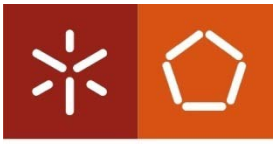

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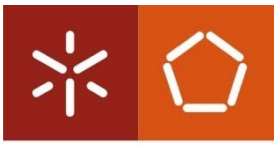
OPTFLUX 2 BEGINNER'S TUTORIAL

BEGINNER'S TUTORIAL

FOR

OPTFLUX 2

METABOLIC ENGINEERING WORKBENCH



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LICENSES

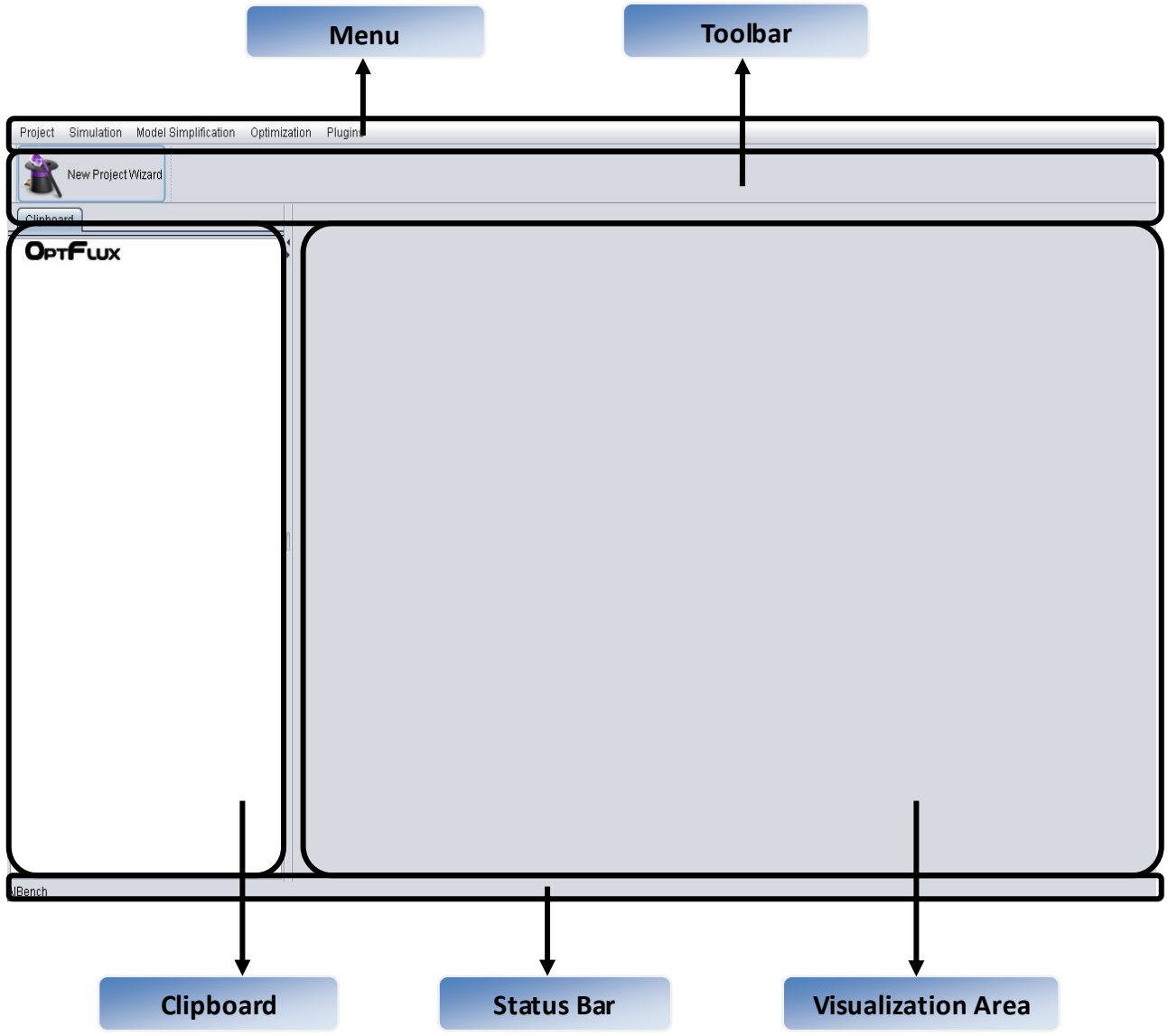
- **For this tutorial:**

- **For the OptFlux software:**

FIRST THINGS FIRST!

Hello and welcome to the OptFlux 2 beginner's tutorial. If you haven't already downloaded the software please do it here: www.OptFlux.org.

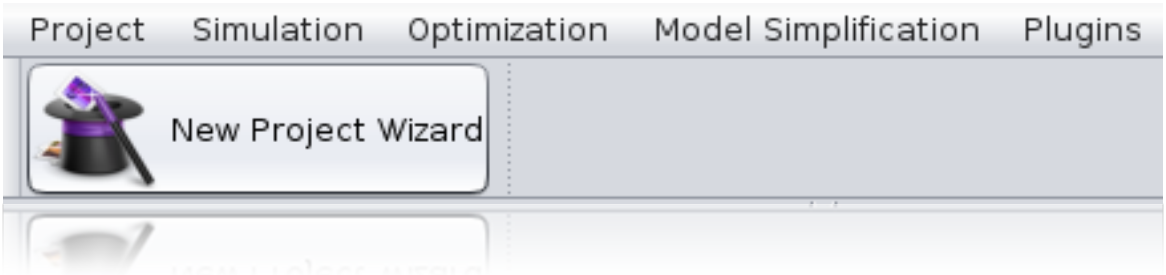
After launching the software you'll be presented with the layout depicted in the image below. Most of OptFlux main features and operations will be accessible to you either through the **Menu** or the **Toolbar**. You can also have access to them by right-clicking in the **Clipboard** area. Your data types i.e., the project, metabolic models, simulation/optimization results, etc., will always be placed in the **Clipboard** area**.** The **Visualization Area** is the place where you can examine those data types in greater detail.
Click around to get familiar with it and after that jump to the next step.



1ST STEP – CREATING A NEW PROJECT

To follow the steps in this tutorial you need to download the file *gomGG.zip*, available in www.OptFlux.org/tutorial/gomGG.zip. The model therein contained is a simplified model for growth of Saccharomyces cerevisiae [Forster, J. and Gombert, A.K. and Nielsen, N. ***A functional genomics approach using metabolomics and in silico pathway analysis.*** Biotechnology and Bioengineering, 2002].
Extract the contents of that file to a directory of your choice.

To begin the creation of a new project, you have to start the **New Project Wizard.** You can access it either through the *File Menu* or the *Toolbar.*

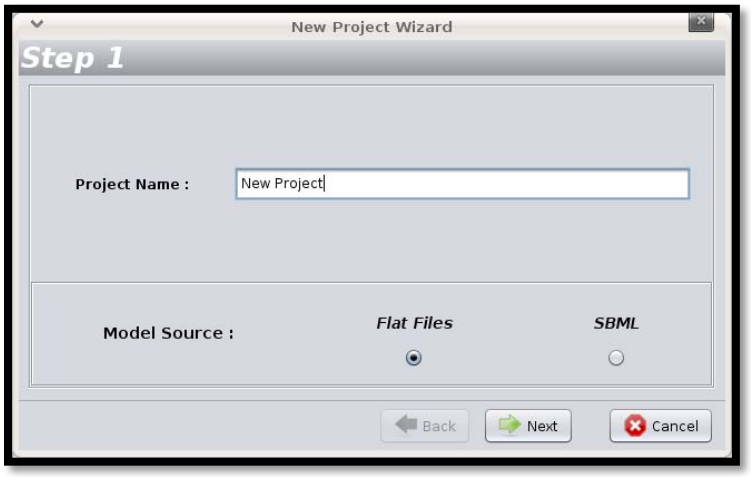


You have the option to create the new project from two different sources: flat files and SBML file.

