# **SeqSense Analysis Solution**

**User Guide** 

Version 2.1



# **SeqSense Analysis Solution**

**User Guide** 

Version 2.1

### **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 click the Contact us link at <u>www.bio-rad.com</u>.

### **Legal Notices**

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage or retrieval system, without permission in writing from Bio-Rad Laboratories, Inc.

Bio-Rad reserves the right to modify its products and services at any time. This guide is subject to change without notice. Although prepared to ensure accuracy, Bio-Rad assumes no liability for errors or omissions, or for any damage resulting from the application or use of this information.

BIO-RAD is a trademark of Bio-Rad Laboratories, Inc.

All trademarks used herein are the property of their respective owner.

Copyright © 2023 by Bio-Rad Laboratories, Inc. All rights reserved.

### **Revision History**

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

iv | SeqSense Analysis Solution

# Table of Contents

Front Cover	i
Revision History	iii
Chapter 1 Getting Started	
Recommended Browsers	
Prerequisites	
Creating a Bio-Rad Website Account	
Creating a User Account in SeqSense	
Signing In	14
Downloading User Documents	
Viewing Your User Profile	
Resetting Your Password	
Logging Out	
Chapter 2 SeqSense Analysis Solution Overview	
Home Page	
Experiments Page	
Samples Page	
Tag Management Page	
Adding Tags in the Tag Management Page	
Adding Tags in the Experiments and Samples Pages	
Removing Tags	
SeqSense Tools	
Searching for Files	
Downloading a List in .CSV Format	
Printing Files and Metadata	
Showing or Hiding Columns	
Filtering Files	
Deleting Experiments and Samples	
Help and Support	

Chapter 3 Creating an Experiment	
Naming the Experiment and Selecting a Prep Kit	
Adding Samples to Your Experiment	
Naming Your Samples	
Uploading Samples	
Selecting Existing Samples	44
Adding or Assigning Tags During Experiment Setup	45
Setting the Pipeline Parameters	46
Starting the Pipeline Run	
Chapter 4 Reporting and Analysis	51
Opening a File from a Completed Pipeline Run	
Reports Generated from the Pipeline Run	
RNA Sequencing Report	54
Batch Summaries	
Other Files Generated from the Pipeline Run	
Back Cover	67

# **Chapter 1 Getting Started**

SeqSense Analysis Solution is a purpose-built workflow for analyzing and visualizing the next-generation sequencing (NGS) data. SeqSense uses a bioinformatics pipeline that is 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. You can run the SeqSense analysis pipeline using 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 click the Contact us link at <a href="https://www.bio-rad.com">https://www.bio-rad.com</a>.

### **Recommended Browsers**

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

Chapter 1 Getting Started

### **Prerequisites**

To use SeqSense Analysis Solution, 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)

**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 Analysis Solution 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 Analysis Solution 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.

Sign In		Register	
Username		$\sim$	
1		Manage Your Orders	:
Please enter a username	Forgot Username		
Password			
		D 12	
	Forgot password?	NUM year color reaction any and	
Sign In		Benefits of Registration	
		Account pricing     Invoices	
		Order status     Online quote re	quests
		Tracking numbers     Hot lists (wish	ists)
		Product availability     Literature requ	ests

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

Registration	8
* Indicates required field.	
* User Name:	* Location:
	USA
* Password:	* Confirm Password:
٢	

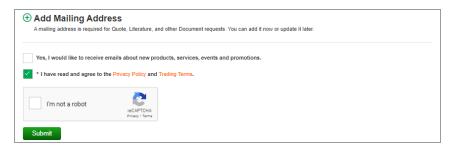
- 4. Complete the Registration section, as follows:
  - a. Enter a unique user name. You will enter this user name when you create your SeqSense account.
  - b. The location defaults to USA. Select a different location where applicable.
  - c. Enter and confirm a password. Your password must contain 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.

Contact Information	
Title:	
Select one 🗸	
* First Name:	* Last Name:
• Email Address:	Confirm Email Address:
* Company:	Daytime Phone: (including area code)     Ext:

6. .Optionally, enter the remaining information.

Job Roles:	Interests:	
Select one	Life Science Research	*
Area of Research:	Industry:	
<b>•</b>	Select one	~

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.
- 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.

Welcome to Bio-Rad Inbox x
noreply@bio-rad.com to me
Welcome to Bio-Rad
Dear John Smith
Thank you for registering for a Bio-Rad online account.
$eq:Please keep this email for your reference. Your Bio-Rad online account username is \ JohnSmith.$
As a registered user, you have quick access to online technical support, saved hot lists, software downloads, and more.
Please do not reply to this email. If you have any questions, call us at 1-800-4-BIORAD (1-800-424-6723).
Thank you, The Bio-Rad Web Services Team

### Creating a User Account in SeqSense

Complete the steps below to create a SeqSense Analysis Solution user account.

#### To create a SeqSense user account

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

The SeqSense Analysis Solution landing page appears.

