SeqSense Cloud

Frequently Asked Questions

Version 2.2

BIO-RAD

SeqSense Cloud

Frequently Asked Questions

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 Frequently Asked Questions Software Version 2.2 DIR No. 10000154023 Ver C	January 2025	Updated to support Omnition pipeline
SeqSense Analysis Solution Frequently Asked Question Software Version 2.1 DIR No. 10000154023 Ver B	June 2023	Update FAQ with new Q & A and separate information by application; change three-hour timeout to one hour
SeqSense Analysis Solution Frequently Asked Questions Software Version 2.0 DIR No. 10000154023 Ver A	June 2022	Create new FAQ

Table of Contents

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.

Chapter 2 Questions and Answers

The information in the following sections is intended to answer specific questions about SeqSense Cloud.

Application-Specific Q & A

All Applications

1. **What new features are included in SeqSense Cloud v2.2?**

   SeqSense Cloud v2.2 now includes the Omnition pipeline. For more information, click here to view the v2.2 Release Notes.

2. **What is the accepted file naming convention?**

   SeqSense Cloud accepts only the Illumina naming convention for FASTQ and GZ files, 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: Ensure that you replace SampleName with a unique name. SeqSense does not accept duplicates. Do not use spaces or hyphens.

   Click here more information on Illumina naming conventions.

3. **What are the most important parameters when setting up a run?**

   The passing value for each parameter is recommended for inclusion. Bio-Rad recommends using the default values as a starting point and then make changes as applicable; increasing the stringency of parameters results in fewer hits.

4. **Is it possible to download or upload my RNA sample files into SeqSense Cloud using command-line?**

   This functionality is not available if you are using the SeqSense Cloud.

5. **Is SeqSense Cloud compatible with data from library prep kits other than SEQuoia Complete, SEQuoia Express, and ddSEQ 3' scRNA-Seq Library Prep Kits?**

   Non-proprietary libraries are not supported at this time. SeqSense Cloud is compatible only with data generated from the SEQuoia Complete Stranded RNA Library Prep Kit, the SEQuoia Express Stranded RNA Library Prep Kit, and the ddSEQ 3' scRNA-seq Library Prep Kit.

6. **Is there a demo data set that I can use to test drive SeqSense Cloud?**

   Yes, two demo data sets are available for SEQuoia Express and SEQuoia Complete:

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

   For a ddSEQ 3' scRNA-seq Omnition example reports, see the Analysis and Reporting section of the Omnition Analysis Software user guide.

7. **Will other reference genomes be added to SeqSense Cloud in the future? How can I request other genomes?**

   If you have reference genomes of interest, contact Bio-Rad Technical Support

8. **How Do I Create a SeqSense Cloud account?**

   a. Navigate to https://seqsense.bio-rad.com/.

   b. Click New User Form.

   c. Complete the form and click Get Access.

   d. Bio-Rad sends an email confirming your account.

   For more information, see the SeqSense Cloud user guide.

9. **Is SeqSense Cloud free to use?**

   Access to SeqSense Cloud is included with the purchase of SEQuoia Express Stranded RNA Library Prep Kit, the SEQuoia Complete Stranded RNA Library Prep Kit, or the ddSEQ Single-Cell 3’ RNA-Seq Kit.

10. **How do I retrieve my SeqSense Cloud username or password if I've forgotten it?**

    To retrieve your SeqSense Cloud user name or reset your password, click the Forgot username? or Forgot your password? link on the SeqSense Cloud Login page or the sign-in page at www.bio-rad.com.

11. **How can I access the SeqSense Cloud Frequently Asked Questions?**

    To access the SeqSense Cloud User Guide, go to bio-rad.com, type "SeqSense Cloud User Guide" in the search, then press enter.

Omnition

1. **What data is compatible with Omnition on SeqSense Cloud?**

   Sequencing data from libraries created with Bio-Rad ddSEQ 3' scRNA-Seq Library Prep Kits.

2. **What is the key difference between the downloadable version of Omnition and SeqSense Cloud?**

   - The downloadable version of Omnition Analysis Software is designed to run on a local Linux server, high performance computing (HPC) cluster, or cloud virtual machine. You can run Omnition on additional Linux distributions or versions if they are able to run the required dependencies, but Bio-Rad supports only the tested versions of the specified operating system. This version of Omnition gives you greater flexibility to configure parameters compared to SeqSense Cloud. To use Omnition, see to the Omnition user guide.
   - You can use the Omnition pipeline on SeqSense Cloud, a web-based solution that applies the same algorithms and performs the same analyses as the downloadable version. Omnition eliminates local infrastructure or bioinformatics expertise, though familiarity is recommended. The cloud version supports only human and mouse reference genomes, without the option for custom references, which are available in the desktop version of the software.
   - SeqSense Cloud only lets you select your reference genome and decide whether to include introns in the analysis. To configure additional parameters, use the downloadable (desktop) version of Omnition Analysis Software.

