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. Metagene-based Deep Mining Using AM

Amplitude matrix (AM), a matrix with genes in rows and metagenes in columns, describes gene-level relationships. In current version of easyMF, users can make use of AM for functional gene discovery and pathway activity inference.

This module consists of two functions: **Functional Gene Discovery**, and **Pathway Activity Inference**.

Functions/Tools	Description	Inputs	Outputs	Time (test data)	Program	References
Functional Gene Discovery	Calculate gene score and rank genes based on the probability of their association with a specific biology function	Amplitude matrix; A set of genes with a specific characteristic	Gene score and rank; Area under the self-ranked curve (AUSR) plot	~ 10s	In-house scripts	*Fehrmann et al.*, 2015
Pathway Activity Inference	Examine the pathway activity for any gene set of interest	Amplitude matrix; Pathway annotation; A set of genes with a specific characteristic	Actived pathways	~ 1 mins	In-house scripts	This study

1. Functional Gene Discovery

Functional gene discovery can be used to calculate gene score and rank genes based on the probability of their association with a specific biology function.

Inputs

- **Amplitude matrix**: An amplitude matrix of AM coefficients with genes in rows and metagenes in columns. Here is an example:

	Metagene 1	Metagene 2	Metagene 3	...	Metagene n
Zm00001d053636	0.080	-0.889	1.504	...	2.029
Zm00001d053632	1.338	0.729	-0.113	...	-0.049
...	...	...	...	...	...
Zm00001d053635	-1.674	0.036	-0.047	...	-0.494

- **Functional genes**: A set of genes associated with a specific biology function, such as enriched in a phenotype of interest. If users select **Upload a file with functional gene IDs from local disk**, a newline-delimited file containing gene IDs needs to be provided; if users select **Enter functional gene IDs**, gene IDs need to be separated by comma. Here are two examples:

A newline-delimited file containing gene IDs for **Upload a file with functional gene IDs from local disk**:

```
Zm00001d053636
Zm00001d053632
Zm00001d053630
...
Zm00001d053635
```

Comma-separated gene IDs for **Enter functional gene IDs**:

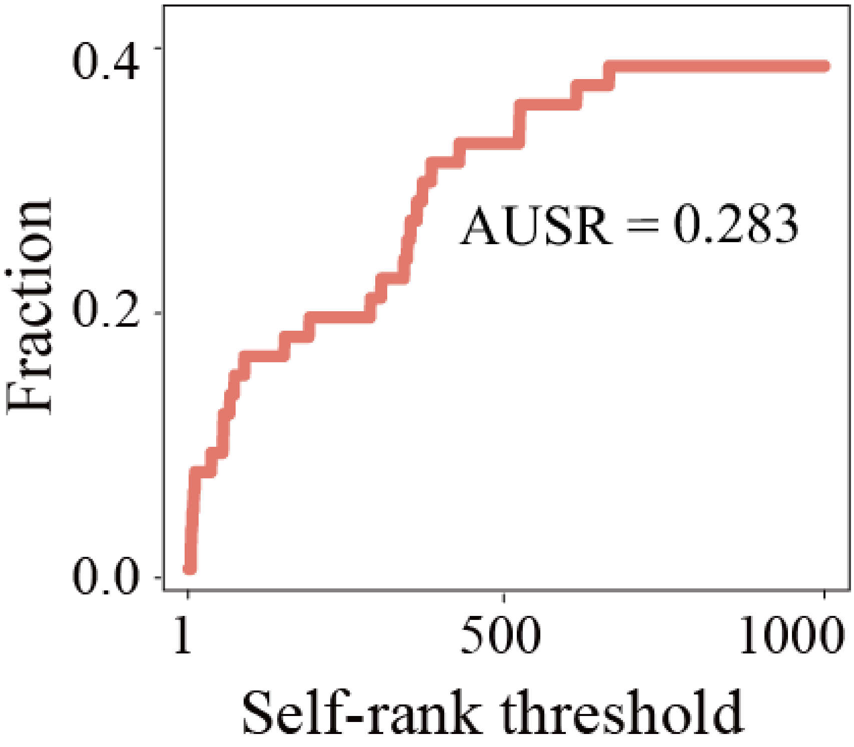
```
Zm00001d053636,Zm00001d053632,Zm00001d053630,...,Zm00001d053635
```

