# easyMF User Mannual

(version 1.0)

easyMF is a user-friendly web platform that aims to facilitate biological discovery from large-scale transcriptome data through matrix factorization (MF). It offers several functional tools for gene expression matrix generation, expression matrix factorization, and metagene-based exploratory analysis including sample clustering, signature gene identification, functional gene discovery, subtype cell detection, and pathway activity inference.

- easyMF project is hosted on https://github.com/cma2015/easyMF.
- easyMF docker image is available in https://hub.docker.com/r/malab/easymf.
- easyMF demo server can be accessed via http://easymf.omicstudio.cloud.
- The following part shows installation of easyMF docker image and detailed documentation for each function in easyMF.

0. Matrix Preparation

Matrix factorization is typically started with an input of a gene expression matrix (genes in rows and individual samples in columns), which prompt us to design this module including three functions to prepare a high-quality gene expression matrix for downstream analysis.

The gene expression matrix can be automatically generated from raw reads using a bioinformatics pipeline (see following figure).
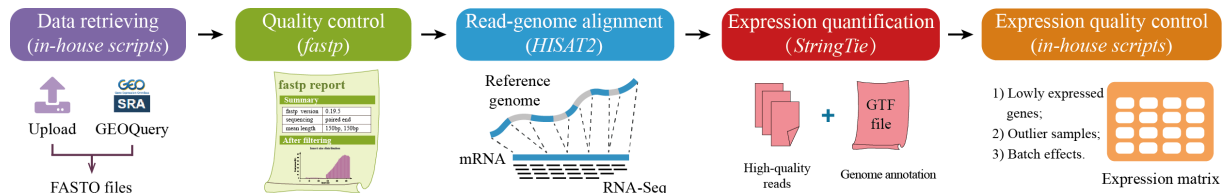
This module consists of three functions: **Data Retrieval**, **Expression Matrix Generation** and **Expression Quality Control**.

Functions/Tools	Description	Inputs	Outputs	Time (test data)	Program	References
Data Retrieval	Retrieve genome sequences, genome annotation, and RNA-Seq data automatically from public databases	Select a species; Database version; Data type	Genome sequences (in terms of Reference genome sequence); Genome annotation (in terms of Reference genome annotation); RNA-Seq data (in terms of Raw RNA-Seq data)	Depends on network speed	In-house scripts	This study
Expression Matrix Generation	Generate a gene expression matrix (genes in rows and individual samples in columns) through raw RNA-Seq quality control, read-genome alignment, and gene expression abundance calculation	Genome sequence and annotation; RNA-Seq data	Gene expression matrix	~ 2 mins	fastp (Raw RNA-Seq quality control)	Chen et al., 2018
					HISAT2 (Read-genome alignment)	Kim et al., 2015
					StringTie (Gene expression abundance calculation)	Pertea et al., 2015
					In-house scripts	This study
Expression Quality Control	Generate a high-quality gene expression matrix through removing lowly expressed genes, outlier samples, or batch effects	Raw gene expression matrix	High-quality gene expression matrix	~ 10s	In-house scripts (Removing lowly expressed genes and outlier samples)	This study
					sva (Removing batch effects)	Leek et al., 2012

1. Data Retrieval

Data Retrieval can be used to retrieve **Genome sequences** and **Genome annotation** from [Ensembl Plants](#), **RNA-Seq data** from [NCBI](#) (National Center for Biotechnology Information) GEO (Gene Expression Omnibus) or SRA (Short Read Archive) databases.

Inputs

For retrieving **genome sequences and annotation**, users need to select option **Obtain Genome Sequences and Annotation**.

- **Select a species**: This option provides the Latin name of 61 species.
- **Database version**: Ensembl releases from 25 to 47 are listed.
- **Data type**: Genome sequences (.fasta) or annotation (.gtf).

For retrieving **RNA-Seq** data, users need to select option **Obtain RNA-Seq data**.

