

A Tutorial for MetaOomics

Contents

1	Introduction	2
1.1	Abbreviation terms	2
1.2	Statistical method explanations	3
2	Preliminaries	3
2.1	Citing MetaOomics	3
2.2	How to start MetaOomics	4
2.2.1	Requirement	4
2.2.2	How to install the software	5
2.2.3	How to start the software	5
2.3	MetaOomics setting page	5
2.4	Question and bug report	6
3	Prepare data	6
3.1	Raw data	6
3.2	Clinical data	7
3.3	Example data in the MetaOomics software	7
4	MetaPreprocess	9
4.1	Preprocessing page	9
4.2	Saved Data page	10
5	Toolsets	12
5.1	MetaQC	12
5.1.1	Procedure	13
5.1.2	Results for MetaQC on AML example	14
5.1.3	Results for MetaQC on prostate cancer example	15
5.2	MetaDE	15
5.2.1	Procedure of differential expression analysis	16
5.2.2	Results of differential expression analysis	17
5.2.3	Procedure of downstream pathway analysis	18
5.2.4	Result of downstream pathway analysis	19
5.3	MetaPath	19
5.3.1	Procedure	19
5.3.2	Results	21
5.4	MetaNetwork	23
5.4.1	Procedure	24
5.4.2	Results	27
5.5	MetaPredict	29
5.5.1	Procedure	30
5.5.2	Results	32
5.6	MetaClust	32
5.6.1	Procedure	32
5.6.2	Results	35
5.7	MetaPCA	35

5.7.1	Procedure	35
5.7.2	Results	36
6	Complete list of options	36
6.1	MetaQC	37
6.2	MetaDE	37
6.3	MetaPath	38
6.4	MetaNetwork	38
6.5	MetaPredict	39
6.6	MetaClust	39
6.7	MetaPCA	40

1 Introduction

MetaOomics is a browser-based software suite for transcriptomic meta-analysis with R shiny-based graphical user interface (GUI). Many state-of-the-art meta-analysis tools are available in this software, including MetaQC for quality control, MetaDE for differential expression analysis, MetaPath for pathway enrichment analysis, MetaNetwork for differential co-expression network analysis, MetaPredict for classification analysis, MetaClust for sparse clustering analysis and MetaPCA for principal component analysis.

In this tutorial, we will go through the installation and usage of MetaOomics step by step using real data examples. The MetaOomics software suite is publicly available at <https://github.com/metaOomics/metaOomics>. This tutorial can be found at https://github.com/metaOomics/tutorial/blob/master/metaOomics_tutorial.pdf. Each MetaOomics module will be introduced in later sections and the associated R packages are available on GitHub at <https://github.com/metaOomics>.

1.1 Abbreviation terms

- General terms:
 - CV: Cross validation
 - DE: Differentially expressed
 - FDR: False discovery rate
 - FC: Fold change
 - FPKM: Fragments Per Kilobase Million mapped reads
 - QC: Quality control
 - RPKM: Read Per Kilobase Million mapped reads
 - TPM: Transcripts Per Kilobase Million mapped reads
- Methods or tools:
 - AW-Fisher: Adaptively weighted Fisher's method
 - CPI: Comparative pathway integrator
 - FEM: Fixed effects model
 - K-S test: Kolmogorov-Smirnov test
 - MAPE: Meta analysis pathway enrichment method
 - PCA: Principal component analysis
 - REM: Random effects model
 - SMR: Standardized mean ranks
 - TSP: Top scoring pair algorithm

1.2 Statistical method explanations

- Think about including a table to briefly introduce (like what we did in the Supplementary information) the statistical terms such as KNN algorithm, Fisher's exact test, KS test, FDR, consensus clustering, simulated annealing algorithm, etc. (ask George if necessary)
- Ask the users to refer to our MetaOmics paper (Supplementary information Box S2) for detailed explanation/interpretation of statistical learning approaches/terms, e.g. DE analysis, pathway analysis, differential co-expression network analysis, etc.

2 Preliminaries

2.1 Citing MetaOmics

MetaOmics software suite implements many meta-analysis methods from different authors. Please cite appropriate papers if you use MetaOmics, by which the authors will receive professional credits for their work.

- MetaOmics software suite itself can be cited as:
 - Ma, T., Huo, Z., Kuo, A., Zhu, L., Fang, Z., Zeng, X., Lin, C.-W., Liu, S., Wang, L., Rahman, T., Chang, L.-C., Kim, S., Li, J., Park, Y., Song, C., Oesterreich, S., Sibille, E. and Tseng, G. C. MetaOmics: Comprehensive Analysis Pipeline and Browser-based Software Suite for Transcriptomic Meta-Analysis.
- Review, comparative papers and published R packages:
 - Tseng, G. C., Ghosh, D., and Feingold, E. (2012). Comprehensive literature review and statistical considerations for microarray meta-analysis. *Nucleic acids research*, 40(9):3785–3799.
 - Chang, L.-C., Lin, H.-M., Sibille, E., and Tseng, G. C. (2013). Meta-analysis methods for combining multiple expression profiles: comparisons, statistical characterization and an application guideline. *BMC bioinformatics*, 14(1):368.
 - Wang, X., Kang, D. D., Shen, K., Song, C., Lu, S., Chang, L.-C., Liao, S. G., Huo, Z., Tang, S., Ding, Y., Kaminski, N., Sibille, E., Lin, Y., Li, J., and Tseng, G. C. (2012). An r package suite for microarray meta-analysis in quality control, differentially expressed gene analysis and pathway enrichment detection. *Bioinformatics*, 28(19):2534–2536.
- MetaQC:
 - (MetaQC) Kang, D. D., Sibille, E., Kaminski, N., and Tseng, G. C. (2012). Metaqc: objective quality control and inclusion/exclusion criteria for genomic meta-analysis. *Nucleic acids research*, 40(2):e15–e15.
- MetaDE:
 - (Fisher) Fisher, R. A. (1925). *Statistical methods for research workers*. Genesis Publishing Pvt Ltd.
 - (AW-Fisher) Li, J. and Tseng, G. C. (2011). An adaptively weighted statistic for detecting differential gene expression when combining multiple transcriptomic studies. *The Annals of Applied Statistics*, 5(2A):994–1019.
 - (AW-Fisher) Huo, Z., Tang, S., Park, Y., and Tseng, G. (2017). P-value evaluation, variability index and biomarker categorization for adaptively weighted fisher's meta-analysis method in omics applications. *arXiv preprint arXiv:1708.05084*.
 - (REM/FEM) Choi, J. K., Yu, U., Kim, S., and Yoo, O. J. (2003). Combining multiple microarray studies and modeling interstudy variation. *Bioinformatics*, 19(suppl 1):i84–i90.
 - (rOP) Song, C. and Tseng, G. C. (2014). Hypothesis setting and order statistic for robust genomic meta-analysis. *The annals of applied statistics*, 8(2):777.

- (minMCC) Lu, S., Li, J., Song, C., Shen, K., and Tseng, G. C. (2009). Biomarker detection in the integration of multiple multi-class genomic studies. *Bioinformatics*, 26(3):333–340.
- (Stouffer) Stouffer, S. A., Suchman, E. A., DeVinney, L. C., Star, S. A., and Williams Jr, R. M. (1949). The american soldier: adjustment during army life.(studies in social psychology in world war ii, vol. 1.)
- (RankProd) Hong, F., Breitling, R., McEntee, C. W., Wittner, B. S., Nemhauser, J. L., and Chory, J. (2006). Rankprod: a bioconductor package for detecting differentially expressed genes in meta-analysis. *Bioinformatics*, 22(22):2825–2827
- MetaPath:
 - (MAPE) Shen, K. and Tseng, G. C. (2010). Meta-analysis for pathway enrichment analysis when combining multiple genomic studies. *Bioinformatics*, 26(10):1316–1323.
 - (CPI) Fang, Z., Zeng, X., Lin, C.-W., Ma, T., and Tseng, G. C. (2017). Comparative pathway integrator: a framework of meta-analytic integration of multiple transcriptomic studies for consensual and differential pathway analysis. *In preparation*.
- MetaNetwork:
 - (MetaDCN) Zhu, L., Ding, Y., Chen, C.-Y., Wang, L., Huo, Z., Kim, S., Sotiriou, C., Oesterreich, S., and Tseng, G. C. (2016). Metadcn: meta-analysis framework for differential co-expression network detection with an application in breast cancer. *Bioinformatics*, 33(8):1121–1129.
- MetaPredict:
 - (MetaKTSP) Kim, S., Lin, C.-W., and Tseng, G. C. (2016). MetaKTSP: A Meta-Analytic Top Scoring Pair Method for Robust Cross-Study Validation of Omics Prediction Analysis. *Bioinformatics*, 32(13):1966–73.
- MetaClust:
 - (MetaSparseKmeans) Huo, Z., Ding, Y., Liu, S., Oesterreich, S., and Tseng, G. (2016). Meta-analytic framework for sparse k-means to identify disease subtypes in multiple transcriptomic studies. *Journal of the American Statistical Association*, 111(513):27–42.
- MetaPCA:
 - (MetaPCA) Kim, S., Kang, D., Huo, Z., Park, Y., and Tseng, G. C. (2017). Meta-analytic principal component analysis in integrative omics application. *Bioinformatics*, 1:8.

2.2 How to start MetaOmics

The full instruction of how to install and start MetaOmics software suite is also available at <https://github.com/metaOmics/metaOmics>.

2.2.1 Requirement

- R >= 3.3.1
- Shiny >= 0.13.2

Note:

- We recommend users to use R 3.3 to implement our tool. If you are using R 3.4 (released on 4/24/2017), you may encounter errors in installing dependencies of the modules. You can manually install the dependencies by running the following commands in R:

```
install.packages(c('GSA', 'combinat', 'samr', 'survival', 'cluster', 'gplots', 'ggplot2', 'irr', 'shape', 'snow',
'snowfall', 'igraph', 'doMC', 'PMA'))
source('https://bioconductor.org/biocLite.R')
biocLite(c('multtest', 'Biobase', 'edgeR', 'DESeq2', 'impute', 'limma', 'AnnotationDbi', 'Consensus-
ClusterPlus', 'genefilter', 'GSEABase', 'Rgraphviz'))
```

- For Windows, users need to run the following command in R to install the package 'doMC':

```
install.packages('doMC', repos='http://R-Forge.R-project.org')
```

2.2.2 How to install the software

- At MetaOmics home page <https://github.com/metaOmics/metaOmics>, clone the project by clicking on “Clone or download” and extract to a working directory, or type in the following in command line:

```
git clone https://github.com/metaOmics/metaOmics
```

2.2.3 How to start the software

- Run from docker image.

1. Install docker (if not installed).

2. In terminal

```
docker pull metaomics/app
```

```
docker run -rm -name metaOmics -p 3838:3838 metaomics/app
```

- Run from R.

1. Open R

2. Set the working directory such that metaOmics folder included.

```
install.packages('shiny')
```

```
shiny::runApp('metaOmics', port=9987, launch.browser=T)
```

2.3 MetaOmics setting page

After starting MetaOmics, the first page is the MetaOmics setting page as shown in Figure 1. There are four tabs on the top of this page (See Figure 1 (1)), which will direct users to specific functional modules of the software including Setting, Preprocessing, Saved Data and Toolsets. Below these tabs is a **Welcome to MetaOmics** section, which briefly introduces the software and other information about the authors and maintainers. Further below, there are two sections: Session Information and Directory for Saving Output Files (See Figure 1 (2)). By clicking “...”, users can set working directory, in which all the meta-analysis results will be saved. The current working directory is displayed on the top right corner (See Figure 1 (3)). There is one more section with the header Toolsets (See Figure 1 (4)), where users can click the blue button to install the desired modules if the “Status” shows “not installed”. If the packages are already installed, an icon of “checked installed” will show up in “Status”. The installation progress may take up to a few minutes for each module. A notification icon will pop up at the bottom right corner upon finishing the installation process. After the modules are installed, restart the MetaOmics software suite so that the Shiny application interface is updated with the installed modules. (See Figure 1 (5)) shows the current active dataset, which is introduced in Section 4.1 **Step 2**.

