

Supported instruments and chemistries

SMRT Link 26.2 supports the following:

Instruments	Instrument software
Revio® system	Instrument software v13.5, SPRQ™ and SPRQ-Nx chemistries
Vega™ system	Instrument software v1.2.0, 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 v26.2** for detailed hardware and software requirements and installation instructions.

Users upgrading from 25.3 or earlier should also review the SMRT Link v26.1 release notes, which include changes not reflected in this document.

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 storage (including Oracle Cloud Object Storage and Cloudflare R2).

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

New features and updates

SMRT Link v26.2 introduces support for the new SPRQ-Nx chemistry, shorter runtimes, and support for 21 CFR Part 11 compliance.

- Support for the Vega SPRQ-Nx reagents for use with the Vega SMRT® Cell tray. Multi-use SMRT Cells remain Revio-only.
- Added support for 4- and 2-hour runs to enable single-shift sequencing of 0.5–2 kb inserts.
- SMRT Link now includes 5hmC methylation reports for Vega. 5hmC calls and per-read modification-probability distributions, introduced in SMRT Link 26.1 for Revio, are now available for Vega collections.
- Manage on-instrument login mode for Vega, supporting 21 CFR Part 11 compliance. When enabled, users must set a Personal Identification Code (PIC) before performing on-instrument actions, and login and logout events are recorded in the instrument's audit trail. See the SMRT Link user guide for configuration details.
- New tamper detection for audit logs. SMRT Link admins can reset the audit trail once tampering has been detected and resolved.

Sample Setup and Run Design

- Removed Revio v1 chemistry from Run Design and Sample Setup, including the ABC Calculator. Historical runs and ABC Calculator records that use v1 chemistry remain viewable.
- **Feature Request:** Added application-specific warnings in Run Design when the entered insert size falls outside the recommended range for the selected application.
- **Feature Request:** Added a warning in Run Design when a 4-reaction sequencing plate is being reused for a second time with unused wells remaining.
- **Feature Request:** The movie acquisition time for the Full-length 16S rRNA sequencing application on Vega now defaults to 4 hours.

SMRT® Analysis

- **Feature Request:** Added the Redo Demultiplexing workflow, allowing users to re-run demultiplexing on an already demultiplexed parent dataset without having to run Undo demultiplexing first. The new workflow is packaged in the wdl-v2.zip. Direct execution requires `cromwell` and workflow-specific JSON input file.
- Legacy analysis workflow versions (previous implementations of Demultiplex Barcodes, Undo Demultiplexing, Read Segmentation, and Export Reads) are no longer shown in the standard SMRT Analysis application list, to reduce confusion between current and legacy pipeline versions.

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 functional updates are as follows:

- `pbbmm2` (v26.2) now supports outputting secondary alignments via a new `--secondary` flag (with `--max-secondary-als` to cap the number retained per read). This feature is for use with downstream RNA quantification tools that require transcriptome-level secondary alignments. Secondary alignments remain suppressed by default.
- **Feature Request:** `pbmarkdup` (v26.2) output BAM files now include a `@PG` header line recording the command invocation.
- `isoseq refine` (v26.2) poly-A trimming algorithm has been improved, replacing the previous HMM-based method with a heuristic approach, to reduce overtrimming at the 3' ends of transcripts in Iso-Seq® analysis. Users can expect small differences in read count and length report metrics.

Fixed Issues

- **Sample Setup:** Corrected an error message in Sample Setup that incorrectly stated a newly created Loading Calculator sample could not be imported; the message now correctly states that these samples cannot be exported.
- **Sample Setup:** Fixed an issue where the pooling calculator's "Pooled library concentration" field displayed unexpected input behavior.
- **Run Design:** Fixed an issue where, in some instances, when setting up auto analysis with the Iso-Seq Analysis workflow and the Iso-Seq Analysis and Read Segmentation workflow, if the optional reference dataset is left empty, the error "Object is missing required member 'uuid'" was encountered.
- **Run Design:** Fixed an issue where creating a new Vega run design could become unresponsive while loading Applications.
- **Run Design:** Fixed an issue where a run design could incorrectly allow duplicate sample wells (for example, multiple A01 wells) to be assigned.