BIO-RAD SeqSense	Login   New User Form
SeqSense Analys Solution Make sense of your sequencing data The SeqSense Web Application offers a user-friendly in that brings transparency and efficiency to data analysis	

2. Click New User Form.

New User Please complete the form below to gain access to SeqSense. You'll receive a confirmation email once your SeqSense account has been activated. All fields are required.	
Point Name John	Smith
Bio-Rad Account Namber	_EAsi Addess John_smith@company.com
Usename frem Bio-radiosen account john_smith (Enter usename created from Bio-radiosen account page)	GET ACCESS

- 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
- User name

**Note:** You must enter the email address and Bio-Rad user name 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.

	Welcome to SeqSense Inbox ×
•	Seqsense Support <seqsense-support@bio-rad.com> to me 💌</seqsense-support@bio-rad.com>
	Dear Customer,
	Your SeqSense Analysis Solution Account has been activated. SeqSense Platform   Home (bio-rad.com)
	Here are some resources that you may be interested in:
	SeqSense NGS Data Analysis Software   Bio-Rad     SeqSense Analysis Solution User Guide (bio-rad.com)
	Thank You,
	SeqSense Product Team

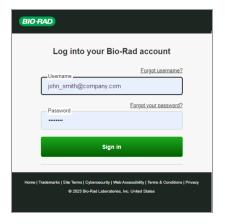
### Signing In

#### To sign into SeqSense Analysis Solution

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



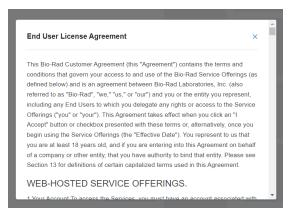
3. When the Login page appears, enter your email address and password and click Sign in.



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

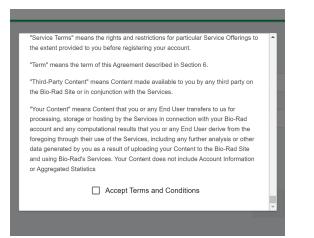
On first use, the Terms and Conditions appear in a scrollable pop-up.

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.



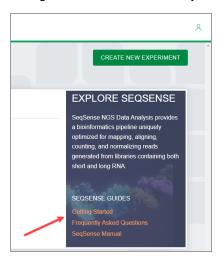
The pop-up closes and the application opens to the Home page.

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

BIO RAD	SeqSense	Link to user profile $\longrightarrow \Re$ and Logout option
	Tabs to SeqSense functional pages	CREATE NEW EXPERIMENT
SAMPLES Tras HELP & SUPPORT K	WELCOME TO SEQSENSE           Your most recent experiments will display here for quick and easy access.           To view all of your experiments, go to "Experiments" in the left navigation panel.	EXPLORE SEQSENSE SeqSense NGS Data Analysis provides a bioinformatics pipeline uniquely optimized for mapping, aligning, courting, and normalizing reads generated from libraries containing both short and long RNA SEQSENSE GUIDES Getting Started Frequently Asked Questions SeqSense Manual

### **Downloading User Documents**

Before you create your first experiment, the Explore SeqSense menu appears in the right pane and associated SeqSense Analysis Solution user documents are linked under SeqSense Guides. The pane is unavailable after you create your first experiment; therefore Bio-Rad recommends downloading and storing each document immediately after you log in.



### **Viewing Your User Profile**

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

BIO RAD	SeqSense	8	

#### To view your user profile

1. Click the  $\stackrel{\text{$>}}{\xrightarrow{}}$  icon to display the pop-up.



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

Profile Information
Name: John Smith User Name: john_smith@company.com Email: john_smith@company.com
Forgot Password?

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

## **Resetting Your Password**

You can reset your password from the Login page.

BIO RA	۵
	Log into your Bio-Rad account
	Username Eorgot username?
	john_smith@company.com
	Password EorgoLyour.password2
	Sign in
Home   Trad	emarks   Sile Terms   Cybenaecurky   Web Accessibility   Terms & Conditions   Privacy © 2023 Bio-Rad Laboratories, Inc. United States

#### To reset your password

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

The Forgot Password page appears.

Forgot Password			
Verify Your Identity			
Enter your username and click Contin	ue.		
* Username:			
john_smith@company.com			
I'm not a robot	reCAPTCHA Privacy - Tarma		
Continue Forgot Username			

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.

Forgot Password		
Verify Your Identity		
Enter your username and click Con	tinue.	
* Username:		
john_smith@company.com		
✓ I'm not a robot	reCAPTCHA Privacy - Terms	
Continue		

4. Click Continue.

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

Forgot Password	
if you have entered a valid username, you will receive an email with your password reset instructions. Please check your email and click on the link it contains to complete the password reset process.	

### **Logging Out**

#### To log out of SeqSense

1. Click the  $\stackrel{ ext{$\wedge$}}{\to}$  icon in the upper-right corner to display the pop-up.



2. Click Logout.

You are immediately logged out of SeqSense.

Chapter 1 Getting Started

# Chapter 2 SeqSense Analysis Solution Overview

Use SeqSense Analysis Solution 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:

П НОМЕ	
EXPERIMENTS	
SAMPLES	
TAGS	
HELP & SUPPORT	
<	

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

### **Home Page**

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

			CREATE NEW EXPERIM
RECENT EXPERIMENTS	STATUS	UPDATED	TAGS
Experiment No_04	COMPLETED	9/15/2022	MARKER2 × 🕀
TestExperiment_SEQuoiaComplete	COMPLETED	9/15/2022	
Experiment_No_03	COMPLETED	8/23/2022	
Experiment_No02	COMPLETED	8/23/2022	MARKER4 × 🕀
Experiment_No_01	Not Started	8/23/2022	MARKER3 × +

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, in progress, completed, failed)
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.

BIO RAD	SeqSense					٨
п номе	Experiments					CREATE NEW EXPERIMENT
					Q Search	<b>6</b> ⊕ ₩ ₹
TAGS	Name	Prep Kit Type	Status	Created $\downarrow$	Modified	Tags
HELP & SUPPORT	Experiment No_04	Sequola Express	COMPLETED	9/15/2022	9/15/2022	
I<	TestExperiment_SEQuolaComplete	Sequoia Complete	COMPLETED	9/15/2022	9/15/2022	
	Experiment_No03	SEQuoia Complete	COMPLETED	8/23/2022	8/23/2022	
	Experiment_No02	Sequoia Complete	COMPLETED	8/23/2022	8/23/2022	
	Experiment_No01	SEQuoia Complete	Not Started	8/23/2022	8/23/2022	
					Rows per p	oage: 5 → 1-5 of 9 < 🗲

The default display contains five 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
	<b>Note:</b> While sample files must adhere to the Illumina FASTQ naming convention, experiments can be named without restrictions. The name must not exceed 250 characters.
Prep Kit Type	Chemistry kit type associated with the experiment (SEQuoia Express or SEQuoia Complete)
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.

