**MADRID:** a pipeline for **MetAbolic Drug Repurposing IDentification**

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**Abstract**

**Summary:** Human metabolic pathways offer numerous therapeutic targets to treat complex diseases such as autoimmunity and cancers. Metabolic modeling can help predict potential drug targets using *in silico* gene or reaction perturbations. However, systematic analyses of metabolic models require the integration of different modeling methods. MADRID is an easy-to-use integrated pipeline for developing metabolic models, running simulation, investigating gene inhibition’s effect on reactions, identifying repurposable drugs, and *in fine* predicting drug targets. It can be installed as a *Docker* image and includes easy to use steps in a *jupyter* notebook.

**Availability and implementation:** The source code of the MADRID pipeline and *Docker* image are available at https://github.com/HelikarLab/MADRID. Contact: bpuniya2@unl.edu and thelikar2@unl.edu

1. **Introduction**

Complex human diseases trigger metabolomic reprogramming, making cellular metabolism a potential source of targets for treatment (DeBerardinis and Thompson, 2012). Genome-scale metabolic network models (GSMNs) are useful computational tools to explore the effect of drugs on a cell’s phenotype and generate testable hypotheses (Jerby and Ruppin, 2012; Uhlen *et al.*, 2017). In recent years, the development of sophisticated tools such as the *COBRA* toolbox in
Matlab and Python facilitated the construction and analysis of metabolic models (Heirendt et al., 2019; Ebrahim et al., 2013).

Constructing tissue- and cell-type-specific GSMNs and performing systems-level studies require collecting various tools based on different programming languages, application programming interfaces (APIs), data resources, and data formats. For example, R/Bioconductor is most suitable for analyzing high-throughput datasets, and Matlab is for metabolic modeling and analysis. Furthermore, model integration with external databases such as DrugBank (Wishart et al., 2018) to run simulations under various conditions requires additional manual, tedious, and error-prone steps. Herein, we present a pipeline for MetAbolic Drug Repurposing IDentification, MADRID, which integrates these tools in one place, and the entire analysis can be run with a few commands in a homogeneous environment.

MADRID analyzes and integrates multi-omics data to build GSMNs for different cell types and tissues. It uses these models together with transcriptomics and proteomics data and existing knowledge from drug databases to identify potential therapeutic targets for user-specified diseases. The pipeline was used to identify and rank drug targets to treat CD4+ T cell-mediated immune disorders (Puniya et al., 2020).

2. Design

MADRID is composed of four major steps for drug target identification (Figure 1). The first step includes the analysis of microarray and proteomics data. The tool defines genes' activity by making decisions based on multiple transcriptomics and proteomics datasets provided by the user. The pipeline uses GEOparser, affy, limma with a custom-built program for data merging.

For transcriptomics datasets, the user provides a configuration file containing the GEO database's accession numbers (i.e., GSE, GSM, GPL) and the name of the platform used (i.e., Affymetrix and Agilent) grouped by cell types. The pipeline will automatically download datasets from the GEO database and analyze them. For proteomics datasets, users need to provide a matrix with protein abundance (expression) across different cell types and a configuration file indicating which samples to use for each cell type.

The second step uses gene activity data generated in the first step and a reference model (e.g., Recon3D (Brunk et al., 2018)) provided in the pipeline and builds cell-type-specific models using COBRApy (Ebrahim et al., 2013), Troppo (Ferreira et al., 2020), CoBAMP (Vieira and
Rocha, 2019). Users can also provide their version of the reference model and input datasets. In case both the reference model and input data are user-provided, they must have the same gene annotations (e.g., Entrez IDs or HGNC symbols). If users in this step supply only the reference model, the model must contain Entrez gene IDs.

The third step analyzes disease-specific data from a user-provided case-control transcriptomics study. It requires a user-supplied configuration file containing GEO accessions (i.e., GSE, GSM, and GPL) and the name of the platform (e.g., Affymetrix, Agilent) with patient and control annotation. The data are downloaded from GEO and analyzed using the *affy* and *limma* packages based on the configuration file. MADRID uses the platform-based standard pipeline to preprocess, normalize, and identify differentially expressed genes by comparing control groups with patients groups. In an ideal situation, the disease datasets are from the same cell-types. However, if the specific cell type data are unavailable, mixed cell populations data such as CD4+ T cells for a particular T cell subtype can be used.

Step 4 consists of multiple substeps. (i) Drug targets obtained from the repurposing tool of the ConnectivityMap database (Corsello *et al.*, 2017) are mapped to metabolic genes in the model. (ii) It performs systematic knockouts of each mapped gene. (iii) Differential fluxes are identified by comparing flux profiles of perturbed and control models. (iv) Differentially regulated fluxes are compared with differentially expressed genes identified in Step 3. This comparison determines the number of up- and down-regulated genes in a disease reversed by each mapped drug. (v) Perturbation effect score (PES; Puniya *et al.*, 2020) is computed for each drug target. PES combines two values: The first value is the number of down-regulated fluxes controlled by up-regulated genes minus up-regulated fluxes controlled by up-regulated genes. The second value is the number of up-regulated fluxes controlled by down-regulated genes minus down-regulated fluxes controlled by down-regulated genes. These two values are normalized with the total number of fluxes and then combined to compute PES. The output of the pipeline will include PES based ranks of drug targets and mapped repurposable drugs for the studied disease.
3. Installation and implementation

MADRID is available as a Docker image and can be run using a jupyter lab notebook. Users need to install Docker to use this pipeline. A Docker image of MADRID can be pulled from GitHub and executed using instructions provided in the Readme file in the repository. The instructions to run the four steps of the pipeline are included in the jupyter notebook.

4. Conclusion

MADRID is an integrative and easy-to-run pipeline that combines various tools analyzing gene and protein expression data, and constructing and simulating metabolic models to identify drug targets and repurposable drugs in human metabolism. The pipeline will help predict drug targets and drugs for complex human diseases such as immune-mediated disorders and cancers that have patients data available. This pipeline's prediction will facilitate drug-discovery for hard-to-treat diseases by providing testable drug targets with repurposed drugs. Predicted new indications for already approved drugs will be indeed cost-effective.
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