Metabolic Reaction Analysis Tool - MRAT
Documentation

Metabolic Reaction Analysis Tool (MRAT) is a web-based platform to obtain automatically curated reactions from database(s). Currently version 1.0 includes whole database of MetaCyc (Version: 14.6). MRAT can be used (i) to search all the reactions where mass is not conserved, (ii) to calculate the appropriate balancing coefficients of reactants and products, wherever possible (feasible reactions), (iii) to identify the set of reactions which cannot be stoichiometrically balanced with the present set of substrates and products (infeasible reactions) and (iv) to provides a SBML (a standard for representing models of biochemical and gene-regulatory networks) format of feasible set of reactions with correct stoichiometry. It can provide suggestion to turn an infeasible reaction into a feasible one by inserting filler metabolite(s). One of the best feature of MRAT is - it will generate a Systems Biology Markup Language(SBML) format which includes all feasible balanced reactions with empirical formula and balancing coefficients. One can also correct stoichiometry of any reaction by uploading it as a SBML format. Output of MRAT can be obtained as a zip file containing unique, multiple, infeasible, error report, readme and a SBML format. One can download sample input files from MRAT.

Contact: skbmbg@caluniv.ac.in; rahul.nutron@gmail.com.

Screen-shots:

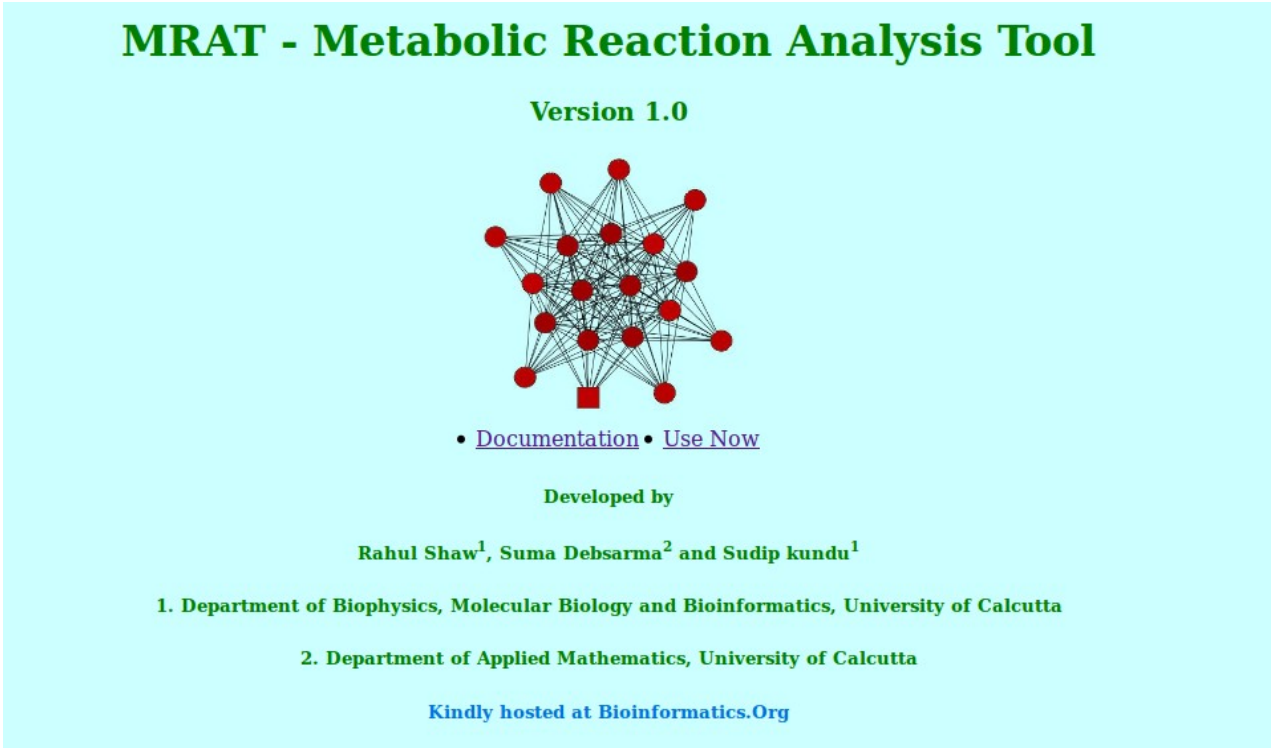

Figure 1 : Screenshot of MRAT

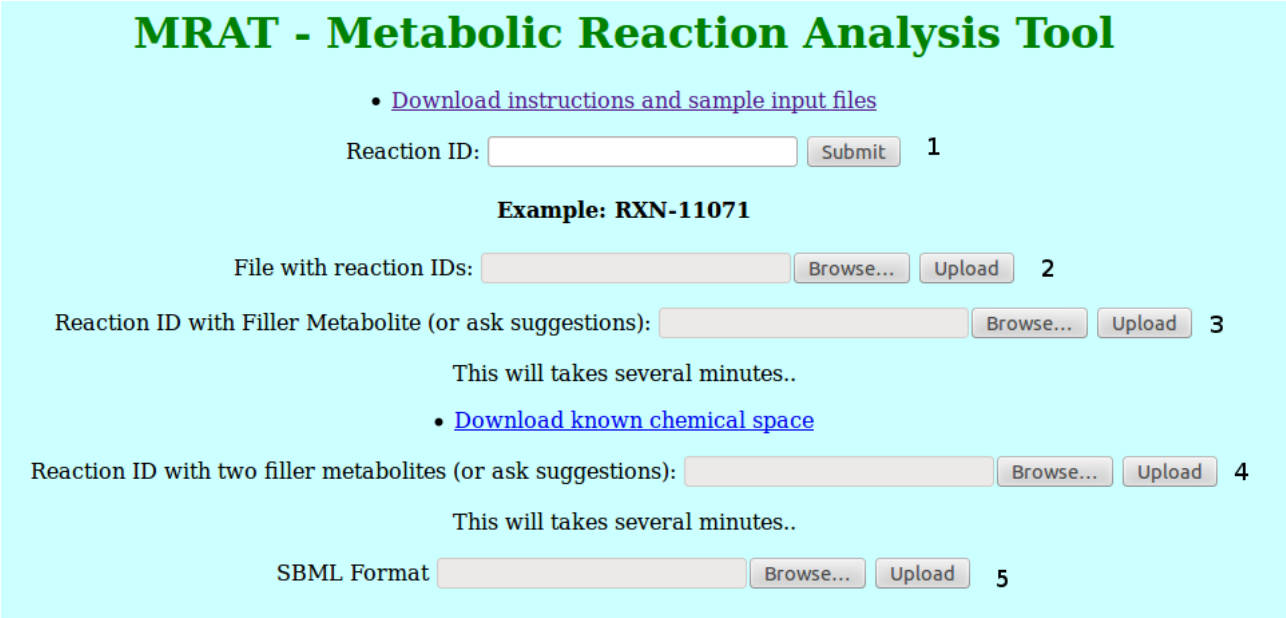

Figure 2 : Screenshot of input page

Figure 3 : Screenshot of tag 1 in Figure 2

Input and Output

Figure 1 shows a screenshot of the main window of MRAT. Figure 2 shows a screenshot of input page of MRAT with tags 1,2,3,4 and 5.

Tag 1. Takes one reaction ID at a time. Figure 3 shows a screenshot of tag 1. Output of tag 1 shown as Attribute-value and SBML format in Figures 7 and 8 respectively.

Tag 2. Upload a file containing reaction IDs. Write the reaction IDs in each line of the file as shown in Figure 4. Output of this submission provides a zip file containing reaction category files and a SBML format (described in the section ' Output SBML Format ') of all feasible reactions. We request users to limit themselves within 5 to 10 reaction IDs at a time due to server limitations.

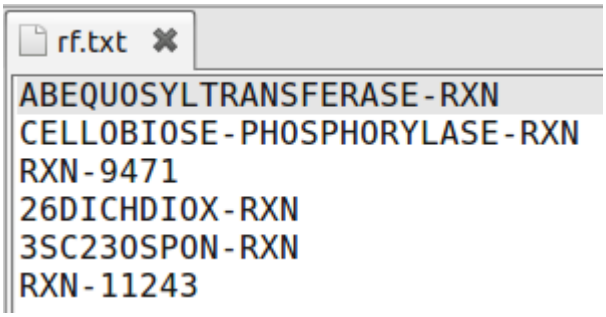

Figure 4: Input file with reaction IDs

Tag 3. Upload file containing infeasible reaction.