1.1 – FROM FLAT FILES

Step 1

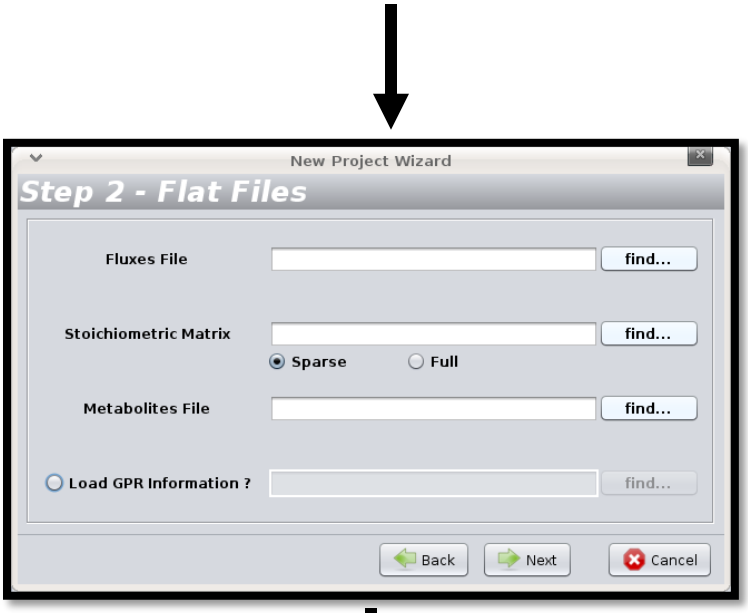
In the first step, the user must input a valid project name
In the picture the name selected was “New Project”



Step 2

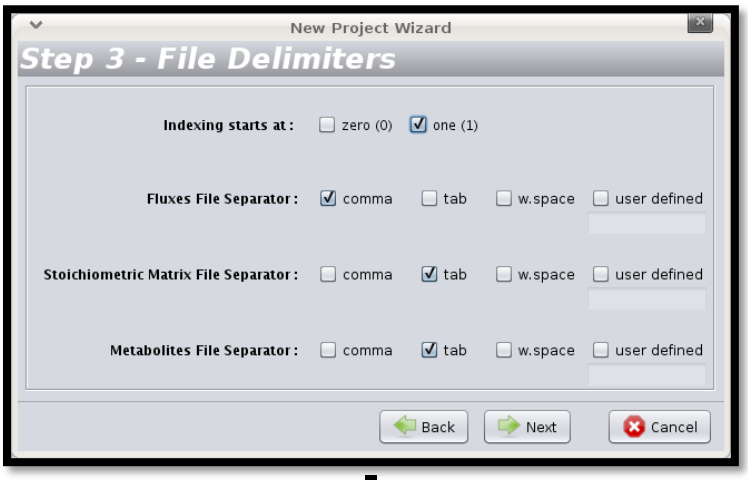
In the second step, the user must select three files, which were provided along with this tutorial:

- The first contains the reactions names and their flux limits – select *gomper.fluxes*;
- The second contains the stoichiometric matrix – select *gomper.matrix*;
- The third contains the metabolite names (optional) – select *gomper.compounds*



Step 3

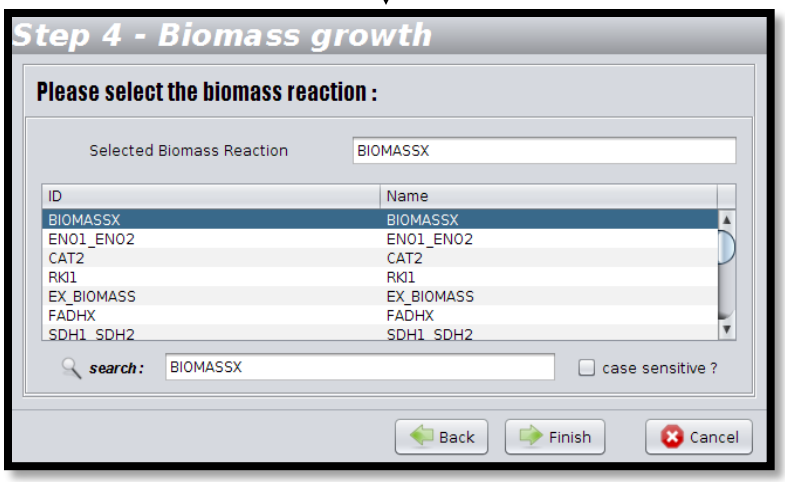
In step 3, the first option concerns the indexing used in the stoichiometric matrix if the SPARSE option was selected. The user must select indexing starting at **zero**. For the remaining files, the user should select the **comma separator** for the Fluxes File and the **tab separator** for the matrix and metabolites file.



Step 4

In the fourth step, OptFlux automatically tries to find the biomass growth associated flux, since this information is essential for both simulation and optimization procedures.

A heuristic method will automatically identify the reaction **"BIOMASSX"**.



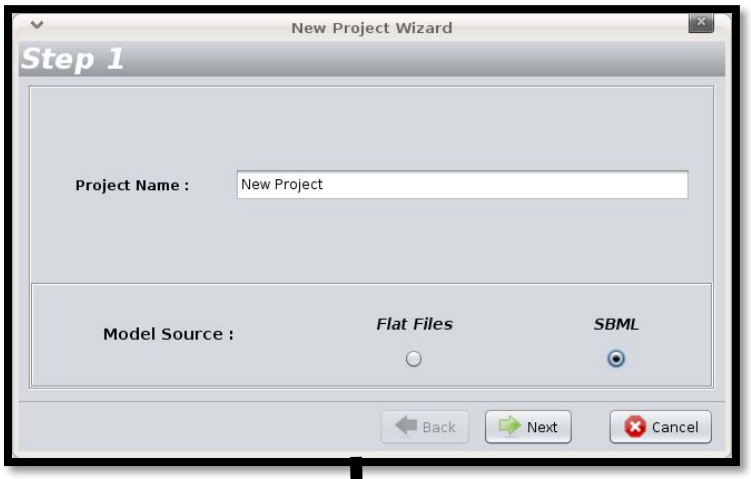
1.2 – FROM A SBML FILE

Step 1

In the first step, the user must input a valid project name.

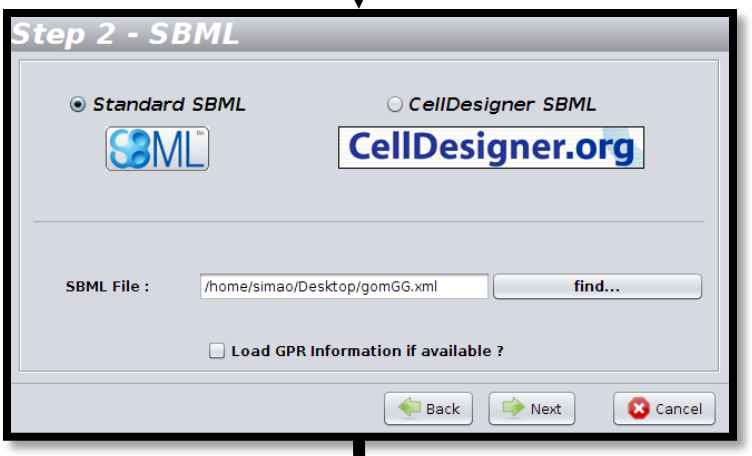
In the picture the name selected was “New Project”

The user must select the SBML option in the bottom as the model source.