Outputs

- **Gene score and rank**: Summary of gene prioritization results containing **Gene ID**, **Score**, **Rank**, and **Annotation**. The higher ranking of a gene, the more related to the biological function. Here is an example:

Gene ID	Score	Rank	Annotation
Zm00001d053636	1	1	Label
Zm00001d053632	0.888	3	Label
Zm00001d004839	1	1	Unlabel
...	...	...	...
Zm00001d053635	0.92	2	Unlabel

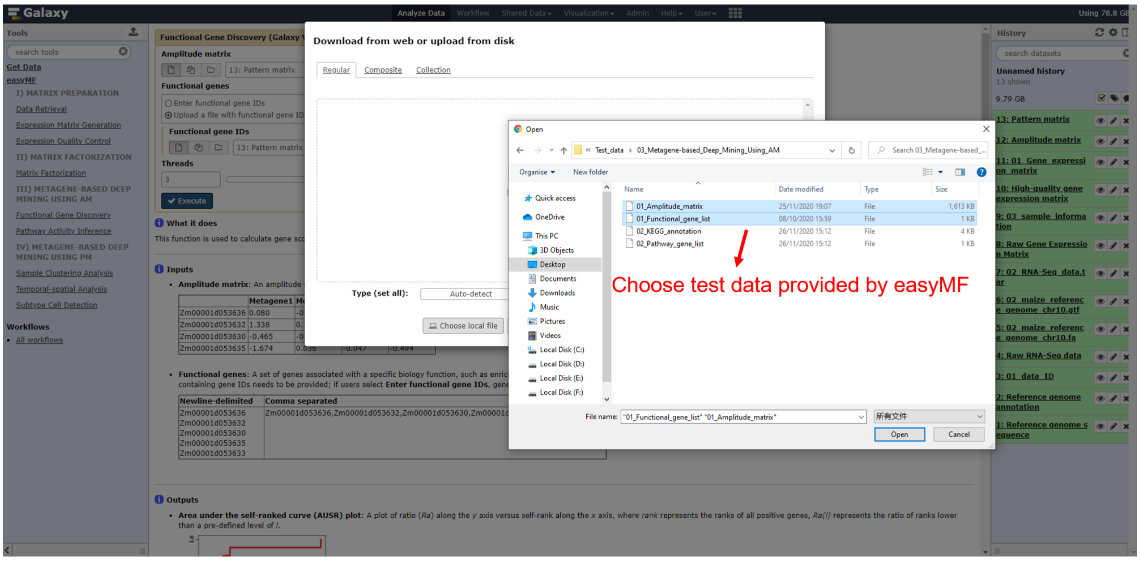
- **Area under the self-ranked curve (AUSR) plot**: A plot of ratio (*Ra*) along the *y* axis versus self-rank along the *x* axis, where rank represents the ranks of all positive genes, *Ra*(*l*) represents the ratio of ranks lower than a pre-defined level of *l*.



How to use this function

- Test data for this function are in directory `Test_data/03_Metagene-based_Deep_Mining_Using_AM` including.
- The following screenshots show us how to implement functional gene discovery using easyMF.

  Step 1: upload test data in directory `Test_data/03_Metagene-based_Deep_Mining_Using_AM` to history panel;



Functional Gene Discovery (Galaxy V

Amplitude matrix: 13: Pattern matrix

Functional genes

○ Enter functional gene IDs
● Upload a file with functional gene ID

Functional gene IDs: 13: Pattern matrix

Threads: 3

What it does

This function is used to calculate gene sc

Inputs

- **Amplitude matrix**: An amplitude

	Metagene1	Me		
Zm00001d053636	0.080	-0		
Zm00001d053632	1.338	0.		
Zm00001d053630	-0.465	-0		
Zm00001d053635	-1.674	0.036	-0.047	-0.494

- **Functional genes**: A set of genes associated with a specific biology function, such as enric containing gene IDs needs to be provided; if users select **Enter functional gene IDs**, gene

Newline-delimited	Comma separated
Zm00001d053636 Zm00001d053632 Zm00001d053630 Zm00001d053635 Zm00001d053633	Zm00001d053636,Zm00001d053632,Zm00001d053630,Zm00001d

Outputs

- **Area under the self-ranked curve (AUSR) plot**: A plot of ratio (*Ra*) along the *y* axis versus self-rank along the *x* axis, where *rank* represents the ranks of all positive genes, *Ra(l)* represents the ratio of ranks lower than a pre-defined level of *l*.