- **Fecth data through data ID or ftp address**: easyMF provides two ways to download RNA-Seq data.

  If users select **Fetch data through data ID**, easyMF downloads RNA-Seq data by NCBI's tool *sratoolkit* (version 2.3.5) through RNA-Seq IDs (such as SRR1765337).

  If users select **Fetch data through data address**, easyMF downloads RNA-Seq data by wget using HTTP/FTP addresses.

Outputs

For **Obtain Genome Sequences and Annotation**

- **Reference genome sequence**
- **Reference genome annotation**

For **Obtain RNA-Seq data**
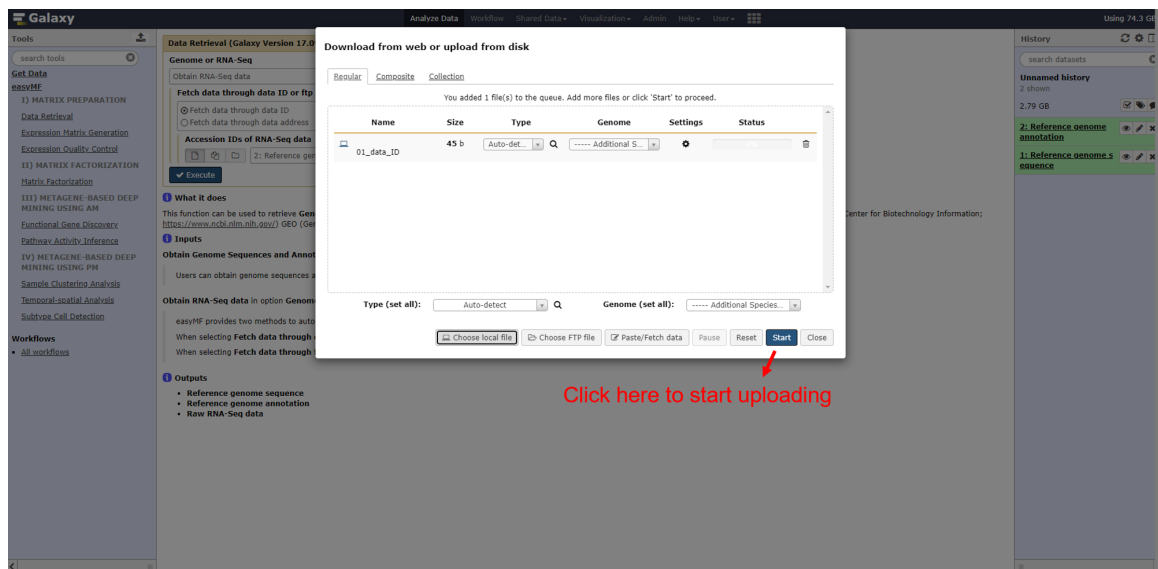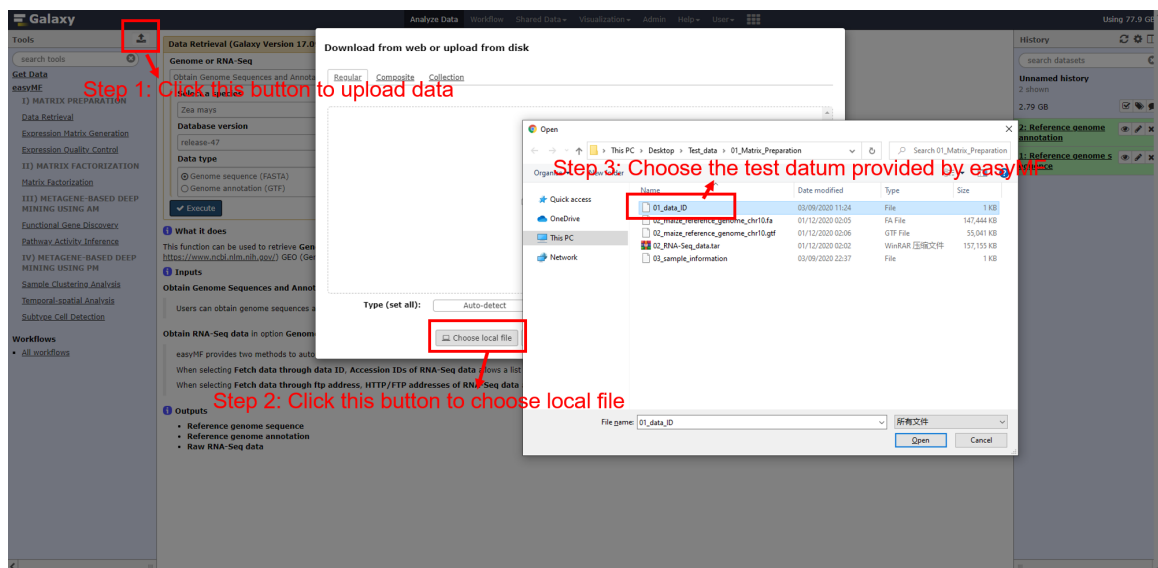
- **Raw RNA-Seq data**

