

Universidade do Minho

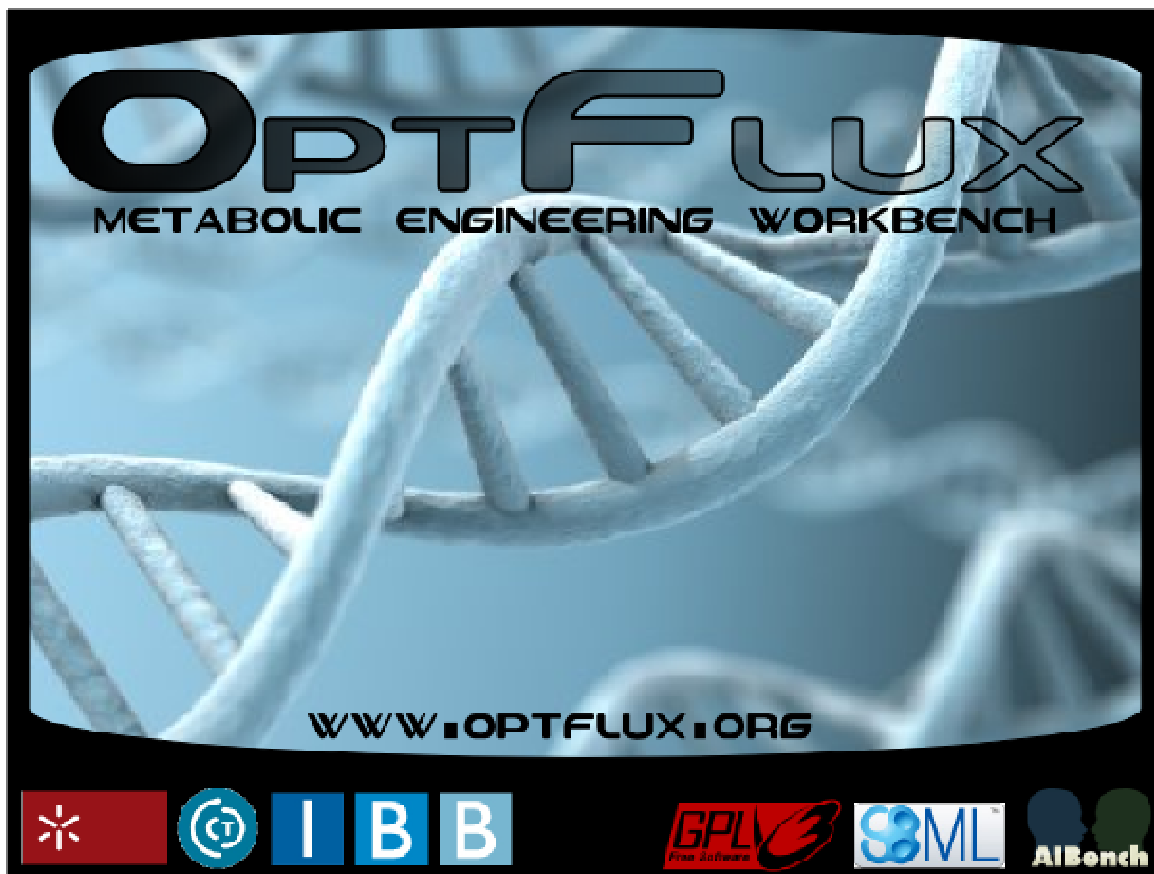
**IBB-CEB** – INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING – CENTRE OF BIOLOGICAL ENGINEERING

**CCTC**– COMPUTER SCIENCE AND TECHNOLOGY CENTER

SCHOOL OF ENGINEERING

UNIVERSITY OF MINHO

## BEGINNER'S TUTORIAL



*Version 1.2*

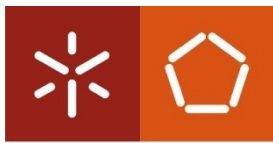


# **BEGINNER'S TUTORIAL**

## **FOR**

# **OPTFLUX**

### **METABOLIC ENGINEERING WORKBENCH**



Universidade do Minho



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- **For the OptFlux software:**

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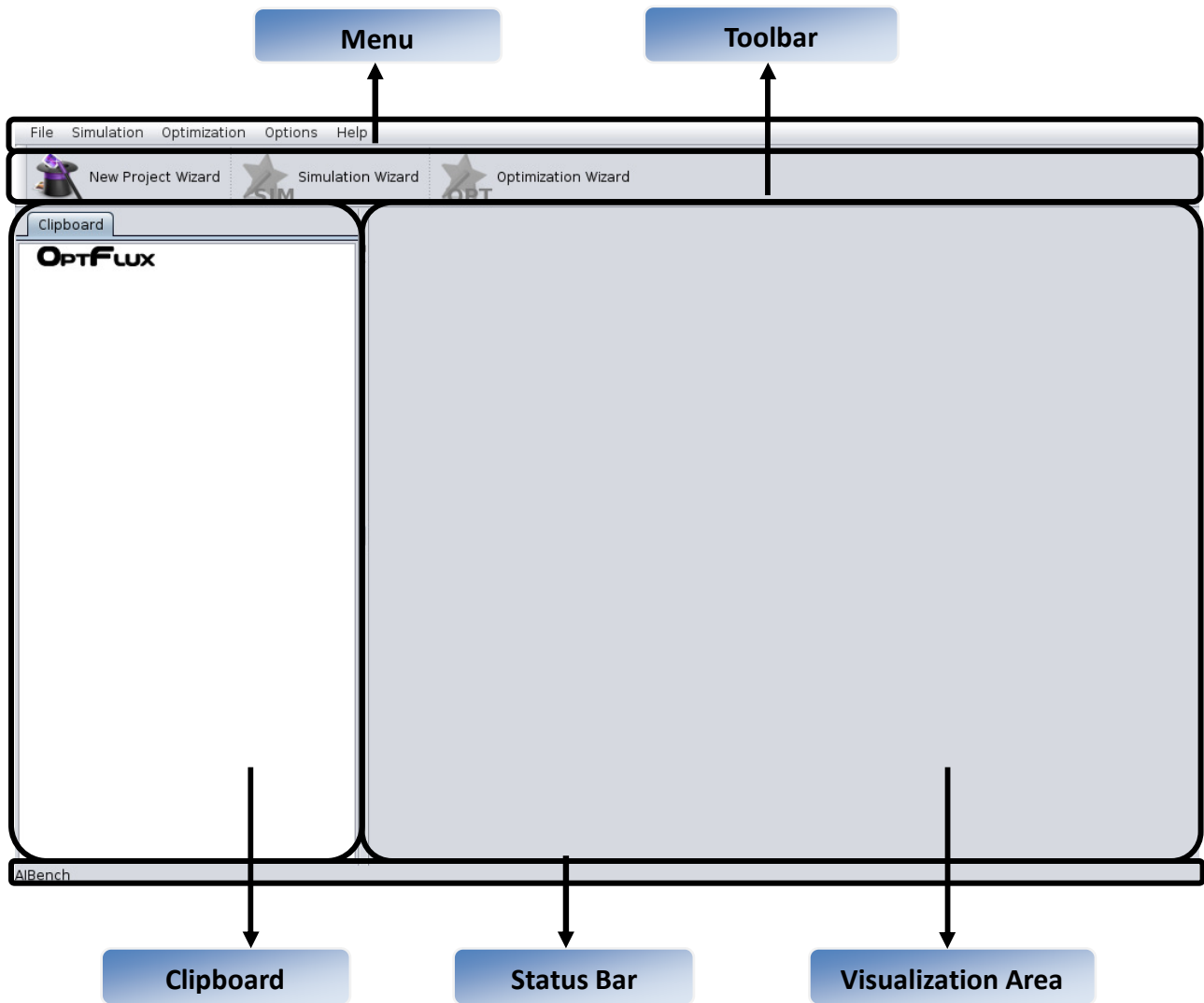
Created inside the SysBio Research Group (<http://sysbio.di.uminho.pt>)



