Metabolomic Data Analysis with MetaboAnalyst 5.0

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1. Background

Metabolite Set Enrichment Analysis (MSEA) is an approach used to identify biologically meaningful patterns significantly enriched in quantitative metabolomics data. In conventional approaches, metabolites are evaluated individually for their significance under the conditions of the study. Next, the compounds that have passed a certain significance level are combined to see if any meaningful pattern can be found. In contrast, MSEA directly investigates a set of functionally related metabolites without the need to preselect compounds based on some arbitrary cut-off threshold.In addition, MSEA contains a collection of metabolites set libraries and the implementation of user-friendly web interfaces. As a result, it has the potential to identify subtle but significant changes among a group of related compounds, which may go undetected with the conventional approaches. MSEA is a metabolomic version of the popular Gene Set Enrichment Analysis (GSEA) software. GSEA is widely used in genomics data analysis and has proven to be a powerful alternative to conventional approaches. For more information, refer to the original paper by Subramanian A1. and a review paper by Nam D, Kim SY2.

1. MSEA Overview

MSEA consists of four steps - data input, data processing, data analysis, and results download. Different analysis procedures are performed based on different input types. In addition, users can browse and search the metabolite set libraries and upload their self-defined metabolite sets for enrichment analysis. Finally, users can also map metabolite names among compound names, synonyms, and major database identifiers.

1. Data Input

MSEA offers three enrichment analysis algorithms. Accordingly, three different types of data inputs are required by these three approaches:

A list of compound names - entered as a one-column data (*Over Representation Analysis (ORA)*);

A single measured biofluid (urine, blood, CSF) sample- entered as tab-separated two-column data with the first column for compound name, and the second for concentration values (*Single Sample Profiling (SSP)*);

1Subramanian*Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles.*, Proc Natl Acad Sci USA. 2005 102(43): 15545-50

2Nam D, Kim SY. *Gene-set approach for expression pattern analysis*, Briefings in Bioinformatics. 2008 9(3): 189-197.

A compound concentration table - entered as a comma-separated (.csv) file with each sample per row and each metabolite concentration per column. The first column is sample names, and the second column is for sample phenotype labels (*Quantitative Enrichment Analysis (QEA)*). For this type of analysis, users should select “Over Representation Analysis” (ORA), which requires a list of compound names as input.

1. Data Processing

The first step is to standardize the compound labels. It is an essential step since the compound labels will subsequently be compared with compounds in the metabolite set library. MSEA has a built-in tool to convert common compound names, synonyms, identifiers used in HMDB ID, PubChem, ChEBI, BiGG, METLIN, KEGG, or Reactome. **Table 1** shows the conversion results. Note: *1* indicates an exact match, *2* indicates an approximate match, and *0* indicates no match. A text file containing the results can be found in the downloaded file *name map.csv*

Table 1: Result from Compound Name Mapping

Query Match HMDB PubChem KEGG SMILES Comment

1 Lactate L-Lactic acid HMDB0000190 61503 C00186 C[C@@H](C(=O)O)O 1

2 Glucose D-Glucose HMDB0000122 5793 C00221 C([C@@H]1[C@H]([C@@H]([C@H](C(O1)O)O)O)O)O 1

3 Isoleucine L-Isoleucine HMDB0000172 6306 C00407 CC[C@H](C)[C@@H](C(=O)O)N 1

4 Proline L-Proline HMDB0000162 145742 C00148 C1C[C@H](NC1)C(=O)O 1

The second step is to check concentration values. The concentration must be measured in “*umol”* for blood and CSF samples, thus enabling SSP analysis. The urinary concentrations must be first converted to *umol/mmol creatinine* to compare with reported concentrations in the literature. No missing or negative values are allowed in SSP analysis. The concentration data for QEA analysis is more flexible. Users can upload either the original concentration data or normalized data. Missing or negative values are allowed (coded as *NA*) for QEA.

1. Selection of Metabolite Set Library

Before proceeding to enrichment analysis, a metabolite set library has to be chosen. MSEA offers seven built-in libraries:

Metabolic pathway associated metabolite sets (*currently contains 99 entries*);

Disease-associated metabolite sets (reported in the blood) (*currently contains 344 entries*);

Disease-associated metabolite sets (reported in urine) (*currently contains 384 entries*)

Disease-associated metabolite sets (reported in the CSF) (*currently contains 166 entries*)

Metabolite sets associated with SNPs (*currently contains 4598 entries*)

Predicted metabolite sets based on computational enzyme knockout model (*currently contains 912 entries*)

Metabolite sets based on locations (*currently contains 73 entries*)

Drug pathway associated metabolite sets (*currently contains 461 entries*)

In addition, MSEA also allows user-defined metabolite sets to be uploaded to perform enrichment analysis on arbitrary groups of compounds. The metabolite set library is a two-column comma-separated text file with the first column for metabolite set names and the second column for its compound names (**must use HMDB compound name**) separated by "; ". Please note that the built-in libraries are mainly from human studies. Therefore, users must upload their self-defined metabolite set libraries for enrichment analysis using data from other organisms.

