SMRT® Link release notes (v13.1)

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Supported instruments and chemistries

SMRT Link v13.1 and SMRT Link Lite v13.1 support the following:

Instruments	SMRT Link v13.1	SMRT Link Lite v13.1
Revio™ system	ICS v13.1, all chemistries	ICS v13.1, all chemistries
Sequel® IIe system	ICS v11.0.1+, all chemistries	ICS v11.0.1+, all chemistries
Sequel® II system	ICS v11.0.1+, all chemistries	**Not supported**
Sequel® system	**Not supported**	**Not supported**

Supported operating systems

SMRT Link server software is supported on English-language distributions of the following:

– Rocky Linux 8.x and 9.x.
– Ubuntu 20.04 Linux® (until end-of-life on 4/1/2025).
– Ubuntu 22.04 Linux®.

- See **SMRT Link software installation guide (v13.1)** for detailed hardware and software requirements and installation instructions.
- SMRT Link v13.1 **requires** use of the newer Keycloak API gateway for all Revio/Sequel II/Sequel IIe systems; SMRT Link v13.1 will **not** work with WSO2 API manager. Users who have not yet migrated to the Keycloak API gateway must do so to use SMRT Link v13.1 with all systems. See **SMRT Link software installation guide (v13.1)** for migration instructions.

New Features

New PureTarget™ repeat expansion pipeline

- New **PureTarget repeat expansion** pipeline analyzes multiplexed samples prepared with the targeted sequencing PureTarget repeat expansion panel.
- Files output for each sample include statistics on each panel target, TRVZ plots, and tandem repeat genotypes for all barcodes.

Sample Setup

- Added support for **Revio polymerase kit 96**. Use **Revio polymerase kit 96** when creating new calculations for use when running high-throughput library preparations where the annealing, binding and cleanup steps have already been completed.
- Added support for the Kinnex™ single-cell RNA kit.
- Removed **HiFi Reads** from the Sample Setup **Application** menu; changed and divided the WGS sequencing applications into **Human WGS**, **Microbial assembly** and **Other WGS**.
- Updated the protocols for **Revio polymerase kit** and **Sequel II binding kit 3.2** to use an increased concentration of polymerase for most applications. This does **not** impact reaction times or the number of reactions per kit.

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Run Design

- Added support for the **PureTarget repeat expansion** application.
- Added support for the Kinnex single-cell RNA kit.
- Removed **HiFi Reads** from the Runs **Application** menu; changed and divided the WGS (Whole Genome Sequencing) applications into **Human WGS**, **Microbial assembly**, and **Other WGS**.
- Locked Revio calculations (created in Sample Setup) can now be imported into Revio run designs.
- Moved the **Use Adaptive Loading** setting toggle to the run level from the collection level for clarity, as the setting applies to the **entire run** - not just to one collection.

Run Details

- Updated the **Read Length Distribution** plot to include fail reads and HiFi reads.

Data Management

- Can now import zipped dataset files even if some of the datasets already exist in SMRT Link.

SMRT® Analysis

- Added **known gene** to the **Iso-Seq® Summary** table and renamed the tabs for clarity.
- The filtering behavior of the **Demultiplex Barcodes** utility (including its use in Sequel II auto-analysis) was changed to demultiplex all reads found in the input dataset **regardless** of read quality, instead of applying a default cutoff of QV=20 (RQ=0.99). The filtering QV can still be set in the **Advanced Parameters** dialog if you prefer the old behavior (the new default value of -1 indicates that filtering should be skipped).
- Added separate settings to the **Export Reads** data utility for exporting FASTQ and FASTA files.

Known issues

- **SMRT Analysis:** When the **Single-Cell Iso-Seq Analysis** application is run with an incorrect kit selection (such as 3' instead of 5'), the workflow will fail with an uninformative error message: `KeyError: median_genes_per_cell`. If this happens, select the correct kit and rerun the analysis.
- **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.
- **Instruments**: The **Active ZMWs** plot displays instantaneous ZMW metrics. This differs from the Preview metrics that estimate overall productivity values across the entire movie.
- **Runs**: The Chemistry bundle updates that introduce new consumable part numbers require a services restart **before** these parts can be used in the **Runs** module. The restart should be executed manually **after** the GUI chemistry bundle update is completed.
- For local SMRT Link installations, the **Variant Calling** and **Target Enrichment** workflows fail when executing tasks that require the Singularity software. Error messages may include `Could not localize`. A Job Management System (JMS) can be installed on single-node local computers to ensure that SMRT Link can execute these workflows. Additional SMRT Link configuration may be required after JMS installation.

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- The **Export Reads** workflow fails FASTQ/FASTA generation when used with datasets created using the Kinnex 16S rRNA kit. To generate FASTQ files for this type of data, use the command-line utility `bam2fastq` available in SMRT Tools, or use the GitHub `pbtk` package available [here](#).
- **Runs**: For Sequel II and IIe run designs, the **Pre-extension time** field has incorrect placeholder text "Enter Insert Size above to Calculate." The time is not calculated from insert size and should be entered directly into the input field.
- **Runs**: Pre-extension settings are not included when exporting Sequel II/IIe run design CSV files. To add **N** hour pre-extension settings after export, modify the CSV file to add a column named **Automation Parameters** with value "`ExtensionTime=double:N|ExtendFirst=Boolean:True`".

Fixed issues

Sample Setup

- Fixed an issue in Sample Setup where the wrong binding kit was selected by default.
- Fixed an issue where importing a calculation using a v3.1 or v3.2 Binding Kit was incorrectly recognized as a **Classic** calculation instead of a **High-Throughput** calculation.

Runs

- Fixed an issue where setting up a new run and selecting **MAS-Seq single cell** was not populating the default values.
- Fixed an issue where setting up a new Run and selecting **Kinnex single-cell RNA** failed.
- Fixed an issue where creating a new Run and specifying **Pre-extension** caused an error.
- Fixed an issue with creating Runs where the GUI allowed an illegal combination of options.
- Fixed an issue where creating a new Run and switching applications incorrectly populated fields.
- Fixed an issue where Run Details page did not load the run if any report JSON files were removed.
- Fixed an issue where exporting a CSV file from the Run Details page exported two files named `run-qc-export.csv`.
- Fixed an issue that caused problems with duplicated runs.
- Fixed an issue that caused the Kinnex applications to not display as options in the Sample Setup and Run Design GUIs.
- Fixed an issue where unbarcoded Revio run designs automatically generated a data export job.

SMRT Analysis

- Fixed an issue that caused the **Variant Calling** application to fail and display an error message.
- Fixed an issue that caused the `lima` step of the **Single-Cell Iso-Seq Analysis** application to run out of memory.
- Fixed an issue that caused the **HiFi Mapping** application to generate Mapping and Summary Metrics reports that include incorrect information.
- Fixed an issue that caused an error when deleting an existing analysis.
- Fixed an issue that could result in SMRT Analysis accepting potentially dangerous parameters. The SMRT Link analysis job API now **rejects** string parameters containing any of the `|;&$><'!#` characters, except for the advanced IPA2 options in assembly workflows.

SMRT Link

- Fix an issue that caused display errors with units and rounding in SMRT Link-generated PDF reports.
- Fixed an issue that could result in a Keycloak vulnerability.
- Fixed an issue where the SMRT Link collection metrics JSON file contained duplicate `movie_id` fields. This impacted the reporting of CCS metrics.
- Fixed an issue that caused SMRT Link to not generate PDF dataset reports on multiple datasets.