A software platform for genome-scale metabolic models simulation, reconstruction and visualization

User manual

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GEMSiRV

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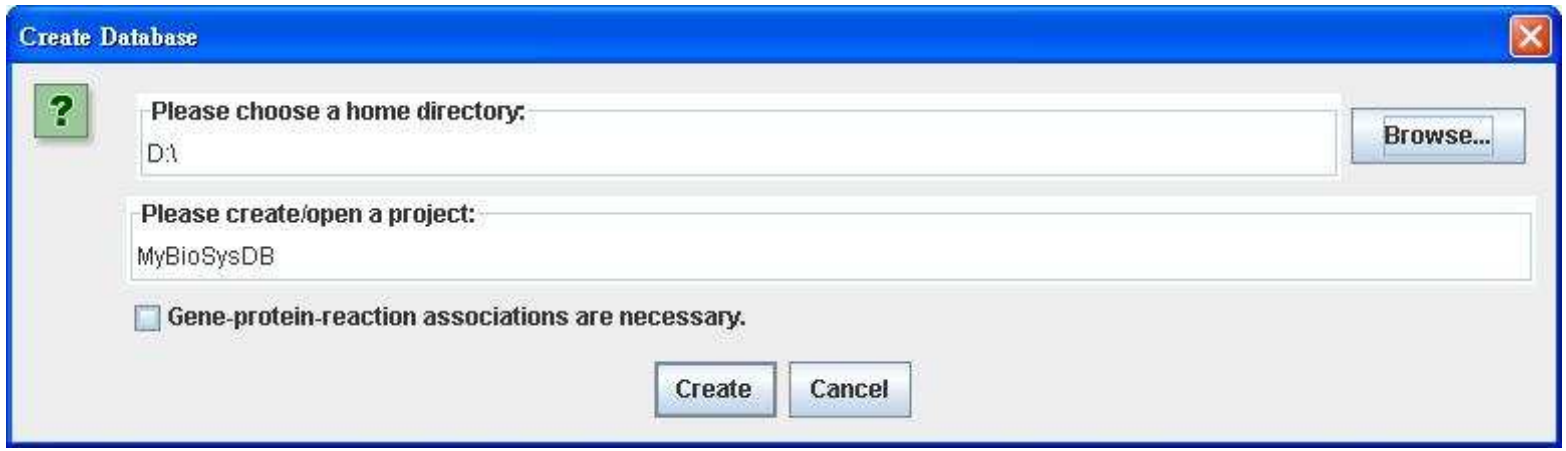
Basically, a metabolic network is an assembly of biochemical reactions. While information about reactions is sufficient for modeling the network, the more information on associated genes or proteins, the more useful for the investigation of cellular responses in gene or protein level. Gene-protein-reaction (GPR) associations can be described in two-layer relations: “gene and protein” and “protein and reaction”, which are usually saved in spreadsheet format, the required information and available models are summarized in http://sb.nhri.org.tw/GEMSiRV/en/Metabolic_Models. On the other hand, published genome-scale metabolic models are commonly exchanged in Systems Biology Markup Language (SBML) format, but the protein information is lost or can not be recovered to the two-layer relation. Therefore, GEMSiRV provides different schema for these two types of metabolic reconstructions. If you want to create a project with clear two-layer relations of GPR associations, please check the checkbox of Gene-protein-reaction associations are necessary to generate the three-index schema (gene, protein and reaction indices) for reconstruction. Otherwise, GEMSiRV will generate the two-index schema (gene and reaction indices) automatically.

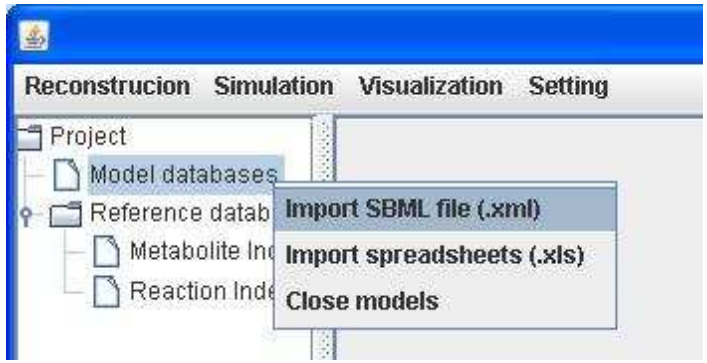


Reconstruction

Click on Reconstruction in the menu bar to open **Model databases** and **Reference databases**.

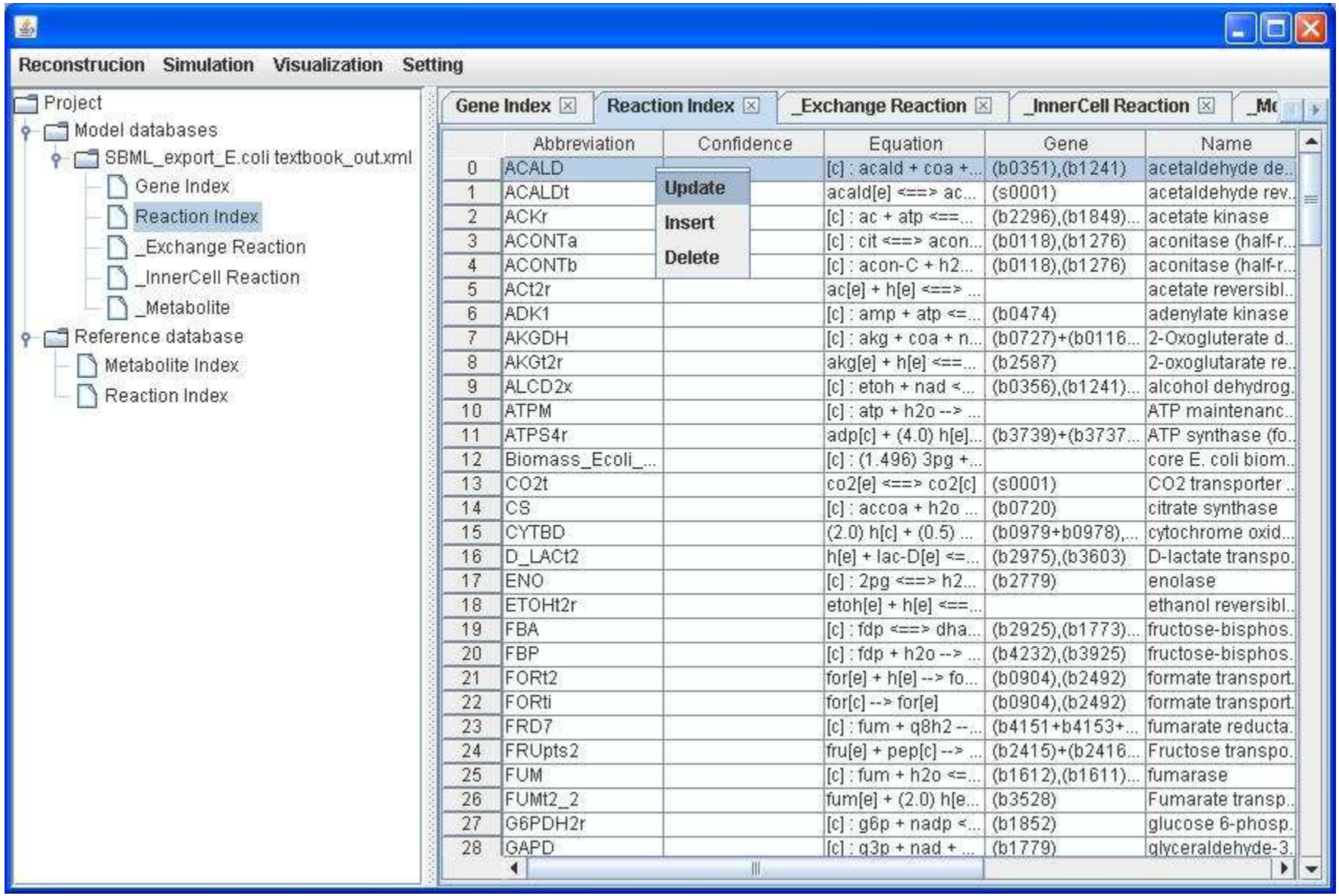
Model importing and editing

Right click on **Model databases** to Import SBML file (.xml) or to Import spreadsheets (.xls), you can import a metabolic model in SBML/spreadsheet format. Some existing metabolic models can be found and downloaded from http://sb.nhri.org.tw/GEMSiRV/en/Metabolic_Models



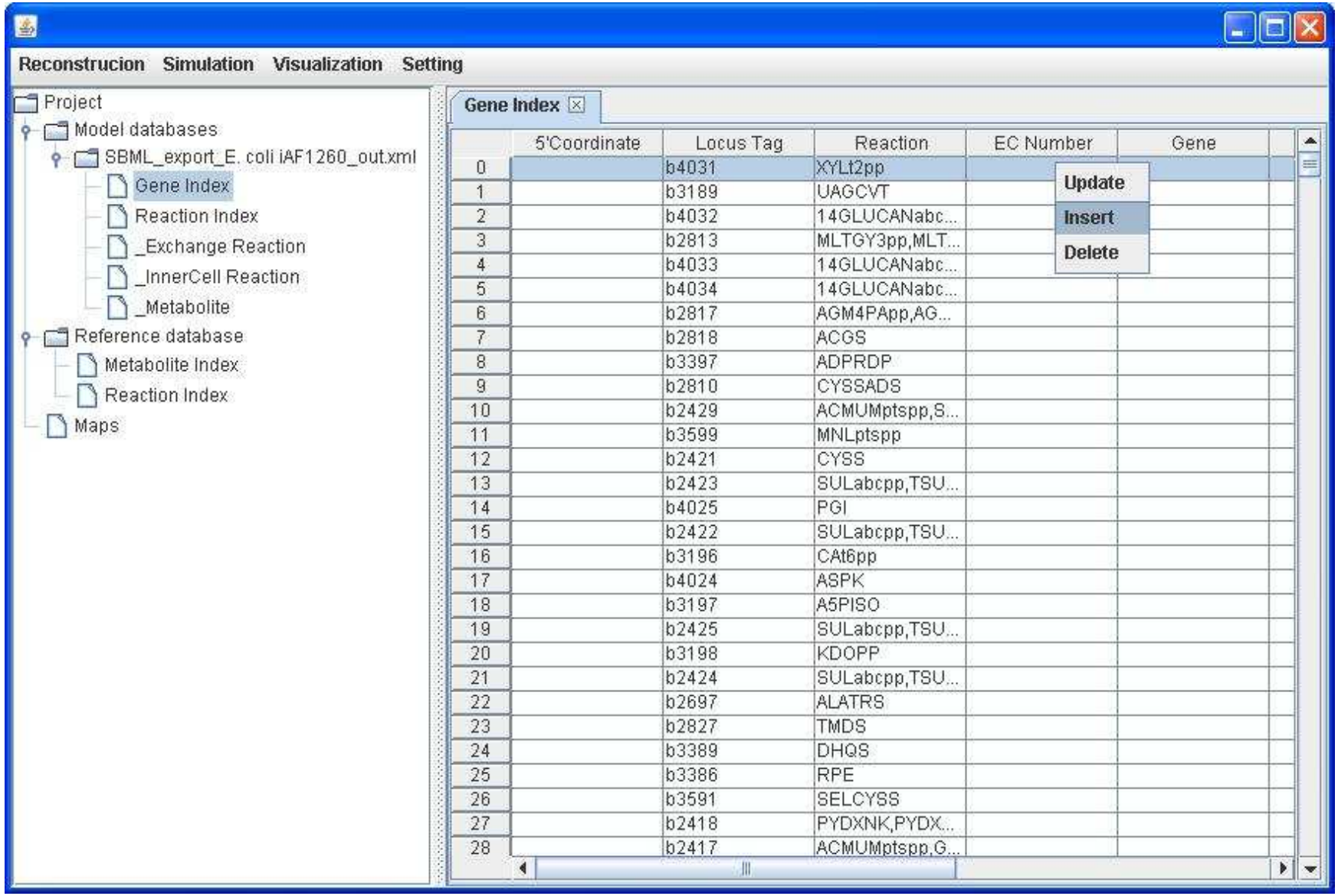
You can directly edit/update the content of the imported model by right clicking on a cell.

In table of Reaction Index:



	Abbreviation	Confidence	Equation	Gene	Name
0	ACALD		[c] : acald + coa +...	(b0351),(b1241)	acetaldehyde de..
1	ACALDt		acald[e] <==> ac...	(s0001)	acetaldehyde rev..
2	ACKr		[c] : ac + atp <==...	(b2296),(b1849)...	acetate kinase
3	ACONTa		[c] : cit <==> acon...	(b0118),(b1276)	aconitase (half-r...
4	ACONTb		[c] : acon-C + h2...	(b0118),(b1276)	aconitase (half-r...
5	ACt2r		ac[e] + h[e] <==> ...		acetate reversibl..
6	ADK1		[c] : amp + atp <=...	(b0474)	adenylate kinase
7	AKGDH		[c] : akg + coa + n...	(b0727)+(b0116...	2-Oxogluterate d..
8	AKGt2r		akg[e] + h[e] <==...	(b2587)	2-oxoglutarate re..
9	ALCD2x		[c] : etoh + nad <...	(b0356),(b1241)...	alcohol dehydrog..
10	ATPM		[c] : atp + h2o --> ...		ATP maintenanc..
11	ATPS4r		adp[c] + (4.0) h[e]...	(b3739)+(b3737...	ATP synthase (fo..
12	Biomass_Ecoli_...		[c] : (1.496) 3pg +...		core E. coli biom..
13	CO2t		co2[e] <==> co2[c]	(s0001)	CO2 transporter ..
14	CS		[c] : accoa + h2o ...	(b0720)	citrate synthase
15	CYTBD		(2.0) h[c] + (0.5) ...	(b0979+b0978),...	cytochrome oxid...
16	D_LACt2		h[e] + lac-D[e] <=...	(b2975),(b3603)	D-lactate transpo..
17	ENO		[c] : 2pg <==> h2...	(b2779)	enolase
18	ETOHt2r		etoh[e] + h[e] <==...		ethanol reversibl..
19	FBA		[c] : fdp <==> dha...	(b2925),(b1773)...	fructose-bisphos..
20	FBP		[c] : fdp + h2o --> ...	(b4232),(b3925)	fructose-bisphos..
21	FORt2		for[e] + h[e] --> fo...	(b0904),(b2492)	formate transport..
22	FORti		for[c] --> for[e]	(b0904),(b2492)	formate transport..
23	FRD7		[c] : fum + q8h2 --...	(b4151+b4153+...	fumarate reducta..
24	FRUpts2		fru[e] + pep[c] --> ...	(b2415)+(b2416...	Fructose transpo..
25	FUM		[c] : fum + h2o <=...	(b1612),(b1611)...	fumarase
26	FUMt2_2		fum[e] + (2.0) h[e...	(b3528)	Fumarate transp..
27	G6PDH2r		[c] : g6p + nadp <...	(b1852)	glucose 6-phosp..
28	GAPD		[c] : g3p + nad + ...	(b1779)	glyceraldehyde-3..

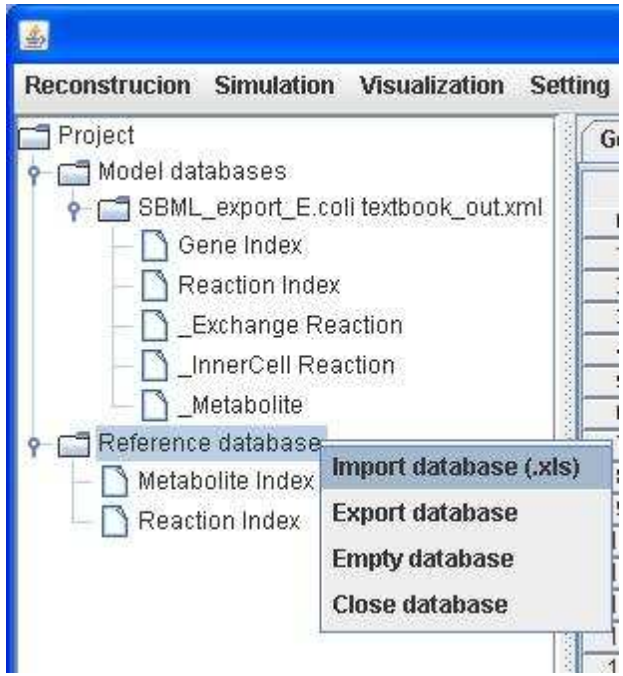
In table of Gene Index:



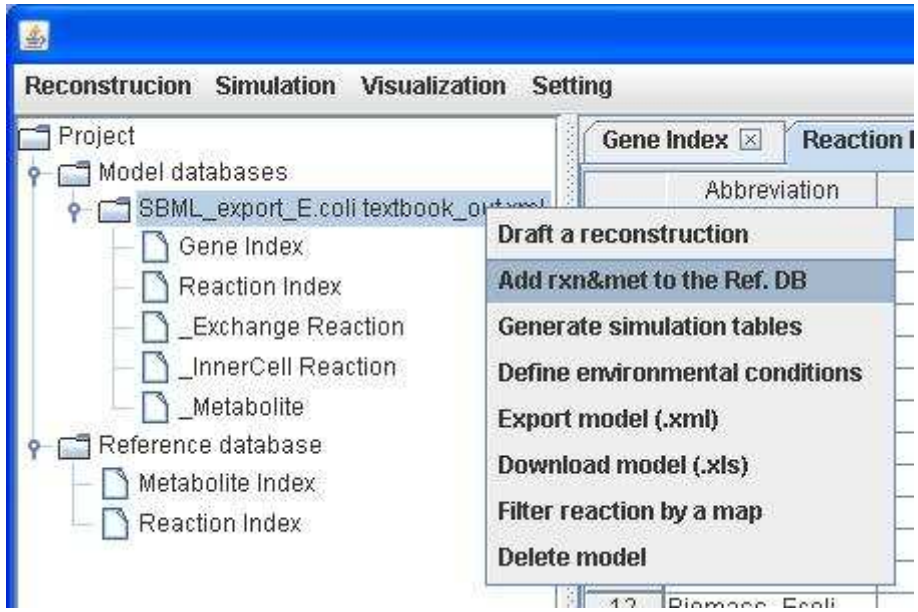
	5'Coordinate	Locus Tag	Reaction	EC Number	Gene
0		b4031	XYLt2pp		
1		b3189	UAGCVT		
2		b4032	14GLUCANabc...		
3		b2813	MLTGY3pp,MLT...		
4		b4033	14GLUCANabc...		
5		b4034	14GLUCANabc...		
6		b2817	AGM4PApp,AG...		
7		b2818	ACGS		
8		b3397	ADPRDP		
9		b2810	CYSSADS		
10		b2429	ACMUMptspp,S...		
11		b3599	MNLptspp		
12		b2421	CYSS		
13		b2423	SULabcpp,TSU...		
14		b4025	PGI		
15		b2422	SULabcpp,TSU...		
16		b3196	CAt6pp		
17		b4024	ASPK		
18		b3197	A5PISO		
19		b2425	SULabcpp,TSU...		
20		b3198	KDOPP		
21		b2424	SULabcpp,TSU...		
22		b2697	ALATRS		
23		b2827	TMDS		
24		b3389	DHQS		
25		b3386	RPE		
26		b3591	SELCYSS		
27		b2418	PYDXNK,PYDX...		
28		b2417	ACMUMptspp,G...		

