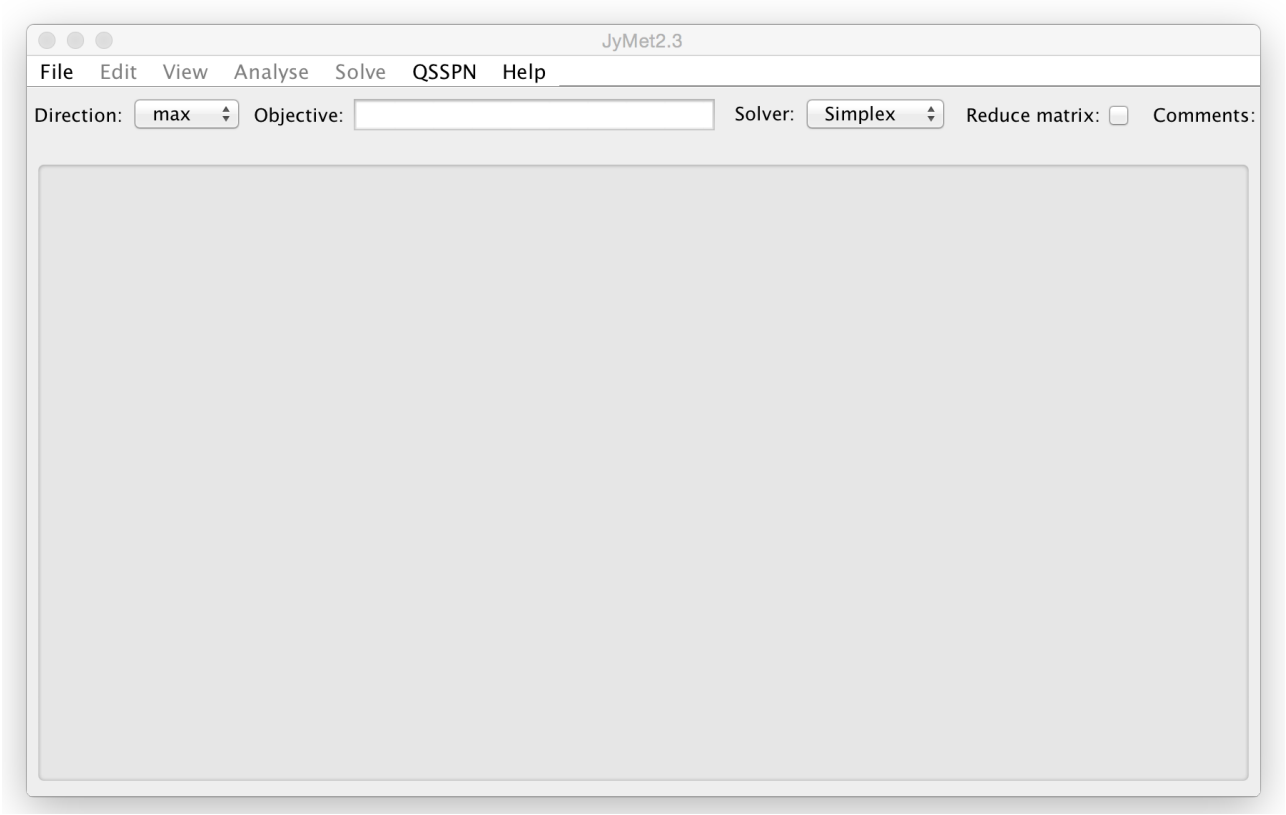
Getting Started with MUFINS

Exercises below provide quick introduction to basic Constraint Based Modelling with MUFINS. Follow steps within each exercise.

Exercise 1. Calculation of growth rate on different media

Download and install MUFINS.
Download MUFINS**.zip file from software distribution. Save the file in directory of your choice. Unzip the file and enter MUFINS** directory. Click JyMet and wait one or two minutes until JyMet Graphics User Interface appears:



*Note: If JyMet does not open or you get error message make sure that you start JyMet from un-zipped file. On Windows the zip file can be viewed as directory and it is not always clear whether it is still zipped or not. You need to extract MUFINS** directory before running JyMet.*

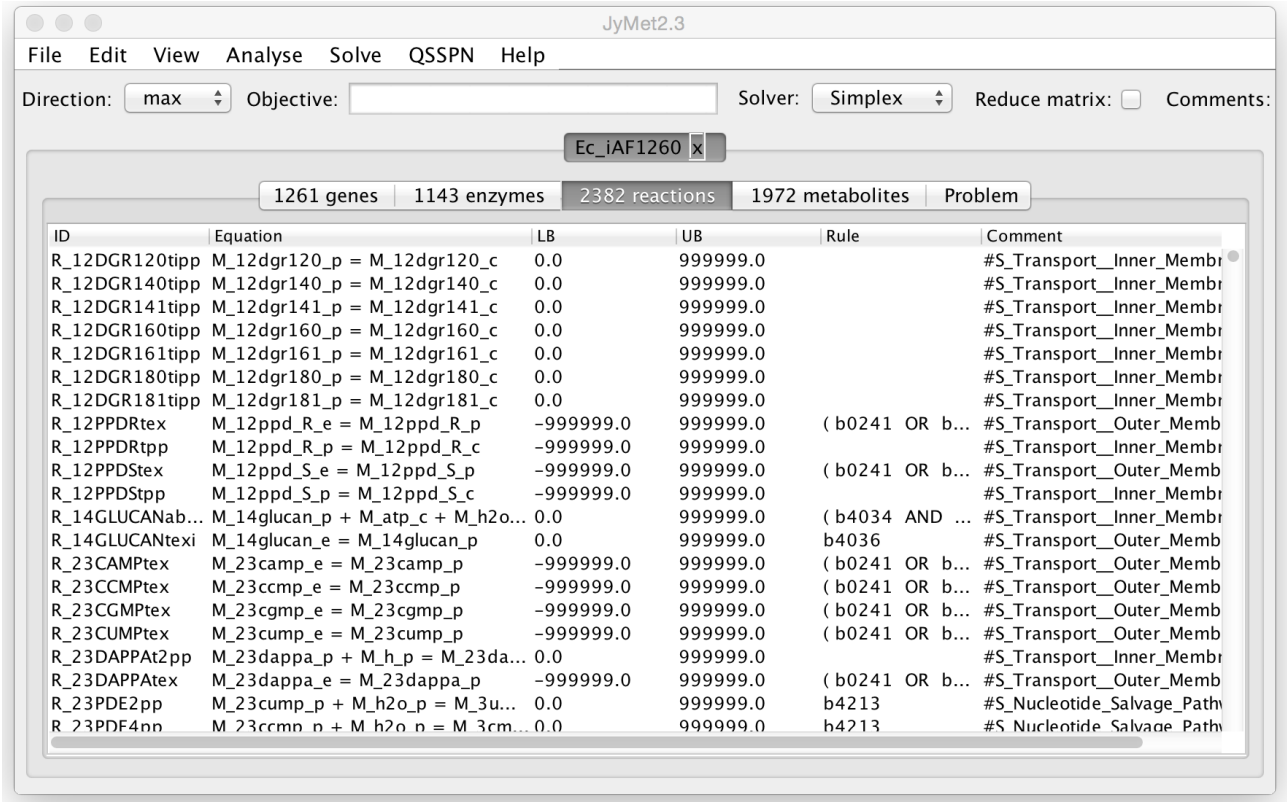
*Note: On Windows, if JyMet does not open and you are sure that MUFINS** directory is un-zipped, try to move it to shorter directory path (e.g. c:\MUFINS.33). Java virtual machine on Windows may not work with very long directory path.*

Genome Scale Metabolic Reaction Network of *Escherichia coli*.
In this exercise we use the Genome Scale Metabolic Network of Escherichia coli developed by the group of Berhard Palsson:

Feist AM, Henry CS, Reed JL, Krummenacker M, Joyce AR, Karp PD, Broadbelt LJ, Hatzimanikatis V, Palsson BØ. A genome-scale metabolic reconstruction for Escherichia coli K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information. Mol Syst Biol. 2007 3:121.

