Octopus_Docs Documentation

Release stable

Taemook

Apr 13, 2020

Contents

Octopus-toolkit

Note: `2018-09-05` : Octopus-toolkit supports Ubuntu18.04 version. Please feel free to contact us if you have any problems in use.

- Please cite the following paper :
  - Kim T, Seo HD, Hennighausen L, Lee D, Kang K. Octopus-toolkit: a workflow to automate mining of public epigenomic and transcriptomic next-generation sequencing data. Nucleic Acids Res. 2018 Feb 6. doi: 10.1093/nar/gky083. PubMed PMID: 29420797

<2017-04-06 17:02:12 by Prof. Keunsoo Kang, Taemook Kim in the Kangklab>

Octopus-toolkit is a stand-alone application for retrieving and processing large sets of next-generation sequencing (NGS) data with a single step. Octopus-toolkit is an automated set-up-and-analysis pipeline utilizing the Aspera, SRA Toolkit, bwtool, Samtools FastQC, Trimmomatic, HISAT2, STAR, and HOMER applications. All the applications will be installed on the user's computer when the program starts. Upon the installation, it can automatically retrieve original files (.SRA) of various data sets, including ChIP-seq, ATAC-seq, DNase-seq, MeDIP-seq, MNase-seq, and RNA-seq, from the gene expression omnibus data repository. The downloaded files can then be sequentially processed to generate BAM and BigWig files, which are used for advanced analyses and visualization. Currently, it can process NGS data from popular model genomes such as, human (Homo sapiens), mouse (Mus musculus), dog (Canis lupus familiaris), Fruit fly (Drosophila melanogaster), Zebrafish (Danio rerio), Arabidopsis (Arabidopsis thaliana), budding yeast (Saccharomyces cerevisiae), and Worm (c.elegans) genomes. With the processed files from Octopus-toolkit, the meta-analysis of various data sets, motif searches for DNA-binding proteins, and the identification of differentially expressed genes and/or protein-binding sites can be easily conducted with few commands by users. Octopus-toolkit can allow biologist and other researchers to run NGS analysis without understanding of computation behind the tools.

CHAPTER 1

Download

`Latest Version (2.2.0)`:(`Octopus-toolkit`), release 04/11/2020

- Version(`2.2.0`) is a major release with the following changes.
- Upgraded versions of some tools
- Changed the tool used to download raw data from NCBI
- Fixed the issue of Err006-1 what raw data was not downloaded.

`Latest Mac Version (2.2.0)`:(`Octopus-toolkit_mac`), release 04/11/2020

- Version(`2.2.0`) is a major release with the following changes.
- Upgraded versions of some tools
- Changed the tool used to download raw data from NCBI
- Fixed the issue of Err006-1 what raw data was not downloaded.

CHAPTER 2

Hardware/Software Requirement

Minimum Memory (RAM):

- `32Gb` memory for RNA-Seq.
- `8Gb` memory for Others (ChIP,ATAC,MNase,DNase,MeDIP)

Operating System:

- 32-64bit `Linux`, 64bit `MacOS`, 64bit `Window(Alpha Version).`

Operating System Version (tested):

- Linux : Ubuntu (`14.04`),(`16.04`, highly recommend), (`18.04`)
- Fedora (`22`),(`25`)
- Mint (`18`)
- CentOS (`7`)
- MacOS (`Sierra.10.12.6`)

CHAPTER 3

Program development

- Eclipse : Neon.1a Service Release(4.6.1)
- Language : Java Programming language (JDK1.8)
- Graphic User Interface(GUI) : Swing & Windowbuilder

CHAPTER 4

Previous version

`Latest Version (2.1.3)`, release 02/20/2019

- Version(`2.1.3`) is a minor release with the following changes.
- Optimized the analysis package for each operating system.
- Adjusted threshold of the read min length in Trimming process.

`Latest Mac Version (2.1.3)`, release 02/20/2019

- Version(`2.1.3`) is a minor release with the following changes.
- Optimized the analysis package for each operating system.
- Adjusted threshold of the read min length in Trimming process.

`Version (2.1.2)`, release 09/05/2018

- Version(`2.1.2`) is a minor release with the following changes.
- Added new operating system to support Ubuntu `18.04` version.
- Upgraded versions of R packages.
- Optimized the analysis package for each operating system.

`Mac Version (2.1.2)`, release 09/05/2018

- Version(`2.1.2`) is a minor release with the following changes.
- Upgraded versions of R packages.
- Optimized the analysis package for each operating system.

`Version (2.1.1)`, release 06/21/2018

- Version(`2.1.1`) is a minor release with the following changes.

- Changed source code (Download) of Octopus-toolkit to install Homer tool.

`Mac Version (2.1.1)`, release 06/21/2018

- Version(`2.1.1`) is a minor release with the following changes.
- Changed source code (Download) of Octopus-toolkit to install Homer tool.

`Version (2.1.0)`, release 02/13/2018

- Version(`2.1.0`) is a minor release with the following changes.
- Periodic inspection of source code
- Change the server storage and link

`Mac Version (2.1.0)`, release 02/13/2018

- Version(`2.1.0`) is a minor release with the following changes.
- Periodic inspection of source code
- Change the server storage and link

`Version (2.0.9)`, release 01/03/2018

- Version(`2.0.9`) is a minor release with the following changes.
- Optimized Paired-end classfication Method in Private Data.
- Changed source code (Private Table) of Octopus-toolkit to make maintenance easier.

`Mac Version (2.0.9)`, release 01/03/2018

- Version(`2.0.9`) is a minor release with the following changes.
- Optimized Paired-end classfication Method in Private Data.
- Changed source code (Private Table) of Octopus-toolkit to make maintenance easier.

`Version (2.0.8)`, release 12/22/2017

- Version(`2.0.8`) is a minor release with the following changes.
- Modified a list of the private table.
- Changed source code (Private Table) of Octopus-toolkit to make maintenance easier.

`Mac Version (2.0.8)`, release 12/22/2017

- Version(`2.0.8`) is a minor release with the following changes.
- Modified a list of the private table.
- Changed source code (Private Table) of Octopus-toolkit to make maintenance easier.

`Version (2.0.7)`, release 12/11/2017

- Version(`2.0.7`) is a minor release with the following changes.
- modified a parsing code because the format of the NCBI's GEO Accession display is changed.

`Mac Version (2.0.7)`, release 12/11/2017

- Version(`2.0.7`) is a minor release with the following changes.
- modified a parsing code because the format of the NCBI's GEO Accession display is changed.

`Version (2.0.6)`, release 11/28/2017

- Version(`2.0.6`) is a minor release with the following changes.
- Changed a method which to obtain modified url of the raw data in NCBI.
- Display Microarray in unsupported list (Err004-2) (NULL -> Microarray)

`Mac Version (2.0.6)`, release 11/28/2017

- Version(`2.0.5`) is a minor release with the following changes.
- Changed a method which to obtain modified url of the raw data in NCBI.
- Display Microarray in unsupported list (Err004-2) (NULL -> Microarray)

`Version (2.0.5)`, release 11/08/2017

- Version(`2.0.5`) is a minor release with the following changes.
- Periodic inspection of source code.

`Mac Version (2.0.5)`, release 11/08/2017

- Version(`2.0.5`) is a minor release with the following changes.
- Periodic inspection of source code.

`Version (2.0.4)`, release 11/07/2017

- Version(`2.0.4`) is a minor release with the following changes.
- Applied the modified url of raw data in GEO Dataset. (Issue : changed FTP path of SRA experiment data in NCBI)

`Mac Version (2.0.4)`, release 11/07/2017

- Version(`2.0.4`) is a minor release with the following changes.
- Applied the modified url of raw data in GEO Dataset. (Issue : changed FTP path of SRA experiment data in NCBI)

`Version (2.0.3)`, release 10/23/2017

- Version(`2.0.3`) is a minor release with the following changes.
- Updated `CentOS` version
- Optimized Mapping process.
- Changed source code of Octopus-toolkit to make maintenance easier.

`Mac Version (2.0.3)`, release 10/23/2017

- Version(`2.0.3`) is a minor release with the following changes.
- Optimized Mapping process.

- Changed source code of Octopus-toolkit to make maintenance easier.

`Version (2.0.2)` , release 09/09/2017

- Version(`2.0.2`) is a minor release with the following changes.
- Modified a Full parameter Option.
- Changed source code of Octopus-toolkit to make maintenance easier.

`Mac Version (2.0.2)`, release 09/09/2017

`Version (2.0.1)`, release 08/23/2017

- Version(`2.0.1`) is a minor release with the following changes.
- Change 3rd party tools to be installed without password.
- When analysis is completed/failed, notify the path of results in a terminal window.
- Changed source code of Octopus-toolkit to make maintenance easier.
- Changed User Interface(UI) of the installation progressbar.
- Added Ubuntu 14.04, Refer to *"How to install libraries"*, *"How to install R"*
- Added the `Tutorial` about how to use a custom adapter sequence generated by ownself and how to discover de novo and known motif using the output file of Octopus-toolkit.

`Beta Version (2.0.0)`, release 07/29/2017

4.1 Table of contents

4.1.1 0.Quick Start

To use the Octopus-toolkit right away, please follow these tutorials:

0-1. Installation Movie Clip

Tutorial for installation. (Youtube)

0-2. Ubuntu(16.04), Mint(18) (We highly recommend to use Ubuntu)

- Commands (`Quick_Start(Ubuntu,mint).txt`):

```
sudo apt-get update
sudo apt-get install openjdk-8-jdk
sudo apt-get install zlib1g-dev libpng12-dev libncurses5-dev build-essential r-
↪base
wget http://octopus-toolkit2.readthedocs.io/en/latest/_downloads/Octopus-toolkit.
↪zip -O Octopus-toolkit.zip
unzip Octopus-toolkit.zip
cd Octopus-toolkit/
java -jar Octopus-toolkit.jar
```

0-3. Fedora(25)

- Commands (`Quick_Start(Fedora).txt`):

```
sudo yum update
sudo yum install java-1.8.0-openjdk
sudo yum install zlib-devel.x86_64 libpng-devel.x86_64 libpng12-devel.x86_64␣
↪ncurses-devel.x86_64 gcc-c++ bzip2-devel xz-devel R
wget http://octopus-toolkit2.readthedocs.io/en/latest/_downloads/Octopus-toolkit.
↪zip -O Octopus-toolkit.zip
unzip Octopus-toolkit.zip
cd Octopus-toolkit/
java -jar Octopus-toolkit.jar
```

0-4. CentOS(7)

- Commands (`Quick_Start(CentOS).txt`):

```
sudo yum update
sudo yum install java-1.8.0-openjdk
sudo yum install zlib-devel.x86_64 libpng-devel.x86_64 libpng12-devel.x86_64␣
↪ncurses-devel.x86_64 gcc-c++ bzip2-devel xz-devel
sudo yum install epel-release
sudo yum install R
wget http://octopus-toolkit2.readthedocs.io/en/latest/_downloads/Octopus-toolkit.
↪zip -O Octopus-toolkit.zip
unzip Octopus-toolkit.zip
cd Octopus-toolkit/
java -jar Octopus-toolkit.jar
```

0-5. MacOS(Sierra 10.12.6)

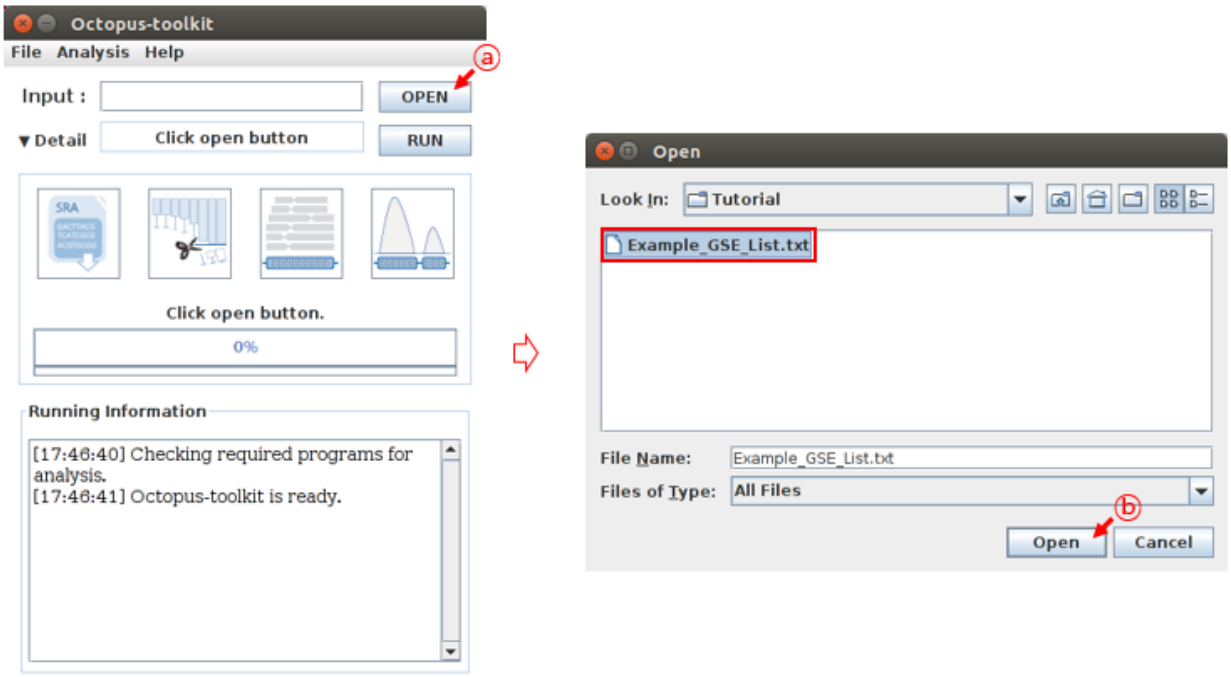
Note:

Please refer to the link below for MacOS Link : *1.Installation 1-6.MacOS(Sierra_10.12.6)*

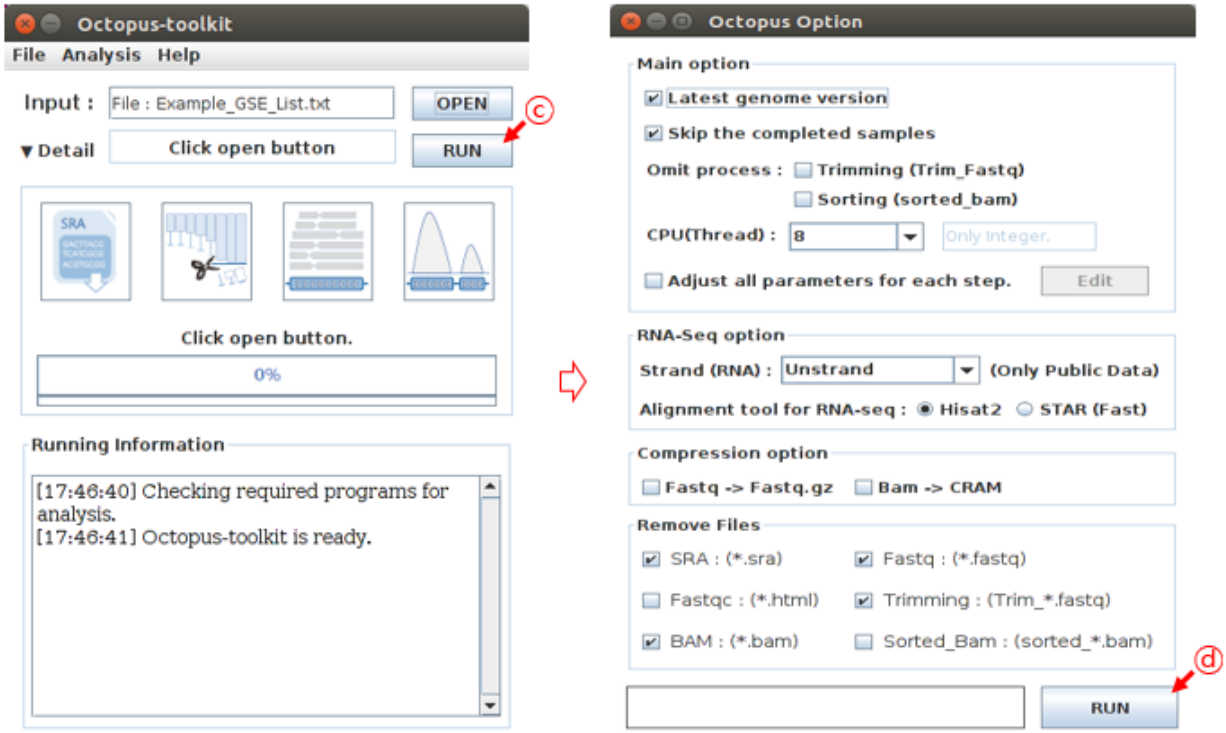
0-6. Quick Run (Public data)

Example GSE file (`Example_GSE_List.txt`)

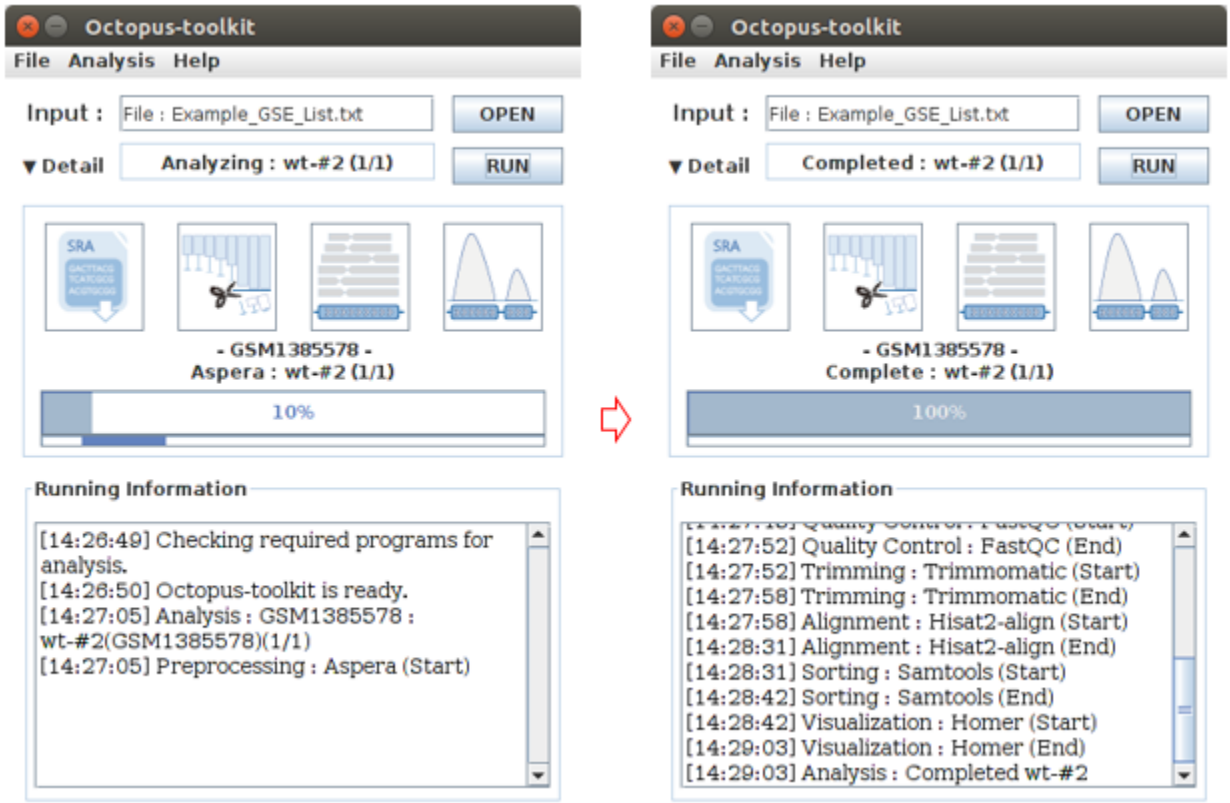
- `A` : Click the OPEN button.
- `B` : Select the `Example_GSE_List.txt` file.



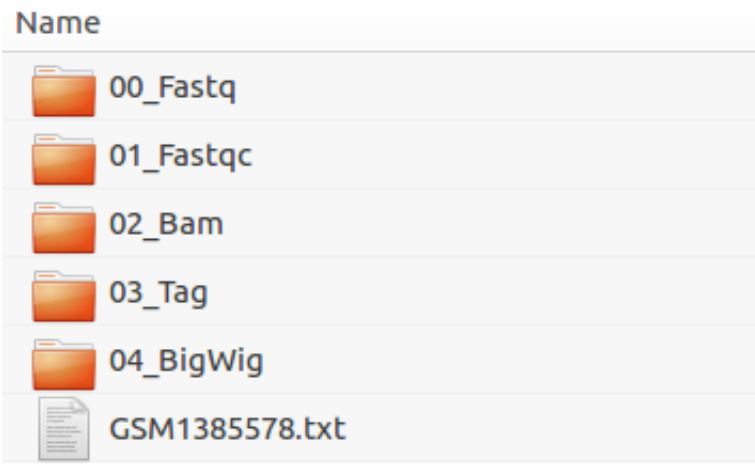
- C : Click the RUN button.
- D : Set parameters. Then, click the RUN button.



- Octopus-toolkit will sequentialy download and process the data specified in the list file. The analysis may take some time.



- Below shows output file of Octopus-toolkit



4.1.2 1.Installation

1-1.Download

Note: Applications in the `Requirement` section must be installed on your computer before running the Octopus-toolkit.

1. Octopus-toolkit_2.2.0 : (`Octopus-toolkit`) (64bit)
2. Octopus-toolkit_mac_2.2.0 : (`Octopus-toolkit_mac`)

1-2.Installation Movie Clip

- Tutorial for installation
- Ubuntu `16.04` version. (Youtube - Ubuntu 16.04)
- CentOS `6.9` version. (Youtube - CentOS 6.9) - Currently not supported.
- CentOS `7` version. (Youtube - CentOS 7)
- MacOS `Sierra 10.12.6` version (Youtube - MacOS Sierra 10.12.6)

1-3.Requirement

To run the Octopus-toolkit, `Java 8` (JDK, Java Development ToolKit) or higher, must be installed on your computer.

- Ubuntu, Mint (`Ubuntu 16.04 or Mint18`):

```
sudo apt-get update
sudo apt-get install openjdk-8-jdk
```

- Ubuntu (`14.04`):

```
sudo add-apt-repository ppa:openjdk-r/ppa
sudo apt-get update
sudo apt-get install openjdk-8-jdk
sudo update-alternatives --config java
sudo update-alternatives --config javac
```

- Fedora, CentOS(`Fedora 22~25 or CentOS 7`):

```
sudo yum update
sudo yum install java-1.8.0-openjdk
```

Octopus-toolkit utilizes several libraries for analysis. Each operating system such as ubuntu, mint and fedora differ in ways to install the applications. Please follow the installation guide below.

1-4.Ubuntu(14.04,16.04), Mint(18)

To run the Octopus-toolkit, you must install the following libraries: `zlib1g`, `libpng12`, `libncurses5`, `g++`, `liblzma`, `libbz2`

- `zlib1g-dev`

```
sudo apt-get install zlib1g-dev
```

- `libncurses5-dev`

```
sudo apt-get install libncurses5-dev
```

- `g++`

```
sudo apt-get install build-essential
```

- `liblzma-dev`

```
sudo apt-get install liblzma-dev
```

- `libbz2-dev`

```
sudo apt-get install libbz2-dev
```

OR

```
sudo apt-get install zlib1g-dev libpng12-dev libncurses5-dev build-essential liblzma-
↪dev libbz2-dev
```

In the Ubuntu version (`18.04`)

- `libpng-dev`

```
sudo apt-get install libpng-dev
```

Another Ubuntu version (`14.04``16.04`), Mint (`18`)

- `libpng12-dev`

```
sudo apt-get install libpng12-dev
```

1-5.Fedora(22~25), CentOS(7)

To run the Octopus-toolkit, you must install the following libraries: `zlib`, `libpng` , `libpng12`, `ncurses`, `gcc-c++`, `libbz2`, `liblzma`

- `zlib-devel`

```
sudo yum install zlib-devel.x86_64
```

- `libpng-devel`

```
sudo yum install libpng-devel.x86_64
```

- `libpng-devel12`

```
sudo yum install libpng12-devel.x86_64
```

- `ncurses-devel`

```
sudo yum install ncurses-devel.x86_64
```

- `gcc-c++`

```
sudo yum install gcc-c++
```

- `libbz2`

```
sudo yum install bzip2-devel
```

- `liblzma`

```
sudo yum install xz-devel
```

OR

```
sudo yum install zlib-devel.x86_64 libpng-devel.x86_64 libpng12-devel.x86_64 ncurses-
↪devel.x86_64 gcc-c++ bzip2-devel xz-devel
```

1-6.MacOS(Sierra_10.12.6)

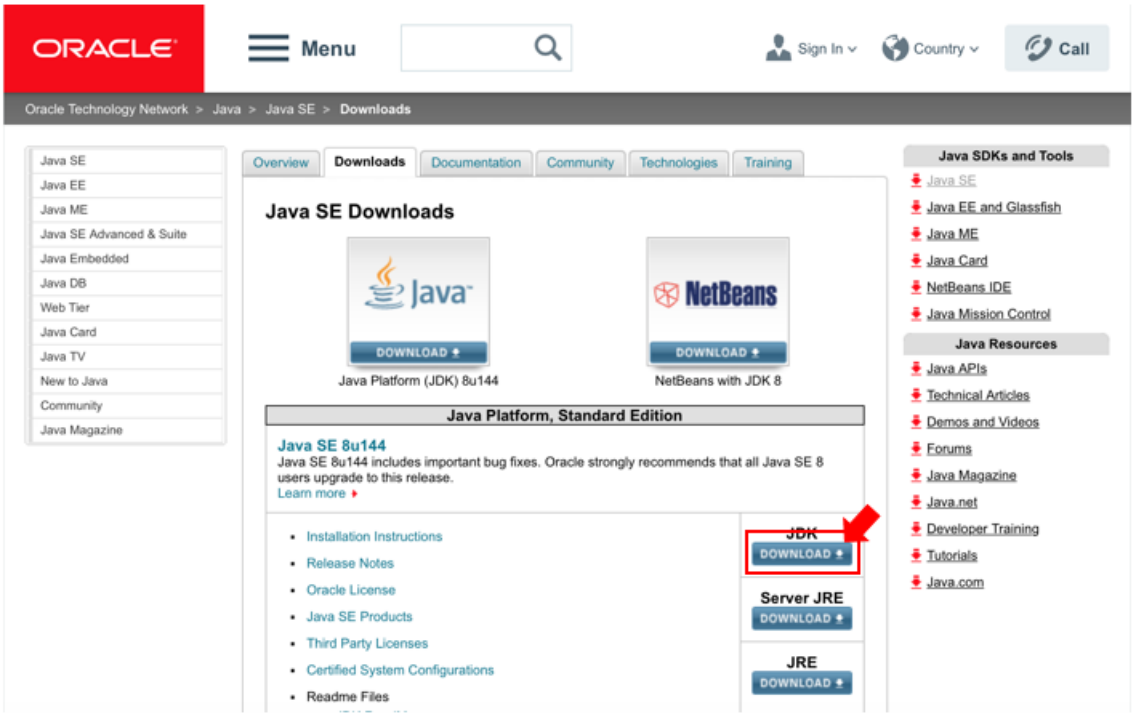
Note: Applications in the `Requirement` section must be installed on your computer before running the Octopus-toolkit (Mac version).

To run the Octopus-toolkit, `Java 8` (JDK, Java Development ToolKit) or higher, must be installed on your computer. (`Octopus-toolkit_mac_2.1.3`)

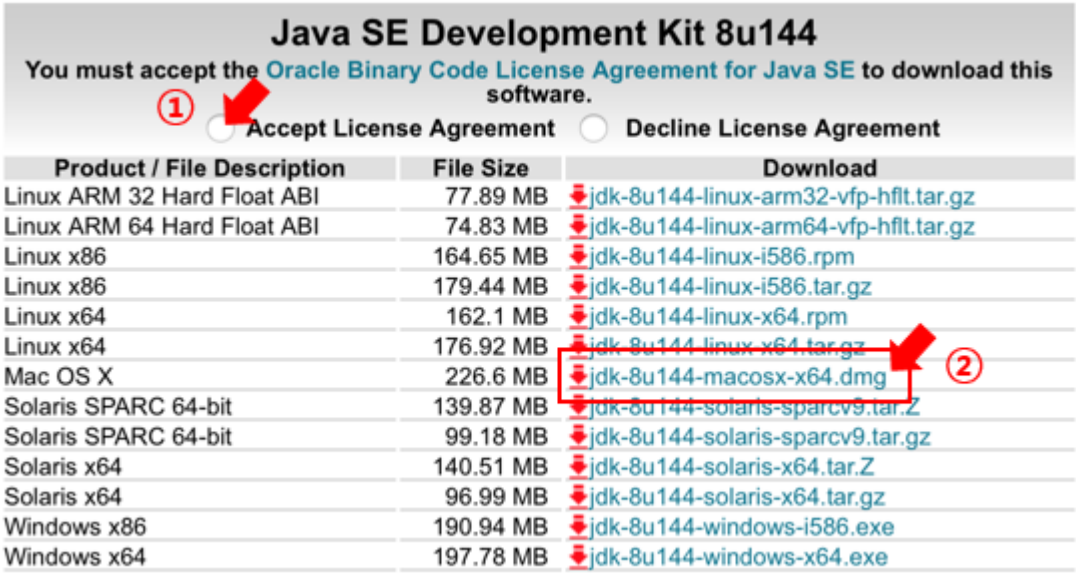
- `Java 8` (JDK, Java Development ToolKit) or higher:

```
Link : http://www.oracle.com/technetwork/java/javase/downloads/index.html
```

0. Click the JDK DOWNLOAD Button



1. Click the Accept License Agreement radio button.
2. Click the jdk-(version)-macosx-x64.dmg



3. Go to the Download folder. Execute the downloaded installation file.
4. Double click on icon to install.

Octopus-toolkit utilizes several libraries for analysis. Please follow the installation guide below.

- Xcode Update:

```
xcode-select --install
```

- Library (wget, liblzma,libpng):

```
/usr/bin/ruby -e "$(curl -fsSL https://raw.githubusercontent.com/Homebrew/install/
↪master/install)"
brew install wget
brew install xz
brew install libpng
```

1-7.R (3.1)

To draw heatmap and Line plot, `R (3.1)` or higher version of R must be installed on your computer.

