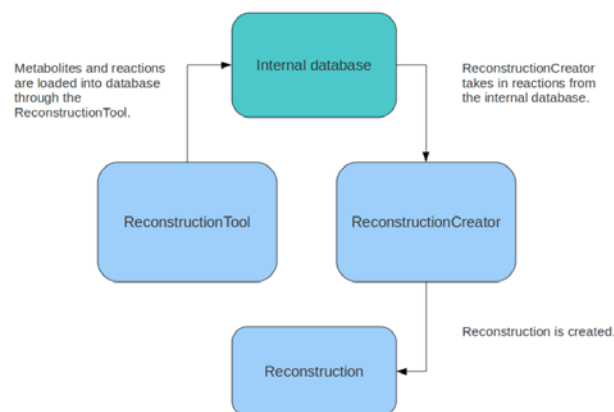


Generation and manipulation of reconstructions with rBioNet

Author(s): Ines Thiele, Ronan M. T. Fleming, Systems Biochemistry Group, LCSB, University of Luxembourg.

Reviewer(s): Catherine Clancy, Stefania Magnúsdóttir, LCSB, University of Luxembourg.

rBioNet is a reconstruction tool that lets you assemble reconstruction in a user friendly environment. In this tutorial you shall learn how you can use this tool to either start a new reconstruction or load in an existing one, followed by, its analysis. The tool consists of 3 main parts, i.e., metabolite creator, reaction creator and reconstruction creator. The metabolite creator is used to add in metabolites and its associated information, i.e., its elemental formula, charge, identifiers (for e.g., KEGG ID, PubChem ID etc.) and other associated attributes. Alternatively, a text file containing all the necessary information in the same order as in the metabolite database can be loaded directly. The reaction creator is used to formulate reactions and as stated before a text file containing all the necessary information about the reaction abbreviation, description, formula, reversibility, confidence score, notes, references. Alternatively, a text file containing all the necessary information in the same order as in the reaction database can be loaded on to the reaction creator directly. The reconstruction creator is used to load in reactions from the reactions database and then assign GPRs (gene-protein-reaction association), subsystem, add in more information in the notes and reference section. Once you have completed your reconstruction you can look at the S-matrix, identify dead ends, look for neighboring reaction to a particular reaction and plot metabolite connectivity in the reconstruction creator with its statistics function.



Features of rBioNet:

Environment to assemble reconstruction that consists of 3 parts

1. Metabolite creator
2. Reaction creator
3. Reconstruction creator

Metabolite creator

- Associated with a metabolite database.
- Used to create a new metabolite in one of three possible ways:
 1. Uploading from a text file that contains all the information in the same order as in the database.
 2. Manually filling in all the information.
 3. Loading from other COBRA reconstructions.
- Checks for duplicate entries.
- Checks the metabolite abbreviation and charged formula.
- Metabolites are organism and compartment independent.

Reaction creator

- Associated with a reaction database.
- Methods to create a reaction is same as for metabolites.
- Reactions contain metabolites pre-existing in the metabolite database.
- Checks for duplicate entries, mass and charge balance.
- Reactions are organism independent but compartment specific. The same reaction can occur in different compartments.
- Either start from scratch or load pre-existing reconstruction.
- Primarily used to assign GPRs.
- Also to add notes, subsystem etc.

Add-ons

- Reconstruction analyzer.
- Checks for dead-end metabolites.
- Provides suggestions for exchange reactions.
- S-matrix visualization.
- Neighbor Reactions & Metabolite connectivity.

EQUIPMENT SETUP

Initialize the COBRA Toolbox.

Initialize The Cobra Toolbox using the `initCobraToolbox` function.

```
%initCobraToolbox
```

Setting the optimization solver.

This tutorial will be run with a 'glpk' package, which is a linear programming ('LP') solver. The 'glpk' package does not require additional installation and configuration.

```
solverName='glpk';
solverType='LP';
changeCobraSolver(solverName,solverType,1);
```

However, for the analysis of large models, such as Recon 3, it is not recommended to use the 'glpk' package but rather an industrial strength solver, such as the 'gurobi' package. For detailed information, refer to The Cobra Toolbox [solver installation guide](#).

A solver package may offer different types of optimization programmes to solve a problem. The above example used a LP optimization, other types of optimization programmes include; mixed-integer linear programming ('MILP'), quadratic programming ('QP'), and mixed-integer quadratic programming ('MIQP').

```
warning off MATLAB:subscripting:noSubscriptsSpecified
```

```
if usejava('desktop') % This line of code is to avoid execution of this tutorial in non gui-environments
```

Steps to load and initiate the Reconstruction Tool

Start up: rBioNet needs a pre-existing database to start up. Stored in the rBioNet is a database. The database consists of the ten reactions of the glycolysis pathway. Hence, you will see the glycolysis reactions in the reaction creator window, the metabolites participating in these reactions in the metabolite creator window.

