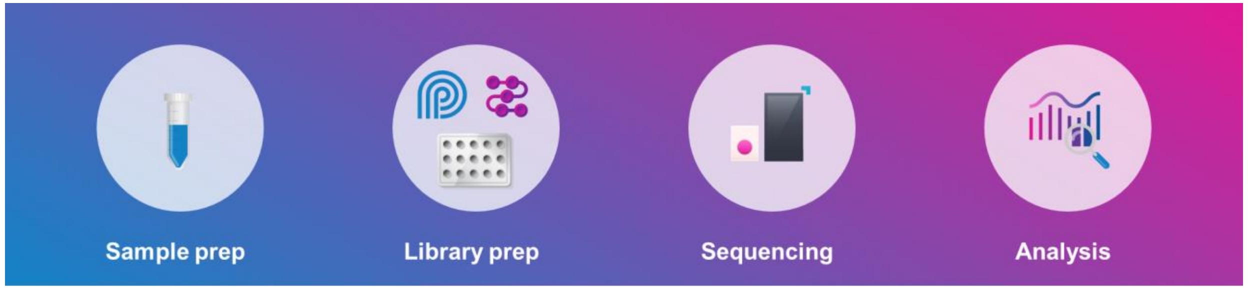
Get started with HiFi sequencing—checklist

BUYER'S GUIDE WITH AUTOMATION PLATFORMS

GETTING STARTED WITH HIFI SEQUENCING:
PLANT AND ANIMAL RESEARCH

PacBio HiFi sequencing project builder tools

- Automated workflow selector tool [Link]

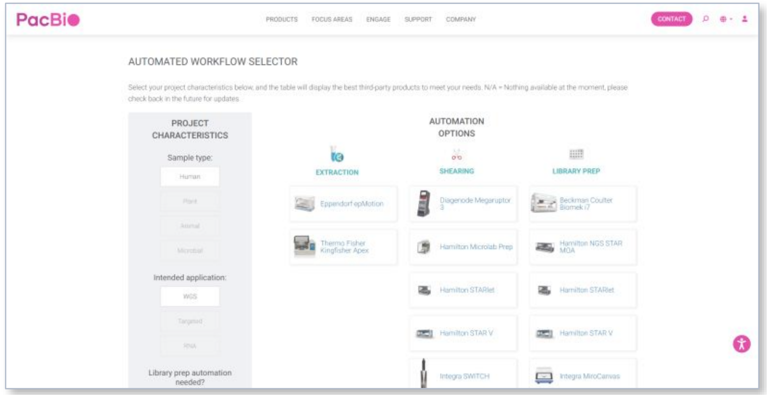


Nanobind DNA kits enable extraction of HMW gDNA from a variety of samples: saliva, cultured cells, bacteria, whole blood, tissue, plant nuclei, and insect.

Automated-friendly and manual library prep kits for WGS, full-length RNA-seq, targeted panels, and many more.

Simple, load-and-go consumable reagents makes sequencing runs effortless.

Advanced algorithms from Google Health and cutting-edge NVIDIA GPUs allow for on-board processing of sequencing data, all with reduced output file sizes to facilitate downstream analysis.



AUTOMATED WORKFLOW SELECTOR

Select your project characteristics below, and the table will display the best third-party products to meet your needs. N/A = Nothing available at the moment, please check back in the future for updates.

PROJECT CHARACTERISTICS

Sample type: Human

Intended application: WGS

Library prep automation needed?