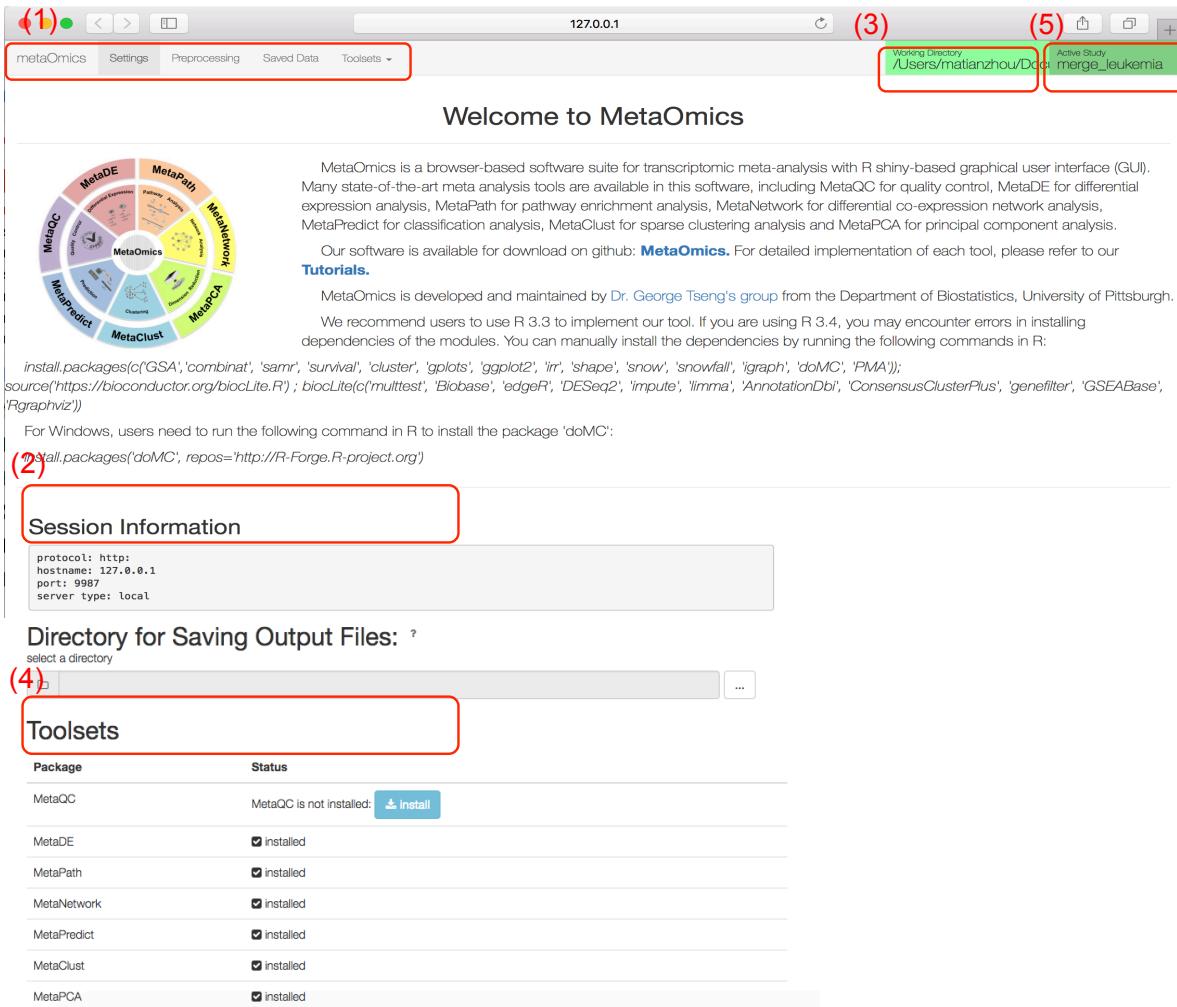


Figure 1: MetaOmics software suite GUI setting page

2.4 Question and bug report

If you encounter errors or bugs, please report to maintainer Tianzhou Ma <tim28@pitt.edu>.

3 Prepare data

In this section, we will introduce how to prepare gene expression data matrix as well as the clinical data files for the MetaOmics software suite.

3.1 Raw data

Gene expression matrix should be prepared as tab-delimited “.txt” or comma-separated “.csv” files, (See an example in Figure 2). The first column corresponds to the feature ID (e.g. gene symbol, probe id or entrez ID) and the rest of columns are the expression data from all the samples. The first row contains the sample ID. Valid data types include microarray data, RNA-seq FPKM/RPKM data, RNA-seq count data.

	A	B	C	D	E	F	G	H	I	J	K
1	ID	GSM445939	GSM445940	GSM445952	GSM445965	GSM445966	GSM445995	GSM446005	GSM446015	GSM446019	GSM446020
2	COX1	14.1741845	14.5190482	13.8179896	14.1805909	14.7791613	14.3450467	14.68766	14.7869009	14.7574207	14.1582959
3	COX2	13.8544454	14.1854915	13.4474018	13.6646626	14.4244321	13.9044761	14.2370772	13.9931093	14.0432901	13.4166744
4	ND4	13.840222	14.4856644	13.5612402	13.8816752	14.5739527	14.1081131	14.5813899	14.2519264	14.2616291	13.8095574
5	RPL41	14.4218804	13.4484882	14.1035968	14.1046225	14.2929066	13.9955247	14.1029454	14.5718506	14.5623457	14.0077579
6	RPS2	14.1384864	13.3737668	13.8091098	13.8294958	13.897014	13.7186942	13.9696975	14.2643786	14.135146	13.7457779
7	RPL23A	13.9851543	13.0577958	13.807726	13.7652435	13.5068014	13.4619198	13.6286114	14.0471201	13.8060203	13.5260356
8	TPT1	14.2015622	13.4487804	13.8933327	13.9124043	14.197062	14.0453267	14.2141676	14.4791302	14.5081582	13.8800374
9	RPL39	14.1331827	13.1026579	13.6928306	13.8217088	14.1705206	13.8267709	14.069521	14.3923098	14.3014678	13.7313433
10	ND2	11.8044506	14.1266472	12.3268843	13.3365085	14.1230073	13.8853862	14.2394535	13.835649	13.6857053	13.4025025

Figure 2: A example input data format

3.2 Clinical data

Clinical data should be prepared as the example in Figure 3. The first column corresponds to the sample ID and the rest of columns contain the clinical information of the samples (e.g. case/control labels). Sample IDs of the clinical data (on rows) should be ordered in the same way as the gene expression data (on columns) to avoid any mismatch issues.

3.3 Example data in the MetaOmics software

We collected three multi-cohort example datasets for the MetaOmics software, including an acute myeloid leukemia (AML) example, a breast cancer (BRCA) example and a prostate cancer example. Table 1 summarizes the first example dataset including three studies on acute myeloid leukemia (AML). Table 2 summarizes the second example dataset including four studies on breast cancer, where the first study contains both count data and FPKM data (continuous). Table 3 summarizes the example dataset including eight studies on prostate cancer. The leukemia data is used to demonstrate MetaPreprocess and all the seven analytical modules (MetaQC, MetaDE, MetaPath, MetaNetwork, MetaPredict, MetaClust and MetaPCA). Considering that all the three studies in leukemia data are of high quality, we further run MetaQC on the prostate cancer data with eight studies to show its capability to identify low quality studies.

Table 1: Multi-study acute myeloid leukemia (AML) gene expression profiles. All three studies are from Affymetrix Human Genome U133plus2 with 5,135 genes. Three subtypes of leukemia are defined as the chromosomal translocation, including inversion of chromosome 16 - inv(16), translocation of chromosome 15 and 17 - t(15;17) and translocation of chromosome 8 and 21 - t(8;21).

Study	source	# samples	# samples by subtypes inv(16)/t(15;17)/t(8;21)
Study 1	Verhaak et al. (2009)	89	33/21/35
Study 2	Balgobind et al. (2010)	74	27/19/28
Study 3	Kohlmann et al. (2008)	105	28/37/40

Table 2: Multi-study breast cancer gene expression profiles. Each gene expression profiles of all four studies contain 10,330 genes. Study 1 contains both count data and FPKM (continuous) data so user should **select only one of them**. The other three studies contain only continuous data. The phenotype of interest is estrogen-receptor (comparing ER+ vs ER-).

Study	source	scale	# samples	# samples by ER ER+/ER-
Study 1	Weinstein et al. (2013)	count	406	319/87
		continuous		
Study 2	Desmedt et al. (2007)	continuous	198	134/64
Study 3	Wang et al. (2005)	continuous	286	209/77
Study 4	Ivshina et al. (2006)	continuous	245	211/34

Table 3: Multi-study prostate cancer dataset information. Eight prostate cancer gene expression profiles were measured by different microarray platforms.

study	source	# platform	# samples	# samples by label Normal/Primary	# genes
Study 1	Welsh et al. (2001)	HG-U95A	34	9/25	8798
Study 2	Yu et al. (2004)	HG-U95Av2	146	81/65	8799
Study 3	Lapointe et al. (2004)	cDNA	103	41/62	13579
Study 4	Varambally et al. (2005)	HG-U133 Plus 2	13	6/7	19738
Study 5	Singh et al. (2002)	HG-U95Av2	102	50/52	8799
Study 6	Wallace et al. (2008)	HG-U133A2	89	20/69	12689
Study 7	Nanni et al. (2006)	HG-U133A	30	7/23	12689
Study 8	Tomlins et al. (2006)	cDNA	57	27/30	9703

4 MetaPreprocess

In this section, we introduce how to upload the datasets into the MetaOomics software suite, which is the first step in order to run any of the seven analytical modules. The R package for MetaPreprocess module can be found at <https://github.com/metaOmics/preproc>.

4.1 Preprocessing page

Step 1 Uploading data:

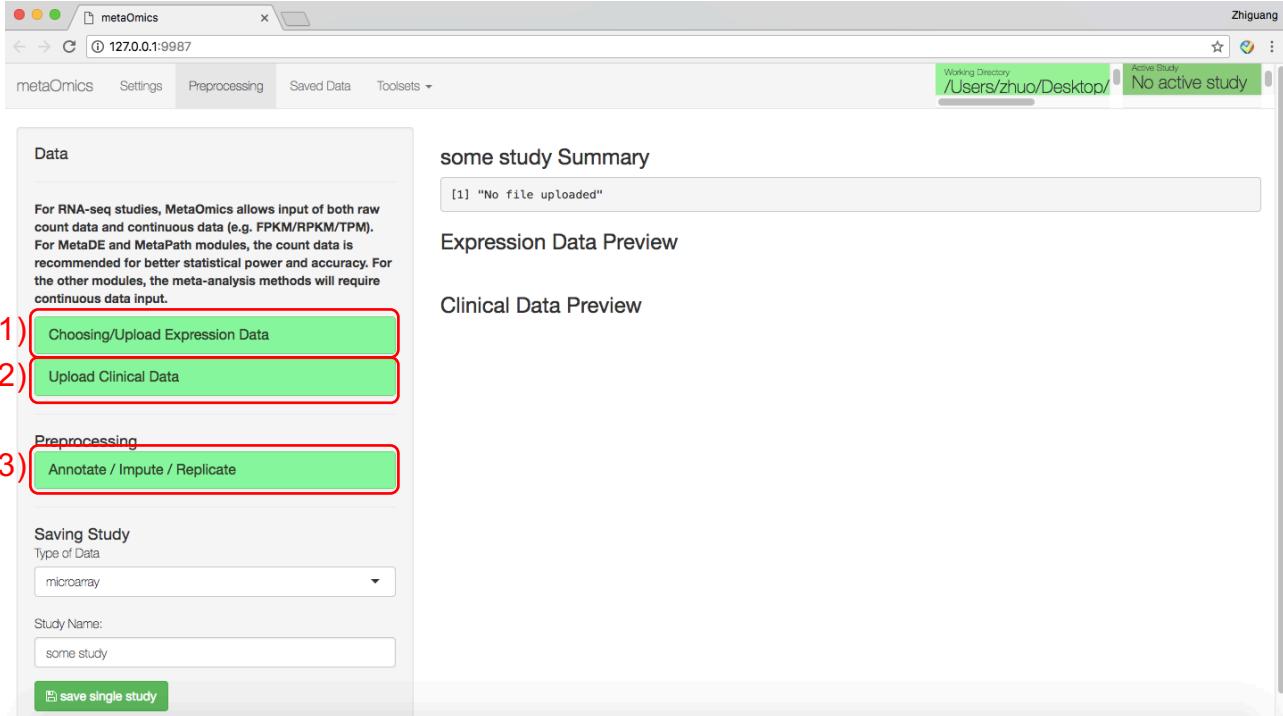


Figure 4: GUI Preprocessing page

In the Preprocessing page, by clicking on the “Choosing/Upload Expression Data” tab, users can upload individual expression data files (i.e., data file from each study) or choose the existing saved data files as in Figure 4 (1). The data should be prepared according to Section 3. Users may optionally upload Clinical Data (See Figure 4 (2)), depending on their biological purposes. Note that all MetaOomics modules require external clinical labels except for MetaClust module. The three example datasets are available under MetaOomics folder “/metaOomics/data/example/”, and we will mainly focus on the AML dataset (“/metaOomics/data/example/leukemia”) throughout this tutorial. By clicking “save single study”, the gene expression profile will be uploaded and the data can be previewed on the right side of the page.

Step 2 Preprocessing:

The MetaOomics software suite also provides handlers (See Figure 4 (3)) for feature annotation, missing value imputation and multiple probes reduction for the same gene. For preprocessing, click on “Annotate/Impute/Replicate” to

1. Annotate the probe ID/reference sequence ID/Entrez ID of individual dataset (choose Gene Symbol if the input data rows are already annotated).
2. Impute missing value using k-nearest neighbors (KNN) algorithm. Note that missing value will be automatically detected. If there is no missing value, this function will be disabled.

- Handle the multiple probes matching to the same gene issue. If each input feature represents a unique gene symbol, this function will be disabled.

A complete introduction of these options is available at the end of this subsection. The right side of Figure 5 will show the summary statistics of uploaded data and preview of the data matrix. There is a search box where users can search for their genes of interest.

The screenshot shows the metaOmics web interface. On the left, there's a sidebar with sections for 'Data' (instructions for RNA-seq studies), 'Choosing/Upload Expression Data' (with a 'Browse...' button for 'study1.csv'), 'Upload Clinical Data', 'Preprocessing' (with 'Annotate / Impute / Replicate'), and 'Saving Study' (with dropdowns for 'Type of Data' set to 'microarray' and 'Study Name' set to 'study1.csv', plus a 'save single study' button). The main area is titled 'study1.csv Summary' and displays statistical summary tables for four samples (GSM158714, GSM158725, GSM158742, GSM158791) across various metrics (Min., 1st Qu., Median, Mean, 3rd Qu., Max.). Below this is the 'Expression Data Preview' section, which shows a table of expression values for 10 samples (COX1, COX2, ND4, RPL41, RPS2, RPL23A, TPT1, RPL39, ND2, RPS18) across 12 genes (GSM158714, GSM158725, GSM158742, GSM158791, GSM158803, GSM158830, GSM158839, GSM158877). A 'Clinical Data Preview' section is also visible at the bottom. A success message 'Study study1.csv saved.' is shown in the bottom right corner.

Figure 5: Uploading individual studies

Step 3 Save single study: In the next step, specify the data type (microarray data; RNA-seq FPKM/RPKM data; RNA-seq count data) and study name, click “save single study”. To upload RNA-seq data, the count data and FPKM/TPM data should be uploaded separately and saved using different names.

Step 4 Upload datasets for all studies: Repeat the steps above for all studies in the meta-analysis. All uploaded studies are now available in the “Saved Data” page.

