iModMix: Integrative Module Analysis for Multi-omics Data

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Abstract

Summary: The integration of metabolomics with other omics ("multi-omics") offers complementary insights into disease biology. However, this integration remains challenging due to the fragmented landscape of current methodologies, which often require programming experience or bioinformatics expertise. Moreover, existing approaches are limited in their ability to accommodate unidentified metabolites, resulting in the exclusion of a significant portion of data from untargeted metabolomics experiments. Here, we introduce *iModMix*, a novel approach that uses a graphical lasso to construct network modules for integration and analysis of multi-omics data. *iModMix* uses a horizontal integration strategy, allowing metabolomics data to be analyzed alongside proteomics or transcriptomics to explore complex molecular associations within biological systems. Importantly, it can incorporate both annotated and unidentified metabolites, addressing a key limitation of existing methodologies. *iModMix* is available as a user-friendly R Shiny application that requires no programming experience (https://imodmix.moffitt.org), and it includes example data from several publicly available multi-omic studies for exploration. An R package is available for advanced users (https://github.com/biodatalab/iModMix).

Availability and implementation: Shiny application: https://imodmix.moffitt.org. The R package and source code: https://github.com/biodatalab/iModMix.

Introduction

Integration of metabolomics with other omics modalities, such as proteomics and transcriptomics, is essential for a comprehensive understanding of complex biological systems and disease mechanisms. The scale and complexity of these so-called "multi-omics" data necessitate computational approaches for effective integration and analysis. However, multi-omics analysis presents several challenges. These include the need for computational tools that are adaptable to diverse experimental setups and data types, as well as user-friendly enough to be used without extensive programming experience (Jendoubi 2021). Moreover, many approaches are unable to incorporate unidentified metabolites, resulting in the exclusion of large portions of data from untargeted metabolomics experiments, thereby limiting the potential for novel discoveries. Current pathway tools often restrict the inclusion of metabolite identifications due to naming mismatches and inconsistent use of standard identifiers like KEGG or HMDB IDs. This results in exclusion of some identified metabolites which cannot be fully utilized for analysis and further narrowing and hindering comprehensive insights (Jendoubi 2021).

In general, approaches for integrating metabolomics with other omics can be categorized into vertical or horizontal integration. Vertical integration takes multiple omics datasets as input and produces a single output, such as clustering assignments or a merged dataset, which no longer reflects the individual features of the original data. iClusterPlus (Q et al. 2013) is an example of vertical integration: it takes multiple omics datasets from the same samples as input, extracts latent factors across omics datasets, clusters samples in latent space, and outputs the clustering assignments by sample. In contrast, horizontal integration utilizes multiple omics datasets in parallel while maintaining the context of the original inputs. PIUmet (Benedetti et al. 2023) is an example of horizontal integration: it takes metabolomics and proteomics data as input, and the resulting network output contains both metabolites and proteins.

iModMix is a horizontal integration framework that constructs network modules from two input omics datasets (*e.g.*, metabolomics and proteomics, metabolomics and transcriptomics). It first uses graphical lasso to estimate a sparse Gaussian Graphical Model (GGM) (Friedman, Hastie, and Tibshirani 2008) for each input omics dataset. GGMs capture direct associations within the input omics datasets, which is an improvement over Weighted Gene Correlation Network Analysis (WGCNA) (Langfelder et al. 2008) module creation that includes both direct and indirect associations. Similar to WGCNA, a Topological Overlap Matrix (TOM) is next calculated (Yip et al. 2007), which quantifies the extent to which pairs of features share common neighbors. Hierarchical clustering is then performed on TOM dissimilarity (1 – TOM), using a dynamic tree cutoff to group related features into modules. *iModMix* next takes the first principal component of the abundances of the features in the module, called an eigenfeature (*e.g.*, eigenmetabolites for metabolomics, eigenproteins for proteomics). These eigenfeatures are representative of the contents of the module, and they can be used in place of normal metabolite abundance or protein expression for testing for differential expression or association with experimental conditions. Finally, integration between omics types is achieved by correlating the eigenfeatures from different omics experiments.

