SMRT® Link Release Notes (v10.2)

SMRT Link Server Installation

- SMRT Link server software is supported on:
  - English-language CentOS 7.x, supported until end-of-life 6/30/2024.
  - English-language CentOS 8.x, supported until end-of-life 12/31/2021.
  - Ubuntu 18.04 and 20.04 64-bit Linux® distributions.
  - These supported versions **also** apply to SMRT Link compute nodes.
- **Note**: PacBio advises **against** new installations of CentOS for use with SMRT Link.
- 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 SMRT Link v10.2 features are computationally-intensive and require adherence to the computational and storage requirements listed in the document **SMRT Link Software Installation (v10.2)**.
- 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 v10.2 supports all chemistry versions for **Sequel® II Systems** and chemistry v2.1 and later for the **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_v2
- Sequel_384_barcodes_v1
- Sequel_64_M13barcodes_v1
- HiFiViral_SARS-CoV-2_M13barcodes
- SMRTbell® Barcoded Adapter Plate 3.0 (bc2001-bc2096)

Reference Sequences:

- Sequel DNA Internal Control
- HIV_HXB2
- HLA_11locus_clustering_guide

- lambdaNEB
- SARS-CoV-2 Genome NC_045512.2
- SARS-CoV-2 Guide for PBAA

SMRT Link Database Note

- SMRT Link v10.2 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.

New Features

SMRT Link - SMRT® Analysis

- New **HiFiViral SARS-CoV-2 Analysis** Application supports the **HiFiViral SARS-CoV-2 Kit.** This application analyzes multiplexed viral samples for SARS-CoV-2, and uses HiFi Reads as input. The previous **SARS-CoV-2 Analysis** application is discontinued.
- **Resequencing** Application is discontinued. Use the **Mapping** Application to performs read mapping and generate coverage reports.
- **Iso-Seq®** Application now only accepts HiFi Reads as input.
- **Minor Variants Analysis** Application now only accepts HiFi Reads as input.
- **Microbial Assembly** Application now accepts **either** Continuous Long Reads or HiFi Reads as input.
- **Structural Variant Calling** Application no longer reports CNV variants.
- Added support for importing/exporting analysis settings.
- `pbservice`: New `import-run` command for importing Run Design CSV files.
- `pbmm2`: Updated default parameters to filter with gap-compressed identity instead of BLAST identity (`-c 0 -y 70`).
- `pbsv`: Updated default parameters for call to require three supporting reads to call a variant (`-A 3 -O 3`).
- `pbsv discover`: Added `--hifi` preset to provide parameters optimized for HiFi Reads.

SMRT Link - Sample Setup

- Simplified application list.
- Updated to support the **HiFiViral SARS-CoV-2 Analysis** Application.
- Updated annealing and binding conditions for Whole Genome Sequencing applications.

SMRT Link - Run Design

- Simplified application list.
- Updated to support the **HiFiViral SARS-CoV-2 Analysis** Application.
- Updated run conditions for Whole Genome Sequencing applications.

SMRT Link

- Searching for analyses, Data Sets, Reference/Barcode files was simplified.

Fixed Issues

- SMRT Link installation now includes the recommended 16s Barcode FASTA file `Sequel_96_barcodes_v2.fasta`.
- Fixed an issue with the Iso-Seq `summary.csv` file. For separately clustered samples (or single samples), the per-sample transcript counts now correctly specifies the sample name.

Known Issues

- When sorting a Data Set table in Data Management, the number of records shown is inconsistent, and may change after sorting with different attributes. This has no impact on the underlying data, and is solely an issue with the display of Data Set records.
- When creating new analyses using the **Copy From** mechanism, users are incorrectly given an option to copy from parent analyses, which will produce an error. Users should **only** copy from child analyses, which should work as intended.
- Demultiplexing does not correctly clip HiFi kinetics tags (`fi`, `fp`, `ri`, `rp`). This results in tags that are longer than the read sequence, which impacts analyses that use the HiFi kinetics tags including Base Modification analysis.
- Some SMRT Link workflows that use data chunking fail with demultiplexed CCS BAM files due to incorrect read group identifiers assigned in demultiplexing.