- Ubuntu, Mint (`Ubuntu 16.04 or Mint18`):

```
sudo apt-get install r-base
```

- Ubuntu (`14.04`):

```
sudo apt-get update
sudo apt-get install r-base
sudo apt-key adv --keyserver keyserver.ubuntu.com --recv-keys E084DAB9
sudo add-apt-repository ppa:marutter/rdev
sudo apt-get update
sudo apt-get upgrade
sudo apt-get install r-base
```

- Fedora (`Fedora 22~25`):

```
sudo yum install R
```

- CentOS (`CentOS 7`):

```
sudo yum install epel-release
sudo yum install R
```

- MacOS (`Sierra`):

```
/usr/bin/ruby -e "$(curl -fsSL https://raw.githubusercontent.com/Homebrew/install/
↪master/install)"
brew install r
```

4.1.3 2.Run

Note: `Requirements` must be installed on a computer before running the Octopus-toolkit. (*Installation*)

2-1.How to run the Octopus-toolkit

Please follow the movie clip (Youtube)

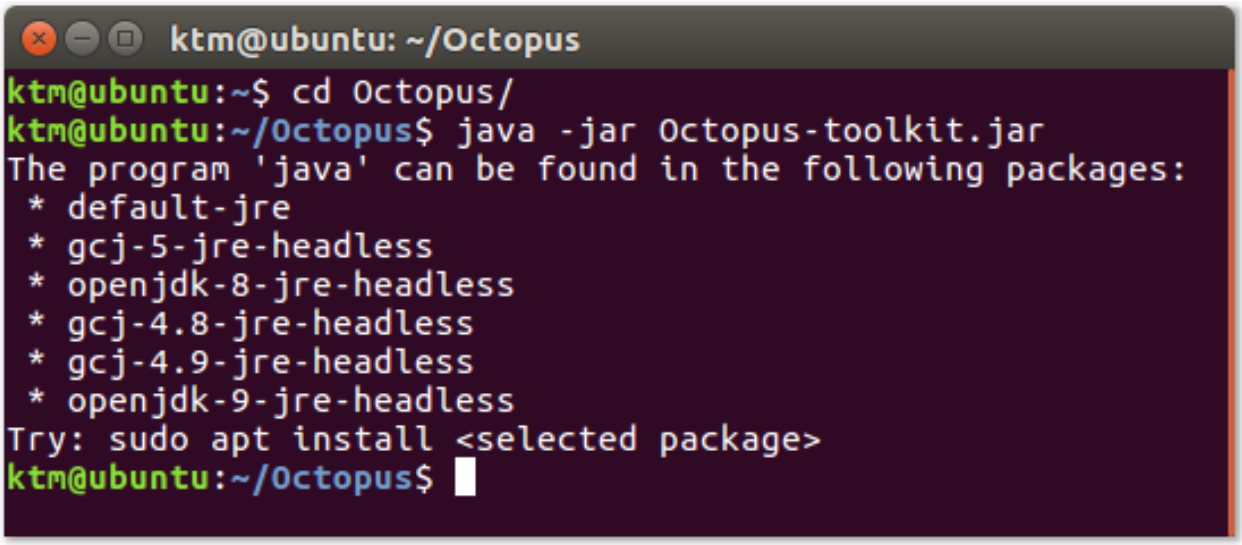
- Download (`Octopus-toolkit`) and uncompress it to the `folder` where you want it to be installed.
- Open the terminal application (cmd) and type the below command in

```
cd Octopus-toolkit/
java -jar Octopus-toolkit.jar
```

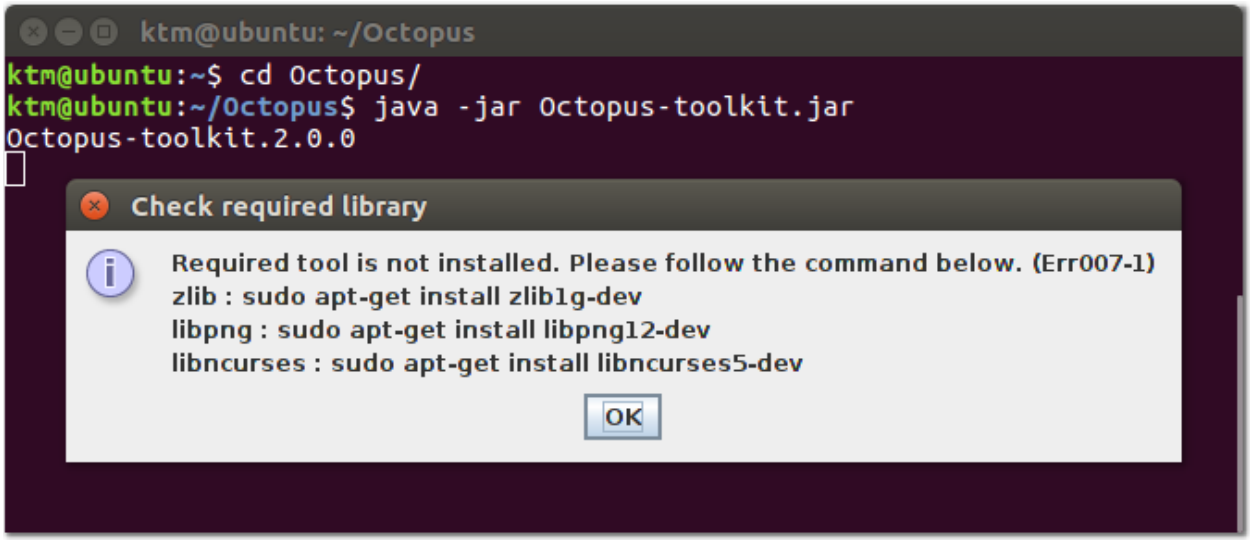
Or Command (Download ~ Run)

```
wget http://octopus-toolkit2.readthedocs.io/en/latest/_downloads/Octopus-toolkit.zip -
↪O Octopus-toolkit.zip
unzip Octopus-toolkit.zip
cd Octopus-toolkit/
java -jar Octopus-toolkit.jar
```

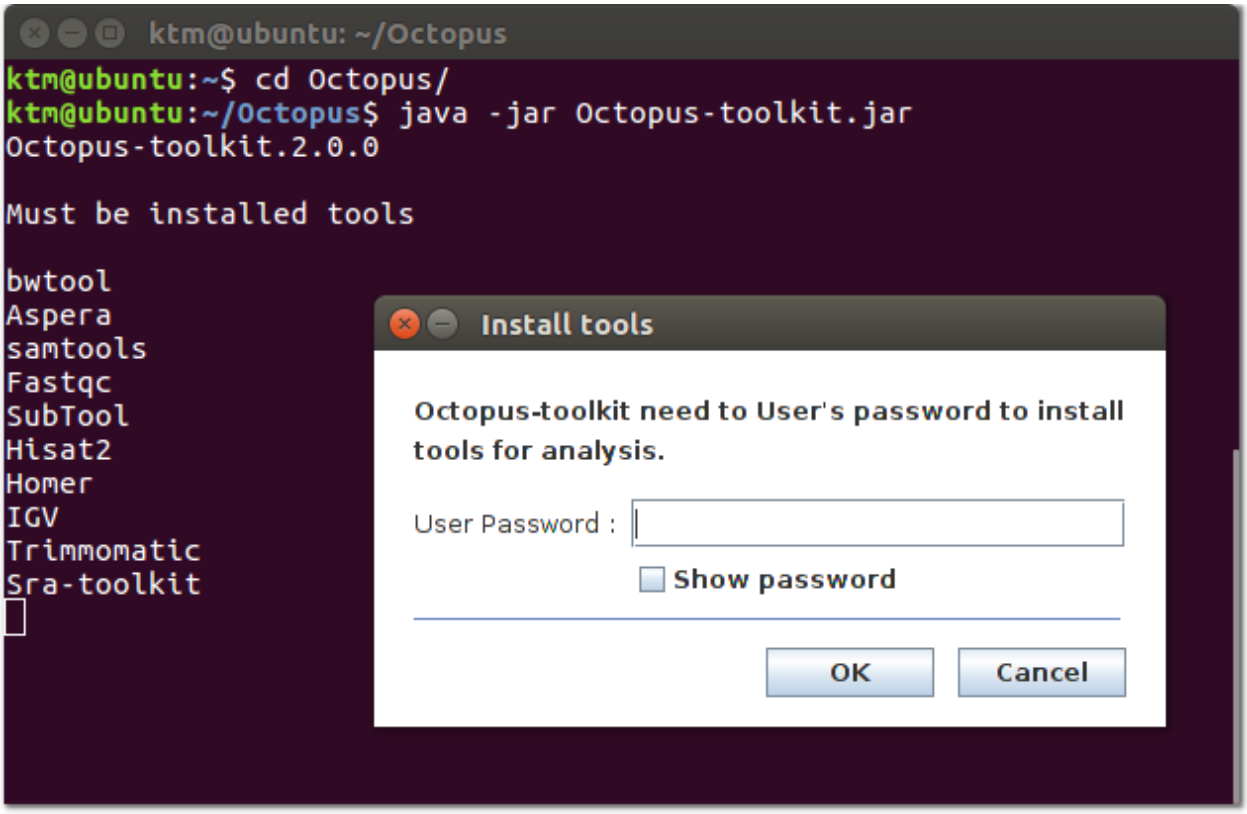
- No Java installation could be found. (Java 8 version) : (*Err007-1*)



- No prerequisite were found. (Libraries in system) : (*Err007-1*)



- Password for permission



2-2.Java Virtual Machine(VM) heap memory limited

Octopus-toolkit requires at least 8 Gb of the memory (32 Gb of memory for processing human/mouse RNA-seq) (Recommend : `32+` Gb RAM).

If you get errors related to running out of memory, please increase the heap memory of the Java Virtual Machine.

- If your memory is less than 16Gb:

```
java -jar Octopus-toolkit.jar -Xms2G -Xmx16G -XX:MaxPermSize=16G -XX:PermSize=2G
```

Argument	Description	Recommend
`Xms`	Initial Heap Size	`2Gb`
`Xmx`	Max Heap Size	Maximum of RAM
`XX:PermSize`	Initial Permanent Size	`2Gb`
`XX:MaxPermSize`	Max Permanent Size	Maximum of RAM

4.1.4 3.Octopus-toolkit output directory

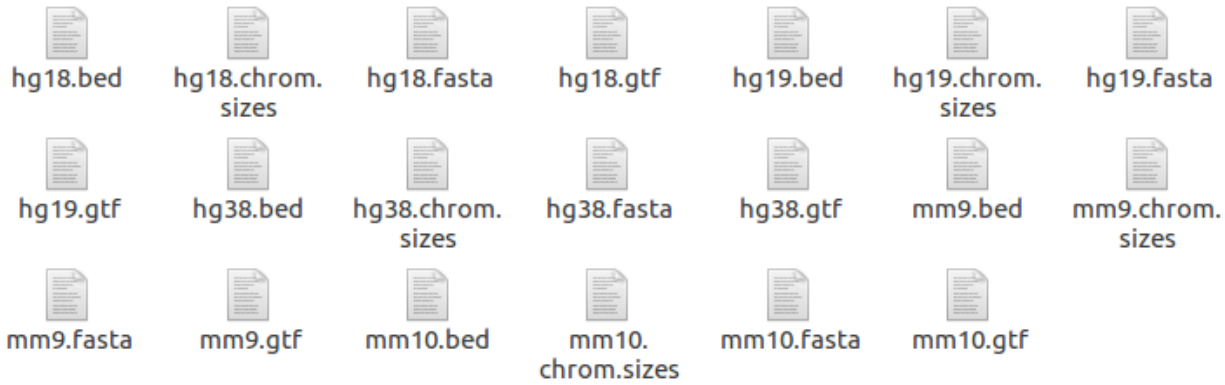
Octopus-toolkit creates five directories when you run the program.

- Octopus-toolkit main directory.

Main Folder	Sub Folder	Description
`Index`	Reference, Hisat2, STAR	Reference genome sequence and annotation files for analysis and alignment tools.
`Log`	Command, Run	Log file containing the commands used for analysis.
`Result`	GSE_Folder, P_Folder	The output files.
`Script`		Scripts used for analysis.
`Tools`	Analysis tools	Store the 3rd party tools used by Octopus-toolkit.

3-1.Index-Reference

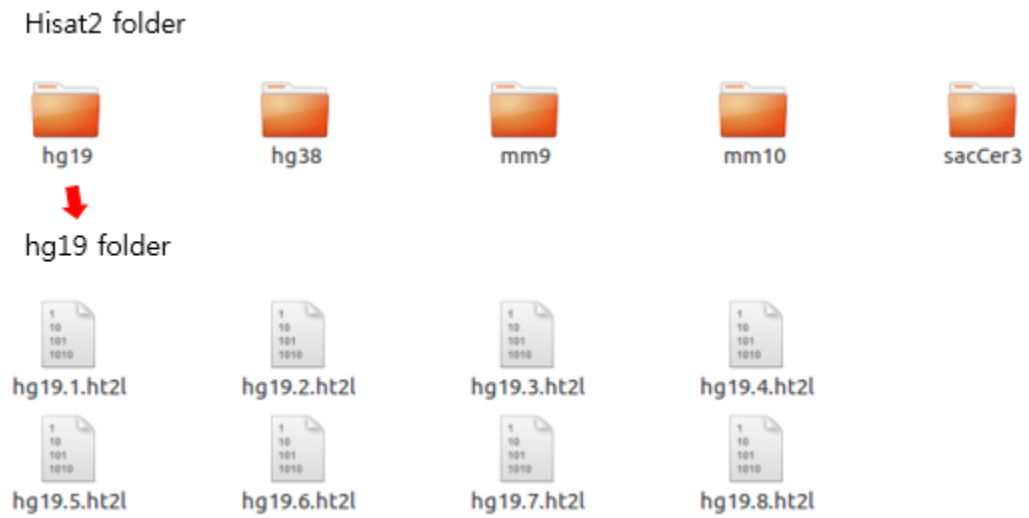
- `Reference folder`



The reference folder contains several reference files required for analysis.

Before starting each process, Octopus-toolkit checks the folder whether the reference files are prepared. If not, it automatically prepares the files.

3-2.Index-Hisat2

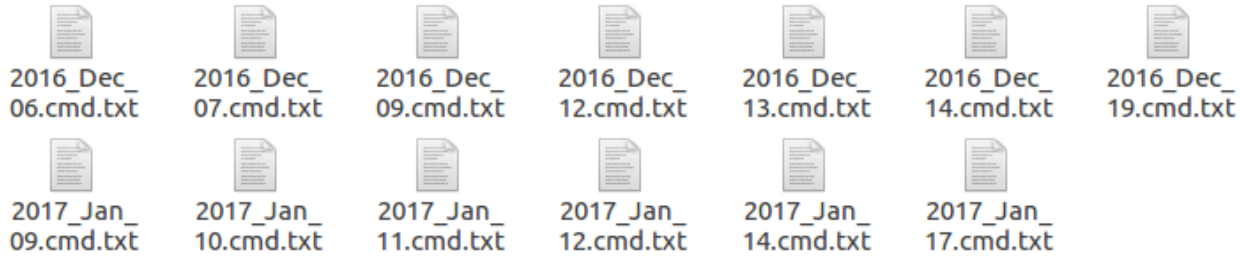


The reference genome sequence file should be indexed at least once before proceeding to the alignment step. The folder contains indexed genome sequence files used by the Hisat2 tool.

Octopus-toolkit inspects the index file of the genome before running the alignment process and runs the indexing step if it does not exist.

3-3.Log-Command

- `Command folder`

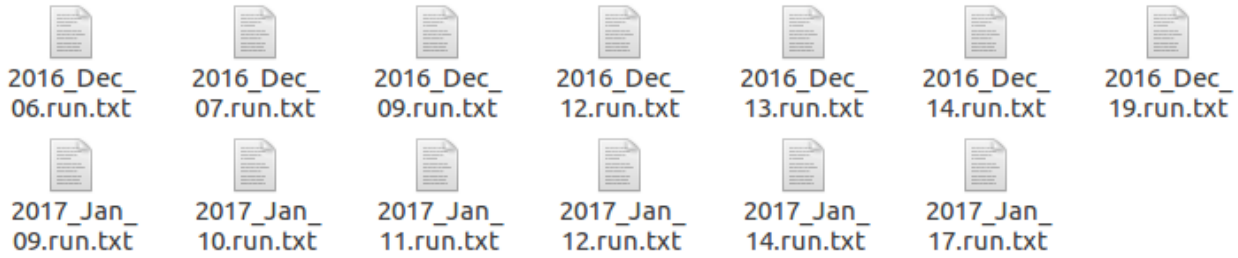


The Command directory contains log files containing the commands used during the analysis.

The file name adopts the date it is created. (`2016_Dec_06.cmd.txt`)

3-4.Log-Run

- `Run folder`

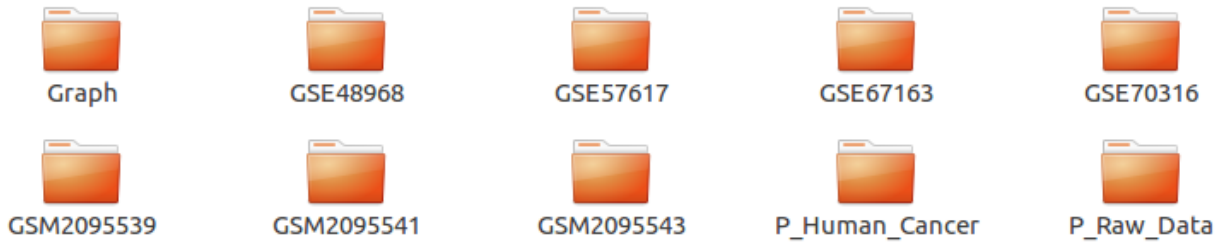


The Run directory contains log files containing running information recorded during analysis.

The file name adopts the date it is created. (`2016_Dec_06.run.txt`)

3-5.Result

- `Result folder`



The Result folder stores the output of Octopus-toolkit.

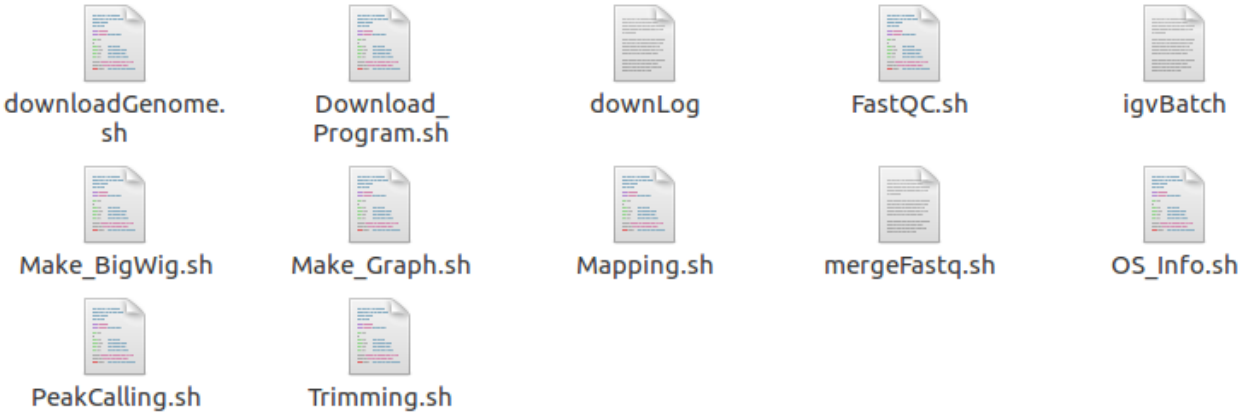
The folder name is based on the GEO accession number you entered. For the private data, the folder name begins with *P_*.

The Graph folder stores Heatmaps and Lineplots when you run the Graph function.

The detailed information regarding the output can be founded : *Output Link*

3-6.Script

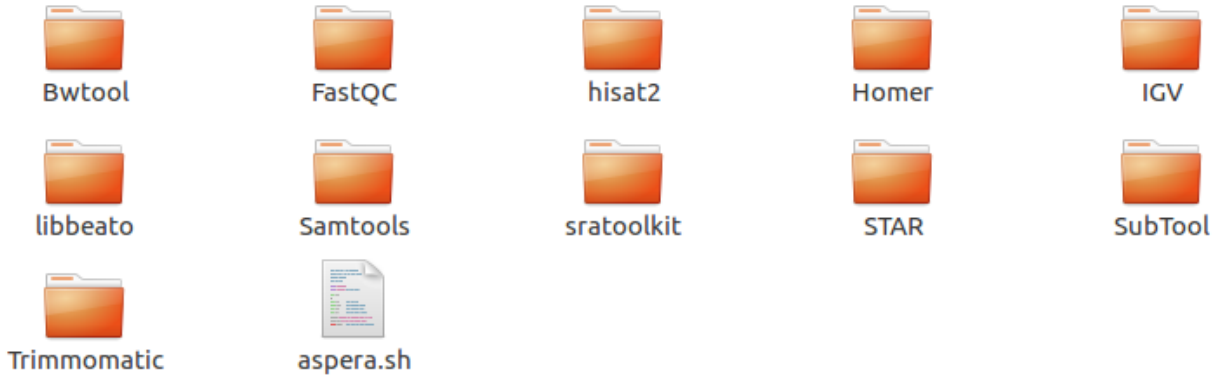
- `Script folder`



The Script folder stores the script files used by Octopus-toolkit.

3-7.Tools

- `Tools folder`

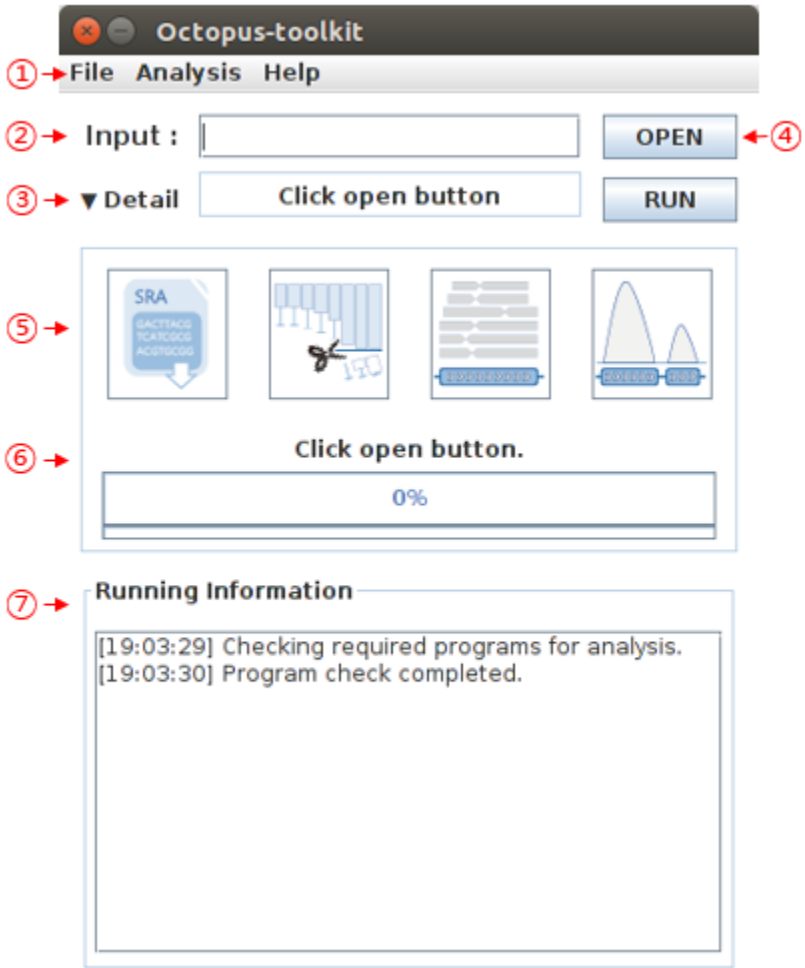


The Tools directory contains binary versions of 3rd party tools used in the Octopus-toolkit.

4.1.5 4.User Interface

4-1.Main UI

- The image below is the main UI of Octopus-toolkit



- The description of each part are as follows.

No	Name	Description
1	`Menu Bar`	Functions such as Private analysis (your data).
2	`Input`	Input GEO accession number (GSE or GSM) or a text file containing GEO accession numbers (one GSE or GSM per line).
3	`Status`	brief information regarding steps and errors.
4	`Open and Run`	Run the anlaysis.
5	`Full parameters`	Setting up the parameters for each tool.
6	`Progress bar`	Progress bar.
7	`Running information`	Status window

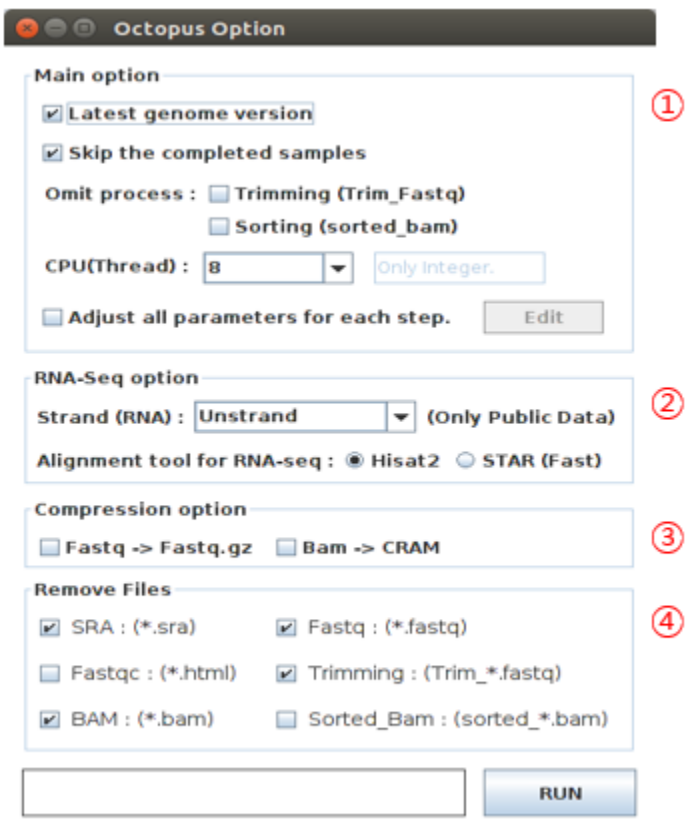
4-2.Menu Bar

- Below are the details.

Menu	Sub Menu	Description
File	`Exit`	Exit the Octopus-toolkit
Analysis	`Private Data`	Analyze your data
	`Peak Calling`	Find peaks (enriched regions) for ChIP-seq MNase-seq MeDIP-seq and ATAC-seq with HOMER.
	`Graph`	Draw the Heatmap and Line Plot from the output.
	`IGV`	Visualization using IGV (Integrative Genomics Viewer)
Help	`Manual(Tutorial)`	Go to the Octopus-toolkit manual page.
	`Error Code`	Go to the Octopus-toolkit *Error code* page.
	`Homepage`	Go to the Octopus-toolkit homepage

4-3.Octopus Option

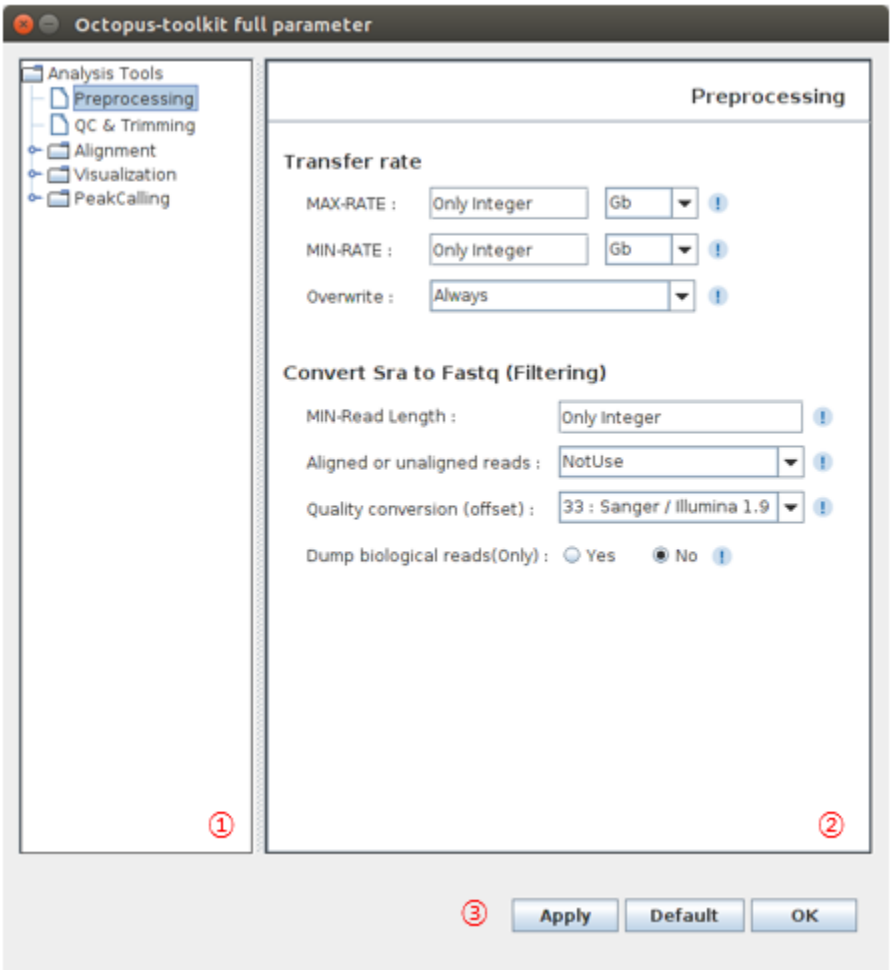
- Octopus-toolkit options



No	Name	Description
1	`Main option`	Main options.
2	`RNA-Seq option`	Options for RNA-seq data only.
3	`Compression option`	To save disk space.
4	`Remove Files`	Delete selected intermediate files after each process.

4-4.Full parameters

- The following image shows Full parameters window.

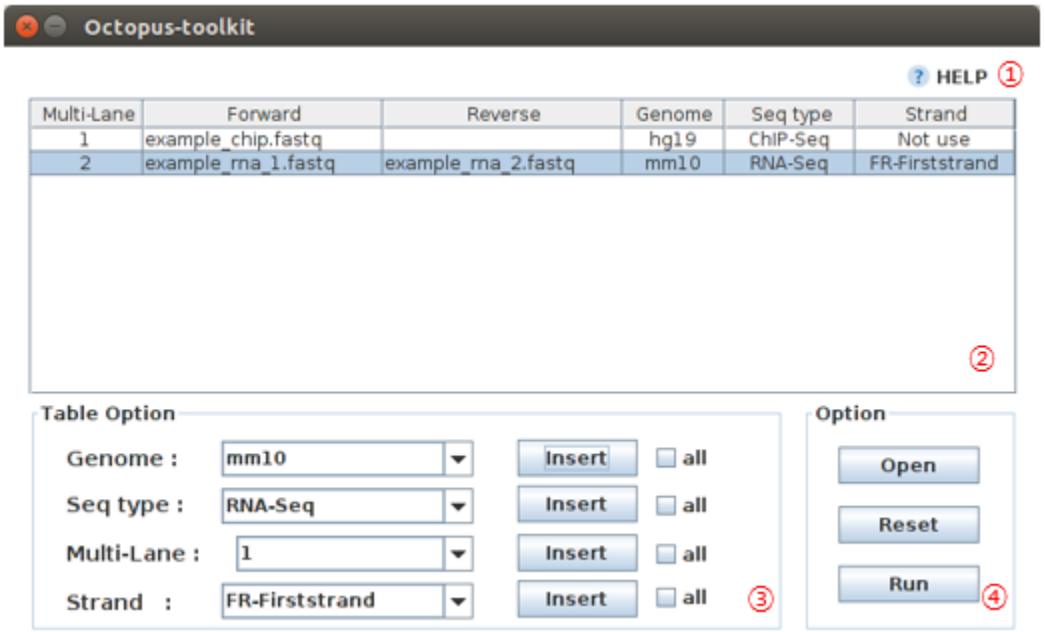


No	Name	Description
1	`Analysis tree`	Select one of steps
2	`Paremeter window`	Change parameters for the process you selected
3	`Apply`	You can apply or reset the option.

4-5.Private Table

You can analyze your own data (Fastq) using Octopus-toolkit (Analysis - Private Data). The private Table is a setup window for your data.

To analyze your own data, you must select appropriate information as follows.

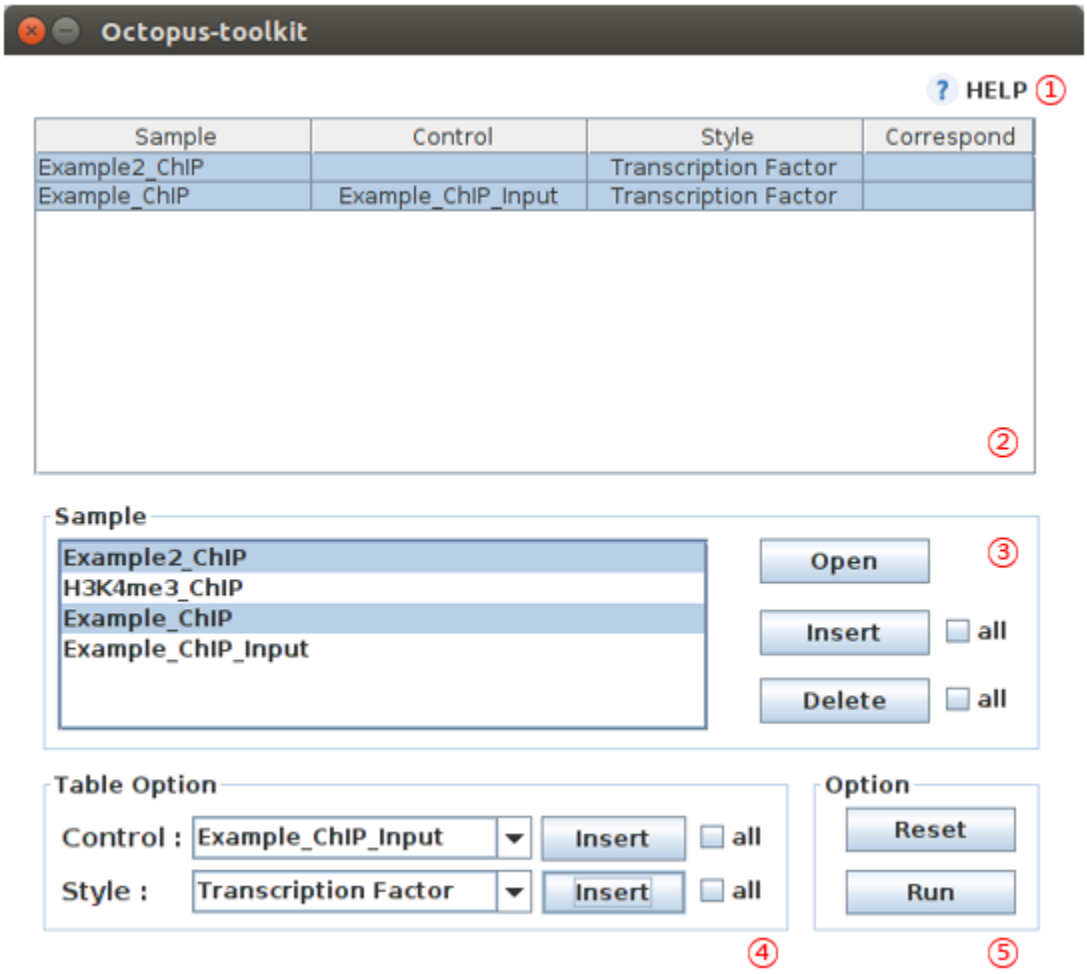


No	Name	Description
1	`Help`	Go to the tutorial page.
2	`Private table`	Files with related information.
3	`Setup`	The option window is used to set appropriate information needed for processing given files.
4	`Apply`	You can apply or reset the option.