Reference database construction

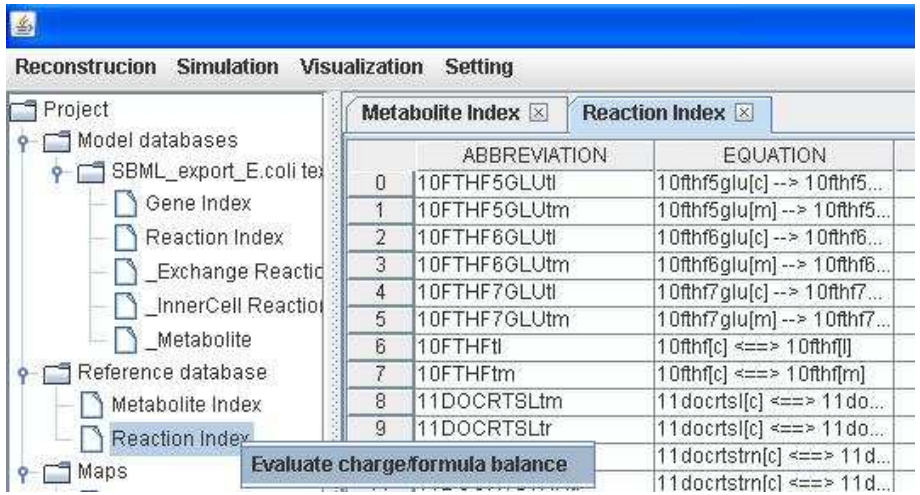
Right click on **Reference database** to Import database (.xls), you can import a reference database to construct your own reference database. Available reference databases including BiGG, KEGG and Model SEED databases can be found and downloaded from http://sb.nhri.org.tw/GEMSiRV/en/Reference_Databases.



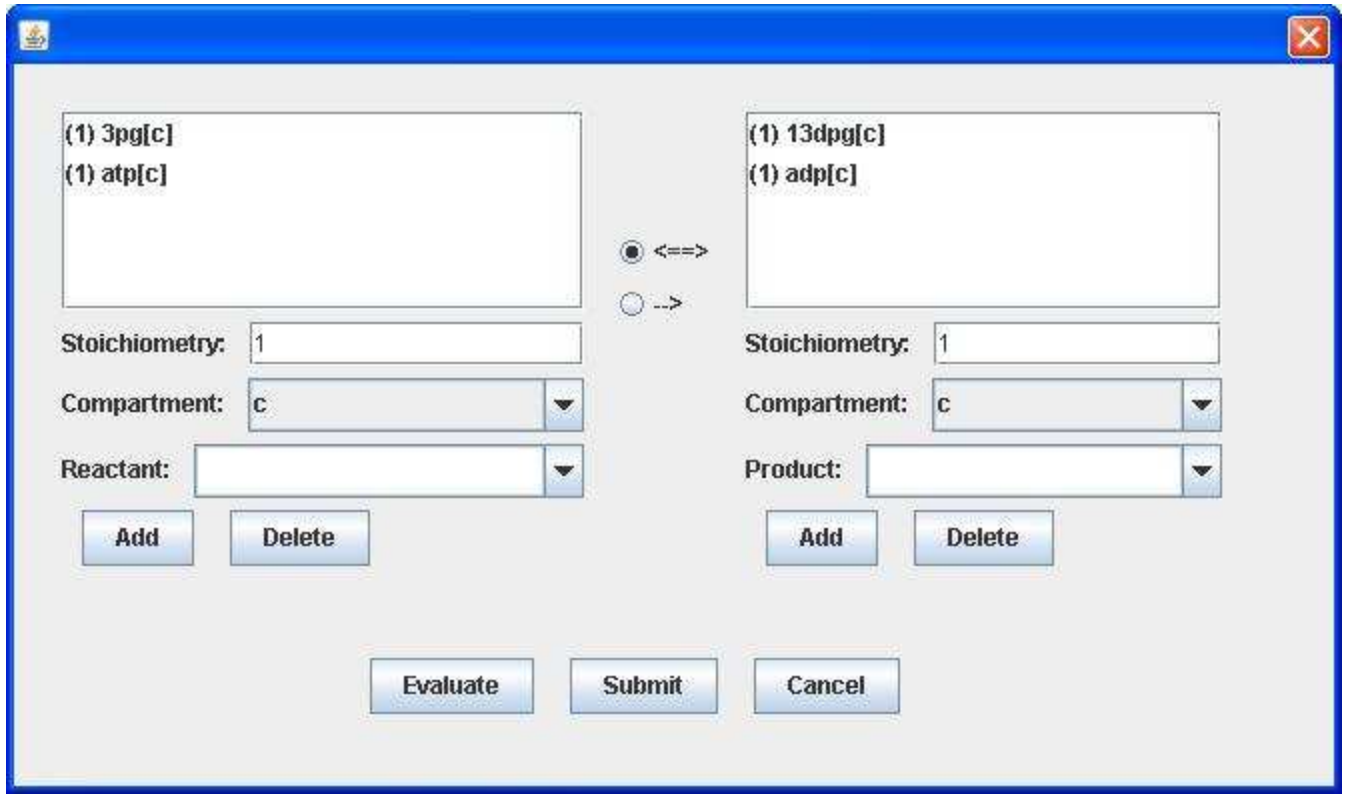
Or, you can right click on the model you imported to Add rxn&met to the Ref. DB, so that you can add the information about metabolites and reaction described in the model to the reference database that you have created.



You can right click on the Reaction Index of **Reference database** to evaluate charge/mass balance of equation.



You can add/edit the equation of reaction by using equation dialog or type directly. For example, for reaction PGK (phosphoglycerate kinase), you can type “[c] : 3pg + atp <==> 13dpg + adp” in its equation or you can enter the EQUATION dialog to edit its content.



Draft reconstruction generation

Firstly, you need to have a close related model organism whose metabolic reconstruction has been built already.

Then you need to prepare a blank reconstruction containing gene information of your interest strain. This file can be generated by GBKParser (http://sb.nhri.org.tw/GEMSiRV/en/GBKParser). However, you need to add the corresponding orthologous genes to the column of Ref-BLAST.

For example, we want to draft a reconstruction of *Salmonella enteric subsp. Enteric serovar Typhimurium str.* LT2 (SLT2) by mapping to

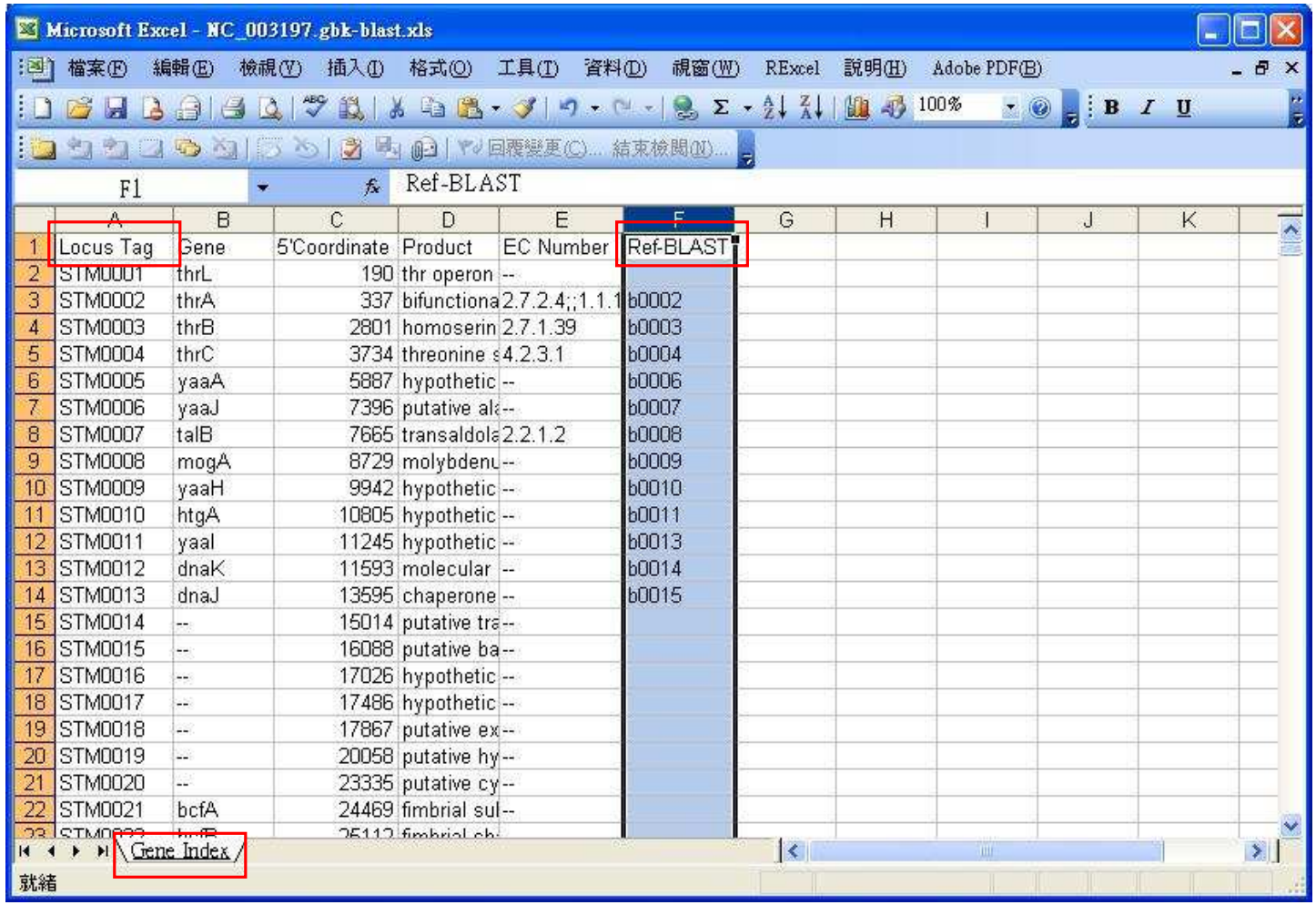
the reconstruction model *i*AF1260 of *Escherichia coli str. K-12 substr.* MG1655 (ECO).

Therefore, we download the gbk files of these two strains from RefSeq (http://www.ncbi.nlm.nih.gov/RefSeq). With available NC_003197.gbk and NC_000913.gbk files for SLT2 and ECO respectively, we then use GBKParser to parse basic gene information and amino acid sequences. In addition, we download the metabolic model *i*AF1260 from BiGG (http://bigg.ucsd.edu/) and modify it with TextReplacer (http://sb.nhri.org.tw/GEMSiRV/en/TextReplacer). The ready-to-use model can be found and downloaded from http://sb.nhri.org.tw/GEMSiRV/en/Metabolic_Models.

The amino acid sequence files for SLT2 and ECO can be used to generate the reciprocal orthologous-gene pairs by BLASTP or other available software. For example, MrBac (http://sb.nhri.org.tw/MrBac) can be used to generate the needed file. However, the detailed procedure is not described here.

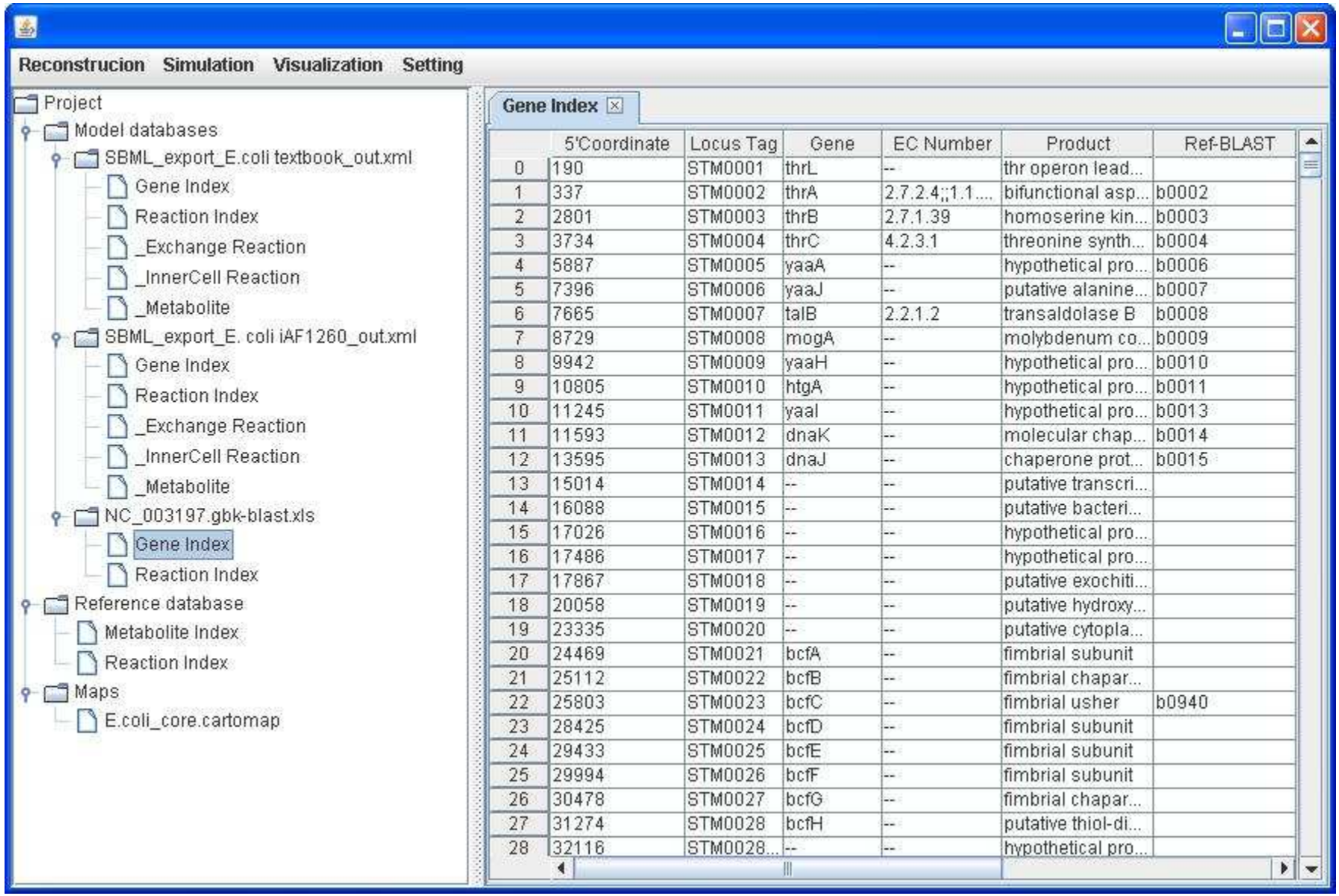
The basic gene information parsed from the gbk file is outputted to a spreadsheet file, e.g. NC_003197.gbk.xls, which can be imported into GEMSiRV directly. Right click on **Model Databases** to Import spreadsheet (.xls).

