mlPEA User Manual

(version 1.0)

- mlPEA is a user-friendly, full-functionality pipeline specifically designed to enhance the processing, analysis, and interpretation of m6A-Seq in non-model plants by leveraging machine learning.
- mlPEA comprises four functional modules: **Data Preprocessing, Transcriptome Construction, m^{6}A Calling, and Functional Exploration**.
- mlPEA was powered with an advanced packaging technology, which enables compatibility and portability.
- mlPEA project is hosted on http://github.com/cma2015/mlPEA
- mlPEA docker image is available at http://hub.docker.com/r/malab/mlpea

Data Preprocessing Module

This module provides four funcitons (see following table for details) to prepare epitranscriptome data.

Tools	Description	Input	Output	Time (test data)	Reference
Download File	Directly fetch epitranscriptome sequencing 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
Sequence Data Preprocessing	Convert epitranscriptome sequencing reads from SRA to FASTQ format	Epitranscriptome sequencing reads in SRA format	Epitranscriptome sequencing reads in FASTQ format	~2 mins	SRA Toolkit
Assess Reads Quality	Check m ^{6}A-Seq reads quality and obtain high-quality reads	m ^{6}A-Seq reads in FASTQ format and adapter sequences in FASTA format	m ^{6}A-Seq reads in FASTQ format;multiQC report in HTML	~2 mins	fastp;MultiQC
Differential *K*-mer Analyses	Perform m ^{6}A-Seq differential *k*-mer analyses	m ^{6}A-Seq reads in FASTQ format	m ^{6}A-Seq reads in FASTA format	Depends on the file size	kmdiff

Download File

This function is designed to download epitranscriptome sequencing 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**:

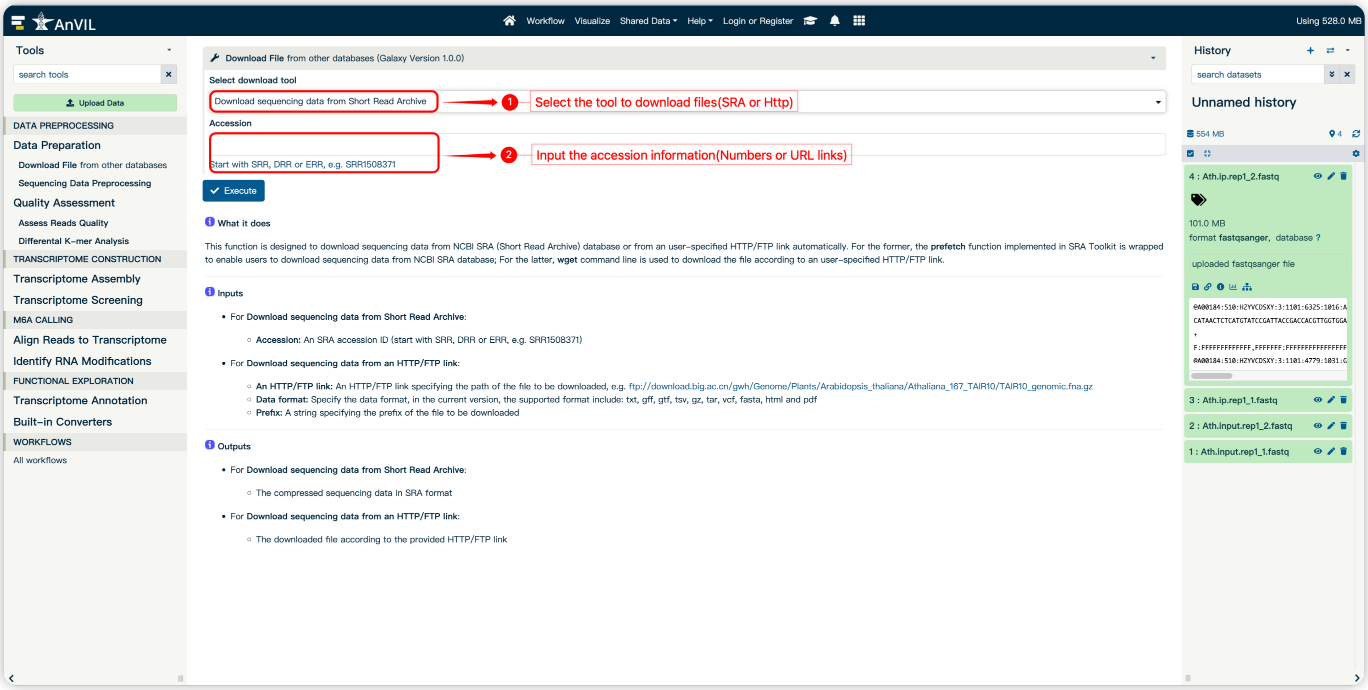
    - **Accession:** An SRA accession ID (start with SRR, DRR or ERR, e.g. SRR1508371)
- For **Download sequencing data from an HTTP/FTP link**:
    - **An HTTP/FTP link**: An HTTP/FTP link specifying the path of the file to be downloaded, e.g. ftp://download.big.ac.cn/gwh/Genome/Plants/Arabidopsis_thaliana/Athaliana_167_TAIR10/TAIR10_genomic.fna.gz
    - **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

