SeqSense Cloud

User Guide

Version 2.2

BIO-RAD

SeqSense Cloud

User Guide

Version 2.2

Bio-Rad Technical Support Department

The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.

Phone: 1-800-424-6723, option 2

Email: Support@bio-rad.com (U.S./Canada Only)

For technical assistance outside the U.S. and Canada, contact your local technical support office or navigate to the Contact us link at www.bio-rad.com.

Legal Notices

Revision History

Document	Date	Description of Change
SeqSense Cloud User Guide Software Version 2.2 DIR No. 10000124696 Ver D	January 2025	Updated to support Omnition pipeline
SeqSense Analysis Solution User Guide Software Version 2.1 DIR No. 10000124696 Ver C	June 2023	Update guide with information on new features and improvements to existing functionality
SeqSense Analysis Solution User Guide Software Version 2.0 DIR No. 10000124696 Ver B	June 2022	Update content for web application
SeqSense Analysis Solution User Guide Software Version .1.0 DIR No. 10000124696 Ver A	2021	New user guide for web application

Table of Contents

Chapter 1 Getting Started

SeqSense Cloud is a purpose-built workflow for analyzing and visualizing the next-generation sequencing (NGS) data. SeqSense uses three bioinformatics pipelines that are optimized for mapping, aligning, counting, and normalizing reads generated from libraries containing both short and long RNA.

Using SeqSense, you can analyze and visualize NGS data from your FASTQ sample files using the following pipelines and kits:

- Omnition analysis pipeline using the ddSEQ Single-Cell 3' RNA-Seq Kit to streamline whole transcriptome analysis (WTA) at the single-cell level by capturing hundreds to thousands of single cells with high sensitivity and recovery.
- SeqSense analysis pipeline with the SEQuoia Complete Stranded RNA Library Prep Kit and the SEQuoia Express Stranded RNA Library Kit.

Note: If you need assistance and are a U.S. or Canada customer, contact Bio-Rad Technical Support using the contact methods cited at the front of this document. For technical assistance outside the U.S. and Canada, contact your local technical support office or navigate to the Contact us link at www.bio-rad.com.

Recommended Browsers

SeqSense supports the latest versions of the Google Chrome and Apple Safari browsers.

Prerequisites

To use SeqSense Cloud, you must

- Purchase from Bio-Rad one of the following prep kits:
  - SEQuoia Express Stranded RNA Library Prep Kit (Catalog No. 12017265)
  - SEQuoia Complete Stranded RNA Library Prep Kit (Catalog No. 17005710)
  - Bio-Rad ddSEQ Single-Cell 3’ RNA-Seq Kit (Catalog No. 17009671)

  Important: When the checkout process concludes, your purchase confirmation includes a Bio-Rad account number. Be sure to save the number, since it is required when you create a SeqSense Cloud user account. The purchase confirmation is separate from the account creation confirmation.
- Create a Bio-Rad website user account

  Note: You can create the account before or after adding items to your shopping cart. If after, you are prompted to create the account before the checkout process begins.
- Create a SeqSense Cloud user account

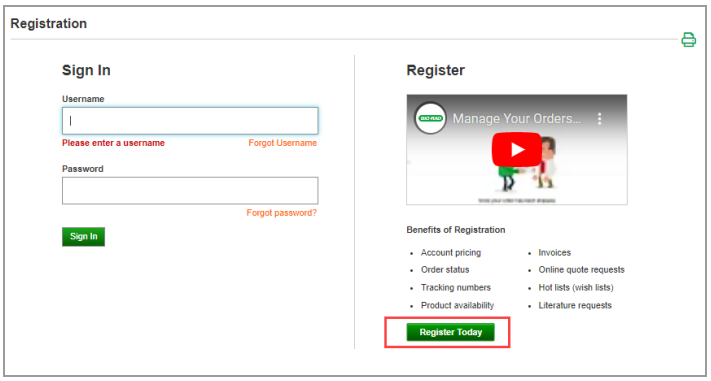
Creating a Bio-Rad Website Account

Complete the steps below to create a Bio-Rad website user account.

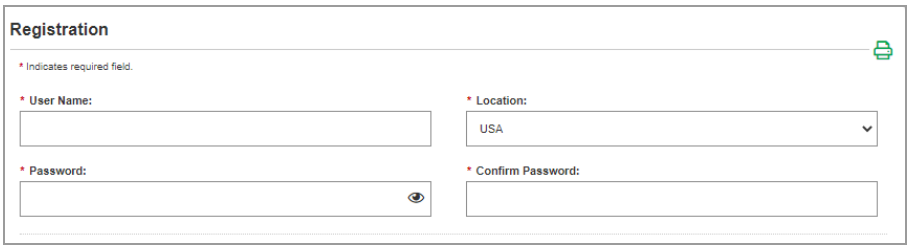
To create a Bio-Rad website user account

1. Enter http://www.bio-rad.com into your browser address field.
2. Click the Log In/Register link at the top of the page.

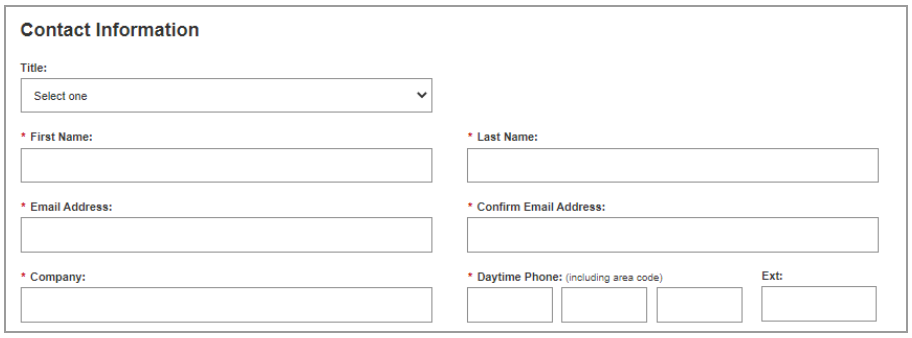
3. Click Register Today.



The registration form opens. Required fields throughout the form are identified by an asterisk (*).

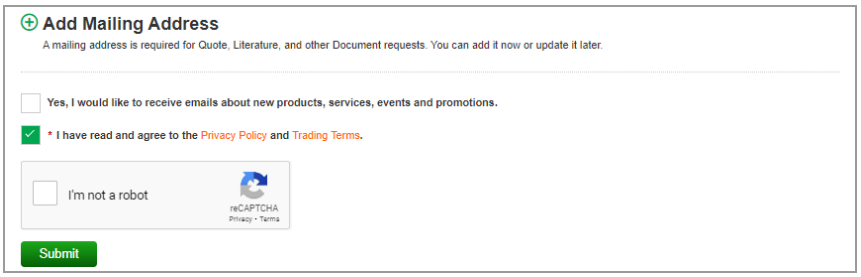


4. Complete the Registration section, as follows:
    a. Enter a unique username. You will enter this username when you create your SeqSense account.
    b. The location defaults to USA. Select a different location where applicable.
    c. Enter and confirm a password. Your password must contain at least one of each of the following:
        - Upper case letter
        - Lower case letter
        - Number
        - Special character
5. Complete the Contact Information section, as follows:
    a. Enter your first and last name. Optionally, select a title.
    b. Enter and confirm your email address. You will enter this email address when you create your SeqSense account.
    c. Enter your company name and daytime phone number.



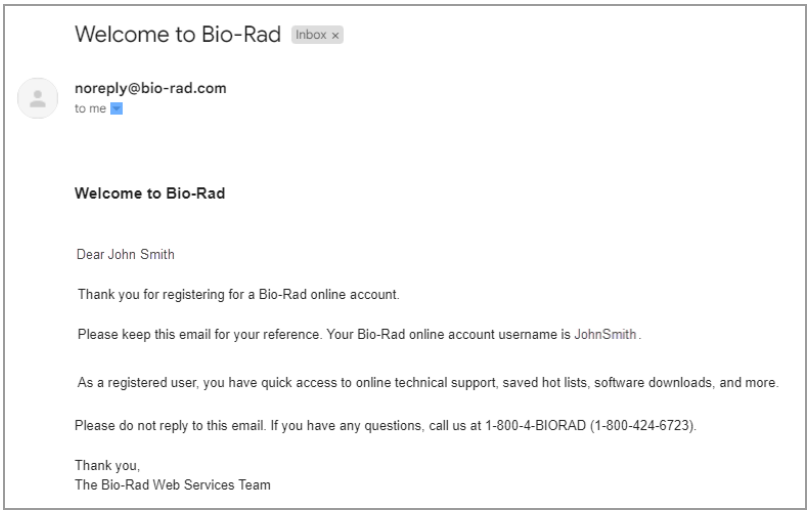
6. (Optional) enter the remaining information.

7. If you are requesting a quote, literature, or documents, click the ⊕ icon and enter your mailing address.



8. Optionally, select the checkbox to receive promotional items from Bio-Rad.
9. Select the checkbox indicating you have read and agree to the Bio-Rad privacy policy and trading terms.
10. Select the *I'm not a robot* checkbox, and then Identify the requested items in the captcha pictures and then click Submit.

    If your registration is successful, Bio-Rad creates your user account and sends an email confirming that your user account is created. If not, an error message appears in the account creation form. Correct the errors and resubmit.



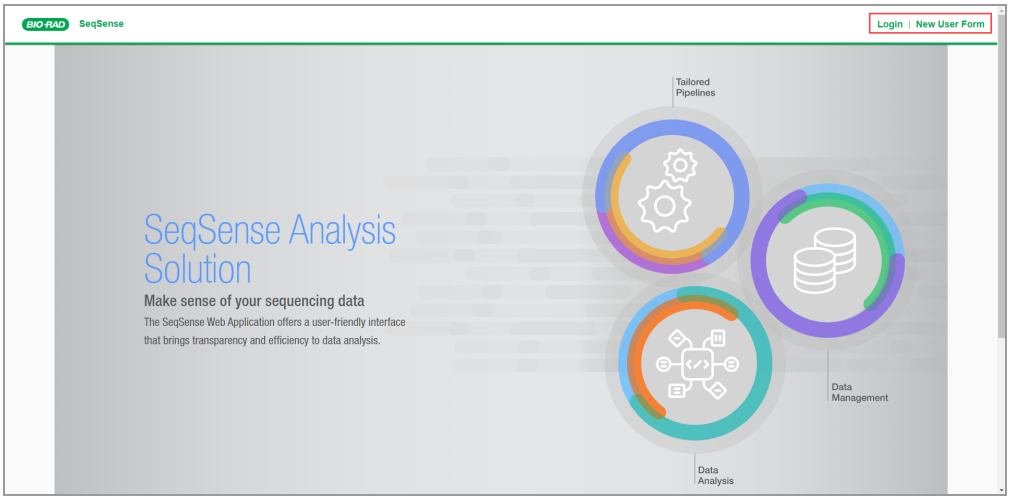
Creating a User Account in SeqSense

Complete the steps below to create a SeqSense Cloud user account.

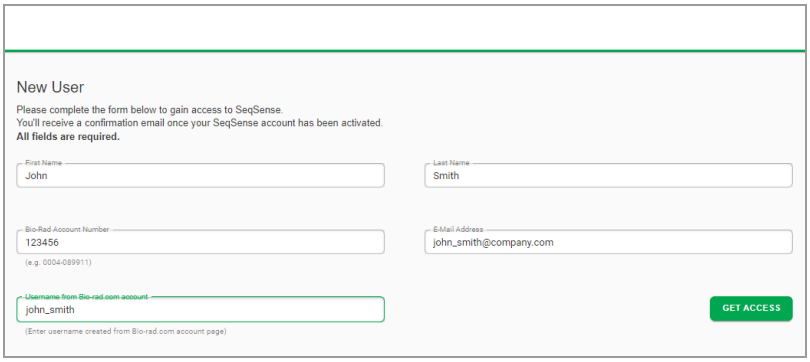
To create a SeqSense user account

1. Enter https://seqsense.bio-rad.com into your browser address field. Optionally, save the URL as a browser bookmark.

   The SeqSense Cloud landing page appears.



2. Click New User Form.

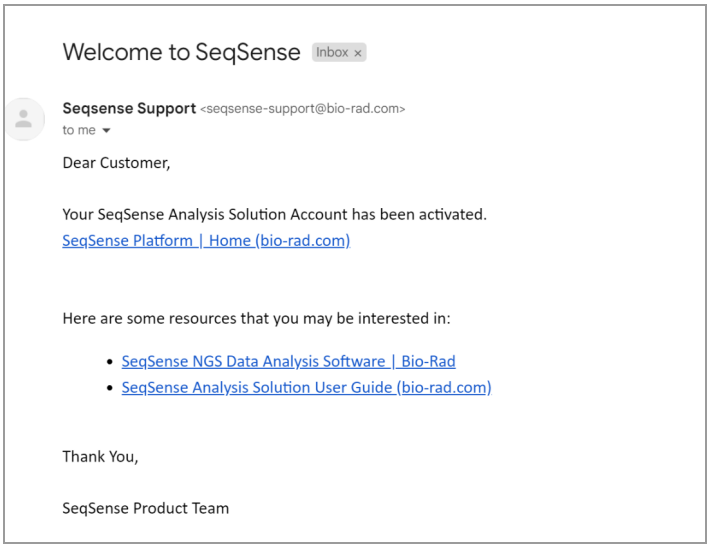


3. Enter the following information:
   - First and last name
   - Bio-Rad account number

     Note: Your Bio-Rad account number is listed on the confirmation you received when you purchased a SEQuoia Express or SEQuoia Complete library prep kit from Bio-Rad (required).
   - Email address
   - Username

     Note: You must enter the email address and Bio-Rad username you entered when you registered for a Bio-Rad website account. See Creating a Bio-Rad Website Account on page 9.
4. Click Get Access.

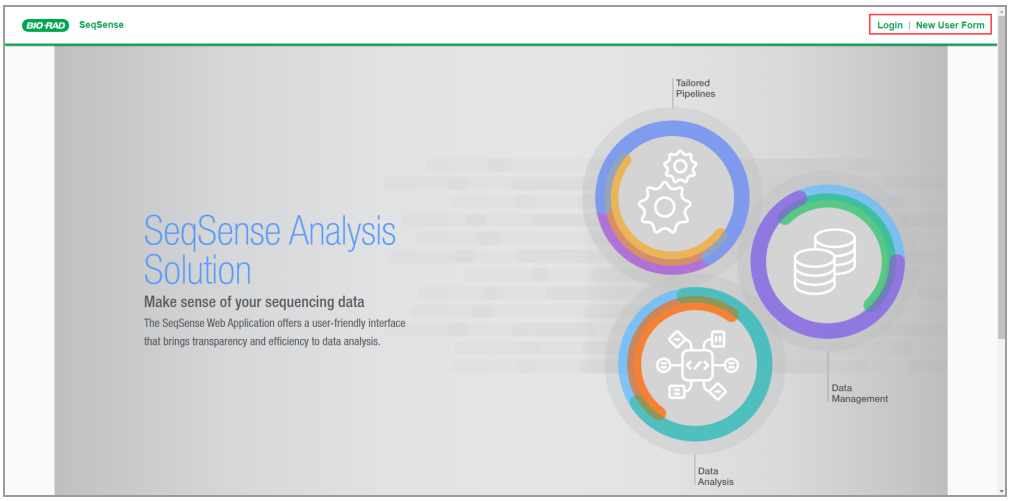
   Bio-Rad sends an email confirming your account.



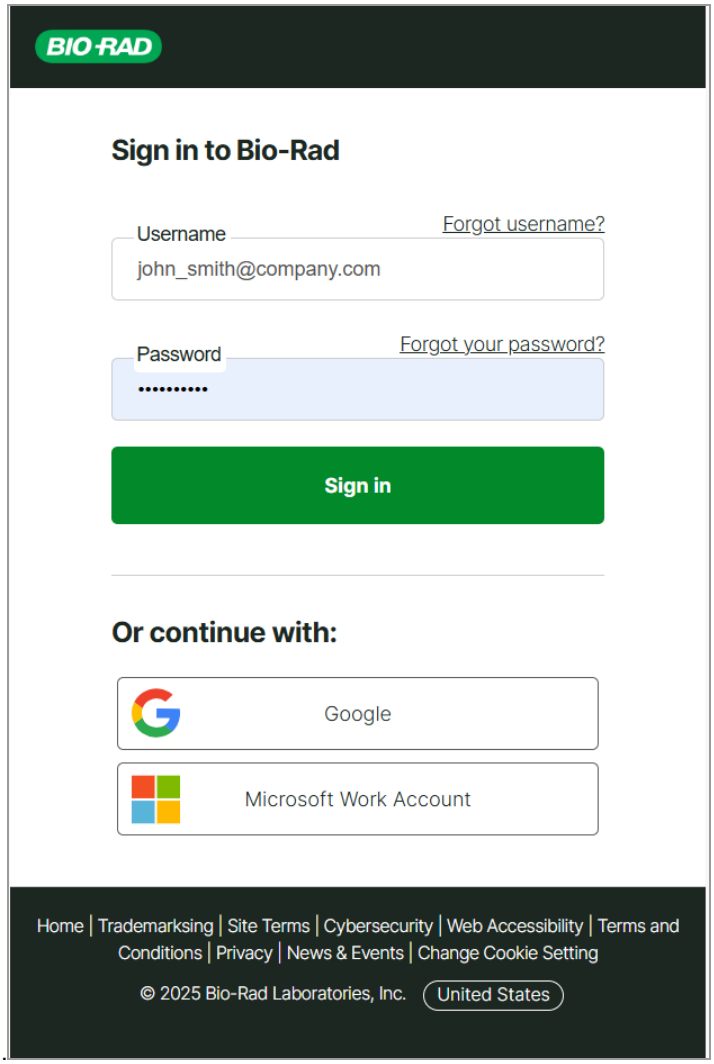
Signing In

To sign into SeqSense Cloud

1. Enter https://seqsense.bio-rad.com in the browser address field to open the landing page.
2. Click Login.



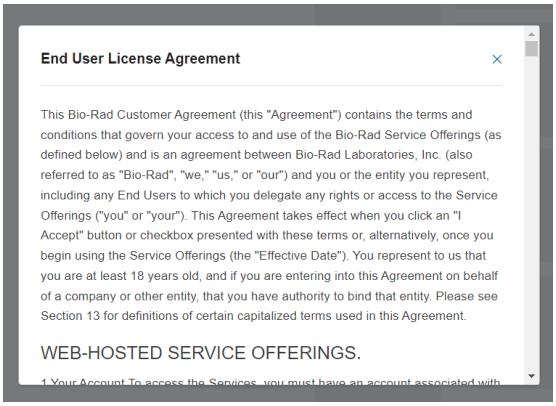
3. When the Login page appears, enter your email address and password and click Sign in



Important: Your email address serves as your username. Use the password you created when you registered your Bio-Rad website account.

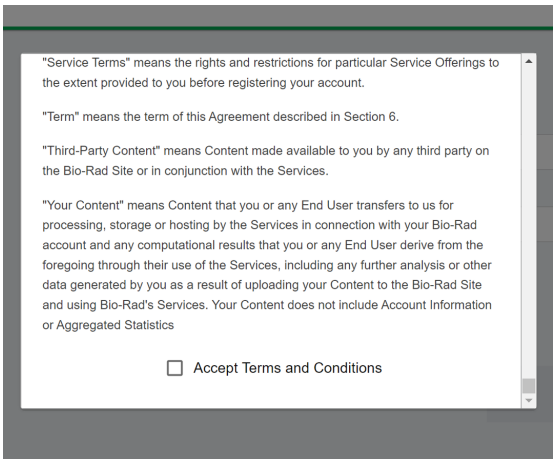
On first use, the Terms and Conditions appear in a scrollable pop-up.

