# PanGraphRNA User Manual

# (version 1.0)

- PanGraphRNA is an efficient, flexible and web-based Galaxy platform that can be easily used to construct graph pangenomes from genetic variations at individual, subpopulation, and population levels. It can assist researchers to select appropriate graph pangenomes using various performance metrics for both real and simulation experiments.
- Currently, PanGraphRNA is composed of four functional modules: **Graph Pangenome Preparation Module, Construction Module, Evaluation Module, and Application Moudule**.
- PanGraphRNA was powered with an advanced packaging technology, which enables compatibility and portability.
- PanGraphRNA project is hosted on https://github.com/cma2015/PanGraphRNA
- PanGraphRNA docker image is available at https://hub.docker.com/r/malab/pangraphrna

## Graph Pangenome Preparation Module

This module prepares input files required for subsequent graph pangenome-based analysis.

| Tools | Description | Input | Output | Time (test data) | Reference |
|---|---|---|---|---|---|
| **Upload File** | Upload input files required for all modules | Files or links | / | Depends on the file size | Galaxy |
| **Download File** | Directly fetch RNA-seq reads from NCBI's SRA database or other databases | SRR accession or HTTP/FTP link | Sequencing reads in SRA format | Depends on the network speed | SRA Toolkit |
| **Sequencing Data Preparation** | Convert RNA-seq reads from SRA to FASTQ format | RNA-seq reads in SRA format | RNA-seq reads in FASTQ format | ~2 mins | SRA Toolkit |

| Tools | Description | Input | Output | Time (test data) | Reference |
|---|---|---|---|---|---|
| **Quality Control for Sequencing Data** | Check RNA-seq reads quality and obtain high-quality reads | RNA-seq reads in FASTQ format and adapter sequences in FASTA format | RNA-seq reads in FASTQ format | ~2 mins | fastp |

# Upload File

This function is designed to upload input files required for all modules.

## Input

- **Input file and data format**: Specify the data format and upload a single-genome FASTA file reference genome to delineate primary paths, a GTF (general transfer format) file containing the gene annotations, a VCF (variant call format) file detailing genetic variations, a collection of RNA-seq FASTQ files and any of other file required in functions.
- **An HTTP/FTP link**: An HTTP/FTP link specifying the path of the file to be downloaded, e.g. ftp://download.big.ac.cn/gwh/Genome/Plants/Arabidopsis_thaliana/Athaliana_167_TAIR10/TAIR10_genomic.fna.gz

# Download File

This function is designed to download RNA-seq reads from NCBI SRA (Short Read Archive) database or from an user-specified HTTP/FTP link automatically. For the former, the **prefetch** function implemented in SRA Toolkit is wrapped to enable users to download sequencing data from NCBI SRA database; For the latter, **wget** command line is used to download the file according to an user-specified HTTP/FTP link.

