

Universidade do Minho

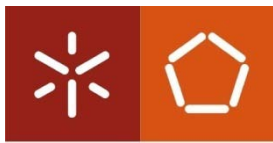
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**CCTC**– COMPUTER SCIENCE AND TECHNOLOGY CENTER  
SCHOOL OF ENGINEERING  
UNIVERSITY OF MINHO

# OPTFLUX 2 BEGINNER'S TUTORIAL



# BEGINNER'S TUTORIAL FOR OPTFLUX 2

METABOLIC ENGINEERING WORKBENCH



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- **For the OptFlux software:**

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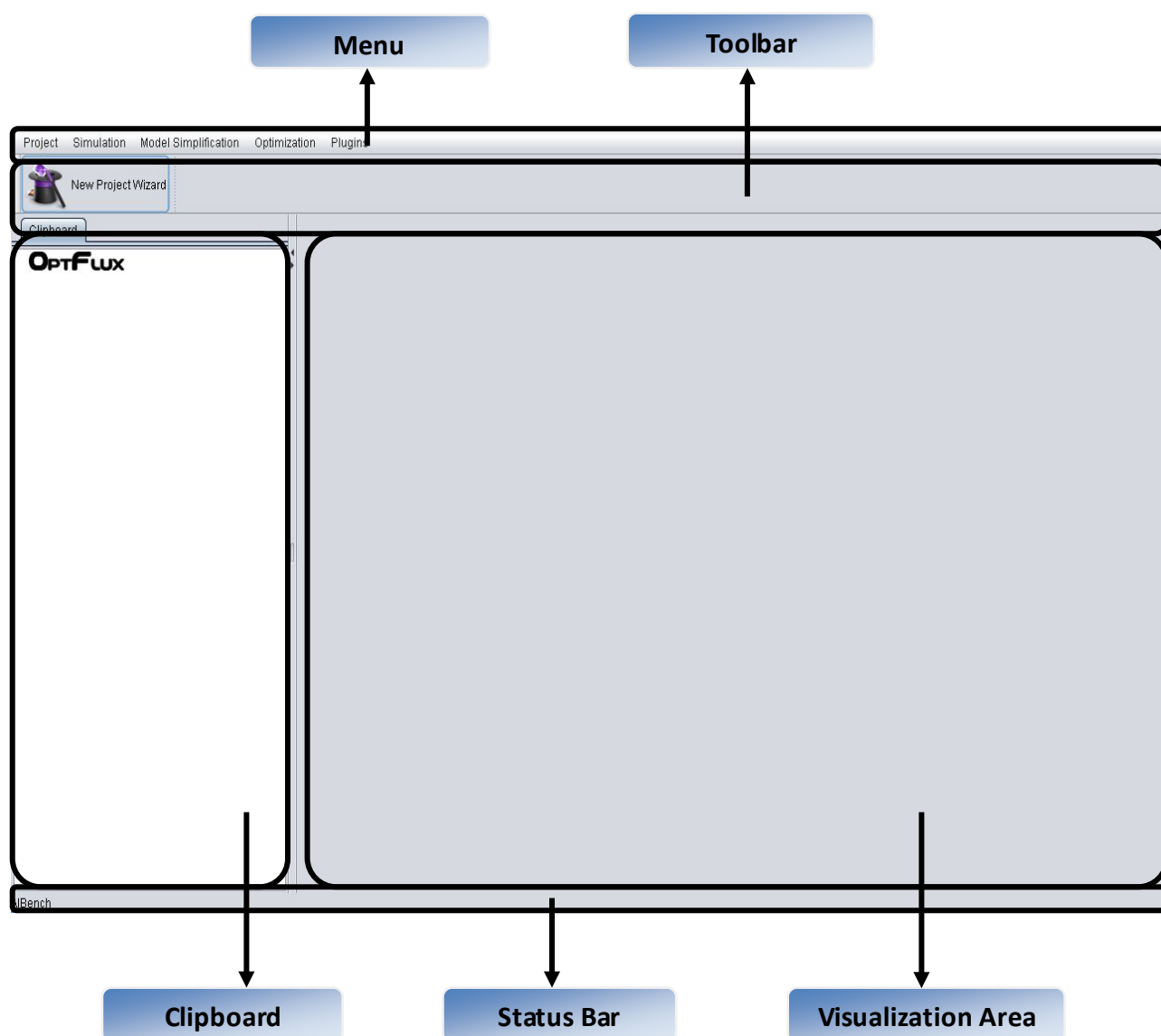
Created inside the SysBio Research Group (<http://sysbio.di.uminho.pt>)



# FIRST THINGS FIRST!

Hello and welcome to the OptFlux 2 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.