Download from web or upload from disk

Type (set all): Auto-detect

« Test_data › 03_Metagene-based_Deep_Mining_Using_AM

Search 03_Metagene-based_...

Name	Date modified	Type	Size
01_Amplitude_matrix	25/11/2020 19:07	File	1,613 KB
01_Functional_gene_list	08/10/2020 15:59	File	1 KB
02_KEGG_annotation	26/11/2020 15:12	File	4 KB
02_Pathway_gene_list	26/11/2020 15:12	File	1 KB

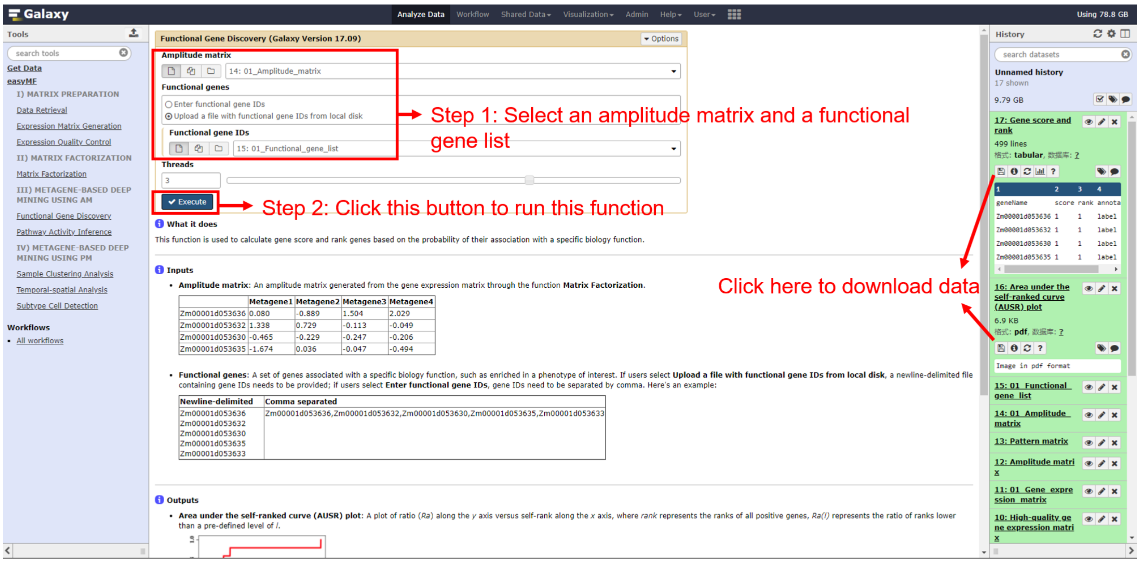
Choose test data provided by easyMF

File name: "01_Functional_gene_list" "01_Amplitude_matrix"   所有文件

Unnamed history
13 shown
9.79 GB

13: Pattern matrix
12: Amplitude matrix
11: 01_Gene_expression_matrix
10: High-quality gene expression matrix
9: 03_sample_information
8: Raw Gene Expression Matrix
7: 02_RNA-Seq_data.tar
6: 02_maize_reference_genome_chr10.gtf
5: 02_maize_reference_genome_chr10.fa
4: Raw RNA-Seq data
3: 01_data_ID
2: Reference genome annotation
1: Reference genome sequence

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



Functional Gene Discovery (Galaxy Version 17.09)

Amplitude matrix: 14: 01_Amplitude_matrix

Functional genes

○ Enter functional gene IDs
● Upload a file with functional gene IDs from local disk

Functional gene IDs: 15: 01_Functional_gene_list

Threads: 3

✓ Execute

Step 1: Select an amplitude matrix and a functional gene list

Step 2: Click this button to run this function

Click here to download data

What it does

This function is used to calculate gene score and rank genes based on the probability of their association with a specific biology function.

Inputs

- **Amplitude matrix**: An amplitude matrix generated from the gene expression matrix through the function **Matrix Factorization**.

