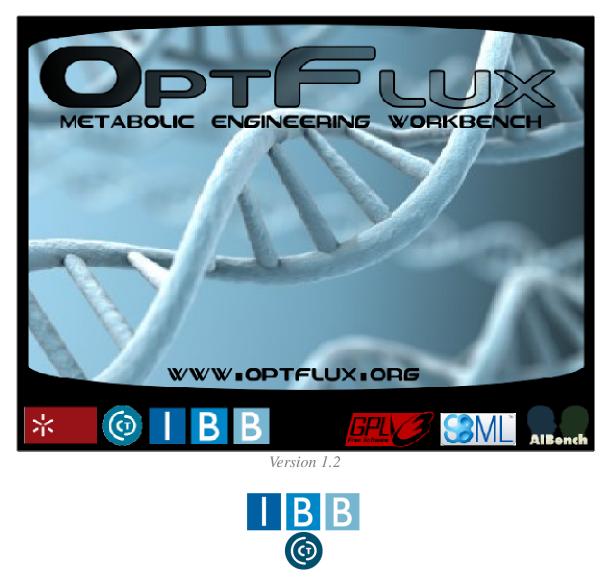


**Universidade do Minho** 

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**



## **BEGINNER'S TUTORIAL**

FOR



METABOLIC ENGINEERING WORKBENCH





**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

> © *IBB-CEB/CCTC* All rights reserved.

### LICENSES

#### • For this tutorial:

This work is licensed under the Creative Commons Attribution-Share Alike 3.0 Unported License. To view a copy of this license, visit <u>http://creativecommons.org/licenses/by-sa/3.0</u> or send a letter to Creative Commons, 171 Second Street, Suite 300, San Francisco, California, 94105, USA.



#### • For the OptFlux software:

Copyright 2009 IBB-CEB - Institute for Biotechnology and Bioengineering - Centre of Biological Engineering CCTC - Computer Science and Technology Center University of Minho

This is free software: you can redistribute it and/or modify it under the terms of the GNU Public License as published by the Free Software Foundation, either version 3 of the License, or (at your option) any later version.

This code is distributed in the hope that it will be useful, but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE. See the GNU Public License for more details.

You should receive a copy of the GNU Public License along with the code. If not, see <a href="http://www.gnu.org/licenses/">http://www.gnu.org/licenses/</a>

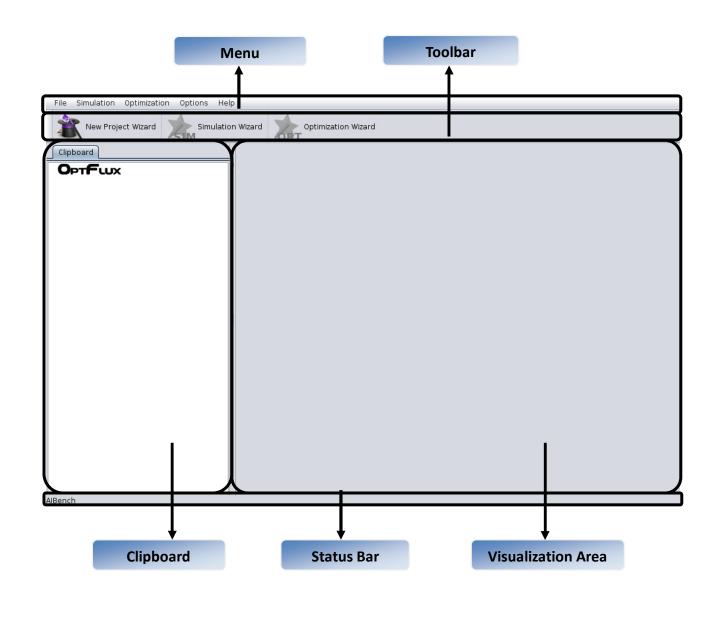
Created inside the SysBio Research Group (http://sysbio.di.uminho.pt)



## **FIRST THINGS FIRST!**

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

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.

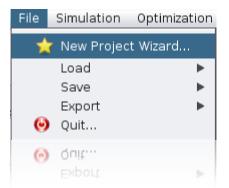


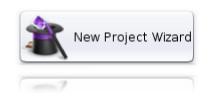
## **1 – CREATING A NEW PROJECT**

To follow the steps in this tutorial you need to download the file smallSC.zip, available in <u>www.optflux.org/tutorial/smallSC.zip</u>. 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**

#### <u>Step 1</u>

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.

New Proje General Proje			step 1/4
Project Name :	Small S. cerevisiae		
Proxy :	<ul> <li>None</li> <li>HTTP</li> <li>SOCKS</li> </ul>	Host : Port :	
Model Source :	Iat Files	O SBML	⊖ BioModels
		<b>4</b> Bar	sk 💽 🌳 Next 🔇 Cancel

#### <u>Step 2</u>

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 <u>smallSCFluxes.txt</u>;

• The second contains the stoichiometric matrix – select <u>smallSCMatrix.txt</u>;

• The third contains the metabolites names (optional) – select <u>smallSCMetabolites.txt</u>

#### <u>Step 3</u>

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.

#### <u>Step 4</u>

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 Projec	step 2/4
Please select the	files for the following fields:
Fluxes File	a/data/small scerevisiae/new/smallSCFluxes.txt find
Stoichiometric Matrix	a/data/small scerevisiae/new/smallSCMatrix.txt find
Metabolites File	a/small scerevisiae/new/smallSCMetabolites.txt find
	🗲 Back 📄 Next 🔇 Cancel

 New Project
 step 3/4

 Select the file options please:
 Indexing starts at: 
 Zero (0) one (1)

 Huxes Hile Separator:
 Zero (0) one (1)
 Indexing starts at: 
 Zero (0) one (1)

 Huxes Hile 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

 Metabolites File Separator:
 comma
 Tab
 W.space
 user defined

New Project	step 4/4
Please select the bioma	iss flux :
Selected Diomass Flux	R_DIOMASSX
ID R_PFK R_TALL R_ATPX R_FRDS2 R_KGD1KGD2 R_CAR R_FADHX R_BIOMASSX	
Search: R_BIOMASSX	Case sensitive ?
	Rack Einish Cancel

### **1.2 – FROM A LOCAL SBML FILE**

#### <u>Step 1</u>

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.

eneral Proje	ct Options :		
Project Name :	Small S. cerevisiae		
Ргоху :	None     HIIP     SOCKS	Husl : Port :	
Model Source :	⊖ Flat Files	SBML	⊖ BioModels

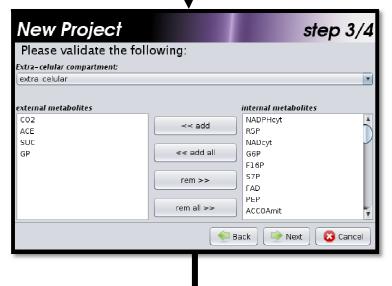
#### Step 2

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 <u>smallSC.xml</u> 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.



#### <u>Step 4</u>

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"**.