- To check the feasibility of an infeasible reaction with a filler metabolite as a substrate upload a file as shown in Figure 5(a). It needs one filler metabolite ID from user.
- To check the feasibility of an infeasible reaction with a filler metabolite as a product upload a file as shown in Figure 5(b). It needs one filler metabolite ID from user.
- To check the feasibility of an infeasible reaction with all possible filler metabolites as substrate upload a file as shown in Figure 5(c). Possible filler metabolites are taken from known chemical space.
- To check the feasibility of an infeasible reaction with all possible filler metabolites as product upload a file as shown in Figure 5(d). Possible filler metabolites are taken from known chemical space.

User can download known chemical space from MRAT. Filler metabolite's coefficient is denoted as ' RIGHT/LEFT FILLER'.

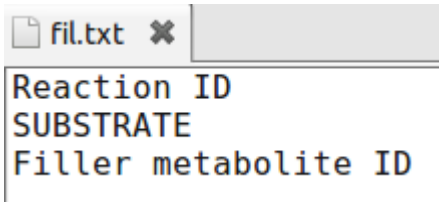

Figure 5(a)

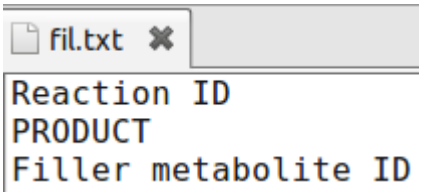

Figure 5(b)

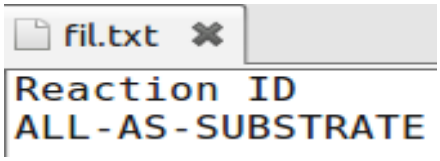

Figure 5(c)

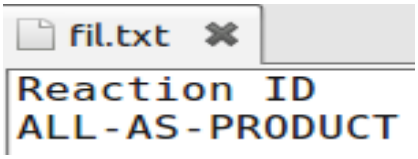

Figure 5(d)

Figure 5

Tag: 4. Upload a file which should include an infeasible reaction ID and two filler metabolites (one as substrate and other as product). If 'ALL' [see Figures 6(b) and 6(c)] is written for substrate or product, all combinations for one entered substrate with all possible products and *vice versa* are obtained. For two filler entries (inserted by the user) as substrate and product, enter one as substrate and other as product [Figure 6(a)] and a negative coefficient in the output shall indicate the required change in the position. Examples of three possible input here are shown in Figure 6 below.

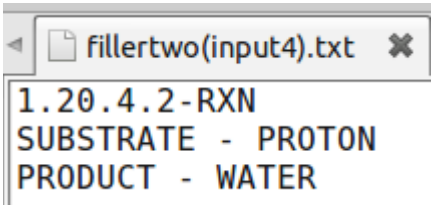

Figure 6(a)

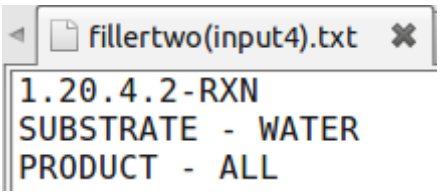

Figure 6(b)

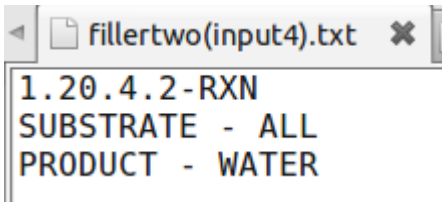

Figure 6(c)

Figure 6

Asking suggestions of filler metabolite (8869 are available) [Figures 5(c), 5(d), 6(b) and 6(c)] is time consuming due to server limitations as for each filler ID, MRAT will have to generate the stoichiometric matrix. If anything goes wrong, please use our "Live ip" server for MRAT by clicking the dialogue "Problem? click here".

