Check for updates

Biotechnology and Bioengineering

WILEY

BIOTECHNOLOGY AND BIOENGINEERING

ARTICLE OPEN ACCESS

RSEA: A Web Server for Pathway Enrichment Analysis of Metabolic Reaction Sets

Merve Yarıcı[1] | Furkan Cantürk[2] | Serdar Dursun[3] | Hatice Nur Aydın[1] | Muhammed Erkan Karabekmez[1]

[1]Department of Bioengineering, Istanbul Medeniyet University, Istanbul, Turkey | [2]Department of Artificial Intelligence, Özyeğin University, Istanbul, Turkey | [3]AVL Research & Engineering, Istanbul, Turkey

Correspondence: Muhammed Erkan Karabekmez (erkan.karabekmez@medeniyet.edu.tr)

Received: 7 June 2024 | **Revised:** 10 February 2025 | **Accepted:** 28 April 2025

Keywords: functional enrichment | Genome-Scale metabolic models | GPR rules | metabolic pathways

ABSTRACT

Changes in biological pathways provide essential clues about metabolism. Genome-scale metabolic models (GEM) are network-based templates that computationally describe all stoichiometric associations and gene-protein reaction (GPR) relations found in an organism for all its metabolic genes and metabolites. Using reaction stoichiometry as input, GEMs mathematically simulate metabolic reaction fluxes occurring in an organism and predict changes in the metabolic system under the relevant condition. Multiple tools and approaches in the literature can capture fluxes sensitive to a given condition by using GEMs. However, functional enrichment analysis of these reaction lists in a systems biology perspective is not straightforward. Here, we introduce RSEA to annotate given reaction sets to significantly related metabolic pathways: Reaction Set Enrichment Analysis web server tool. RSEA converts given reaction list derived from GEMs into proper reaction identifiers and statistically analyze its enrichment in metabolic pathways. RSEA is designed to provide researchers with a practical and user-friendly platform to explore and interpret sets of reactions in biological pathways and freely available online (https://rseatool.com/).

1 | Introduction

Genome-scale metabolic models (GEMs) provide a framework within which systems biology approaches can be applied by contextualizing different types of big data, such as genomics, metabolomics, and transcriptomics (Passi et al. 2021). GEMs are computational representations that mathematically simulate metabolic processes of organisms (Edwards et al. 2002). GEMs can be used to simulate cell metabolism and predict cell phenotypes and can also be tailored to create context-specific GEMs using omics data integration approaches (Kim et al. 2015; Zhang and Hua 2016). These models offer a broad scope for understanding genotype-phenotype relationships and biological processes. Genome-scale metabolic networks are derived from the organism's genome and systematically express the relationships between metabolic reactions, enzymes, and genes (Antonakoudis et al. 2020).

Reaction lists obtained from GEMs can be functionally interpreted by converting them into corresponding gene lists using gene-protein-reaction (GPR) associations. GPR rules define the relationships between genes, proteins, and the reactions they catalyze in metabolic networks. These rules can describe isoenzymes (where one gene can catalyze a reaction) or enzyme complexes (where multiple genes are required for the reaction), leading to OR or AND rules, respectively (Di Filippo et al. 2021). There is no direct and one-to-one correspondence between genes and metabolic reactions. When one analyzes GEMs and finds a list of reactions with significant change in flux values, it is common to use over-representation analysis (ORA) to statistically associate the reaction list to known pathways as a systems biology approach. Existing tools tend to map reactions to gene lists first, then conduct ORA. However, these gene lists are subject to various limitations. The primary reason for these limitations is that genes are not limited to a single function but can take on multiple biological roles and interact in various

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2025 The Author(s). *Biotechnology and Bioengineering* published by Wiley Periodicals LLC.

cellular processes and organismal development (Khatri et al. 2012). These versatile gene functions can complicate the interpretation of gene lists, presenting a challenge that hinders an in-depth understanding of biological processes at the cellular level and the organism's development. Traditional methods are insufficient to understand gene expression analysis, regulations, and interactions (Du et al. 2016).

Pathway enrichment analysis is a statistical method used to determine whether genes, proteins, or reactions are over-represented within specific biological pathways based on existing knowledge stored in biological databases. This approach identifies the biological processes or pathways most likely involved in the related condition (Liu et al. 2022). In this field, currently used web-based software tools such as gProfiler (Reimand et al. 2007), Gene Set Enrichment Analysis (GSEA) (Subramanian et al. 2005), DAVID (Dennis et al. 2003), PANTHER (Thomas et al. 2003), hiPATHIA (Hidalgo et al. 2017; Rian et al. 2021), ToppGene (Chen et al. 2009), FunRich (Pathan et al. 2015), ClusterProfiler (Yu et al. 2012; Wu et al. 2021), and Reactome (Milacic et al. 2024) include statistical methods used to identify overrepresented biological processes and pathways in a set of genes or proteins. These tools for enrichment analysis, investigate the pathways and biological processes present for a given specific gene list by performing statistical pathway enrichment analysis on existing information obtained from databases such as Gene Ontology (GO) (Ashburner et al. 2000), PathwayCommons (Cerami et al. 2010) and, Reactome (Milacic et al. 2024). However, enrichment analyses using gene sets fall short because there is no one-to-one relationship between genes and reactions.

Another frequently used tool is Kyoto Genes and Genomes Encyclopedia (KEGG) Mapper (Kanehisa and Sato 2020), a collection of tools created by KEGG for mapping and analyzing biological pathways, modules, and other functional elements. While KEGG Mapper is a powerful and widely used tool, it does not work with reaction IDs. This limitation is critical when studying metabolic models, where the focus is on reactions rather than just genes or proteins. Therefore, pathway enrichment with KEGG Mapper also has a gene-centric approach without considering GPR rules.

