



## SMRT Link Server Installation

- SMRT Link server software is supported on English-language CentOS 6.x; 7.x and Ubuntu 14.04; 16.04 64-bit Linux<sup>®</sup> distributions. (This also applies to SMRT Link compute nodes.)
- SMRT Link is **not** guaranteed to work on Linux versions that are no longer supported by the Operating Systems' vendors.
- SMRT Link server software **cannot** be installed on macOS<sup>®</sup> or Windows<sup>®</sup> systems.
- Several new SMRT Link v6.0.0 features are computationally-intensive and require adherence to the computational and storage requirements listed in the document **SMRT Link Software Installation (v6.0.0)**.
- To install **only** command-line SMRT Tools, use the `--smrttools-only` option with the installation command, whether for a new installation or an upgrade. Examples:

```
smrtlink-*.run --rootdir smrtlink --smrttools-only  
smrtlink-*.run --rootdir smrtlink --smrttools-only --upgrade
```

## Included with the SMRT Link Installation

### Barcode Sequences:

- IsoSeqPrimers (**New**)
- RSII\_384\_barcodes
- RSII\_96\_barcodes
- Sequel\_RSII\_16\_Barcodes\_Overhang\_Adapter (**New**)
- Sequel\_RSII\_16\_barcodes\_v1
- Sequel\_RSII\_16\_barcodes\_v2 (**New**)
- Sequel\_RSII\_384\_barcodes\_v1
- Sequel\_RSII\_96\_barcodes\_v1

### Reference Sequences:

- Sequel DNA Internal Control
- HIV\_HXB2
- HLA\_11locus\_clustering\_guide (**New**)
- lambdaNEB

## New Features

### SMRT Link

- Increased security for web services.
- Ability to opt-in for secondary analysis usage tracking.
- Faster loading time for Data Management and SMRT Analysis pages by restricting default display to only the most recent 10,000 Data Sets (Data Management) and 6,000 analyses (SMRT Analysis). Older Data Sets and analyses can be searched using **Advanced Search**.

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- Required user-entry fields in Run Design and SMRT Analysis are indicated by bold font rather than red boxes around the fields or asterisks.

### SMRT Link - SMRT Analysis

- **Structural Variant Calling Application**
  - Support for additional variant calls: Inversions, translocations, and insertions and deletions  $\geq 20$  bp. (The previous version detected insertions and deletions  $\geq 50$  bp.)
  - Base pair-precise variant calls instead of approximate variant calls.
  - Significant optimization of the analysis workflow.
  - Improved scalability for large cohort sizes.
- **New Iso-Seq 3 Application**
  - Significantly faster time-to-results compared to Iso-Seq 1.
  - Maintains the transcript recovery performance of previous implementations, while reducing the false positives rate.
  - Improved support for barcode and cDNA primer detection, and increased detection of incorrect primer combinations and library artifacts.
  - Native support of customized cDNA primer and barcode combinations.
  - Iso-Seq 3 **replaces** Iso-Seq 2. Iso-Seq 1 is still available.
- **Assembly (HGAP 4) Application**
  - Improved microbial assembly performance using optimized default HGAP parameters.
- **General**
  - Added the ability to define file names for the downloadable analysis output files.
  - File names of barcoded CCS and LAA outputs inside zip downloads now include the Bio Sample Name.
  - In the Resequencing and CCS Mapping applications, added an advanced analysis parameter to generate and display a consolidated BAM file in the **Results** page **File Downloads** section.

### SMRT Link - Data Management

- Demultiplexed Data Sets are now accessible from the parent Data Set's row in the Data Sets table by clicking the number displayed in a new **Demultiplexed Subsets** column.
- The Data Set details pages of demultiplexed Data Sets now include subread length statistics and distribution histograms.
- Displays customer-uploaded GMAP references.
- Ability to browse the local file system in addition to the SMRT Link server for files to import.
- In the **Create Data Set** page, added a Data Set Filter for subread length.

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### SMRT Link - Sample Setup

- Enabled mechanism for easy update of Sample Setup parameters.

### SMRT Link - Run Design

- Enabled import of Sample Setup results into Run Design.
- The Run Design CSV template file is now downloadable from SMRT Link and supports barcoded Run Designs.

