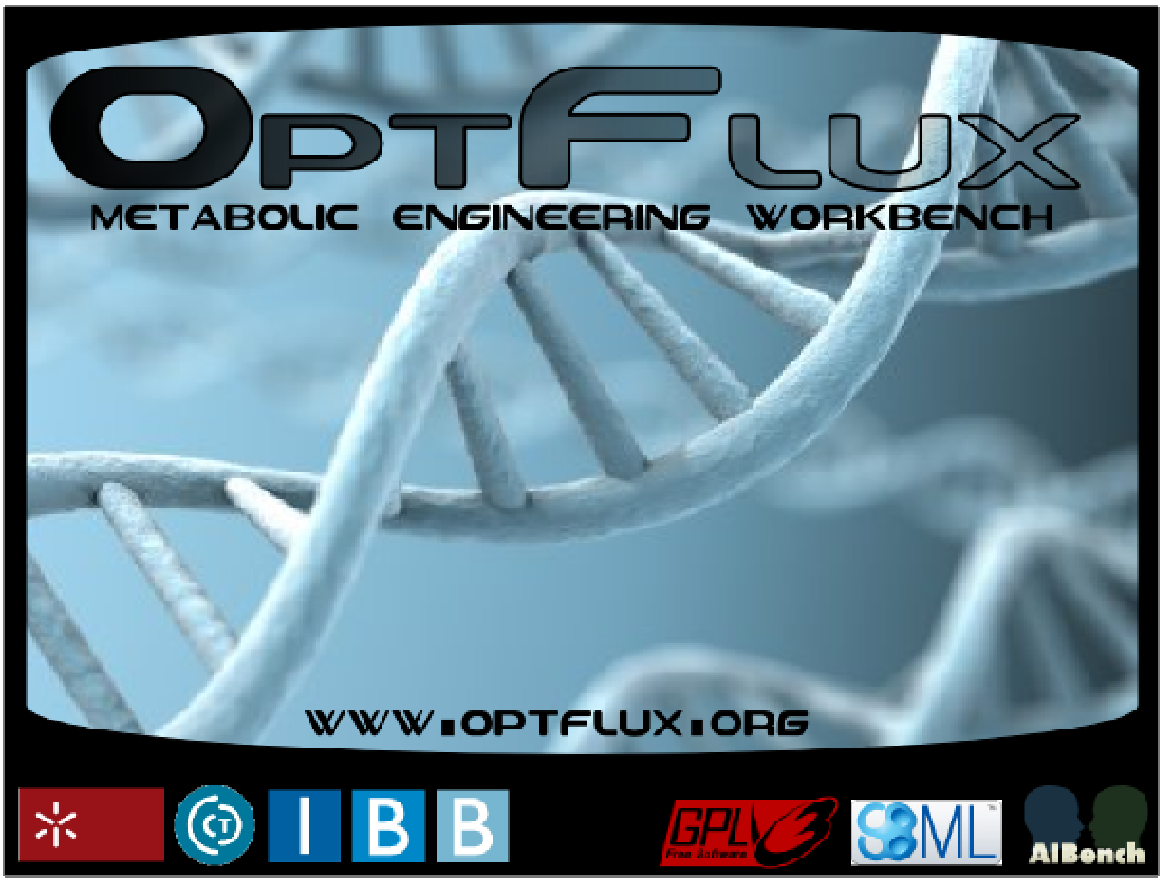
Universidade do Minho

IBB-CEB – INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING – CENTRE OF BIOLOGICAL ENGINEERING
CCTC– COMPUTER SCIENCE AND TECHNOLOGY CENTER
SCHOOL OF ENGINEERING
UNIVERSITY OF MINHO

BEGINNER'S TUTORIAL



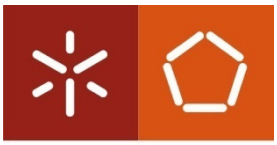
Version 1.2

BEGINNER'S TUTORIAL

FOR

OPTFLUX

METABOLIC ENGINEERING WORKBENCH



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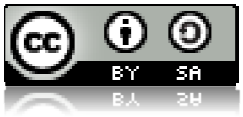
LICENSES

- **For this tutorial:**



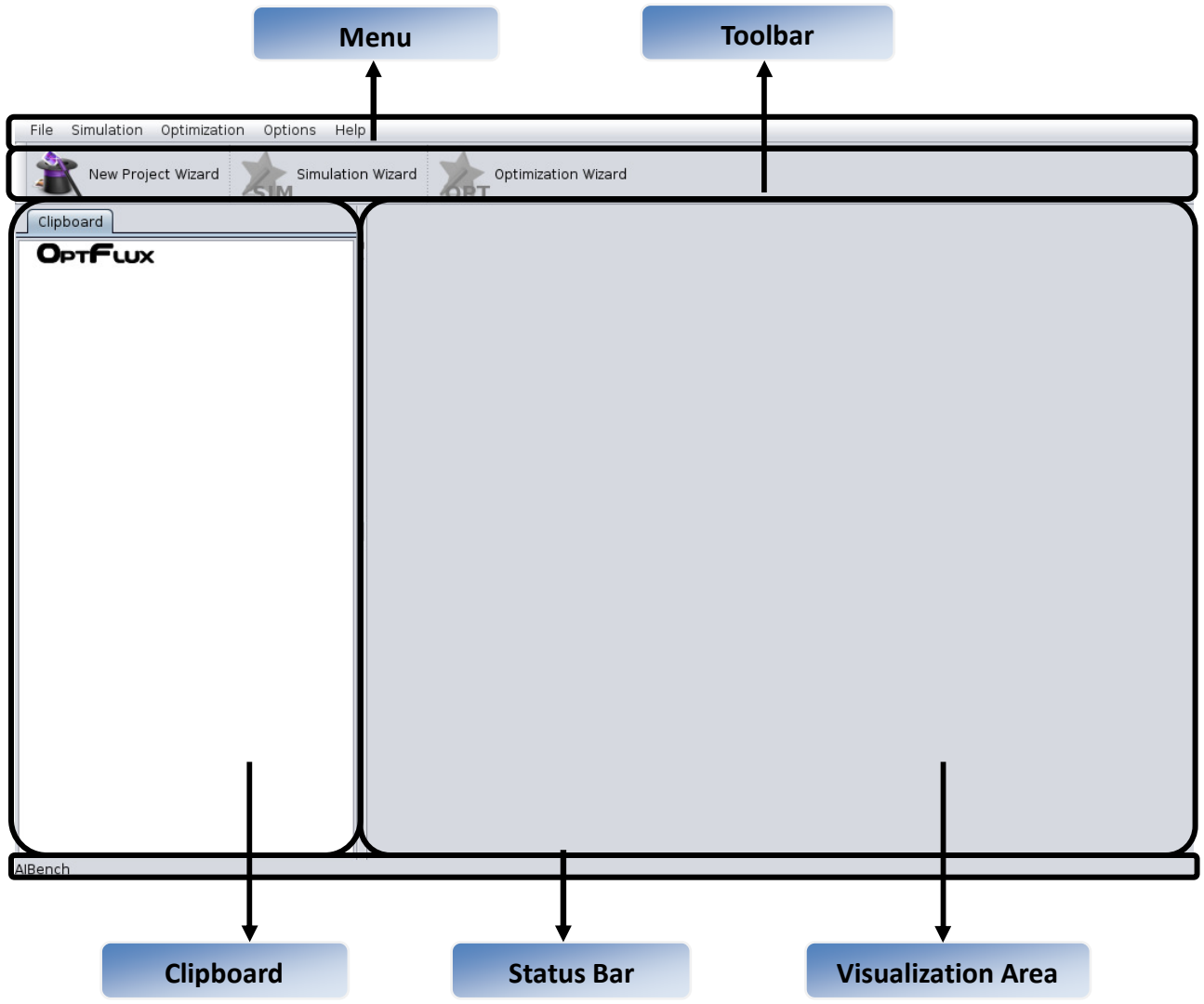
- **For the OptFlux software:**

Created inside the SysBio Research Group (http://sysbio.di.uminho.pt)

FIRST THINGS FIRST!