BIO RAD	SeqSense				۸
🕞 номе	Samples				
ES SAMPLES				Q,  Search	<b>△ ⊕ Ⅲ</b> ऱ
🔊 tas	Name	Prep Kit Type	Created $\downarrow$	Tags	
K HELP'S SUPPORT	NS45M_S4_L001_R1_001.fastq.gz	SEQuola Complete	5/12/2023		1 A C
IC.	NS45M_S4_L001_R2_001.fastq.gz	SEQuoia Complete	5/12/2023		- 1 C
	Test5M_S1_L001_R1_001.fastq.gz	SEQuola Express	5/9/2023	MARKER4 × 📀	
	Test5M_S1_L001_R2_001.fastq.gz	SEQuoia Express	5/9/2023	MARKER4 ×	- F
				Rows per page:	5 - 1-4 of 4 < >

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 41.
- 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 42 for information.

The default display contains five 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.

BIO RAD	SeqSense
HOME	Tag Management
E SAMPLES	Add Tag marker1 🕲 marker2 🕲 marker3 🕲 marker4 😒 marker5 🕲 marker6 🕲
TAGS	Max 20 characters
HELP & SUPPORT	

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.

BIO RAD	SeqSense		
	Tag Management		
SAMPLES	Add Tag MARKER6	marker1 🔕 marker2 🔕 marker3 🔕	marker4 🙁 marker5 😒
TAGS	Max 20 characters		
HELP & SUPPORT			

- 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.

BIO RAD	SeqSense
	Tag Management
B samples	Add Tag marker1 🕲 marker2 🕲 marker3 🕲 marker3 🕲 marker5 🕲 marker5 🕲
TAGS	Max 20 characters
K	

### 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  $\textcircled{\bullet}$  icon in the Tags column.

The Add Tag dialog box appears.

Add Tag				
Tag Name MARKER				
Max. 20 Chara		)		
	MARKER1		CANCEL	ок
	MARKER2			

**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 Analysis Solution.

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.		
Q Search	Search for existing experiments and samples; this field is available from the Experiments and Samples pages. See Searching for Files on page 31.		
0	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.		
Ŧ	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.		
$\oplus$	Add a tag; this button is available from the Home, Experiments, and Samples pages. See Tag Management Page on page 27.		
Î	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.		
	Rows per page: 10 ▼ 1-2 of 2 < >		

### **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.

Q Search	6	ē		Ŧ	
----------	---	---	--	---	--

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

С номе	Samples					
EXPERIMENTS						
SAMPLES					Q, r2	<b>∆ ⊕ Ⅲ</b> ऱ
TAGS	Name	Prep Kit Type	Created $\downarrow$	Tags		
HELP & SUPPORT	Test5M_S1_L001_R2_001.fastq.gz	SEQuoia Complete	5/9/2022	TAGID_1 $\times$ TAGID_5 $\times$ TAGID_3 $\times$		
IK	NS45M_S4_L001_R2_001.fastq.gz	SEQuola Express	5/2/2022	TAGID_3 × 🕀		
	N\$45M_\$4_L001_R2_001.fastq.gz	SEQuoia Complete	4/29/2022	TAGID_5 × 🕀		
					Rows per page: 5 🚽	1-3 of 3 < >

### 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.
- Open your Downloads directory on your computer. The .csv file automatically appears in the directory.

	A	В	С	D	E	F
1	Name	Prep Kit Type	Status	Created	Modified	Tags
2	Experiment_No03	SEQuoia Complete	COMPLETED	8/23/2022	8/23/2022	NewTag
3	Experiment_No02	SEQuoia Complete	Not Started	8/23/2022	8/23/2022	AnotherNewTag
4	Experiment_No01	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.

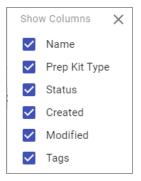
3. Click Print.

### **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.

FILTERS RESET	×
Name	Prep Kit Type
Status	Created
Modified	Tags

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 ( $\overline{\bullet}$ ) 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.

Samples					
		Q r2		<b>0 6 8</b> 3	Ŧ
Name	Ргер Кіt Туре	Created $\downarrow$	Tags		
Test5M_S1_L001_R2_001.fastq.gz	SEQuoia Complete	5/2/2022	æ		
NS45M_S4_L001_R2_001.fastq.gz	SEQuoia Express	5/2/2022	Ð	•	
NS45M_S4_L001_R2_001.fastq.gz	SEQuoia Complete	4/29/2022	Ð	•	
		Rows per page	: 5 🚽	1-3 of 3 < >	>

# Help and Support

The Help and Support tab provides a link to the SeqSense Analysis Solution 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 facilitates the upload process to your experiment.

#### To create a new experiment

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

BIO-RAD	SeqSense	٨
П номе	CREATE NEW EXPERIMENT	*

The Create New Experiment page opens.

BIO RAD	SeqSense	٨
п номе	CREATE NEW EXPERIMENT	
EUPERMENTS      EUPERMENTS      Supres      tag      tag      HELP & SUPPORT      K	EXPERMENT ITLE           My New Experiment <ul></ul>	
	× Cancel	NEXT: ADD SAMPLE FILES

2. Continue to Naming the Experiment and Selecting a Prep Kit on page 38.

## 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, which include a proprietary engineered enzyme (SEQzyme) that combines cDNA synthesis with adapter addition in a continuous synthesis reaction.