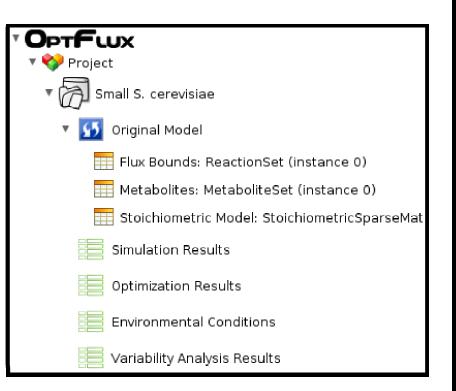
			•	
	lew Pro	oject		step 4/4
F	Please sele	ct the biom	ass flux :	
	Selected	Biomass Flux	R_BIOMASSX	
	ID		Name	
	R PFK		R PFK	<b>A</b>
	R_TAL1		R_TAL1	
	R_ATPX		R_ATPX	
	R_FRDS2		R_FRDS2	
	R_KGD1KGD2		R_KGD1KGD2	
	R_CAR		R_CAR	
	R_FADHX		R_FADHX	¥
	R_BIOMASSX		R_BIOMASSX	<b>⊻</b> _
	$\subseteq$ search :	R_BIOMASSX		case sensitive ?
			Back	Finish 🔀 Cancel

### **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 "Original Metabolic named 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.