4. Scroll through and read the Terms and Conditions.



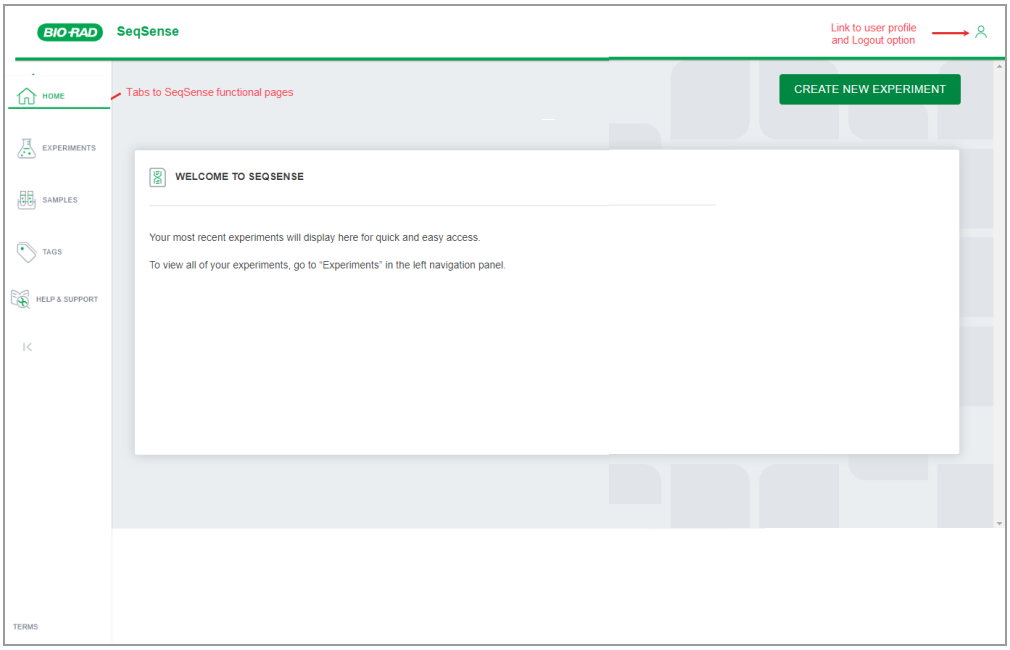
5. Select the Accept Terms and Conditions checkbox.

You must accept the terms and conditions before you can use the application.



The pop-up closes and the application opens to the Home page. To review the Terms and Conditions again at a later time, click Terms and Conditions at the bottom of the left navigation bar.

Important: The Explore SeqSense pane does not appear after you create your first experiment. Continue to the next section to download the SeqSense Cloud documentation now.



Downloading User Documents

Before you create your first experiment, download the SeqSense Cloud from bio-rad.com.

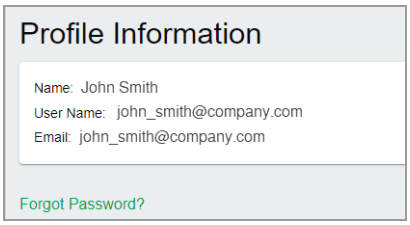
Viewing Your User Profile

From the User Profile page, you can view your current profile information.

To view your user profile

1. Click the icon to display the pop-up.

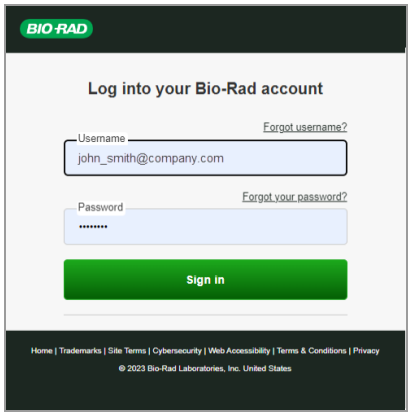
2. Click the email address hyperlink.
3. The Profile Information page opens.



4. Click a tab in the left pane to exit Profile Information.

Resetting Your Password

You can reset your password from the Login page.



To reset your password

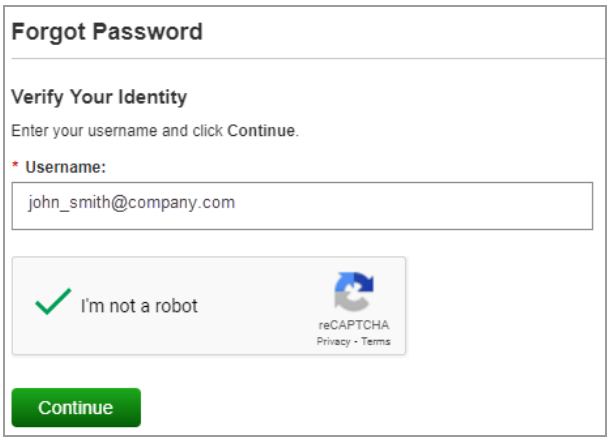
1. In the Login page, click Forgot your password?

   The Forgot Password page appears.

2. In the Forgot Password dialog box, enter your email.

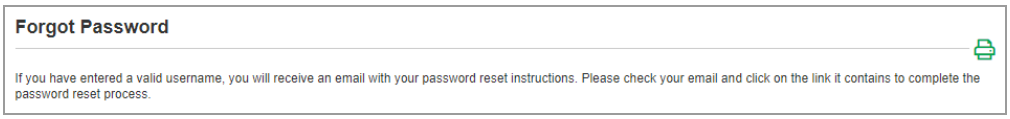
3. Select the *I'm not a robot* checkbox and identify the requested item in the reCAPTCHA pictures.

   When you have correctly identified the items, a check mark appears.



4. Click Continue.

   Bio-Rad displays the following message and sends an email to the validated user account.



Logging Out

To log out of SeqSense

1. Click the icon in the upper-right corner to display the pop-up.

2. Click Logout.

   You are immediately logged out of SeqSense.

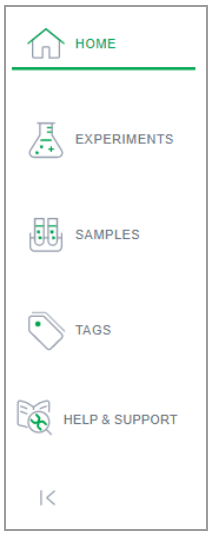
Chapter 2 SeqSense Cloud Overview

Use SeqSense Cloud to do the following:

- Create experiments for pipeline runs
- Upload and manage your FASTQ samples
- Manage your experiment files
- Perform related analysis

Tip: To practice in the application before you begin creating actual experiments, you can download and use the demo data sets from Bio-Rad for SEQuoia Complete and SEQuoia Express. For information, see Help and Support on page 36.

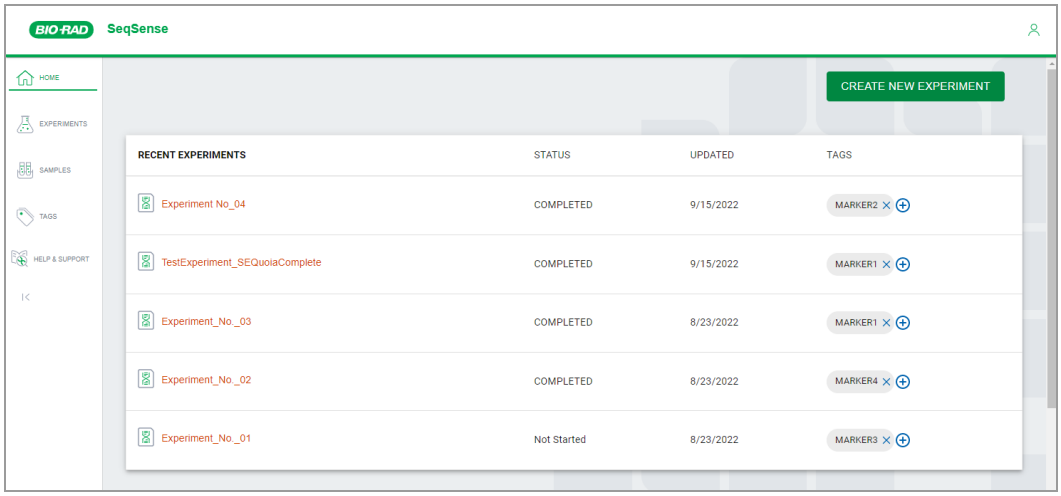
SeqSense functionality is available from the following tab layout:



Tip: Click the |< icon to collapse the panel to show icons only.

Home Page

When you log into SeqSense, the Home page appears by default.



From the Home page you can

- View your most recent experiments, sorted by date

  Note: To see the full list of your experiments, select the Experiments tab on the left.

- Create a new experiment
- Add tags to a pending or completed experiment

Table 1 explains the columns on the Home page.

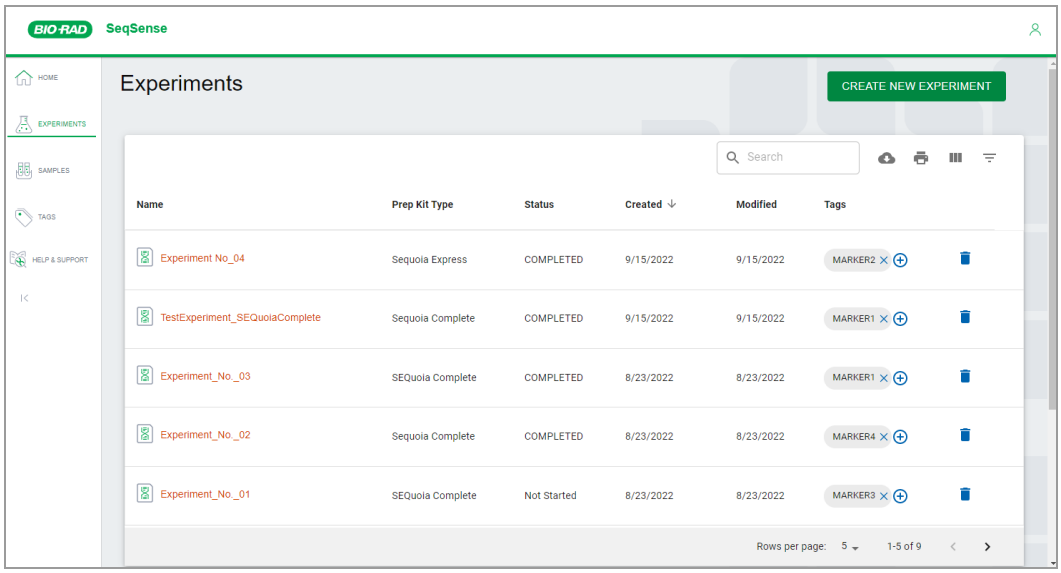
Table 1. Home page column display

Column name	Description
Experiment name	Unique name for your experiment
Status	Current status of the pipeline run (Not Started, RUNNING, COMPLETED)
Updated	Date the experiment was updated
Tags	Identifiers associated with the experiment

Experiments Page

The Experiments page provides a scrolling list of all your experiments, and also provides options to create a new experiment, add tags to an experiment, remove tags from an experiment, and delete an experiment.

Note: Deleting tags from an experiment does not remove the tags from the application. Removing a tag entirely is only allowed from the Tags Management page.



The default display contains fifteen rows. To increase the number, select the Rows per page dropdown arrow in the bottom-right corner and select the number of rows to display.

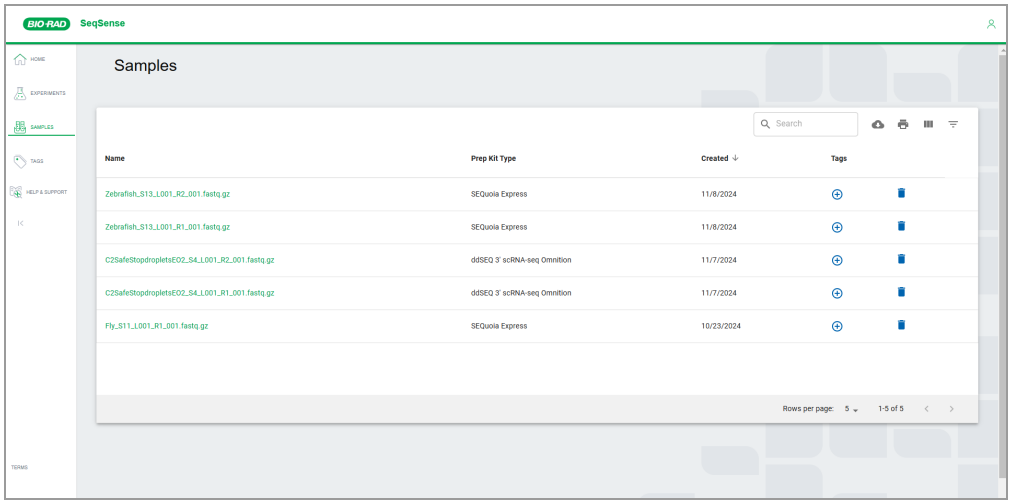
Table 2 explains the columns on the Experiments page.

Table 2. Experiments page column display

Column name	Description
Name	Experiment name **Note:** While sample files must adhere to the Illumina FASTQ naming convention, experiments can be named without restrictions. The name must not exceed 250 characters. **Important:** Do not use Personal Protected Information (PPI) when creating a file name.
Prep Kit Type	Chemistry kit type associated with the experiment (SEQuoia Express, SEQuoia Complete, or ddSEQ 3' scRNA-seq Omnition)
Status	The current status of the pipeline run (Not Started, In Progress, Completed, or Failed)
Created	Date the experiment was created
Modified	Date the experiment was updated
Tags	Identifiers associated with the experiment ■ Click ⊕ to create a tag and automatically associate it with the experiment or sample. ■ Click ✕ to delete a tag from an experiment or sample.

Samples Page

The Samples page displays a list of your uploaded sample (FASTQ) files.



Note the following:

- Your sample names must be unique, and the file name must follow the Illumina naming convention. For information, see Naming Your Samples on page 43.
- You can view and delete samples from the Samples page, but you can upload and save sample files only when you are creating an experiment. See Uploading Samples on page 45 for information.

The default display contains fifteen rows. To increase the number, select the Rows per page dropdown arrow in the bottom-right corner and select the number of rows to display.

From the Samples page, you can

- Add tags to a sample. For information on tags, see Tag Management Page on page 27.
- Delete a sample. For information, see Deleting Experiments and Samples on page 35.

  Important: There is no recovery option after you delete a sample. To reuse the sample, you must upload it again when you create your experiment.

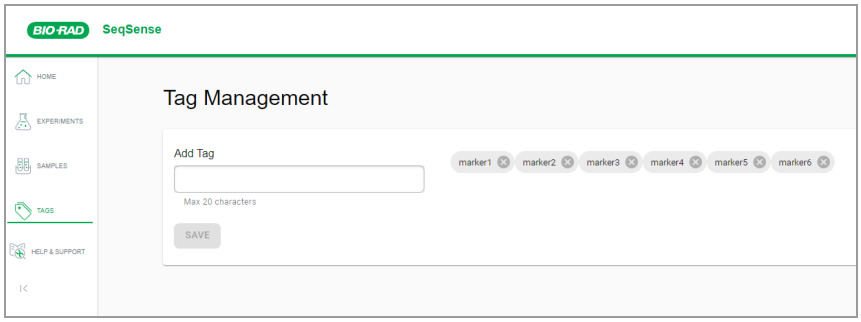
Table 3 explains the columns on the Samples page.

Table 3. Samples page column display

Column name	Description
Name	Contains the sample name
Prep Kit Type	Chemistry kit type associated with the sample
Created	Date the sample was uploaded
Tags	Identifiers associated with the sample

Tag Management Page

Tags are descriptive identifiers that allow you to organize and categorize your files. Examples include the project name, experiment name, variables, methods, and so forth.



You can add tags on the Tag Management page or to an experiment as you create it.

Note: When you add tags to an experiment, they are automatically associated with the samples selected or uploaded. Currently, there is a limit of 20 tags that can be added to an experiment.

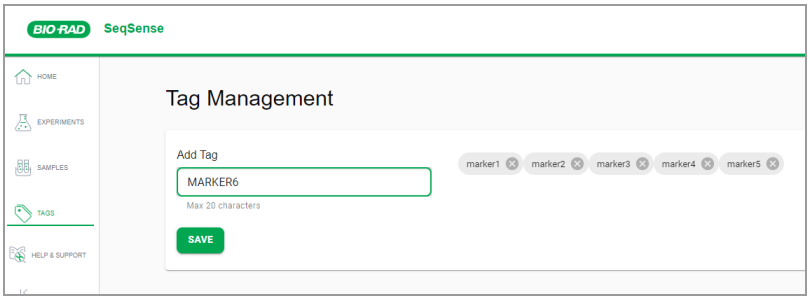
You can also add tags to pending or completed experiments from the Home and Experiments pages, and to samples from the Samples page.

Adding Tags in the Tag Management Page

When you add tags in the Tag Management page, they are available to apply to any experiment or sample. Tags can represent any identifying feature of the experiment or sample, and you can group and sort experiments and samples based on the tags assigned.

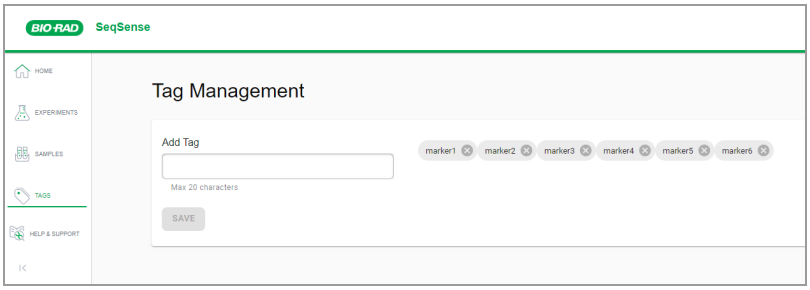
To add tags to the application

1. Select the Tags tab to open the Tags Management page.



2. Enter a tag name in the Add Tag field.
3. Click Save.

   The tag appears on the Tag Management page, and also in the Available Tags column when you create an experiment.



Adding Tags in the Experiments and Samples Pages

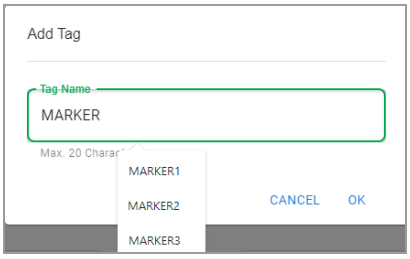
You can add tags to experiments and samples in the associated pages.

Important: When you add a tag to an *experiment*, it is also added to the samples uploaded to the experiment. If you add a tag to a *sample*, it applies to the sample only.

To assign a tag in the Experiments or Samples page

1. Open the applicable page and select an experiment or sample.
2. Click the ⊕ icon in the Tags column.

   The Add Tag dialog box appears.



   Important: When you click in the Tag Name field, a dropdown list containing the available tags is displayed. As shown below, if you type all or part of an existing tag name in the field, the selection narrows to the tags fitting the criteria.

3. After clicking in the field, do one of the following:
   - Select an existing tag from the dropdown menu. If you don't see your tag, you can enter part of the tag name to narrow the list.
   - Type a unique tag name into the field.
4. Click OK to assign the tag. New tags are added to the Tag Management page for future use.

Removing Tags

Before you remove tags, note the following:

- If you remove a tag from an experiment, it is still available to assign to other experiments.
- If you remove a tag from the Tag Management page, the tag is deleted from the application.

To remove a tag

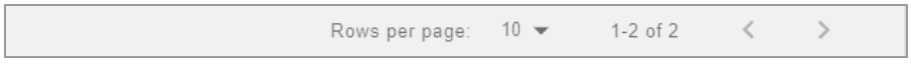
▶ Click the X in the applicable tag to remove it.

SeqSense Tools

The following application tools are available in SeqSense Cloud.

Table 4. Buttons, fields, and icons

Use this...	To do this...
CREATE NEW EXPERIMENT	Create a new experiment; this button is available from the Home and Experiments pages. See Creating an Experiment on page 37.
Search	Search for existing experiments and samples; this field is available from the Experiments and Samples pages. See Searching for Files on page 31.
	Download a .csv file containing experiment or sample information; this button is available from the Experiments and Samples pages. See Downloading a List in .CSV Format on page 32.
	Print the .csv data. You can print a paper copy or save the data as a PDF. This button is available from the Experiments and Samples pages. See Printing Files and Metadata on page 33.
	Add or remove columns from the page display; this button is available from the Experiments and Samples pages. See Showing or Hiding Columns on page 33.
	Add a tag; this button is available from the Home, Experiments, and Samples pages. See Tag Management Page on page 27.
	Delete the associated experiment or sample file; this button is available from the Experiments page and Samples page. See Deleting Experiments and Samples on page 35.
	Delete a tag from the Tag Management page or from an experiment or sample. See Removing Tags on page 29.

