PacBio

SMRT® Link Cloud user guide (v26.2)

Revio® and Vega™ systems

Research use only. Not for use in diagnostic procedures.

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PacBio

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SMRT® Link Cloud user guide (v26.1)

Introduction

This document describes how to use the PacBio® SMRT Link Cloud software.

SMRT Link Cloud features include:

- **Instruments** module for viewing information about connected instruments.
- **Sample Setup** module for calculating library loading concentrations.
- **Runs** module for designing and monitoring sequencing runs and viewing run performance metrics.
- **Data Management** module for viewing datasets and dataset reports.
- **SMRT® Analysis module** for executing select analysis workflows.
- **Instrument settings** administration tools for setting up data transfer schemes and connecting instruments.
- **User management** administration tools.
- **Audit logs** administration tool for viewing and downloading audit logs.

SMRT Link Cloud does not include built-in secondary analysis workflows by default. Select SMRT Analysis workflows can be configured to execute in customer-managed AWS compute environments. Please see the SMRT Analysis section for more details.

Your ecosystem, analysis needs, and compute resources should inform which SMRT Link option is the best fit. To learn more about analysis features, visit https://pacb.com/smrt-link/ or refer to the SMRT Link user guide at https://www.pacb.com/support/software-downloads/.

This document also describes:

- Details about dataset report metrics
- SMRT Link Cloud networking and requirements

Supported instruments:

- Revio® systems with v13.5 instrument software.
- Vega™ systems with v1.2 instrument software.

Sequel®, Sequel II, and Sequel IIe systems are **not supported** and require a separate local installation of SMRT Link v13.1.

Terms and Conditions: https://www.pacb.com/wp-content/uploads/SMRTlink-Cloud-Services-Agreement.pdf

Contact information

For additional technical support, contact PacBio at support@pacb.com or 1-877-920-PACB (7222).

Getting started

To request SMRT Link Cloud, please inform your Field Applications Bioinformatics Support (FABS) scientist. They will submit a workspace request to our Global Technical Support team.

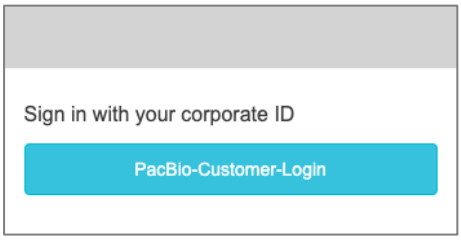
To create a SMRT Link Cloud workspace, you will need to provide the following information.

- **SMRT Link Cloud admin email address and name**
  The admin will receive an email invitation to join your private cloud workspace.
- **SMRT Link Cloud workspace name**
  The workspace name should be up to 32 alphanumeric characters (no spaces) and usually references an account name, e.g., MyPBCloud.

Requests are typically fulfilled within seven business days before installations for new customers and within three to four business days for current customers switching to SMRT Link Cloud from their customer managed server.

Your SMRT Link Cloud admin will be invited to join SMRT Link Cloud via an email from support@pacb.com.

SMRT Link Cloud is accessible at https://smrtlink.pacbcloud.com/. Please **sign in by clicking the PacBio-Customer-Login button.**



Before you begin sequencing, please ensure you have completed the following:

- ☐ Verify storage and networking requirements.
- ☐ Configure data transfer to your storage (transfer scheme).
- ☐ Connect your instrument.
- ☐ Add users.

These steps must be completed to use your instrument. Please reference this guide or your system specific site prep guide for additional details.

After accessing SMRT Link Cloud, the **Instruments module** displays.

- Click the **PacBio logo** at the top left to navigate back to the Instruments page from within the application.
- Select a module name from the **Module** menu (next to the PacBio logo) to access that module.
- Click **Settings** to configure your system or perform administrative functions (Admins **only**).
- Click **User Name > Sign Out** to log out of SMRT Link Cloud.

Settings menu

- **General**
  - **Admin users only**: Use the **Time Zone** control to specify the time zone to use with **all** instruments connected to this instance of SMRT Link.
  - To specify how numbers are formatted, click **Number Formatting** and select **Period** or **Comma** as the decimal separator.
- **Instrument Settings**
  - The **Instrument Management** table displays information about the Revio system(s) connected to this SMRT Link Cloud workspace.
  - **Admin users only:** Connect a new instrument and specify the file transfer scheme to use for moving sequencing data from the instrument to your local or cloud storage.
- **Notification settings**
  - Specify the number of days for when to automatically archive notifications.
  - Specify the type(s) of notifications to include in the red notification count on the main page: **Informational**, **Warning**, **Error,** and **Critical**. (**Note**: **All** notification types still display in the **Notification Center** dialog; only the checked types are reflected in the red notification count.)
- **User management**
  - **Admin users only**: Add/delete SMRT Link users and specify their roles.
- **API Keys**
  - **Admin users only**: Create and manage API keys.
- Audit Logs
  - **Admin users only:** View and filter audit events and export audit reports as PDF files. Note: Only Admins will have Audit Logs as a menu option.
- Set Instrument Login
  - Create a Personal Identification Code (PIC) to use for instrument login. Only required for Vega systems where login mode has been configured.

- Instrument Login Management
  - **Admin users only:** Configure and manage the instrument login feature. Login mode is available for Vega systems only.
- **About**
  - Displays information about SMRT Link Cloud, as well as component versions.

Notifications menu

- Displays SMRT Link system-level notifications. Click a notification to see additional information.

Instrument settings

Specifying a file transfer location

A **file transfer location** is the **cloud or network** location where sequencing data generated by the instrument is stored. The instrument sends data to the designated location after sequencing and post-processing. Once you have defined a new file transfer location, it displays in the File Transfer Location table and becomes available when adding new instruments.

There are five different transfer schemes that you can choose from:

- **ssh (srs):** This method provides transfers to your network storage over an encrypted connection provided by SSH. Your FSE or Tech Support can provide the public key from the instrument, which must then be installed on the storage server by your network/IT administrator to allow transfer.
- **Amazon S3**: Provides secure file transfers between your Revio system and your Amazon S3 cloud storage bucket.
- **Google Cloud Storage:** Provides secure file transfers between your Revio system and your Google Cloud Storage.
- **Microsoft Azure Blob storage:** Provides secure file transfers between your Revio system and your Microsoft Azure Blob storage.
- **S3-compatible storage:** Supports transfer for cloud-based systems that use an S3-compatible API, including Oracle Cloud Object Storage and Cloudflare R2.

Setting up a file transfer location

1. Go to Settings > Instrument Settings.
2. Click + New File Transfer Location.
3. Select the desired Scheme.
4. Fill in the required fields.
5. Name: User-specified text string that displays in the Data directory dialog to identify the transfer scheme.
6. Description: User-specified text string that describes the transfer scheme.

7. Scheme-specific fields (see below).
8. Click Save.
9. Once the transfer scheme is associated with a connected instrument, click Test settings to ensure that the scheme works.

ssh (srs) scheme specific fields

- Host: DNS name or IP address of your storage server. This may be the SMRT Link server or another storage location on the network. The name of this system can be obtained from your system administrator. Example: mp-srs.
- Destination path: File system location that contains all data transferred via srs.
- (Optional) Relative path: Path used to place run data in a specific sub-directory underneath the location specified in Destination path, on a per-instrument basis. A common value for this field is the instrument serial number or name. This field can contain only alphanumerics, "-", "_", and "/". This field allows separation of run data from different instruments, which allows for easier location of particular run data when browsing the file system.
- Username: Name of the service account used for transferring datasets to the remote file server.
- SSH Key: Full path to the SSH private key. The SSH key must be manually installed on your instrument server by your FSE or Tech Support.

Amazon S3 scheme specific fields

- Bucket: Bucket name without the "s3://" prefix. Example: mp-s3
- Region: Region where the bucket is hosted. To find your region run `curl --silent --head https://s3.amazonaws.com/<your-bucket> | grep 'x-amz-bucket-region'`
- (Optional) Path: Path used to place run data in a specific sub- directory within the specified bucket, This field can contain only alphanumerics and "-", "_", and "/" characters. This field allows separation of run data from different instruments, which allows for easier location of particular run data when browsing the file system.
- Access key ID.
- Secret access key.

S3 Bucket configuration

1. Create a private S3 bucket, e.g. mylabname-pacbio-instrument-data in your preferred geographical region.
2. Create an IAM policy pacbio-instrument-file-transfer granting the necessary permissions on the bucket, including the ability to read, write, and delete objects.
3. Create an IAM user with the new policy attached.
4. Create static access credentials for the new IAM user and save them for step 5.
5. Log in to SMRT Link as an admin user, navigate to Instrument Settings, and create a new Amazon S3 transfer scheme with the bucket name and region from step 1, and access credentials from step 4.

Example IAM policy, where $BUCKET is replaced by the actual destination bucket name (e.g. `mylabname-pacbio-instrument-data`).

```
{
  "Version": "2012-10-17",
  "Statement": [
        {
              "Sid": "VisualEditor0",
              "Effect": "Allow",
              "Action": [
                    "s3:PutObject",
                    "s3:GetObject",
                    "s3:GetBucketWebsite",
                    "s3:GetObjectVersionTagging",
                    "s3:ListBucketVersions",
                    "s3:GetObjectAttributes",
                    "s3:GetObjectTagging",
                    "s3:ListBucket",
                    "s3:GetBucketVersioning",
                    "s3:GetObjectVersionAttributes",
                    "s3:GetObjectVersion",
                    "s3:DeleteObject"
              ],
              "Resource": [
                    "arn:aws:s3:::$BUCKET",
                    "arn:aws:s3:::$BUCKET/*"
              ]
        }
  ]
}
```

Google Cloud Storage specific fields

- Bucket: Bucket name with no “gs://” prefix
- Path: /path/to/subfolder in bucket to which to post
- Access key: Create HMAC key in GCP console (see https://cloud.google.com/storage/docs/authentication/managing-hmackeys#console)
- Secret key: Create HMAC key in GCP console (see https://cloud.google.com/storage/docs/authentication/managing-hmackeys#console)

Microsoft Azure Blob Storage specific fields

- Account name: Account name, for example <account-name> from https://<account-name>.blob.core.windows.net
- Container: The container name from https://<account-name>.blob.core.windows.net/<container>
- Path: /path/to/subfolder in container
- Account key: Key associated with account

S3-compatible storage specific fields

Note: Follow S3 Bucket configuration requirements when setting up S3-compatible storage.