4.2 Saved Data page

After uploading multiple studies with or without clinical data, users can turn to the Saved Data page.

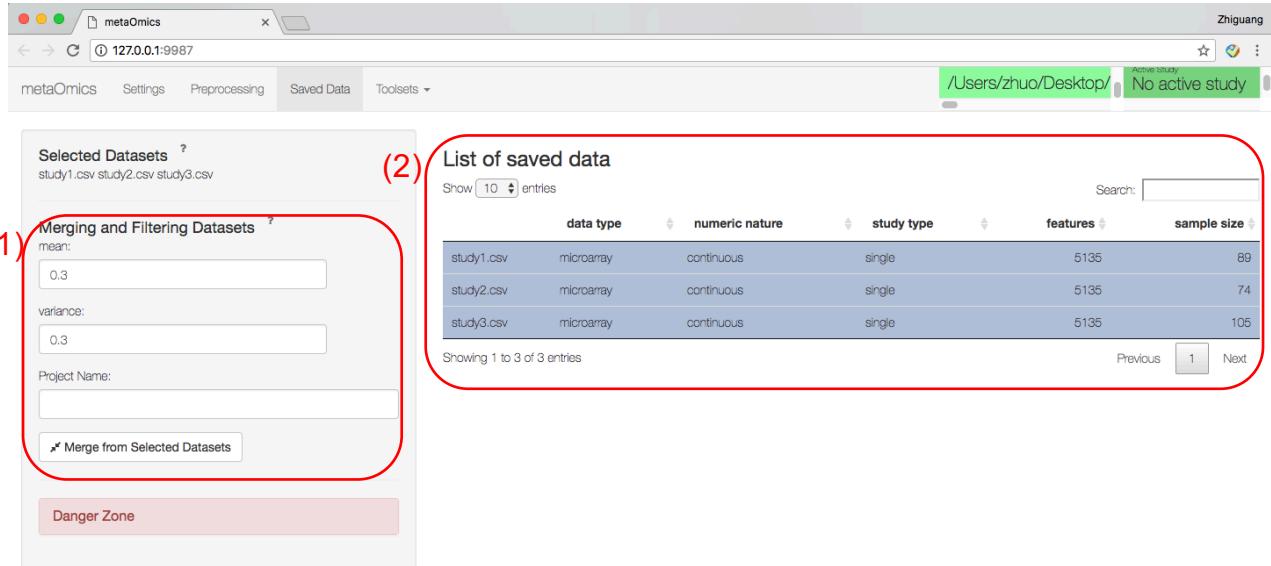


Figure 6: Merge from selected datasets

Step 1 Merging and Filtering: All saved datasets from the previous step will be found in Figure 6 (2). Users should select multiple datasets for further meta-analysis. Users can filter out genes with low expression level (by default, mean expression lower than 30th percentile) or low variance (by default, variance lower than 30th percentile). After specifying filtering criteria, enter “Project Name” and click on the “Merge from Selected Datasets” (See Figure 6 (1)). A merged dataset (study type = “multiple”) will appear on the “List of saved data” panel (Figure 6 (2)). Creating multiple projects with varying preprocessing criteria is useful. For example, the user can start from a project with harsh filtering criteria (maintain 500-1000 genes) and give a test run through all modules to save time. If successful, a larger project can be created by including more genes. If users want to delete any dataset, they can click on the red “danger zone” button and delete the selected datasets.

Step 2 Make active dataset: The last thing to do before using any of the meta-analytic modules is to select the merged data and click on “Make your dataset Active Dataset” – a big green button in Figure 7. Then the merged data will become the active study that shows up on the top right corner of the page. The active dataset serves as the input for all the analytical modules in MetaOmics.

The screenshot shows the metaOmics software interface. At the top, there are tabs for 'metaOmics', 'Settings', 'Preprocessing', 'Saved Data', and 'Toolsets'. The 'Saved Data' tab is active. In the top right corner, it says 'Zhiguang' and shows the path '/Users/zhuo/Desktop/'. Below the tabs, there's a 'Selected Datasets' section with 'merge05' listed. A 'Merging and Filtering Datasets' section below it says 'You need to select more than one dataset'. A red 'Danger Zone' button is visible. The main area is titled 'List of saved data' and contains a table with the following data:

	data type	numeric nature	study type	features	sample size
study1.csv	microarray	continuous	single	5135	89
study2.csv	microarray	continuous	single	5135	74
study3.csv	microarray	continuous	single	5135	105
merge05	continuous	continuous	multiple	1283	268

At the bottom of the table area, it says 'Showing 1 to 4 of 4 entries'. Below the table, there are buttons for 'Previous', '1', and 'Next'. A large green button at the bottom of the page says 'Make merge05 Active Dataset'.

Figure 7: Make merged Dataset Active

5 Toolsets

All the preparatory steps are done after running the MetaPreprocess module. It's time to apply the seven analytical modules in MetaOmics by clicking on the "Toolsets" tab and select the tool for your research question. In the next few subsections, we will introduce in details how to run each of these modules. For each module, a summary table of studies and sample sizes is shown on the top left corner. There is an "about" drop-down menu which contains a brief introduction and tutorial of the module. The "options" drop-down menu contain common options users can select or tune in the analysis. The "advanced options" menu contains more technical options which we generally do not recommend users to change unless they are familiar with the methods. After applying the modules, all result files will be automatically saved in the working directory which is specified in Section 2.3. For computationally demanding methods, the procedure may take minutes or up to hours depending on size of datasets. Users can keep track of the progress by checking the R console.

5.1 MetaQC

MetaQC package provides an objective and quantitative tool to help determine the inclusion/exclusion of studies for meta-analysis. More specifically, MetaQC provides users with six quantitative quality control (QC) measures: including IQC, EQC, AQCg, CQCg, AQCP and CQCP. Details of how each measure is defined and computed can be found in Kang et al. (2012). In addition, visualization plots and summarization tables are generated using principal component analysis (PCA) biplots and standardized mean ranks (SMR) to assist in visualization and decision. "MetaQC" package itself can be downloaded at (<https://github.com/metaOmics/MetaQC>).

5.1.1 Procedure



Figure 8: “MetaQC” options

There are three main options available for the “MetaQC” package as shown in Figure 8. The complete list of MetaQC options are available in Section 6.1.

Step 1 Options: Under the drop-down menu “options” (Figure 8 (2)), users can

- perform gene filtering. Gene filtering is suggested to reduce computational cost. Once “Yes” is chosen for gene filtering, users are further asked to specify the filtering cutoffs by mean or by variance. In the demo example, the merged data have already had gene filtering in the merging step, so no further filtering is performed.
- specify the approach (either by raw p-value or adjusted p-value) and cutoff to select potentially DE genes needed in the computation of IQC, EQC, AQCg and CQCg.
- specify the approach (either by raw p-value or adjusted p-value) and cutoff to select potentially enriched pathways needed in the computation of AQCp and CQCp.

Step 2 Advanced options:

Under the drop-down menu “Advanced Options” ((3) in Figure 8), users are allowed to tune other parameters of MetaQC. In particular, it includes the selection of pathways by pathway size and the number of permutations to run to obtain the six measures. A detailed list of all options available for the package can be found at the end of the section. However, this is advanced options and users are suggested not to modify the option setting in this section without knowing the method.

Step 3 Run MetaQC Analysis:

Once all the above options are specified, users can click on “Run MetaQC Analysis” (Figure 8 (4)) to implement the tool.

5.1.2 Results for MetaQC on AML example

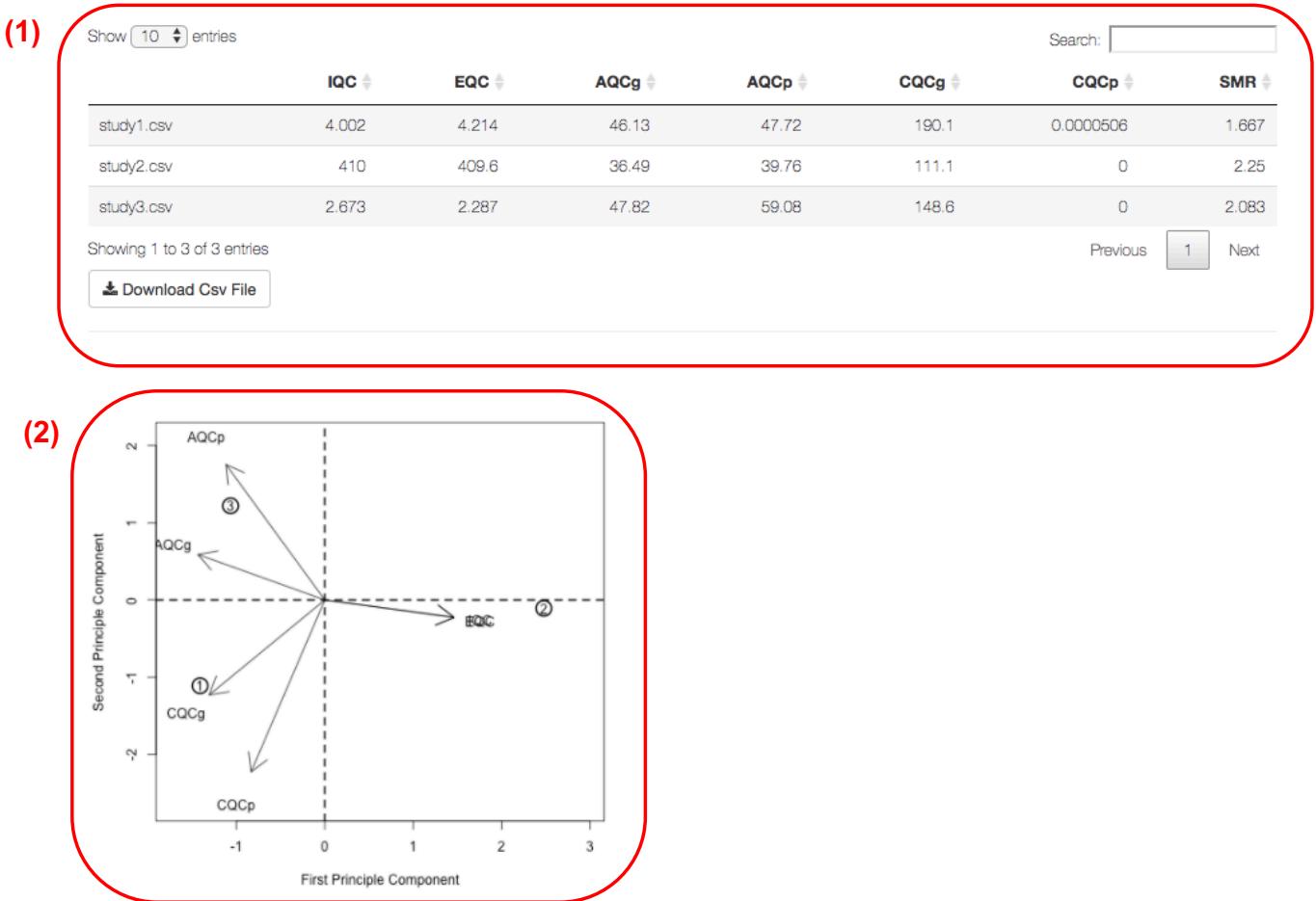


Figure 9: “MetaQC” Results for AML example

The performance of “MetaQC” in the AML example is shown in Figure 9. Detailed descriptions of these studies can be found in Table 1. As shown in Figure 9, MetaQC module generates a summary table of MetaQC results (9 (1)) as well as a PCA biplot (9 (2)). The table includes seven columns, with the first six columns corresponding to the six quantitative quality control measures of all studies (a larger value indicates a better quality) and the seventh column is the rank summary statistics of all the six quality measures (a lower rank indicates a better quality). Users can download the full table as a csv file by clicking on “Download Csv File”. In addition to the tabular results, “MetaQC” also generated a PCA biplot based on the six quality control measures, where the circled number is the study index and arrows indicate different measures. Generally, studies with larger SMR values, and studies more off from the other studies and a majority of the measures are considered lower quality. Since the AML example has very good data quality, we won’t be able to demonstrate how to use MetaQC to identify studies of low quality. Therefore, we further performed “MetaQC” on the prostate cancer example, where we will give suggestions on excluding studies with low quality or incompatibility with other studies.