Original spreadsheet file:



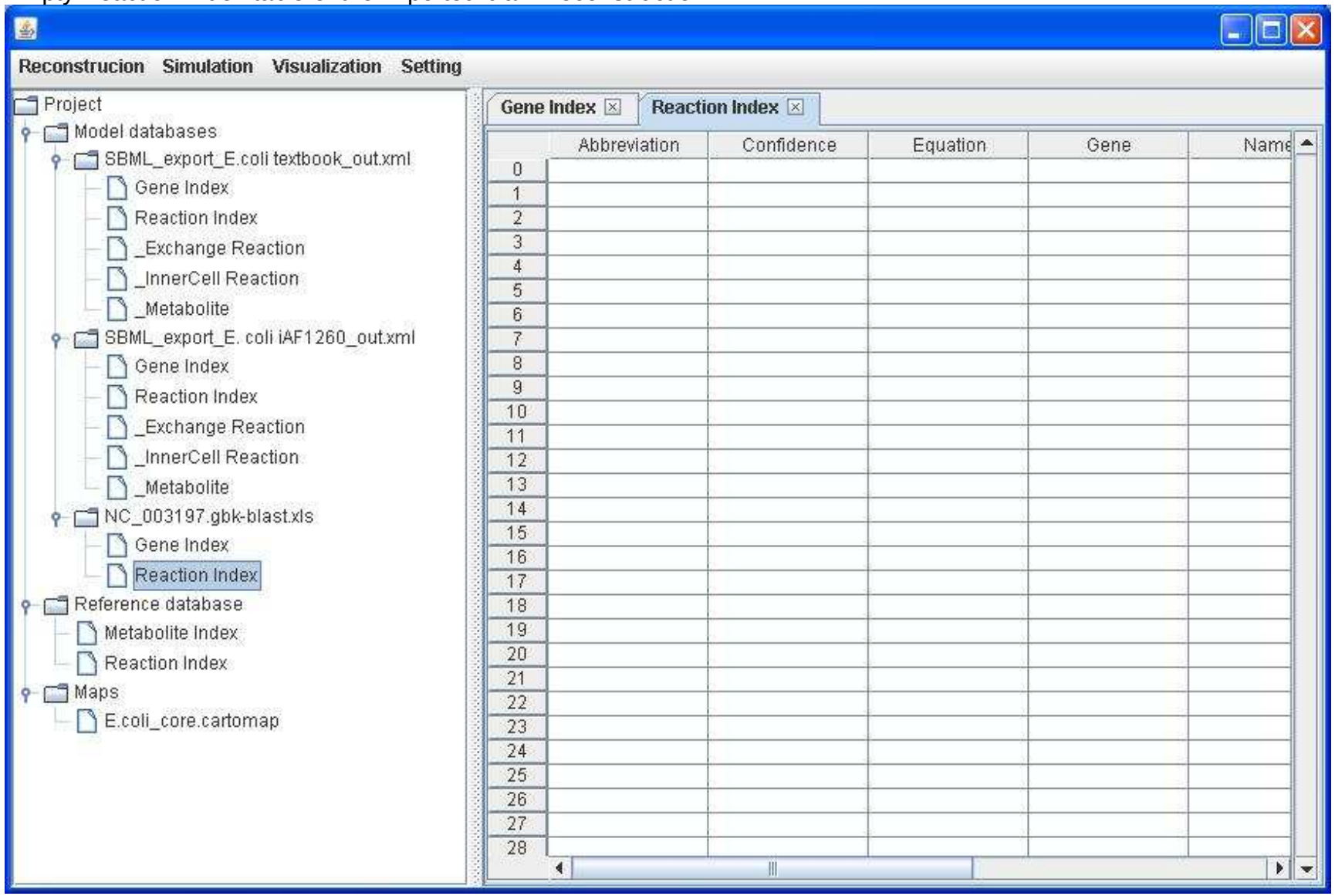
Required fields

Gene Index table of the imported blank reconstruction (NC_003197.gbk-blast.xls):

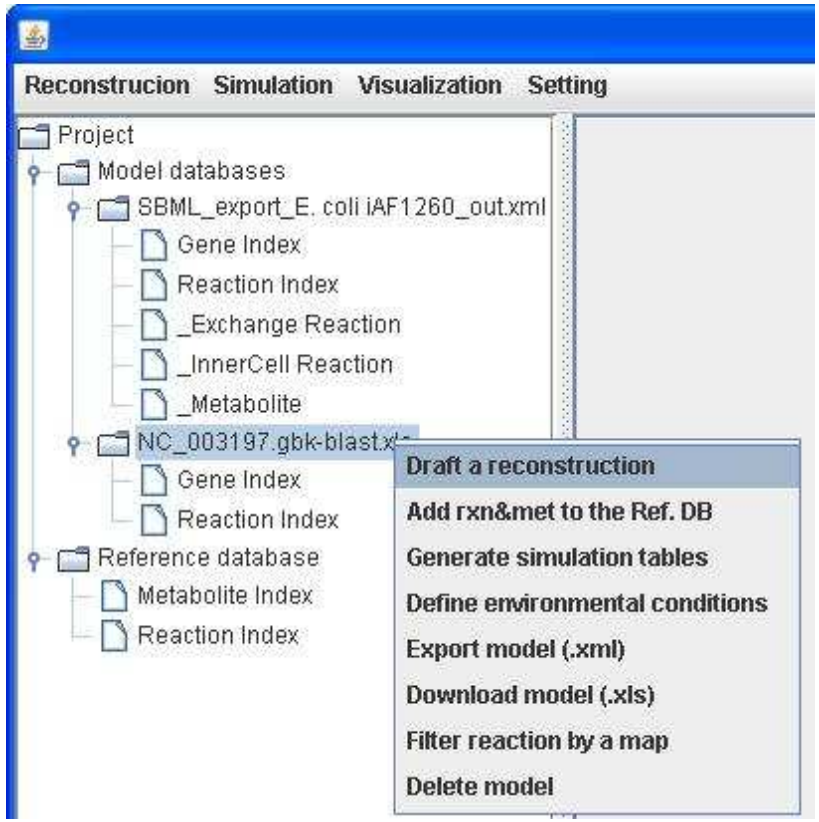


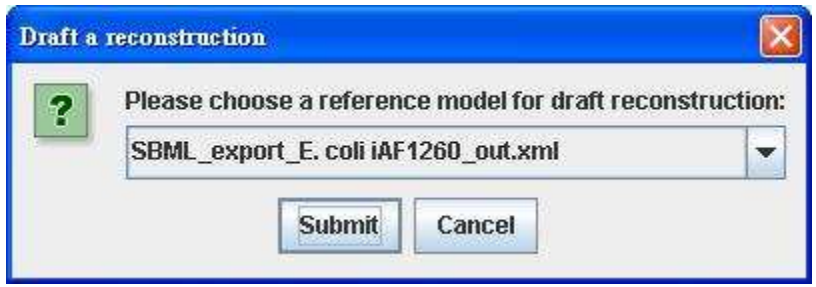
	5'Coordinate	Locus Tag	Gene	EC Number	Product	Ref-BLAST
0	190	STM0001	thrL	--	thr operon lead...	
1	337	STM0002	thrA	2.7.2.4;;1.1....	bifunctional asp...	b0002
2	2801	STM0003	thrB	2.7.1.39	homoserine kin...	b0003
3	3734	STM0004	thrC	4.2.3.1	threonine synth...	b0004
4	5887	STM0005	yaaA	--	hypothetical pro...	b0006
5	7396	STM0006	yaaJ	--	putative alanine...	b0007
6	7665	STM0007	talB	2.2.1.2	transaldolase B	b0008
7	8729	STM0008	mogA	--	molybdenum co...	b0009
8	9942	STM0009	yaaH	--	hypothetical pro...	b0010
9	10805	STM0010	htgA	--	hypothetical pro...	b0011
10	11245	STM0011	yaaI	--	hypothetical pro...	b0013
11	11593	STM0012	dnaK	--	molecular chap...	b0014
12	13595	STM0013	dnaJ	--	chaperone prot...	b0015
13	15014	STM0014	--	--	putative transcri...	
14	16088	STM0015	--	--	putative bacteri...	
15	17026	STM0016	--	--	hypothetical pro...	
16	17486	STM0017	--	--	hypothetical pro...	
17	17867	STM0018	--	--	putative exochiti...	
18	20058	STM0019	--	--	putative hydroxy...	
19	23335	STM0020	--	--	putative cytopla...	
20	24469	STM0021	bcfA	--	fimbrial subunit	
21	25112	STM0022	bcfB	--	fimbrial chapar...	
22	25803	STM0023	bcfC	--	fimbrial usher	b0940
23	28425	STM0024	bcfD	--	fimbrial subunit	
24	29433	STM0025	bcfE	--	fimbrial subunit	
25	29994	STM0026	bcfF	--	fimbrial subunit	
26	30478	STM0027	bcfG	--	fimbrial chapar...	
27	31274	STM0028	bcfH	--	putative thiol-di...	
28	32116	STM0028...	--	--	hypothetical pro...	

Empty Reaction Index table of the imported blank reconstruction:

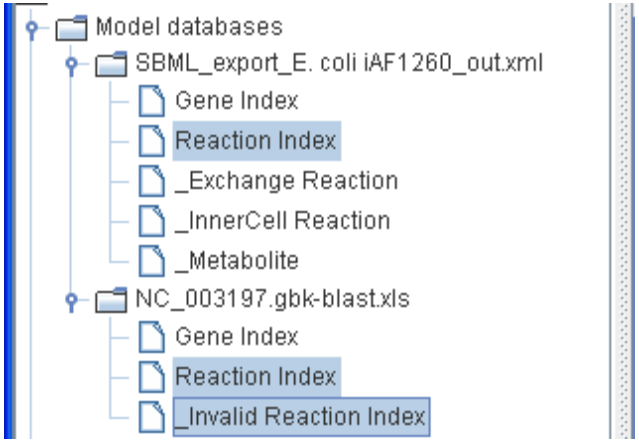


Right click on the blank reconstruction to Draft a reconstruction by choosing SBML_export_E.coli *i*AF1260_out.xml as the reference model.



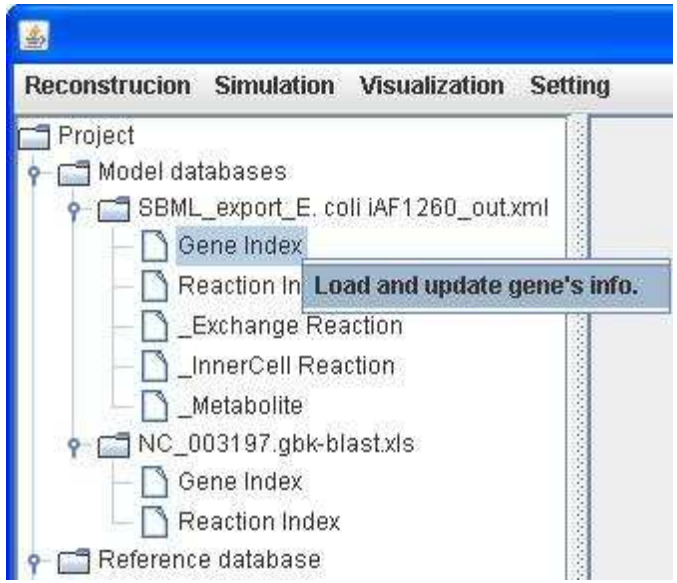


The reactions in the reference reconstruction are classified into two indices (Reaction Index and _Invalid Reaction Index) for the draft reconstruction: one list containing reactions whose associated orthologous genes are present in the blank reconstruction and conform to Boolean statements as described in the reference reconstruction, the other containing those reactions with unknown gene-reaction associations or reactions whose orthologous genes are absent and let to disagree Boolean statements.

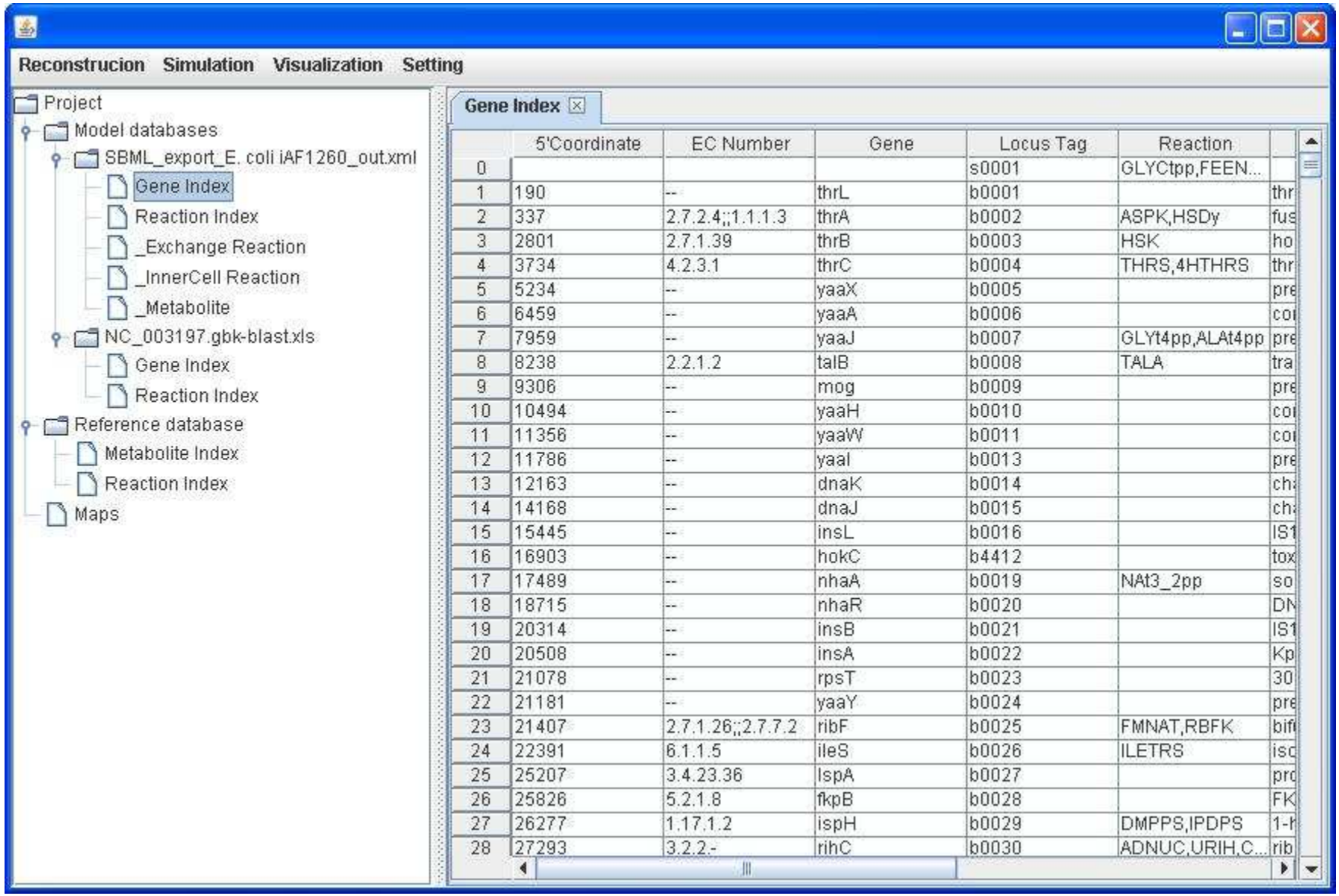


Model refinement

Based on the draft reconstruction generated from Model SEED (http://seed-viewer.theseed.org/seedviewer.cgi?page=ModelView) or GEMSiRV, users can curate and refine the reconstruction in GEMSiRV. However, the lack of gene information in imported models may hinder the progress. We, therefore, provide a function to load and update the gene information in GEMSiRV. You can right click on the Gene Index of a model to Load and update gene's info., and upload the spreadsheet file generated by GBKPaser (http://sb.nhri.org.tw/GEMSiRV/en/GBKParser), e.g. NC_000913.gbk.xls for ECO.