0. Open MATLAB

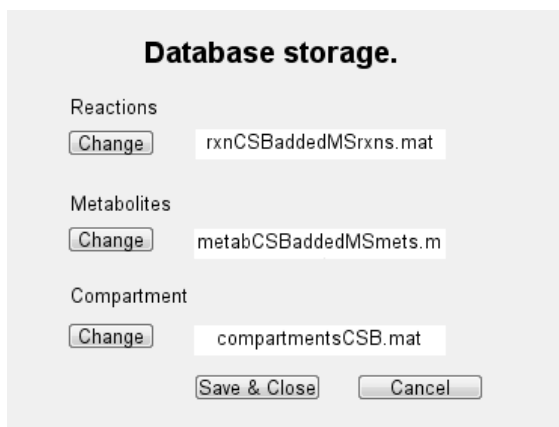
1. Add The CobraToolbox provided to you in your path:

In MATLAB window go to *file-> setpath-> add with subfolders-> cobra toolbox folder -> save & close*

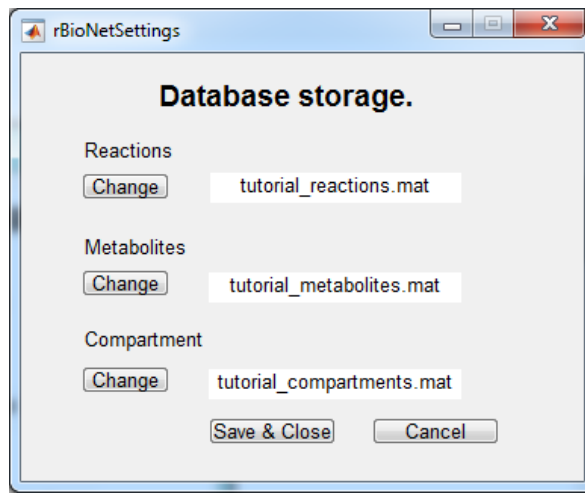
2. Initiate rBioNet by linking the database files.

- Note there are three .mat files, corresponding to the reaction database, metabolite database, and the compartment database.

```
rBioNetSettings
```



- This window below appears:



Click on the change tab for reactions and locate to the tutorial_reactions.mat file, which is provided to you in the rBioNet tutorial folder.

Reactions-> Change-> tutorial_reactions.mat->save

Click on the change tab under metabolites and locate to the tutorial_metabolites.mat file, which is provided to you in the rBioNet tutorial folder.

Metabolites->Change-> tutorial_metabolites.mat->save

Click on the change tab under compartment and locate to the tutorial_compartments.mat file, which is provided to you in the rBioNet tutorial folder.

Compartment->Change-> tutorial_compartments.mat -> save

This is the pre-existing database with glycolysis reactions and you saved it as your current database. You can modify it or remove the reactions as per your requirements.

Open and navigating in rBioNet

Let's open the rBioNet tool:

ReconstructionTool

A window appears called the 'Reaction and Metabolite Editor'.

Click on the *Reaction View Table* and then *Show All*, which shall show all the glycolysis reactions.

Reaction view table: *Reaction creator -> Refresh/Show All*

Reaction And Metabolite Editor

File Edit Help

Reaction View Table Metabolite View Table

	Abbreviation	Description	Formula
1	ENO	enolase	$2pg[c] \rightleftharpoons h2o[c] + pep[c]$
2	Ex_glc-L(e)	Ex_glc-L(e)	$glc-L[e] \rightarrow$
3	FBA	fructose-bisphosphate aldolase	$fdp[c] \rightleftharpoons dhap[c] + g3p[c]$
4	GAPD	glyceraldehyde-3-phosphate dehydrogenase	$g3p[c] + nad[c] + p[c] \rightleftharpoons 13dp[c] + h[c] + nadh[c]$
5	Glc-Dt	Glc-Dt	$glc-D[e] \rightleftharpoons glc-D[c]$
6	HEX1	hexokinase (D-glucose:ATP)	$atp[c] + glc-D[c] \rightarrow adp[c] + g6p[c] + h[c]$

Search

Abbreviation ☐ Exact Match

Metabolite / Reaction

New Reaction Load Reaction Save Reaction

Reaction

Reaction Abbreviation

Direction : Irreversible

Reaction Description

Confidence Score : 0

Metabolite

Abbreviation

Compartment Acidocalcisome (a)

Reaction Side Substrate

Coefficient 1

Metabolite

Add Remove

	Abbreviation	Description	Coefficient	Compartment
1				
2				
3				
4				

Click on the *Metabolite View Table* and then *Show All*, which shall let you see all the glycolysis metabolites

Metabolite view table: *Metabolite creator* -> *Refresh/Show All*

Reaction And Metabolite Editor

File Edit Help

Reaction View Table Metabolite View Table

	Abbreviation	Description	Neutral formula	Charged formula	Charge	KeggID	PubChemI
1	13dpg	3-Phospho-D-glyceroyl phosphate	C3H8O10P2	C3H4O10P2	-4	C00236	3535
2	2pg	D-Glycerate 2-phosphate	C3H7O7P	C3H4O7P	-3	C00631	3904
3	3pg	3-Phospho-D-glycerate	C3H7O7P	C3H4O7P	-3	C00197	3497
4	adp	ADP	C10H15N5O10P2	C10H12N5O10P2	-3	C00008	3310
5	atp	ATP(4-)	C10H16N5O13P3	C10H12N5O13P3	-4	C00002	
6	dhap	Dihydroxyacetone phosphate	C3H7O6P	C3H5O6P	-2	C00111	3411

Search

Abbreviation ☐ Exact Match

Metabolite / Reaction

New Reaction Load Reaction Save Reaction

Reaction

Reaction Abbreviation

Direction : Irreversible

Reaction Description

Confidence Score : 0

Metabolite

Abbreviation

Compartment Acidocalcisome (a)