5.1.3 Results for MetaQC on prostate cancer example

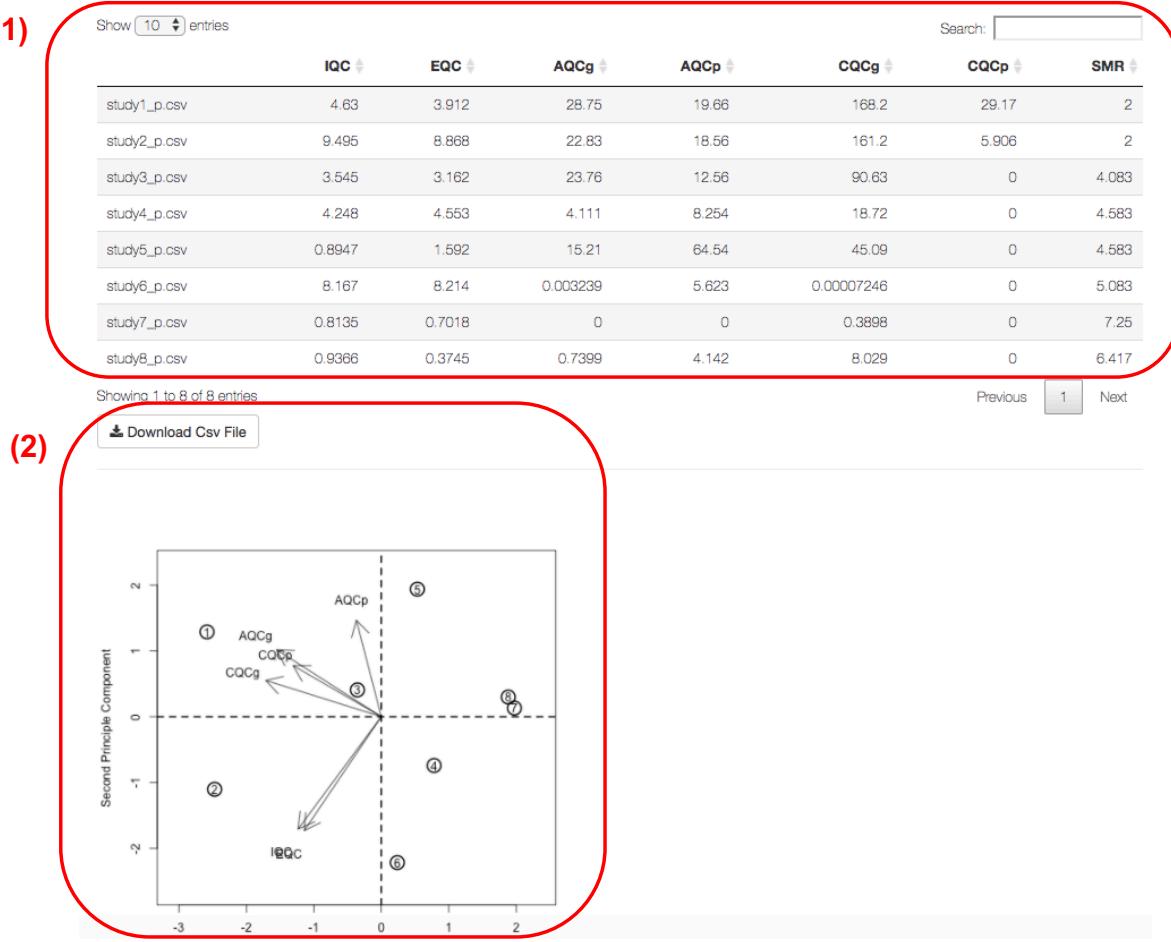


Figure 10: “MetaQC” Results for prostate cancer example.

The test data used to demo the “MetaQC” package here is merged from eight prostate cancer studies with 30% of genes filtered out by mean and 30% filtered out by variance (2077 genes remained for the MetaQC analysis). Detailed descriptions of these studies can be found in Table 3. As shown in Figure 10, MetaQC module generates a summary table (Figure 10 (1)) of MetaQC results as well as a PCA biplot (Figure 10 (2)). The table includes seven columns, with the first six columns corresponding to the six quantitative quality control measures of all studies (a larger value indicates a better quality) and the seventh column is the rank summary statistics of all the six quality measures (a lower rank indicates a better quality). Users can download the full table as a csv file by clicking on “Download Csv File”. In addition to the tabular results, “MetaQC” also generated a PCA biplot based on the six quality control measures, where the circled number is the study index and arrows indicate different measures. Generally, studies with larger SMR values, and studies more off from the other studies and a majority of the measures are considered lower quality. In this case, the 7th and the 8th studies have relatively poorer quality. Both tabular summary and biplot are automatically saved to the working directory.

5.2 MetaDE

MetaDE package implements 12 major meta-analysis methods with 22 variations for differential expression analysis that fall into 3 main categories: combining p-values, combining effect sizes and others (e.g. combining ranks, etc.). Depending on the type of outcome, the package can perform two class comparison, multi-class comparison, association with continuous or survival outcomes. The package allows the input of either

microarray (continuous intensity) and/or RNA-seq data (count) for individual study analysis. The R package for MetaDE module can be found at <https://github.com/metaOmics/MetaDE>. After obtaining DE genes from the differential expression analysis, users can further perform post-hoc pathway enrichment analysis using the declared DE genes. In the two subsections below we will go over how to perform (1) differential expression analysis and (2) pathway enrichment analysis based on (1). Note that MetaDE involves many sophisticated meta-analysis methods, please refer to the MetaOmics paper (Supplementary information: Box S3) for a detailed description of each method.

5.2.1 Procedure of differential expression analysis

The figure shows a user interface for the MetaDE software. At the top are links for 'About' and 'Glossary'. Below these are ten numbered red boxes corresponding to the steps in the figure caption:

- (1) Meta Method Type: A dropdown menu currently set to 'Combine P-Value'.
- (2) Meta Method: A dropdown menu currently set to 'Fisher'.
- (3) mixed data types?: A radio button group where 'No' is selected.
- (4) Response Type: A dropdown menu currently set to 'Individual Study Option'.
- (5) Individual Study Option: A dropdown menu.
- (6) Advanced Options: A section containing several checkboxes.
- (7) Run: A green button with a play icon.
- (8) Pathway Databases: A section with checkboxes for KEGG, GO Biological Process, GO Cellular Component, GO Molecular Function, Reactome, and BioCarta.
- (9) Pathway Analysis Options: A section containing several checkboxes.
- (10) Run Pathway Analysis: A green button with a play icon.

Figure 11: “MetaDE” options

In Figure 11, Red boxes (1) - (7) are the options and steps to run the differential expression analysis. A detailed list of all options available in Section 6.2.

Step 1 Choose the type of meta-analysis method: There are three types of meta-analysis to choose: combining p-values, combining effect size and others.

Step 2 Choose a meta-analysis method:

- For “combining p-values” category, users can choose from “Fisher”, “AW-Fisher*”, “maxP”, “minP”, “rOP*” and “Stouffer”, where some of them have the one-sided corrected versions.
- For “combining effect size” category, users can choose from “FEM” and “REM*”, where “REM*” has choice of six analytical algorithms for implementation.
- For “others”, there are three rank-based method (PR, SR and rankProd) and minMCC for multi-class meta-analysis. To choose from the overwhelmingly many meta-analysis methods, we follow Chang et al. (2013) and mark * for top performing methods AW-Fisher, REM (HO option) and rOP as recommendation for users.

Step 3 Mixed data type: If this option is selected, MetaDE will allow partial studies with count data from RNA-seq and remaining studies with continuous intensities from microarray.

Step 4 Choose the response type: Under the drop-down menu, users can specify types of outcome (response) variable to be two-class, continuous, multi-class or survival. By choosing “two class comparison”, users can specify the group label name for the Label Attribute (from the column names of your clinical data). Then for group label (a factor of at least two levels), specify the name for the “Control Label” and “Experimental Label”, respectively. For the other types, only group label name is needed.

Step 5 Choose Study Design for individual study:

- Individual data type can be either discrete (count) or continuous.
- Under drop-down menu “Setting Individual Study method”, user can specify individual study method according to individual data type. For continuous data (e.g. microarray), available options include LIMMA (default method) and SAM. For discrete data (e.g. RNA-seq count), available options include edgeR, DESeq2 and voom.
- The users can also specify whether each study is paired design or not.

Step 6 Advanced Options

- Use complete options: other uncommonly used options will become available. Again, this is not suggested if you are not familiar with the method.
- Parametric: if No is selected, permutation will be performed instead of parametric closed form solution.
- Covariate: indicate if any covariate will be adjusted.
- Alternative Hypothesis: two-sided or one-sided.

Step 7 Run: Once all the above options are specified, users can click on “Run” button to perform MetaDE analysis

5.2.2 Results of differential expression analysis

We used the AML example to demonstrate the MetaDE module. After merging the three datasets and filtering out 50% of genes by mean and 50% by variance, 1283 genes remained. In this example we only compare two phenotypes: inv(16) and t(15;17). Detailed descriptions of these studies can be found in Table 1. Two main outputs from the first “meta differential analysis” step in the procedure are shown in Figure 12. Heatmap of DE genes is rendered after specifying the FDR cutoff for selection of DE genes and clicking on “Plot DE Genes Heatmap”. The “image size” can be adjusted by dragging the scroll bar. In the heatmap, rows refer to the declared DE genes under the specified FDR cutoff, columns refer to samples, and solid white lines are used to separate different studies and the dashed white lines are used to separate groups. Colors of the cells correspond to scaled expression level as indicated in the color key below. For the results generated by “AW-Fisher*”, there is one additional column of cross-study weight distribution on the left end of the heatmap and the genes in the heatmap are sorted by their weight distribution. Summary of meta analysis results is on bottom, including information of individual test statistics, individual study p-value, meta-analysis p-value, FDR, etc.

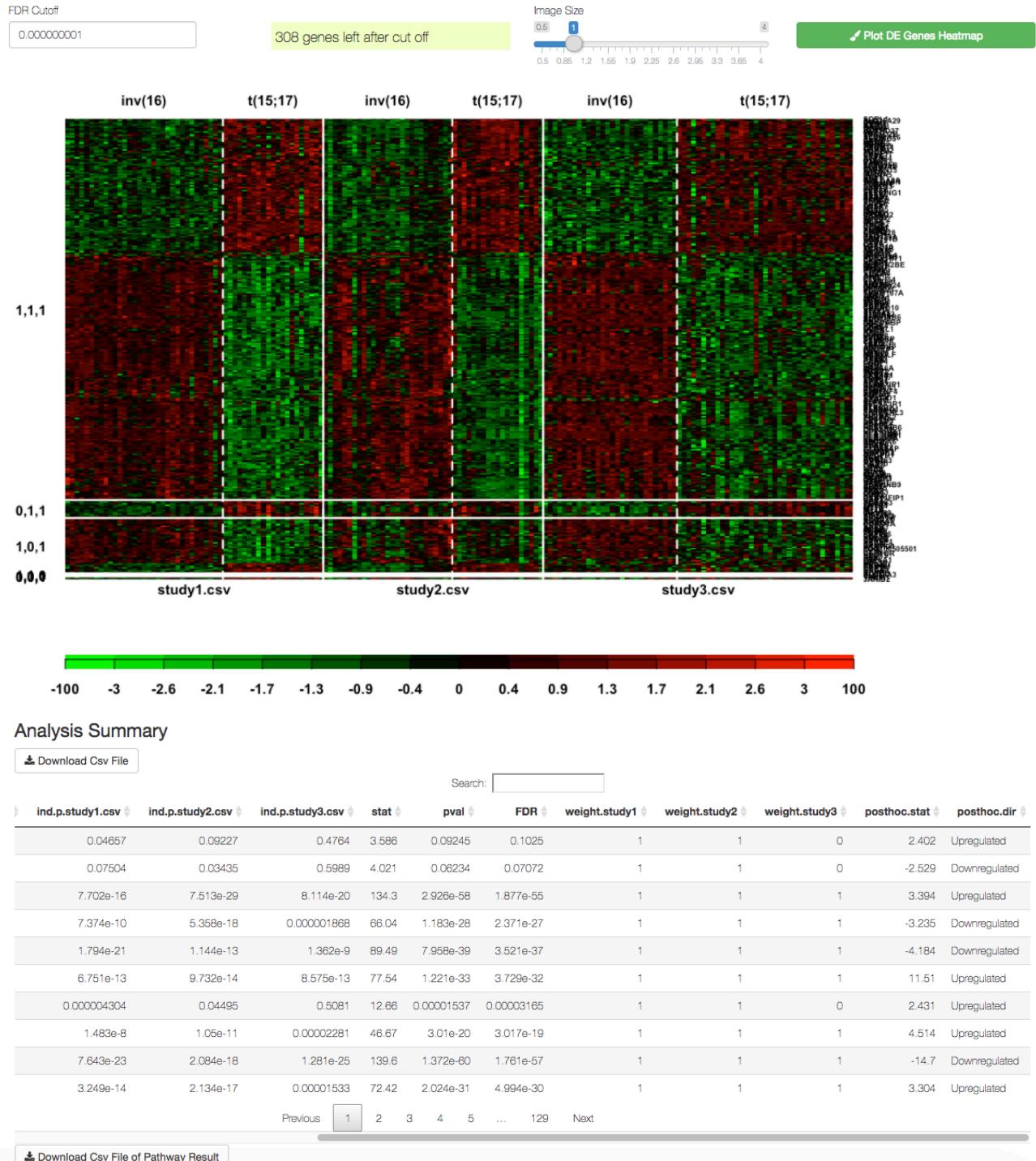


Figure 12: “MetaDE” Results

5.2.3 Procedure of downstream pathway analysis

Users can then perform pathway enrichment analysis on the declared DE genes from the previous step, the options and steps of which are shown in **Red boxes (8) - (10)** in Figure 11. Procedure is outlined as below.

Step 1 Choose the pathway database: Users can select from 25 available pathway databases to perform the pathway enrichment analysis.

Step 2 Choose the pathway enrichment method and the pathway size range: In this step users can choose pathway enrichment options with Kolmogorov-Smirnov (KS) test as default option, or Fisher's exact test by specifying number of input genes for pathway analysis. For Fisher's exact test, the input genes can be obtained by either specifying a MetaDE p-value cutoff, or specifying number of top DE genes. Users can also specify the minimum/maximum gene size of pathways to be included for pathway enrichment analysis.

Step 3 Run: Once all the above options are specified, users can click on "Run Pathway analysis" button to perform pathway enrichment analysis.

5.2.4 Result of downstream pathway analysis

The result for downstream pathway analysis is shown in Figure 13. The summary includes the pathway names, the corresponding enrichment p-value and FDR. In addition to the results shown in the browser, users can download the result by clicking on "Download Csv File" on the top left of the summary table.

	Search:	pvalue	qvalue
GO:BP immune system process		0.0003076	0.4141
KEGG Leishmania infection		0.0006526	0.4141
KEGG Cell adhesion molecules (CAMs)		0.0007405	0.4141
Reactome MHC class II antigen presentation		0.0009559	0.4141
Reactome Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell		0.001333	0.4227
Reactome PD-1 signaling		0.002083	0.4227
KEGG Systemic lupus erythematosus		0.002226	0.4227
Reactome Phosphorylation of CD3 and TCR zeta chains		0.0023	0.4227
Reactome MyD88:Mal cascade initiated on plasma membrane		0.00233	0.4227
KEGG Antigen processing and presentation		0.002608	0.4227
Showing 1 to 10 of 1,901 entries			
Previous 1 2 3 4 5 ... 191 Next			

Figure 13: Downstream pathway analysis based on MetaDE genes

5.3 MetaPath

In MetaDE package, following the detection of biomarkers, pathway analysis (a.k.a. gene set enrichment analysis) is usually performed for functional annotation and biological interpretation. Beyond that, the MetaPath module provides two advanced meta-analytic pathway analysis tools: Meta-Analysis for Pathway Enrichment (MAPE) and Comparative Pathway Integrator (CPI) (Shen et al., 2010; Fang et al., 2017). It also includes advanced pathway clustering diagnostics and pathway clustering with text mining to circumvent abundant pathway redundancy in the databases and improve interpretation. The R package for MetaPath module can be found at <https://github.com/metaOmics/MetaPath>.