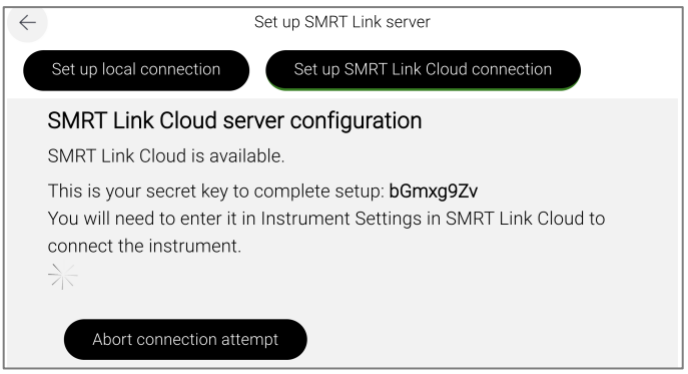
- Endpoint: URL for object storage endpoint, for example https://storage.googleapi.com
- Bucket: Bucket name with no prefix, for example /bucketname.
- Region (Optional): Region name for cloud-based services.
- Path (Optional): Path used to place run data in a specific sub- directory underneath the location specified by your Bucket, on a per-instrument basis. A common value for this field is the instrument serial number or name. This field can contain only alphanumerics, “-“, “_”, and “/”. This field allows separation of run data from different instruments, which allows for easier location of particular run data when browsing the file system.
- Access key: Access key for cloud-based services.
- Secret key: Secret key.

Connecting a Revio system

Note: The following procedure is available **only** for SMRT Link Cloud users whose role is **Admin**. Accessing your secret setup key will require access to the instrument user interface (IUI) of your Revio system.

1. At your Revio system, access the IUI home screen and click Continue.
2. On the next screen, click the hamburger menu in the top right corner and select Instrument Details
3. Under Instrument Details, click Change Server in the bottom left corner.
4. Choose Set up SMRT Link Cloud Connection.

   If SMRT Link Cloud is available, select Connect to SMRT Link Cloud. If the connection is not available, ensure network configuration requirements are met (Appendix A).
5. On the next screen, record the secret key. The key is needed to connect your Revio system to SMRT Link Cloud.

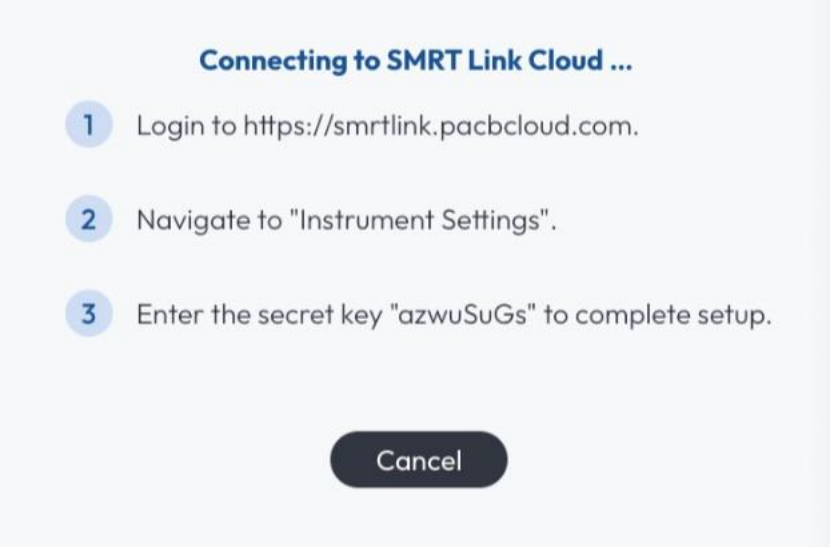


6. **In SMRT Link Cloud,** choose Settings > Instrument Settings.
7. Click + Connect New Instrument.
8. Enter the Secret Key of the new Revio system.
9. Enter the name of the new instrument, then click Continue.
10. Note: The name must contain only alphanumeric characters, spaces, hyphens (-), underscores (_), or apostrophes ('). In addition, the instrument name must be unique for a given SMRT Link installation.
11. Select a File Transfer Location. Note: If a run is currently in progress, the file transfer location will be updated after the run is completed.
12. Click Apply.

Connecting a Vega system

Note: The following procedure is available **only** for SMRT Link Cloud users whose role is **Admin**. Accessing your secret setup key will require access to the instrument user interface (IUI) of your Vega system.

1. At your Vega system, select Menu on your IUI home screen
2. Under Instrument Details, click Change Server and select the “Cloud” button to connect to SMRT Link Cloud.
3. On the next screen, follow the instructions for connecting to SMRT Link Cloud and copy the secret key. The key is needed to connect your Vega system to SMRT Link Cloud.



4. **In SMRT Link Cloud,** choose Settings > Instrument Settings.
5. Click + Connect New Instrument.
6. Enter the Secret Key of the new Vega system.
7. Enter the name of the new instrument, then click Continue.
8. The name must only contain alphanumeric characters, spaces, hyphens (-), underscores (_), or apostrophes ('). In addition, the instrument name must be unique for a given SMRT Link installation.
9. Select a File Transfer Location. Note: If a run is currently in progress, the file transfer location will be updated after the run is completed.
10. Click Apply.

Instruments

Viewing instrument information

Use the SMRT Link **Instruments** module to:

- **View information about Revio systems connected to SMRT Link, as well as any ongoing runs.** If three or more instruments are connected to SMRT Link, click **Expand All** to view information about all instruments. Click **Collapse All** for a more compact display.
- **For each instrument** connected to the instance of SMRT Link Cloud, the Instruments page displays:
  - The instrument name, as defined by the user.
  - The instrument type (Revio and Vega).
  - The instrument's current status (Starting, WarmUp, SelfTest, Ready, Running, ShuttingDown, Problem.) A red alarm bell symbol displays next to the instrument status if any system-critical errors appear during a sequencing run.
  - The time until preload available, which is the time when you can access the work deck and queue up the next run while the current run is sequencing.
  - The individual run name. Click the run name link to see information about the specific run in the Runs module.
- SMRT® Cell status (**Pending**, **Loading**, **Sequencing**, **Complete**.) This also displays the number of SMRT Cells in each status category, as well as the total number of SMRT Cells used in the run.

  Click a SMRT Cell to view sequencing productivity plots. Note: A red box with an exclamation point indicates that the SMRT Cell failed. A crossed out red box indicates that the run was stopped by the user.

  - Run Completion displays the estimated time remaining to complete sequencing run or the time elapsed since the sequencing run completed. Also displays the date (in YYYY-MM-DD format) when the last sequencing run was completed.

Multi-use SMRT Cell Status (Revio-only)

For Revio systems using multi-use SMRT Cells, when multi-use SMRT Cells are present on the instrument, the Instruments page also displays Multi-use SMRT Cell Status information.

- SMRT Cell: The Cell ID.
- Start within: The remaining time to start the next use or Expired if no time remains.
- Remaining Uses for Next Run: The number of uses remaining for the SMRT Cell.
- Information: Status or warning message for the SMRT Cell

Sample Setup

Creating a new calculation

To prepare your samples for sequencing, use the Sample Setup module to calculate the final Product dilution for prepared polymerase-bound SMRTbell® libraries.

To start a calculation:

1. Select Sample Setup from the Module menu.
2. Click +Add Calculation.

The **Loading Calculator** should be used with the **Revio polymerase kit 96, Revio SPRQ and Revio SPRQ–Nx polymerase kit,** and **Vega polymerase kit.** The annealing, binding and cleanup steps should have already been completed. The Loading calculator provides instructions for the final loading dilution for previously-prepared polymerase-bound SMRTbell libraries. Input the number of sample wells being prepared, followed by the input concentration, average insert size, and loading concentration of each sample. The tool will return instructions for making the final dilution for each sample well.

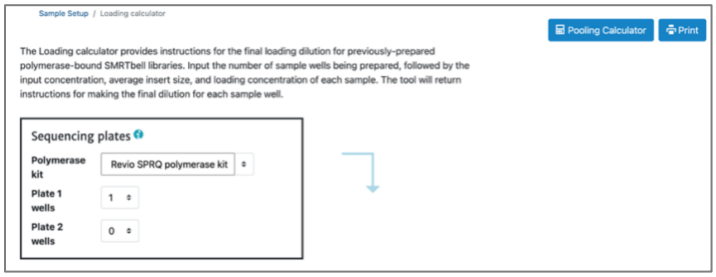
Using the Loading Calculator for Revio polymerase kit 96, Revio SPRQ and SPRQ–Nx polymerase kit, and Vega polymerase kit

1. Specify the **number** (1–4) of sample wells to use per sequencing plate. **Note**: If you are using only **one** sequencing plate, specify 0 for Plate 2.
2. For **each** well ID, enter the sample name, the concentration (ng/µL), the average insert size (in base pairs), the loading concentration (in picomolars), and any comments. Use the tab key to move between fields. **Note**: If using a partially-used Revio sequencing plate, change a Well ID by clicking on it and using the drop-down menu. For example, wells A and B are used and you want to start with C01.

3. Repeat Step 2 for any additional sample wells.
   Note: **All** sample wells must be filled in for the instructions to display.

Note: Samples created by the Loading Calculator cannot be exported or re-imported in Sample Setup.

If using the **Revio polymerase kit 96** setting **or** pooling multiple biological samples together in one well, click **Pooling Calculator**:



1. Enter the number of samples to be multiplexed together (between 2 and 384.)
2. Specify the pooled library target volume (in µL).
3. Specify the unit to use for the output concentration.
4. Specify the pooled library concentration, using the unit specified.
5. For each sample, specify the concentration and the sample volume. (Use the tab key to move between fields.)
6. Repeat Step 6 for the rest of the samples to be multiplexed.

Editing or printing calculations

1. On the **Sample Setup** screen, select one or more calculation names.
2. Click **Edit**. (**Note**: If the samples use different versions of chemistry, a warning message displays.)
3. Edit the sample(s) as necessary.
4. To print the calculation(s), use the **Print** button.

Deleting calculations

1. On the **Sample Setup** screen, select one or more calculation names to delete.
2. Click **Delete**.

Importing and exporting calculations

Loading Calculator calculations cannot be imported or exported. Import/export is a legacy feature for ABC calculations from earlier SMRT Link versions that will be removed in a future release. We don't recommend exporting or importing Sample Setup calculations.