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

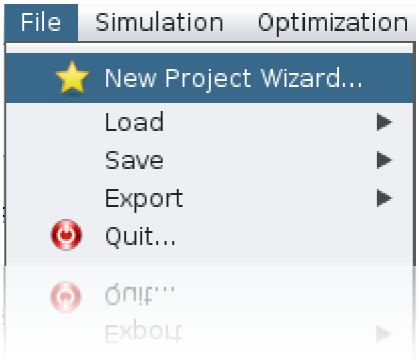
After launching the software you'll be presented with the layout depicted in the image below. Most of OptFlux main features and operations will be accessible to you either through the **Menu** or the **Toolbar**. You can also have access to them by right-clicking in the **Clipboard** area. Your data 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.

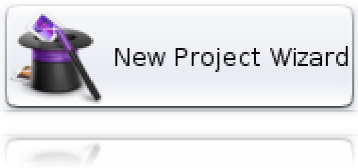


1 – CREATING A NEW PROJECT

To follow the steps in this tutorial you need to download the file smallSC.zip, available in www.optflux.org/tutorial/smallSC.zip. The stoichiometric 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, Vol 79, 703-712].
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 now have the option to create the new project from three different sources: local flat files, local SBML file or through the remote BioModels repository. This tutorial will only cover the first two.

1.1 – FROM LOCAL FLAT FILES

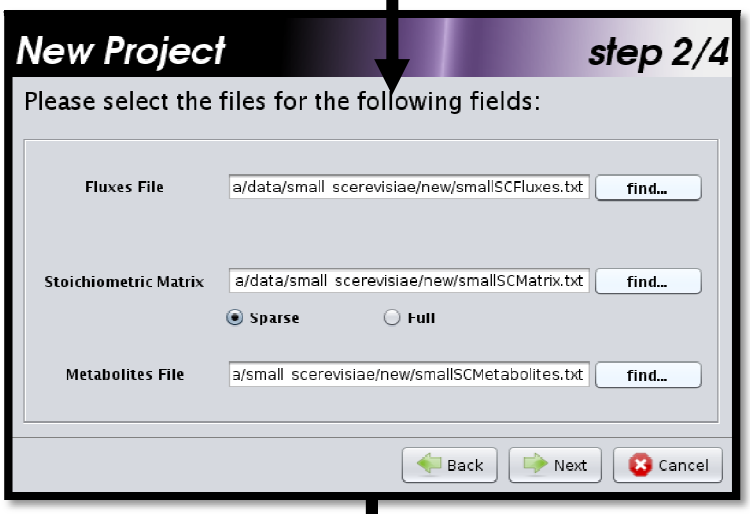
Step 1

In the first step, the user must input a valid project name
In the picture the name selected was "Small S. cerevisiae"
No proxy information is necessary in this step.

Step 2

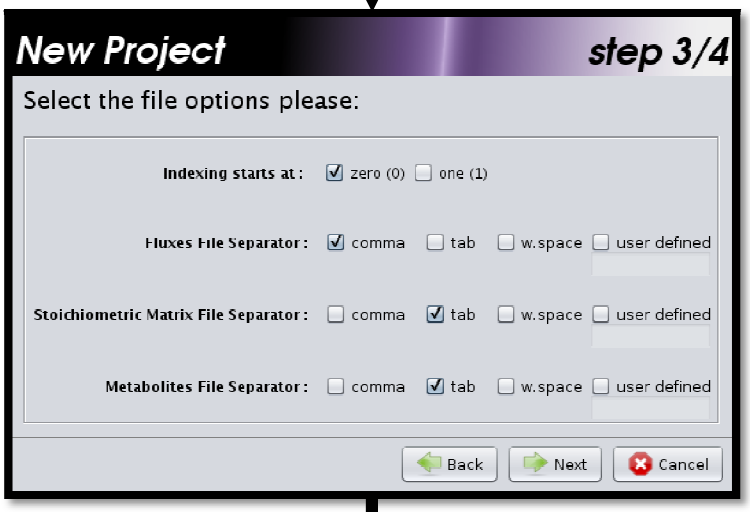
In the second step, the user must select three files, which were provided along with this tutorial:

- The first contains the reactions names and their flux limits – select *smallSCFluxes.txt*;
- The second contains the stoichiometric matrix – select *smallSCMatrix.txt*;
- The third contains the metabolites names (optional) – select *smallSCMetabolites.txt*



Step 3

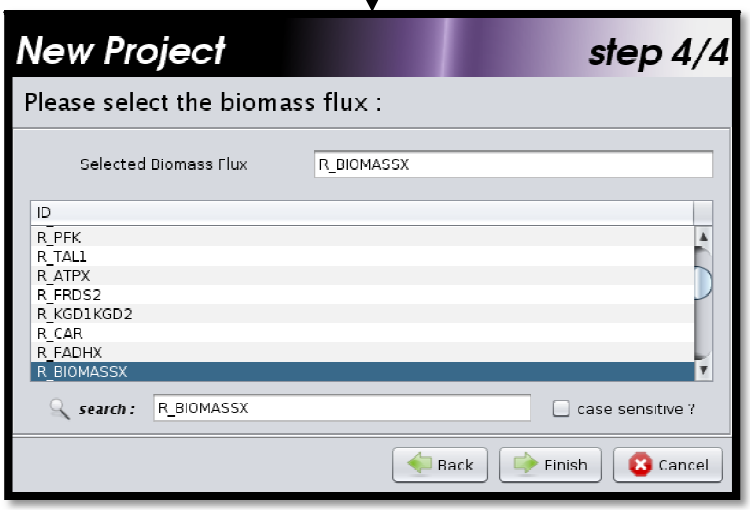
In step 3, the first option concerns the indexing used in the stoichiometric matrix if the SPARSE option was selected. The user must select indexing starting at **zero**. For the remaining files, the user should select the **comma separator** for the Fluxes File and the **tab separator** for the matrix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



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 **"R_BIOMASSX"**.



1.2 – FROM A LOCAL SBML FILE

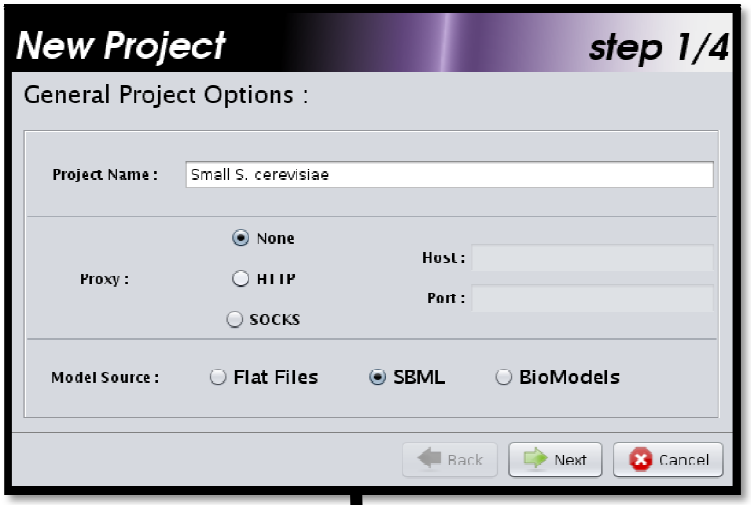
Step 1

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

In the picture the name selected was “Small S. cerevisiae”

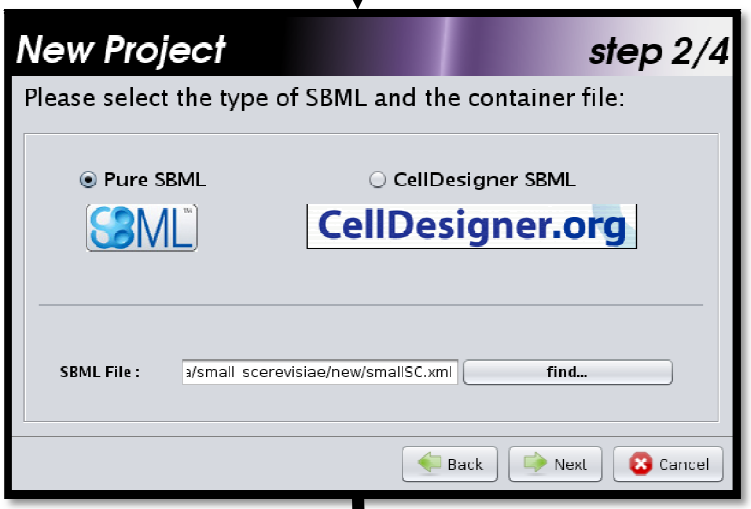
No proxy information is necessary in this step.