Reaction Side Substrate

Coefficient 1

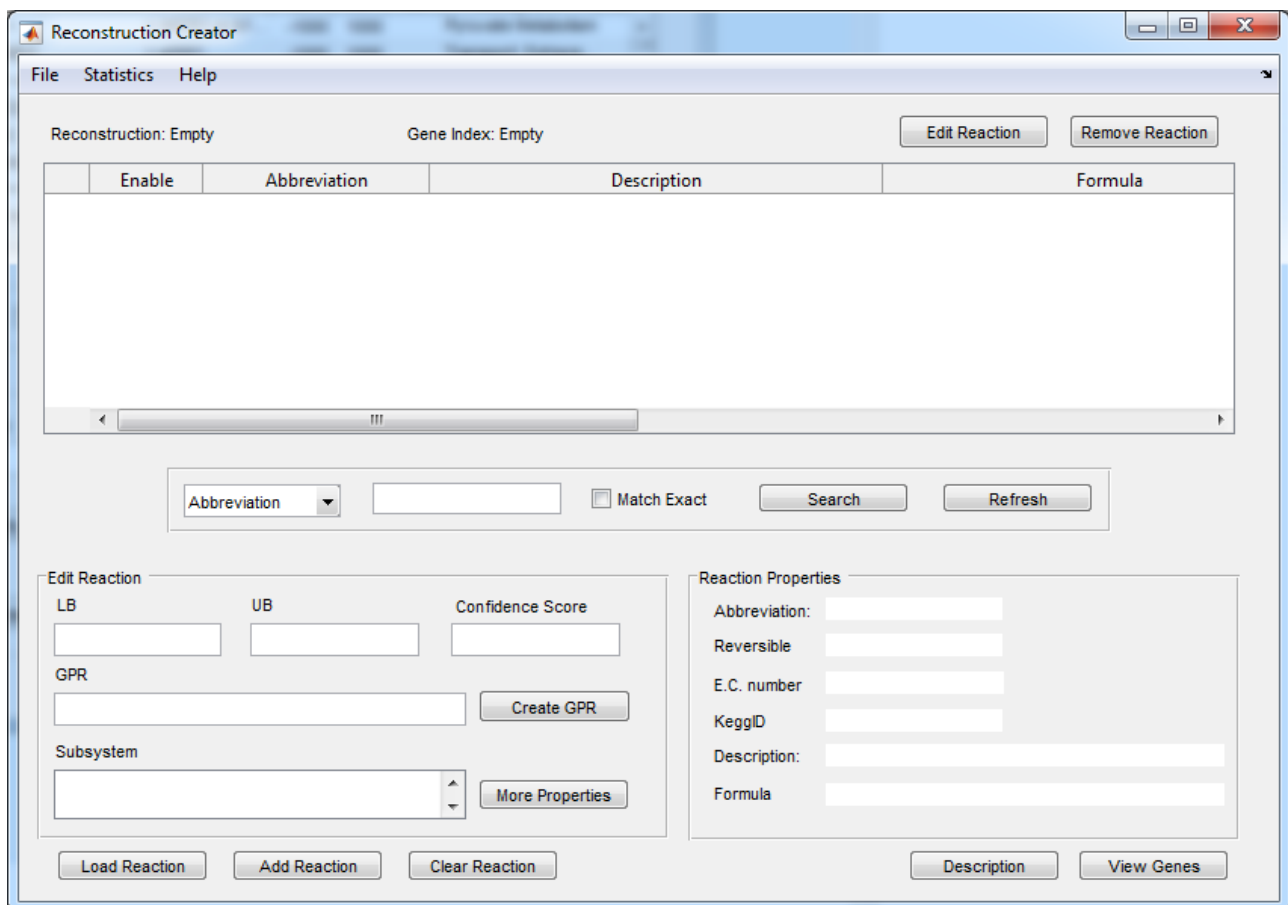
Metabolite

Add Remove

	Abbreviation	Description	Coefficient	Compartment
1				
2				
3				
4				

To visualize the 'Reconstruction Creator' window go to File and Open the model creator.