How to use this function

- The following screenshot shows us how to download sequencing reads in SRA format


AnVIL
Workflow
Visualize
Shared Data
Help
Login or Register
Using 528.0 MB
Tools
search tools
Upload Data
DATA PREPROCESSING
Data Preparation
Download File from other databases
Sequencing Data Preprocessing
Quality Assessment
Assess Reads Quality
Differental K–mer Analysis
TRANSCRIPTOME CONSTRUCTION
Transcriptome Assembly
Transcriptome Screening
M6A CALLING
Align Reads to Transcriptome
Identify RNA Modifications
FUNCTIONAL EXPLORATION
Transcriptome Annotation
Built–in Converters
WORKFLOWS
All workflows
History
search datasets
Unnamed history
554 MB
4
Execute

Download File from other databases (Galaxy Version 1.0.0)

Select download tool: Download sequencing data from Short Read Archive

① Select the tool to download files(SRA or Http)

Accession: Start with SRR, DRR or ERR, e.g. SRR1508371

② Input the accession information(Numbers or URL links)

What it does

This function is designed to download sequencing data 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.

Inputs

- 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

Outputs

- 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

4 : Ath.ip.rep1_2.fastq

101.0 MB

format **fastqsanger**, database ?

uploaded fastqsanger file

```
@A00184:510:H2YVCDSXY:3:1101:6325:1016:A
CATAACTCTCATGTATCCGATTACCGACCACGTTGGTGGA
+
F:FFFFFFFFFFFFF,FFFFFFF:FFFFFFFFFFFFFFF
@A00184:510:H2YVCDSXY:3:1101:4779:1031:G
```