			Fluxes				
eaction Nam	ne	Lower Boun	d Uppe	er Bound	Туре		
SDHcomple	ex	0.0	1000		INTERNAL		
ZWF		0.0	1000	0.0	INTERNAL		
FBA		-10000.0	1000		INTERNAL		
LSC1LSC2		-10000.0	1000		INTERNAL		
SUC		0.0	1000		EXTERNAL		
PDC NADHX		0.0	1000		INTERNAL		
ACETR		0.0	1000		EXTERNAL		
CIT		0.0	1000		INTERNAL		
PDH		0.0	1000		INTERNAL		
FUM1		-10000.0	1000	0.0	INTERNAL		
PFK		0.0	1000		INTERNAL		
TAL1		-10000.0	1000		INTERNAL		
ATPX		0.0	1000		INTERNAL		
FRDS2 KGD1KGD2		0.0	1000		INTERNAL		
CAR		0.0	1000		FXTERNAL		
	search:	0.0	TUA		1 211 1004		
BIOMASSX				Motabolitos			
ACO				Metabolites			
DAR	Abbreviation		Complete Name	Compartment Nam	ne	Compartment Location	
ACS GND	NADPHcyt		NADPHcyt	internal		INTERNAL	<b>A</b>
MAE1	R5P		R5P	internal		INTERNAL	
PYC	NADcyt		NADcyt	internal		INTERNAL	
TPI	G6P		G6P	internal		INTERNAL	
ENO	F16P		F16P	internal		INTERNAL	
РСК	C02		C02	extra_celular		EXTERNAL	
PGI	S7P		S7P	internal		INTERNAL	
HXK	FAD		FAD	internal		INTERNAL	
GLD	PEP		PEP	internal		INTERNAL	
PGK CAT2	ACCOAmit		ACCOAmit	internal		INTERNAL	
TKI1TKI2b	NADHcyt ACE		NADHcyt	internal ortra colular			
PGL	SUC		ACE SUC	extra_celular extra_celular		EXTERNAL	
ADUB	DHAP		DHAP	internal		INTERNAL	
	SUCCOA		SUCCOA	internal		INTERNAL	
leactions	OAA			internal		INTERNAL INTERNAL	
	NADmit	search:					
_	P13G			Reacti	ons		
	P3G						
	ACCOAcyt	Name	Reactants			Products	
		R_SDHcompl	FAD + SUC		>	FADH2 + FUM	
	FADH2						
	PYR	R_ZWF	G6P + NADPcyt		>	NADPHcyt + G15L	
	PYR NADPHmit	R_ZWF R_FBA	F16P		<>	NADPHcyt + G15L DHAP + GA3P	
	PYR NADPHmit P6G	R_ZWF R_FBA R_LSC1LSC2	F16P SUCCOA + ADP			NADPHcyt + G15L	
	PYR NADPHmit P6G GA3P	R_ZWF R_FBA R_LSC1LSC2 R_SUC	F16P SUCCOA + ADP SUC		<> <>	NADPHcyt + G15L DHAP + GA3P SUC + ATP	
	PYR NADPHmit P6G GA3P ATP	R_ZWF R_FBA R_LSC1LSC2 R_SUC R_PDC	F16P SUCCOA + ADP SUC PYR	mit	<> <>	NADPHcyt + G15L DHAP + GA3P SUC + ATP CO2 + ACA	
	PYR NADPHmit P6G GA3P ATP MAL	R_ZWF R_FBA R_LSC1LSC2 R_SUC R_PDC R_NADHX R_ACETR	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE	mit	<> <> >	NADPHcyt + G15L DHAP + GA3P SUC + ATP	
	PYR NADPHmit P6G GA3P ATP MAL G15L	R_ZWF R_FBA R_LSC1LSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + OAA	imit	<> <> > > >	NADPHcyt + GISL DHAP + GA3P SUC + ATP CO2 + ACA 20.0 × NADmit + 24.0 × ATP CI	
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI	R_ZWF R_FBA R_LSC1LSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_PDH	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + OAA NADmit + PYR	mit		NADPHcyt + G15L DHAP + GA3P SUC + ATP C02 + ACA 20.0 × NADmit + 24.0 × ATP CI C02 + ACCOAmit + NADHmit	
	PYR NADPHmit P6G GA3P ATP MAL G15L	R_ZWF R_FBA R_LSC1LSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_PDH R_FUM1	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + OAA NADmit + PYR FUM	mit		NADPHcyt + G15L DHAP + GA3P SUC + ATP C02 + ACA 20.0 × NADmit + 24.0 × ATP CI C02 + ACCOAmit + NADHmit MAL	
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA	R_ZWF R_FBA R_LSC1LSC2 R_SUC R_NADHX R_ACETR R_CIT R_CIT R_FDH R_FUM1 R_PFK	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + OAA NADmit + PYR FUM ATP + F6P	mit		NADPHcyt + GISL DHAP + GA3P SUC + ATP CO2 + ACA 20.0 × NADmit + 24.0 × ATP CI CO2 + ACCOAmit + NADHmit MAL FISP + ADP	
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RU5P	R_ZWF R_FBA R_LSCILSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_PDH R_FUM1 R_FK R_TAL1	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P	mit		NADPHcyt + G15L DHAP + GA3P SUC + ATP C02 + ACA 20.0 x NADmit + 24.0 x ATP CI C02 + ACCOAmit + NADHmit MAL F16P + ADP F6P + E4P	
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RU5P NADPmit	R_ZWF R_FBA R_LSCLSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_PDH R_FUM1 R_PFK R_TAL1 R_ATPX	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP	mit		NADPHcyt + GISL DHAP + GA3P SUC + ATP CO2 + ACA 20.0 x NADmit + 24.0 x ATP CI CO2 + ACCOAmit + NADHmit MAL FIGP + ADP FGP + E4P ADP	
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCILSC2 R_SUC R_PDC R_NADHX R_ACETR R_CT R_PDH R_FUM1 R_PFK R_TALL R_ATPX R_FRDS2	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM	mit		NADPHcyt + G15L DHAP + GA3P SUC + ATP C02 + ACA 20.0 x NADmit + 24.0 x ATP CI C02 + ACCOAmit + NADHmit MAL F16P + ADP F6P + E4P	
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCLSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_PDH R_FUM1 R_PFK R_TAL1 R_ATPX	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP	mit		NADPHcyt + GISL DHAP + GA3P SUC + ATP CO2 + ACA 20.0 × NADmit + 24.0 × ATP CI CO2 + ACCOAmit + NADHmit MAL FISP + ADP F6P + E4P ADP FAD + SUC	
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCILSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_PDH R_FUM1 R_FK R_TALL R_ATPX R_FRDS2 R_KGD1KGD2	F16P SUCCOA + ADP SUC PYR 24.0 x ADP + 20.0 x NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG			NADPHcyt + GISL DHAP + GA3P SUC + ATP CO2 + ACA 20.0 × NADmit + 24.0 × ATP CI CO2 + ACCOAmit + NADHmit MAL FISP + ADP F6P + E4P ADP FAD + SUC	
Ī	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_ISCILSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_FDH R_FUM1 R_FFK R_TAL1 R_ATPX R_FRDS2 R_CAR R_FADHX R_BIOMASSX	F16P SUCCOA + ADP SUC PYR 24.0 x ADP + 20.0 x NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG C02 20.0 x FADH2 + 24.0 x ADI 0.011004741 x NADPHcyt			NADPHcyt + GISL DHAP + GA3P SUC + ATP CO2 + ACA 20.0 × NADmit + 24.0 × ATP CI CO2 + ACCOAmit + NADHmit MAL FIGP + ADP F6P + E4P ADP FAD + SUC CO2 + SUCCOA + NADHmit 20.0 × FAD + 24.0 × ATP 0.001956398 × NADHcyt + 0.C	002690048 × NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCILSC2 R_SUC R_NADHX R_ACETR R_CIT R_PDH R_FUM1 R_FFK R_TAL1 R_ATPX R_FRDS2 R_KGD1KGD2 R_CAR R_FADHX R_BIOMASSX R_ACO	F16P SUCC0A + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG CO2 20.0 × FADH2 + 24.0 × ADI 0.011004741 × NADPHcyt Cl	Ρ		NADPHcyt + G15L DHAP + GA3P SUC + ATP CO2 + ACA 20.0 × NADmit + 24.0 × ATP CI CO2 + ACCOAmit + NADHmit MAL F16P + ADP F6P + E4P FAD + SUC CO2 + SUCCOA + NADHmit 20.0 × FAD + 24.0 × ATP 0.001956398 × NADHcyt + 0.0	002690048 × NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCILSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_PDH R_FUM1 R_FK R_TAL1 R_ATPX R_FADS2 R_KGD1KGD2 R_CAR R_FADHX R_BIOMASSX R_AC0 R_DAR	F16P SUCCOA + ADP SUC PYR 24.0 x ADP + 20.0 x NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG CO2 20.0 x FADH2 + 24.0 x ADI 0.011004741 x NADPHcyt Cl NADHcyt + DHAP	Ρ		NADPHcyt + GISL DHAP + GA3P SUC + ATP C02 + ACA 20.0 × NADmit + 24.0 × ATP CI C02 + ACCOAmit + NADHmit MAL FIGP + ADP F6P + E4P ADP F6P + E4P ADP F02 + SUC C02 + SUCCOA + NADHmit 20.0 × FAD + 24.0 × ATP 0.001956398 × NADHcyt + 0.0 ICI NADcyt + GP	002690048 × NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSC1LSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_PDH R_FUM1 R_FFK R_TAL1 R_FFK R_TAL1 R_ATPX R_FRDS2 R_CAR R_FADHX R_BIOMASSX R_ACO R_DAR R_ACS	F16P SUCC0A + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG C02 20.0 × FADH2 + 24.0 × ADI 0.011004741 × NADPHcyt CI NADHcyt + DHAP ACE + 2.0 × ATP	Ρ		NADPHcyt + G15L DHAP + GA3P SUC + ATP CO2 + ACA 20.0 x NADmit + 24.0 x ATP CI CO2 + ACCOAmit + NADHmit MAL F16P + ADP F6P + E4P ADP FAD + SUC CO2 + SUCCOA + NADHmit 20.0 x FAD + 24.0 x ATP 0.001955398 x NADHcyt + 0.0 ICI NADcyt + GP ACCOAcyt + 2.0 x ADP	002690048 × NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCILSC2 R_SUC R_NADHX R_ACETR R_CT R_PDH R_FUM1 R_PFK R_TALL R_ATPX R_FRDS2 R_KGD1KGD2 R_CAR R_FADHX R_FADHX R_FADHX R_ACS R_GND	F16P SUCC0A + ADP SUC PYR 24.0 x ADP + 20.0 x NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F0P S7P + GA3P ATP FADH2 + FUM NADmit + AKG C02 20.0 x FADH2 + 24.0 x ADI 0.011004741 x NADPHcyt CI NADHcyt + DHAP ACE + 2.0 x ATP P6G + NADPcyt	Ρ		NADPHcyt + GISL           DHAP + GA3P           SUC + ATP           C02 + ACA           20.0 x NADmit + 24.0 x ATP           CI           C02 + ACCOAmit + NADHmit           MAL           F16P + ADP           F6P + E4P           ADP           FAD + SUC           C02 + SUCCOA + NADHmit           20.0 x FAD + 24.0 x ATP           0.01956398 x NADHcyt + 0.0           ICI           NADCHcyt + GP           ACCOAcyt + 2.0 x ADP           NADPHcyt + C02 + RUSP	002690048 x NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_ISCILSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_FDH R_FUM1 R_FFK R_TAL1 R_ATPX R_FRDS2 R_CAR R_FADHX R_BIOMASSX R_ACO R_DAR R_ACS R_GND R_MAE1	F16P SUCCOA + ADP SUC PYR 24.0 x ADP + 20.0 x NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG C02 20.0 x FADH2 + 24.0 x ADI 0.011004741 x NADPHcyt Cl NADHcyt + DHAP ACE + 2.0 x ATP P6G + NADPcyt MAL + NADPmit	Ρ		NADPHcyt + G15L DHAP + GA3P SUC + ATP CO2 + ACA 20.0 × NADmit + 24.0 × ATP CI CO2 + ACCOAmit + NADHmit MAL F16P + ADP F6P + E4P ADP FAD + SUC CO2 + SUCCOA + NADHmit 20.0 × FAD + 24.0 × ATP 0.001956398 × NADHyt + 0.0 ICI NADCyt + GP ACCOAcyt + 2.0 × ADP NADPHcyt + CO2 + RUSP CO2 + PVP + NADPHmit	202690048 x NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCLSC2 R_SUC R_NADHX R_ACETR R_CT R_PDH R_FUM1 R_PFK R_TALL R_FROS2 R_KGD1KGD2 R_CAR R_FADHX R_BIOMASSX R_ACO R_DAR R_GND R_MAE1 R_PYC	F16P SUCC0A + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG CO2 20.0 × FADH2 + 24.0 × ADI 0.011004741 × NADPHcyt CI NADHcyt + DHAP ACE + 2.0 × ATP P6G + NADPcyt MAL + NADPmit CO2 + PYR + ATP	Ρ		NADPHcyt + G15L           DHAP + GA3P           SUC + ATP           C02 + ACA           20.0 x NADmit + 24.0 x ATP           CI           C02 + ACCOAmit + NADHmit           MAL           F16P + ADP           F6P + E4P           ADP           FAD + SUC           C0.0 x FAD + 24.0 x ATP           0.01956398 x NADHcyt + 0.0           ICI           NADCyt + GP           ACCOAcyt + 2.0 x ADP           NADPHcyt + C02 + RUSP           C02 + FVR + NADPHmit           OAA + ADP	002690048 × NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCILSC2 R_SUC R_NADHX R_ACETR R_CTT R_PDH R_FUM1 R_FFK R_TALL R_ATPX R_FRDS2 R_KGD1KGD2 R_CAR R_FADHX R_FADHX R_FADHX R_FADHX R_FADHX R_ACS R_GND R_MAE1 R_PYC R_TPI	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG C02 20.0 × FADH2 + 24.0 × ADI 0.011004741 × NADPHcyt Cl NADHcyt + DHAP ACE + 2.0 × ATP P6G + NADPcyt MAL + NADPmit C02 + PYR + ATP DHAP	Ρ		NADPHcyt + GISL DHAP + GA3P SUC + ATP CO2 + ACA 20.0 × NADmit + 24.0 × ATP CI CO2 + ACCOAmit + NADHmit MAL FISP + ADP F6P + E4P ADP FAD + SUC CO2 + SUCCOA + NADHmit 20.0 × FAD + 24.0 × ATP 0.001956398 × NADHcyt + 0.0 ICI NADcyt + GP ACCOAcyt + 2.0 × ADP NADPHcyt + CO2 + RUSP CO2 + PYR + NADPHmit OAA + ADP GA3P	002690048 x NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSC1LSC2 R_SUC R_PDC R_NADHX R_ACETR R_CT R_FDH R_FUM1 R_FFK R_TAL1 R_FFK R_TAL1 R_ATPX R_FRDS2 R_CAR R_FADHX R_BIOMASSX R_ACO R_DAR R_ACS R_ACS R_ACS R_MAE1 R_PYC R_TPI R_ENO	F16P SUCCOA + ADP SUC PYR 24.0 x ADP + 20.0 x NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG C02 20.0 x FADH2 + 24.0 x ADI 0.011004741 x NADHcyt Cl NADHcyt + DHAP ACE + 2.0 x ATP P6G + NADPrit C02 + PYR + ATP DHAP P2G	Ρ		NADPHcyt + G15L           DHAP + GA3P           SUC + ATP           C02 + ACA           20.0 x NADmit + 24.0 x ATP           CI           C02 + ACCOAmit + NADHmit           MAL           F16P + ADP           F6P + E4P           ADP           FAD + SUC           C0.0 x FAD + 24.0 x ATP           0.01956398 x NADHcyt + 0.0           ICI           NADCyt + GP           ACCOAcyt + 2.0 x ADP           NADPHcyt + C02 + RUSP           C02 + FVR + NADPHmit           OAA + ADP	002690048 × NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCILSC2 R_SUC R_NADHX R_ACETR R_CIT R_PDH R_FUM1 R_FPK R_TAL1 R_ATPX R_FRDS2 R_KGD1KGD2 R_CAR R_FADHX R_FADHX R_FADHX R_FADHX R_GND R_ACS R_GND R_MAE1 R_PYC R_TPI R_EN0 R_PCK	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG C02 20.0 × FADH2 + 24.0 × ADI 0.011004741 × NADPHcyt Cl NADHcyt + DHAP ACE + 2.0 × ATP P6G + NADPcyt MAL + NADPmit C02 + PYR + ATP DHAP	Ρ		NADPHcyt + G15L DHAP + GA3P SUC + ATP C02 + ACA 20.0 x NADmit + 24.0 x ATP C1 C02 + ACCOAmit + NADHmit MAL F16P + ADP F6P + E4P ADP FAD + SUC C02 + SUCCOA + NADHmit 20.0 x FAD + 24.0 x ATP 0.001956398 x NADHcyt + 0.0 ICI NADcyt + GP ACCOAcyt + 2.0 x ADP NADPHcyt + C02 + RUSP C02 + PYR + NADPHmit 0AA + ADP GA3P PEP	002690048 × NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSC1LSC2 R_SUC R_PDC R_NADHX R_ACETR R_CT R_FDH R_FUM1 R_FFK R_TAL1 R_FFK R_TAL1 R_ATPX R_FRDS2 R_CAR R_FADHX R_BIOMASSX R_ACO R_DAR R_ACS R_ACS R_ACS R_MAE1 R_PYC R_TPI R_ENO	F16P SUCC0A + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG C02 20.0 × FADH2 + 24.0 × ADI 0.011004741 × NADPHcyt CI NADPKyt + DHAP ACE + 2.0 × ATP P6G + NADPcyt MAL + NADPmit C02 + PYR + ATP DHAP P2G 0AA + ATP	Ρ		NADPHcyt + GISL           DHAP + GA3P           SUC + ATP           C02 + ACA           20.0 x NADmit + 24.0 x ATP           CI           C02 + ACCOAmit + NADHmit           MAL           F16P + ADP           F6P + EAP           ADP           FAD + SUC           C0.0 x FAD + 24.0 x ATP           0.001956398 x NADHcyt + 0.0           ICI           NADCyt + GP           ACCOAcyt + 2.0 x ATP           0.001956398 x NADHcyt + 0.0           ICI           NADCYt + CP           NADPHcyt + C02 + RUSP           C02 + PYR + NADPHmit           OAA + ADP           GA3P           PEP           C02 + PEP + ADP	002690048 x NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_ISCILSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_FDH R_FUM1 R_FFK R_TAL1 R_ATPX R_FRDS2 R_CAR R_FADHX R_FADHX R_BIOMASSX R_ACO R_DAR R_ACS R_GND R_MAE1 R_PYC R_FIN R_FIN R_FIN R_FIN R_PCK R_PCK R_PCK R_PCK	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG CO2 20.0 × FADH2 + 24.0 × ADI 0.011004741 × NADPHcyt CI NADHcyt + DHAP ACE + 2.0 × ATP P6G + NADPcyt MAL + NADPmit CO2 + PYR + ATP DHAP P2G OAA + ATP G6P	Ρ		NADPHcyt + GISL           DHAP + GA3P           SUC + ATP           C02 + ACA           20.0 x NADmit + 24.0 x ATP           CI           C02 + ACCOAmit + NADHmit           MAL           FIGP + ADP           F6P + E4P           ADP           FAD + SUC           C0.0 x FAD + 24.0 x ATP           0.001 y56398 x NADHcyt + 0.00           ICI           NADCyt + GP           ACCOAcyt + 2.0 x ADP           NADPHcyt + C02 + RUSP           C02 + PYE + NADPHmit           OAA + ADP           GA3P           PEP           C02 + PEP + ADP           F6P	002690048 × NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSC1LSC2 R_SUC R_PDC R_NADHX R_ACETR R_CT R_FDH R_FUM1 R_PFK R_TAL1 R_FRDS2 R_CAR R_FADHX R_FADHX R_BIOMASSX R_ACO R_DAR R_ACS R_ACS R_ACS R_ACS R_ACS R_ACS R_PYC R_TPI R_ENO R_PCK R_PCK R_PCK	F16P SUCCOA + ADP SUC PYR 24.0 x ADP + 20.0 x NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + GA3P ATP FADH2 + FUM NADmit + AKG CO2 20.0 x FADH2 + 24.0 x ADI 0.011004741 x NADPHcyt CI NADHcyt + DHAP ACE + 2.0 x ATP P6G + NADPcyt MAL + NADPmit CO2 + PYR + ATP DHAP P2G OAA + ATP G6P ATP NADcyt + GA3P P13G + ADP	Ρ		NADPHcyt + GISL           DHAP + GA3P           SUC + ATP           C02 + ACA           20.0 x NADmit + 24.0 x ATP           CI           C02 + ACCOAmit + NADHmit           MAL           FI6P + ADP           F6P + E4P           ADP           FAD + SUC           0.00 x FAD + 24.0 x ATP           0.001 y 55398 x NADHcyt + 0.00           ICI           NADCyt + GP           ACCOAcyt + 2.0 x ADP           NADPHcyt + C02 + RUSP           C02 + PYR + NADPHmit           QA3P           PEP           G04 + ADP           G64 + ADP           F6P           G6P + ADP           NADHcyt + P13G           P3G + ATP	002690048 x NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCLSC2 R_SUC R_NADHX R_ACETR R_CT R_PDH R_FUM1 R_PFK R_TAL1 R_ATPX R_FRDS2 R_CAR R_FADHX R_FRDS2 R_CAR R_FADHX R_FADHX R_FADHX R_ACS R_GND R_ACS R_GND R_MAE1 R_PGK R_GLD R_PGK R_CAT2	F16P           SUCCOA + ADP           SUC           SUC           PYR           24.0 × ADP + 20.0 × NADH           ACE           ACCOAmit + 0AA           NADmit + PYR           FUM           ATP + F6P           S7P + GA3P           ATP           FADH2 + FUM           NADmit + AKG           CO2           20.0 × FADH2 + 24.0 × ADI           0.011004741 × NADPHcyt           CI           NADHcyt + DHAP           ACE + 2.0 × ATP           P6G + NADPcyt           MAL + NADPmit           CO2 + PYR + ATP           P4G           OAA + ATP           G6P           ATP           NADcyt + GA3P           P13G + ADP           ACCOAcyt	Ρ		NADPHcyt + GISL           DHAP + GA3P           SUC + ATP           C02 + ACA           20.0 × NADmit + 24.0 × ATP           CI           C02 + ACCOAmit + NADHmit           MAL           F16P + ADP           F6P + E4P           ADP           FAD + SUC           C0.0 × FAD + 24.0 × ATP           0.001956398 × NADHcyt + 0.0           ICI           NADCYt + GP           ACCOAcyt + 2.0 × ATP           NADPHcyt + 2.0 × ADP           NADPHcyt + 2.0 × ADP           NADPHcyt + 2.0 × ADP           OAA + ADP           G6P + ADP           F6P           G6P + ADP           NADHcyt + P13G           P36 + ATP           ACCOAmit	002690048 x NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_ISCILSC2 R_SUC R_PDC R_NADHX R_ACETR R_CIT R_FDH R_FIN R_FRDS2 R_CAR R_FADHX R_ATPX R_FADHX R_FADHX R_ACO R_CAR R_ACO R_DAR R_ACS R_GND R_MAEI R_PYC R_FIN R_ENO R_PCK R_PGI R_PGK R_PGI R_PGK R_CAT2 R_TMITKI2b	F16P SUCCOA + ADP SUC PYR 24.0 × ADP + 20.0 × NADH ACE ACCOAmit + 0AA NADmit + 0AA NADmit + PYR FUM ATP + F6P S7P + 6A3P ATP FADH2 + FUM NADmit + AKG CO2 20.0 × FADH2 + 24.0 × ADI 0.011004741 × NADPHcyt CI NADHcyt + DHAP ACE + 2.0 × ATP P6G + NADPcyt MAL + NADPmit CO2 + PYR + ATP DHAP P2G OAA + ATP G6P ATP NADcyt + GA3P P13G + ADP ACCOAcyt XSP + E4P	Ρ		NADPHcyt + G15L           DHAP + GA3P           SUC + ATP           C02 + ACA           20.0 x NADmit + 24.0 x ATP           CI           C02 + ACCOAmit + NADHmit           MAL           F16P + ADP           F6P + E4P           ADP           FAD + SUC           C02 + SUCCOA + NADHmit           20.0 x FAD + 24.0 x ATP           0.001 956398 x NADHcyt + 0.00           ICI           NADCyt + GP           ACCOAcyt + 2.0 x ADP           NADPHcyt + C02 + RUSP           C02 + PYR + NADPHmit           OAA + ADP           GA3P           PEP           C02 + PEP + ADP           F6P           G6P + ADP           NADHcyt + P13G           P3G + ATP           ACCOAmit           GA3P + F6P	002690048 × NADPmit
	PYR NADPHmit P6G GA3P ATP MAL G15L ICI ACA RUSP NADPmit CI	R_ZWF R_FBA R_LSCLSC2 R_SUC R_NADHX R_ACETR R_CT R_PDH R_FUM1 R_PFK R_TAL1 R_ATPX R_FRDS2 R_CAR R_FADHX R_FRDS2 R_CAR R_FADHX R_FADHX R_FADHX R_ACS R_GND R_ACS R_GND R_MAE1 R_PGK R_GLD R_PGK R_CAT2	F16P           SUCCOA + ADP           SUC           SUC           PYR           24.0 × ADP + 20.0 × NADH           ACE           ACCOAmit + 0AA           NADmit + PYR           FUM           ATP + F6P           S7P + GA3P           ATP           FADH2 + FUM           NADmit + AKG           CO2           20.0 × FADH2 + 24.0 × ADI           0.011004741 × NADPHcyt           CI           NADHcyt + DHAP           ACE + 2.0 × ATP           P6G + NADPcyt           MAL + NADPmit           CO2 + PYR + ATP           P4G           OAA + ATP           G6P           ATP           NADcyt + GA3P           P13G + ADP           ACCOAcyt	Ρ		NADPHcyt + GISL           DHAP + GA3P           SUC + ATP           C02 + ACA           20.0 × NADmit + 24.0 × ATP           CI           C02 + ACCOAmit + NADHmit           MAL           F16P + ADP           F6P + E4P           ADP           FAD + SUC           C0.0 × FAD + 24.0 × ATP           0.001956398 × NADHcyt + 0.0           ICI           NADCYt + GP           ACCOAcyt + 2.0 × ATP           NADPHcyt + 2.0 × ADP           NADPHcyt + 2.0 × ADP           NADPHcyt + 2.0 × ADP           OAA + ADP           G6P + ADP           F6P           G6P + ADP           NADHcyt + P13G           P36 + ATP           ACCOAmit	002690048 × NADPmit