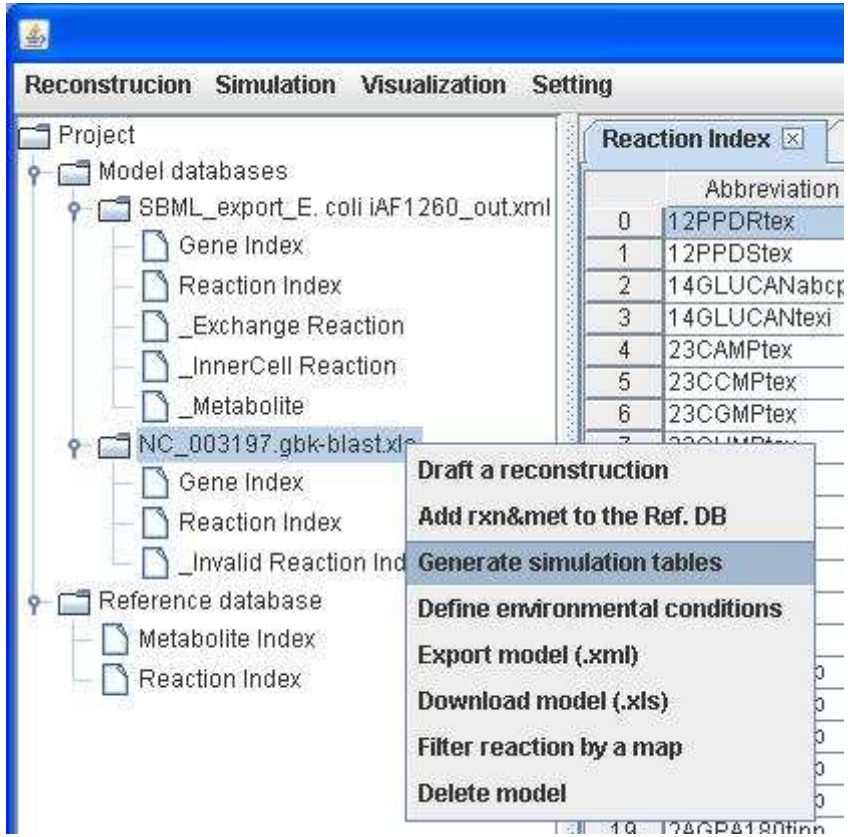
Gene information can be loaded and updated accordingly:



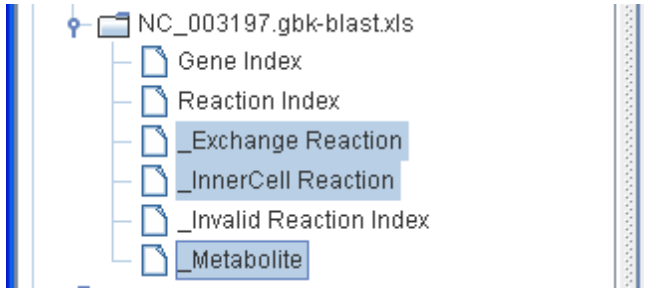
	5'Coordinate	EC Number	Gene	Locus Tag	Reaction	
0				s0001	GLYCtpp,FEEN...	
1	190	--	thrL	b0001		thr
2	337	2.7.2.4;;1.1.1.3	thrA	b0002	ASPK,HSDy	fus
3	2801	2.7.1.39	thrB	b0003	HSK	ho
4	3734	4.2.3.1	thrC	b0004	THRS,4HTHRS	thr
5	5234	--	yaaX	b0005		pre
6	6459	--	yaaA	b0006		cor
7	7959	--	yaaJ	b0007	GLYt4pp,ALAt4pp	pre
8	8238	2.2.1.2	talB	b0008	TALA	tra
9	9306	--	mog	b0009		pre
10	10494	--	yaaH	b0010		cor
11	11356	--	yaaW	b0011		cor
12	11786	--	yaaI	b0013		pre
13	12163	--	dnaK	b0014		cha
14	14168	--	dnaJ	b0015		cha
15	15445	--	insL	b0016		IS1
16	16903	--	hokC	b4412		tox
17	17489	--	nhaA	b0019	NAt3_2pp	so
18	18715	--	nhaR	b0020		DN
19	20314	--	insB	b0021		IS1
20	20508	--	insA	b0022		Kp
21	21078	--	rpsT	b0023		30
22	21181	--	yaaY	b0024		pre
23	21407	2.7.1.26;;2.7.7.2	ribF	b0025	FMNAT,RBFK	bif
24	22391	6.1.1.5	ileS	b0026	ILETRS	isc
25	25207	3.4.23.36	lspA	b0027		pro
26	25826	5.2.1.8	fkpB	b0028		FK
27	26277	1.17.1.2	ispH	b0029	DMPPS,IPDPS	1-h
28	27293	3.2.2.-	rihC	b0030	ADNUC,URIH,C...	rib

With the aids of simulation and visualization, users can readily identify dead-end metabolites and blocked reactions in the models. Prior to

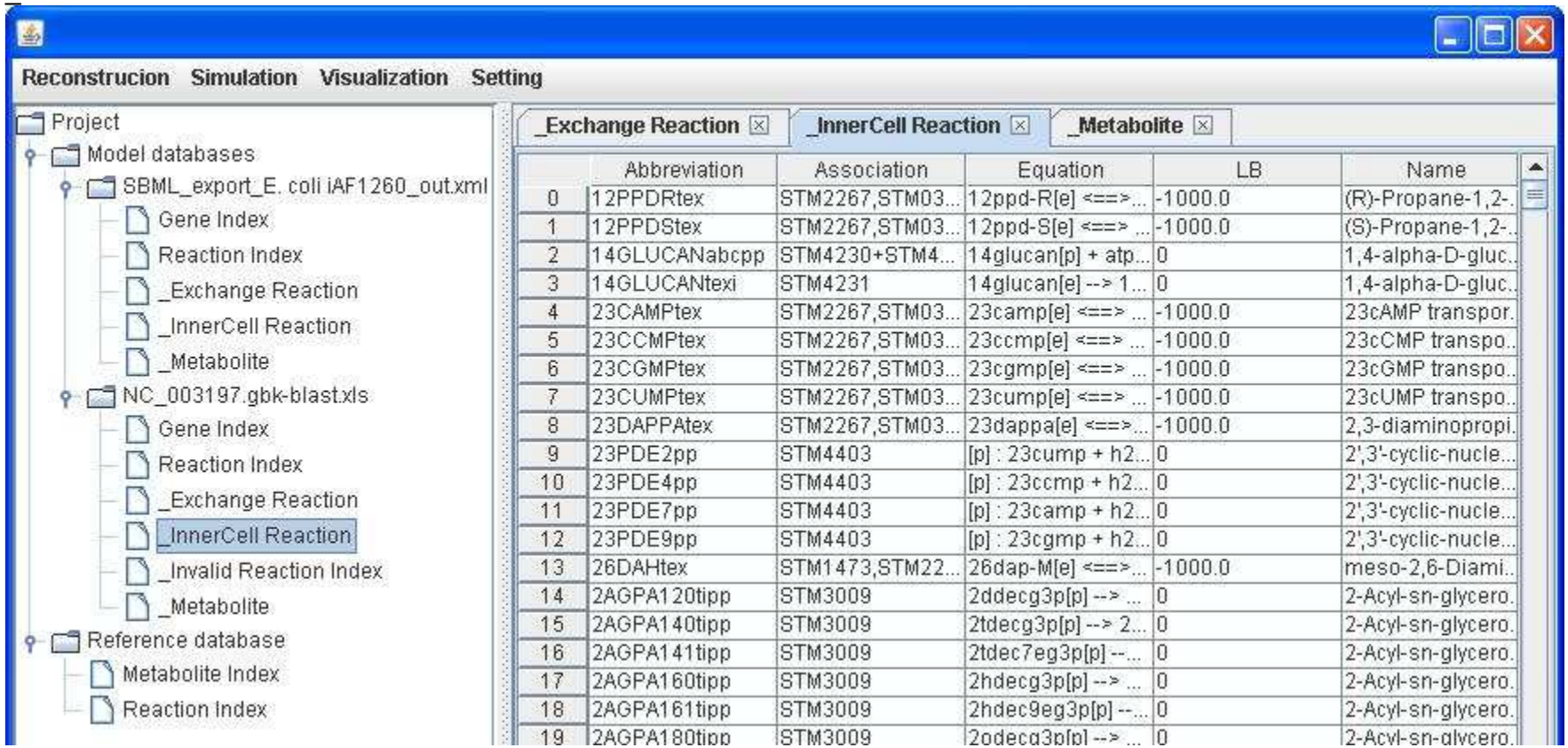
perform simulation, users need to convert the reconstruction into a mathematical model. Therefore, you can right click on a model to Generate simulation tables to generate a model containing a stoichiometric matrix as well as default systems boundaries.



After clicking on Generate simulation tables, three tables including InnerCell Reaction, Exchange Reaction and Metabolite are generated. The prefix "_" used in these three tables for easily distinguishing from the tables required for reconstruction, e.g. Gene Index, Protein Index (optional) and Reaction Index.



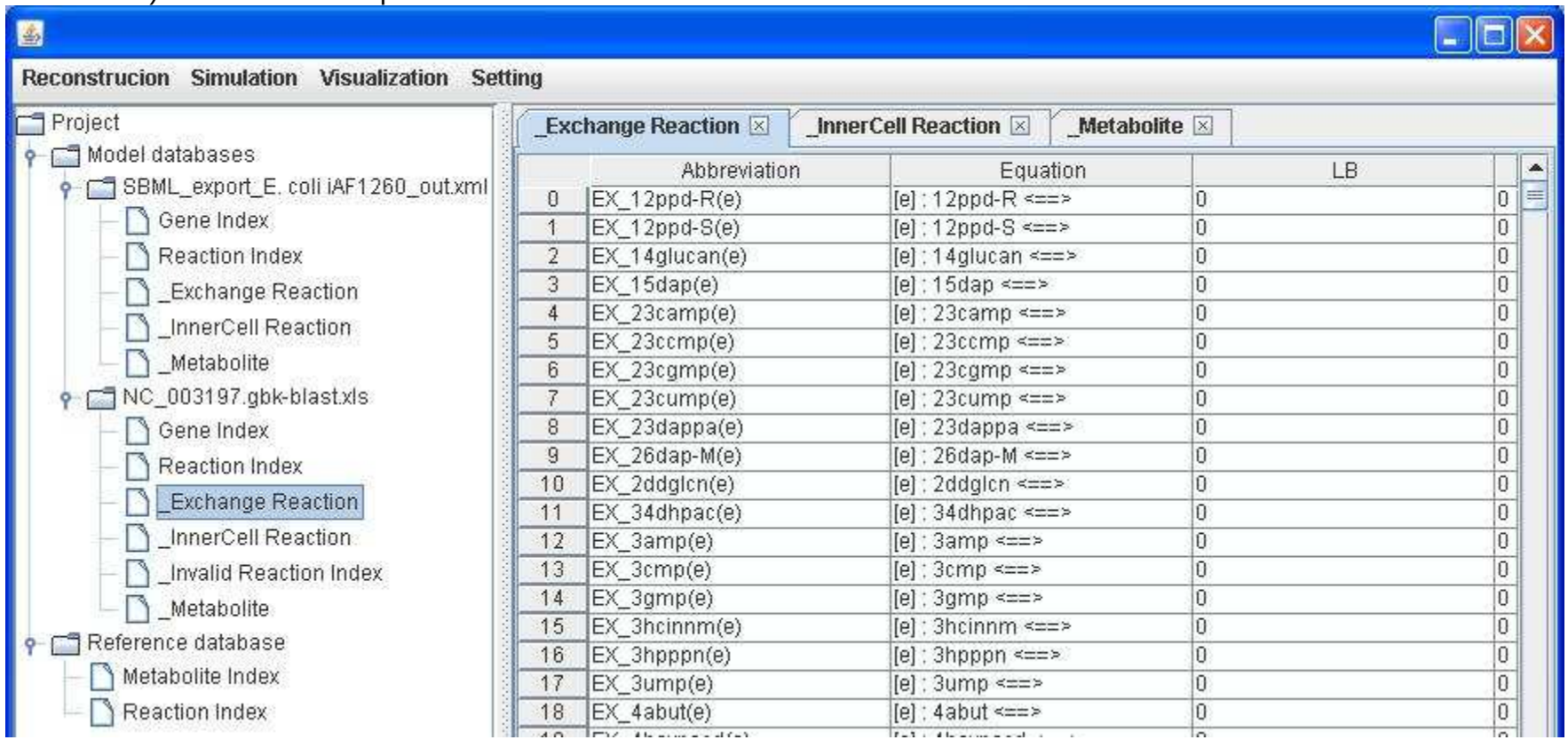
_InnerCell Reaction:



	Abbreviation	Association	Equation	LB	Name
0	12PPDRtex	STM2267,STM03...	12ppd-R[e] <==>...	-1000.0	(R)-Propane-1,2-.
1	12PPDStex	STM2267,STM03...	12ppd-S[e] <==> ...	-1000.0	(S)-Propane-1,2-.
2	14GLUCANabcpp	STM4230+STM4...	14glucan[p] + atp...	0	1,4-alpha-D-gluc.
3	14GLUCANtexi	STM4231	14glucan[e] --> 1...	0	1,4-alpha-D-gluc.
4	23CAMPtex	STM2267,STM03...	23camp[e] <==> ...	-1000.0	23cAMP transpor.
5	23CCMPtex	STM2267,STM03...	23ccmp[e] <==> ...	-1000.0	23cCMP transpo.
6	23CGMPtex	STM2267,STM03...	23cgmp[e] <==> ...	-1000.0	23cGMP transpo.
7	23CUMPtex	STM2267,STM03...	23cump[e] <==> ...	-1000.0	23cUMP transpo.
8	23DAPPAtex	STM2267,STM03...	23dappa[e] <==>...	-1000.0	2,3-diaminopropi.
9	23PDE2pp	STM4403	[p] : 23cump + h2...	0	2',3'-cyclic-nucle...
10	23PDE4pp	STM4403	[p] : 23ccmp + h2...	0	2',3'-cyclic-nucle...
11	23PDE7pp	STM4403	[p] : 23camp + h2...	0	2',3'-cyclic-nucle...
12	23PDE9pp	STM4403	[p] : 23cgmp + h2...	0	2',3'-cyclic-nucle...
13	26DAHtex	STM1473,STM22...	26dap-M[e] <==>...	-1000.0	meso-2,6-Diami.
14	2AGPA120tipp	STM3009	2ddecg3p[p] --> ...	0	2-Acyl-sn-glycero.
15	2AGPA140tipp	STM3009	2tdecg3p[p] --> 2...	0	2-Acyl-sn-glycero.
16	2AGPA141tipp	STM3009	2tdec7eg3p[p] --...	0	2-Acyl-sn-glycero.
17	2AGPA160tipp	STM3009	2hdecg3p[p] --> ...	0	2-Acyl-sn-glycero.
18	2AGPA161tipp	STM3009	2hdec9eg3p[p] --...	0	2-Acyl-sn-glycero.
19	2AGPA180tipp	STM3009	2odecg3p[p] --> ...	0	2-Acyl-sn-glycero.

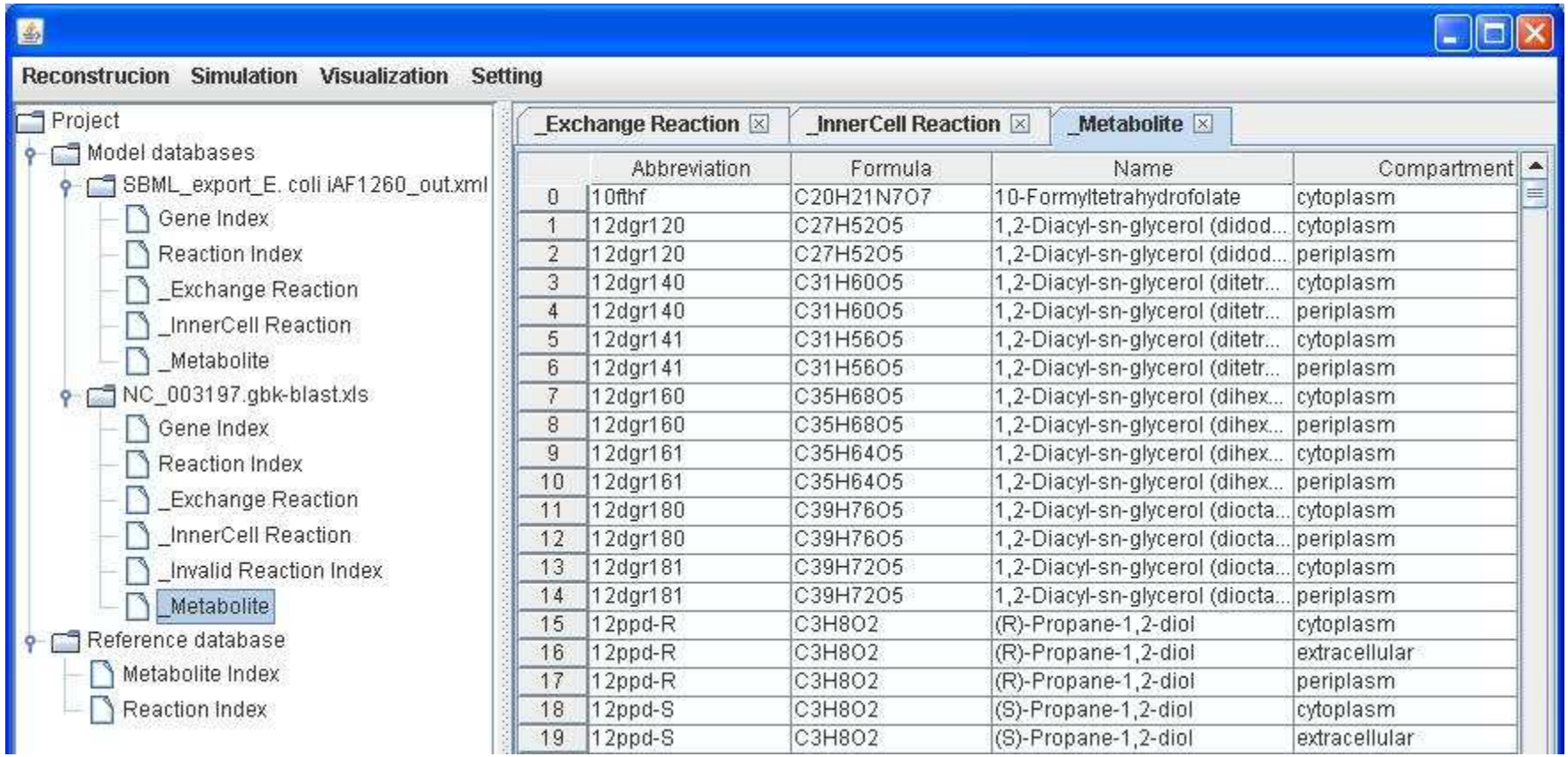
_Exchange Reaction:

Please note that the _Exchange Reaction table will be generated only when you have exchanging metabolites (i.e. extracellular metabolites) in the reaction equations.



