SMRT® Link release notes (v26.1)

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”.

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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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- 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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- 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 Analysis: Microbial Genome Analysis jobs will complete with a successful status even if tasks within the job may have failed. Any failed tasks may produce empty output files and warning messages in workflow or task logs. This behavior is intended to ensure that, when only some tasks complete successfully, outputs for all completed tasks are accessible. If the failed task is an incomplete or empty assembly, using 3rd party assembly tools such as `hifiasm` is recommended.
- SMRT Tools: When using `pbcromwell configure` to generate a `cromwell.conf` for command-line execution of a pbcromwell workflow, the configuration file is missing the `submit-docker` config section that allows the Variant Calling workflow to call Singularity. Run SMRT Link server configuration for a full SMRT Link installation to generate `$SMRT_ROOT/userdata/generated/config/cromwell_cli.conf`.
- 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.
- Administration: In some cases, when an SSO user logs out and then back in, login will fail with the error "Your login attempt timed out. Login will start from the beginning." Attempting to sign-in with SSO again should be successful.
- Administration: If more than 7 JMS configurations are in use, SMRT Link services will not start properly. Please use less than 7 compute configs to avoid this issue.
- Administration: LDAP users without roles, when logging in, are directed to a page without content but are not shown "Invalid Credentials" as expected.
- Administration: If using the `generate-cron-backup` script, backups for new versions of SMRT Link are not made after upgrading. Users should run `generate-cron-backup` after upgrading.
- Administration: Users with the Bioinformatician role are not able to select the Instruments module and cannot view or create Projects.