PacBio

SMRT® Link Cloud user guide (v25.1)

Research use only. Not for use in diagnostic procedures.

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PacBio

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SMRT® Link Cloud user guide (v25.1)

Introduction

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

SMRT Link Cloud features include:

- **Instruments** module for view 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.
- **Instrument settings** administration tools for setting up data transfer schemes and connecting instruments.
- **User management** administration tools.

SMRT Link Cloud does not include analysis or API access. 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

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.

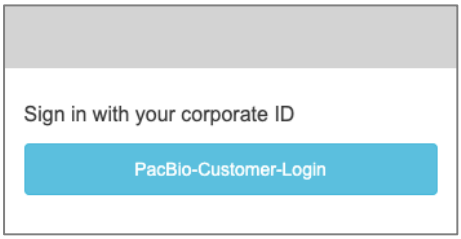
To create a SMRT Link Cloud workspace, you will need to provide the following information.

- **SMRT Link Cloud admin email address and name**
  The admin will receive an email invitation to join their 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.

The 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.

Using SMRT® Link Cloud

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 Revio® system or perform administrative functions (Admins **only**).
- Click **User Name > Sign Out** to log out of SMRT Link Cloud.

Settings menu commands

- **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 types(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.
- **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.
  - To clear **all** notifications, click **Mark All As Read**. You can also select individual notifications and mark them as **Read** or **Unread**.
  - To **save** the notification log as a text file, click **View Notification Log**.

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.

Note: While setting up transfer schemes, using the rsync daemon will continue to be available for current Revio customers currently using this method. This transfer scheme is deprecated and will not be supported in future software, as it does not provide in-transit data encryption.

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 "/". 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.

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

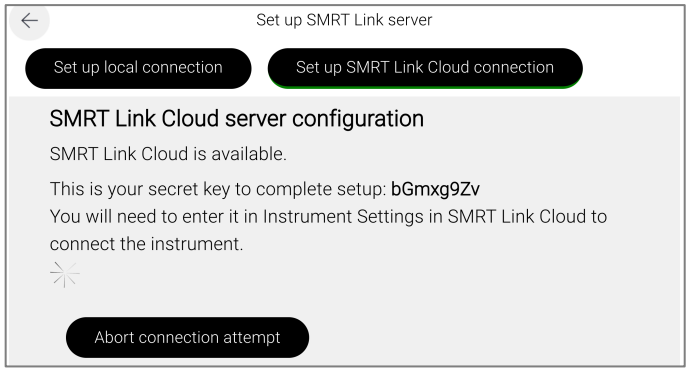
S3-compatible storage specific fields

- 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 Revio systems

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



7. **In SMRT Link Cloud,** choose Settings > Instrument Settings.
8. Click + Connect New Instrument.
9. Enter the Secret Key of the new Revio system.
10. Enter the name of the new instrument, then click Continue.
11. 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.
12. 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.
13. 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).
  - 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 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.

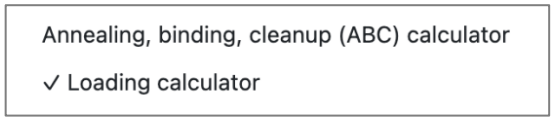
Sample Setup

Creating a new calculation

To prepare your samples for sequencing, use the Sample Setup module to calculate the final loading dilution for prepared polymerase-bound SMRTbell® libraries. Sample Setup for annealing, polymerase binding, and cleanup (ABC) can only be used for Revio (non-SPRQ™) and Vega™ polymerase kits. You can print the instructions for use in the lab.

To start a calculation:

1. Select Sample Setup from the Module menu.
2. Click +Add Calculation and select one of two choices from the drop-down menu. After you select a choice from the drop-down menu, it becomes the default value for adding new calculations. You can also export the calculated values to a CSV file for laboratory automation.