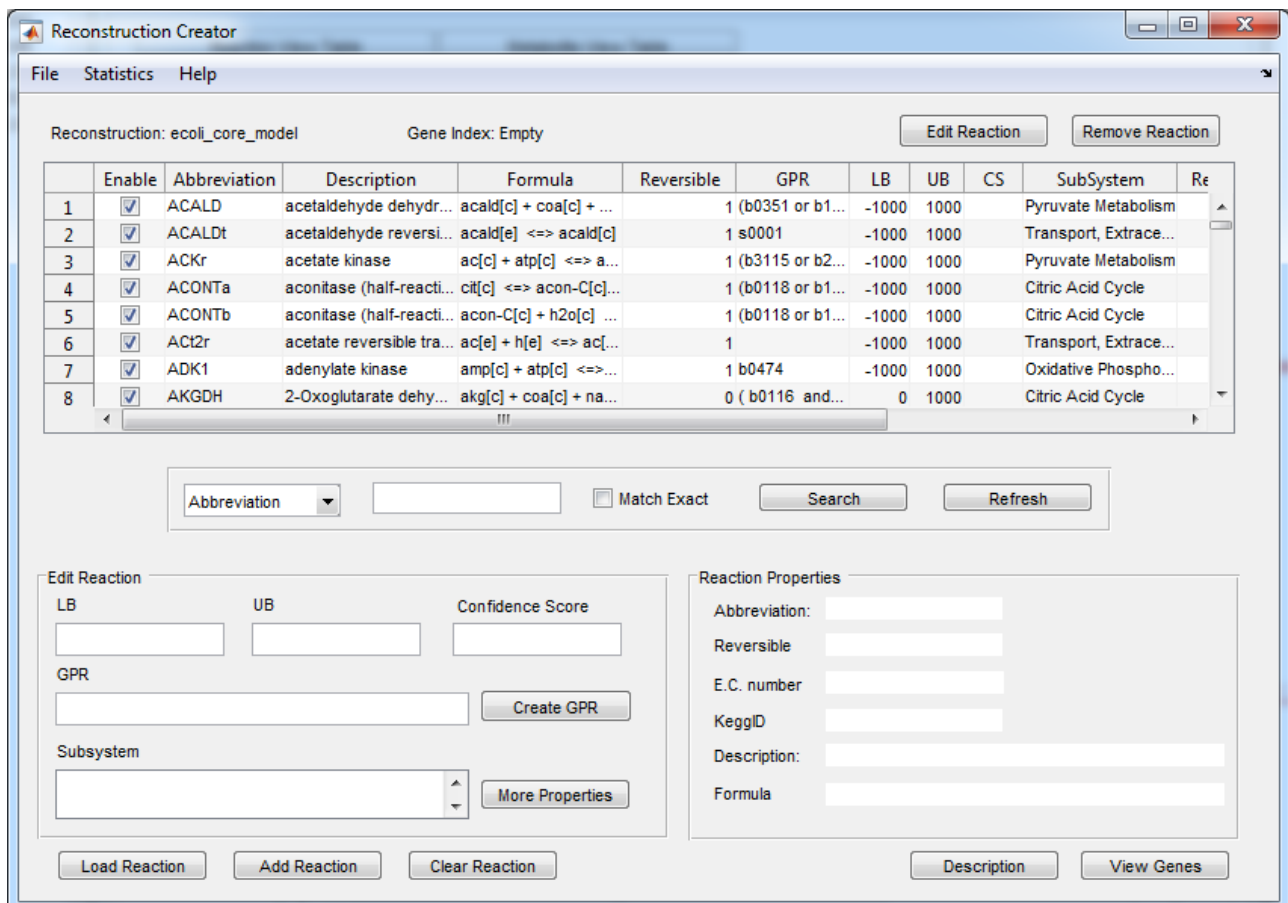
File -> Open Model Creator -> Reconstruction Creator



Load in the *E.coli* core model into the reconstruction creator

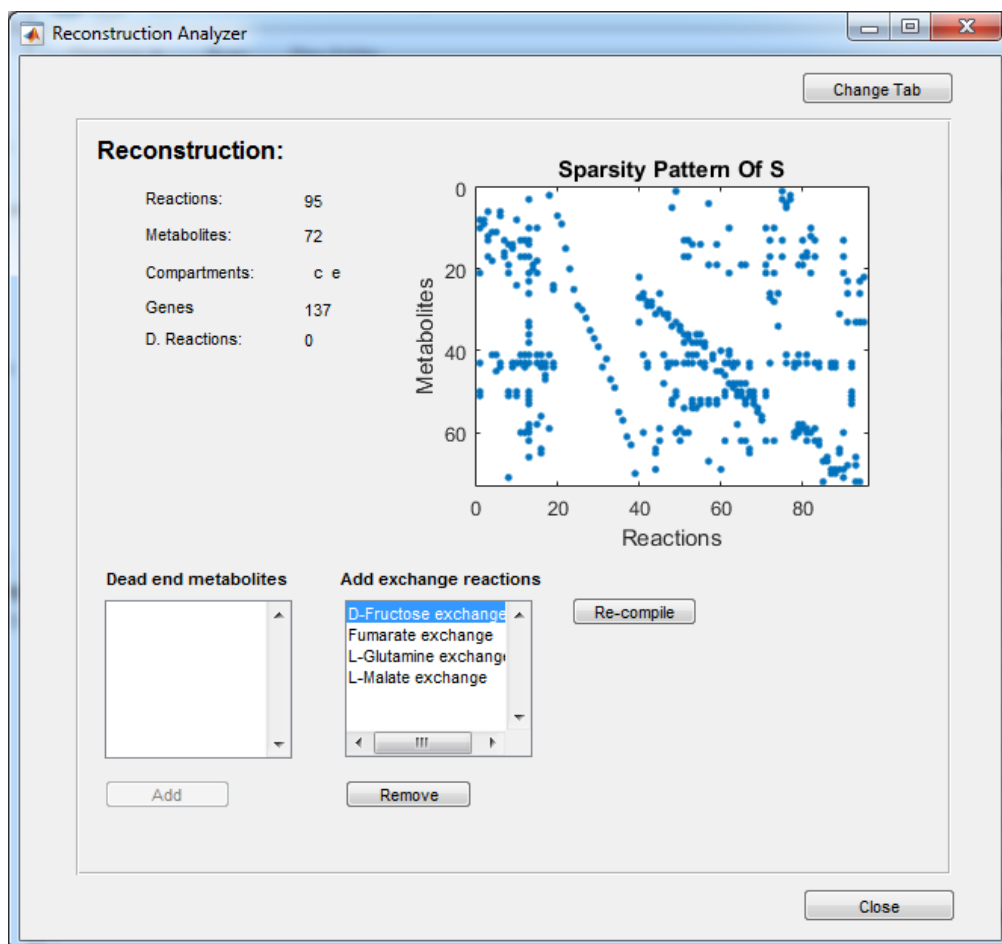
In the Reconstruction Creator do as follows:

File -> open model -> complete reconstruction -> select the *E. coli* core model provided in the tutorial folder (tutorial_Ecoli_core_model.mat) -> click yes on the reconstruction description bar -> click no on the load gene index bar.