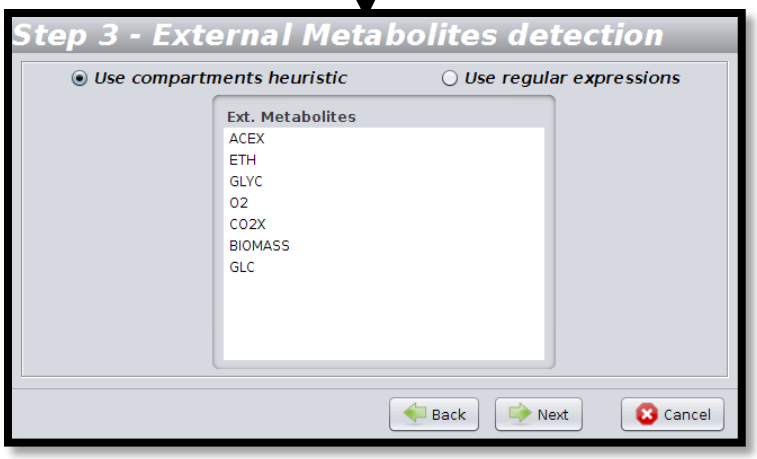
Step 2

In the second step the user must select the file to load and the type of SBML therein contained. In this example, the user must select the file *gomGG.xml* provided in the attached zip file. The type of file to select can be **Standard SBML** or CellDesigner SBML (this example contains CD annotations for visualization).



Step 3

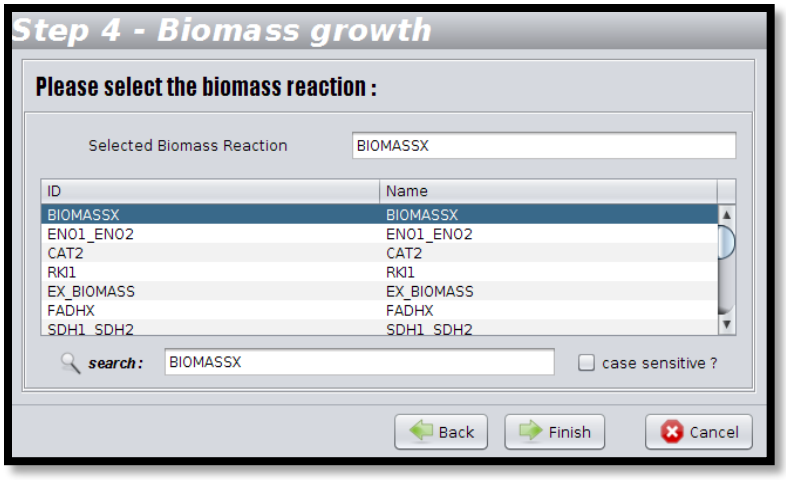
The third step is relative to the extra-cellular environment. *OptFlux* will automatically try to find the extra-cellular compartment and the respective metabolites, using the compartments heuristics it will detects external metabolites based on the definitions of the compartments contained in the SBML file.



Step 4

In the fourth step, OptFlux automatically tries to find the biomass growth associated flux, since this information is essential for both simulation and optimization procedures.

A heuristic method will automatically identify the reaction **"BIOMASSX"**.

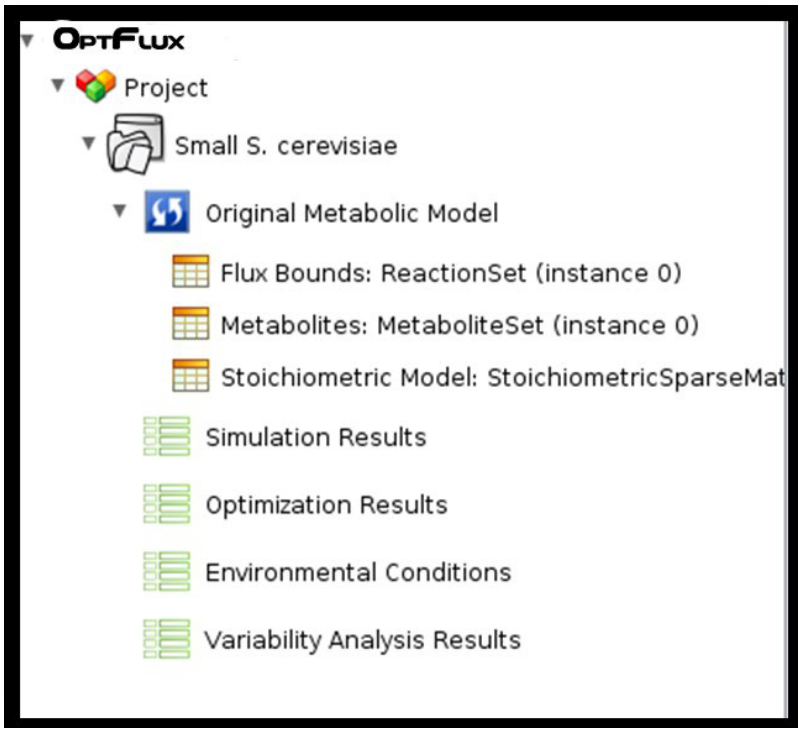


1.3 – PROJECT CREATED IN THE CLIPBOARD

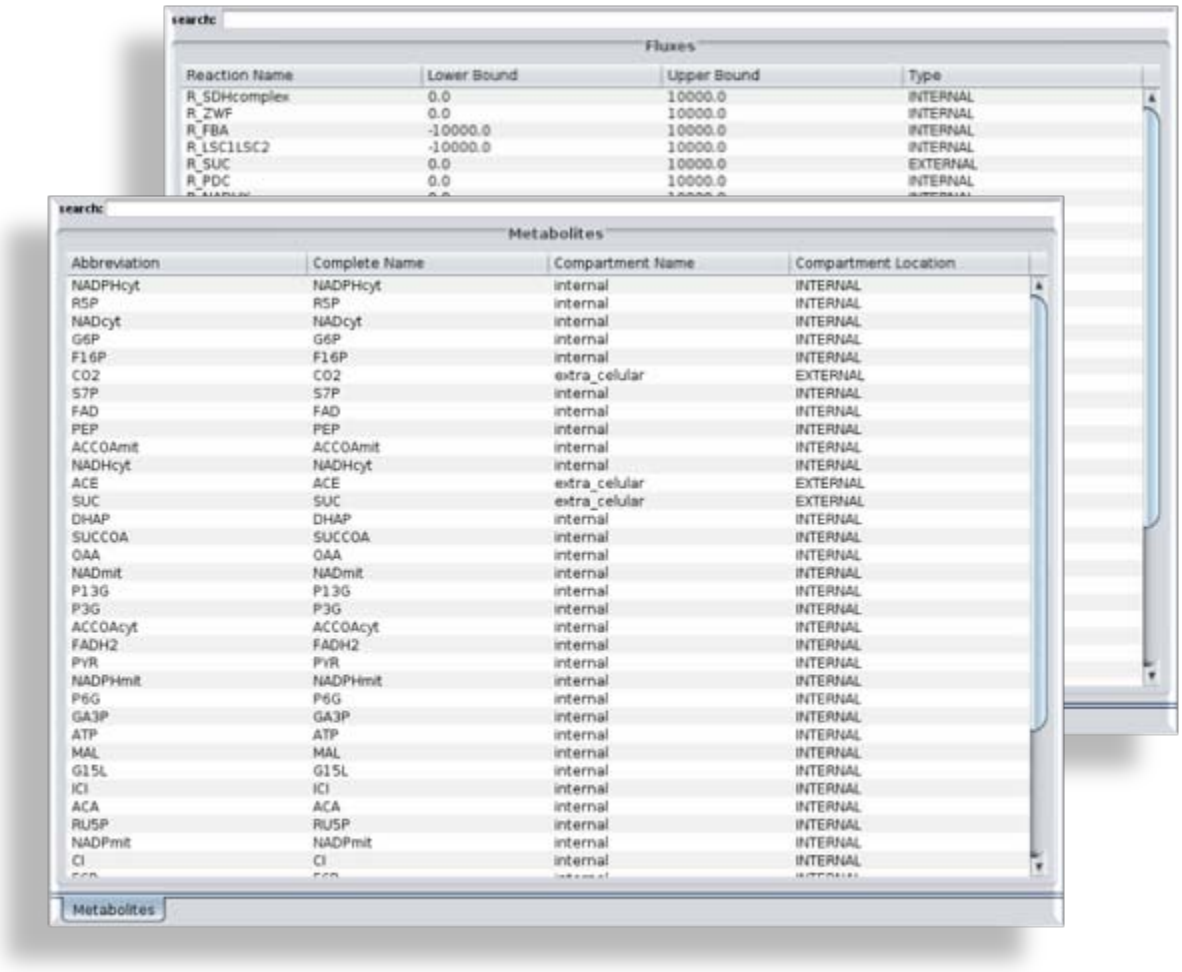
After following either **step 1.1** or **step 1.2**, the user will now be presented with the scenario depicted in the following screenshot:

In the image, one can see the default structure of any OptFlux project. The central data type is named "Original Metabolic Model". Inside, one can access the flux limits information, metabolites information and also the stoichiometric coefficients in a human-readable fashion.