## **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*.

Simulation	Optimization	Visualizati
📩 Simu	lation Wizard	
WildT	ype Simulation	
Muta	nt Simulation	
Flux \	√ariability Analy	sis
Envir	onmental Cond	itions
Envir	onmental Cond	itions



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).

#### <u>Step 1</u>

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.

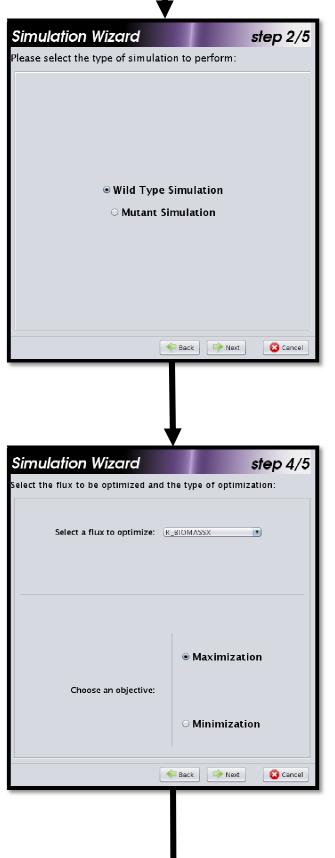
In the context of this tutorial, leaving the default selection is just fine.

Simulation Wizard	step 1/5
lease select the model to use in the simu	ulation:
Project:	
Small S. cerevisiae	
Mudel:	
Original Metabolic Model	×
Tack (	Next 🛛 🕄 Cancel

#### <u>Step 2</u>

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.** 

#### <u>Step 5</u>

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.

Simulatic	on Wizard	step 5/5
efine enviro	nmental/external cond	itions if necessary:
search:		
	external fluxe	s
Name		
R_SUC R_ACETR R_CAR R_GPP		
name	edit lower upper	🏺 add to env. conditions
	modified flux	es
Name	Lower	Upper
	. emove from environme	ntal conditions
Use Conditions		T
	🗲 Ва	ck 😥 Finish 🛛 😵 Cancel

#### <u>Step 6</u>

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.

