#### 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 2 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 assion GPRs (sene-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 5-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. 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
- 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 crosnism independent but compartment specific. The same reaction can occur in different compartments. . Dither start from scratch or load pre-existing reconstruction.
  - . Primarily used to assign GPRs · Also to add notes, subsystem etc.
- Add-one

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

## EQUIPMENT SETUP

Initialize the COBRA Toolbox.

DESIGN OF COURSE

initCobraToolbox global CETDIR; WGet the folder of the toolbox.

Setting the optimization solver.

quadratic programming (NCOP).

This tutorial will be run with a "q1pk" package, which is a linear programming ('LP') solver. The "q1pk" package does not require additional installation and configuration.

source year (in );
change (obtrast liver (solverflane, solver Type, 1);
However, for the analysis of large models, such as Recon 3, 8 is not recommended to use the "slick" package but rather an industrial

stereigh solver, such as the " guroùá" padage. For detailed information, refer to The Cobra Toobox <u>solver institution quido</u>.

A solver padage may offer different types of opinization programmes to solve a pobliers. The above example used a LP opinization, other house of colinization recomments include: missel-integer are consuments (Intel<sup>®</sup>), anadatic programming (Intel<sup>®</sup>), and intel<sup>®</sup> comparaming (Intel<sup>®</sup>), and intel<sup>®</sup> consuments (Intel<sup>®</sup>) and intel<sup>®</sup> consuments (Intel<sup>®</sup>) and intel<sup>®</sup> consuments (Intel<sup>®</sup>) and intel<sup>®</sup> consuments (Intel<sup>®</sup>).

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if weejawa('desktop') % This line of code is to avoid execution of this turcrial in non qui-environments.

Skeps to load and initiate the Reconstruction Tool

Start up: Childred meets a pre-existing database to start up. Start up: Childred in a database. The database consists of the ten reactions of the glocylopia present, Priesco, you will see the glocylopia reactions in the reaction creator window, the metabolities participating in these reactions in the metabolities creator window.

In Initiation Gibbler to history has database files.

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

NGet the path of the tutorial to store the reliables Sutabases in this fold wif you want to some another folder jout change the pathen. comp\_anh of [tutorialDuth filessy "sutarial\_compartments.nam"]; met tash of futorialDuth filessy "sutarial\_compartments.nam"];

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'compath', 'ren\_path', 'ren\_path'

Note there are there are files corresponding to the maction database, metabolite database, and the concentract database.

#### • .....

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Click on the change tab for reactions and locate to the tutorial\_reactions.met file, which is provided to you in the stilloblet state of price.

Reactions -- Change -- tutorial / reactions.mail-serve

Click on the change tab under metabolities and locate to the tutorial\_metabolities.mat file, which is provided to you in the rillioNet tutorial folder.

Metabolities-Changes-butorial\_metabolities.mat-ourse

Click on the change tab under compartment and locate to the tutorial\_compartments mat file, which is provided to you in the rillioNet tutorial felder.

Compartment-oChange-o tutorial\_compariments.mat -o save

This is the pre-existing distalase with plycolysis reactions and you saved it as your current database. You can modify it or remove the reactions as per your requirements. Open and naight/asting in riblioNets Linft open the distribution:

ReconstructionTool

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

vindow 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

	Edit Help									
	Reaction View	Table	Melabolite View Table							
	Abbreviation		Description		Formula					
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Click on the Metabolite View Table and then Show All, which shall let you see all the glycolysis metab Metabolite view table: Metabolite creator <- Refresh/Show All

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Reaction And Metabolite Editor



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

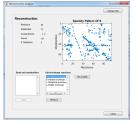
In the Reconstruction Creator do as follows:

File -> gen model -> compiler reconstruction -> select the E. coll core model provided in the tutorial folder (tutorial, Ecol., core, model.mati) -> click year or the reconstruction describe that -> click year or the reconstruction described that -> click year of the r

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## 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 NewLoadSave Metabolite view.



 Limiter the required information for a new metabolitie, including: accrevation (cop), description (currently) photiphase), charged formula (CICRASCOP), and charge (-2).
 Then click Sawe Metabolitie.

#### Adding metabolites from a text file

Adding metaboses from a text six

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

Assertances, load a set tie circosy into the Heaction and serapose Lotor.

Go to file -> add test file -> with metabolite -> select the file tutorial\_uneacycle\_meta.bd (provided in the tutorial folder)

Click yes on each window that appears.

• When you are using this appreach to create your reconstruction, make sure that all the information is abealusely correct and throcosthic checked before you make the addition.



## Manually adding reactions

Go to the Reaction and Metabolite Editor window and click on the Metabolite Reaction tab to switch to the NewLoad/Save Reaction view. Then, click New Reaction and enter the reaction information including the reaction abbreviation (ARGN), description (anginase), direction (Investible), and confidence score (4). Click on More Properties to add additional information (Notes, References, ED Number, KeapID).



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, Compariment: Oytoplasm (c), Reaction Side: Substrate, Coefficient: 1. -> Click Add to add the metabolite to the

 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.



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

Now, the tool checks for elemental and change 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 test file

thoroughly checked before you make the addition.

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

Go to file > add feet file > with reactions > select the file habols/uneacycle\_nons.bit (provided in the tutorial folder).

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

• When you are using this accreach to create your reconstruction, make sure that all the information is absolutely correct and



### 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 substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index" > select the file substitution creator window go to File > Click on "Load gene index
- Un the Reconstruction cleator window go to File -> Click on Load the tutorial folder)
- Add a reaction from the reaction database to the model by clicking on "Load reaction"



icalica III

- · Click on Create GPR



Add an ANDIOR 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 colled (i.e., CPR) 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 addrive metabolities or reactions manually, you can load the reaction or metabolite database and files directly into the

MATLASI workspace and delete<sup>1</sup> edit in the variable editor in the MATLASI and save it. This shall be your new delabase from now on. Make sure that you never make errors while filling in the information in the database.

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

fclose allyk close all open windows
delete(CGTDIR filesep 'tutorials' filesep 'rBioNet' filesep 'rBioNetSettingu00.mat'])

end Reference

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