AUTOMATION OPTIONS

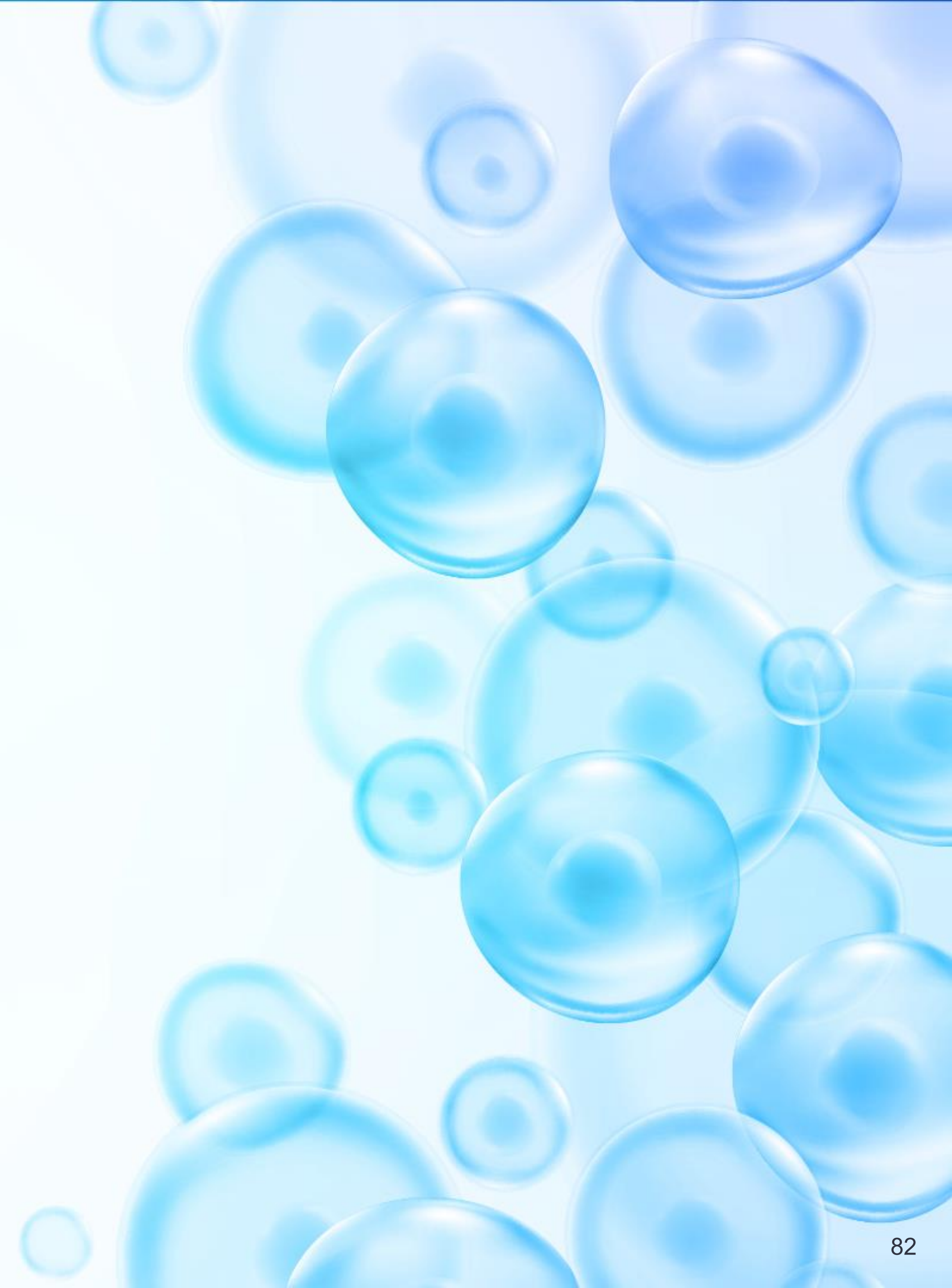
EXTRACTION	SHEARING	LIBRARY PREP
Eppendorf epMotion	Diagenode Megaruptor 3	Beckman Coulter Biomek i7
Thermo Fisher Kingfisher Apex	Hamilton Microlab Prep	Hamilton NGS STAR MOA
	Hamilton STARlet	Hamilton STARlet
	Hamilton STAR V	Hamilton STAR V
	Integra SWITCH	Integra MiroCanvas

Refer to the PacBio website for the latest HiFi sequencing project support documentation.