Now, we see the content of the *E. coli* core model in the reconstruction creator.

On the reconstruction creator, click on *Statistics -> Reconstruction analyzer*. A window called the 'Reconstruction Analyzer' appears and is used to visualize the S-matrix and identifies dead end metabolites.



Adding new metabolites

Manually adding a new metabolite

Go to the Reaction and Metabolite Editor window and click on the Metabolite/Reaction tab to switch to the New/Load/Save Metabolite view.

Reaction And Metabolite Editor

File Edit Help

Reaction View Table Metabolite View Table

	Abbreviation	Description	Neutral formula	Charged formula	Charge	KeggID	PubChemI
1	13dpg	3-Phospho-D-glyceroyl phosphate	C3H8O10P2	C3H4O10P2	-4	C00236	3535
2	2pg	D-Glycerate 2-phosphate	C3H7O7P	C3H4O7P	-3	C00631	3904
3	3pg	3-Phospho-D-glycerate	C3H7O7P	C3H4O7P	-3	C00197	3497
4	adp	ADP	C10H15N5O10P2	C10H12N5O10P2	-3	C00008	3310
5	atp	ATP(4-)	C10H16N5O13P3	C10H12N5O13P3	-4	C00002	
6	dhap	Dihydroxyacetone phosphate	C3H7O6P	C3H5O6P	-2	C00111	3411

Search

Abbreviation ☐ Exact Match

Abbreviation* Description*

Neutral Formula Inchi String

Charged formula*

Charge* Smile

KeggID

PubChemID HMDB CheBIID

* Required

- Enter the required information for a new metabolite, including: abbreviation (cbp), description (Carbamoyl phosphate), charged formula (CH2NO5P), and charge (-2).
- Then click Save Metabolite.

Adding metabolites from a text file

Alternatively, load a text file directly into the Reaction and Metabolite Editor.

Go to file -> add text file -> with metabolite -> select the file tutorial_ureacycle_mets.txt (provided in the tutorial folder)

Click yes on each window that appears.

- When you are using this approach to create your reconstruction, make sure that **all** the information is **absolutely correct** and thoroughly checked before you make the addition.

addmetabolites

Text file: Columns separated by tabs and in same order as in table. Make sure your data is correct after loading. Data in table can be edited here before adding to database. Columns marked with star (*) are mandatory.

	Abbreviation*	Description*	Neutral formula	Charged formula*	Charge*	KeggID	PubChemID	CheBIID	Inchi String	Smil
1	cbp	Carbamoyl p...	CH4NO5P	CH2NO5P	-2	C00169				
2	hco3	Bicarbonate		CHO3	-1	C00288				
3	nh4	Ammonium		H4N	1	C01342				
4	orn	Ornithine	C5H12N2O2	C5H13N2O2	1	C01602				
5	citr-L	L-Citrulline	C6H13N3O3	C6H13N3O3	0	C00327				
6	asp-L	L-Aspartate	C4H7NO4	C4H6NO4	-1	C00049				
7	ppi	Diphosphate	H4O7P2	HO7P2	-3	C00013				
8	amp	AMP	C10H14N5O7P	C10H12N5O7P	-2	C00020				
9	argsuc	N(omega)-(L...	C10H18N4O6	C10H17N4O6	-1	C03406				

Adding new reaction

Manually adding reactions

Go to the Reaction and Metabolite Editor window and click on the Metabolite/Reaction tab to switch to the New/Load/Save Reaction view.

Then, click New Reaction and enter the reaction information including: the reaction abbreviation (ARGN), description (arginase), direction (Irreversible), and confidence score (4). Click on More Properties to add additional information (Notes, References, EC Number, KeggID).

Next go the "Metabolite View Table" and select a metabolite belonging to the reaction.

Enter the metabolite's coefficient, compartment and reaction side (substrate or product).

- Metabolite: arg-L, Compartment: Cytoplasm (c), Reaction Side: Substrate, Coefficient: 1. -> Click Add to add the metabolite to the reaction.
- Metabolite: h2o, Compartment: Cytoplasm (c), Reaction Side: Substrate, Coefficient: 1. -> Add.
- Metabolite: orn, Compartment: Cytoplasm (c), Reaction Side: Product, Coefficient: 1. -> Add.
- Metabolite: urea, Compartment: Cytoplasm (c), Reaction Side: Product, Coefficient: 1. -> Add.

Save the reaction.

Reaction And Metabolite Editor

File Edit Help

Reaction View Table Metabolite View Table

	Abbreviation	Description	Neutral formula	Charged formula	Charge	
25	orn	Ornithine	C5H12N2O2	C5H13N2O2	1	C01
26	pep	Phosphoenolpyruvate	C3H5O6P	C3H2O6P	-3	C00
27	pi	hydrogenphosphate	H3O4P	HO4P	-2	C00
28	ppi	Diphosphate	H4O7P2	HO7P2	-3	C00
29	pyr	pyruvate	C3H4O3	C3H3O3	-1	C00
30	urea	Urea	CH4N2O	CH4N2O	0	C00

Search

Abbreviation ☐ Exact Match

Metabolite / Reaction

New Reaction Load Reaction Save Reaction

Reaction

Reaction Abbreviation: Direction: Irreversible

Reaction Description: Confidence Score: 4

Save reaction in database.

