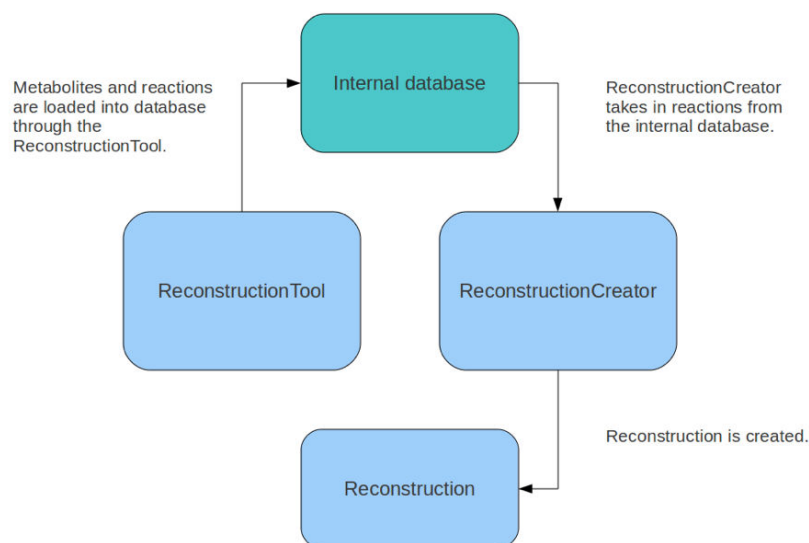


# Generation and manipulation of reconstructions with rBioNet

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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***

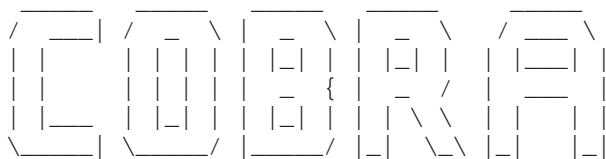
- Reconstruction analyzer.
- Checks for dead-end metabolites.
- Provides suggestions for exchange reactions.
- S-matrix visualization.
- Neighbor Reactions & Metabolite connectivity.

## **EQUIPMENT SETUP**

### **Initialize the COBRA Toolbox.**

Initialize The Cobra Toolbox using the `initCobraToolbox` function.

```
initCobraToolbox(false) % false, as we don't want to update
```



COntstraint-Based Reconstruction and Analysis  
The COBRA Toolbox - 2017

Documentation:  
<http://opencobra.github.io/cobratoolbox>

```
> Checking if git is installed ... Done.
```

```

> Checking if the repository is tracked using git ... Done.
> Checking if curl is installed ... Done.
> Checking if remote can be reached ... Done.
> Initializing and updating submodules ... Done.
> Adding all the files of The COBRA Toolbox ... Done.
> Define CB map output... set to svg.
> Retrieving models ... Done.
> TranslateSBML is installed and working properly.
> Configuring solver environment variables ...
- [---*] ILOG_CPLEX_PATH: C:\Program Files\IBM\ILOG\CPLEX_Studio1271\cplex\matlab\x64_win64
- [----] GUROBI_PATH : --> set this path manually after installing the solver ( see instructions )
- [---*] TOMLAB_PATH: C:\Program Files\tomlab\
- [----] MOSEK_PATH : --> set this path manually after installing the solver ( see instructions )
Done.
> Checking available solvers and solver interfaces ... Done.
> Setting default solvers ... Done.
> Saving the MATLAB path ... Done.
- The MATLAB path was saved in the default location.

> Summary of available solvers and solver interfaces

```

	Support	LP	MILP	QP	MIQP	NLP
cplex_direct	active	0	0	0	0	-
dqqMinos	active	0	-	-	-	-
glpk	active	1	1	-	-	-
gurobi	active	1	1	1	1	-
ibm_cplex	active	1	1	1	-	-
matlab	active	1	-	-	-	1
mosek	active	0	0	0	-	-
pdco	active	1	-	1	-	-
quadMinos	active	0	-	-	-	0
tomlab_cplex	active	1	1	1	1	-
qpng	passive	-	-	1	-	-
tomlab_snopt	passive	-	-	-	-	1
gurobi_mex	legacy	0	0	0	0	-
lindo_old	legacy	0	-	-	-	-
lindo_legacy	legacy	0	-	-	-	-
lp_solve	legacy	1	-	-	-	-
opti	legacy	0	0	0	0	0
Total	-	7	4	5	2	2

