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.