Download and uncompress the *MUFINS.**_Examples.zip* file from the journal supplementary material. Uncompress the file. Directory *MUFINS.**_Examples/Ec_iAF1260* contains *Ec_iAF1260_flux1.xml* file downloaded from supplementary material of original publication is in Systems Biology Markup Language (SBML, http://sbml.org/Main_Page) format. The bounds of transport reactions were set to represent minimal medium with glucose as a carbon source. The model is called iAF1260 in an attempt to create naming convention of "*in silico"* microbial strains. The "i" stands for "in silico", AF are initials of the first author and 1260 is the number of genes covered by the model.

Import SBML file into JyMet.
Use "File->Import SBML" menu of JyMet to open *Ec_iAF1260_flux1.xml*. You may need to wait a minute or two again. You will see the model loaded into the spreadsheets of the JyMet interface.



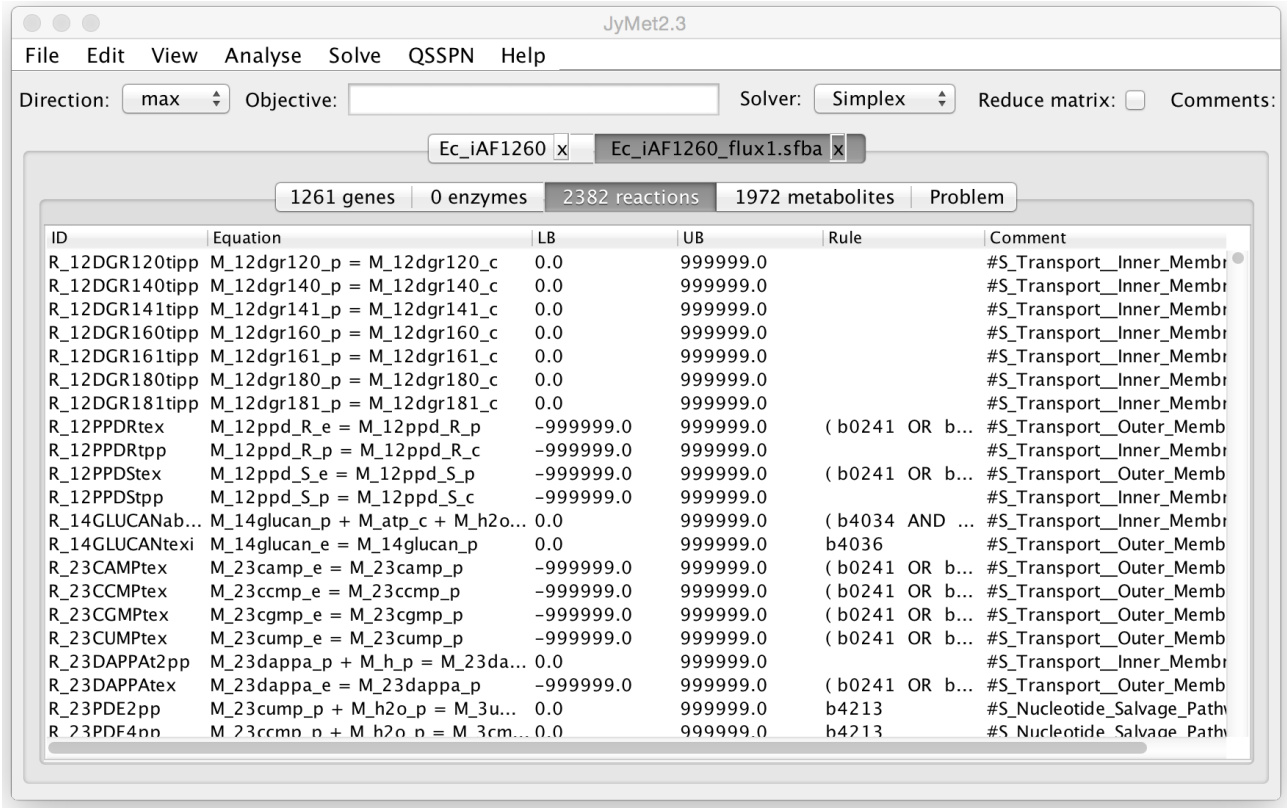
The reaction table presented on the picture above shows the following: The name of the reaction, reaction formula with abbreviated metabolite names, lower flux bound, upper flux bounds and the rule linking genes to reactions. Comments section contains any free text description of the reaction. Comments can be used to tag reactions of interest, so they can be quickly found in simulation results tables with "Edit->search".

Save and examine MUFINS reaction table.
Any of JyMet spreadsheets can be saved in tab-separated text file with "File->Save table". The contents of active spreadsheet, visible on the screen is saved. In particular, this can

be used to save reaction, problem and results tables. Make sure that reaction tab is active and us “File->Save table” to create *Ec_iAF1260_flux1.sfba* file. Open this file with Excel and text editor. As you can see this is FBA model representation that can be easily edited outside of JyMet. As long as it is saved as tab-separated text file it can be loaded back to JyMet. The sfba and qsspn command line tools use this table as their native representation of the GSMN.

Note: Mac users may experience a problem when working with reaction tables saved from Excel. Excel uses Windows rather than Mac end of line format. You need to open and save the text file with other editor that corrects end of line problem.