5.3.1 Procedure

The MetaPath package requires the input of raw expression data as in MetaDE. There are three major steps to implement the package: pathway analysis, pathway clustering diagnostics and pathway clustering with text mining. As shown in Figure 14, there are nine major options that need to be specified to implement the package. Detailed list of all options available for the package can be found in Section 6.3

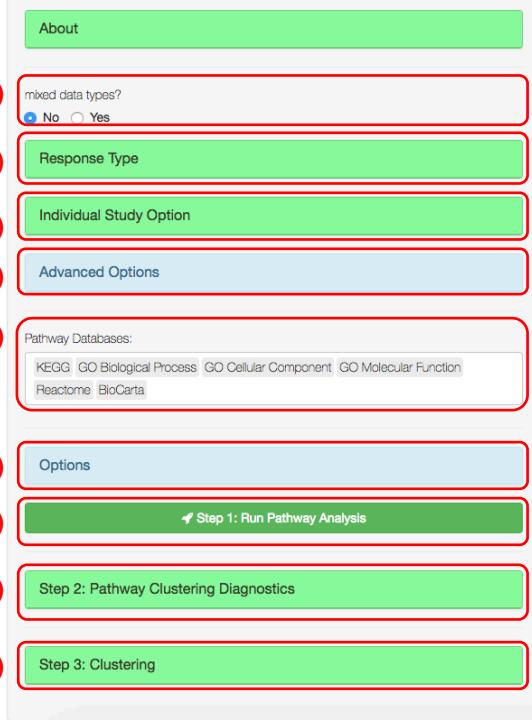


Figure 14: “MetaPath” options

Setup pathway analysis parameters: As shown in Figure 14, users need to specify (1) whether the input gene expression profile is mixed of continuous data and discrete data; (2) response type, case/control labels (similar to MetaDE); (3) individual study option (similar to MetaDE); (4) advanced options including whether to adjust for covariates or the direction of hypothesis testing;. In (5), users can select from 25 available pathway databases for the enrichment analysis. In (6), users can select MetaPath method (either MAPE or CPI). By default, the “MAPE” approach is used. Other options include the pathway enrichment method (the Fisher’s exact test or KS test), the minimum and maximum pathway size. If “Fisher’s exact test” is chosen for the enrichment method, users need to further specify the criteria for selection of DE genes, e.g. the number of top ranked genes. On the other hand, if “KS test” is chosen, one needs to further specify whether to use permutation to obtain enrichment p-value.

Step 1 Run Pathway Analysis: Once the above options are specified, users can click on (7) to “Run Pathway Analysis”.

Step 2 Pathway clustering diagnostics: From the previous step (**Step 1**), users can choose the top enriched pathways for further clustering. One can expand the drop-down menu and use FDR cutoff to choose top pathways and click on (8) “Pathway Clustering Diagnostics” to implement the second step.

Step 3 Pathway clustering with text mining: From the previous step (**Step 2**), users can determine the optimal number of clusters in the pool of pathways selected. Now, one can specify the number of clusters and click on (9) to get pathway “Clustering” results. Note that you may not want to select too large K since you wish to have a certain amount of pathways in each cluster for the validity of text mining algorithm. We generally suggest users to specify K no larger than 7 for fewer than 100 pathways.

5.3.2 Results

Analysis Summary

Show 10 entries

Search:

	q_value_meta	p_value_meta	study1.csv	study2.csv	study3.csv
Reactome MHC class II antigen presentation	0.001039	5.998e-7	0.001702	0.001254	0.0002194
Reactome Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	0.006311	0.000007284	0.01204	0.0007369	0.0008623
KEGG Cell adhesion molecules (CAMs)	0.007497	0.00001298	0.00781	0.0041	0.0004607
KEGG Intestinal immune network for IgA production	0.01223	0.00004152	0.002608	0.01782	0.001208
KEGG Asthma	0.01223	0.00004938	0.004537	0.005154	0.002933
KEGG Systemic lupus erythematosus	0.01223	0.00004124	0.002968	0.006707	0.002797
GO:BP immune system process	0.01223	0.00004558	0.0006081	0.003762	0.02733
KEGG Autoimmune thyroid disease	0.0123	0.00006387	0.009055	0.00174	0.00587
KEGG Allograft rejection	0.0123	0.00006387	0.009055	0.00174	0.00587
Reactome Phosphorylation of CD3 and TCR zeta chains	0.01482	0.00008549	0.002917	0.01788	0.002491

Showing 1 to 10 of 1,733 entries

Previous 1 2 3 4 5 ... 174 Next

Figure 15: “MetaPath” Results (1), this table shows analysis results of all pathways, including individual study association analysis p-value, meta pathway analysis p-value/FDR, etc. Users can sort these pathways by clicking the p_value_meta up arrow button. In addition, users can search the pathway name in the “Search” bar.

We used the AML data to demonstrate the usage of MetaPath module, with the same filtering criteria and the phenotype of interest as in MetaDE module. Detailed descriptions of these studies can be found in Table 1. After **Step 1** is finished, a summary table was generated as shown in Figure 15 (based on the CPI method). The full table is automatically saved in the working directory specified before.

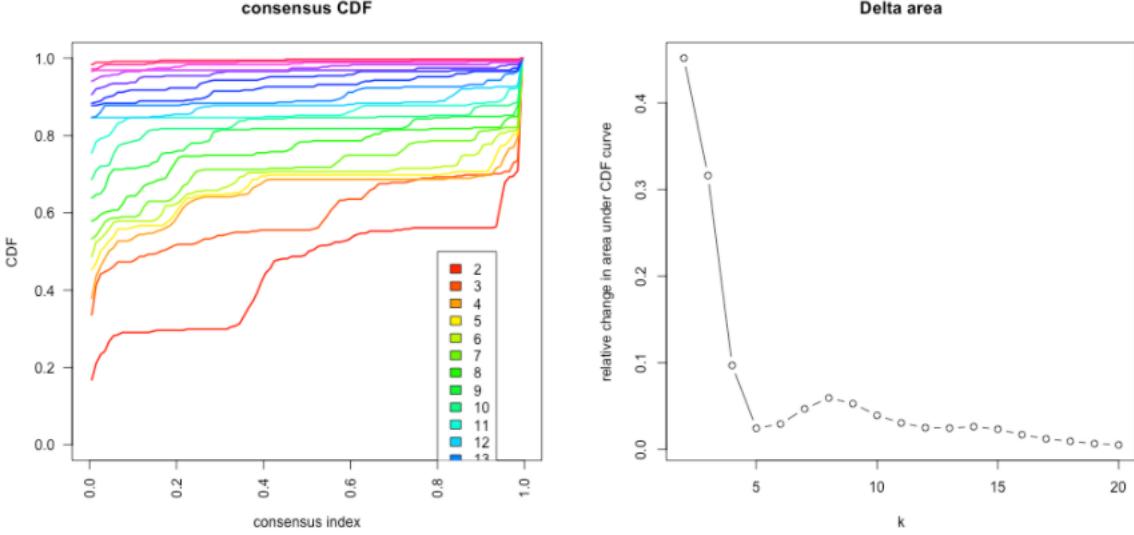


Figure 16: “MetaPath” Results (2): both plots assist users in finding the optimal number of clusters K and users may refer to Monti et al. (2003) for detailed interpretation of the two plots. To be brief, the CDF of the consensus matrix for each K (indicated by colors) is estimated by a histogram of 100 bins. The CDF reaches an approximate maximum implying consensus and cluster confidence is at a maximum at this K . The delta area shows the relative change in area under the CDF curve comparing K and $K - 1$, thus allows users to determine K at which there is no appreciable increase in CDF (which drops as the number of cluster increases). In the example, $K = 5$ is chosen since it locates at the elbow turning point (i.e. where the magnitude of incremental decrease in delta area diminishes).

In order to perform pathway clustering analysis, we need to determine the number of clusters K . By clicking “Pathway Cluster Diagnostics” (**Step 2**), two plots are generated on the right panel (Figure 16): including a consensus CDF plot and a Delta area plot (both from the “ConsensusClusterPlus” package). Note that in this specific example, we set FDR cutoff value in **Step 2** as 0.5, and 28 pathways left under this cutoff. Both plots assist users in finding the optimal number of clusters K and users may refer to Monti et al. (2003) for detailed interpretation of the two plots. To be brief, the CDF of the consensus matrix for each K (indicated by colors) is estimated by a histogram of 100 bins. The CDF reaches an approximate maximum implying consensus and cluster confidence is at a maximum at this K . The delta area shows the relative change in area under the CDF curve comparing K and $K - 1$, thus allows users to determine K at which there is no appreciable increase in CDF (which drops as the number of cluster increases). In the example, $K = 5$ is chosen since it locates at the elbow turning point (i.e. where the magnitude of incremental decrease in delta area diminishes).

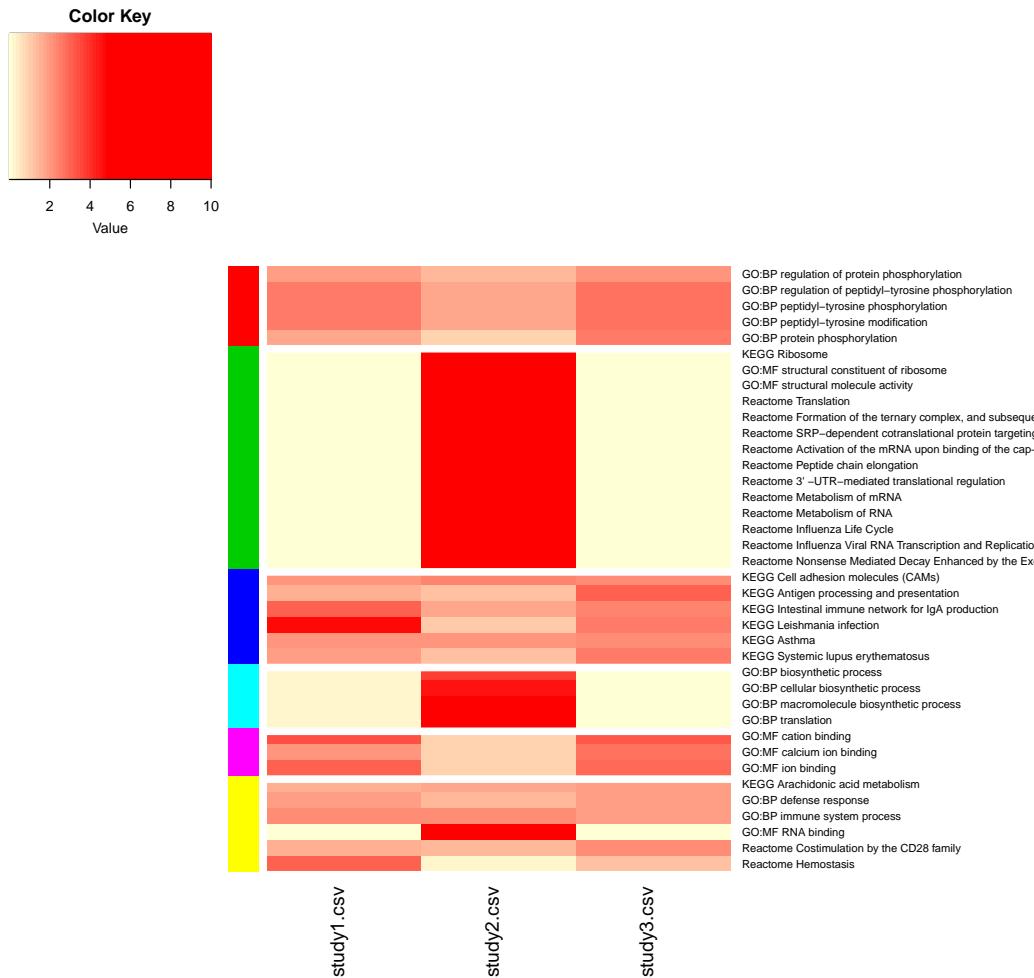


Figure 17: “MetaPath” Results (3): The heatmap in Figure 17 shows the $-\log_{10}$ transformed p-value of enrichment analysis in each study from **Step 1**. Studies are on columns and the selected pathways are on rows, red means more enriched. The pathways are sorted by the pathway clusters as indicated by the colors on the left side of the heatmap.

The heatmap in Figure 17 shows the $-\log_{10}$ transformed p-value of enrichment analysis in each study from **Step 1**. Studies are on columns and the selected pathways are on rows, red means more enriched. The pathways are sorted by the pathway clusters as indicated by the colors on the left side of the heatmap. In addition, key words of each cluster of pathways are extracted and analyzed by a built-in text mining algorithm and one file named “Clustering_Summary.csv” is saved to the working directory which shows a summary of the text mining results.

5.4 MetaNetwork

By clicking toolsets and then MetaNetwork, users are directed to MetaNetwork home page as Figure 18. The R package for MetaNetwork module can be found <https://github.com/metaOmics/MetaNetwork>.

MetaNetwork

Summary Table

#Genes	#Samples
1283	89
1283	74
1283	105

About

Glossary

Case Name: inv(16)

Control Name: t(15;17)

Number of Permutations: 20

Edge Cutoff: 0.1

Generate Network

Advanced Options

Figure 18: MetaNetwork homepage

MetaNetwork includes three steps to get differentially co-expressed networks, including (1) generate network, (2) search for basic modules, and (3) assemble super-modules. The left side of Figure 18 is the control panel of step 1. The control panel for step 2 and step 3 will show up after the previous step is done. The explanation of the complete list of all options are in Section 6.4

5.4.1 Procedure

Step 1 Generate Network The first step of MetaNetwork is to generate co-expression network. In this step, the network for permuted data will also be generated. Users need to select case and control names, the number of permutations, and edge cut-off which determines the proportion of edges to be kept in the network. After clicking **Generate Network** button, screen will show message indicating the algorithm is running to generate network.