Given the importance of reactions in metabolic network analysis, there is a need for network-based software tools that statistically evaluate the enrichment of reaction sets by directly using labels associated with reaction sets. This approach provides a reaction-centric perspective by explaining complex relationships between genes, proteins, and the reactions they catalyze. In GEMs, the description of how the associated reactions are catalyzed is often included as logical expressions connecting associated genes for each reaction (GPR rules) (Di Filippo et al. 2021).

Ravi and Gunawan (2021) performed reaction set enrichment analysis by applying hypergeometric test to reactions identified using human GEM subsystems. (Ravi and Gunawan 2021). However, this manual process was labor-intensive and time-consuming. It is also possible to perform flux enrichment analysis in Cobra toolbox, a software tool widely used in metabolic engineering studies (Heirendt et al. 2019). This method analyzes reaction enrichment through metabolic network models and their subsystems. However, it is limited to the subsystems included in the user-provided model, potentially skipping important subsystems (Gu et al. 2019). While metabolic network models are a powerful tool for simulating and understanding cellular processes, they are often incomplete due to the complexity of biological systems, leading to potential gaps in analysis. Furthermore, reaction enrichment analyses can be challenging regarding computational and processing load when working with large-scale metabolic network models (Passi et al. 2021).

In this study, we present RSEA: Reaction Set Enrichment Analysis Web Server Tool for correlating reaction sets with biological pathways. This web-based software tool converts reaction lists obtained from GEMs into reaction labels found in KEGG database (Kanehisa 2000), then identifies enriched metabolic pathways. Designed to provide a deeper understanding of the relationships between biological pathways through metabolic model-based reactions, this tool stands out as a web-server allowing us to explore metabolic interactions in a more reaction-centric way.

2 | Materials and Methods

2.1 | Selection of GEMs

The human metabolic model Recon 3D (Brunk et al. 2018), which is available in the Virtual Metabolic Human (VMH) (Noronha et al. 2019) and BiGG Models (King et al. 2016) databases, as well as metabolic models of some model organisms such as *E. coli* (iAF1260) and YeastGEM_v9 (Zhang et al. 2023), was used as the template models for the first version of RSEA.

2.2 | Matching Reaction Identifiers

The reaction IDs received from the user are categorized into BiGG, MetaNetX, and KEGG formats according to the available reaction IDs in these three databases. Using the MetaNetX file of cross-references available online (Moretti et al. 2021), BiGG reaction identifiers are converted to MetaNetX identifiers. Then, MetaNetX reaction identifiers are converted to KEGG reaction identifiers using the cross-reference datasets available online (Capela et al. 2022). If the models already had reaction identifiers in MetaNetX or KEGG format, they were used directly. For models that did not have pre-existing identifiers in these formats, the reactions with the appropriate IDs from these databases were manually matched.

2.3 | Processing Input Reactions

Reactions on all pathways in the KEGG database are captured with the KEGG API and stored in a file on the server of RSEA, resulting in a list of reactions for each pathway and a list of pathways containing each reaction—the reaction-pathway lists. After converting the input reaction identifiers to KEGG identifiers, RSEA generates sets of input reactions where each set is

10970290, 2025, 8, Downloaded from https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.29020 by Ozyegin University, Wiley Online Library on [10/09/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

linked to a KEGG pathway, using the pathway-reaction lists stored in the server.

2.4 | FrontEnd Software

The front end was developed using the React JavaScript library, chosen for its stability and compatibility with various web environments. Users can interact with the system by entering reaction identifiers or uploading text or.csv files, and can view and download enrichment analysis results.

2.5 | BackEnd Software

The application was implemented in Python, and the FastAPI library was utilized to process user requests efficiently, and push results back to the web interface. This robust architecture ensures the responsiveness and reliability of the system.

Given a list of reactions, if the user selects a specific model, the enrichment analysis is restricted to the reactions and pathways defined within that chosen model. This means that only the reactions and pathways available in the model are considered for the analysis, ensuring that the results are relevant to the specific organism or system represented by the model. However, if the user does not select a model, the tool defaults to analyzing the reaction list against all pathways and reactions available in the KEGG database. This broader approach considers all reactions in KEGG, which may provide a more general enrichment result but might include pathways or reactions not directly relevant to the user's specific system of interest.

Additionally, if an input reaction identifier is unavailable in MetaNetX, VMH, or KEGG databases or a cross-reference is not available for it, those reactions are listed as "Unmatched Reactions" and are not considered in the enrichment analysis at all.

Using the pathway-reaction lists in the server, hypergeometric test is performed with "hypergeom" method in Scipy library. The implemented hypergeometric test in RSEA aims to identify pathways that exhibited notable representation within the compilation of input reactions. Adjusted *p*-values are calculated using multiple testing methods over the *p*-values calculated with the hypergeometric tests (Figure 1).

2.6 | Case Studies

We conducted two different case studies to demonstrate the effectiveness and usefulness of RSEA. For the first case study, the dataset is taken from the GEO database (Barrett et al. 2005) via GSE21942 accession code (Kemppinen et al. 2011). Differentially expressed genes (DEGs) were identified using the limma R package (Ritchie et al. 2015) Genes with adjusted *p*-values below 0.05 were considered DEG. These DEGs were then matched to corresponding genes in the Recon3D (Brunk et al. 2018). For the matched DEGs, pathway enrichment analysis was performed using the gProfiler database (Reimand et al. 2007).