4-6.Peak Calling Table

You can identify peaks using the Peak Calling function. You have to select appropriate options for each file from the setting window.

This function is not applicable for RNA-seq data.

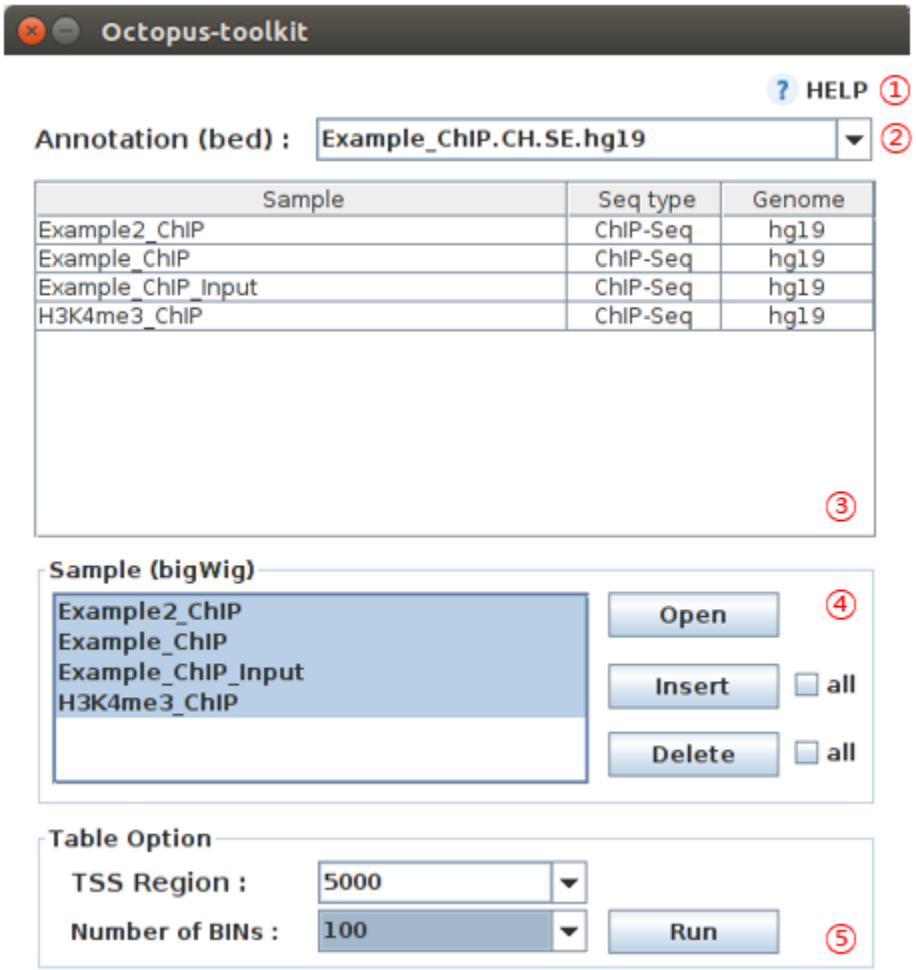


No	Name	Description
1	`Help`	Go to the tutorial.
2	`Set up table`	Parameters for peak calling
3	`Files`	Select files for analysis
4	`Setup`	Select appropriate options for given files
5	`Apply`	You can apply or reset the parameters

4-7.Graph Table

To draw heatmap and line plots with the identified regions.

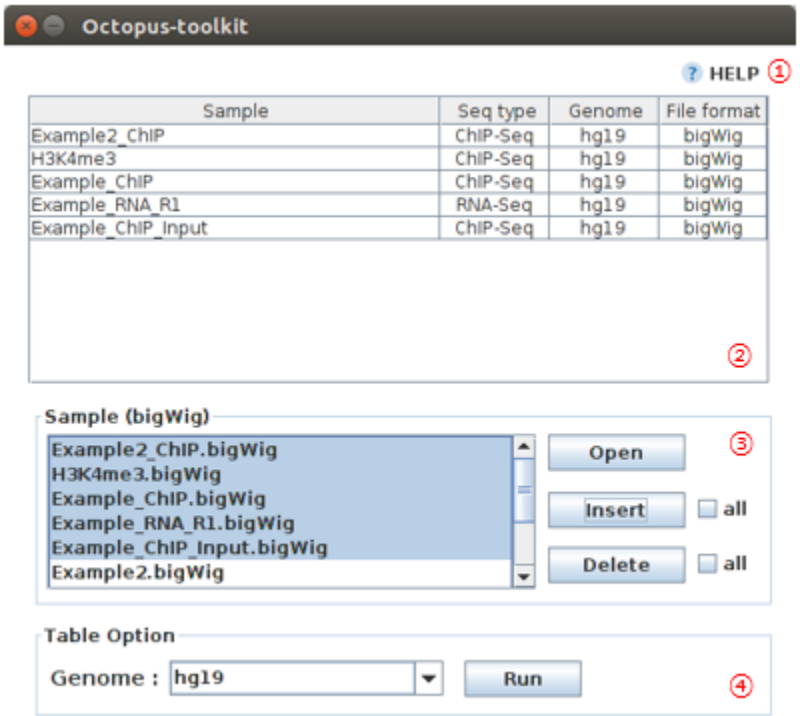
This function is not applicable for RNA-seq data.



No	Name	Description
1	`Help`	Go to the tutorial.
2	`Annotation`	Choose a peak file.
3	`Samples`	Status window
4	`Sample bigWig files`	Select bigWig files of samples you want to draw over the identified regions in the peak file.
5	`Option`	Define the range (bp) relate to the center of peaks.

4-8.IGV Table

You can visualize your data with bigWig files via IGV (IGV, Integrative Genomics Viewer).



No	Name	Description
1	`Help`	Go to the tutorial.
2	`Samples`	Status window.
3	`Sample bigWig files`	Select bigWig files for visualization.
4	`Genome`	Choose the reference genome.

4.1.6 5.User Guide

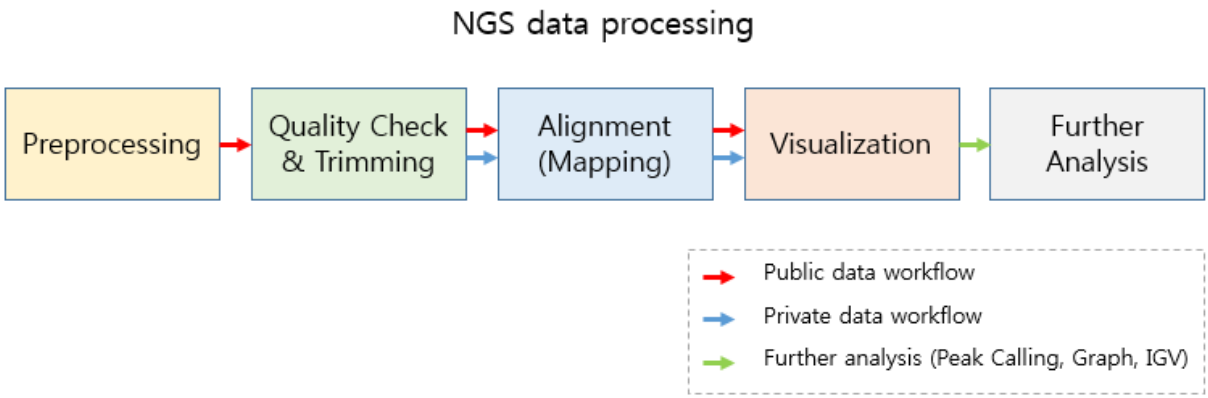
Octoput-toolkit can analyze a number of publicly available next-generation sequencing (NGS) data in with a single step. In addition, you can also analyze your own data (.fastq) using the same analysis pipeline that is provided by the Octopus-toolkit.

- `Supported NGS types` : RNA-seq, ChIP-seq, ATAC-seq, DNase-seq, MeDIP-seq, and MNase-seq
- `Public data` : NGS data released from gene expression omnibus (GEO).
- `Private data` : NGS data stored in your computer (.fastq or .fastq.gz)

Octopus-toolkit provides several additional functions for further analysis. * `Peak Calling` : Identification of read enriched regions (.bed) * `Drawing Graph` : Drawing line plot and heatmap on specified regions (.bed) * `Visualization` : Explore genome with bigWig files through IGV

Basically, Octopus-toolkit processes NGS data by the following steps.

- `NGS data processing`



5-1.3rd party tools used in Octopus-toolkit

Octopus-toolkit utilizes the following 3rd party tools during the process.

NGS Process	Function	3rd party tool	Sub-tools
`Preprocessing`	Download SRA files from NCBI	`Aspera`	ascp
	Convert SRA files to Fastq files	`SRAToolkit`	fastq-dump
`Quality check`	Quality check for raw data	`FastQC`	fastqc
`Trimming`	Trimming for adapter sequence and portions of low-quality reads	`Trimmomatic`	
`Alignment`	Indexing a reference genome	`Hisat2`, `STAR`	hisat2-build, STAR
	Mapping reads to the reference genome	`Hisat2`, `STAR`	hisat2-align, STAR
`Visualization`	Create bigWig files for visualization	`Homer`	makeTagDirectory,makeUCSCFile,analyzeRe
`Peak calling`	Detect enriched regions by mapped reads	`Homer`	findPeak, pos2bed,annotatePeaks
`Graph`	Calculate normalized values from bigWig files	`Bwtool`	matrix
	Draw the heatmap and line plot	`R`	pheatmap, ggplot2
`IGV`	Explore the genome with processed data (bigWig files)	`IGV`	

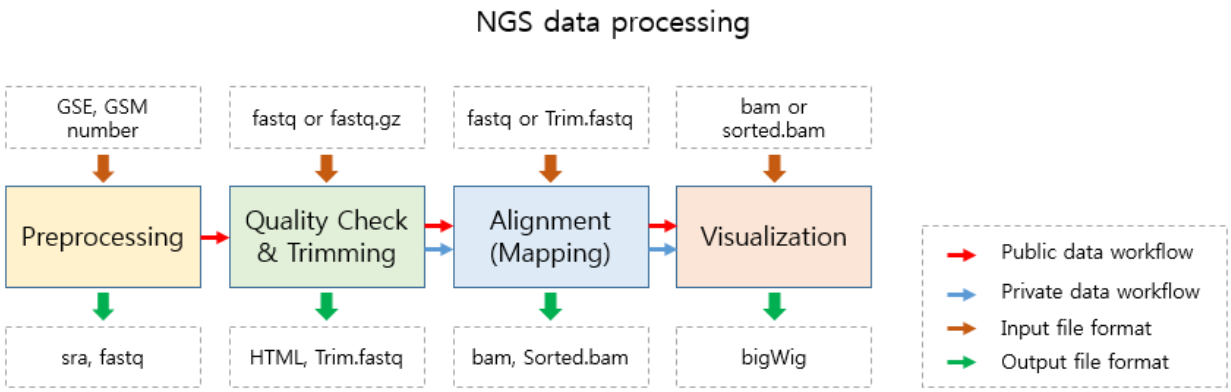
5-2.Public data

Quick Start

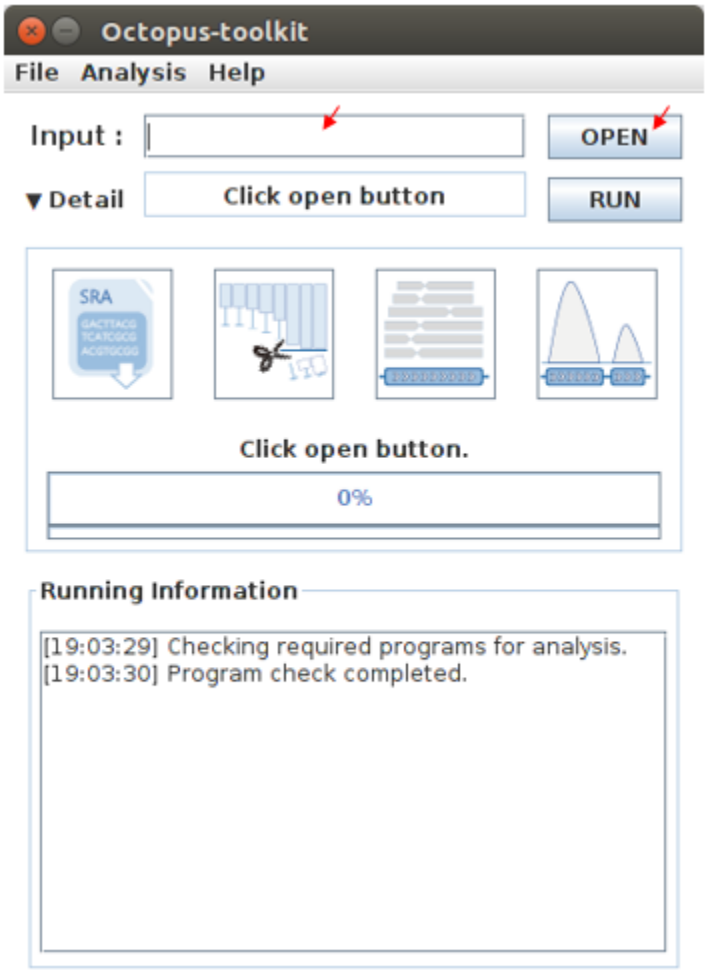
Note:

1. Enter a GEO (Gene Expression Omnibus) accession number or click the open button to load a list of GEO accession numbers.
2. Click the Run button.
3. Select appropriate options you want to use.
4. Click the Run button to begin the analysis.

Work flow



To analyze a single or a set of publicly available NGS data from GEO.



Please enter a single GEO Accession number or open a text file containing GEO Accession numbers.

- Input : `GEO Accession number`

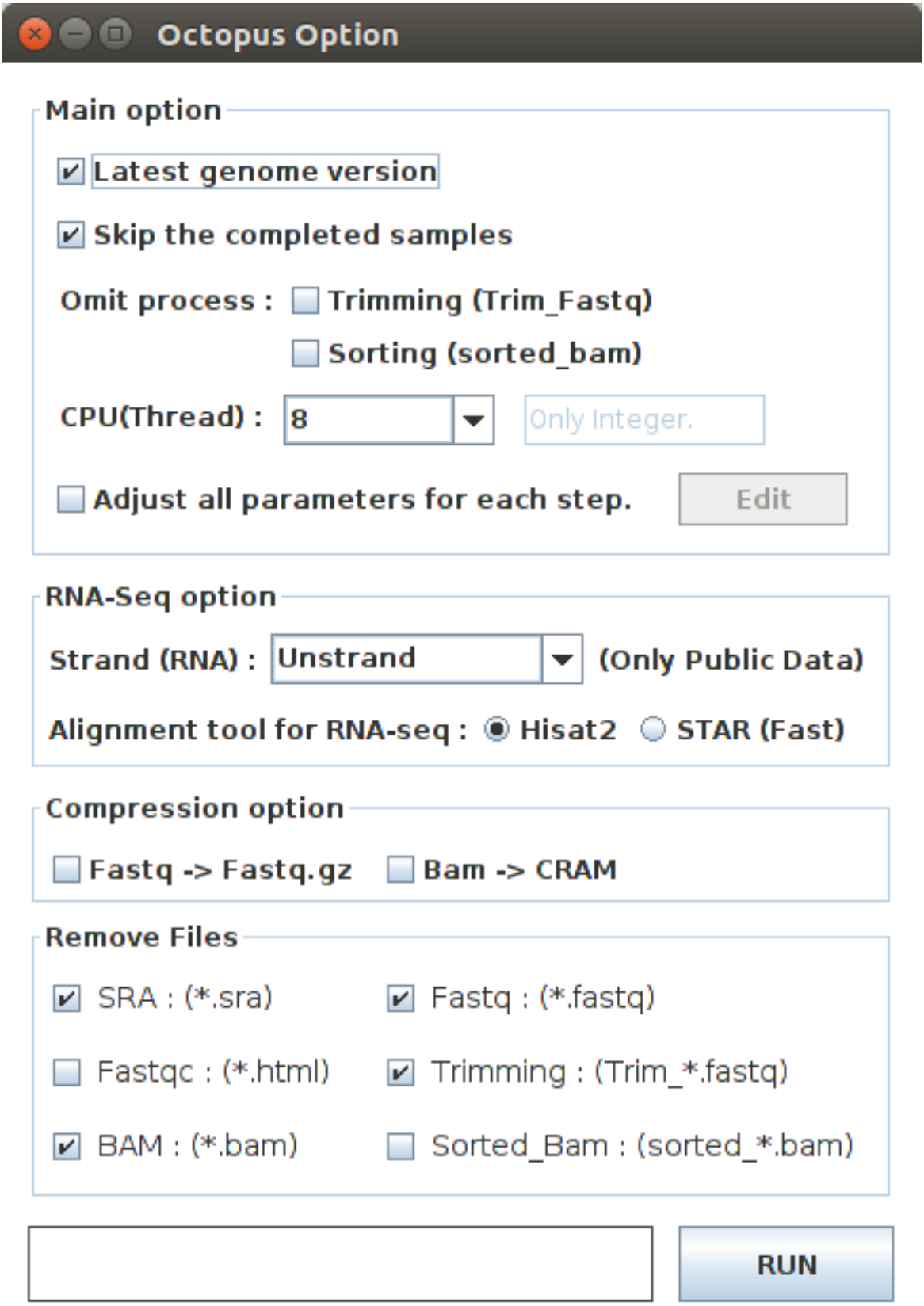
```
GSExxx : Each GSE (study) record is assigned to a single study which contains at
↪least a NGS data (GSM).
GSMxxx : Each GSM (sample) record is assigned to a single NGS data.
```

- Input : `GEO Accession number list` (`example.list`)

1. Enter a single GEO accession number or click the open button to load a list GEO accession numbers.
2. Click the Run button.

Octopus option

You can change a number of parameters provided by Octopus-toolkit or the integrated tools.


Octopus Option
Main option
Latest genome version
Skip the completed samples
Omit process :
Trimming (Trim_Fastq)
Sorting (sorted_bam)
CPU(Thread) :
8
Only Integer.
Adjust all parameters for each step.
Edit
RNA-Seq option
Strand (RNA) :
Unstrand
(Only Public Data)
Alignment tool for RNA-seq :
Hisat2
STAR (Fast)
Compression option
Fastq -> Fastq.gz
Bam -> CRAM
Remove Files
SRA : (*.sra)
Fastq : (*.fastq)
Fastqc : (*.html)
Trimming : (Trim_*.fastq)
BAM : (*.bam)
Sorted_Bam : (sorted_*.bam)
RUN

Option	Decription
`Latest genome version`	Use the latest genome rather than the genome used for the study.
`Skip the completed samples`	Skip the samples that have already been analyzed.
`Omit process`	Omit the selected processes such as trimming and sorting steps.
`CPU(Thread)`	Set the number of CPUs to use.
`Adjust all parameters for each step`	Change full parameters in each step.
`Edit`	Open the Full parameter option window.
`Strand (RNA)`	Set the library strand for RNA-Seq.
`Alignment tool for RNA-seq`	Set the alignment tool for RNA-seq.
`Fastq -> Fastq.gz`	Compress Fastq to Fastq.gz.
`Bam -> CRAM`	Compress Bam to CRAM.
`Remove Files`	Delete selected intermediate files once each process is completed to save space.

- `Latest genome version`

Octopus-toolkit can analyze the genomes of Homo sapiens, Mus musculus, Drosophila melanogaster, Saccharomyces cerevisiae, and Canis lupus familaris.

Table 1: Available analysis genome version

Organism	Genome version
Homo sapiens	hg38, hg19, hg18
Mus musculus	mm10, mm9
Drosophila melanogaster	dm6, dm3
Saccharomyces cerevisiae	sacCer3
Canis lupus familaris	canFam3
Arabidopsis thaliana	tair10
Danio rerio	danRer10
Caenorhabditis elegans	ce11

The latest genome version uses the latest version of the genome for analysis. If you don't select this option, Octopus-toolkit uses the genome defined by submitter.

- Latest genome (O) : hg38, mm10, dm6, sacCer3, canFam3, tair10, danRer10, ce11
- Latest genome (X) : hg19, mm9, dm3, sacCer3, canFam3, tair10, danRer10, ce11
- `Skip the completed samples`

While analyzing a number of GSE/GSM data, you can stop the analysis and resume it later.

Octoput-toolkit will skip the samples that have been analyzed completely.

If you have the samples that have been analyzed completely and you want to analyze it again, please do not check this option.

- `Omit process`

The omit process allows you to skip the trimming step and/or the sorting step. This shortens the anaysis time.

During the trimming process, all reads will be discarded if all of the reads have bad sequencing quality. Octopus-toolkit will analyze the original raw data (.fastq) in this case by skipping the trimming step.

During the sorting process, BAM file will be sorted by using Samtools. In general, many applications uses sorted BAM files. If you are not interested in analyzing the sorted BAM files, you may skip this process.

- `CPU(Thread)`

You can set the number of CPUs for analysis. (Default : Maximum number of cores depending on your computer)

- `Adjust all parameters for each step`

You can adjust many parameters for each stop. Check the box and click the Edit button. The parameter window will pop up.

Please refer to the link for details : *Full Parameter*

- `Edit`

When you click the Edit button, the parameter window will appear.

- `Strand (RNA)`

The strand option allows you to choose whether or not to take the stranded information into account. This is only available for stranded-specific RNA-seq.

Octopus-toolkit extracts information from the GEO website when analyzing the public data. However, stranded-specific information of RNA-seq is not well documented. Therefore, this may or may not be applicable depending on the data.

You can select either non-strand library or the strand-specific library such as FR-Firststrand, FR-Secondstrand using this option.

- `RNA-Seq alignment tools`

You can select an alignment tool to be used during the alignment process for RNA-seq: HISAT2 or STAR.

HISAT2 uses less memory (RAM) than STAR, but STAR is generally faster than HISAT2.

- `Fastq->Fastq.gz` or `Bam->CRAM`

You can compress intermediate files to save your disk space.

- `Remove Files`

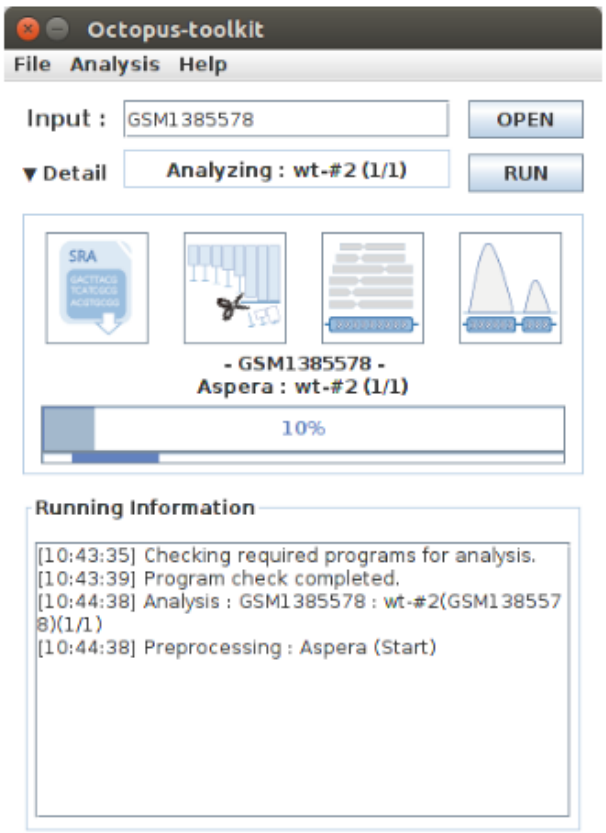
Each step creates intermediate files which may or may not be used. If you want to further analyze the processed data, you might want to keep those intermediate files. If not, you can remove intermediate files (up to few hundread gigabytes) by selecting the boxes in Reomve Files window.

Option	Extension	Description
`SRA`	sra	Compressed raw data downloaded from NCBI. (Sequence Read Archive)
`Fastq`	fastq or fq	Raw data converted during preprocessing. (A short read sequence file)
`Fastqc`	html or text	Output generated during Quality Check. (output of FastQC)
`Trimming`	fastq or fq	Output generated during Trimming. (Trimmed raw file(Fastq))
`BAM`	bam	Output generated during Alignment. (Mapped read to the genome)
`Sorted_Bam`	bam	Output generated during Sorting. (Sorted mapped read)

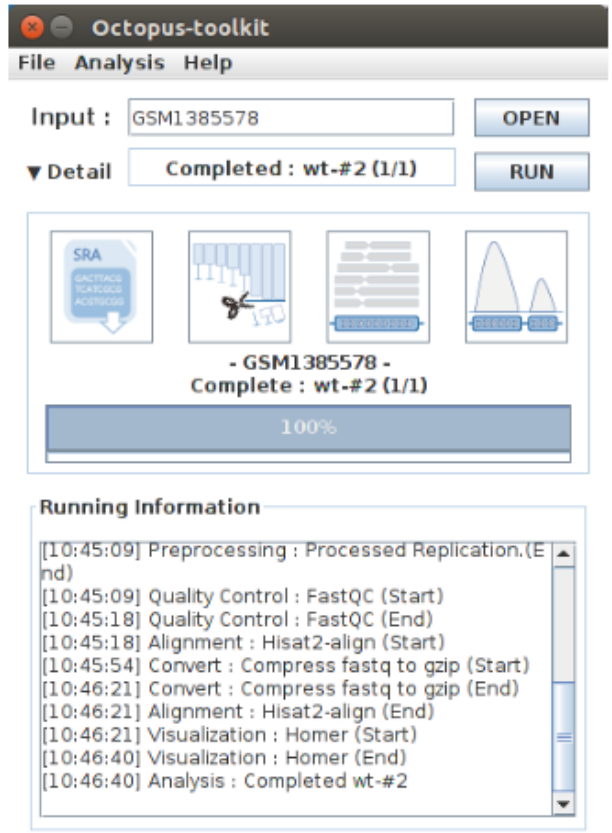
3. Set the paramters and options.
4. Click the Run button to begin the anlaysis.

Run

- Below shows progress bar and status window (GSM1385578).



< The start of analysis >



< The end of analysis >

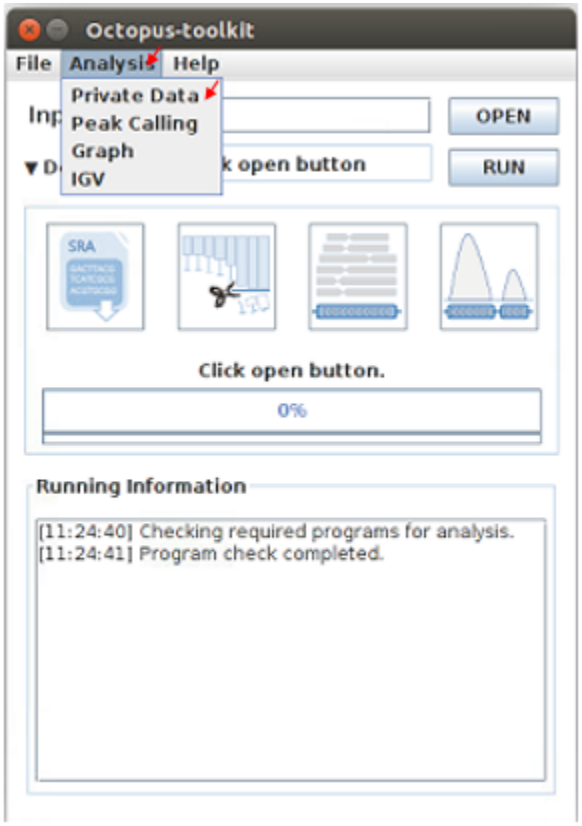
5-3.Private data

Quick Start

Note:

1. Select the analysis tab -> Select the Private Data function in the Menu bar.
2. Select raw files (.fastq) in your computer.
3. Add appropriate information for each sample in the private table.
4. Click the Run button in the private table.

Analyzing your data (private data)



Unlike the public data analysis, private data analysis does not require the converting step (.sra to .fastq).

Input files can be fastq (.fastq or .fq) files or compressed fastq (.fastq.gz or .fq.gz) files.

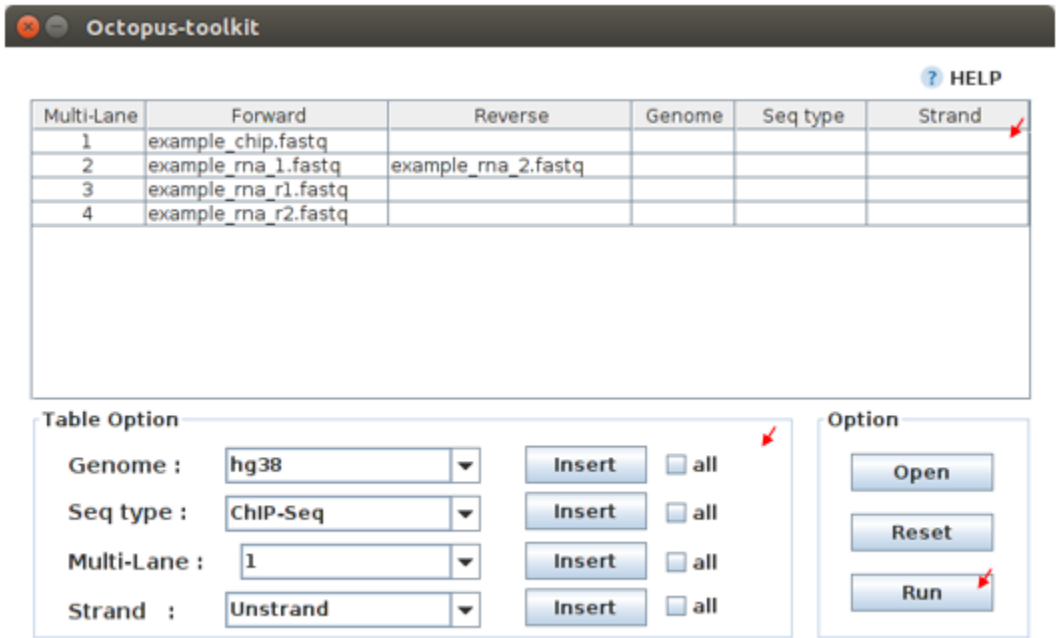
Files must follow the rules below.

Note:

- Raw data : Sample `.fastq` or Sample `.fq`
- compressed Raw data : Sample `.fastq.gz` or Sample `.fq.gz`
- Single-End data : Sample `.fastq` (or fq, fastq.gz, fq.gz)
- Paired-End data : Sample `_1.fastq`, Sample `_2.fastq`

Octopus-toolkit only loads files that match the above rules.

Private table



Octopus-toolkit requires appropriate sample information for each file. You need to specify the required information.

If any of the selected files does not appear in the list, please check the file name and format of your files.

You must specify the following information for each sample.

Option	Decription
`Genome`	Select the genome.
`Seq type`	Select the experimental type such as ChIP-seq.
`Multi-Lane`	Set the Multi-lane option.
`Strand`	Select the strand strategy if applicable.

- `Genome`

The following genomes are available in the Octopus-toolkit:

Species	Genome version
`Homo sapiens`	`hg38` (Dec.2013, GRCh38), `hg19` (Feb.2009,GRCh37), `hg18` (Mar.2006 NCBI36)
`Mus musculus`	`mm10` (Dec.2011 GRCm38), `mm9` (July.2007 NCBI37)
`Drosophila melanogaster`	`dm6` (Aug.2014 BDGP Release 6+ ISO1 MT), `dm3` (Apr.2006 BDGP R5)
`Saccharomyces cerevisiae`	`sacCer3` (Apr.2011 SacCer_Apr2011)
`Canis lupus familaris`	`canFam3` (Sep.2011 Broad CanFam3.1)
`Arabidopsis thaliana`	`tair10`
`Danio rerio`	`danRer10` (Sep.2014 GRCz10)
`Caenorhabditis elegans`	`ce11` (Feb.2013 WBcel235)

- `Seq type`

Octopus-toolkit supports the following experimenatal types: ChIP-Seq, RNA-Seq, MeDIP-Seq, ATAC-Seq, DNase-Seq and MNase-Seq.

- `Multi-Lane`

A single sample can be obtained from muliple lanes in a sequencing instrument. In this case, files from multe lanes can be merged by setting the same number in the Multi-Lane column.

Multi-lane files generally have the following filenames.

```
Sample.L001.fastq, Sample.L002.fastq, Sample.L003.fastq ... Sample.L008.fastq
```

To merge the above files, you must set the number of 'Multi-Lane columns' to the same number for each file.

- `Strand`

This option is to set the library strategy for RNA-seq.

