

A tutorial for metaOmic

Contents

1 Introduction

MetaOmics is a GUI for meta-analysis implemented using R shiny. Current version includes MetaQC for quality control, MetaDE for differential expression analysis, MetaPath for pathway enrichment analysis, MetaClust for sparse clustering analysis, MetaPCA for principal component analysis, MetaKTSP for classification analysis, MetaDCN for differential co-expression network analysis, MetaLA for liquid association analysis.

In this tutorial, we will go through installation and usage step by step using a real example.

The metaOmics suit software is publicly available at <https://github.com/metaOmic/metaOmics>. Individual R packages are also available on GitHub and the url will be introduced in each individual package section.

2 Preliminaries

2.1 Citing MetaOmics

MetaOmics implements many meta-analytic methodology by their authors. Please cite appropriate papers when you use result from MeteOmics suit, by which the authors will receive professional credit for their work.

- MetaOmics suit itself can be cited as:
- 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, R. A. (1925). *Statistical methods for research workers*. Genesis Publishing Pvt Ltd.
 - Li, J., Tseng, G. C., et al. (2011). An adaptively weighted statistic for detecting differential gene expression when combining multiple

transcriptomic studies. *The Annals of Applied Statistics*, 5(2A):994–1019.

- 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.
- and many more

- MetaPath:

- Shen, K. and Tseng, G. C. (2010). Meta-analysis for pathway enrichment analysis when combining multiple genomic studies. *Bioinformatics*, 26(10):1316–1323.
- .

- MetaClust: 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: not published yet.
- MetaKTSP: not published yet.
- MetaDCN: not published yet.
- MetaLA: not published yet.

2.2 Installation

The full instruction of how to install, start are available at <https://github.com/metaOmic/metaOmics>.

2.2.1 Requirement

- R >= 3.3.1
- Shiny >= 0.13.2

2.2.2 How to start the app

- First, clone the project
- git clone <https://github.com/metaOmic/metaOmics>
- in R (suppose the application directory is metaOmics),
> install.packages('shiny')
> shiny::runApp('metaOmics', port=9987, launch.browser=T)

2.2.3 How to start the documentation

- Install rmarkdown for R
- Inside ‘doc’ directory, start R console, and:
- in R

```
1 rmarkdown::run(shiny_args=list(port=9988, launch.browser=T))
```

- or in command line

```
1 R -e "rmarkdown::run(shiny_args=list(port=9988, launch.browser =T))"
```

If you run into an issue with something like ‘pandoc version 1.12.3 or higher is required and was not found.?’, just install pandoc manually. For example, on Mac, it would be ‘brew install pandoc’. If you have Rstudio, you can also get rstudio’s pandoc environment. Go to rstudio console and find the system environment variable for ‘RSTUDIO_PANDOC’

In R:

```
1 Sys.getenv("RSTUDIO_PANDOC")
```

2.3 Question and bug report

Ask Anzhe what is the appropriate way to maintain the package?

3 Prepare data

3.1 Raw data

Data should be prepared as the example in Figure ???. First column should be feature ID (e.g. gene symbol) and the rest of the columns are samples. The first row is sample ID. Valid data type includes continuous, count.

The screenshot shows a Microsoft Excel spreadsheet with the following structure:

	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	13.6644626	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	14.0007759
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	14.1997062	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	13.8835649	13.6857053	13.4025025
11	RPS18	14.1950914	13.2245529	13.8789651	13.9155682	13.9672183	13.8135139	14.1093296	14.3927609	14.3095881	13.8317787
12	RPL37	13.7058004	12.8119102	13.3801223	13.5777508	13.6655865	13.4866264	13.5917687	13.8567646	13.7736878	13.3617574
13	RPL30	13.4054998	12.1211517	13.2228422	13.383714	13.2426155	13.250811	13.4838896	13.7547287	13.5276746	13.101915
14	RPS4X	13.8333138	13.0225864	13.5383624	13.7282801	13.300111	13.3981243	13.7100845	13.9321655	13.7211005	13.5440807
15	RPL32	13.9604926	12.8106502	13.6758375	13.7287171	13.7165548	13.594741	13.9769265	14.0313074	13.9445242	13.3819729
16	TMSB4X	13.3246885	12.1018215	13.1277736	13.3929776	13.9258423	13.5067522	12.9406726	13.7856005	13.8576944	12.8216926
17	RPS17	14.004012	12.8680591	13.7092862	13.7209076	13.472394	13.3000626	13.6710495	14.0922747	13.9272016	13.5751354
18	RPL9	13.7682089	12.7355572	13.4851269	13.6074655	13.3794251	13.3715674	13.6789654	14.0369392	13.7989794	13.3794219
19	RPL11	13.1068926	11.8041819	12.959188	13.2304038	12.6737969	12.8629437	13.2297796	13.531635	13.3865164	12.8034242
20	RPL3	13.1003076	11.2308104	12.6676873	12.856598	11.8035135	12.066841	12.5966984	13.0618903	12.6732755	12.4201737
21	TMSB10	13.4992692	12.4847027	13.3053195	13.9229064	13.4893535	13.4303906	13.1984362	13.2277138	13.676856	12.8385526
22	UBC	12.6877469	11.2673769	12.428891	12.6531995	12.8093268	13.0569176	12.772718	13.1046039	12.4465834	12.4462248
23	RPL34	13.6748654	12.6004251	13.435718	13.5799487	13.4795839	13.4485159	13.715027	13.9986572	13.7915361	13.4117338
24	RPS3	13.377261	11.6797357	13.2251255	13.2240022	12.8373728	12.4130461	13.1883117	13.57352	13.3897875	12.9368834
25	GAPDH	11.7615563	10.6091352	12.090135	12.7600258	12.0082746	12.6371621	13.0494016	12.9957249	12.7918573	12.375633
26	UBB	12.9585862	11.8361919	12.7529098	12.6796118	12.394406	11.9336763	12.8433033	13.1560767	12.7851394	12.6930262
27	MPO	11.7578693	10.2667543	11.9584299	12.5560562	10.8735194	11.2210145	10.6698364	12.7304432	12.0959163	11.807057
28	RPL19	13.241946	11.4920457	12.95958	12.9573326	12.4867549	12.8390422	12.8650221	13.2425224	13.0159003	12.5675945
29	RPL6	13.1265705	11.7239338	13.063908	13.2136254	12.6273555	12.8178965	12.9838201	13.3099411	13.1238109	12.7874825
30	EEF2	12.1472604	9.70071474	11.7571483	12.0628499	11.3676495	11.69021	11.7508785	12.2203233	11.7522107	11.5744148

Figure 1: A example data format

3.2 Clinical data

Clinical data should be prepared as the example in Figure ???. First column should be sample ID and each row represents a sample. The rest of the columns are clinical information.

	A	B	C	D	E
1	label				
2	GSM445939	inv(16)			
3	GSM445940	inv(16)			
4	GSM445952	inv(16)			
5	GSM445965	inv(16)			
6	GSM445966	inv(16)			
7	GSM445995	inv(16)			
8	GSM446005	inv(16)			
9	GSM446015	inv(16)			
10	GSM446019	inv(16)			
11	GSM446020	inv(16)			
12	GSM446030	inv(16)			
13	GSM446032	inv(16)			
14	GSM446033	inv(16)			
15	GSM446035	inv(16)			
16	GSM446036	inv(16)			
17	GSM446037	inv(16)			
18	GSM446038	inv(16)			
19	GSM446039	inv(16)			
20	GSM446047	inv(16)			
21	GSM446056	inv(16)			
22	GSM446088	inv(16)			
23	GSM446102	inv(16)			
24	GSM446119	inv(16)			
25	GSM446120	inv(16)			
26	GSM446127	inv(16)			
27	GSM446143	inv(16)			
28	GSM446147	inv(16)			
29	GSM445923	t(15;17)			
30	GSM446023	t(15;17)			
31	GSM446027	t(15;17)			

Figure 2: A example clinical data format

4 Toolsets

4.1 MetaQC

4.2 MetaDE

MetaDE package implements 12 major meta-analysis methods for differential expression analysis, and now it allows the analysis of both microarray and RNA-seq data. In this tutorial, we will demonstrate the MetaDE pipeline step by step using two meta-analysis methods: Fisher’s method and Adaptively weighted Fisher’s method (AW-Fisher). Please refer to ? and ? for details of these two methods. Individual MetaDE package is also available on GitHub at <https://github.com/metaOmic/MetaDE>.