	Abbreviation	Equation	LB	
0	EX_12ppd-R(e)	[e] : 12ppd-R <==>	0	0
1	EX_12ppd-S(e)	[e] : 12ppd-S <==>	0	0
2	EX_14glucan(e)	[e] : 14glucan <==>	0	0
3	EX_15dap(e)	[e] : 15dap <==>	0	0
4	EX_23camp(e)	[e] : 23camp <==>	0	0
5	EX_23ccmp(e)	[e] : 23ccmp <==>	0	0
6	EX_23cgmp(e)	[e] : 23cgmp <==>	0	0
7	EX_23cump(e)	[e] : 23cump <==>	0	0
8	EX_23dappa(e)	[e] : 23dappa <==>	0	0
9	EX_26dap-M(e)	[e] : 26dap-M <==>	0	0
10	EX_2ddglcn(e)	[e] : 2ddglcn <==>	0	0
11	EX_34dhpac(e)	[e] : 34dhpac <==>	0	0
12	EX_3amp(e)	[e] : 3amp <==>	0	0
13	EX_3cmp(e)	[e] : 3cmp <==>	0	0
14	EX_3gmp(e)	[e] : 3gmp <==>	0	0
15	EX_3hcinnm(e)	[e] : 3hcinnm <==>	0	0
16	EX_3hpppn(e)	[e] : 3hpppn <==>	0	0
17	EX_3ump(e)	[e] : 3ump <==>	0	0
18	EX_4abut(e)	[e] : 4abut <==>	0	0

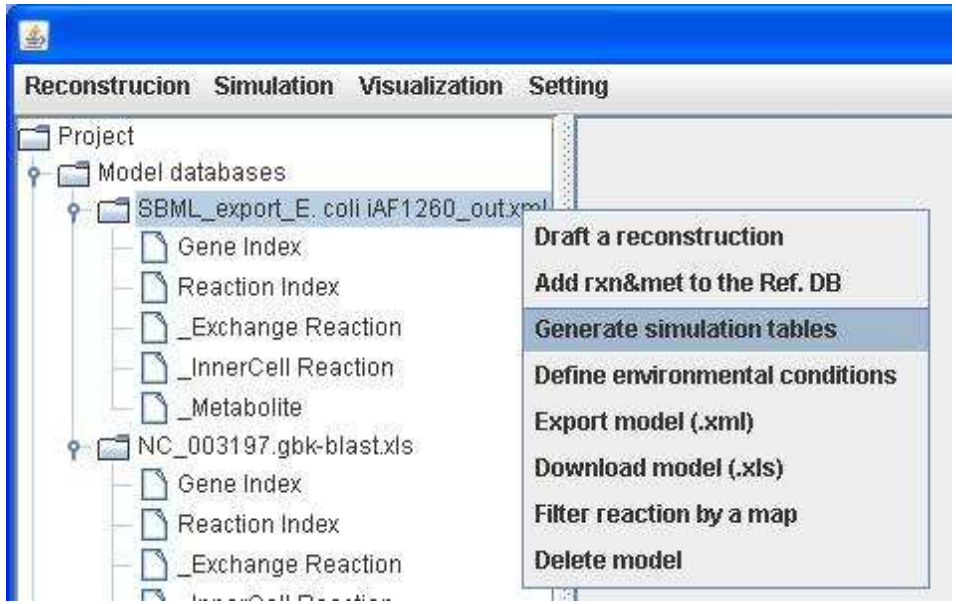
_Metabolite:



	Abbreviation	Formula	Name	Compartment
0	10fthf	C20H21N7O7	10-Formyltetrahydrofolate	cytoplasm
1	12dgr120	C27H52O5	1,2-Diacyl-sn-glycerol (didod...	cytoplasm
2	12dgr120	C27H52O5	1,2-Diacyl-sn-glycerol (didod...	periplasm
3	12dgr140	C31H60O5	1,2-Diacyl-sn-glycerol (ditetr...	cytoplasm
4	12dgr140	C31H60O5	1,2-Diacyl-sn-glycerol (ditetr...	periplasm
5	12dgr141	C31H56O5	1,2-Diacyl-sn-glycerol (ditetr...	cytoplasm
6	12dgr141	C31H56O5	1,2-Diacyl-sn-glycerol (ditetr...	periplasm
7	12dgr160	C35H68O5	1,2-Diacyl-sn-glycerol (dihex...	cytoplasm
8	12dgr160	C35H68O5	1,2-Diacyl-sn-glycerol (dihex...	periplasm
9	12dgr161	C35H64O5	1,2-Diacyl-sn-glycerol (dihex...	cytoplasm
10	12dgr161	C35H64O5	1,2-Diacyl-sn-glycerol (dihex...	periplasm
11	12dgr180	C39H76O5	1,2-Diacyl-sn-glycerol (diocta...	cytoplasm
12	12dgr180	C39H76O5	1,2-Diacyl-sn-glycerol (diocta...	periplasm
13	12dgr181	C39H72O5	1,2-Diacyl-sn-glycerol (diocta...	cytoplasm
14	12dgr181	C39H72O5	1,2-Diacyl-sn-glycerol (diocta...	periplasm
15	12ppd-R	C3H8O2	(R)-Propane-1,2-diol	cytoplasm
16	12ppd-R	C3H8O2	(R)-Propane-1,2-diol	extracellular
17	12ppd-R	C3H8O2	(R)-Propane-1,2-diol	periplasm
18	12ppd-S	C3H8O2	(S)-Propane-1,2-diol	cytoplasm
19	12ppd-S	C3H8O2	(S)-Propane-1,2-diol	extracellular

Because growth media for modeled organisms may be similar, an environmental condition can be easily set to a model by right clicking on the model to Define environmental conditions.

Here we use the *in silico* (computational) minimal media for the model *i*AF1260 as an example (the text file can be downloaded in http://sb.nhri.org.tw/GEMSiRV/en/Manual). In order to set the system boundaries to the default values, we right click on the model to Generate simulation tables.



The new simulation tables are generated and replace the previous tables. We set a growth medium for modeling the model. We prepare a text file containing the user-defined boundaries and objective, and then right click on the model to Define environmental conditions.

In silico minimal media for the model *i*AF1260.

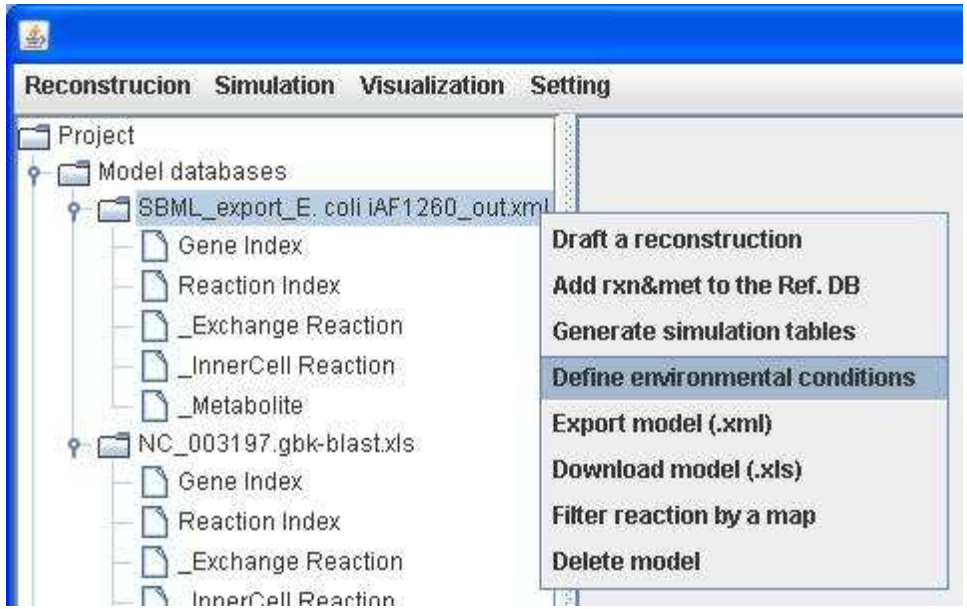
M9 medium_comp.TXT

```
1  [REACTIONS]
2  EX_ca2(e):LB=-1000
3  EX_cl(e):LB=-1000
4  EX_co2(e):LB=-1000
5  EX_cobalt2(e):LB=-1000
6  EX_cu2(e):LB=-1000
7  EX_fe2(e):LB=-1000
8  EX_fe3(e):LB=-1000
9  EX_h2o(e):LB=-1000
10 EX_h(e):LB=-1000
11 EX_k(e):LB=-1000
12 EX_mg2(e):LB=-1000
13 EX_mn2(e):LB=-1000
14 EX_mobd(e):LB=-1000
15 EX_na1(e):LB=-1000
16 EX_nh4(e):LB=-1000
17 EX_pi(e):LB=-1000
18 EX_so4(e):LB=-1000
19 EX_tungs(e):LB=-1000
20 EX_zn2(e):LB=-1000
21 EX_o2(e):LB=-18.5
22 ATPM:LB=8.39,UB=9.39
23 Biomass_Ecoli_core_N(w/GAM)_Nmet2:Objective Coefficient=1
24 EX_glc-D(e):LB=-8
```

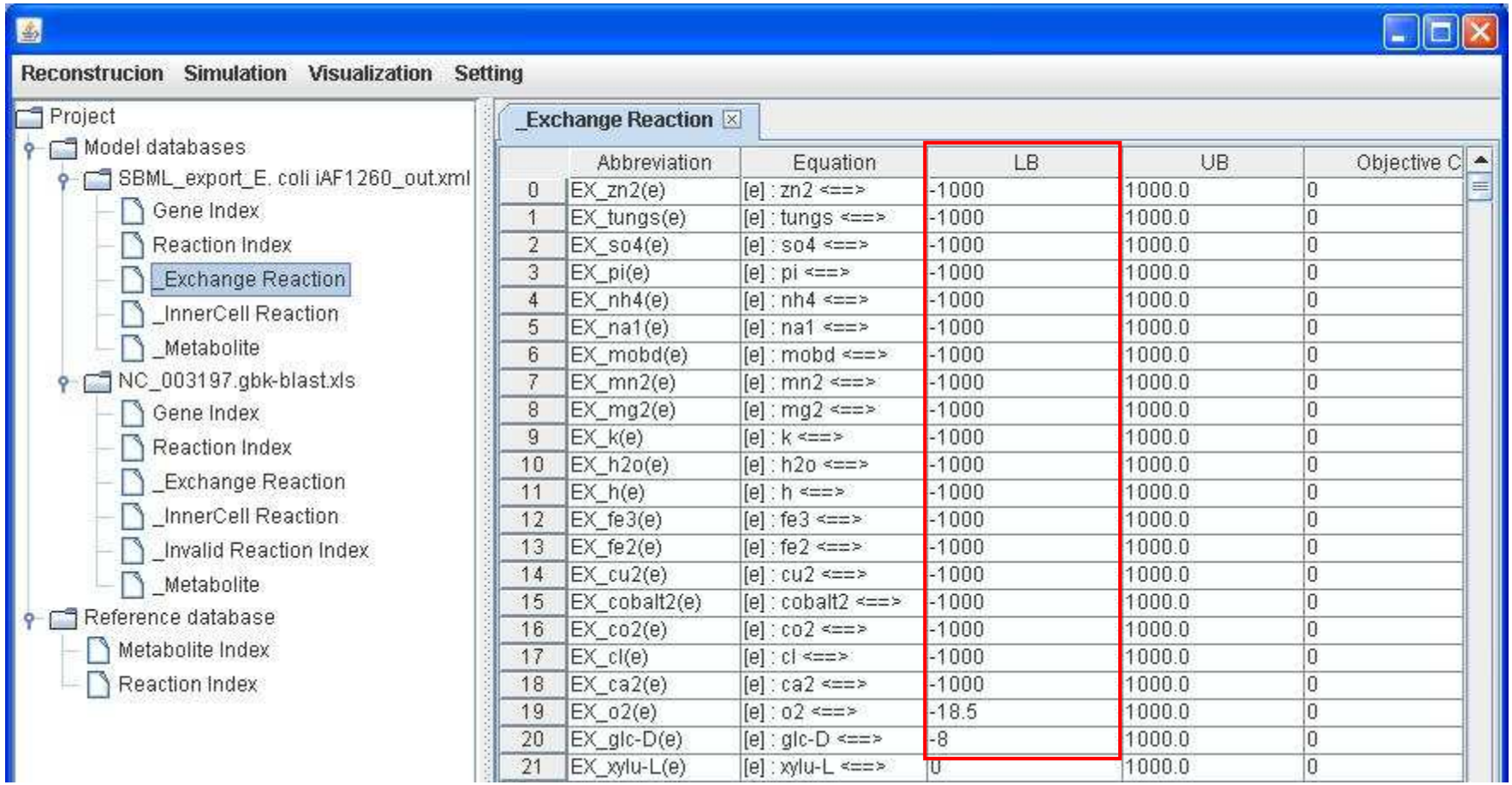
A complete medium to simulate all extracellular metabolites can enter/exit the cell freely.

complete medium.TXT

```
1  [ALL EXCHANGE REACTIONS]
2  LB=-1000
3  UB=1000
4  Objective Coefficient=0
5
```

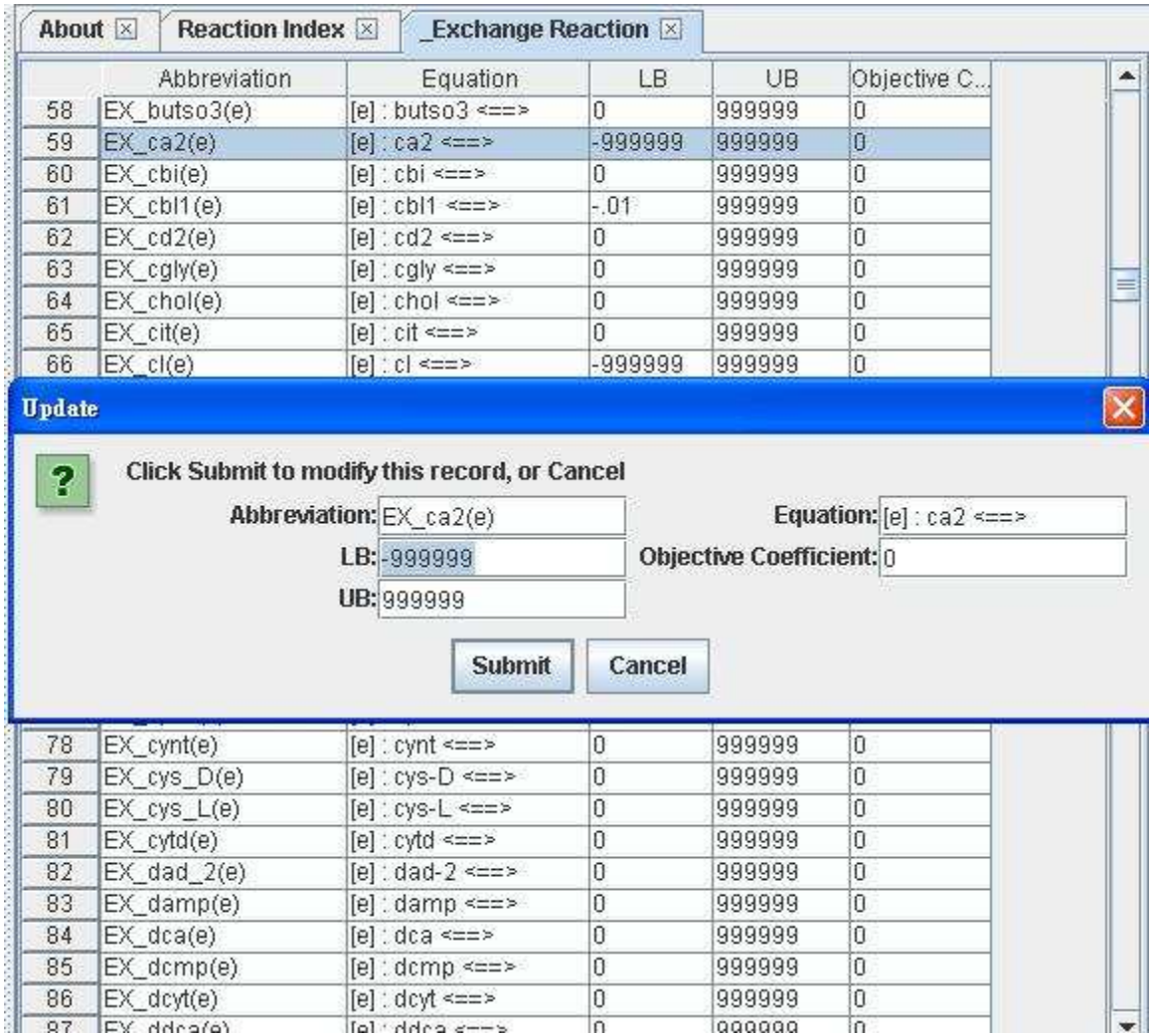


The user-defined system boundaries and the objective are set in the reconstruction model accordingly.



