SMRT® Link release notes (v25.3)

Supported instruments and chemistries

SMRT Link v25.3 and SMRT Link Lite v25.3 support the following:

Instruments	SMRT Link v25.3
Revio® system	Instrument software v13.3, all chemistries.
Vega™ system	Instrument software v1.1.0, all chemistries.

The **Sequel® II and IIe system** is not supported by SMRT Link v25+. An installation of SMRT Link v13.1 is required. Please see https://www.pacb.com/support/software-downloads/ for details.

See **SMRT Link software installation guide (v25.3)** 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 v25.3 will be the last release with the unencrypted rsync daemon transfer for Revio. Once disabled, file transfers using this option will fail. PacBio strongly recommends transitioning to a supported transfer option.

Who should upgrade?

- PureTarget™ users.
- Current customers interested in new administration features such as SSO.
- Current customers interested in new features or impacted by now fixed issues.

New Features and Updates

Sample Setup

- Improved Loading Calculator now displays samples in fixed order and allows viewing of all samples without having to resize the table.
- The Loading Calculator now includes the lock feature for each sample, enabling "Import from Sample Setup" during Run Design. In Run Design, the sequencing plate must be selected before a sample can be imported.

Runs

- New PureTarget carrier application for use with the new Pure Target carrier panel 1.0.
- For Hybrid Capture and PureTarget carrier applications, the Analysis Options default analysis workflow Target Enrichment supports application specific Advanced Parameters. Please reference the Target Enrichment application in the SMRT Link user guide for more details on recommendations.
  - The Hybrid Capture application sets Mark PCR duplicates = ON.
  - The PureTarget carrier application includes Fail reads and sets Padding around regions to 0.

Data Management

- New Target regions bed files are provided to support updated PureTarget products. Specifications for these BED files are provided in the SMRT Link user guide.
  - "PureTarget repeat expansion panel 2.0" BED contains the target region repeat definitions and is used with the PureTarget repeat expansion workflow.
  - "PureTarget repeat expansion panel 1.0" BED was formerly "PureTarget repeat expansion panel" BED, contains the target region repeat definitions and is used with the PureTarget repeat expansion workflow.
  - "PureTarget carrier panel 1.0" BED contains the target region cut sites and is used with the Target Enrichment workflow.

SMRT Analysis

- Target Enrichment workflow updates:
  - Removed `picard` dependency, which was previously required for generating coverage stats. This workflow no longer requires configuration with Singularity and containerized tools when variant calling is turned off.
  - Updated Target Enrichment Sample Summary header names to be more descriptive.
  - Updated BED file format now requires a fourth column with the region name in the format of ID=GENE1. Please see the SMRT Link user guide Appendix E for BED file format guidelines.
- PureTarget repeat expansion workflow updates:
  - Updated workflow to use the latest version of `trgt` v3.0.0. Please reference the TRGT GitHub documentation for additional details.
  - Updated Target Enrichment Sample Summary header names to be more descriptive.
- Updated SAT test now performs HiFi mapping of an example *E.coli* reference dataset.

Administration

- Keycloak has been updated to v24.0.4 for enhanced user management and improved security. The Keycloak console is now accessed at `https://<hostname>:9443/admin/` where previously it was accessed at `https://<hostname>:9443/auth/admin/.` Please reference the SMRT Link installation guide for updated documentation.
- Added Single Sign-On (SSO) and SAML support. Please see the SMRT Link installation guide for configuration details.

Fixed issues

- **Run Design:** Fixed an issue where if "Assign Data to Project" is not the default General Project, assignments are ignored, and data is automatically assigned to the General Project.
- **Run Design:** Fixed an issue where for in-progress or completed Runs, the Data Options and Analysis Options menus could not be expanded in a run design.
- **Run Design:** Fixed an issue where when using “Import from Sample Setup” the Application, Bio Sample name, and application specific analysis defaults were not always populated.
- **Run Design:** Fixed an issue, where when importing Run Design CSV, Bio Sample names with invalid characters such as spaces would create a run design with empty Bio Sample names instead of producing an error as expected.
- **Run Design:** Fixed an issue where automatic demultiplexing jobs were not scheduled in SMRT Analysis when the Barcoded M13 Primer Plate is selected. Automatic demultiplexing of the asymmetric Twist Universal Adapters with UDI and Barcoded M13 Primer Plate barcode sets in SMRT Link is supported. All other custom asymmetric barcode sets must be demultiplexed manually in SMRT Analysis or using the command line.
- **Data Management**: Fixed an issue where if a demultiplexed ‘child’ dataset was imported before the ‘parent’ dataset, it would cause an error when the ‘parent’ dataset was imported.
- **Data Management**: Fixed an issue where a moved dataset references the original data path and subsequently fails analysis.
- **SMRT Analysis:** Fixed an issue where workflows using `lima` (such as SMRT Analysis workflow Demultiplex Barcodes) failed when run on high memory compute nodes.
- **SMRT Analysis:** Iso-Seq® Analysis workflow and Read Segmentation and Iso-Seq Analysis workflow now default to the setting “Cluster reads separately” for Cluster of Barcoded Samples option.
- **SMRT Analysis:** FASTA outputs from Iso-Seq workflows are now compressed using blocked GZIP. This format allows the compressed files to be indexed with `samtools faidx`.
- **SMRT Analysis:** Fixed an issue where, when setting up a Single-Cell Iso-Seq job, switching Kit Types would result in the job failing to submit.
- **SMRT Analysis**: Fixed an issue in the Variant Calling workflow where VCF files were returned for the gVCF outputs.
- **SMRT Analysis:** Fixed an issue where pbcromwell workflows failed on parentheses in the `parameter_meta` block of the wdl file.
- **Settings:** Fixed an issue where on some customer servers, Update Genomes and Annotations would fail to fetch reference datasets.
- **Settings:** Fixed an issue where on some customer servers, run data was not automatically imported into SMRT Link due to the presence of trailing right-hand-side slashes in the file transfer location path.
- **Administration**: SMRT Link user administration does not require using the KeyCloak console and can be accomplished by admins using the GUI.

Known issues

- **Sample Setup:** For Loading Calculator samples, Sample Setup CSVs cannot be imported or exported.
- **Run Design:** By design, Plate 1 and Plate 2 must be the same part number for Revio run designs. When using both the 4 rxn Revio SPRQ™ sequencing plate and 1 rxn Revio SPRQ sequencing plate, separate run designs must be created for each product.
- **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 Design:** 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. The default Bio Sample names must be edited to exclude spaces to save the run design.
- **Run Design:** When interactively assigning Bio Sample names to barcodes, selecting multiple cells and pressing DEL will delete the Run Design. Only one cell should be deleted at a time.
- **Run Design:** In some cases, when setting up auto analysis for the 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’” may be encountered. This can be resolved by selecting an optional reference and then unselecting it, so the field is blank.
- **Run Details:** The Run Details table reports “Read quality (median)” however, the CSV export of the run details table erroneously has column header “Mean QV” instead. Please correct the header after export, if desired.
- **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:** 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 Management:** Users with the Bioinformatician role cannot view or create Projects.
- **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.
- **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`.
- **SMRT Tools**: The `dataset create` tool does not support creating an XML for `--type BedSet`. When using a `pbcromwell` to run a workflow that requires a `bedset.xml` please import the target region BED file into SMRT Link and point to the resulting XML file when executing a workflow.

- **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.

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