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.

#### Table 5. Available chemistry (prep) kits

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

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

BIO-RAD	SeqSense	
П номе	CREATE NEW EXPERIMENT	
	EXPERIMENT TITLE Sample_Experiment_EXPRESS	·
SAMPLES		Select a Sample Prep Kit for this experiment (Required).
TAGS		

**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.

BIORAD	SeqSense	^ ^
П номе	CREATE NEW EXPERIMENT	
	EXPERIMENT TITLE Sample_Experiment_EXPRESS Select a Sample Prep Kit for this experiment (Required).	
TAGS	Express	
K HELP & SUPPORT	Stundard RMA-Seq           Standard RMA-Seq           Interview of the second se	
	× Cancel NEX	(T: ADD SAMPLE FILES

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 40.

### **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.

BIO RAD	SeqSense	8
🗇 номе	CREATE NEW EXPERIMENT / SEQuola Express	
EXPERIMENTS	Sample_Experiment_EXPRESS	
TAGS	ADD SAMPLE FILES To add samples to your experiment, upload local files on the UPLOAD tab or choose existing files on the EXISTING SAMPLE FILES tab. If you are uploading multiple large file application times out.	ARKER4 (+) MARKERS (+)
HELP & SUPPORT	UPLOAD EXISTING SAMPLE FILES	
K	Click or drop FastQ files on the group boxes to updated them to your experiment. Be sure to use standard the naming conventions to keep R1 and R2 files as sample pars.	
	Citick or drop your Fast12 Bes here to upload. 💿	
		'
	© Back: Edit Title & Prep Kit Type SAVE CHANGE	S NEXT: SET PARAMETERS

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 41.

**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 42.
- If you are using previously uploaded files, select the Existing Sample Files tab and continue to Selecting Existing Samples on page 44.

### **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

OR

FirstSampleName\_S1\_L001\_R1\_001.fq.gz FirstSampleName\_S1\_L001\_R2\_001.fq.gz

Table 6 describes each segment of the 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
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.

#### Table 6. FASTQ sample file naming convention

### **Uploading Samples**

**Important:** SeqSense Analysis Solution 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 41 and <u>Illumina BaseSpace Sequence</u> <u>Hub</u>.
- SeqSense Analysis Solution 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.

#### 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.

ADD SAMPLE FILES	
Add sample files to your experiment by uploading local files or transferrin	ig existing sample files.
UPLOAD EXISTING SAMPLE FILES	
Click or drop FastQ files on the group boxes to upload them to your ex	kperiment.
Be sure to use standard file naming conventions to keep R1 and R2 fil	les as sample pairs.
	Click or drop your FastQ files here to upload.

As the files are uploaded, SeqSense shows the progress.

	Nertin Th	^
NS45M_S4_L001_R1_001.fastq.gz	231.54 MB 75 % X	
NS45M_S4_L001_R2_001.fastq.gz	226.88 MB 56 % — ×	
		*
	Click or drop your FastQ files here to upload.	
X		

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

ADD SAMPLE FILES To add samples to your experiment, upload local files on the UPLOAD tab or cho application times out.	ose existing files on the EXISTING SAMPLE FILES tab. If you are uploading multiple large files, save your progress frequently to prevent loss of data
UPLOAD EXISTING SAMPLE FILES	
Click or drop FastQ files on the group boxes to upload them to your experimer Be sure to use standard file naming conventions to keep R1 and R2 files as so	
NS45M_S4_L001_R1_001.fastq.gz	231.54 MB 100 %
NS45M_S4_L001_R2_001.fastq.gz	228.86 MB 100 % ×
	Click or drop your FastD files here to upload.
·	
✓ 2 Files uploaded successfully!	
Sack: Edit Title & Prep Kit Type	SAVE CHANGES NEXT: SET PARAMETERS

2. Click Save Changes.

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

ADD SAMPLE FILES				
UPLOAD EXISTING SAMPLE FILES The Existing Sample Files panel displays recently uploaded sample files from your previous e	xperiments. You can use any of these	samples in your new experiment.		
NAME	SIZE	DATE 🛧	PROGRESS	
NS45M_S4_L001_R1_001.fastq.gz	231.54 MB	Thu, May 25, 2023 at 11:04 AM	$\otimes$	
NS45M_S4_L001_R2_001.fastq.gz	226.86 MB	Thu, May 25, 2023 at 11:04 AM	×	
✓ SuccessI Your files are ready for your next experiment.			Rows per page: 10 ▾ 1-2 of 2 < >	
S Back: Edit Title & Prep Kit Type		sa	AVE CHANGES NEXT: SET PARAMETERS	

- 3. Do one of the following:
  - Assign tags. Continue to Adding or Assigning Tags During Experiment Setup on page 45.
  - Click Next: Set Parameters and see Setting the Pipeline Parameters on page 46.

### **Selecting Existing Samples**

**Important:** SeqSense Analysis Solution 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.

ADD SAMPLE FILES To add samples byour experiment, upload local files on the UPLOAD tab or choose existing files o UPLOAD EXISTING SAMPLE FILES	on the EXISTING SAMPLE FILES tab.	If you are uploading multiple large files, save your progress frequen	ifly to prevent loss of data if the application times out.
The Existing Sample Files panel displays recently uploaded sample files from your previous	experiments. You can use any of these	samples in your new experiment.	
NAME	SIZE	DATE 🛧	PROGRESS
NS45M_S4_L001_R1_001.fastq.gz	231.54 MB	Thu, May 25, 2023 at 11:04 AM	$\otimes$
NS45M_S4_L001_R2_001.fastq.gz	226.86 MB	Thu, May 25, 2023 at 11:04 AM	×
<ul> <li>Success! Your files are ready for your next experiment.</li> </ul>			
			Rows per page: 10 👻 1-2 of 2 < >
Back: Edit Title & Prep Kit Type			SAVE CHANGES NEXT: SET PARAMETERS

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 45.
  - Click Next: Set Parameters and see Setting the Pipeline Parameters on page 46.