Abbreviation: Compartment: Cytoplasm (c)

Reaction Side: Product Coefficient: 1

Metabolite

Add Remove

	Abbreviation	Description	Coefficient	Compartment	Side	Charged Formu
1	arg-L	L-Arginine	1	Cytoplasm (c)	Substrate	C6H15N4O2
2	h2o	water	1	Cytoplasm (c)	Substrate	H2O
3	orn	Ornithine	1	Cytoplasm (c)	Product	C5H13N2O2
4	urea	Urea	1	Cytoplasm (c)	Product	CH4N2O

- For the assignment of GPRs to reaction ('Create GPR') please refer to the next section.

Now, the tool checks for elemental and charge balancing, and provides a warning if there is an error.

If everything is correct in the follow up window, click yes to save your reaction.

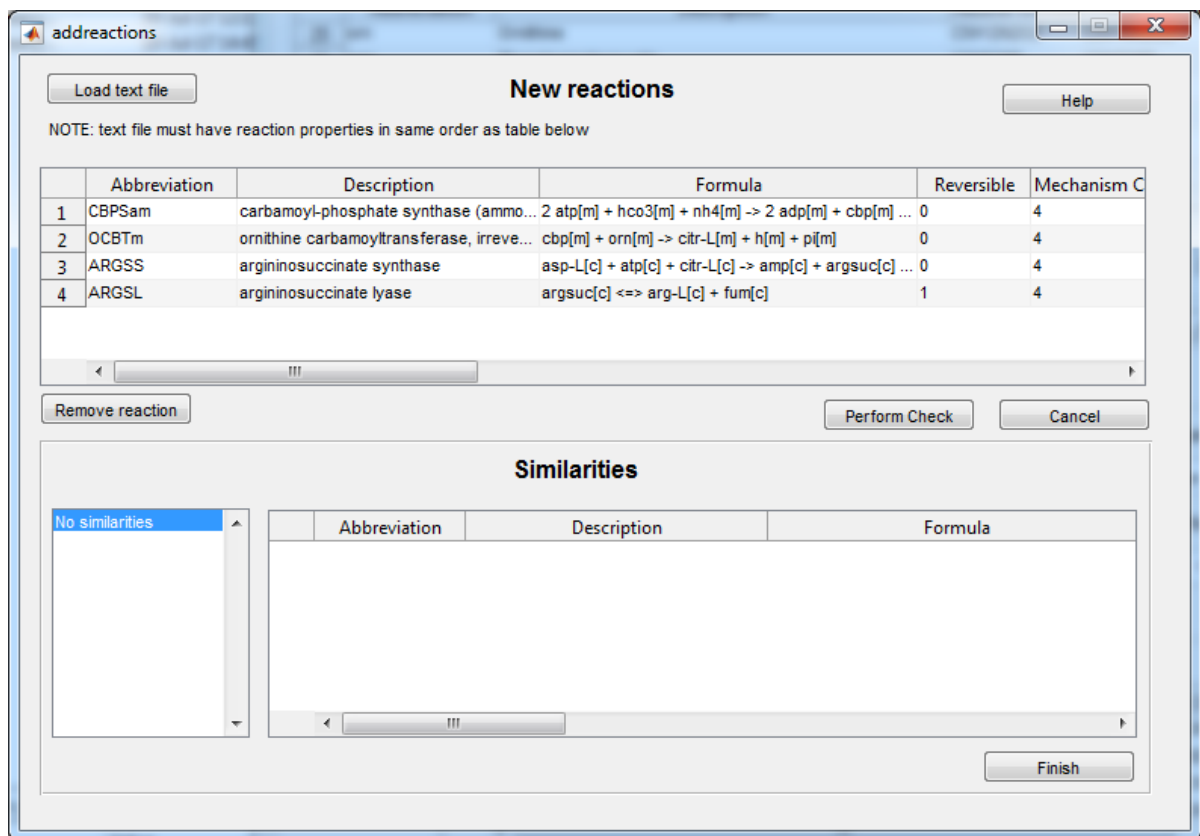
Adding reactions from a text file

Alternatively, load a text file directly into the Reaction and Metabolite Editor.

Go to file -> add text file -> with reactions -> select the file *tutorial_ureacycle_rxns.txt* (provided in the tutorial folder)

Click on Perform Check to make sure all reactions are mass and charge balanced.

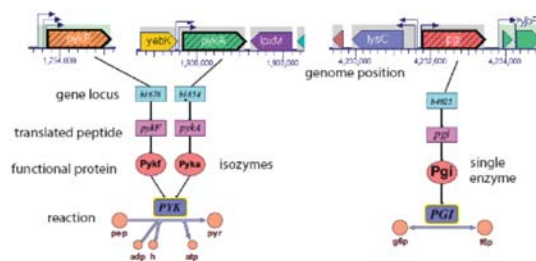
- When you are using this approach to create your reconstruction, make sure that all the information is **absolutely correct** and thoroughly checked before you make the addition.