Runs

Use the SMRT Link **Runs** module to:

- View run status and metrics.
- Create, edit, or import run designs. A **run design** specifies:
  - The samples, reagents, and SMRT Cells to include in the sequencing run.
  - The run parameters such as movie time and consensus mode to use for the sample.

Viewing information about runs

Click **Expand All** to expand **all** of the table columns. Click **Collapse All** to collapse the table columns

- Runs can be sorted and searched for:
  - To sort runs, click a **column title**.
  - To search for a run, enter a unique search string into the **Search** field.
  10. To view **basic** information about a run, click the **magnifying glass** next to the run name. This displays instrument and run details including run state and SMRT Cell status.
- Run information displayed in the table includes:
  - The name of the run.
  - The status of the run: **Ready, Running, Stopped, Terminated** or **Complete.**
  - Who created the run, when it was created, when it was started, and when it was completed.
  - Any run comments.
  - The number of samples in the run.
  - The number of SMRT Cells used in the run.
  - The number of files successfully transferred to the network; one per SMRT Cell.
- To **export** run information to a CSV file: Click the checkbox next to the run to export, then click **Export Selected**.
- If the run is **not completed**, this displays summary information about the run design used for the run.
- If the run is completed, this displays information used to monitor progress and perform run QC remotely:

Run details

If a run is completed, view run specific settings and metrics by clicking the Run Name. This will take you to a new page containing the following per run details.

Overview

- **Run Created:** The date and time when the run was created.
- **Run Start**: The date and time when the run was started.
- **Run Complete**: The date and time the run was completed.
- **Created By:** The name of the user who started the run.
- **Instrument Name**: The name of the instrument.
- **Completed Cells:** The number of successfully completed SMRT Cells**.**
- **Failed Cells:** The number of SMRT Cells that did not successfully generate data.
- **Time remaining for Post Processing:** The time needed, after movie acquisition ends, to convert sequencing data to HiFi reads.
- **Transfer Status:** Whether or not the data was successfully transferred from the instrument to the network. Possible values are: **Transferring**, **Failed**, **Complete**, or blank if the SMRT Cell is still acquiring or has not started acquiring yet.
- **Run ID:** An internally-generated ID number identifying the run. (This is different from a UUID, which identifies individual Datasets.)
- **Instrument SN**: The serial number of the instrument.
- **Instrument software**: The version of the instrument software installed on the instrument
- **Transfer Directory:** The path to run sequencing data determined by the configured transfer scheme, and the transfer subdirectory specified during run design.

Consumables

Click the **>** arrow at the top of the **Consumables** table to see, for each sample well used, the consumable type, lot number, expiration date, and other information.

Table fields

- **Well**
  - **Plate well:** The plate number and well ID of an individual well used for this sample.
  - **Well name**: The name of the individual well used for this sample.
  - **Well comment**: User-specified comment for the individual well.
- **Run**
  - **Status**: The current collection status for the SMRT Cell, which can be one of the following: **Complete**, **Collecting**, **Paused**, **Queued**, **Stopped**, **Failed**, **Running**, or **Pending**.
  - **Movie time**: The length of the movie, in hours, associated with this SMRT Cell.
  - **Loading concentration:** The on-plate loading concentration, in picomolarity.
  - **Workflow:** The instrument robotics workflow used for the run.

  - **Loading time**: The time the system took for loading to progress before proceeding to sequencing.

- **SMRT Cells**

  The SMRT Cells section is displayed in the Run Details table only when at least one sample/collection in the run used a multi-use SMRT Cell.
  - **SMRT Cell type:** Indicates whether the SMRT Cell is Single use or Multi-use.
  - **Use:** For multi-use SMRT Cells, displays the use count (for example, 1, 2, or 3). For single-use SMRT Cells, this field displays "-".
  - **SMRT Cell ID:** The identifier for the SMRT Cell.

- **Productivity**
  - **Total bases**: Calculated by multiplying the number of productive (P1) ZMWs by the mean polymerase read length; displayed in Gigabases.
  - **P0**: Empty ZMW; no signal detected (Revio only).
  - **P1**: ZMW with a high quality read detected (Revio only).
  - **P2**: Other, signal detected but no high quality read (Revio only).
  - **Loading level**: Estimated percent loading on a Vega SMRT Cell (Vega only).

- **HiFi reads**: CCS reads whose quality value is equal to or greater than 20.
  - **Reads:** The total number of CCS reads whose quality value is equal to or greater than 20.
  - **Yield:** The total yield (in base pairs) of the CCS reads whose quality value is equal to or greater than 20.
  - **Length (mean):** The mean read length of the CCS reads whose quality value is equal to or greater than 20.
  - **Read quality (median):** The median QV of the CCS reads whose quality value is equal to or greater than 20.
  - **Q30+ bases**: The percentage of bases in CCS reads whose quality value is equal to or greater than 30.

- **Polymerase reads**: Polymerase reads are trimmed to the high-quality region and include bases from adapters, as well as potentially multiple passes around a SMRTbell template.
  - **Pol. read length (mean)**: The mean high-quality read length of all polymerase reads. The value includes bases from adapters as well as multiple passes around a circular template.
  - **Pol. read length (N50)**: The length L such that 50% of all sequenced bases from polymerase reads are contained in reads of length ≥.
  - **Longest subread (mean)**: The mean subread length, considering only the longest subread from each ZMW.
  - **Longest subread (N50):** The length L such that 50% of all bases (considering only the longest subread per ZMW) are in subreads of length ≥ L.
  - **Base rate**: The average base incorporation rate, excluding polymerase pausing events.

- **Control reads**
  - **Reads:** The number of control reads obtained.
  - **Read length (mean):** The mean read length of the control reads.
  - **Concordance (mean)**: The average concordance (agreement) between the control raw reads and the control reference sequence.
  - **Concordance (mode)**: The median concordance (agreement) between the control raw reads and the control reference sequence.

- **Basic preview (estimates)**

  Note: Estimate is displayed at 4 hours after acquisition begins. This preview is not generated for 2-hour or 4-hour movies; short collections receive only the Full preview below.

  - **P1**: An estimate of the percentage of ZMWs rated as P1, meaning that a high-quality read was detected. **Note**: The P1% in the run preview table will be **greater** than the percent active ZMWs in the Sequencing ZMWs plot in the same table. This is because the Sequencing ZMWs plot reports percent active ZMWs at a given time point, while the run preview estimates the percent of ZMWs that will produce a high-quality read over the **entire** acquisition.
  - **HiFi read length, mean**: An estimate of the mean length of the HiFi reads per SMRT Cell for the sequencing run. **Note**: This value is typically an underestimate, as some of the longer molecules require more time to be fully sequenced.

- **Full preview (estimates)**

  Note: Estimate is displayed 1 hour before the end of acquisition

  - **HiFi yield**: An estimate of HiFi yield generated per SMRT Cell for the sequencing run. **Note**: This value is based on a subsample of ZMWs and may be an overestimate or an underestimate.
  - **HiFi read length, mean**: An estimate of the mean length of the HiFi reads per SMRT Cell for the sequencing run.
  - **HiFi read quality, median:** An estimate of the median HiFi read quality per SMRT Cell for the sequencing run.

- **File Transfer**
  - **Status**: Whether or not the data was successfully transferred from the instrument to the network. Possible values are: **Transferring**, **Failed**, **Complete**, or blank if the SMRT Cell is still acquiring or has not started acquiring yet.
  - **Action**: If the Status is **Failed**, the **Retry File Transfer** button becomes available. Click the button to retry the file transfer. (If the file transfer doesn't work after several tries, contact PacBio Technical Support for help.)

Preview metrics

Run previews are **estimates** of run performance at two different time points: 4 hours after sequencing acquisition begins for collections 12 hours or longer and 1 hour before the end of acquisition. These estimates are based on a subsample of ZMWs. This information is **approximate** and intended to guide future runs by providing early information on loading, library fragment size, and representation of barcodes in the pool.

Click the **>** arrow at the top of the **Preview metrics** table to see estimated metrics for **all** SMRT Cells within a given run. **Note**: These preview metrics can also be seen by clicking on a SMRT Cell on the Instruments page.

Barcode counts

Click the > arrow at the top of the **Barcode Counts** table to see **estimated** metrics for **all** barcoded and unbarcoded reads included in the run. Select the desired SMRT Cell and time point under the **Well name** and **Time point** drop-down menus, respectively.

Note: The values displayed may **overestimate** the number of unbarcoded reads. In addition, all estimates may be **less accurate** for barcodes at low frequency (<10%) due to sample size. Any barcodes below a 1% frequency are **not** displayed, and are grouped into the "Other" category.

- **Barcode**: An individual barcode detected in the sample, as well as unbarcoded reads.
- **HiFi reads**: An estimate of the percent of reads with each barcode, as well as the percent of unbarcoded reads.
- **HiFi read length, mean**: An estimate of the average HiFi read length for each barcode or for unbarcoded reads.

Plots

View plots for each SMRT Cell where data was successfully transferred. Clicking on an individual plot displays an expanded view. These plots include:

- **Polymerase Read Length**: Plots the number of reads against the polymerase read length.
- **Control Polymerase RL**: Displays the polymerase read length distribution of the control, if used.
- **Control Concordance**: Maps control reads against the known control reference and reports the concordance.
- **Base Yield Density**: Displays the number of bases sequenced in the collection, according to the length of the read in which they were observed. Values displayed are per unit of read length (i.e. the base yield density) and are averaged over 2000 bp windows to gently smooth the data. Regions of the graph corresponding to bases found in reads longer than the N50 and N95 values are shaded in medium and dark blue, respectively.

- **Read Length Density:** Displays a density plot of reads, hexagonally binned according to their high-quality read length and median subread length. For very large insert libraries, most reads consist of a single subread and will fall along the diagonal. For shorter inserts, subreads will be shorter than the HQ read length, and will appear as horizontal features. This plot is useful for quickly visualizing aspects of library quality, including insert size distributions, reads terminating at adapters, and missing adapters.
- **HiFi Read Length Distribution**: Displays a histogram distribution of HiFi reads (QV ≥20), other CCS reads (three or more passes, but QV<20), and other reads, by read length. **Note: Other reads** means single-pass subreads and anything else for which the software could not determine a consensus sequence.
- **Read Quality Distribution:** Displays a histogram distribution of HiFi reads (QV ≥20) and other CCS reads by read quality.
- **Read Length vs Predicted Accuracy:** Displays a heat map of CCS read lengths and predicted accuracies. The boundary between HiFi reads and other CCS reads is shown as a dashed line at QV 20.
- **5mC Detections**: If 5mC calling in CpG motifs was performed, this plot displays a reverse cumulative distribution of all detected CpG motifs according to their predicted probability of methylation.