# 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  $\textcircled{\bullet}$  icon.

CREATE NEW EXPERIMENT / SEQuoia Complete				
EXPERIMENT TITLE	ADD TAG	@	ADDED TAGS (2/Mew All	AVAILABLE TAGS(4) Wew All
Sample_Experiment_EXPRESS	MARKER7	]	MARKERT X MARKER4 X	
	Max 20 characters			

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

ADDED TAGS (2)View All	AVAILABLE TAGS(4) VIEW AII
MARKER1 × MARKER4 ×	MARKER7 (+) MARKER6 (+) MARKER5 (+) MARKER3 (+) MARKER2 (+)

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.

ADD TAG	(+)	ADDED TAGS (3)View All
		MARKER1 X MARKER4 X MARKER7 X

3. Continue to Setting the Pipeline Parameters on page 46.

## **Setting the Pipeline Parameters**

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 in the scrollable page.

BIO RAD	SeqSense	٩
номе	PIPELINE PARAMETERS / SEQuoia Express	
	EXPERIMENT TITLE ADD TAG Sample_Experiment_EXPRESS Max 20 char	ADOED TAGS (2/Wer All     AVAILABLE TAGS(7) Wer All     MARKER4 X MARKER1 X     MARKER5      MARKER7      MARKER7      MARKER5      MARKER7
TAGS	REFERENCE GENOME Species  Spike in type ( hg38 NONE	*
IK	Select a species type SECONDARY ANALYSIS SETTINGS	Hide 🛪
	Skip UMI Processing?  Skip Read Trimming? Yes No Yes No	
	Minimum MapQ Score To Count  Reset	Min Base Pairs Per Read  Pofault  255  Min Base  0  500  Control  Control
	3' Read Quality Cutoff 🌒	5' Read Quality Cutoff  Perfault Reset to Default
	Back: Add Sample Files	SAVE CHANGES START PIPELINE

#### 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:

SECONDARY ANALYSIS SETTINGS				Hide 🛪
Skip UMI Processing? 0 O Yes 🔹 No	Skip Read Trimming?  Ves  No			
Minimum MapQ Score To Count		Reset to Default 255	Min Base Pairs Per Read 0 101 0	Reset to Default 500
3' Read Quality Cutoff () 3' Read Quality 14 0		Reset to Default 42	5'Read Quality Cutof 0	Reset to Default 42

- 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.

SECONDARY ANALYSIS ADVANCED	SETTINGS			
Sequencing Read Type Used 🐧		Gene Expression Units (1)		Minimum Threshold Genes Reported 🕦
PE	Ψ.	RPKM	Ŧ	0
Default: Paired-end (PE) reads. Select SE Single end reads	E for			Enter a numerical value from 0 to 9e7 for RPKM or TPM, or selected

- 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.
- 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 Analysis Solution 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.

BIO-RAD	SeqSense			8
斺 номе	EXPERIMENT DE	TAIL / Sequoia Complete		
	EXPERIMENT TITLE			
55 SAMPLES	Experiment No_04			
	Status	RUNNING	Pipeline Run In-Progress.	
TADS	Created	Thu, 15 Sep 2022 18:30:56 GMT		
🛞 HELP & SUPPORT	Pipeline	Sequoia Complete View Files >		
K	Associated Tags(1)			
	Available Tags(3)	MARKER2 (+) View All	-	
		Experiment Notes Typing a note here would look like this.		
			SAVE CHAN	GES

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)
- Failed (pipeline run failed)

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.

50 | SeqSense Analysis Solution

# **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.

BIO RAD	SeqSense		8
П номе	EXPERIMENT DETAIL / SEQuola Complete		
	EXPERANENT TITLE Sample_Experiment		
TAGS	Status COMPLETED Created Wed, 24 May 2023 16:35:35 GMT	REPORTS O OTHER FILES O	
R HELP & SUPPORT	Pipeline SEQuoia Complete View Output File(s) > View Input File(s) >	HTML Reports for each sample file will open in a new browser tab	
	Associated Tags(2) MARKER1 X MARKER4 X View All	pdfReport.pdf	
	Available Tags(6) MARKERS  MARKERS  MARKERS  View All Experiment Notes Typing a note here would look like this.		
		SAVE CHANGES	

Reports and other files appear on two tabs, Reports and Other Files, as follows:

- 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 from basic statistics and sequencing content and quality, as well as length distribution, duplication levels, strings of overrepresented data, and adapter content.

# **Opening a File from a Completed Pipeline Run**

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 the 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.

### **Reports Generated from the Pipeline Run**

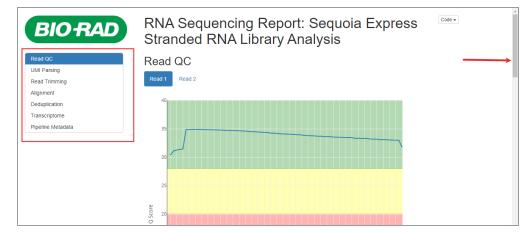
This section describes the files generated from the pipeline run that appear on the Reports tab.