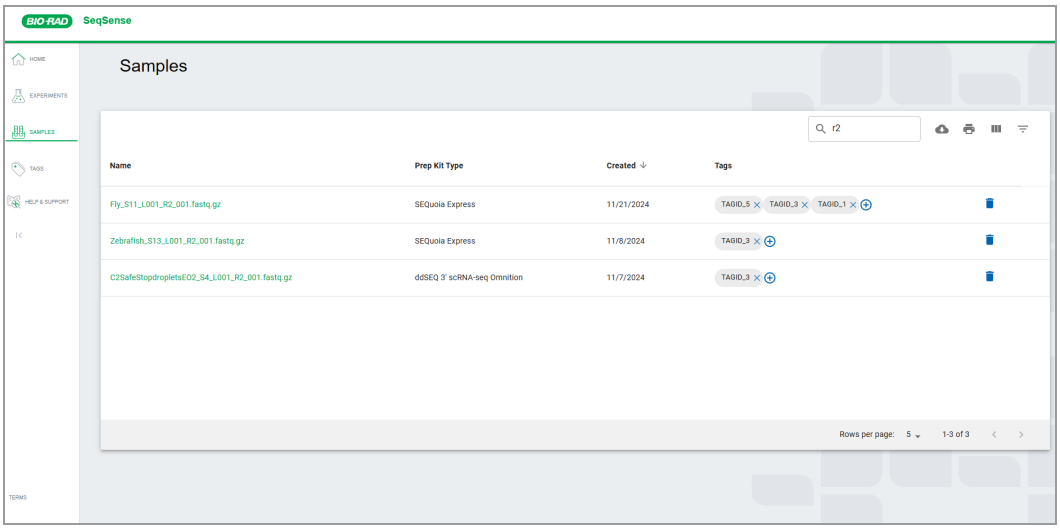


Searching for Files

To use the search feature

1. In the left panel, click Experiments or Samples.
2. Enter your search criteria in the Search field and then click the Search icon.

3. As you type, the search results narrow to those that specifically apply.

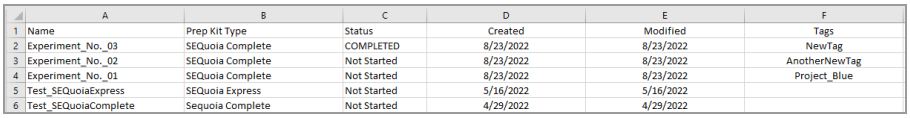


Downloading a List in .CSV Format

You can download a list of your sample files or your experiment files in .csv format.

To download a list to a .csv file

1. In the left panel, click Samples or Experiments.
2. Click the Download icon in the toolbar.
3. Open your Downloads directory on your computer. The .csv file automatically appears in the directory.



	A	B	C	D	E	F
1	Name	Prep Kit Type	Status	Created	Modified	Tags
2	Experiment_No._03	SEQuoia Complete	COMPLETED	8/23/2022	8/23/2022	NewTag
3	Experiment_No._02	SEQuoia Complete	Not Started	8/23/2022	8/23/2022	AnotherNewTag
4	Experiment_No._01	SEQuoia Complete	Not Started	8/23/2022	8/23/2022	Project_Blue
5	Test_SEQuoiaExpress	SEQuoia Express	Not Started	5/16/2022	5/16/2022	
6	Test_SEQuoiaComplete	Sequoia Complete	Not Started	4/29/2022	4/29/2022	

4. Double-click the file to open it and view the results.

   Note: When you first open the file, the columns are collapsed into a static width, but you can click and drag the column edge to expand its width.

   The file contains information under the following headings:

   - Name
   - Prep Kit Type
   - Status (experiments only)
   - Created
   - Modified (experiments only)
   - Tags

Printing Files and Metadata

To print the file list and metadata for sample and experiment files

1. In the left panel, click Samples or Experiments.
2. Click the Print icon in the toolbar.

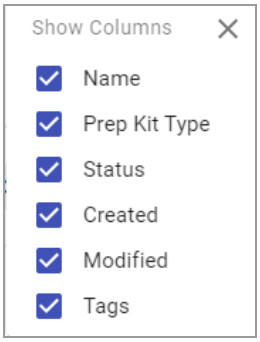
   The Print screen opens, displaying the information and format that will be printed.
3. Click Print.

Showing or Hiding Columns

To show or hide columns in the Samples grid

1. In the left panel, click Samples.
2. Click the View Columns icon in the toolbar.

   A pop-up opens, displaying the current list of columns.



3. Select or clear checkboxes:
   - When you clear a checkbox, the column is immediately removed from the display.
   - When you select a checkbox, the column is immediately added to the display.

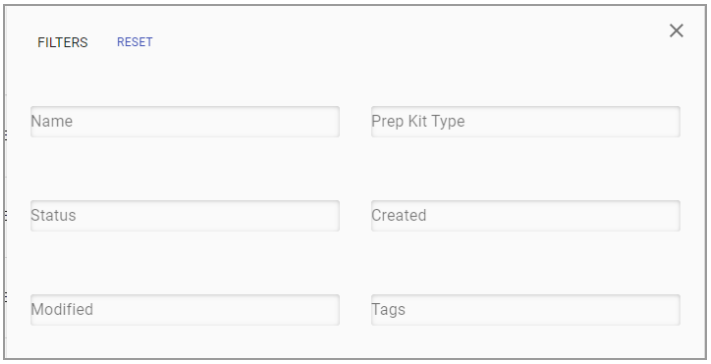
Filtering Files

As you add more files, filtering is useful to display a particular subset. You can filter sample files or experiment files in the display, based on the columns configured for the page. You can filter on any column descriptor (for example, Name or Prep Kit) or combination, whether the columns are displayed or hidden.

To filter the list of files

1. In the left panel, click Experiments or Samples.
2. Click the Filter icon in the toolbar.

   The Filter dialog box opens and contains a field for each available column on the page.



3. Enter your filter criteria in one or more of the fields.

   As you type, the following occurs:

   - The criteria you enter also appears in the upper-left corner.
   - The list filters per the text or value you enter in the filter dialog box.

4. (Optional) To remove a filter, click the × next to the filter text or delete the text in the field. To reset the screen, click Reset.

Deleting Experiments and Samples

Complete the following steps to delete an experiment or sample.

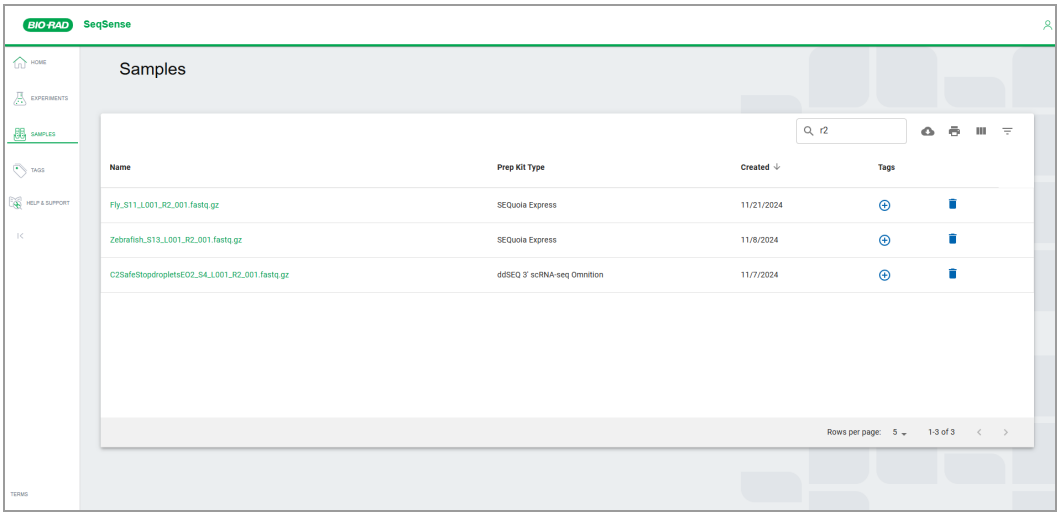
Important: There is no recovery option. To reuse the sample, you must upload it again when you create the experiment.

To delete an experiment or sample

1. In the left panel, click Experiments or Samples.
2. Under Name, select an experiment or sample and click the trash can ( ) icon on the right.

   SeqSense deletes the experiment or sample.

   Note: The following graphic shows the Samples page, but the procedure is the same on the Experiments page.



Help and Support

The Help and Support tab provides a link to the SeqSense Cloud FAQ in PDF format.

To display the PDF in your browser

▶ Select the Help & Support tab.

The Frequently Asked Questions PDF opens in your browser.

To test the application, demo data sets are available for download as follows:

- Click here to download the demo data set for SEQuoia **Express**.
- Click here to download the demo data set for SEQuoia **Complete**.

If you have additional questions, contact Bio-Rad Technical Support using the information at the front of this document.

Chapter 3 Creating an Experiment

To create an experiment, you will

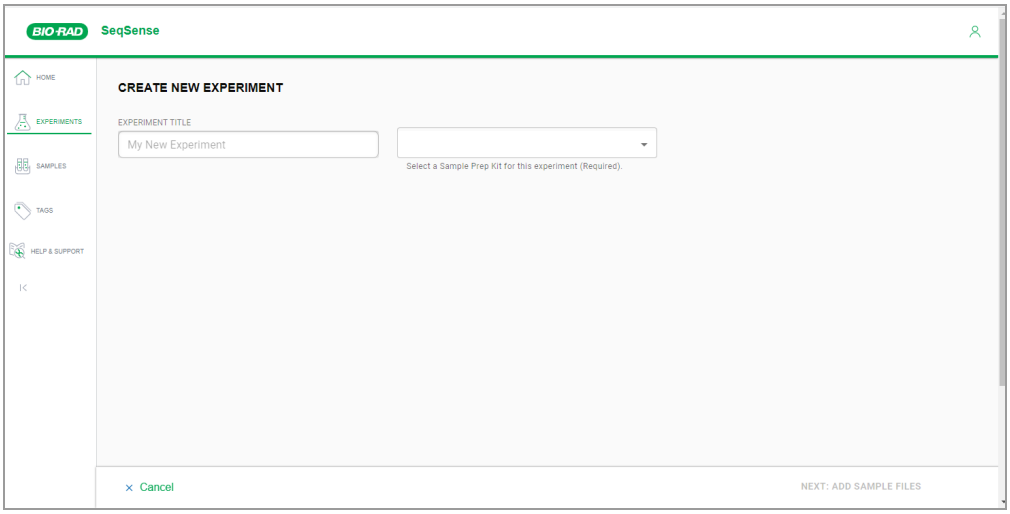
- Name the experiment and select a chemistry (prep) kit
- Upload sample files
- Add and assign tags
- Set the pipeline parameters

Tip: If you are uploading samples, store the applicable FASTQ files in an easily navigable folder on your computer before you begin. This streamlines the process of uploading the file to your experiment.

To create a new experiment

1. On the Home page or Experiments page, click Create New Experiment.

The Create New Experiment page opens.



2. Continue to Naming the Experiment and Selecting a Prep Kit on page 39.

Naming the Experiment and Selecting a Prep Kit

From the Create New Experiment page, you can name your experiment and select a stranded RNA library prep kit. The chemistry (prep) kits described in Table 5 are high-performance stranded RNA sequencing kits.

Note: SEQuoia Express and SEQuoia Complete include a proprietary engineered enzyme (SEQzyme) that combines cDNA synthesis with adapter addition in a continuous synthesis reaction.

Important: Do not use Personal Protected Information (PPI), such as your first name or initials, in the experiment name.

Table 5. Available chemistry (prep) kits

Express	The SEQuoia Express Stranded RNA Library Prep Kit captures long RNA transcripts, including mRNA and long non-coding transcripts (greater than 200 bp). The streamlined three-tube workflow enables high-throughput library construction from high-quality samples in less than three hours.
Complete	The SEQuoia Complete Stranded RNA Library Prep Kit captures long and short RNAs in a single library, even from limited and low-quality samples. The unique enzymatic properties effectively capture all types and sizes of RNA species in a novel enzymatic reaction, significantly improving the diversity and quality of RNA libraries, even from limited or degraded RNA samples.
ddSEQ Single-Cell 3’ RNA-Seq	The ddSEQ Single-Cell 3’ RNA-Seq Kit enables streamlined whole transcriptome analysis (WTA) at the single-cell level. Leveraging Bio-Rad’s ddSEQ droplet technology, this assay is able to capture and profile hundreds to thousands of single cells with high sensitivity and recovery.

To name the experiment and select a chemistry (prep) kit

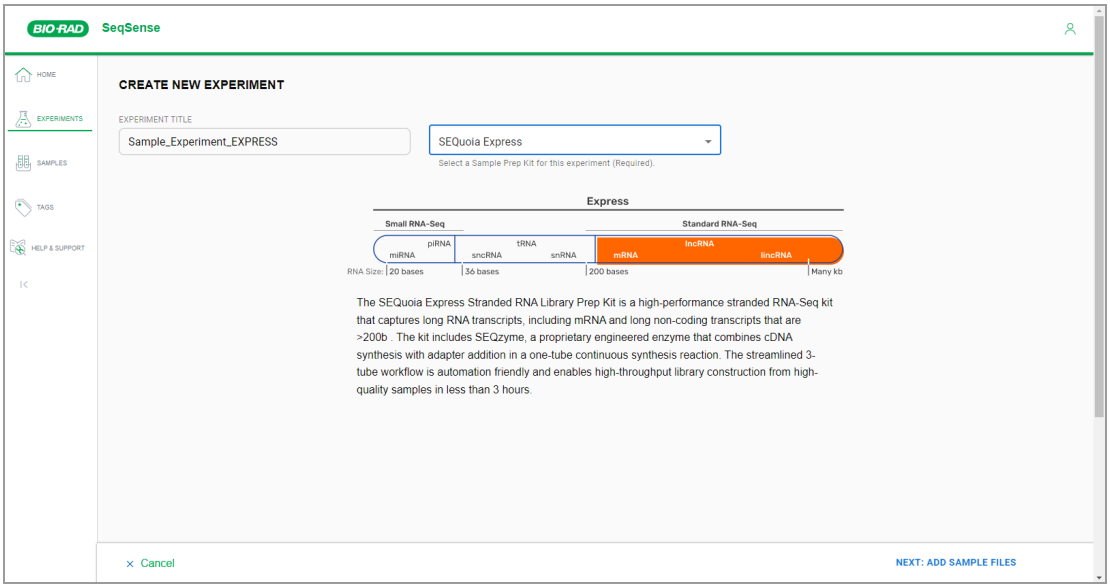
1. In the Experiment Title field, enter a name for your experiment.

   Note: An advisory message appears below the Experiment Title field if the name is already in use. Enter a unique name for the experiment.

2. From the dropdown list, select a sample prep kit.

   Important: If you choose SEQuoia Complete as the pipeline, the application can process only one sample at a time (R1 or R1+R2). To ensure reports are generated, upload only one sample for the pipeline run. The application can run multiple SEQuoia Express and Omnition samples simultaneously.

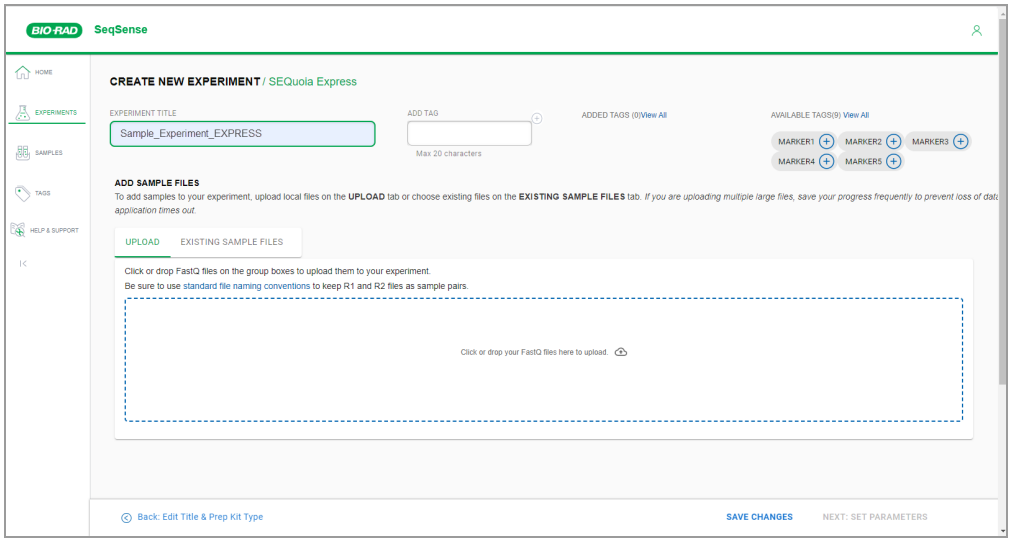
The page expands to show related RNA sequencing information.



3. Click Next: Add Sample Files and continue to Adding Samples to Your Experiment on page 42.

Adding Samples to Your Experiment

When you click the Next: Add Sample Files link, the Add Sample Files section appears in the Create New Experiment page.



The Add Sample Files section contains the Upload tab and the Existing Sample Files tab. You can upload one or more samples to SeqSense to use in your experiment, or you can select files you have already uploaded in previous experiments.

Important: Your sample files must conform to the Illumina naming convention. See Naming Your Samples on page 43.

Tip: On the Existing Sample Files tab, only samples that are compatible with the prep kit you selected appear for selection.

Do one of the following:

- By default, the Upload tab is selected. If you are uploading the samples you will use in the pipeline run, continue to Uploading Samples on page 45.
- If you are using previously uploaded files, select the Existing Sample Files tab and continue to Selecting Existing Samples on page 47.

Naming Your Samples

Important: SeqSense does not accept duplicate sample names. Before you upload samples to your experiment, check the Samples tab to ensure your sample names are unique. Rename any duplicates.

To ensure that SeqSense knows to pair R1 and R2 as one sample, all samples must have names that comply with the Illumina FASTQ naming convention, as shown below:

SampleName_S1_L001_R1_001.fastq.gz

SampleName_S1_L001_R2_001.fastq.gz

SampleName_S1_R1_001.fastq.gz

SampleName_S1_R2_001.fastq.gz

OR

SampleName_S1_L001_R1_001.fq.gz

SampleName_S1_L001_R2_001.fq.gz

SampleName_S1_R1_001.fq.gz

SampleName_S1_R2_001.fq.gz

Important: Do not use Protected Personal Information (PPI) (for example, your first name or initial) in your sample name.

Table 6 describes each segment of the naming convention.

Table 6. FASTQ sample file naming convention

Segment Name	Description
SampleName	Name of the sample provided in the sample sheet. If a sample name is not available, you can use the sample ID provided in the sample sheet or rename the sample if its current name is a duplicate of an existing sample file.
Sx	Sample number based on sample order in the sample sheet (S1, S2, and so forth). **Important:** Reads that cannot be assigned to any sample are written to a FASTQ file for sample number 0 and excluded from downstream analysis.
Lxxx	Lane number (optional)

Segment Name	Description
Rx	Read number For a single-end run, the corresponding read number is R1 and for a paired-end run, the corresponding read numbers are R1 and R2. (When generated, the index reads are I1 and I2).
001	Static identifier for the last segment.

Uploading Samples

Important: SeqSense Cloud times out after one hour. Bio-Rad recommends that you start the pipeline run within one hour of uploading or selecting the sample files.

In the Add Sample Files section, the Upload tab is selected by default.

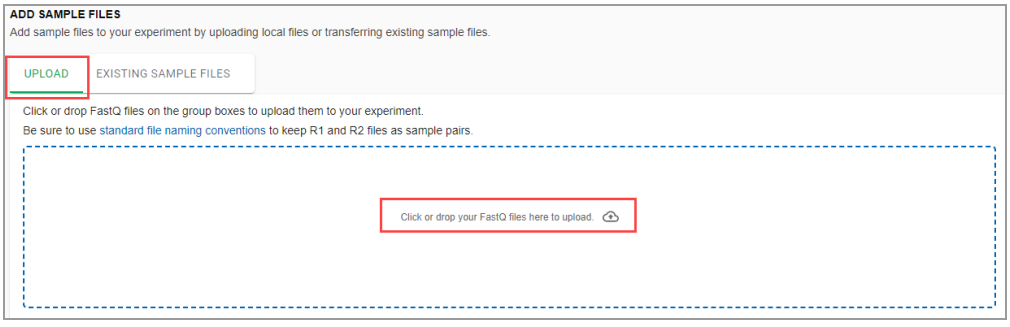
Note the following:

- FASTQ files must be named using the naming convention established by Illumina (shown below) in Gzip (.gz) format. SeqSense does not support FASTQ files saved with a different naming convention. For more information, see Naming Your Samples on page 43 and Illumina BaseSpace Sequence Hub.
- SeqSense Cloud does not accept duplicate file names. Before you upload samples to your experiment, check the Samples tab to ensure your samples have unique names. Rename any duplicates.
- You must use the FASTQ files you upload in an experiment before they appear permanently on the Existing Sample Files tab.
- If you are using the SEQuoia Complete prep kit, Bio-Rad recommends uploading only one sample (R1 or R1+R2). If more samples are uploaded SeqSense might not generate reports for the pipeline run.
- You can upload multiple Omnition samples simultaneity.