- **Annealing, binding, and cleanup (ABC) calculator:** This setting should be selected for ABC and can only be used for Revio non-SPRQ/Vega polymerase kits**.** Process multiple samples with similar library properties (such as mean insert size and DNA concentration) in parallel. (This was called **Revio polymerase kit** mode in the previous releases). This setting calculates the amount of sample required to load a specified number of cells; it outputs protocols for primer annealing, polymerase binding and cleanup. The entire volume of the prepared sample is loaded.
- **Loading calculator:** This setting should be used with the **Revio polymerase kit 96, Revio SPRQ 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 Annealing, binding, and cleanup (ABC) calculator for the Revio polymerase kit

1. Enter the sample name.
2. Select a sequencing application for the sample. The following fields are auto-**populated** and display in green:
    - Library type
    - Polymerase binding kit
3. Enter the **number of samples** for this calculation. Samples should be substantially equivalent to each other; all should have insert sizes and concentrations within +/- 15% of the specified values.
4. Enter the **number of SMRT Cells** to bind per sample.
5. Enter the available **volume per sample**, in µL. When preparing multiple samples, this should be the **minimum** volume available for any sample.
6. Specify an **insert size,** in base pairs. The insert size is the length of the double-stranded nucleic acid fragment in a SMRTbell template, excluding the hairpin adapters. This matches the mean insert size for the sample; the size range boundaries are described in the library preparation protocol. Enter the mean insert size of the sample(s).
7. Enter the sample **concentration(s)**, in ng/µL. Note that the acceptable range of input concentrations depends on insert size:
8. If necessary, edit the **Cleanup anticipated yield**. Adjust this percentage based on previous experience. (Cleanup removes excess primers/polymerase from bound complexes, which results in higher quality data.)
9. Specify the concentration on plate, in pM.
10. Specify the **Minimum pipetting volume**, in µL. This allows you to set a lower limit on pipetting volumes to use in certain protocol steps, such as sample annealing and binding. We recommend setting this to 1 µL, though in some cases (for example if sample availability is very limited), it may be appropriate to set a value below 1 µL. Some protocol steps include fixed values of 1 µL that will **not** be affected by this setting.
11. **(Optional**) Enter a comment, such as a batch identifier for a LIMS, or information about the sample.
12. Optionally, do one of the following:

— Click **Copy** to start a **new** sample group using the information entered. Then, edit specific fields for each sample group.

— Click **Remove** to delete the current calculation.

- Click **Lock** to lock the calculation. This is **required** before samples can be imported into the Runs module, and also sends a finalized version of the instructions to the server for use in Data Set reports. **Note**: You can lock calculations, but you **cannot** import sample calculations into Revio Run Designs.

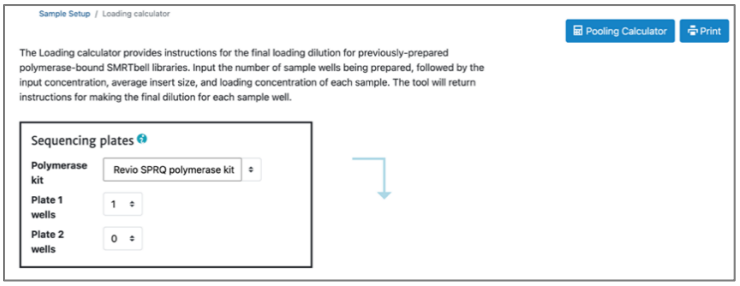
13. After locking, no further changes can be made to a calculation. Click **View** to see the locked Sample Setup instructions. Locking ensures that calculations are always synchronized with their run time state if a report is generated at a later date.
14. **Lock** is **only** available If there are one or more samples visible **and** field value have been entered.

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

Use this setting to calculate the final sample dilution for the Revio sequencing plate when you are starting with polymerase-bound SMRTbell libraries prepared using liquid handler automation or libraries that were previously prepared manually. The setting produces instructions for making the final dilution for each sample well.

1. Specify the **number** (1–4) of sample wells to use per Revio sequencing plate. **Note**: If you are using only **one** Revio 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.

If using the **Revio polymerase kit 96** setting **and** 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.
7. To **print** the pooling calculation, click **Print**.
8. To **export** the pooling calculations as a CSV file, click **Export**.