+ Legend: - = not applicable, 0 = solver not compatible or not installed, 1 = solver installed.

```

> You can solve LP problems using: 'glpk' - 'gurobi' - 'ibm_cplex' - 'matlab' - 'pdco' - 'tomlab_cplex' -
> You can solve MILP problems using: 'glpk' - 'gurobi' - 'ibm_cplex' - 'tomlab_cplex'
> You can solve QP problems using: 'gurobi' - 'ibm_cplex' - 'pdco' - 'tomlab_cplex' - 'qpng'
> You can solve MIQP problems using: 'gurobi' - 'tomlab_cplex'
> You can solve NLP problems using: 'matlab' - 'tomlab_snopt'

> Checking for available updates ...
--> You cannot update your fork using updateCobraToolbox(). [0b7c0f @ rBio-tutorial].
Please use the MATLAB.devTools (https://github.com/opencobra/MATLAB.devTools).

```

```
global CBTDIR; %Get the folder of the toolbox.
```

## 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 instalation 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.

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.

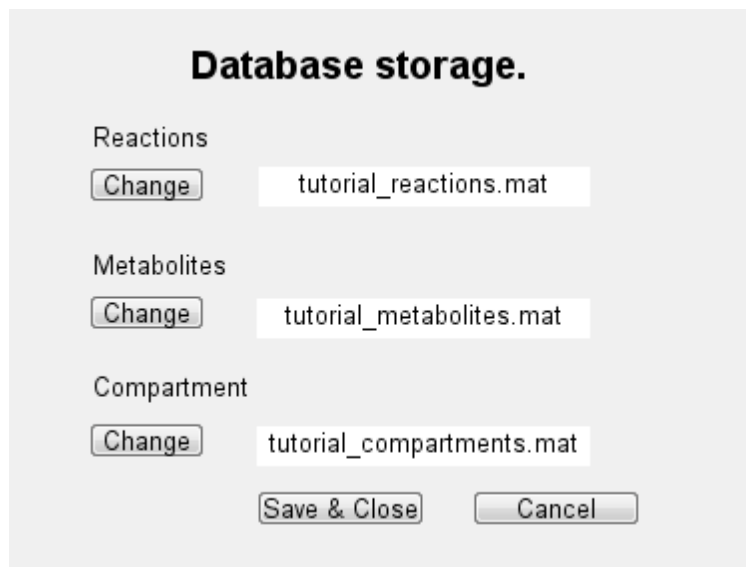
1. Initiate rBioNet by linking the database files.

- For the tutorial, we will create a file "rBioNetSettingsDB.mat" that contains the paths to the tutorial reaction, metabolite, and compartment database files.

```
%Get the path of the tutorial to store the rBioNet Databases in this folder.  
%If you want to use another folder just change the pathes.  
tutorialPath = fileparts(which('tutorial_rBioNet'));  
comp_path = [tutorialPath filesep 'tutorial_compartments.mat'];  
met_path = [tutorialPath filesep 'tutorial_metabolites.mat'];  
rxn_path = [tutorialPath filesep 'tutorial_reactions.mat'];  
save([tutorialPath filesep 'rBioNetSettingsDB.mat'],...  
    'comp_path', 'met_path', 'rxn_path')
```

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

```
rBioNetSettings
```



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 nagivating in rBioNet

Let's open the rBioNet tool:

ReconstructionTool



Reaction And Metabolite Editor

File Edit Help

Reaction View Table Metabolite View Table

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

Search

Abbreviation  ☐ Exact Match

Metabolite / Reaction

New Reaction Load Reaction Save Reaction

Reaction

Reaction Abbreviation

Reaction Description

Direction : Irreversible

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

Reaction Description

Direction : Irreversible

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



Reconstruction Creator

File Statistics Help

Reconstruction: Empty Gene Index: Empty

Edit Reaction Remove Reaction

Enable	Abbreviation	Description	Formula

Abbreviation  ☐ Match Exact Search Refresh

Edit Reaction

LB  UB  Confidence Score

GPR  Create GPR

Subsystem  More Properties

Load Reaction Add Reaction Clear Reaction

Reaction Properties

Abbreviation:

Reversible

E.C. number

KeggID

Description:

Formula

Description View Genes

### 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.

Reconstruction Creator

File

Statistics

Help

Reconstruction: *ecoli\_core\_model*

Gene Index: Empty

Edit Reaction

Remove Reaction

	Enable	Abbreviation	Description	Formula	Reversible	GPR	LB	UB	CS	SubSystem	Re
1	<input checked="" type="checkbox"/>	ACALD	acetaldehyde dehydr...	acald[c] + coa[c] + ...	1	(b0351 or b1...	-1000	1000		Pyruvate Metabolism	
2	<input checked="" type="checkbox"/>	ACALDt	acetaldehyde reversi...	acald[e] <=> acald[c]	1	s0001	-1000	1000		Transport, Extrac...	
3	<input checked="" type="checkbox"/>	ACKr	acetate kinase	ac[c] + atp[c] <=> a...	1	(b3115 or b2...	-1000	1000		Pyruvate Metabolism	
4	<input checked="" type="checkbox"/>	ACONTa	aconitase (half-reacti...	cit[c] <=> acon-C[c]...	1	(b0118 or b1...	-1000	1000		Citric Acid Cycle	
5	<input checked="" type="checkbox"/>	ACONTb	aconitase (half-reacti...	acon-C[c] + h2o[c] ...	1	(b0118 or b1...	-1000	1000		Citric Acid Cycle	
6	<input checked="" type="checkbox"/>	Act2r	acetate reversible tra...	ac[e] + h[e] <=> ac[...	1		-1000	1000		Transport, Extrac...	
7	<input checked="" type="checkbox"/>	ADK1	adenylate kinase	amp[c] + atp[c] <=>...	1	b0474	-1000	1000		Oxidative Phospho...	
8	<input checked="" type="checkbox"/>	AKGDH	2-Oxoglutarate dehy...	akg[c] + coa[c] + na...	0	( b0116 and...	0	1000		Citric Acid Cycle	

Abbreviation

☐ Match Exact

Search

Refresh

Edit Reaction

LB

UB

Confidence Score

GPR

Create GPR

Subsystem

More Properties

Load Reaction

Add Reaction

Clear Reaction

Reaction Properties

Abbreviation:

Reversible

E.C. number

KeggID

Description:

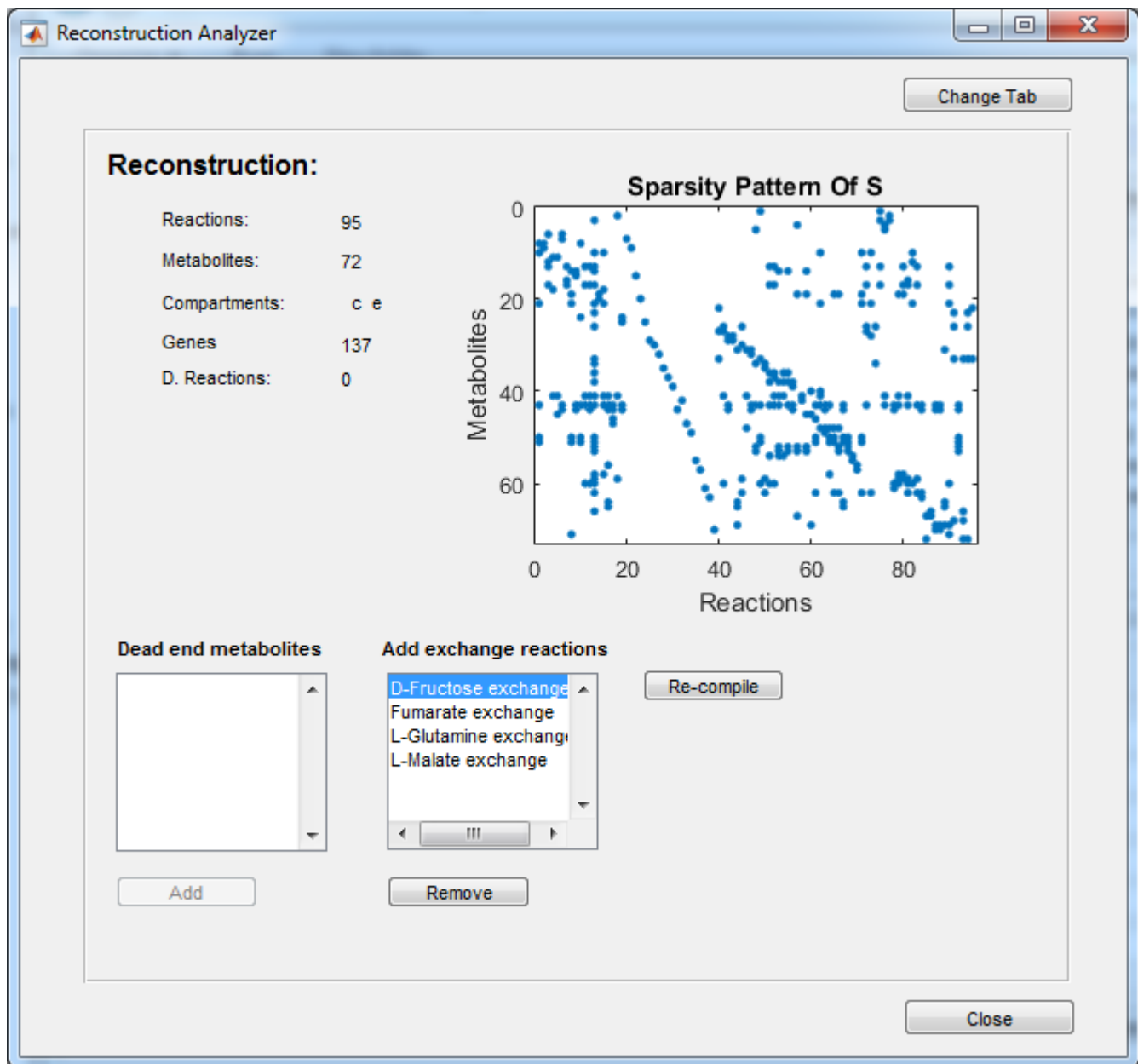
Formula

Description

View Genes

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\*

Neutral Formula

Charged formula\*

Charge\*

KeggID

PubChemID

Description\*

Inchi String

Smile

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.

metabolites

Load text file

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				

Remove line

Continue

Cancel

## 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).

Reaction And Metabolite Editor

File Edit Help

Reaction View Table Metabolite View Table

	Abbreviation	Description	Neutral formula	Charged formula	Charge	Compartment
4	adp	ADP	C10H15N5O10P2	C10H12N5O10P2	-3	C00
5	amp	AMP	C10H14N5O7P	C10H12N5O7P	-2	C00
6	arg-L	L-Arginine	C6H14N4O2	C6H15N4O2	1	C00
7	argsuc	N(omega)-(L-Arginino)succinate	C10H18N4O6	C10H17N4O6	-1	C03
8	asp-L	L-Aspartate	C4H7NO4	C4H6NO4	-1	C00
9	atp	ATP(4-)	C10H16N5O13P3	C10H12N5O13P3	-4	C00

Search

Abbreviation  ☐ Exact Match

Metabolite / Reaction

New Reaction Load Reaction Save Reaction

Reaction

Reaction Abbreviation  Direction:

Reaction Description  Confidence Score:

Metabolite

Abbreviation  Compartment

Reaction Side  Coefficient

Metabolite

Abbreviation	Description	Coefficient	Compartment
<input type="button" value="Add"/>			
<input type="button" value="Add metabolite from database to reaction table."/>			

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

Reaction Side  Coefficient

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.

The screenshot shows a software window titled 'addreactions'. It has a 'Load text file' button and a 'Help' button. A note states: 'NOTE: text file must have reaction properties in same order as table below'. Below this is a table with 6 columns: an index, Abbreviation, Description, Formula, Reversible, and Mechanism C. The table contains 4 rows of reaction data. Below the table is a 'Remove reaction' button, a 'Perform Check' button, and a 'Cancel' button. At the bottom is a 'Similarities' section with a list box on the left showing 'No similarities' and a table on the right with columns for Abbreviation, Description, and Formula. A 'Finish' button is at the bottom right.

