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### 1. smallRNA Pipeline

## 1.1 Can I upload 3' adapter clipped input files?

Yes, the pipeline accepts 3' adapter clipped input files.

- \* If your 3' adapter sequence is clipped, then you should choose the "NO 3' ADAPTER (already removed from these reads)" option in the "3' Adapter Sequence Options" section.
- \* If your 3' adapter sequence is NOT clipped, then follow the directions below:
- \*\* If you know the 3' adapter sequence for your sample(s), it is highly recommended to provide that adapter sequence by choosing the "MANUALLY SPECIFY 3' ADAPTER" option and then typing your adapter sequence in the "Manual Input of 3' Adapter Sequence" text box.
- \*\* However, by default, the pipeline will attempt to find your 3' adapter sequence and remove it from your sample(s), so you are not required to provide your 3' adapter sequence or clip your 3' adapter sequence beforehand.

## 1.2 How many mismatches are allowed for mapping the reads against the human genome?

The exceRpt pipeline allows anywhere from 0 to 3 endogenous mismatches.

You can find this option under the "Advanced Endogenous Alignment Options" section (you will have to maximize the section, as its contents are hidden by default).

**1.3** How many reads should we have minimum to be able to cover low abundant targets with confidence in sabout 1-10 million usable reads per sample (after filtering rRNAs, calibrators, etc.) is ideal to get a good profile of the sequenced small RNAs.

#### 1.4 What are the sources of the various small RNA libraries used in this pipeline?

The pipeline currently uses the following small RNA libraries from the source given below:

- \* rRNAs from 45S, 5S, and mt\_rRNA sequences for human and mouse
- \* miRNAs from "miRBase":http://www.mirbase.org/ version 21
- \* tRNAs from "gtRNAdb":http://gtrnadb.ucsc.edu/
- \* piRNAs from "piRNABank":http://pirnabank.ibab.ac.in/ (removed duplicate sequences)
- \* Annotations from "Gencode":http://www.gencodegenes.org/ version 24 (hg38), version 18 (hg19), version M9 (mm10)
- \* CircularRNAs from "circBase":http://www.circbase.org/

## 1.5 What are the various output files generated by the pipeline?

Learn more about the output files generated by the exceRpt pipeline by visiting the [[exRNA Data Analysis]] page.

## 1.6 What type of input files can be used for running the exceRpt Small RNA-seq pipeline?

This pipeline takes one or more single end "FASTQ":http://en.wikipedia.org/wiki/FASTQ\_format or "SRA" input file(s).

The input file(s) can be compressed.

Any SRA file will be converted to FASTQ by the pipeline for further processing.

You can upload multiple archives, each containing any number of samples, to the Genboree Workbench to launch a single job.

## 2. Long RNASeq Pipeline

## 2.1 What type of input files can be used for running the long RNA-seq pipeline?

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The long RNA-Seq pipeline accepts a single-end (one FILE) or paired end (TWO FILES) "FASTQ":http://en.wikipedia.org/wiki/FASTQ\_format files.

The input file(s) can be compressed.

#### 3. Genboree Workbench

3.1 Once I upload my files into the Genboree Workbench, are they public or not by default? Do I need to do so All files in the Genboree Workbench are private by default, so only +you+ can see the files you upload to your database.

However, you have the option to add people to your group, +if+ you would like to share the data with them. This "FAQ":http://genboree.org/theCommons/ezfaq/show/public-commons?faq\_id=494 provides information on how to add members to your group.

## 4. Network and Pathway Analysis

### 4.1 What types of pathways and biological processes are my exRNA of interest involved in?

Use the Pathway Finder tool (in the Visualization menu) to identify pathways that contain your exRNA of interest or their protein targets. You can also explore exRNA-related pathways at WikiPathways (http://exrna.wikipathways.org).

#### 4.2 Which proteins are targeted by my exRNA of interest?

Use the Target Interaction Finder tool (in the Visualization menu) to identify validated protein targets for any list of miRNAs based on miRTarBase (http://mirtarbase.mbc.nctu.edu.tw). The tool generates a network of miRNA-protein interactions that you can visualize in Cytoscape (http://cytoscape.org).

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