# 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](http://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.

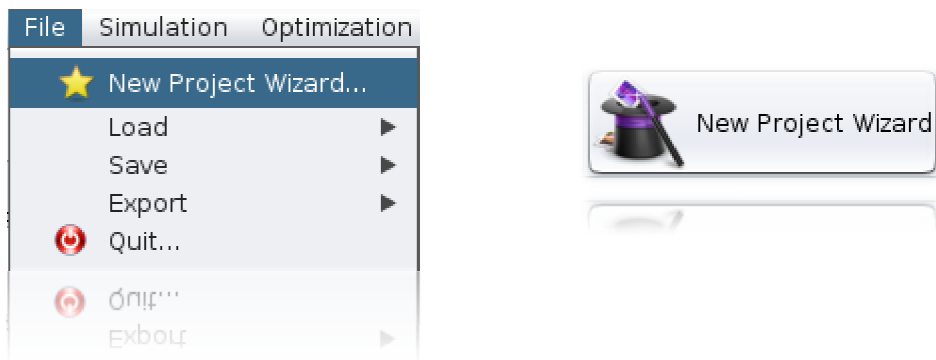


# 1 – CREATING A NEW PROJECT

To follow the steps in this tutorial you need to download the file `smallSC.zip`, available in [www.optflux.org/tutorial/smallSC.zip](http://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.

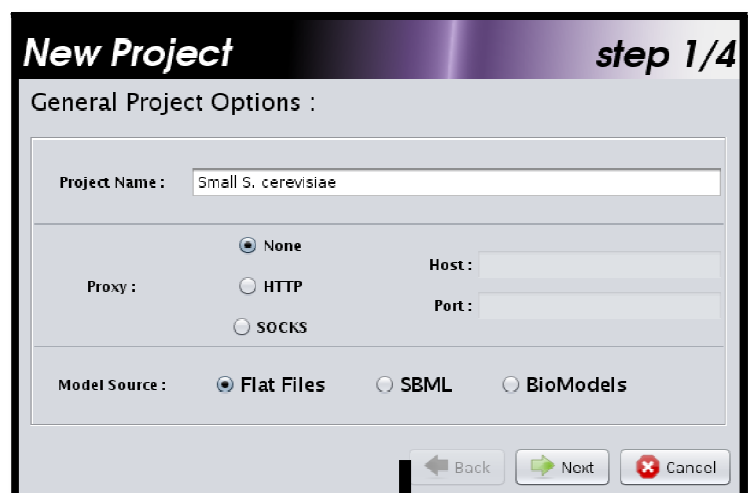
## 1.1 – FROM LOCAL FLAT FILES

### 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

**New Project** step 2/4

Please select the files for the following fields:

Fluxes File:  

Stoichiometric Matrix:  

☒ Sparse ☐ Full

Metabolites File:

### 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.

**New Project** step 3/4

Select the file options please:

Indexing starts at: ☒ zero (0) ☐ one (1)

Fluxes File Separator: ☒ comma ☐ tab ☐ w.space ☐ user defined

Stoichiometric Matrix File Separator: ☐ comma ☒ tab ☐ w.space ☐ user defined

Metabolites File Separator: ☐ comma ☒ tab ☐ w.space ☐ user defined

### 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".

**New Project** step 4/4

Please select the biomass flux :

Selected Biomass Flux:

ID
R_PFK
R_TAL1
R_ATPX
R_FRDS2
R_KGD1KGD2
R_CAR
R_FADHX
<b>R_BIOMASSX</b>

search:  ☐ case sensitive ?

## 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.

**New Project** step 1/4

General Project Options :

Project Name :

Proxy : ☒ None ☐ HTTP ☐ SOCKS

Host :   
Port :

Model Source : ☐ Flat Files ☒ SBML ☐ BioModels


### 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**.

**New Project** step 2/4

Please select the type of SBML and the container file:

☒ Pure SBML ☐ CellDesigner SBML

SBML File :

### 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.

**New Project** step 3/4

Please validate the following:

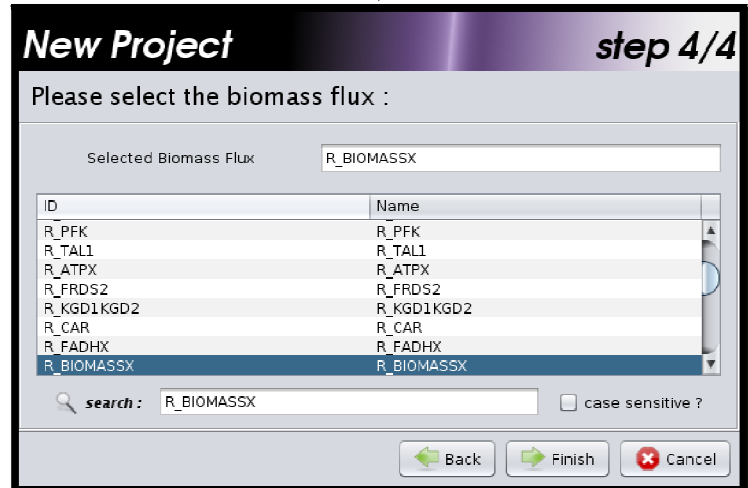
Extra-cellular compartment:

external metabolites		internal metabolites
CO2	<input type="button" value=" &lt;&lt; add"/> <input type="button" value=" &lt;&lt; add all"/> <input type="button" value=" rem &gt;&gt;"/> <input type="button" value=" rem all &gt;&gt;"/>	NADPHcyt
ACE		R5P
SUC		NADcyt
GP		G6P
		F16P
		S7P
		FAD
		PEP
		ACCOAmit

#### 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”.

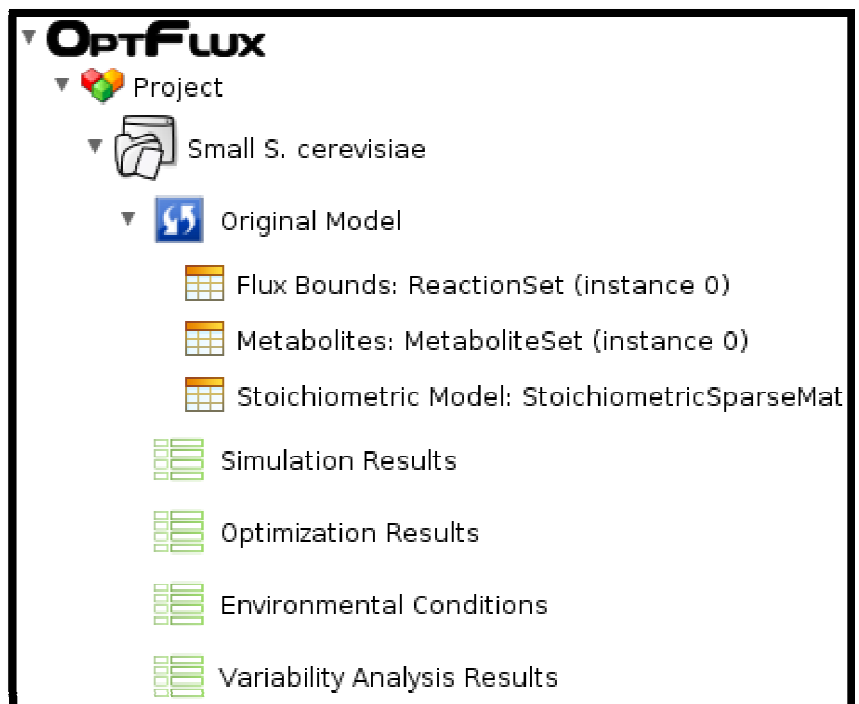


### 1.3 – PROJECT CREATED IN THE CLIPBOARD

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.

search:

Reaction Name	Lower Bound	Upper Bound	Type
R_SDHcomplex	0.0	10000.0	INTERNAL
R_ZWF	0.0	10000.0	INTERNAL
R_FBA	-10000.0	10000.0	INTERNAL
R_LSC1LSC2	-10000.0	10000.0	INTERNAL
R_SUC	0.0	10000.0	EXTERNAL
R_PDC	0.0	10000.0	INTERNAL
R_NADHX	0.0	10000.0	INTERNAL
R_ACETR	0.0	10000.0	EXTERNAL
R_CIT	0.0	10000.0	INTERNAL
R_PDH	0.0	10000.0	INTERNAL
R_FUM1	-10000.0	10000.0	INTERNAL
R_PFK	0.0	10000.0	INTERNAL
R_TAL1	-10000.0	10000.0	INTERNAL
R_ATPX	0.0	10000.0	INTERNAL
R_FRDS2	0.0	10000.0	INTERNAL
R_KGD1KGD2	0.0	10000.0	INTERNAL
R_CAR	0.0	10000.0	EXTERNAL

search:

Abbreviation	Complete Name	Compartment Name	Compartment Location
NADPHcyt	NADPHcyt	internal	INTERNAL
R5P	R5P	internal	INTERNAL
NADcyt	NADcyt	internal	INTERNAL
G6P	G6P	internal	INTERNAL
F16P	F16P	internal	INTERNAL
CO2	CO2	extra_celular	EXTERNAL
S7P	S7P	internal	INTERNAL
FAD	FAD	internal	INTERNAL
PEP	PEP	internal	INTERNAL
ACCOAmit	ACCOAmit	internal	INTERNAL
NADHcyt	NADHcyt	internal	INTERNAL
ACE	ACE	extra_celular	EXTERNAL
SUC	SUC	extra_celular	EXTERNAL
DHAP	DHAP	internal	INTERNAL
SUCCOA	SUCCOA	internal	INTERNAL

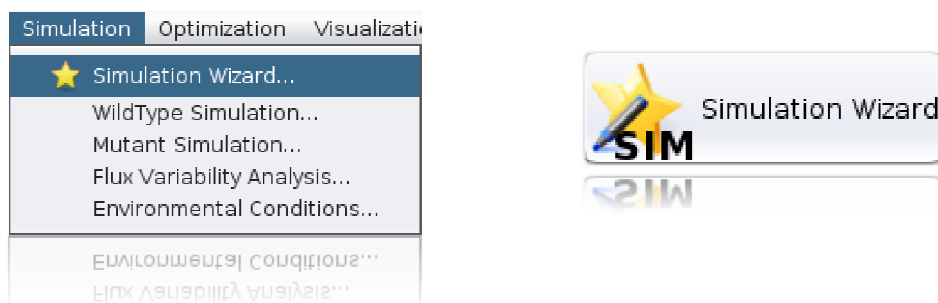
search:

Name	Reactants	Direct...	Products
R_SDHcompl...	FAD + SUC	----->	FADH2 + FUM
R_ZWF	G6P + NADPcyt	----->	NADPHcyt + G15L
R_FBA	F16P	<-----	DHAP + GA3P
R_LSC1LSC2	SUCCOA + ADP	<-----	SUC + ATP
R_SUC	SUC	----->	
R_PDC	PYR	----->	CO2 + ACA
R_NADHX	24.0 x ADP + 20.0 x NADHmit	----->	20.0 x NADmit + 24.0 x ATP
R_ACETR	ACE	----->	
R_CIT	ACCOAmit + OAA	----->	CI
R_PDH	NADmit + PYR	----->	CO2 + ACCOAmit + NADHmit
R_FUM1	FUM	<-----	MAL
R_PFK	ATP + F6P	----->	F16P + ADP
R_TAL1	S7P + GA3P	<-----	F6P + E4P
R_ATPX	ATP	----->	ADP
R_FRDS2	FADH2 + FUM	----->	FAD + SUC
R_KGD1KGD2	NADmit + AKG	----->	CO2 + SUCCOA + NADHmit
R_CAR	CO2	----->	
R_FADHX	20.0 x FADH2 + 24.0 x ADP	----->	20.0 x FAD + 24.0 x ATP
R_BIOMASSX	0.011004741 x NADPHcyt + 3.66825E-4 x R5P + 0.0...	----->	0.001956398 x NADHcyt + 0.002690048 x NADPmit ...
R_ACO	CI	<-----	ICI
R_DAR	NADHcyt + DHAP	----->	NADcyt + GP
R_ACS	ACE + 2.0 x ATP	----->	ACCOAcyt + 2.0 x ADP
R_GND	P6G + NADPcyt	----->	NADPHcyt + CO2 + RU5P
R_MAE1	MAL + NADPmit	----->	CO2 + PYR + NADPHmit
R_PYC	CO2 + PYR + ATP	----->	OAA + ADP
R_TPI	DHAP	<-----	GA3P
R_ENO	P2G	<-----	PEP
R_PCK	OAA + ATP	----->	CO2 + PEP + ADP
R_PGI	G6P	<-----	F6P
R_HXK	ATP	<-----	G6P + ADP
R_GLD	NADcyt + GA3P	<-----	NADHcyt + P13G
R_PGK	P13G + ADP	<-----	P3G + ATP
R_CAT2	ACCOAcyt	<-----	ACCOAmit
R_TK1TK2b	X5P + E4P	<-----	GA3P + F6P
R_PGL	G15L	<-----	P6G

Reactions Steady-State Equations

## 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

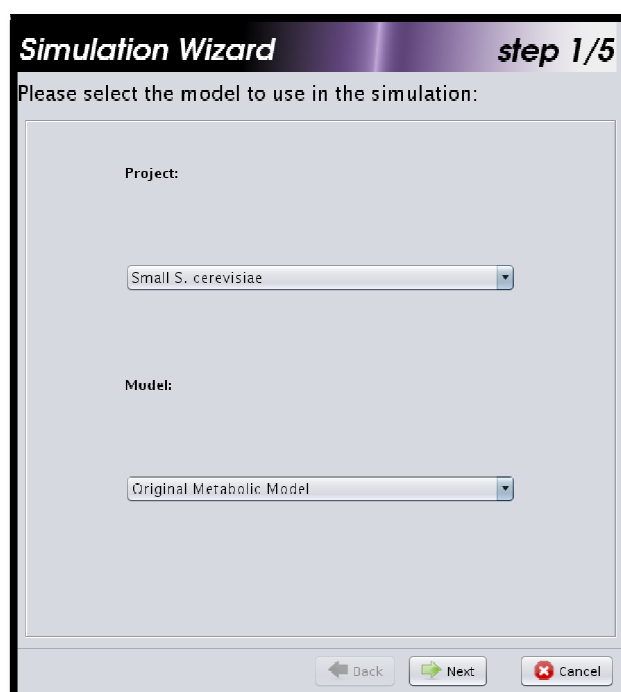
All the wild-type simulations are performed using Flux-Balance Analysis (FBA), where a Linear Programming problem is defined by the maximization of one flux of the model, usually corresponding to the reaction of biomass formation. For more information about this and other methods/ algorithms used by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




The image shows a screenshot of the 'Simulation Wizard' dialog box at step 2/5. The title bar reads 'Simulation Wizard' and 'step 2/5'. The main text says 'Please select the type of simulation to perform:'. Below this, there are two radio button options: 'Wild Type Simulation' (which is selected) and 'Mutant Simulation'. At the bottom, there are three buttons: 'Back' (with a left arrow), 'Next' (with a right arrow), and 'Cancel' (with a red X).

## 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**.



The image shows a screenshot of the 'Simulation Wizard' dialog box at step 4/5. The title bar reads 'Simulation Wizard' and 'step 4/5'. The main text says 'Select the flux to be optimized and the type of optimization:'. Below this, there is a dropdown menu labeled 'Select a flux to optimize:' with 'R\_BIOMASSX' selected. To the right of the dropdown, there are two radio button options: 'Maximization' (which is selected) and 'Minimization'. At the bottom, there are three buttons: 'Back' (with a left arrow), 'Next' (with a right arrow), and 'Cancel' (with a red X).

## 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.

The Simulation Wizard window is at step 5/5, titled "Define environmental/external conditions if necessary:". It features a search bar at the top. Below it, there are two main sections: "external fluxes" and "modified fluxes". The "external fluxes" section has a table with columns "Name", "lower", and "upper", and a list of fluxes: R\_SUC, R\_ACETR, R\_CAR, and R\_GPP. The "modified fluxes" section has a similar table but is currently empty. At the bottom of the "external fluxes" section is a button labeled "add to env. conditions". At the bottom of the "modified fluxes" section is a button labeled "remove from environmental conditions". There is also a checkbox labeled "Use Conditions" and buttons for "Back", "Finish", and "Cancel".

## 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.

The OptFlux software interface is shown. The left sidebar contains a tree view with "OptFlux" at the top, followed by "Project", "Original Model", "Simulation Results", and "Wild Type". The "Simulation Results" section is expanded, showing "Wild Type" selected. The main window displays the "Wild Type" simulation results. It shows the "maximized flux (R\_BIOMASSX)" as 87.6551 and the "simulation method" as FRA. Below this, there is a "knockouts" section which is currently empty. To the right, a table titled "Flux Values" lists various fluxes and their values. At the bottom, there is a search bar, a checkbox for "show only non zero (0) values", and a button for "export all values...".