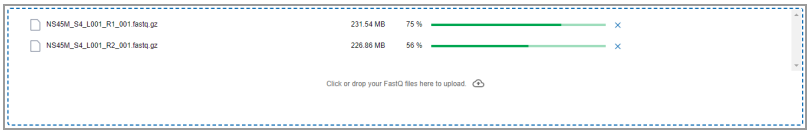
To upload samples

1. Click the *Click or drop your FASTQ files here to upload* link. Follow the prompts to select the files.

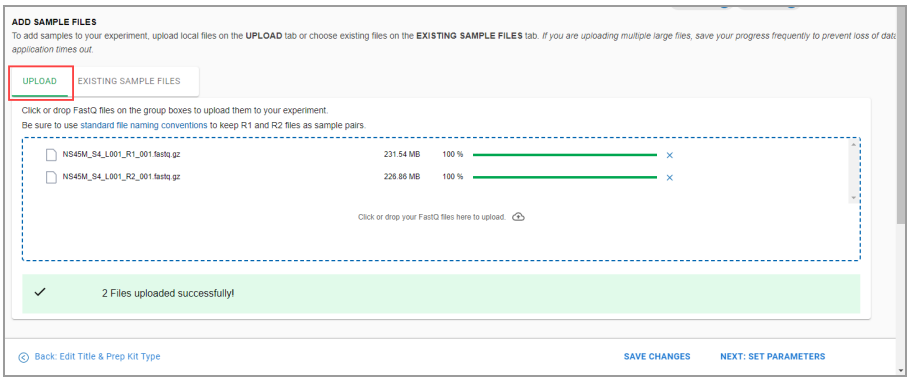
   Tip: You can also drag and drop the files from your computer to the application.



As the files are uploaded, SeqSense shows the progress.

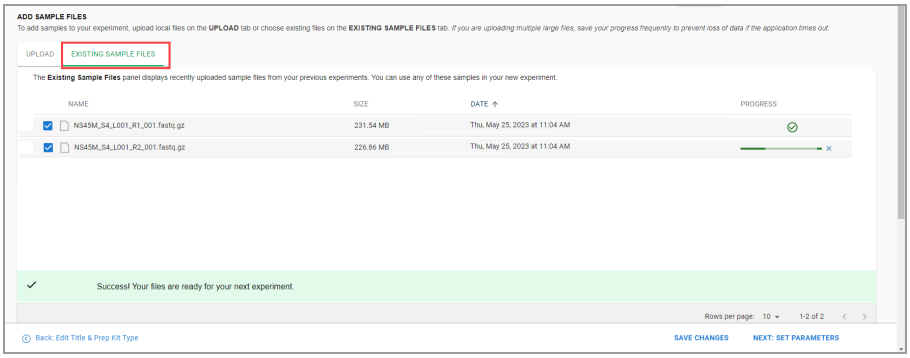


When fully uploaded, the files appear in the box and a message appears, confirming the upload was successful.



2. Click Save Changes.

   The samples appear on the Existing Sample Files tab with the checkboxes selected.



3. Do one of the following:

   - Assign tags. Continue to Adding or Assigning Tags During Experiment Setup on page 48.
   - Click Next: Set Parameters and see Setting the Pipeline Parameters (Express and Complete) on page 49.

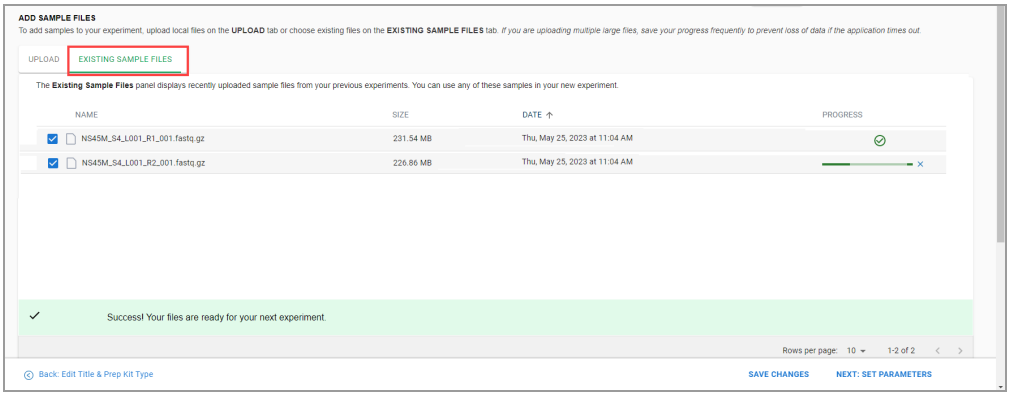
Selecting Existing Samples

Important: SeqSense Cloud times out after one hour. Bio-Rad recommends that you start the pipeline run within one hour of uploading or selecting the sample files.

After you upload the sample files and click Save Changes, they appear on the Existing Sample Files tab, where you can select them for future pipeline runs.

To select existing samples

1. Select the Existing Sample Files tab to display the list of available samples that are compatible with your selected prep kit.



2. Select the checkboxes for the samples to use in the pipeline run.

   Note: The progress bar on the right appears immediately after you select the checkbox, and a green check mark appears when the files are ready.

3. Click Save Changes.

4. Do one of the following:

   - Assign tags. Continue to Adding or Assigning Tags During Experiment Setup on page 48.
   - Click Next: Set Parameters and see Setting the Pipeline Parameters (Express and Complete) on page 49.

Adding or Assigning Tags During Experiment Setup

You can add one or more tags to the experiment as you create it, or you can assign one or more existing tags that you created on the Tag Management page. Tags you add and save as part of creating an experiment are saved to the Tag Management page.

To add or assign a tag to the experiment during experiment setup

1. Do one of the following:

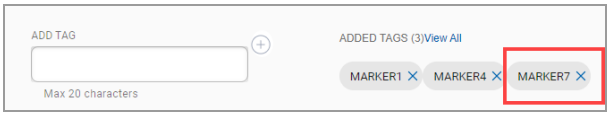
   - In the Add Tag field, enter a tag name and click the plus icon.

   - Under Available Tags, locate the tag to assign to the experiment and click the plus icon.

2. Click Save Changes.

   SeqSense adds the tag to the experiment and displays it under the Added Tags heading.

   New tags are also saved to the Tag Management page.



3. Continue to Setting the Pipeline Parameters (Express and Complete) on page 49.

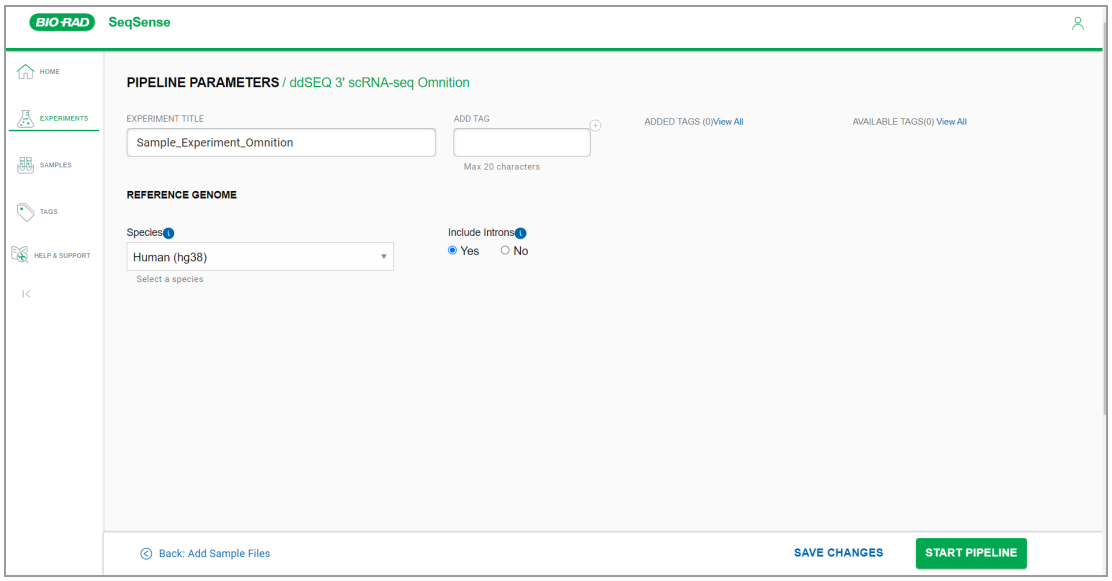
Setting the Pipeline Parameters (Express and Complete)

After you click Next: Set Pipeline Parameters, the Pipeline Parameters page appears. You select a reference genome and then define quality parameters for your data on the scrollable page. The process of setting pipeline parameters differs between Express and Complete experiments and Omnition experiments.

This section explains how to set parameters for Express and Complete pipelines, and for Omnition pipelines.

Setting the Pipeline Parameters (Omnition)

You can set the pipeline's reference genome parameters for ddSEQ 3' scRNA-seq Omnition pipelines as well as rename the experiment and add a tag.



To set the pipeline parameters

1. Select a species type to align to and annotate against.

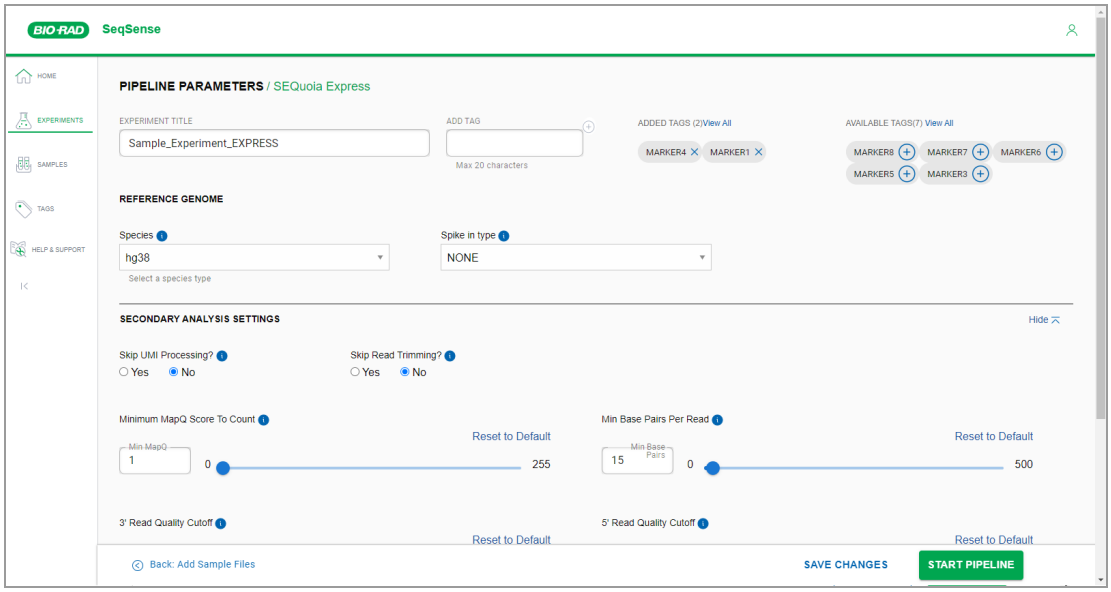
   The Omnition kit provides

   - Human (hg38) — GRCh38 Homo sapiens (human)
   - Mouse (mm10) — GRCm38 Mus musculus (house mouse)

2. Indicate whether or not reads that map to introns should be included in gene counts by clicking Yes or No.

Setting the Pipeline Parameters (SEQuoia Express and SEQuoia Complete)

You can select the pipeline's reference genome, set secondary analysis settings, and configure advanced secondary analysis settings for SEQuoia Express and SEQuoia Complete pipelines.



To set the pipeline parameters

1. Under Reference Genome, do the following to select a genome assembly:

   a. Select a species type to align to and annotate against.

      The Complete kit provides

      - hg38 — GRCh38 Homo sapiens (human)
      - mm10 — GRCm38 Mus musculus (house mouse)
      - mor6 — Rattus norvegicus (rat)

      The Express kit provides

      - ce11 — Caenorhabditis elegans/C. elegans (roundworm)
      - danRer11 — Danio rerio (zebrafish)
      - dm6 — Drosophila melanogaster (fruit fly)

- hg38 — GRCh38 Homo sapiens (human)
- mm10 — GRCm38 Mus musculus (house mouse)
- mor6 — Rattus norvegicus (rat)
- sacCer3 — Saccharomyces cerevisiae S288C (baker's yeast)
- tair10 — Arabidopsis thaliana (thale cress)

b. (Optional) Select a spike-in type.

Bio-Rad currently supports the ERCC RNA Spike-in Control Mix (ERCC) only. The mix provides a set of external RNA controls that enable the performance assessment applied to gene expression experiments. Use the data from the spike-ins for normalization.

2. Under Secondary Analysis Settings, do the following:

c. Skip UMI Processing? — This selection is applicable only when R1 and R2 are present. Unique molecular identifiers (UMIs) are strings of random nucleotides attached to the start of reads. They are used to remove read duplicates, which improves the accuracy of count results.

- To remove UMIs before they are mapped (while keeping the sequence), select Yes.
- To keep UMI processing, keep the default setting (No).

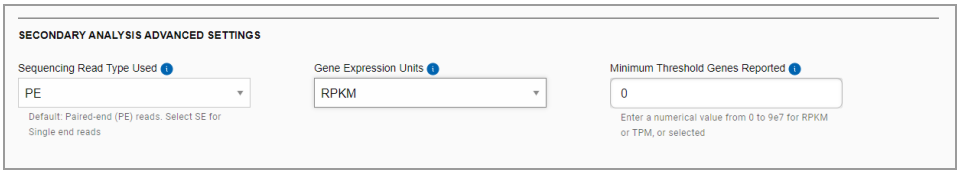
d. Skip Read Trimming? — Read trimming removes low-quality bases at the 3' end of a read, reads containing too many N-bases along their length, and so forth. To remove the excess noise, keep the default setting of No. Otherwise, change Skip Read Trimming to Yes.

e. For the following, move the slider to the appropriate value. Selected values appear to the right.

- Minimum MapQ Score to Count — MapQ quantifies the probability that a read is misplaced. It equals −10 log10 Pr {mapping position is wrong}, rounded to the nearest integer. Using this option, you can potentially filter out the poorly aligned reads. Reads below this specified score are not used in the mapping
- Min Base Pairs Per Read — removes reads that are shorter than a specific number of base pairs, after trimming of adapters and bad regions

- 3’ Read Quality Cutoff — removes 3’ end with low-quality, based on a quality cutoff, after adapter removal (if any)
- 5’ Read Quality Cutoff — removes 5’ end with low-quality, based on a quality cutoff, after adapter removal (if any)

3. (Optional) If you selected the Express chemistry (prep) kit, you can also specify advanced settings.



   a. Sequencing Read Type Used — for paired-end read sequencing, select PE; for single-end, select SE. For quantification, either process is acceptable; for all other studies, paired-end is recommended.

   b. Gene Expression Units — select the metric that will be used for gene expression quantification; select from reads RPKM (reads per kilobase of transcript, per million), TPM (transcripts per million), or None.

      - Minimum Threshold Genes Reported — enabled only when RPKM or TPM is selected; enter a double value to cut off how many reads are minimally required for a gene to be counted.

4. Click Save Changes.

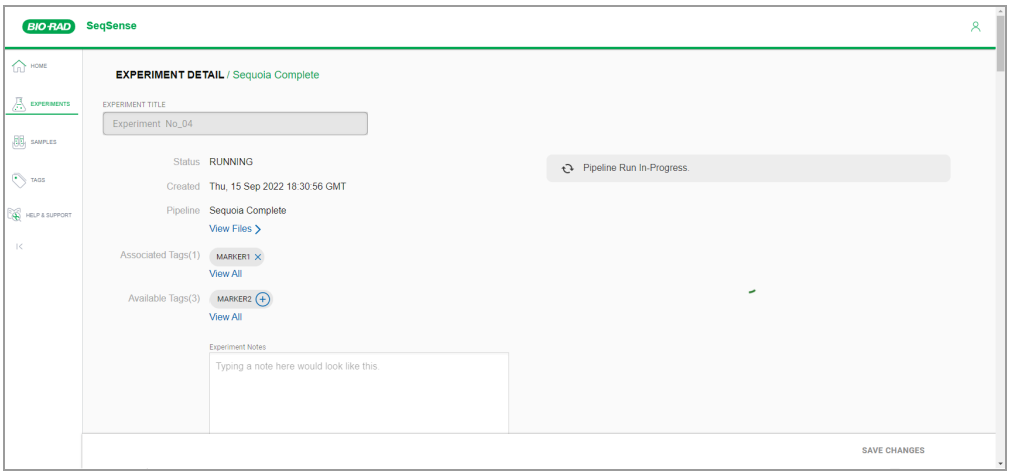
Starting the Pipeline Run

Important: SeqSense Cloud times out after one hour. Bio-Rad recommends that you start the pipeline run within one hour of uploading or selecting the sample files.

To start and run the pipeline

▶ Click Start Pipeline.

The Experiment Detail page appears and shows the run in progress.



Four potential statuses can appear in the Status field:

- Not Started (pipeline run is initializing)
- RUNNING (pipeline run is in progress)
- COMPLETED (pipeline run is finished)

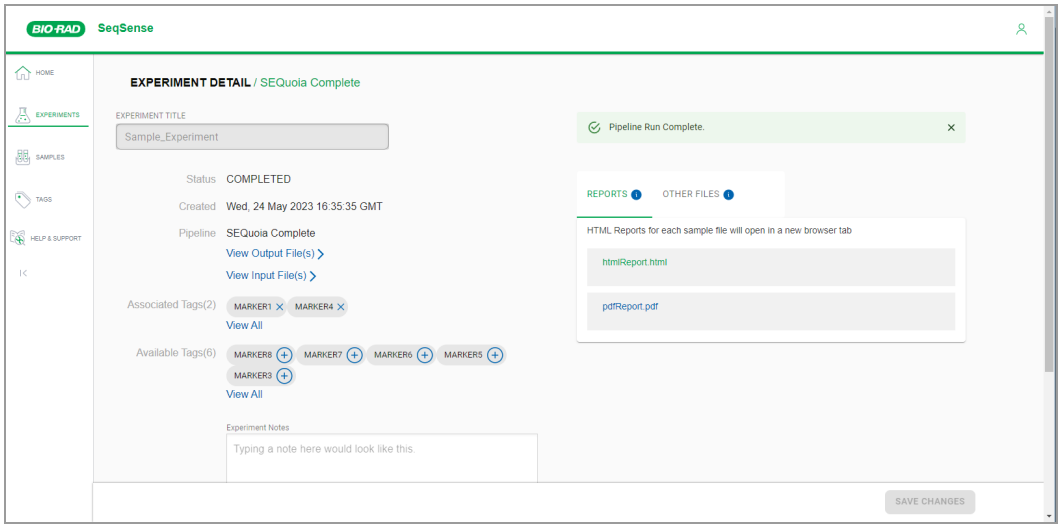
When the pipeline run status is complete, the experiment file appears in the list on the Experiments page, and also on the Home page under Recent Experiments.

Chapter 4 Reporting and Analysis

When the experiment is finished, the Experiment Detail page displays links to files and charts that were generated during the pipeline run.

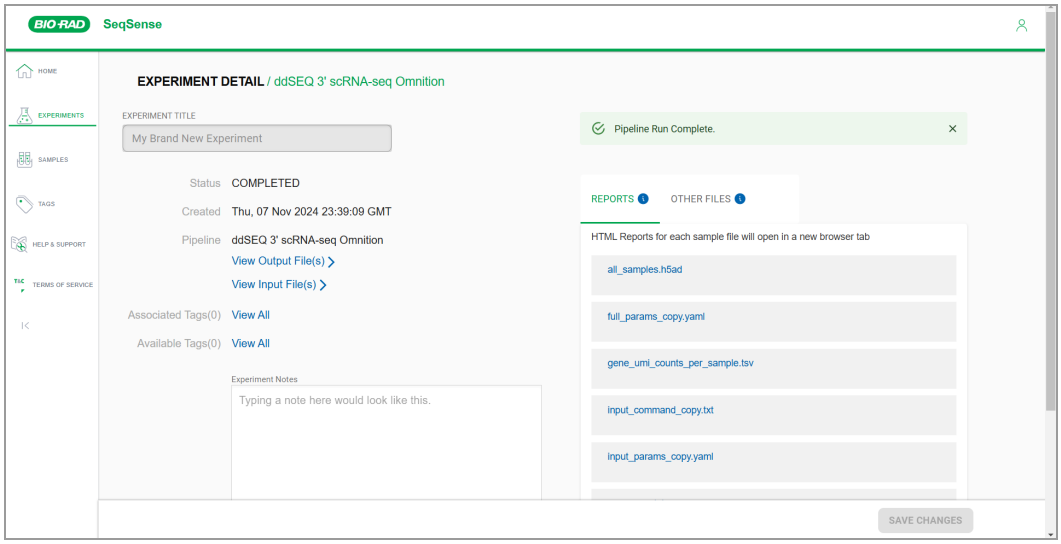
Reports and other files appear on two tabs, Reports and Other Files.