	Abbreviation	Equation	LB	UB	Objective C
0	EX_zn2(e)	[e] : zn2 <==>	-1000	1000.0	0
1	EX_tungs(e)	[e] : tungs <==>	-1000	1000.0	0
2	EX_so4(e)	[e] : so4 <==>	-1000	1000.0	0
3	EX_pi(e)	[e] : pi <==>	-1000	1000.0	0
4	EX_nh4(e)	[e] : nh4 <==>	-1000	1000.0	0
5	EX_na1(e)	[e] : na1 <==>	-1000	1000.0	0
6	EX_mobd(e)	[e] : mobd <==>	-1000	1000.0	0
7	EX_mn2(e)	[e] : mn2 <==>	-1000	1000.0	0
8	EX_mg2(e)	[e] : mg2 <==>	-1000	1000.0	0
9	EX_k(e)	[e] : k <==>	-1000	1000.0	0
10	EX_h2o(e)	[e] : h2o <==>	-1000	1000.0	0
11	EX_h(e)	[e] : h <==>	-1000	1000.0	0
12	EX_fe3(e)	[e] : fe3 <==>	-1000	1000.0	0
13	EX_fe2(e)	[e] : fe2 <==>	-1000	1000.0	0
14	EX_cu2(e)	[e] : cu2 <==>	-1000	1000.0	0
15	EX_cobalt2(e)	[e] : cobalt2 <==>	-1000	1000.0	0
16	EX_co2(e)	[e] : co2 <==>	-1000	1000.0	0
17	EX_cl(e)	[e] : cl <==>	-1000	1000.0	0
18	EX_ca2(e)	[e] : ca2 <==>	-1000	1000.0	0
19	EX_o2(e)	[e] : o2 <==>	-18.5	1000.0	0
20	EX_glc-D(e)	[e] : glc-D <==>	-8	1000.0	0
21	EX_xylu-L(e)	[e] : xylu-L <==>	0	1000.0	0

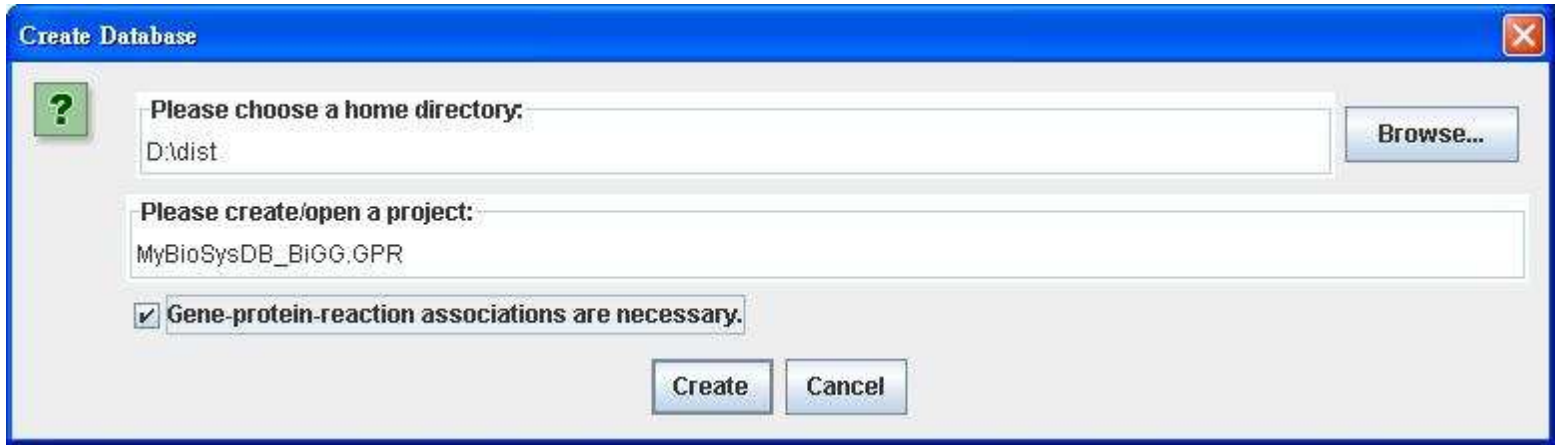
Or you can simply right click on the reaction to update the lower bound (LB), upper bound (UB) or objective coefficient.



You can freely export or save a metabolic model in SBML format or in spreadsheet format by right clicking on a model to Export model (.xml) or to Download model (.xls). Such models generated by GEMSiRV are fully compatible to GEMSiRV for later importing and simulation.

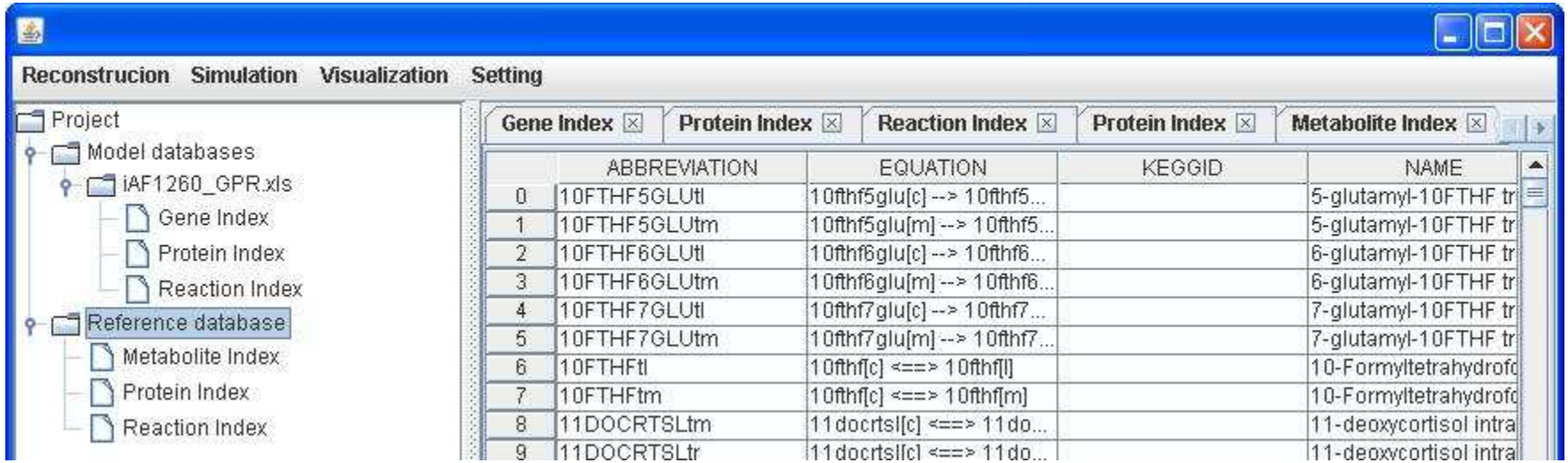
In addiction to the metabolic models saved in SBML format, metabolic reconstructions can be stored in spreadsheet format. The spreadsheet format can store the two-layer relation for gene-protein and protein-reaction associations in network reconstructions. We provide available reconstruction models (GPR) in http://sb.nhri.org.tw/GEMSiRV/en/Metabolic_Models and demonstrate how we use GEMSiRV to reconstruct metabolic networks with GPR relationships.

From reconstruction to model



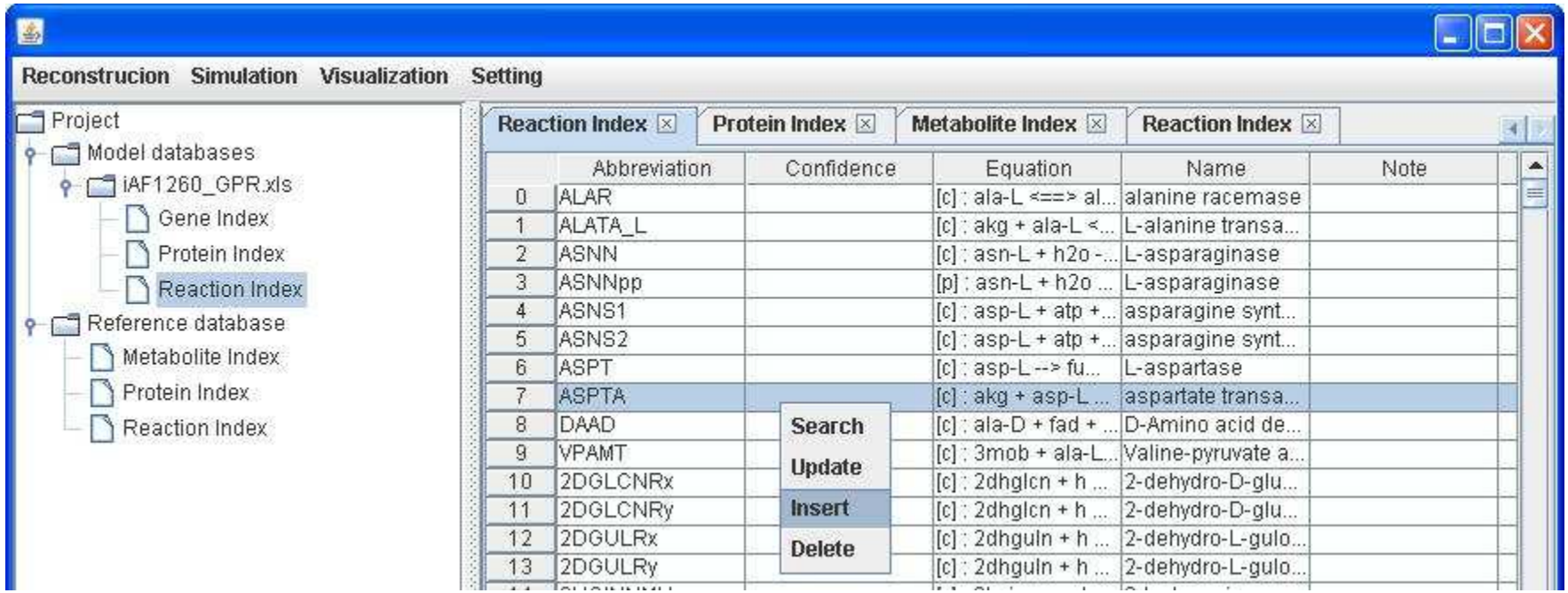
After clicking on Reconstruction in the menu bar, right click on the **Model databases** to Import spreadsheets (.xls) for importing the reconstruction file of iAF1260_GPR.xls (download from http://sb.nhri.org.tw/GEMSiRV/en/Metabolic_Models). This reconstruction contains three indices: Gene, Protein and Reaction Index.

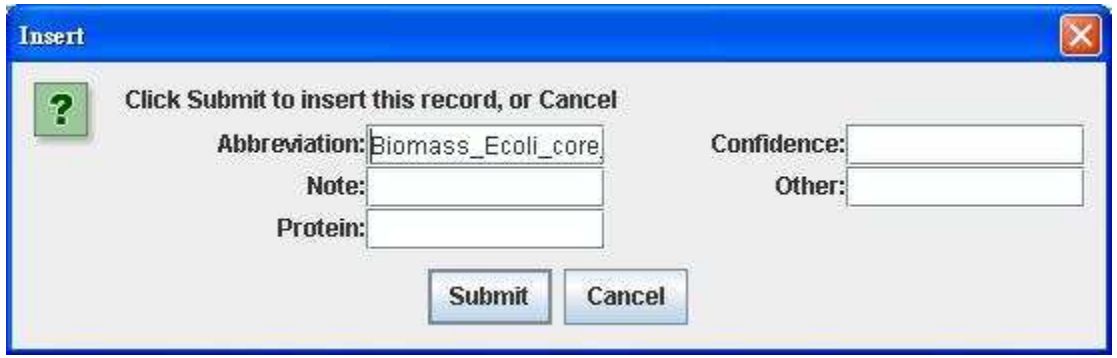
Then right click on **Reference database** to Import database (.xls) for importing the reference database file Ref_BiGG_GPR.xls which is provided in http://sb.nhri.org.tw/GEMSiRV/en/Reference_Databases.

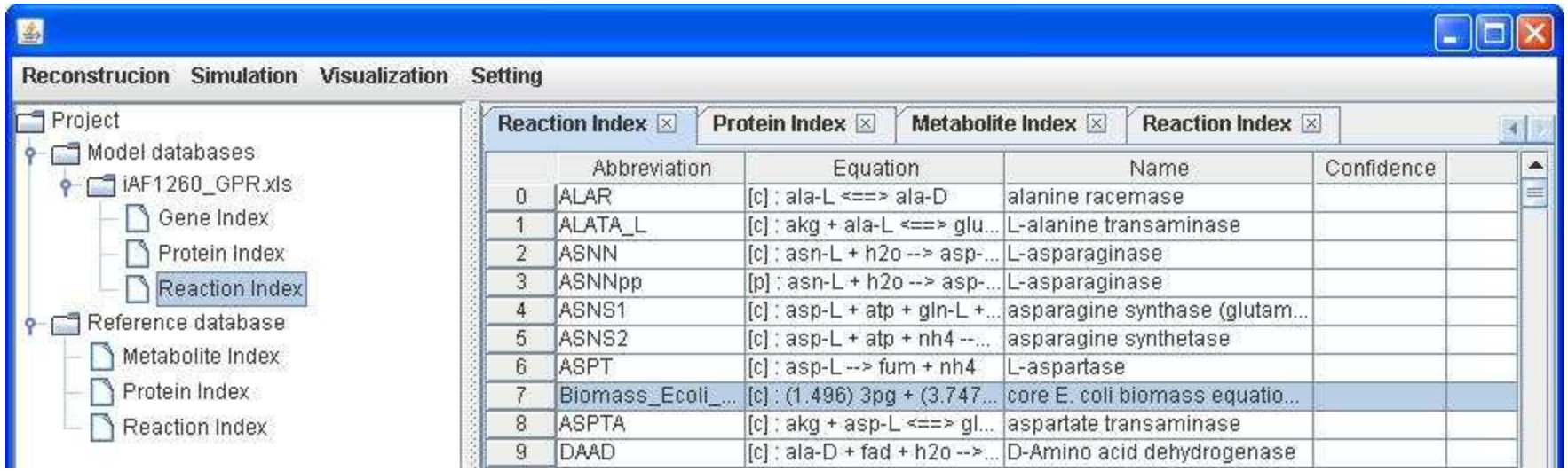


A biomass for E. coli, Biomass_Ecoli_core_N (w/ GAM)-Nmet2, is available in the reference database, you can add the reaction to the

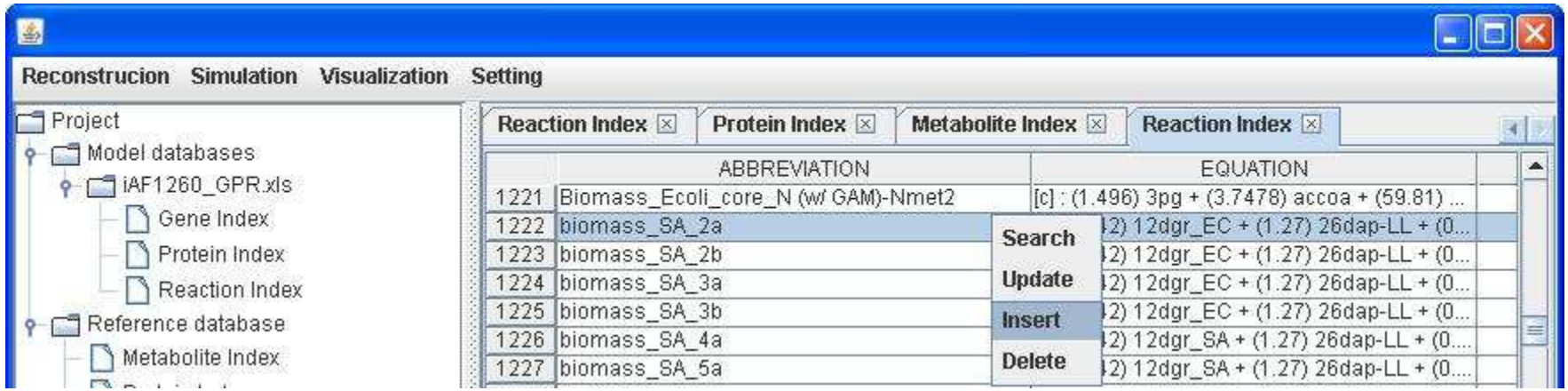
reconstruction by right clicking on the main window of Reaction Index to Insert. After submitting the abbreviation of reaction “Biomass_Ecoli_core_N (w/ GAM)-Nmet2”, the related information including reaction name and equation will be conveyed to the reconstruction from the reference database.





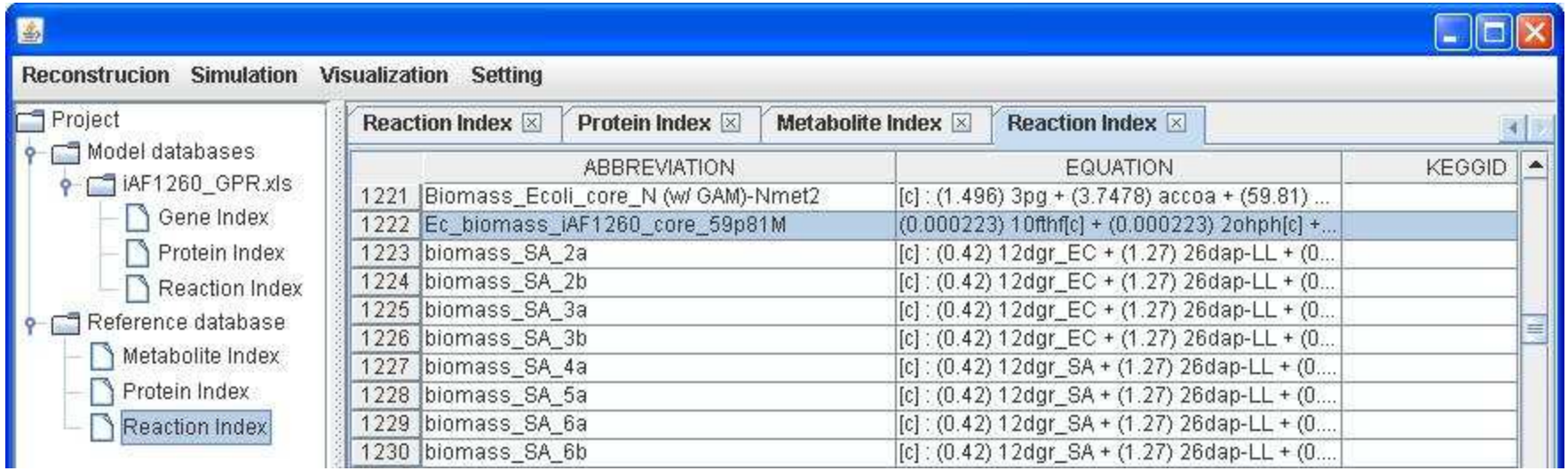


Likewise, you can add a new reaction into the reference.