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



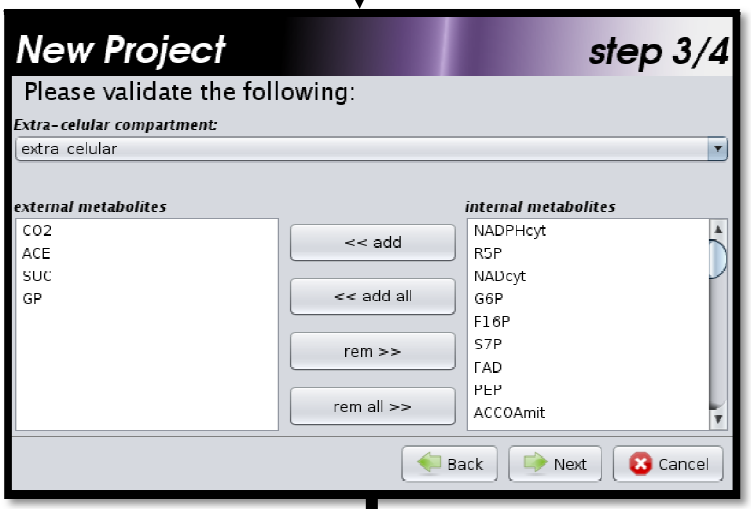
Step 2

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 *smallSC.xml* provided in the attached zip file. The type of file to select must be **Pure SBML**.



Step 3

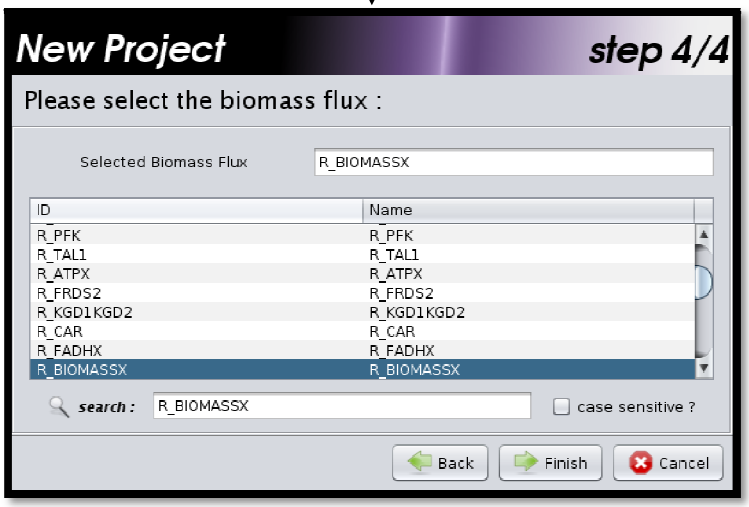
The third step is relative to the extra-cellular environment. *OptFlux* will automatically try to find the extra-cellular compartment and the respective metabolites. If everything goes smoothly, the user should have “**extra-cellular**” selected as the extra-cellular compartment and the metabolites **CO2**, **ACE**, **SUC** and **GP** detected as the external metabolites.



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 **"R_BIOMASSX"**.

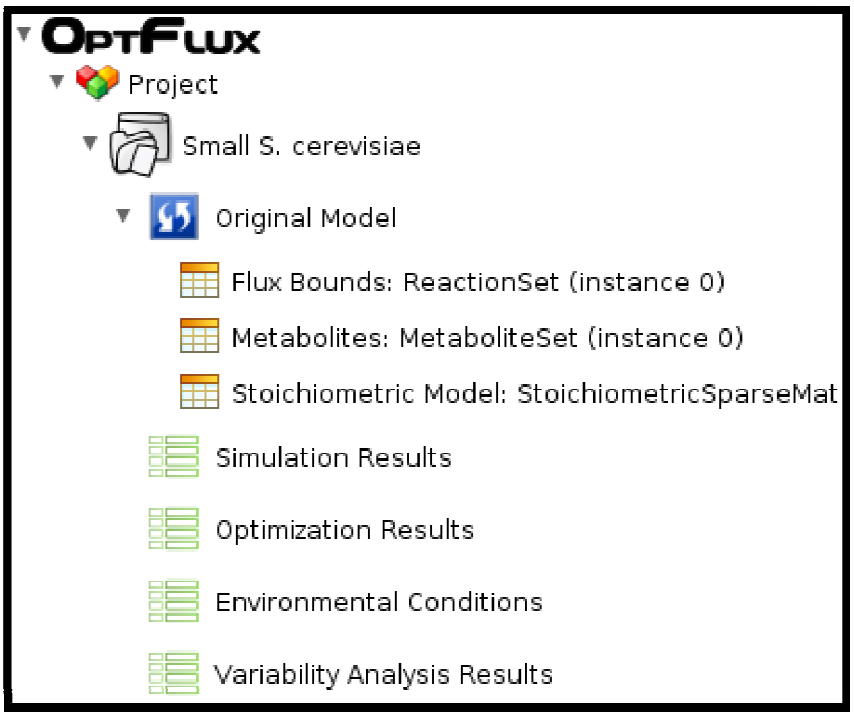


1.3 – PROJECT CREATED IN THE CLIPBOARD

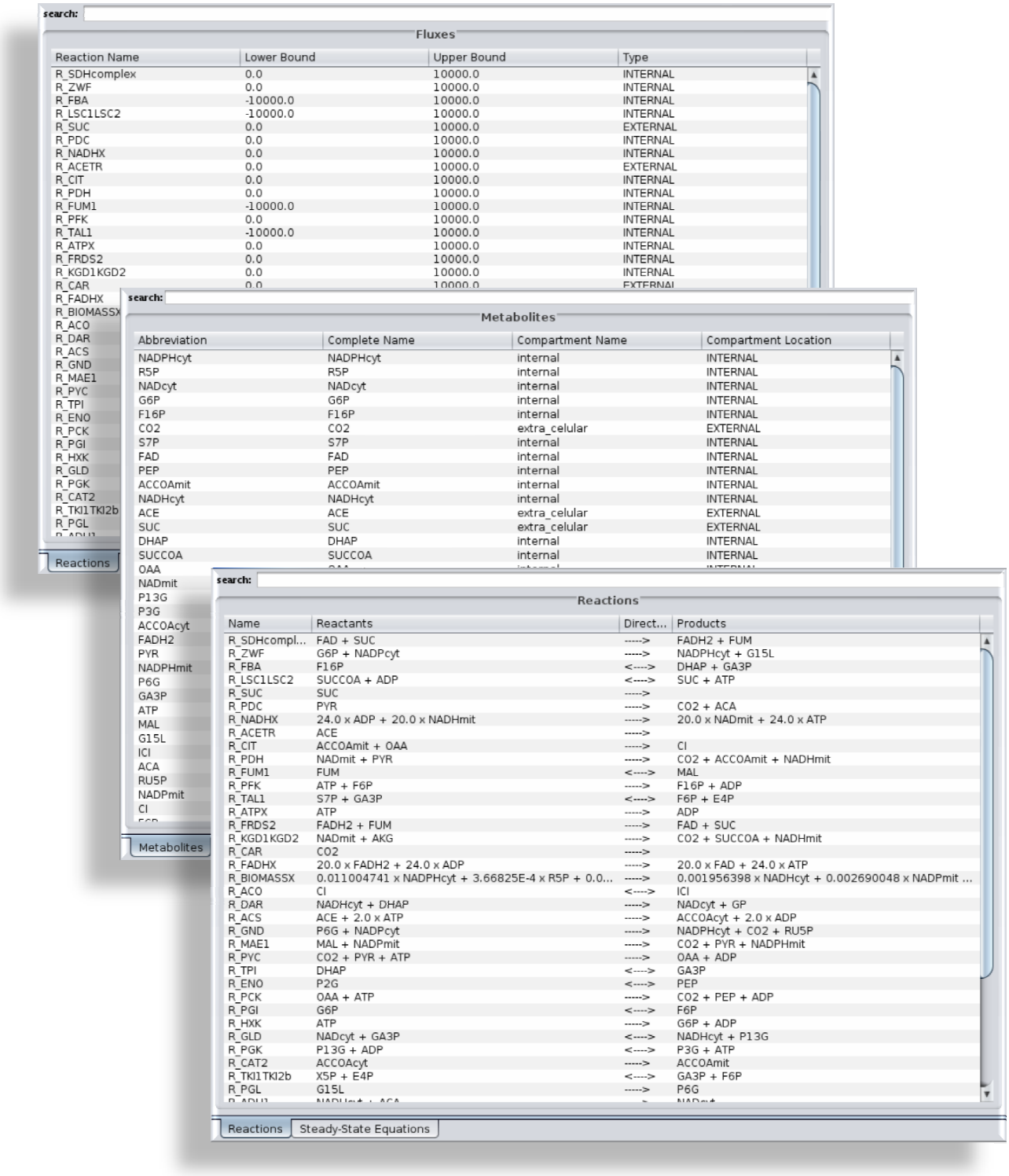
After following either **step 1.1** or **step 1.2**, the user will now be presented with the scenario depicted in the following screenshot:

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 information on flux limits, metabolites 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.