1	A	В	C	D	E	F	G	н	1	J.	K	L	M	N	0
	Read QC														
		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
	1	1	30.36017892	32	32	32	32	32	1	31,14421379	32	32	32	32	32
t	2	2	31,10110432	32	32	32	32	32	2	31.09305343	32	32	32	32	32
t	3	3	31.26647582	32	32	32	32	32	3	31.12443906	32	32	32	32	32
	4	4	31.35389384	32	32	32	32	32	4	31.1605661	32	32	32	32	32
3	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
0	7	7	34.8727263	36	36	36	36	36	7	34.8230383	36	36	36	36	36
1	8	8	34,86504473	36	36	36	32	36	8	34,89286275	36	36	36	36	36
2	9	9	34.88252523	36	36	36	36	36	9	35.01419965	36	36	36	36	36
3	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
85	32	32	34.34060441	36	36	36	32	36	32	34.33671953	36	36	36	32	36
36	33	33	34.24165994	36	36	36	32	36	33	34.28433577	36	36	36	32	36
87	34	34	34.25699609	36	36	36	32	36	34	34.25092584	36	36	36	32	36
		NICHTAN CA.	LOOC_csvReport	(+)											

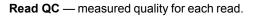
Note: Files that appear with a .csv extension contain raw data, as shown in the following example.

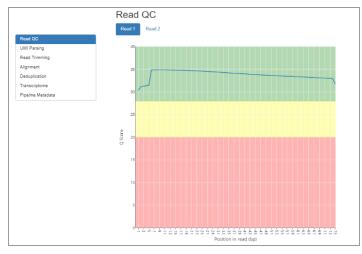
Files in .html and .pdf formats contain the same data as the .csv file, but in more organized displays. The following report example contains data for a paired end run. For Express or Complete kits, you can click a link to the report section on the left. For Express kits, you can also scroll through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



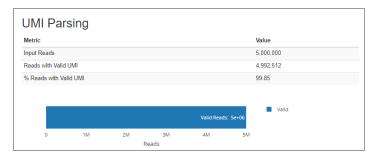
### **RNA Sequencing Report**

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





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



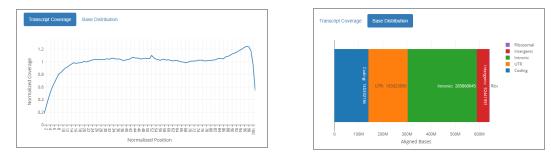
Metric	Value
Total read pairs processed	4,992,512
Read 1 with adapter	0
Read 2 with adapter	0
Pairs that were too short	6,174
Pairs written (passing filters)	4,986,338
Total basepairs processed	700,348,230
Read 1 basepairs	369,729,191
Read 2 basepairs	330,619,039
Quality-trimmed	0
Read 1 basepairs	0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

#### Read trimming — modification of raw read sequences in a pipeline

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

/letric	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

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

Metric	Value
Weuto	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

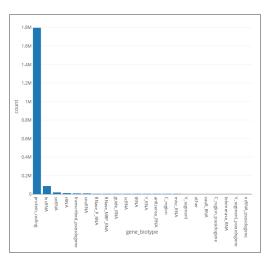
**Transcriptome**—readout of statistics for the genome, including counts for genes (raw), with the length and biotype, overall summary stats, and the breakdown of the gene biotypes (including a histogram)

Transcriptome			
Long RNA Count Summary	RNA Count Summary appendix	Gene Bioty	rpes
Show 10 🗸 entries			Search:
Gene	⇒ Length ≑	Count	Biotype ≑
WASH7P	1769	27	transcribed_pseudogene
FAM138A	1130	1	IncRNA
LOC100996442	4894	10	misc_RNA
LOC729737	5474	18	IncRNA
LOC102723897	1761	36	transcribed_pseudogene
MIR6859-2	68	2	
LOC112268260	6593	3	protein_coding
LOC100132287	4370	12	IncRNA
LOC100133331	4273	15	IncRNA
LOC107984841	926	8	protein_coding
Showing 1 to 10 of 19,518 entrie	s Prev	ious 1	2 3 4 5 1,952 Next

#### Transcriptome (continued)

Transcriptome					
Long RNA Count Summary RNA Coun	t Summary appendix	Gene Blotypes			
Show 20 v entries		Sea	rch:		
	Result	Ţ	c	ount	
Total Alignments			4,403,903		
Assigned			1,936,690		
Unassigned Unmapped			0		
Unassigned MappingQuality			0		
Unassigned Chimera			0		
Unassigned FragmentLength			0		
Unassigned Duplicate			0		
Unassigned MultiMapping			0		
Unassigned Secondary			0		
Unassigned NonSplit			0		
Unassigned NoFeatures			2,365,735		
Unassigned Overlapping Length			0		
Unassigned Ambiguity			101,478		
Genes with >0 Counts			19,518		

Long RNA Count Summary	RNA Count Summary appendix	Gene Biotypes			
how 20 🗸 entries			Search:		
	Biotype	¢		Count	÷
protein_coding			1,797,542		
IncRNA			87,288		
snRNA			18,867		
rRNA			11,102		
transcribed_pseudogene			7,845		
snoRNA			5,533		
RNase_P_RNA			2,836		
RNase_MRP_RNA			2,274		
guide_RNA			1,354		
scRNA			541		
IRNA			406		
Y_RNA			221		
antisense_RNA			183		
C_region			98		
misc_RNA			69		
V_segment			10		
other			6		
vault_RNA			5		
C_region_pseudogene			3		
telomerase_RNA			3		