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

Sample Setup supports importing and exporting calculations in CSV format.

To **import** a new calculation, first find (or create) a calculation **similar** to that you wish to import, then export it in CSV format. You can then customize the exported CSV file as needed, then **import** the modified CSV file.

Note: The content of the CSV file generated using the **Export** button in the Sample Setup home screen is **different** from the content of the CSV file generated using the **Annealing, binding, and cleanup (ABC)** setting's **Download CSV** button used for lab automation.

1. Access SMRT Link using the Chrome web browser.
2. Select **Sample Setup** from the Module menu.

3. Select an existing calculation.
4. Click **Export**, then click **Download**.
5. Edit the exported calculation in Excel (changing sample names, concentrations, and so on), then save it under a new name.
6. In Sample Setup, click **Import**.
7. Click **Browse**, then select the CSV file you previously modified in Step 5 and click **Open**. If everything is correct, click **Continue**. The imported calculation displays.

Note:

- You can select **multiple** calculations to export to the same CSV file.
- You can also **import** multiple calculations by adding rows to the CSV file.

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

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.
  - 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 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 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 Data Sets.)
- **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.
- **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.
  - **P1**: ZMW with a high quality read detected.
  - **P2**: Other, signal detected but no high quality read.
- **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)**: 50% of all read bases came from polymerase reads longer than this value.
  - **Longest subread (mean)**: The mean subread length, considering only the longest subread from each ZMW.
  - **Longest subread (N50):** 50% of all read bases came from subreads longer than this value when considering only the longest subread from each ZMW.
  - **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 begin

  - **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 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 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.)

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Preview metrics

Run previews are **estimates** of run performance at two different time points: 4 hours after sequencing acquisition begins 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.

Creating 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 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.

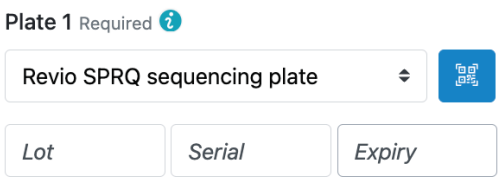
SMRT Link includes **two** different ways to create a run design:

- 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.

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 **Revio** is selected as the instrument type.
5. Enter a **Run Name**. (The software creates a new run name based on the current date and time; edit the name as needed.)
6. Select a sequencing plate to specify **Plate 1** as the sequencing plate for position 1 on the Revio work deck associated with this new run design. 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.



7. (**Optional**) 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.
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>.
10. **Use Adaptive Loading should be set to YES** for all Applications except PureTarget repeat expansion.
11. **In Sample Information,** specify the Application.
12. Specify the plate and well **position**. Use wells on each plate sequentially and do **not** leave unused wells between samples.
13. Specify the **Well Name** and any Well comments.
14. Specify the **Standard** Library type. **Note**: This can be set to **Kinnex** or **Adeno-associated Virus** based on the application you select in Step 4.

— **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 end or on both ends, and 2) either complementary or unique forward and reverse strand sequences.

15. 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.
16. Specify the **Library Concentration** in picomoles.
17. Movie Acquisition Time defaults to the recommendation for the Application selected. Options include 12, 24, and 30 hours.

18. Under **Samples**, specify whether the sample is **indexed**. Note: in SMRT Link Cloud only the default SMRTbell adapter indexes and MAS SMRTbell barcoded adapters (v2) are available. For demultiplexing using other barcodes please select No and demultiplex your sample off-instrument using lima.

    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.

    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**. Edit the file and enter the biological sample names associated with the barcodes in the second column, then save the file. Use alphanumeric characters, hyphens, or underscores **only.** The maximum number of characters is 40.
    - **Note**: Open the CSV file in a text editor and check that the columns are separated by **commas**, not semicolons or tabs.

19. Select Data Options.

- **Include Base Kinetics** set 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 methylation detection and 6mA for FiberSeq chromatin accessibility detection.
- **Consensus mode** by default is per molecule from AAV and some custom applications may require generating CCS reads per strand.

