SMRT Link Server Installation

- SMRT Link server software is supported on English-language CentOS 6.x; 7.x and Ubuntu 16.04; 18.04 64-bit Linux® 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® or Windows® systems.
- Several new SMRT Link v7.0.1 features are computationally-intensive and require adherence to the computational and storage requirements listed in the document SMRT Link Software Installation (v7.0.1).
- 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:

- Sequel_16_barcodes_v1
- Sequel_16_Barcodes_v3
- Sequel_96_barcodes_v1
- Sequel_384_barcodes_v1
- RSII_96_barcodes
- RSII_384_barcodes
- IsoSeqPrimers_v2 (Includes the content of IsoSeqPrimers as well as support for NEB and Clontech primers.)

Reference Sequences:

- Sequel DNA Internal Control
- HIV_HXB2
- HLA_11locus_clustering_guide
- lambdaNEB

New Features

SMRT Link

- Scalability and robustness improvements.
- Optimization of analysis pipelines.
- In addition to the existing Sequencing mode generating Continuous Long Reads (CLR), SMRT Link now supports CCS Sequencing.
- Support for the new data type: CCS Reads.
- The following three data types are now supported by SMRT Link:
  - Sequel data: Subreads from Sequel or Sequel II instruments.
– CCS data: Single Molecule consensus reads generated from the CCS analysis.
– RS II data: Subreads from PacBio RS II instruments.

• **Automated Analysis:** Auto Analysis and Pre Analysis allow a specific analysis to be automatically run after a sequencing run has finished and the data is transferred to the SMRT Link Server. Auto Analysis can be set up in Run Design or SMRT Analysis after the Run Design is saved and before the run is loaded on the instrument. Auto Analysis can be run on CCS or CLR data, and includes all analysis applications available for the corresponding data type. Auto Analysis works with both Sequel® and Sequel II Systems, but with the following limitations:

– **Sequel II System:** Works with Sequel data, CCS data, and demultiplexed data.
– **Sequel System:** Can perform Auto Analysis or Pre Analysis, but not both.

**Pre Analysis** is set up at Run Design as the run is defined and includes demultiplexing of CLR and CCS sequencing modes and CCS analysis for CLR reads.
– Pre Analysis is available for both Sequel and Sequel II Systems.

• PDF report generation for a sample/Data Set with extensive information including sample set up, run design and run QC details, analysis parameters and results.
• Supports configuration and use of compute cluster templates for analysis execution, based on the available computing resources.

**Note:** If the SMRT Link instance connected to your sequencing instrument is not communicating directly with your compute cluster, turn off the default setting “Generate CCS Data” when designing your run in Run Design for CCS Sequencing mode.

**SMRT Link - SMRT Analysis**

• **General**
  – New significantly faster and more efficient CCS algorithm.
  – All analysis applications: blasr replaced by minimap2 resulting in faster mapping (>20-fold speed up) for all applications that include a mapping step.
  – Mapping, Demultiplexing and Structural Variant Calling applications now accept CCS data as input.
  – New analysis wizard for setting up analyses.

• **Iso-Seq Application**
  – Iso-Seq 3 is renamed Iso-Seq, and includes additional efficiency optimizations.
  – The Iso-Seq 1 application is deprecated; its functionality is available in the Iso-Seq application.
  – Gmap replaced by minimap2 for mapping. Gmap reference files are no longer used.
• **Structural Variant Calling Application**
  – Can call duplications and copy number variants (CNVs) in addition to insertions, deletions, inversions and translocations available in the previous SMRT Link version.
  – Improved sensitivity for larger insertions and deletions.

**SMRT Link - Data Management**
• Improved usability:
  – Select and view Data Sets, Projects, barcode files and reference sequence files.
  – Export barcode and reference files.

**SMRT Link - Sample Setup**
• Ability to generate and review protocols for Sequel or Sequel II Systems.
• Initial values now set based on the choice of System.
• Support for the upcoming Iso-Seq Express method.
• Support for the low DNA input protocol.
• Support for protocol variations specific to the use of the Predictive Loading feature. (Beta feature, Sequel II System only.)

**SMRT Link - Run Design**
• For the CCS Sequencing mode, added the option to automatically generate CCS data after sequencing data is transferred from the instrument to the SMRT Link server.
• Ability to create and review runs for Sequel or Sequel II Systems.
• Initial values now set based on the choice of System.
• Predictive loading (Beta feature, Sequel II System only) uses active monitoring of the ZMW loading process to predict a favorable loading end point.
• Run and immobilizations times are now specified in hours.

**Fixed Issues in SMRT Link v7.0.0**
• When analyzing multiplexed samples, selecting subsets from different collections is now enabled.
• If the input Data Set for an imported analysis was originally created by merging multiple sub-Data Sets, re-running the imported analysis now works correctly.
• In SMRT Analysis, the Structural Variant Calling application no longer fails for some combinations of submitted Data Sets and reference genomes where the reference contains small contigs <~200 kb.
• In an Advanced Search of Data Sets and analyses, the search operator in now works correctly for date, numeric, and string fields.

**Known Issues**
• When an Analysis or Data Sets page includes thumbnail plots, the displayed plots do not expand correctly when clicked.
• Selecting more than 384 Data Sets using the GUI, then clicking the List button, causes an error.
• If you create an analysis using multiple sub-Data Sets, then delete all sub-Data Set analyses, the combined analysis is not shown as deleted.
• In SMRT Analysis, unchecking the “Failed” filter when the table of analyses is still loading (and says “Loading...”) causes an error.
• After the initial installation, pressing the Browser refresh button before replying to the “Notify PacBio of successful installation” dialog causes a “No pb-modal-underlay has been registered!” error.
• 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.
• Auto Analysis cannot be copied to reuse the same analysis parameters.
• In Run Design, the Pre-Extension Time field may not allow entry of a value less than 1.0 hours. A value less than 1.0 may be achieved through .csv file import if required. Using a value of 1.0 hour when the recommended time is less than 1.0 should not adversely affect sequencing results.
• The Run Design .csv template file bundled with SMRT Link is an older version, and is not compatible with this version of the software. A replacement may be obtained by contacting your local FAS representative.

Fixed issues in SMRT Link v7.0.1

SMRT Link v7.0.1 fixes minor issues found in SMRT Link v7.0.0. All SMRT Link v7.0.0 documentation apply to SMRT Link v7.0.1.

• Using the browser’s Back button after visiting an analysis details page in SMRT Analysis no longer causes an error.
• Editing a Run Design that contains a customer’s immobilization time no longer causes that immobilization time to be multiplied by 60.
• The Estimated Run Duration in Run Design for custom immobilization times is now correct.
• Changed the text used in Steps 4 and 7 for the AMPure/ProNex protocol in Sample Setup.
• The Sequel Additive volume in Sample Setup was adjusted for the Sequel/Sequel II System use case.
• The CLR Predictive Loading default target is now 60%.
• Resequencing analysis no longer fails on machines with no system zlib installed, or with zlib earlier than v1.2.8 installed.