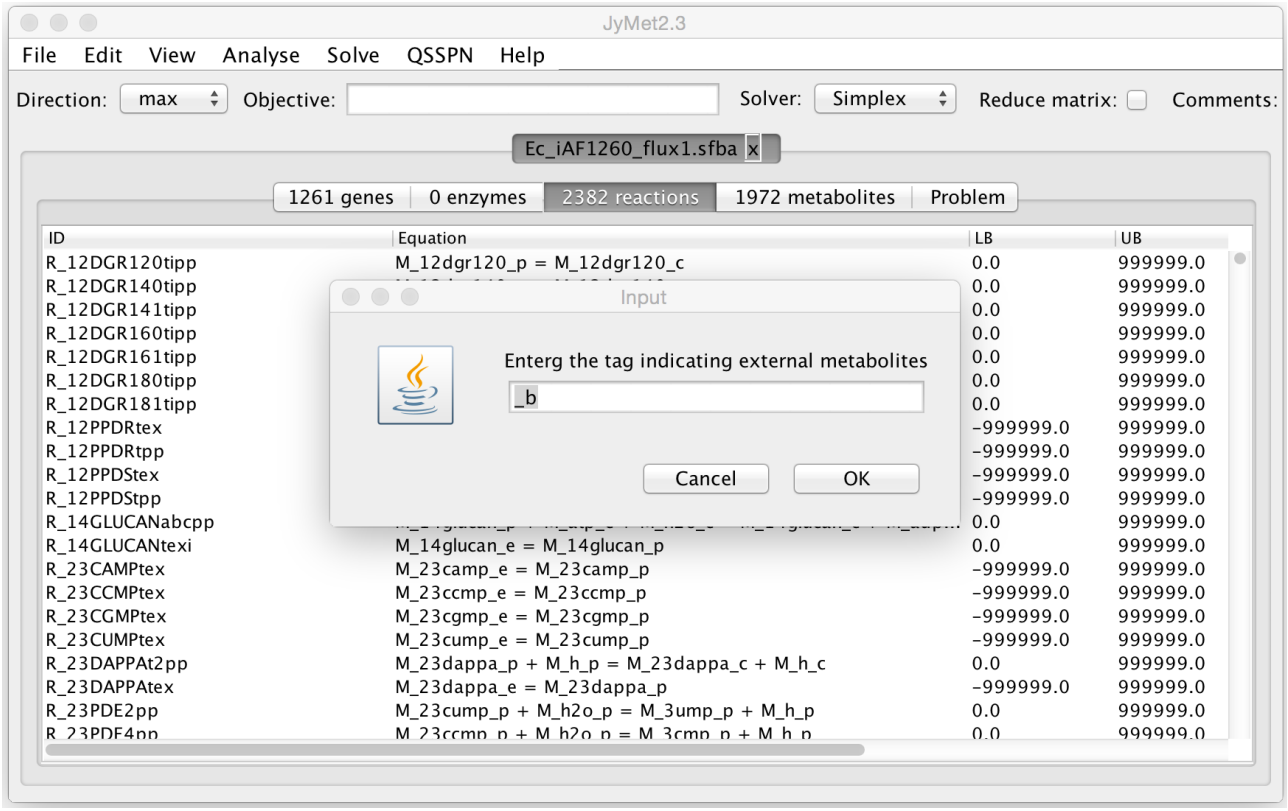
Open GSMN in reaction table format.
In JyMet use “File->Open model” to open Ec_iAF1260_flux1.sfba file. You will see dialog box warning that external metabolites are not defined. Click OK to acknowledge this message. You will see the following screen.



Note, that compared to previous screen information about “enzymes” is lost. Reaction table uses gene-reaction association rules (rule column). In some SBML gene-enzyme-reaction association may be defined. We consider reaction table to be native, working format of MUFINS. It is an input file of command line tool and qsspn engine. It is also more convenient for JyMet as SBML parsing takes time. We recommend to use SBML for import/export of final model distributions, rather than model development and simulation.

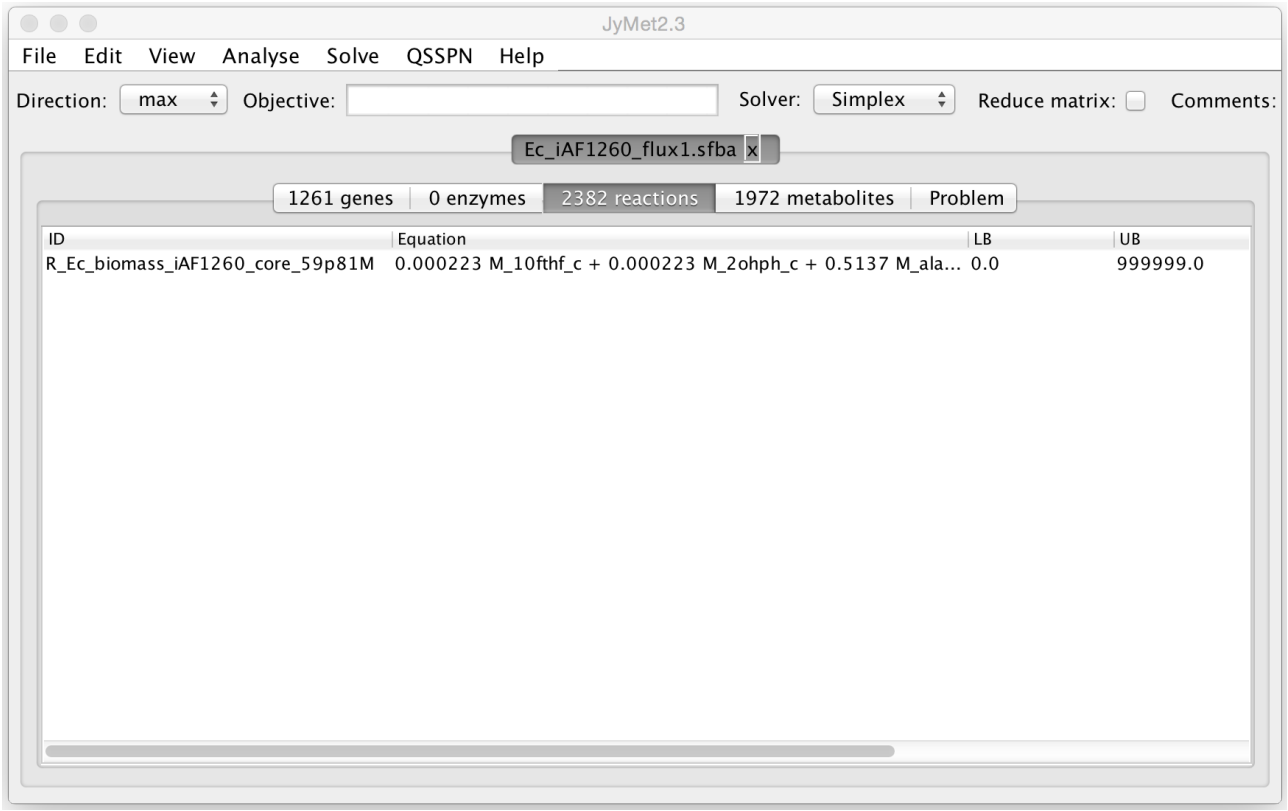
Close Ec_iAF1260 tab
JyMet allows working with multiple models. The models are represented as separate tabs in upper bar, lower bar represents genes, reaction, metabolite and results tables for the particular model. Close the model tag Ec_iAF1260 where SBML model was loaded (click on x). We will work in reaction table format from now on.

Define external metabolites.
External metabolites represent sources and sinks of metabolic flux. Without external metabolites the FBA model will not calculate any other value of the maximal flux than 0, because there will be no source of metabolic flux. The models originating from Palsson's group usually use "_b" tag at the end of metabolite name to indicate external metabolite. To make MUFINS use this tag to define external metabolites click "Solve->Externality tag" in JyMet menu and type _b in the dialogue box.



Biomass reaction and growth rate.
One of the reactions in the system defines biosynthethic and energy demand of the synthesis of cell components. The flux through this "biomass" reaction represents growth rate of the population of bacterial cells.