1. Enrichment Analysis

Over Representation Analysis (ORA) is performed when a list of compound names is provided. The list of compounds can be obtained by conventional feature selection methods, a clustering algorithm, or compounds with abnormal concentrations detected in SSP to investigate if some biologically meaningful patterns can be identified.

ORA was implemented using the *hypergeometric test* to evaluate whether a specific metabolite set is present more than expected by chance compared to a specific list. Also, one-tailed p-values, corrected for multiple comparisons, are provided. **Figure 1 and Table 2** summarize the results.

**Metabolite Sets Enrichment Overview**

Gluconeogenesis

Warburg Effect

Lactose Degradation Glucose-Alanine Cycle Lactose Synthesis

Transfer of Acetyl Groups into Mitochondria

Glycolysis Galactose Metabolism Sphingolipid Metabolism

P-value

7e-03

|  |
| --- |
|  |
|  |
|  |
|  |

1e-01

2e-01

Pyruvate Metabolism

Arginine and Proline Metabolism

Valine, Leucine and Isoleucine Degradation

0 10 20

Enrichment Ratio

Figure 1: Summary Plot for Over Representation Analysis (ORA)

Table 2: Result from Over Representation Analysis

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | total | expected | hits | Raw p | Holm p | FDR |
| Gluconeogenesis | 35 | 0.14 | 2 | 6.53E-03 | 6.40E-01 | 6.40E-01 |
| Warburg Effect | 58 | 0.23 | 2 | 1.76E-02 | 1.00E+00 | 8.61E-01 |
| Lactose Degradation | 9 | 0.04 | 1 | 3.47E-02 | 1.00E+00 | 1.00E+00 |
| Glucose-Alanine Cycle | 13 | 0.05 | 1 | 4.99E-02 | 1.00E+00 | 1.00E+00 |
| Lactose Synthesis | 20 | 0.08 | 1 | 7.60E-02 | 1.00E+00 | 1.00E+00 |
| Transfer of Acetyl Groups into Mitochondria | 22 | 0.09 | 1 | 8.33E-02 | 1.00E+00 | 1.00E+00 |
| Glycolysis | 25 | 0.10 | 1 | 9.43E-02 | 1.00E+00 | 1.00E+00 |
| Galactose Metabolism | 38 | 0.15 | 1 | 1.41E-01 | 1.00E+00 | 1.00E+00 |
| Sphingolipid Metabolism | 40 | 0.16 | 1 | 1.48E-01 | 1.00E+00 | 1.00E+00 |
| Pyruvate Metabolism | 48 | 0.19 | 1 | 1.75E-01 | 1.00E+00 | 1.00E+00 |
| Arginine and Proline Metabolism | 53 | 0.21 | 1 | 1.92E-01 | 1.00E+00 | 1.00E+00 |
| Valine, Leucine and Isoleucine Degradation | 60 | 0.23 | 1 | 2.15E-01 | 1.00E+00 | 1.00E+00 |

1. Appendix: R Command History
   * "mSet<-InitDataObjects(\"conc\", \"msetora\", FALSE)"
   * "cmpd.vec<-c(\"Lactate\",\"Glucose\",\"Isoleucine\",\"Proline\")"
   * "mSet<-Setup.MapData(mSet, cmpd.vec);"
   * "mSet<-CrossReferencing(mSet, \"name\");"
   * "mSet<-CreateMappingResultTable(mSet)"
   * "mSet<-SetMetabolomeFilter(mSet, F);"
   * "mSet<-SetCurrentMsetLib(mSet, \"smpdb\_pathway\", 2);"
   * "mSet<-CalculateHyperScore(mSet)"
   * "mSet<-PlotORA(mSet, \"ora\_0\_\", \"net\", \"png\", 72, width=NA)"
   * "mSet<-PlotEnrichDotPlot(mSet, \"ora\", \"ora\_dot\_0\_\", \"png\", 72, width=NA)"
   * "mSet<-CalculateHyperScore(mSet)"
   * "mSet<-PlotORA(mSet, \"ora\_1\_\", \"net\", \"png\", 72, width=NA)"
   * "mSet<-PlotEnrichDotPlot(mSet, \"ora\", \"ora\_dot\_1\_\", \"png\", 72, width=NA)"
   * "mSet<-SaveTransformedData(mSet)"

[15] "mSet<-PreparePDFReport(mSet, \"guest5723476134630056340\")\n"

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