## Input

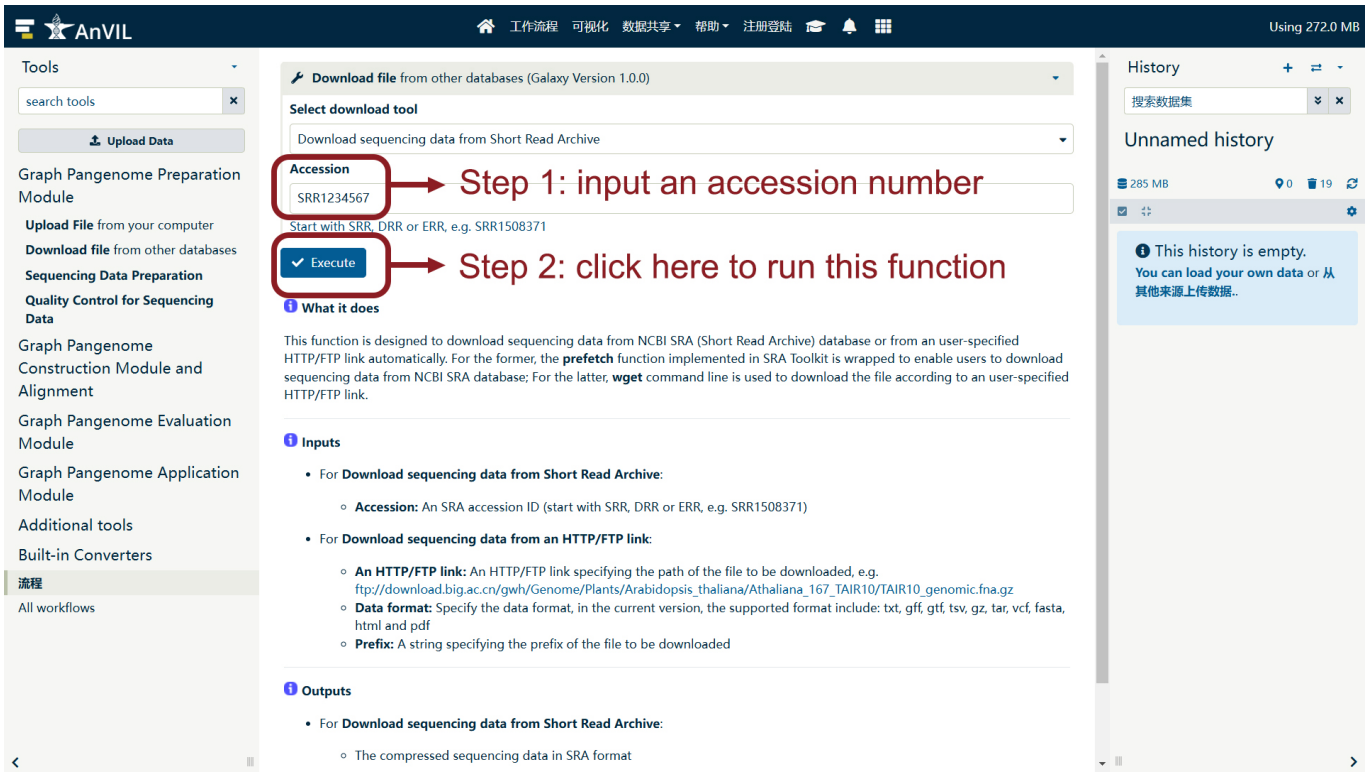
- For **Download sequencing data from Short Read Archive**:
    - **Accession:** An SRA accession ID (start with SRR, DRR or ERR, e.g. SRR1508371)
- For **Download sequencing data from an HTTP/FTP link**:
    - **An HTTP/FTP link**: An HTTP/FTP link specifying the path of the file to be downloaded, e.g.

ftp://download.big.ac.cn/gwh/Genome/Plants/Arabidopsis_thaliana/Athaliana_167_TAIR10/TAIR10_genomic.fna.gz

- **Data format**: Specify the data format, in the current version, the supported format include: txt, gff, gtf, tsv, gz, tar, vcf, fasta, html and pdf
- **Prefix**: A string specifying the prefix of the file to be downloaded

## Output

- For **Download sequencing data from Short Read Archive**:
  - The compressed sequencing data in SRA format
- For **Download sequencing data from an HTTP/FTP link**:
  - The downloaded file according to the provided HTTP/FTP link




# Sequencing Data Preparation

This function wrapped **fastq-dump** function implemented in **SRA Toolkit**.
See http://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=software for details.