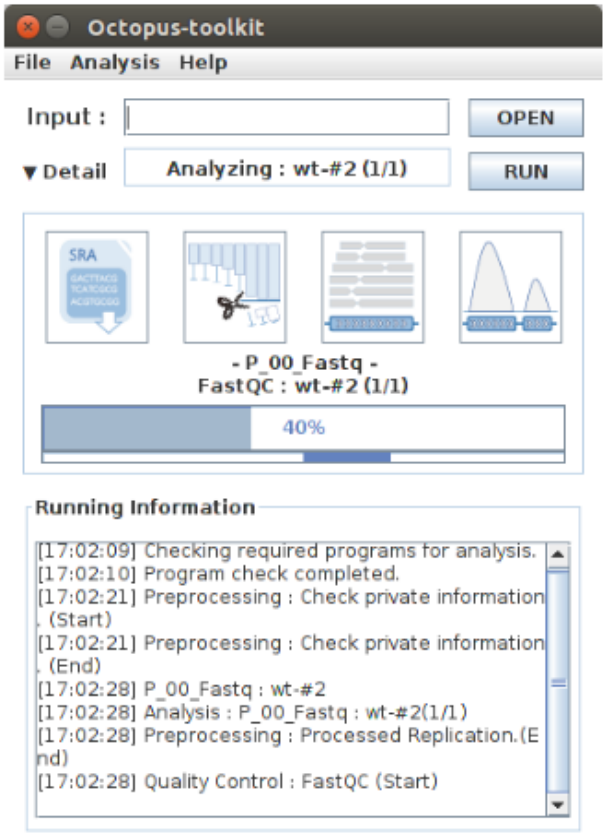
1. Unstranded library : `Unstrand` (Default)
2. Strand-specific library : `FR-Firststrand` or `FR-secondstrand`

Options

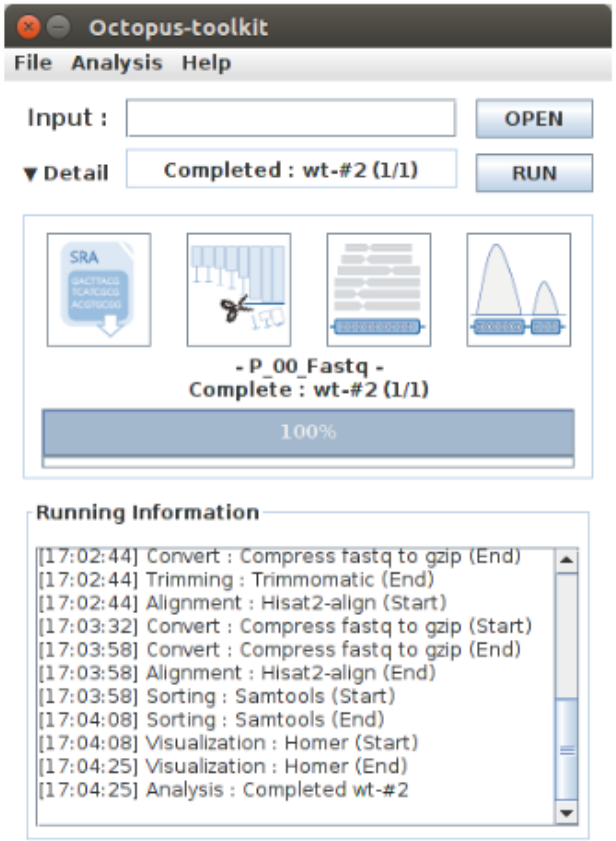
Options for private analysis is the same as public data analysis. Please refer to the public data analysis. (*Octopus option*)

Run

- Snapshots (Private data analysis)



< The start of analysis >



< The end of analysis >

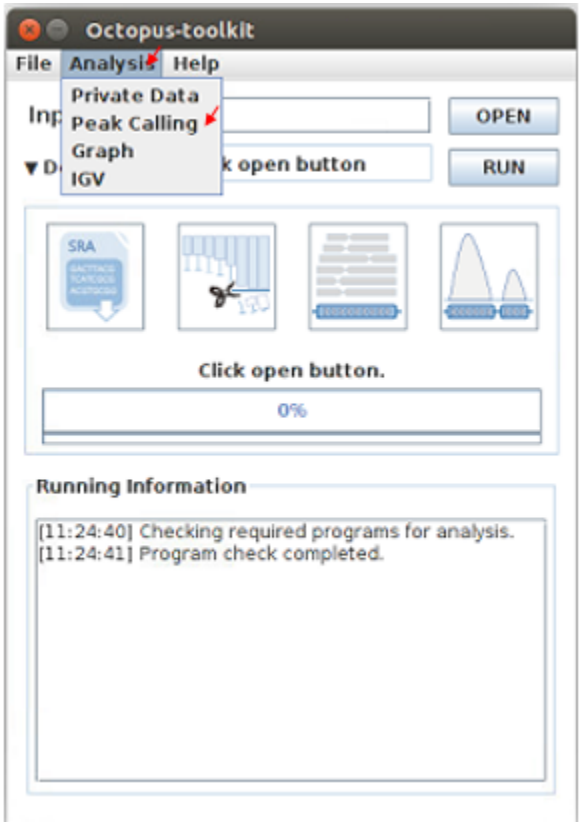
5-4.Peak Calling

Quick Start

Note:

1. Select the Analysis tab -> Click the Peak Calling function in the Menu bar.
2. Select the output folder (Result/GSExxxxx) in the Result directory generated by Octopus-toolkit.
3. Add information of each sample in the peak calling table.
4. Click the Run button.

Peak calling anlaysis

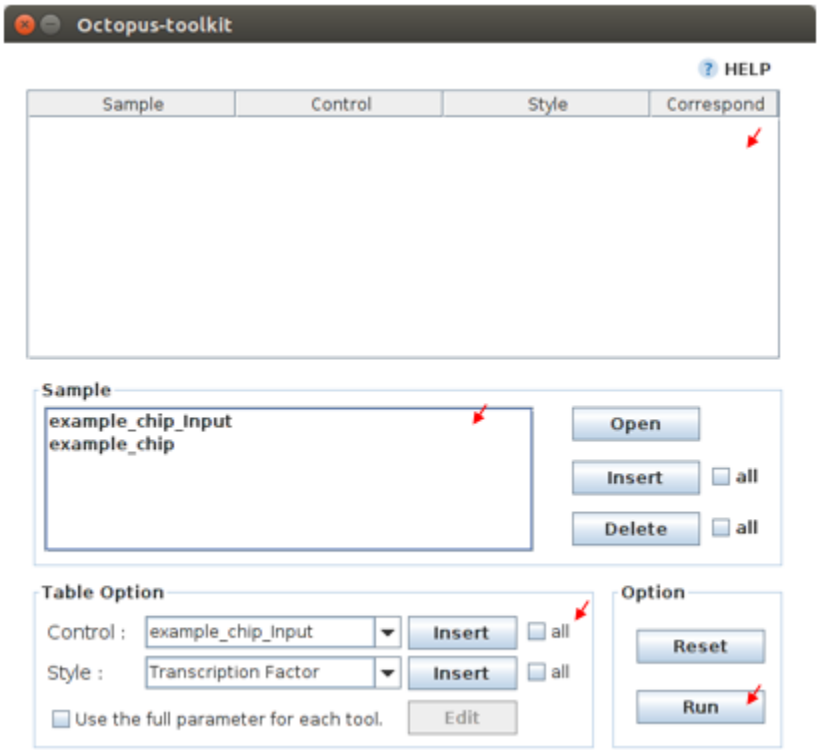


The purpose of the peak calling anlaysis is to identify regions enriched by mapped reads.

In order to perform the peak calling analysis, you must have the Octopus-toolkit output folders.

1. Select the Analysis tab -> Click the Peak Calling function in the Menu bar.
2. Select output directories generated by the Octopus-toolkit.

Peak calling table



To run the peak calling analysis, please select output folders (Result/GSExxxxx). Then, fill in the blanks using the Table Option functions.

- `Control`

If available, please select an appropriate control (IgG or input) per sample to filter out the background noise. (Recommended)

- `Style`

Based on experimental types, you can select a predefined paramter (by HOMER) for the Peak calling process.

option	Seq type	Description
`Transcription Factor`	ChIP-Seq, DNase-Seq	Peak finding for single contact or focal ChIP-Seq experiments or DNase-Seq.
`Histone`	ChIP-Seq	Peak finding for broad regions of enrichment found in ChIP-Seq experiments for various histone marks.
`DNase`	DNase-Seq	Adjusted parameters for DNase-Seq peak finding.
`mC`	MeDIP-Seq	DNA methylation analysis.

Please select a style option that meets your analysis needs.

3. Add appropriate information for each sample in the private table.
4. Click the Run button in the private table.

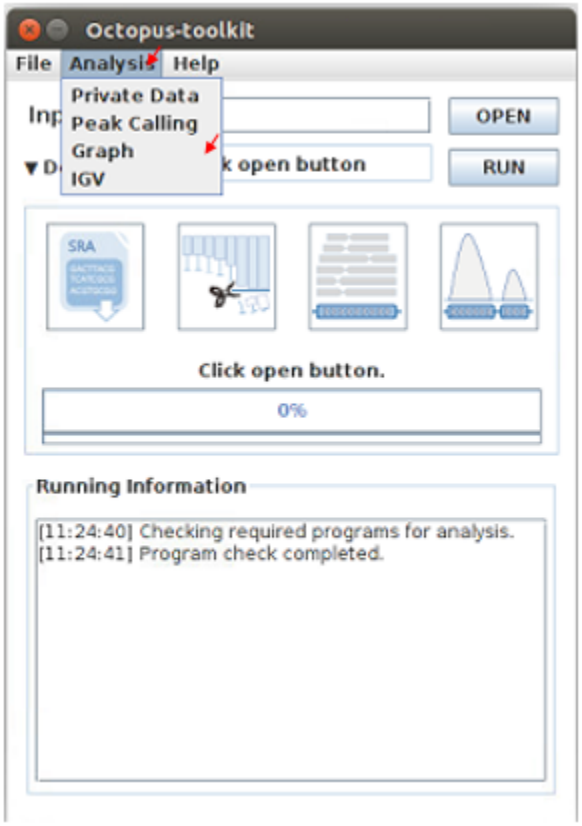
5-5.Graph

Quick Start

Note:

1. Select the Analysis tab -> Click the Graph function in the Menu bar.
2. Select output folders (Result/GSExxxxx). Multiple output folders can be selected.
3. Set the range of transcription start site (TSS) region and BIN size in the Graph table.
4. Click the Run button.

Start analyzing Graph



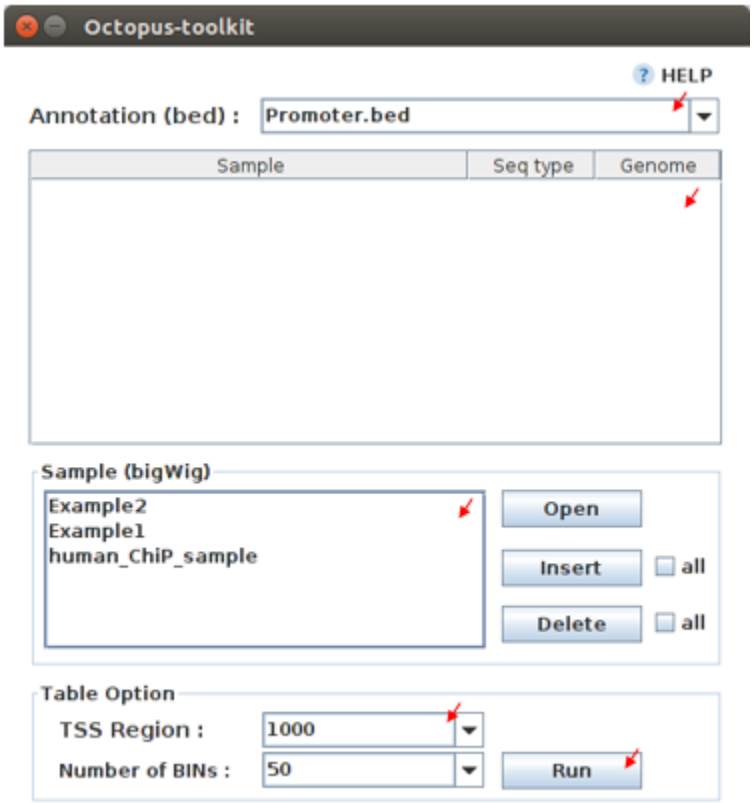
The Graph function is to draw average signal pattern on specificed regions which are defined by the user. Signals are extracted from bigWig (normalized to ten million mapped reads) files.

If you would like to draw plots on peaks, you need to complete the peak calling analysis for a sample of your interest.

- `Previous steps` : Public data or Private data analysis -> Peak Calling.

1. Select the Analysis tab -> Click the Graph function in the Menu bar.
2. Select output folders generated by either Public analysis or Private analysis.

Graph table



To draw graphs, Octopus-toolkit requires bigWig (signal) files, which are generated by either Public anlaysis or Private analysis.

- `bigWig` : Output of the Public data or Private data analysis.
- `bed` : Output of the Peak calling analysis.

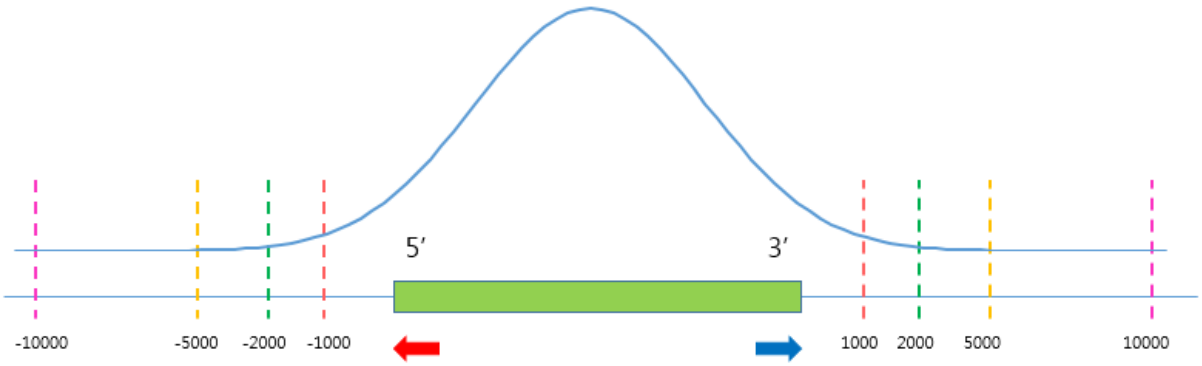
Finally, hit the Run button. The output (plots) will be stored in the Graph directory under the Result folder.

- `Annotation(bed)`

First, select loci (.bed) of your interest from the Annotation (bed) function. Second, select samples (.bigWig) of your interest from the Sample list.

- `TSS Region`

Third, set appropriate paramters from the Table option. The unit for this option is basepair (bp).



The default ranges of TSS-regions are 1000, 2000, 5000 and 10000 bp.

- `Number of BINs`

The region selected in the TSS region option is divided into n (number of BINs) BINs

The lager the bin size, the smoother the graph can be drawn.

3. set the TSS region and BIN size in the Graph table.
4. Click the Run button.

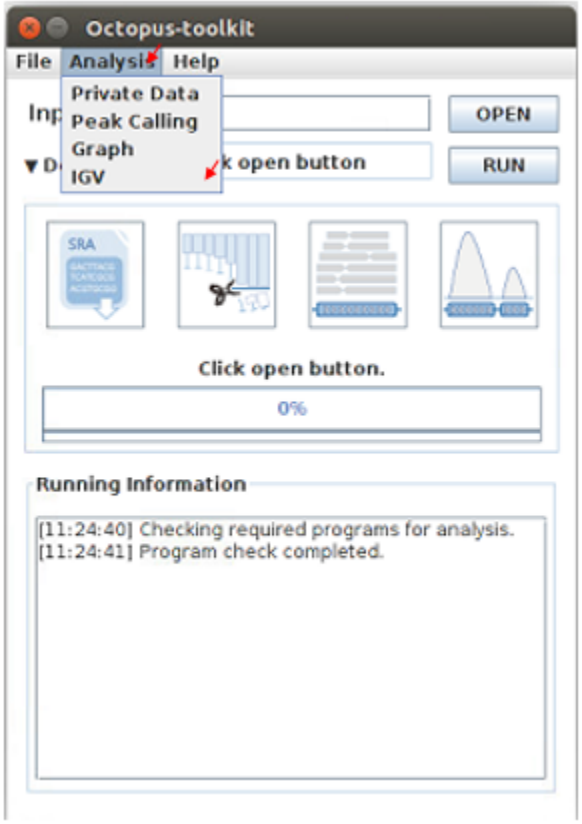
5-6.Visualization

Quick Start

Note:

1. Select the Analysis tab -> Click the IGV function in the Menu bar.
2. Select output folders (Result/GSExxxxx) of your interest.
3. In the sample window, select samples and then, click the Insert button.
4. Check see if all genomes are the same. Only data in the same genome can be loaded into the IGV.
5. Set the same genome in the Table option.
6. Click the Run button.

Start analyzing IGV

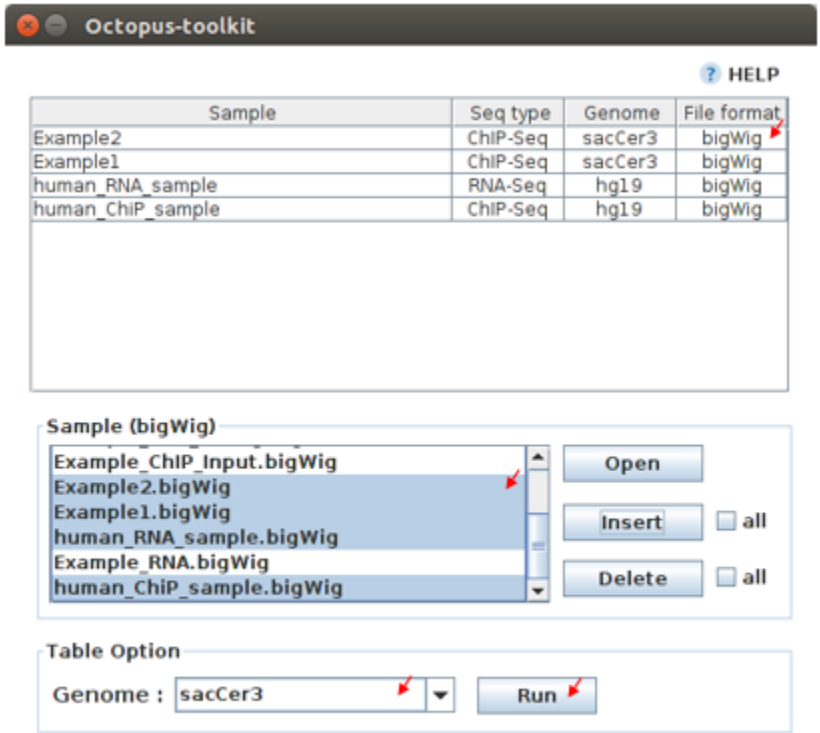


The IGV function is a process of visualizing analyzed data through IGV, a visualization tool.

IGV uses bigWig files.

1. Select the Analysis tab -> Click the IGV function in the Menu bar.
2. Select output folders (Result/GSExxxxx) of your interest.

IGV table



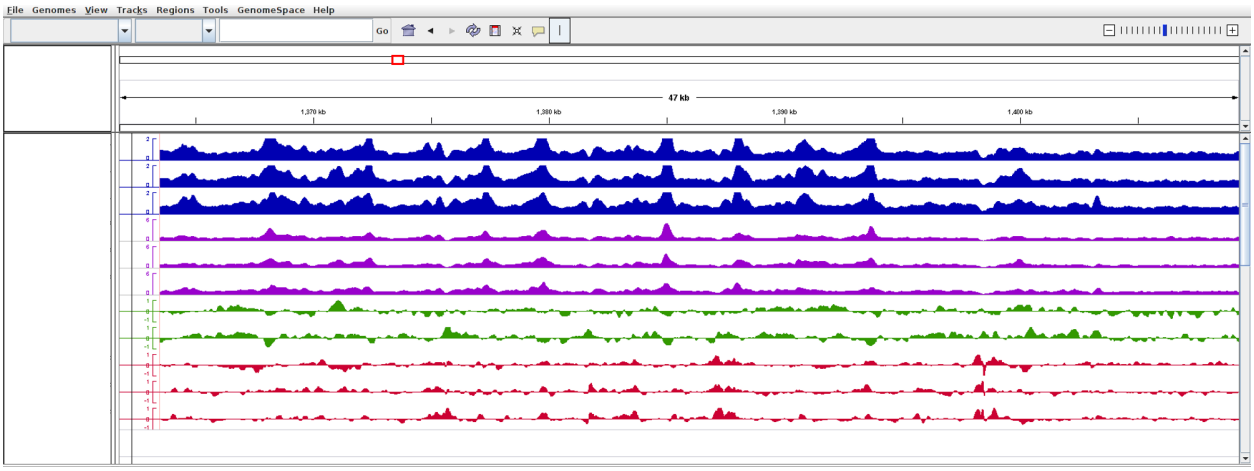
- `Genome`

Genome option shows the genome of the samples.

3. In the sample window, select samples and then, click the Insert button.
4. Check if all genomes are the same. Only the data in the same genome can be loaded into the IGV.
5. Set the same genome in the Table option.
6. Click the Run button.

Run

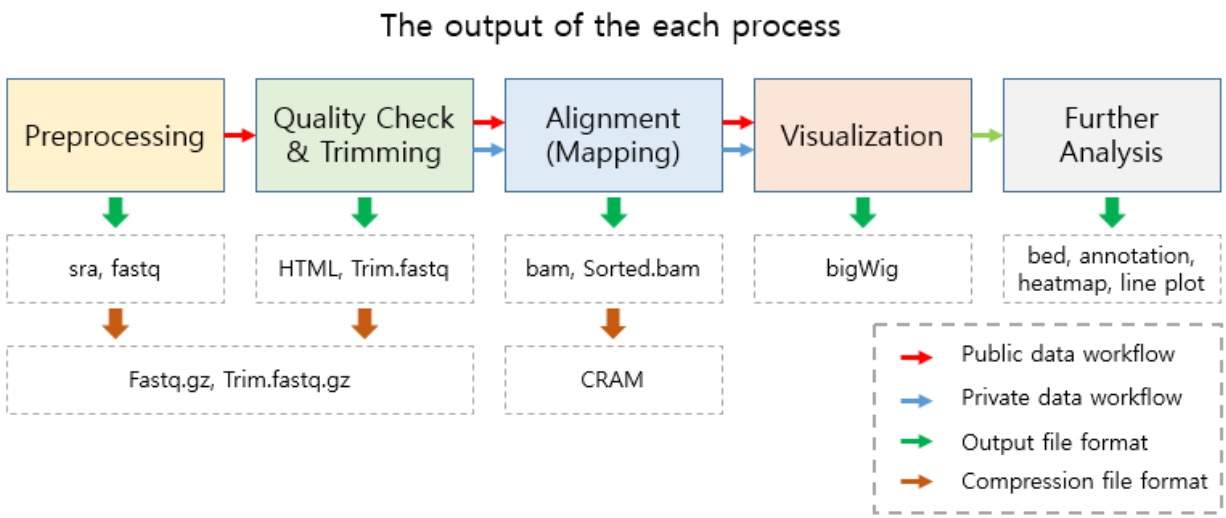
- Below shows run screen of IGV.



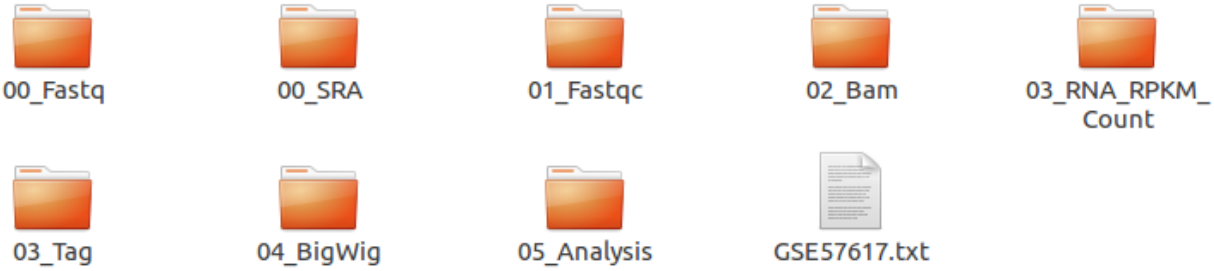
Unlike other tools integrated in Octopus-toolkit, the IGV tool runs separately from the Octopus-toolkit.

5-7.Output (important!)

The output files generated by each process are as follows:

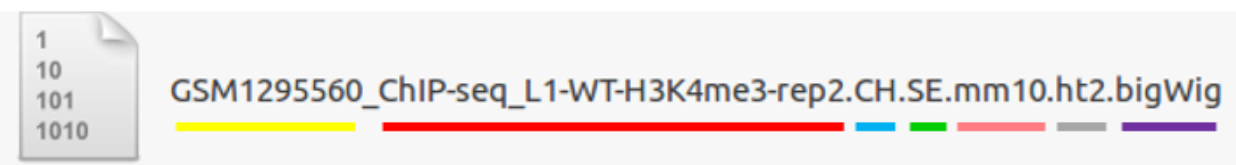


- In the Result folder



Folder name	Process	File format	Description
`00_Fastq`	Preprocessing,Trimming	`fastq`, `Trim.fastq`	Save the raw file and trimmed file.
`00_SRA`	Preprocessing	`sra`	Store the SRA file downloaded from NCBI
`01_Fastqc`	Quality check	`html`, `txt`	Save the result of the Quality check.
`02_Bam`	Alignment	`bam`, `sorted.bam`, `bai`	Save the Alignment and sorted files.
`03_RNA_RPKM_Count`	Normalization	`RPKM`, `Count`	Save the calculated RPKM and raw read count tables for the RNA-Seq data.
`03_Tag`	Downstream (motif) analyses by HOMER	`Tag folder`	Save the Tag folders created by the Homer tool.
`04_BigWig`	Visualization	`bigWig`	Save the bigWig files for visualization
`05_Analysis`	Peak Calling,Annotation	`bed`, `annotation`	Save the peak (.bed) and annotation files.
`GSE57617.txt`	Log file	`txt`	Sample.txt is a file that stores the analysis status and information.

5-8.File Naming



- **`Yellow`** [GSM Accession number] Only applicable for the public data.
- **`Red`** [ChIP-Seq_L1-WT-H3K4me3-rep2] Sample file name (title) defined on the GEO website.
- `Blue` : Experimental types described below.

Experimental types	Abbreviation	Experimental types	Abbreviation	Experimental types	Abbreviation
ChIP-Seq	`CH`	RNA-Seq	`RN`	MeDIP-Seq	`ME`
ATAC-Seq	`AT`	DNase-Seq	`DN`	MNase-Seq	`MN`

- **`Green`** [Sequencing strategy] SE : Single-End, PE : Paired-End
- **`Pink`** [Reference genome] Reference Genome
- **`Gray`** [Alignment tool] RNA-Seq alignment tools. (ht2 : Hisat2, str: STAR)
- **`Purple`** [File extension] Output Format

5-9.Full Parameters

You can adjust the parameters of 3rd party tools integrated into the Octopus-toolkit.

The 3rd party tools used in Octopus-toolkit : *3rd party tools*

Preprocessing

In the preprocessing step, Octopus-toolkit downloads selected NGS data from NCBI and converts the downloaded (.sra) files to FASTQ files. The 3rd party tools used in the preprocessing step are Aspera and SRAToolkit(fastq-dump)

- **Transfer rate**

  `MAX-RATE` : MAX transfer rate (Only Integers)

  `MIN-RATE` : MIN transfer rate (Only Integers)

  `Overwrite` : Overwrite-Method, Always(Default), Never, Older, Diff

- **Convert Sra to Fastq (Filtering)**

  `MIN-Read Length` : Filter by sequence length >= <Value> (Only Integers)

  `Aligned or unaligned reads` : Dump only aligned sequence or unaligned sequences, NotUse(Default), Both, Aligned, Unaligned

  `Quality conversion` (offset) : Offset to use for quality conversion, 33(Default), 64

  `Dump biological reads(Only)` : Dump only biological reads, No(Default)

QC & Trimming

QC & Trimming is the process of assessing the quality of the reads. If bad sequencing quality are detected, portions of low-quality reads are trimmed. The 3rd party tools used in QC & Trimming are FastQC and Trimmomatic.

- **Determining the quality of DNA Sequence**

  `K-Mer` : Specifies the length of Kmer to look for in the Kmer content module, Specified Kmer length must be between 2 and 10. Default length is 7 if not specified.

  `Allocated memory` : Set the momory available on your computer for Quality check. Provides a measure of currently available memory . (Octopus-toolkit option)

- **Trimmed DNA sequence data**

  `Illumina adapt Sequence` : Cut adapter and other illumina-specific sequences from the read.

  `Seed mismatches` : Specifies the maximum mismatch count which will still allow a full match to be performed

  `Palindrome clip threshold` : Specifies how accurate the match between the two 'adapter ligated' reads must be for PE palindrome read alignment.

  `Simple clip threshold` : Specifies how accurate the match between adapter or any sequence must be against a read.

  `Window size` : specifies the number of bases to be averaged.

  `Average quality` : Specifies the average quality required.

  `LEADING` : Specifies the minimum quality required to keep a base.

  `TRAILING` : Specifies the minimum quality required to keep a base.

  `HEADCROP` : The number of bases to keep, from the start of the read.

  `TAILCROP` : The number of bases to remove from the start of the read.

`Minimum length of reads to be kept` : Specifies the minimum length of reads to be kept.

Alignment-Hisat2

Alignment is the process of mapping reads to a reference genome. The 3rd party tool used in Alignment is Hisat2.

- **Input**

  `Skip N read` : Skip the first <int> reads/pairs in the input (none)

  `Stop after aligning N reads` : Stop after first <int> reads/pairs (no limit)

  `Trim N bases 5' end` : Trim <int> bases from 5'/left end of reads (0)

  `Trim N bases 3' end` : Trim <int> bases from 3'/right end of reads (0)

- **Scoring**

  `Ambiguous read penalty` : Penalty for non-A/C/G/Ts in read/ref

  `Mismatch penalty` : Max and min penalties for mismatch; lower qual = lower penalty <2,6>

  `Soft-Clipping penalty` : Max and min penalties for soft-clipping; lower qual = lower penalty <1,2>

  `Read gap penalty` : Read gap open, extend penalties (5,3)

  `Reference gap penalty` : Reference gap open, extend penalties (5,3)

- **Alignment**

  `Ignore all quality values` : Treat all quality values as 30 in Phred scale (no)

  `Do not align reverse of read` : Do not align forward (original) version of read (no)

  `Do not align forward of read` : Do not align reverse-complement version of read (no)

- **Spliced alignment**

  `Do not spliced alignment` : Disable spliced alignment

  `Canonical` : Penalty for a canonical splice site (0)

  `Non-canonical` : Penalty for a non-canonical splice site (12)

  `MIN-Length` : Minimum intron length (20)

  `MAX-Length` : Maximum intron length (500000)

Alignment-STAR

Alignment is the process of mapping reads to a reference genome. The 3rd party tool used in Alignment is STAR (RNA-Seq only).

- **Alignment**

  `AlignIntronMin` : Minimum intron size: genomic gap is considered intron if itsnlength>=alignIntronMin, otherwise it is considered as Deletion (21)

  `AlignIntronMax` : Maximum intron size, if 0, max intron size will be determined byn(2^winBinNbits)*winAnchorDistNbins (0)

`AlignMatesGapMax` : Maximum gap between two mates, if 0, max intron gap will be determined by (2^winBinNbits)*winAnchorDistNbins (0)

- **Output Filtering**

  `OutFilterMultimapNmax` : int: maximum number of loci the read is allowed to map to. Alignments (all of them) will be output only if the read maps to no more loci than this value. Otherwise no alignments will be output, and the read will be counted as "mapped to too many loci" in the Log.final.out. (10)

  `OutFilterMismatchNmax` : int: alignment will be output only if it has no more mismatches than this value. (10)

  `OutFilterMismatchNoverLmax` : float: alignment will be output only if its ratio of mismatches to *mapped* length is less than or equal to this value.(0.3)

Visualization-TagDirectory

Visualization-TagDirectory is the process of creating Tag directories. The 3rd party tool used in TagDirectory is Homer.

- **Create tag directory**

  `Fragment-Length` : (Set estimated fragment length - given: use read lengths), By default treats the sample as a single read ChIP-Seq experiment

  `Maximum tags per bp` : Maximum tags per bp, default: no maximum

  `Flip the strands of each read` : Flip strand of each read, i.e. might want to use with some RNA-seq

  `Length of the read to keep` : Filter reads with lengths outside this range

Visualization-MakeBigWig

MakeBigWig is the process of creating bigWig files from the Tag directories. The 3rd party tool used in MakeBigWig is Homer.

- **Make visualization data**

  `Size of the bigWig files` : Size of file, when gzipped, default: 1e10, i.e. no reduction

  `Fragment Length` : Approximate fragment length, default: auto

  `Resolution` : Resolution, in bp, of file, default: 1, `avg` report average coverage if resolution is larger than 1bp, default: max is reported

  `Tags per bp to count` : Minimum and maximum tags per bp to count, default: no limit

  `Plot negative values` : Plot negative values, i.e. for - strand transcription

- **Normalization**

  `Normalize the total number of reads` : Total number of tags to normalize experiment to, default: 1e7

  `Set the standard length` : Expected length of fragment to normalize to [0=off], default: 100

PeakCalling-ChIP-Seq/Histone

PeakCalling is the process of detecting enriched regions (peaks) by mapped reads. The 3rd party tool used in PeakCalling is Homer.

