SMRT® Link release notes (v13.0)

Supported instruments and chemistries

SMRT Link v13.0 and SMRT Link Lite v13.0 support the following:

Instruments	SMRT Link v13.0	SMRT Link Lite v13.0
Revio™ system	ICS v13.0, all chemistries	ICS v13.0, all chemistries
Sequel® IIe system	ICS v11.0+, all chemistries	ICS v11.0+, all chemistries
Sequel® II system	ICS v11.0+, all chemistries	**Not supported**
Sequel® system	**Not supported**	**Not supported**

Supported operating systems

SMRT Link v13.0 is supported on:

- English-language CentOS 7.x, supported until end-of-life 6/30/2024.
- English-language Rocky Linux 8.x supported until end-of-life 5/31/2029.
- Ubuntu 20.04 and 22.04 64-bit Linux® distributions.

- See **SMRT Link software installation guide (v13.0)** for detailed hardware and software requirements and installation instructions.
- SMRT Link v13.0 **requires** use of the newer Keycloak API gateway for **all** Revio/Sequel II/Sequel IIe systems; SMRT Link v13.0 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 v13.0 with **all** systems. See **SMRT Link software installation guide (v13.0)** for migration instructions.

New Features

Support for new Kinnex™ kits

- Added support for the new Kinnex kits to the Sample Setup and Runs modules:
  - Kinnex single-cell RNA kit
  - Kinnex 16S rRNA kit
  - Kinnex full-length RNA kit

SMRT Link Lite

- New **SMRT Link Lite** can be installed for users who do not require SMRT Analysis and need more minimal compute requirements.
- Use the same installer for SMRT Link or SMRT Link Lite.
- **Note**: SMRT Link Lite works **only** with Revio and Sequel IIe systems. On Revio systems, it **requires** ICS v13.0. On Sequel IIe systems, it **requires** ICS v11.0+.

Metrics preview estimates (Revio-only)

- Added new estimates of Revio run performance at two different time points: 4 hours after sequencing acquisition begins and 1 hour before the end of acquisition.
- Guides future runs by providing early information on loading, library fragment size, and representation of barcodes in the pool.

New HiFi Target Enrichment pipeline

- New **HiFi Target Enrichment** pipeline analyzes multiplexed samples prepared with a target enrichment workflow.
- Files output for each sample include mapped BAM files, target enrichment statistics, and variant call sets.

New Read Segmentation and Iso-Seq® Analysis pipeline

- New **Read Segmentation and Iso-Seq Analysis** pipeline enables analysis and functional characterization of full-length isoform (Iso-Seq) data generated with Iso-Seq express 2.0 kit plus Kinnex full-length RNA kit.
- File outputs include full-length (FLNC) reads and clustered transcript sequences and (if genome and annotation are available) mapped BAM files, collapsed transcript GFF, and transcript classification results.

Updated Read Segmentation and Single-Cell Iso-Seq Analysis pipeline

- Updated **Read Segmentation and Single-Cell Iso-Seq Analysis** pipeline to additionally support the Kinnex single-cell RNA kit (for 10x 5' kit support).

Sample Setup changes

- All samples created now display in one table on the Sample Setup home page.
- Selecting **Sequel II binding kit 3.1/3.2, Revio polymerase kit** displays what was known as **High Throughput mode** in previous releases.
- Selecting **Sequel II binding kit 2.1/2.2** displays what was known as **Classic mode** in earlier releases.
- Protocols now display in a format that matches the PacBio library preparation protocols, with numbered steps and colors to indicate the correct tubes. The protocols are **unchanged**.

Instruments module GUI changes

- Click on a SMRT Cell icon to display sequencing activity plots and run preview during a movie collection. This replaced the **View sequencing ZMWs** text link, which is no longer displayed.
- **Time until pre-load available** was changed to **Time until door locks** to more accurately reflect the information relayed by the countdown timer. Generally, preloading a subsequent run will be available when the door unlocks, except in cases where there is an instrument issue that requires attention.

Changes to Run Design CSV file format and GUI

Revio systems only

- The Revio Run Design CSV file format was extensively restructured to improve usability. Revio run designs will be exported from SMRT Link using **only** this new format. The older SMRT Link v12.0 Run Design CSV format is **deprecated** but can still be imported into SMRT Link v13.0, though changes to settings and fields described below must still be applied.
- Multiplexing is now **optional** and can be disabled for a Well Sample by setting the new **Sample is indexed** field to **No**. The **default--default** barcode previously used for non-multiplexed samples is now **obsolete** and should be removed from run designs.
- **Insert size (bp)** is now a **required** field for each Well Sample.
- **Use adaptive loading** was added as a field for each Well Sample. This option must be consistently enabled or disabled for **all** Well Samples in the same run.
- **Consensus mode** was added as a field to control CCS behavior. Valid options are **molecule** (default) or **strand**.

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- **Full resolution base qual** and **Subread to HiFi pileup** were added as optional advanced settings in the Run Design CSV template.

Revio/Sequel II/Sequel IIe systems

- **CSV version** was added as a field to support future backwards compatibility.
- **Compute settings** was added as a field to control cluster options for auto-analysis jobs.
- **Adapters / Barcodes** was changed to **Indexes** in the GUI and CSV templates.
- **Experiment ID** was changed to **Transfer subdirectory** in the GUI and CSV templates.
- **Experiment name**, a field that appeared in run metadata, is now **removed** from the run design GUI and CSV templates. Older templates that contain **Experiment name** are still valid, and the Experiment name will still appear in the run metadata.
- **Library type** and **Same barcode on both ends** are new run design options.