The biomass reaction in iAF1260 model has long name which is difficult to remember. We will use Search function of JyMet to find it by "biomass" substring. In "Edit->Search", enter biomass as a query. The table will show one row with biomass reaction:



Click in “Equation” field and scroll biomass definition. It contains substances which are considered to be biomass components. The coefficients of these substances reflect experimentally determined biomass composition of the E. coli cells under media conditions of interest.

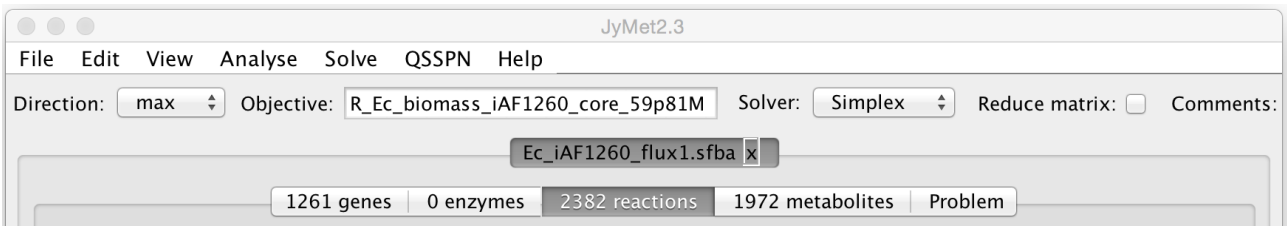
Finally, to return to the all reactions view, click View->Show->All.

Note: While using Search function make sure that one of the following is true: i) no field in the spreadsheet you are searching is selected ii) whole area you want to search is selected. It frequently happens that only one of the fields is selected. The search function looks only into this field and may not return result you expect.

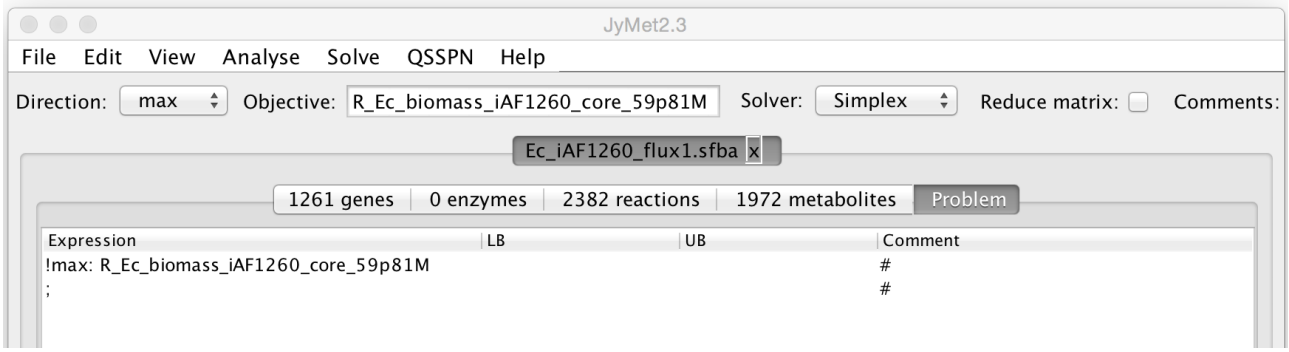
Calculate maximal growth rate.

To run simulations with JyMet you need to define objective function, choose analysis method and write definition of the problem. Writing of the problem file makes execution of simple task a bit more complicated than necessary, but gives a lot of flexibility for definition of complex analysis protocols and media conditions.

Select objective function: Type biomass reaction name in the “Objective:” field of JyMet:

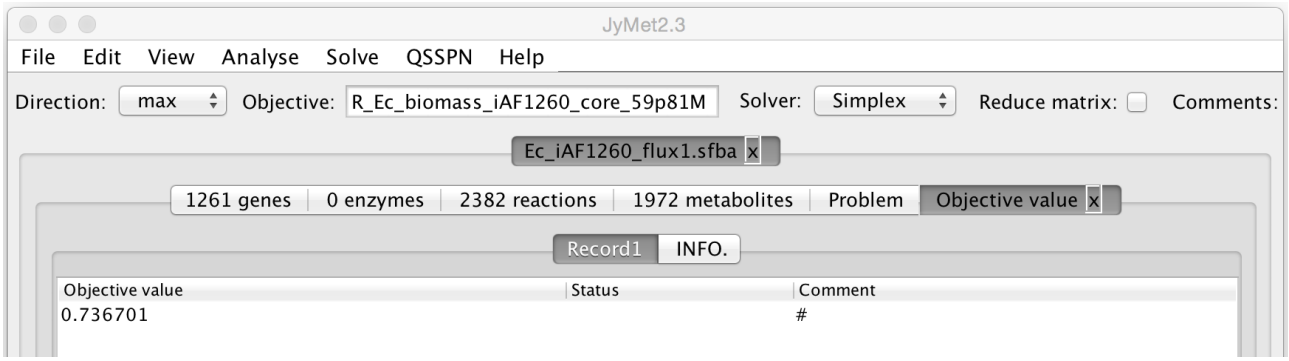


Select analysis method: In Analyse menu choose: “Objective value” (default selection). *Create problem definition:* In Solve menu click “Write problem”. See definition of the problem in the Problem window of JyMet.



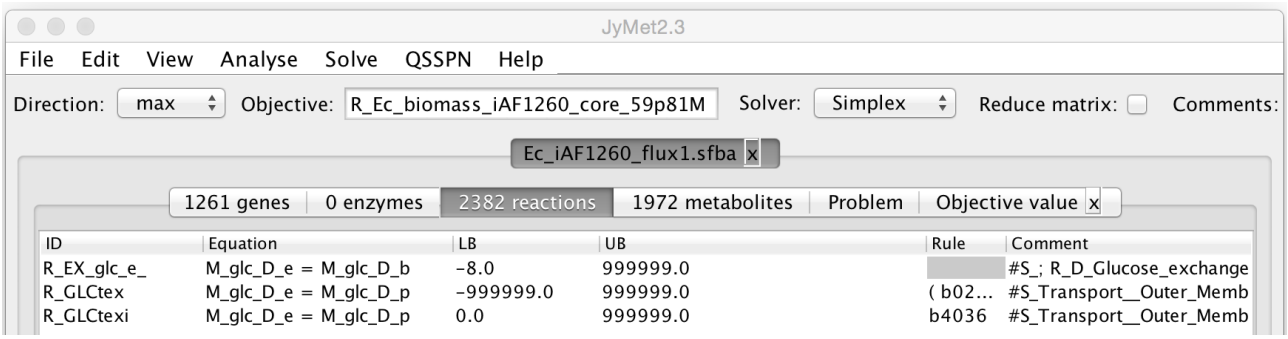
The expression in problem file window instructs the software to calculate maximal theoretical flux through reaction R_Ec_biomass_iAF1260_core_59p81M.