Tag 5. Upload a SBML format of reaction(s) having 'empiricalFormula' as a component of species and the format should include 'listOfReactants' and 'listOfProducts'. Described in the section 'Input SBML Format'.

```
UNIQUE-ID - RXN-1082
LEFT - R-2-HYDROXYGLUTARATE
CHEMICAL FORMULA - (H 6)
CHEMICAL FORMULA - (C 5)
CHEMICAL FORMULA - (O 5)
^COEFFICIENT(1) - 1.0
^COEFFICIENT(2) - 4.0
^COEFFICIENT(3) - 5.0
LEFT - ACETYL-COA
CHEMICAL FORMULA - (C 23)
CHEMICAL FORMULA - (H 34)
CHEMICAL FORMULA - (O 17)
CHEMICAL FORMULA - (N 7)
CHEMICAL FORMULA - (P 3)
CHEMICAL FORMULA - (S 1)
^COEFFICIENT(1) - 1.0
^COEFFICIENT(2) - 6.0
^COEFFICIENT(3) - 1.0
LEFT - PROTON
CHEMICAL FORMULA - (H 1)
^COEFFICIENT(1) - 4.0
^COEFFICIENT(2) - 21.0
^COEFFICIENT(3) - 10.0
RIGHT - 2-HYDROXYGLUTARYL-COA
CHEMICAL FORMULA - (C 26)
CHEMICAL FORMULA - (H 41)
CHEMICAL FORMULA - (O 20)
CHEMICAL FORMULA - (N 7)
CHEMICAL FORMULA - (P 3)
CHEMICAL FORMULA - (S 1)
^COEFFICIENT(1) - 1.0
^COEFFICIENT(2) - 6.0
^COEFFICIENT(3) - 1.0
RIGHT - ACET
CHEMICAL FORMULA - (H 3)
CHEMICAL FORMULA - (C 2)
CHEMICAL FORMULA - (O 2)
^COEFFICIENT(1) - 1.0
^COEFFICIENT(2) - 1.0
^COEFFICIENT(3) - 11.0
```

Figure 7: Output in Attribute-Value format

```
<?xml version="1.0" encoding="UTF-8"?>
<sbml xmlns="http://www.sbml.org/sbml/level2" version="1" level="2">
    <model id="model" name="MRAT-MODEL">
        <listOfCompartments>
            <compartment id="compartment" name="compartment" size="1.0"/>
        </listOfCompartments>
        <listOfSpecies>
            <species id="R-2-HYDROXYGLUTARATE" name="--i-R--i---2-hydroxyglutarate" compartment="compartment"
empiricalFormula="H6C5O5">
            </species>
            <species id="ACETYL-COA" name="acetyl-CoA" compartment="compartment" empiricalFormula="C23H34O17N7P3S1">
            </species>
            <species id="PROTON" name="H-SUP----SUP-" compartment="compartment" empiricalFormula="H1">
            </species>
            <species id="2-HYDROXYGLUTARYL-COA" name="-R--2-hydroxyglutaryl-CoA" compartment="compartment"
empiricalFormula="C26H41O20N7P3S1">
            </species>
            <species id="ACET" name="acetate" compartment="compartment" empiricalFormula="H3C2O2">
            </species>
        </listOfSpecies>
        <listOfReactions>
            <reaction id="RXN-1082" name="name" reversible="false">
                <listOfReactants>
                    <speciesReference species="R-2-HYDROXYGLUTARATE" coefficient="1.0"/>
                    <speciesReference species="PROTON" coefficient="4.0"/>
                    <speciesReference species="ACETYL-COA" coefficient="1.0"/>
                </listOfReactants>
                <listOfProducts>
                    <speciesReference species="2-HYDROXYGLUTARYL-COA" coefficient="1.0"/>
                    <speciesReference species="ACET" coefficient="1.0"/>
                </listOfProducts>
            </reaction>
        </listOfReactions>
    </model>
</sbml>
```

Figure 8: Output SBML format

Output SBML Format

```
<listOfSpecies>
    <species id="WATER" name="H-SUB-2--SUB-O" compartment="compartment" empiricalFormula="H2O1">
    </species>
    <species id="TRICHLOROETHENE" name="trichloroethylene" compartment="compartment" empiricalFormula="H1C2CL3">
    </species>
    <species id="CPD-9675" name="trichloroacetate" compartment="compartment" empiricalFormula="C2O2CL3">
    </species>
    <species id="PROTON" name="H-SUP----SUP-" compartment="compartment" empiricalFormula="H1">
    </species>
    <species id="CL-" name="chloride" compartment="compartment" empiricalFormula="CL1">
    </species>
    <species id="OXALATE" name="oxalate" compartment="compartment" empiricalFormula="C2O4">
    </species>
</listOfSpecies>
```