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

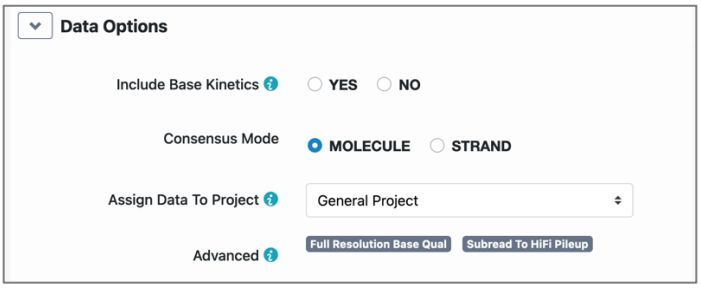
To obtain the sample CSV files

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
Run Name	**Yes**	A human-readable name used to refer to the run in SMRT Link interfaces. Allowed characters: alphanumeric, space, hyphen, underscore, colon, period, apostrophe. **Example**: 20240530_A6_VVnC
Run Comments	No	A human-readable comment attached to run. Allowed characters: alphanumeric, space, hyphen, underscore, colon, period, apostrophe. **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")
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, apostrophe
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. Time is in hours.

Application	No	• Human WGS • Microbial assembly • 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 • HiFi target enrichment • <3kb amplicons • ≥3kb amplicons • PureTarget™ repeat expansion • 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**	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 (used for epigenetics analysis.) **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
```

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)

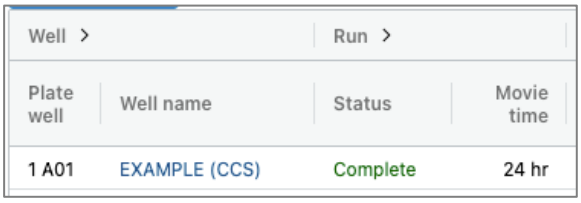
The full plate identifier is entered as a concatenation of its components, for example “10349670003617500347202503 21”)

Dataset reports

Dataset reports contain a per-collection performance metrics.

Viewing reports

1. Select the **Runs** module. From runs click the **Run Name** for the desired run**.** This will redirect you the run specific Run Details page.
2. In the Run Details, table click the **Well name** for any completed run. This will take you the dataset report for the selected library. Contents of the dataset report are described in the next section.