Run calculations. Click Solve->Solve and see the result:



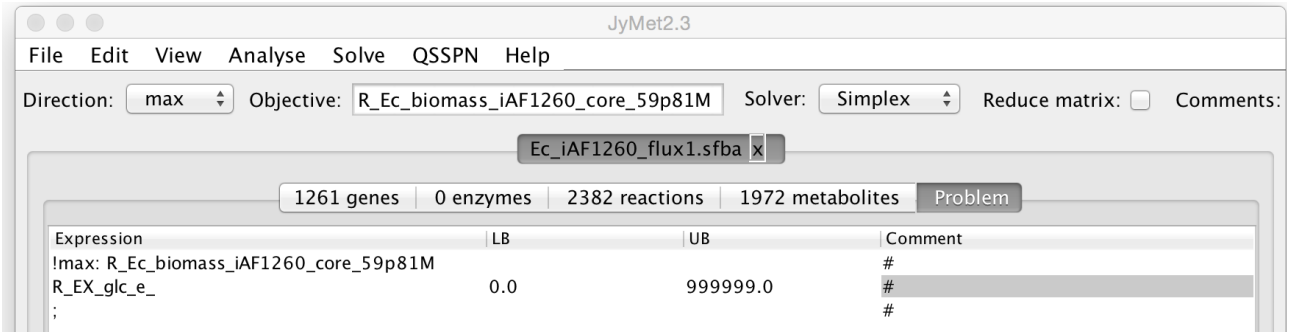
The maximal growth rate chemically feasible under media conditions of this experiment is 0.736701 1/h. The units of the flux in this model are mmol / hr/ gDW (milimol per hour per gram dry weight). However, coefficients in biomass reaction are calculated in units of mmol/gDW. The biomass reaction sums the mole fraction of each precursor necessary to produce 1 g dry weight of cells. Therefore, biomass flux is calculated in units of 1/h and represents growth rate of *E. coli* culture in log phase.

Change media conditions.
In iAF1260 model the glucose exchange with the external environment is defined by the reaction R_EX_glc_e_ :



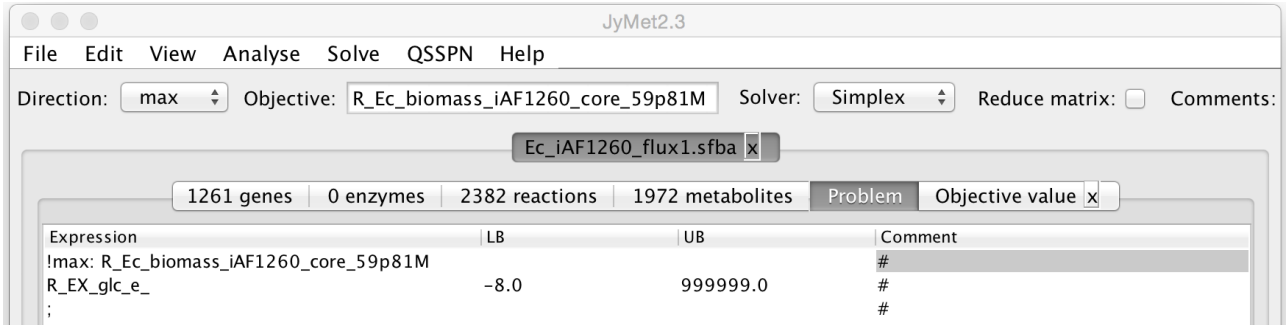
As you can see the reaction is written in the direction of secretion. The glucose M_glc_D_e is further transported into periplasm of the bacterial cell. It is converted into external glucose M_glc_D_b. However, the flux bounds make this reaction opened into transport direction. If the flux is set to -8.0 the reaction will be capable to transport 8 mM of extracellular M_glc_D_b into the cell in one hour in 1 g of cellular dry weight.

To change media conditions you need to open or close some of the exchange reactions by changing their flux bounds. You can change reaction bounds in the model, but better way of doing this is to set new reaction bounds in problem file. Edit the the problem file in the following way:



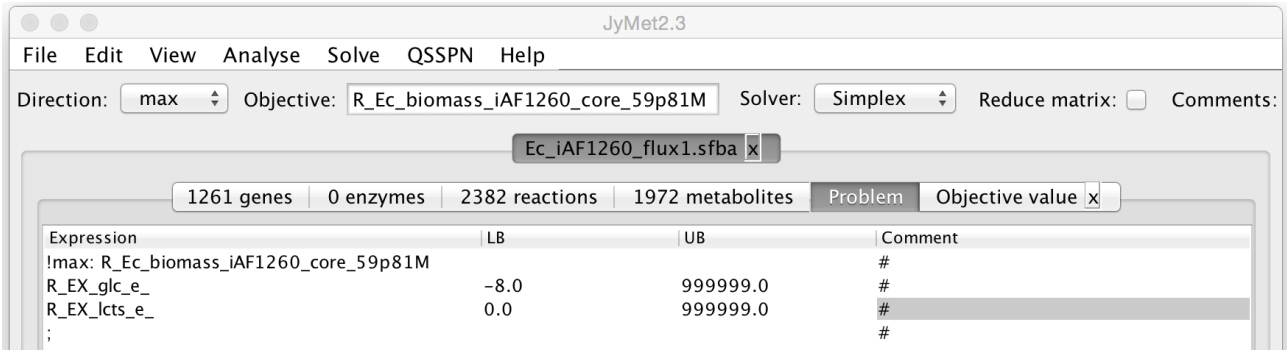
Use “Edit->Insert rows” to add rows to problem file table. Make sure to place “;” in the last row. You could define several problems, separated by “;” which would be sequentially evaluated.

The new bounds of reaction R_EX_glc_e_ **overwrite** setting in the reaction table. To make sure that it works calculate maximal growth rate again. Since the transport of the sole carbon source is closed the maximal growth rate should be 0. Set glucose transport bound back to original settings:



and make sure that the maximal growth rate is now back to 0.736701 1/h. If it works you can control glucose transport by changing settings in the problem file.

Add lactose transport reaction to the problem file.
Lactose exchange is defined by the reaction R_EX_lcts_e_. To enable changing of carbon source between glucose and lactose, add a row to problem file table (“Edit -> Insert rows”) and add lactose exchange:

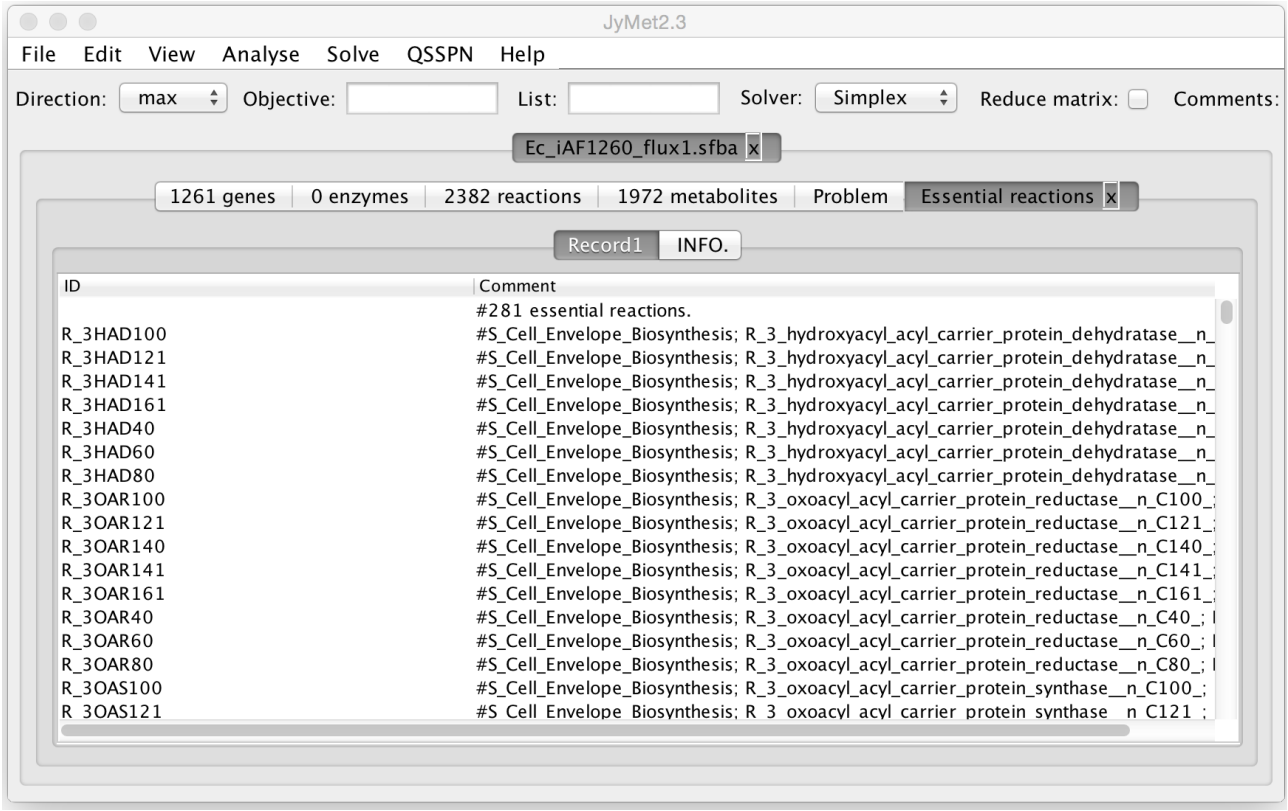


Now you can control the carbon source by changing bounds of these two reactions.

Exercise 2. Essential reactions.

The “Essential reactions” method of MUFINS checks essentiality of every reaction in the system. For each reaction the program constraints the flux through this reaction to 0 and calculates maximal value of the objective function. If inactivation of particular reaction results in the objective function flux equal to 0, reaction is reported as essential. In other words, the software reports reactions which have to be active to make certain metabolic objective (e.g. growth) chemically feasible.

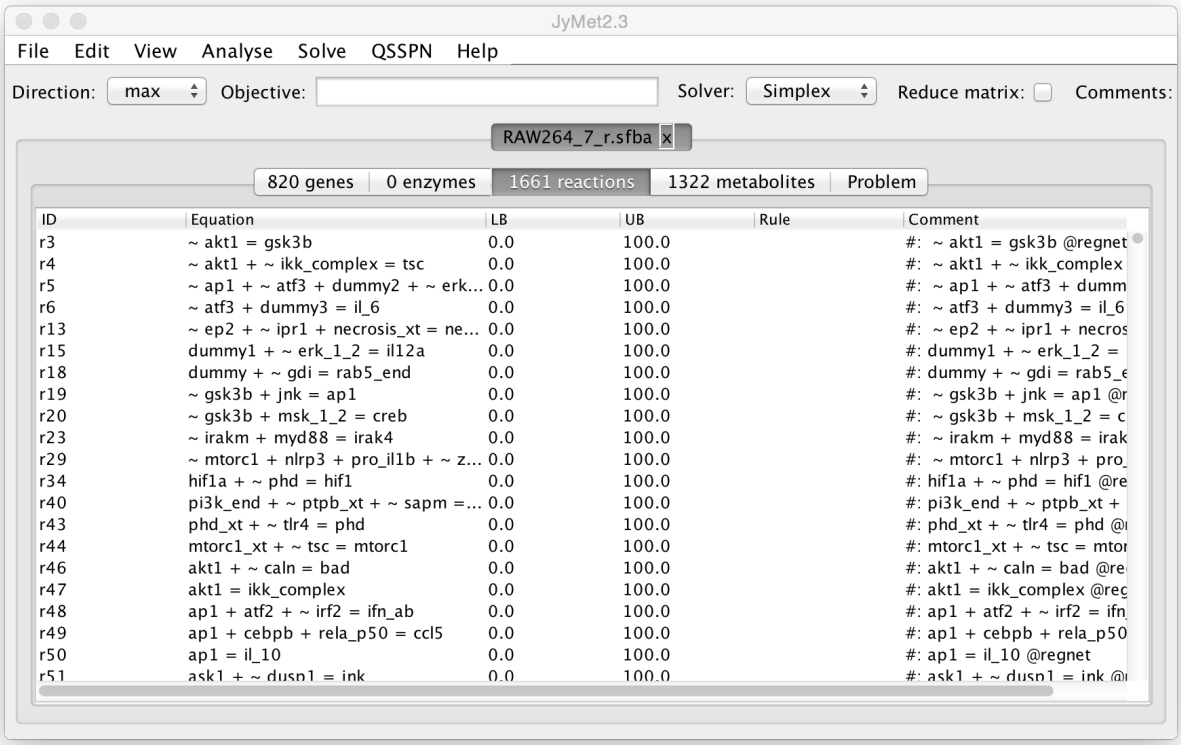
Find reactions essential for growth on minimum medium with glucose as a carbon source. Reset your problem file to represent minimal medium with glucose as a carbon source. To run reaction essentiality scan click “Analyse->Essential” reactions. The **calculations will take about 10 minutes**. You will see the following screen:



Results can be saved to a file using “File->Save table”. If you use “.xls” extension you will be able to open this file is Excel.

Exercise 3. Visualisation of signalling pathways reprogramming mammalian macrophage metabolism.

Open the model.
Download and uncompress the *MUFINS**_Examples.zip* file from the journal supplementary material. Uncompress the file. Open *RAW264_7_r/RAW264_7_r.sfba* file in JyMet (see Exercise 1 for basic JyMet operation). You will see the following window:



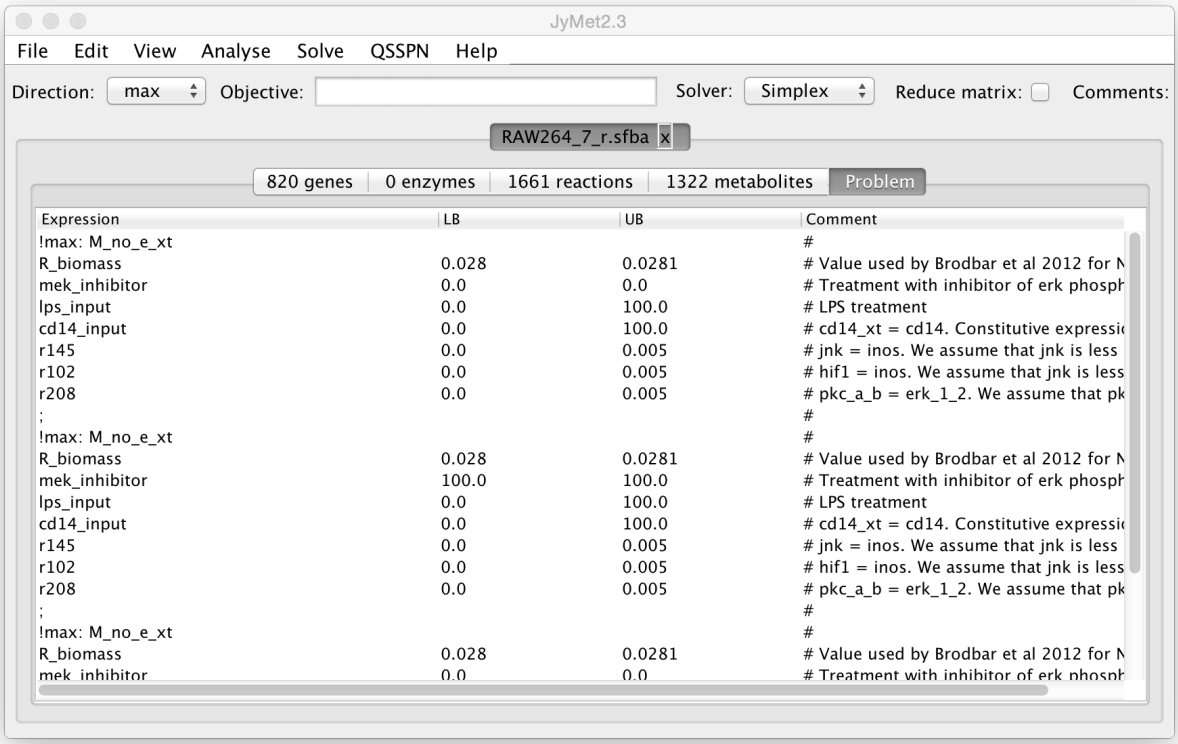
Set external metabolites with externality tag "_xt" (Solve->Externality tag). This is the model of cell signalling, gene regulation and whole-cell metabolism in RAW264.7 macrophage used as a Use Case 3 in MUFINS manuscript. The model of gene regulation and signalling described in the Additional File 2 of the manuscript has been integrated with the RAW264.7 mouse macrophage GSMN published in:

Bordbar A, Mo ML, Nakayasu ES, Schrimpe-Rutledge AC, Kim YM, Metz TO, Jones MB, Frank BC, Smith RD, Peterson SN, Hyduke DR, Adkins JN, Palsson BO. Model-driven multi-omic data analysis elucidates metabolic immunomodulators of macrophage activation. Mol Syst Biol. 2012 Jun 26;8:558. doi: 10.1038/msb.2012.21.

Note reaction formulas with "~" symbol proceeding inhibitors. MUFINS is the only general Constraint Based Modelling software implementing linear inhibition constraints (see the manuscript).

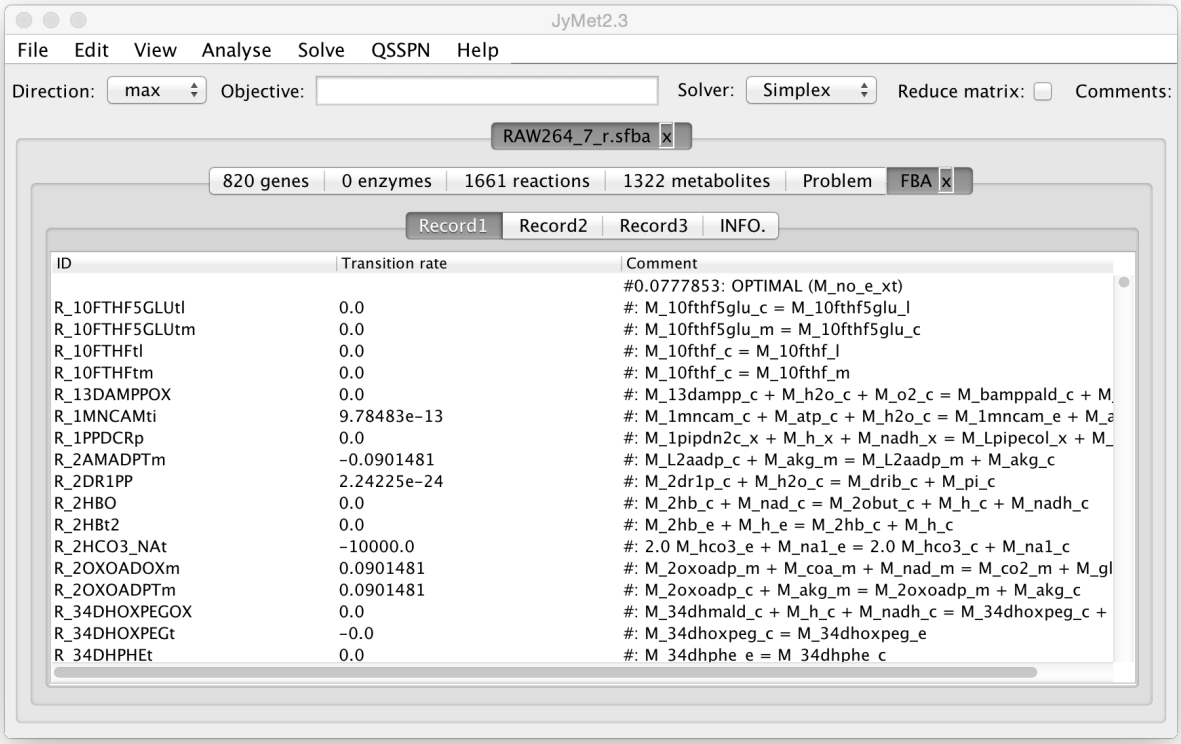
All the reactions in regulatory part of the model are "tagged" with "@regnet" in comments section. As "#" symbol separates comment part of the line, the "hashtag" cannot be used. Here we use @regnet as it is a unique tag for "Edit->Search" function. Combination of multiple tags can be used too.

Open the problem file.
Use “File->Open problem” to load *RAW264_7_r/simulate.pfile*. from *MUFINS.**_Examples:*



The file defines multiple simulations separated by “;”. These “in silico” experiments investigate influence of LPS and Mek1 inhibitor on the maximal rate nitric oxide production rate.