3 : Ath.ip.rep1_1.fastq

2 : Ath.input.rep1_2.fastq

1 : Ath.input.rep1_1.fastq

Sequence Data Preprocessing

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

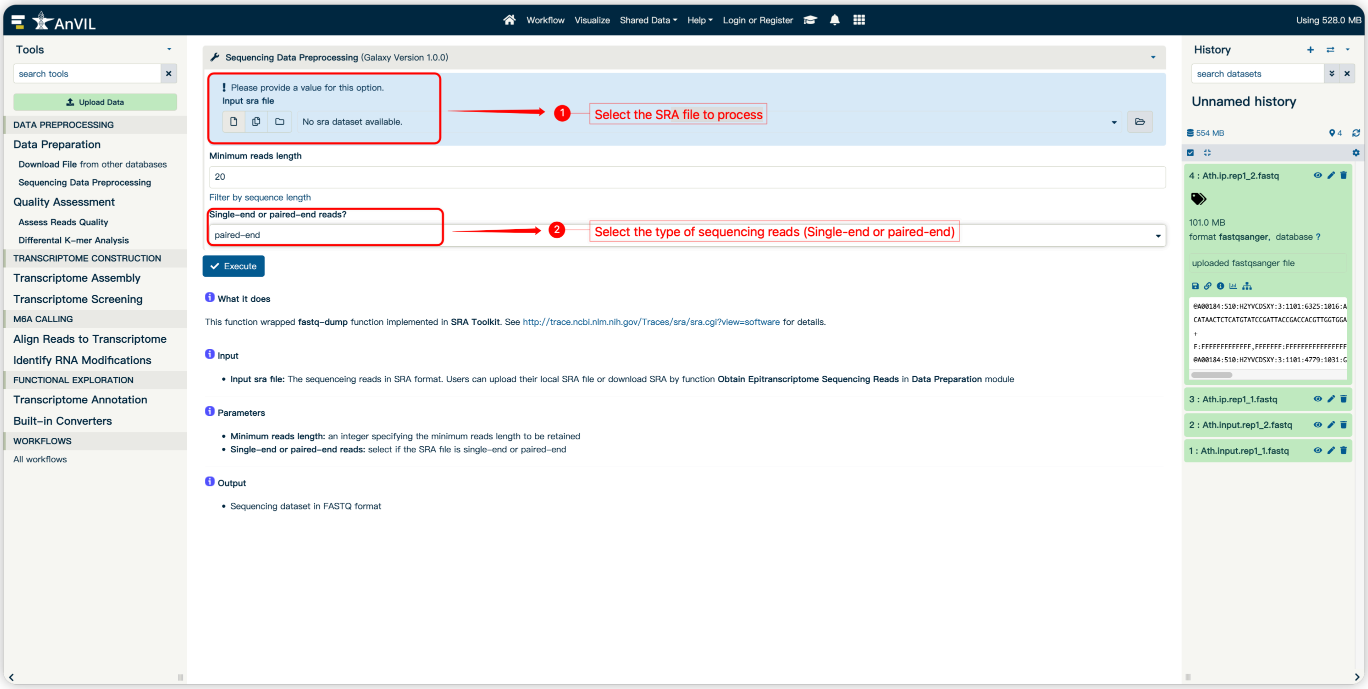
- **Input sra file:** The sequenceing reads in SRA format. Users can upload their local SRA file or download SRA by function **Download File** in **Data Preparation** module

Output

- Sequencing dataset in FASTQ format

How to use this function

- The following screenshot shows us how to use this function to convert sequencing reads in SRA format to FASTQ format


AnVIL
Workflow
Visualize
Shared Data
Help
Login or Register
Using 528.0 MB
Tools
search tools
Upload Data
DATA PREPROCESSING
Data Preparation
Download File from other databases
Sequencing Data Preprocessing
Quality Assessment
Assess Reads Quality
Differental K–mer Analysis
TRANSCRIPTOME CONSTRUCTION
Transcriptome Assembly
Transcriptome Screening
M6A CALLING
Align Reads to Transcriptome
Identify RNA Modifications
FUNCTIONAL EXPLORATION
Transcriptome Annotation
Built–in Converters
WORKFLOWS
All workflows
History
search datasets
Unnamed history
554 MB
4
Execute

Sequencing Data Preprocessing (Galaxy Version 1.0.0)

! Please provide a value for this option.

Input sra file

No sra dataset available.

① Select the SRA file to process

Minimum reads length

20

Filter by sequence length

Single–end or paired–end reads?

paired–end

② Select the type of sequencing reads (Single-end or paired-end)

What it does

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 sra file:** The sequenceing reads in SRA format. Users can upload their local SRA file or download SRA by function **Obtain Epitranscriptome Sequencing Reads** in **Data Preparation** module

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

4 : Ath.ip.rep1_2.fastq

101.0 MB

format **fastqsanger**, database ?

uploaded fastqsanger file

```
@A00184:510:H2YVCDSXY:3:1101:6325:1016:A
CATAACTCTCATGTATCCGATTACCGACCACGTTGGTGGA
+
F:FFFFFFFFFFFFF,FFFFFFF:FFFFFFFFFFFFFFFF
@A00184:510:H2YVCDSXY:3:1101:4779:1031:G
```

3 : Ath.ip.rep1_1.fastq

2 : Ath.input.rep1_2.fastq

1 : Ath.input.rep1_1.fastq

Differential *K*-mer Analyses

In this function, two existing NGS tools MultiQC and fastp are integrated to check sequencing reads quality and obtain high-quality reads, respectively.