Using the same gene expression data, expression data associated with each reaction in the human template model RECON3D (Brunk et al. 2018) was determined. For this, the "mapExpressionToReactions" function in Cobra Toolbox (Heirendt

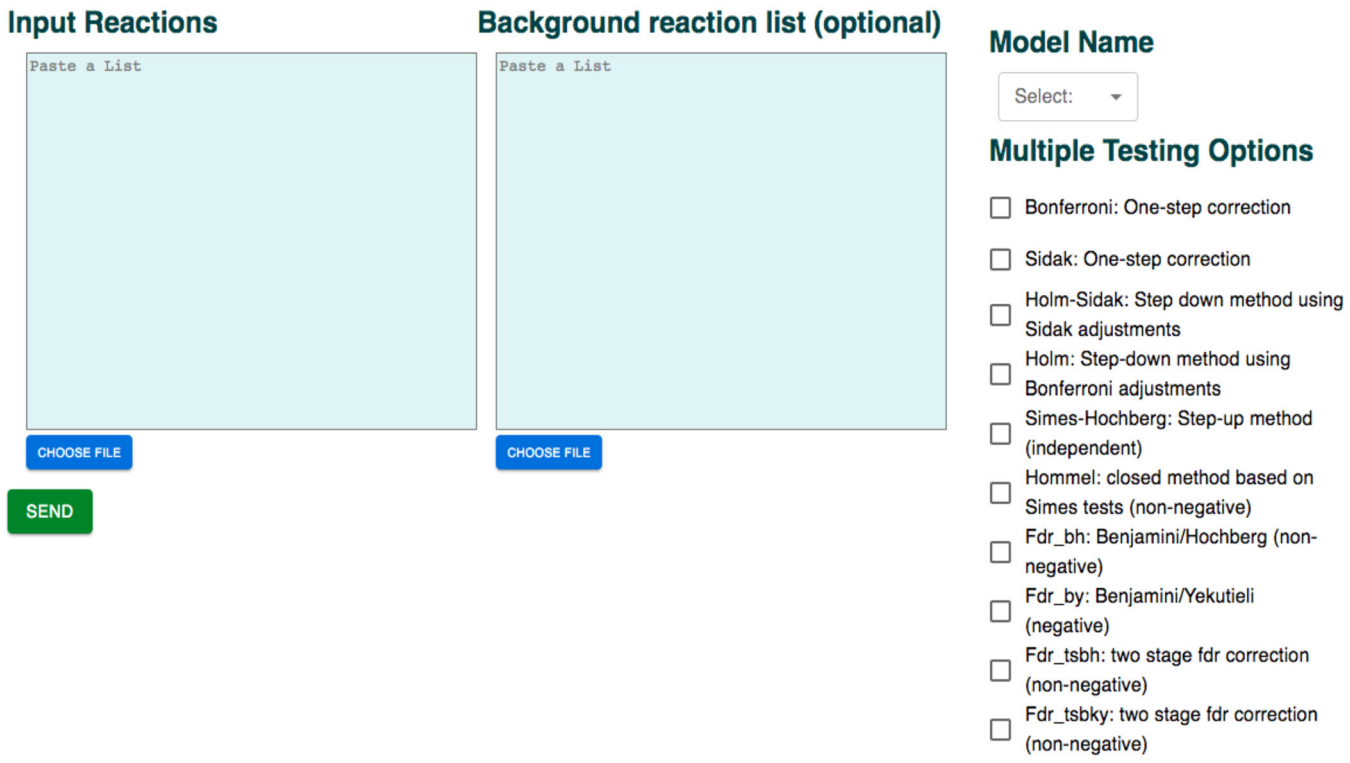


FIGURE 1 | RSEA tool website preview.

10970290, 2025, 8, Downloaded from https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.29020 by Ozyegin University, Wiley Online Library on [10/09/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

et al. 2019) was used by utilizing MATLAB 2023 platform. The limma R package (Ritchie et al. 2015) was used to find differentially expressed reactions (DERs) between MS patients and healthy samples in the dataset. Reactions with adjusted *p*-values below 0.05 were considered DER. Reaction set enrichment analyses were performed using RSEA.

For the other case study, results from Cesur et al. (2022) were repeated with RSEA.

3 | Results

In RSEA, enrichment analysis is performed for KEGG metabolic pathways. Users enter reaction IDs as input, and RSEA converts these reaction identifiers into corresponding reaction identifiers from MetaNetX, VMH, or KEGG, for further analysis (Figure 2). Since different metabolic models use different reaction identifier formats, the algorithm matches the input identifiers with the appropriate reaction IDs from the databases. However, if an input reaction or its associated metabolites cannot be matched with any identifier in the available databases, it will be listed as an "Unmatched Reaction" and excluded from the enrichment analysis. This often occurs because different databases, such as BiGG and GEMs, may use different chemical compound identification systems. For example, the BiGG database typically derives its identifiers from the KEGG compound database, while GEMs from other sources may manually select identifiers from different databases. As a result, when a metabolite or reaction is unavailable in the reference database, the matching process may not succeed. While the frequency of unmatched reactions varies depending on the model, this issue could lead to a partial or biased pathway enrichment. To mitigate this, users are encouraged to manually review unmatched reactions and attempt to reconcile them with the appropriate database identifiers.

3.1 | Case Study: Multiple Sclerosis (MS)

We investigated the gene expression dataset of peripheral blood mononuclear cells in MS patients and healthy controls. MS is a complex neurological disorder characterized by diverse genetic alterations contributing to its pathogenesis. In this case study, we examined the pathways related to MS disease with GSEA and reaction set enrichment analysis.