4.2.1 Meta analysis

After opening the MetaDE page, as shown in Figure ??, there are 2 drop-down menus (“Meta Method Type” and “Meta Method”) and 4 tabs on the left of the page (“Response Type”, “Setting Individual Study Method”, “Advanced Options” and “Run”). We generally suggest users not to change any parameter setting in the “Advanced Options” unless users know the underlying methodology well.

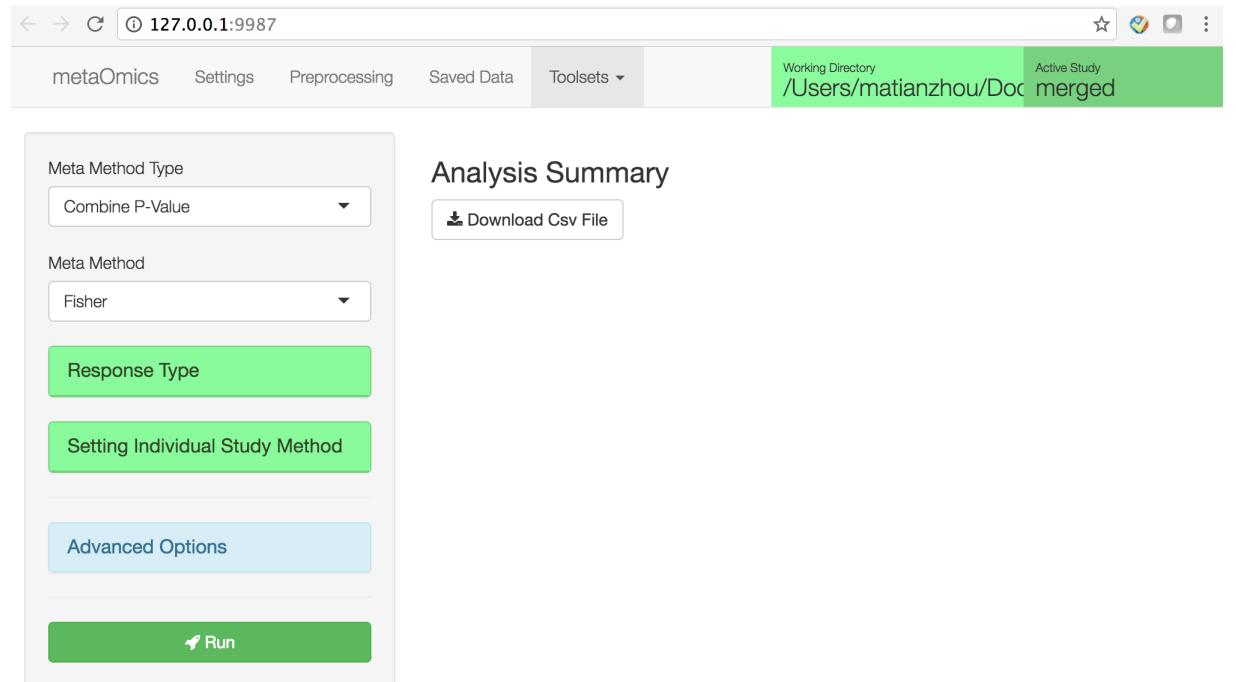


Figure 3: Homepage of MetaDE

For Fisher’s method, we will choose “Combine P-value” and “Fisher” (Figure ??); and for AW Fisher’s method, we will choose “Combine P-value” and “AW Fisher” (Figure ??).

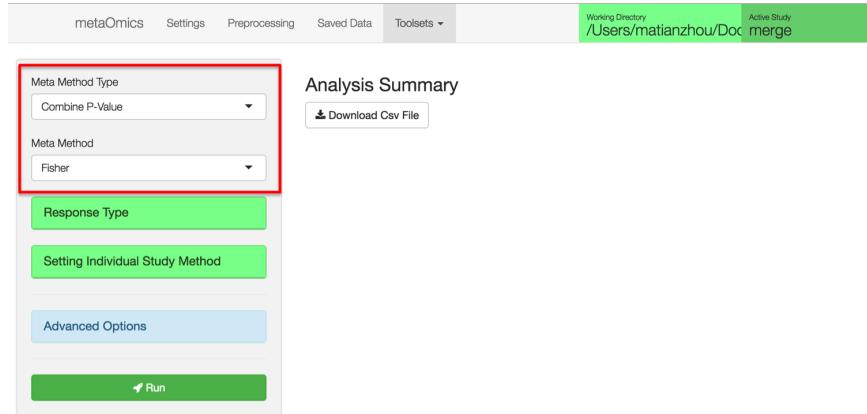


Figure 4: Fisher's method setting

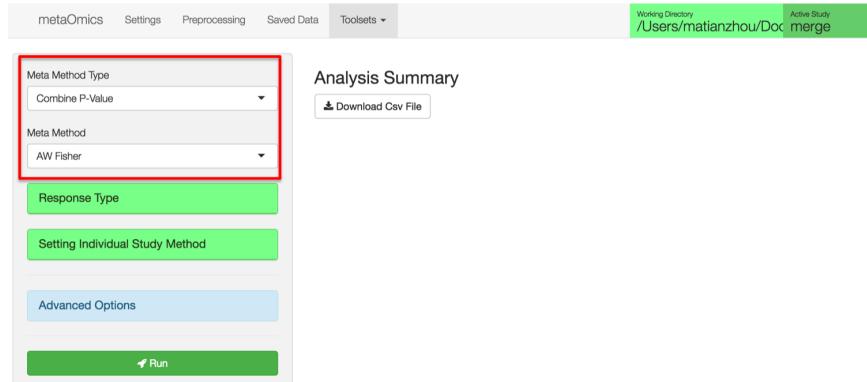


Figure 5: AW Fisher's method setting

Then, in the next step, we click on “Response Type”, for two-class DE analysis, choose “Two Class Comparison”, choose the group label name for the Label Attribute (from the column names of your clinical data). Then for the group label (a factor of at least two levels), choose a name for the “Control Label” and “Experimental Label”, respectively (Figure ??).

Two Class Comparison

Label Attribute: label

Control Label: inv(16)

Experimental Label: t(15;17)

Setting Individual Study Method

Advanced Options

Run

Figure 6: Response type setting

Next, we click on “Setting Individual Study Method” and choose “LIMMA” to perform DE analysis in each individual study (Figure ??). Available options include “LIMMA” and “SAM” for continuous data (e.g. microarray), “edgeR”, “DESeq2” and “limmaVoom” for discrete data (e.g. RNA-seq count). Details of the above mentioned DE methods can be found in XXX (reference).

study1.csv

LIMMA (Linear Model for Microarray data)

study2.csv

LIMMA (Linear Model for Microarray data)

study3.csv

LIMMA (Linear Model for Microarray data)

Advanced Options

Run

Figure 7: Individual study DE analysis method

** Optionally, we can click on “Advanced Options”, choose “Parametric=yes” so parametric methods will be used for inference. We can choose whether to

adjust for any important “Covariates” (e.g. potential confounders) and we set the alternative hypothesis to be “abs”, i.e. two-sided test (Figure ??).

Advanced Options

Use complete options
 Yes No

Parametric
 No Yes

Covariate:
 None

Alternative Hypothesis:
 abs

Figure 8: Advanced Options

After we click on “Run”, we will see a summary table generated on the right of the page as shown in Figure ???. The “Analysis Summary” includes the analysis results of all genes, including individual study statistics/p-value, meta-analysis statistics/p-value/FDR, etc. Users can search the gene name in the “Search” bar, and download the full table as a csv file by clicking on “Download Csv File”.

metaOmics Settings Preprocessing Saved Data Toolsets Working Directory /Users/matianzhou/Doc merge Active Study

	ind.stat.study1.csv	ind.stat.study2.csv	ind.stat.study3.csv	ind.p.study1.csv
COX1	0.0126812082556715	-0.0352823378450787	-0.0404256450138021	0.26892893736200
COX2	0.012086567005725	-0.0170330767053708	-0.0479332961282468	0.28994810474082
ND4	0.0115738612113951	-0.014945675881802	-0.0211095447931435	0.32474691664235
RPL41	-0.0160595156310522	-0.0707063583417713	-0.00180747617572272	0.16285867176410
RPS2	0.00377184273132259	-0.06680558060008396	0.000591323212125763	0.73724578494079
RPL23A	-0.0183077872427684	-0.0838415811836129	-0.0106412941765993	0.10654656869934
TPT1	-0.00071750453329344	-0.0539417110424626	0.017762992241745	0.94846427751892
RPL39	-0.010755961519947	-0.0616472700124989	0.00807756500336784	0.33301945214041

Figure 9: Summary table of Meta-analysis results

4.2.2 Visualization

In addition to tabular output, for better visualization, users can also plot heatmap of DE genes at specified FDR cutoff. Users can enter the “FDR Cutoff” (number of genes selected are shown interactively), then click on “Plot DE Genes Hetamap” to draw the heatmap. The “image size” can be adjusted by dragging the scroll bar (Figure ??).