## Input

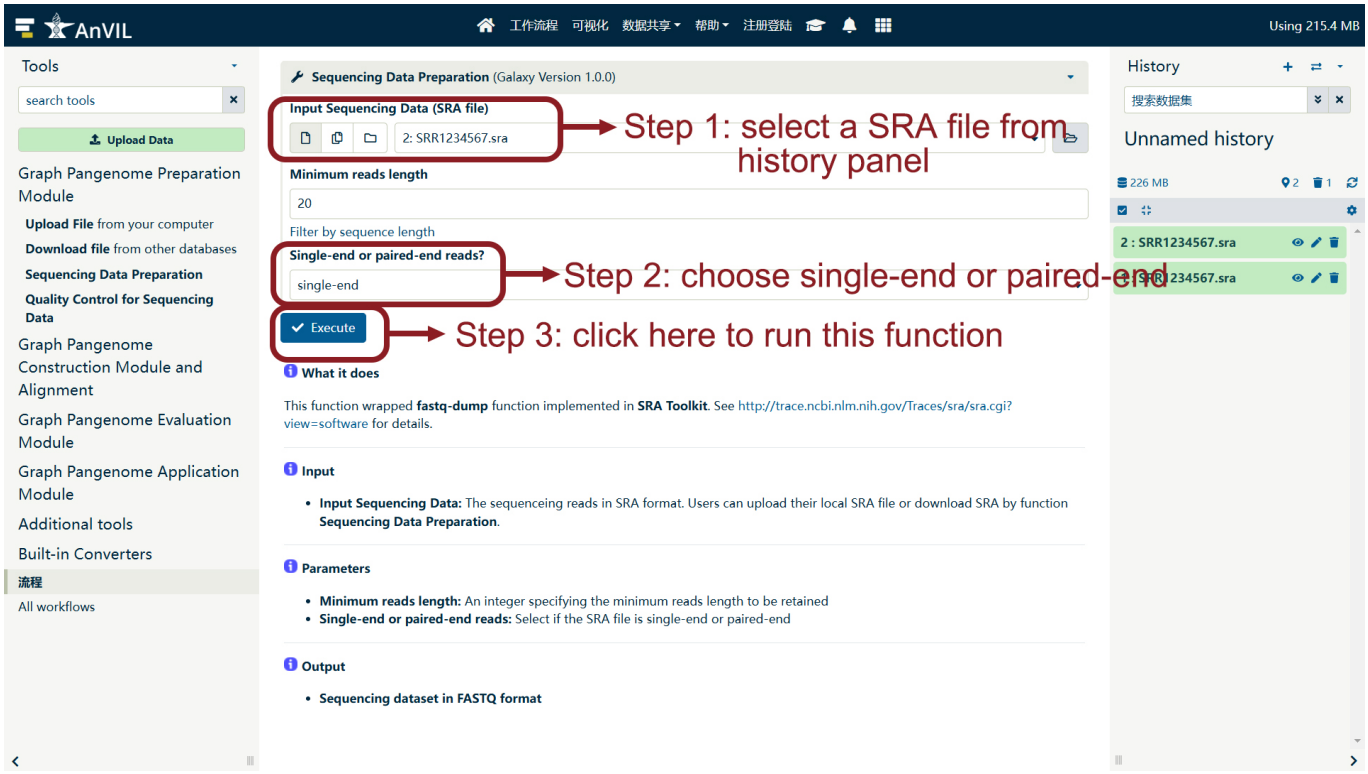
- **Input Sequencing Data:** The sequenceing reads in SRA format. Users can upload their local SRA file or download SRA by function **Sequencing Data Preparation**.

## Parameters

- **Minimum reads length:** An integer specifying the minimum reads length to be retained
- **Single-end or paired-end reads:** Select if the SRA file is single-end or paired-end

## Output

- **Sequencing dataset in FASTQ format**




# Quality Control for Sequencing Data

This function is designed to check RNA-seq reads quality and obtain high-quality reads.