Run Design

A **run design** specifies:

- The samples, reagents, and SMRT Cells to include in the sequencing run.
- The run parameters such as movie time and loading to use for the sample.

After a run design is created, it becomes selectable from the instrument touchscreen.

Run designs created in SMRT Link are accessible from **all** sequencing systems linked to the same SMRT Link server.

SMRT Link includes **two** different ways to create a run design:

- Use the SMRT Link **New Run Design** screen to create a new run design.
- Create a CSV file, then import it using the SMRT Link **Runs** module.

Character requirements:

Several fields in Run Design have character requirements. This ensures a consistent user experience and compatibility with downstream SMRT Link and 3rd party tools and workflows. Please reference the Run Design CSV file structure table for each field's requirements. Requirements apply when creating or editing via GUI or CSV import.

Creating a run design for Vega and Revio systems

Note: To create a run design, **either** use the New Run Design screen, **or** import a CSV file. Do **not** mix the two methods.

1. Select **Runs** from the Module menu.
2. Click **+ Create New Run**.
3. Set Run level settings on the left.
4. Ensure that the appropriate instrument type (Revio or Vega) is selected.
5. Enter a **Run Name**. By default, software creates a new run name based on the current date and time; edit the name as needed. Supported characters: alphanumeric, space, hyphen, underscore, colon, period, and apostrophe. Comma and newline are not permitted.
6. Select your sequencing plate from the Plate 1 drop down menu. You can locate the lot number, serial number and expiration dates on the sequencing plate label.

   You can also scan the QR code on the sequencing plate label using a laptop or webcam camera, then clicking the **Scan** button. This fills in the **Lot**, **Serial** and **Expiry** fields.

   On Revio this is the sequencing plate for position 1 on the Revio work deck associated with this new run design.

Note: Revio SPRQ-Nx sequencing plates are compatible with both single-use Revio SMRT Cell tray and multi-use Revio SMRT Cell tray – Nx. The Revio SPRQ sequencing plate is not compatible with the multi-use Revio SMRT Cell tray–Nx

7. (**Optional**) For Revio only, specify **Plate 2** as the sequencing plate for position 2 on the Revio work deck associated with this run design.
8. (**Optional**) Enter **Run Comments** as needed. Supported characters: alphanumeric, space, hyphen, underscore, colon, period, and apostrophe. Comma and newline are not permitted.
9. (**Optional**) Enter a **Transfer Subdirectory**, which specifies a subdirectory within the transfer location. Run files are transferred to

   <TransferRoot>/<SubDirectory>/<RunDirectory> instead of <TransferRoot>/<RunDirectory>.

   11. **For Revio only,** Adaptive Loading should be set to YES for all Applications except PureTarget™ repeat expansion. Adaptive Loading is not included on the Vega system.
10. In **Sample Information,** specify the Application.
11. **For Revio only,** specify the plate and well **position**. Use wells on each plate sequentially and do **not** leave unused wells between samples.
12. Specify the **Well Name** and any Well comments.
13. Specify the **Standard** Library type. **Note**: This can be set to **Kinnex™** or **Adeno-associated Virus** based on the application you select in Step 11.

    Library Type identifies the structure of the molecules to be sequenced, which determines how the instrument performs adapter calling and consensus read generation.

— **Standard** specifies a single sequence for adapter calling. Standard libraries consist of a single DNA insert with the same SMRTbell adapter loop on each end of the molecule.

— **Kinnex** specifies two sequences for adapter calling. Kinnex libraries consist of concatenated smaller inserts with different SMRTbell adapter loops on each end of the molecule. (For a video on using all Kinnex kits with the Runs module, click here.)

— **Adeno-associated Virus** disables adapter correction (that is, it disables splitting molecules with an adapter on only one end) and enables generating a consensus sequence per strand, considering only passes from that strand (by-strand mode). Adeno-associated Virus libraries include a variety of structures, which may have: 1) A SMRTbell adapter loop on only one end or on both ends, and 2) either complementary or unique forward and reverse strand sequences.

14. Specify an **insert size** (500 base pairs minimum). The insert size is the length of the double-stranded nucleic acid fragment in a SMRTbell template, excluding the hairpin adapters. This matches the average insert size for the sample; the size range boundaries are described in the library preparation protocol.

Note: For libraries <5 kb, do not use Revio SPRQ sequencing plate–Nx with multi-use Revio SMRT Cell tray – Nx. Use Revio SPRQ sequencing plate–Nx with single-use Revio SMRT Cell tray.

15. Specify the **Library Concentration** in picomoles.
16. Movie Acquisition Time defaults to the recommendation for the Application selected. Options include 12, 24, and 30 hours for the Revio and 2, 4, 12, or 24 hours for the Vega system.
17. Under **Samples**, specify whether the sample is **indexed**. **Note**: Demultiplexing on-instrument is only supported for symmetric indexes. When demultiplexing using asymmetric barcodes please select No and demultiplex your sample off-instrument using lima.

    SMRT Analysis beta users: Asymmetric barcode sets (for example, Twist Universal Adapters with UDI v2 or Barcoded M13 Primer Plate) are processed off-instrument in SMRT Link. A Demultiplexing Barcodes job will be scheduled to run in SMRT Analysis once sequencing is complete.

    If **No**, enter a **Bio Sample Name**. This is the name of the biological sample contained in the sequencing library, such as HG002. Go to Step 20. Allowed characters: alphanumeric, hyphen, underscore. Character limit: ≤40 characters.

    If **Yes**, proceed. Specify an **Index file** and select **Biosample Names**, either interactively or by downloading a file:

    Interactively:

    - Click **Interactively**, then drag barcodes from the **Available Barcodes** column to the **Included Barcodes** column. (Use the check boxes to select multiple barcodes.)
    - **(Optional)** Click a **Bio Sample** field to edit the Bio Sample Name associated with a barcode. **Note**: Avoid using spaces in Bio Sample Names as they may lead to third-party compatibility issues.
    - **(**Optional) Click Download as a file for later use.
    - Click **Save** to save the edited barcodes/Bio Sample names. You see **Success** on the line below, assuming the file is formatted correctly.

    From a File:

    - Click **From a File**, then click **Download File**.
    - Enter the Bio Sample Name associated with the barcodes in the second column, then save the file. Allowed characters: alphanumeric, hyphen, underscore. Character limit: ≤40 characters.

      If you did not use all barcodes in the Autofilled Barcode Sample file in the sequencing run, delete those rows.

      Note: Open the CSV file in a text editor and check that the columns are separated by **commas**, not semicolons or tabs.

18. Select Data Options.

    - **Include Base Kinetics: No by default.** Setting to YES will specify that CCS analysis output includes kinetics information (used for epigenetic analysis). Note: Adding kinetics information can increase the amount of storage used by the output BAM files by up to 5 times, and is not needed for 5mC/5hmc methylation detection and 6mA for Fiber-seq chromatin accessibility detection.
    - **Consensus mode:** Per molecule by default. For AAV and some custom applications generating CCS reads per strand may be desired to identify differences between the two strands.

Editing or deleting run designs

1. Select **Runs** from the Module menu.
2. Click the name of the run design to edit or delete.

Duplicating run designs

1. Select **Runs** from the Module menu.
2. Click the name of the run design to duplicate.
    - If the run status is **Ready**: Click **Duplicate**.
    - If the run status is **Completed**: Click **View Run Design**, then click **Duplicate**.
3. Edit the Run Name. (The default name is Copy of...).
4. Click **Save**.

Creating run designs by importing a CSV

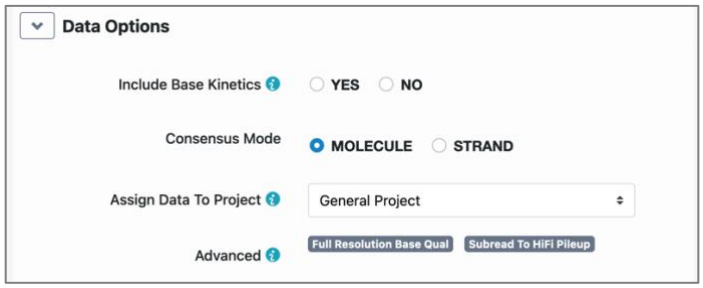
To obtain the sample CSV files

1. Select **Runs** from the Module menu.
2. Click **Import Run**.
3. Click **Download Template**. The ZIP file contains a template for Revio systems and downloads to your local machine

To update and import the CSV file

1. Update the appropriate CSV file as necessary for the run design using the definitions of the run design fields in the tables below.
2. Save the edited CSV file.
3. Import the file into SMRT Link by selecting the Runs module and clicking Import Run.
4. Select the saved CSV file designed for the run and click **Open**, then click **Done**. The file is now imported and available for selection on the instrument.
5. If Full Resolution Base Qual or Subread To HiFi Pileup is TRUE, the

imported run design will display these options under Advanced in Data Options.



The Revio Run Design CSV file format is divided into three main sections:

- [Run Settings] - Settings that apply to the entire run.
- [SMRT Cell Settings] - Settings that apply to a specific collection, plate well, or SMRT Cell.
- [Samples] - Settings that apply to a specific barcoded sample.

Each section begins with a line that starts with the name of the section surrounded by square brackets.

Run Settings section

Each line in this section (after the [Run Settings] line) represents a **single** setting, and includes the setting name followed by a comma separator, and then the setting value.