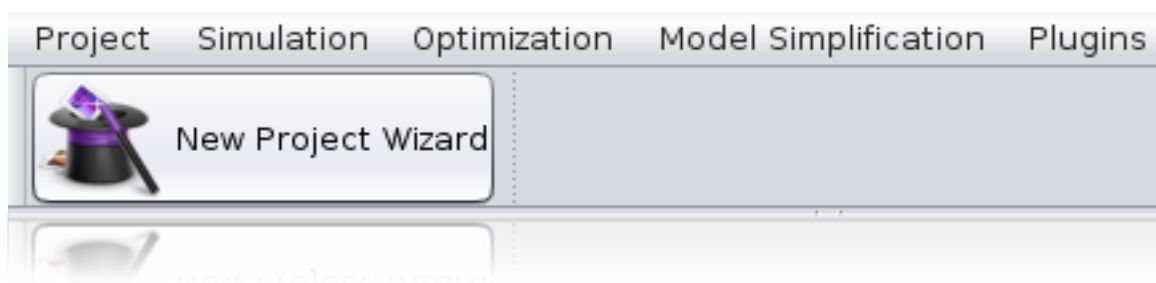


# 1<sup>ST</sup> STEP – CREATING A NEW PROJECT

To follow the steps in this tutorial you need to download the file [gomGG.zip](http://www.OptFlux.org/tutorial/gomGG.zip), available in [www.OptFlux.org/tutorial/gomGG.zip](http://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*.



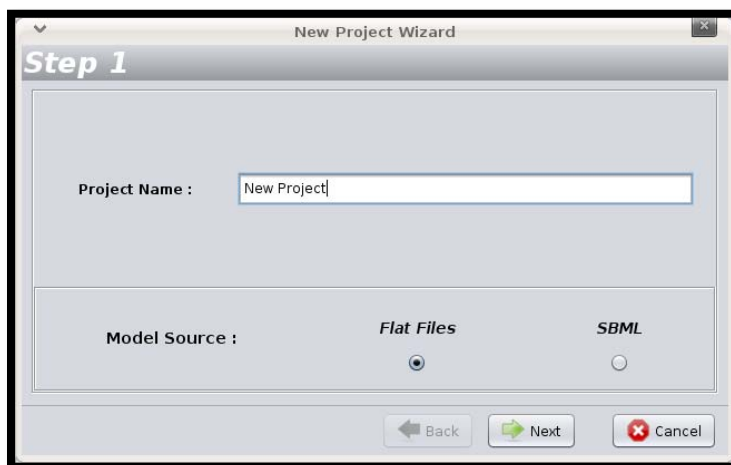
You have the option to create the new project from two different sources: flat files and SBML file.

## 1.1 – FROM FLAT FILES

### Step 1

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

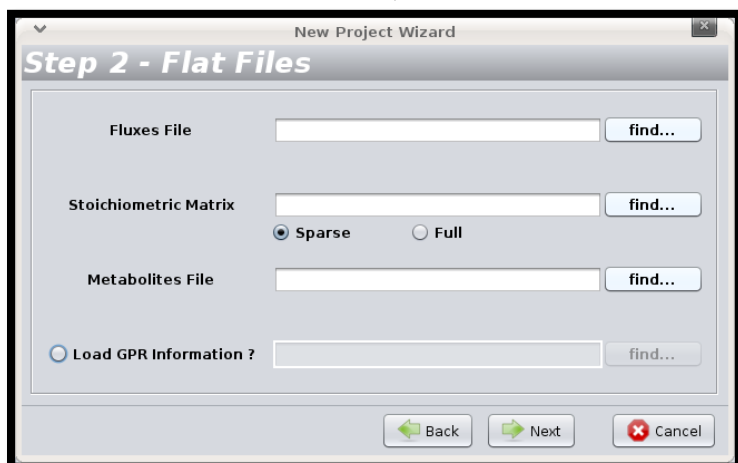
In the picture the name selected was “New Project”



## Step 2

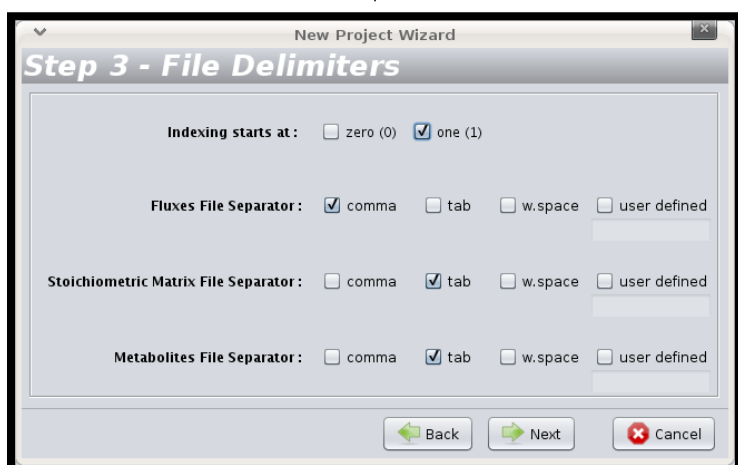
In the second step, the user must select three files, which were provided along with this tutorial:

- 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

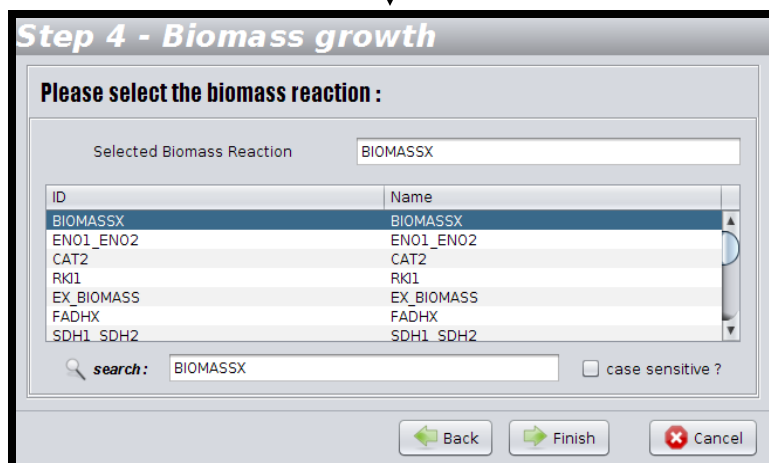
In step 3, the first option concerns the indexing used in the stoichiometric matrix if the SPARSE option was selected. The user must select indexing starting at **zero**. For the remaining files, the user should select the **comma separator** for the Fluxes File and the **tab separator** for the matrix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



ID	Name
BIOMASSX	BIOMASSX
EN01_EN02	EN01_EN02
CAT2	CAT2
RK1	RK1
EX_BIOMASS	EX_BIOMASS
FADHX	FADHX
SDH1_SDH2	SDH1_SDH2

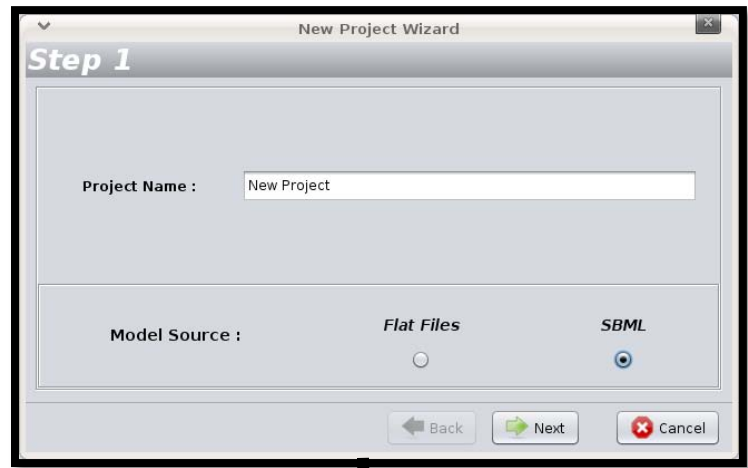
## 1.2 – FROM A SBML FILE

### 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.



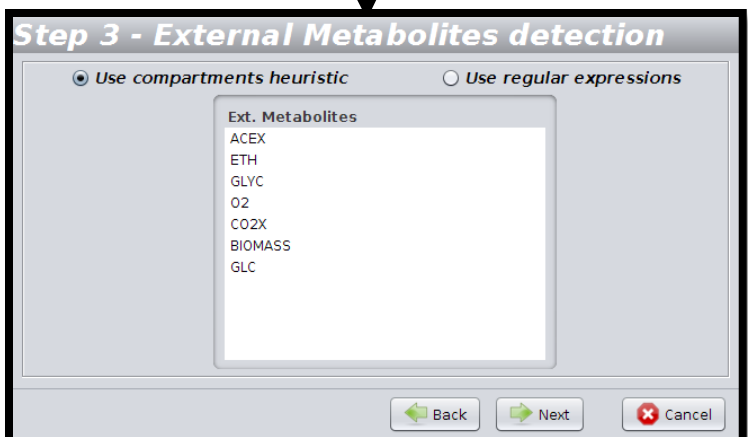
### Step 2

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

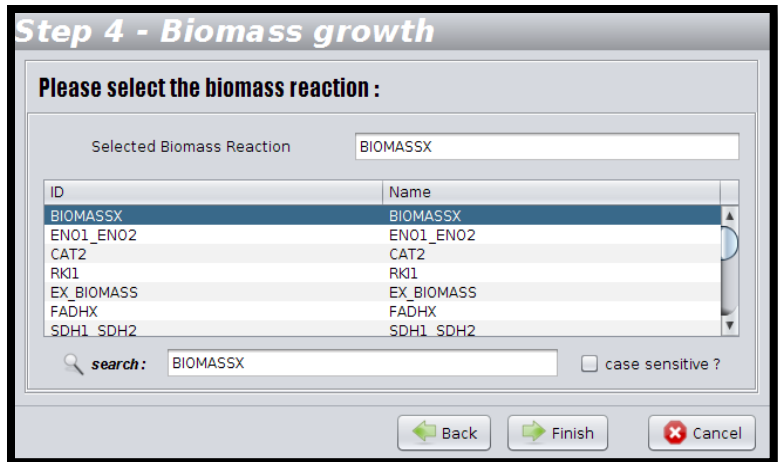
The third step is relative to the extra-cellular environment. *OptFlux* will automatically try to find the extra-cellular compartment and the respective metabolites, using the compartments heuristics it will detects external metabolites based on the definitions of the compartments contained in the SBML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