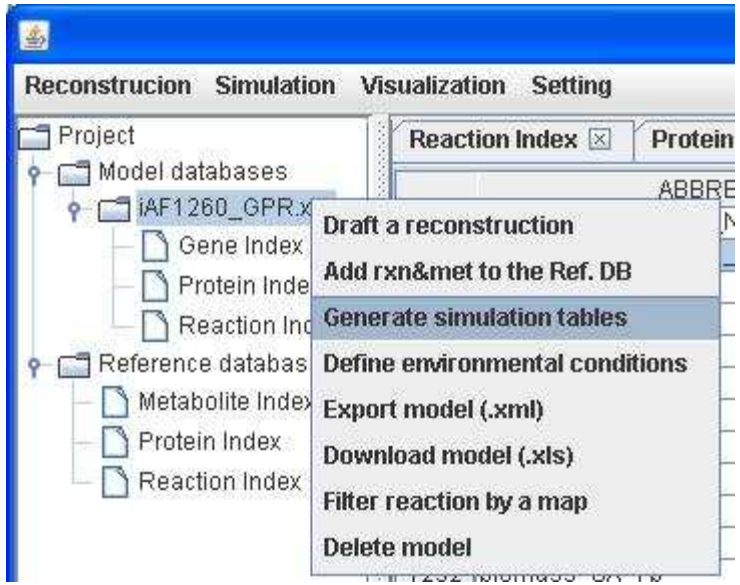


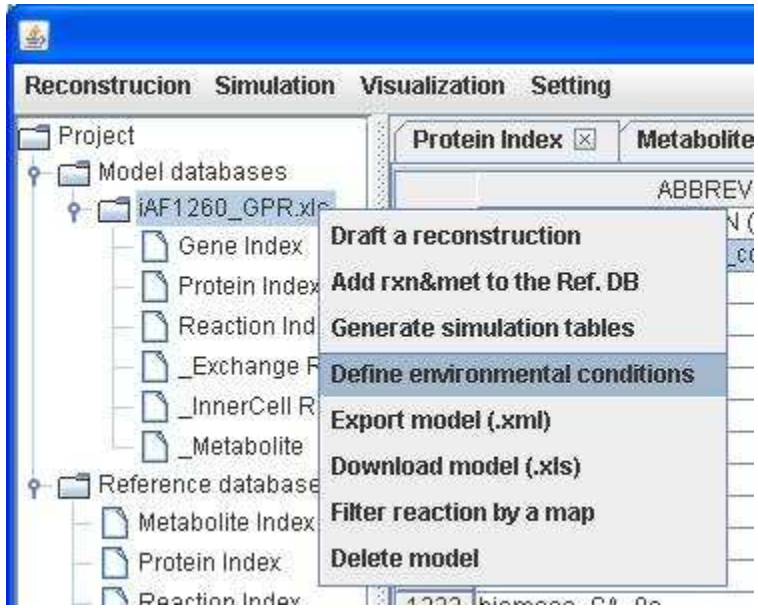
The reaction of Ec_biomass_iAF1260_core_59p81M can be added into the reference database.
Abbreviation: Ec_biomass_iAF1260_core_59p81M
Equation:

(0.000223) 10fthf[c] + (0.000223) 2ohph[c] + (0.5137) ala-L[c] + (0.000223) amet[c] + (0.2958) arg-L[c] + (0.2411) asn-L[c] + (0.2411) asp-L[c] + (59.984) atp[c] + (0.004737) ca2[c] + (0.004737) cl[c] + (0.000576) coa[c] + (0.003158) cobalt2[c] + (0.1335) ctp[c] + (0.003158) cu2[c] + (0.09158) cys-L[c] + (0.02617) datp[c] + (0.02702) dctp[c] + (0.02702) dgtp[c] + (0.02617) dttp[c] + (0.000223) fad[c] + (0.007106) fe2[c] + (0.007106) fe3[c] + (0.2632) gln-L[c] + (0.2632) glu-L[c] + (0.6126) gly[c] + (0.2151) gtp[c] + (54.462) h2o[c] + (0.09474) his-L[c] + (0.2905) ile-L[c] + (0.1776) k[c] + (0.01945) kdo2lipid4[e] + (0.4505) leu-L[c] + (0.3432) lys-L[c] + (0.1537) met-L[c] + (0.007895) mg2[c] + (0.000223) mlthf[c] + (0.003158) mn2[c] + (0.003158) mobd[c] + (0.01389) murein5px4p[p] + (0.001831) nad[c] + (0.000447) nadp[c] + (0.011843) nh4[c] + (0.04148) pe160[p] + (0.02233) pe160[c] + (0.02632) pe161[c] + (0.04889) pe161[p] + (0.1759) phe-L[c] + (0.000223) pheme[c] + (0.2211) pro-L[c] + (0.000223) pydx5p[c] + (0.000223) ribflv[c] + (0.2158) ser-L[c] + (0.000223) sheme[c] + (0.003948) so4[c] + (0.000223) thf[c] + (0.000223) thmpp[c] + (0.2537) thr-L[c] + (0.05684) trp-L[c] + (0.1379) tyr-L[c] + (0.000055) udcpdp[c] + (0.1441) utp[c] + (0.4232) val-L[c] + (0.003158) zn2[c] --> (59.81) adp[c] + (59.81) h[c] + (59.806) pi[c] + (0.7739) ppi[c]



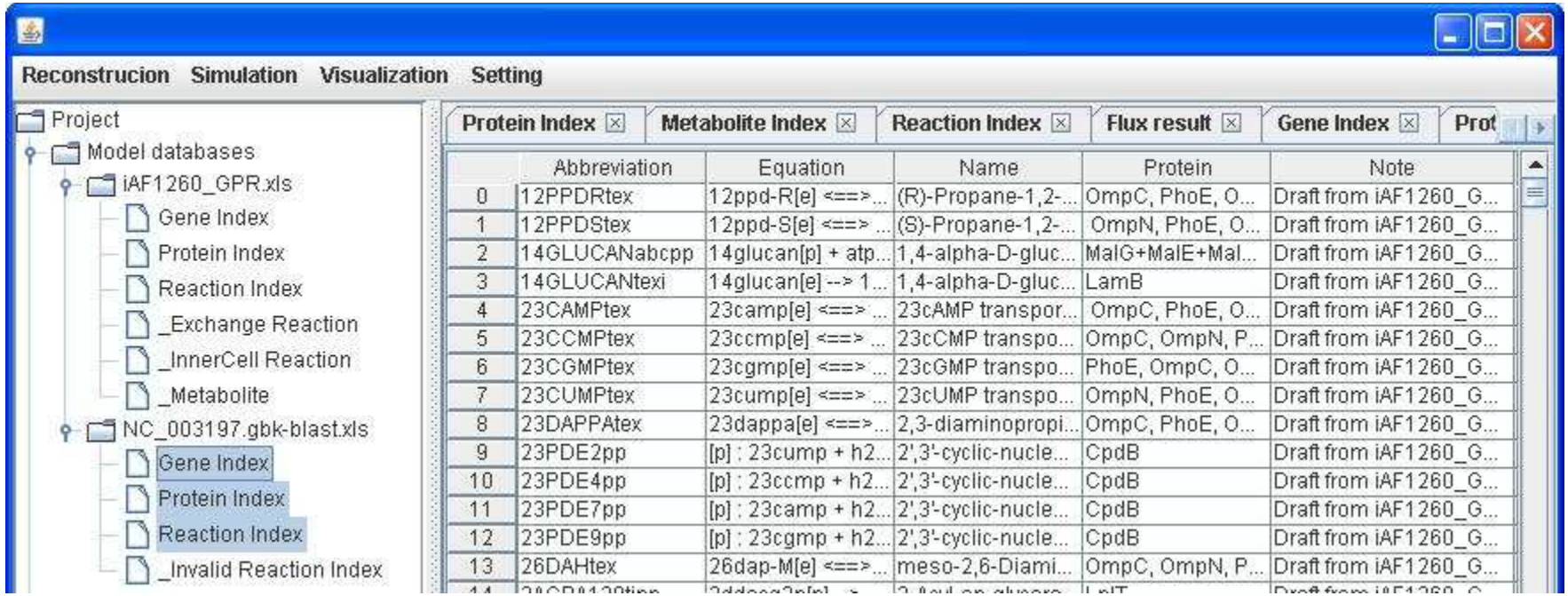
Right click on a reconstruction to Generate simulation tables can convert the reconstruction to a model. Then you can set the system boundaries for simulation.


Reconstrucion
Simulation
Visualization
Setting
Project
Model databases
iAF1260_GPR.x
Gene Index
Protein Inde
Reaction Ind
Reference databas
Metabolite Index
Protein Index
Reaction Index
Reaction Index
Protein
ABBRE
Draft a reconstruction
Add rxn&met to the Ref. DB
Generate simulation tables
Define environmental conditions
Export model (.xml)
Download model (.xls)
Filter reaction by a map
Delete model

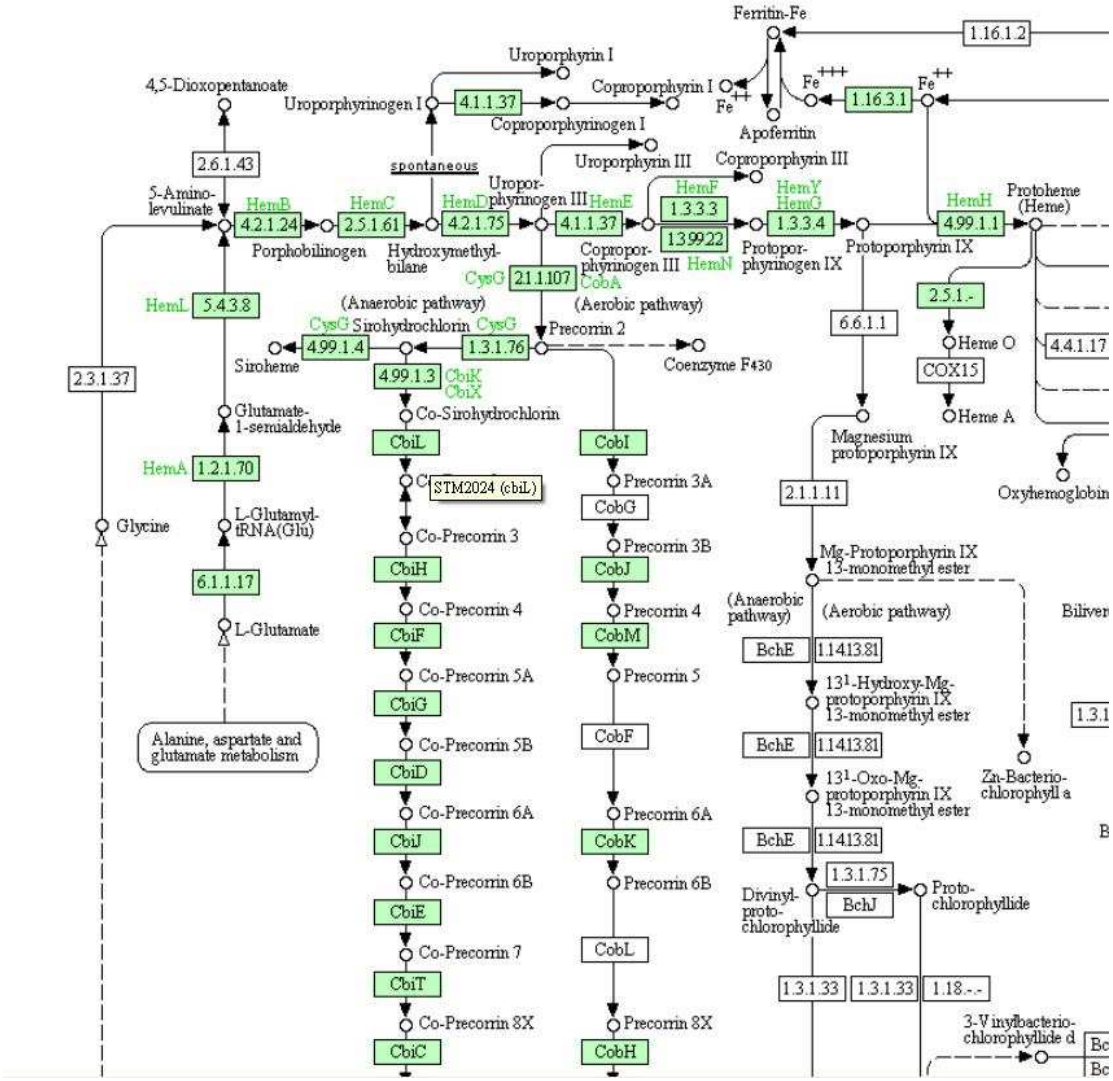

Reconstrucion
Simulation
Visualization
Setting
Project
Model databases
iAF1260_GPR.xls
Gene Index
Protein Index
Reaction Ind
_Exchange R
_InnerCell R
_Metabolite
Reference database
Metabolite Index
Protein Index
Protein Index
Metabolite
ABBREV
Draft a reconstruction
Add rxn&met to the Ref. DB
Generate simulation tables
Define environmental conditions
Export model (.xml)
Download model (.xls)
Filter reaction by a map
Delete model

Draft reconstruction and network refinement

As described previously, we can draft a reconstruction for a genetically related species (e.g. *Salmonella*) with the existing *E. coli* model in GEMSiRV. Therefore, we import the file NC_003197.gbk-blast.xls and draft a reconstruction with reference to *i*AF1260_GPR.



Then we can refine the draft reconstruction by adding metabolic reactions with gene-protein-reaction associations, some existing reactions in the reference database can be conveyed to the reconstruction. For example, *Salmonella* is reported to be able to synthesize cobalamin due to its metabolic genes (operon) STM2016-STM2035. Therefore, we can manually add those associated reactions and proteins to the draft reconstruction.



Locus STM2024

Gene cbiL

Protein CbiL

Reaction CPC2MT

Reaction CPC2MT

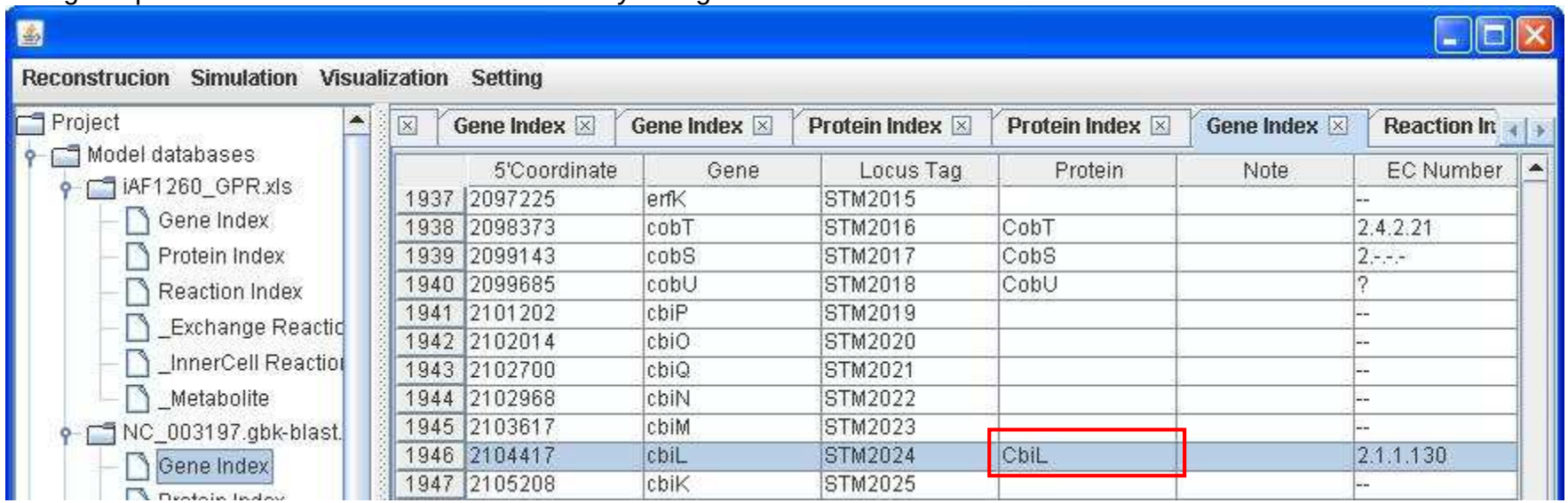
Name precorrin-2 C20-methyltransferase

Equation [c] : amet + copre2 --> ahcys + copre3 + h

After clicking into the Protein Index of NC_003197.gbk-blast.xls, right click on the main window of protein index to insert the protein

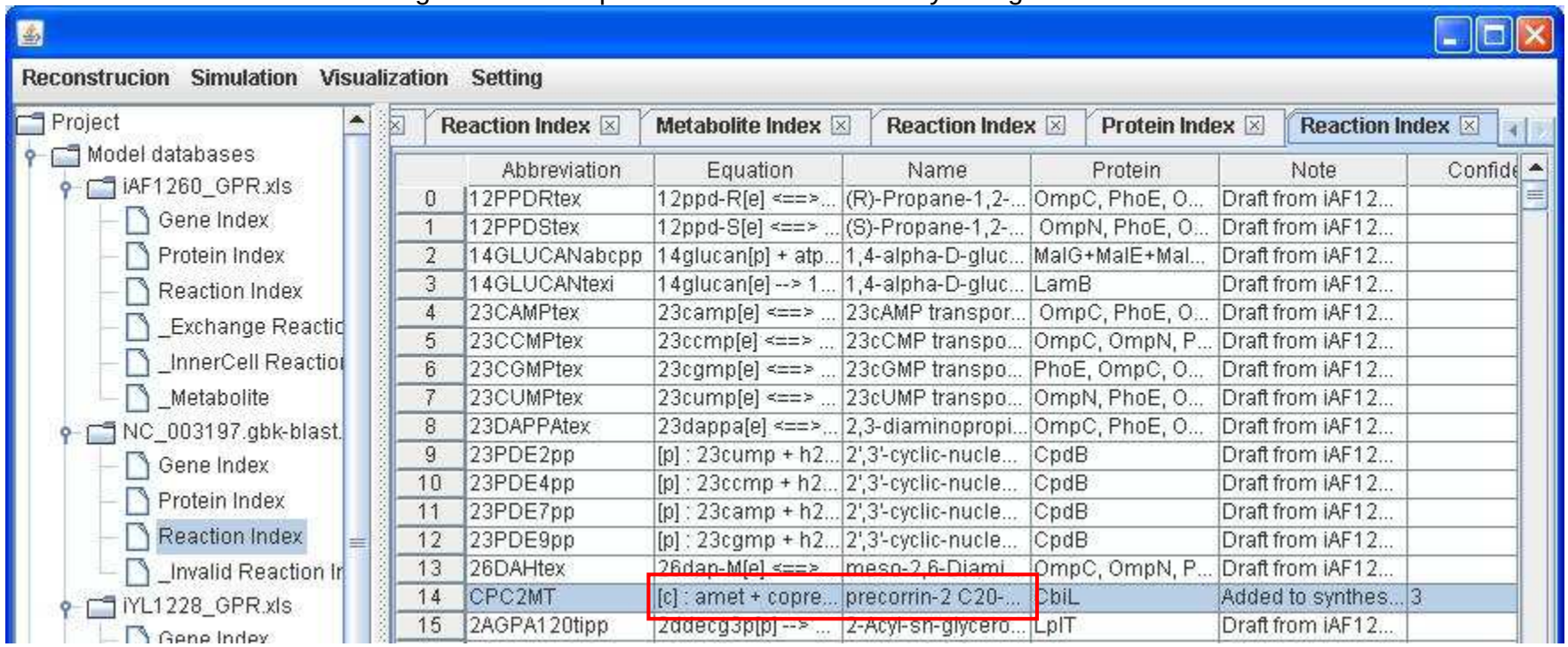
abbreviation CbiL, the associated gene STM2024 and a note Added to synthesize cobalamin.