	Abbreviation	Description	Formula	Reversible	Mechanism C
1	CBPSam	carbamoyl-phosphate synthase (ammo...	2 atp[m] + hco3[m] + nh4[m] -> 2 adp[m] + cbp[m] ...	0	4
2	OCBTm	ornithine carbamoyltransferase, irreve...	cbp[m] + orn[m] -> citr-L[m] + h[m] + pi[m]	0	4
3	ARGSS	argininosuccinate synthase	asp-L[c] + atp[c] + citr-L[c] -> amp[c] + argsuc[c] ...	0	4
4	ARGSL	argininosuccinate lyase	argsuc[c] <=> arg-L[c] + fum[c]	1	4

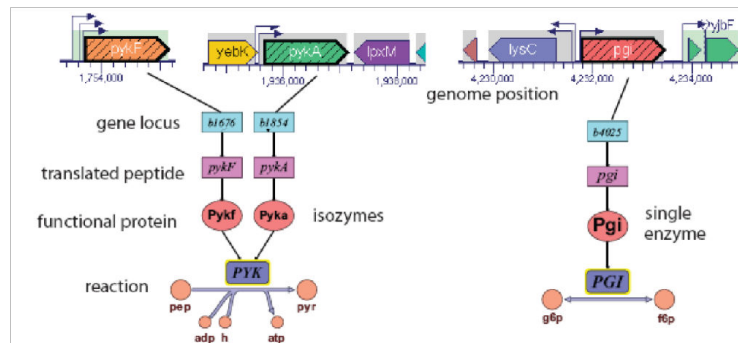
Similarities			
	Abbreviation	Description	Formula
No similarities			

## 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'

Load Reaction

Search

Abbreviation  ☐ Exact Match

	Abbreviation	Description	Formula	Reversible	Confidence Score
3	ARGSS	argininosuccinate synthase	asp-L[c] + atp[c] + citr-L[c] -> amp[c] + argsuc[c] ...	0	4
4	CBPSam	carbamoyl-phosphate synthase (ammo...	2 atp[m] + hco3[m] + nh4[m] -> 2 adp[m] + cbp[m] ...	0	4
5	ENO	enolase	2pg[c] <=> h2o[c] + pep[c]	1	0
6	Ex_glc-L(e)	Ex_glc-L(e)	glc-L[e] ->	0	1
7	FBA	fructose-bisphosphate aldolase	fdp[c] <=> dhap[c] + g3p[c]	1	0
8	GAPD	glyceraldehyde-3-phosphate dehydrog...	g3p[c] + nad[c] + pi[c] <=> 13dpg[c] + h[c] + nadh...	1	0
9	Glc-Dt	Glc-Dt	glc-D[e] <=> glc-D[c]	1	0
10	HEX1	hexokinase (D-glucose:ATP)	atp[c] + glc-D[c] -> adp[c] + g6p[c] + h[c]	0	2
11	OCBTm	ornithine carbamoyltransferase, irreve...	cbp[m] + orn[m] -> citr-L[m] + h[m] + pi[m]	0	4
12	PFK	phosphofructokinase	atp[c] + f6p[c] -> adp[c] + fdp[c] + h[c]	0	2
13	PGI	glucose-6-phosphate isomerase	g6p[c] <=> f6p[c]	1	0
14	PGK	phosphoglycerate kinase	3pg[c] + atp[c] <=> 13dpg[c] + adp[c]	1	0
15	PGM	phosphoglycerate mutase	2pg[c] <=> 3pg[c]	1	0
16	PYK	pyruvate kinase	adp[c] + h[c] + pep[c] -> atp[c] + pyr[c]	0	0

- 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.

CreateGPR

Create GPR.