- **ChIP-Seq/Histone**

  `Peak size` : Peak size, default: 0

  `MIN-Distance` : Minimum distance between peaks, default: 0 (peak size x2)

  `Genome Size` : Set effective mappable genome size, default: 2e9

  `Fragment Length` : Approximate fragment length, default: auto

  `Input Fragment Length` : Approximate fragment length of input tags, default: auto

  `Tag` : Maximum tags per bp to count, 0 = no limit, default: auto

  `Input tag` : Maximum tags per bp to count in input, 0 = no limit, default: auto

  `Tag count to normalize` : Tag count to normalize to, default 10000000

  `Region Resolution` : Extends start/stop coordinates to cover full region considered "enriched" (YES), `Resolution` number of fractions peaks are divided in when extending 'regions', def: 4

PeakCalling-Peak Filter

- **Peak Filter**

  `Fold Enrichment(Input)` : Fold enrichment over input tag count, default: 4.0

  `Poisson p-value threshold(Input)` : Poisson p-value threshold relative to input tag count, default: 0.0001

  `Fold Enrichment(Local)` : Fold enrichment over local tag count, default: 4.0

  `Poisson p-value threshold(Local)` : Poisson p-value threshold relative to local tag count, default: 0.0001

  `Fold Enrichment(Unique Tag)` : Fold enrichment limit of expected unique tag positions, default: 2.0

  `Local Size(Local tag)` : Region to check for local tag enrichment, default: 10000

  `Input Size(Input tag)` : Size of region to search for control tags, default: 0

  `` `False Discovery Rate `` : False discovery rate, default = 0.001

  `Poisson p-value cutoff` : Set poisson p-value cutoff, default: 0.001

  `Set # of tags` : Set # of tags to define a peak, default: 25

  `Set # of normalized tags` : Set # of normalized tags to define a peak, by default uses 1e7 for norm

PeakCalling-Other analysis

- **MethylC-Seq/BS-Seq**

`Find Region` : Find unmethylated/methylated regions, default: -unmethyC

`Methyl Threshold` : Methylation threshold of regions, default: avg methylation/2

`Min cytosine per Methyl` : Minimum number of cytosines per methylation peak, default: 6

4.1.7 6.Tutorial

A case by case tutorial.

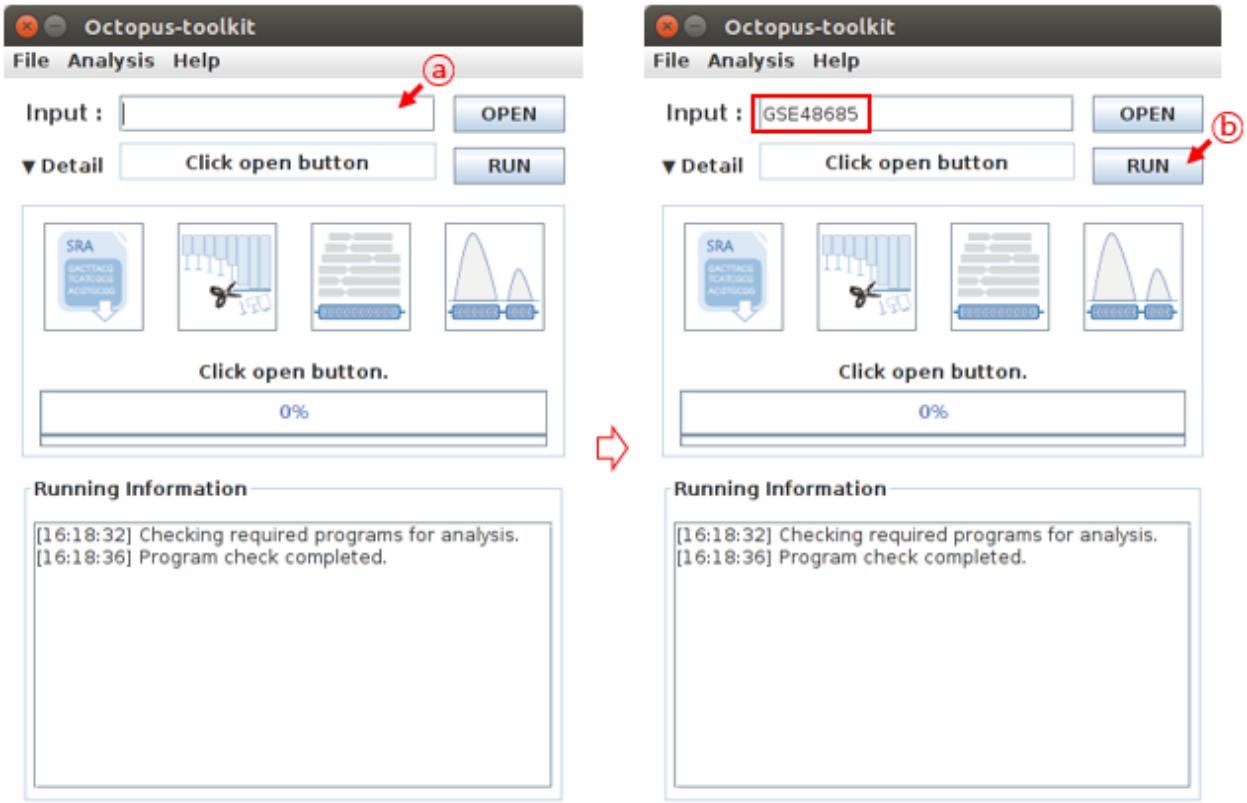
Tutorial ID	Description
6-1.Public data	How to analyze a public data with `GEO accession number`.
6-2.Public data	How to analyze a list of public data with a text file containing the `list` of GEO accession numbers.
6-3.Private data	How to setup Private table for `user's data`.
6-4.Private data	How to setup Private table when you have multiple files for a single sample: `Multi-lane`.
6-5.Peak Calling	How to identify peaks using `Peak Calling` with the output.
6-6.Graph	How to draw `Graph` with the output.
6-7.IGV	How to explore genome using `IGV` with the output.
6-8.Custom adapter sequence	how to use a `custom adapter sequence` generated by ownself.
6-9.Motif analysis	how to discover `de novo and known motif` using the output file of Octopus-toolkit.

6-1.Public data (Single GSE/GSM)

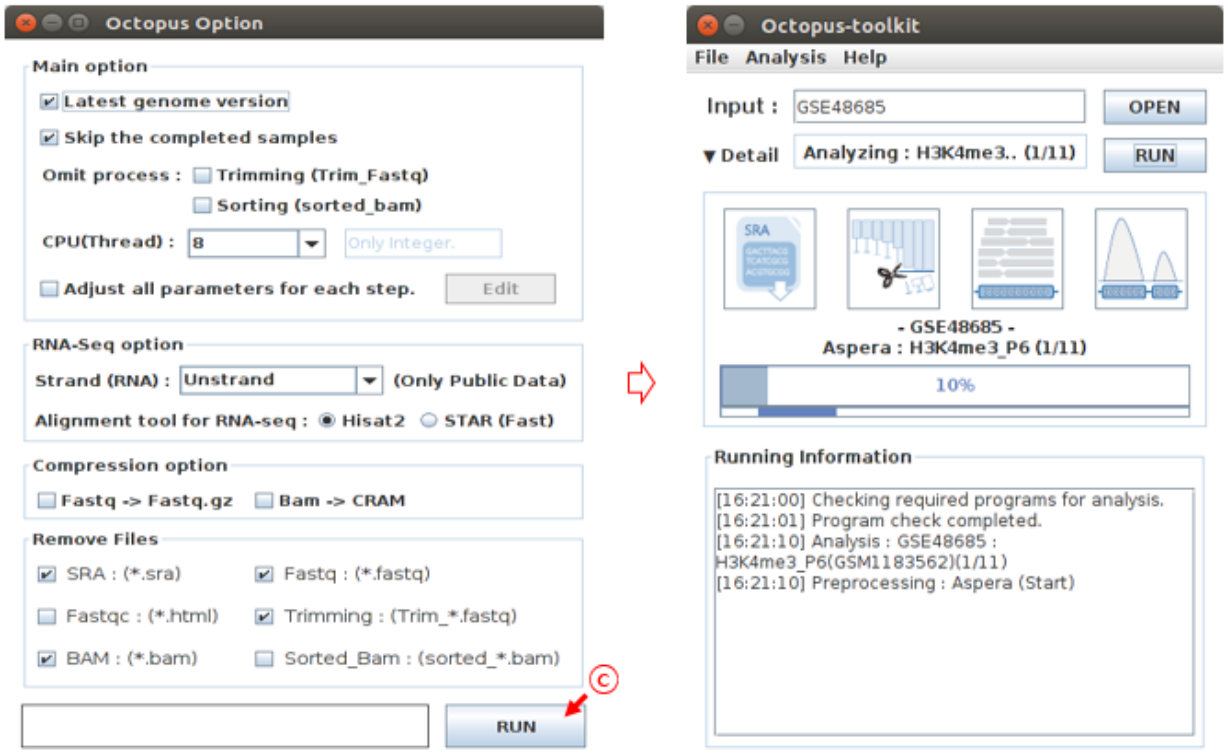
Note: `6-1.Public data (Single GSE/GSM)` describes how to process publicly available data by entering a single GEO accession number.

Analyzing published data is a simple process. Enter a GEO accession number in the input text area. Then click the Run button and Octopus-toolkit option window will appear. In the Option window, set the parameters for the analysis and click the RUN button to begin the analysis.

- `GEO accession number` : GSE48685 (ChIP-Seq:10, RNA-Seq:1)



- `A` : Enter GSE48685 in the input text area.
- `B` : Click the Run button



- `C` : Select the options to analyze and click the Run button. (Option : Defalut)

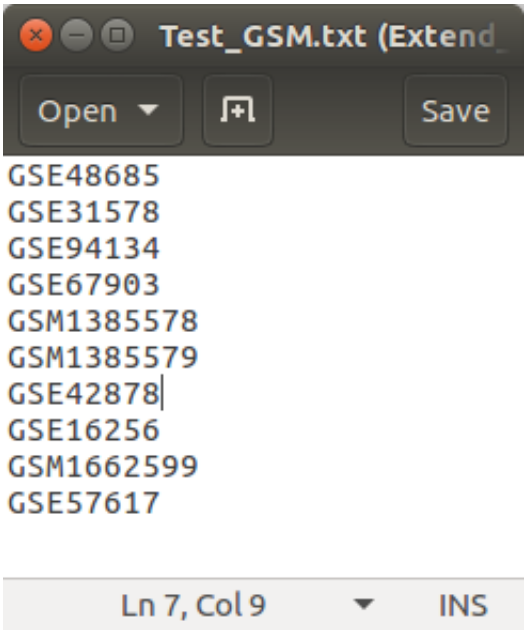
Finally, Octopus-toolkit will automatically download raw files in the GSE48685 ftp directory and subsequenty analyze the data. The output will be stored in a specified directory. No other action is required.

6-2.Public data (Multi GSE/GSM)

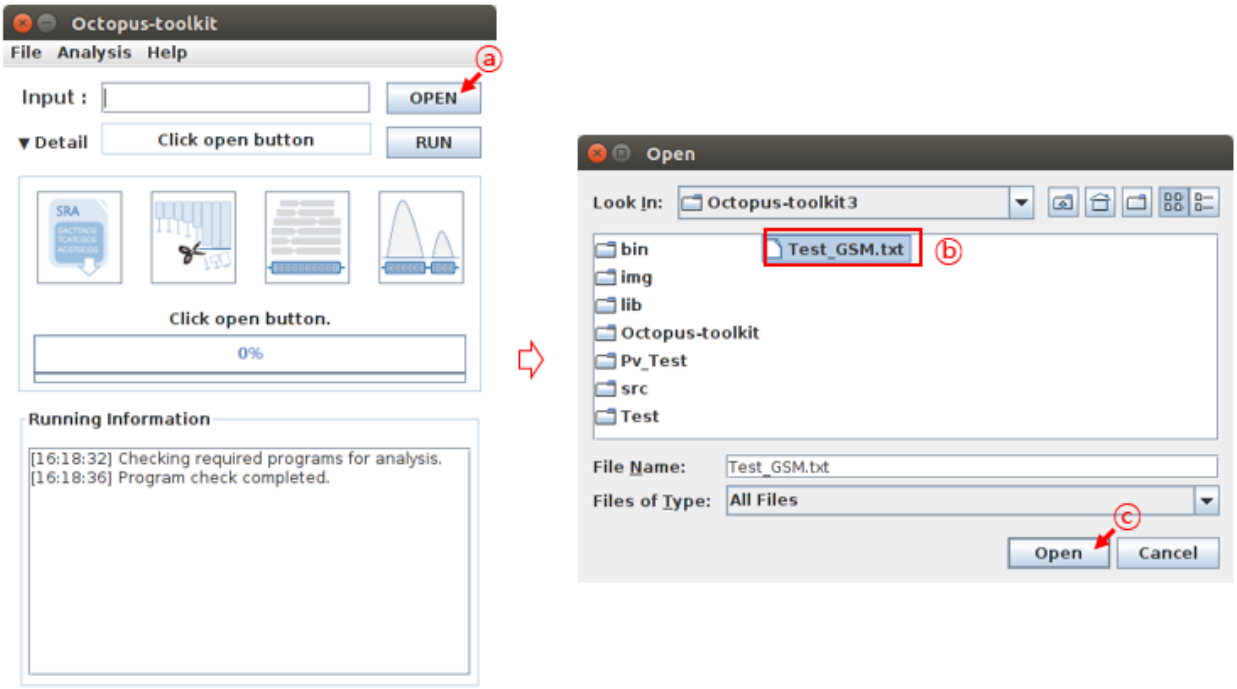
Note: `6-2.Public data (Multi GSE/GSM)` describes how to sequentially analyze a set of public data (a list of GSE accession numbers).

You may want to analyze samples (GSM) in a study (GSE) with several other studies (GSEs) altogether. In this case, you need to create a text file containing GSM ids for samples and GSE ids for studies.

An example is shown below. (`example.list`)

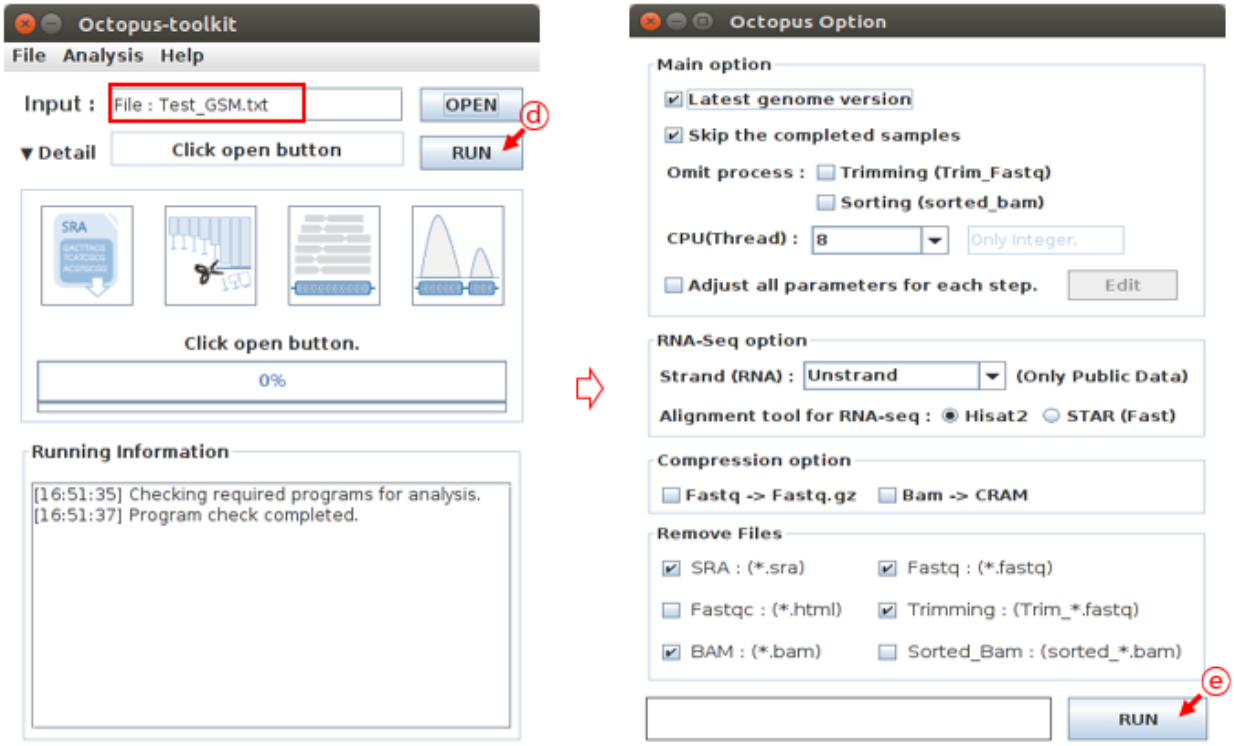


Then, click the OPEN button and select the list file you prepared.

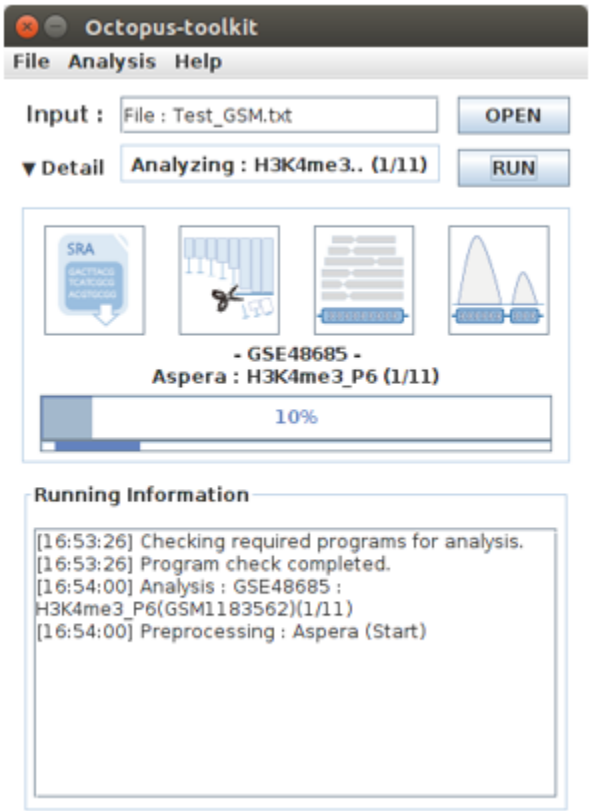


- A : Click the Open button
- B : Select the GEO accession number list file.
- C : Click the Open button

Then, click the RUN button. Octopus-toolkit option window will appear. In the Option window, set the parameters for the analysis and click the RUN button to begin the analysis.



- D : Click the RUN button
- E : Select the options to analyze and click the RUN button. (Option : Defalut)



Finally, Octopus-toolkit will automatically analyze the list of data. Sit back and relax until the results are out.

6-3.Private data (Basic)

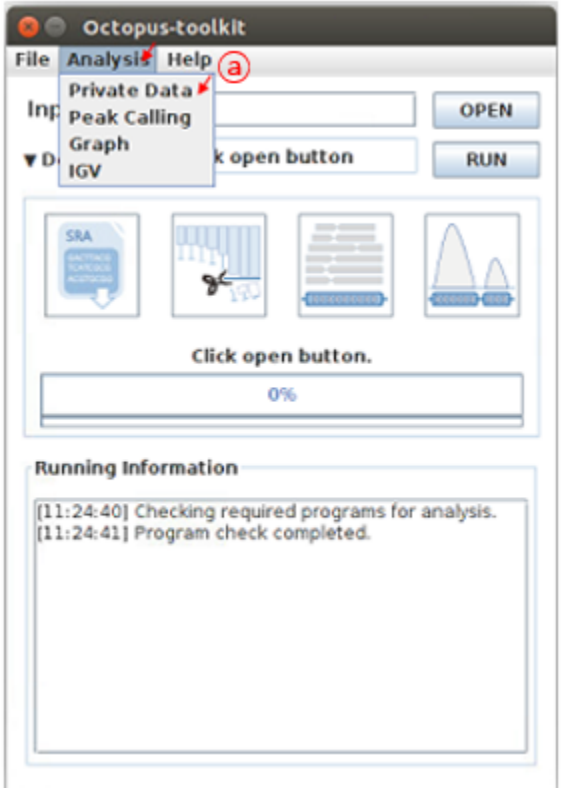
Note: `6-3.Private data (Basic)` describes how to analyze your own data using the same analysis pipeline for the public data.

Let's assume that you have the following data.

Table 2: Analysis situation.

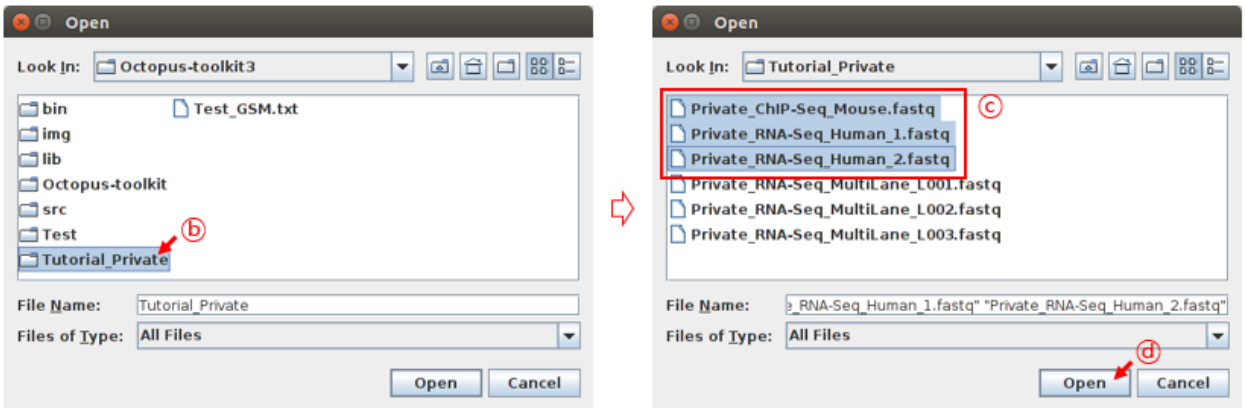
NO	File name	Genome	Seq Type	SE or PE	Strand
1	Private_ChIP-Seq_Mouse.fastq	mm10	ChIP-Seq	Single-End	Not use
2	Private_RNA-Seq_Human_1.fastq	hg38	RNA-Seq	Paired-End	FR-Firststrand
3	Private_RNA-Seq_Human_2.fastq	hg38	RNA-Seq	Paired-End	FR-Firststrand

First, open the Analysis tab and then, click Private data function.



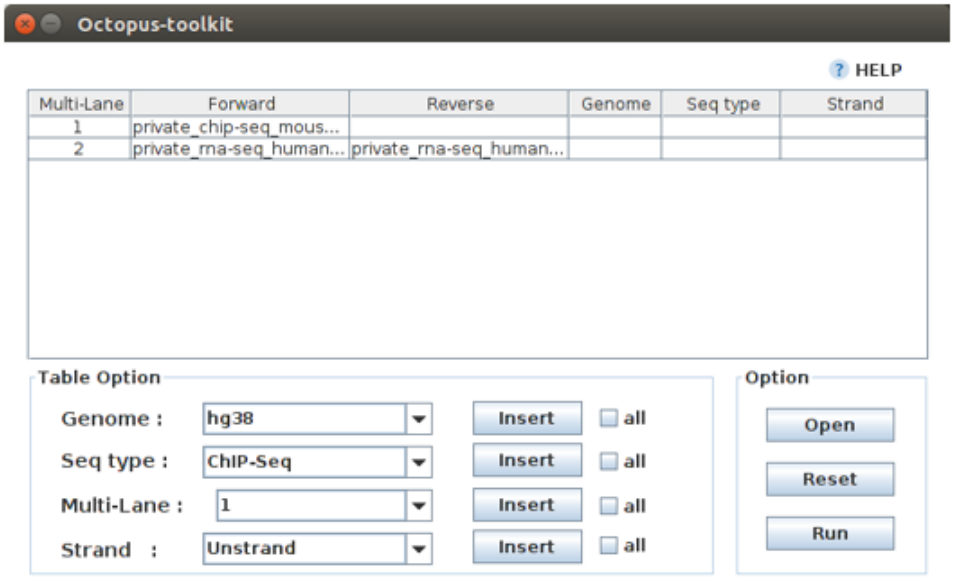
- A : Click the Private Data function in the Analysis menu bar.

Select your fastq files and click the Open button.

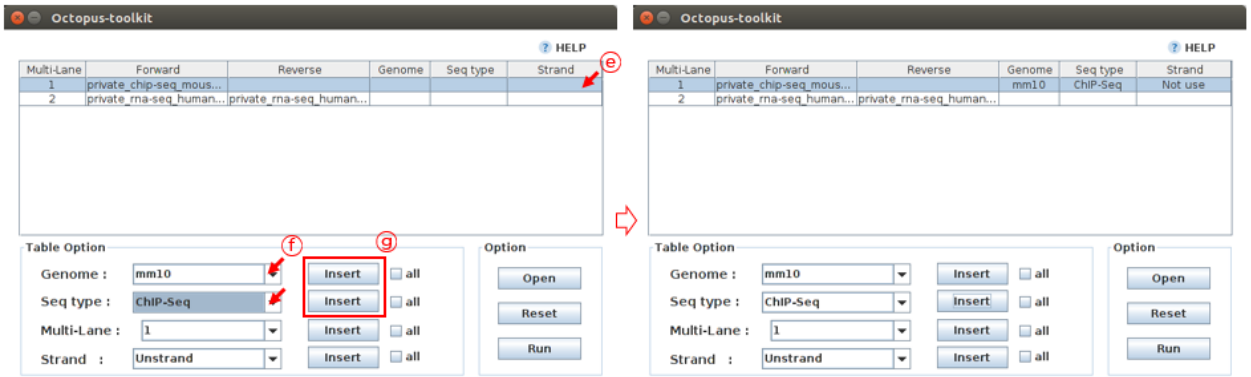


- B : select the folder
- C : Select the files
- D : Click the Open button

The following Private Table window will appear.



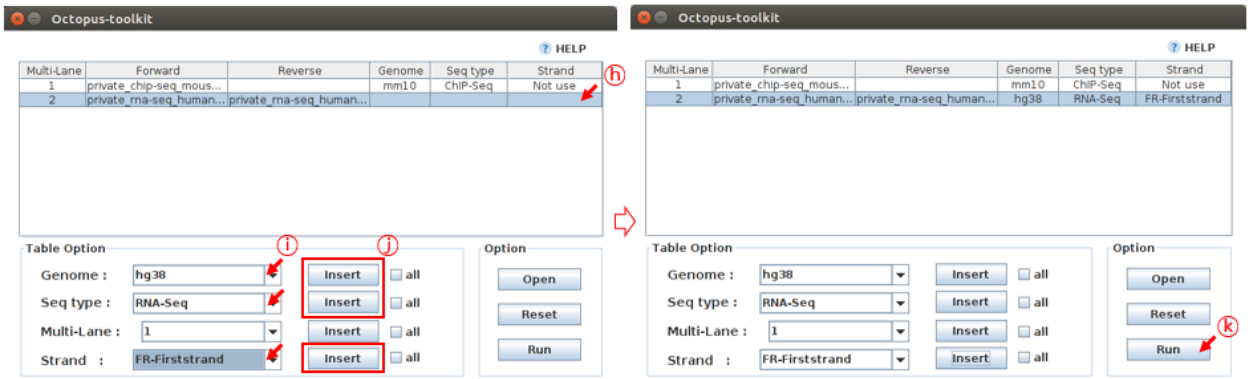
Case 1. Fill in the blank for the 1.Private_ChIP-Seq_Mouse fastq file. Reads in this ChIP-seq file (single-end) should be mapped to the mm10 genome.



- `E` : Select the Private_ChIP-Seq_Mouse.fastq sample.
- `F` : Select appropriate parameters regarding this sample. (Genome : `mm10`, Seq-Type : `ChIP-Seq`)
- `G` : Click the Insert button

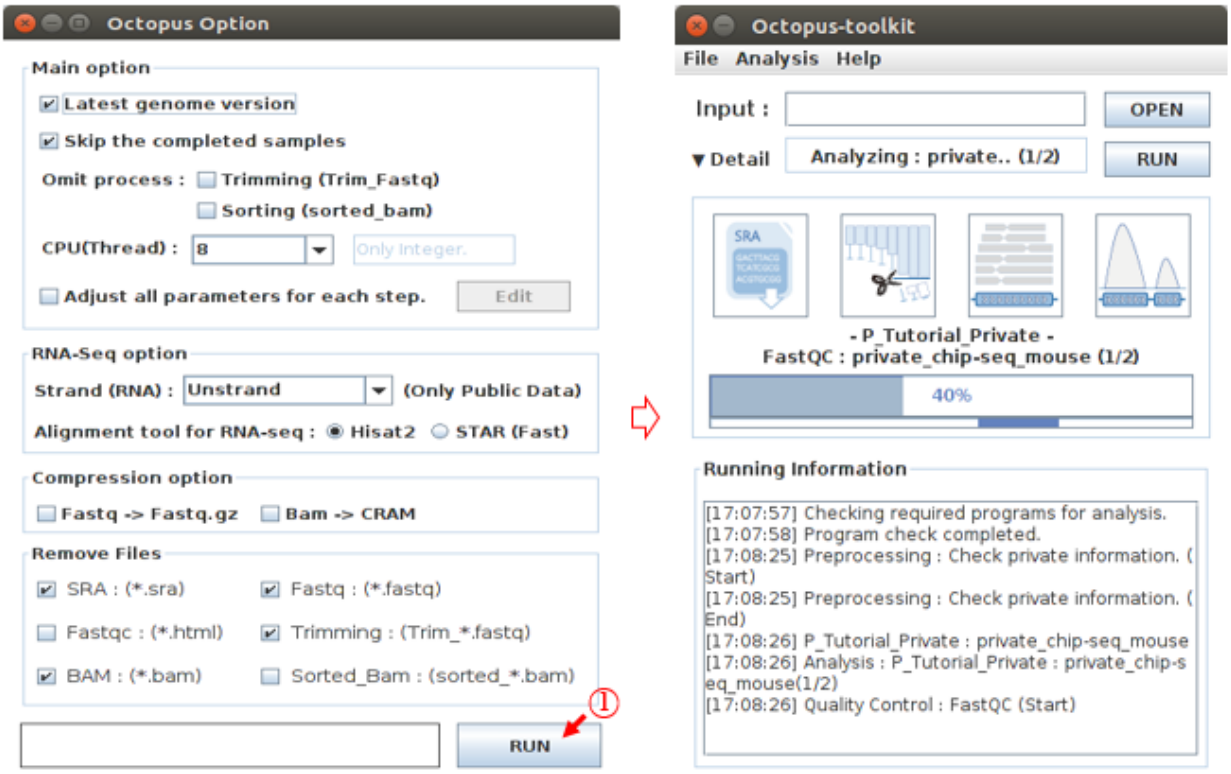
Case 2. Fill in the blank for the 2 and 3.Private_RNA-Seq_Human fastq files. Reads in this RNA-seq files (paired-end, FR-Firststrand) should be mapped to the hg38 genome.

Octopus-toolkit automatically recognizes Paired-End files. The name of the files must be the same and end with the suffix _1.fastq and _2.fastq



- H : Select the Private_RNA-Seq_Human.fastq sample.
- I : Select information about this sample. (Genome : hg38, Seq-Type : RNA-Seq, Strand : FR-Firststrand)
- J : Click the Insert button
- K : Click the Run button

The Octopus-toolkit option window will appear. In the Option window, set the parameters for the analysis and click the RUN button to begin the analysis.



- L : Click the Run button.

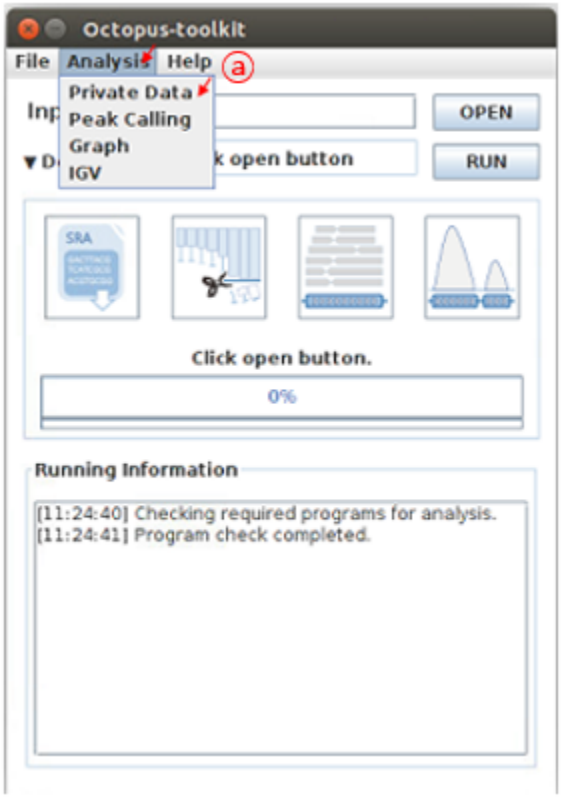
6-4.Private data (Multi-lane)

Note: `6-4.Private data (Multi-lane)` describes how to process your samples from multe lanes.