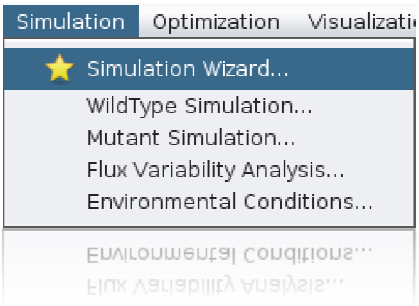


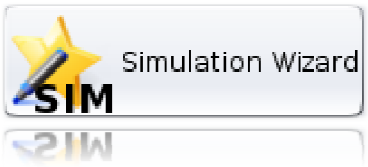
The viewers for the Reactions, Metabolites and the Stoichiometric Matrix are depicted in the screenshots below.



2 – PERFORMING SIMULATIONS

To begin a simulation process the easy way, the user should use the **Simulation Wizard** available either through the *File Menu* or the *Toolbar.*





The wizard to perform simulations has an internal map of possible paths to follow. This tutorial will not explore every one of them; instead, some specific examples will be presented.

2.1 – PERFORMING A WILD-TYPE SIMULATION

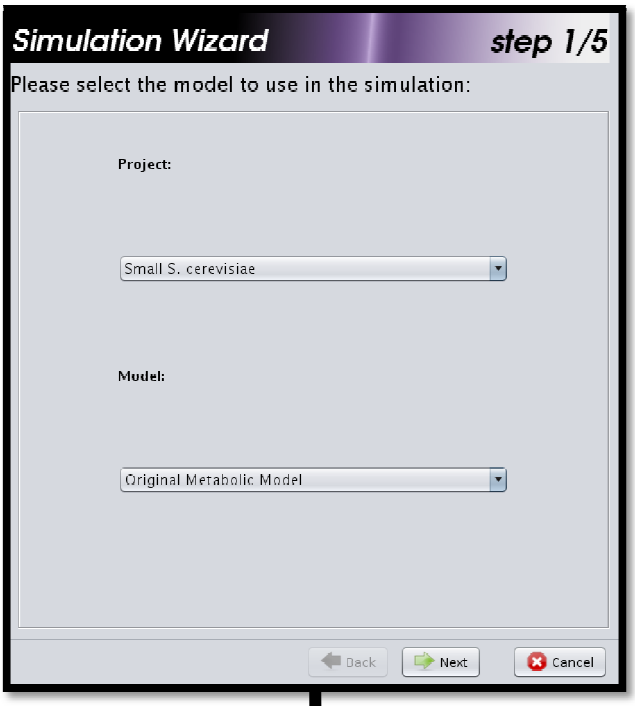
All the wild-type simulations are performed using Flux-Balance Analysis (FBA), where a Linear Programming problem is defined by the maximization of one flux of the model, usually corresponding to the reaction of biomass formation. For more information about this and other methods/ algorithms used by *OptFlux*, please refer to the **OptFlux Manual** (www.OptFlux.org/manual/OptFluxManual.pdf).

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.

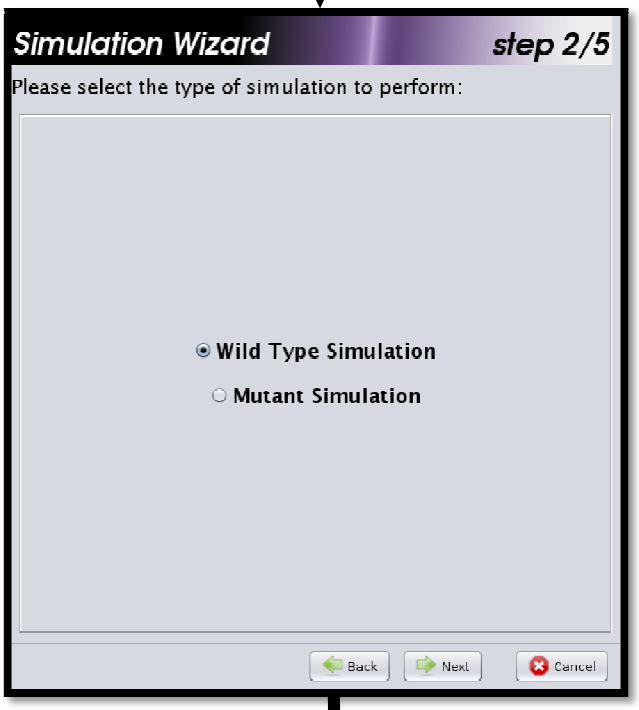
In the context of this tutorial, leaving the default selection is just fine.



Step 2

The second step allows the user to select either a wild-type or a mutant simulation.

The user must now select the wild-type simulation (the default).



Step 4

When the user selects the wild-type simulation in step 2, the wizard automatically jumps to step 4, since step 3 does not apply in this context.

In this step, the user can select a flux to optimize (typically the biomass growth associated flux, which should be selected by default) and the optimization objective, in this case **Maximization.**

Step 5

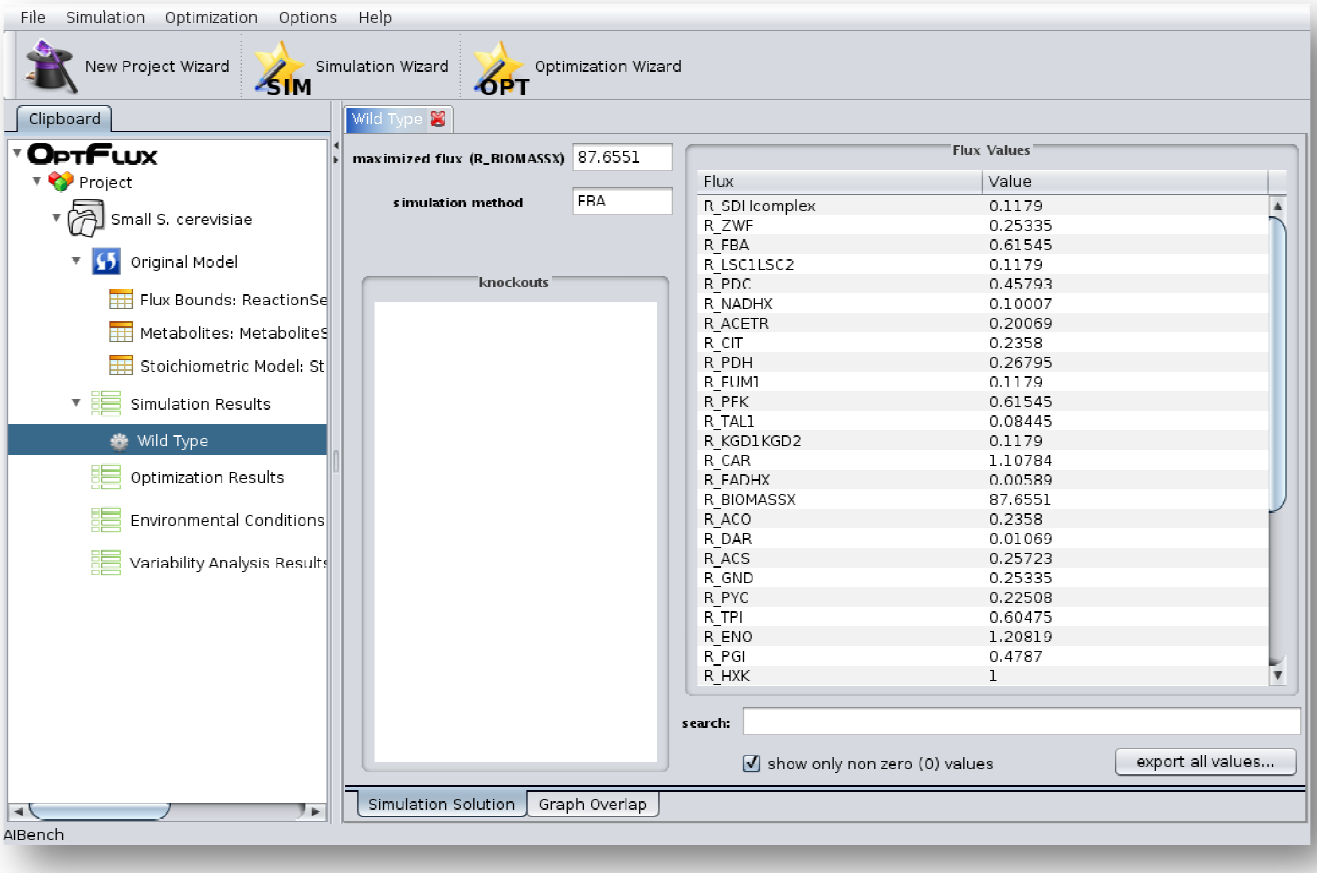
The environmental conditions step can be used to define specific condition in which this simulation must be performed. An example is the absence of oxygen or a different setting in the carbon source flux (thus providing a different intake of substrate to the organism).