## Input

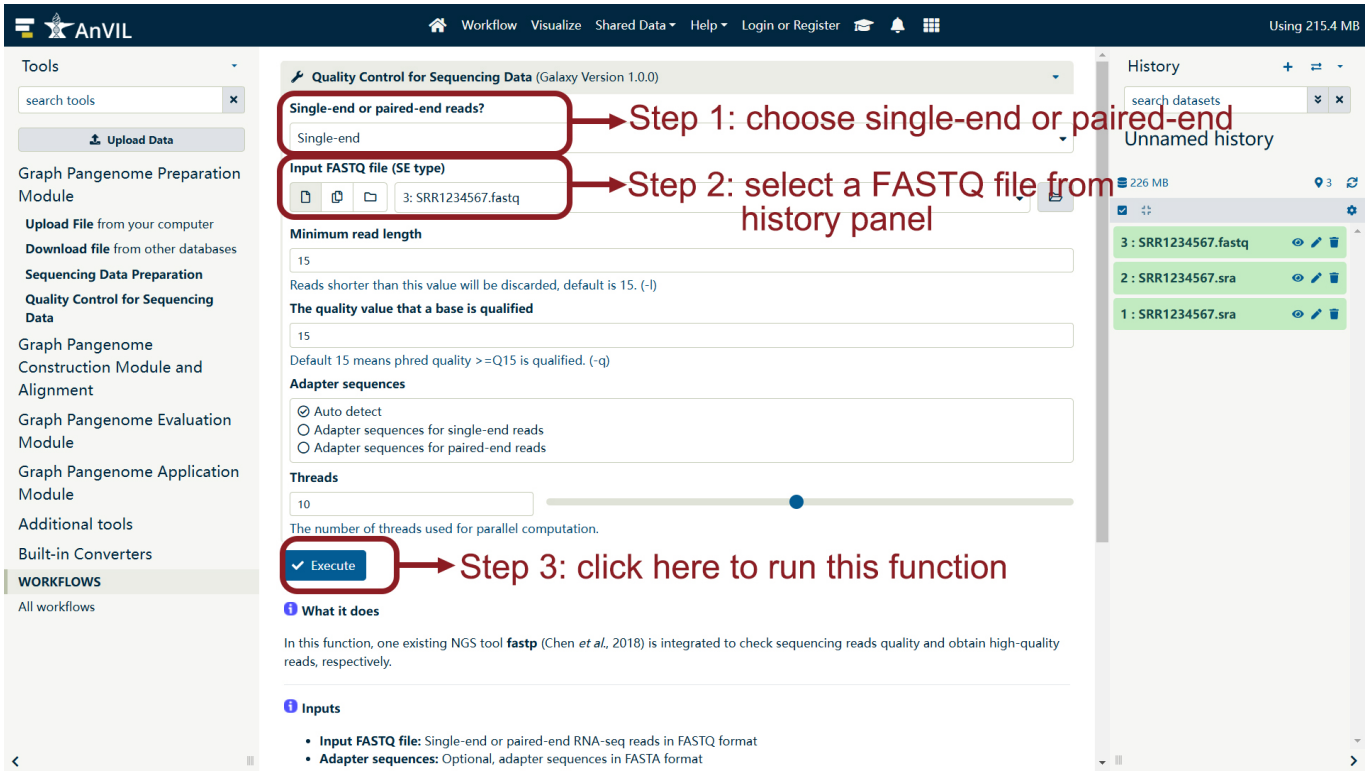
- **Input FASTQ file:** Single-end or paired-end RNA-seq reads in FASTQ format
- **Adapter sequences:** Optional, adapter sequences in FASTA format

## Parameters

- **Minimum read length:** Reads shorter than this value will be discarded, default is 15 (-l)
- **The quality value that a base is qualified**

## Output

- **Clean reads in FASTQ format**
- **Clean reads fastp report in HTML format**


AnVIL
Workflow
Visualize
Shared Data
Help
Login or Register
Using 215.4 MB
Tools
search tools
Upload Data
Graph Pangenome Preparation Module
Upload File from your computer
Download file from other databases
Sequencing Data Preparation
Quality Control for Sequencing Data
Graph Pangenome Construction Module and Alignment
Graph Pangenome Evaluation Module
Graph Pangenome Application Module
Additional tools
Built-in Converters
WORKFLOWS
All workflows
Quality Control for Sequencing Data (Galaxy Version 1.0.0)
Single-end or paired-end reads?
Single-end
Step 1: choose single-end or paired-end
Input FASTQ file (SE type)
3: SRR1234567.fastq
Step 2: select a FASTQ file from history panel
Minimum read length
15
Reads shorter than this value will be discarded, default is 15. (-l)
The quality value that a base is qualified
15
Default 15 means phred quality >=Q15 is qualified. (-q)
Adapter sequences
Auto detect
Adapter sequences for single-end reads
Adapter sequences for paired-end reads
Threads
10
The number of threads used for parallel computation.
Execute
Step 3: click here to run this function
What it does
In this function, one existing NGS tool fastp (Chen et al., 2018) is integrated to check sequencing reads quality and obtain high-quality reads, respectively.
Inputs
Input FASTQ file: Single-end or paired-end RNA-seq reads in FASTQ format
Adapter sequences: Optional, adapter sequences in FASTA format
History
search datasets
Unnamed history
226 MB
3
3 : SRR1234567.fastq
2 : SRR1234567.sra
1 : SRR1234567.sra