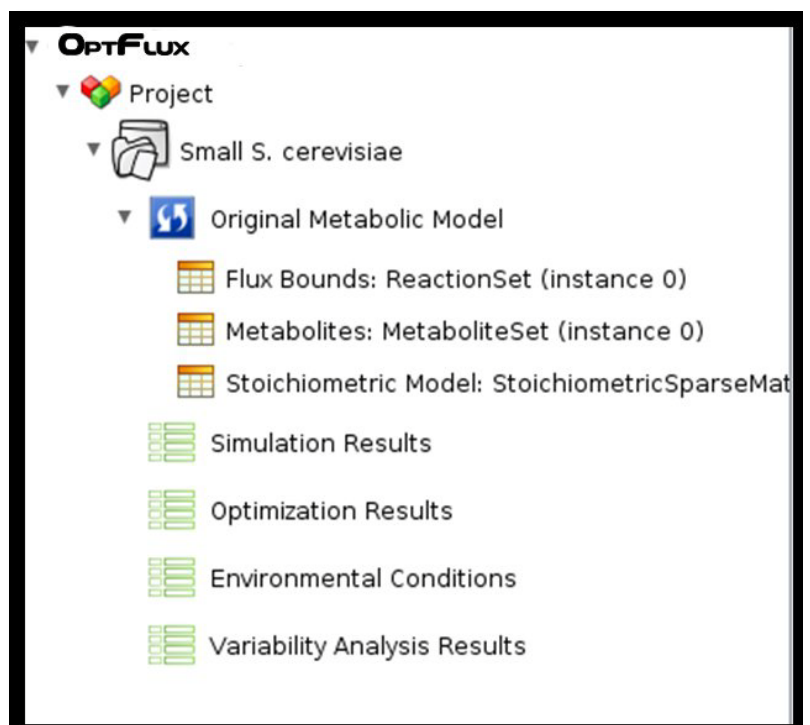


### 1.3 – PROJECT CREATED IN THE CLIPBOARD

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.

**Fluxes**

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

**Metabolites**

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
OAA	OAA	internal	INTERNAL
NADmit	NADmit	internal	INTERNAL
P13G	P13G	internal	INTERNAL
P3G	P3G	internal	INTERNAL
ACCOAcyt	ACCOAcyt	internal	INTERNAL
FADH2	FADH2	internal	INTERNAL
PvR	PvR	internal	INTERNAL
NADPHmit	NADPHmit	internal	INTERNAL
P6G	P6G	internal	INTERNAL
GA3P	GA3P	internal	INTERNAL
ATP	ATP	internal	INTERNAL
MAL	MAL	internal	INTERNAL
G15L	G15L	internal	INTERNAL
ICI	ICI	internal	INTERNAL
ACA	ACA	internal	INTERNAL
RU5P	RU5P	internal	INTERNAL
NADPmit	NADPmit	internal	INTERNAL
CI	CI	internal	INTERNAL
CCO	CCO	internal	INTERNAL

## 2<sup>ST</sup> 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.

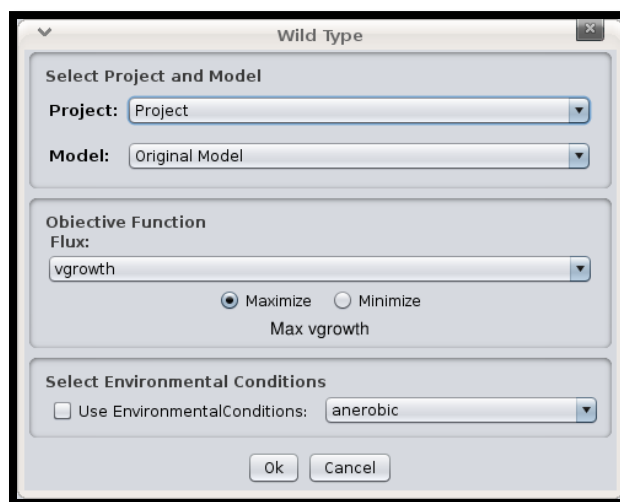
### 2.1 – PERFORMING A WILD-TYPE SIMULATION