Setting name	Required	Description
Instrument Type	**Yes**	Must be Revio or Vega.
Run Name	**Yes**	A human-readable name used to refer to the run in SMRT Link interfaces. Must contain only alphanumeric, space, hyphen, underscore, colon, period, and apostrophe characters. Comma and newline are not permitted. **Example**: 20240530_A6_VVnC
Run Comments	No	A human-readable comment attached to run. Must contain only alphanumeric, space, hyphen, underscore, colon, period, and apostrophe characters. Comma and newline characters are not permitted. **Example**: My first Revio run1
Plate 1	**Yes**	Enter or scan a part number or plate identifier that specifies the sequencing plate in workdeck position 1 (See "Plate identifier requirements")
Plate 2	No	Enter or scan a part number or plate identifier that specifies the sequencing plate in workdeck position 2 (See "Plate identifier requirements"). Not applicable to Vega.
Transfer Subdirectory	No	Specifies a subdirectory within the transfer location. Run files are transferred to <TransferRoot>/<TransferSubdirectory>/<RunDirectory> instead of <TransferRoot>/<RunDirectory>. Enter alphanumeric characters, hyphens, underscores, or forward slash **only**.
CSV Version	**Yes**	Must be 1 for SMRT Link Cloud.

SMRT Cell Settings section

This section is represented as a table. As a Revio run may contain between 1 and 8 SMRT Cells, the identifier of the well sample associated with each SMRT Cells used in the run **must** be listed on the first line of the section following [SMRT Cell Settings]. This forms the section table header.

Well sample identifiers are written in the format <plate number>_<plate well name> where:

- <plate number> is either 1 or 2, representing plate 1 and plate 2, respectively.
- <plate well name> is one of the following: A01, B01, C01, D01.

For example, 1_A01 is the identifier for the well sample that is loaded on plate 1 well A01.
Example section start line for a run containing the maximum of 8 SMRT Cells:

[SMRT Cell Settings],1_A01,1_B01,1_C01,1_D01,2_A01,2_B01,2_C01,2_D01

Each subsequent line in this section represents a **single** setting and includes the setting name followed by a comma separator, and then the setting value for the respective well sample as indicated in the section start line.

Setting name	Required	Description
Well Name	**Yes**	Allowed characters: alphanumeric, hyphen, underscore **Example**: A6_3230046_A01_SB_ChemKitv2_8rxnKit
Well Comment	No	Allowed characters: alphanumeric, space, hyphen, underscore, colon, period, and apostrophe characters only. Comma and newline are not permitted.
Library Type	**Yes**	Must be Standard, Kinnex, or Adeno-associated virus. **Library Type** identifies the structure of the molecules to be sequenced, which determines how the instrument performs adapter calling and consensus read generation.
Insert Size (bp)	**Yes**	Enter an integer greater than 500.
Movie Acquisition Time (hours)	**Yes**	Enter 12, 24, or 30 (Revio only). Time is in hours.
Application	No	• Human WGS • Microbial assembly • Ampli-Fi • Other WGS • Iso-Seq method • MAS-Seq single cell • Kinnex single-cell RNA • Kinnex full-length RNA • Adeno-associated virus • Kinnex 16S rRNA • Full-length 16S rRNA sequencing • Shotgun metagenomic profiling or assembly • Hybrid Capture

		• <3kb amplicons • ≥3kb amplicons • PureTarget repeat expansion • PureTarget custom • PureTarget carrier • Other If blank or contains invalid values, default is Other.
Sample is indexed	No	Enter TRUE or FALSE. Default = TRUE.
Bio Sample Name	No	Required **only** if Sample is indexed is FALSE for a given collection. Enter Bio Sample Names in the same row as their associated Barcode Names. Use alphanumeric characters, hyphens, or underscores **only**. Bio Sample Names **cannot** be longer than 40 characters. **Example**: sample1 **Note**: This field is used for collections for non-multiplexed data

Setting name	Required	Description
Indexes	**Yes**	Must be a UUID for an Index Set present in the database. 43f950a9-8bde-3855-6b25-c13368069745 for SMRTbell adapter indexes cf5d7a6e-d95b-3360-cde5-d579f9abf06b for MAS SMRTbell barcoded adapters (v2)
Library Concentration (pM)	**Yes**	Enter a positive integer. Units are in parts per million.
Use Adaptive Loading	**Yes**	Revio only. Enter TRUE or FALSE. **Note**: All collections **must** use the same value.
Include Base Kinetics	**Yes**	Enter TRUE or FALSE. Enter TRUE to specify that CCS analysis output includes kinetics information. **Note**: Adding kinetics information can increase the amount of storage used by the output BAM files by up to **5 times**.
Consensus mode	**Yes**	Enter molecule to generate a consensus sequence per ZMW, considering passes from **both** strands. Enter strand to generate a consensus sequence per strand, considering only passes from that strand (--by-strand option).
Full Resolution Base Qual	No	Enter TRUE or FALSE. Default = FALSE. Enter TRUE to output base quality values (BAM QUAL column) without binning. If TRUE, Full Resolution Base Qual will be shown under Advanced Data Options in the Edit Run Design menu.
Subread To HiFi Pileup	No	Enter TRUE or FALSE. Default = FALSE. Enter TRUE to output three tags – sa, sm, and sx – that summarize the subread-to-HiFi consensus read alignment. If TRUE, Subread To HiFi Pileup will be shown under Advanced Data Options in the Edit Run Design menu. • sa: Number of subread alignments that span each position in the consensus read. Represented as a series of run-length encoded spans sa:B:I:<LENGTH1>,<COVERAGE1>,...,<LENGTHN>,<COVERAGEN> • sm: Number of aligned subread bases that match the consensus base at each position in the consensus read. Output as one value per position: sm:B:C,<MATCH1>,<MATCH2>,...,<MATCHN> • sx: Number of aligned subread bases that differ from the consensus base at each position in the consensus read. Output as one value per position: sm:B:C,<MISMATCH1>,<MISMATCH2>,...,<MISMATCHN>

Sample Settings section

This section is represented as a table. The first line after the [Samples] line is the table header and includes these columns:

Column name	Required	Description
Bio Sample Name	**Yes**	Enter Bio Sample Names in the same row as their associated Barcode Names. Use alphanumeric characters, hyphens, or underscores **only**. Bio Sample Names **cannot** be longer than 40 characters. **Example**: sample1 **Note**: This field is used for collections for barcoded samples in multiplexed data.
Plate well	**Yes**	Enter alphanumeric characters, hyphens, or underscores only. The well sample identifier that a given barcoded sample belongs to. **Example**: 1_A01
Adapter	**Yes**	Enter alphanumeric characters, hyphens, or underscores only. This is the name of the **left** adapter used for a barcoded sample. Example: bc2001.
Adapter2	**Yes**	Enter alphanumeric characters, spaces, hyphens, underscores, colons, or periods only. This is the name of the **right** adapter used for a barcoded sample. It should be the same as Adapter for symmetric indexing. Example: bc2001

Each row following the table header line represents a single barcoded sample in the run. The comma-separated values in each row correspond to the column names described in the table above.

Example Revio run design CSV file

```
[Run Settings]
Instrument Type,Revio
Run Name,Example Revio Run
Plate 1,102118800
Plate 2,102118800
Transfer Subdirectory,Example_Transfer_Subdirectory
CSV Version,1

[SMRT Cell Settings],1_A01,1_B01,2_A01
Well Name,Sample1,Sample2,Sample3
Application,Other,Other,Other
Library Type,Standard,Standard,Standard
Movie Acquisition Time (hours),24,24,24
Insert Size (bp),15000,15000,15000
Assign Data To Project,1,1,1
Library Concentration (pM),200,200,200
Include Base Kinetics,FALSE,FALSE,FALSE
Indexes,43f950a9-8bde-3855-6b25-c13368069745,,
Sample is indexed,TRUE,FALSE,FALSE
Bio Sample Name,,BioSampleB,BioSampleC
Use Adaptive Loading,TRUE,TRUE,TRUE
Consensus Mode,molecule,molecule,molecule

[Samples]
Bio Sample Name,Plate Well,Adapter,Adapter2
BioSample1,1_A01,bc2001,bc2001
BioSample2,1_A01,bc2002,bc2002
```

Example Vega run design CSV file

```
[Run Settings]
Instrument Type,Vega
Run Name,Example Vega Run
Plate 1,102118800
Transfer Subdirectory,Example_Transfer_Subdirectory
CSV Version,1

[SMRT Cell Settings]
Well Name,A01
Application,Human WGS
Library Type,Standard
Movie Acquisition Time (hours),24
Insert Size (bp),15000
Assign Data To Project,1
Library Concentration (pM),200
Include Base Kinetics,FALSE,
Indexes,43f950a9-8bde-3855-6b25-c13368069745,,
Sample is indexed,TRUE
Consensus Mode,molecule

[Samples]
Bio Sample Name,Plate Well,Adapter,Adapter2
BioSample1,1_A01,bc2001,bc2001
BioSample2,1_A01,bc2002,bc2002
```

CSV file general requirements

- Each line in the CSV file represents **one** sample.
- The CSV file may **only** contain ASCII characters. Specifically, it must satisfy the regular expression /^[\x00-\x7F]*$/g

Boolean values

- Valid boolean values for **true** are: true, t, yes, or y.
- Valid boolean values for **false** are: false, f, no, or n.
- Boolean values are **not** case-sensitive.

Plate identifier requirements

Sequencing plates are designed in run designs through a "plate identifier" string. There are two acceptable formats: part number only ("anonymous run"), and full plate identifier ("linked run").

Part number

9-digit part number without dashes (Example: 103496700)

Plate identifier

9-digit part number without dashes (Example: 103496700)
6-digit lot number (Example: 036175)
5-digit serial number (Example: 00347)
8-digit expiration date as YYYYMMDD (Example: 20250321)

The full plate identifier is entered as a concatenation of its components, for example "10349670003617500347202503 21".

Data Management

Use the Data Management module to view Dataset information for:

- **HiFi reads**: Reads generated with CCS analysis whose quality value is equal to or greater than 20.
- **Barcodes**: Predefined, PacBio provided barcode sets.
- **References**: PacBio and user provided reference sets, e.g. GRCh38. Reference dataset features will be available in a future SMRT Link Cloud release.
- **Target Regions:** Predefined, PacBio provided target region BED sets. Target region dataset features will be available in a future SMRT Link Cloud release.

Click **View > Data** and select the type of Dataset to view.

Dataset report contents

The Dataset reports are generated on-instrument. These reports are designed to provide information collection metrics prior to data analysis, and are useful for data QC purposes.

For a detailed description of all dataset reports contents please consult Appendix B.

Importing Dataset reports

Selecting Import Data allows users to import dataset reports in the format of a report.zip.

A report.zip file is typically found in a collection's **statistics** subdirectory. To use this file in SMRT Link, download it locally and upload it using the **Import Dataset Report** button. After upload, the dataset becomes available for SMRT Analysis.