3. **Can the Omnition pipeline process multiple samples simultaneously?**

   Yes, you can run multiple samples using the Omnition pipeline at the same time.

4. **Why can't I see a report after an Omnition pipeline run?**

   The pipeline run might take a few hours to complete. If reports are still unavailable after a successful pipeline run, contact Bio-Rad Customer Support.

5. **Does Omnition support bulk or single-cell RNA sequencing?**

   Omnition supports single-cell RNA sequencing (scRNA-seq).

6. **What reference genomes are supported?**

   The following reference genomes are available for ddSEQ 3' scRNA-seq Omnition:

   - hg38

- mm10

SEQuoia Complete and SEQouia Express

1. **What are the key differences between SEQuoia Express and SEQuoia Complete Analysis pipelines?**

   - The SEQuoia Complete Stranded RNA Library Prep Kit captures long and short RNAs in a single library, making it ideal for low-quality or limited samples. It supports construction of >99% stranded libraries for Illumina platforms and includes access to SeqSense Cloud f or secondary data analysis.
   - The SEQuoia Express Stranded RNA Library Prep Kit focuses on preparing libraries from messenger RNA and long non-coding RNA. It uses SEQzyme, a proprietary enzyme that combines cDNA synthesis and adapter addition, reducing steps and protocol time to less than three hours.
   - SEQuoia Complete processes only one set of FASTQ files at a time, while SEQuoia Express handles multiple. If you run multiple FASTQ files simultaneously with SEQuoia Complete, the pipeline will fail, and the application will not generate reports

2. **What is the normal range of mapped reads for a SEQuoia Complete library and a SEQuoia Express library?**

   There is no set normal range, since some parameters involve dependencies (for example, read quality, read depth and sample quality).

3. **How should I trim the read if I don’t use SeqSense Cloud**?

   In addition to quality trimming (FastQC), SeqSense Cloud offers adapter trimming and you can skip read trimming. There are several bioinformatic tools available to perform the related tasks, but Bio-Rad has no specific recommendations.

4. **What is the suggested configuration when using the SEQuoia Complete Stranded RNA Library Kit?**

   For SEQuoia Complete, Bio-Rad recommends that you use 2 x 75 bp PE sequencing. The 8bp UMI is read in R2.

5. **Why can't I see a report after a SEQuoia Complete pipeline run?**

   Because a SEQuoia Complete pipeline can process only one sample at a time. and might not generate reports if more than one sample is uploaded. To ensure reports are generated, upload only one sample (R1 or R1+R2) for the pipeline run.

6. **How many reports can I run at a time SeqSense Cloud when I use the SEQuoia Complete prep kit?**

   If you select SEQuoia Complete, SeqSense Cloud can process only one sample at a time and might not generate reports if more than one sample is uploaded. To ensure reports are generated, upload only one sample (R1 or R1+R2) for the pipeline run.

7. **What reference genomes are supported?**

   The following reference genomes are available for SEQuoia Express:

   - ce11
   - danRer11
   - dm6
   - hg38
   - mm10
   - rnor6
   - sacCer3
   - tair10

   The following reference genomes are available for SEQuoia Complete:

   - hg38
   - mm10
   - rnor6

Secondary Analysis Q & A

1. **How do I interpret a FastQC report?**

   Review the information provided using the following hyperlink:

   https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

   The Babraham Bioinformatics website provides FastQC documentation from the authors, with examples of good and bad data. To access a helpful FastQC tutorial, open the following hyperlink:

   https://rtsf.natsci.msu.edu/genomics/tech-notes/fastqc-tutorial-and-faq/

2. **Is it OK for my quality score to initially be below 30?**

   Yes, but a quality score of Q30 or better is ideal.

3. **What happens if the score in a SeqSense Cloud report falls outside the green region? Any fixes suggested?**

   Bio-Rad recommends that you troubleshoot the issue with your sequencing provider.

4. **What are possible reasons for seeing a higher fraction of unmapped (unaligned) reads?**

   This is typically attributed to low quality sequencing, poor sample quality, contamination, polyA, or a viral or bacterial sequence.

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*

10000154023 Ver C US/EG

0225 Sig 0125