A major advantage of *iModMix* is its ability to generate network modules and their associated eigenfeatures independently of feature annotation. This allows *iModMix* to incorporate annotated and unidentified metabolites into its results. Since this method does not rely on existing pathway databases like KEGG, it can uncover new associations among features. *iModMix* is available as an R Shiny application (https://imodmix.moffitt.org) that provides an easy to use graphical interface and detailed documentation for users without programming expertise, and it is available as an R package (https://github.com/biodatalab/iModMix). *iModMix* can run on standard desktop computers with at least 8GB of RAM and a multi-core processor. The running time depends on the size and complexity of the dataset, but typical data sizes of 20,000 variables, the analysis can be completed in under an hour.

Implementation

Data Upload

The *iModMix* workflow accepts two omics datasets at a time (Fig. 1A). If available, users can provide feature-level annotation corresponding to the input omics dataset. A separate metadata file containing sample IDs and at least one sample grouping column (*e.g.*, treatment, control) are necessary for conducting PCA, heatmaps, and boxplots. Once uploaded, the tool scales the data by centering each feature to have a mean of zero and a standard deviation of one. Missing values are imputed using the k-nearest neighbors' algorithm, or users can also provide their own imputed data if a different method is preferred. We provide ten datasets encompassing gene expression and metabolomics data to illustrate *iModMix's* capabilities. These datasets, from (Benedetti et al. 2023), have been formatted to meet *iModMix* specifications, with variable columns labeled as "Feature_ID" and sample columns labeled as "Samples". These datasets are designed to facilitate user engagement with *iModMix* and are readily accessible for download on Zenodo (https://doi.org/10.5281/zenodo.13988161). We also provide an in-house dataset of 20 lung adenocarcinoma mouse samples (10 wild type, 10 knockout), with 7353 metabolomics features and 7928 protein groups, to highlight *iModMix's* functionality in handling unidentified metabolites.

Module Construction

iModMix first estimates partial correlations using the glassoFast R package (Friedman, Hastie, and Tibshirani 2008) (Fig. 1B) to build a GGM. Next, the TOM is computed and TOM dissimilarity (1 – TOM) is used for hierarchical clustering (Yip et al. 2007). Modules are created using the dynamic tree cut method, specifically the 'hybrid' method, which refines assignments derived from a static cut height by analyzing the shape of each branch (Langfelder, Zhang, and Horvath 2008). Finally, *iModMix* calculates eigenfeatures for each module using the moduleEigengenes function from the WGCNA R package (Langfelder et al. 2008) (Fig. 1B) that are used for downstream omics analyses.

