SeqSense Analysis Solution

Frequently Asked Questions

Version 2.1

BIO-RAD

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Bio-Rad Technical Support Department

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Revision History

Document	Date	Description of Change
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

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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 https://www.bio-rad.com.

Chapter 2 Questions and Answers

The information in the following sections is intended to answer specific questions that were received regarding the SeqSense Analysis Solution application.

Reference Genome and Demo Data Set Q & A

- **Is there a demo data set that I can use to test drive the SeqSense Analysis Solution?**

  Yes, demo data sets are available:

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

- **What 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

- **Will other genomes be added to SeqSense Analysis Solution in the future? How can I request other genomes?**

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

Application-Specific Q & A

- **What are key features released in the latest release of the SeqSense Analysis Solution web application?**

  For more information, click here to view the v2.1 Release Notes.

- **Does the web app time out? If so, what is the time limit?**

  The SeqSense Analysis Solution web app times out after one hour. Bio-Rad recommends that you start an analysis within one hour of uploading or selecting the sample files.

- **What are the key differences between the SEQuoia Express Analysis Toolkit and the SeqSense Analysis Solution applications?**

  - The SEQuoia Express Analysis Toolkit is a Docker container that is used with a Linux command line interface to run scripts and libraries on the infrastructure for your organization. To use the toolkit, Bioinformatics expertise is required.
  - The SeqSense Analysis Solution uses the same algorithms and performs the same analysis, but is web-based. The application does not require local infrastructure or bioinformatics expertise, although familiarity is recommended.

- **What is the accepted file naming convention?**

  The SeqSense Analysis Solution 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
  OR
  SampleName_S1_L001_R1_001.fq.gz
  SampleName_S1_L001_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.

- **What is the suggested configuration for the SEQuoia Express Stranded RNA Library Kit?**

  For the Express chemistry (prep) kit, Bio-Rad recommends that you use 2 x 75 bp PE sequencing. The 8bp UMI is read in R2.

- **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.

- **How should I trim the read if I don't use SeqSense Analysis Solution?**

  In addition to quality trimming (FASTQC), the SeqSense Analysis Solution 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.

- **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).

- **For alignment, must I use the STAR aligner?**

  Yes, the application offers the STAR aligner only.

- **Is it possible to download or upload my RNA sample zip files into SeqSense using command lines?**

  This functionality is not available if you are using the SeqSense Analysis Solution web application but you can use a command line structure with the SEQuoia Express Analysis Toolkit.

- **Is the application compatible with libraries constructed with kits other than SEQuoia?**

  Non-proprietary libraries are not supported at this time. The SeqSense Analysis Solutionapplication is compatible only with the SEQuoia Complete Stranded RNA Library Prep Kit and the SEQuoia Express Stranded RNA Library Prep Kit.

- **Why are no reports generated in SeqSense Analysis Solution when I use the SEQuoia Complete prep kit?**

  If you select SEQuoia Complete, SeqSense Analysis Solution 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.

Secondary Analysis Q & A

- **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/

- **What is an acceptable quality score?**

  With all Illumina sequencers, it is normal for the median quality score to start out lower over the first five to seven bases, and then rise. The average quality score steadily drops over the length of the read. With paired-end reads, the average quality scores for read 1 is almost always higher than for read 2.

- **What happens if the score in a SeqSense Analysis Solution report falls outside the green region? Any fixes suggested?**

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

- **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.

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