	Metagene1	Metagene2	Metagene3	Metagene4
Zm00001d053636	0.080	-0.889	1.504	2.029
Zm00001d053632	1.338	0.729	-0.113	-0.049
Zm00001d053630	-0.465	-0.229	-0.247	-0.206
Zm00001d053635	-1.674	0.036	-0.047	-0.494

- **Functional genes**: A set of genes associated with a specific biology function, such as enriched in a phenotype of interest. If users select **Upload a file with functional gene IDs from local disk**, a newline-delimited file containing gene IDs needs to be provided; if users select **Enter functional gene IDs**, gene IDs need to be separated by comma. Here's an example:

Newline-delimited	Comma separated
Zm00001d053636 Zm00001d053632 Zm00001d053630 Zm00001d053635 Zm00001d053633	Zm00001d053636,Zm00001d053632,Zm00001d053630,Zm00001d053635,Zm00001d053633

Outputs

- **Area under the self-ranked curve (AUSR) plot**: A plot of ratio (*Ra*) along the *y* axis versus self-rank along the *x* axis, where *rank* represents the ranks of all positive genes, *Ra(l)* represents the ratio of ranks lower than a pre-defined level of *l*.

17: Gene score and rank
499 lines
格式: tabular, 数据库: ?

1	2	3	4
geneName	score	rank	annota
Zm00001d053636	1	1	label
Zm00001d053632	1	1	label
Zm00001d053630	1	1	label
Zm00001d053635	1	1	label

16: Area under the self-ranked curve (AUSR) plot
6.9 KB
格式: pdf, 数据库: ?
Image in pdf format

15: 01_Functional_gene_list

14: 01_Amplitude_matrix

13: Pattern matrix

12: Amplitude matrix

11: 01_Gene_expression_matrix

10: High-quality gene expression matrix

Running time

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

2. Pathway Activity Inference

Pathway activity inference can be used to examine the pathway activity for any gene set of interest.

Inputs

- **Amplitude matrix**: An amplitude matrix of AM coefficients with genes in rows and metagenes in columns. Here is an example:

	Metagene 1	Metagene 2	Metagene 3	…	Metagene n
Zm00001d053636	0.080	-0.889	1.504	…	2.029
Zm00001d053632	1.338	0.729	-0.113	…	-0.049
…	…	…	…	…	…
Zm00001d053635	-1.674	0.036	-0.047	…	-0.494

- **Pathway annotation**: A pathway annotation file, which contains **Gene ID**, **Pathway ID**, and **Pathway name** separated by a tab character. Here is an example:

Gene ID	Pathway ID	Pathway name
Zm00001d042869	zma00010	Glycolysis / Gluconeogenesis
Zm00001d025586	zma00010	Glycolysis / Gluconeogenesis
Zm00001d039089	zma00020	Citrate cycle (TCA cycle)
Zm00001d037278	zma00030	Pentose phosphate pathway

- **Gene set**: A list of genes used to estimate pathway activity.

Outputs

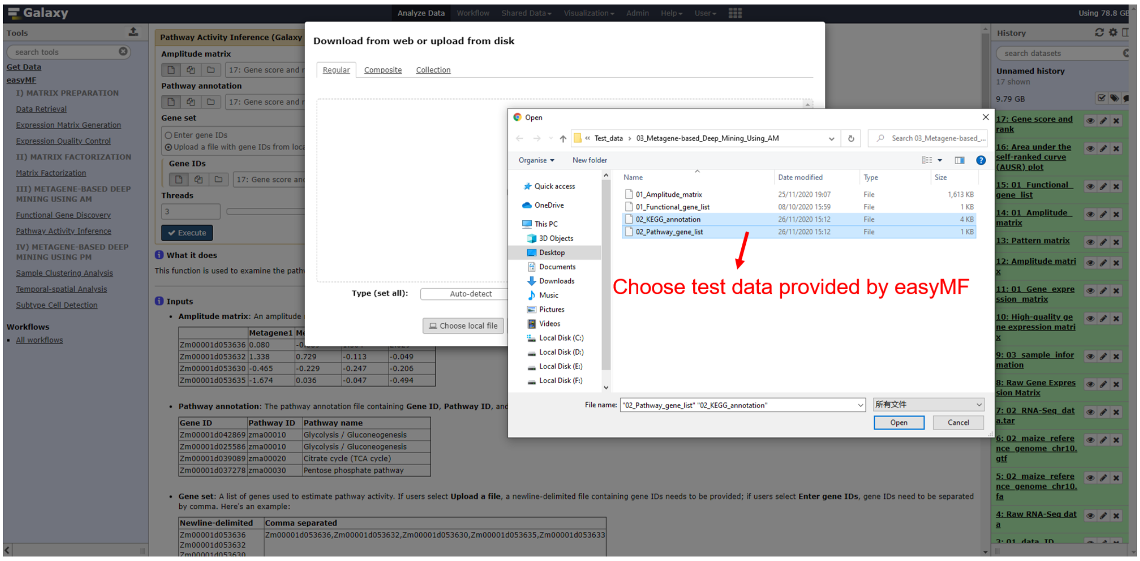
- **Pathway activity**: The activity of each pathway. Each column shows **Pathway**, ***P*-value**, **FDR**, **Term**, **Significant**, **Annotate** and AM coefficient in each metagene. In the result, active pathway can be obtained through a *p*-value filtration of each pathway information.