PacBio

www.pacb.com

§ Part 4: Appendix



Revio system v13.5 on-plate loading concentration recommendations

Appendix

Revio system v13.5 on-plate loading concentration (OPLC) recommendations

OPLC recommendations[1] to achieve SMRT Cell target loading ranges are the same for Revio SPRQ and Revio SPRQ-Nx chemistry

Application	Application subtype	Library insert size	OPLC	Run time	Target *P1* loading	HiFi reads or yield per cell
Whole genome sequencing	**Human WGS**	15 – 20 kb	200 – 300 pM	24 hours	50 – 75%	100 – 120 Gb
	Microbial assembly	15 – 20 kb	200 – 300 pM	24 hours	50 – 75%	100 – 120 Gb
	Other WGS	15 – 20 kb	200 – 300 pM	24 hours	50 – 75%	100 – 120 Gb
Low DNA input	**Ampli-Fi sequencing**	5 – 10 kb	100 – 140 pM	24 hours	50 – 75%	35 – 70 Gb
RNA sequencing	**Kinnex single-cell RNA**	15 – 20 kb	130 – 160 pM	24 hours	50 – 75%	100 – 120 Gb
	Kinnex full-length RNA	15 – 20 kb	130 – 160 pM	24 hours	50 – 75%	100 – 120 Gb
Viral sequencing	**AAV sequencing**	2 – 5 kb	200 – 300 pM	24 hours	50 – 75%	6 – 8 M reads
Metagenomics	**Kinnex 16S rRNA**	15 – 20 kb	130 – 160 pM	24 hours	50 – 75%	100 – 120 Gb
	Shotgun metagenomic profiling or assembly	7 – 10 kb	200 – 300 pM	24 hours	50 – 75%	35 – 70 Gb
Targeted sequencing	**<1 kb amplicons**	500 bp – 1 kb	200 – 400 pM	12 hours	50 – 75%	6 – 8 M reads
	1 – 5 kb amplicons	1 – 5 kb	200 – 300 pM	12 hours	50 – 75%	6 – 8 M reads
	≥5 kb amplicons	5 – 10 kb	200 – 300 pM	24 hours	50 – 75%	35 – 70 Gb
	Hybrid capture	5 – 10 kb	200 – 300 pM	24 hours	50 – 75%	35 – 70 Gb

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[1] **Note:** Refer to appropriate ***Procedure & checklist*** protocol documentation for the latest OPLC recommendations for different applications.