Figure 9(a)

```
<listOfReactions>
    <reaction id="RXN-9150" name="name" reversible="false">
        <listOfReactants>
            <speciesReference species="WATER" coefficient="2.0"/>
            <speciesReference species="CPD-9675" coefficient="1.0"/>
        </listOfReactants>
        <listOfProducts>
            <speciesReference species="CL-" coefficient="3.0"/>
            <speciesReference species="OXALATE" coefficient="1.0"/>
            <speciesReference species="PROTON" coefficient="4.0"/>
        </listOfProducts>
    </reaction>
    <reaction id="RXN-9149" name="name" reversible="false">
        <listOfReactants>
            <speciesReference species="WATER" coefficient="2.0"/>
            <speciesReference species="TRICHLOROETHENE" coefficient="1.0"/>
        </listOfReactants>
        <listOfProducts>
            <speciesReference species="CPD-9675" coefficient="1.0"/>
            <speciesReference species="PROTON" coefficient="5.0"/>
        </listOfProducts>
    </reaction>
</listOfReactions>
```

Figure 9(b)

Figure 9: MRAT output in SBML format

Figure 9 shows MRAT output in SBML format . Feasible input reaction's information will automatically converts in SBML format including participating metabolite's empirical formulas, also each of these reactions are mass balanced. Figure 9(a) and Figure 9(b) shows a SBML format of two reactions RXN-9149 and RXN-9150 (shown below).

RXN-9149:
trichloroethylene + 2Water(H2O) = trichloroacetate + 5Proton(H) (1)

RXN-9150:
trichloroacetate + 2Water(H2O) = 3chloride + oxalate + 4Proton(H) (2)

Figure 9(a) shows the portion which shows specie's empirical formulas and Figure 9(b) shows the portion which shows balancing coefficients.

Input SBML Format

Figure 10 shows a portion of the input SBML file where all metabolite's (species) empirical formulas are declared. Figure 11 shows the part of input SBML format which includes reaction list without metabolite's balancing coefficients and Figure 12 shows the output after obtaining balancing coefficients by MRAT.

```
<listOfSpecies>
    <species id="NITRIC-OXIDE" name="nitric-oxide" compartment="compartment" empiricalFormula="H1O1N1">
    </species>
    <species id="OXYGEN-MOLECULE" name="oxygen" compartment="compartment" empiricalFormula="O2">
    </species>
    <species id="NITROGEN-MOLECULE" name="N2" compartment="compartment" empiricalFormula="N2">
    </species>
    <species id="PROTON" name="H" compartment="compartment" empiricalFormula="H1">
    </species>
    <species id="CPD-12498" name="7-oxateasterone" compartment="compartment" empiricalFormula="H48C28O5">
    </species>
    <species id="CPD-12497" name="7-oxatyphasterol" compartment="compartment" empiricalFormula="H48C28O5">
    </species>
</listOfSpecies>
```

Figure 10: Input SBML format including specie's empirical formulas

```
<listOfReactions>
    <reaction id="RXN-11489" name="name" reversible="false">
        <listOfReactants>
            <speciesReference species="NITRIC-OXIDE"/>
        </listOfReactants>
        <listOfProducts>
            <speciesReference species="OXYGEN-MOLECULE"/>
            <speciesReference species="PROTON"/>
            <speciesReference species="NITROGEN-MOLECULE"/>
        </listOfProducts>
    </reaction>
    <reaction id="RXN-11539" name="name" reversible="false">
        <listOfReactants>
            <speciesReference species="CPD-12498"/>
        </listOfReactants>
        <listOfProducts>
            <speciesReference species="CPD-12497"/>
        </listOfProducts>
    </reaction>
</listOfReactions>
```

Figure 11: Input reaction list without species’s coefficients

```
<listOfReactions>
    <reaction id="RXN-11489" name="name" reversible="false">
        <listOfReactants>
            <speciesReference species="NITRIC-OXIDE" coefficient="2.0"/>
        </listOfReactants>
        <listOfProducts>
            <speciesReference species="OXYGEN-MOLECULE" coefficient="1.0"/>
            <speciesReference species="PROTON" coefficient="2.0"/>
            <speciesReference species="NITROGEN-MOLECULE" coefficient="1.0"/>
        </listOfProducts>
    </reaction>
    <reaction id="RXN-11539" name="name" reversible="false">
        <listOfReactants>
            <speciesReference species="CPD-12498" coefficient="1"/>
        </listOfReactants>
        <listOfProducts>
            <speciesReference species="CPD-12497" coefficient="1"/>
        </listOfProducts>
    </reaction>
</listOfReactions>
```

Figure 12: MRAT output reaction list with species’s coefficients