Step 2 Search for basic modules

The next step is to search basic modules. Advanced options (recommended not to change) include the number of repeats used for each initial seed modules (“Number of repeat”), the maximum Monte Carlo steps for simulated annealing algorithm (MC Steps), and the maximum pairwise Jaccard index allowed for basic modules (Jaccard Cutoff), as shown in Figure 19. Explanation of these technical

terms are omitted in this tutorial, but readers can refer to Zhu et al. (2016) for details. After clicking **Search for basic modules** button in Figure 19, screen will show message indicating the algorithm is running to search for basic modules. This step is computationally demanding depending the on gene size. After this step is done, the screen will show a table of basic modules highly connected in cases but lose connections in control and vice versa.

Case Name
inv(16)

Control Name
t(15;17)

Number of Permutations:
20

Edge Cutoff
0 0.1 1

Generate Network

Advanced Options

Number to repeat:
3

MC steps:
500

Jaccard Cutoff
0.8

Search for basic modules

Figure 19: MetaNetwork control panel for search for basic modules

Search for basic modules can be time consuming, especially if a large number of genes are used. After this step is done, the screen will show a table of basic modules higher correlated in case and a table of basic modules higher correlated in control as in Figure 20.

Basic modules higher correlated in case:

Basic modules higher correlated in case:			
Module.Index	Component.Number	Repeat.Index	Gene.Set
1 H1	2	1	PSAP/CTSS/CECR1/LGALS3/AP1S2/TLR2/HEXB/SMIM24/CTSB/OGFRL1/MYO1F/CPXM1/SERPINA1/MNDA/TNF
2 H2	2	2	SERPINA1/AP1S2/CECR1/SMIM24/PSAP/TRBV27/RGS10/CPXM1/TLR2/CAPN2/OGFRL1/MS4A6A
3 H3	2	3	CTSB/TLR2/RGS10/HEXB/SERPINA1/CECR1/LGALS3/AP1S2/SMIM24/S100A9/CPXM1/MAP2K1/CAPN2
4 H4	6	1	NFIL3/IER3/CD83/CPXM1/EZR/RIPK2/SLC2A3
5 H5	6	2	NFIL3/IER3/LCP1/CDKN1A/TCEAL4/TGFB1
6 H6	6	3	NFIL3/LYN/IER3/LCP1/UCP2/MARCKSL1/RAB11FIP1

Showing 1 to 6 of 6 entries

Previous 1 Next

Basic modules higher correlated in control:

Basic modules higher correlated in control:			
Module.Index	Component.Number	Repeat.Index	Gene.Set
1 L1	2	1	GCA/FABP5/PRR11/PCNA/C1QBP/MAFF/FAM107B/PRDX3/TNFSF13B/PRTN3/PRKACB/CBF/CAT/RAB10/AN
2 L2	2	2	ICAM3/TGFB1/TYROBP/PLP2/BIN2/HCST/MYO1F/TIMP1/LAPTM5/S100A9
3 L3	2	3	PCNA/FAM107B/GCA/PRTN3/C1QBP/KIAA0101/EZR/RAB10/CAT/PRDX3/TNFSF13B/ANP32E/DYNLL1
4 L4	6	1	SLC2A3/JUP/LYN/PPP1R15A/EZR/PCNA/RAB10/FLNA/PIM3/KLF6
5 L5	6	2	STAM/JUP/LYN/PIM3/PDLIM1/HLA-DPA1/ITM2A/TPSAB1/CSTA
6 L6	6	3	SLC2A3/STAM/JUP/LYN/PPP1R15A/PCNA/FLNA/PLP2/HLA-DPA1

Showing 1 to 6 of 6 entries

Previous 1 Next

Figure 20: MetaNetwork output from search for basic modules step

Step 3 Assemble supermodules

After search for basic modules step is done, the control panel becomes that in Figure 21. The last step is to assemble supermodules. Users can decide the FDR cut-off to select basic modules for supermodule assembly. After clicking **Assemble supermodules** button, screen will show message indicating the algorithm is running to assemble supermodules. A table for basic modules, supermodules and their network visualization will be shown on the right panel of the screen. MetaNetwork automatically creates files of top supermodules designed to input to a Cytoscape plug-in “MetaDCNEexplorer” (<http://tsenglab.biostat.pitt.edu/software.htm>) for improved visualization and dynamic exploration.

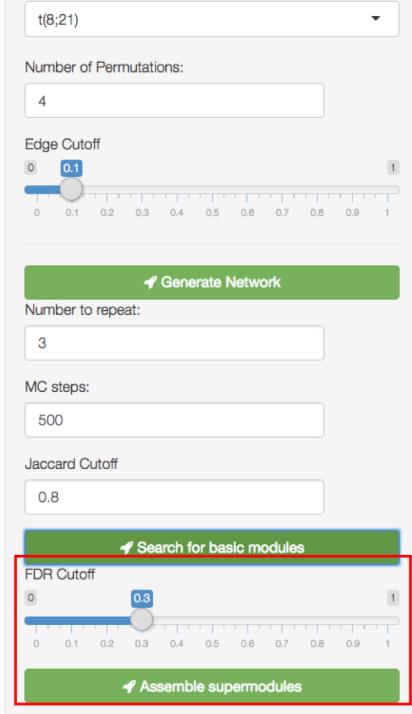


Figure 21: MetaNetwork control panel for assemble supermodules step

5.4.2 Results

We used the leukemia data to demonstrate the MetaNetwork module. After merging the three datasets by filtering out 80% of genes by mean and 80% by variance, 206 genes remained. In this example we only compare two phenotypes: inv(16) and t(8;21). Detailed descriptions of these studies can be found in Table 1. In general, the MetaNetwork tool is time consuming for large datasets (for both network generation and search for basic modules steps). We generally suggest users to carefully restrict the number of genes (e.g. less than a thousand) for a test run before implementing large gene set. By default, all outputs and several interim RData files will be automatically saved to the folder named “MetaNetwork” under the working directory specified in Section 2.3.

After the **Generate Network** step is done, no output will show up in the screen. Instead, a message box will show up indicating several Rdata files are saved in the MetaNetwork folder. After **Search for basic modules** step is done, the screen will show a table of basic modules higher correlated in case or control, as in Figure 20. After **Assemble supermodules** assembly is done, screen will show a table of supermodules (Figure 22). Users can also select basic modules to plot (Figure 23). Meanwhile several files will be saved in the folder MetaNetwork. Detailed explanation of all these intermediate files are described in Section 6.4. Note that because the module searching procedure is a random process, users may not be able to repeat the exact same results each time.

MetaDCN pathway-guided supermodules

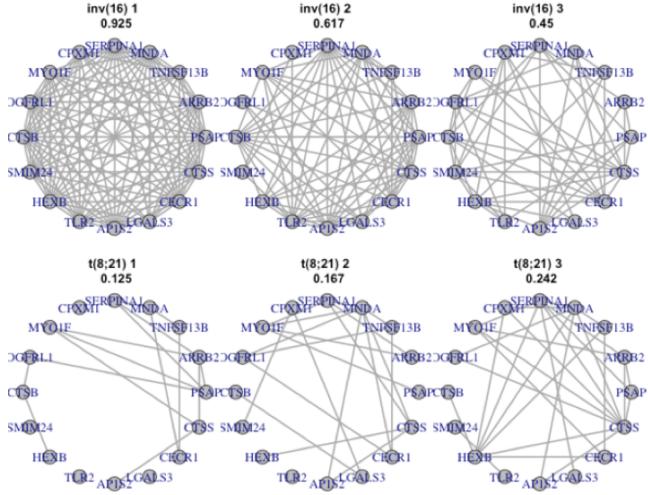
Show <input type="button" value="10"/> entries	Search: <input type="text"/>			
pathway_name	pathway_size	p_value	q_value	size
KEGG_LYSOSOME	121	9.73e-05	0.01	20
REACTOME_MHC_CLASS_II_ANTIGEN_PRESENTATION	91	0.00697	0.0393	20
REACTOME_PLATELET_ACTIVATION_SIGNALING_AND_AGGREGATION	208	0.00495	0.0393	25
REACTOME_RESPONSE_TO_ELEVATED_PLATELET_CYTOSOLIC_CA2_	89	0.00281	0.0393	22
BIOCARTA_IMCALPAIN_PATHWAY	25	0.00283	0.0393	30
GO_CYTOSKELETAL_PROTEIN_BINDING	159	0.00185	0.0393	13
REACTOME_COSTIMULATION_BY_THE_CD28_FAMILY	63	0.00431	0.0393	14
GO_ACTIN_FILAMENT_BINDING	25	0.00369	0.0393	13
BIOCARTA_CFTR_PATHWAY	12	0.00725	0.0393	18
GO_ACTIN_CYTOSKELETON_ORGANIZATION_AND_BIOPROCESS	105	0.00697	0.0393	20

Showing 1 to 10 of 103 entries

Previous 2 3 4 5 ... 11 Next

Figure 22: MetaNetwork supermodules table

3 modules higher correlated in case under FDR 0.3, select modules to plot:



6 modules higher correlated in control under FDR 0.3, select modules to plot:

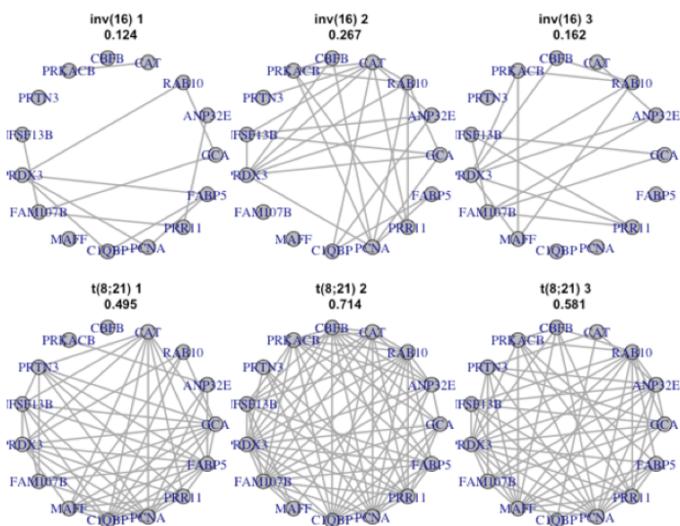


Figure 23: MetaNetwork select basic modules to plot. Each dot represents a gene. An edge represents the two genes are highly correlated.

5.5 MetaPredict

Top scoring pairs (TSP) is a robust algorithm for predicting gene expression profiles, which adopts non-parametric rank-based prediction rule. The MetaPredict is a meta-analysis version of the TSP algorithm that combines multiple transcriptomic studies to build a prediction model and shows improved prediction accuracy as compared to single study analysis. The R package for MetaPredict module can be found at <https://github.com/metaOmics/MetaPredict>.

The homepage for MetaPredict is shown in Figure 24. Under advanced options, there are 1 drop-down menu (“Methods for MetaPredict”) (1), three number entries (“Max number of top scoring pairs (K)” (2), “Number of cores for parallel computing” (3). These will be introduced shortly but the users are not suggested to change them unless they know about the algorithm. The necessary parameters include “Number of top scoring pairs (K)” (7), three character entries (“Please select TWO labels to cluster” (4), “Please select studies for training” (5), and “Please select studies for testing” (6), and two executing tabs (“Train model”

and “Predict”).

5.5.1 Procedure

The screenshot shows the MetaPredict homepage with several input fields and buttons. The fields are numbered 1 through 7:

- (1) Methods for MetaPredict: A dropdown menu set to "Mean score".
- (2) Max number of top scoring pairs (K): An input field containing "29".
- (3) Number of cores for parallel computing: An input field containing "1".
- (4) Please select TWO labels to cluster: A text input field.
- (5) Please select studies for training: A text input field.
- (6) Please select ONE study for testing: A text input field.
- (7) Number of top scoring pairs (K): An input field containing "28".

Below the input fields are two green buttons: "Train model" and "Predict".

Figure 24: Homepage of MetaPredict

Step 1 Building prediction model based on meta-analysis

First, we need to decide a method to select K top scoring gene pairs from multiple studies (Figure 24). Second, we need to provide the maximum number of top scoring pairs K (algorithm will search from 1 up to K with default $K = 29$) and the number of cores for parallel computing. Next, we need to select only two labels to build the classification model. In other words, if there exists more than two kinds of labels, we need to choose two from them. If you click on (4), all available labels will pop up. Then, select the dataset as training data and testing respectively, and click the “Train model” button to run the MetaPredict program. It may take a while to run the model.

Step 2 MetaPredict prediction

After the model training is finished, on the top right it will show up a “Gene pair table” (Figure 25) which present the top K gene pairs statistics. A diagnostic plot (Figure 26) is outputted to assist users decide which K to use in the final prediction model. The suggested value is shown in the plot as green line, which is decided by VO method we introduced in the original paper. Users may also decide K on their own to predict the class label of testing data. After deciding K , then hit the button “Predict” (Figure 24). Finally, a confusion matrix is output to show the prediction results (Figure 27). The prediction results are also saved in the working directory. A complete list of options is available in Section 6.5.