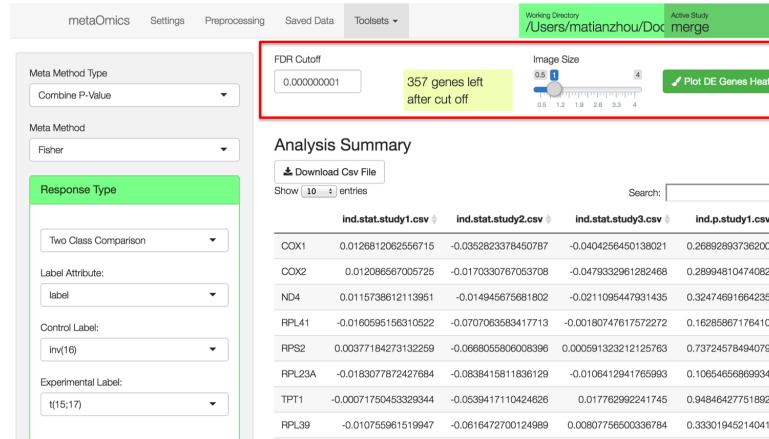


Figure 10: Plot heatmap setting

Shown in Figure ?? and Figure ?? are two heatmaps generated from Fisher and AW Fisher results. Note that one additional column of weight distribution across studies is added in the heatmap of AW-Fisher, and all genes in the heatmap are sorted based on their weight distribution.

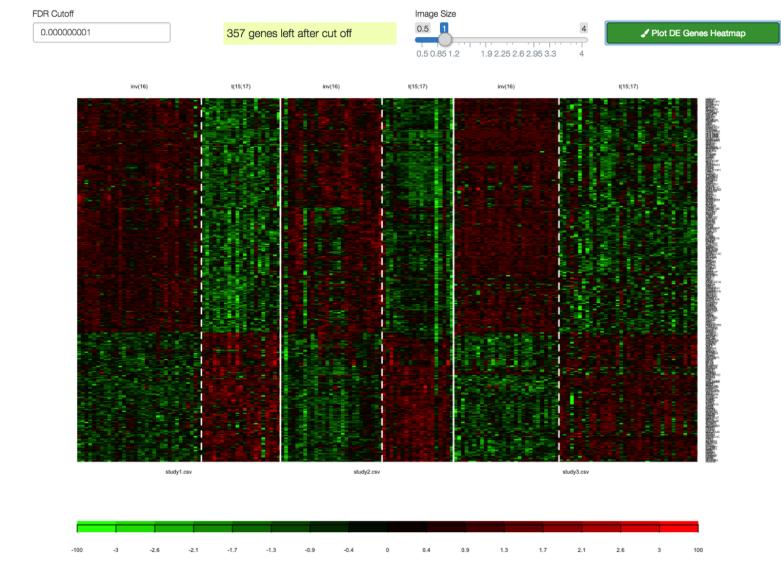


Figure 11: Heatmap based on Fisher results

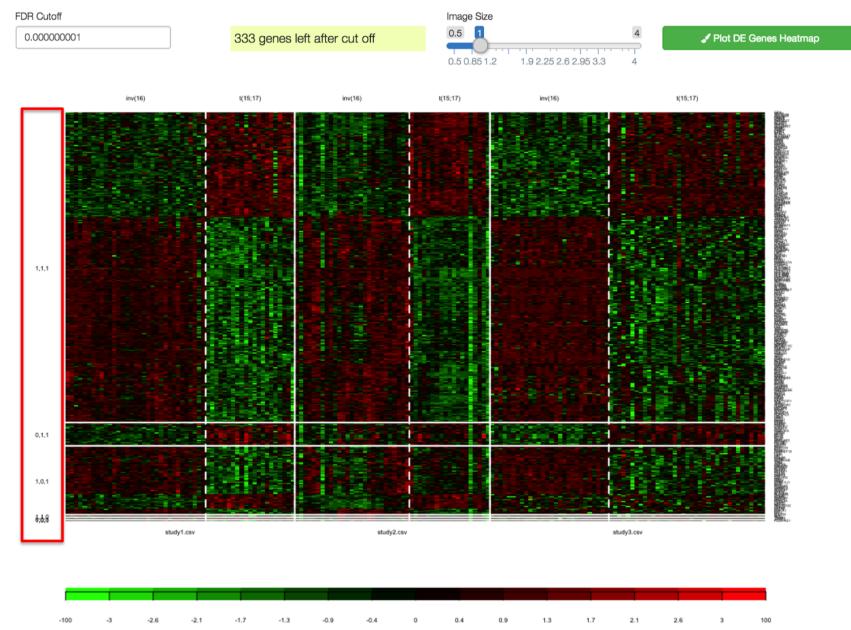


Figure 12: Heatmap based on AW Fisher results

4.2.3 Downstream pathway analysis

Upon getting the meta differential analysis results (users have to perform meta DE analysis first before pathway analysis), we will further perform downstream pathway analysis. As shown in Figure ??, we first need to choose the Pathway databases to be used as shown in the highlighted box. Next we will choose the pathway enrichment method, the default is the Kolmogorov-Smirnov test (KS test, Figure ??). Alternatively, one can choose to use Fisher's exact test, once this method is selected, you need to pick a DE gene set using a hard threshold, either by p-value cutoff or the number of top ranked genes (Figure ??). Lastly, we will specify the minimum/maximum pathway size of pathways we wish to include for functional analysis, then click “Run Pathway Analysis”.

The screenshot shows a user interface for pathway analysis. On the left, a sidebar titled "Pathway Dayabases:" lists several options: KEGG GO Biological Process, GO Cellular Component, GO Molecular Function, Reactome, and BioCarta. The "BioCarta" option is highlighted with a red box. To the right, there is a table of pathway analysis results and a "Pathway Analysis Options" panel.

	TPT1	-0.00071750453329344	-0.0539417110424626	0.017762992241745	0.94846
RPL39	-0.010755961519947	-0.0616472700124989	0.00807756500336784	0.33301	
RPS18	-0.0200442322714253	-0.0718688592766959	-0.00513492219189818	0.096352	
ND2	0.0104555540876066	0.00327594223827794	-0.0410391011942624	0.35294	

Showing 1 to 10 of 2,515 entries Previous 1 2 3 4 5 ... 252 Next

[Download Csv File of Pathway Result](#)

Pathway Analysis Options

Pathway Enrichment Method: Kolmogorov-Smirnov test

pathway min gene size: 15

pathway max gene size: 500

Run Pathway Analysis

Figure 13: Selection of pathway database

Pathway Dayabases:

- KEGG GO Biological Process
- GO Cellular Component
- GO Molecular Function
- Reactome
- BioCarta

Pathway Analysis Options

Pathway Enrichment Method:

pathway min gene size

pathway max gene size

TPT1	-0.00071750453329344	-0.0539417110424626	0.017762992241745	0.94846	
RPL39	-0.010755961519947	-0.0616472700124989	0.00807756500336784	0.33301	
RPS18	-0.0200442322714253	-0.0718668592766959	-0.00513492219189618	0.096352	
ND2	0.0104555540876066	0.00327594223827794	-0.0410391011942624	0.35294	