### SMRT Link - Run QC

- Added a plot displaying the Base Yield Density; bases read, per unit Read Length.
- Added a plot displaying the Insert Read Length Density; the density of reads according to their longest subread and total HQ read length.
- Plots now display in magnified windows when clicked.

### Fixed Issues

- In Run Design, clicking **Edit** on a saved barcoded Run Design with a CSV-format Barcoded Sample Name File now works correctly.
- In Run Design, when omitting a comma in the CSV-format Barcoded Sample Name File to separate fields, the Bio Sample Names are now correctly assigned.
- In Run Design, setting “Sample Is Barcoded” to “Yes” **without** specifying a barcode set no longer causes the run to fail.
- Run Design tooltip help messages now display correctly.
- In a Run Design that contains multiple SMRT Cells with barcoded samples, automatic demultiplexing of each SMRT Cell’s sequencing data starts as soon as it is transferred to the SMRT Link server instead of waiting for the entire set of SMRT Cells to be transferred.
- Automatic demultiplexing of the **last** cell in a run containing multiple SMRT Cells with barcoded samples is now launched automatically.
- In Data Management, reordering table columns by using the column selector now persists after leaving the page.
- In SMRT Analysis, copying an analysis no longer fails if any advanced parameters in the copied analysis were set to non-default values.
- In SMRT Analysis, exporting an analysis no longer leaves a gray overlay on the browser page.
- In SMRT Analysis, data download file sizes now display in KB, MB, and GB instead of bytes.
- In SMRT Analysis, in the results page of a completed Demultiplex Barcodes analysis, the **Summary Metrics > Mean Longest Subread Length** is now calculated correctly as the mean over all barcodes of the lengths of the longest individual subreads within each barcoded sample. **Summary Metrics > Mean Read Length** is now calculated correctly as the mean polymerase read length.

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## Known Issues

- In the pop-up window that displays immediately after installing or upgrading SMRT Link, clicking “Do not notify PacBio of the successful installation” for the first question **disables** all services for sending PacBio installation and analysis log files for troubleshooting, acceptance of new chemistry bundle updates, and data about software usage.
- When an Analysis or Data Sets page includes thumbnail plots, the displayed plots do **not** expand when clicked. Click on the **title** of a plot to expand it.
- In an Advanced Search of Data Sets and analyses, the search operator `in` fails for date and numeric fields. It works correctly for string fields.
- If the input Data Set for an imported analysis was originally created by merging multiple sub-Data Sets, re-running the imported analysis fails.
- SMRT View can fail on Windows clients running older versions of Java. Update to the latest 64-bit Java for SMRT View support.
- The Active Use time for the Sequencing Kit 3.0, 8-reaction version was incorrectly set to 72 hours. The correct value is 100 hours. Users may see a warning that this reagent has expired when setting up a run more than 72 hours after initial use of this item. Please disregard this warning if the elapsed time is less than 100 hours.
- In SMRT Analysis, the Structural Variant Calling application fails for some combinations of submitted Data Sets and reference genomes where the reference contains small contigs < ~200 kb. The **Analysis Overview > Status** section of the results page displays a status of **FAILED: Task pbsv.tasks.call-0 FAILED**. The failure is due to an incompatibility in how the application chunks computations when running on a compute cluster. Contact PacBio Technical support for assistance in implementing a workaround.
- Sample Setup protocols generated using prior versions of SMRT Link **cannot** be displayed in the v6.0.0 software. Please consider printing out any critical records before upgrading.
- Creating a Data Set by including individual barcoded samples from different cells cannot be performed using the GUI. Use the command-line `dataset merge` command instead.
- The HGAP 4 advanced analysis parameter **Save Output for Falcon Unzip** is not included in the GUI, but is always **On**. This produces additional files with `.las` file extensions that can fill your disk space. We suggest monitoring and purging these files periodically, if you don't use them.
- HGAP and Resequencing analyses fail with **BamPostService returned a non-zero exit status 1** messages when the SMRT Link installation is not configured correctly. Please contact PacBio Technical Support for help with troubleshooting.

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