How to use this function

- The following screenshot shows us how to download genome sequences and annoatation using easyMF.
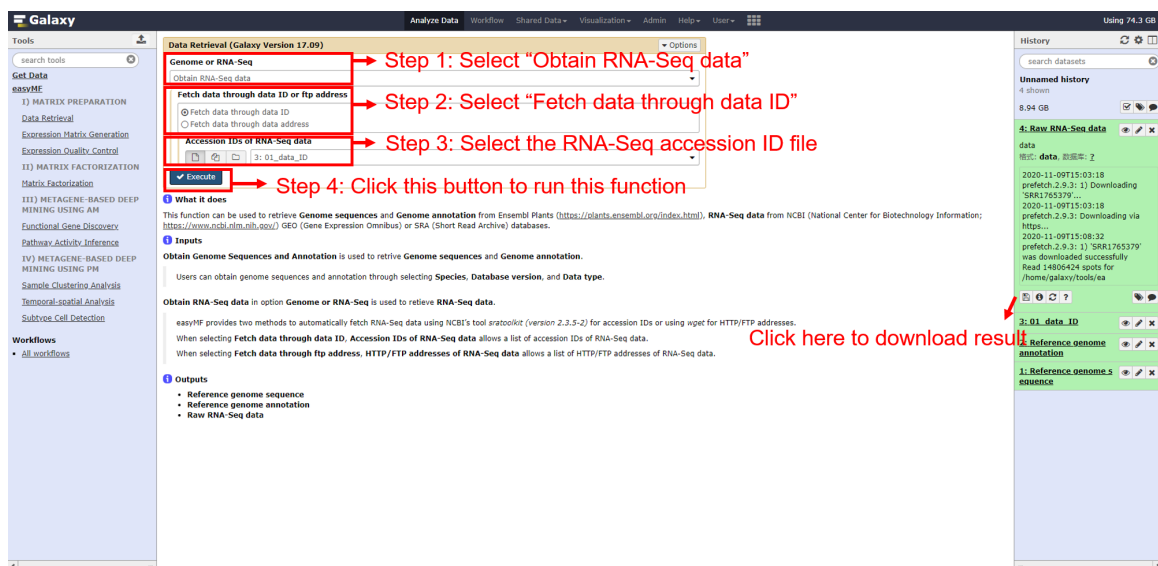

- The following screenshots show us how to download RNA-Seq data using easyMF.

  Step 1: download test data provided by easyMF;

  Step 2: upload test datum `01_data_ID` in directory `Test_data/01_Matrix_Preparation` to history panel;

Step 3: input the corresponding file, and run the function.

Running time

Running time for the test data depends on network speed.