The commonly used gene set enrichment tool gProfiler (Reimand et al. 2007) was employed to conduct pathway enrichment analysis for 1700 DEGs we found, revealing a spectrum of pathways associated with MS. gProfiler provides insights into general pathways, these methods may miss key metabolic processes. While this analysis provided valuable insights into the broader molecular context of MS, we sought to

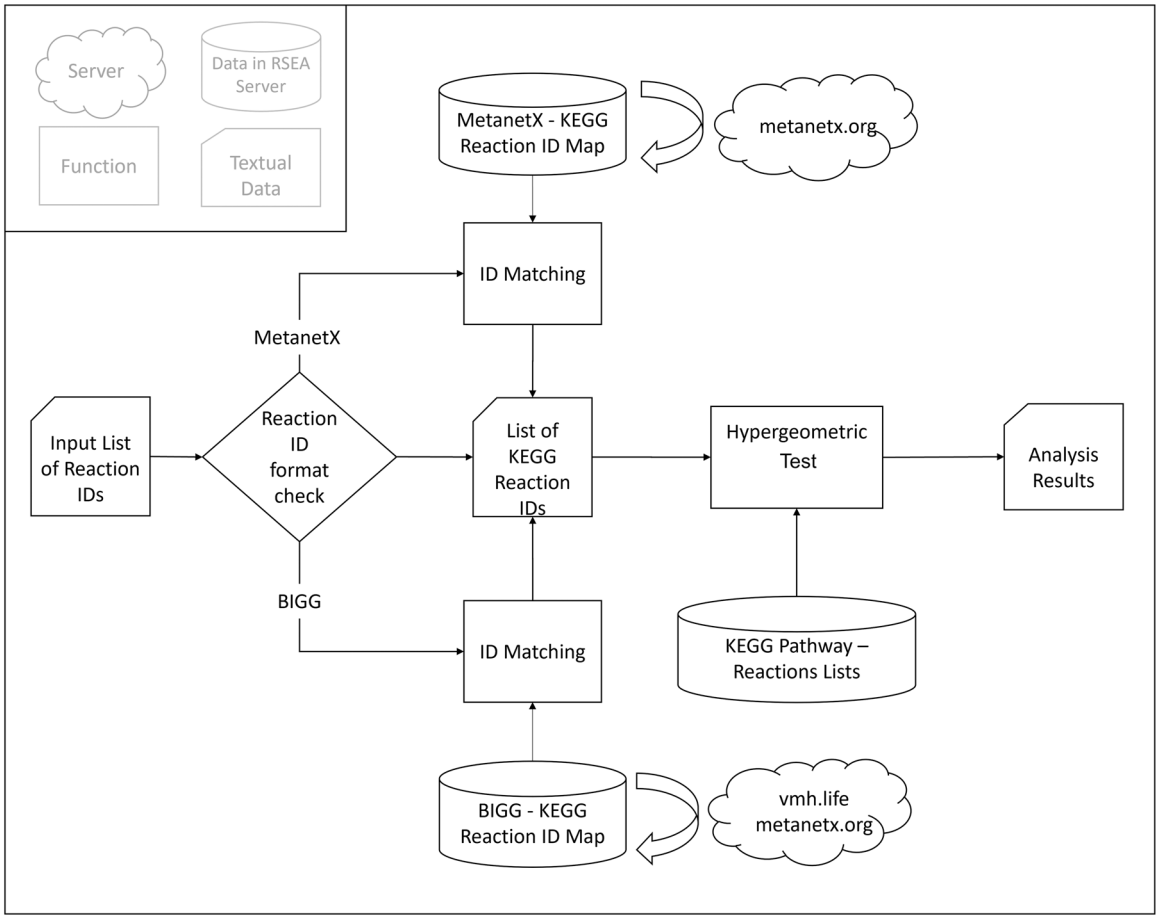


FIGURE 2 | Overview of the back end of the RSEA tool.

10970290, 2025, 8, Downloaded from https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.29020 by Ozyegin University, Wiley Online Library on [10/09/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

enhance our understanding by employing RSEA for DERs. That is why, we converted the DEGs to DERs and detected 1182 DERs (Supporting Information S1).

When we perform pathway enrichment of DEGs, if the genes that are related to the metabolism are few in the DEG list, KEGG metabolic pathways cannot be captured. However, when we convert these DEGs into DERs, we can investigate a subset of these DEGs that are associated with metabolic reactions. Therefore, we can identify metabolic pathways that are statistically meaningful as we altered the background. One can also select metabolic DEGs out of all DEG list and perform conventional pathway enrichment, which could be more practical in some instances. However, DER analysis offers an alternative approach that focuses on reactions rather than genes, enabling the identification of enriched reaction sets that provide an insights avoiding biases led by redundant gene-reaction matches.

The RSEA analysis uncovered specific reaction sets enriched in MS, offering a more targeted perspective on the molecular alterations associated with the disease. Notable pathways related to MS identified through RSEA included tyrosine metabolism, pyrimidine metabolism, purine metabolism, aminoacyl-tRNA biosynthesis and terpenoid backbone biosynthesis (Rispoli et al. 2021; Oppong et al. 2024) (Figure 3). Among the pathways identified for MS through RSEA, purine metabolism and pyrimidine metabolism were also identified through metabolic DEG enrichment analysis (Supporting Information S1).

Comparing the results of GSEA and reaction set enrichment analysis with RSEA appeared to perform better in providing more detailed insights into the molecular complexities of MS. While pathway enrichments of gene sets elucidated important pathways, RSEA revealed complex details of the metabolic reactions involved in the disease and demonstrated its high sensitivity in capturing MS-associated genetic alterations.

In conclusion, this study highlights the practicability of reaction set enrichment analysis in unraveling the molecular mechanism of biological contexts. For this case study, the RSEA tool's capacity to identify specific metabolic reaction sets associated with the disease positions it as a valuable asset in understanding the complexities of MS at the genetic level. Consequently, RSEA has the potential to identify mechanisms of diseases and underline the precise targets for therapeutic interventions.