Note: SMRT Analysis is supported only for runs that were designed within the same SMRT Link workspace.

Understanding Barcode Sets

Barcode Types:

- **Symmetric:** Each barcode is paired with itself, specifically barcode pairs use the same barcode ID on both ends, for example bc1001,bc1001.
- **Asymmetric:** Barcode pairs include at least one allowed pair with different barcode IDs on the two ends, for example bc1001_F,bc1001_R.
- **Iso-Seq and Other:** Reserved classifications for preloaded barcode sets that are not used for demultiplexing on-instrument or using SMRT Link Demultiplex Barcodes.

Demultiplexing behavior depends on barcode type:

- Symmetric barcode sets are demultiplexed automatically on-instrument.
- Asymmetric barcode sets are demultiplexed manually by the user after the run completes. If SMRT Link Cloud is configured for SMRT Analysis, users will be able to schedule automatic demultiplexing when creating a run design.

Understanding Projects

- Projects are collections of Data Sets, and can be used to restrict access to Data Sets to a subset of SMRT Link users.
- Projects are intended for organizing and limiting visibility of Data Sets, not as a comprehensive data access control mechanism.
- By default, all Data Sets and data belong to the General Project and are accessible to all users of SMRT Link.
- Any SMRT Link user can create a Project and be the owner. Projects must have an owner, and can have multiple owners.
- Unless a Project is shared with other SMRT Link users, it is only accessible by the owner.
- Only owner(s) can delete a Project; deleting a Project deletes all Data Sets and analyses that are part of the Project.

  Important: Projects do not restrict access to run-level information (such as run metrics, QC plots, or details) available in the Runs module.
  As a result, users will still be able to view run performance data even if they do not have access to the associated Project for datasets created from that run.

  Projects include:

- One or more Data Sets and associated Quality Control information.
- One or more analysis results and the associated Data Sets, including information for all analysis parameters and reference sequence (if used).

  Data Sets and Projects

- Once created, a Data Set always belongs to at least one project; either the General project or another project the user has access to.
- Data Sets can be associated with multiple projects.
- The data represented by a Data Set can be copied into multiple projects using the Data Management report page Copy button. Any changes made to a particular copy of a Data Set affect only that copy, not any other copies in other Projects. If a Data Set is to be used with multiple Projects, PacBio recommends that you make a separate copy for each Project.
- Use the Projects menu (located at the top-right of the Data Management page) to filter the Data Sets displayed; this is based on which Projects the Data Sets are associated with.

SMRT Analysis *beta*

SMRT Analysis enables the execution of select workflows via the GUI. The module is visible to all SMRT Link Cloud users.

Execution of SMRT Analysis workflows currently requires a customer-managed AWS environment. Configuration of SMRT Analysis for SMRT Link Cloud is a limited-access beta feature and is only available for a select number of **Revio** SMRT Link Cloud accounts.

At initial release, supported workflows include:

- Demultiplex Barcodes
- Undo Demultiplexing
- Read Segmentation
- Export FASTQ
- Redo Demultiplexing

Please contact PacBio Support for additional information regarding participation and enablement.

User Management

SMRT Link Cloud supports the user roles **Admin** and **Lab Tech**. Roles define which SMRT Link Cloud modules a user can access. The following table lists the privileges associated with the three user roles:

Note: There can be **multiple** users with the Admin role; but there **must** always be at least **one SMRT Link Cloud** Admin user.

Tasks/privileges	Admin	Lab Tech	Bioinformatician
Add/delete SMRT Link users	Y	N	N
Assign roles to SMRT Link users	Y	N	N
Update SMRT Link software	Y	N	N
Add/update instruments	Y	N	N
Access **Instruments** module	Y	Y	N
Access **Sample Setup** module	Y	Y	N
Access **Runs** module	Y	Y	N
Access **Data Management** module	Y	Y	Y
Access **SMRT Analysis** module	Y	Y	Y

Invite new users

1. Choose **Settings > User Management**.
2. Click Invite **New User**. In a pop-up window you will be prompted to provide
   - First name of user
   - Last name
   - Email address
   - Role
3. Click **Send Invitation.** This user will be sent an email invitation from PacBio to create a password for SMRT Link Cloud.

Edit user details

1. Choose **Settings > User Management**.
2. There are two ways to find users:
   - To display **all** SMRT Link users: Click **Display all Enabled Users**.
   - To find a specific user: Enter a user name, or partial name, and click Search By Name.
3. Click the desired user. If the user status is **Enabled**, the user has access to SMRT Link; **Disabled** means the user **cannot** access SMRT Link.
   - To **add** a SMRT Link user: Click the **Enabled** button, then assign a role.
   - To **disable** a SMRT Link user: Click the **Disabled** button.
4. Click **Save Changes**.

API Keys

Generate new API keys within the SMRT Link Cloud user interface (Settings > API Keys). For more details on the SMRT Link API please reference the SMRT Link API use cases document found at https://www.pacb.com/support/software-downloads/.

- SMRT Link Cloud API endpoints are documented at https://smrtlink.pacbcloud.com/docs/services/.
- API Key names should only include alphanumeric characters, dashes, and underscores.
- Once a key is generated, it is displayed on the screen at creation, but it will not be shown again. If the key is lost, please regenerate the key.

Appendix A – Network and compute requirements

Please reference your systems site prep guide for additional details.

Sequencing data storage

All Revio systems require customer-provided local or cloud storage for sequencing data at system installation. Direct-to-cloud data transfer is supported for major cloud vendors including Amazon S3, Google Cloud Storage, and Microsoft Azure Blob Storage. The amount of storage required for sequencing data is dependent on system utilization.

Storage requirements

– For the **Revio** system, the sequencing data storage required is up to 78 TB/year, assuming approximately 60 GB of HiFi data per SMRT Cell and utilization at 1,300 SMRT Cells per year.
– For the **Vega** system, the sequencing data storage required is up to 6TB/year, assuming approximately 45 GB of HiFi data per SMRT Cell and utilization at 200 SMRT Cells per year.

Revio and Vega SMRT Link Cloud networking

Ports and firewalls

SMRT Link Cloud communicates on port 443 for both GUI and API users. The IT/Network administrator must open port 443/TCP outbound from the instrument.

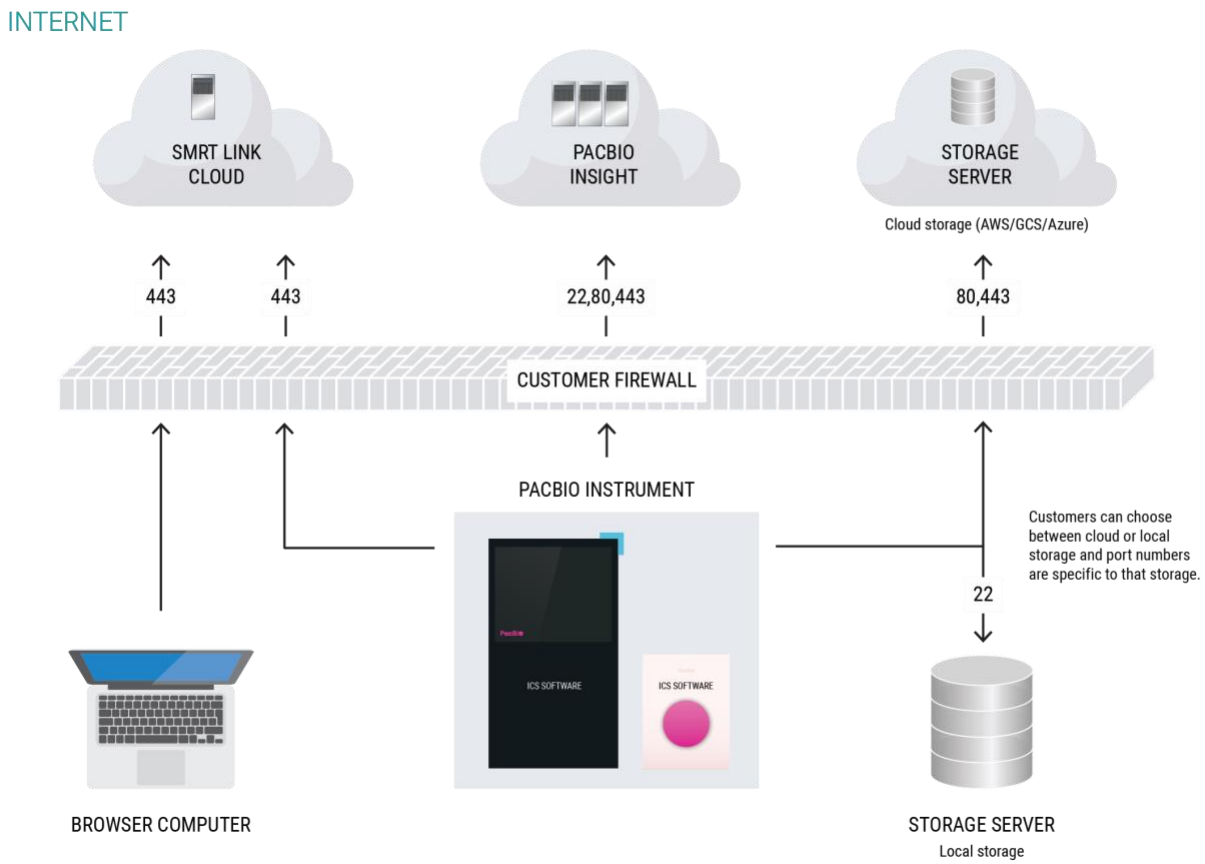
For robustness and scalability, AWS IP addresses change over time. Some firewall appliances using IP caching may block access if they store outdated IP information or can't refresh DNS lookups. Consult your IT administrator for details on your network setup.

If working on a restricted network with IP-filtering firewalls, please contact PacBio Support at support@pacb.com for additional configuration options.

SMRT Link Cloud is not compatible when proxy servers are in-place.