Showing 1 to 10 of 2,515 entries Previous ... Next

Figure 14: KS test setting

Pathway Analysis Options

Pathway Enrichment Method:

p-value cutoff

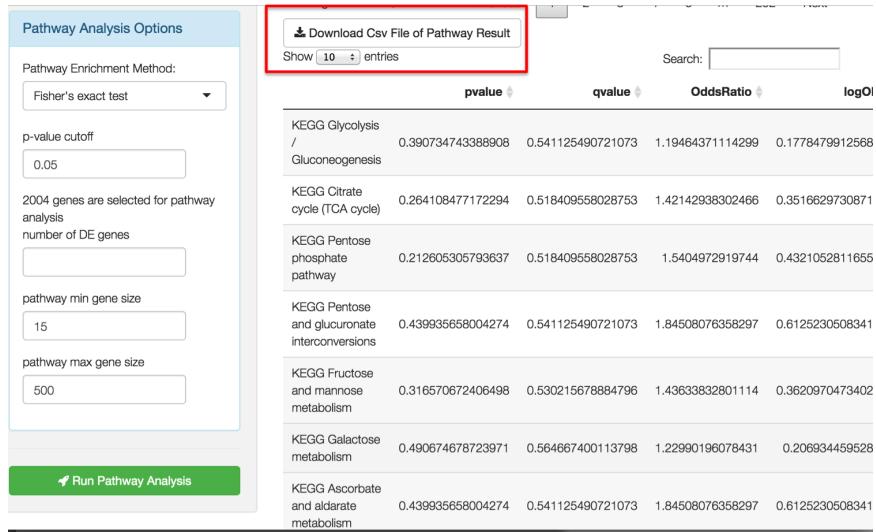
2004 genes are selected for pathway analysis
 number of DE genes

pathway min gene size

pathway max gene size

Figure 15: Fisher's exact test setting

We will then see a summary table of pathway analysis results generated on the right of the page as shown in Figure ???. Users can search the pathway name in the “Search” bar, and download the full table as a csv file by clicking on “Download Csv File of Pathway Result” as highlighted.



The screenshot shows the 'Pathway Analysis Options' interface. On the left, there are input fields for 'Pathway Enrichment Method' (set to 'Fisher's exact test'), 'p-value cutoff' (set to '0.05'), 'number of DE genes' (set to '2004'), 'pathway min gene size' (set to '15'), and 'pathway max gene size' (set to '500'). A green 'Run Pathway Analysis' button is at the bottom. On the right, a table displays pathway results with columns: pvalue, qvalue, OddsRatio, and logO. The first row shows KEGG Glycolysis / Gluconeogenesis with pvalue 0.390734743388908, qvalue 0.541125490721073, OddsRatio 1.19464371114299, and logO 0.1778479912566. Other rows include KEGG Citrate cycle (TCA cycle), KEGG Pentose phosphate pathway, KEGG Pentose and glucuronate interconversions, KEGG Fructose and mannose metabolism, KEGG Galactose metabolism, and KEGG Ascorbate and aldarate metabolism.

	pvalue	qvalue	OddsRatio	logO
KEGG Glycolysis / Gluconeogenesis	0.390734743388908	0.541125490721073	1.19464371114299	0.1778479912566
KEGG Citrate cycle (TCA cycle)	0.264108477172294	0.518409558028753	1.42142938302466	0.3516629730871
KEGG Pentose phosphate pathway	0.212605305793637	0.518409558028753	1.5404972919744	0.4321052811655
KEGG Pentose and glucuronate interconversions	0.439935658004274	0.541125490721073	1.84508076358297	0.6125230508341
KEGG Fructose and mannose metabolism	0.316570672406498	0.530215678884796	1.43633832801114	0.3620970473402
KEGG Galactose metabolism	0.490674678723971	0.564667400113798	1.22990196078431	0.206934459528
KEGG Ascorbate and aldarate metabolism	0.439935658004274	0.541125490721073	1.84508076358297	0.6125230508341

Figure 16: Summary of pathway analysis result

4.3 MetaPath

4.3.1 Run pathway analysis

After opening the MetaPath page, as shown in Figure ??, there are 3 main steps to implement MetaPath. We generally suggest users not to change any parameter setting in “Advanced” and “Advanced Options” unless users know the underlying methodology well.

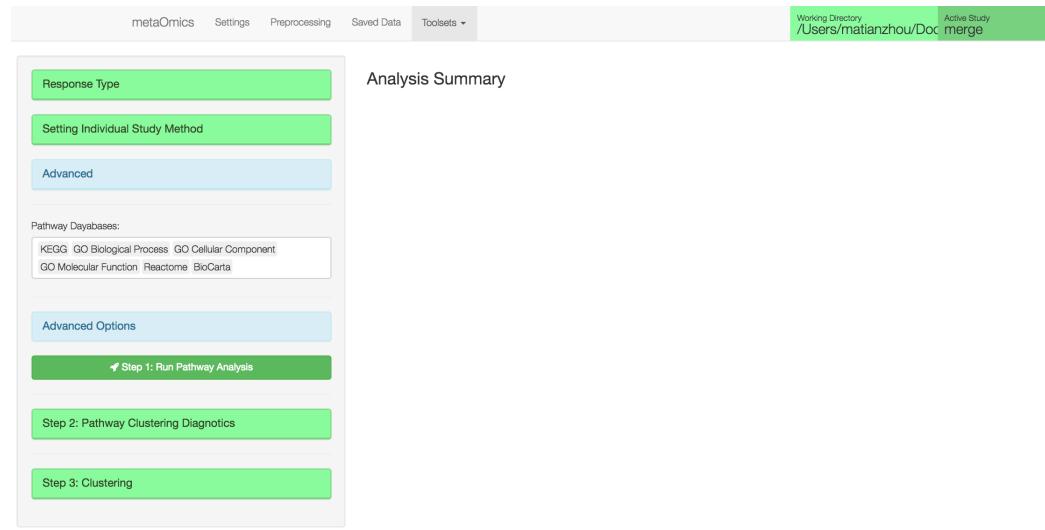


Figure 17: Homepage of MetaPath

To start, we need to perform DE (association) analysis in each individual study before performing pathway analysis for functional annotation. We click on “Response Type”, for two-class DE analysis, choose “Two Class Comparison”, choose the group label name for the Label Attribute (from the column names of your clinical data). Then for the group label (a factor of at least two levels), choose a name for the “Control Label” and “Experimental Label”, respectively (Figure ??).

The screenshot shows the 'Response Type' settings dialog box. A red box highlights the input fields for 'Two Class Comparison', 'Label Attribute', 'Control Label', and 'Experimental Label'. The 'Label Attribute' field contains 'label', 'Control Label' contains 'inv(16)', and 'Experimental Label' contains 't(15;17)'.

Setting	Value
Two Class Comparison	Selected
Label Attribute:	label
Control Label:	inv(16)
Experimental Label:	t(15;17)

Figure 18: Response type setting

Then we click on “Setting Individual Study Method” and choose “LIMMA” to perform DE analysis in each individual study (Figure ??). Available options include “LIMMA” and “SAM” for continuous data (e.g. microarray), “edgeR”, “DESeq2” and “limmaVoom” for discrete data (e.g. RNA-seq count). Details of the above mentioned DE methods can be found in XXX (reference).

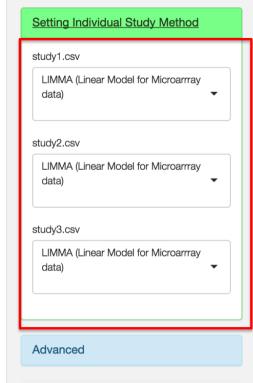


Figure 19: Individual study DE analysis method

Optionally, we can click on “Advanced” and choose whether to adjust for any important “Covariates” (e.g. potential confounders) and we set the alternative hypothesis to be “abs”, i.e. two-sided test (Figure ??).

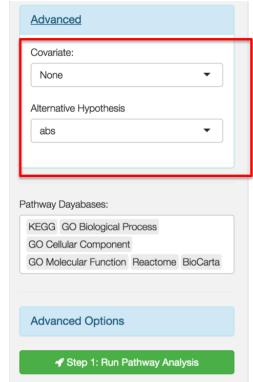


Figure 20: Advanced Options for single study association analysis

Next, we need to specify the parameters for pathway analysis. As shown in Figure ??, we first need to choose the Pathway databases to be used as shown in the highlighted box. Then optionally, we can click on the “Advanced Options” tab and choose the software to be used: CPI or MAPE. For CPI, we

can use the Kolmogorov-Smirnov test (Figure ??). CPI implements AW Fisher's method for meta p-value method in order to see both consensus and differential enrichment patterns. Alternatively, one can choose to use Fisher's exact test, once this method is selected, we need to pick the number of top ranked genes as the DE gene set for pathway analysis (Figure ??). For MAPE, the two tests are also available. There is one additional option of meta p-value method, available options include Fisher's method, maxP, minP, rOP and AW Fisher' method (Figure ??). When rOP is selected, additional option for order statistic of p-value (rth ordered p-value) will be available. Instead of closed-form distributions, users can use permutation of gene labels to get pathway p-value forthe Kolmogorov-Smirnov test (Figure ??). When "Permutation to get p-value" is choosen to be "YES", additional option for number of permutations will be available. However, we generally suggest users not to run permutation because it may take up to hours to compute. Lastly, we will specify the minimum/maximum pathway size of pathways we wish to include for functional analysis, then click "Step 1: Run Pathway Analysis".