Input

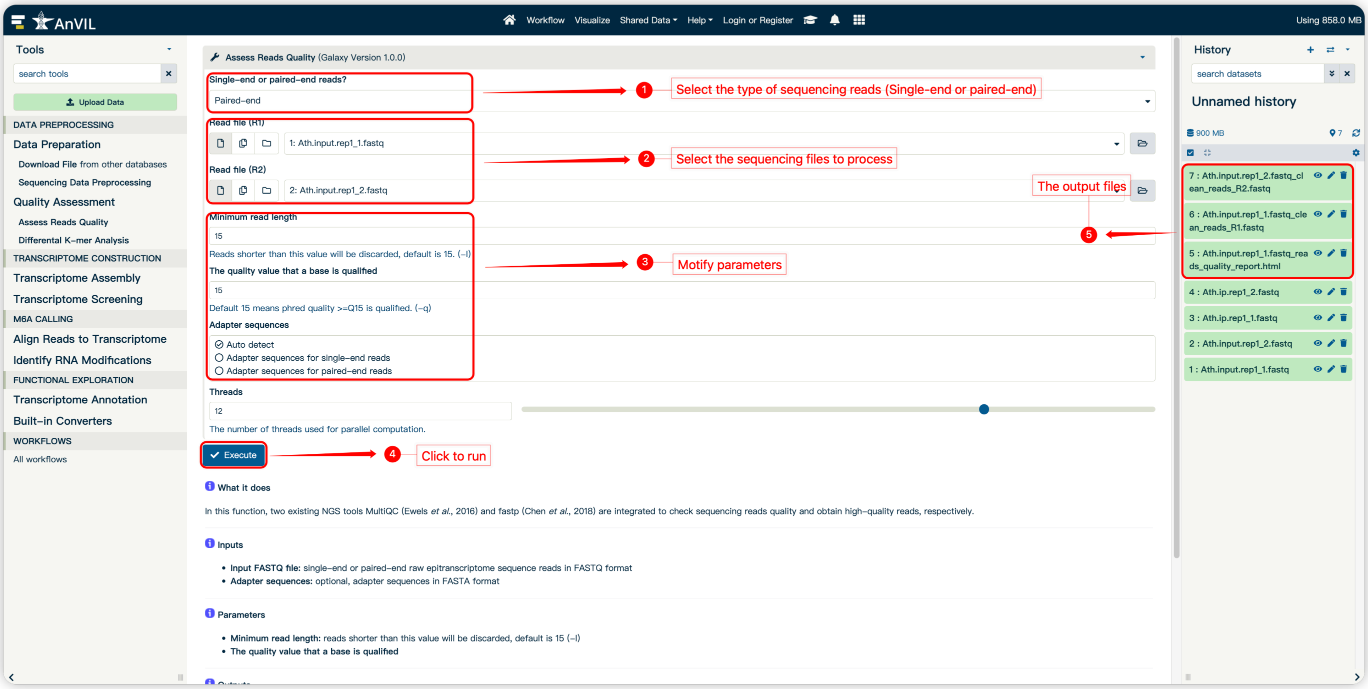
- **Input FASTQ file:** single-end or paired-end raw epitranscriptome sequence reads in FASTQ format
- **Adapter sequences:** optional, adapter sequences in FASTA format

Output

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

How to use this function

- The following screenshot shows us how to use this function to check m ^{6}A-Seq reads quality and obtain high-quality reads.