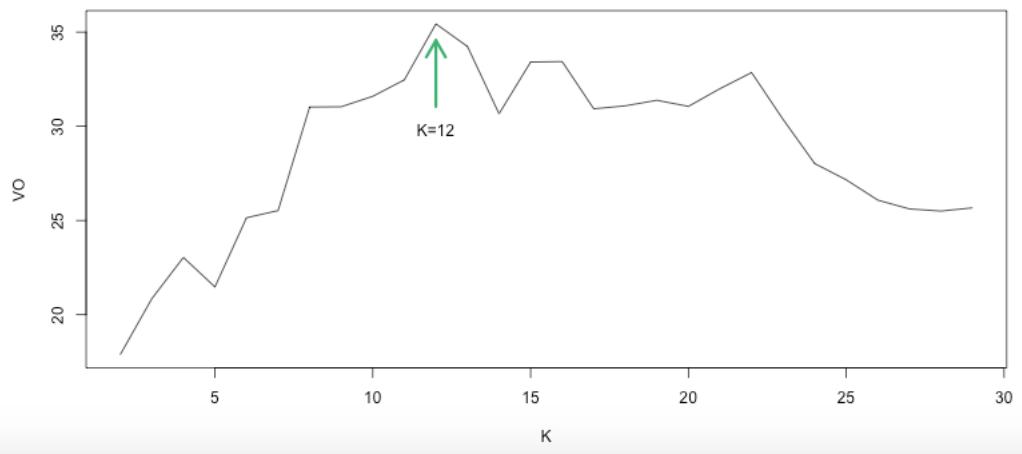
Gene pair table

GeneIndex1	GeneIndex2	Gene1	Gene2	Score_overall	Score_study1	Score_study2
83	409	RNASE3	KDM4B	-1.97	-0.97	-1.00
170	239	ANPEP	P2RX5	-1.94	-0.94	-1.00
86	321	LST1	TM7SF3	-1.94	-0.94	-1.00
103	156	VEGFA	TNFSF13	1.93	1.00	0.93
229	1028	FAM101B	ADRM1	-1.89	-0.89	-1.00
111	146	CD96	GLIPR2	1.88	0.91	0.96
109	164	DEPTOR	C1orf162	1.88	0.91	0.96
286	879	PLXNB2	MFSD10	-1.87	-0.94	-0.93
20	427	CD9	CPXM1	-1.87	-0.94	-0.93
263	613	RASSF5	SHC1	-1.87	-0.94	-0.93
420	814	RASSF2	CBFB	-1.87	-0.94	-0.93
110	188	RGS10	NFIL3	1.85	0.89	0.96
44	426	ITGB2	IL2RG	-1.85	-0.88	-0.96
139	175	LY86	OAT	-1.85	-0.88	-0.96
13	39	TRH	GPR183	1.84	0.88	0.96
349	970	PLP2	HLA-E	-1.84	-0.91	-0.93
186	221	AP1S2	RAB37	-1.83	-0.94	-0.89
484	678	SASH3	PTPN7	-1.83	-0.94	-0.89
246	458	SPRY2	SLC39A8	-1.83	-0.94	-0.89
93	1115	LAT2	ZBTB4	-1.83	-0.97	-0.85

Figure 25: Results for MetaPredict, top predictive pairs of genes.

K diagnostic plot

The recommended K is the K maximizing variance optimization (VO) t statistics

Figure 26: Results for MetaPredict, select K by maximizing variance optimizaiton (VO) t statistics.

Confusion Table	
Original_inv(16)	Original_t(8;21)
28	14
0	26

Sensitivity = 28/42 = 0.6666666666666667
Specificity = 26/26 = 1

Figure 27: Confusion table for MetaPredict testing cohort.

5.5.2 Results

We used the leukemia data to demonstrate the MetaNetwork module. After merging the three datasets by filtering 50% of genes by mean and 50% by variance, 1283 genes remained. In this example we only compare two phenotypes: inv(16) and t(8;21). Detailed descriptions of these studies can be found in Table 1. The top predictive pairs of genes are available in Figure 25. The diagnostic plot showing the optimum K is shown in Figure 26. A confusion matrix is output showing the prediction results is in Figure 27). The prediction results are also saved in the working directory.

5.6 MetaClust

By clicking on the Toolset tab and then choose MetaClust, users are directed to MetaClust home page as in Figure 28. MetaClust (Hu et al., 2016) aims to perform sample clustering analysis combining multiple transcriptomic studies. By integrating information from multiple studies of similar biological purposes, MetaClust can identify an unified intrinsic gene set among all studies, perform weighted clustering analysis using the common intrinsic gene set and match the clustering patterns across studies to define disease subtypes/cluster types. The resulting clustering from meta-analysis is more robust and accurate than single study analysis. The R package for MetaClust module can be found at <https://github.com/metaOmics/MetaSparseKmeans>.

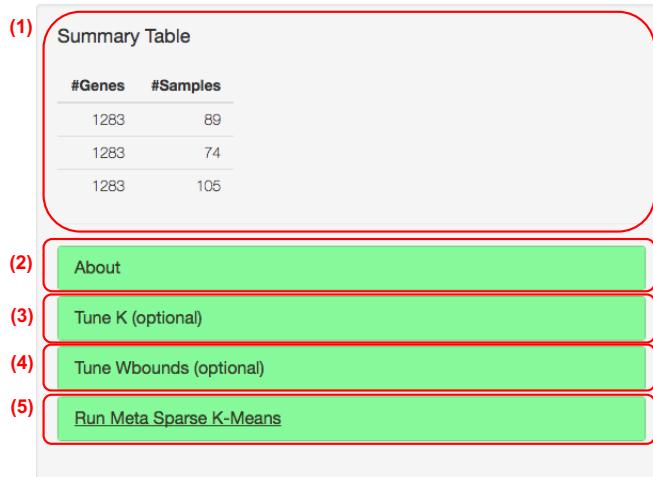


Figure 28: MetaClust home page

5.6.1 Procedure

Again, probably need to use a table to briefly introduce e.g. "Gap statistics"

Figure 28 shows the home page of MetaClust. On the top left panel users can see the data summary Table (1). Below there are four tabs. About tab (2) which includes basic introduction of MetaClust, Two tabs for Tune K as well as Wbounds, and the Run Meta Sparse K-Means tab. Starting with multiple studies, we could run MetaSparseKmeans (5) with pre-specified number of clusters (K) and gene selection tuning parameter (Wbounds). If you are not sure about what are good K and Wbounds, please try Tune K (3) and Tune Wbounds (4) panel. A complete list of options is available in Section 6.6.

Step 1 Tune K :

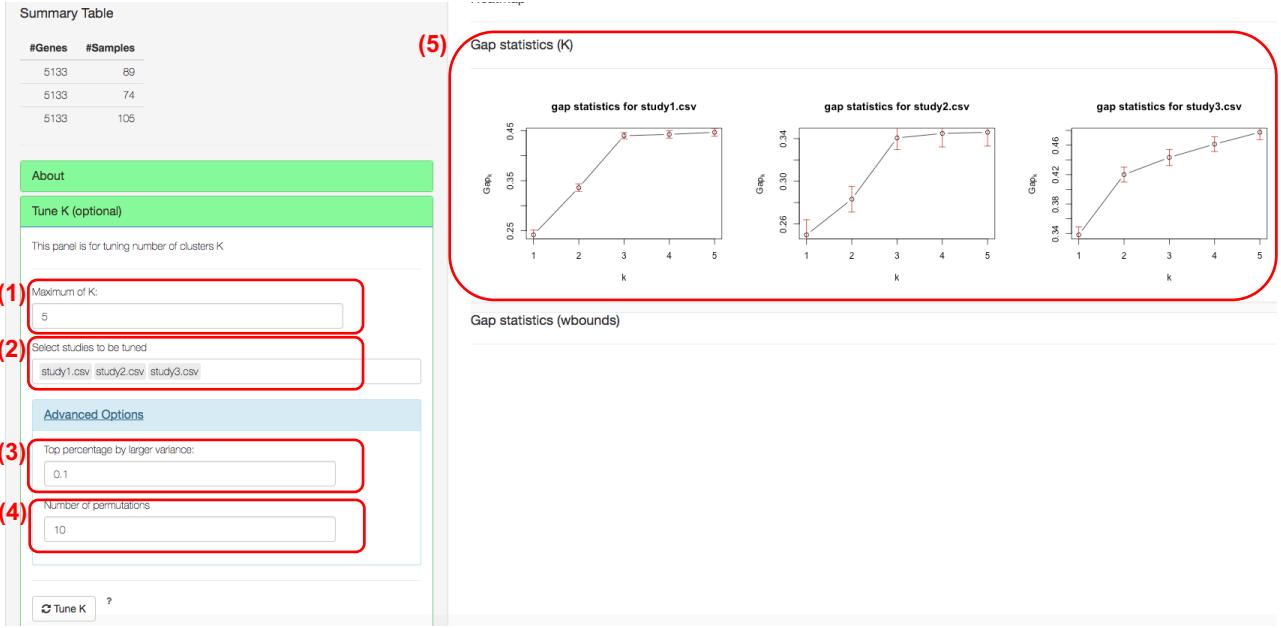


Figure 29: Tuning parameter selection for number of clusters

If the users are not sure what is the number of clusters, they can start to use the Tune K panel as in Figure 29. Gap statistics will be used to get optimal K for each individual study. Detailed description about Gap statistics can be found Tibshirani et al. (2001). Users need to specify maximum number of K (1), which the algorithm will search number of studies from 1 to K . Studies to be tuned can be selected (4). In advanced options, users can further specify number of top variance genes to be included and number of permutations. But if users don't know the algorithm, please leave them as default. Top percentage p% by larger variance means that we will use top p% larger variance genes to perform gap statistics (3). Number of permutation is the number of bootstrap samples for gap statistics (4). At least 50 bootstrap samples are suggested for a stable result of number of clusters. By clicking button "Tune K ", we will obtain gap statistics as in Figure 29. A good K is selected such that the Gap_k is maximized or stabilized across all studies. From the figure, $K = 3$ is preferred since the gap statistics from all three studies become flat (5).

Step 2 Tune Wbounds:

Wbounds directly control number of features selected by metaClust. A larger number of Wbounds will result in more number of selected genes. If the users are not sure what is a good Wbound, they can start to use the Tune Wbounds panel as in Figure 30.

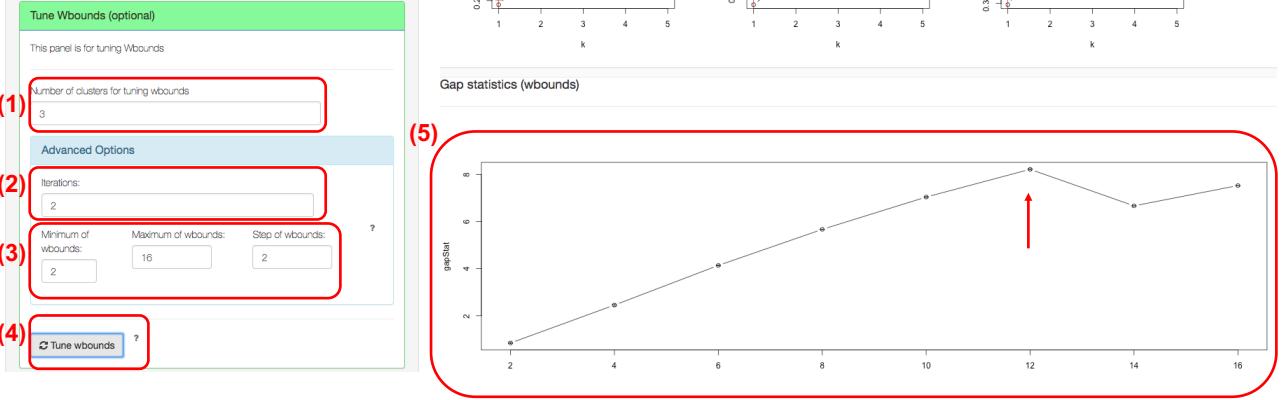


Figure 30: Wbound selection

Again, gap statistics will be used for tuning Wbounds. Users will specify number of clusters for tuning Wbounds (1), which could be obtained from the previous step. In advanced options, users can further specify the number of iterations and the range of candidate Wbounds. But if users don't know the algorithm, please leave them as default. Iterations (2) specify number of bootstrap samples for gap statistics. Users also need to specify the searching space of Wbounds by minimum of Wbounds, maximum of Wbounds and Step of Wbounds (3). After all these steps are set, user can click on "Tune Wbounds" button (4). The results will be shown in Figure 30 (5). Wbound=12 is preferred since the corresponding gap statistics is maximized (where the red arrow indicates).

Step 3 Run MetaClust:

Under Run Meta Sparse K-Means panel, user can specify the number of clusters (1), Wbounds (2) and run MetaClust (5) as in Figure 31.

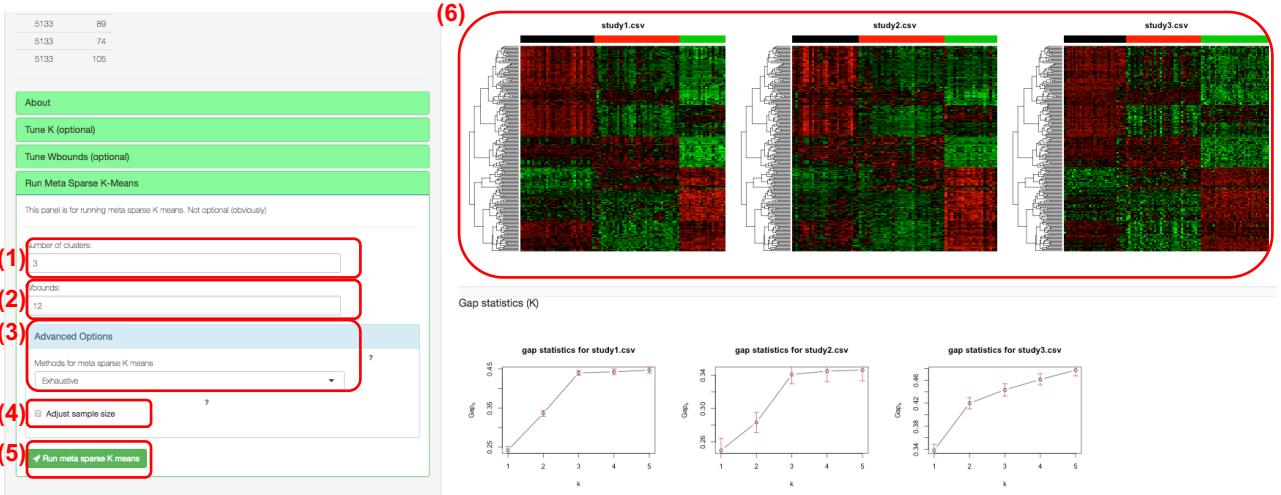


Figure 31: Result for MetaClust

In advanced options (for which users are not suggested to change if they are not familiar with the algorithm), There are three clustering matching methods (3): Exhaustive, linear, MCMC. Exhaustive is suggested if the data is not large. Currently, only Exhaustive search method is implemented. Adjust sample size checkbox (at position (5)) allows users to adjust sample size effect. After number of clusters and Wbounds are specified, users can click on Run meta sparse K means and obtain results as Figure 31.