For the present example, these settings should be left as they are by default.

Press “finish” to perform the simulation.

Step 6

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 obtained with the simulation method and can even export the list of values to a text file.



2.2 – PERFORMING A DELETION MUTANT SIMULATION

A mutant simulation can be performed by using the FBA algorithm or two other alternative formulations: minimization of metabolic adjustment (MOMA) or Regulatory on/off minimization of metabolic flux changes (ROOM), for which more information is available in the user's manual (www.OptFlux.org/manual/OptFluxManual.pdf). For the purpose of this tutorial, only the FBA approach will be used.

To perform a mutant simulation, steps 1, 4 and 5 are the same as in the wild-type simulation (2.1). Therefore, only steps 2, 3 and 6 will be presented in detail.

Step 2

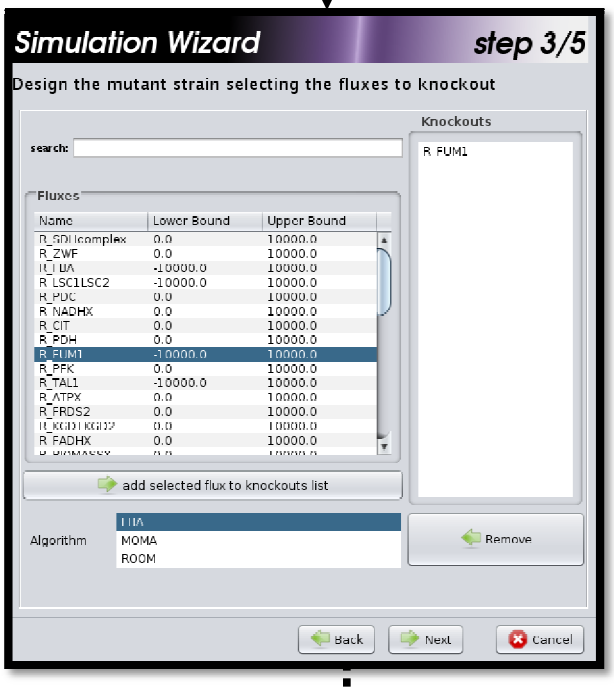
The second step allows the user to select either a wild-type or a mutant simulation.

Instead of the wild-type simulation, this time the user should select the mutant simulation option.

Step 3

In step 3, the user has the possibility to manually specify which deletions to perform in the simulation. To achieve that, the user must select the reactions to be deleted from the table in the left and use the "add selected flux to knockouts list" button to add them to the list in the right. In this case, the **R_FUM1** reaction should be selected. The user can also choose the simulation method from the list bellow (FBA, MOMA or ROOM).

Select FBA (Flux Balance Analysis), and follow to step 4 in the wild-type simulation tutorial above.

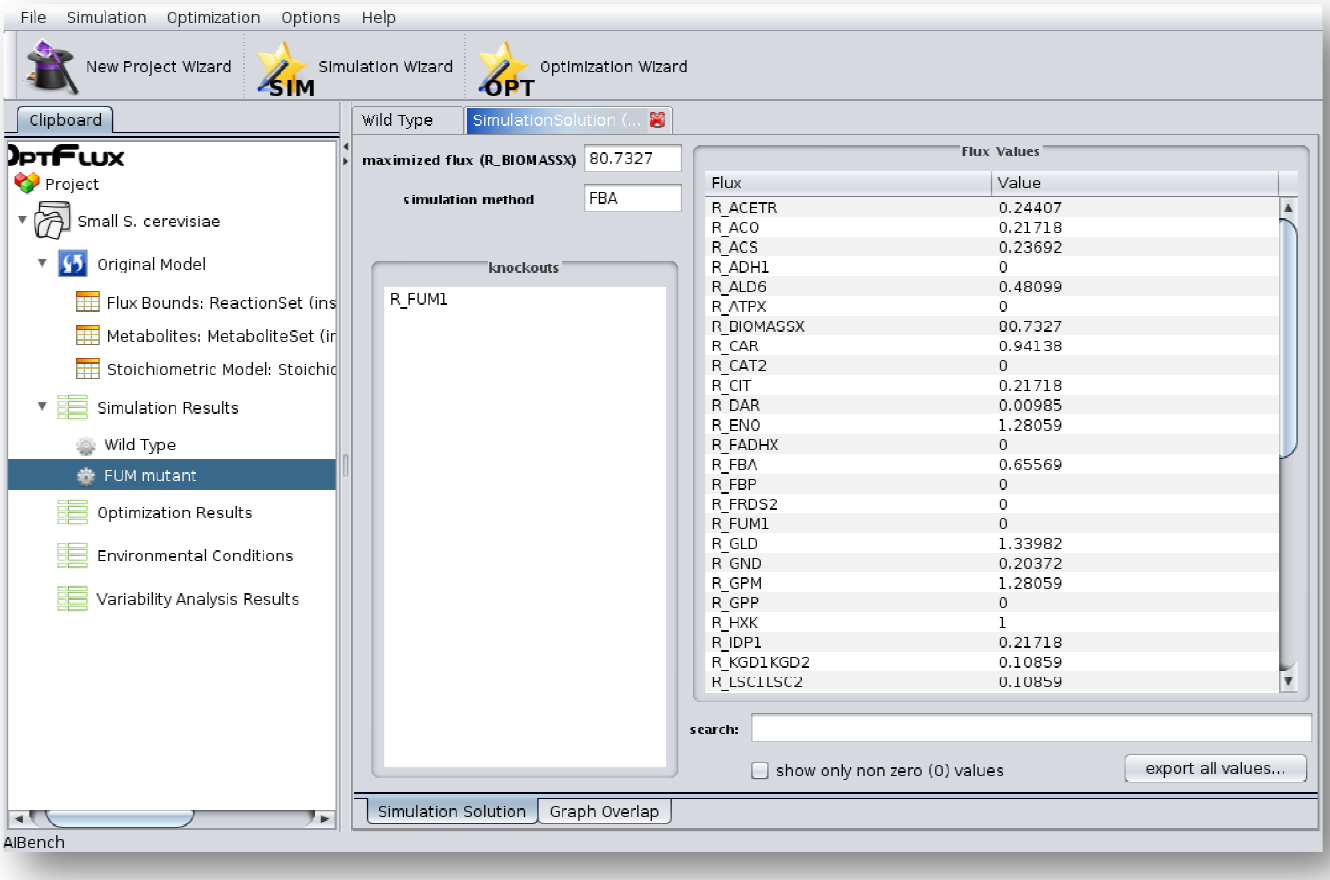


to step 4

Step 6

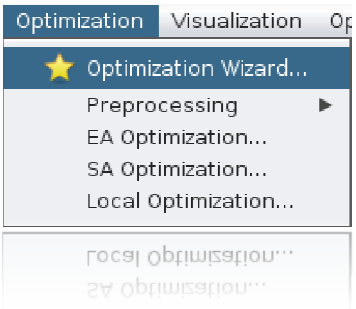
If all the previous steps were completed successfully, a new object will be placed in the **simulation results** list. The name of that object can be changed to whatever the user wants by right-clicking that object and selecting the *"Rename"* option or by pressing F2 when the object is selected. In this case, the name was changed to **FUM mutant**.

