

## 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 <sup>®</sup> Ile system	ICS v11.0+, all chemistries	ICS v11.0+, all chemistries
Sequel <sup>®</sup> II system	ICS v11.0+, all chemistries	<b>Not supported</b>
Sequel <sup>®</sup> system	<b>Not supported</b>	<b>Not supported</b>

**Note:** SMRT Link v13.0 and SMRT Link Lite v13.0 do **not** support the Kinnex single-cell RNA kit for Revio, Sequel II, or Sequel II systems.

## 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<sup>®</sup> 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 Ile systems; SMRT Link v13.0 will **not** work with WS02 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

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### Support for new Kinnex™ kits

- Added support for the new Kinnex kits to the Sample Setup and Runs modules:
  - 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 Ile systems. On Revio systems, it **requires** ICS v13.0. On Sequel Ile 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.

## 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, Revo 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

### Revo systems only

- The Revo Run Design CSV file format was extensively restructured to improve usability. Revo 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**.
- **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**.

## 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

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- **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.
- **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.
- **Sample Setup:** The Kinnex single-cell RNA kit is incorrectly listed in the application list for Revio, Sequel II, and Sequel II systems. The Kinnex single-cell RNA kit is **not** supported in SMRT Link v13.0.

## Fixed issues

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- 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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