At this point the user should click around a bit to get familiar with this structure and the information therein contained.



The viewers for the Reactions, Metabolites and the Stoichiometric Matrix are depicted in the screenshots below.



2ST STEP – PERFORMING SIMULATIONS

OptFlux allows the user to perform a simulation of the "wild-type" strain, i.e. of the model with no genetic modifications.
Access the "Wild Type" option either through the "Simulation" menu or right clicking on the model icon in the clipboard.

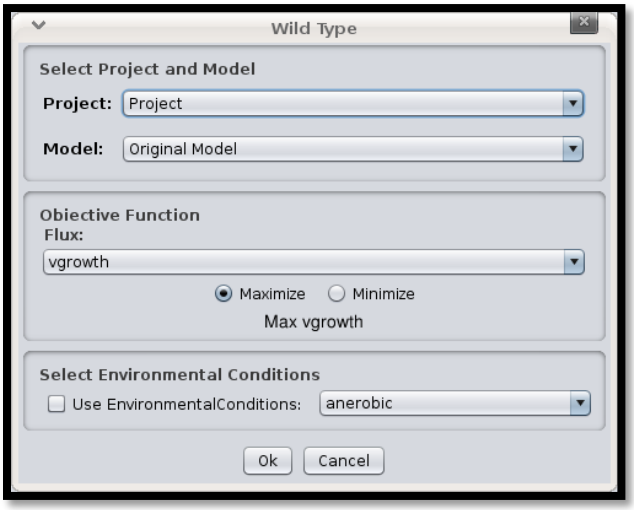
2.1 – PERFORMING A WILD-TYPE SIMULATION

Step 1

In the first step, the user must select the Project and the Metabolic Model to which the simulation will refer.

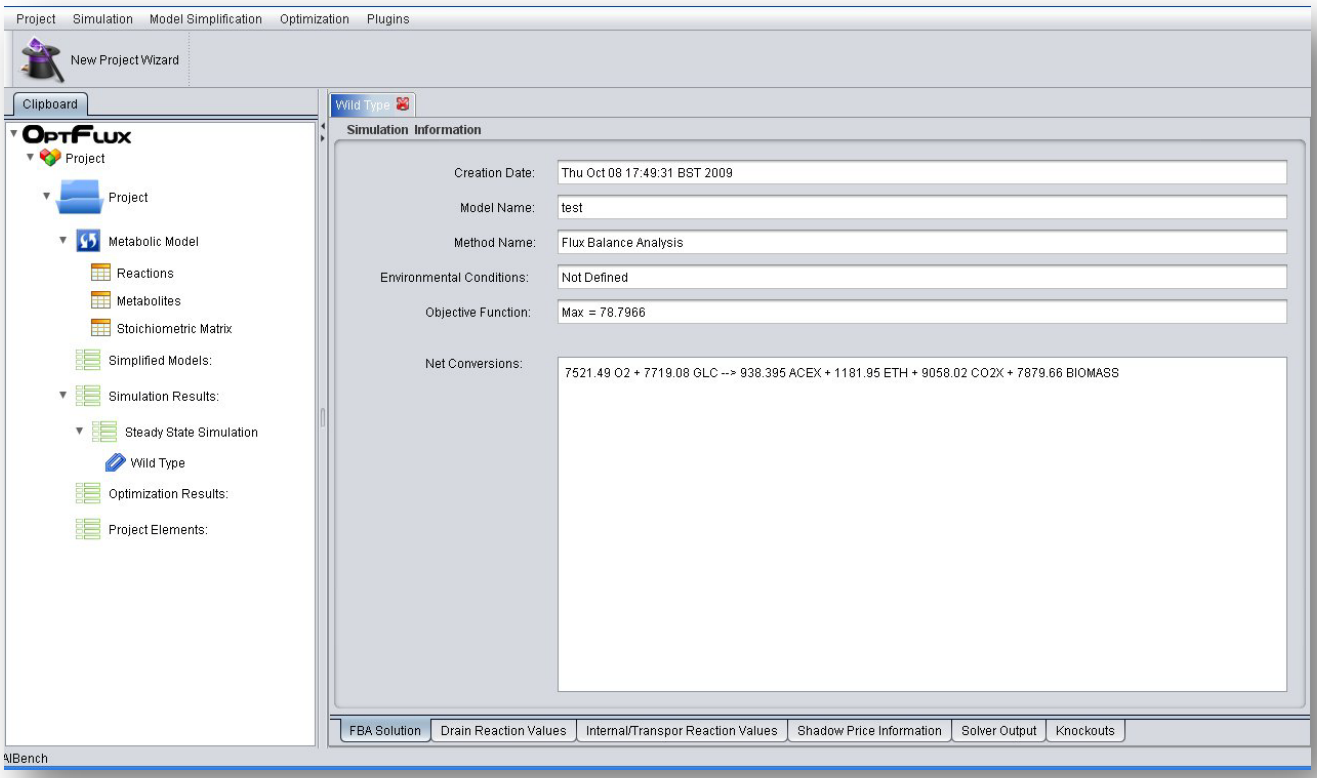
This step is necessary since OptFlux supports multiple-projects and each project can contain an Original Metabolic Model and a Simplified Metabolic Model.

In the context of this tutorial, leaving the default selection is just fine.



Step 2

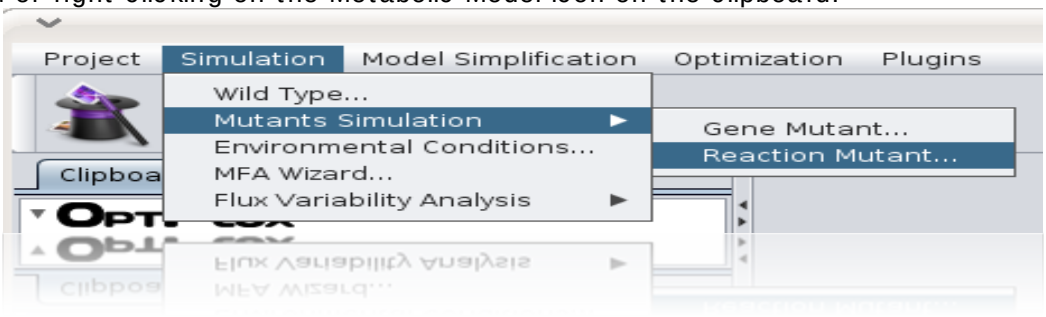
After completing all the previous steps, a new object named “Wild Type” is placed within the **Simulation Results** list. By left-clicking this object the user has access to detailed information about the performed simulation. The user can, for instance, see the values for all the fluxes the simulation method calculated and can even export the list of values to a text file.



2.2 – PERFORMING A MUTANT SIMULATION – REACTION DELETIONS

Step 1

You can access the "Reaction Mutant" option under the "Simulation -> Mutants Simulation" menu or right clicking on the Metabolic Model icon on the clipboard.