Image: State in the state	fild Type 🛎			Wild Type 💐	Clipboard
* Small S. cerevisiae       simulation method       FBA       R_SDHcomplex       0.1179         * Small S. cerevisiae       R_FBA       0.61545       0.1179         * Small S. cerevisiae       0.1179       R_FBA       0.61545         * Metabolites: ReactionSe       R_DC       0.45793         * Metabolites: Metabolites: Metabolites: Stoichiometric Model: St       R_CIT       0.2069         * Wild Type       0.01179       R_PPK       0.61545         * Optimization Results       PFK       0.61545         * Optimization Results       R_CAR       1.10784         * Variability Analysis Results       R_ACS       0.2338         * Variability Analysis Results       R_DAR       0.0069         * TPI       0.020179       R_ROD         * Proc       0.22535       0.1179         * Proc       0.226795       R_FUMI         * Wild Type       0.00589       R_CAR       1.10784         * R_ADHX       0.00589       R_BIOMASSX       87.6551         R_ACO       0.2338       R_DAR       0.0069         R_CS       0.25723       R_GND       0.25335         R_PYC       0.22508       R_FIPI       0.60475         R_ENO       1.20819	aximized flux (R_BIOMASSX) 87.6551 Flux Values		87.6551	maximized flux (R_BIOMASSX)	ΟρτΓιυχ
simulation method       FBA       R_SDHcomplex       0.1179         minimized of the section Set or section Set of the set of the section Set of the section Set of the section Set of the set of the section Set of the set of the section Set of the section Set of the section Set of the set of the set of the set of the sect of the set of the set of the set of the set		Flux			
Visional Model       R FBA       0.61545         Flux Bounds: ReactionSe       R_ISC1L5C2       0.1179         Metabolites: Metabolites       Metabolites: Metabolites       R_PPC       0.45793         Stoichiometric Model: St       R_ODH       0.20069         Simulation Results       R_FHA       0.00174         Optimization Results       R_FHA       0.001545         Proc       0.2358       0.1179         R_FIM1       0.1179       0.1179         R_FIM1       0.1179       0.1179         R_FIM1       0.1179       0.08445         R_FOH       0.26795       0.1179         R_FIM1       0.1179       0.08445         R_FOH       0.26795       0.1179         R_FOH       0.26795       0.1179         R_FOH       0.26795       0.1179         R_FOH       0.26795       0.1179         R_CAR       1.10784       0.008445         R_FBDHX       0.0089       0.2358         R_CAR       0.1069       0.2358         R_ACS       0.25723       0.25723         R_GND       0.25335       0.25723         R_GND       0.225335       0.25723         R_ENO </td <td>simulation method FBA R SDHcomplex 0.1179</td> <td>R SDHcomplex</td> <td>FBA</td> <td>s imulation method</td> <td></td>	simulation method FBA R SDHcomplex 0.1179	R SDHcomplex	FBA	s imulation method	
• Model           R_LSC1LSC2           0.1179             Flux Bounds: ReactionSe           R_PDC           0.45793             Metabolites: Metabolites           R_IACETR         0.20069           0.20069             Stoichiometric Model: St           R_FIM           0.61545             Simulation Results           PFK           0.61545             Optimization Results           R_ACB           0.00794             Optimization Results           R_ACA           0.008445             Variability Analysis Results           R_ACO           0.20535             Variability Analysis Results           R_ACS           0.25335             R_FIP           0.025335             R_FIP           0.22508             R_FIP           0.225335             R_FIP           0.225335             R_FIP           0.22508             R_FIP           0.22535             R_FIP	R_ZWF 0.25335	R_ZWF			Small S. cerevisiae
Image: Section Set Flux Bounds: Reaction Set Flux Bounds: Set Flux Bound: Set Flux Bounds: Set Flux Bounds: Set Flux	R_FBA 0.61545	R_FBA			
Flux Bounds: ReactionSe       R_NADHX       0.10007         Metabolites: Metabolites       R_ACETR       0.20069         Stoichiometric Model: St       R_CIT       0.2358         Simulation Results       R_PDH       0.26795         Muid Type       0ptimization Results       R_FK       0.61545         Privation Results       R_CAR       1.10784         Privation Results       R_FADHX       0.00589         Privation Results       R_ACO       0.2358         R_ENVERTION Results       R_CAR       1.10784         R_ACO       0.2358       R_ACO         R_ACO       0.2358       R_DAR         Matabolity Analysis Results       R_ACS       0.25723         R_FIN       0.60475       R_ENO         R_ENO       1.20819       R_ENO					🔻 🎦 Original Model
Image: Metabolites: Metabo					
Implementation Results       R_CIT       0.2358         Implementation Results       R_PDH       0.26795         Implementation Results       R_FIM1       0.1179         Implementation Results       R_ACD       0.008445         Implementation Results       R_ACAR       1.10784         Implementation Results       R_ACAR       0.00589         Implementation Results       R_ACO       0.2358         Implementation Results       R_ACO       0.25723         Implementation Results       R_ACS       0.25723         Implementation Results       R_FIPIC       0.60475         R_ENO       1.20819       1.20819					Flux Bounds: ReactionSe
Image: Stoichiometric Model: St       R_Cli       0.2358         Image: Stoichiometric Model: St       R_PDH       0.26795         Image: Simulation Results       R_FIMI       0.1179         Image: Optimization Results       R_KGD1KGD2       0.1179         Image: Optimization Results       R_FADHX       0.00589         Image: Province Results       R_ACO       0.2358         Image: Variability Analysis Results       R_ACS       0.26795         Image: Variability Analysis Results       R_R       R_IPK         Image: Variability Analysis Results       R_R       R_IPK         Image: Variability Analysis Results       R_R       0.01699         R_R       R_IPK       0.20723         R_R       R_IPK       0.201699         R_IPK       0.201699       R_IPK         R_IPK       0.201699       R_ROB         R_IPK       0.225335       R_ROB         R_IPK       0.60475       R_ROB         R_IPK       0.60475       R_ENO         R_IPK       0.22508       R_IPK					Metabolites: Metabolites
Image: Standard Holder Hold					
* invitation Results       R       PFK       0.61545         * Wild Type       R_TAL1       0.08445         Optimization Results       R_KGD1KGD2       0.110784         Environmental Conditions       BIOMASSX       87.6551         Variability Analysis Results       PDAR       0.01669         R_ACS       0.25723       R_GND         R_TPI       0.60475       R_FI         BIOMASSX       87.651       R_ACS         BOD       0.25335       R_PYCC         R_FI       0.60475       R_ENO         R_ENO       1.20819       R_ENO					🧮 Stoichiometric Model: St
Wild Type       R_TAL1       0.08445         Optimization Results       R_KOD1KGD2       0.1179         Environmental Conditions       R_FADHX       0.00589         Variability Analysis Results       R_ACO       0.2388         R_ACS       0.01669         R_ACS       0.25723         R_FPVC       0.22508         R_TPI       0.60475         R_ENO       1.20819					
Wild Type         R_KGD1KGD2         0.1179           Optimization Results         R_CAR         1.10784           Environmental Conditions         R_BIOMASSX         87.6551           Variability Analysis Results         R_CAR         0.0169           Variability Analysis Results         R_GND         0.25335           R_PYC         0.22508         R_PYC           R_ENO         1.20819         0.00175					Simulation Results
Optimization Results         R         1.10784           Environmental Conditions         R-BIOMASSX         87.6551           Variability Analysis Results         R-ACO         0.2358           PYC         0.01069         R-ACS         0.25723           R_GND         0.25335         R-PYC         0.22508           R_TPI         0.60475         R-ENO         1.20819					🦀 Wild Type
Optimization Results         R=FADHX         0.00589           Environmental Conditions         R=BIOMASSX         87.6551           Variability Analysis Results         R=ACO         0.2358           Variability Analysis Results         R=ACS         0.25723           R=PYC         0.22508         R=PYC           R=TPI         0.60475         R=ENO           R=ENO         1.20819         1.20819				0	👹 wiid Type
R       BIOMASSX       87.6551         Environmental Conditions       R_ACO       0.2358         Variability Analysis Results       R_DAR       0.01069         R_CS       0.25723       R_GND       0.25335         R_PYC       0.22508       R_PYC       0.26075         R_ENO       1.20819       R_ENO       1.20819					Optimization Besults
Environmental Conditions         R_ACO         0.2358           Variability Analysis Results         R_DAR         0.01069           Variability Analysis Results         R_GND         0.25723           R_FYC         0.22508         R_TPI           R_ENO         1.20819         0.2010					
R_DAR         0.01069           R_ACS         0.25723           R_GND         0.25335           R_PYC         0.22508           R_TPI         0.60475           R_ENO         1.20819					Environmental Conditions
Variability Analysis Result         R_ACS         0.25723           R_GND         0.25335           R_PYC         0.22508           R_TPI         0.60475           R_ENO         1.20819					
R_GND 0.25335 R_PYC 0.22508 R_TPI 0.60475 R_ENO 1.20819					🔲 Mariahility Apolysis Results
R_PYC 0.22508 R_TPI 0.60475 R_ENO 1.20819					Variability Analysis Results
R_TPI 0.60475 R_ENO 1.20819					
R_ENO 1.20819					
K_FGI 0.4787	R_PGI 0.4787				
R_HXK 1					
search:	search:	search:			

### **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 (<u>www.OptFlux.org/manual/OptFluxManual.pdf</u>). 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.

#### <u>Step 2</u>

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.



#### <u>Step 3</u>

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.

			Knockouts
earch:			R_FUM1
Fluxes			_
Name	Lower Bound	Upper Bound	
R_SDHcomplex	0.0	10000.0	T I
R_ZWF	0.0	10000.0	
REIBA	-10000.0	10000.0	
R LSC1LSC2	-10000.0	10000.0	
R_PDC	0.0	10000.0	
R NADHX	0.0	10000.0	
R_CIT R_PDH	0.0	10000.0	
R FUM1	-10000.0	10000.0	
R PEK	0.0	10000.0	
R TAL1	-10000.0	10000.0	
R ATPX	0.0	10000.0	
R FRDS2	0.0	10000.0	
R_KGD1KGD2	0.0	10000.0	
R FADHX	0.0	10000.0	
D DIMMACOV	0.0	10000.0	
📫 ad	d selected flux to	knockouts list	
111	٨		
Algorithm MO	MA		Remove
RO	ом		
			-
		🔶 Back	📫 Next 🛛 🙆 Cancel

#### <u>Step 6</u>

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**.

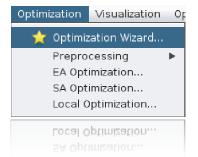
Left-clicking the object will launch the simulation solution viewer in the visualization area on the right. This time one can notice, for instance, that the biomass growth associated flux has a value lower than the one in the wild-type simulation.