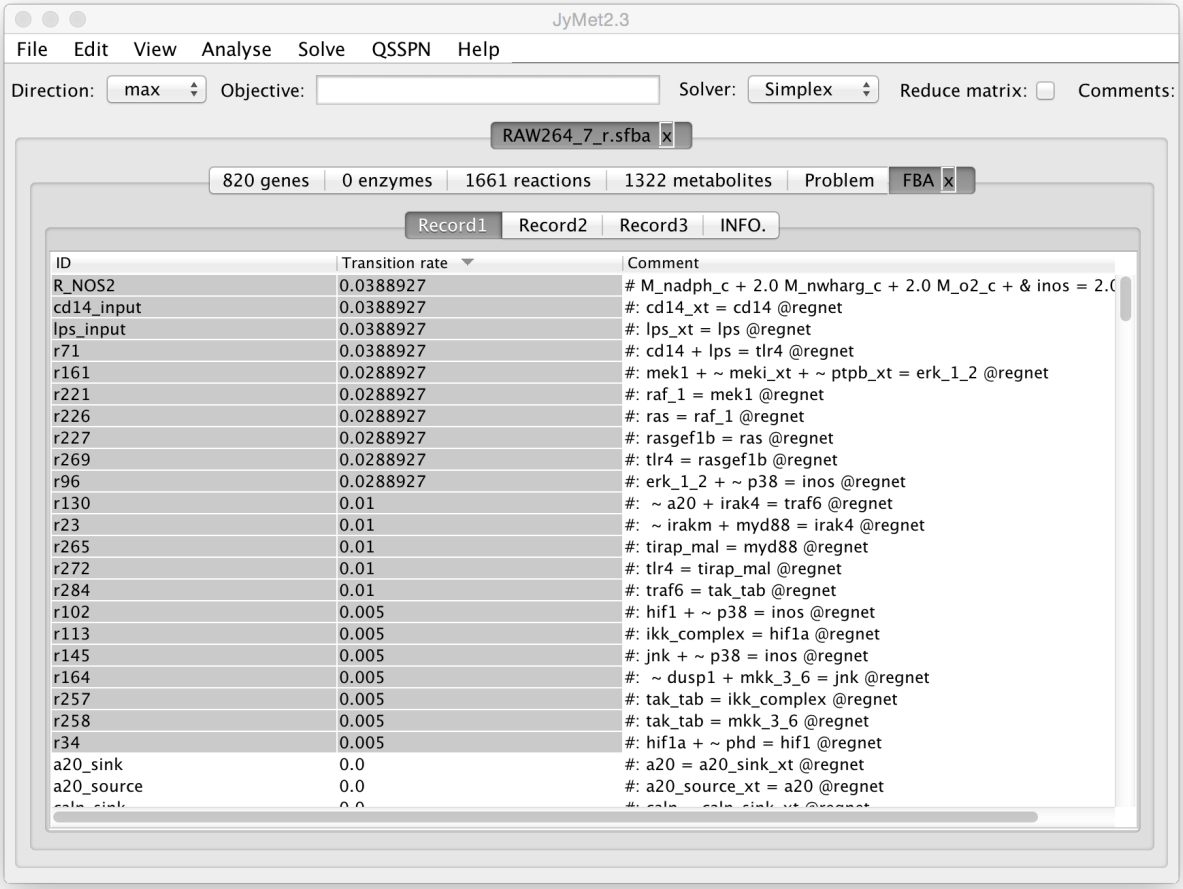
Run Flux Balance Analysis
Select “Analyse->FBA” and then “Solve->solve”. You will see maximal objective function value and example flux distribution. You will see the following window:



Note, that results for the three simulations in the problem file are available in 3 tags of “FBA”. The “INFO” tag contains simulation log including command lines used to run standalone tool. The command lines can be used as examples for stand-alone simulations or scripts.

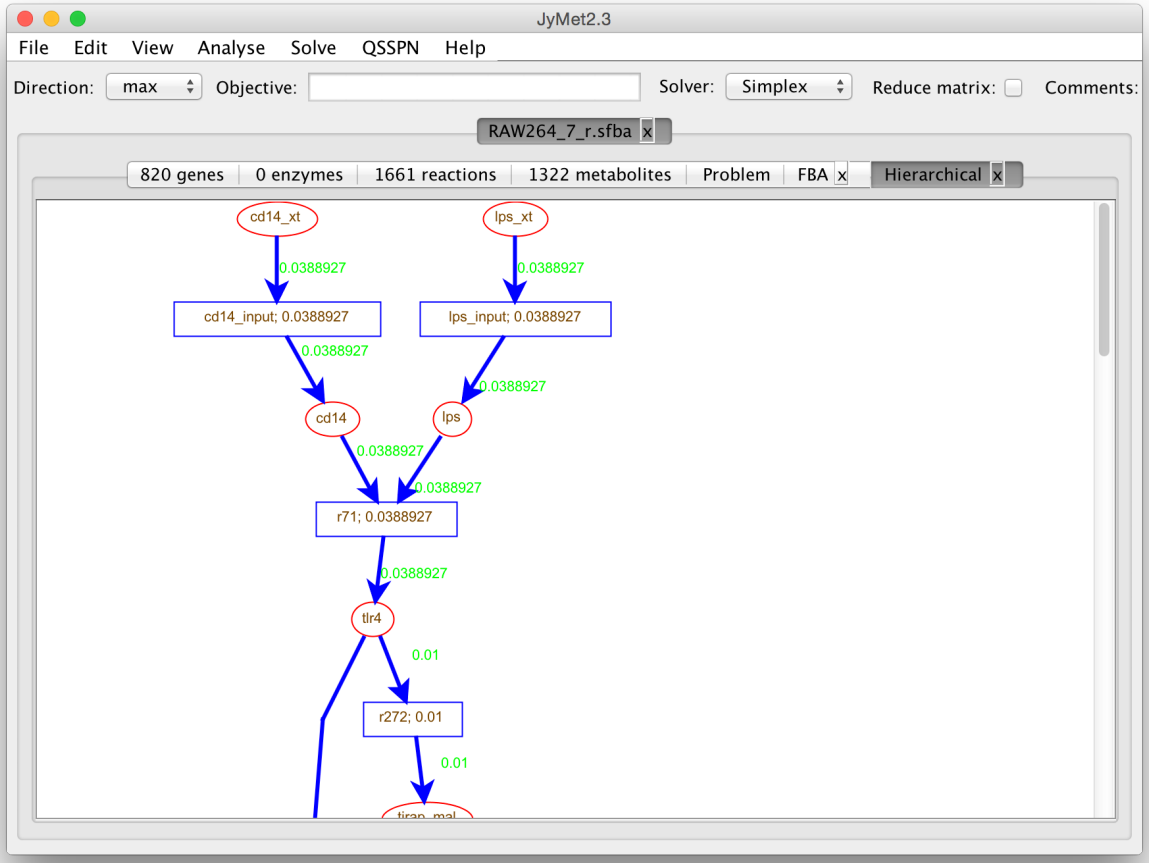
Filter and sort regulatory network fluxes.
Use “Edit->search” to filter all reactions tagged by “@regnet” string. Subsequently, click “Transition rate” header to sort these reactions by flux. This will identify all regulatory network reactions with non-zero flux in this particular example solution.

Select all non-zero reaction names and fluxes in example solution:



Use “View->Layout->hierarchical” to visualise these fluxes, with automatic, hierarchical layout. The network will be visualised in Petri Net (bipartite graph) notation with rectangles representing transitions and circles representing places (figure below). Flux values are written within reaction rectangle, line thickness is used to visualise fluxes. You can right click on the window and zoom the graph. You can also adjust layout manually and save layout with “File->Save graph” function. The graph can then be used to visualise results with “Visualise->Layout->custom”. In this case reaction names in the graph are matched against reaction IDs in current results table. Therefore, the graph layout also acts as a reaction name filter. This mode of visualisation is ideal for visualising effective pathways emerging in genome scale networks under particular conditions. For example, here (figure below), we see signalling pathway activating nitric oxide production as the results of LPS input.

The interactive FBA and network visualisation has been used for simulations described in Use Case 1 of the manuscript.


JyMet2.3
File
Edit
View
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Direction:
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Solver:
Simplex
Reduce matrix:
Comments:
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1661 reactions
1322 metabolites
Problem
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