Run Design

- Add **Duplicate** button to copy a Run design.
- Run comments entered when creating a Run design can be searched from the Runs module table.

Run Details

- Improve column layout and default columns in the run details tables, and now use human-readable units (kb, Gb, M reads).
- Simplify run plots and label all plots with the movie name. All plots are available in the Data Management module.
- **Movie name** (previously known as **Movie ID** or **Metadata context ID**) is the `<instrument-number_date_time>` format, for example `m64263e_211008_213305`. **Movie name** is now displayed on the top right corner of all Run QC plots.

Configurable movie times for Revio systems

- Movie times can now be configured to be 12, 24, or 30 hours.

Adeno-Associated Virus support for Revio systems

- **Adeno-Associated Virus** is now an option when creating a new run design or a new Sample Setup calculation.

Inferred Barcodes report for Undo Demultiplex utility

- The **Undo Demultiplex** utility now includes an **Inferred barcodes** report to help with proper demultiplexing. The report identifies known barcode sequences at the end of reads.

Audit logging

- An audit log can now be downloaded from SMRT Link using **Settings > About**.

Notification badge

- The notification badge (the red counter showing how many notifications there are) can be configured from **Settings > Notification Settings**.

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SMRT Link API Python reference

- New documentation for the SMRT Link API using Python examples. See **SMRT Link Python API reference (v13.0)** for details.

SMRT Link web services API

- **New endpoint**: `GET /smrt-link/runs/{runId}/collections/{collectionId}/reports`
- **New endpoint**: `GET /smrt-link/runs/{runId}/reports`
- Added a `maxAge` query term to run, job, and dataset searches. The query term is internally converted to a search of the corresponding date range.
- Allow users to query using just a date string (such as `"2020-02-18"`) instead of the UTC string (such as `"2020-02-18T05:41:43.663Z"`).

Known issues

- **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. The workaround is to manually edit the Output Data Set Name.
- **SMRT Analysis:** The **HiFi Target Enrichment** analysis workflow does **not** progress when the read number is too low.
- **SMRT Analysis:** To ensure compatibility with third-party tools and formats, the **HiFi Target Enrichment** analysis workflow replaces space characters in the Bio Sample Name of input datasets with underscores.
- **SMRT Link**: When connecting a new instrument or switching to a different SMRT Link server, it may take a minute or two for the update to display in the GUI.
- **Data Management**: When merging barcoded Iso-Seq datasets for later downstream analysis, the sample names of the input datasets should be the **same** so that the summary metrics can display in the output. When sample names do **not** match, the summary metrics may not display correctly.
- **Data Management**: The **Number of Passes** plot incorrectly shows many **Other Reads** as having 25+ passes.
- **Runs**: For Revio runs, the **Pre-extension time** column on the run information page displays as empty.
- **Runs**: When a run is specified with **Adaptive loading** set to **NO**, the **Loading time** column on the run information page displays as empty.
- **Runs**: When creating a new run design, **Adaptive loading** is a run-level setting, but is displayed as a sample-level setting in the GUI. If Adaptive loading is set differently for different samples, only **one** value is saved.
- **Runs**: When duplicating a run design, some analysis names and associated workflows may not be copied correctly.
- **Runs**: When creating a new run design with **Adeno-associated Virus** as the application and selecting a Consensus mode, the **Include base kinetics** option is incorrectly grayed out. The workaround is to select **Unspecified** as the application, then select **Adeno-associated Virus** as the library type. You can then select a Consensus Mode, and the **Include base kinetics** option will be available.
- **Preview**: The 4-hour preview metrics are missing for runs that use a Consensus mode of **Strand**. The preview timepoint is also displayed incorrectly as 1-hr before the end of acquisition instead of 4-hrs in the run information table.
- **Preview**: The **Latest** value in the **Active, %** plot may be incorrect for some runs.
- **Preview**: Updating the SMRT Link server during an active run may sometimes cause the loss of preview metrics.

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- **SMRT Link user guide**: In the **HiFi Target Enrichment** report section, the **Percent On-Target Bases** field is equivalent to `1 - PCT_EXC_OFF_TARGET` in `hs_metrics` of Picard Tools.

Fixed issues

- Fixed an issue that sometimes caused reserved characters to be incorrectly encoded when used in the **Edit output filename prefix** dialog. This made it impossible for the output files to be downloaded.
- Fixed an issue that sometimes caused the Iso-Seq workflow to request an incorrect amount of memory.
- Fixed an issue that sometimes caused a Microbial Genome Analysis job with **Find modified base motifs** set to **ON** to fail.
- Fixed an issue that sometimes caused an edited run design to not be saved.
- Fixed an issue where sometimes clicking **Display all enabled users** caused an error.
- Fixed an issue that sometimes caused the `pbservice import-run` command to not post a Run Design CSV file.
- **Revio, Runs module**: Fixed an issue that sometime caused **Time remaining for postprocessing** to not display correctly.
- **Revio, Runs module**: Fixed an issue with support of 2D barcode scanners for scanning Revio sequencing plate QR codes. Previously, the entire barcode information was entered into a single field. The barcode information is now properly split across part number, lot, expiration date, and serial number.
- **Revio**: Improved the reliability of communications between a Revio system and a connected SMRT Link server over DHCP or static IP network connections. SMRT Link **automatically** updates Revio system IP addresses when the instrument network connection settings are changed.
- **Revio**: Added a timeout to the file transfer test performed when loading and launching a run design.

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