AnVIL
Workflow
Visualize
Shared Data
Help
Login or Register
Using 858.0 MB
Tools
search tools
Upload Data
DATA PREPROCESSING
Data Preparation
Download File from other databases
Sequencing Data Preprocessing
Quality Assessment
Assess Reads Quality
Differental K–mer Analysis
TRANSCRIPTOME CONSTRUCTION
Transcriptome Assembly
Transcriptome Screening
M6A CALLING
Align Reads to Transcriptome
Identify RNA Modifications
FUNCTIONAL EXPLORATION
Transcriptome Annotation
Built–in Converters
WORKFLOWS
All workflows
History
search datasets
Unnamed history
900 MB
7
Execute

Assess Reads Quality (Galaxy Version 1.0.0)

Single–end or paired–end reads?
Paired–end

Read file (R1)
1: Ath.input.rep1_1.fastq

Read file (R2)
2: Ath.input.rep1_2.fastq

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
12
The number of threads used for parallel computation.

① Select the type of sequencing reads (Single–end or paired–end)

② Select the sequencing files to process

③ Motify parameters

④ Click to run

⑤ The output files

7 : Ath.input.rep1_2.fastq_clean_reads_R2.fastq
6 : Ath.input.rep1_1.fastq_clean_reads_R1.fastq
5 : Ath.input.rep1_1.fastq_reads_quality_report.html
4 : Ath.ip.rep1_2.fastq
3 : Ath.ip.rep1_1.fastq
2 : Ath.input.rep1_2.fastq
1 : Ath.input.rep1_1.fastq

What it does

In this function, two existing NGS tools MultiQC (Ewels *et al.*, 2016) and fastp (Chen *et al.*, 2018) are integrated to check sequencing reads quality and obtain high–quality reads, respectively.

Inputs

- **Input FASTQ file:** single–end or paired–end raw epitranscriptome sequence 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**

Assess Reads Quality

In this function, one existing disk-based program kmdiff is integrated to count k-mers from (possibly gzipped) FASTQ/FASTA files.

Input

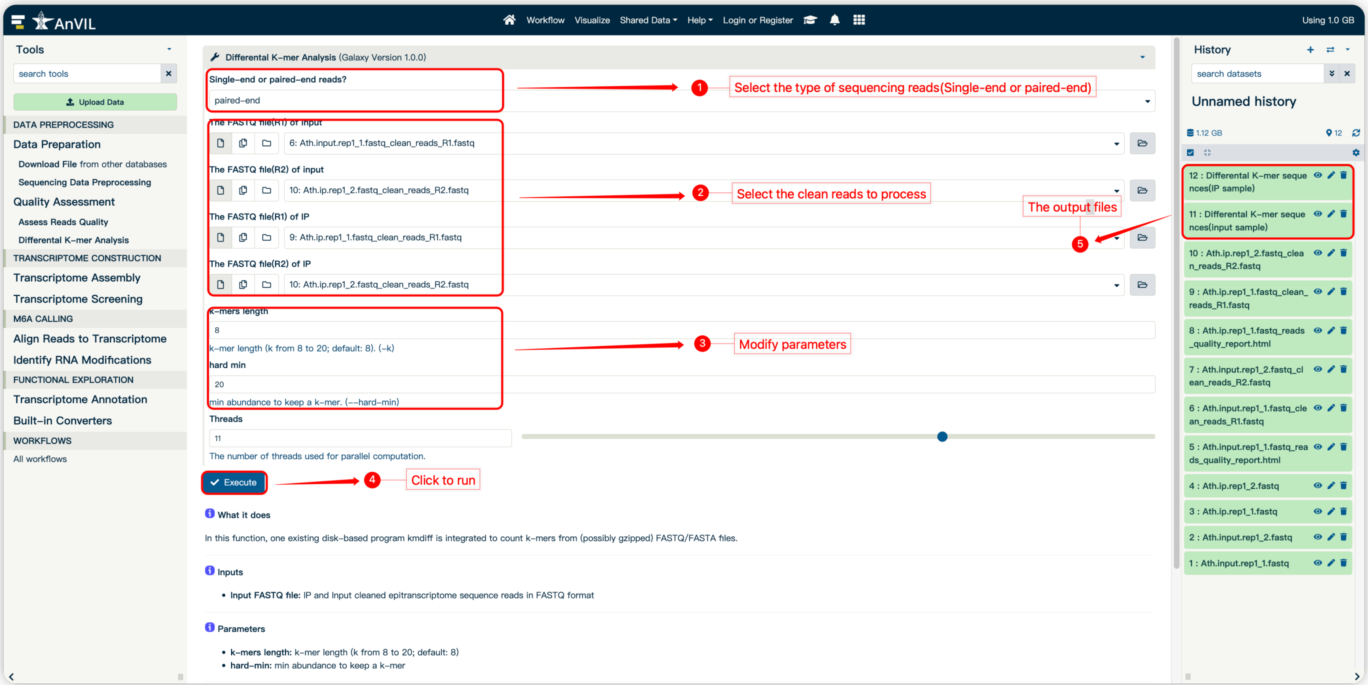
- **Input FASTQ file:** IP and Input cleaned epitranscriptome sequence reads in FASTQ format