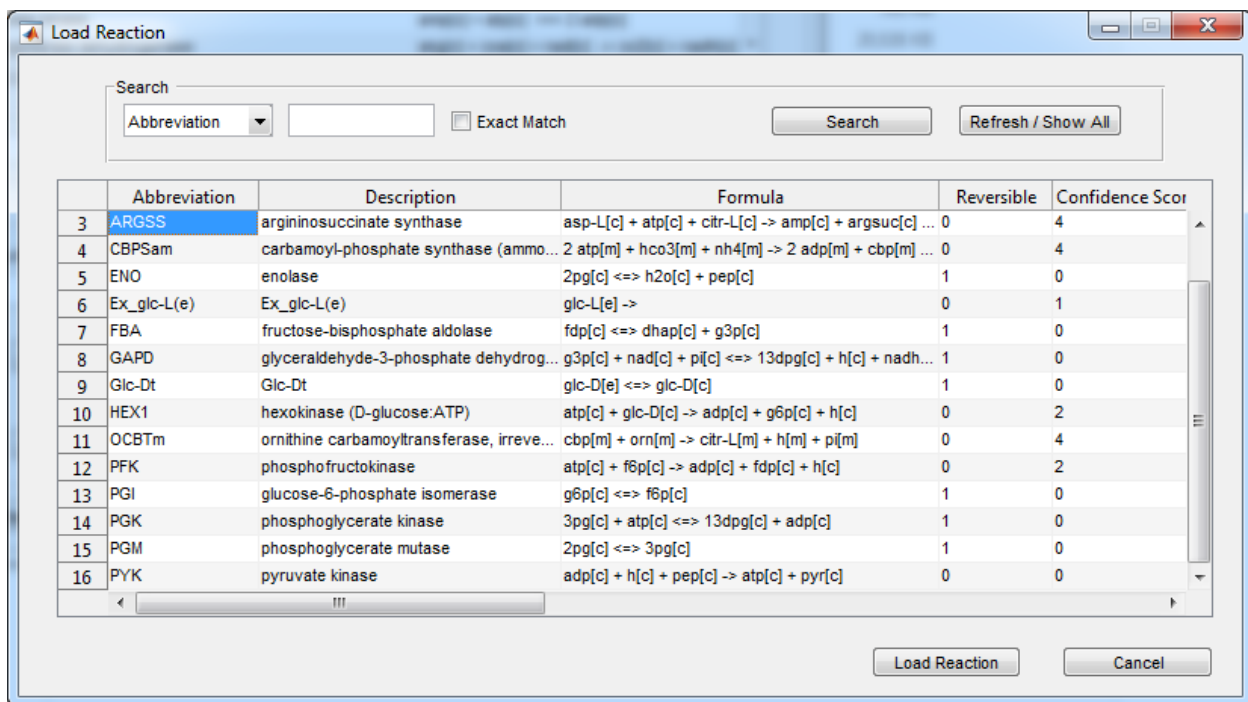
Assigning GPRs to the reactions

Gene protein reaction associations

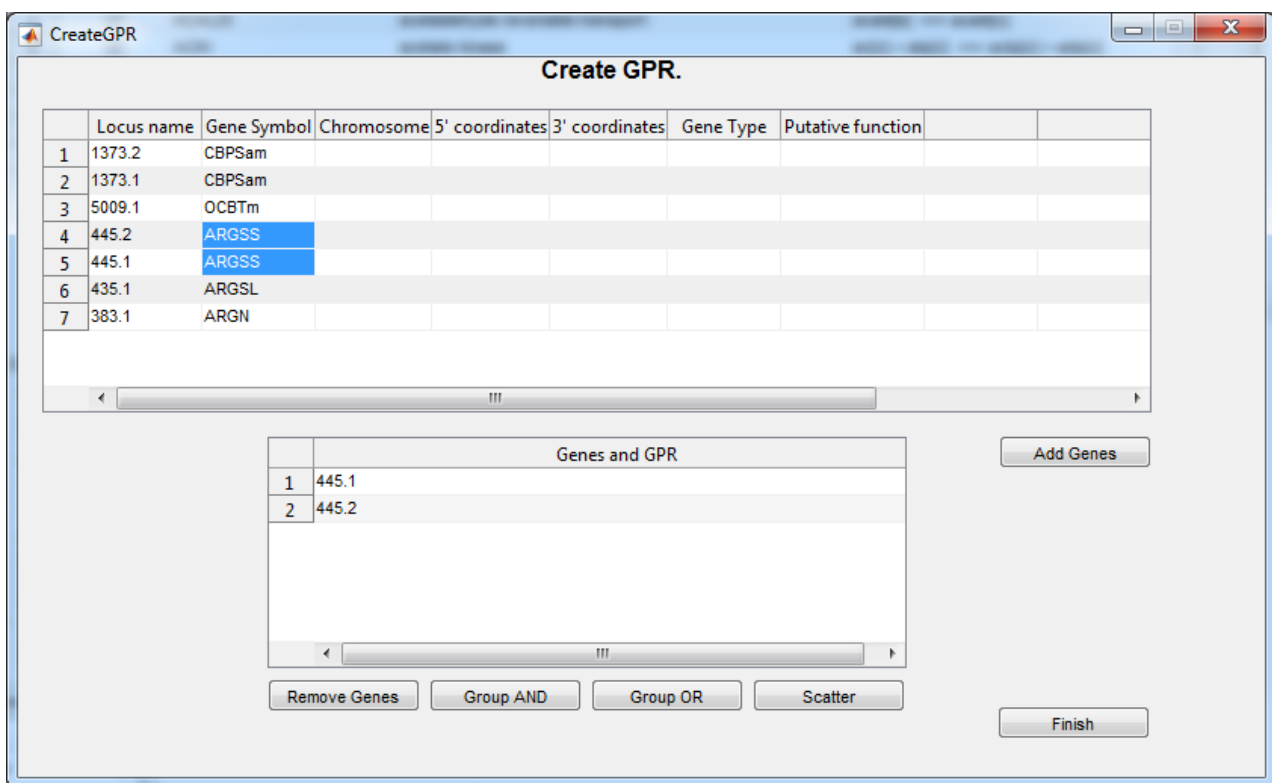
Some reactions are catalyzed by more than one enzyme