Pathway	P-value	FDR	Term	Significant	Annotate	Metagene1	Metagenen2
zma00592	0.077	0.977	alpha-Linolenic acid metabolism	3	33	0.001	0.022
zma04146	0.101	0.934	Peroxisome	2	67	0.016	0.035
zma00010	0.177	0.864	Glycolysis / Gluconeogenesis	2	102	0.031	0.112
zma00906	0.227	0.981	Carotenoid biosynthesis	2	27	0	0.012

How to use this function

- Test data for this function are in directory `Test_data/03_Metagene-based_Deep_Mining_Using_AM` including `01_Amplitude_matrix` and `01_Functional_gene_list`.
- The following screenshots show us how to examine the pathway activity using easyMF.

  Step 1: upload test data in directory `Test_data/03_Metagene-based_Deep_Mining_Using_AM` to history panel;



Pathway Activity Inference (Galaxy

Amplitude matrix: 17: Gene score and

Pathway annotation: 17: Gene score and

Gene set: ○ Enter gene IDs ● Upload a file with gene IDs from loca

Gene IDs: 17: Gene score and

Threads: 3

What it does

This function is used to examine the path

Inputs

- **Amplitude matrix**: An amplitude

	Metagene1	M		
Zm00001d053636	0.080	-0.		
Zm00001d053632	1.338	0.729	-0.113	-0.049
Zm00001d053630	-0.465	-0.229	-0.247	-0.206
Zm00001d053635	-1.674	0.036	-0.047	-0.494

- **Pathway annotation**: The pathway annotation file containing **Gene ID**, **Pathway ID**, an

Gene ID	Pathway ID	Pathway name
Zm00001d042869	zma00010	Glycolysis / Gluconeogenesis
Zm00001d025586	zma00010	Glycolysis / Gluconeogenesis
Zm00001d039089	zma00020	Citrate cycle (TCA cycle)
Zm00001d037278	zma00030	Pentose phosphate pathway

- **Gene set**: A list of genes used to estimate pathway activity. If users select **Upload a file**, a newline-delimited file containing gene IDs needs to be provided; if users select **Enter gene IDs**, gene IDs need to be separated by comma. Here's an example:

Newline-delimited	Comma separated
Zm00001d053636 Zm00001d053632 Zm00001d053630	Zm00001d053636,Zm00001d053632,Zm00001d053630,Zm00001d053635,Zm00001d053633

Download from web or upload from disk

Type (set all): Auto-detect

« Test_data › 03_Metagene-based_Deep_Mining_Using_AM

Search 03_Metagene-based_...

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01_Amplitude_matrix	25/11/2020 19:07	File	1,613 KB
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02_KEGG_annotation	26/11/2020 15:12	File	4 KB
02_Pathway_gene_list	26/11/2020 15:12	File	1 KB