SEQuoia Complete and SEQuoia Express



- On the Reports tab, you can access RNA sequencing data. You can scroll through the HTML file to view QC reads, UMI parsing, read trimming, alignment, deduplication, transcriptome, and pipeline metadata, or you can print the same data in PDF format.
- On the Other Files tab, you can access FastQC quality data, including basic statistics sequencing content and quality, length distribution, duplication levels, strings of overrepresented data, and adapter content.

Omnition



- On the Reports tab, you can access RNA sequencing data. You can scroll through the .html file that opens the Omnition environment, which displays the experiment summary (summary, pipeline messages, and software configuration), analysis statistics (including cell and mapping metrics), pipeline results, deconvolution, and QC reads.
- On the Other Files tab, you can access filtered and unfiltered count matrices, alignment data, and information on the clustering and inter-cell distances for each sample.

Opening a File from a Completed Pipeline Run

After a pipeline run completes, raw data appears in various file types, depending on the pipeline type (SEQuoia or Omnition).

Opening a Completed SEQuoia Complete or SEQuoia Express File

Typically, raw data appears in .csv and .txt formats and formatted data appears in .html and .pdf formats. Files either open in your browser or are downloaded to your computer. To open downloaded files, you must have the corresponding or compatible application installed.

To open a file containing results from a SEQuoia pipeline run

1. In the Experiment Details page for the completed run, select either the Reports tab or Other Files tab.
2. Scroll to and click a file hyperlink.

3. Click a hyperlink. Depending on the file type, the file will open immediately in the applicable application, or be downloaded to your default folder.

   Important: The application to open the file must be installed on your computer.

Opening a Completed Omnition File

A completed Omnition pipeline workflow produces multiple output types, including .html , .csv, BAM, .gz, h5Ad, .txt, .tsv, and yaml file types. For more information, see Reports Generated from an Omnition Pipeline run.

Important: 3’ RNA analysis pipeline uses Seurat version 4.3.0 to generate output Seurat files. To check compatibility with later releases of Seurat, refer to the Seurat documentation at https://satijalab.org/seurat/.

Table 7. Pipeline outputs

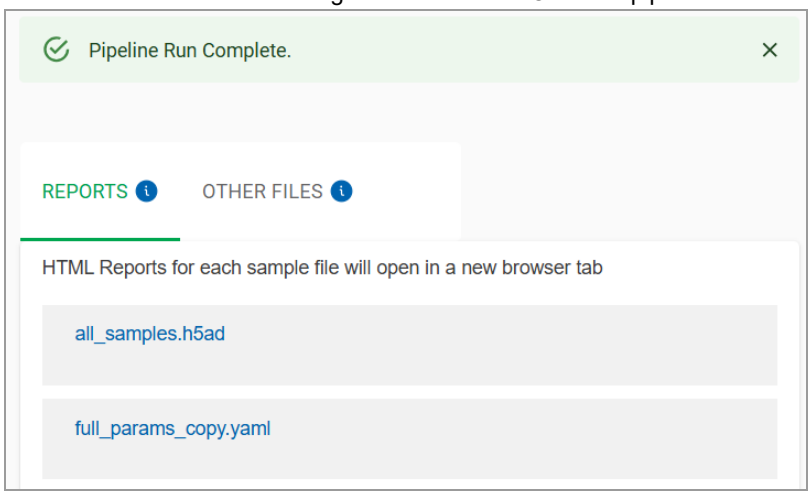
Output	Description
HTML report	*A single HTML report with quality control metrics* If Omnition performed a multiple sample batch analysis, you can select individual samples from the dropdown list to view the populated metrics and results.
Metric Summary file	*A CSV file containing metrics for all analyzed samples* Omnition tracks many metrics throughout the course of the pipeline that are recorded in the metric_summary.csv file.
BAM files	*A BAM file for each sample in a pipeline run* The BAM file is named with the sample name and has the extension .final.bam. It contains the primary alignment for each read aligned in the experiment, deduplicated at the cell level where PCR duplicates within the same partition are represented by a single representative fragment.
BAM tags	*A BAM tag reports the primary alignment for each representative fragment from each partition* BAM tags are annotated on the records present in the BAM files. Each alignment has the NH, HI, AS, and nM tags generated by STAR. Omnition uses the tags, as follows: ■ XT tag to label the gene name ■ XF tag to label the genic feature ■ XN tag to label the number of targets ■ XS tag for assignment status for each alignment ■ XB tag for the bead barcode ■ XC tag for the cell barcode ■ XM tag is used for the UMI for each alignment
Filtered Count matrix	*A sparse matrix file with the extension .filtered.mtx.gz* The counts matrix captures the per-cell UMI counts for each gene and for each cell above the knee, where columns represent the row index, the column index, and the value at that coordinate. The row and column names are the gene names and cell barcodes, respectively, and stored in the accompanying .filtered.genes.tsv and .filtered.barcodes.tsv files.

Table 7. Pipeline outputs, continued

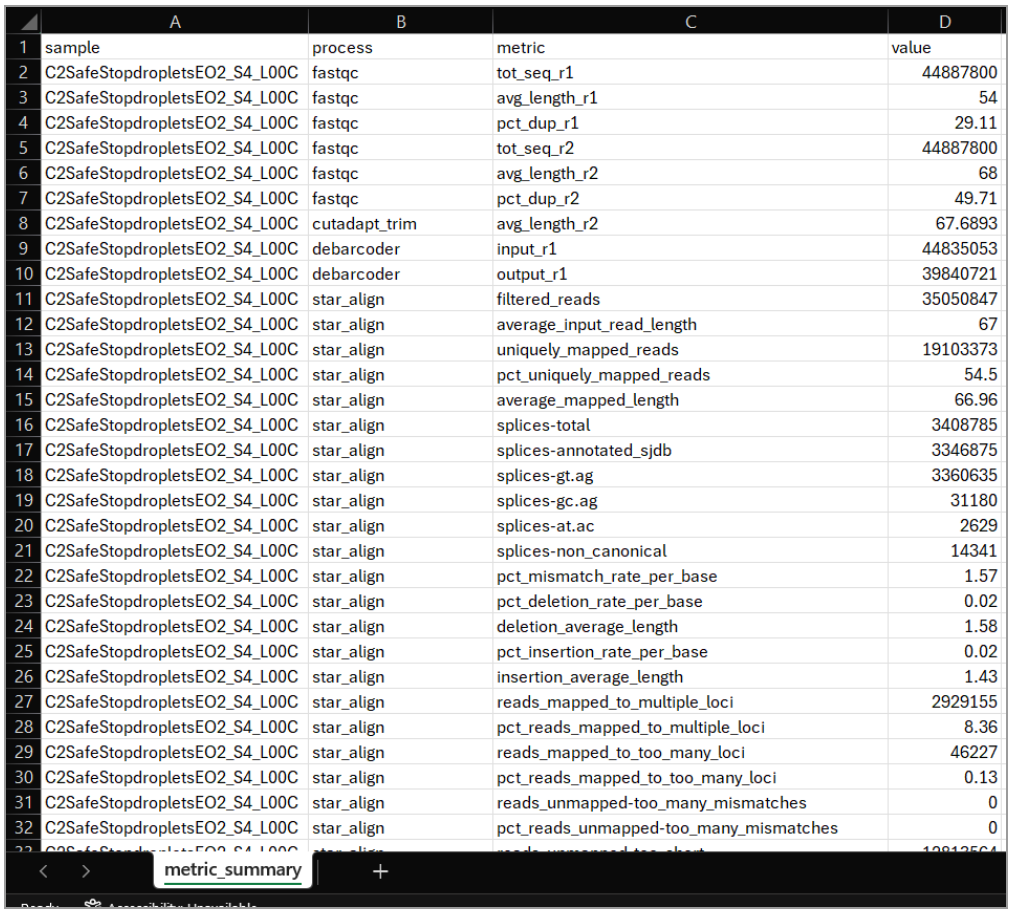
Output	Description
Unfiltered Count matrix	*A sparse matrix file with the extension .unfiltered.mtx.gz* The counts matrix captures the per barcode UMI counts for each gene and for all barcodes, where columns represent the row index, the column index, and the value at that coordinate. The row and column names are the gene names and cell barcodes, respectively, and stored in the accompanying .unfiltered.genes.tsv and .unfiltered.barcodes.tsv files.
Seurat files	*An RDS file for each sample containing the object generated by Seurat* The Seurat object contains information on the clustering and inter-cell distances for each sample.
H5AD files	*An H5AD file is created for each sample and a single H5AD file is created for all samples in the pipeline run* The H5AD files store the filtered cell matrix in AnnData format.
Software Configuration (YAML) file	Software Configuration (YAML) file. YAML is a data serialization language that is human readable and uses white-space indentation to indicate nesting in the formatted configuration file structure. For more information about setting up Omnition configurations, see the Omnition user guide

Reports Generated from an Omnition Pipeline Run

This section describes the files generated from an Omnition pipeline run that appear on the Reports tab.



Note: Files that appear with a .csv extension are metric summary files that contain metrics for all analyzed samples. Omnition tracks many metrics throughout the course of the pipeline that are recorded in this file.



	A	B	C	D
1	sample	process	metric	value
2	C2SafeStopdropletsEO2_S4_L00C	fastqc	tot_seq_r1	44887800
3	C2SafeStopdropletsEO2_S4_L00C	fastqc	avg_length_r1	54
4	C2SafeStopdropletsEO2_S4_L00C	fastqc	pct_dup_r1	29.11
5	C2SafeStopdropletsEO2_S4_L00C	fastqc	tot_seq_r2	44887800
6	C2SafeStopdropletsEO2_S4_L00C	fastqc	avg_length_r2	68
7	C2SafeStopdropletsEO2_S4_L00C	fastqc	pct_dup_r2	49.71
8	C2SafeStopdropletsEO2_S4_L00C	cutadapt_trim	avg_length_r2	67.6893
9	C2SafeStopdropletsEO2_S4_L00C	debarcoder	input_r1	44835053
10	C2SafeStopdropletsEO2_S4_L00C	debarcoder	output_r1	39840721
11	C2SafeStopdropletsEO2_S4_L00C	star_align	filtered_reads	35050847
12	C2SafeStopdropletsEO2_S4_L00C	star_align	average_input_read_length	67
13	C2SafeStopdropletsEO2_S4_L00C	star_align	uniquely_mapped_reads	19103373
14	C2SafeStopdropletsEO2_S4_L00C	star_align	pct_uniquely_mapped_reads	54.5
15	C2SafeStopdropletsEO2_S4_L00C	star_align	average_mapped_length	66.96
16	C2SafeStopdropletsEO2_S4_L00C	star_align	splices-total	3408785
17	C2SafeStopdropletsEO2_S4_L00C	star_align	splices-annotated_sjdb	3346875
18	C2SafeStopdropletsEO2_S4_L00C	star_align	splices-gt.ag	3360635
19	C2SafeStopdropletsEO2_S4_L00C	star_align	splices-gc.ag	31180
20	C2SafeStopdropletsEO2_S4_L00C	star_align	splices-at.ac	2629
21	C2SafeStopdropletsEO2_S4_L00C	star_align	splices-non_canonical	14341
22	C2SafeStopdropletsEO2_S4_L00C	star_align	pct_mismatch_rate_per_base	1.57
23	C2SafeStopdropletsEO2_S4_L00C	star_align	pct_deletion_rate_per_base	0.02
24	C2SafeStopdropletsEO2_S4_L00C	star_align	deletion_average_length	1.58
25	C2SafeStopdropletsEO2_S4_L00C	star_align	pct_insertion_rate_per_base	0.02
26	C2SafeStopdropletsEO2_S4_L00C	star_align	insertion_average_length	1.43
27	C2SafeStopdropletsEO2_S4_L00C	star_align	reads_mapped_to_multiple_loci	2929155
28	C2SafeStopdropletsEO2_S4_L00C	star_align	pct_reads_mapped_to_multiple_loci	8.36
29	C2SafeStopdropletsEO2_S4_L00C	star_align	reads_mapped_to_too_many_loci	46227
30	C2SafeStopdropletsEO2_S4_L00C	star_align	pct_reads_mapped_to_too_many_loci	0.13
31	C2SafeStopdropletsEO2_S4_L00C	star_align	reads_unmapped-too_many_mismatches	0
32	C2SafeStopdropletsEO2_S4_L00C	star_align	pct_reads_unmapped-too_many_mismatches	0

Omnition Analysis Software provides an HTML report that contains all samples processed in a pipeline run. The report includes the following sections:

- Experiment Summary Tab on page 63
- Analysis Statistics Tab on page 65
- Results Tab on page 69
- Deconvolution Tab on page 70
- ReadQC Tab on page 71

▶ To access tool tips, pause on the ⓘ icon.

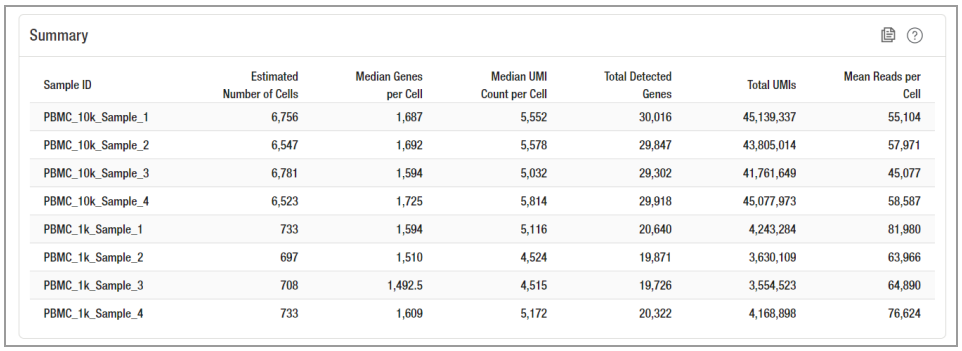
Follow the tabs on the top of the report page to view each section for a given sample. Switch between samples via the sample ID dropdown list found in the following sample-level sections:

- Analysis Statistics
- Results
- Deconvolution

Experiment Summary Tab

The Experiment Summary tab provides an experiment-level summary of key metrics, pipeline messages and configurations for all samples analyzed in the same Omnition Analysis Software run.

Summary Report



Summary

Sample ID	Estimated Number of Cells	Median Genes per Cell	Median UMI Count per Cell	Total Detected Genes	Total UMIs	Mean Reads per Cell
PBMC_10k_Sample_1	6,756	1,687	5,552	30,016	45,139,337	55,104
PBMC_10k_Sample_2	6,547	1,692	5,578	29,847	43,805,014	57,971
PBMC_10k_Sample_3	6,781	1,594	5,032	29,302	41,761,649	45,077
PBMC_10k_Sample_4	6,523	1,725	5,814	29,918	45,077,973	58,587
PBMC_1k_Sample_1	733	1,594	5,116	20,640	4,243,284	81,980
PBMC_1k_Sample_2	697	1,510	4,524	19,871	3,630,109	63,966
PBMC_1k_Sample_3	708	1,492.5	4,515	19,726	3,554,523	64,890
PBMC_1k_Sample_4	733	1,609	5,172	20,322	4,168,898	76,624

Table 8. Summary, single species

Table field	Tool tip
Estimated Number of Cells	Calculated cell count after quality control and deconvolution
Median Genes per Cell	Median number of genes detected per cell
Median UMI Counts per Cell	Median number of UMIs detected per cell
Total Genes Detected	Total number of genes detected per sample
Total UMIs	Total number of deduplicated aligned reads associated with cells
Mean Reads per Cell	Average number of sequenced read pairs per cell

Messages Field

Table 9. Status messages

Text box field	Tool tip
Messages	Status messages displayed by Omnition Analysis Software

Software Configuration Field

Table 10. Software configuration

Text box field	Tool tip
Software Configuration	Configuration (YAML) file used to perform the analysis for the data in this report

Analysis Statistics Tab

Barcode Rank Plot

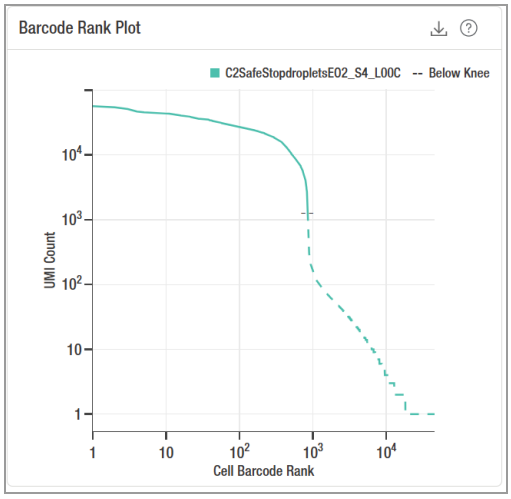


Table 11. Barcode Rank

Plot	Tool tip
Barcode Rank	Shows the distribution of UMIs across individual cell barcodes; The signal below the inflection point is assumed to be background.

Median Genes per Cell Plot

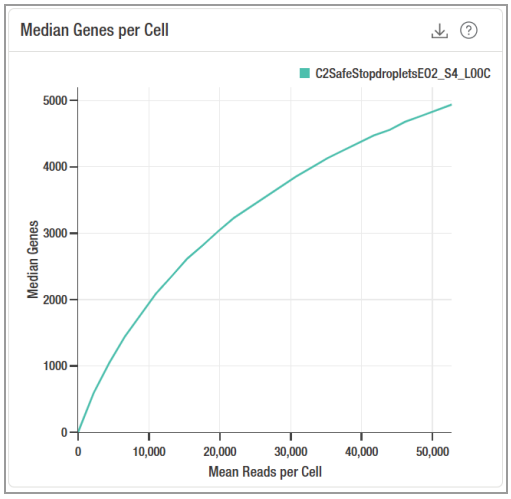


Table 12. Median Genes per Cell

Plot	Tool tip
Median Genes per Cell	Compares the number of median genes per cell to the mean reads per cell; this shows the effect of sequencing depth on median genes per cell, in order to assess data yield as a function of sequencing depth

Cell Metrics Tables

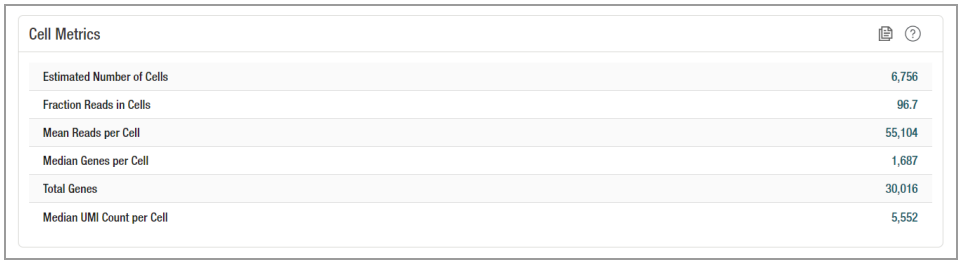


Table 13. Cell Metrics

Table field	Tool tip
Estimated Number of Cells	Calculated cell count after quality control and deconvolution
Fraction of Reads in Cells	Percentage of deduplicated read pairs that are associated with cells
Mean Reads per Cell	Average number of sequenced read pairs per cell
Median UMI Count per Cell	Median number of UMIs detected per cell
Median Genes per Cell	Median number of genes detected per cell
Total Genes Detected	Total number of genes detected per sample

Mapping Metrics Table

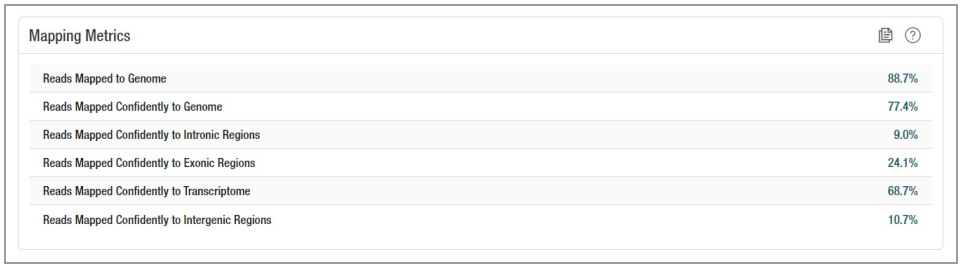


Table 14. Mapping Metrics

Table field	Tool tip
Reads Mapped to Genome	Percent of reads that mapped to the genome
Reads Mapped Confidently to the Genome	Percent of reads uniquely mapped to the genome
Reads Mapped Confidently to the Transcriptome	Percentage of reads uniquely mapped to the transcriptome
Reads Mapped Confidently to Intronic Regions	Percent of reads uniquely mapped to intronic regions of the genome
Reads Mapped Confidently to Exonic Regions	Percent of reads uniquely mapped to exonic regions of the genome
Reads Mapped Confidently to Intergenic Regions	Percent of reads uniquely mapped to intergenic regions of the genome
Reads Mapped Antisense to Gene	Percentage of reads that are mapped to the transcriptome but antisense to the gene and does not map to any sense genes