3. A PDF version of the report is available for download by clicking **Data > File Downloads** on the dataset report section menu.

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.

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:

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

Note: There can be **multiple** users with the Admin role; but there **must** always be at least **one SMRT Link Cloud** Admin user. The Bioinformatician role is available but only allows access to the Instruments module and is not recommended for most use cases

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**.

Appendix A – Network and compute requirements

Please reference your Revio system 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

Sequencing data (assuming approximately 60 GBytes of HiFi data per SMRT Cell and utilization at 1,300 SMRT Cells per year) is up to 78TB/year

Revio and SMRT Link Cloud networking

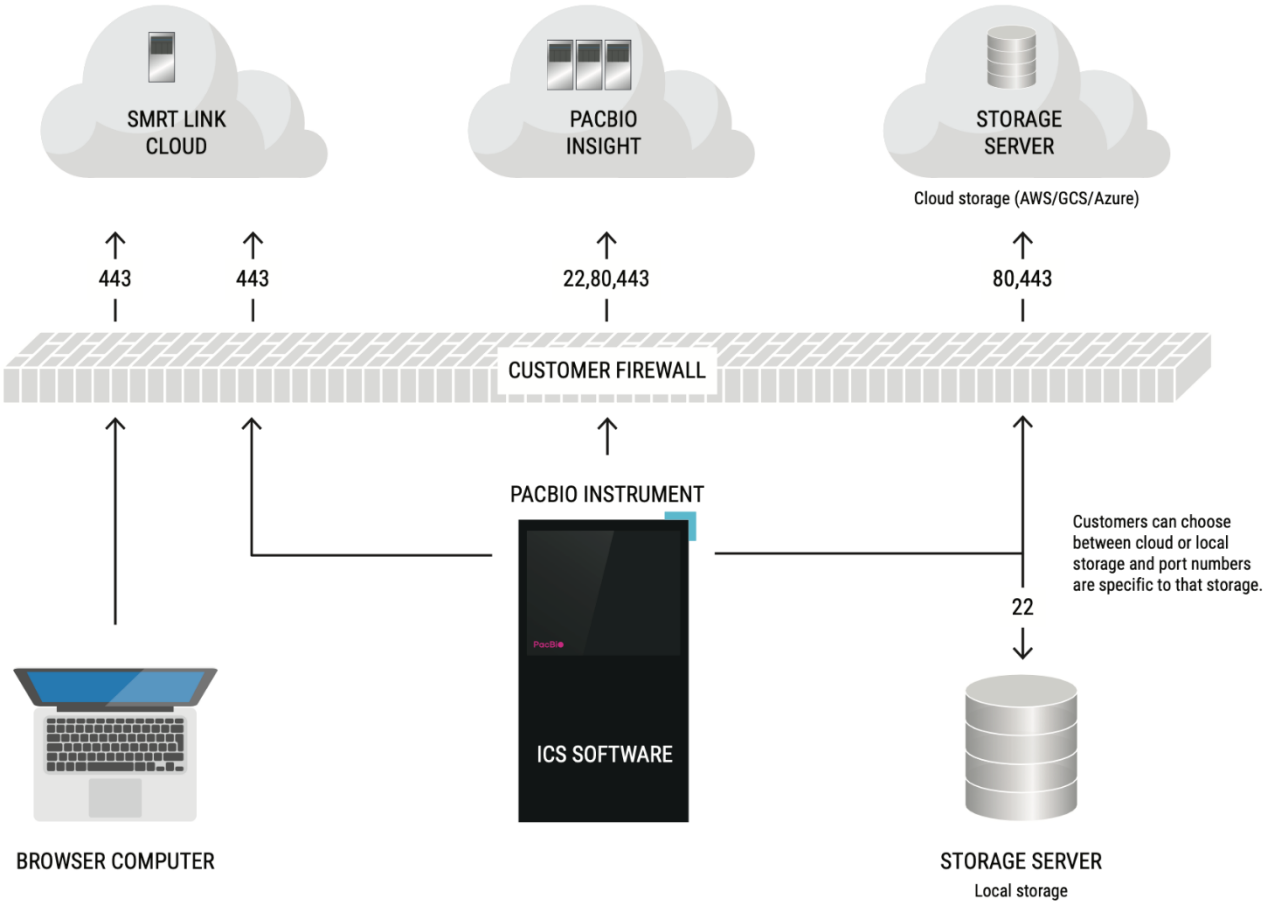
Ports and firewalls

SMRT Link Cloud communicates exclusively on port 443.

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

INTERNET



Appendix B - Additional information included in the dataset report

Dataset Overview

Displays the following information about the Data Set:

- The Data Set 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

4hr Run Preview

- See “Basic preview” from Runs section on page 20 for a description of report fields.

23hr Run Preview

- See “Basic preview” from Runs section on page 20 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

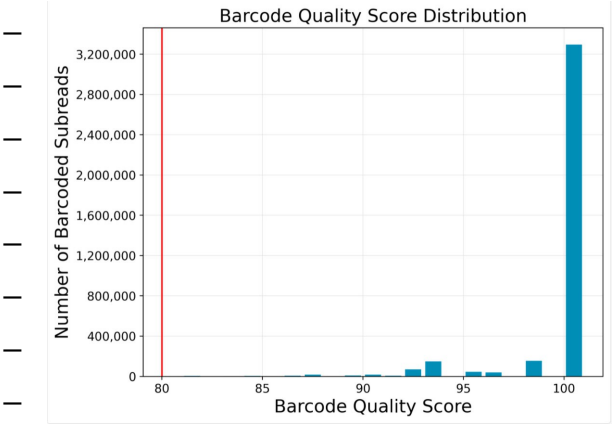
- **Number of Reads Per Barcode**: Line graph displays the number of sorted reads per barcode.

- **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.

Raw Data Report

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