5.6.2 Results

We used the leukemia data to demonstrate the MetaClust module. After merging the three datasets, we didn't filter out any genes (filter 0% genes by mean and 0% by variance) thus 5133 genes remained. Detailed descriptions of these studies can be found in Table 1. In this example, we do not need the extra label information. The result is shown in Figure 31 (5). We obtained unified feature selection across all studies. The clusters are well separated in each study and the cluster patterns are consistent across all studies. The clustering heatmaps and labels are saved in the MetaClust folder.

5.7 MetaPCA

Dimension reduction is a popular data mining approach for transcriptomic analysis. MetaPCA aims to combine multiple omics datasets of identical or similar biological hypothesis and perform simultaneous dimensional reduction in all studies. The results show improved accuracy, robustness and better interpretation among all studies. By clicking on Toolsets tab and then choose MetaPCA, users are directed to MetaPCA home page as Figure 32. The R package for MetaPCA module can be found at <https://github.com/metaOmics/metaPCA>.

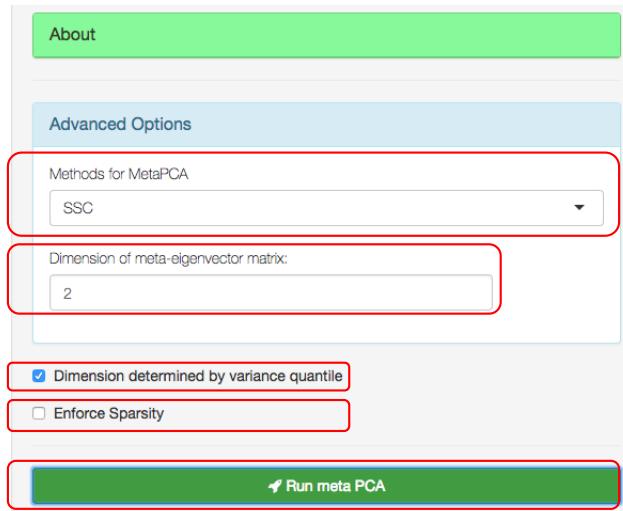


Figure 32: MetaPCA settings

5.7.1 Procedure

The procedure how to use metaPCA is described below. A complete list of options is available in Section 6.7.

Step 1 Specify parameters

There are very few parameters need to be specified for MetaPCA, as in Figure 32. Advanced options are not suggested to be changed unless the users are familiar with the algorithm. There are two methods for MetaPCA (at position (1)). SSC represents MetaPCA via sum of squared cosine (SSC) maximization. SV represents MetaPCA via sum of variance decomposition (SV). Details of SSC and SV can be found in metaPCA manuscript (Kim et al., 2017). SSC has better performance and is suggested. Dimension of meta-eigenvector matrix option (2) allows users to specify dimension of the output meta-eigenvector matrix. The checkbox of “dimension determined by variance quantile” is suggested to be checked (3). When checked, the dimension size of each study’s eigenvector matrix (SSC) is determined by the pre-defined level of variance quantile 80%. If the checkbox of “sparsity encouraged” is checked (at position (4)), users can perform MetaPCA. After clicking on search for optimal tuning parameter button, the optimum tuning parameter will be returned to the box “tuning parameter for sparsity”, which may be time consuming.

Step 2 Perform metaPCA

By clicking the “Run meta PCA” button, the MetaPCA module will be performed.

5.7.2 Results

The input dataset is the same as the input for MetaDE module. Detailed descriptions of these studies can be found in Table 1.

The result of MetaPCA is shown in Figure 33. For each study, only first two studies are visualized. The results show nice separations between three groups. These figures and eigenvectors are saved to the MetaPCA folder.

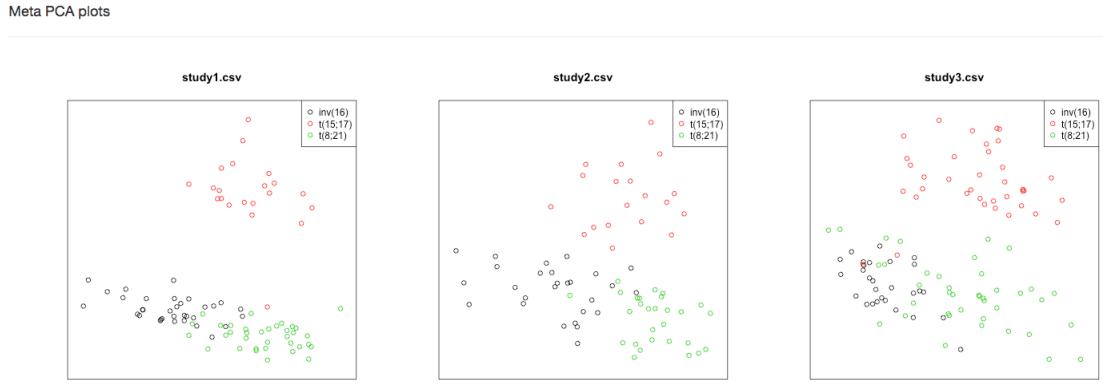


Figure 33: MetaPCA result

6 Complete list of options

MetaPreprocess

Complete List of Options:

1. Upload expression data:
 - Header: should be checked if the input file includes a header.
 - Separator: indicates what type of separator is used for the data matrix.
 - Quote for String: how is the data matrix quoted.
 - Log transforming data: if you want to perform log transformation of your data, check yes.
 - Use existing datasets: if you want to load a dataset previously uploaded, you can choose from the checklist.
2. Annotation/impute/Replicate:
 - Annotation: possible ID type can be Gene Symbol (default), Probe ID, reference sequence ID, entrez ID.
 - Impute: if selected, missing value imputation will be performed by k-nearest neighbor (KNN) algorithm.
 - Replicate Handling: if selected and if the same gene symbol maps to multiple probes, the probe with the largest interquartile range (IQR) will be selected as a representative for this gene.
3. Saved Data, Merging and Filtering Datasets:
 - Mean: the percentage of genes being filtered out based on the mean expression levels (e.g. 0.3 represent 30%).
 - Variance: the percentage of genes being filtered out based on the variance of expression (e.g. 0.3 represent 30%).
 - Study Name: dataset name after merging. This name will appear in the list of saved data table.

- Merge from Selected Datasets: perform filtering and merging.

4. Danger zone:

- Delete Selected Data: the selected data will be deleted permanently if clicked, so please be cautious.

6.1 MetaQC

Complete List of Options:

1. Options

- Perform gene filtering: If yes: cut lowest percentile by mean, cut lowest percentile by variance.
- Use adjusted p-value for selecting DE genes
- p-value cutoff for selecting DE genes
- Use adjusted p-value for selecting pathways
- p-value cutoff for selecting pathways

2. Advanced Option (**Optional):

- Pathway min gene size
- Pathway max gene size
- Number of permutations

3. Run MetaQC Analysis

6.2 MetaDE

Complete List of Options:

1. Meta Method Type: Combining p-value, Combining effect size, Others.

2. Meta Method: Fisher, AW-Fisher, FEM, REM, Sum of Rank, Product of Rank, multi-class correlation

3. Mixed data type: selected if both count data and continuous data exist.

4. Response Type:

- Two class comparison, Multi-class comparison, Continuous outcome, Survival outcome.
- Label Attribute: select the label name of the outcome.
- Control Label & Experimental Label: specify the case/control label for two-class comparison.

5. Individual Study Option:

- Setting individual study method
- Setting individual study paired option

6. Advanced Option (**Optional):

- Use complete options
- Parametric
- Covariate
- Alternative hypothesis

7. Run

8. Pathway Databases

9. Pathway Analysis Option:

- Pathway enrichment method
- Pathway min gene size
- Pathway max gene size

10. Run Pathway Analysis

6.3 MetaPath

Complete List of Options:

1. mixed data types: whether the input data is a mixture of count data and continuous data.
2. Response Type:
 - Two class comparison, Multi-class comparison, Continuous outcome, Survival outcome.
 - Label Attribute: select the label name of the outcome.
 - Control Label & Experimental Label: specify the case/control label for two-class comparison.
3. Individual Study Option:
 - Setting individual study method
 - Setting individual study paired option
4. Advanced Option (**Optional):
 - Covariate
 - Alternative hypothesis
5. Pathway Databases
6. Pathway Analysis Option:
 - Software
 - Pathway enrichment method
 - Pathway min gene size
 - Pathway max gene size
7. Run Pathway Analysis
8. Pathway Clustering Diagnostics
9. Get Clustering Result

6.4 MetaNetwork

Complete List of Options:

1. Generate Network:
 - Case Name: specify case group label.
 - Control Name: specify control group label.
 - Number of Permutations: the number of permutations used for generating network.
 - Edge Cutoff: edge cut-off determines the proportion of edges to be kept in the network.
2. Search for basic modules:
 - Number to repeat: the number of repeats used for each initial seed modules.
 - MC steps: the maximum Monte Carlo steps for simulated annealing algorithm.
 - Jaccard cutoff: maximum pairwise Jaccard index allowed for basic modules.
3. Assemble supermodules:
 - FDR cutoff: FDR cut-off to select basic modules for supermodule assembly.

Intermediate results

1. Generate Network

- `AdjacencyMatrices.Rdata` is a list of adjacency matrices for case and control subjects in each study. The order is study1 case, study2 case, ..., studyS case, study1 control, study2 control, ..., studyS control.
- `CorrelationMatrices.Rdata` is a list of correlation matrices for case and control subjects in each study.
- `AdjacencyMatricesPermutationP.Rdata` is a list of adjacency matrices for permuted datasets in permutation P.

2. Search for basic modules

- `basic_modules_summary_forward_weight_w1.csv` is a summary table of basic modules that are higher correlated in case, detected using w1.
- `basic_modules_summary_backward_weight_w1.csv` is a summary table of basic modules that are higher correlated in control, detected using w1.
- `threshold_forward.csv` is a table of number of basic modules higher correlated in case, detected under different w1 values and FDR cut-offs.
- `threshold_backward.csv` is a table of number of basic modules higher correlated in control, detected under different w1 values and FDR cut-offs.
- `permutation_energy_forward_P.Rdata` is a list of energies for basic modules that higher correlated in case, detected from permutation P.
- `permutation_energy_backward_P.Rdata` is a list of energies for basic modules that higher correlated in control, detected from permutation P.

3. Assemble supermodules

- `module_assembly_summary_weight_w1.csv` is summary table of supermodules using w1 weight.
- CytoscapeFiles folder contains the input files for Cytoscape to visualize supermodules.

6.5 MetaPredict

Complete List of Options:

1. Model trainings:

- Methods for MetaPredict: include Mean score, Fisher, Stouffer.
- Max number of top scoring pairs (K)
- Number of cores for parallel computing
- TWO labels to cluster: labels for MetaPredict
- Please select studies for training
- Please select studies for testing
- Number of top scoring pairs (K): Number of top scoring pairs (K) for prediction.

6.6 MetaClust

Complete List of Options:

1. Tune K (** optional)

- Maximum of K: the maximum number of K that gap statistics will step through.
- Top percentage by larger variance: Top percentage p% by larger variance means that we will use top p% larger variance genes to perform gap statistics.
- Number of permutations: Number of permutation is number of bootstrap samples for gap statistics.

- Select studies to be tuned: Studies to be tuned.
 - Tune K : start tuning K .
2. Tune Wbounds (** optional)
 - Number of clusters for tuning wbounds: number of clusters for tuning Wbounds.
 - Iterations: Iterations are number of bootstrap samples for gap statistics.
 - Minimum of wbounds: lower bound of the searching space of Wbounds.
 - Maximum of wbounds: upper bound of the searching space of Wbounds.
 - Step of of wbounds: stepsize of the searching space of Wbounds.
 - Tune wbounds: start tuning wbounds.
 3. Run Meta Sparse K -means:
 - Number of clusters: number of clusters. Can be tuned from Tune K option.
 - Wbounds: control numbers of selected features. Can be tuned from Tune Wbounds option.
 - Methods for MetaClust: Exhaustive is suggested if the data is not large. Linear will perform smart search and get solution much faster than Exhaustive, but it may yield less accuracy. MCMC might be very time consuming.
 - Adjust sample size: adjust sample size effect.
 - Run meta sparse Kmeans: start tuning wbounds.

6.7 MetaPCA

Complete List of Options:

1. Common MetaPCA parameters:
 - Methods for MetaPCA: SSC represent MetaPCA via sum of squared cosine (SSC) maximization. SV represent MetaPCA via sum of variance decomposition (SV).
 - Dimension of meta-eigenvector matrix: dimension of the output meta-eigenvector matrix.
 - Dimension determined by variance quantile: the dimension size of each study's eigenvector matrix (SSC) is determined by the pre-defined level of variance quantile 80%.
2. If sparsity encouraged is selected, there are extra tuning parameter (λ) that may need to be tuned.
 - Min λ : lower bound of the searching space of λ .
 - Max λ : upper bound of the searching space of λ .
 - Step of of λ : stepsize of the searching space of λ .
 - Tuning parameter for sparsity: Tuning parameter for sparsity that will be used for sparse MetaPCA.

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