Flux	Value
R_SDIcomplex	0.1179
R_ZWF	0.25335
R_FBA	0.61545
R_LSC1LSC2	0.1179
R_PDC	0.45793
R_NADHX	0.10007
R_ACETR	0.20069
R_CIT	0.2358
R_PDH	0.26795
R_FUM1	0.1179
R_PFK	0.61545
R_TAL1	0.08445
R_KGD1KGD2	0.1179
R_CAR	1.10784
R_FADHX	0.00589
R_BIOMASSX	87.6551
R_ACO	0.2358
R_DAR	0.01069
R_ACS	0.25723
R_GND	0.25335
R_PVC	0.22508
R_TPI	0.60475
R_ENO	1.20819
R_PGI	0.4787
R_HXK	1

## 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](http://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 below (FBA, MOMA or ROOM).

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

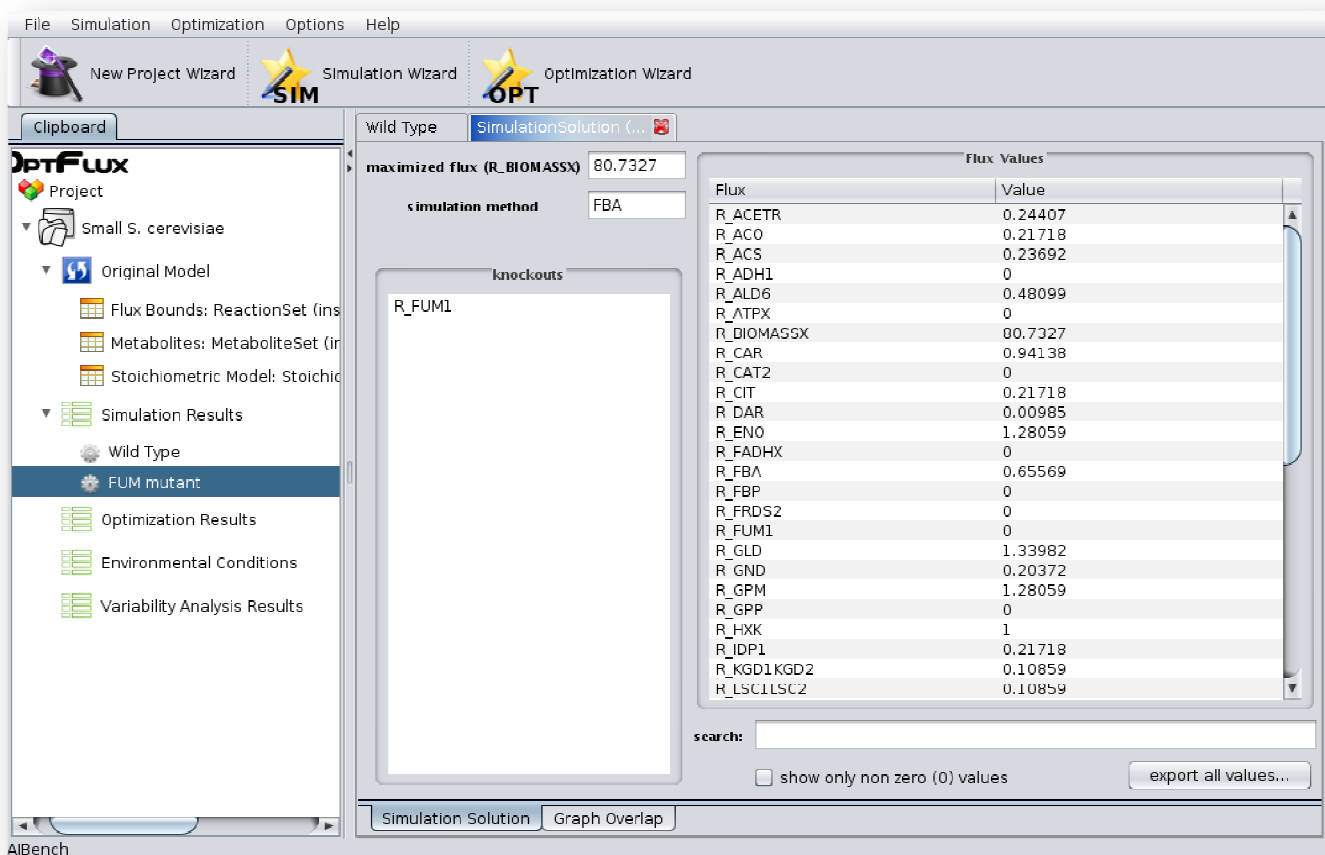


to step 4

### 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**.

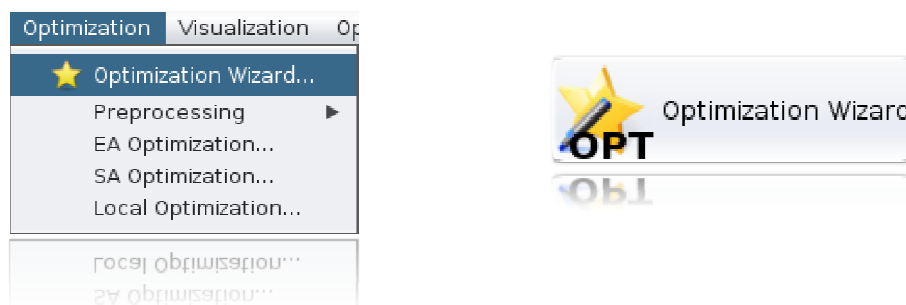
Left-clicking the object will launch the simulation solution viewer in the visualization area on the right. This time one can notice, for instance, that the biomass growth associated flux has a value lower than the one in the wild-type simulation.