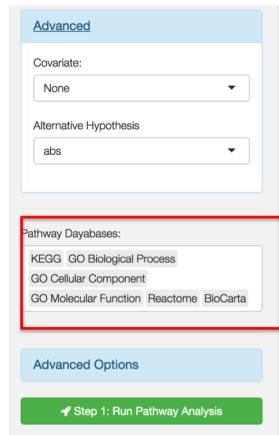


Figure 21: Selection of pathway database

Advanced Options

Software: CPI (Comparative Pathway Integrator)

Pathway Enrichment Method: Kolmogorov-Smirnov test

Permutation to get p-value
 YES
 No

pathway min gene size: 15

pathway max gene size: 500

Step 1: Run Pathway Analysis

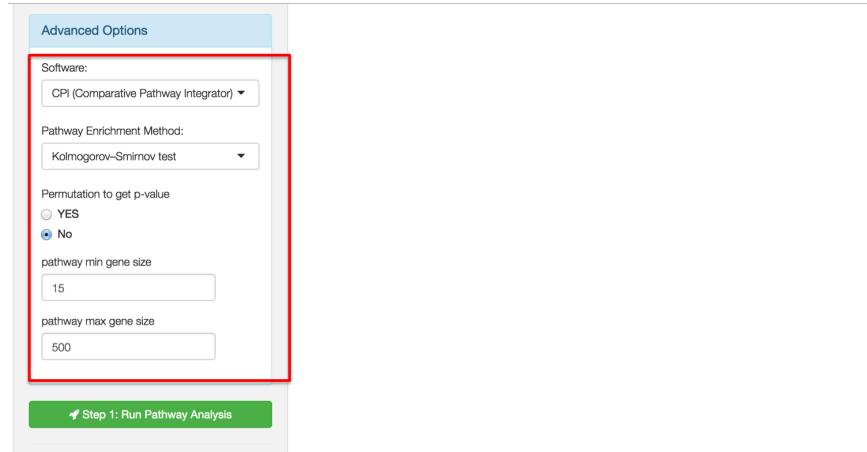


Figure 22: CPI setting

Advanced Options

Software: CPI (Comparative Pathway Integrator)

Pathway Enrichment Method: Fisher's exact test

number of DE genes: 500

pathway min gene size: 15

pathway max gene size: 500

Step 1: Run Pathway Analysis

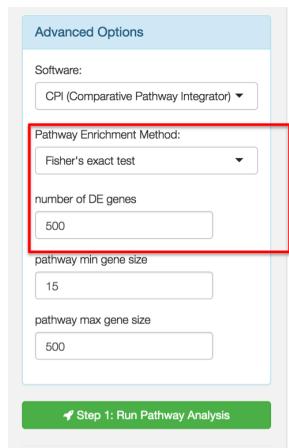


Figure 23: Fisher's Exact Test

Advanced Options

Software: MAPE (Meta Analysis Pathway Enrichment)

meta p-value method: Fisher's method

Pathway Enrichment Method: Kolmogorov-Smirnov test

Permutation to get p-value: YES No

pathway min gene size: 15

pathway max gene size: 500

Step 1: Run Pathway Analysis

Figure 24: MAPE setting

We will see a summary table generated on the right of the page as shown in Figure ?? (based on the default CPI method). The “Analysis Summary” includes the analysis results of all pathways, including individual study association analysis p-value, meta pathway analysis p-value/FDR, etc. Users can search the gene name in the “Search” bar, and the full table is automatically saved in the working directory specified before.

Analysis Summary

Show: 10 entries Search:

	q_value_meta	p_value_meta	study1.csv	study2.csv	st
KEGG Glycolysis / Gluconeogenesis	0.999891275288248	0.964947989936307	0.768190803653848	0.676076162705849	0.7284606
KEGG Citrate cycle (TCA cycle)	0.891811044872201	0.175518611360224	0.057352554842899	0.970668185433847	0.8046806
KEGG Pentose phosphate pathway	0.7913969668697601	0.116064687783132	0.150287056538356	0.805413213928083	0.03869676
KEGG Pentose and glucuronate interconversions	0.951646614020744	0.2711446005587264	0.557913386174218	0.0956407642622913	0.4242034
KEGG Fructose and mannose metabolism	0.936196204581523	0.218775012548455	0.41175995909966	0.0741963837197967	0.3142786
KEGG Galactose metabolism	0.802434783787353	0.122637091749799	0.0521931958940879	0.906116662919474	0.1200277
KEGG Ascorbate and aldarate metabolism	0.999891275288248	0.560695412961379	0.741610716462521	0.240307883811889	0.4251336

Figure 25: Summary table of CPI analysis results

4.3.2 Pathway clustering

In the next step, we performed clustering diagnostics of pathways at specified FDR cutoff (Figure ??). After clicking on “Pathway Cluster Diagnostics”, we will see two plots generated on the right panel: consensus CDF and Delta area plots (Figure ??), we can use these two plots to determine the optimal number of clusters, to be used in the third clustering step.



Figure 26: Pathway clustering diagnostics

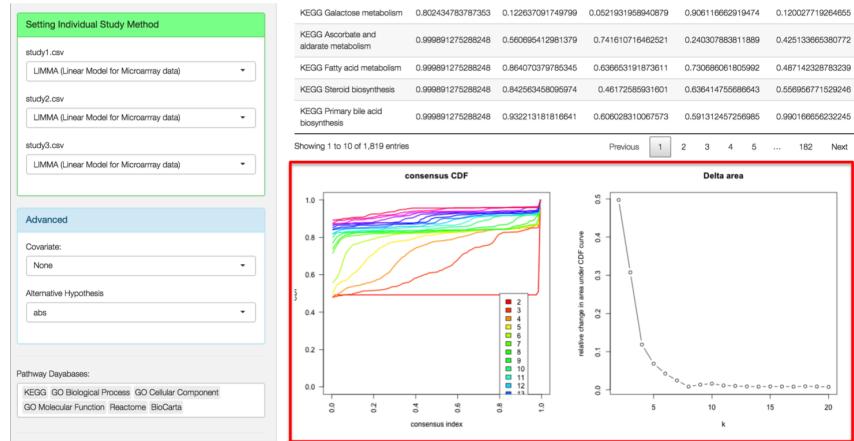


Figure 27: Diagnostic plots

4.4 MetaClust

By clicking toolsets and then metaClust, users are directed to metaClust home page as Figure ??.

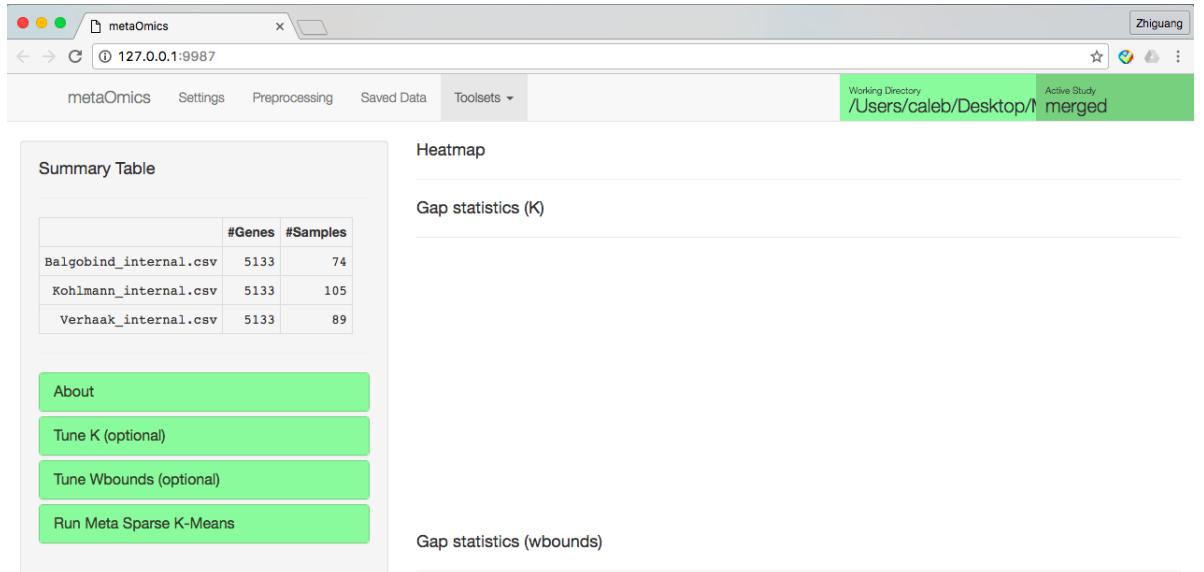


Figure 28: GUI Preprocessing page

On the top left panel users can see data summary Table. Below there are 4 tabs.