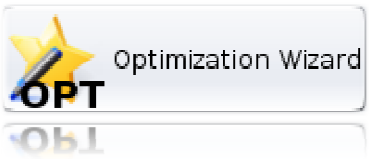
Left-clicking the object will launch the simulation solution viewer in the visualization area on the right. This time one can notice, for instance, that the biomass growth associated flux has a value lower than the one in the wild-type simulation.



3 – PERFORMING STRAIN OPTIMIZATION

In OptFlux, strain optimization allows to automatically discover sets of gene deletions that maximize a given objective function related with a desired industrial objective. Two meta-heuristic optimization methods, Evolutionary Algorithms (EAs) and Simulated Annealing (SA) are available. The example chosen here to illustrate some of OptFlux's capabilities envisages the maximization of succinate production using Evolutionary algorithms.
To begin an optimization procedure, the **Optimization Wizard** available should be used. The user can launch it either through the *File Menu* or the *Toolbar.*





The wizard to perform optimization has an internal map of possible paths to follow. This tutorial will not explore every one of them; instead, some specific examples will be presented.

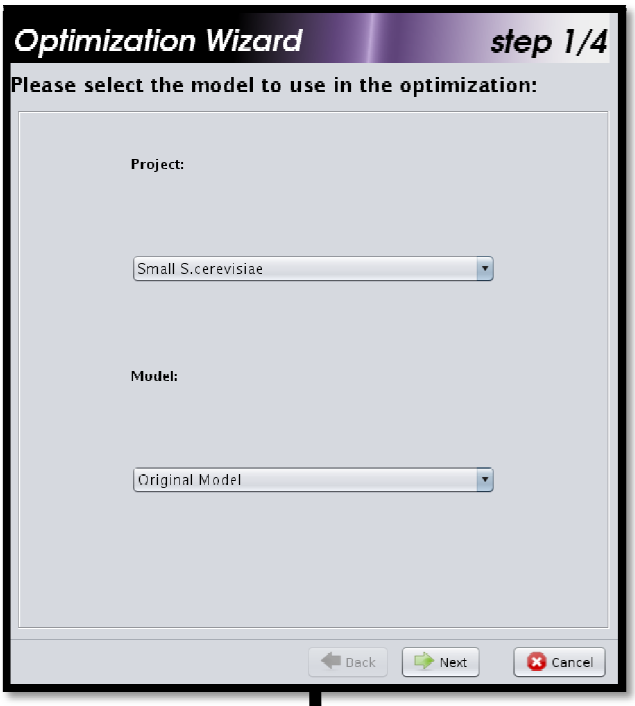
3.1 – PERFORMING AN OPTIMIZATION USING THE EVOLUTIONARY ALGORITHM

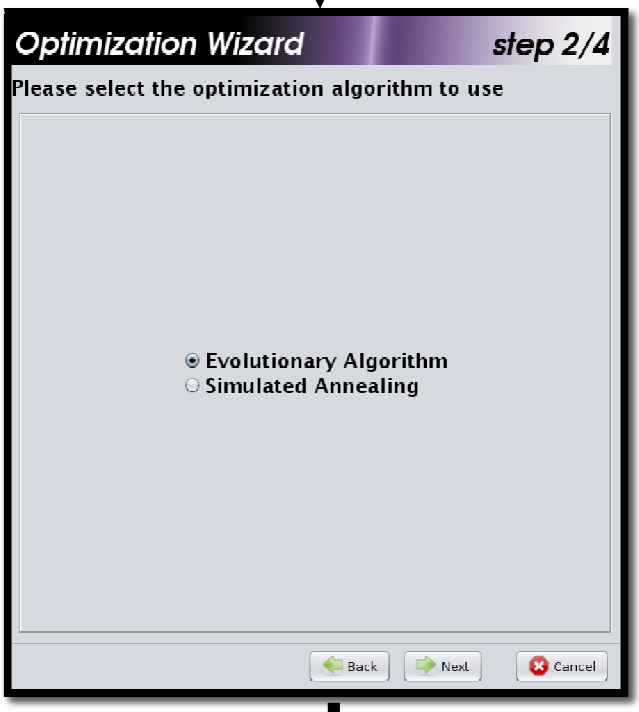
Step 1

In the first step the user must select the Project and the Metabolic Model to which the optimization procedure 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

The second step allows the user to select between the use of the Evolutionary Algorithm (EA) or the Simulated Annealing (SA).

The user should now select the Evolutionary Algorithm option (the default).

Step 3

In this step all the parameters for both the Evolutionary Algorithm and the simulation must be set. In this tutorial, and since the model used is relatively small, some parameters distinct from the default will be set.

The **number of generations** should be set to, at least, 20 generations**.** The **desired flux** is the flux we intend to maximize in the mutant strain. The user must select the **R_SUC** (succinate secretion) reaction here.

In the **substrate field**, the **R_HXK** reaction must also be selected, which corresponds to the glucose uptake reaction. The remaining parameters should be kept in their **default values**.

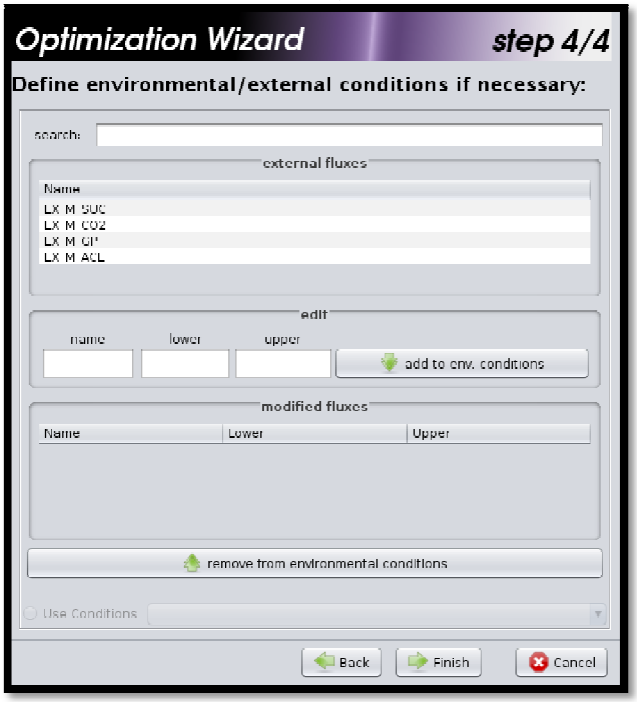
With this configuration, *OptFlux* will try to find the best deletion mutant strains that are optimum at the production of succinate, using glucose as the carbon source.

Step 4

The environmental conditions step can be used to define specific conditions in which this simulation must be performed. An example is the absence of oxygen or a different setting in the carbon source flux (thus providing a different intake of substrate to the organism).

For the purpose of this tutorial, these settings should be left as they are by default.

Press "finish" to start the optimization procedure.