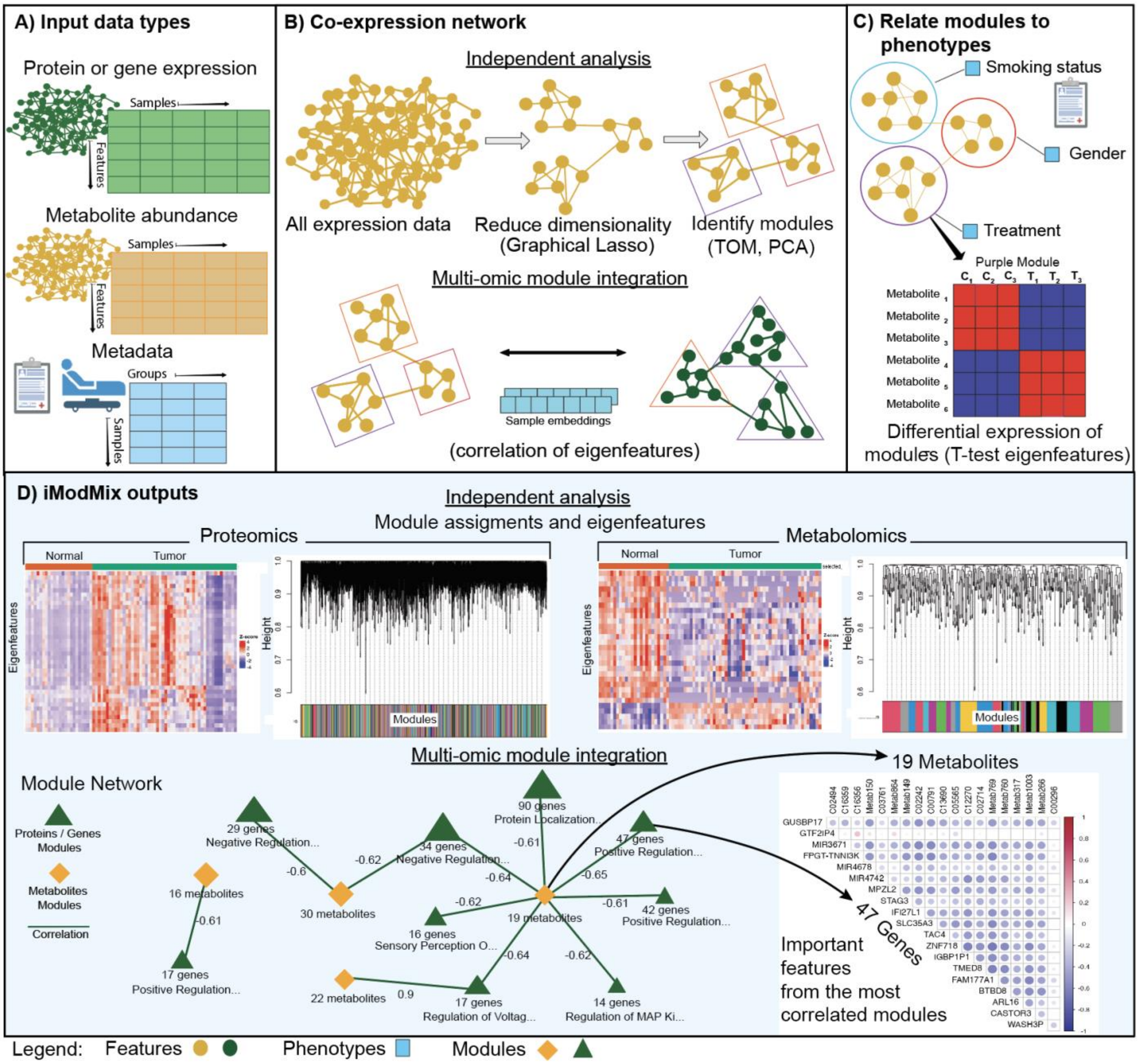
A) Input data types
Protein or gene expression
Samples
Features
Metabolite abundance
Samples
Features
Metadata
Groups
Samples
B) Co-expression network
Independent analysis
All expression data
Reduce dimensionality (Graphical Lasso)
Identify modules (TOM, PCA)
Multi-omic module integration
Sample embeddings
(correlation of eigenfeatures)
C) Relate modules to phenotypes
Smoking status
Gender
Treatment
Purple Module
C1 C2 C3 T1 T2 T3
Metabolite 1
Metabolite 2
Metabolite 3
Metabolite 4
Metabolite 5
Metabolite 6
Differential expression of modules (T-test eigenfeatures)
D) iModMix outputs
Independent analysis
Module assigments and eigenfeatures
Proteomics
Normal
Tumor
Eigenfeatures
Height
Modules
Metabolomics
Normal
Tumor
Eigenfeatures
Height
Modules
Multi-omic module integration
Module Network
Proteins / Genes Modules
Metabolites Modules
Correlation
90 genes Protein Localization...
29 genes Negative Regulation...
34 genes Negative Regulation...
47 genes Positive Regulation...
16 metabolites
30 metabolites
19 metabolites
22 metabolites
16 genes Sensory Perception O...
42 genes Positive Regulation...
17 genes Positive Regulation...
17 genes Regulation of Voltag...
14 genes Regulation of MAP Ki...
-0.61
-0.62
-0.6
-0.64
-0.65
-0.61
-0.62
-0.61
-0.64
-0.62
0.9
19 Metabolites
47 Genes
Important features from the most correlated modules
C02494 C16359 C16356 Metab150 C03761 Metab864 Metab149 C02242 C00791 C13690 C05565 C12270 C02714 Metab769 Metab760 Metab317 Metab1003 Metab266 C00296
GUSBP17
GTF2IP4
MIR3671
FPGT-TNNI3K
MIR4678
MIR4742
MPZL2
STAG3
IFI27L1
SLC35A3
TAC4
ZNF718
IGBP1P1
TMED8
FAM177A1
BTBD8
ARL16
CASTOR3
WASH3P
1 0.8 0.6 0.4 0.2 0 -0.2 -0.4 -0.6 -0.8 -1
Legend: Features Phenotypes Modules

Figure 1: Overview of *iModMix*. (A) *iModMix* integrates transcriptomics, proteomics, metabolomics, and clinical data to understand biological systems comprehensively. (B) Co-expression network: The tool performs independent analyses of each omics dataset to identify biologically relevant modules, and it then integrates the datasets by correlating eigenfeatures. (C) *iModMix* evaluates associations between phenotype-related modules across different data types. (D) Results of the integrated analysis of transcriptomics and metabolomics data from normal and tumor samples using *iModMix*. The network interaction and most correlated modules are also visualized.