#### Step 1

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

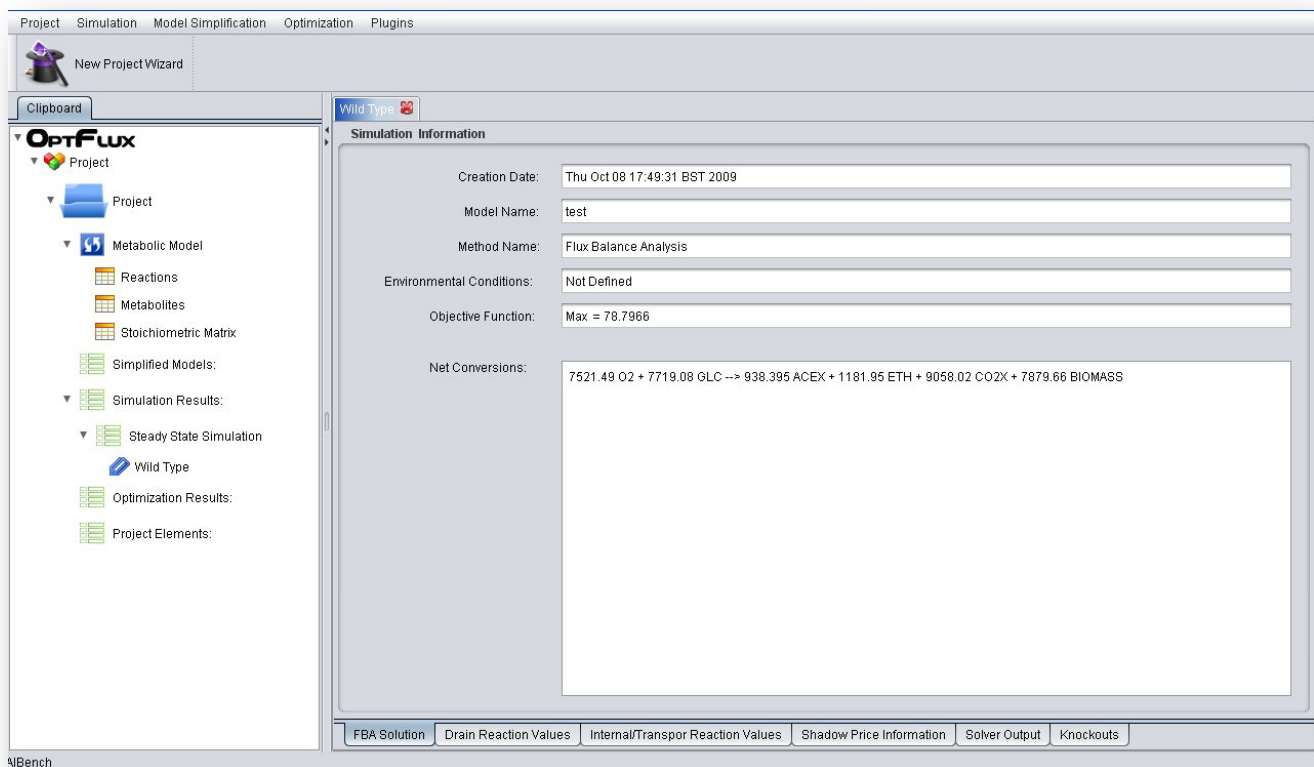
This step is necessary since OptFlux supports multiple-projects and each project can contain an Original Metabolic Model and a Simplified Metabolic Model.

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



## Step 2

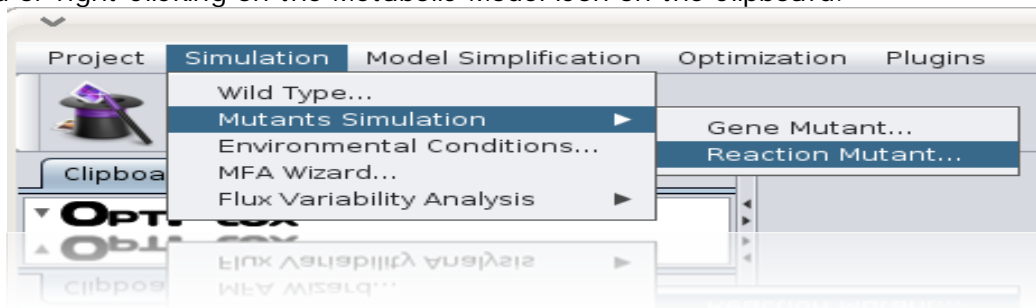
After completing all the previous steps, a new object named “Wild Type” is placed within the **Simulation Results** list. By left-clicking this object the user has access to detailed information about the performed simulation. The user can, for instance, see the values for all the fluxes the simulation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

A screenshot of the 'Reaction Mutant' dialog box in OptFlux. The dialog has several sections: 'Select Project and Model' with dropdowns for 'Project' and 'Metabolic Model'; 'Reaction' and 'Knockout' lists with arrows for moving reactions between them; 'Select Simulation Method' with a dropdown set to 'Flux Balance Analysis'; 'Objective Function' with a dropdown set to 'BIOMASSX' and radio buttons for 'Maxim...' (selected) and 'Minim...'; and 'Select Environmental Conditions' with a checkbox for 'Use EnvironmentalConditions:'. At the bottom are 'Ok' and 'Cancel' buttons.