## 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.

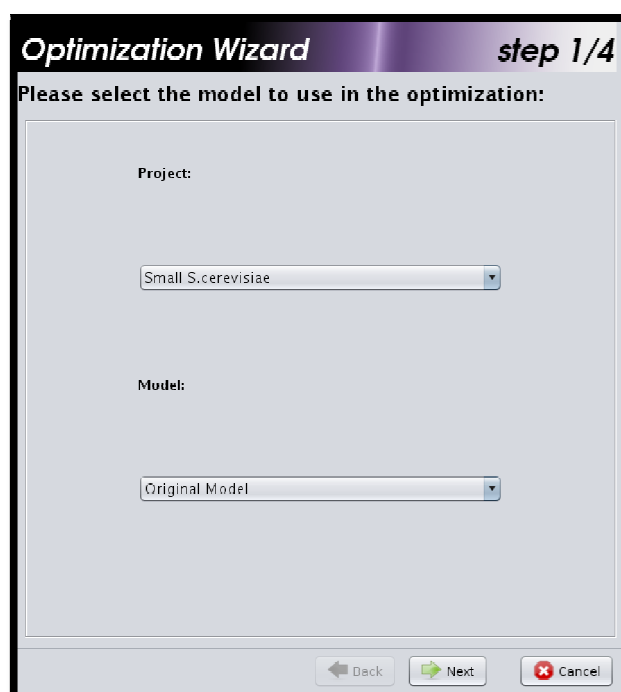
### 3.1 – PERFORMING AN OPTIMIZATION USING THE EVOLUTIONARY ALGORITHM

#### 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.

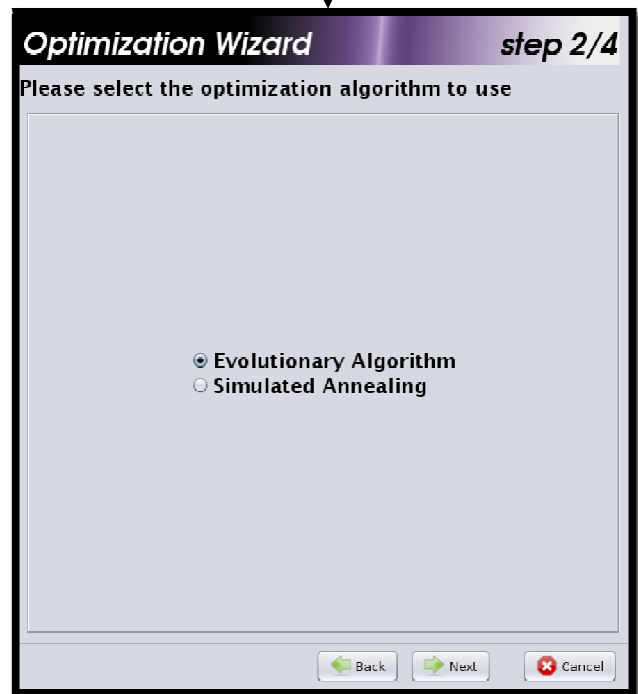
In the context of this tutorial, leaving the default selection is just fine.



## 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).



**Optimization Wizard** step 2/4

Please select the optimization algorithm to use

☒ Evolutionary Algorithm  
☐ Simulated Annealing

Back Next Cancel

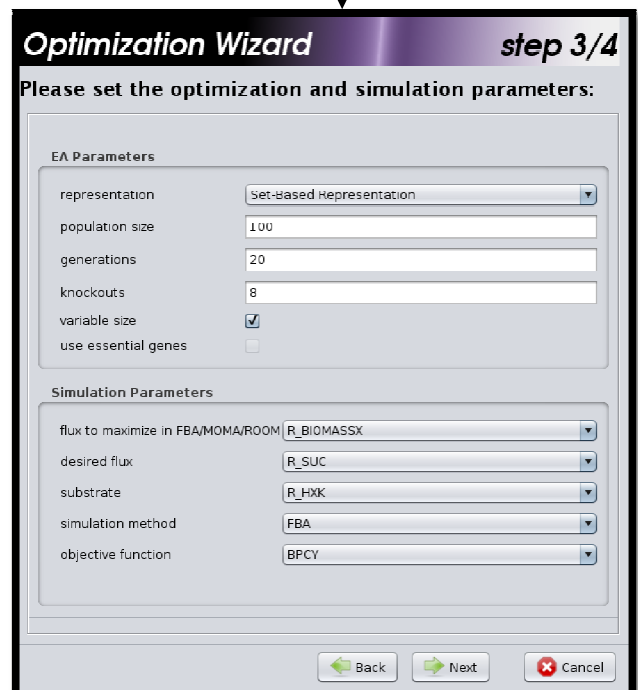
## Step 3

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.



**Optimization Wizard** step 3/4

Please set the optimization and simulation parameters:

**EA Parameters**

representation: Set-Based Representation  
population size: 100  
generations: 20  
knockouts: 8  
variable size: ☒  
use essential genes: ☐

**Simulation Parameters**

flux to maximize in FBA/MOMA/ROOM: R\_BIOMASSX  
desired flux: R\_SUC  
substrate: R\_HXK  
simulation method: FBA  
objective function: BPCY

Back Next Cancel

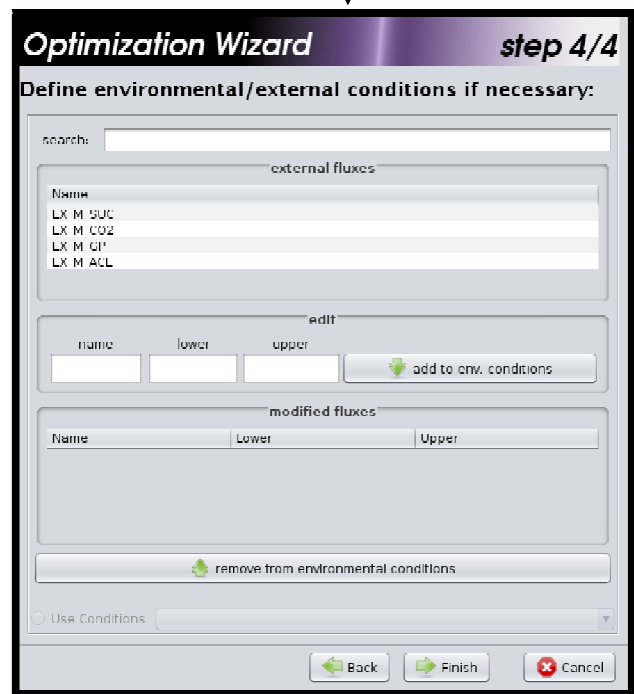


#### Step 4

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.



The screenshot shows the 'Optimization Wizard' window at 'step 4/4'. The title bar is purple with the text 'Optimization Wizard' and 'step 4/4'. Below the title bar, the text 'Define environmental/external conditions if necessary:' is displayed. The interface includes a search bar at the top. Below it, there are two main sections: 'external fluxes' and 'modified fluxes'. The 'external fluxes' section has a table with columns 'Name', 'Lower', and 'Upper'. It lists four items: 'LX M SUC', 'LX M CO2', 'LX M GI\*', and 'LX M ACL'. Below this table is an 'edit' section with input fields for 'name', 'lower', and 'upper', and a button 'add to env. conditions'. The 'modified fluxes' section has a similar table with columns 'Name', 'Lower', and 'Upper', but it is currently empty. Below this table is a button 'remove from environmental conditions'. At the bottom of the window, there is a checkbox labeled 'Use Conditions' and three buttons: 'Back', 'Finish', and 'Cancel'.