Table 3: Analysis situation.

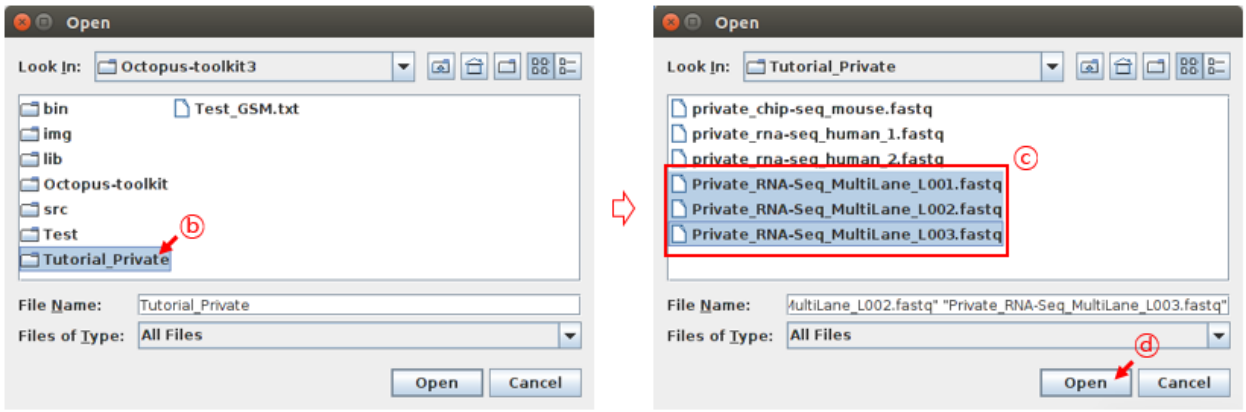
NO	File name	Genome	Seq Type	SE or PE	Strand
1	Private_ChIP-Seq_MultiLane_L001.fastq	hg38	ChIP-Seq	Single-End	Not use
2	Private_ChIP-Seq_MultiLane_L002.fastq	hg38	ChIP-Seq	Single-End	Not use
3	Private_ChIP-Seq_MultiLane_L003.fastq	hg38	ChIP-Seq	Single-End	Not use

First, open the Analysis tab and then, click Private data function.



- `A` : Click the Private Data in the Analysis menu bar.

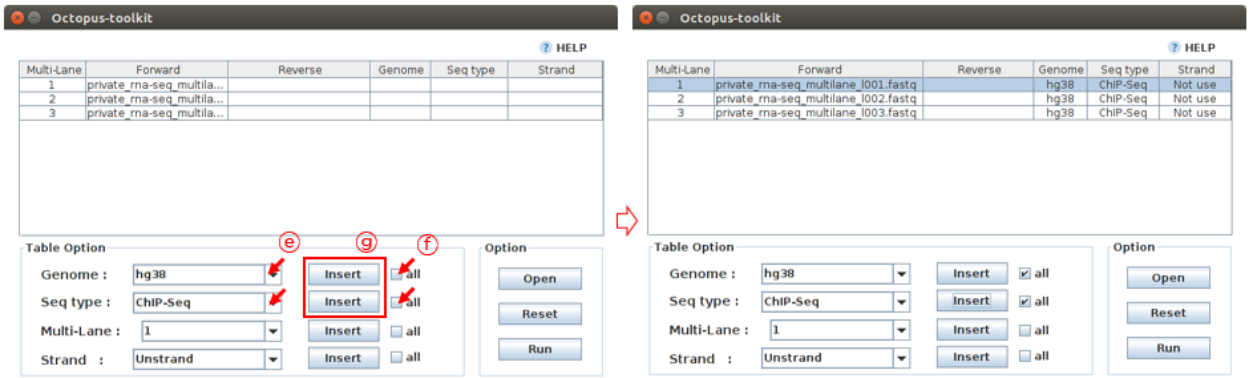
Select your fastq (multi-lane) files and click the Open button.



- B : select the folder
- C : Select the files
- D : Click the Open button

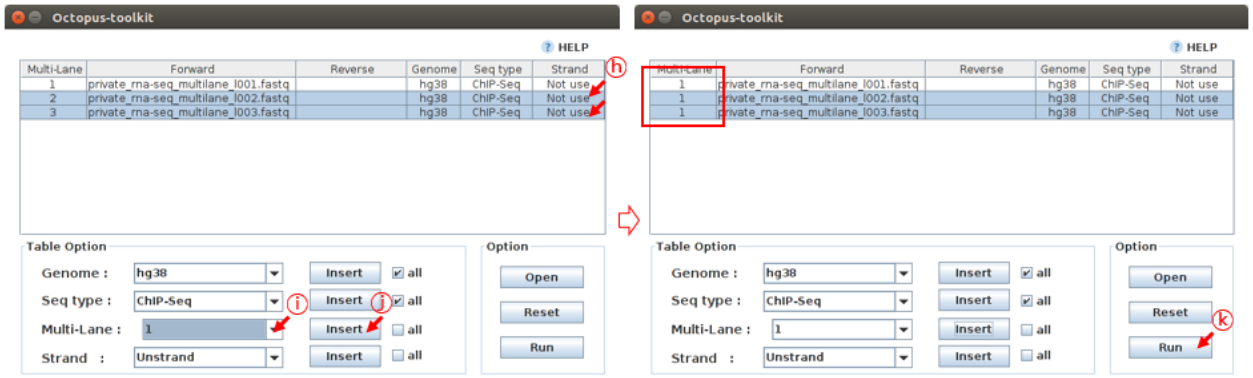
The following Private Table window will appear.

Case 1. let's fill in the blank for the Private_ChIP-Seq_MultiLane fastq file. Reads in these ChIP-seq files (single-end) should be mapped to the hg38 genome. Since all samples have the same information, you can use the all button to enter the same information at once.



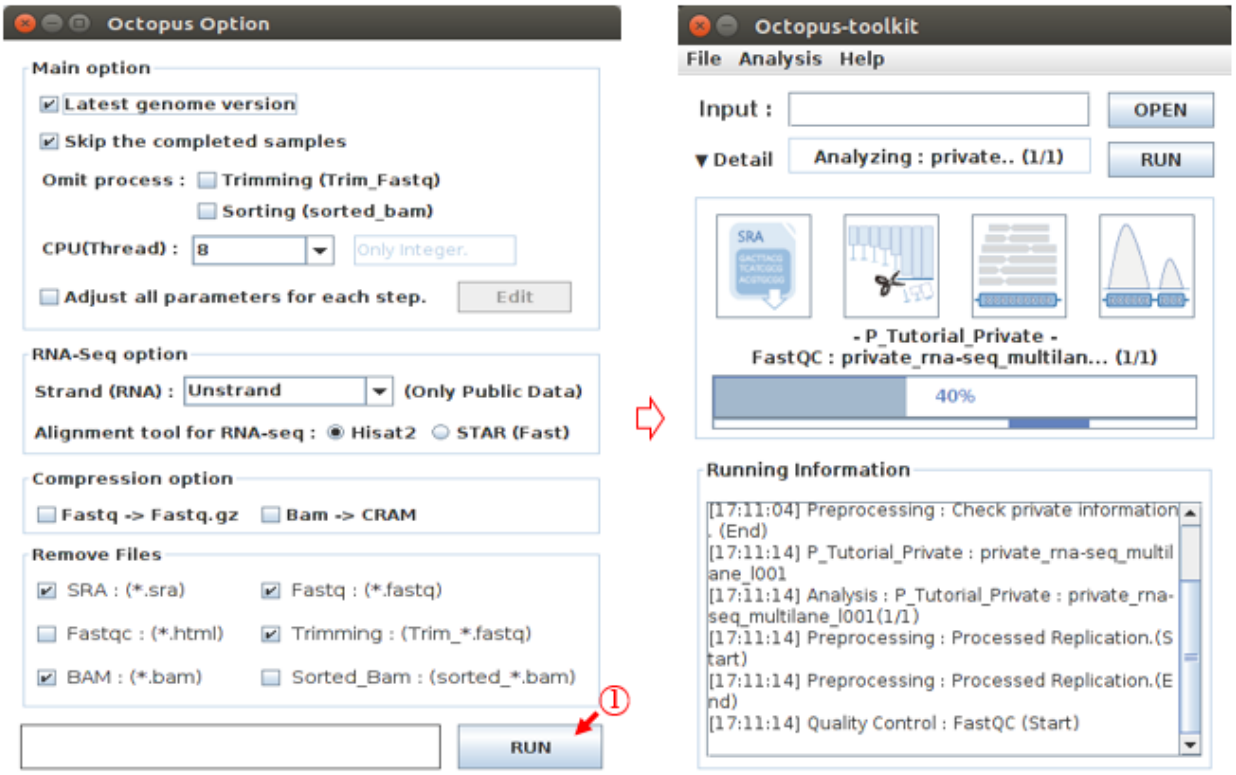
- E : Select information about this sample. (Genome : `hg38`, Seq-Type : `ChIP-Seq`)
- F : Click the all button
- G : Click the Insert button

Octopus-toolkit will merge the files with the same number in the Multi-Lane column prior to analysis. Please carefully assign the same number to multi-lane fastq files.



- H : Select the Private_RNA-Seq_MultiLane Files.
- I : Select the number 1 (Multi-Lane)
- J : Click the Insert button
- K : Click the Run button

The Octopus-toolkit option window will appear. In the Option window, set the parameters for the analysis and click the RUN button to begin the analysis.



- L : Click the Run button

6-5.Peak Calling

Note: `6-5.Peak Calling` describes how to identify peaks (enriched regions by mapped reads) with the Octopus-toolkit output.

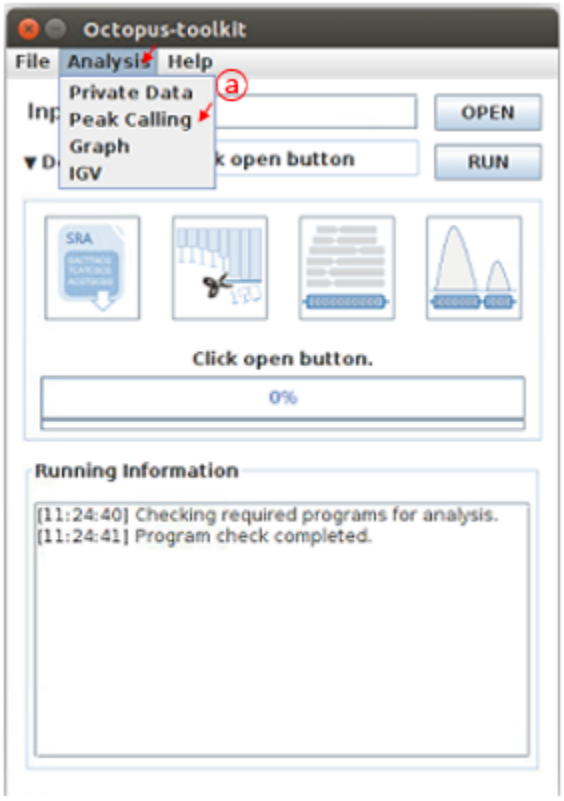
You can identify peaks from the output: 6-1 ~ 6-4.

Let's say you have the following ChIP-seq data.

Table 4: Analysis situation.

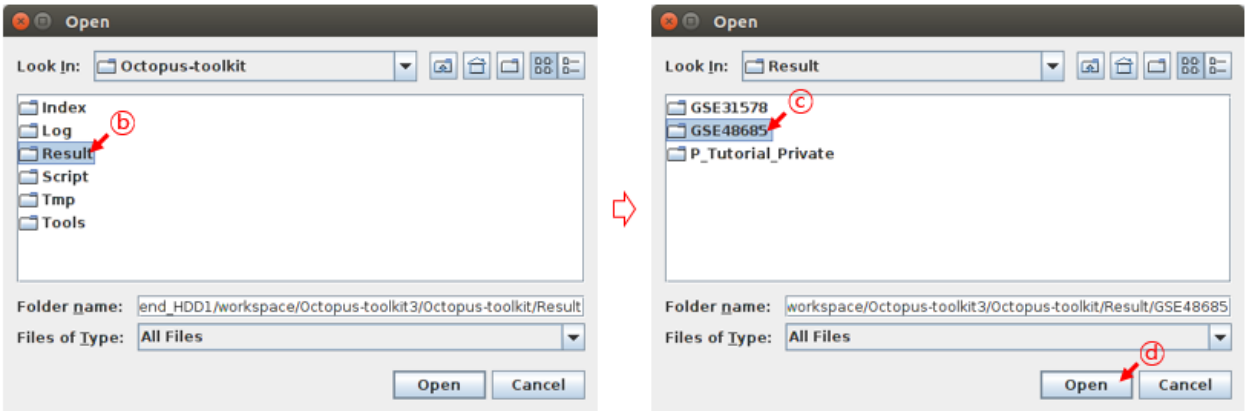
NO	Sample name	Input/Control/IgG	Style	Result Path
1	STAT5A_P6	Input_P6	Transcription Factor	Result/GSE48685

First, open the Analysis tab and then, click the Peak Calling function.



- `A` : Click the Peak Calling in the Analysis menu bar.

Octopus-toolkit output will be stored in the Result folder. You need to select an appropriate study (GSE directory) in the Result folder. For example, select the GSE48685 directory.

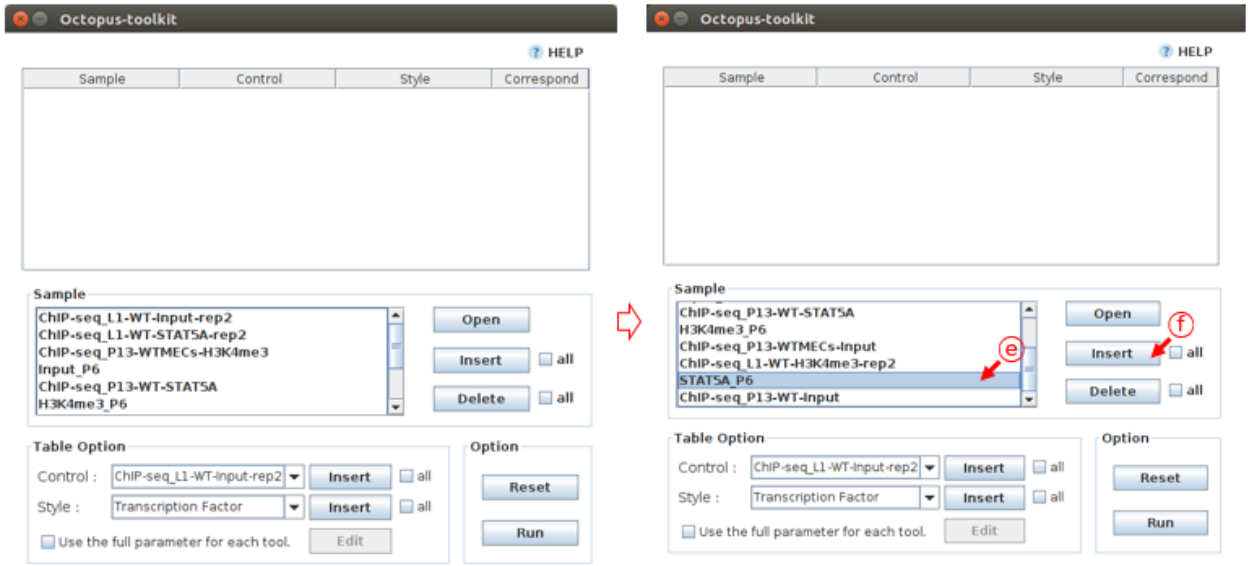


- B : Select the Result folder.
- C : Select the GSE48685 folder.
- D : Click the Open button.

Once you select an GSE folder (not double click), please click the Open button. Then, the Peak Calling Table will appear.

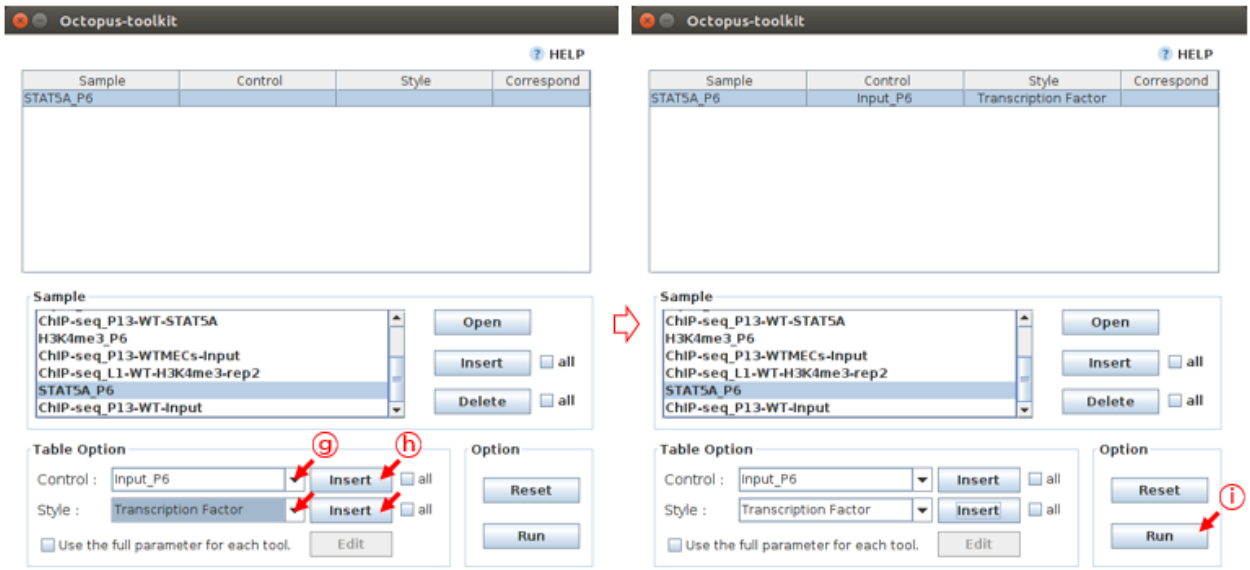
Samples of GSE48685, which were processed by Octopus-toolkit, will appear in the Sample area.

First, you need to add the processed samples to the Peak Calling table using the Insert function.



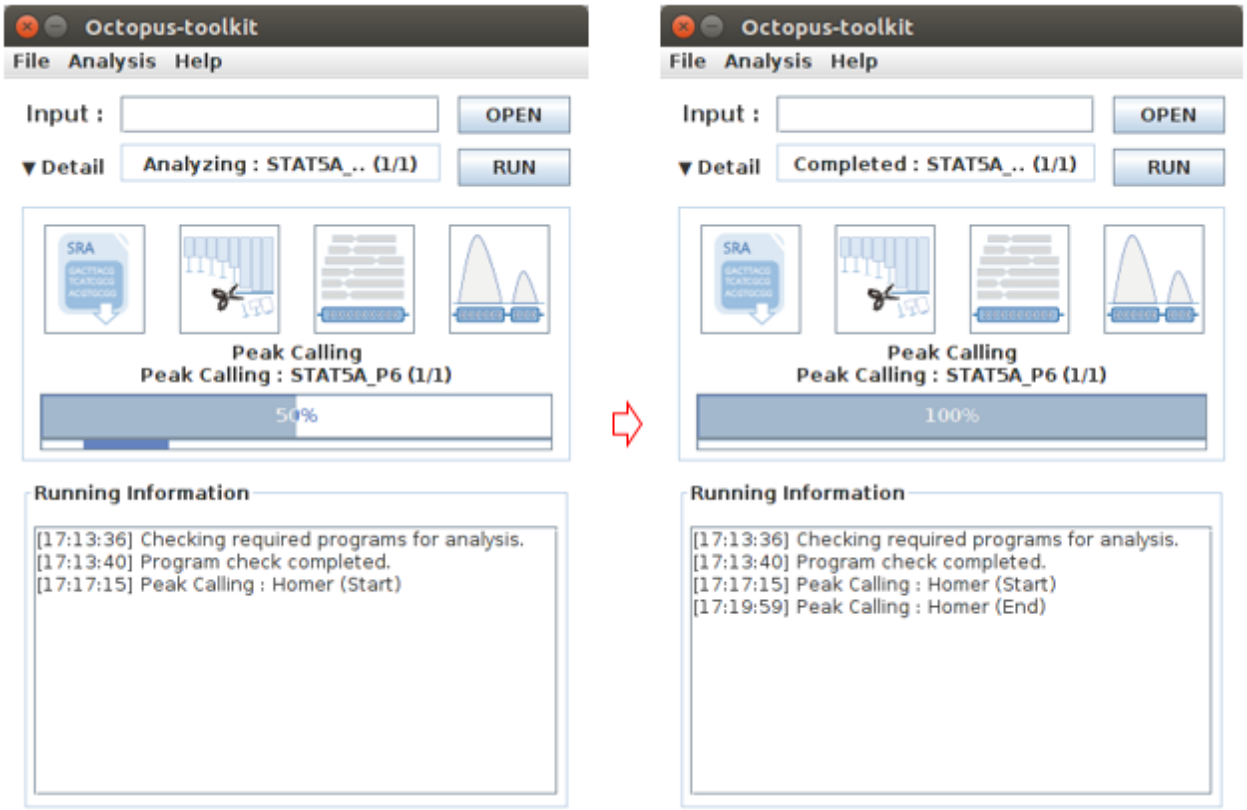
- E : Select the STAT5A_P6
- F : Click the Insert button

Then fill in the blanks for the selected samples using the Table option function. If there is a control (Control) sample to filter out background noise, you also need to add it to the Correspond column.

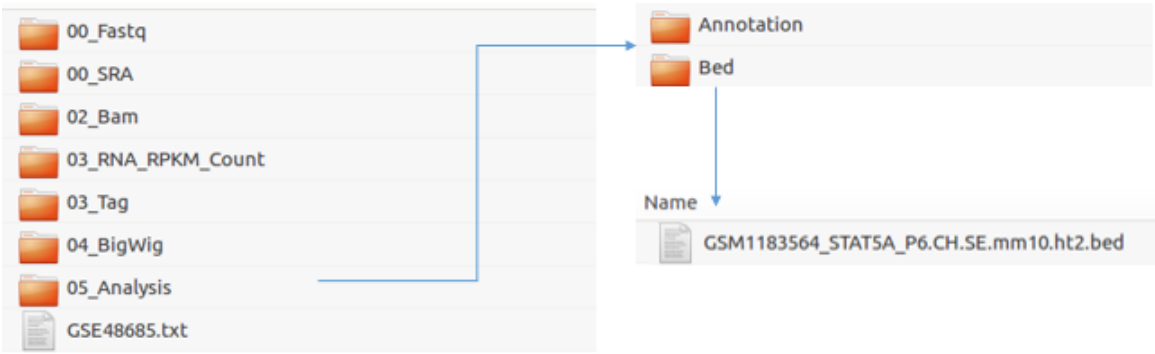


- `G` : Select the information about STAT5A_P6 (Control : `Input_P6`, Style : `Transcription Factor`)
- `H` : Click the Insert button
- `I` : Click the Run button

Peak Calling analysis will start according to the Table information.



Once completed, you can find the result files (.bed for peaks) in the 05_Analysis directory in the Result/GSE48685 directory.



- `Result Path` : Octopus-toolkit/Result/GSE48685

6-6.Graph

Note: `6-6.Graph` describes how to draw plots with the output: 6-1 ~ 6-5.

You can draw a heatmap and line plots with a few clicks.

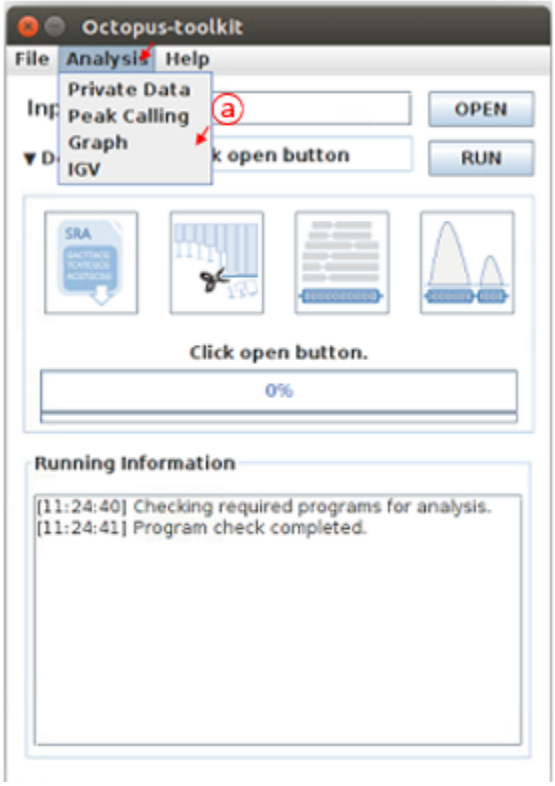
6-6.Graph tutorial describes how to draw plots for multiple outputs. Let say you have the following outputs processed by Octopus-toolkit.

Table 5: Analysis situation.

NO	Sample name	Peak(.bed)
1	STAT5A_P6	O
2	M_Bcl6_rep2_G50	X
3	MH_STAT5_rep2_G41	X

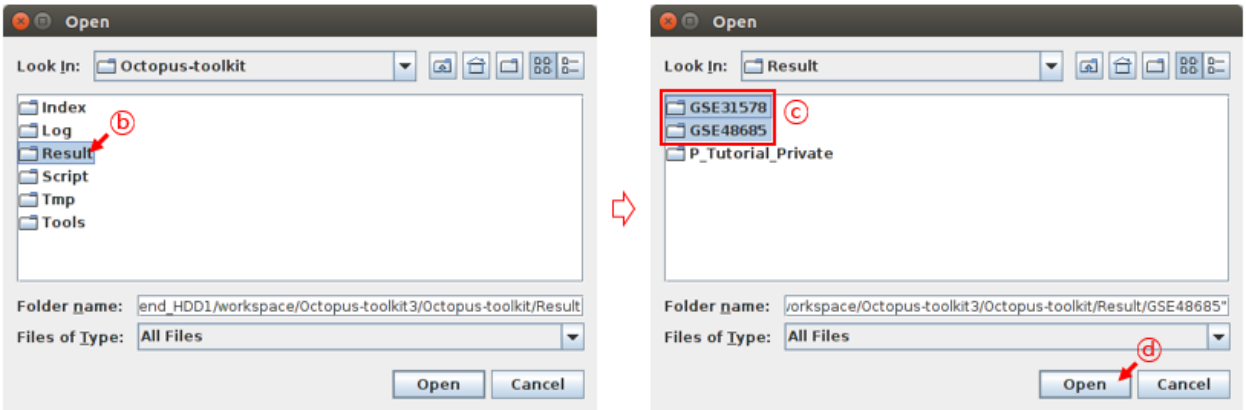
- Option : +- `1000 bp` based on TSS, Bin Size : `100`

First, open the Analysis tab and then, click the Graph function.



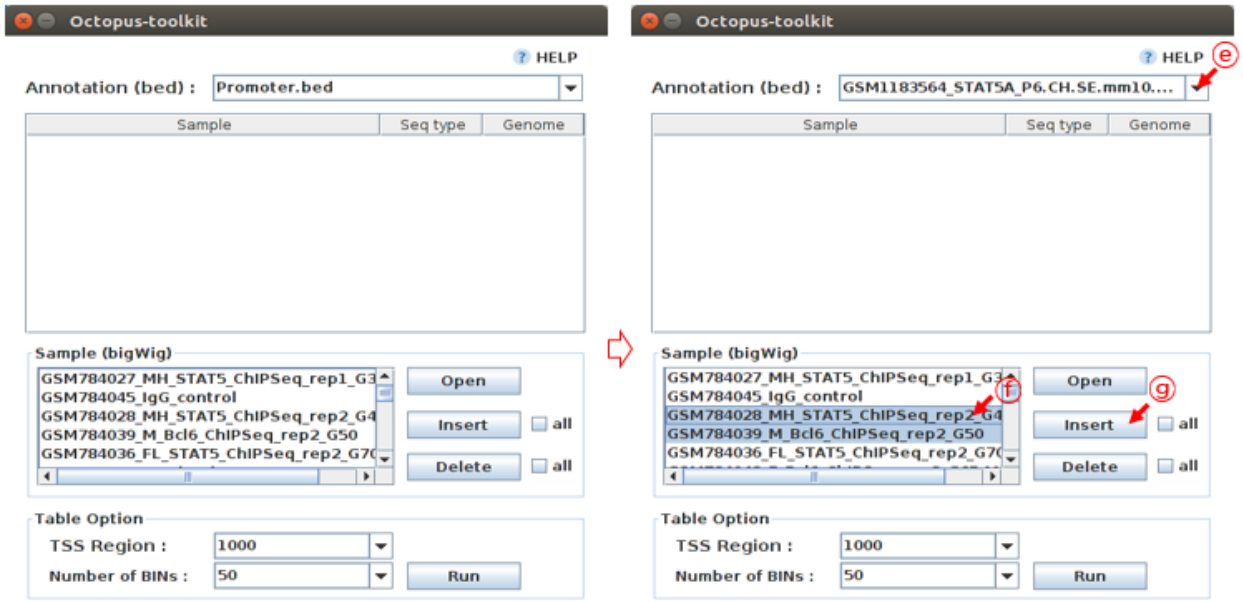
- A : Click the Graph in the Analysis menu bar.

Octopus-toolkit output will be stored in the Result folder. To draw heatmap and plot, you need to select appropriate studies (GSE directories) in the Result folder. For example, select the GSE48685 and GSE31578 directories.



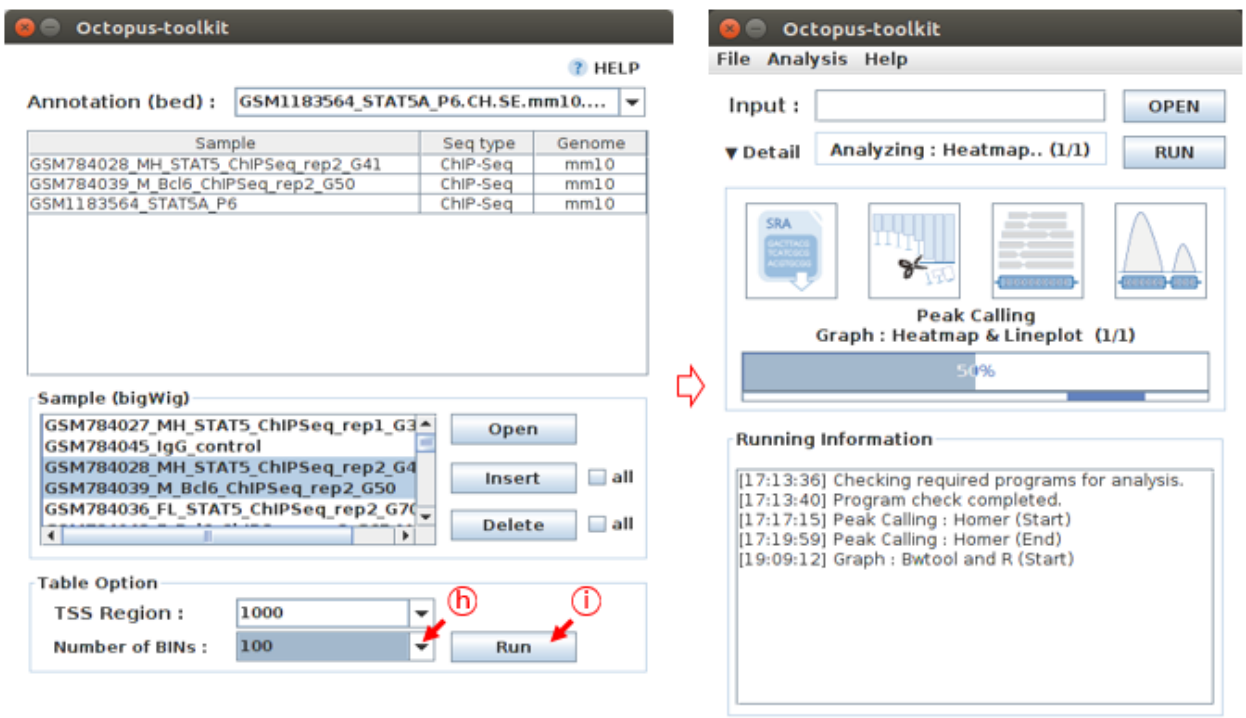
- B : Select the Result folder.
- C : Select the GSE48685 and GSE31578 folders.
- D : Click the Open button.

The heatmap and plot will be drawn based on an annotation file (reference). The default annotation file (.bed) contains promoter regions. You can replace it with peak file (.bed) generated by Octopus-toolkit if you perform the peak calling analysis.