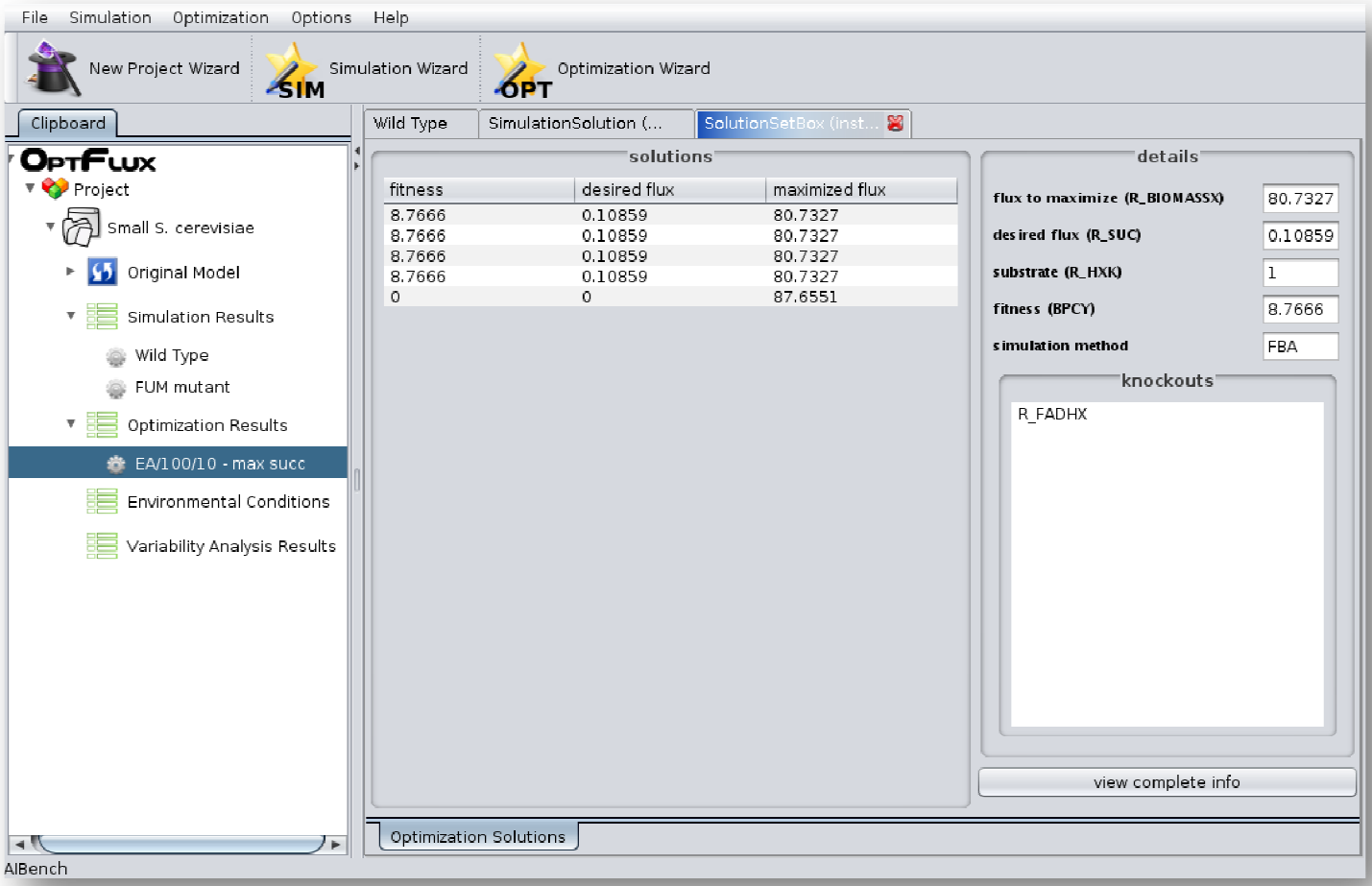


Step 5

After completing all the previous steps, the optimization will be executed and after a while (generally, the time will depend greatly on the population size and number of generations selected), a new object will be placed inside the **Optimization Results** list.

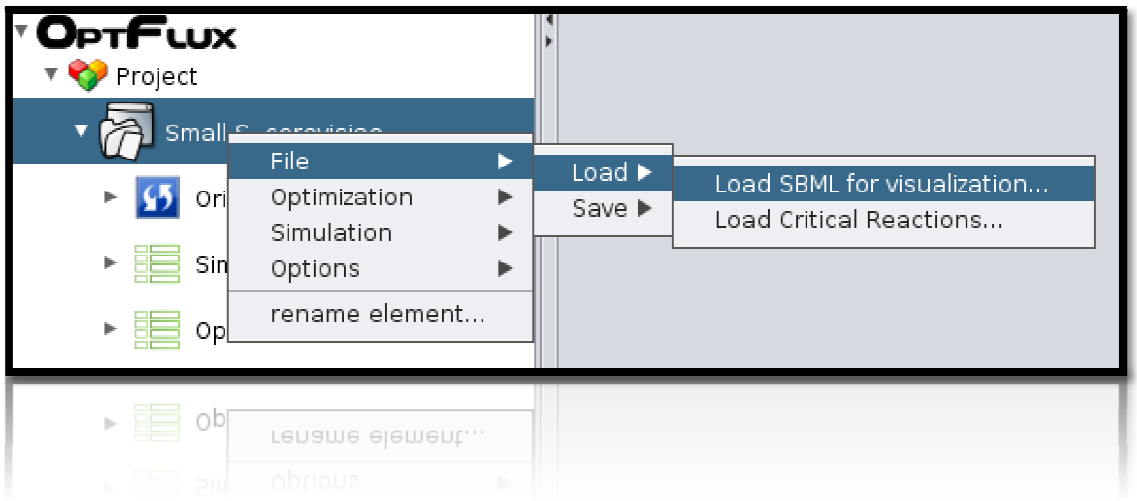
By left-clicking this object the user has access to detailed information about the performed optimization procedure. In the solutions list, all the different mutant strains found will be listed. Please note that at the end of the optimization procedure, an extra simplification step is performed so that all the unnecessary deletions used are removed.

The result for the performed optimization is depicted in the picture bellow.

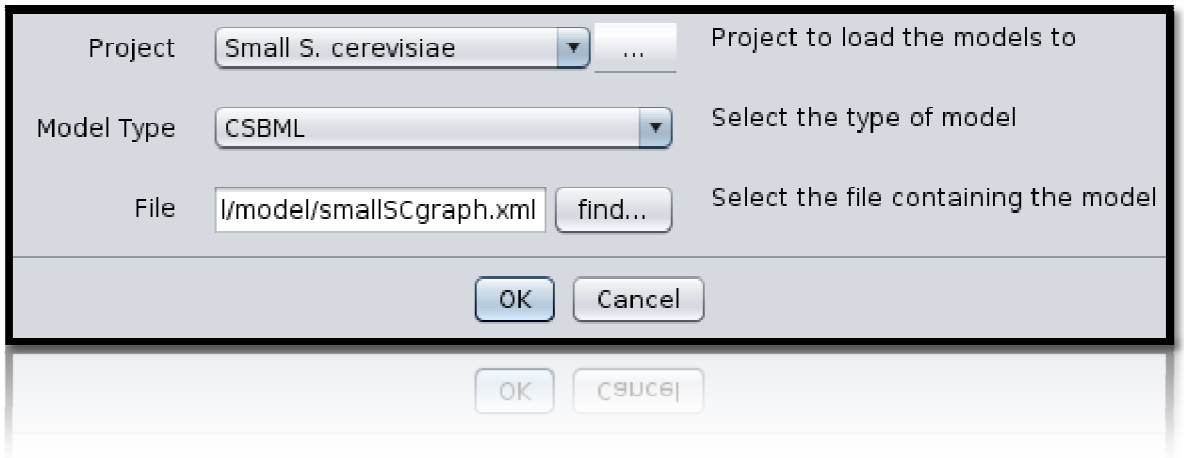

File
Simulation
Optimization
Options
Help
New Project Wizard
Simulation Wizard
Optimization Wizard
Clipboard
OptFlux
Project
Small S. cerevisiae
Original Model
Simulation Results
Wild Type
FUM mutant
Optimization Results
EA/100/10 - max succ
Environmental Conditions
Variability Analysis Results
Wild Type
SimulationSolution (...
SolutionSetBox (inst...
solutions
fitness
desired flux
maximized flux
8.7666
0.10859
80.7327
8.7666
0.10859
80.7327
8.7666
0.10859
80.7327
8.7666
0.10859
80.7327
0
0
87.6551
details
flux to maximize (R_BIOMASSX)
80.7327
desired flux (R_SUC)
0.10859
substrate (R_HXK)
1
fitness (BPCY)
8.7666
simulation method
FBA
knockouts
R_FADHX
view complete info
Optimization Solutions
AlBench