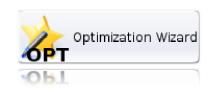
ipboard	Wild Type SimulationSol	lution ( 📓			
r <b>F</b> lux	maximized flux (R_BIOMASSX)	80.7327		Flux Values	
Project		FBA	Flux	Value	
Small S. cerevisiae	s imulation method	FBA	R_ACETR	0.24407	4
Small S. cerevisiae			R_ACO	0.21718	
			R_ACS	0.23692	
🛚 🚮 Original Model	knockouts		R_ADH1	0	
Flux Bounds: ReactionSet (ins	R FUM1		R_ALD6	0.48099	
E Flux Bounds: ReactionSet (ins	1.001		R_ATPX	0	
🥅 Metabolites: MetaboliteSet (ir			R_BIOMASSX	00.7327	
			R_CAR	0.94138	
📰 Stoichiometric Model: Stoichic			R_CAT2	0	
			R_CIT	0.21718	
Simulation Results			R_DAR R ENO	0.00985 1.28059	
🍙 Wild Type			R FADHX	0	
🥁 wild Type			R_FBA	0.65569	
🌼 FUM mutant	U I		R FBP	0	
			R FRDS2	õ	
Optimization Results			R FUM1	0	
			R GLD	1.33982	
Environmental Conditions			R GND	0,20372	
			R GPM	1.28059	
🔚 Variability Analysis Results			R_GPP	0	
			R_HXK	1	
			R_IDP1	0.21718	
			R_KGD1KGD2	0.10859	
			R_LSCIESC2	0.10859	1
			search:		
			Jea chi		

## **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**

#### <u>Step 1</u>

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.

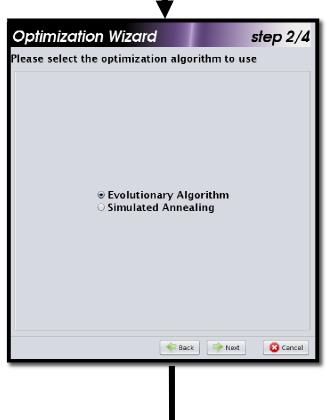
In the context of this tutorial, leaving the default selection is just fine.

Optimization Wizard	step 1/4				
lease select the model to use in the optimization:					
Project:					
Small S.cerevisiae	×				
Mudel:					
Original Model					
deck	Next Cancel				

#### <u>Step 2</u>

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).



#### <u>Step 3</u>

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.

EA Parameters		
representation	Set-Based Representation	<b>T</b>
population size	100	
generations	20	
knockouts	8	
variable size		
use essential genes		
Simulation Parameters		•
desired flux	R_SUC	
substrate	R_HXK	
simulation method	FBA	
objective function	BPCY	•

#### <u>Step 4</u>

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.

Optimiza	tion Wizard	step 4/4			
efine enviro	nmental/external o	onditions if necessary:			
search:					
	external flux	ies			
Name		()			
LX M SUC LX M CO2					
LX M CO2					
LX M ACL					
	edit				
name	lower upper				
		💗 add to env. conditions			
	modified flux	es			
Name	Lower	Upper			
	A				
🎂 remove tram environmental conditions					
Use Conditions		Y			
	🔶 B	ack 🛛 🧼 Finish 👘 🔀 Cancel			

#### <u>Step 5</u>

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.

New Project Wizard	imulation Wizard 🔰	Optimization Wizard			
board	Wild Type Sim	nulationSolution ( So	olutionSetBox (inst 🞇		
эт <b>F</b> ωх	]	solutions		details	
Project	fitness	desired flux	maximized flux	flux to maximize (R_BIOMASSX)	80.732
	8.7666	0.10859	80.7327		80.732
Small S. cerevisiae	8.7666	0.10859	80.7327	desired flux (R_SUC)	0.1085
	8.7666	0.10859	80.7327		
🕨 🎦 Original Model	8.7666	0.10859	80.7327	substrate (R_HXK)	1
	0	0	87.6551	fitness (BPCY)	8.7666
<ul> <li>Simulation Results</li> </ul>				nuicss (bi city	0.7000
ild Type				simulation method	FBA
100				knockouts	
🎡 FUM mutant				KHOCKOULS	
🔻 🔚 Optimization Results				R_FADHX	
🍅 EA/100/10 - max succ					
Environmental Conditions					
🔚 Variability Analysis Results					
				view complete info	

## **4 – GRAPH VISUALIZATION**

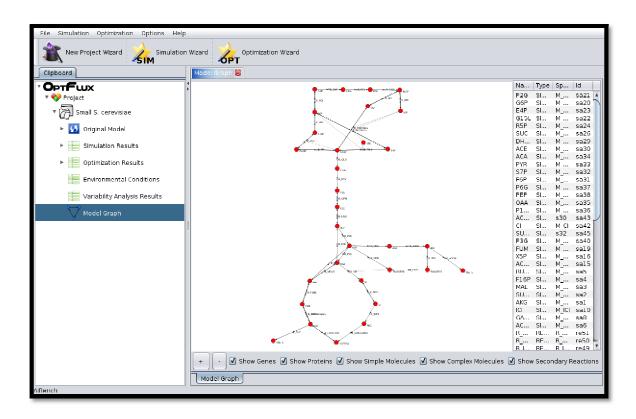
For an improved visualization of the results, the user can also load a CellDesigner SBML file which provides a graphical representation of the metabolism.

Continue     Project     Small     Small     Small     Sin     Sin     Op	File Optimization Simulation Options rename element	Load ► Save ►	Load SBML for visualization Load Critical Reactions
► Bee ob	options rename element		

In the provided archive, a file named <u>"smallSCgraph.xml"</u> 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.

Project	Small S. cerevisiae	Project to load the models to				
Model Type	CSBML	Select the type of model				
File	l/model/smallSCgraph.xml find	Select the file containing the model				
OK Cancel						
	OK Cancel					

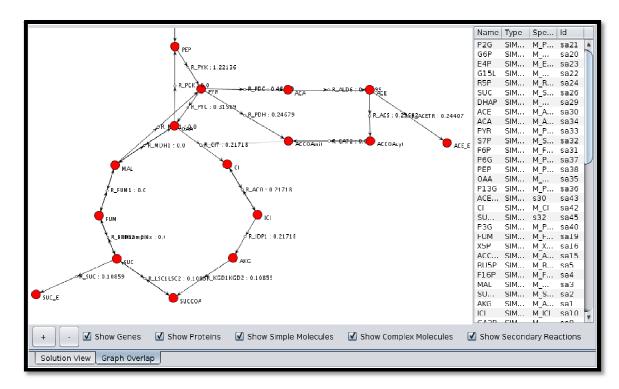
A new object will be placed in the Project tree under the name Model Graph.



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

File Simulation Optimization Options Help						
New Project Wizard Simulation Wizard Optimization Wizard						
_ Clipboard	Model Graph	EA/100/10 - max succ 😫				
		solutions deta		details	s	
🔻 💝 Project	fitness	desired flux	maximized flux	Hux to maximize (R_BIOMASSX)	00.7327	
Small S. cerevisiae	8.7666 8.7666 8.7655	0.10859 0.10859 0.10859	80.7327 80.7327 80.7327	desired flux (R_SUC)	0.10859	
Griginal Model	8.7666	0.10859	90.7327 87.6551	substrate (R_HXK)	1	
Simulation Results		ŭ	A7.0.1.1	fitness (BPCY)	8.7666	
🔻 🔚 Optimization Results				simulation method	FBA	
🎄 EA/100/10 - max succ				knockou	its	
Environmental Conditions				R_FADHX		
Variability Analysis Results						
Model Graph						
•						
					]	
				view complet	l # info	
	Uptimization :	Solutions				
AlBench	_					

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.