Results Tab

UMAP Cluster Plot

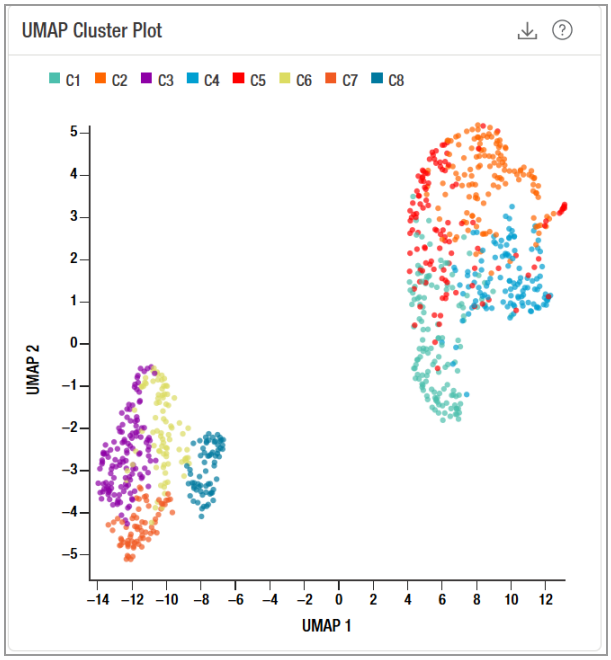


Table 15. UMAP

Plot	Tool tip
UMAP by Cluster	Identifies clusters based on differential gene expression **Note:** Clustering is done by embedding cells in a graph structure, with edges drawn between cells with similar feature expression patterns. The algorithm attempts to partition this graph into highly interconnected clusters.
UMAP by UMI Count	Shows the total UMI counts per cell

Top Features by Cluster Table

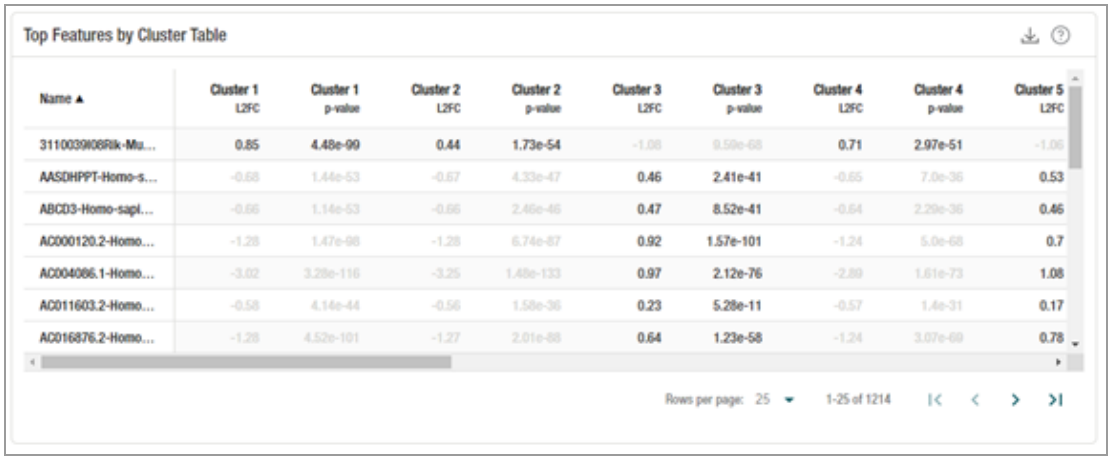


Table 16. Top Features by Cluster table

Table field	Tool tip
Top Features by Cluster	Highlights the top 250 differentially expressed genes per cluster by Log2 Fold-Change (L2FC) that have a p-value < 0.01. **Note:** Grayed out genes are genes with a L2FC < 0 or adjusted p-value >= 0.10.

Deconvolution Tab

Deconvolution Statistics Table

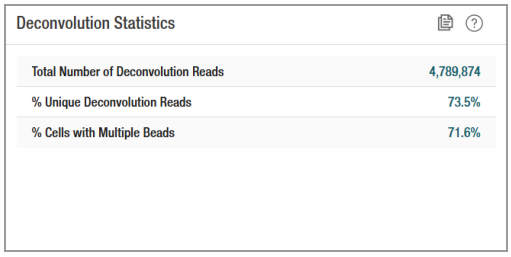


Table 17. Deconvolution Statistics

Table field	Tool tip
Number of Deconvolution Reads	Calculated number of reads used for grouping cDNA reads across beads to recreate cells
% Unique Deconvolution Reads	Percentage of unique deconvolution reads out of total deconvolution reads

Table 17. Deconvolution Statistics, continued

Table field	Tool tip
% Cells with Multiple Beads	Percentage of cells with reads that were split across multiple beads, and then merged to recreate the cell

Beads per Partition Plot

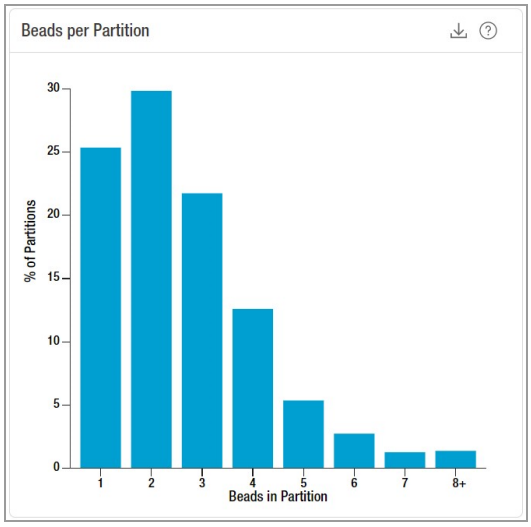


Table 18. Beads per Partition

Plot	Tool tip
Beads per Partition	Histogram showing the distribution of the bead barcodes contained in each partition.

ReadQC Tab

Sequencing Table

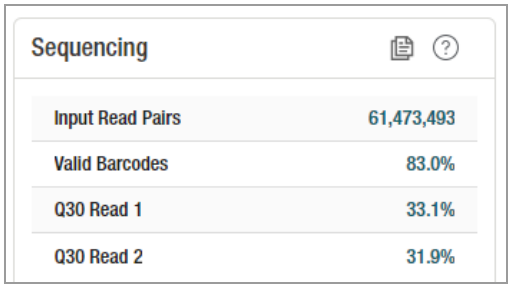


Table 19. Sequencing

Table field	Tool tip
Number of reads	Number of read pairs input into alignment
Valid Barcodes	Percent of read pairs containing complete barcode sequences
Average Q score for Read 1 (percent)	The average quality (Q) score across all bases for Read 1. A base call with a Q score of 30 has a base call accuracy of 99.9%.
Average Q score for Read 2 (percent)	The average quality (Q) score across all bases for Read 2. A base call with a Q score of 30 has a base call accuracy of 99.9%.

Pipeline Summary Table

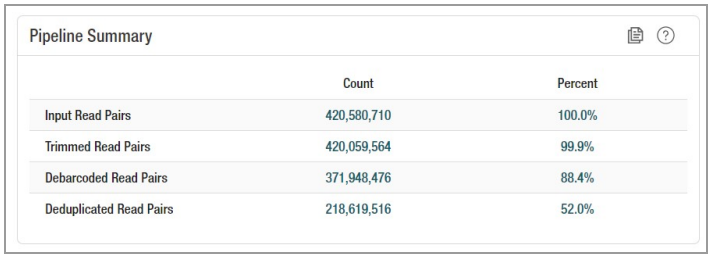


Table 20. Pipeline Summary

Table field	Tool tip
Input Read Pairs	Number of read pairs input into alignment
Trimmed Read Pairs	Read pairs that passed the trimming process that might have been trimmed
Debarcoded Read Pairs	Read pairs that have been assigned to at least one bead barcode
Mapped Read Pairs	Read pairs that mapped to the genome.
Deduplicated Read Pairs	Read pairs with distinct UMI.

Quality Score Plot

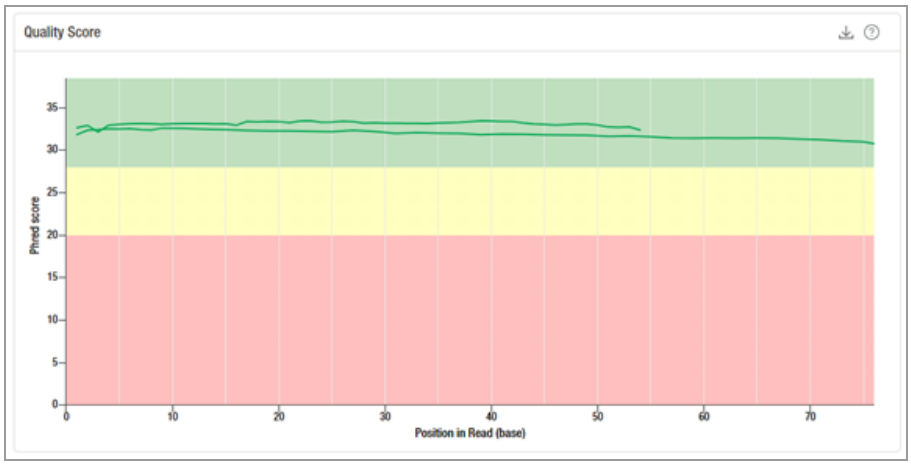


Table 21. Quality Score

Plot	Tool tip
Quality Score	Mean Phred quality score at each base position, as an indication of the base call accuracy

Sequence Trace Heat Map Plot

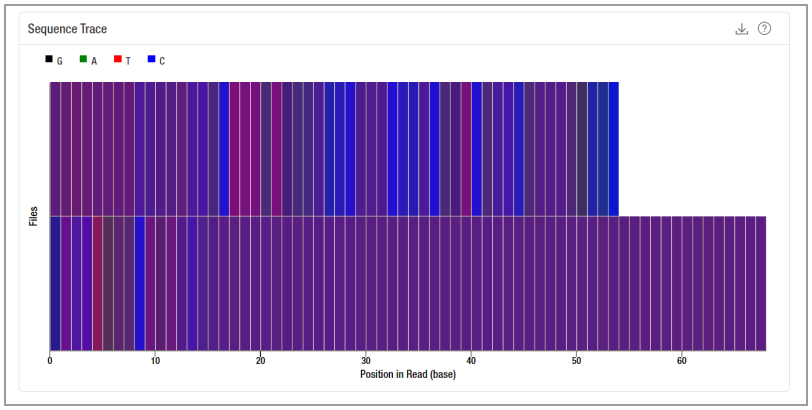
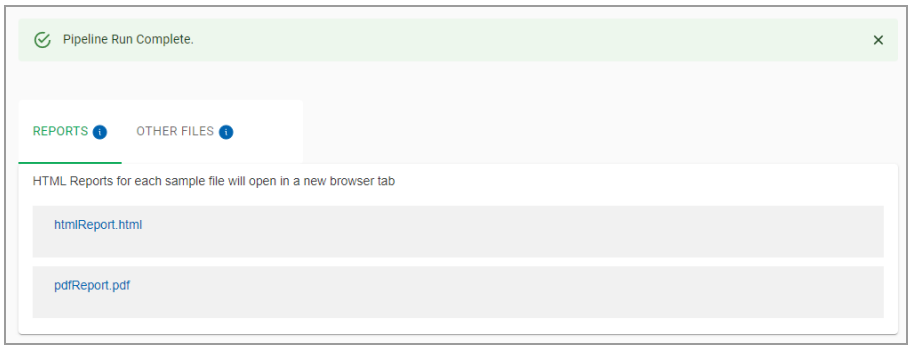


Table 22. Sequence Trace Heat Map

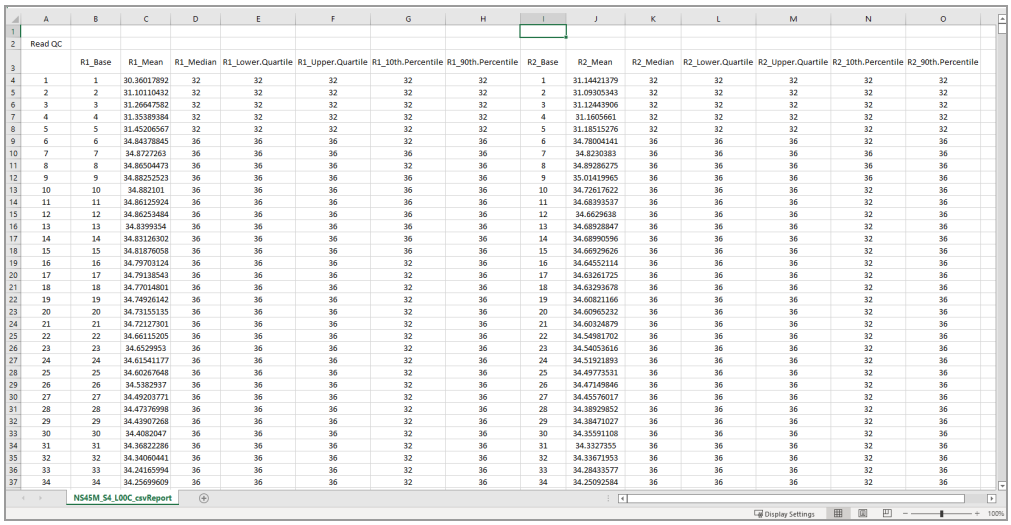
Plot	Tool tip
Sequence Trace Heat Map	Proportion of each of the four DNA bases being called at each base position

Reports Generated from a SEQuoia Complete or SEQuoia Express Pipeline Run

This section describes the files generated from a SEQuoia Complete or SEQuoia Express pipeline run that appear on the Reports tab.



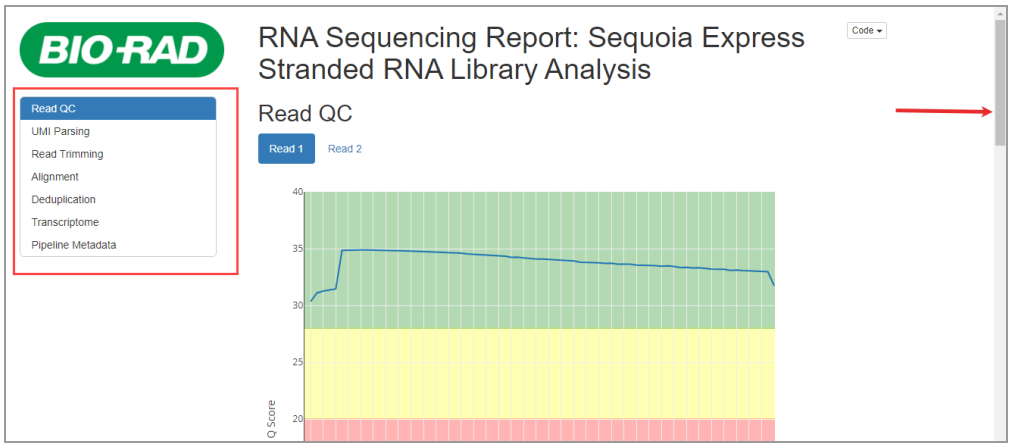
Note: Files that appear with a .csv extension contain raw data, as shown in the following example.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1															
2	Read QC														
3		R1_Base	R1_Mean	R1_Median	R1_Lower.Quartile	R1_Upper.Quartile	R1_10th.Percentile	R1_90th.Percentile	R2_Base	R2_Mean	R2_Median	R2_Lower.Quartile	R2_Upper.Quartile	R2_10th.Percentile	R2_90th.Percentile
4	1	1	30.36017892	32	32	32	32	32	1	31.14421379	32	32	32	32	32
5	2	2	31.10110432	32	32	32	32	32	2	31.09305343	32	32	32	32	32
6	3	3	31.26647582	32	32	32	32	32	3	31.12443906	32	32	32	32	32
7	4	4	31.35389384	32	32	32	32	32	4	31.1605661	32	32	32	32	32
8	5	5	31.45206567	32	32	32	32	32	5	31.18515276	32	32	32	32	32
9	6	6	34.84378845	36	36	36	32	36	6	34.78004141	36	36	36	32	36
10	7	7	34.8727263	36	36	36	36	36	7	34.8230383	36	36	36	36	36
11	8	8	34.86504473	36	36	36	32	36	8	34.89286275	36	36	36	36	36
12	9	9	34.88252523	36	36	36	36	36	9	35.01419965	36	36	36	36	36
13	10	10	34.882101	36	36	36	36	36	10	34.72617622	36	36	36	32	36
14	11	11	34.86125924	36	36	36	36	36	11	34.68393537	36	36	36	32	36
15	12	12	34.86253484	36	36	36	36	36	12	34.6629638	36	36	36	32	36
16	13	13	34.8399354	36	36	36	36	36	13	34.68928847	36	36	36	32	36
17	14	14	34.83126302	36	36	36	36	36	14	34.68990596	36	36	36	32	36
18	15	15	34.81876058	36	36	36	36	36	15	34.66929626	36	36	36	32	36
19	16	16	34.79703124	36	36	36	32	36	16	34.64552114	36	36	36	32	36
20	17	17	34.79138543	36	36	36	32	36	17	34.63261725	36	36	36	32	36
21	18	18	34.77014801	36	36	36	32	36	18	34.63293678	36	36	36	32	36
22	19	19	34.74926142	36	36	36	32	36	19	34.60821166	36	36	36	32	36
23	20	20	34.73155135	36	36	36	32	36	20	34.60965232	36	36	36	32	36
24	21	21	34.72127301	36	36	36	32	36	21	34.60324879	36	36	36	32	36
25	22	22	34.66115205	36	36	36	32	36	22	34.54981702	36	36	36	32	36
26	23	23	34.6529953	36	36	36	32	36	23	34.54053616	36	36	36	32	36
27	24	24	34.61541177	36	36	36	32	36	24	34.51921893	36	36	36	32	36
28	25	25	34.60267648	36	36	36	32	36	25	34.49773531	36	36	36	32	36
29	26	26	34.5382937	36	36	36	32	36	26	34.47149846	36	36	36	32	36
30	27	27	34.49203771	36	36	36	32	36	27	34.45576017	36	36	36	32	36
31	28	28	34.47376998	36	36	36	32	36	28	34.38929852	36	36	36	32	36
32	29	29	34.43907268	36	36	36	32	36	29	34.38471027	36	36	36	32	36
33	30	30	34.4082047	36	36	36	32	36	30	34.35591108	36	36	36	32	36
34	31	31	34.36822286	36	36	36	32	36	31	34.3327355	36	36	36	32	36
35	32	32	34.34060441	36	36	36	32	36	32	34.33671553	36	36	36	32	36
36	33	33	34.24165994	36	36	36	32	36	33	34.28433577	36	36	36	32	36
37	34	34	34.25699609	36	36	36	32	36	34	34.25092584	36	36	36	32	36

NS45M_S4_L00C_csvReport

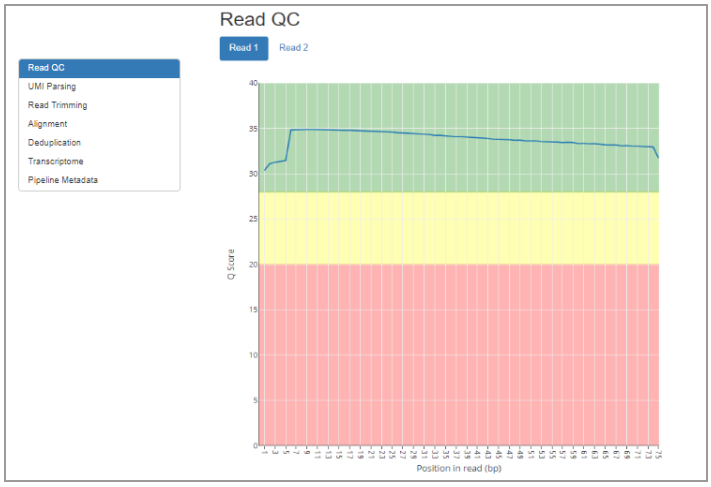
Files in .html and .pdf formats contain the same data as the .csv file, but in more organized displays. The following report example contains data for a paired end run. For Express or Complete kits, you can click a link to the report section on the left. For Express kits, you can also scroll through the report sections.



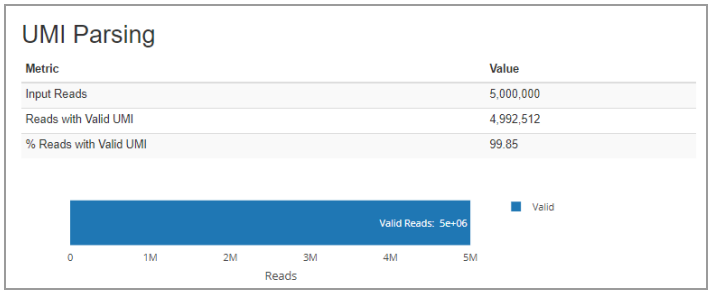
RNA Sequencing Report

The RNA Sequencing report contains the data described in the following sections.