#### 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.

FileSimulation 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

EA100/10 - 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

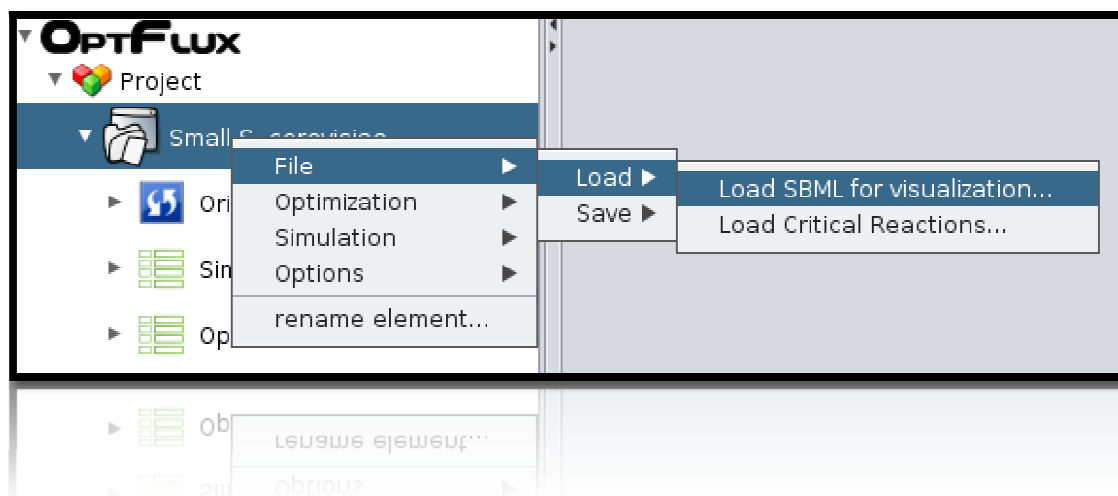
view complete info

Optimization Solutions

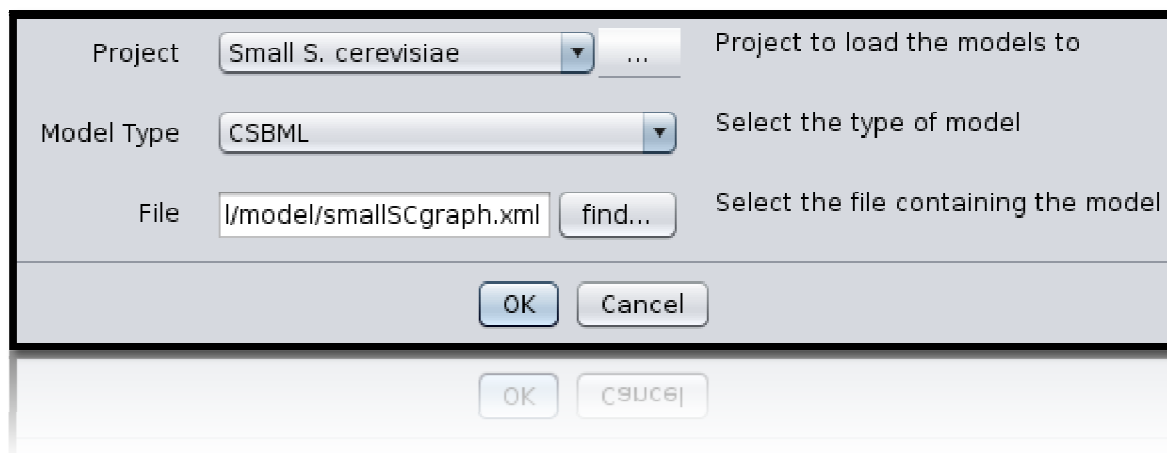
AltBench

## 4 – GRAPH VISUALIZATION

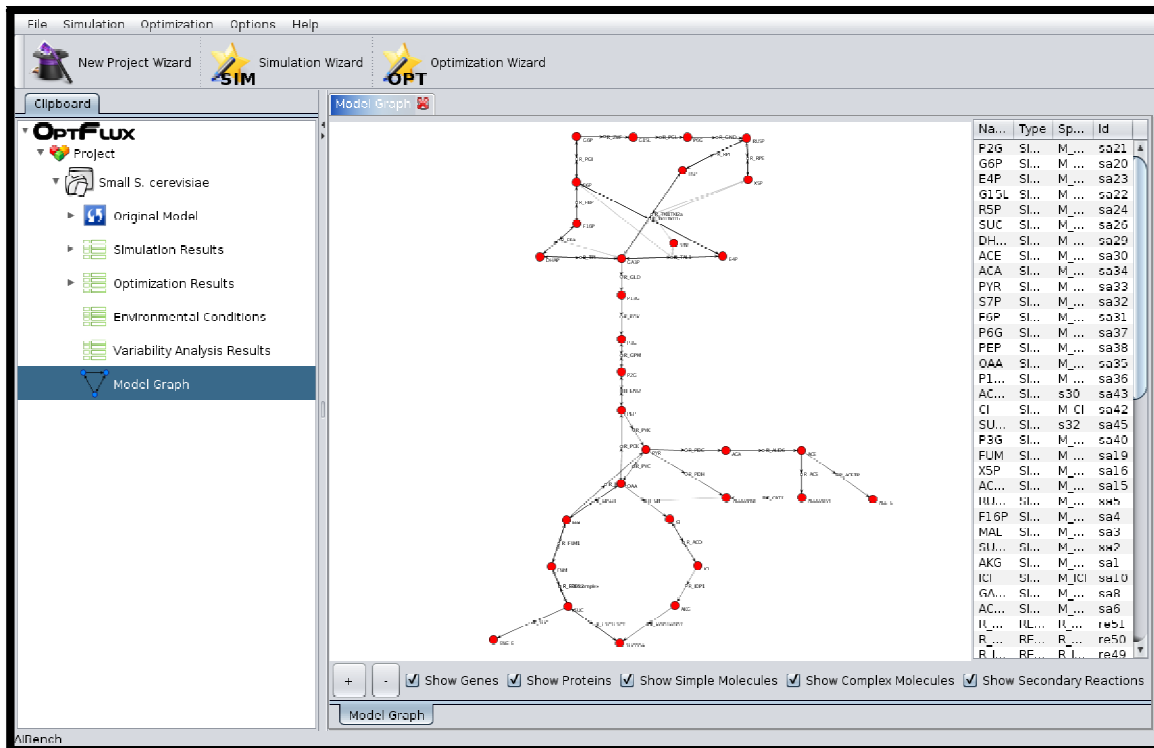
For an improved visualization of the results, the user can also load a CellDesigner SBML file which provides a graphical representation of the metabolism.