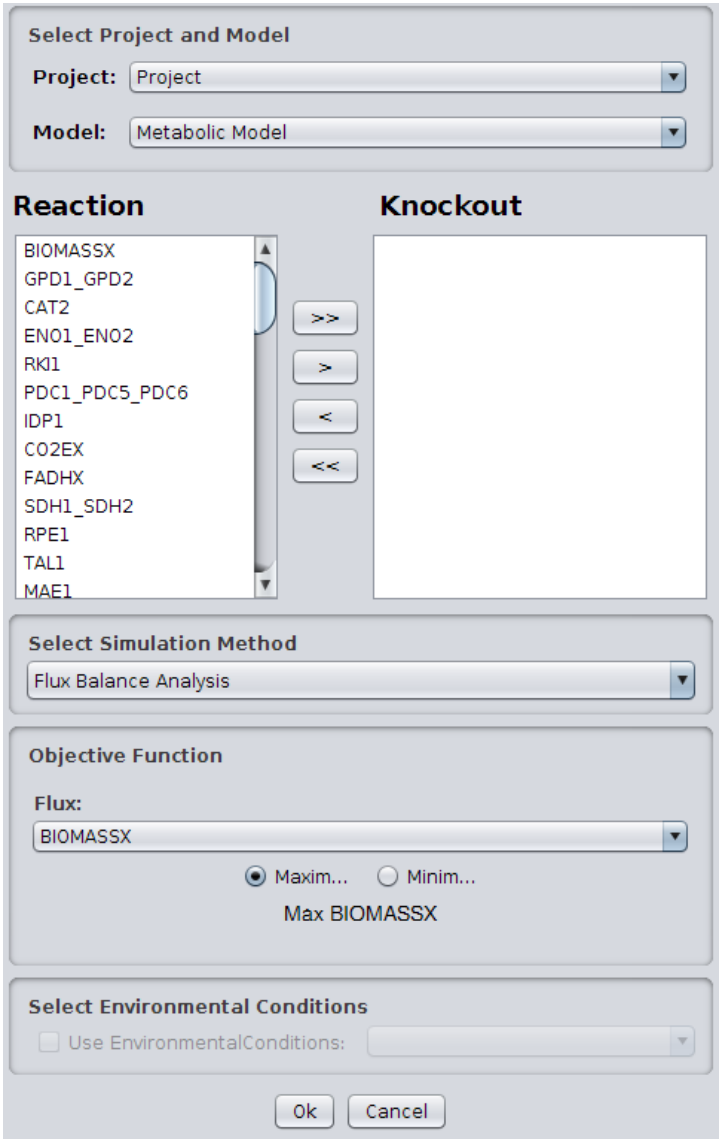
Step 2

1. Reaction knockout list
Selecting in the Reaction list you can add/remove (using the arrows buttons) reactions to the knockout list (the list of reactions to be deleted, on the right).

2. Select Simulation Method
OptFlux can use several simulation methods for knockout simulations, namely: Flux-Balance Analysis, ROOM-LP, ROOM-MILP, MOMA

ROOM-LP stands for the Regulatory On-Off Minimization Method (ROOM), using a linear programming (LP) relaxation; ROOM-MILP is the original ROOM that uses a Mixed Integer LP (MILP) method; MOMA stands for the Minimization of Metabolic Adjustment method that uses quadratic programming.

3. Objective Function Configuration
Here you can select the reaction to optimize (biomass, by default), and you can also define if you will be maximizing or



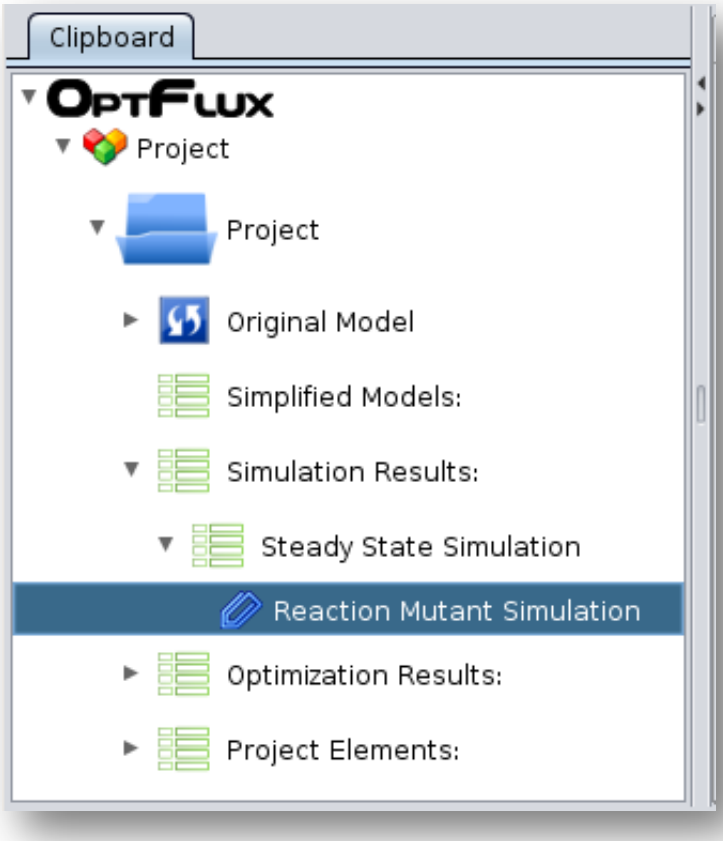
minimizing the flux.

4. Select Environmental Conditions

If you have created environmental conditions you can select them to be used as constrains in the simulation. These can be used to define the values of drain fluxes, i.e. the rates at which metabolites are consumed or produced.

Step 3

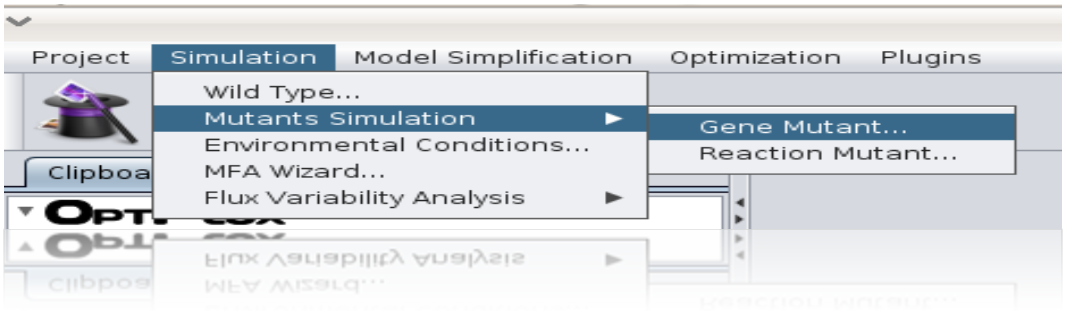
And that's all!! You can press OK and the results will be loaded into the clipboard..