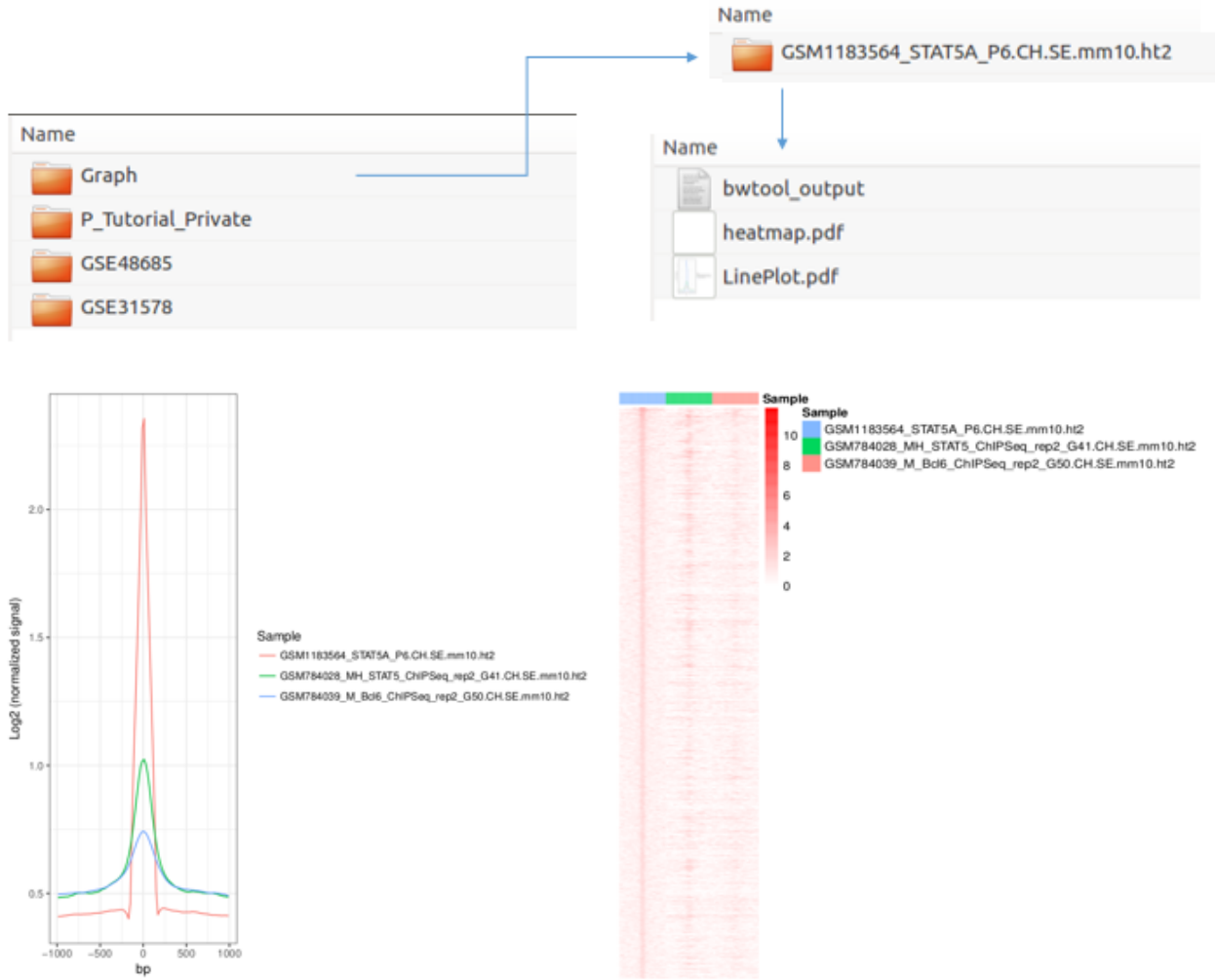
- E : Select STAT5A_P6_CH.SE.mm10 as the reference.
- F : Select samples of your interest from the list.
- G : Click the Insert button.

In the Table option, Adjust TSS region (bp) and Number of BINs (resolution) parameters. Click the Run button to perform the Graph analysis.



- H : Select the 1000 in TSS region and 100 in Number of BINs
- I : Click the Run button

Heatmap and plot will be stored in the Result/Graph folder.

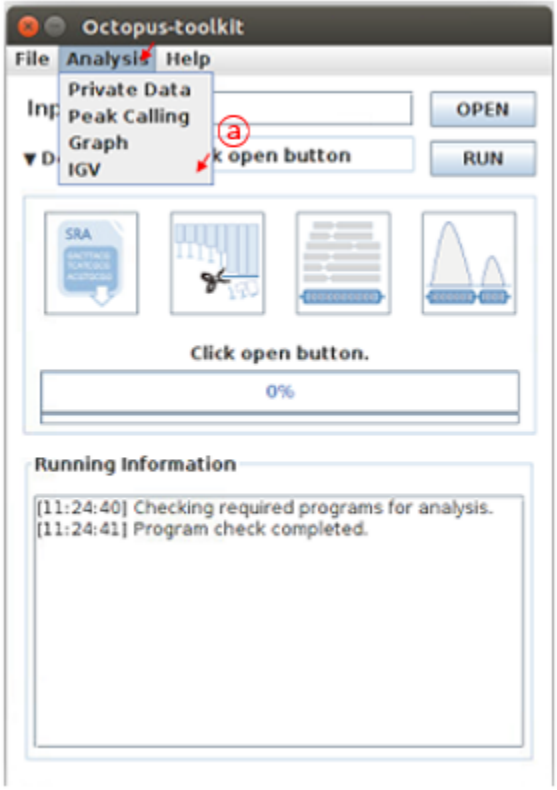


6-7.IGV

Note: `6-7.IGV` describes how to visualize genomes with data (bigWig files) via IGV.

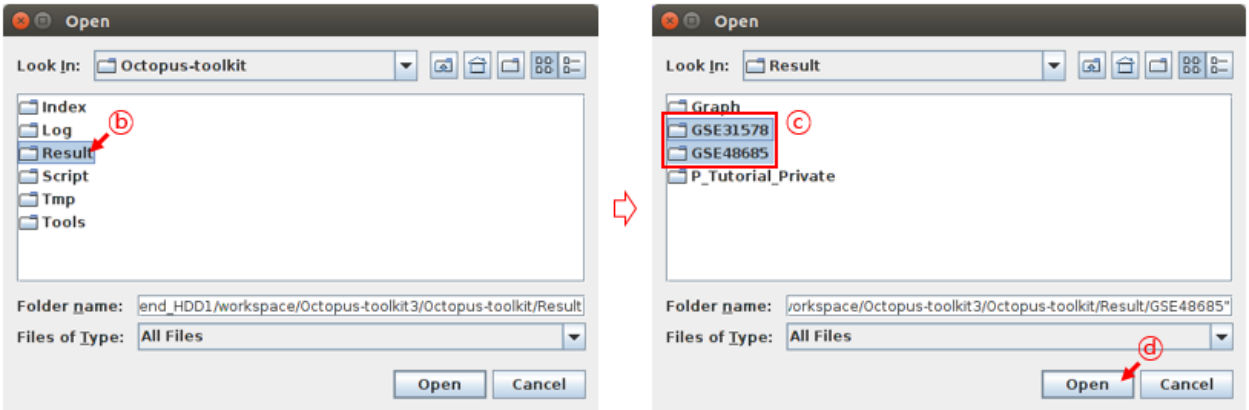
Octopus-toolkit generates bigWig files which can be visualized using Integrative Genomics Viewer(IGV).

First, open the Analysis tab and then, click the IGV function.



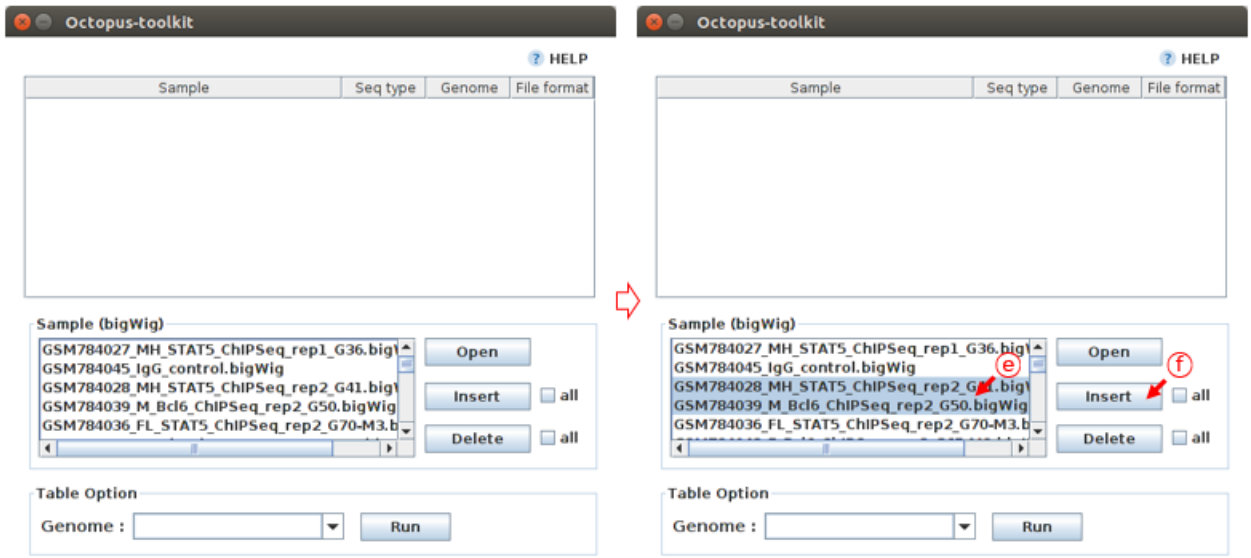
- A : Click the IGV in the Analysis menu bar.

You need to select appropriate studies (GSE directories) in the Result folder. For example, select the GSE48685 and GSE31578 directories. It will load all bigWig files in the selected directories.



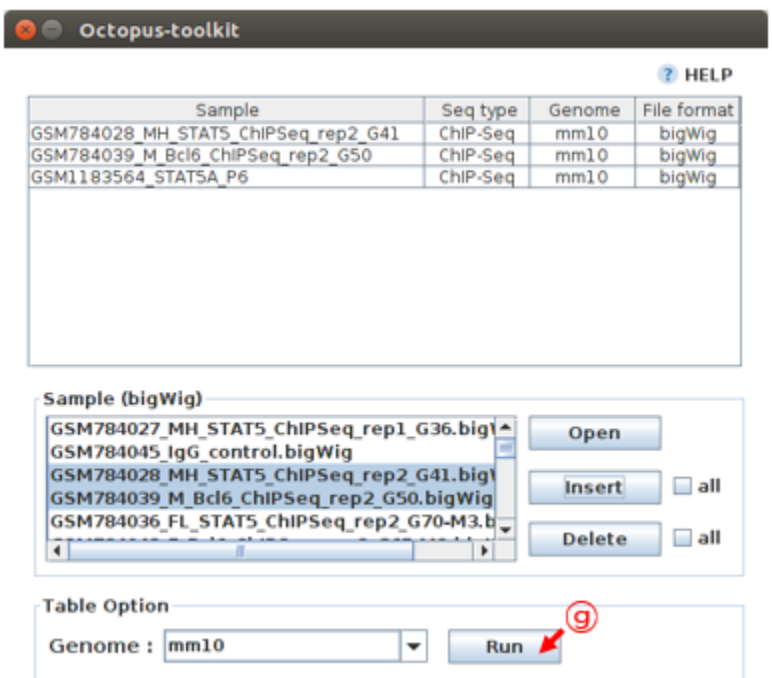
- B : Select the Result folder.
- C : Select the GSE48685 and GSE31578 folders.
- D : Click the Open button.

Let's say you select the following samples. You must select an appropriate genome for visualization. Obviously, you cannot load bigWig files from different genomes.



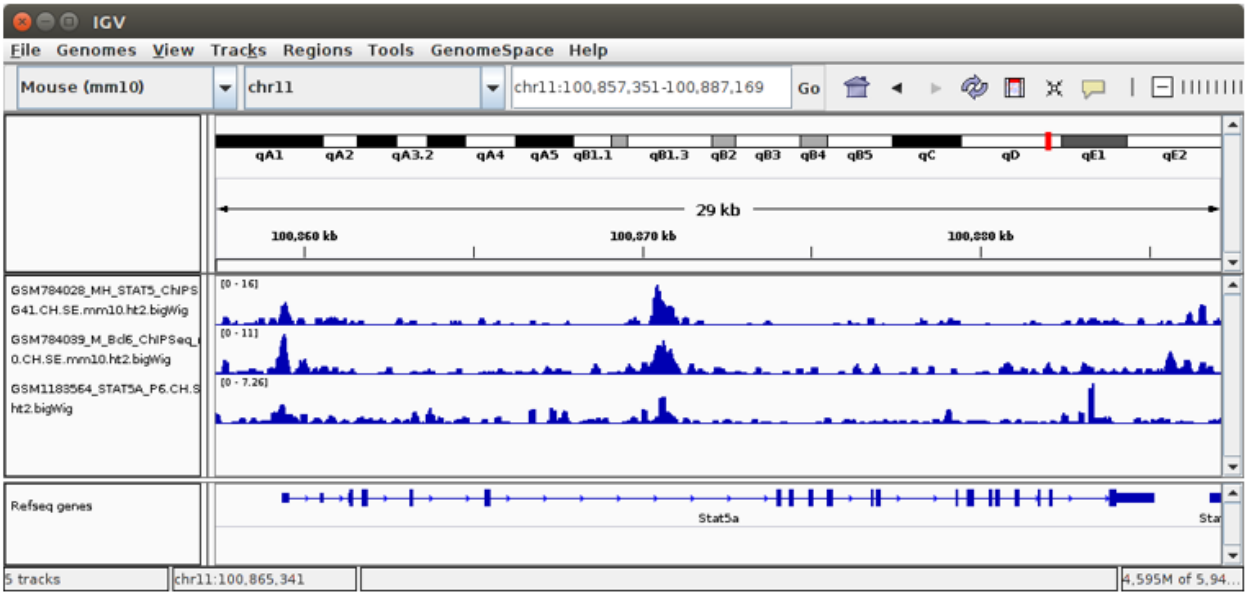
- E : Select samples.
- F : Click the Insert button.

Click the Run button to start the Graph analysis.



- G : Click the Run button.

Depending on the number and size of data, it may take some time to load those files onto the IGV. Please take your time.



6-8.User's custom adapter sequence(Trimming)

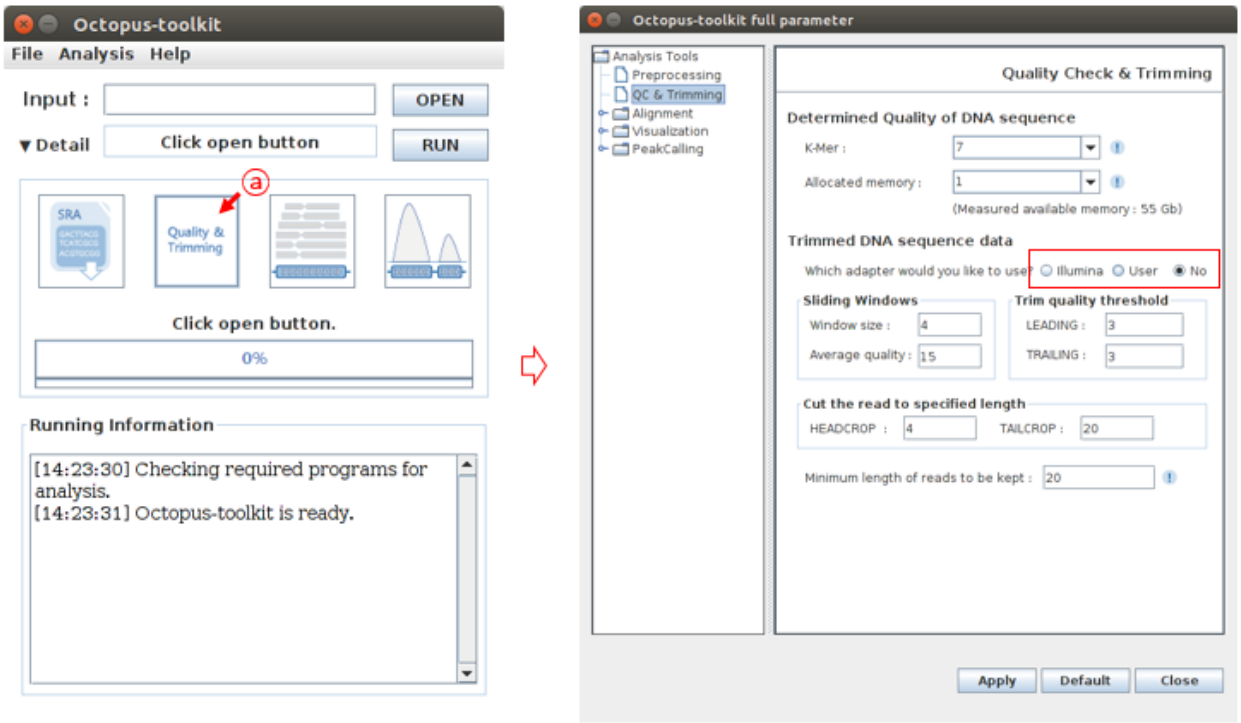
Note: `6-8.IGV` describes how to use a custom adapter sequence generated by ownself.

Typically, The user uses the adapter sequence provided Trimmomatic. But some users want to use custom adapter sequence generated by ownself.

First, The user should make a custom adapter sequence file. The format of files equals a single or multiple sequence file. (File name extension is `.fasta` and `.fa`) (`Custom_adapter.fasta`)

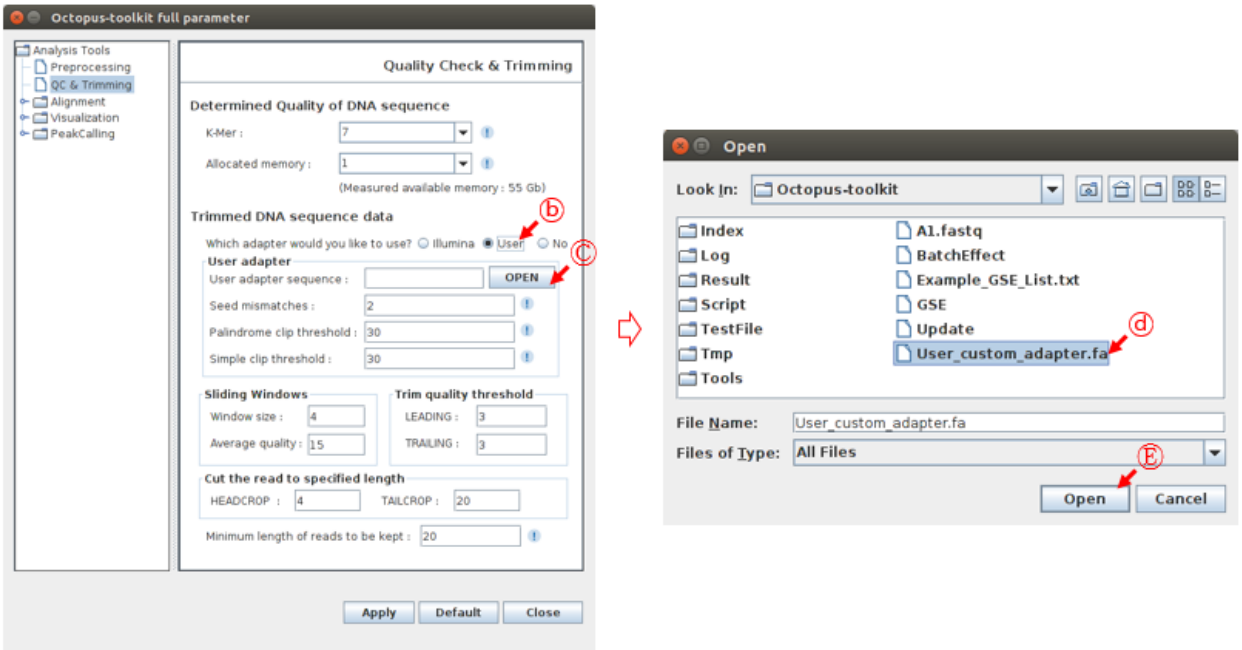
For more detailed information, please refer to the link below. (Trimmomatic : How to make the adapter fasta)

Second, click the Quality & Trimming button in Octopus-toolkit.



- A : Click the Quality & Trimming button.

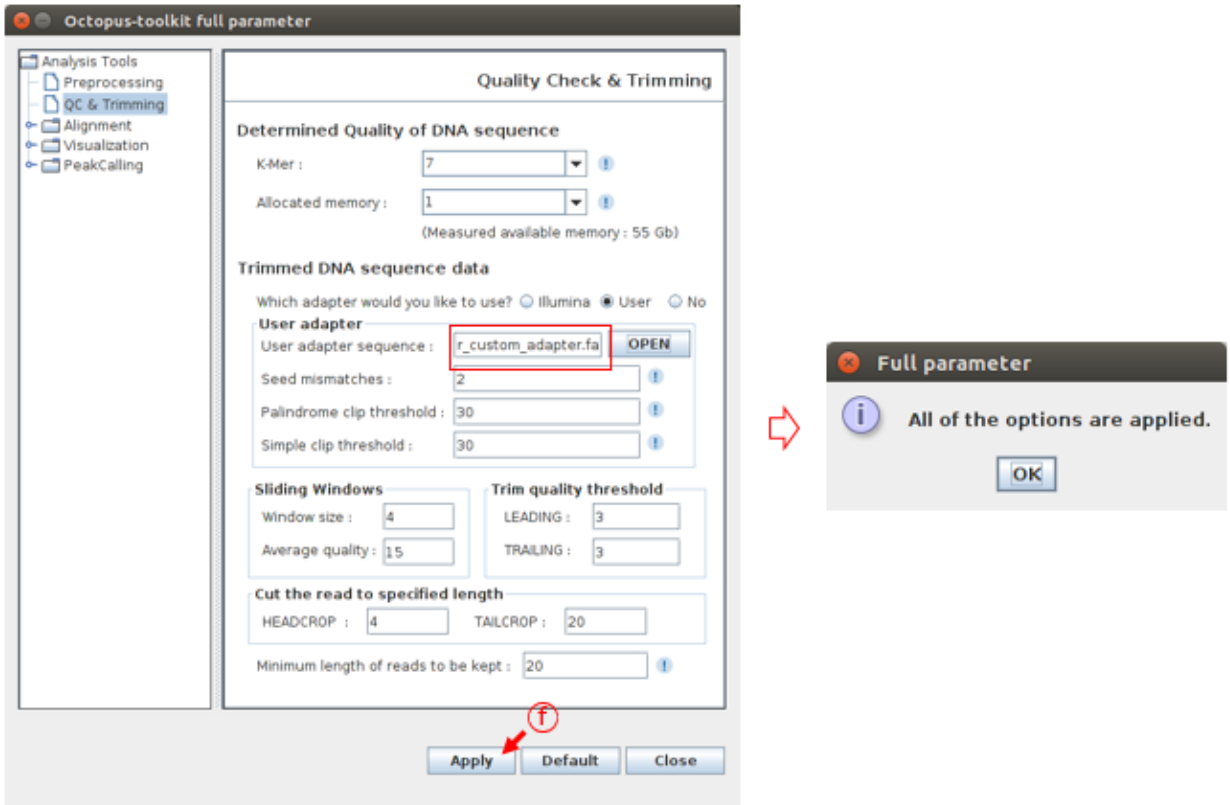
To open the custom adapter sequence file, Select a User radio button and click the Open button. You need to select your adapter sequence file in your computer.



- B : Click the User radio button.
- C : Click the Open button.
- D : Select the custom adapter sequence file generated by user.

- `E` : Click the Open button

Click the Apply button to apply the custom adapter sequence.



- `F` : Check the Apply button.

6-9.Motif analysis

Note: `6-9.Motif analysis` describes how to discover de novo and known motif using the output file of Octopus-toolkit : 6-1 ~ 6-5.

Octopus-toolkit is not supporting a motif analysis yet.

The user can analyze de novo and known motif using below command before to be completing development about motif analysis.

We will use a bed format file, which is generated by peak calling in Octopus-toolkit, for discovering motif.

Table 6: Test environment.

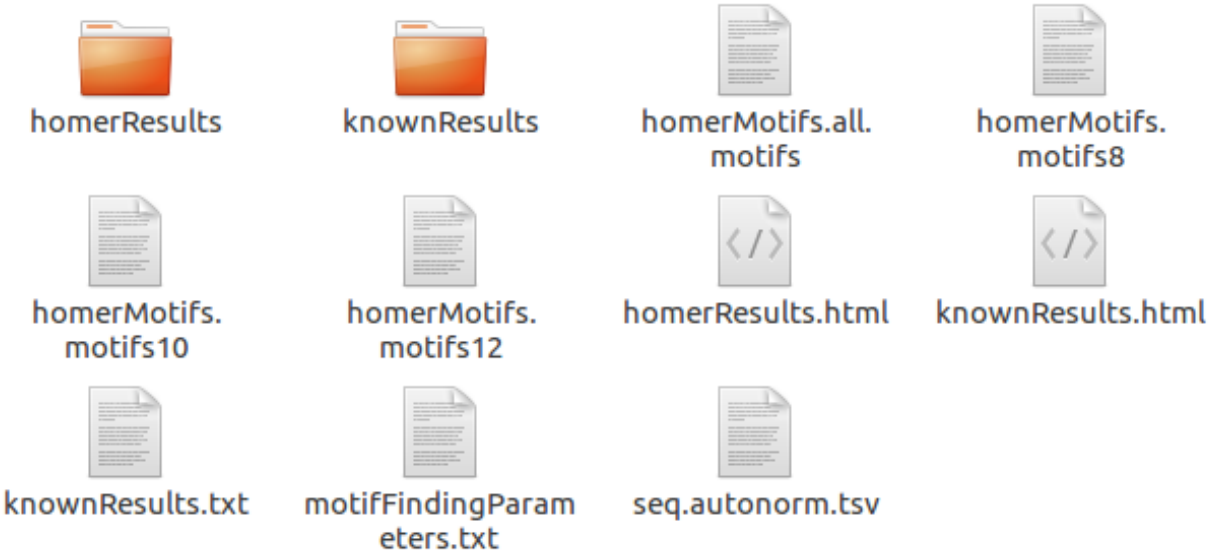
NO	command	Description
1	Pathway of Octopus-toolkit	/home/user_id/Octopus-toolkit/
2	user_id	octopus
3	Output of Octopus-toolkit	GSE48685
4	Input file like bed format file	05_Analysis/Bed/GSM1183564_STAT5A_P6.CH.SE.mm10.ht
5	Genome	mm10

- The `command` for `Motif` analysis:

```
// Add the Homer pathway
export Octopus_Homer="/home/user_id/Octopus-toolkit/Tools/Homer/bin"
export PATH=$PATH:$Octopus_Homer

cd /home/octopus/Octopus-toolkit/Result/GSE48685/
mkdir 06_Motif_output
/home/octopus/Octopus-toolkit/Tools/Homer/bin/findMotifsGenome.pl 05_Analysis/Bed/
↪GSM1183564_STAT5A_P6.CH.SE.mm10.ht2.bed mm10 06_Motif_output/
```

- The output of the Motif analysis.

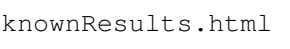


The output of Motif analysis provides a motif's letter-probability matrix, list of a detected motif, statistical value and best-matched gene symbol.

- `homerResults.html : De novo Motif`

Homer *de novo* Motif Results (06_Motif_output//)

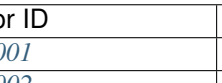
Known Motif Enrichment Results
Gene Ontology Enrichment Results
If Homer is having trouble matching a motif to a known motif, try copy/pasting the matrix file into STAMP
More information on motif finding results: HOMER | Description of Results | Tips
Total target sequences = 150014
Total background sequences = 150190
* - possible false positive

Rank	Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)	Best Match/Details	Motif File
1		1e-668	-1.538e+03	7.09%	4.00%	50.5bp (58.0bp)	Stat3(Stat)/mES-Stat3-ChIP-Seq(GSE11431)/Homer(0.942) More Information | Similar Motifs Found	motif file (matrix)
2		1e-293	-6.747e+02	38.67%	34.14%	55.5bp (56.8bp)	Rbpj1(?)/Panc1-Rbpj1-ChIP-Seq(GSE47459)/Homer(0.785) More Information | Similar Motifs Found	motif file (matrix)
3		1e-231	-5.335e+02	42.69%	38.58%	55.1bp (57.9bp)	NFIA/MA0670.1/Jaspar(0.880) More Information | Similar Motifs Found	motif file (matrix)
4		1e-139	-3.217e+02	43.41%	40.21%	56.0bp (58.3bp)	RUNX1(Runt)/Jurkat-RUNX1-ChIP-Seq(GSE29180)/Homer(0.802) More Information | Similar Motifs Found	motif file (matrix)
5		1e-114	-2.639e+02	32.95%	30.22%	55.6bp (58.1bp)	Fox:Ebox(Forkhead,bHLH)/Panc1-Foxa2-ChIP-Seq(GSE47459)/Homer(0.711) More Information | Similar Motifs Found	motif file (matrix)
6		1e-102	-2.352e+02	26.99%	24.58%	54.9bp (57.0bp)	PB0165.1_Sox11_2/Jaspar(0.599) More Information | Similar Motifs Found	motif file (matrix)
7		1e-96	-2.233e+02	1.67%	1.07%	53.5bp (57.7bp)	NeuroD1(bHLH)/Islet-NeuroD1-ChIP-Seq(GSE30298)/Homer(0.621) More Information | Similar Motifs Found	motif file (matrix)

- `knownResults.html : known Motif`

Homer Known Motif Enrichment Results (06_Motif_output/)

Homer *de novo* Motif Results
Gene Ontology Enrichment Results
Known Motif Enrichment Results (txt file)
Total Target Sequences = 149985, Total Background Sequences = 150264

Rank	Motif	Name	P-value	log P-pvalue	q-value (Benjamini)	# Target Sequences with Motif	% of Targets Sequences with Motif	# Background Sequences with Motif	% of Background Sequences with Motif	Motif File	PDF
1		STAT5(Stat)/mCD4+-Stat5-ChIP-Seq(GSE12346)/Homer	1e-618	-1.425e+03	0.0000	9734.0	6.49%	5469.6	3.64%	motif file (matrix)	pdf
2		STAT1(Stat)/HelaS3-STAT1-ChIP-Seq(GSE12782)/Homer	1e-469	-1.082e+03	0.0000	8066.0	5.38%	4647.3	3.09%	motif file (matrix)	pdf
3		Stat3(Stat)/mES-Stat3-ChIP-Seq(GSE11431)/Homer	1e-378	-8.719e+02	0.0000	10427.0	6.95%	6832.7	4.55%	motif file (matrix)	pdf
4		STAT4(Stat)/CD4-Stat4-ChIP-Seq(GSE22104)/Homer	1e-336	-7.744e+02	0.0000	19630.0	13.09%	14922.6	9.93%	motif file (matrix)	pdf
5		Stat3+il21(Stat)/CD4-Stat3-ChIP-Seq(GSE19198)/Homer	1e-314	-7.236e+02	0.0000	14402.0	9.60%	10486.8	6.98%	motif file (matrix)	pdf
6		EHF(ETS)/LoVo-EHF-ChIP-Seq(GSE49402)/Homer	1e-179	-4.131e+02	0.0000	23596.0	15.73%	19798.5	13.17%	motif file (matrix)	pdf
7		ERG(ETS)/VCaP-ERG-ChIP-Seq(GSE14097)/Homer	1e-148	-3.418e+02	0.0000	27368.0	18.25%	23680.7	15.75%	motif file (matrix)	pdf

4.1.8 7.Error Code

7-1.Summary

Error ID	Description
Err001	Octopus-toolkit cannot access the web page.
Err002	Incorrect GEO accession number.
Err003	The experiment type cannot be handled with Octopus-toolkit.
Err004	The data cannot be processed.
Err005	Not enough disk space.
Err006	Related to each processing step.
Err007	Some analytics tools are not installed.
Err008	Incorrect password.
Err009	Octopus-toolkit can't read/write files from your computer.
Err010	Incorrect number of Paired-End data.

If you have any questions, Please contact us at Octopustoolkit@gmail.com

7-2.Detail

Err001

Octopus-toolkit attempts to access the NCBI server (National Center for Biotechnology Information) to obtain sample information.

If `your network connection is unstable`, or `the NCBI server is tempararily unavailable`, Octopus-toolkit cannot get information for GSE and/or GSM.

First, `check the network connection` of your computer. If it is ok, please `check the` NCBI and whether the server is operating normally.

If the above cannot solve the problem, the connection to the NCBI may be `timed out` due to unknown reasons. Please re-run Octopus-toolkit after some time (`temporary phenomenon`).