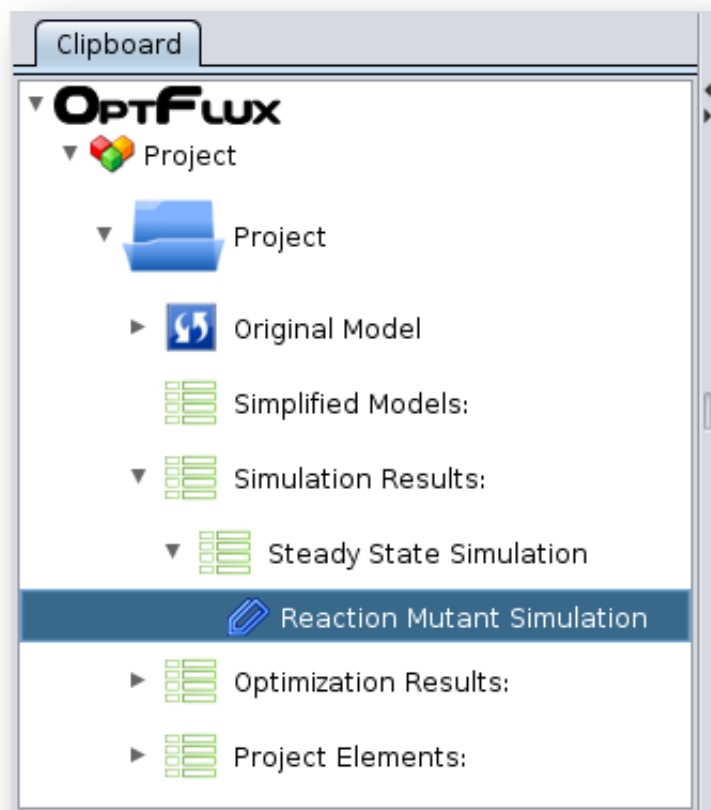
minimizing the flux.

#### **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**

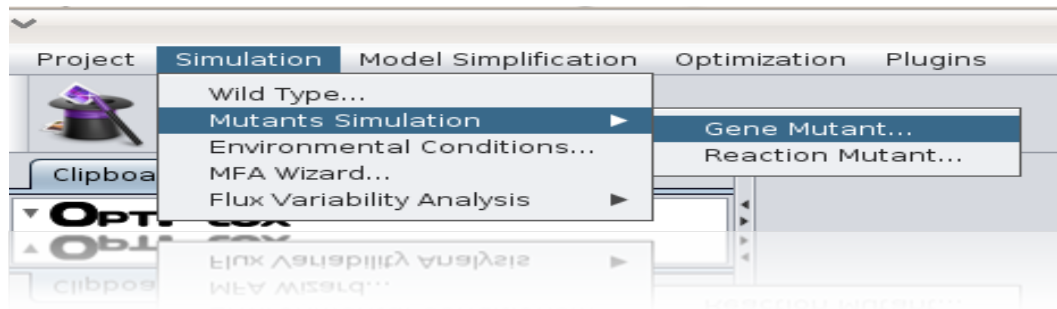
And that's all!! You can press OK and the results will be loaded into the clipboard..



## **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

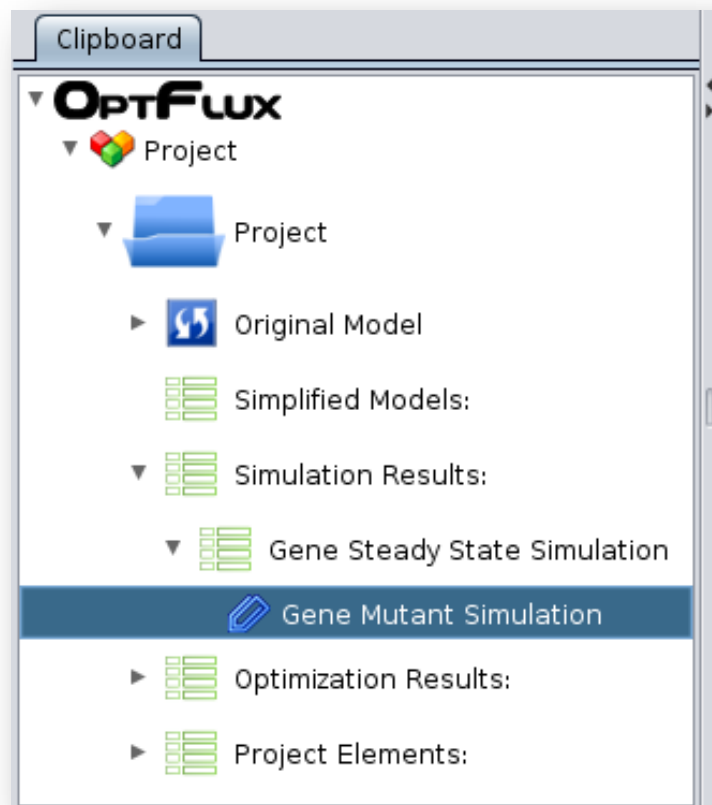
Here you can select the reaction to optimize (biomass, by default), and you can also define if you will be maximizing or minimizing that flux.