3.2 | Case Study: Yeast Metabolic Cycle

The study conducted by Cesur et al. (2022) aimed to investigate the contribution of epigenomic information to metabolic predictions in the yeast metabolic cycle and characterize metabolic

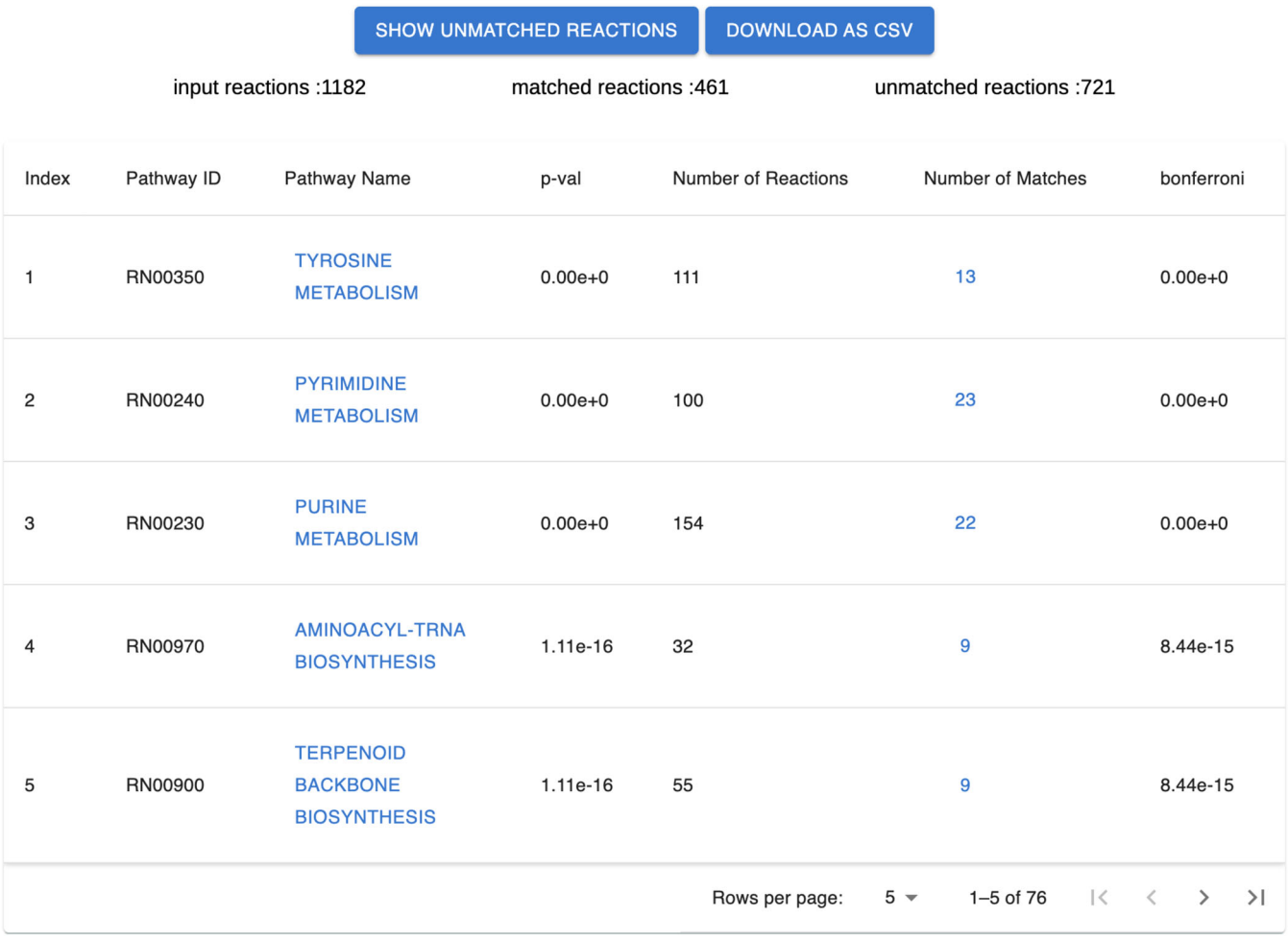


FIGURE 3 | Part of output page of RSEA for case Study 1.

10970290, 2025, 8, Downloaded from https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.29020 by Ozyegin University, Wiley Online Library on [10/09/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

alterations during the yeast metabolic cycle using ATAC-seq and RNA-seq datasets in condition-specific metabolic modeling. As a result of this study, the reactions in Yeast8 GEM (Lu et al. 2019) regulated by phase transitions were identified using the ΔFBA approach (Ravi and Gunawan 2021). The study indicates that the enrichment analysis performed with the relevant genes showed that these genes were enriched in fundamental pathways such as amino acid, fatty acid, carbohydrate, and purine metabolism. Also, it is seen that yeast metabolism is altered in the biosynthesis of secondary metabolites, Lysine biosynthesis, and oxidative phosphorylation pathways in different oxygen consumption levels (Cesur et al. 2022).

When we performed enrichment analysis with RSEA without converting the reactions obtained by the authors with GEMs into gene sets, we obtained the same analysis results as they did with gene sets (Supporting Information S1). This shows that RSEA automates the enrichment process and produces identical results more efficiently, without the need for manual conversion of reaction data to gene sets.

The main advantage of RSEA is that it automates and simplifies the process while providing the same level of insight. This proves that RSEA can streamline analyses by directly using reaction sets, saving time and eliminating intermediate steps required by other methods.