2.3 – Performing a mutant simulation – GENE KNOCKOUT

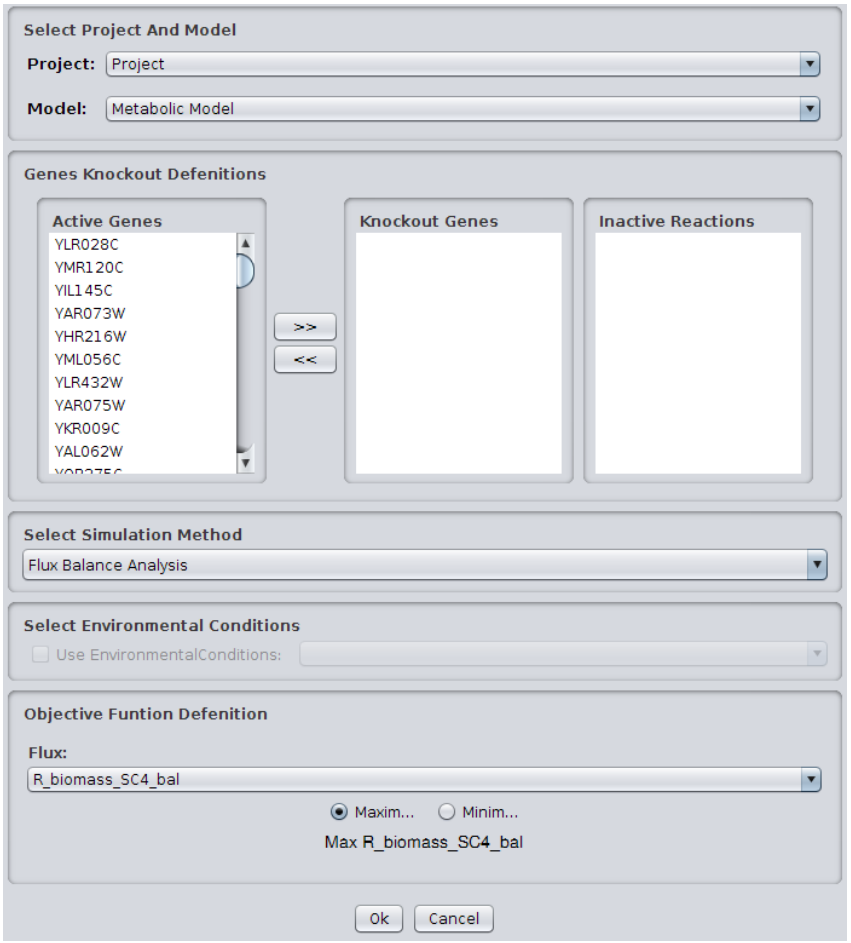
Step 1

You can access the "Gene Mutant" option under the "Simulation -> Mutants Simulation" menu or right clicking on the Metabolic Model icon on the clipboard.



Step 2

In the Gene Mutant Simulation you can select the model/project to work, and set up your configuration.



1. Gene knockout list

Selecting in the Gene list you can add/remove (using the arrows buttons) genes to the knockout list (the list of genes to be knocked out, in the right). In the Inactive Reactions list you can see the reactions that will be turned off knocking out that set of genes.

2. Select Simulation Method

OptFlux can use several simulation methods for knockout simulations, namely:
Flux-Balance Analysis, ROOM-LP, ROOM-MILP, MOMA

ROOM-LP stands for the Regulatory On-Off Minimization Method (ROOM), using a linear programming (LP) relaxation; ROOM-MILP is the original ROOM that uses a Mixed Integer LP (MILP) method; MOMA stands for the Minimization of Metabolic Adjustment method that uses quadratic programming.

3. Objective Function Configuration

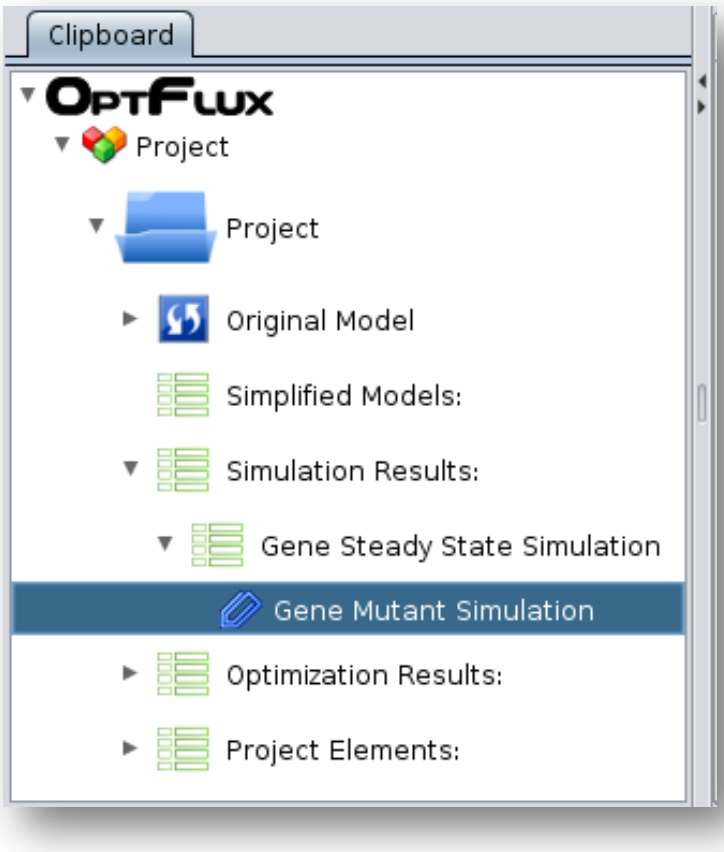
Here you can select the reaction to optimize (biomass, by default), and you can also define if you will be maximizing or minimizing that flux.

4. Select Environmental Conditions

If you have created environmental conditions you can select them to be used as constraints in the simulation. These can be used to define the values of drain fluxes, i.e. the rates at which metabolites are consumed or produced.

Step 3

And that's all !! You can press OK and the results will be loaded into the clipboard..

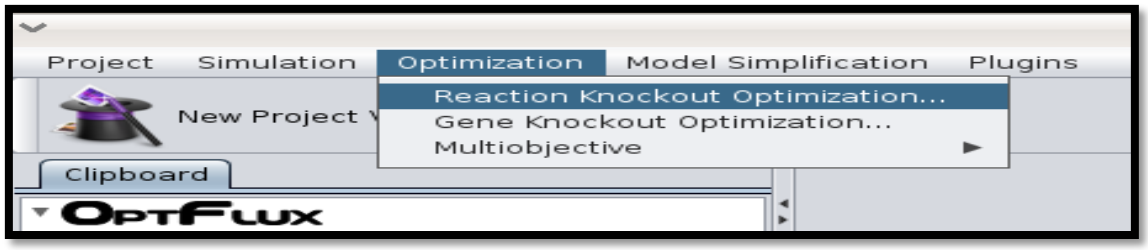


3ST STEP – PERFORMING OPTIMIZATION

3.1 – OPTIMIZE THE KNOCKOUT SET - REACTION DELECTIONS

Step 1

You can access the "Reaction Knockout Optimization" option under the "Optimization" menu.



Step 2

You can select the model/project to work, and set up your optimization configuration.

1.Select Project and Model

In the Project combo box select the project where you want to perform the optimization, in the Model combo box select the model within the project that you want to use (if you have performed model simplification, the simplified models will be easier to handle by the optimization algorithms).

2.Select Simulation Method

OptFlux can use several simulation methods, namely Flux-Balance Analysis, ROOM-LP, ROOM-MILP, MOMA. Check the Simulation How To's to find out more about these methods...

