SMRT® Link Release Notes (v9.0)

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.)
  Note: SMRT Link v9.0 is the **last** version that will be supported on CentOS 6.x.

- 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 systems running other versions of UNIX, macOS® or Windows®.

- Several new SMRT Link v9.0 features are computationally-intensive and require adherence to the computational and storage requirements listed in the document **SMRT Link Software Installation (v9.0)**.

- 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
  ```

Supported Chemistry

- SMRT Link v9.0 supports all chemistry versions for **Sequel II System** and chemistry v2.1 and later for **Sequel System**.

Included with the SMRT Link Installation

Barcode Sequences:

- gDNA_Sample_Amplification_Adapter
- IsoSeq_Primers_12_Barcodes_v1
- IsoSeqPrimers_v2 (Includes the content of IsoSeqPrimers as well as support for NEB and Clontech primers.)
- Sequel_16_barcodes_v1
- Sequel_16_barcodes_v3
- Sequel_96_barcodes_v1
- Sequel_384_barcodes_v1

Reference Sequences:

- Sequel DNA Internal Control
- HIV_HXB2
- HLA_11locus_clustering_guide
- lambdaNEB

SMRT Link Database Note

- SMRT Link v9.0 no longer includes weekly automatic database backups. A database backup is still automatically performed once, during installation or upgrade. Failure to back up the SMRT Link database on a regular schedule risks losing **all** records in SMRT Link

(including users, Data Sets, analyses, barcodes, and references) if a file system or reconfiguration error occurs. The underlying sequencing or analysis files, such as BAM files, are **not** affected. We **strongly** recommend asking your local Linux System Administrator to schedule regular weekly backups of the SMRT Link database using standard Linux utilities. For additional details, please contact PacBio Technical Support.

Iso-Seq® Note:

- The recommended analysis workflow for Iso-Seq experiments is to generate the CCS data and then analyze it with the Iso-Seq application. For details on suggested analysis parameters, see the **SMRT Link User Guide**, Iso-Seq Analysis Application section.

New Features

SMRT Link

- Added support for sample traceability through the various SMRT Link modules by using the **Bio Sample Name** field.
- Added analysis support for the Ultra Low DNA input protocol. (Protocol release: 2020.)
- Added GUI multiplexing support for up to 10,000 barcodes.

SMRT Link - Run Design

- The Run Design CSV template can be downloaded using Run **Design > Import Run Design > Download Template**.
- Run Design now contains a required **Bio Sample Name** field for both single and multiplexed samples.
  - For multiplexed experiments, SMRT Link provides default names for **one** Bio Sample Name per barcode. The default name can be edited as needed in the **Barcoded Sample Name File**.
  - For multiplexed **Iso-Seq experiments and analysis only**, Bio Sample Names are **not** required.
- For multiplexed experiments, **Well Sample Name** and **Bio Sample Name** entered at Run Design:
  - Are displayed as column values in the Data Management and SMRT Analysis modules.
  - Are displayed as Data Set attributes in the Data Set details page.
  - Are populated in the LB and SM tags in read group headers of the basecalling BAM files.

SMRT Link - Data Management

- To preserve sample traceability, editing a Data Set's Well Sample Name and Bio Sample Name is **no longer** available in Data Management.

SMRT Link - SMRT Analysis

- Demultiplex Barcodes Application

– The Barcoded Sample Name File is a **required** parameter. Only the barcodes and corresponding sample names listed in this file appear in the Demultiplex Barcodes report.
– The **Infer Barcodes Used** parameter is **On** by default and therefore no longer included in the GUI. All completed demultiplexed analyses include a file called **Inferred Barcodes** (in the **Data > File Downloads** section) which identifies the barcodes inferred to be present in the sample.

- Base Modification Analysis Application
  – Two new analysis graphs are available: Quality value scores per-base, and quality scores per-motif.
- Support for the Ultra low-DNA input protocol
  – Added the new **Mark PCR Duplicates** application.
  – Added the new **Trim gDNA Amplification Adapters** application.
- Improvements to Microbial Assembly
  – Added the ability to downsample coverage using the **Downsample Coverage** advanced parameter.
  – Added new pre-assembly metrics to the analysis report.
  – Added new coverage statistics to the Contigs table.
  – Memory requirements were reduced.
- CCS analysis
  – Faster time to results – 15-20%.

Fixed Issues

- Selection of Data Sets using the **Select All** checkbox now works correctly within any existing filters.
- Sorting by date in tables now works correctly, including for entries created on the same day. Sorting no longer treats the date as a text string.
- Successful upload of a Bio Sample Name file in the Run Design editor now displays a confirmation message.
- Invalid characters in the Bio Sample Name File are now stripped out automatically.
- It's no longer possible to create Run Designs that incorrectly use more than two distinct sequencing kits.
- For insert sizes, entering a decimal value no longer causes an error. Integer values must be used.
- For runs created for Sequel instruments, the summary list of reagents and consumables no longer fails to distinguish between SMRT Cells and SMRT Cells LR.
- In the SMRT Link GUI, the Structural Variant Calling application no longer fails when launched with demultiplexed Data Sets.
- SMRT Analysis no longer fails on compute environments using LSF as a job scheduler.

Known Issues

Data Management

- Creating a new Data Set from filtered data sets fails. The workaround is to create a new Data Set from the original, unfiltered Data Sets, then filter the resulting Data Set.