4.4.1 About

About tab includes basic introduction of metaClust. Starting with multiple studies, we could run MetaSparseKmeans 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 and Tune Wbounds panel.

4.4.2 Tune K

If the users are not sure what is number of clusters, they can start to use the Tune K panel as in Figure ??.

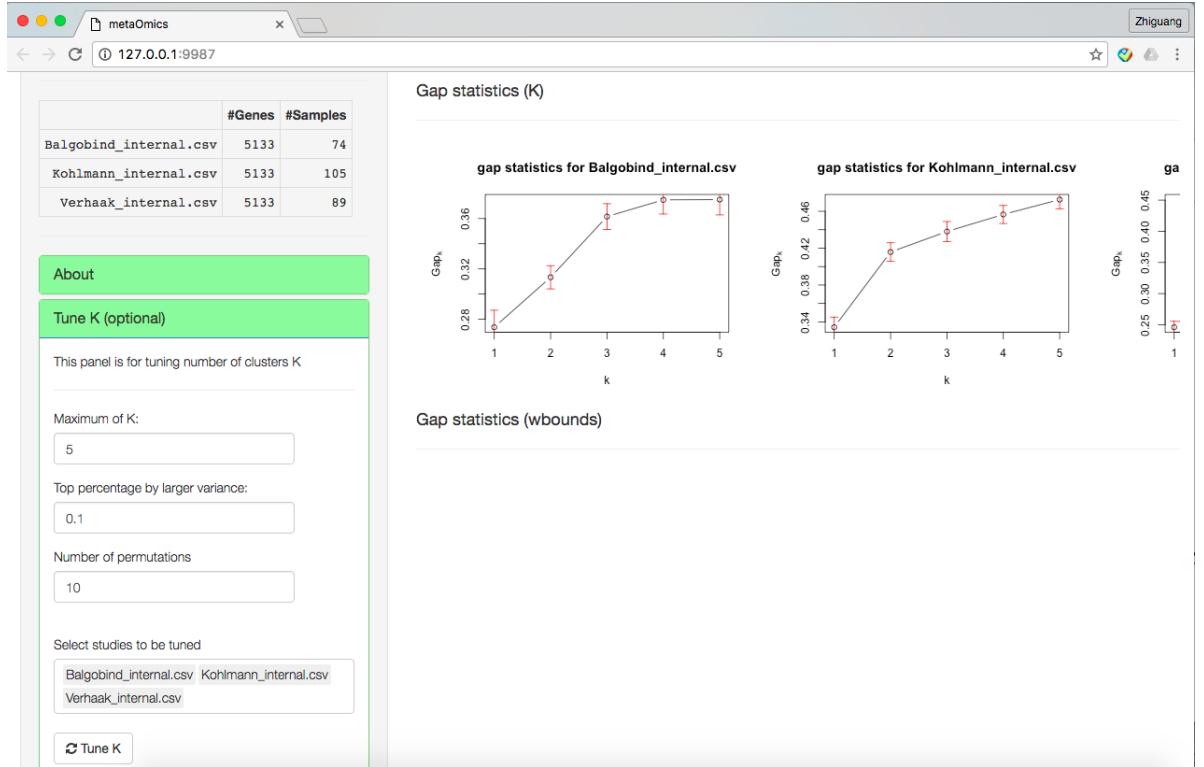


Figure 29: GUI Preprocessing page

Users will use gap statistics to get optimal K for each individual study. Users need to specify maximum number of K, which the algorithm will search number of studies from 1 to K. Top percentage p% by larger variance means that we will use top p% larger variance genes to perform gap statistics. Number of permutation is number of bootstrap samples for gap statistics. After selecting studies to be tuned and clicking button “Tune K”, we will obtain gap statistics plot as in Figure ???. A good K is selected such that the Gap_k is maximized or stabilized. From the figure, K=3 is preferred.

4.4.3 Tune Wbounds

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

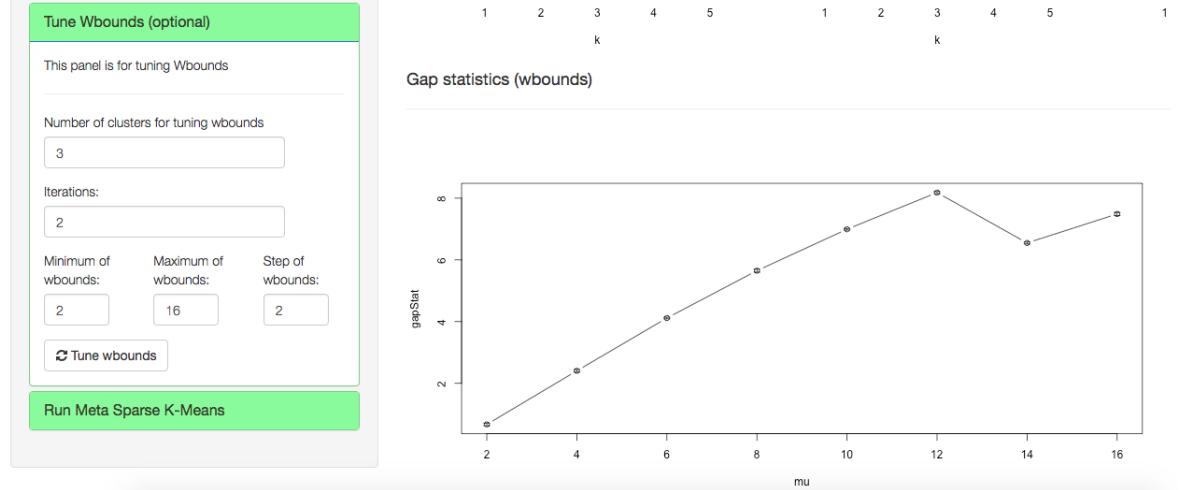


Figure 30: GUI Preprocessing page

Again, gap statistics will be used for tuning Wbounds. Users will specify number of clusters for tuning Wbounds, which could be obtained from the previous step. Iterations is the same thing as 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. After all these steps are set, user can click on “Tune Wbounds” button. The results will be shown in Figure ???. Wbound=12 is preferred since the corresponding gap statistics is maximized.

4.4.4 Run Meta Sparse K-Means

Under Run Meta Sparse K-Means panel, user can specify number of clusters, Wbounds and run meta sparse K means, as in Figure ??.