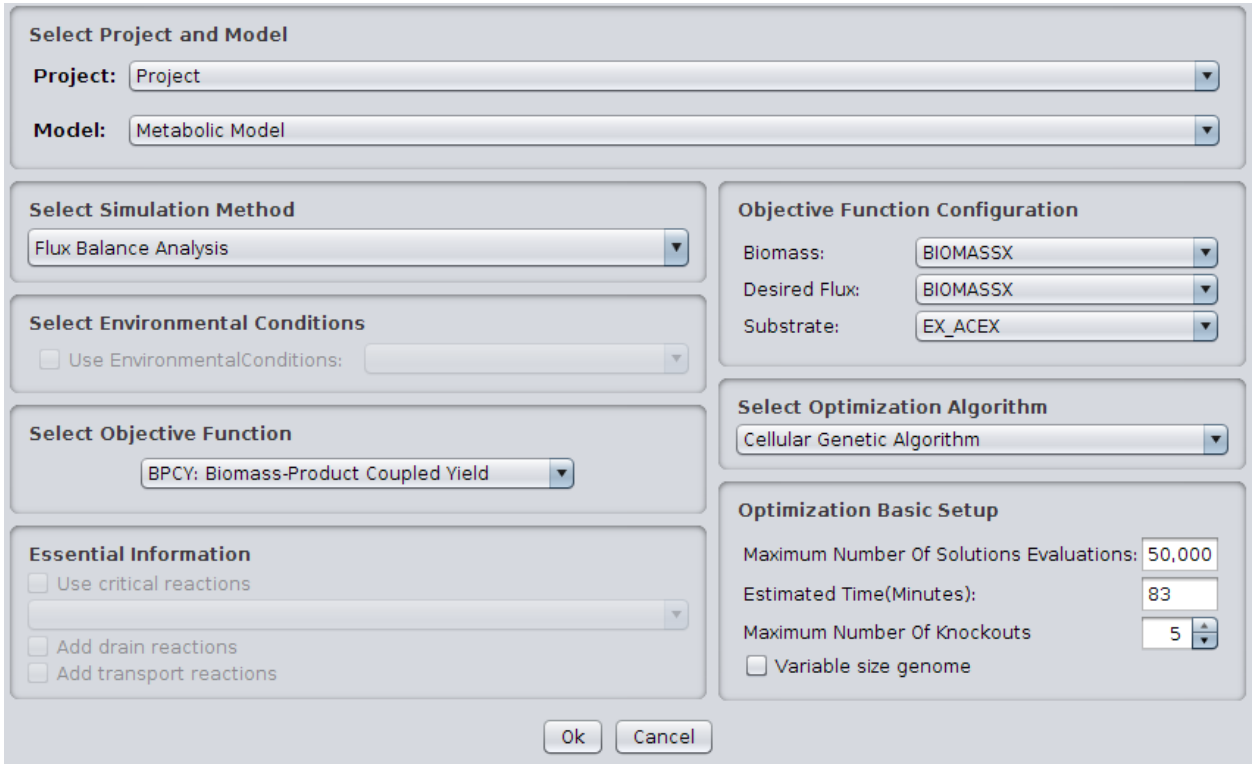
3.Select Environmental Conditions

If you have created environmental conditions you can select them to be used as constraints in the simulation.

4.Select Objective Function

OptFlux can use two types of objective function:
BPCY - Biomass-Product Coupled Yield, YIELD - Product Yield with Minimum Biomass.
The first calculates the product of the biomass flux and the compound production flux; the second, returns the value of the target compound production flux divided by the substrate consumption flux, if the biomass is larger than a minimum value, defined by the user.

5.Objective Function Configuration
Here you can select the flux to be optimize (the compound you wish to produce), the flux that represents the biomass and the substrate that is in use.



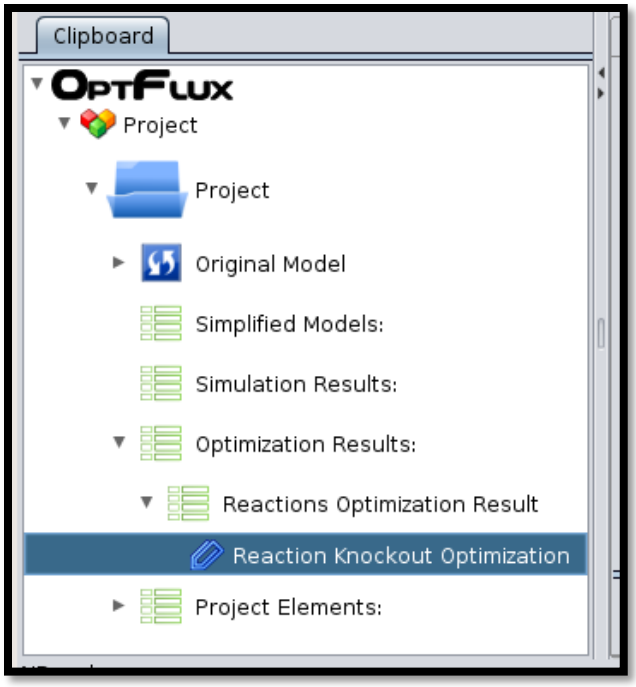
6.Select Optimization Algorithm
OptFlux allows you to perform optimizations using one of the following algorithms: Cellular Genetic Algorithm, Evolutionary Algorithm, Simulated Annealing.

7.Optimization Basic Setup
Here you can configure the maximum number of solution evaluations (Simulations) and check the expected time to perform that number of evaluations.br> You can also set up the maximum number of knockouts and if the set of knockouts should be static or have a variable size.

8.Essential information
You can define if it is possible to knockout some special type of reactions like drains, transport and critical reactions (if you have loaded/created this information).

Step 3

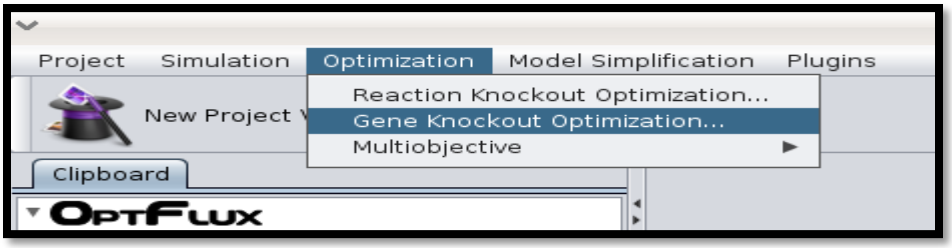
And that's all, now you can press OK and check the results in the clipboard.



3.2 – OPTIMIZE THE KNOCKOUT SET – GENE KNOCKOUTS

Step 1

You can access the "Gene Knockout Optimization" option under the "Optimization" menu. ***To use this optimization you have to use with a model with GPR (gene-protein-reaction) information.***

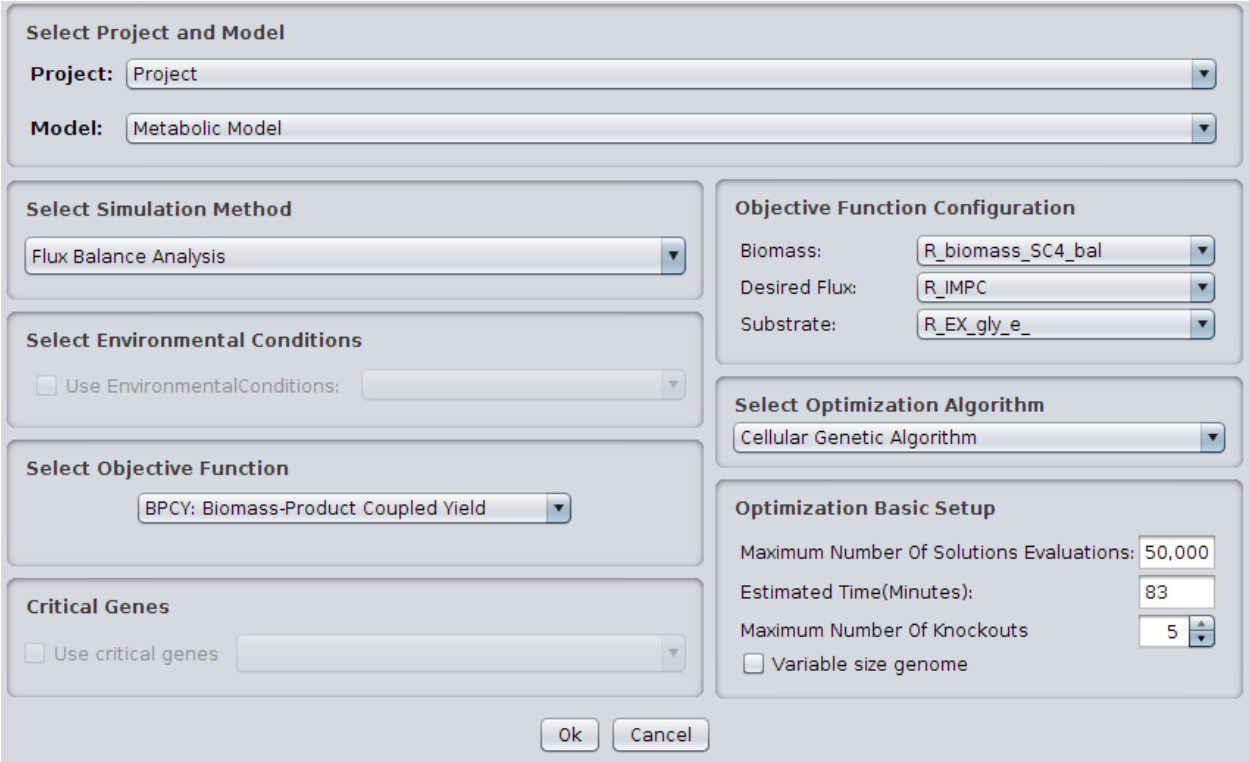


Step 2

You can select the model/project to work, and set up your optimization configuration.