2. Expression Matrix Generation

This function can be used to generate a gene expression matrix (genes in rows and individual samples in columns) through raw RNA-Seq quality control, read-genome alignment, and gene expression abundance calculation.

Inputs

In **Data** section

- **Reference genome sequence**: Reference genome sequence in FASTA format used for read-genome alignment.
- **Reference genome annotation**: Reference genome annotation in GTF format used to estimate gene expression abundance.
- **Raw RNA-Seq data**: A compressed file containing RNA-Seq data in tar.gz format.

In **Parameters** section, easyMF needs users set parameters used for "RNA-Seq quality control" and "Read-genome alignment".

For "RNA-Seq quality control"

- **Minimum read length**: A threshold of read length that reads shorter than the length will be discarded.
- **The quality value that a base is qualified**: A threshold of base quality value to trim low-quality reads.

For "Read-genome alignment"

- **Minimum intron length for RNA-Seq alignment**
- **Maximum intron length for RNA-Seq alignment**

Output

- **Raw Gene Expression Matrix**: A gene expression matrix (genes in rows and individual samples in columns).

How to use this function

- Test data for this function are in directory `Test_data/01_Matrix_Preparation` including `02_maize_reference_genome_chr10.fa`, `02_maize_reference_genome_chr10.gtf`, and `02_RNA-Seq_data.tar.gz`.

- The following screenshots show us how to generate a gene expression matrix using easyMF.

   Step 1: upload test data in directory `Test_data/01_Matrix_Preparation` to history panel;

   Step 2: input the corresponding files and appropriate parameters, then run the function.

Running time

This step will cost ~ 2 mins for the test data.

3. Expression Quality Control

Once gene expression matrix generated, to accurately implement MF-based analysis, quality of the gene expression matrix need to be improved, which can be operated through three different dimensions including **removing lowly expressed genes**, **removing outlier samples**, **removing batch effects**.

Inputs

For **Removing lowly expressed genes**

- **Expression value of expressed genes**: Expression value of genes regarded as expressed.
- **Minimum sample number**: The number of samples of expressed genes.

easyMF provides default values for these two parameters: **Expression value of expressed genes** (default as 1) and **Minimum sample number** (default as 3), which means genes regarded as expressed with expression value greater than 1 in at least 3 samples.

For **Removing outlier samples**

- **Threshold of potential repeat samples**: Expression values between two samples are almost identical.
- **Threshold of low-quality samples**: Sample distance between two RNA-Seq data.

For **Removing batch effects**

- **Sample information**: RNA-Seq samples with batch information. In the text, the first column presents sample IDs, and the second column presents batch information distinguished with Arabic numerals.

```
SRR1765379   1
SRR1765380   1
SRR1765337   2
SRR1765338   2
```

Output

- **High-quality gene expression matrix**: A high-quality gene expression matrix (genes in rows and individual samples in columns).

How to use this function

- Test data for this function are `03_sample_information` in `01_Matrix_Preparation` for **Sample information**, and **Raw gene expression matrix** generated by the function **Expression Matrix Generation**.

- The following screenshota show us how to generate a high-quality gene expression matrix using easyMF.