Source	Destination	Port/Protocol	Description
Revio or Vega system	SecureLink Servers	22/tcp, 80/tcp, 443/tcp	Communication for remote support (PacBio Insight)
Revio or Vega system	Storage (cloud or local)	SSH: 22/tcp, Cloud: 80/tcp or 443/tcp	Data transfer from instrument to customer storage
Revio or Vega system	Customer or external NTP servers	123/udp	Used for updating machine time. Defaults to pool.ntp.org
Revio or Vega system	Customer server	53/udp or 53/tcp	Nameservers
Revio or Vega system	SMRT Link Cloud	443/tcp	Communication from instrument to SMRT Link
Customer laptop/ desktop	SMRT Link Cloud	443/tcp	SMRT Link web services and GUI https

Revio and Vega SMRT Link Cloud network diagram



Appendix B – Additional information included in the dataset report

Dataset Overview

Displays the following information about the Dataset:

- The Dataset Name, ID, description, and when it was created and updated.
- The number of reads and their total length in base pairs.
- The names of the run and instrument that generated the data.
- The biological sample name and well sample names of the sample used to generate the data.
- Path to the location where sequencing data is stored. Note: this path is not accessible to SMRT Link Cloud
- Thumbnails of the plots associated with dataset report

Basic preview

- See “Basic preview” from Runs section for a description of report fields.

Full preview

- See “Full preview” from Runs section for a description of report fields.

Barcodes

Barcodes > Summary Metrics

- **Unique Barcodes**: The number of unique barcodes in the sequence data.
- **Barcoded HiFi Reads**: The number of correctly-barcoded reads in the HiFi sequence data.
- **Unbarcoded HiFi Reads**: The number of reads in the HiFi sequence data that do not contain barcodes.
- **Barcoded HiFi Read (%)**: The percentage of reads in the HiFi sequence data that contain barcodes.
- **Barcoded HiFi Yield (Gb)**: The number of bases in HiFi sequence data reads that contain barcodes.
- **Unbarcoded HiFi Yield (Gb)**: The number of bases in HiFi sequence data reads that do not contain barcodes.
- **Barcoded HiFi Yield (%)**: The percentage of bases in HiFi sequence data reads that contain barcodes.
- **Unbarcoded HiFi Yield (%)**: The percentage of bases in HiFi sequence data reads that do not contain barcodes.
- **Mean HiFi Reads per Barcode**: The mean number of HiFi reads per barcode combination.
- **Max. HiFi Reads per Barcode**: The maximum number of HiFi reads per barcode combination.
- **Min. HiFi Reads per Barcode**: The minimum number of HiFi reads per barcode combination.

- **Barcoded HiFi Read Length (mean, kb)**: The mean read length of HiFi reads per barcode combination, in kilobase pairs.
- **Unbarcoded HiFi Read Length (mean, kb)**: The mean read length of HiFi reads not containing barcodes, in kilobase pairs.

Barcodes > Barcode Data

- **Sample Name:** The name of the biological sample associated with the barcode combination.
- **Barcode**: A string containing the pair of barcode indices for which the following metrics apply.
- **Barcode Quality**: The barcode quality (QV) associated with the barcode combination.
- **HiFi Reads**: The number of HiFi reads associated with the barcode combination.
- **HiFi Read Length (mean, bp):** The mean read length of HiFi reads per barcode combination, in base pairs.
- **HiFi Read Quality (mean, QV)**: The mean barcode quality (QV) associated with the barcode combination.
- **HiFi Yield (bp):** The number of bases in HiFi sequence data reads that contain barcodes, in base pairs.
- **Polymerase Read Length (mean, bp)**: The mean read length of polymerase reads associated with the barcode combination, in base pairs.
- **Polymerase Yield (bp)**: The number of bases in polymerase reads associated with the barcode combination, in base pairs.

Barcodes > Inferred Barcodes

- **Barcode:** The barcode name.
- **Reads %:** The percent of reads out of the first 35,000 that are inferred to be assigned to the barcode combination.
- **Barcode score, mean:** The mean barcode score associated with the reads inferred to be associated with the barcode combination.

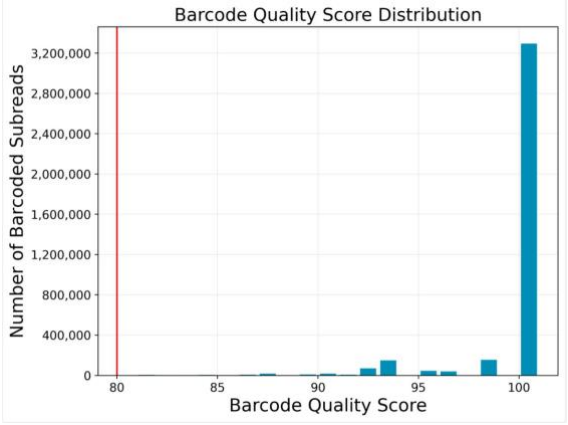
Barcodes > Barcoded Read Statistics

- **Number of Reads Per Barcode**: Line graph displays the number of sorted reads per barcode.
  - **Good performance**: The Number of Reads per Barcode line (blue) should be mostly linear. Note that this depends on the choice of Y-axis scale. The mean Number of Reads per Barcode line (red) should be near the middle of the graph and should not be skewed by samples with too many or too few barcodes.
  - **Questionable performance**: A sharp discontinuity in the blue line, followed by no yield, with the red line way far from the center. Check the output file **Inferred Barcodes**, note the correct barcodes used, and consider reanalyzing the multiplexed samples with the correct Bio Sample names for the barcodes actually used. If you reanalyze the data, ensure that the **Barcode Name** file includes **only** the correct barcodes used.
- **Barcode Frequency Distribution**: Histogram distribution of read counts per barcode.

  - **Good performance**: A uniform distribution, which is most often a fairly tight symmetric normal distribution, with few barcodes in the tails.
  - **Questionable performance**: A large peak at zero. This can indicate use of incorrect barcodes. Check the output file **Inferred Barcodes**, note the correct barcodes used, and consider reanalyzing the multiplexed samples with the correct Bio Sample names for the barcodes actually used. If you reanalyze the data, ensure that the **Barcode Name** file includes **only** the correct barcodes used.
- **Mean Read Length Distribution**: Histogram distribution of the mean polymerase read length for all samples.
  - **Good performance**: The distribution should be normal with a relatively tight range.
  - **Questionable performance**: A spread out distribution, with a mode towards the low end.

Barcodes > Barcode Quality Scores

- **Barcode Quality Score Distribution:** Histogram distribution of barcode quality scores. The scores range from 0–100, with 100 being a perfect match. Any significant modes or accumulation of scores <60 suggests issues with some of the barcode analyses. The red line is set at 80 – the minimum default barcode score.
  - **Good performance**: HiFi demultiplexing runs should have >90% of reads with barcode quality score ≥95



  - **Questionable performance**: A bimodal distribution with a large second peak usually indicates that some barcodes that were sequenced were **not** included in the barcode scoring set.

Barcodes > Barcoded Read Binned Histograms

- **Read Length Distribution By Barcode**: Histogram distribution of the polymerase read length by barcode. Each column of rectangles is similar to a read length histogram rotated vertically, seen from the top. Each sample should have similar polymerase read length distribution. Non-smooth changes in the pattern looking from left to right might indicate suboptimal performance.
- **Barcode Quality Distribution By Barcode**: Histogram distribution of the per- barcode version of the **Read Length Distribution by Barcode** histogram. The histogram should contain a single cluster of hot spots in each column. All barcodes should also have similar profiles; significant differences in the pattern moving from left to right might indicate suboptimal performance.
  - **Good performance**: All columns show a single cluster of hot spots.

- **Questionable performance**: A bimodal distribution would indicate missing barcodes in the scoring set.

CCS Analysis

CCS Analysis Report > Summary Metrics

Note: CCS reads with quality value equal to or greater than 20 are called **HiFi reads**.

- **HiFi Reads**: The total number of CCS reads whose quality value is equal to or greater than 20.
- **HiFi Yield (bp)**: The total yield (in base pairs) of the CCS reads whose quality value is equal to or greater than 20.
- **HiFi Read Length (mean, bp)**: The mean read length of the CCS reads whose quality value is equal to or greater than 20.
- **HiFi Read Length (median, bp)**: The median read length of the CCS reads whose quality value is equal to or greater than 20.
- **HiFi Read Length N50 (bp)**: 50% of all CCS reads whose quality value is equal to or greater than 20 are longer than this value.
- **HiFi Read Quality (median)**: The median number of CCS reads whose quality value is equal to or greater than 20.
- **Base Quality ≥Q30 (%)**: The percentage of CCS reads whose quality value is equal to or greater than 30.
- **HiFi Number of Passes (mean):** The mean number of passes used to generate CCS reads whose quality value is equal to or greater than 20.

CCS Analysis Report > HiFi Read Length Summary

- **Read Length (kb):** The HiFi read length, ranging from ≥0 to ≥40,000 base pairs.
- **Reads:** The number of HiFi reads with the specified read length.
- **Reads (%)**: The percentage of HiFi reads with the specified read length.
- **Yield (Gb):** The number of base pairs in the HiFi reads with the specified read length.
- **Yield (%):** The percentage of base pairs in the HiFi reads with the specified read length.

CCS Analysis Report > HiFi Read Quality Summary

- **Read Quality (Phred):** Phred-scale quality values, ranging from QV ≥20 to QV ≥50.
- **Reads:** The number of HiFi reads with the specified read quality.
- **Reads (%)**: The percentage of HiFi reads with the specified read quality.
- **Yield (Gb):** The number of base pairs in the HiFi reads with the specified read quality.
- **Yield (%):** The percentage of base pairs in the HiFi reads with the specified read quality.

CCS Analysis Report > Read Length Distribution

- **HiFi Read Length Distribution**: Histogram distribution of HiFi reads by read length.
- **Yield by HiFi Read Length**: Histogram distribution of the cumulative yields of CCS reads by read length.
- **Read Length Distribution**: Histogram distribution of all reads by read length.

CCS Analysis Report > Number of Passes

- Histogram of the number of complete subreads in CCS reads, broken down by number of reads.

CCS Analysis Report > Read Quality Distribution

- Histogram distribution of the CCS reads by the Phred-scale read quality.

CCS Analysis Report > Predicted Accuracy vs. Read Length

- Heat map of CCS read lengths and predicted accuracies.

Methylation

HiFi methylation callers estimate the likelihood of a modification at a specific motif. All models have some false positives and negatives, so consider the known modifications in the sample to avoid mistaking false positives for real modifications.