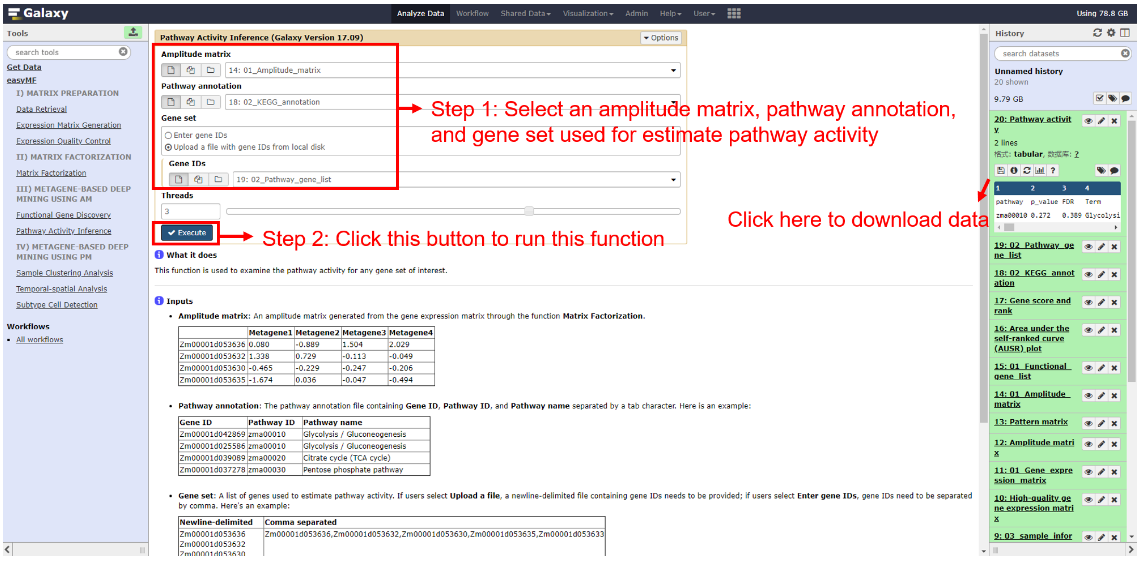
Choose test data provided by easyMF

File name: "02_Pathway_gene_list" "02_KEGG_annotation" 所有文件

Unnamed history, 17 shown, 9.79 GB

17: Gene score and rank; 16: Area under the self-ranked curve (AUSR) plot; 15: 01_Functional_gene_list; 14: 01_Amplitude_matrix; 13: Pattern matrix; 12: Amplitude matrix; 11: 01_Gene_expression_matrix; 10: High-quality gene expression matrix; 9: 03_sample_information; 8: Raw Gene Expression Matrix; 7: 02_RNA-Seq_data.tar; 6: 02_maize_reference_genome_chr10.gtf; 5: 02_maize_reference_genome_chr10.fa; 4: Raw RNA-Seq data; 3: 01_data_ID

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



Pathway Activity Inference (Galaxy Version 17.09)

Amplitude matrix: 14: 01_Amplitude_matrix

Pathway annotation: 18: 02_KEGG_annotation

Gene set

○ Enter gene IDs
● Upload a file with gene IDs from local disk

Gene IDs: 19: 02_Pathway_gene_list

Threads: 3

Step 1: Select an amplitude matrix, pathway annotation, and gene set used for estimate pathway activity

Step 2: Click this button to run this function

Click here to download data

```
1        2        3      4
pathway  p_value  FDR    Term
zma00010 0.272    0.389  Glycolysi
```

What it does

This function is used to examine the pathway activity for any gene set of interest.

Inputs

- **Amplitude matrix**: An amplitude matrix generated from the gene expression matrix through the function **Matrix Factorization**.

	Metagene1	Metagene2	Metagene3	Metagene4
Zm00001d053636	0.080	-0.889	1.504	2.029
Zm00001d053632	1.338	0.729	-0.113	-0.049
Zm00001d053630	-0.465	-0.229	-0.247	-0.206
Zm00001d053635	-1.674	0.036	-0.047	-0.494

- **Pathway annotation**: The pathway annotation file containing **Gene ID**, **Pathway ID**, and **Pathway name** separated by a tab character. Here is an example:

Gene ID	Pathway ID	Pathway name
Zm00001d042869	zma00010	Glycolysis / Gluconeogenesis
Zm00001d025586	zma00010	Glycolysis / Gluconeogenesis
Zm00001d039089	zma00020	Citrate cycle (TCA cycle)
Zm00001d037278	zma00030	Pentose phosphate pathway

- **Gene set**: A list of genes used to estimate pathway activity. If users select **Upload a file**, a newline-delimited file containing gene IDs needs to be provided; if users select **Enter gene IDs**, gene IDs need to be separated by comma. Here's an example:

Newline-delimited	Comma separated
Zm00001d053636 Zm00001d053632 Zm00001d053630	Zm00001d053636,Zm00001d053632,Zm00001d053630,Zm00001d053635,Zm00001d053633

Running time

This step will cost ~ 1 mins for the test data.