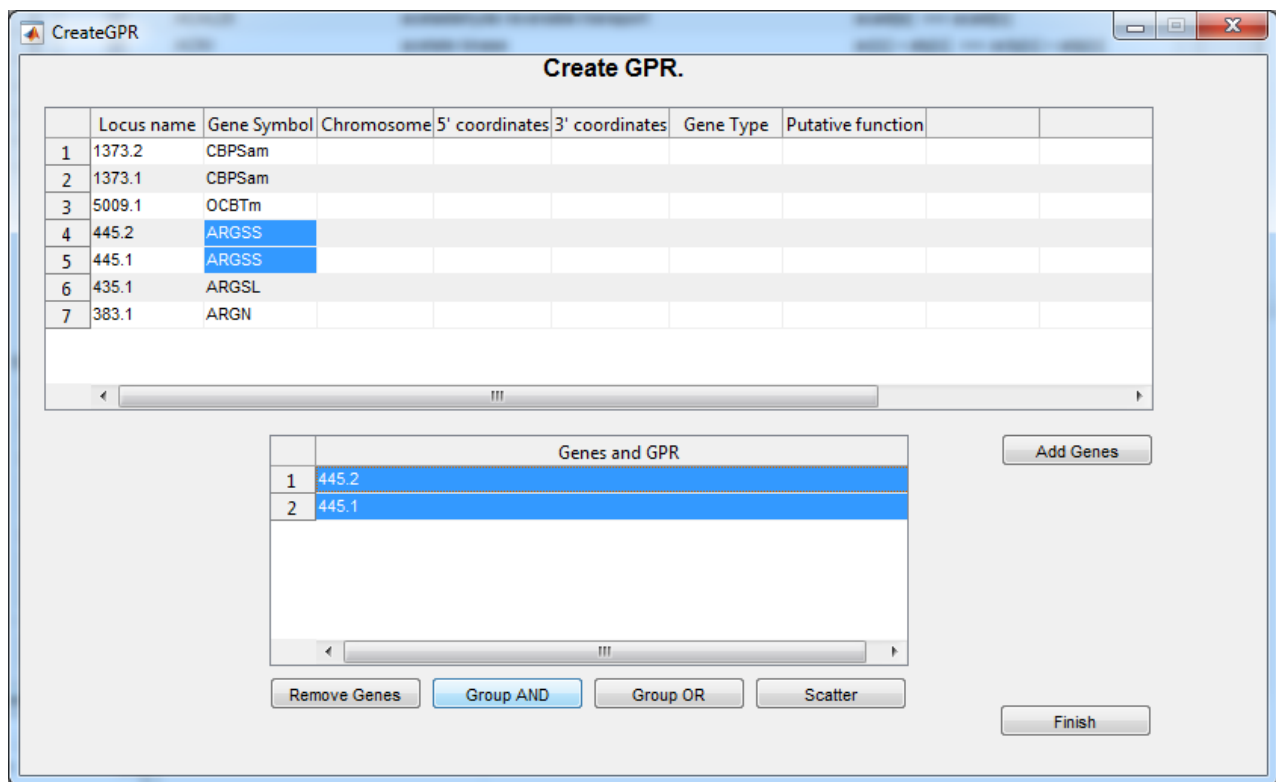
- On the Reaction and Metabolite Editor go to File -> Open Model Creator
- On the Reconstruction creator window go to File -> Click on 'Load gene index' -> select the file *tutorial_ureacycle_genes.txt* (provided in the tutorial folder)
- Add a reaction from the reaction database to the model by clicking on 'Load reaction'



- Select a reaction (ARGSS) that you want to assign a GPR, and then click 'Load reaction'.
- Click on Create GPR
- Click on genes you want to assign -> click add genes.



Add an AND/OR rule to a group of genes: Click 'Group AND' for groups selected genes with and, or click 'Group OR' for groups selected genes with or.



- Once the GPR has been configured correctly with AND/OR rules, click Finish.

Once the GPR has been assigned and other edits done (for e.g., addition of subsystem, further notes and references), click the Add Reaction button in the Reconstruction Creator.

- If editing an existing reaction, a window appears asking if you want to replace the current reaction -> click yes.

Now you should be able to see the new edited (i.e., GPR associated) reaction in the list of reactions on the reconstruction creator.

Once you have finished with all the reactions, go to File -> Save -> As Reconstruction Model.

If you made errors while adding metabolites or reactions manually, you can load the reaction or metabolite database .mat files directly into the MATLAB workspace and delete/ edit it in the variable editor in the MATLAB and save it. This shall be your new database from now on.

Make sure that you never make errors while filling in the information in the database.

end

Reference

[1] Thorleifsson SG, Thiele I. Bioinformatics. 2011 Jul 15;27(14):2009-10.