Module Exploration

The phenotype analysis section provides a framework for classifying phenotypes based on eigenfeatures. Users can upload metadata, select phenotypes of interest, and set significance thresholds for statistical tests. The module performs classification using Student's t-test, comparing selected phenotypes and generating results that include t-statistics and p-values. Significant eigenfeatures are visualized through boxplots. Additionally, users can explore specific modules and view associated features, PCA loading, and heatmap plots to view feature behavior across different phenotypes (Fig. 1C). *iModMix* supports pathway enrichment analysis of genes or proteins by utilizing all available libraries in Enrichr (Kuleshov et al. 2016). This allows users to choose their preferred library, such as KEGG, GO, Human Gene Atlas, or WikiPathways.

Multi-omics analysis

iModMix integrates modules across omics datasets by calculating the Spearman correlation between eigenfeatures. *iModMix* then constructs an interactive network, where protein/gene modules are represented as green triangles and metabolite modules as yellow diamonds, with correlation coefficients shown on connecting arrows. Users can select the number of top multi-omics module correlations to visualize and explore detailed correlations within these modules through corrplots and tables. Lists of metabolites and proteins/genes within highly correlated modules are provided, facilitating further pathway analysis and offering insights into the relationships between different omics layers. Additionally, classification between features of each layer and phenotypes using t-tests and boxplots is provided, enabling users to visualize and identify significant differences. (Fig. 1B).

Case-Study: ccRCC Dataset with Identified Metabolites

We used 24 normal and 52 tumor clear cell renal cell carcinoma (ccRCC) samples (Golkaram et al. 2022; Tang et al. 2023; Benedetti et al. 2023) as a case study. It contained 23001 genes from RNA-seq and 904 identified metabolites from untargeted metabolomics. Applying *iModMix* identified 401 gene modules and 24 metabolite modules. Differential expression analysis of the modules through t-test confirmed changes in metabolite abundance between groups recapitulated those from prior work (Benedetti et al. 2023) highlighting reduced levels of adenosine/C00212 (module ME#443A83FF, p-value = 0.0000), gamma-glutamyltyrosine (module ME#472D7BFF, p-value = 0.0001), creatinine/C00791 and 1-methyladenosine/C02494 (module ME#1F9A8AFF p-value = 0.0000) in tumors compared to normal tissue. Conversely, metabolites with increased abundance in tumors compared to normal tissues included fructose/C00095 (module ME#472D7BFF, p-value = 0.0001), proline/C00148 and glutamine/C00064 (module ME#35B779FF, p-value = 0. 0023) (Benedetti et al. 2023). The top 5 correlated metabolomic modules to transcriptomic modules included pathways such as mitochondria fission and regulation of MAPK activity. Mitochondria fission results in lowered respiratory rates and emphasizes tumor reliance on glucose resulting in higher glycolysis rates common in renal cell carcinoma (Linehan et al. 2019).

