SMRT® Link release notes (v25.1)

Supported instruments and chemistries

SMRT Link v25.1, including SMRT Link Lite v25.1, support the following:

Instruments	SMRT Link v25.1
Revio® system	Instrument software v13.3, all chemistries
Vega™ system	Instrument software v1.0, all chemistries
Sequel® II system	Not supported; use SMRT Link v13.1

SMRT Link is supported on English-language distributions of:

- Rocky Linux 8 and 9
- Ubuntu 22.04 and 24.04

See **SMRT Link software installation guide (v25.1)** for detailed hardware and software requirements and installation instructions.

New Features and Updates

Overall

- Removed the integrated help documentation, instead linking to the written documentation on pacb.com.
- Removed support for Sequel II and Sequel IIe systems in Instrument, Runs, and Sample Setup. SMRT Link v13.1 is recommended for Sequel II/IIe systems.
- Removed support for subread datasets and analyses.

Sample Setup

- Added support for **Revio SPRQ™ polymerase kit** and **Vega polymerase kit**, with simplified protocols. Annealing, binding, and cleanup (ABC) instructions for these kits use fixed reagent volumes and are included in library prep protocols. SMRT Link Sample Setup is used to calculate the final loading dilution of prepared SMRTbell libraries using the "Loading calculator".
- Renamed tools to "ABC calculator" and "Loading calculator". ABC calculator applies to the Revio (non-SPRQ) polymerase kit (24 rxn). Other kits use the Loading calculator.
- Updated Best Practices and clarified protocol wording.

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Runs

- Added Run Design support for Vega system.
- Added Run Design support for the Revio SPRQ sequencing plate and Vega sequencing plate.
- Updated and simplified Run Designs for Revio:
  - Moved “Library Concentration” from Run Options to the main panel.
  - Removed Polymerase Kit field. The polymerase kit is implied by the Sequencing Plate selection. The “Polymerase Kit” row is deprecated (though permitted) in Run Design CSV.
  - Removed the option to choose "Same Barcodes on Both Ends of Sequence". All indexes are treated as symmetric except Twist Universal Adapters with UDI.
- Removed requirement that Bio Sample names must be unique in barcode-to-sample assignment.
- Relaxed handling of used reactions in linked sequencing plates. Reactions that SMRT Link has marked as used are now labeled rather than being restricted from run designs. This prevents issues where SMRT Link state is inconsistent with the physical plate.
- Limited Bio Sample names to ≤40 characters and restricted to alphanumeric, hyphen, and underscore characters. Updated the header in Barcoded_Samples.csv example file to reflect the new requirements. This eliminates downstream issues with unexpected characters in sample names.
- Limited Run Name, Run Comments, Well Comment, and Analysis Name fields to alphanumeric, space, hyphen, underscore, colon, period, and apostrophe characters. Comma and newline are not permitted.
- If “Full Resolution Base Qual" and “Subread To HiFi Pileup” settings are TRUE, they are now shown under Advanced in Data Options in the SMRT Link GUI. The options are set via run design CSV.
- Added “Run Started” column to Runs table.
- Removed the “Print” button in Run Details. Use the browser print option instead.
- Renamed “ICS Software Version” to “Instrument software”, added the “Transfer directory” entry, and eliminated “Instrument chem bundle” entry in Run Details overview table.
- Added Movie column and removed Pre-extension column in Run Details table. Made minor updates to column labels and visibility.
- Constrained axes in the Read Length Density plot to eliminate plotting visual artifacts.

Data Management

- Generalized the “5mC CpG Report” into the Methylation report, which shows the fraction of sites predicted as methylated and the probability distribution for 5mC and 6mA calls.
- Limited barcode names to ≤40 characters and restricted to alphanumeric and underscore characters when uploading a custom barcode FASTA. This eliminates downstream issues with unexpected characters in barcode names.
- Removed per-column sorting in the File > Downloads table.
- Added the absolute directory path on the server for each file in the File > Downloads table to simplify locating files.

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SMRT® Analysis

- Combined Analysis and Data Utility workflows into one menu, simplifying workflow selection.
- Retired workflows:
  - HiFiViral SARS-CoV-2 Analysis – this application has reached its end of life.
  - Genome Analysis – our current recommendation for large genome assembly is the 3rd party tool `hifiasm` used in the PacBio® HiFi-human-assembly-WDL.
  - 5mC CpG Detection – 5mC calling is run on-instrument and is available in `jasmine`.
  - Structural Variant Calling – this functionality is built into the Variant Calling analysis.
- Updated version of `pbmm2` to v.1.16.0. This version improves alignments shorter than 500 bp, affecting Pure Target repeat analysis and other workflows. See the full change log here.
- Updated `isoseq` tools to v.4.2.0. This version updates the logic used in `isoseq collapse` leading to better deduplication of redundant isoforms, among other updates. See the full change log here.
- Updated version of `trgt` from 0.9.0 to v1.1.1. This update includes deterministic output and improved logic for filtering low-quality reads. See the full change log here.
- In the HiFi Target Enrichment workflow, added options to set both "Call SNVs and indels" and "Call structural variants" to ON/OFF.
- PureTarget™ repeat expansion workflow now supports input karyotypes for higher accuracy genotyping of X-linked expansions. This is enabled through CSV import of karyotype data in Advanced Parameters.
- In the Iso-Seq Analysis and Read Segmentation and Iso-Seq workflows, the `pigeon filter` options can now be changed through the GUI using the Advanced Parameters.
- In the Single-Cell Iso-Seq and Read Segmentation and Single-Cell Iso-Seq workflows, the `pigeon filter` and `pigeon make-seurat` options can now be changed through the GUI using the Advanced Parameters.
- Improved performance and memory usage for the HiFi Mapping analysis workflow.
- Defined the "0x80" bit to the fail_reads.bam fail flag (ff tag) as "Reads with segmentation adapters in non-sequential order". This tag is added during Read Segmentation for Kinnex™ libraries.