#### 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..

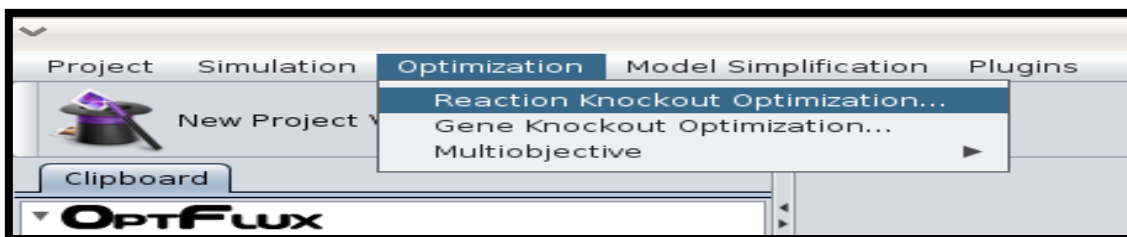


## 3<sup>ST</sup> STEP – PERFORMING OPTIMIZATION

### 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.

The screenshot displays the 'Objective Function Configuration' dialog box in the OptFlux software. The dialog is organized into several sections:

- Select Project and Model:** Contains two dropdown menus. 'Project' is set to 'Project' and 'Model' is set to 'Metabolic Model'.
- Select Simulation Method:** A dropdown menu set to 'Flux Balance Analysis'.
- Select Environmental Conditions:** A checkbox labeled 'Use Environmental Conditions:' is currently unchecked.
- Select Objective Function:** A dropdown menu set to 'BPCY: Biomass-Product Coupled Yield'.
- Essential Information:** Contains three checkboxes: 'Use critical reactions' (unchecked), 'Add drain reactions' (unchecked), and 'Add transport reactions' (unchecked).
- Objective Function Configuration:** Contains three dropdown menus: 'Biomass:' set to 'BIOMASSX', 'Desired Flux:' set to 'BIOMASSX', and 'Substrate:' set to 'EX\_ACEX'.
- Select Optimization Algorithm:** A dropdown menu set to 'Cellular Genetic Algorithm'.
- Optimization Basic Setup:** Contains four input fields: 'Maximum Number Of Solutions Evaluations:' set to '50,000', 'Estimated Time(Minutes):' set to '83', 'Maximum Number Of Knockouts' set to '5' (with up/down arrows), and an unchecked checkbox for 'Variable size genome'.

At the bottom of the dialog are 'Ok' and 'Cancel' buttons.

## 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

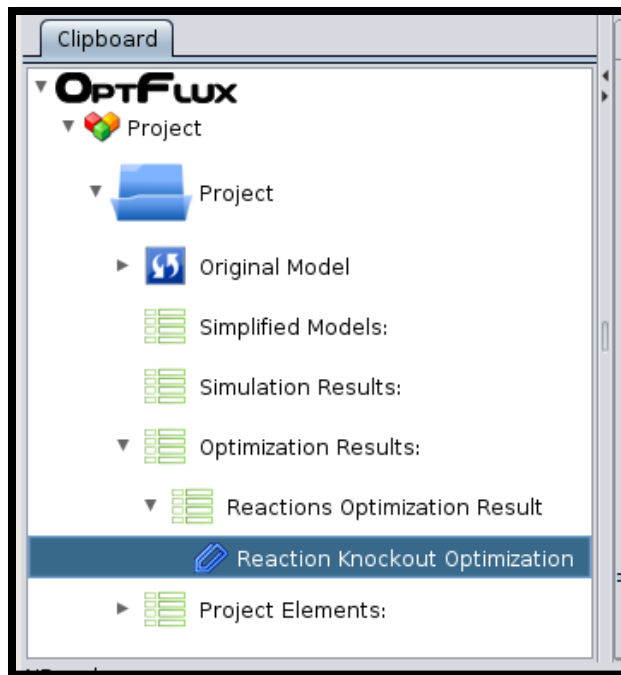
Here you can configure the maximum number of solution evaluations (Simulations) and check the expected time to perform that number of evaluations. You can also set up the maximum number of knockouts and if the set of knockouts should be static or have a variable size.