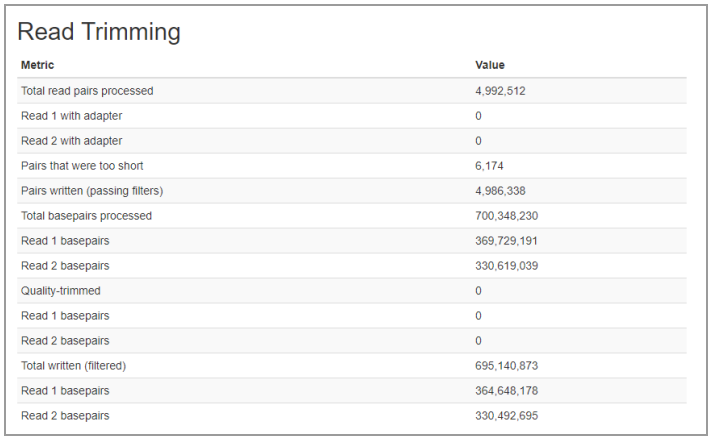
Read QC — measured quality for each read.



UMI (unique molecular identifier) parsing — measure of reads with acceptable UMIs, out of total input reads



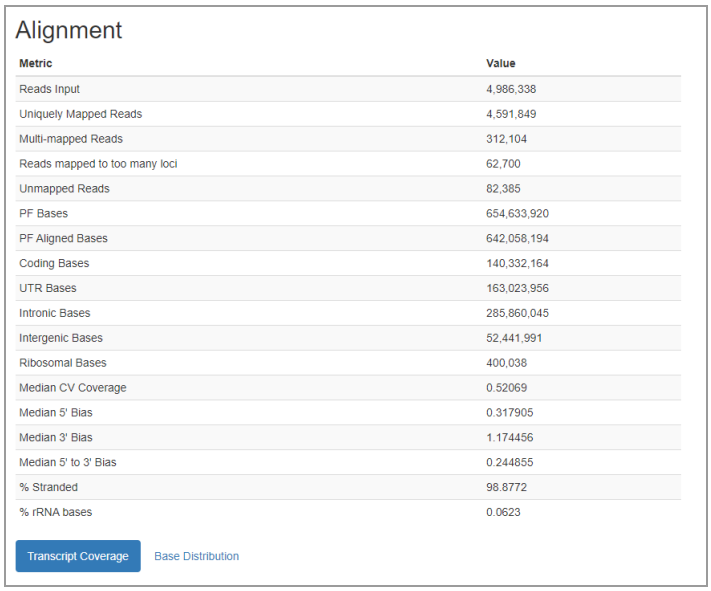
Read trimming — modification of raw read sequences in a pipeline



Read Trimming

Metric	Value
Total read pairs processed	4,992,512
Read 1 with adapter	0
Read 2 with adapter	0
Pairs that were too short	6,174
Pairs written (passing filters)	4,986,338
Total basepairs processed	700,348,230
Read 1 basepairs	369,729,191
Read 2 basepairs	330,619,039
Quality-trimmed	0
Read 1 basepairs	0
Read 2 basepairs	0
Total written (filtered)	695,140,873
Read 1 basepairs	364,648,178
Read 2 basepairs	330,492,695

Alignment — alignment statistics for the reads that pass read trimming and QC, against the specified genome

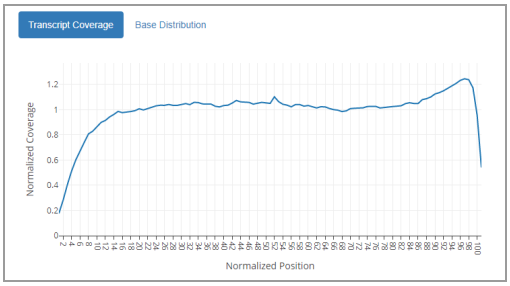


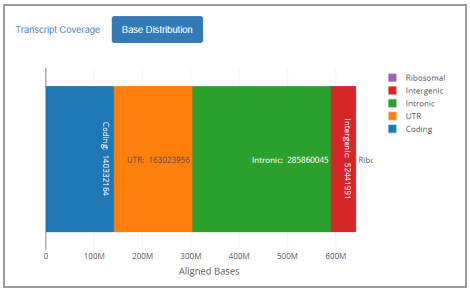
Alignment

Metric	Value
Reads Input	4,986,338
Uniquely Mapped Reads	4,591,849
Multi-mapped Reads	312,104
Reads mapped to too many loci	62,700
Unmapped Reads	82,385
PF Bases	654,633,920
PF Aligned Bases	642,058,194
Coding Bases	140,332,164
UTR Bases	163,023,956
Intronic Bases	285,860,045
Intergenic Bases	52,441,991
Ribosomal Bases	400,038
Median CV Coverage	0.52069
Median 5' Bias	0.317905
Median 3' Bias	1.174456
Median 5' to 3' Bias	0.244855
% Stranded	98.8772
% rRNA bases	0.0623

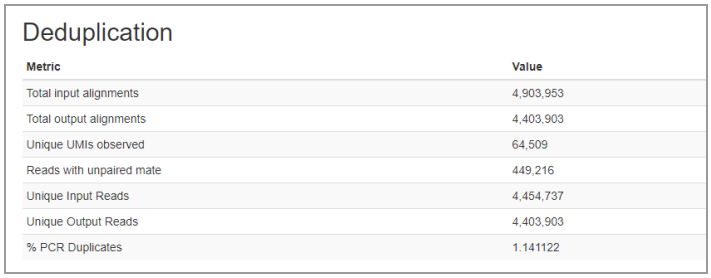
Transcript Coverage Base Distribution

Alignment Transcription Coverage or Base Distribution — transcript size and coverage by position, as well as location in the gene model of those reads





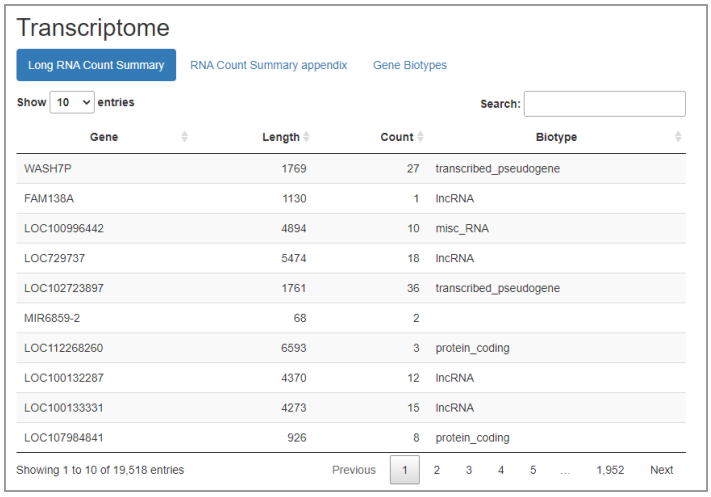
Deduplication — Deduplication statistics of the reads using the UMI to remove duplicates



Deduplication

Metric	Value
Total input alignments	4,903,953
Total output alignments	4,403,903
Unique UMIs observed	64,509
Reads with unpaired mate	449,216
Unique Input Reads	4,454,737
Unique Output Reads	4,403,903
% PCR Duplicates	1.141122

Transcriptome —readout of statistics for the genome, including counts for genes (raw), with the length and biotype, overall summary stats, and the breakdown of the gene biotypes (including a histogram)



Transcriptome

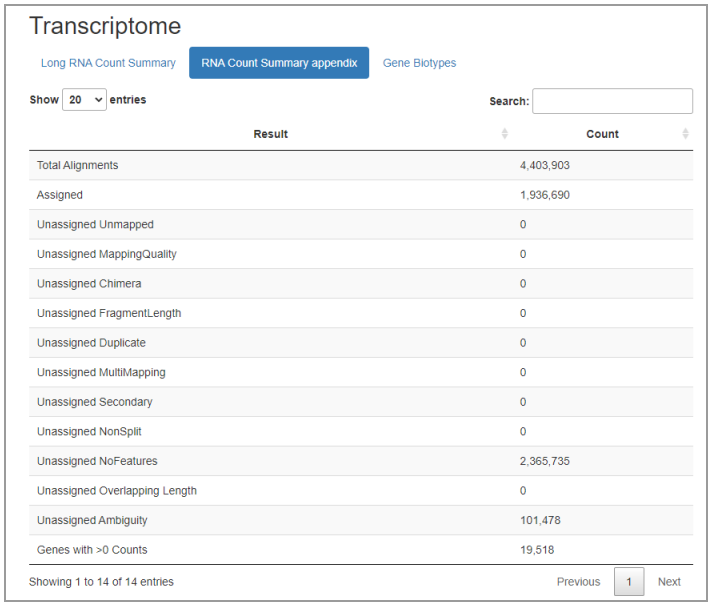
Long RNA Count Summary | RNA Count Summary appendix | Gene Biotypes

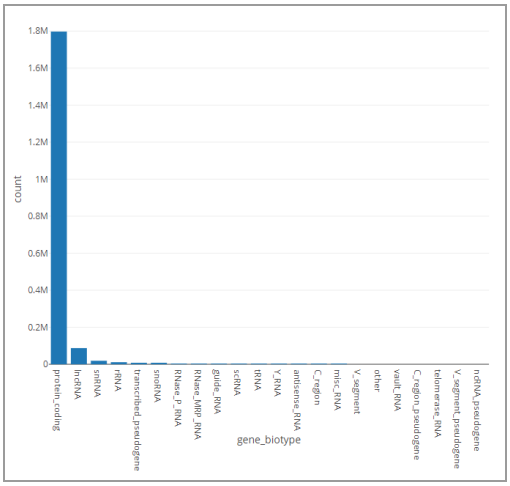
Show 10 entries Search:

Gene	Length	Count	Biotype
WASH7P	1769	27	transcribed_pseudogene
FAM138A	1130	1	lncRNA
LOC100996442	4894	10	misc_RNA
LOC729737	5474	18	lncRNA
LOC102723897	1761	36	transcribed_pseudogene
MIR6859-2	68	2	
LOC112268260	6593	3	protein_coding
LOC100132287	4370	12	lncRNA
LOC100133331	4273	15	lncRNA
LOC107984841	926	8	protein_coding

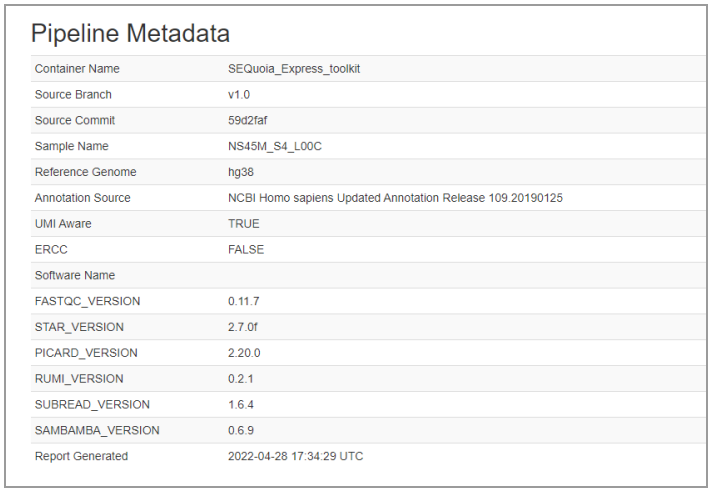
Showing 1 to 10 of 19,518 entries Previous 1 2 3 4 5 … 1,952 Next

Transcriptome (continued)





Pipeline metadata — metadata with the version of software and supporting files for the run



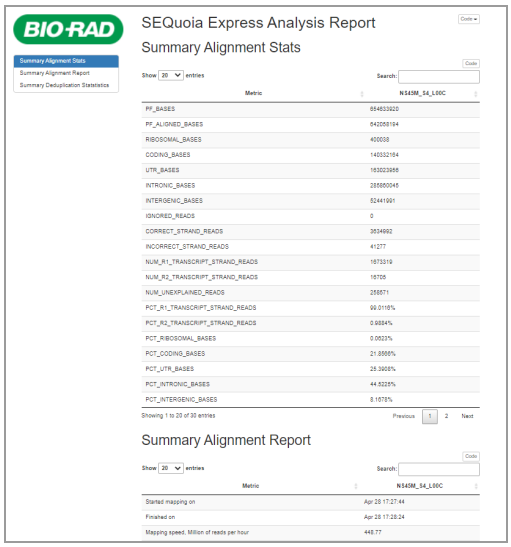
Pipeline Metadata

Container Name	SEQuoia_Express_toolkit
Source Branch	v1.0
Source Commit	59d2faf
Sample Name	NS45M_S4_L00C
Reference Genome	hg38
Annotation Source	NCBI Homo sapiens Updated Annotation Release 109.20190125
UMI Aware	TRUE
ERCC	FALSE
Software Name	
FASTQC_VERSION	0.11.7
STAR_VERSION	2.7.0f
PICARD_VERSION	2.20.0
RUMI_VERSION	0.2.1
SUBREAD_VERSION	1.6.4
SAMBAMBA_VERSION	0.6.9
Report Generated	2022-04-28 17:34:29 UTC

Batch Summaries

The batch summary reports contain data on alignment and deduplication. You can open a file of raw data or a formatted PDF, as shown below.

Note: Batch reports are available for the Express kit only.



BIO-RAD

Summary Alignment Stats
Summary Alignment Report
Summary Deduplication Statistics

SEQuoia Express Analysis Report

Summary Alignment Stats

Show 20 entries Search:

Metric	NS45M_S4_L00C
PF_BASES	654653920
PF_ALIGNED_BASES	642058194
RIBOSOMAL_BASES	400038
CODING_BASES	140332164
UTR_BASES	163023966
INTRONIC_BASES	285860046
INTERGENIC_BASES	52441991
IGNORED_READS	0
CORRECT_STRAND_READS	3634992
INCORRECT_STRAND_READS	41277
NUM_R1_TRANSCRIPT_STRAND_READS	1673319
NUM_R2_TRANSCRIPT_STRAND_READS	16706
NUM_UNEXPLAINED_READS	256571
PCT_R1_TRANSCRIPT_STRAND_READS	99.0116%
PCT_R2_TRANSCRIPT_STRAND_READS	0.9884%
PCT_RIBOSOMAL_BASES	0.0623%
PCT_CODING_BASES	21.8566%
PCT_UTR_BASES	25.3908%
PCT_INTRONIC_BASES	44.5225%
PCT_INTERGENIC_BASES	8.1678%

Showing 1 to 20 of 30 entries Previous 1 2 Next

Summary Alignment Report

Show 20 entries Search:

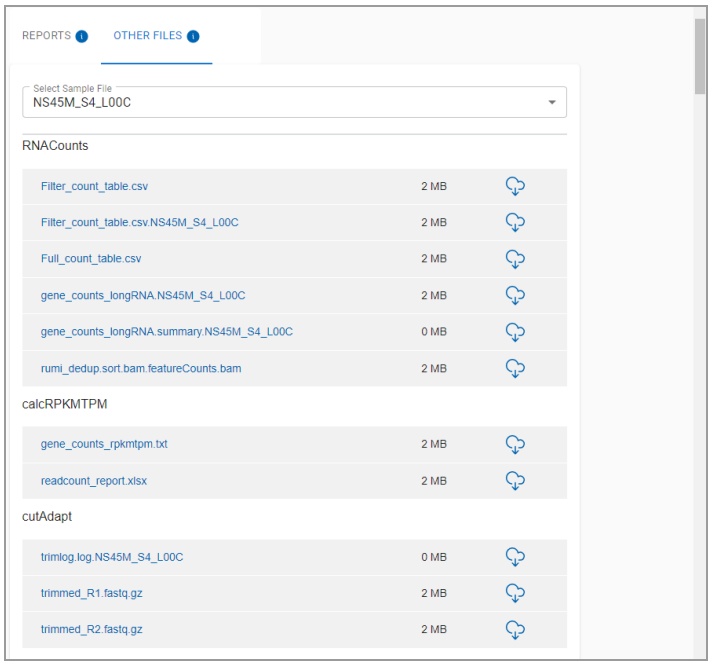
Metric	NS45M_S4_L00C
Started mapping on	Apr 26 17:27:44
Finished on	Apr 26 17:28:24
Mapping speed, Million of reads per hour	448.77

Other Files Generated from the Pipeline Run

This section describes files generated from the pipeline run that appear on the Other Files tab. For the genes sequenced in the experiment, files typically contain

- Counts by type
- Statistical quality data

Most files are downloaded when you click the link, and open in the native application.



Binary count data is displayed either in Notepad, in comma-delimited format or Excel, in tabular format. The following example shows long RNA data in a CSV file opened in Excel, and contains columns for the gene ID, chromosome ID, start, end, strand, length, and duplicates.

	A	B	C	D	E	F	G
1	# Program:featureCounts v1.6.4; Command:"featureCounts" "-p" "-T" "16" "--primary" "-M" "-t" "exon" "-g" "gene_id" "-s" "1" "-Q" "0" "-a" "hg38_longRNA_annotation.gtf" "-o" "./gene_counts_longRNA" "-R" "BAM" "rumi_dedup.sort.bam"						
2	**Geneid**	**Chr**	**Start**	**End**	**Strand**	**Length**	**rumi_dedup.sort.bam**
3	DDX11L1	chr1;chr1;chr1	11874;12613;13221	12227;12721;14409	+;+;+	1652	0
4	WASH7P	chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1	14362;14970;15796;16607;16858;17233;17606;17915;18268;24738;29321	14829;15038;15947;16765;17055;17368;17742;18061;18366;24891;29370	-;-;-;-;-;-;-;-;-;-;-	1769	27
5	MIR6859-1	chr1	17369	17436	-	68	0
6	MIR1302-2HG	chr1;chr1;chr1	29926;30564;30976	30039;30667;31295	+;+;+	538	0
7	MIR1302-2	chr1	30366	30503	+	138	0
8	FAM138A	chr1;chr1;chr1	34611;35277;35721	35174;35481;36081	-;-;-	1130	1
9	OR4F5	chr1	69091	70008	+	918	0
10	LOC100996442	chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1	89006;89006;89006;89006;89006;89006;92091;92091;92091;92091;92091;92091;112700;112700;120721;120775;120847;164705;164705;164705;164705;164705;164705;164705;165884;165884;165884;165884;165884;165884;165884;165884;165884;165884;165884;167959;167959;167964;167964;168100;168100;168100;168100;168100;168100;168100;168610;169049;169049;169049;169049;169049;169049;169049;172557;172557;172557;172557;172557;172557;172557;173753;173753;173753;173753;173753;173753;173753;173753;176076;181037;267303	91629;91629;91629;91629;91629;91629;92240;92240;92240;92240;92240;92240;112804;112804;120814;120798;120932;164791;164791;164791;164791;164791;164791;164791;165942;165942;165942;165942;165942;165942;165942;165942;165942;165942;165942;168165;168165;168093;168093;168165;168165;168165;168165;168165;168165;168165;168649;169264;169264;169264;169264;169264;169264;169264;172688;172688;172688;172688;172688;172688;172688;174412;174410;173862;174659;173862;174412;174414;174659;176116;181130;267354	-;-	4894	10
11	LOC729737	chr1;chr1;chr1	134773;139790;140075	139696;139847;140566	-;-;-	5474	18
12	LOC102725121	chr1;chr1;chr1	182388;183132;183740	182746;183240;184878	+;+;+	1607	0
13	LOC102723897	chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1	184878;185491;186317;187129;187380;187755;188130;188439;188791;195263;199837	185350;185559;186469;187287;187577;187890;188266;188584;188902;195416;199860	-;-;-;-;-;-;-;-;-;-;-	1761	36
14	MIR6859-2	chr1	187891	187958	-	68	2
15	LOC107985721	chr1;chr1;chr1	200442;201078;201496	200555;201181;203983	+;+;+	2706	0
16	LOC112268260	chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;	350706;350706;350706;355425;356685;357515;357515;357515;357515;357515;357515;358049;358049;358049;358049;358049;358049;358049;358284;359140;359345;365565;365565;365565;368251;368251;373144;373144;373144;379769;379769;379769;380897;399041;39	351039;351039;351039;356652;357586;357586;357586;357586;357586;357586;357586;358183;358183;358183;358183;358183;358183;358183;358325;359319;359515;365692;365692;365692;368380;368380;373323;373323;373323;37987	-;-	6593	3

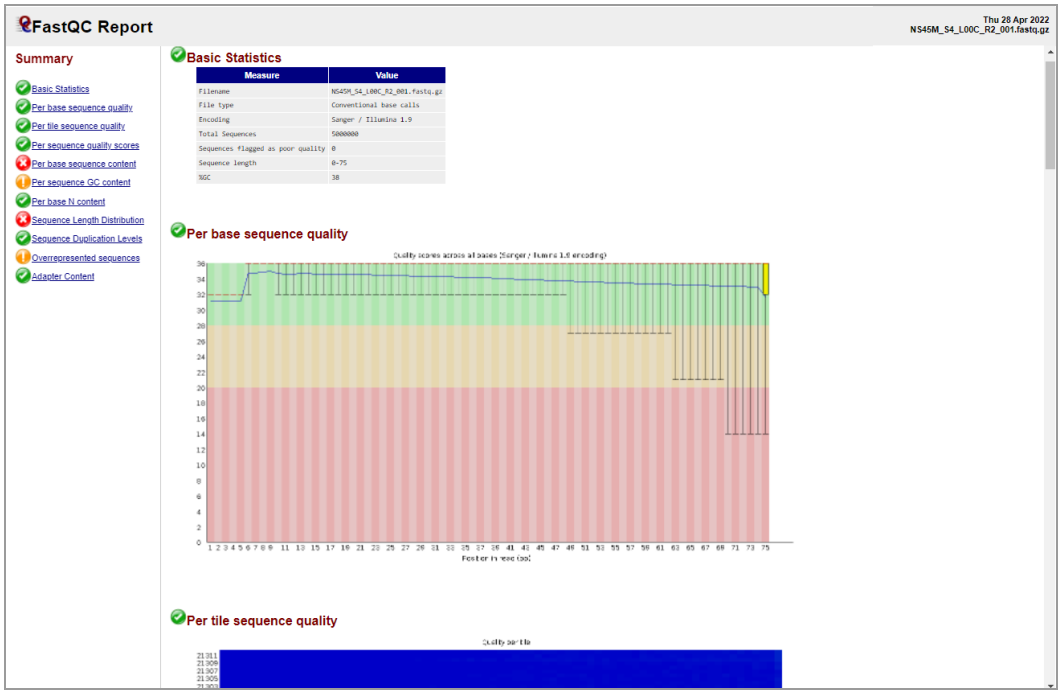
The following example shows, in Notepad comma-delimited format, filtered counts for each gene, count number and expression units.