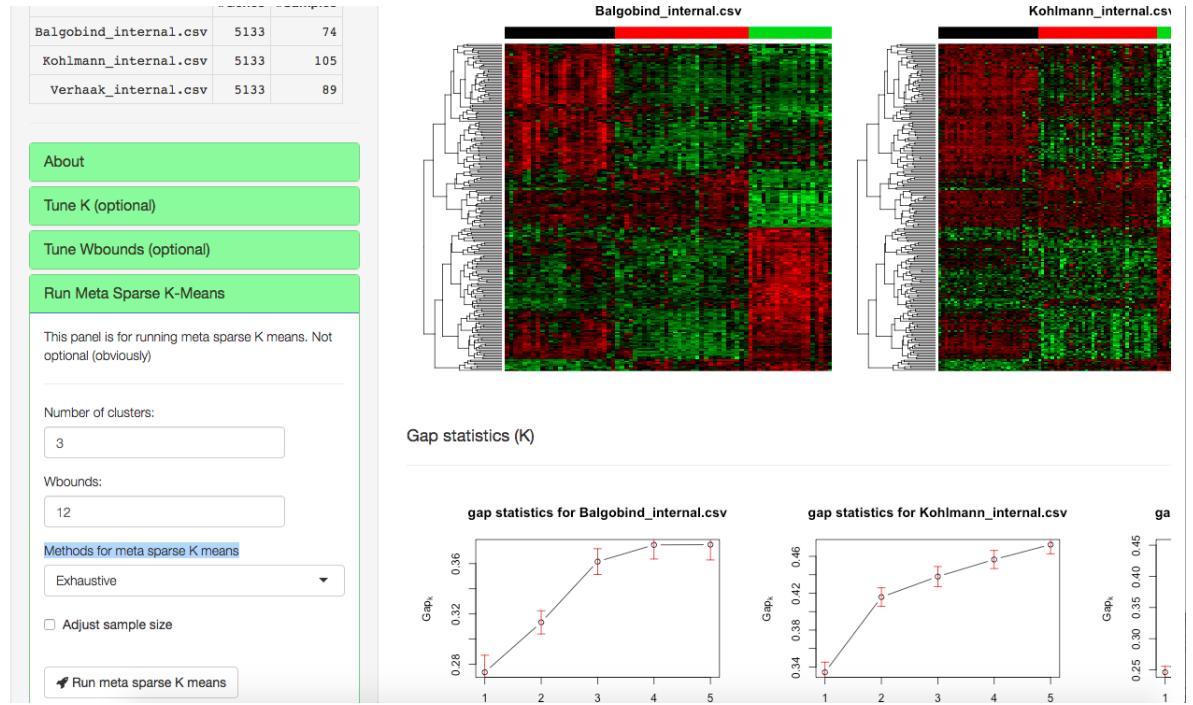


Figure 31: GUI Preprocessing page

There are three clustering matching methods: Exhaustive, linear, MCMC. 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 checkbox 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 ??.

4.5 metaPCA

By clicking toolsets and then metaPCA, users are directed to metaPCA home page as Figure ??.

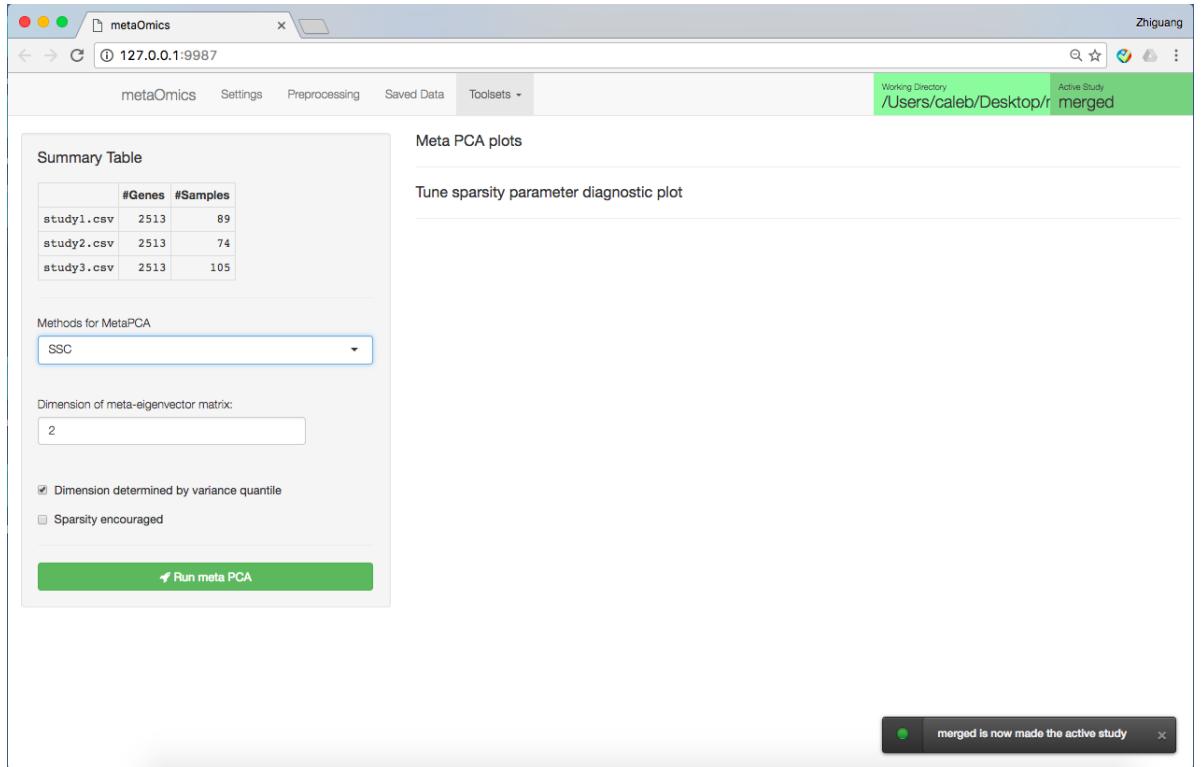


Figure 32: GUI Preprocessing page

On the top left panel users can see data summary Table. Below there are several options.

4.5.1 Methods for MetaPCA

- MetaPCA via sum of variance decomposition (SV)

Let $X^{(m)}$ be an observed $p \times n^{(m)}$ data matrix of sample size $n^{(m)}$ and p features for study m ($1 \leq m \leq M$). Denote by $S^{(m)}$ the maximum likelihood (ML) estimate of the $p \times p$ covariance matrix $\Omega^{(m)}$ of $X^{(m)}$. MetaPCA via sum of variance decomposition (SV) aims to solve the following eigen-value decomposition problem.

$$T^{SV} = \sum_{m=1}^M w^{(m)} S^{(m)}, \quad (1)$$

where $w^{(m)}$ is the reciprocal of the largest eigenvalue of $S^{(m)}$. The common principal components L are calculated from the eigen-decomposition of

$T^{SV} : L^T(T^{SV})L = \Lambda$ and K top common PCs should be retained for down-stream analysis. Selection of the optimal K will be described later in the the section of Parameter selection.

- MetaPCA via sum of squared cosine (SSC) maximization.

the second MetaPCA framework motived by SSC criterion proceeds as below. The top $j^{(m)}$ eigenvectors are calculated from study m to form eigenvector matrix $V^{(m)}$. We then perform eigen-decomposition on $T^{SSC} = \sum_{m=1}^M V^{(m)}V^{(m)^T}$ and select the top K eigenvectors to form the meta-analytic common eigen-space:

$$\left(\sum_{m=1}^M V^{(m)}V^{(m)^T} \right) B^{SSC} = \Lambda^* B^{SSC} \quad (2)$$

where $V^{(m)}$ is a matrix consisting of $j^{(m)}$ leading eigenvectors, Λ^* is a diagonal eigenvalue matrix, and $B^{SSC} = (\beta_1^{SSC}, \dots, \beta_K^{SSC})$ contains the top K eigenvectors.

4.5.2 Dimension of meta-eigenvector matrix

Dimension of meta-eigenvector matrix option allows user to specify dimension of the output meta-eigenvector matrix.

4.5.3 Dimension determined by variance quantile

Logical value whether dimension size of each study's eigenvector matrix (SSC) is determined by the pre-defined level of variance quantile 80%.

4.5.4 Sparsity encouraged

If the Sparsity encouraged checkbox is selected, we are able to tune the best tuning parameter λ and perform sparse metaPCA. After clicking on search for optimal tuning parameter button, the optimum tuning parameter will be returned to the box “tuning parameter for sparsity”

4.5.5 Run meta PCA

If Sparsity encouraged checkbox is selected, sparse meta PCA will be performed. Otherwise, meta PCA will be performed. The result is shown in the following figures.

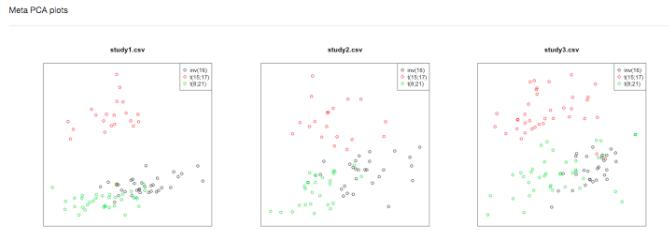


Figure 33: GUI Preprocessing page

4.6 MetaKTSP

4.7 MetaDCN

By clicking toolsets and then metaDCN, users are directed to metaDCN home page as Figure ??.