3.3 | Limitations

When using the RSEA tool model selection is important for the background reaction list. If a model is not selected or does not provide a background reaction list, the tool still works but all reactions in the KEGG generic reaction networks will be used as the background, and some significant pathways might lose. Additionaly, standardization of reaction identifiers is an important factor in the performance of such studies. For example, in this version of RSEA, enrichment analysis is performed only for KEGG metabolic pathways. Enrichment analysis cannot be performed in other common pathway databases such as WikiPathways (Pico et al. 2008), Reactome (Milacic et al. 2024) and Rhea (Bansal et al. 2022).

4 | Discussion

Studying living systems through reactions, proteins, and other molecules can help to understand the underlying mechanisms that drive biological processes. This perspective is compatible with the principles of systems biology, which tries to explain it as a whole rather than genetic elements. Also, focusing on reactions, proteins, and other molecular entities is becoming increasingly important in advancing our understanding of biology and addressing complex biological questions (Powell and Dupré 2009; Mazzocchi 2012).

Gene-reaction connections can be identified with GEMs, and metabolic flux can be predicted. Since the 2000s, metabolic modeling applications have been widely used in studies (Gu et al. 2019). However, there is no available tool to analyze the predicted reactions straightforwardly. Therefore, studies examining metabolic flows using GEMs use approaches that are not straightforward to make sense of the predicted reactions (Lu et al. 2023).

In this study, we designed a web-based analysis tool that researchers working in the field can use to analyze their results. RSEA is emerging as an important tool that performs metabolic pathway enrichment analysis using reaction to determine relevant biological pathways. In RSEA, it is possible to perform functional enrichment analyses for metabolic models of various model organisms, including the most comprehensive human metabolic model Recon3D (Brunk et al. 2018). In addition, the user can analyze models not included in the existing models list with the model background list. In this way, relationships between biological pathways can be examined more deeply and easily through metabolic model-based reactions.

In RSEA, enrichment analysis is performed for KEGG metabolic pathways. However, it is important to note that KEGG pathways are manually curated and represent a subset of reactions that may be incomplete, missing certain reactions and pathways that could be relevant to a particular biological context. This limitation could potentially lead to gaps in the pathway enrichment results, as key reactions might be omitted from the analysis.

We tested the effectiveness of the tool with two different case studies. In the first case study, we aimed to examine the pathways related to MS by performing gene set and reaction set enrichment analysis using the GSE21942 (Kemppinen et al. 2011) transcriptomic dataset of MS disease. As a result of our enrichment analysis with DEGs, we identified a spectrum of MS-related pathways. When we converted DEGs to DERs with CobraToolbox's "mapExpressionToReactions" function (Heirendt et al. 2019), RSEA analysis revealed more specific sets of reactions enriched in MS and pathways such as tyrosine metabolism, pyrimidine metabolism, purine metabolism, aminoacyl-tRNA biosynthesis more directly linked to MS (Rispoli et al. 2021). This demonstrates the tool's ability to identify pathways more directly linked to MS at the molecular level.

Besides, if metabolic enzyme-related genes are few in the DEG list, KEGG cannot capture the pathways related to metabolic reactions. This is why the enrichment result we obtained for 1700 DEGs for the case study (Section 3.1) was different from the enrichment result we obtained for 1182 DERs when we converted these DEGs to DERs. With our DERs enrichment, we are interested in the subset of DEGs associated with metabolic reactions so we can identify metabolic pathways that are more statistically significant.

In the other case study (Section 3.2), we saw that the reaction sets previously determined using GEMs were converted into genes related to GPR rules, and enrichment analysis was performed with the determined gene sets. When we repeated this study's results with RSEA, we obtained the same results and significantly facilitated the process.

RSEA is particularly well-suited to address biological questions involving metabolic pathways, where identifying reactions can offer insights into altered biochemical processes. It is also useful for hypothesis generation in metabolic disease research, where specific

10970290, 2025, 8, Downloaded from https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.29020 by Ozyegin University, Wiley Online Library on [10/09/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

reaction sets may highlight potential therapeutic targets or biomarkers. Additionally, RSEA can be applied in studies requiring a deeper understanding of metabolic network alterations, such as those seen in cancer metabolism or rare metabolic disorders.

The primary advantage of RSEA lies in its reaction-centric approach, which provides a more granular understanding of pathway alterations compared to gene-centric methods. This approach allows for the direct analysis of reaction flux and the identification of pathway perturbations at the enzymatic level, offering a closer representation of the underlying metabolic processes. However, one limitation of this method is that it may not capture the full breadth of biological changes when non-metabolic genes play a critical role in a disease or condition.

As a result, with RSEA, a deeper understanding of the relationships between metabolic model-based reactions and biological pathways and the opportunity for more detailed genetic and metabolic interactions are now possible. For future work, we aim to expand the scope and functionality of the tool by integrating additional databases such as Rhea, WikiPathways, and Reactome. This integration will enhance the tool's utility by providing more comprehensive coverage of biological pathways. Additionaly, RSEA 2.0, will aim to minimize data loss by expanding the database coverage and improving identifier matching algorithms. Standardizations for reaction identifiers like International chemical identifier for reactions (RInChI) (Grethe et al. 2018) can help to eliminate number of unmatched identifiers in the upcoming versions. We are preparing for the release of RSEA 2.0 in an upcoming update, which will further improve the tool's capabilities and address these limitations.

Author Contributions

Merve Yarıcı: conceptualization and methodology, writing – original draft preparation, resources, case studies, validation, data analysis and interpretation. **Furkan Cantürk:** back-end algorithms and software, designing and developing the RSEA web-server. **Serdar Dursun:** front-end software, designing and developing the RSEA web-server. **Hatice Nur Aydın:** conceptualization and methodology, resources, case studies. **Muhammed Erkan Karabekmez:** conceptualization and methodology, designing and developing the RSEA web-server, writing – original draft preparation, writing – proofreading, supervision.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

RSEA is freely available and open to all users without registration or login. It is available at https://rseatool.com/.