Instrument settings

- Added new file transfer schemes to support direct-to-cloud transfer to Amazon S3, Google Cloud Storage, and Microsoft Azure Blob Storage.
- Renamed "s5cmd" file transfer scheme to "S3-compatible storage".
- Renamed "srs" file transfer type to "ssh/srs".
- Renamed "rsync" file transfer scheme to "rsync daemon." The rsync daemon transfer type remains available for Revio but is no longer recommended.
- Updated SMRT Link events server to use port 443 instead of 8083. SMRT Link installations configured to use port 8083 are still new supported. By default new installations will use port 443. Please see your system Site Prep guide on pacb.com for a complete list of network requirements.

APIs

- Added new API endpoints that provide Run Details metrics and paths to plots:
  `GET /smrt-link/runs/{runId}/qc` and
  `GET /smrt-link/runs/{runId}/collections/{collectionId}/qc.`
- Changes to API endpoints may impact existing API workflows. We recommend testing any API based tooling after upgrading SMRT Link.

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Fixed issues

Notifications

- Fixed an issue where hiding a notification did not reduce the unread notification count.

Sample Setup

- Removed minimum insert size requirement in Sample Setup.

Runs

- Fixed an issue where single-character Bio Sample Names in a CSV were skipped during Run Design import.
- Updated component logic to ensure run design defaults are correctly set, regardless of the order in which applications are selected (e.g., Kinnex Single Cell to Kinnex Full-Length).
- Fixed an issue where imported values for "Full Resolution Base Qual" and "Subread To HiFi Pileup" were lost after changes in the GUI.
- Removed the minimum insert size requirement in Run Design, resolving errors related to the "Insert size must be at least 500 bp" message.
- Fixed a bug where sequencing plate lot number, serial number, and expiry date were not included when exporting a run design CSV.

SMRT Analysis

- Fixed an issue where the HiFi Target Enrichment workflow associated incorrect sample names with reads during the mapping task resulting in files being associated with the wrong sample. Please reference PQN-20240718 | for additional details.
- Fixed an issue in Iso-Seq® analysis and Read Segmentation and Iso-Seq workflows, where when a pool of >9 and are jointly analyzed (“Pool reads and cluster together” option), the High Quality isoforms FASTA file name no longer matches the BioSample_[number]. Please reference PQN-20240715 for additional details.
- Corrected an issue where transcripts.bam output file from `isoseq cluster` always reports platform (PM) as SEQUEL in read group header. The platform from the input is now passed through to output.
- Fixed an issue where extra Gs were present on the 5’ end of 5’ Single-Cell reads. The default Single Cell Barcode and UMI Design for 5' was updated to 16B-10U-13X-T, which now matches the 10x Genomics’ Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) protocol.
- Fixed and issue to improve the accuracy of saturation curves in Iso-Seq Analysis.
- Fixed an issue where Imported custom BED files were not found it in the Target Set list when creating a HiFi Target Enrichment analysis job.
- Fixed an issue that caused `trgt` to crash when only one read covered a locus.
- Fixed an issue in HiFi Target Enrichment where workflows failed if user-provided BED files included an empty info column.
- Fixed an issue in Microbial Genome Analysis where the reported contig sizes were for an intermediate output rather than the final polished, rotated assembly.

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- Updated `deepvariant` to v1.6.1 and updated call_variants threads parameters which resolves hanging Variant Calling and HiFi Target Enrichment jobs.
- Fixed an issue where lima FASTQ output contained unexpected outputs because newer PacBio BAM tags of type `B,C` were causing extra line breaks within the FASTQ names.
- Fixed an issue where loaded pages timed out when internet connection was interrupted.

Data Management

- Fixed an issue where the results of Barcode Sets import were not viewable in SMRT Link Lite.

Known issues

- Run Design: Editing a previously saved run design resets Analysis Options to defaults; re-enter fields when editing.
- SMRT Analysis: When HiFi Target Enrichment or PureTarget repeat expansion workflows are run on pools of samples which include failed samples (those with zero mapped reads), it can cause jobs to fail. We recommend excluding failed samples from analysis.
- SMRT Analysis: For high coverage microbial genomes, polishing fails due to high coverage using the default analysis settings. To resolve this issue, add downsampling by using “Downsampled coverage” option in the Advanced Analysis Settings.
- SMRT Analysis: The cluster step in the Read Segmentation and Iso-Seq and Iso-Seq Analysis workflows may fail with an out of memory error when the number of input reads is high. The workaround is to click Advanced Parameters and set Add task memory (MB) to 64000 or higher.
- Run Details: Vega runs may sometimes display the status "SEQUENCING" even after completion. The run completes successfully and shows a Transfer Complete time in the Overview section.

See https://github.com/broadinstitute/cromwell/blob/develop/LICENSE.txt for Cromwell redistribution information.

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