Case-Study: Lung Adenocarcinoma Dataset with Unidentified Metabolites

Matched proteomics and metabolomics dataset was generated using two mouse models for lung adenocarcinoma (10 wild type, 10 knockout), with 7353 metabolomics features (annotated and unidentified) and 7928 protein groups. Applying *iModMix* generated 267 gene modules and 191 metabolite modules. High correlations were found between these modules, starting from a correlation threshold of 0.95. The second most correlated modules (protein module: ME#3ABA76FF with metabolite module: ME#238A8DFF), with a correlation of -0.94, included 14 genes (Gng11, Cnbp, Pkm, Aprt, Pgk1, Rbx1, Baz1b, Pgam1, Mif, Lgals3, Sp110, Map2, Mmrn2, Aldoc) and 21 metabolites, three of which are identified metabolites (Cortisol 21-acetate, Thymine, Deoxyuridine). Pathway analysis of these 14 genes and 3 metabolites using MetaboAnalyst (Pang et al. 2024) revealed significant enrichment in Glycolysis or Gluconeogenesis (P-value: 7.94E-7) and Pyrimidine metabolism (P-value: 0.0043961). Glycolysis and gluconeogenesis are known to be activated in non-small cell lung cancer (NSCLC) in a manner modulated by tumor size and oxygenation, and they differentially correlate with patient outcomes. The frequent co-activation of these pathways in NSCLC suggests their potential as targets for future therapeutic strategies aimed at cancer cell metabolism (Smolle et al. 2020). Additionally, high expression levels of pyrimidine metabolic rate-limiting enzymes have been identified as adverse prognostic factors in lung adenocarcinoma (LUAD). This reprogramming of metabolism is associated with clinical outcomes, indicating that the pyrimidine metabolism signaling pathway plays a significant role in LUAD prognosis (Wang et al. 2020).

Benchmarking

We benchmark *IModMix* to identify the optimal parameters for gene network construction using the *iModMix* algorithm. The process considers three input parameters: num_genes, num_fold, and lambda. Expression data from RC20 and lung adenocarcinoma mouse samples were used, and genes with the highest variance were selected, constrained by num_genes. To identify the best sparsity parameter (lambda), we applied five-fold cross-validation using the Graphical Lasso (Glasso) algorithm, which calculates a sparse Gaussian Graphical Model (GGM) for each input omics dataset. The Glasso model was fitted using the glassoFast function with lambda, and metrics such as the number of non-zero partial correlations, log-likelihood, and execution time were evaluated. Hierarchical clustering analysis was then used to identify gene modules. Stability was achieved in both datasets with an alpha value of 0.25 (Fig. S1A, Fig. S1B), allowing for efficient and accurate model evaluation across various configurations. This benchmarking process demonstrates that *IModMix* offers a refined approach to network construction by focusing on direct associations, making it a robust alternative to WGCNA for complex omics data.

Summary:

The *iModMix* pipeline captures interactions that could not be observable with metabolomics or transcriptomics alone. *iModMix* progresses the field of multi-omic analysis with advantages such as *de novo* network generation, independent of existing pathway databases and the ability to integrate annotated and unidentified metabolites for multi-omic analysis.