	Locus name	Gene Symbol	Chromosome	5' coordinates	3' coordinates	Gene Type	Putative function		
1	1373.2	CBPSam							
2	1373.1	CBPSam							
3	5009.1	OCBTm							
4	445.2	ARGSS							
5	445.1	ARGSS							
6	435.1	ARGSL							
7	383.1	ARGN							

Genes and GPR

1	445.1
2	445.2

Remove Genes

Group AND

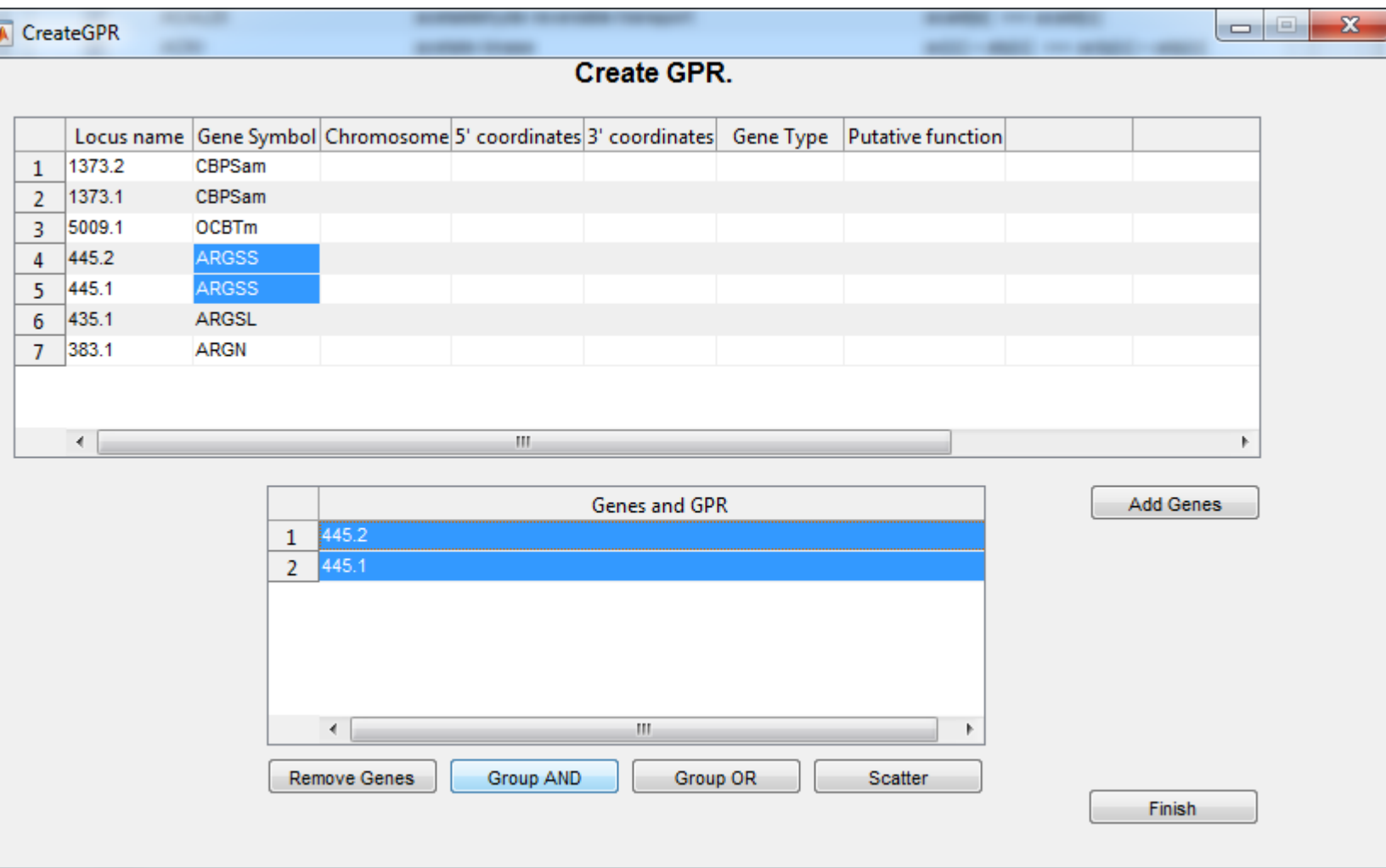
Group OR

Scatter

Add Genes

Finish

**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.**

## Clean-up

Remove "rBioNetSettingsDB.mat" file from the tutorial directory.

```
fclose all;% close all open windows
delete([CBTDIR filesep 'tutorials' filesep 'rBioNet' filesep
'rBioNetSettingsDB.mat'])
```

```
end
```

## Reference

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