Multi-use Revio SMRT Cell run setup workflow for low-throughput run schedules

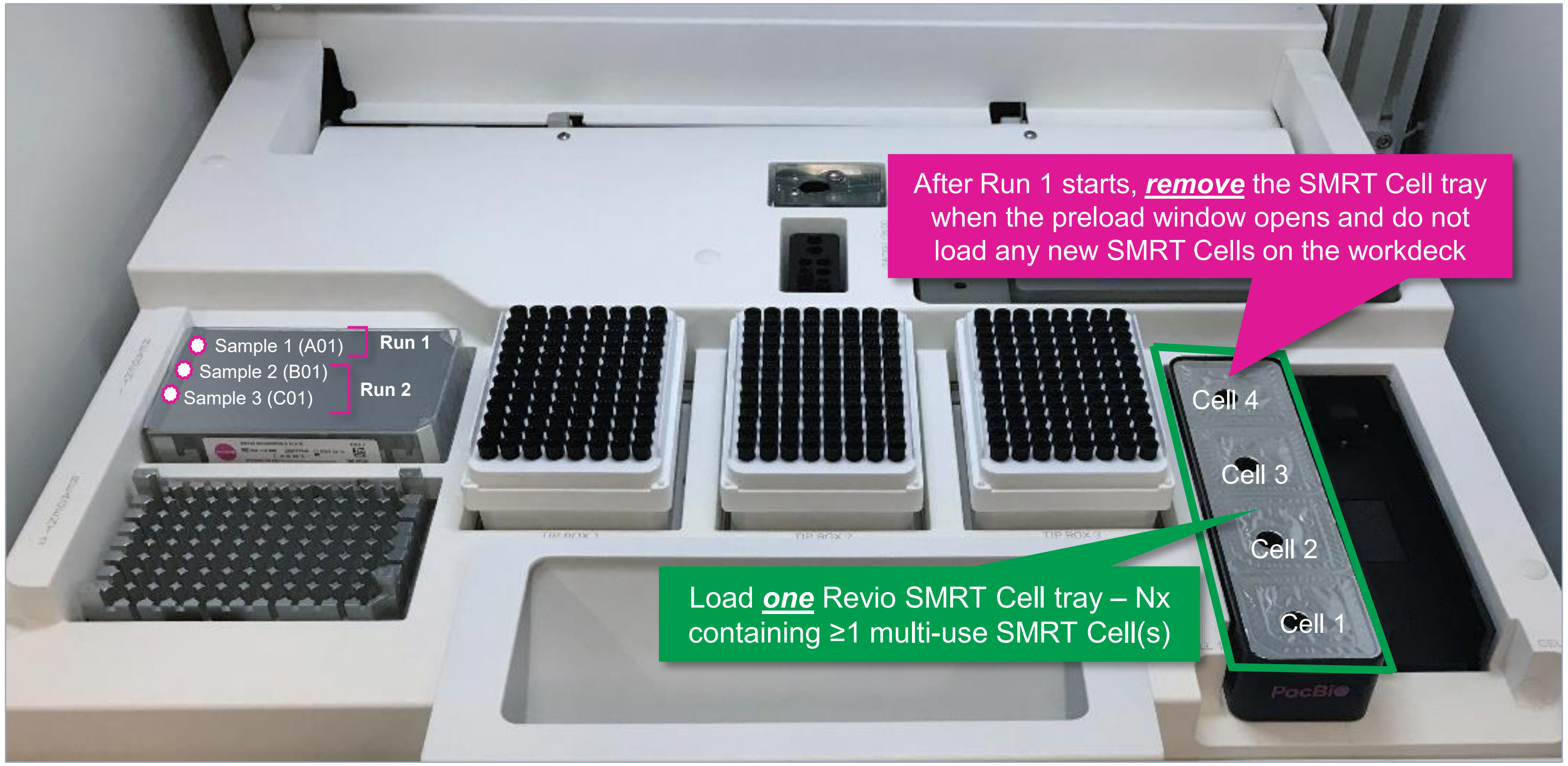
Appendix

Low-throughput workflow using multi-use Revio SMRT Cells

Example: Sequence on *1* multi-use Revio SMRT Cell at a time (three uses per cell)

Example run schedule: Run 1 = 1 sample; Run 2 = 2 samples (total # acquisitions = 3)

→ Perform all three acquisitions using the *same* multi-use Revio SMRT Cell



After Run 1 starts, ***remove*** the SMRT Cell tray when the preload window opens and do not load any new SMRT Cells on the workdeck

Load ***one*** Revio SMRT Cell tray – Nx containing ≥1 multi-use SMRT Cell(s)

Ex. Low-throughput run setup workflow

1. Load sample 1 into **one** Revio SPRQ seq plate – Nx. (For example, load sample 1 into well A01.)
2. Place sequencing plate containing your sample onto the Revio work deck in **Plate slot 1**.
3. Load **one** Revio SMRT Cell tray – Nx containing ≥1 multi-use SMRT Cell(s) onto the Revio work deck in tray slot 1 or slot 2.
4. Create a new run design (**Run 1**) to sequence only **one** sample (e.g., Plate 1 well A01).
5. Start Run 1: Sample 1 will sequence on Use 1 of the multi-use SMRT Cell (e.g., Cell 1).
6. When preload window opens, **remove** the SMRT Cell tray from the work deck and do not load any new cells[1]
7. Create a new run design (**Run 2**) to sequence the 2nd and 3rd samples (e.g., Plate 1 wells B01 + C01).
8. Load the 2nd and 3rd samples into the same sequencing plate and place the plate on the work deck.
9. Start Run 2: Samples 2 + 3 will sequence on Use 2 and Use 3, respectively, on the **same** multi-use SMRT Cell (e.g., Cell 1) as sample 1.

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[1] Once removed from tray, a SMRT Cell is never returned to the tray. **Note:** Expired SMRT Cells and SMRT Cells with no remaining uses are **automatically discarded** into the instrument waste bin. There is **no option** to use expired multi-use SMRT Cells for a sequencing run.