- **Run Design:** Fixed an issue where duplicating a run carried over consumable metadata (plate lot, serial number, and expiry), which could cause unused wells on a new plate to be incorrectly labeled as “Used.”
- **Run Design:** Fixed an issue where SMRT Link could crash when creating a new run design using the “Kinnex 16S rRNA” Application together with the Export FASTQ analysis workflow.
- **Run Design:** Fixed an issue where a crash sometimes occurred when an invalid expiry date (for example, an out-of-range month) was entered for a plate or reagent kit.
- **Run Design:** Fixed an issue where viewing or editing the run design for a previously completed run intermittently displayed an error page instead of the read-only run design.
- **Run Design:** Fixed an issue where a PureTarget repeat expansion run design could be saved without a reference genome selected, instead of being blocked with a missing entry point error.
- **Run Details:** Fixed an issue where Run QC report PDF timestamps were always displayed in UTC; timestamps are now localized to the configured time zone.
- **Run Details:** Fixed an issue where a run name exceeding 255 characters could fail to import or crash the SMRT Link UI. Run names are now validated and limited to 255 characters in Run Design.
- **Data Management:** Fixed an issue where exporting a parent dataset (all samples in a collection) could fail because the export used the local file path instead of the dataset’s canonical location; this also affected the dataset merge and create functions.
- **SMRT Analysis:** Fixed an issue where Demultiplexing Barcodes (1ima) could fail to trim one base present in the barcode when demultiplexing with asymmetric barcodes in some cases.
- **SMRT Analysis:** Increased the default memory allocation for the Variant Calling workflow to reduce out-of-memory failures, particularly for SPRQ-Nx datasets.
- **SMRT Analysis:** Fixed an issue in the Demultiplex Barcodes workflow where users could not rename the Demultiplexed Output Data Set Name.
- **SMRT Analysis:** Fixed an issue where restarting a failed analysis job could cause the job page to remain in the “SUBMITTED” state.
- **SMRT Analysis:** Fixed an issue where, when demultiplexing more than one dataset using the “One Analysis Per Dataset – Custom Parameters” option, the Demultiplexed Output Data Set Name did not update for datasets after the first.
- **SMRT Analysis:** Improved the robustness of the analysis results page file download feature. Large files are now streamed instead of loaded into browser memory, and missing log files or missing output files are now handled gracefully instead of crashing the page.
- **SMRT Tools:** Fixed `pbcromwell` CLI argument parsing so that entry points specified with the `-e` flag are correctly resolved for the Read Segmentation and Iso-Seq workflow, eliminating a false missing entry point error.
- **SMRT Tools:** Fixed `pbservice import-dataset` to correctly update both the path and `remotePath` metadata fields when re-importing a dataset at a new file system location.
- **Administration:** Fixed an issue where exporting the audit log without filters could fail with a layout error.
- **Administration:** The audit log PDF export now lists any filters that were applied, making clear when the export represents a filtered subset of the full audit trail.
- **Administration:** Fixed issues with the SMRT Link API documentation (Swagger) page that incorrectly displayed the SMRT Link version and generated a malformed curl command for the “Try it out” feature.
- **Administration:** Fixed an issue where the password-reset link presented to local Keycloak users with a temporary password redirected to an invalid URL.
- **Administration:** Added input validation for File Transfer Location configuration fields, including checks for invalid characters in destination paths and access keys; fixed a related issue where Azure Blob Storage’s Account Key field was incorrectly validated using AWS access key rules.

- **Administration:** Fixed the SMRT Link installer connectivity check to resolve false failures when checking availability of the SMRT Link Update and Event services during installation or upgrade.
- **Administration:** Fixed the SMRT Link upgrade script to use the bundled python3 interpreter, preventing Keycloak database upgrade failures on hosts where system python3 is not installed.

