SEQuoia Express Analysis Toolkit

Frequently Asked Questions

Version 1.0

BIO-RAD

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 www.bio-rad.com.

Legal Notices

Revision History

Document	Date	Description of Change
SEQuoia Express Analysis Toolkit Frequently Asked Questions DIR No. 10000155690 Ver A	June 2022	Create new FAQ (software version 1.0)

Table of Contents

Table of Contents

Chapter 1 Introduction

The SEQuoia Express Analysis Toolkit is a Linux command line tool that processes FASTQ files as input for secondary analysis, and then produces BAM files count matrices, and reports as downstream output for tertiary analysis.

For questions that are not answered in this document, or if you need user assistance, use the contact methods cited below.

- For U.S. and Canada customers:

  Bio-Rad Technical Support
  1-800-424-6723, option 2
  support@bio-rad.com
  Monday through Friday, 5:00 AM to 5:00 PM, Pacific Time.

- 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.

This FAQ document is intended to assist with using the SEQuoia Express Analysis Toolkit

Chapter 2 Questions and Answers

The information in the following sections is intended to answer specific questions that have been received regarding the SEQuoia Express Analysis Toolkit and SeqSense Analysis Solution applications.

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**.

- **How can I download a reference genome data set for the SEQuoia Express Analysis Toolkit?**

  Click here to download the reference genome data set.

- **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

- **Can I use reference genomes that have not been provided in Dropbox for the SEQuoia Express Analysis Toolkit?**

  Non-proprietary genomes are not supported at this time.

- **Will other genomes be added to SeqSense Analysis Solution or the SeqSense Express Analysis Toolkit 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 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 are key features released in the latest release of SeqSense Web Application?**

  For more information, click here to view the v2.0 Release Notes.

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

  The SeqSense Analysis Solution web app times out after three hours. Bio-Rad recommends that you start analysis within three hours of uploading the sample files.

- **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 be 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.

- **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, sample quality, and so forth).

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

  Yes, the application offers the STAR aligner only.

- **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.

- **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 SeqSense compatible with libraries constructed with kits other than SEQuoia?**

  Non-proprieary libraries are not supported at this time. The SeqSense Express Analysis Toolkit and SeqSense Analysis Solution applications are compatible only with the following chemistry kits:

  - SEQuoia Complete Stranded RNA Library Prep Kit
  - SEQuoia Express Stranded RNA Library Prep Kit

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/

- **For reports provided by either SeqSense Analysis Solution or SeqSense Express Analysis Toolkit, what happens if the score falls outside the green region? Any fixes suggested?**

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

- **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 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.