MBROLE 2.0—functional enrichment of chemical compounds

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ABSTRACT

Metabolites Biological Role (MBROLE) is a server that performs functional enrichment analysis of a list of chemical compounds derived from a metabolomics experiment, which allows this list to be interpreted in biological terms. Since its release in 2011, MBROLE has been used by different groups worldwide to analyse metabolomics experiments from a variety of organisms. Here we present the latest version of the system, MBROLE2, accessible at http://csbg.cnbcsic.es/mbrole2. MBROLE2 has been supplemented with 10 databases not available in the previous version, which allow analysis over a larger, richer set of vocabularies including metabolite–protein and drug–protein interactions. This new version performs automatic conversion of compound identifiers from different databases, thus simplifying usage. In addition, the user interface has been redesigned to generate an interactive, more intuitive representation of the results.

INTRODUCTION

The final step in the analysis of high-throughput ‘omics’ experiments is typically a functional interpretation of the results, for which several bioinformatics methods have been developed. Functional enrichment, one of the most commonly used approaches for the interpretation of transcriptomics and proteomics experiments (1), can be examined with the aid of several online tools (2). These techniques analyse the functional annotations assigned to the set of genes or proteins obtained in the experiment (e.g. over- or under-expressed). Comparison of the frequency of these annotations with those of a background set allows extraction of annotations statistically enriched in the genes/protein being studied, which are used to interpret the experimental outcome in biological terms.

Recent technological advances allow analysis of the small chemical compound repertoires in biological samples, providing the basis of the field known as metabolomics (3). As with other ‘omics’ techniques, there is a need to interpret the results of these experiments in functional terms. The goal of functional analysis in metabolomics is to transform a long list of metabolites identified in an experiment into a reduced set of meaningful biological terms that represent, for example, the biological pathways affected (4). A few years ago, we developed MBROLE, a web-based tool for the interpretation of metabolomics experiments by functional enrichment analysis (5). Most existing tools at that time focused on the functional analysis of genes/proteins; in contrast, MBROLE and a few others focused on the analysis of chemical compounds (6–8). The first version of MBROLE contained functional (biological) data on compounds from several public databases, including the Kyoto Encyclopedia of Genes and Genomes (KEGG) (9), the Human Metabolome Database (HMDB) (10), the Chemical Entities of Biological Interest (ChEBI) database (11) and the PubChem database (pubchem.ncbi.nlm.nih.gov). MBROLE has been used to analyse metabolomics experiments carried out in organisms such as human (12), rat (13), Escherichia coli (14), Thermobifida fusca (15), Synechococcus elongates (16), Phoenix canariensis (17) or Cordyceps bassiana (18).

Here, we present a new version of MBROLE whose main improvements include new metabolite functional annotations, inclusion of compound interactions with emphasis on metabolite–protein and drug–protein interactions, new supported compound identifiers, automatic conversion of identifiers, an improved user interface with a more intuitive presentation of the results and new annotation reports.

MBROLE2 ANALYSIS

MBROLE2 requires users to (i) provide a list of identifiers (IDs) corresponding to a set of chemical compounds (i.e. those found in the experiment), (ii) select the type of annotations they want to analyse and (iii) select a desired background set (e.g. an organism) (Figure 1A). Several input IDs are supported, and a ‘Conversion’ utility also accepts chemical names, CAS-Registry numbers and PubChem compounds.

MBROLE2 outputs an interactive table with the enriched functional annotations and their statistical significance in terms of P-value and false discovery rate (FDR). P-values are calculated with the cumulative hypergeometric distri-
Metabolite annotations

MBROLE2 allows analysis of functional annotations of chemical compounds compiled from several public databases. In addition to the databases available in the first release (KEGG, HMDB, ChEBI and PubChem), we have included data from 10 new resources comprising metabolome databases such as the Yeast Metabolome Database (YMDB) (20) and the *E. coli* Metabolome Database (ECMDB) (21), pathway and metabolic reaction databases such as the BioCyc Database Collection (22), Rhea (23) and UniPathway (24), and resources centred on specific classes of compounds including lipids, such as the LIPID MAPS Structure Database (LMSD) (25), and drugs, such as the Comparative Toxicogenomics Database (CTD) (26), Medical Subject Headings (MeSH) (27), the DrugBank database (28), and the Manually Annotated Targets and Drugs Online Resource (MATADOR) (29).

With the addition of these databases, MBROLE2 enables analysis of a wide variety of functional annotations that describe many different aspects of the chemistry and biology of chemical compounds (see Table 1); these include pathways and sub-pathways, interactions with enzymes, proteins and other types of molecules, physiological locations, chemical classifications and taxonomies, and biological roles, uses and applications (e.g. drug indications).

Automatic ID conversion

As input, the previous version of MBROLE required a list of chemical identifiers from the same database for which the annotations were to be analysed (e.g. a list of KEGG compounds for the analysis of KEGG pathways or a list of ChEBI accessions for analysis of ChEBI roles). Users therefore had to convert the IDs (using the MBROLE conversion utility) if they were not supported by the chosen database.

In MBROLE2, we have automated the conversion of chemical IDs, such that the user provides a single ID list, which are automatically converted to those of the appropriate database, depending on the annotations chosen for analysis. This greatly simplifies input and allows users to provide a mixture of chemical IDs in the same list. Both user IDs and converted IDs are shown in the results table.

To ensure unambiguous conversion, only chemical IDs are supported as input for analysis (and not metabo-
Pathways and sub-pathways

| Interactions | BioCyc, SMPDB, UniPathway, KEGG pathways, KEGG modules |
|--------------|-----------------------------------------------------|
| Physiological location | With enzymes (ECMDB/YMDB/HMDB, KEGG, BioCyc) |
| Chemical classification and taxonomies | With proteins (BioCyc, MATADOR, HMDB/ECMDB/YMDB) |
| Roles, uses and applications | Other molecules: bioactive peptides, lipids, etc. (KEGG) |
| Cellular (HMDB/ECMDB/YMDB) | Cellular (HMDB/ECMDB/YMDB) |
| Tissue (HMDB) | Tissue (HMDB) |
| Biofluid (HMDB) | Biofluid (HMDB) |
| HMBD/YMDB taxonomy, KEGG classifications, LIPID MAPS taxonomy | HMBD/YMDB taxonomy, KEGG classifications, LIPID MAPS taxonomy |
| Biochemical, chemical and application (ChEBI) | Biochemical, chemical and application (ChEBI) |
| Biofunctions (HMDB) | Biofunctions (HMDB) |
| Roles (KEGG) | Roles (KEGG) |
| Drug indications (DrugBank), pharmacological actions (MeSH) | Drug indications (DrugBank), pharmacological actions (MeSH) |
| Anatomical Therapeutic Chemical (ATC) classification system (for drugs) | Anatomical Therapeutic Chemical (ATC) classification system (for drugs) |
| Diseases (HMDB and CTD) | Diseases (HMDB and CTD) |

Output. A single table is provided with the enriched annotations in all the vocabularies selected. Only annotations found for at least three compounds in the input set are included (Figure 1B). Users can sort the table interactively by any column. For example, to focus on annotations for a given vocabulary, the table can be sorted by the ‘Category’ column; to retrieve the most significant annotations regardless of the vocabulary they belong to, the sort can be by P-value or FDR columns, etc. To spot significant annotations conveniently, cells containing P-values and FDRs ≤ 0.001 are coloured green, yellow (for values between 0.001 and 0.05) and red (>0.05). An option is also included to export this results’ table to a spreadsheet program, so the user can perform additional calculations, post-processing and graphical representations.

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