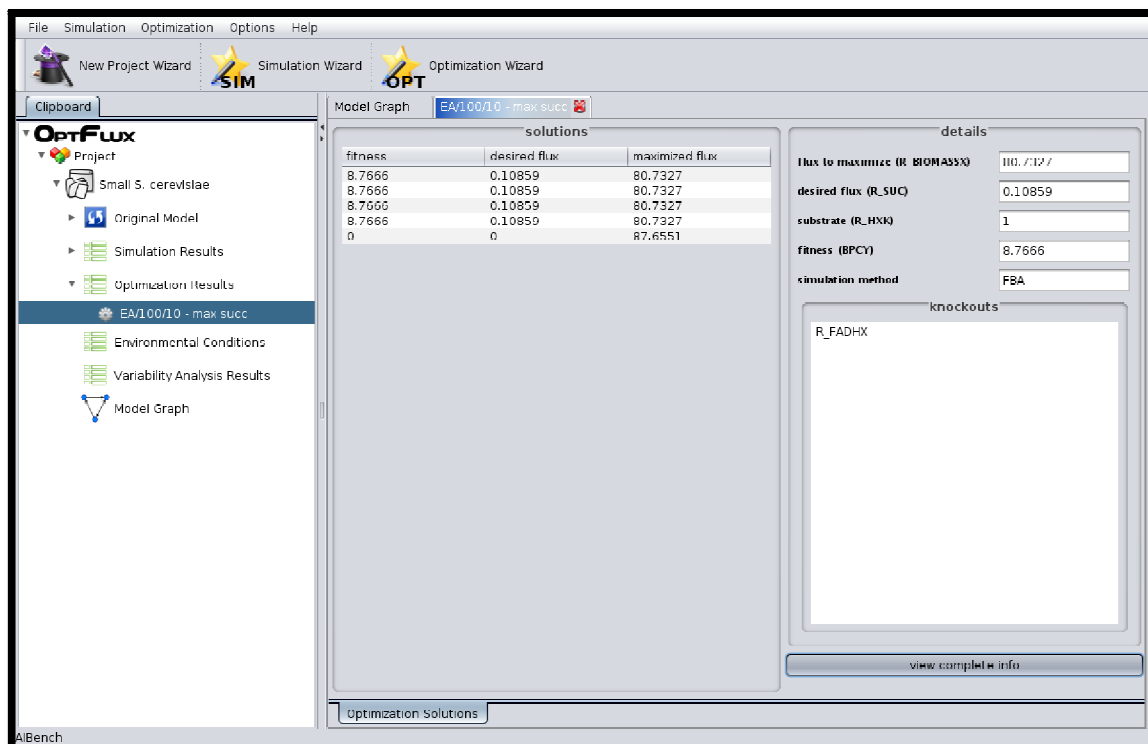
In the provided archive, a file named *“smallSCgraph.xml”* is included. To load it, the user needs to access File Menu -> Load -> Load SBML for visualization. The type of SBML selected must be CSBML.



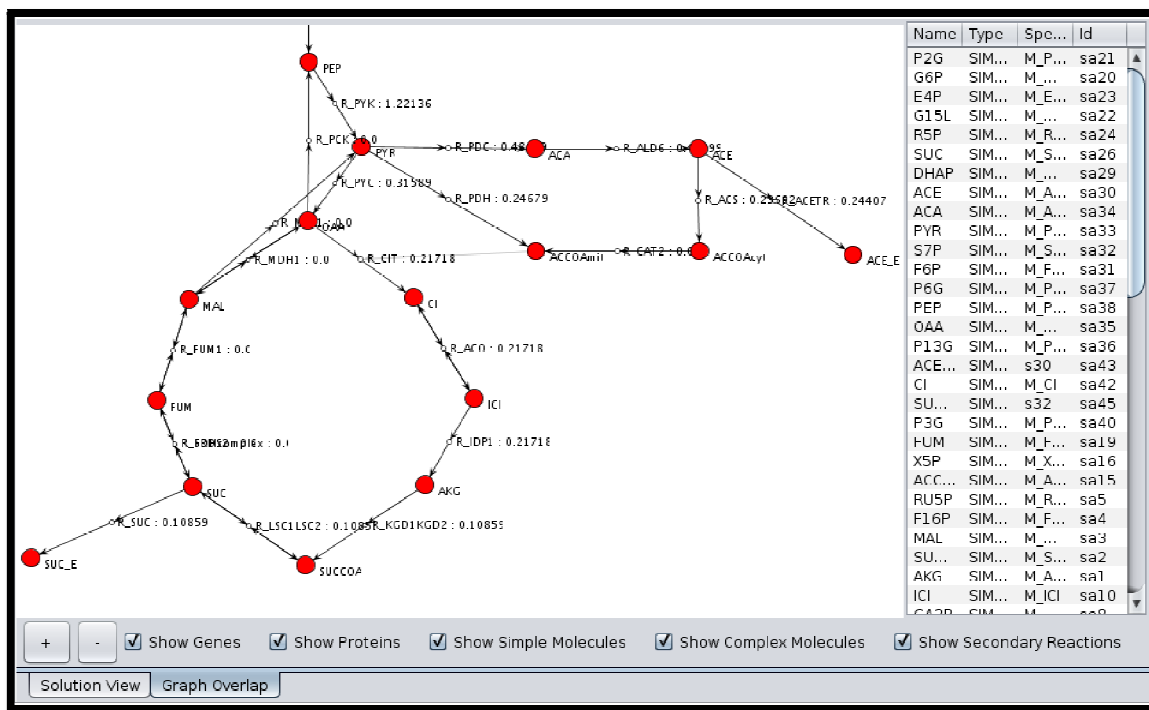
A new object will be placed in the Project tree under the name **Model Graph**.



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 Overlay*, the previously loaded graph will be presented having that particular solution results superimposed in it.