## 8.Essential information

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

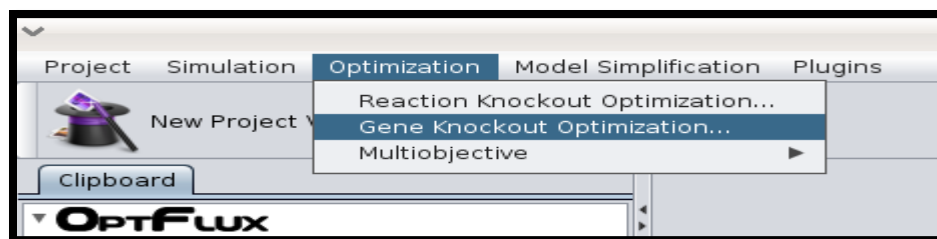
And that's all, now you can press OK and check the results in the clipboard.



## 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).

**Select Project and Model**

**Project:** Project

**Model:** Metabolic Model

**Select Simulation Method**

Flux Balance Analysis

**Select Environmental Conditions**

☐ Use Environmental Conditions:

**Select Objective Function**

BPCY: Biomass-Product Coupled Yield

**Critical Genes**

☐ Use critical genes

**Objective Function Configuration**

**Biomass:** R\_biomass\_SC4\_bal

**Desired Flux:** R\_IMPC

**Substrate:** R\_EX\_gly\_e\_

**Select Optimization Algorithm**

Cellular Genetic Algorithm

**Optimization Basic Setup**

Maximum Number Of Solutions Evaluations: 50,000

Estimated Time(Minutes): 83

Maximum Number Of Knockouts: 5

☐ Variable size genome

Ok Cancel

## 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. If 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.

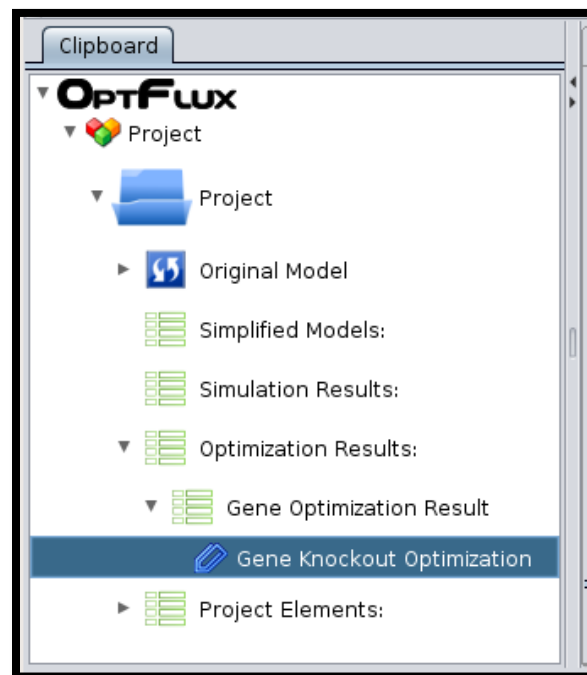
You can also set up the maximum number of knockouts and if the set of knockouts should be static or have a variable size.

### 8. Essential information

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

### Step 3

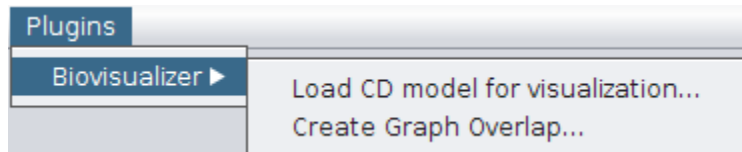
And that's all, now you can press OK and check the results in the clipboard.



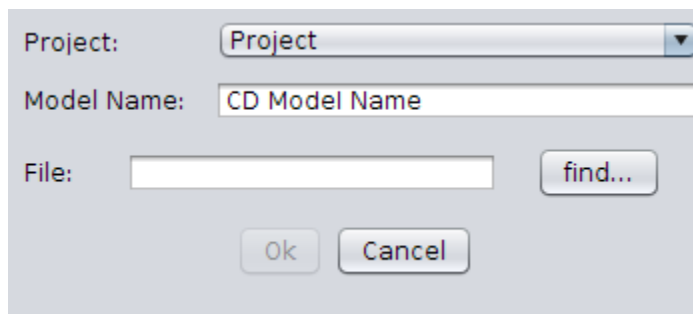
## 4<sup>ST</sup> STEP – INTERACTION WITH CELLDISIGNER

### 3.1 – LOADING A CELLDISIGNER MODEL FOR VISUALIZATION

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

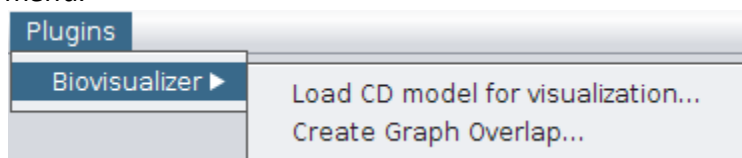


In the project combo box select the project where you want to load the CD into, then choose the file and write the name for the CD visualization object in the clipboard.



### 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.

**Select Project, CD Model and Simulation**

**Project:**

**Model:**

**Simulation:**

**Name:**

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