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	

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

### **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.
---

Summary Align	ment Stats		
Show 20 V entries		Search:	
	Metric	) N\$45M_\$4_L00C	
PF_BASES		654633920	
PF_ALIGNED_BASES		042058194	
RIBOSOMAL_BASES		400038	
CODING_BASES		140332184	
UTR_BASES		163023956	
INTRONIC_BASES		285850045	
INTERGENIC_BASES		62441991	
IGNORED_READS		0	
CORRECT_STRAND_READS		3534992	
INCORRECT_STRAND_READS		41277	
NUM_R1_TRANSCRIPT_STRANC	READS	1673319	
NUM_R2_TRANSCRIPT_STRANC	READS	16705	
NUM_UNEXPLAINED_READS		256571	
PCT_R1_TRANSCRIPT_STRAND	READS	99.0116%	
PCT_R2_TRANSCRIPT_STRAND	READS	0.9884%	
PCT_RIBOSOMAL_BASES		0.0023%	
PCT_CODING_BASES		21.8506%	
PCT_UTR_BASES		25.3908%	
PCT_INTRONIC_BASES		44.5225%	
PCT_INTERGENIC_BASES		8.1078%	
Showing 1 to 20 of 30 entries		Previous 1 2	N
Summary Align	ment Report		13
Show 20 🗸 entries		Search:	
	Metric	NS45M_S4_L00C	
Started mapping on		Apr 28 17:27:44	
Finished on		Apr 28 17:28:24	
Mapping speed, Million of reads pe	rhour	441.77	

# **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.

REPORTS O OTHER FILES O		
Select Sample File NS45M_S4_L00C		Ŧ
RNACounts		
Filter_count_table.csv	2 MB	Ç
Filter_count_table.csv.NS45M_S4_L00C	2 MB	Ç
Full_count_table.csv	2 MB	¢
gene_counts_longRNA.NS45M_S4_L00C	2 MB	Ç
gene_counts_longRNA.summary.NS45M_S4_L00C	0 MB	Ç
rumi_dedup.sort.bam.featureCounts.bam	2 MB	Ç
alcRPKMTPM		
gene_counts_rpkmtpm.txt	2 MB	Ç
readcount_report.xlsx	2 MB	Ç
utAdapt		
trimlog.log.NS45M_S4_L00C	0 MB	Ç
trimmed_R1.fastq.gz	2 MB	Ç
trimmed_R2.fastq.gz	2 MB	Ç

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:featureC	ounts v1.6.4; Command:"featureCounts" "-p" "-T" "16" "prin	nary" "-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.ban
3 DDX11L1	chr1;chr1;chr1	11874;12613;13221	12227;12721;14409	+(+)+	1652	0
WASH7P	chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;	14362;14970;15796;16607;16858;17233;17606;17915;18268;24738;2 9321	14829;15038;15947;16765;17055;17368;17742;18061;1836 6;24891;29370		1769	27
5 MIR6859-1	chr1	17365	17436		68	0
5 MIR1302-2HG	chrischrischri	29926;30564;30976	30039;30667;31295	10101	538	0
MIR1302-2	chr1	30366	30503	+	138	0
FAM138A	chrijchrijchri	34611;35277;35721	35174;35481;36081	-92	1130	1
OR4F5	chr1	69091	70008	+	918	0
LOC100996442	christeristeristeristeristeristeristeriste	9006.80002.90062.90062.90062.90062.9001.2001.2001.2001 2011250021.100021.100021.10012101201201201200000000	9123-9124-9124-9124-9124-9124-9124-9124-9124		4894	10
11 LOC729737	chr1;chr1;chr1	134773;139790;140075	139696;139847;140566	-22	5474	18
2 LOC102725121	chrijchrijchri	182388;183132;183740	182746;183240;184878	+;+;+	1607	0
LOC102723897	chrljchrljchrljchrljchrljchrljchrljchrlj	184878;185491;186317;187129;187380;187755;188130;188439;1887 91;195263;199837	185350;185559;186469;187287;187577;187890;188266;188 584;188902;195416;199860		1761	36
4 MIR6859-2	chr1	187891	187958		68	2
5 LOC107985721	chr1:chr1:chr1	200442;201078;201496	200555;201181;203983	41414	2706	0
LOC112268260	chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;	350706;350706;350706;355425;356685;3575155;3575155;3575155;3575155;3575155;3575515;3575155;3575515;3575155;3575515;3575515;3575515;3575515;3575515;3575515;3575515;3575515;3575555;355555;355555;35555555555	851019;351019;351039;356652;357586;357586;357586;357 586;357586;357586;357586;358183;358183;358183;358183; 358183;358181;358181;35822;339019;359515;365692;36 5692;365692;368380;568380;573323;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	-	$\times$
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		
"AADC" 100 10 1007031030740 AT AAA0300700744		 -

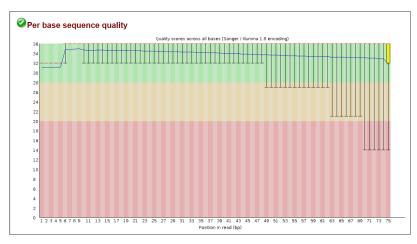
You can also open some files in HTML format. The files are scrolling displays that open in your browser and contain links in the left panel to jump directly to the corresponding chart. The following graphic shows a scrollable HTML FastQC report.

<b><i>R</i></b> FastQC Report			Thu 28 Apr 2022 NS45M_S4_L00C_R2_001.fastq.gz
Summary	Basic Statistics		A
- Cullinary	Measure	Value	
Basic Statistics	Filenane	NS45M_S4_L00C_R2_001.fastq.gz	
Per base sequence quality	File type	Conventional base calls	
Per tile sequence quality	Encoding	Sanger / Illumina 1.9	
	Total Sequences	5000000	
Per sequence guality scores	Sequences flagged as poor qu		
Per base sequence content	Sequence length	e-75	
Per sequence GC content	XGC	38	
Per base N content			
Seguence Length Distribution	<b>0</b>		
Sequence Duplication Levels	Per base sequence		
Overrepresented sequences	361 T T T T T T	Quality scores as	pos al papes (Sengery Tumne 1.8 encoding)
Adapter Content	54 20 20 20 20 20 20 20 20 20 20 20 20 20		
	0 123456789 11 13	15 17 10 21 20 25 27 20 01	2 25 27 26 41 42 45 47 46 51 52 55 57 56 61 62 65 67 68 71 73 75 Poster invescion
			Foster n Reg (30).
	Per tile sequence q	uality	
	21 21 1		Quality part la
	21311 21309 21307 21305 21305		

The report contains the statistical displays described below.