1.Select Project and Model

In the Project combo box select the project where you want to perform the optimization, in the Model combo box select the model in the project that you want to use (if you have performed model simplification, the simplified models will be easier to handle by the optimization algorithms).



2.Select Simulation Method

OptFlux can use several simulation methods, namely Flux-Balance Analysis, ROOM-LP, ROOM-MILP, MOMA.

Check the Simulation How To's to find out more about these methods...

3.Select Environmental Conditions

If you have created environmental conditions you can select them to be used as constraints in the simulationIf you have loaded/created environmental conditions you can select them to use as constraints in the simulation.

4.Select Objective Function

OptFlux can use two types of objective function:

BPCY - Biomass-Product Coupled Yield, YIELD - Product Yield with Minimum Biomass.

The first calculates the product of the biomass flux and the compound production flux; the second, returns the value of the target compound production flux divided by the substrate consumption flux, if the biomass is larger than a minimum value, defined by the user

5.Objective Function Configuration

Here you can select the flux to be optimize (the compound you wish to produce), the flux that

represents the biomass and the substrate that is in use.

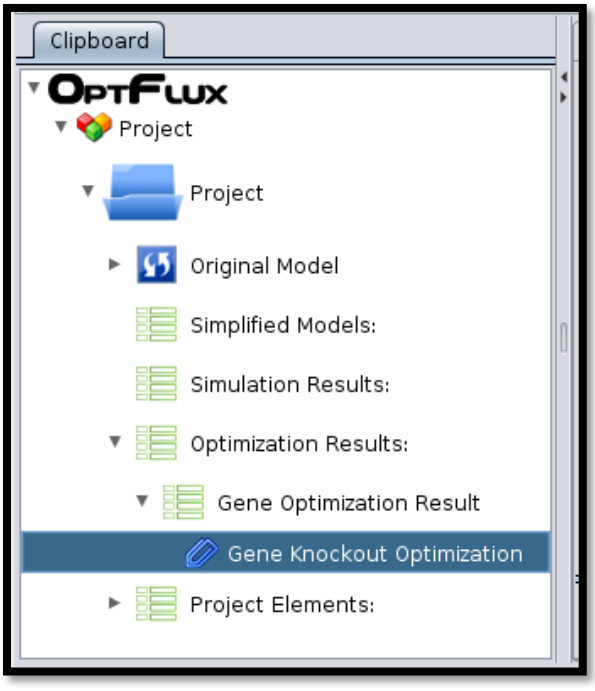
6.Select Optimization Algorithm
OptFlux allows you to perform optimizations using one of the following algorithms:
Cellular Genetic Algorithm, Evolutionary Algorithm, Simulated Annealing

7.Optimization Basic Setup
Here you can configure the maximum number of solution evaluations (simulations) and check the expected time to perform that number of evaluations.
You can also set up the maximum number of knockouts and if the set of knockouts should be static or have a variable size.

8.Essential information
If you loaded/created some essential genes you can define if it is possible to knockout some critical genes.

Step 3

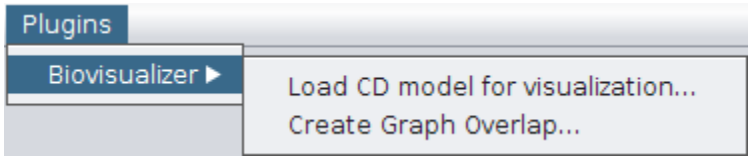
And that's all, now you can press OK and check the results in the clipboard.



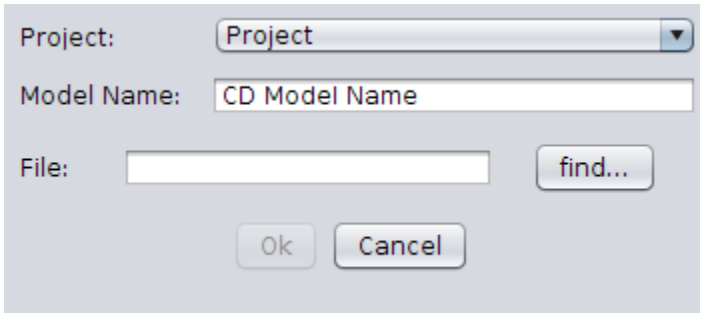
4ST STEP – INTERACTION WITH CELLDESIGNER

3.1 – LOADING A CELLDESIGNER MODEL FOR VISUALIZATION

You can access the "Load CD model for visualization" option under the "Plugins -> Biovisualizer" menu.

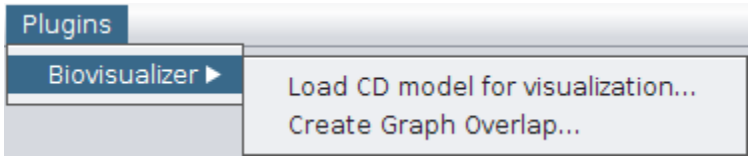


In the project combo box select the project where you want to load the CD into, then choose the file and write the name for the CD visualization object in the clipboard.

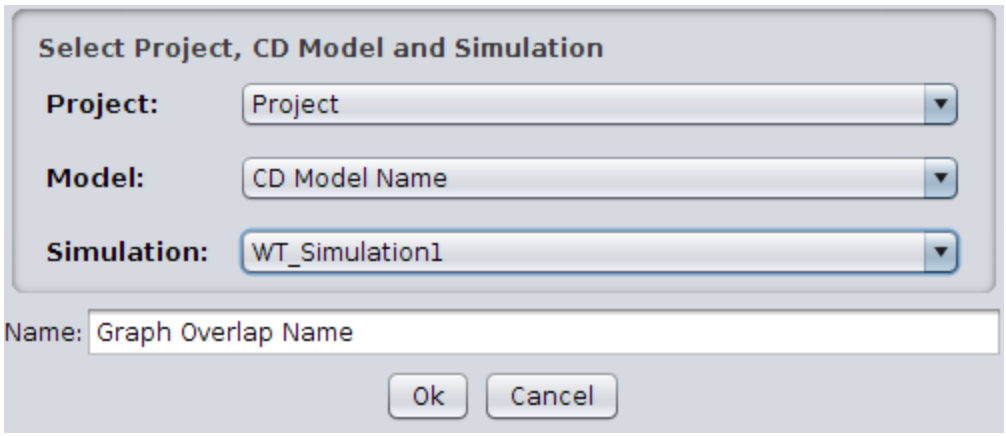


3.2 – SIMULATION RESULT OVERLAP WITH CD MODEL

You can access the "Create a Graph Overlap" option under the "Plugins -> Biovisualizer" menu.



Select the Project to use, and the simulation result and model to use in the graph overlap.



Then you can visualize the generated graph overlap model, present in the Project Elements section of the clipboard.