References

Antonakoudis, A., R. Barbosa, P. Kotidis, and C. Kontoravdi. 2020. "The Era of Big Data: Genome-Scale Modelling Meets Machine Learning." *Computational and Structural Biotechnology Journal* 18: 3287–3300.

Ashburner, M., C. A. Ball, J. A. Blake, et al. 2000. "Gene Ontology: Tool for the Unification of Biology." *Nature Genetics* 25, no. 1: 25–29.

Bansal, P., A. Morgat, K. B. Axelsen, et al. 2022. "Rhea, the Reaction Knowledgebase in 2022." *Nucleic Acids Research* 50, no. D1: D693–D700.

Barrett, T., T. O. Suzek, D. B. Troup, et al. 2005. "NCBI GEO: Mining Millions of Expression Profiles—Database and Tools." *Nucleic Acids Research* 33, no. suppl_1: 562–566.

Brunk, E., S. Sahoo, D. C. Zielinski, et al. 2018. "Recon3D Enables a Three-Dimensional View of Gene Variation in Human Metabolism." *Nature Biotechnology* 36, no. 3: 272–281.

Capela, J., D. Lagoa, R. Rodrigues, et al. 2022. "Merlin, an Improved Framework for the Reconstruction of High-Quality Genome-Scale Metabolic Models." *Nucleic Acids Research* 50, no. 11: 6052–6066.

Cerami, E. G., B. E. Gross, E. Demir, et al. 2010. "Pathway Commons, a Web Resource for Biological Pathway Data." *Nucleic Acids Research* 39, no. suppl_1: D685–D690. https://doi.org/10.1093/nar/gkq1039.

Cesur, M. F., T. Çakır, and P. Pir. 2022. "Genome-Wide Analysis of Yeast Metabolic Cycle Through Metabolic Network Models Reveals Superiority of Integrated ATAC-seq Data Over RNA-seq Data." *mSystems* 7, no. 3: e01347-21.

Chen, J., E. E. Bardes, B. J. Aronow, and A. G. Jegga. 2009. "ToppGene Suite for Gene List Enrichment Analysis and Candidate Gene Prioritization." *Nucleic Acids Research* 37, no. suppl_2: W305–W311.

Dennis, G., B. T. Sherman, D. A. Hosack, et al. 2003. "DAVID: Database for Annotation, Visualization, and Integrated Discovery." *Genome Biology* 4, no. 9: R60.

Du, J., M. Li, Z. Yuan, et al. 2016. "A Decision Analysis Model for KEGG Pathway Analysis." *BMC Bioinformatics* 17, no. 1: 407.

Di Filippo, M., C. Damiani, and D. Pescini. 2021. "GPRuler: Metabolic Gene-Protein-Reaction Rules Automatic Reconstruction." *PLoS Computational Biology* 17, no. 11: e1009550.

Edwards, J. S., M. Covert, and B. Palsson. 2002. "Metabolic Modelling of Microbes: The Flux-Balance Approach." *Environmental Microbiology* 4, no. 3: 133–140. https://doi.org/10.1046/j.1462-2920.2002.00282.x.

Grethe, G., G. Blanke, H. Kraut, and J. M. Goodman. 2018. "International Chemical Identifier for Reactions (RInChI)." *Journal of Cheminformatics* 10: 22.

Gu, C., G. B. Kim, W. J. Kim, H. U. Kim, and S. Y. Lee. 2019. "Current Status and Applications of Genome-Scale Metabolic Models." *Genome Biology* 20: 121.

Heirendt, L., S. Arreckx, T. Pfau, et al. 2019. "Creation and Analysis of Biochemical Constraint-Based Models Using the COBRA Toolbox v. 3.0." *Nature Protocols* 14, no. 3: 639–702.

Hidalgo, M. R., C. Cubuk, A. Amadoz, F. Salavert, J. Carbonell-Caballero, and J. Dopazo. 2017. "High Throughput Estimation of Functional Cell Activities Reveals Disease Mechanisms and Predicts Relevant Clinical Outcomes." *Oncotarget* 8, no. 3: 5160–5178.

Kanehisa, M. 2000. "KEGG: Kyoto Encyclopedia of Genes and Genomes." *Nucleic Acids Research* 28, no. 1: 27–30.

Kanehisa, M., and Y. Sato. 2020. "KEGG Mapper for Inferring Cellular Functions From Protein Sequences." *Protein Science* 29, no. 1: 28–35.

Kemppinen, A. K., J. Kaprio, A. Palotie, and J. Saarela (2011). Systematic Rreview of Genome-Wide Expression Studies in Multiple Sclerosis. *BMJ open*, bmjopen-2011.

Khatri, P., M. Sirota, and A. J. Butte. 2012. "Ten Years of Pathway Analysis: Current Approaches and Outstanding Challenges." *PLoS Computational Biology* 8, no. 2: e1002375.

Kim, B., W. J. Kim, D. I. Kim, and S. Y. Lee. 2015. "Applications of Genome-Scale Metabolic Network Model in Metabolic Engineering." *Journal of Industrial Microbiology and Biotechnology* 42, no. 3: 339–348. https://doi.org/10.1007/s10295-014-1554-9.

King, Z. A., J. Lu, A. Dräger, et al. 2016. "BiGG Models: A Platform for Integrating, Standardizing and Sharing Genome-Scale Models." *Nucleic Acids Research* 44, no. D1: D515–D522.