4 – GRAPH VISUALIZATION

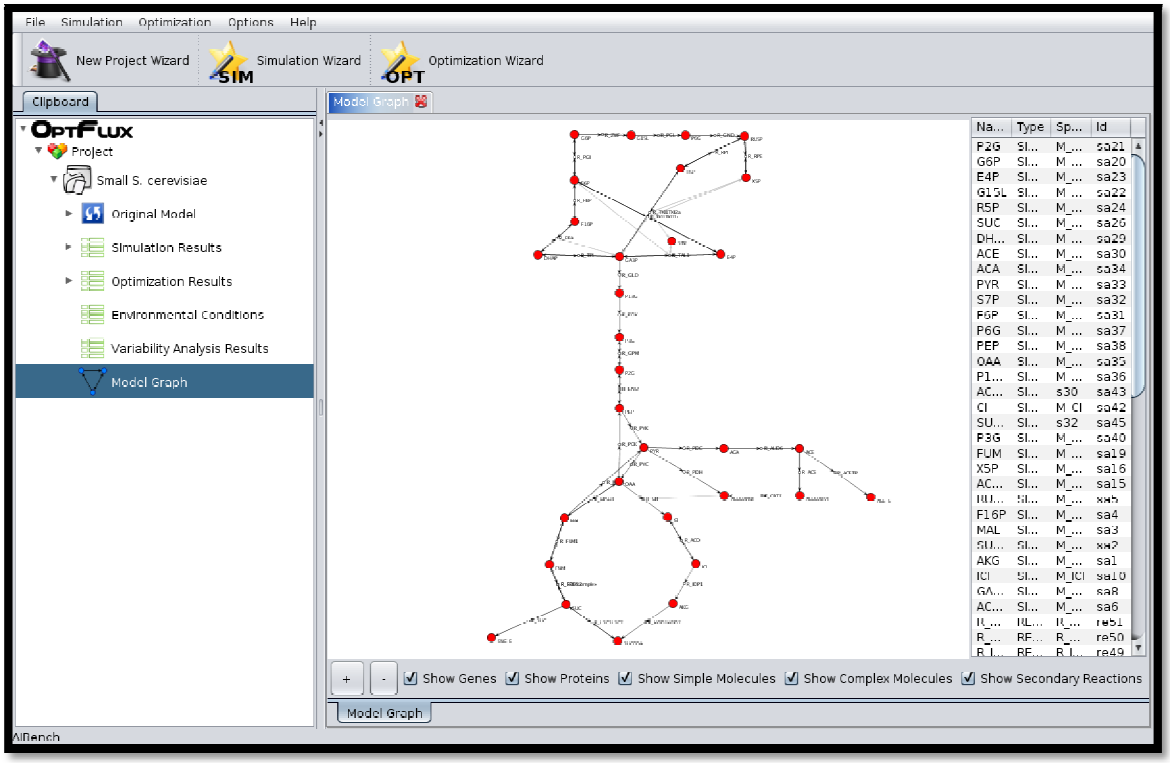
For an improved visualization of the results, the user can also load a CellDesigner SBML file which provides a graphical representation of the metabolism.



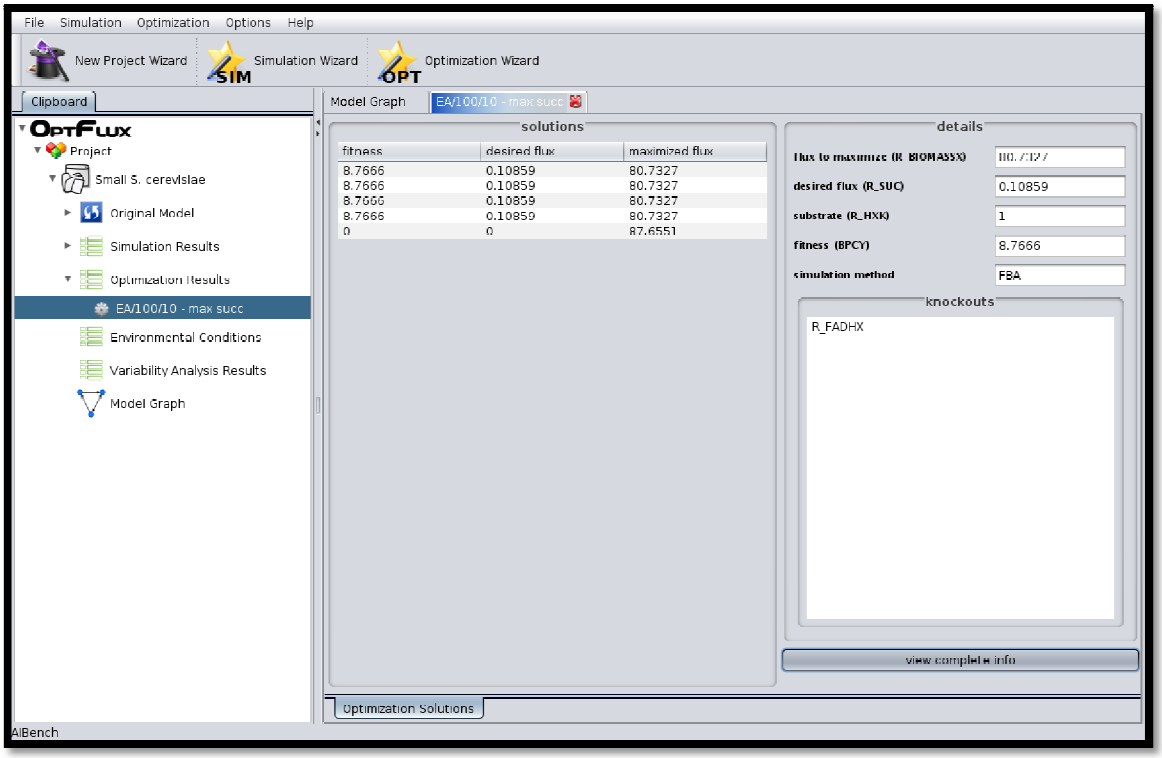
In the provided archive, a file named *"smallSCgraph.xml"* is included. To load it, the user needs to access File Menu -> Load -> Load SBML for visualization. The type of SBML selected must be CSBML.



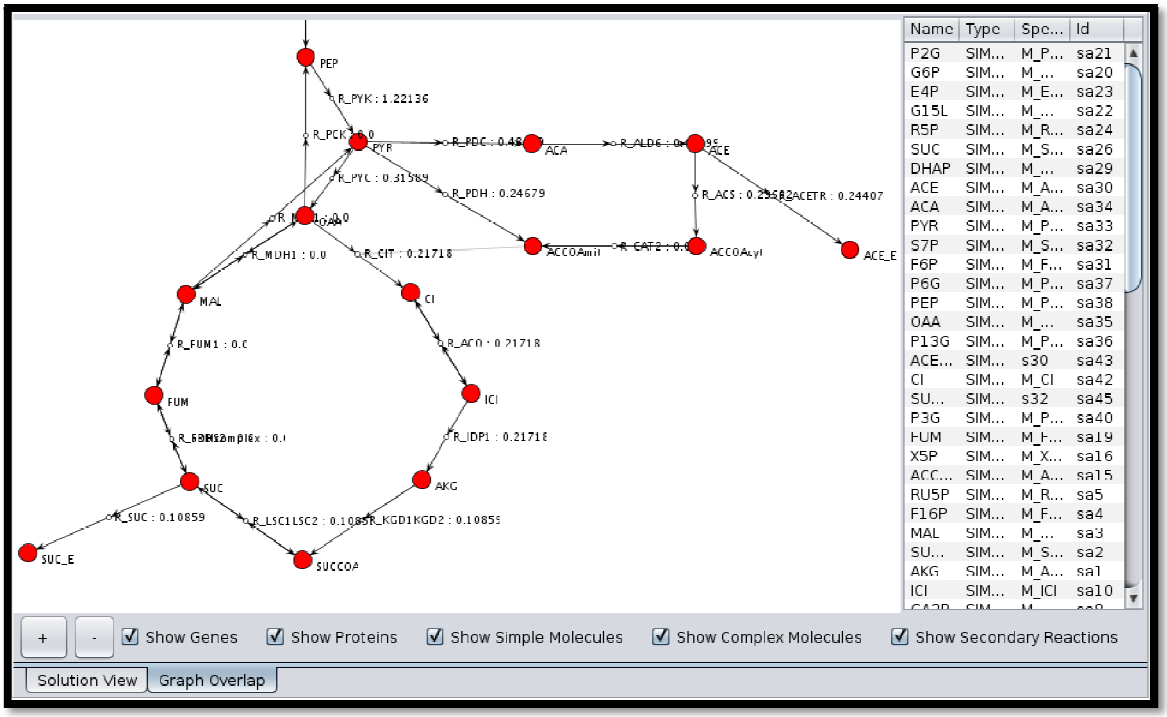
A new object will be placed in the Project tree under the name **Model Graph.**



In this model, some metabolites/reactions were removed in order to improve the visualization experience, thus making it not usable to simulations/optimizations.



Returning to the **Optimization Results** view and by, again, clicking the “view complete info” button, the solution details will be presented.



In that view, by pressing the tab *Graph Overlap*, the previously loaded graph will be presented having that particular solution results superimposed in it.