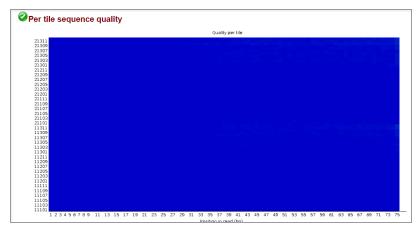
Basic statistics — contains general information on the RNA sequences.

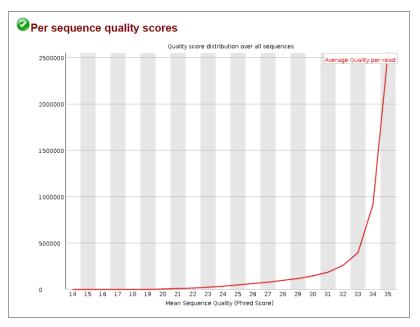
Basic Statistics				
Measure	Value			
Filename	NS45M_S4_L00C_R2_001.fastq.gz			
File type	Conventional base calls			
Encoding	Sanger / Illumina 1.9			
Total Sequences	5000000			
Sequences flagged as poor quality	0			
Sequence length	0-75			
%GC	38			



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

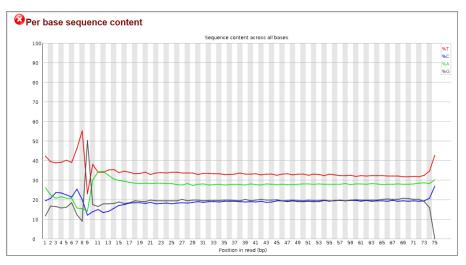
Per tile sequence quality — shows the deviation from the average quality for each tile.

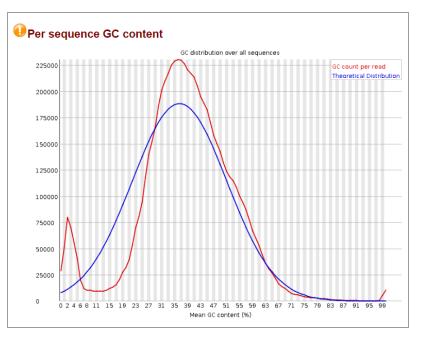




**Per sequence quality scores** — tells you if a subset of the sequences have universally low quality values.

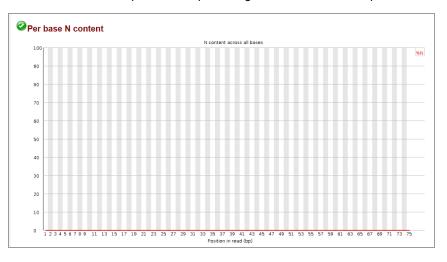
**Per base sequence content** — shows the proportion of each base position where each of the four normal DNA bases has been called.

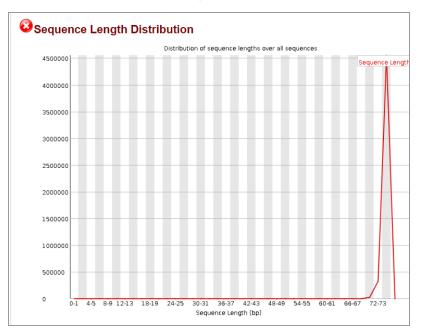




**Per sequence GC content** — measures the GC content across the entire length of each sequence in a file and compares it to a modelled normal distribution of GC content.

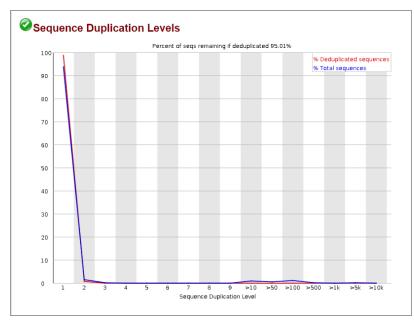
Per base N content —plots out the percentage of base calls at each position for which an N was called.





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

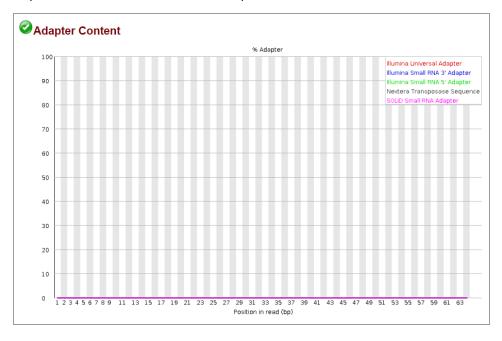
**Sequence duplication levels** — for every sequence in a library, plots the relative number of sequences with different degrees of duplication.



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

Overrepresented sequences				
	Sequence	Count	Percentage	Possible Source
	666666666666666666666666666666666666666	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.





**Bio-Rad** 

Laboratories, Inc.

Life Science Group 
 Website
 bio-rad.com
 USA 1
 800
 424
 6723
 Australia
 61
 2
 9914
 2800
 Austral
 00
 800
 00
 24
 67
 33
 Belgium
 00
 800
 00
 24
 67
 23
 Belgium
 00
 800
 02
 46
 723
 Finand
 00
 800
 02
 46
 723
 India
 91
 24
 00
 800
 12
 402
 9300
 Issue
 13
 86361
 700
 Koos
 13
 86361
 700
 800