Err002

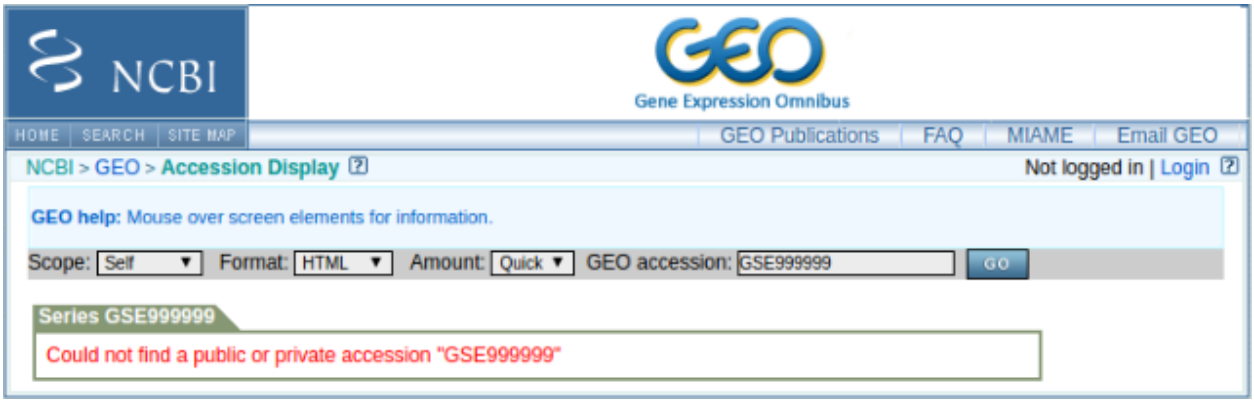
Octopus-toolkit obtains sample information from the GEO (gene expression omnibus) website.

- `GEO Accession Number`

```
A GSExxx is a unique GEO accession number assigned to a study.
A GSMxxx is a unique GEO accession number assigned to a sample. A single GSE␣
↪(study) can have a number of GSM (samples).
```

Octopus-toolkit can only process registered GSE or GSM ids in GEO. `Err002` occurs when you put `unregistered` accession ids or `misspelled accession ids`.

- Unregistered GSE id (Input : `GSE999999`)



- Misspelled or incorrect accession number (Input : `ChIP-Seq`)

Please `check the GEO accession number` whether it is registered in the GEO.

Err003

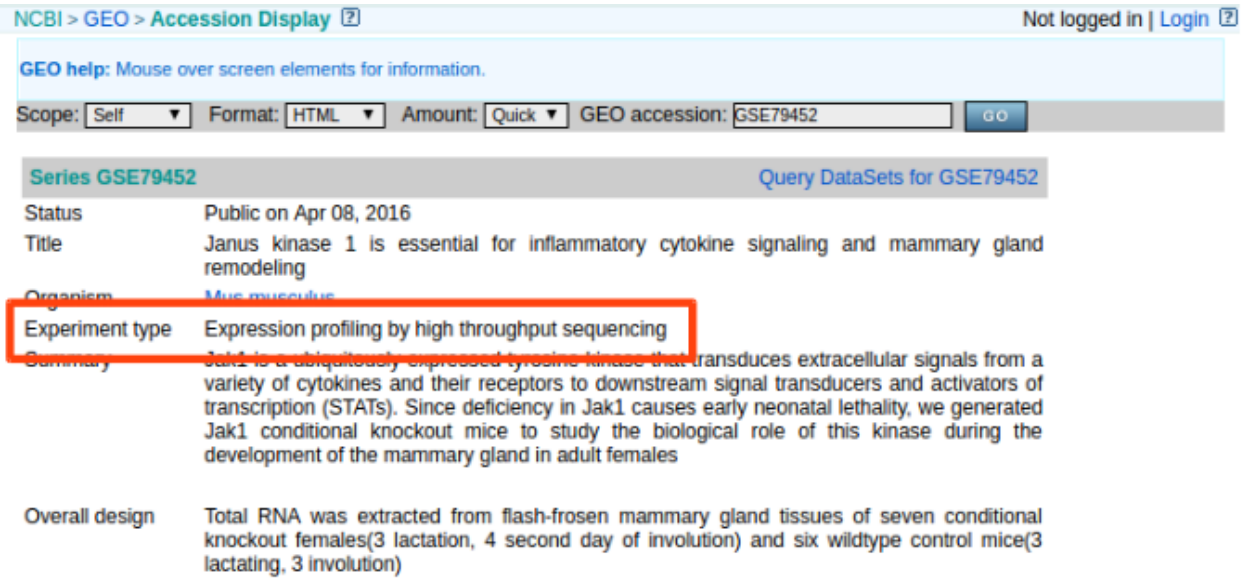
There are many different types of next-generation sequencing (NGS) data. As defined by NCBI (`NGS data` - Study type), genome binding/occupancy profiling by high throughput sequencing indicates ChIP-seq data.

Octopus-toolkit currently supports the following types of NGS data. Other NGS types will be skipped. `expression profiling by high throughput sequencing` (RNA-seq) `genome binding/occupancy profiling by high throughput sequencing` (ChIP-seq / MNase-seq / ATAC-seq / MeDIP-seq / DNase-seq)

(Other NGS types will be added later)

You can check `experiment type` of given GEO accession number through the website. (ex: GSE79452)

- Experiment Type



Err004

Not all data in the GEO can be processed with the Octopus-toolkit. Octopus-toolkit check the following information before the processing. `Organism`, `Library strategy`, `Instrument model`, and `FTP Address(SRA Experiment)`). (Important)

- DataSet for GSE79452 (Ex : GSE79452)

Sample GSM2095539 Query DataSets for GSM2095539

Status	Public on Apr 08, 2016
Title	Jak1_42186_Con_Lactation
Sample type	SRA
Source name	Mammary gland tissue
Organism	Mus musculus
Characteristics	tissue: Mammary gland developmental stage: Day 7 of Lactation
Extracted molecule	total RNA
Extraction protocol	Total RNA was extracted from flash-frozen mammary gland tissues of seven conditional knockout femalesand six wildtype control mice using the RNeasy Mini Kit (Qiagen). RNA samples were processed using the TruSeq RNA Sample kit and sequenced using a HiSeq2000 sequencer (Illumina).
Library strategy	RNA-Seq
Library source	transcriptomic
Library selection	cDNA
Instrument model	Illumina HiSeq 2000
Platform ID	GPL13112
Series (1)	GSE79452 Janus kinase 1 is essential for inflammatory cytokine signaling and mammary gland remodeling

Relations

BioSample	SAMN04571205
SRA	SRX1650911

Supplementary file	Size	Download	File type/resource
SRX/SRX165/SRX1650911		(ftp)	SRA Experiment

Raw data provided as supplementary file

Processed data is available on Series record

`Err004` is divided into the following four subcategories.

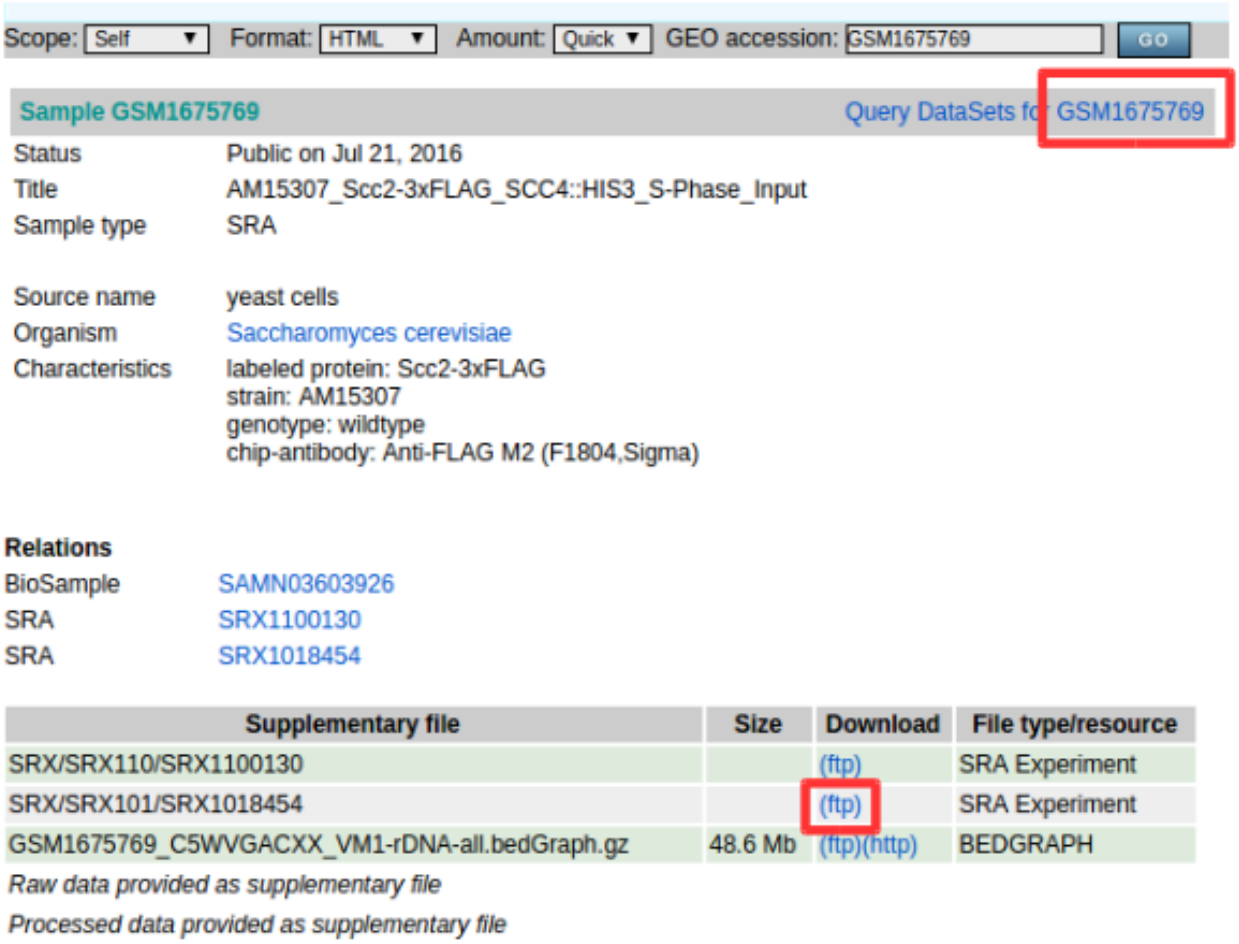
Sub Error ID	Description
`Err004-1`	The organism is not supported.
`Err004-2`	The experiment type is not supported (for example Exome-seq).
`Err004-3`	The instrument is not supported. Octopus-toolkit can only process data generated by Illumina instrument.
`Err004-4`	Raw data (.sra) is currently unavailable (probably newly registered data).

`Err004` is related to unsupported data by Octopus-toolkit. The following data is currently handled with Octopus-toolkit.

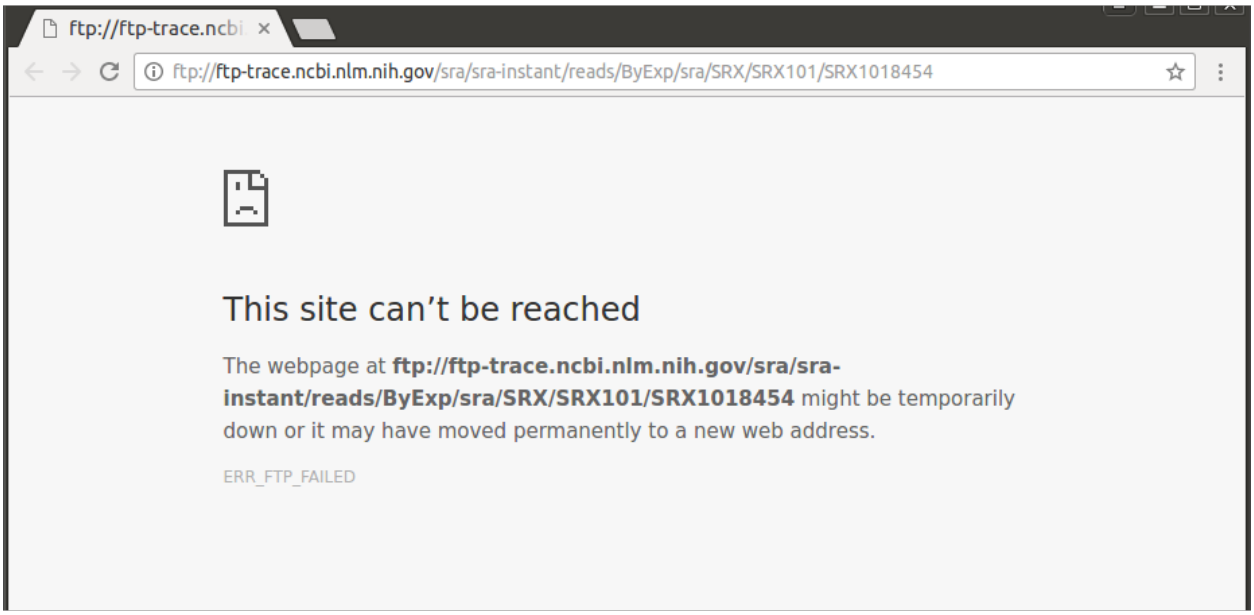
Type	Description
Organism	Homo sapiens, Mus musculus, Drosophila melanogaster, Saccharomyces cerevisiae, Canis lupus familaris, Arabidopsis thaliana, Danio rerio, Caenorhabditis elegans
Library Strategy	ChIP-Seq, RNA-Seq, MeDIP-Seq, ATAC-Seq, DNase-Seq, MNase-Seq
Instrument Model	Illumina GA/HiSeq/MiSeq (Illumina)

`Err004-4` indicates that data has been registered in the GEO, but the raw data (.sra) has not been released yet. Therefore, please check the availability of raw files.

- `Error004-4` example : GSM1675769



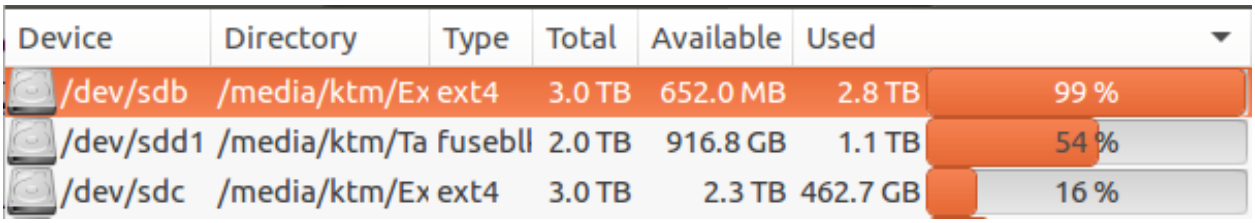
- No raw files (.sra).



Err005

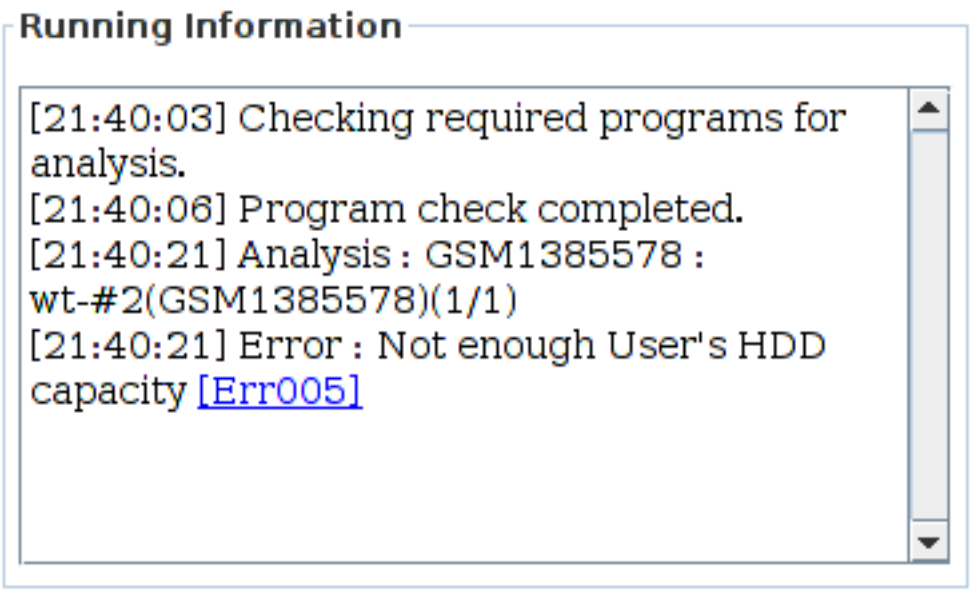
This error is related to disk space. To resolve this issue, obtain `enough free space` (more than 10Gb) and re-run the analysis.

- Check your hard disk space.



Device	Directory	Type	Total	Available	Used	
/dev/sdb	/media/ktm/Ex	ext4	3.0 TB	652.0 MB	2.8 TB	99 %
/dev/sdd1	/media/ktm/Ta	fusebll	2.0 TB	916.8 GB	1.1 TB	54 %
/dev/sdc	/media/ktm/Ex	ext4	3.0 TB	2.3 TB	462.7 GB	16 %

- Status window.



Err006

`Err006` is divided into six subcategories.

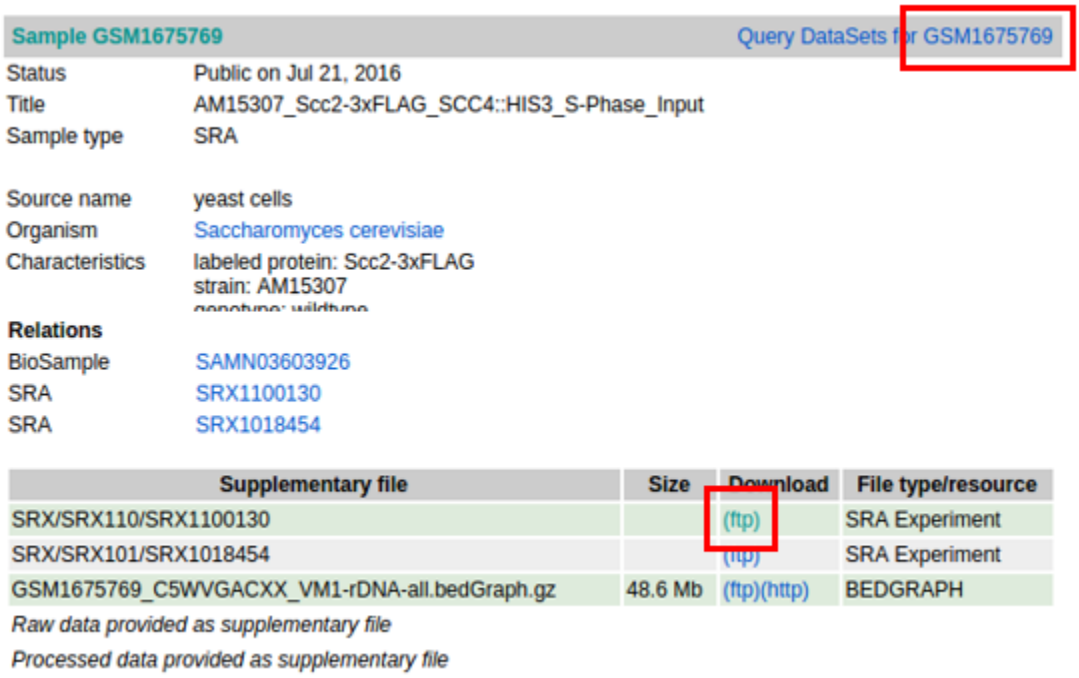
Sub Error ID	Description
Err006-1	Cannot `access` NCBI's FTP server.
Err006-2	File converting error from .sra to .fastq using `fastq-dump`.
Err006-3	Related to the .fastq file while checking the quality using `FastQC`.
Err006-4	No input file (.fastq) for `Trimming`.
Err006-5	Related to the `Mapping` step.
Err006-6	Related to the `Sorting` step (BAM file).

Err006-1

NCBI provides raw data of published sample through `FTP server` to user. If the NCBI homepage is working normally, you can extract the sample information, but if the FTP server does not work, you will not be able to download the data.

To solve this issue, you connect directly to the FTP server of NCBI.

- `Error006-1` example : GSM1675769



Sample GSM1675769 Query DataSets for GSM1675769

Status	Public on Jul 21, 2016
Title	AM15307_Scc2-3xFLAG_SCC4::HIS3_S-Phase_Input
Sample type	SRA
Source name	yeast cells
Organism	Saccharomyces cerevisiae
Characteristics	labeled protein: Scc2-3xFLAG strain: AM15307 genotype: wildtype

Relations

BioSample	SAMN03603926
SRA	SRX1100130
SRA	SRX1018454

Supplementary file	Size	Download	File type/resource
SRX/SRX110/SRX1100130		(ftp)	SRA Experiment
SRX/SRX101/SRX1018454		(ftp)	SRA Experiment
GSM1675769_C5WVGACXX_VM1-rDNA-all.bedGraph.gz	48.6 Mb	(ftp)(http)	BEDGRAPH

Raw data provided as supplementary file

Processed data provided as supplementary file

If you can connect to the FTP server, manually download the published sample.

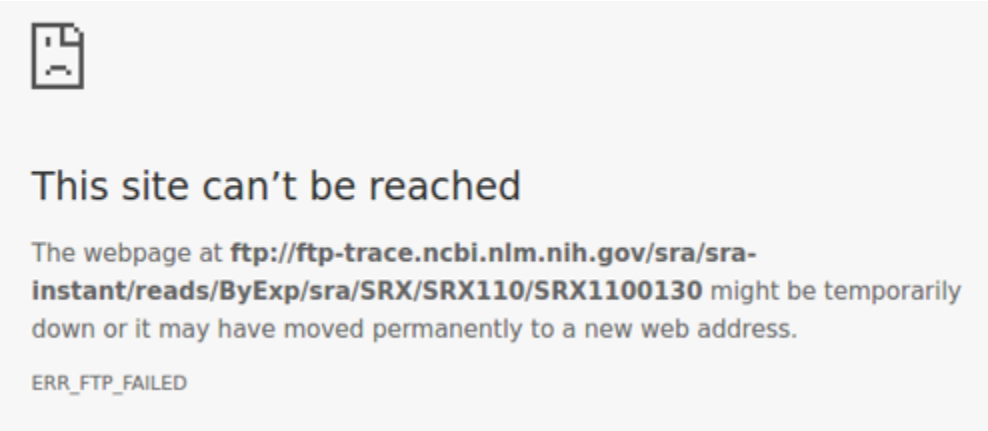
- NCBI Ftp server is running.(`Success`)

Index of /sra/sra-instant/reads/ByExp/sra/SRX/SRX110/SRX1100130

Name	Size	Date Modified
[parent directory]		
SRR2125091/		

If the server is closed or samples can not be downloaded, please contact the NCBI because it is an issue for the NCBI.

- NCBI Ftp server is closed.(`Fail`)



This site can't be reached

The webpage at **ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByExp/sra/SRX/SRX110/SRX1100130** might be temporarily down or it may have moved permanently to a new web address.

ERR_FTP_FAILED

If the above method works normally, please try Octopus-toolkit again.

If you still have an `Err006-1` in the retrial, please contact us at the address below.

Contact us : Octopustoolkit@gmail.com

Err006-2

Raw data of samples downloaded from NCBI is compressed in `SRA format`. For NGS analysis, `SRA` file should be converted to `Fastq` format. The tool used in this step is `Fastq-dump`, a sub tool of `SRA-Toolkit`.

- `Input file` : Sequence Read Archive (Extension : `sra`)
- `Output file` : Short read sequence. (Extension : `fastq`)

`006-2` occurs when there is no or invalid SRA file, which is the input file for executing Fastq-dump.

This error may arise due to an abrupt disconnection during the previous downloading step of the raw data from FTP server, or raw data uploaded to NCBI may be broken.

You should check your `network status`, `free space` on your computer and try the analysis again.

If the above method does not work, please contact us at the address below.

Contact us : Octopustoolkit@gmail.com

Err006-3

`Err006-3` means that the input file(`Fastq`) for the `Quality Check` is invalid or an issue in the system itself during `Quality Check` using `FastQC`.

You should check fastq files on your computer and try the analysis again.

If the above method does not work, please contact us at the address below.

Contact us : Octopustoolkit@gmail.com

After successfully completing the `Quality Check` step, some problems may prevent `FastQC` from generating `Fastqc_data.txt`.

Octopus-toolkit extracts the encoding information of the sample from `fastqc_data.txt` among the outputs of `FastQC`. Therefore, if `Fastqc_data.txt` is not generated, it stores the encoding information of the latest samples. (`Sanger / Illumina 1.9`)

- `Err006-3` Encoding information:

Basic Statistics

Measure	Value
Filename	H3K4me1_ChIPSeq_mb.fastq
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	11308561
Sequences flagged as poor quality	0
Sequence length	35-37
%GC	48

Err006-4

`Err006-4` occurs when there is no input file(`Fastq`) for `Trimming` step or when all reads are removed due to `bad quality`.

You should check fastq files on your computer and try the analysis again.

If the above method does not work, please contact us using address below.

Contact us : Octopustoolkit@gmail.com

If all reads are removed by `bad quality`, Octopus-toolkit will use the non-trimmed input file(`Fastq`) to proceed. (Next step : `Mapping`)

Err006-5

`Err006-5` may arise due to the following reasons.

- The input file (`non_trimmed Fastq`, `Trimmed Fastq`) does not exist.
- A large number of reads are trimmed due to `bad sequencing quality` or `high threshold used`.
- Too few mapped reads (Less than 2 MegaByte).

You should check your input file (`non-trimmed and trimmed fastq files`), `read count`, `file size after timming`.

Err006-6

`Err006-6`: BAM (mapped) file does not exist or the number of mapped reads is too small.

You should check `input file` and `BAM file`.

Err007

`Err007` is related to the installation step.

To use the Octopus-toolkit, your must follow the installation procedure completely: `Requirement(Err007-1)` and `analysis tools(Err007-2)`.

- *Requirement* : Library files must be installed.
- Analysis tools : Tools are installed automatically by Octopus-toolkit. If the installation procedure is interrupted, please remove the Octopus-toolkit directory and rerun it.

Octopus-toolkit download files from the `HOMER` website. `Err007` occurs if the website (http://homer.ucsd.edu/homer/) is unavailable, `Err007` can occur.

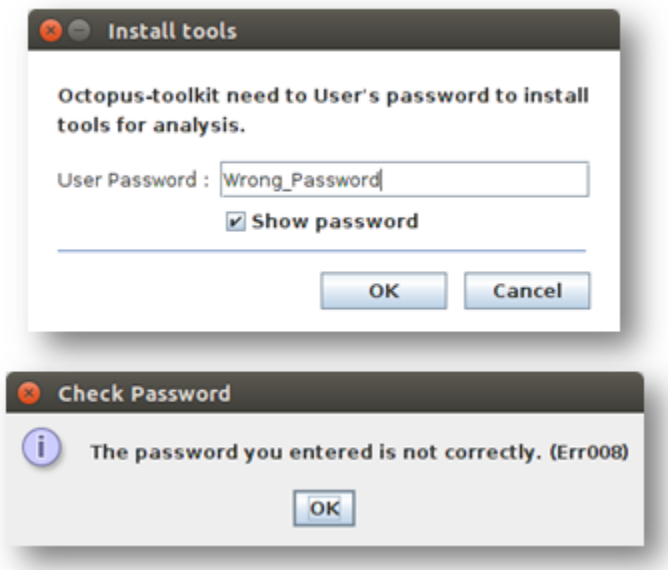
Err008

`Err008` is related to password issue.

- `Password` : You must enter your password once during the installtion step.

Please check your password and try again.

- When you enter incorrect password (Example : My password = ktm123)



Err009

`Err009` is related to script files generated by Octopus-toolkit. If this happens, please rerun it later.

Err010

`Err010` indicates that the number of files (paired-end sample) does not match when merging.

If there are several SRA files in one sample (GSM), Octopus-toolkit will merge them.

Paired-end data must have two files, Sample1_1.fastq and Sample1_2.fastq.

`Err010` occurs if any of these fails.

4.1.9 8.License

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Version 3, 29 June 2007

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4.1.10 9.Help

If you have any questions, please email us to the following address: Octopustoolkit@gmail.com

When sending an email, please include the following text in the title.

- `Recommend`

```
[Question-Type] Title
```

9-1.Example

- [Octopus-toolkit] What is the latest version of Octopus-toolkit?
- [Error] How to resolve the Err001?
- [Question] Is the Octopus-toolkit capable of ChIP-Seq analysis?
- [Request] Can you add a previous mouse genome version (mm8)?
- [Etc] What is your name?

4.1.11 10.Octopus-toolkit for Windows(alpha version)

10-1.Development Environment

- Window version : 7
- Eclipse : Neon.1a Service Release(4.6.1)
- Language : Java Programming language (JDK1.8)
- Graphic User Interface(GUI) : Swing & Windowbuilder

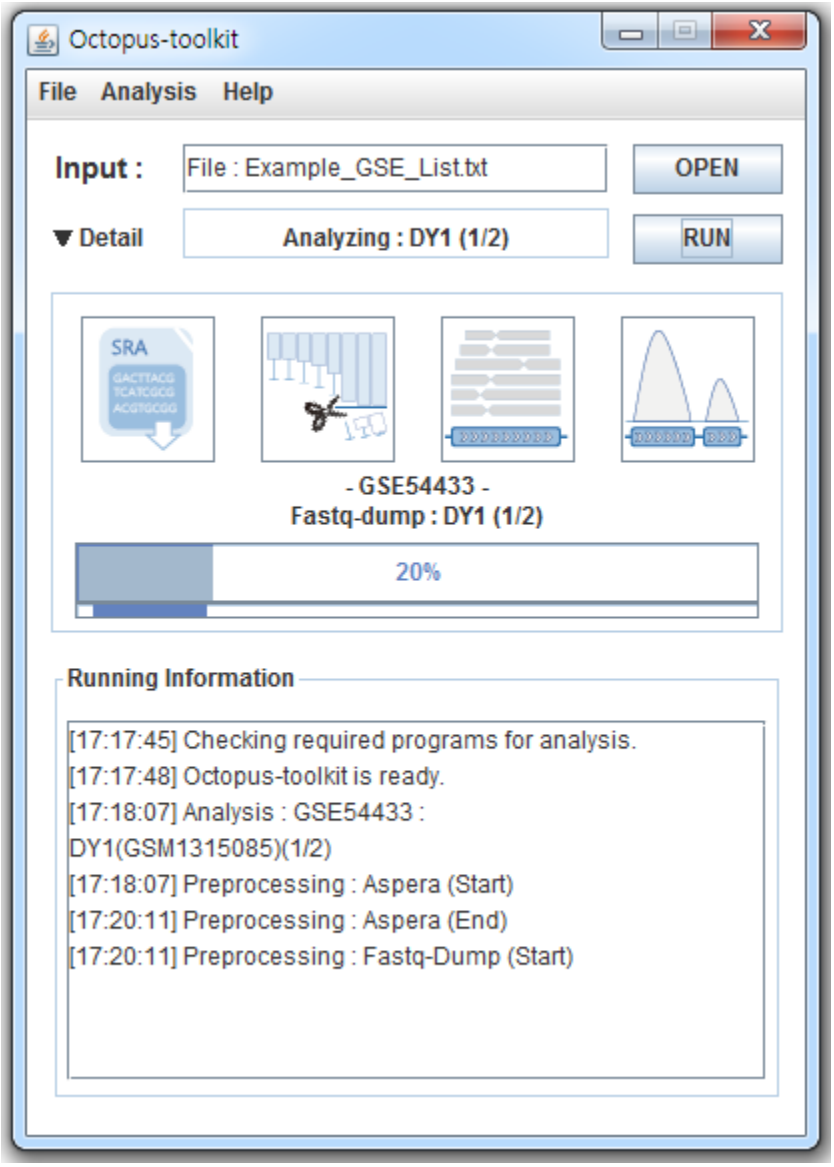
10-2. Requirement

To run the Octopus-toolkit, Java 8 (JDK, Java Development ToolKit) or higher must be installed on your computer.

10-3.Alpha test version

Octopus-toolkit Alpha version : (`Octopus-toolkit_win_test_version.exe`)

- Running window. (Test)



4.1.12 11. 3rd Party Tools

11-1.Version 2.2.0

Octopus-toolkit utilizes the following 3rd party tools during the process.

3rd party tool	Version	Function
`Aspera`	v3.7.2.141527	Download SRA files from NCBI
`SRAToolkit`	v2.9.2	Convert SRA files to Fastq files
`FastQC`	v0.11.5	Quality check for raw data
`Trimmomatic`	v0.36	Trimming for adapter sequence and portions of low-quality reads
`Hisat2`	v2.1.0	Indexing and Mapping to reference genome
`STAR`	v2.5.1	Indexing and Mapping to reference genome for RNA-Seq
`Homer`	v4.10.1	Create bigWig for visualization and Detect enriched regions by mapped reads
`Bwtool`, `libbeato`	v1.0	Calculate normalized values from bigWig files
`R`	v3.1	Draw the heatmap and line plot
`IGV`	v2.7.2	Explore the genome with processed data (bigWig files)
`Samtools`	v1.5	Sorting and Indexing the mapped reads