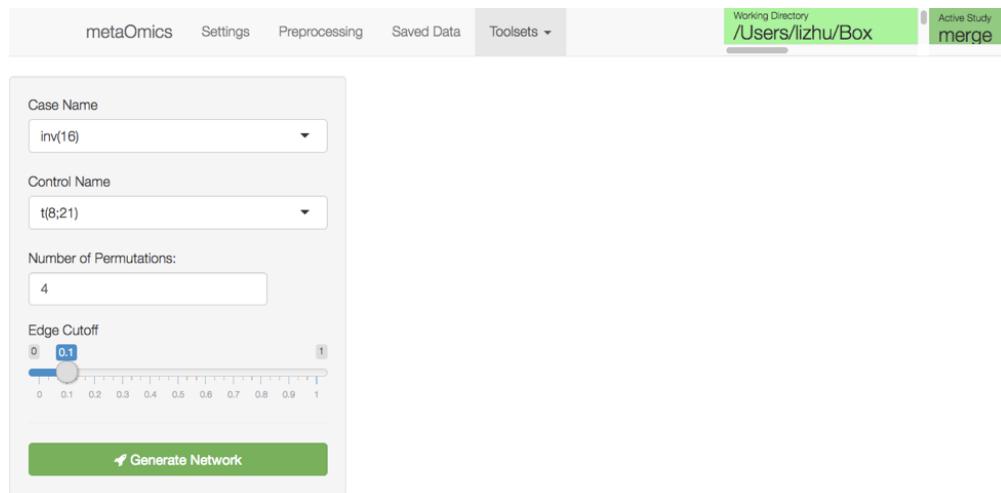


Figure 34: MetaDCN homepage

MetaDCN includes three steps to get differentially co-expressed networks: generate network, search for basic modules, and assemble supermodules. The left screen is the control panel of step 1. The control panel for next step will show up after the previous step is done.

4.7.1 Generate Network

The first step of MetaDCN is to generate 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.

After the generate network step is done, no output will show up in the screen. Instead, a message box indicating several Rdata files are saved in the Meta DCN folder, including:

- `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.

4.7.2 Search for basic modules

After the generate network step is done, the control panel will be as Figure ???. The next step is to search for basic modules. Users need to specify the number of repeats used for each initial seed modules (Number to repeat), the maximum Monte Carlo steps for simulated annealing algorithm (MC Steps), and the maximum pairwise Jaccard index allowed for basic modules (Jaccard Cutoff). After clicking **Search for basic modules** button, screen will show message indicating the algorithm is running to search for basic modules.

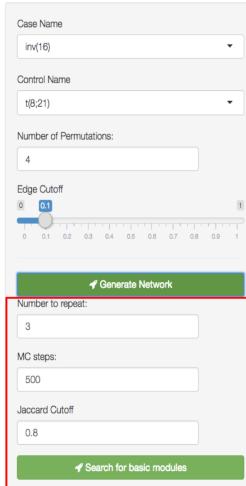


Figure 35: MetaDCN control panel for search for basic modules

Search for basic modules will take minutes, 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 Figure ???. Meanwhile, several files will be saved in the Meta DCN folder:

- 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.

Basic modules higher correlated in case:

Basic modules higher correlated in case:			
Show	10 entries	Search:	
Module.Index	Component.Number	Repeat.Index	Gene.Set
1	H1	2	1 PSAP/CTSS/CECR1/LGALS3/AP1S2/TLR2/HEXB/SMM24/CTSB/OGFRL1/MYO1F/CPXM1/SERPINA1/MNDA/TNF
2	H2	2	2 SERPINA1/AP1S2/CECR1/SMM24/PSAP/TRBV27/RGS10/CPXM1/TLR2/CAPN2/OGFRL1/MS4A6A
3	H3	2	3 CTSB/TLR2/RGS10/HEXB/SERPINA1/CECR1/LGALS3/AP1S2/SMM24/S100A9/CPXM1/MAP2K1/CAPN2
4	H4	6	1 NFL3/IER3/CD83/CPXM1/EZR/RIPK2/SLC2A3
5	H5	6	2 NFL3/IER3/LCP1/CDKN1A/TCEAL4/TGFB1
6	H6	6	3 NFL3/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:			
Show	10 entries	Search:	
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/AN1
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/FNNA/PIM3/KLF6
5	L5	6	2 STAM/JUP/LYN/PIM3/PDLM1/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 36: MetaDCN output from search for basic modules step

4.7.3 Assemble supermodules

After search for basic modules step is done, the control panel will be Figure ???. 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.

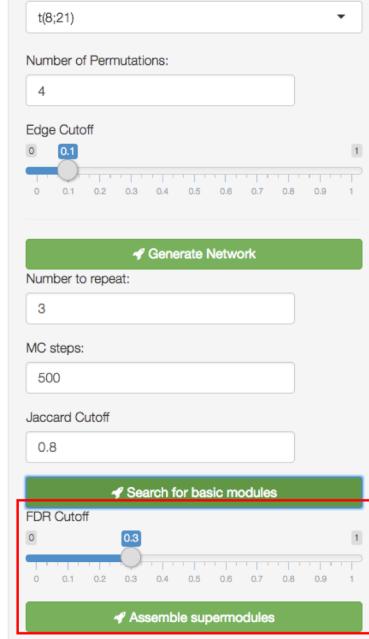


Figure 37: MetaDCN control panel for assemble supermodules step

After supermodule assembly is done, screen will show a table of supermodules (Figure ??). Users can also select basic modules to plot (Figure ??). Meanwhile several files will be saved in the folder MetaDCN:

- 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.

MetaDCN pathway-guided supermodules				
pathway_name	pathway_size	p_value	q_value	size
KEGG_LYSOSOME	121	9.73e-05	0.01	20
REACTOME_MHC_CLASS_I_ANTIGEN_PRESENTATION	91	0.00697	0.093	20
REACTOME_PLATELET_ACTIVATION_SIGNALING_AND_AGGREGATION	208	0.00495	0.093	25
REACTOME_RESPONSE_TO_ELEVATED_PLATELET_CYTOSOLIC_CA2_	89	0.00281	0.093	22
BIOCARTA_MCALPAIN_PATHWAY	25	0.00283	0.093	30
GO_CYTOSKELETAL_PROTEIN_BINDING	159	0.00185	0.093	13
REACTOME_COSTIMULATION_BY_THE_CD28_FAMILY	63	0.00431	0.093	14
GO_ACTIN_FILAMENT_BINDING	25	0.00369	0.093	13
BIOCARTA_CTRP_PATHWAY	12	0.00725	0.093	18
GO_ACTIN_CYTOSKELETON_ORGANIZATION_AND_BIogenesis	105	0.00697	0.093	20

Showing 1 to 10 of 103 entries Previous 1 2 3 4 5 ... 11 Next

Figure 38: MetaDCN supermodules table

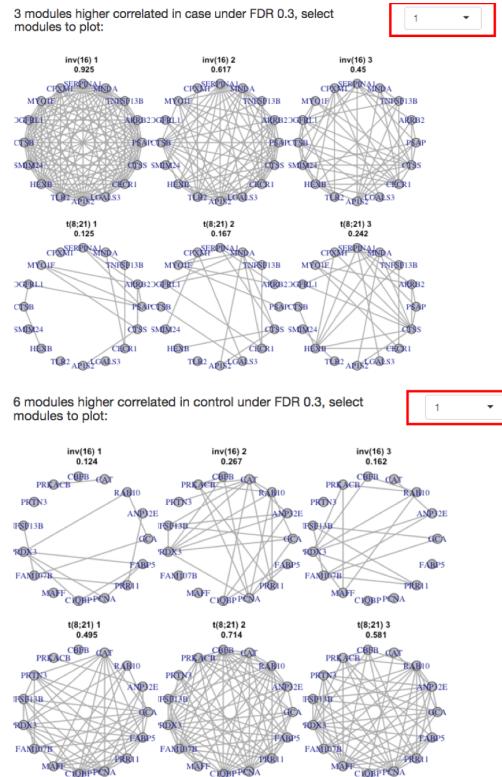


Figure 39: MetaDCN select basic modules to plot

References

- 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.
- Fisher, R. A. (1925). *Statistical methods for research workers*. Genesis Publishing Pvt Ltd.
- 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.
- 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.
- Li, J., Tseng, G. C., et al. (2011). An adaptively weighted statistic for detecting differential gene expression when combining multiple transcriptomic studies. *The Annals of Applied Statistics*, 5(2A):994–1019.
- Shen, K. and Tseng, G. C. (2010). Meta-analysis for pathway enrichment analysis when combining multiple genomic studies. *Bioinformatics*, 26(10):1316–1323.