  Step 1: upload test datum in directory `Test_data/01_Matrix_Preparation` to history panel;
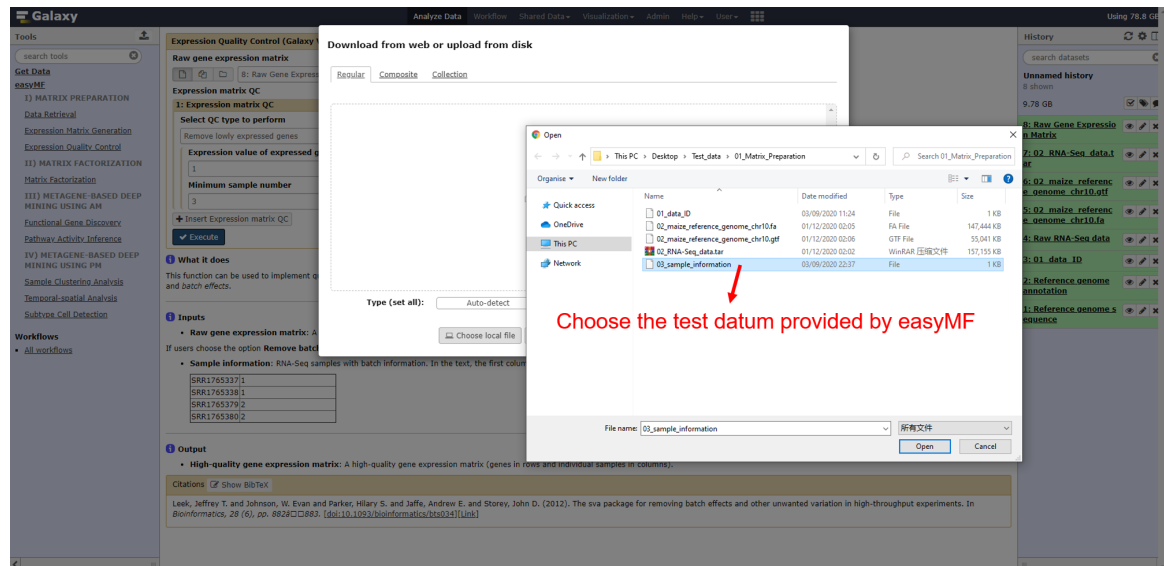
  Step 2: input the corresponding files and appropriate parameters, then run the function.
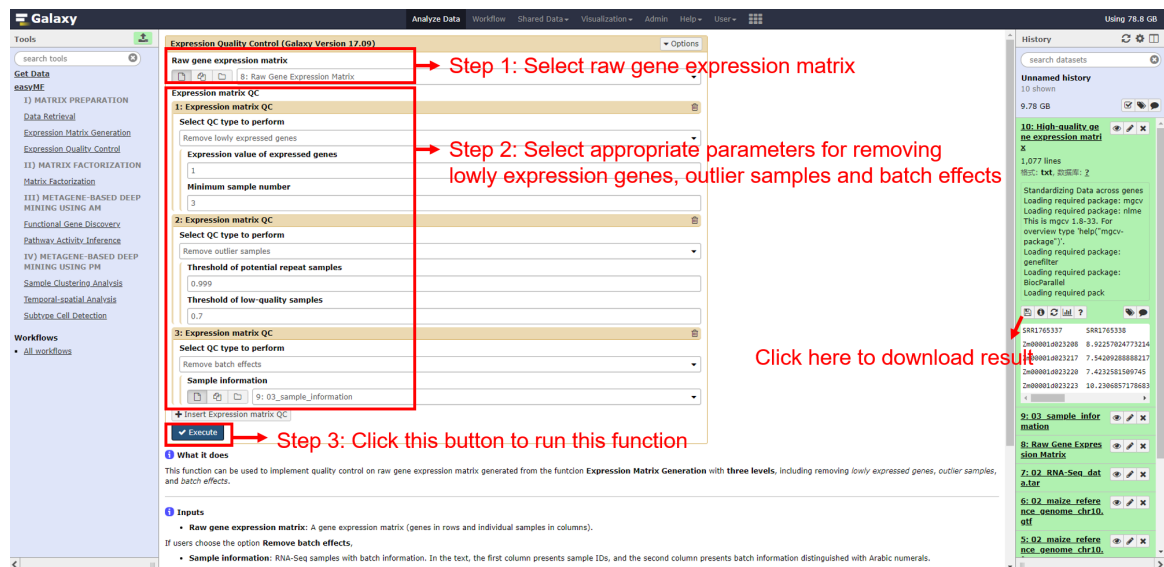
Running time

This step will cost ~ 10s for the test data.