5mC/5hmc/6mA modifications:

- Reports the modification context and the cumulative percentage of modified sites in the sample mapped against the predicted probability of methylation.
- Plots the percentage of CpG or A sites in the sample versus the predicted probability of methylation.
- Plots the distribution of modification percentages per read
- For 6mA, the model only reports 6mAs where probability of modification is >= 0.98 to reduce false positives.
- For 5hmc, the model only reports 5hmc sites where the probability of modification is >= 0.50.

Note: On-instrument 6mA calling is intended to support Fiber-Seq applications and is not intended for use as a general 6mA caller for the microbial genome analysis application.

Raw Data Report

- **Polymerase Read Bases**: The total number of polymerase read bases in the Dataset.
- **Polymerase Reads**: The total number of polymerase reads in the Dataset.
- **Polymerase Read Length (mean)**: The mean read length of all polymerase reads in the Dataset.
- **Polymerase Read N50**: The read length at which 50% of all the bases in the Dataset are in polymerase reads longer than, or equal to, this value.
- **Subread Length (mean)**: The mean read length of all subreads in the Dataset.
- **Subread N50**: The length at which 50% of all the subreads in the Dataset are longer than, or equal to, this value.
- **Insert Length (mean)**: The mean length of all the inserts in the Dataset.
- **Insert N50:** The length at which 50% of all the inserts in the Dataset are longer than, or equal to, this value.

Appendix C – Audit Logs

Audit logs capture system and user actions performed in SMRT Link and on connected instruments, including who performed the action, what action was performed, where it occurred, and when it occurred.

Audit events are recorded starting from the time SMRT Link v26.1 installation or upgrade; events prior to installation/upgrade are not included.

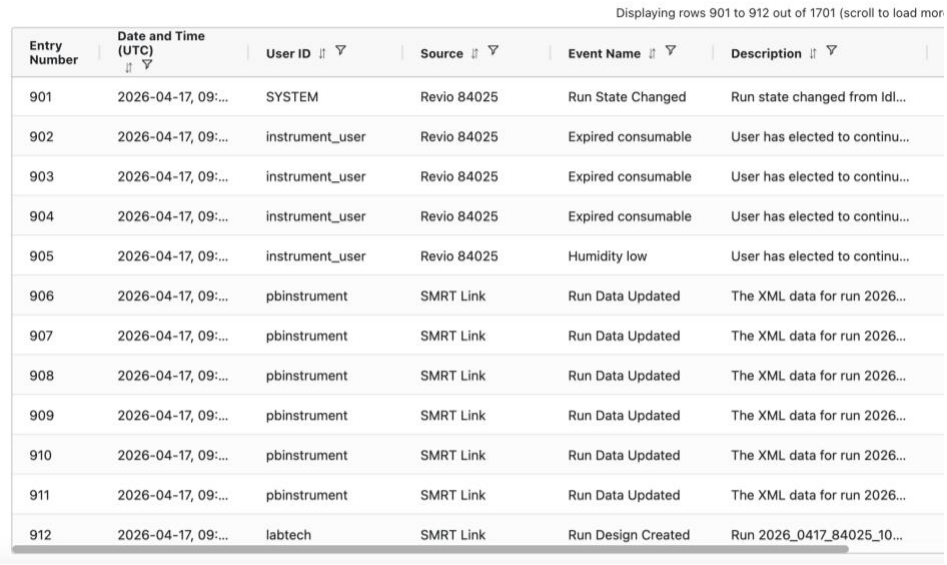
Accessing audit logs

1. Log into SMRT Link with an Admin account.
2. Click Settings.
3. Select Audit Logs.

- **Audit log table**
  The Audit Logs page displays a table of recorded activity across all connected instruments. Each entry includes: Entry Number, Date and Time (UTC), User ID, Source, Event Name, Description, and Software Version

  Audit log entries include both system-generated and user-performed actions.

  User ID values may include:
  - SYSTEM: Actions performed by instrument control software (for example, instrument start, run state changes, or data transfer events).
  - Instrument user: Actions performed on the instrument (for example, starting runs or accepting warnings).
  - SMRT Link user: Actions performed in SMRT Link (for example, creating run designs or submitting analysis jobs).



Displaying rows 901 to 912 out of 1701 (scroll to load mor

Entry Number	Date and Time (UTC)	User ID	Source	Event Name	Description
901	2026-04-17, 09:...	SYSTEM	Revio 84025	Run State Changed	Run state changed from Idl...
902	2026-04-17, 09:...	instrument_user	Revio 84025	Expired consumable	User has elected to continu...
903	2026-04-17, 09:...	instrument_user	Revio 84025	Expired consumable	User has elected to continu...
904	2026-04-17, 09:...	instrument_user	Revio 84025	Expired consumable	User has elected to continu...
905	2026-04-17, 09:...	instrument_user	Revio 84025	Humidity low	User has elected to continu...
906	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
907	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
908	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
909	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
910	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
911	2026-04-17, 09:...	pbinstrument	SMRT Link	Run Data Updated	The XML data for run 2026...
912	2026-04-17, 09:...	labtech	SMRT Link	Run Design Created	Run 2026_0417_84025_10...

- **Audit log filtering**
  - The audit table can be filtered using column-specific filters.
  - Filtering allows users to refine results by user, event type, source, or time range.

- **Downloading audit reports**
  Audit logs can be exported as a PDF report. To download a report:
  1. Click Download on the Audit Logs page.
  2. Wait for the report to be generated (this may take several minutes).

  The exported PDF reflects the filters applied to the audit log table at the time of export.

- **Audit trail tamper detection**
  Every audit trail entry is hash-chained: each new entry's hash incorporates the previous entry's hash, so any retroactive edit or deletion of a past entry breaks the chain from that point forward. This happens automatically for every audit event; there is no separate step to turn it on.
  SMRT Link checks this chain automatically once per night. The check has three possible outcomes:
  - **No tampering detected:** nothing is surfaced to admins; this is the expected nightly outcome.
  - **Tampering detected:** SMRT Link raises a system notification at ERROR severity in the Notification Center.
  - **Detection not possible** (for example, if the chain data itself is unreadable): SMRT Link raises a notification at WARNING severity, with an explanation appended to the message.

- **Tampering notification**
  - Notifications referencing audit tampering in the Notification Center.
  - Selecting Click Reset Audit Trail on the Audit Logs page archives the current audit log table and begins a new one, so normal audit logging can resume with a clean chain.
  - The tamper-detection check currently runs on a nightly schedule only; there is no on-demand or manual trigger in the SMRT Link UI.

Appendix D – Instrument Login Management and PIC (Vega-only)

SMRT Link Cloud v26.2 introduces an on-instrument login mode for Vega systems, to support 21 CFR Part 11 compliance. Login mode is for Vega-only; it is not available on Revio. When login mode is enabled for an instrument, users must authenticate at the instrument touchscreen with a username and PIC (Personal Identification Code) before performing on-instrument actions. Login and logout events are recorded in the instrument's audit trail (see “Appendix C – Audit Logs”).

Setting and managing your PIC

Every SMRT Link user (Admin or Lab Tech) who will log into a Vega instrument with login mode enabled must set their own PIC. This is self-service — administrators cannot see or retrieve another user's PIC.

Setting your PIC for the first time

1. Log in to SMRT Link.

2. From the Settings menu, select Set Instrument Login. (Visible to Admins and Lab Techs; Bioinformaticians do not see this menu item.)

3. If you have not yet set a PIC, enter a code in the PIC field and re-enter it in the Confirm PIC field. The two fields must match, and the code must meet your organization's minimum length requirement (set by your administrator; see “Configuring login mode (Admin)” below). SMRT Link validates this before you can save.

4. Click Save PIC.

Note: Your PIC is stored securely and cannot be retrieved or displayed by SMRT Link or by an administrator once set. If you forget it, you can select Enter a New PIC (see “Changing your PIC”) or an administrator must issue a Force PIC Reset (see “Users tab” below) so you can set a new one.

Changing your PIC

Return to Settings > Set Instrument Login at any time to set a new PIC, following the same steps above. Doing so immediately replaces your previous PIC.

PIC expiration

Your organization's administrator configures a PIC lifetime (in days). The Set Instrument Login page displays your PIC expiration date.

Once your PIC expires, you will need to set a new one, or contact your administrator if you're locked out.

Lockout after failed attempts

If you enter an incorrect PIC at the instrument too many times, your account is locked out for that instrument. The number of allowed attempts, and the time window they are counted over, are configured by your administrator. While locked out, you cannot log in at the instrument until an administrator unlocks your account; self-service unlock is not available, since unlocking requires a documented reason for compliance purposes.

Configuring login mode (Admin)

Note: The following procedures are available only for SMRT Link Cloud users whose role is Admin.

Administrators manage login mode from Settings > Instrument Login Management. This page has three tabs: Users, Configuration, and Instruments.

Users tab

Displays a table of all users who have a PIC-based instrument login configured, including their status (active, expired, or locked out), username, instrument user name, PIC expiration date, and failed attempt count. Each user row has three available actions:

- Unlock: Clears a lockout so the user can attempt to log in again. Requires the administrator to enter a reason, which is recorded in the audit trail.
- Force PIC Reset: Invalidates the user's current PIC and requires them to set a new one the next time they visit Set Instrument Login. Use this when a user has forgotten their PIC or a reset is required for compliance/rotation reasons.
- Remove: Deletes the user's instrument login record.

Configuration tab

Settings apply to all Vega instruments with login mode enabled (each has an inline tooltip in the UI explaining its effect):

- PIC Minimum length (characters) — the shortest PIC users are permitted to set. Only alphanumeric characters are permitted.
- PIC Expiration (days) — how long a PIC remains valid before it must be changed.
- Max failed login attempts — how many consecutive incorrect PIC entries trigger a lockout.
- Observation window (minutes) — the time window over which failed attempts are counted toward the lockout threshold.
- Instrument session timeout (minutes) — how long an authenticated instrument session remains active before automatically logging the user out.

Important: Changing the Minimum PIC length value immediately expires every user's current PIC — all users will need to set a new PIC that meets the new requirement before they can log in again. This is the only one of the five settings with this effect.

Instruments tab

Lists all connected instruments with a Login Mode Enabled toggle.

This toggle only appears for Vega instruments; it does not appear for Revio. Login mode is not supported on Revio.

Toggling this on requires users to enter their PIC at that instrument going forward. The Login Mode toggle is managed here, not from the instrument connection dialog described in “Connecting a Revio system” or “Connecting a Vega system.”