Filter_count_table.csv (2).NS45M_S4_L00C - Notepad

File Edit Format View Help

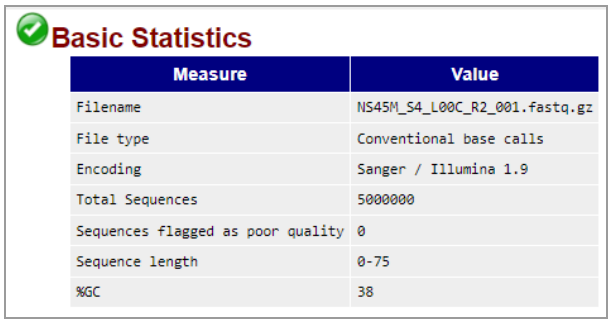
```
"Gene","Count","RPKM","TPM"
"A1BG",0,0,0
"A1BG-AS1",1,0.242415444947368,0.710100350678859
"A1CF",2,0.10837336504101,0.31745487395235
"A2M",985,93.9068914829809,275.07866335705
"A2M-AS1",1,0.224497781625171,0.657614672585205
"A2ML1",36,2.78686901327798,8.16349248726461
"A2MP1",0,0,0
"A3GALT2",0,0,0
"A4GALT",16,2.06074291938296,6.03647292370554
"A4GNT",0,0,0
"AA06",0,0,0
"AAAS",33,8.89784941271565,26.0642055609488
"AACS",18,2.71126259022231,7.94202084160661
"AACSP1",0,0,0
"AADAC",0,0,0
"AADACL2",0,0,0
"AADACL2-AS1",0,0,0
"AADACL3",5,0.636676815953012,1.86499845492721
"AADACL4",0,0,0
"AADACP1",0,0,0
"AADAT",4,0.689839542735996,2.02072644882561
"AAGAB",47,6.4732489180264,18.9618954671807
"AAK1",508,12.3979395968639,36.316915604696
"AAMDC",3,0.829691854961799,2.43039166622277
"AAMP",53,13.4610327496844,39.4310027487144
"AANAT",0,0,0
"AAR2",33,5.32148083240177,15.5880554807049
"AARD",0,0,0
```

You can also open some files in HTML format. The files are scrolling displays that open in your browser and contain links in the left panel to jump directly to the corresponding chart. The following graphic shows a scrollable HTML FastQC report.



The report contains the statistical displays described below.

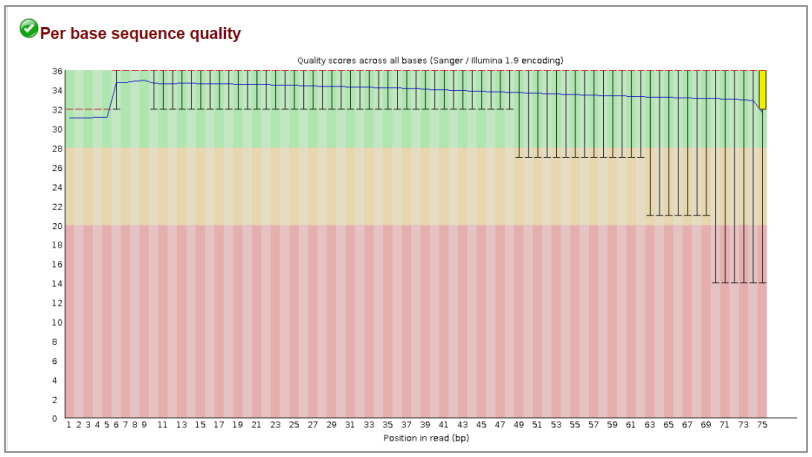
Basic statistics — contains general information on the RNA sequences.



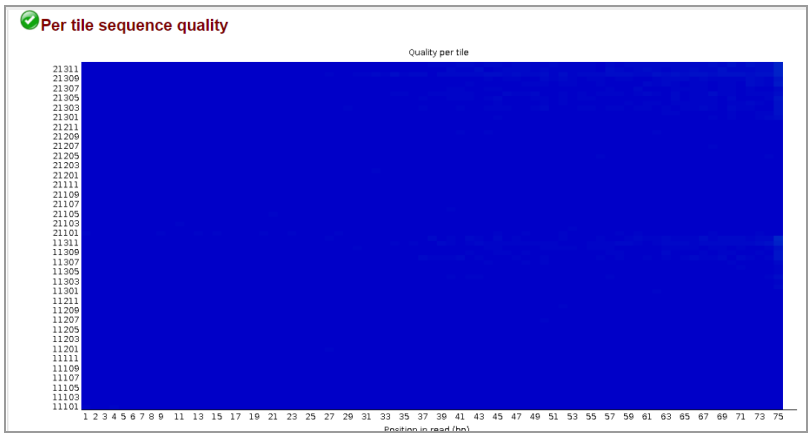
Basic Statistics

Measure	Value
Filename	NS45M_S4_L00C_R2_001.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	5000000
Sequences flagged as poor quality	0
Sequence length	0-75
%GC	38

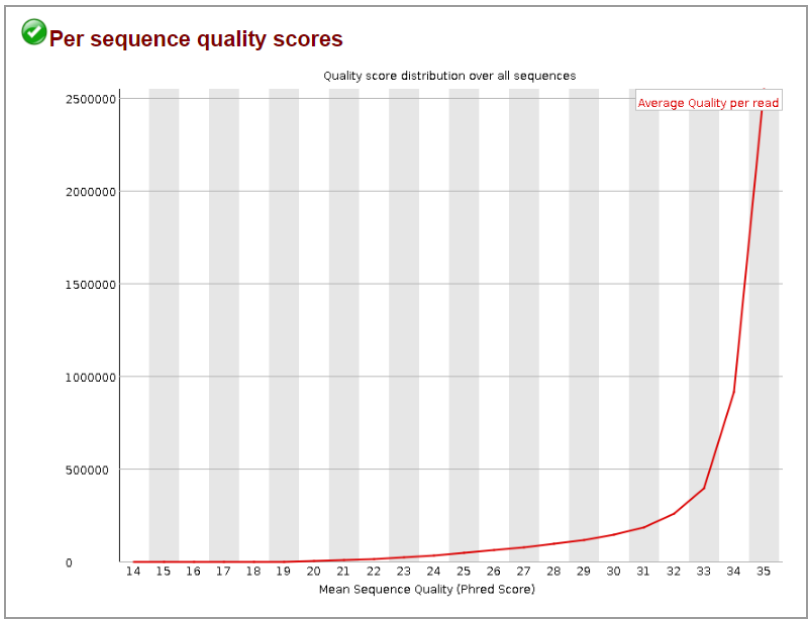
Per base sequence quality — represents quality values across all bases at each position in the FASTQ file.



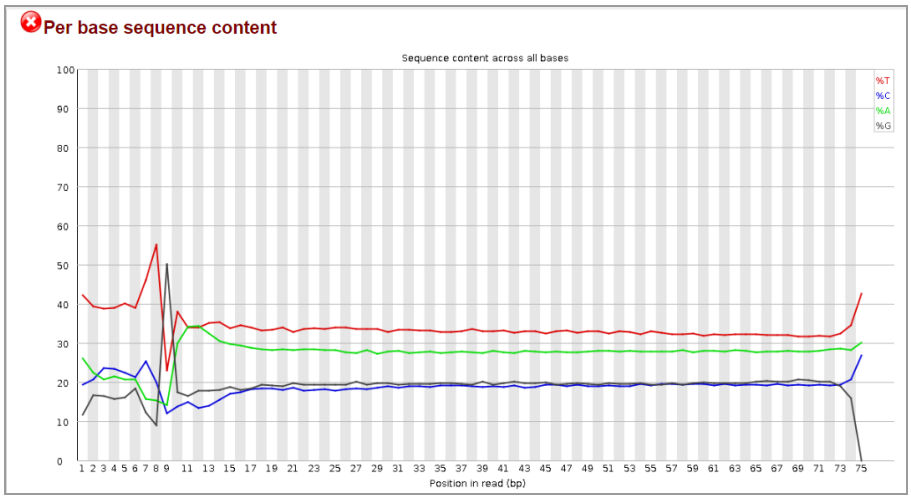
Per tile sequence quality — shows the deviation from the average quality for each tile.



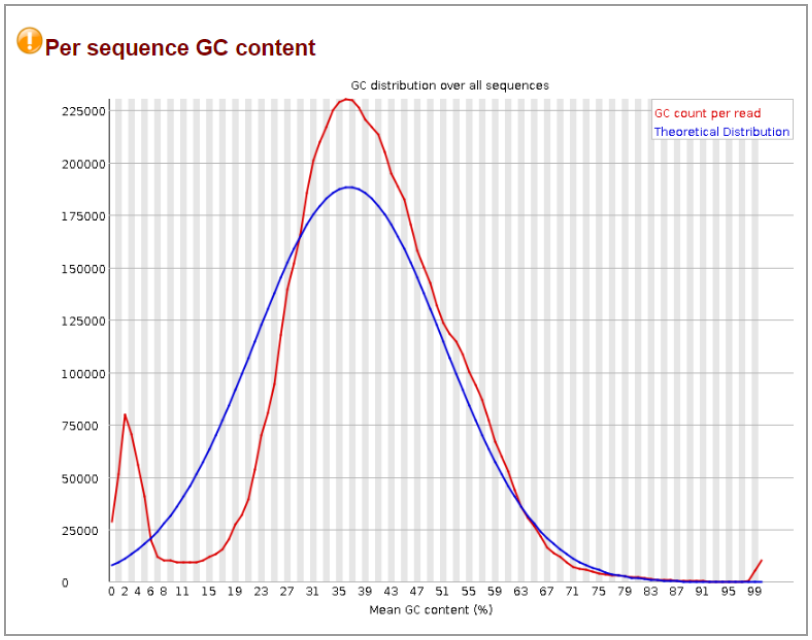
Per sequence quality scores — tells you if a subset of the sequences has universally low-quality values.



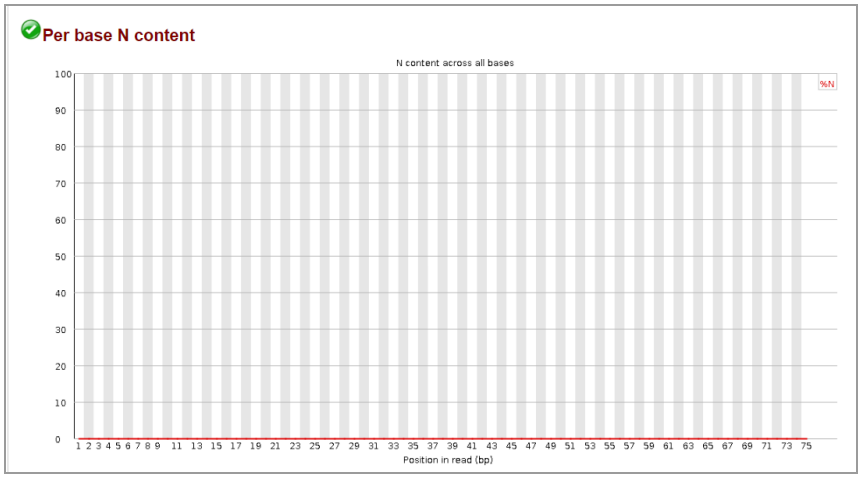
Per base sequence content — shows the proportion of each base position where each of the four normal DNA bases has been called.



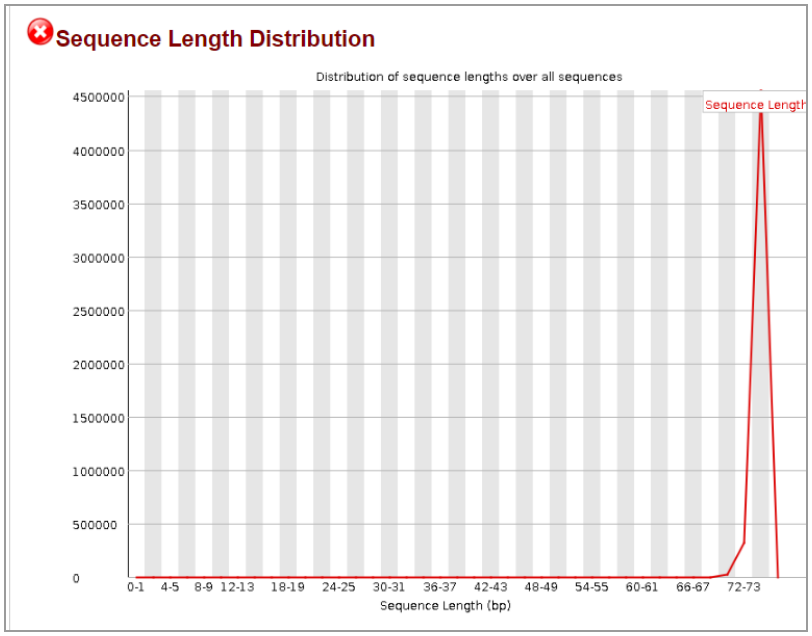
Per sequence GC content — measures the GC content across the entire length of each sequence in a file and compares it to a modelled normal distribution of GC content.



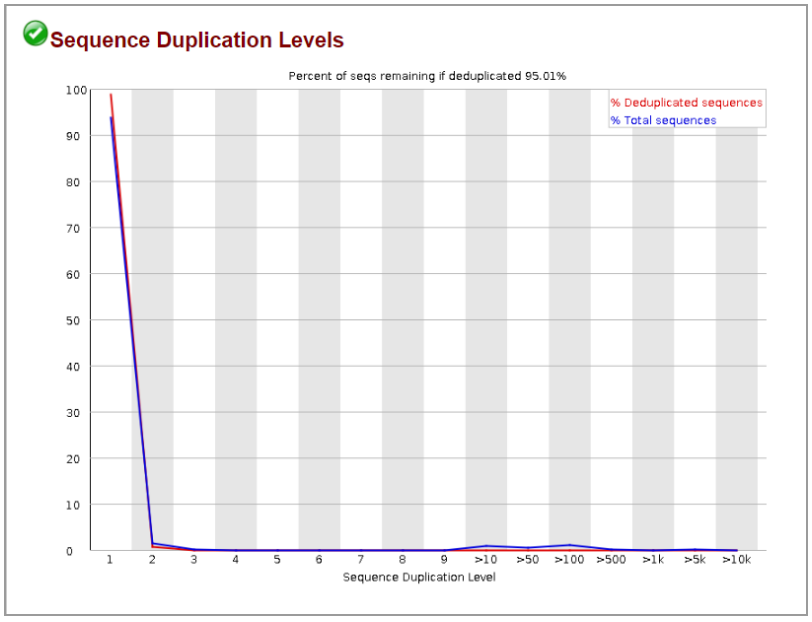
Per base N content —plots out the percentage of base calls at each position for which an N was called.



Sequence length distribution — sequence fragments of either uniform or varying length distributions, which can be used to measure quality.



Sequence duplication levels — for every sequence in a library, plots the relative number of sequences with different degrees of duplication.



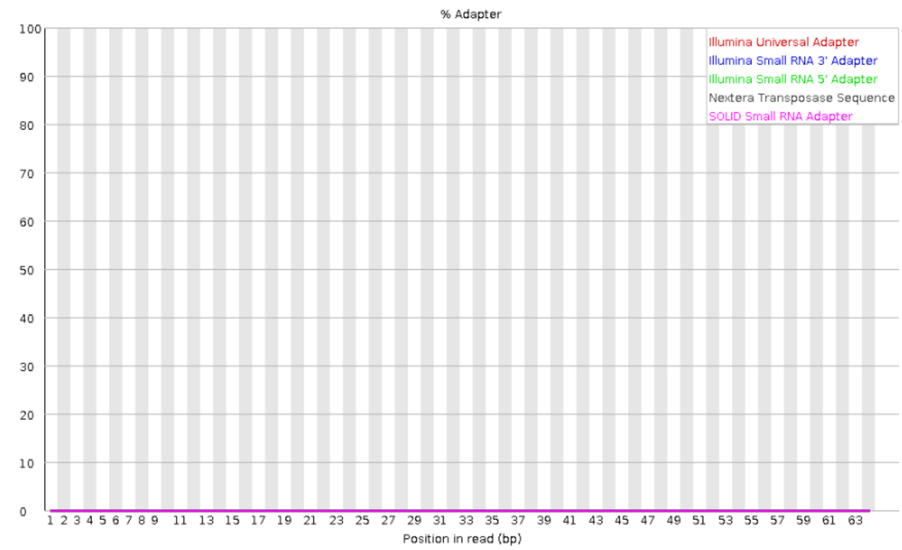
Overrepresented sequences — a list of sequences that appear more than expected in a file.

Overrepresented sequences

Sequence	Count	Percentage	Possible Source
GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	9795	0.1959	No Hit

Adapter content — a cumulative plot of the fraction of reads, where the sequence library adapter sequence is identified at the indicated base position.

Adapter Content



Appendix A Glossary

Table 23 contains definitions for terms used in this document.

Table 23. SeqSense terms

Term	Definition
BAM	Binary sequence Alignment Map Formatted file that stores biological sequences aligned to a reference sequence. For more information, see the Illumina BAM File Format page.
Deconvolution	Identification and assignment of a barcode multiplet to a single-cell data point.
FASTQ	File format, produced by sequencing instruments, which contains read names, base calls, and Phred +33 quality scores encoded as ASCII characters; for more information, see the Illumina FASTQ File Format page.
Feature	Unique type of countable molecule (genes, proteins, and so forth).
H5AD	Hierarchical Data Format 5 AnnData specification A format for efficiently reading from and writing to large datasets without loading into RAM.
Secondary Analysis	Processing FASTQ files to a filtered matrix of cell and feature count. This includes quality metrics.
Tertiary Analysis	Any analysis to form biological conclusions.
UMAP	Uniform Manifold Approximation and Projection A method for dimensionality reduction. For the purposes of this software, UMAP is utilized to cluster cells .
UMI	Unique Molecular Identifier An index added to original template molecules before PCR amplification, and used to identify unique molecules.
YAML	YAML Ain't Markup Language; a human-friendly data serialization language.

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
Canada *1 800 361 1808* ***China*** *86 21 6169 8500* ***Czech Republic*** *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23* ***Finland*** *00 800 00 24 67 23*
France *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 080 007 7373* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23*
Russian Federation *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*

10000124696 Ver D (US/EG)

0225 Sig 0125