

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® lle 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.

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 <code>Could not localize</code>. 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.
- 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.



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.



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