10970290, 2025, 8, Downloaded from https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.29020 by Ozyegin University, Wiley Online Library on [10/09/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Liu, H., M. Yuan, R. Mitra, et al. 2022. "CTpathway: A CrossTalk-Based Pathway Enrichment Analysis Method for Cancer Research." *Genome Medicine* 14, no. 1: 118.

Lu, H., F. Li, B. J. Sánchez, et al. 2019. "A Consensus S. Cerevisiae Metabolic Model Yeast8 and Its Ecosystem for Comprehensively Probing Cellular Metabolism." *Nature Communications* 10, no. 1: 3586.

Lu, Y., Z. Pang, and J. Xia. 2023. "Comprehensive Investigation of Pathway Enrichment Methods for Functional Interpretation of LC–MS Global Metabolomics Data." *Briefings in Bioinformatics* 24, no. 1: bbac553.

Mazzocchi, F. 2012. "Complexity and the Reductionism–Holism Debate in Systems Biology." *WIREs Systems Biology and Medicine* 4, no. 5: 413–427.

Milacic, M., D. Beavers, P. Conley, et al. 2024. "The Reactome Pathway Knowledgebase 2024." *Nucleic Acids Research* 52, no. D1: D672–D678.

Moretti, S., V. D. T. Tran, F. Mehl, M. Ibberson, and M. Pagni. 2021. "MetaNetX/MNXref: Unified Namespace for Metabolites and Biochemical Reactions in the Context of Metabolic Models." *Nucleic Acids Research* 49, no. D1: D570–D574.

Noronha, A., J. Modamio, Y. Jarosz, et al. 2019. "The Virtual Metabolic Human Database: Integrating Human and Gut Microbiome Metabolism With Nutrition and Disease." *Nucleic Acids Research* 47, no. D1: D614–D624.

Oppong, A. E., L. Coelewij, G. Robertson, et al. 2024. "Blood Metabolomic and Transcriptomic Signatures Stratify Patient Subgroups in Multiple Sclerosis According to Disease Severity." *Iscience* 27, no. 3: 109225.

Passi, A., J. D. Tibocha-Bonilla, M. Kumar, D. Tec-Campos, K. Zengler, and C. Zuniga. 2021. "Genome-Scale Metabolic Modeling Enables in-Depth Understanding of Big Data." *Metabolites* 12, no. 1: 14.

Pathan, M., S. Keerthikumar, C. S. Ang, et al. 2015. "FunRich: An Open Access Standalone Functional Enrichment and Interaction Network Analysis Tool." *Proteomics* 15, no. 15: 2597–2601.

Pico, A. R., T. Kelder, M. P. Van Iersel, K. Hanspers, B. R. Conklin, and C. Evelo. 2008. "WikiPathways: Pathway Editing for the People." *PLoS Biology* 6, no. 7: e184.

Powell, A., and J. Dupré. 2009. "From Molecules to Systems: The Importance of Looking Both Ways." *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences* 40, no. 1: 54–64.

Ravi, S., and R. Gunawan. 2021. "ΔFBA—Predicting Metabolic Flux Alterations Using Genome-Scale Metabolic Models and Differential Transcriptomic Data." *PLoS Computational Biology* 17, no. 11: e1009589.

Reimand, J., M. Kull, H. Peterson, J. Hansen, and J. Vilo. 2007. "G:Profiler—A Web-Based Toolset for Functional Profiling of Gene Lists From Large-Scale Experiments." *Nucleic Acids Research* 35, no. suppl_2: 193–200.

Rian, K., M. R. Hidalgo, C. Çubuk, et al. 2021. "Genome-Scale Mechanistic Modeling of Signaling Pathways Made Easy: A Bioconductor/Cytoscape/Web Server Framework for the Analysis of Omic Data." *Computational and Structural Biotechnology Journal* 19: 2968–2978.

Rispoli, M. G., S. Valentinuzzi, G. De Luca, et al. 2021. "Contribution of Metabolomics to Multiple Sclerosis Diagnosis, Prognosis and Treatment." *International Journal of Molecular Sciences* 22, no. 20: 11112.

Ritchie, M. E., B. Phipson, D. Wu, et al. 2015. "limma Powers Differential Expression Analyses for RNA-Sequencing and Microarray Studies." *Nucleic Acids Research* 43, no. 7: e47-e47.

Subramanian, A., P. Tamayo, V. K. Mootha, et al. 2005. "Gene Set Enrichment Analysis: A Knowledge-Based Approach for Interpreting Genome-Wide Expression Profiles." *Proceedings of the National Academy of Sciences* 102, no. 43: 15545–15550.

Thomas, P. D., M. J. Campbell, A. Kejariwal, et al. 2003. "PANTHER: A Library of Protein Families and Subfamilies Indexed by Function." *Genome Research* 13, no. 9: 2129–2141.

Wu, T., E. Hu, S. Xu, et al. 2021. "clusterProfiler 4.0: A Universal Enrichment Tool for Interpreting Omics Data." *The Innovation* 2, no. 3: 100141.

Yu, G., L. G. Wang, Y. Han, and Q. Y. He. 2012. "clusterProfiler: An R Package for Comparing Biological Themes Among Gene Clusters." *Omics: a Journal of Integrative Biology* 16, no. 5: 284–287.

Zhang, C., and Q. Hua. 2016. "Applications of Genome-Scale Metabolic Models in Biotechnology and Systems Medicine." *Frontiers in Physiology* 6. https://doi.org/10.3389/fphys.2015.00413.

Zhang, C., B. J. Sánchez, F. Li, et al. 2023. Yeast9: A Consensus Yeast Metabolic Model Enables Quantitative Analysis of Cellular Metabolism by Incorporating Big Data. *bioRxiv*, 2023-12.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

10970290, 2025, 8, Downloaded from https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.29020 by Ozyegin University, Wiley Online Library on [10/09/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License