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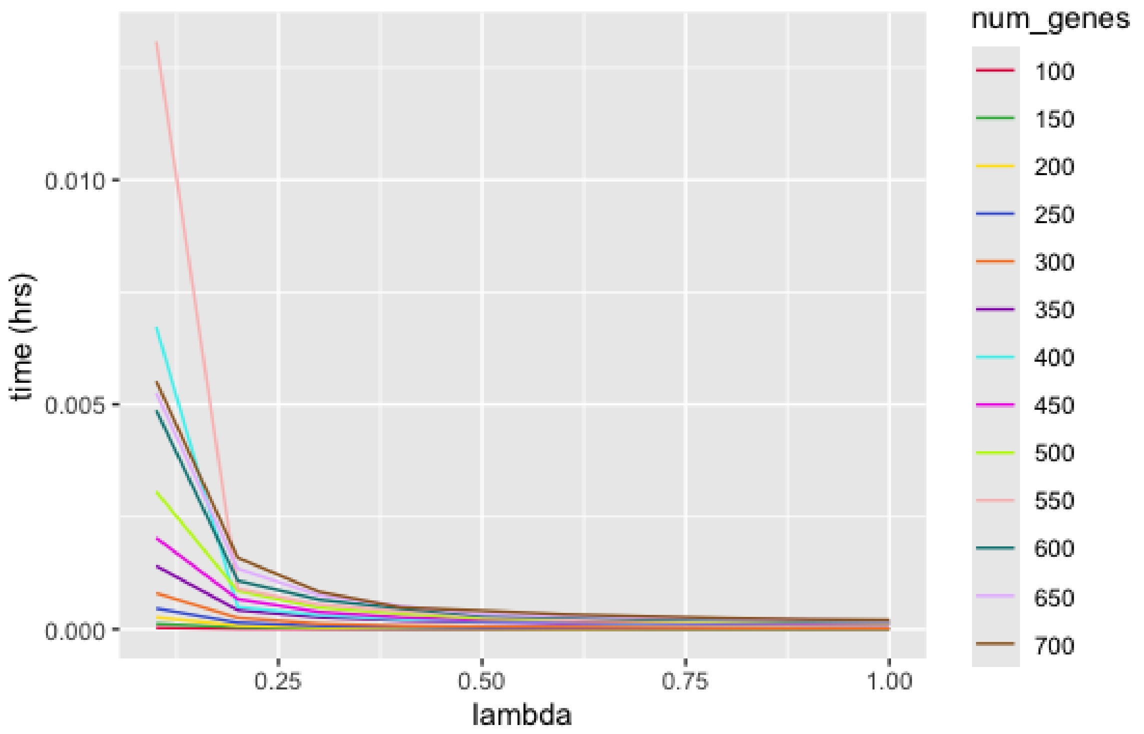
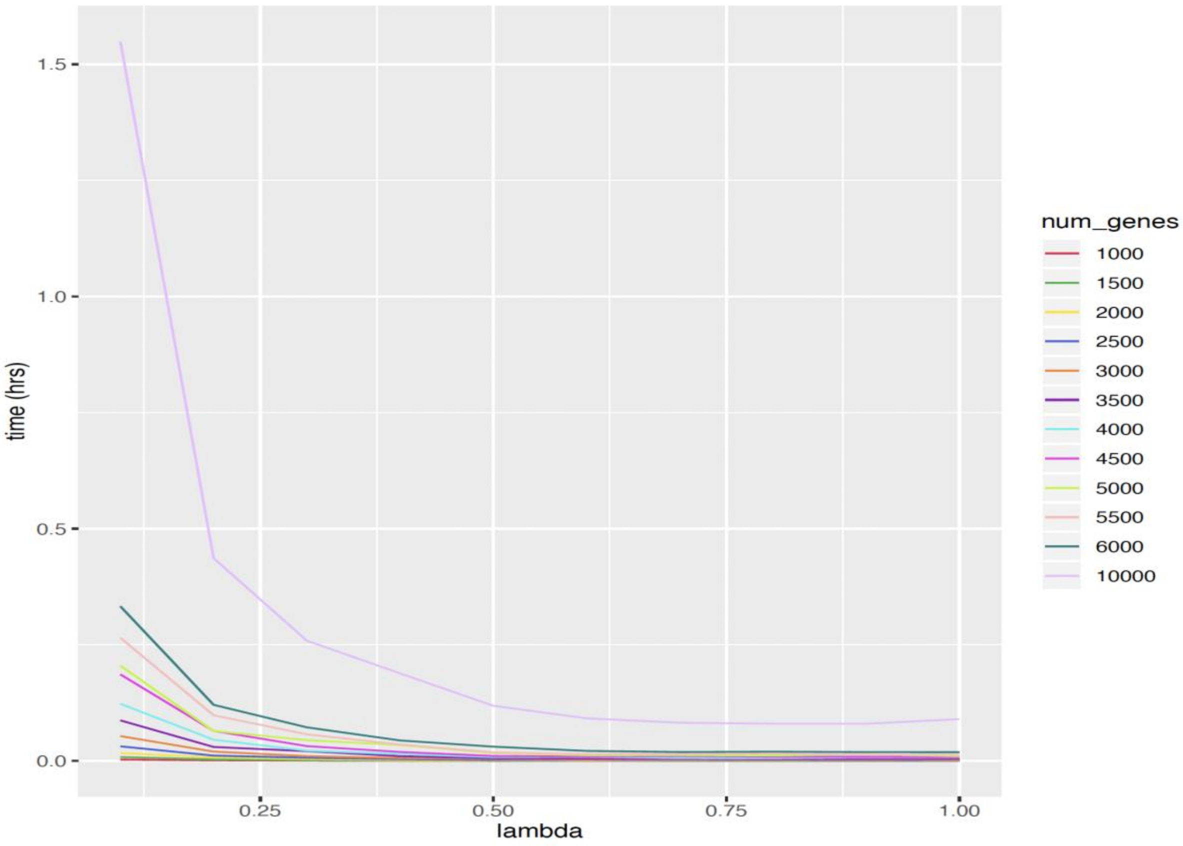
Supplementary Figure 1. Benchmaking results.

Metabolomic abundance RC20 data



Gene expresion data lung adenocarcinoma mouse samples data


time (hrs)
lambda
num_genes