Known Issues

- **Sample Setup:** Loading Calculator Sample Setup CSVs cannot be imported or exported.
- **Sample Setup:** A custom PureTarget™run design cannot be saved using SMRT Link Lite. Workaround: Create a run design using a CSV file with all required analysis fields populated. A template CSV can be provided by PacBio Support upon request.
- **Sample Setup:** The loading calculator produces incorrect calculations if commas are used in the Average Insert Size field. Workaround: enter numeric values without comma separators (for example, enter 15000 instead of 15,000).
- **Run Design:** A sequencing plate with remaining uses may be incorrectly reported as fully used, preventing it from being selected in a new run design. This occurs when the in-use plate was Plate 2 in its previous run. Workaround: Save the run design with the metadata fields (Lot, Serial, and Expiry) blank.
- **Run Design:** When setting up a Run Design with auto-analysis using Read Segmentation, the Segmentation Adapter Set is not automatically set based on the selected application. Workaround: manually set the Segmentation Adapter Set to the appropriate value before saving the run design.
- **Run Design:** When duplicating a sample with incomplete fields, Plate 1 Well A01 may be assigned twice. Workaround: delete the duplicate entry before saving the run design.
- **Run Design:** Run Design may prompt about unsaved changes even when no changes were made or after the design has already been saved. Workaround: click OK on the prompt; the run design has been saved correctly.
- **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:** In the Target Regions view, sorting by some columns may not sort correctly.
- **Data Management:** Sorting the Barcodes page by Barcode Type may fail.
- **SMRT Analysis:** Some fields in job reports are sorted alphabetically rather than numerically, including Demultiplexing's HiFi Reads values in the Barcode Data table, Target Enrichment's Mean Target Coverage values, and PureTarget repeat expansion's Number of Reads values.
- **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.
- **SMRT Analysis:** The PureTarget repeat expansion workflow may report per-target coverage values in `/call-pbreports_target_enrichment_puretarget/execution/target_cov_by_sample.csv` that are too high. Please use the recommended per-allele, per-sample coverage from the `puretarget.repeat_expansion_genotypes.csv` in the job `/outputs` or the Target Enrichment workflow.
- **SMRT Analysis:** Target Enrichment analyses may fail when BED files contain quotation marks. Workaround: remove quotes from column 4, or validate the BED file using `trgt validate`.
- **SMRT Analysis:** Exporting a Read Segmentation and Iso-Seq analysis job may fail. Workaround: export analysis results using command-line tools rather than the SMRT Link job export feature.

- **SMRT Tools:** The default Cromwell configuration does not use the correct syntax for SGE when running Singularity-based workflows. Workaround: configure the Cromwell submit-docker section to write the Singularity command to a script file and submit the script file path; contact PacBio Technical Support for assistance.
- **SMRT Tools:** Running HiFi Mapping with `pbcr Cromwell` using default settings may fail due to the default number of chunks not being appropriate for the compute environment. Workaround: set the maximum number of chunks explicitly using `--task-option max_nchunks=<N>`, where N is appropriate for your compute environment.
- **SMRT Tools:** When using `pbcr Cromwell` `configure` to generate a `cromwell.conf`, 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:** 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.
- **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:** LDAP users without roles, when logging in, are directed to a page without content but are not shown "Invalid Credentials" as expected.
- **Administration:** Querying the "Created By" column fails for Auth0 usernames. No workaround is available.
- **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:** For some SMRT Link modules, using "Copy link address" may omit the `/sl` path segment, producing a broken link.
- **Instrument Settings:** Transfer Scheme Test can only be executed by an admin user and will fail when run by a non-admin user. Workaround: perform Transfer Scheme testing using an administrator account login.
- **Instrument Settings:** Some Revio users utilizing direct to cloud data transfer, e.g. "S3", may need to recreate a new transfer scheme after instrument upgrade.

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