Output

- **Differental K-mer sequences(input sample) in FASTA format**
- **Differental K-mer sequences(IP sample) in FASTA format**

How to use this function

- The following screenshot shows us how to use this function.


AnVIL
Workflow
Visualize
Shared Data
Help
Login or Register
Using 1.0 GB
Tools
search tools
Upload Data
DATA PREPROCESSING
Data Preparation
Download File from other databases
Sequencing Data Preprocessing
Quality Assessment
Assess Reads Quality
Differental K–mer Analysis
TRANSCRIPTOME CONSTRUCTION
Transcriptome Assembly
Transcriptome Screening
M6A CALLING
Align Reads to Transcriptome
Identify RNA Modifications
FUNCTIONAL EXPLORATION
Transcriptome Annotation
Built–in Converters
WORKFLOWS
All workflows
History
search datasets
Unnamed history
1.12 GB
12
Execute

Differental K–mer Analysis (Galaxy Version 1.0.0)

Single–end or paired–end reads?
paired–end

The FASTQ file(R1) of input
6: Ath.input.rep1_1.fastq_clean_reads_R1.fastq

The FASTQ file(R2) of input
10: Ath.ip.rep1_2.fastq_clean_reads_R2.fastq

The FASTQ file(R1) of IP
9: Ath.ip.rep1_1.fastq_clean_reads_R1.fastq

The FASTQ file(R2) of IP
10: Ath.ip.rep1_2.fastq_clean_reads_R2.fastq

k–mers length
8
k–mer length (k from 8 to 20; default: 8). (–k)

hard min
20
min abundance to keep a k–mer. (––hard–min)

Threads
11
The number of threads used for parallel computation.

① Select the type of sequencing reads(Single–end or paired–end)

② Select the clean reads to process

③ Modify parameters

④ Click to run

⑤ The output files

What it does

In this function, one existing disk–based program kmdiff is integrated to count k–mers from (possibly gzipped) FASTQ/FASTA files.

Inputs

- **Input FASTQ file:** IP and Input cleaned epitranscriptome sequence reads in FASTQ format

Parameters

- **k–mers length:** k–mer length (k from 8 to 20; default: 8)
- **hard–min:** min abundance to keep a k–mer

12 : Differental K–mer sequences(IP sample)
11 : Differental K–mer sequences(input sample)
10 : Ath.ip.rep1_2.fastq_clean_reads_R2.fastq
9 : Ath.ip.rep1_1.fastq_clean_reads_R1.fastq
8 : Ath.ip.rep1_1.fastq_reads_quality_report.html
7 : Ath.input.rep1_2.fastq_clean_reads_R2.fastq
6 : Ath.input.rep1_1.fastq_clean_reads_R1.fastq
5 : Ath.input.rep1_1.fastq_reads_quality_report.html
4 : Ath.ip.rep1_2.fastq
3 : Ath.ip.rep1_1.fastq
2 : Ath.input.rep1_2.fastq
1 : Ath.input.rep1_1.fastq