The gene-protein association will be automatically brought into the Gene Index table.

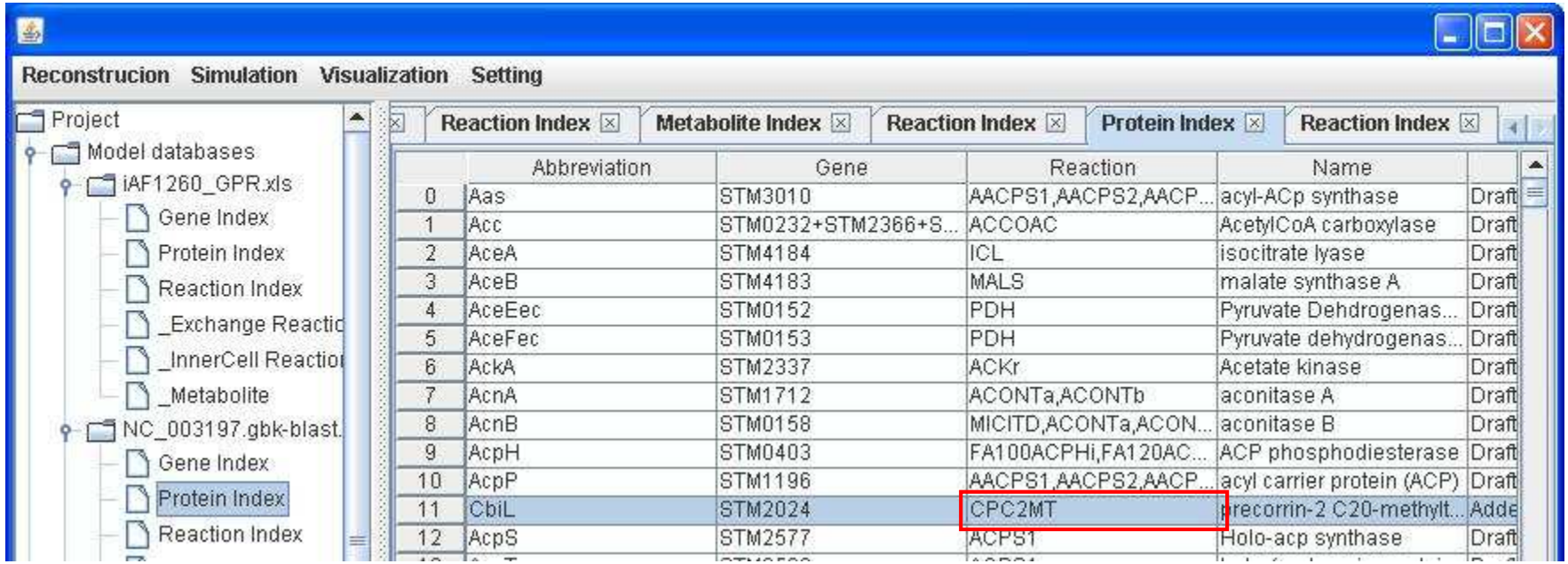


After clicking into the Reaction Index of NC_003197.gbk-blast.xls, right click on the main window of reaction index to insert the reaction abbreviation CPC2MT, the associated protein CbiL, a note Added to synthesize cobalamin and the confidence score 3 for genetic evidence.

The reaction information including name and equation will be automatically brought into the Reaction Index table.



Likewise, the protein-reaction association will be automatically brought into the Reaction Index table.


Reconstrucion
Simulation
Visualization
Setting
Project
Model databases
iAF1260_GPR.xls
Gene Index
Protein Index
Reaction Index
_Exchange Reactic
_InnerCell Reactio
_Metabolite
NC_003197.gbk-blast.
Gene Index
Protein Index
Reaction Index
Reaction Index
Metabolite Index
Reaction Index
Protein Index
Reaction Index
Abbreviation
Gene
Reaction
Name
0 Aas STM3010 AACPS1,AACPS2,AACP... acyl-ACp synthase Draft
1 Acc STM0232+STM2366+S... ACCOAC AcetylCoA carboxylase Draft
2 AceA STM4184 ICL isocitrate lyase Draft
3 AceB STM4183 MALS malate synthase A Draft
4 AceEec STM0152 PDH Pyruvate Dehdrogenas... Draft
5 AceFec STM0153 PDH Pyruvate dehydrogenas... Draft
6 AckA STM2337 ACKr Acetate kinase Draft
7 AcnA STM1712 ACONTa,ACONTb aconitase A Draft
8 AcnB STM0158 MICITD,ACONTa,ACON... aconitase B Draft
9 AcpH STM0403 FA100ACPHi,FA120AC... ACP phosphodiesterase Draft
10 AcpP STM1196 AACPS1,AACPS2,AACP... acyl carrier protein (ACP) Draft
11 CbiL STM2024 CPC2MT precorrin-2 C20-methylt... Adde
12 AcpS STM2577 ACPS1 Holo-acp synthase Draft

Simulation

Before simulation, make sure you have set the path of linear programming solver.

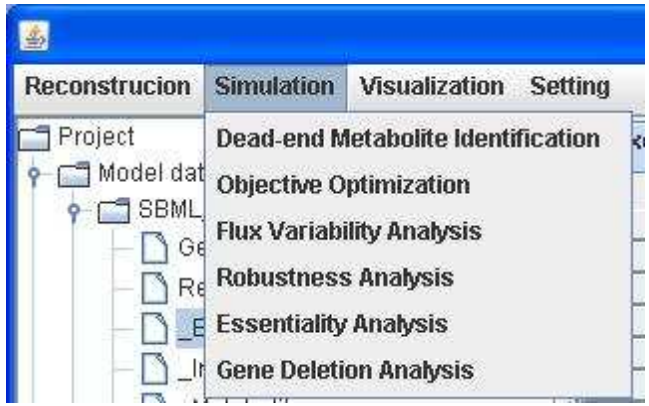
To download GNU Linear Programming Kit (GLPK).

http://sourceforge.net/projects/winglpk/ (for windows) or
http://www.gnu.org/software/glpk/ (for Linux/Mac).

After extracting the file you downloaded (e.g. winglpk-4.45.zip), please add the path of glpsol.exe to your Environment variables.

Open the Control Panel -> Click System -> Click Advanced system setting -> Open Environment variables -> Edit Path -> Add variable value ";the path where glpsol.exe locate" (e.g. ;D:\winglpk-4.45\w64)

Click on Simulation in the menu bar to choose which analysis you want to perform.

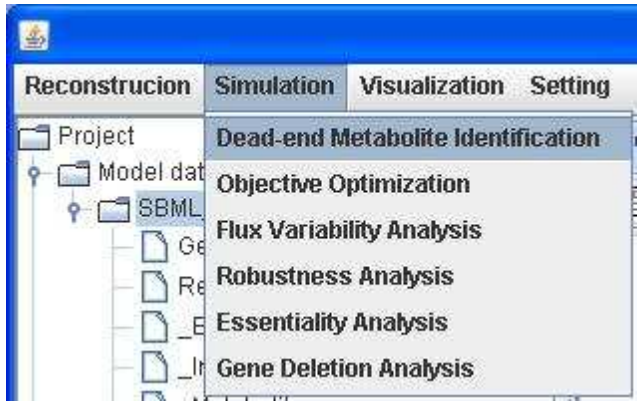


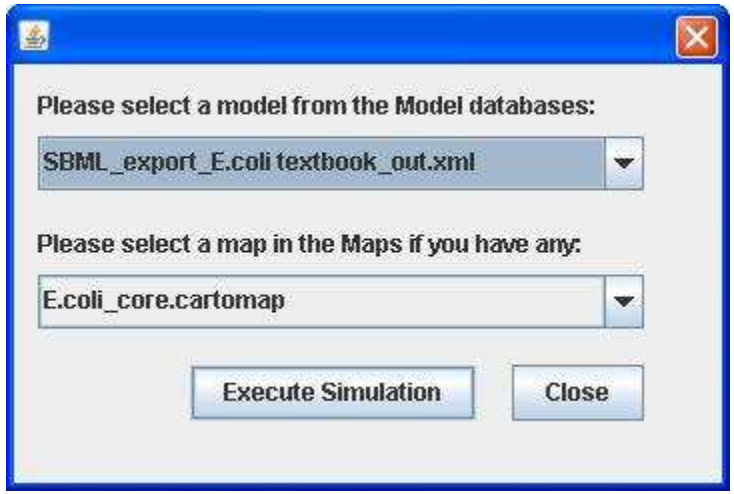
As a case study for demonstration of simulation, we import the E.coli textbook model which was exported from the BiGG into GEMSiRV and use a customized map E.coli_core.cartomap for visualization. You can find and download the model and the map from http://sb.nhri.org.tw/GEMSiRV/en/Metabolic_Models and http://sb.nhri.org.tw/GEMSiRV/en/Metabolic_Maps, respectively.

Dead-end metabolite identification

A network reconstruction is converted into a mathematical model including a stoichiometric matrix which describes the connectivity feature of the network and defined systems boundaries before simulation. GEMSiRV can examine the connectivity of all metabolites in a network for dead-end metabolite identification and tag such metabolic dead ends with crosses in the map.

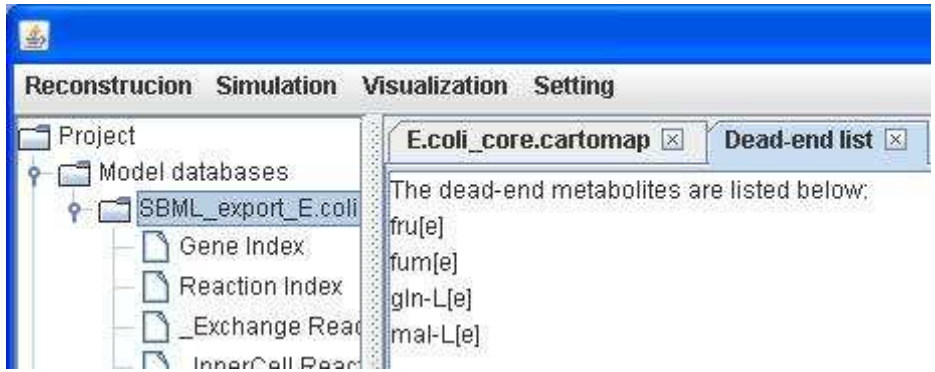
You can select a model and a map (if you have) to perform the examination of network connectivity for dead-end metabolite identification.



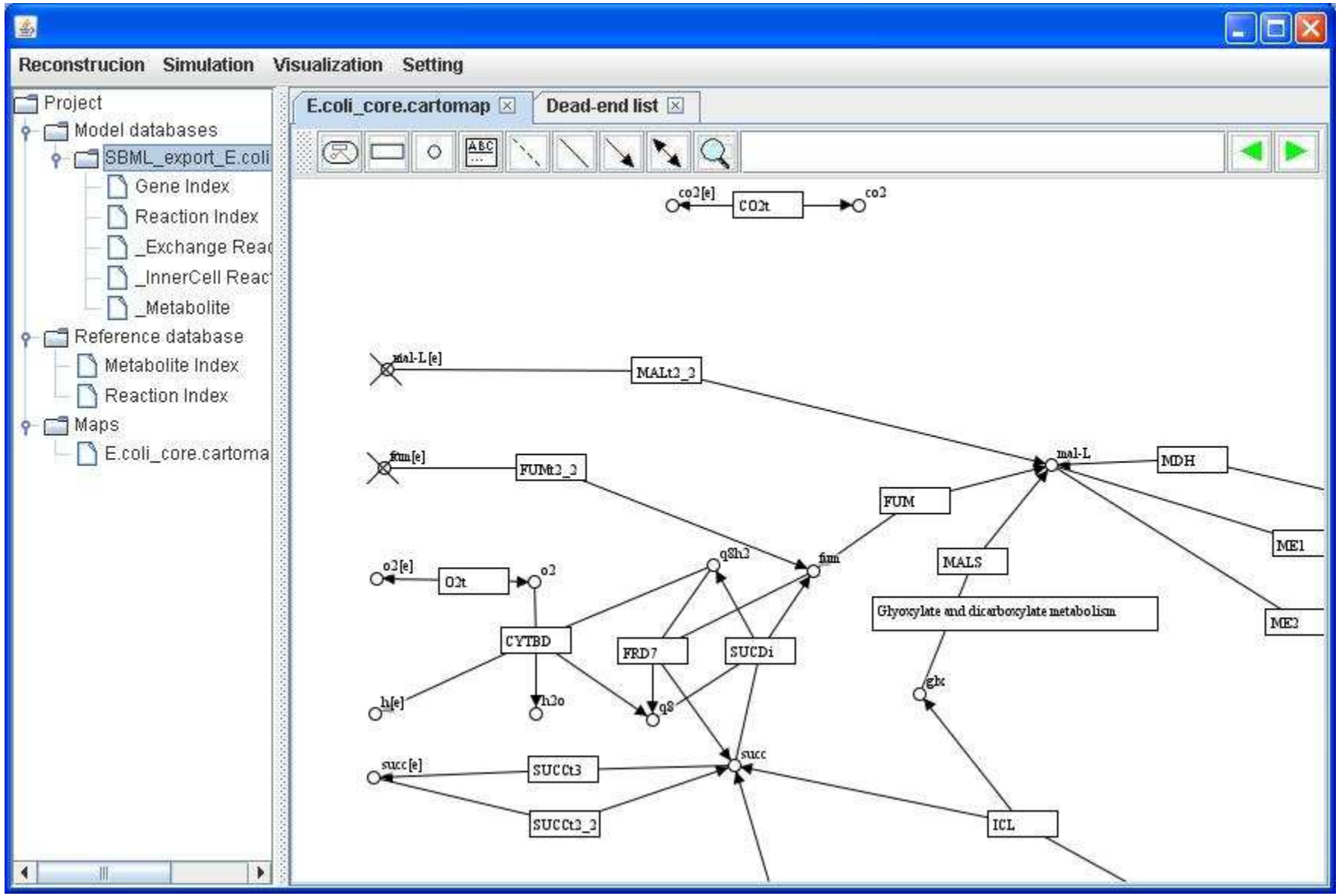


A dead-end metabolite list is generated and those metabolites are tagged with crosses in the map.

A list for dead-end metabolites:



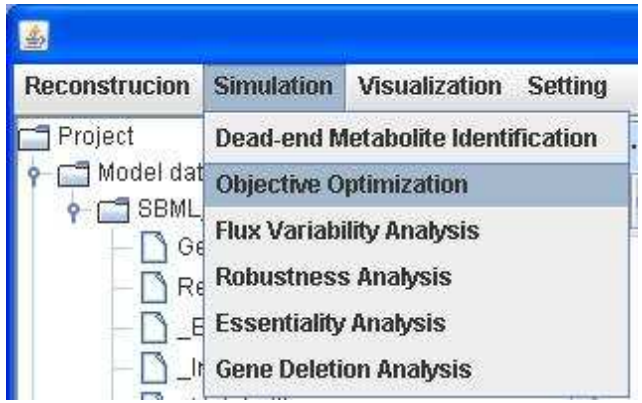
A visualization map with dead-end metabolites:

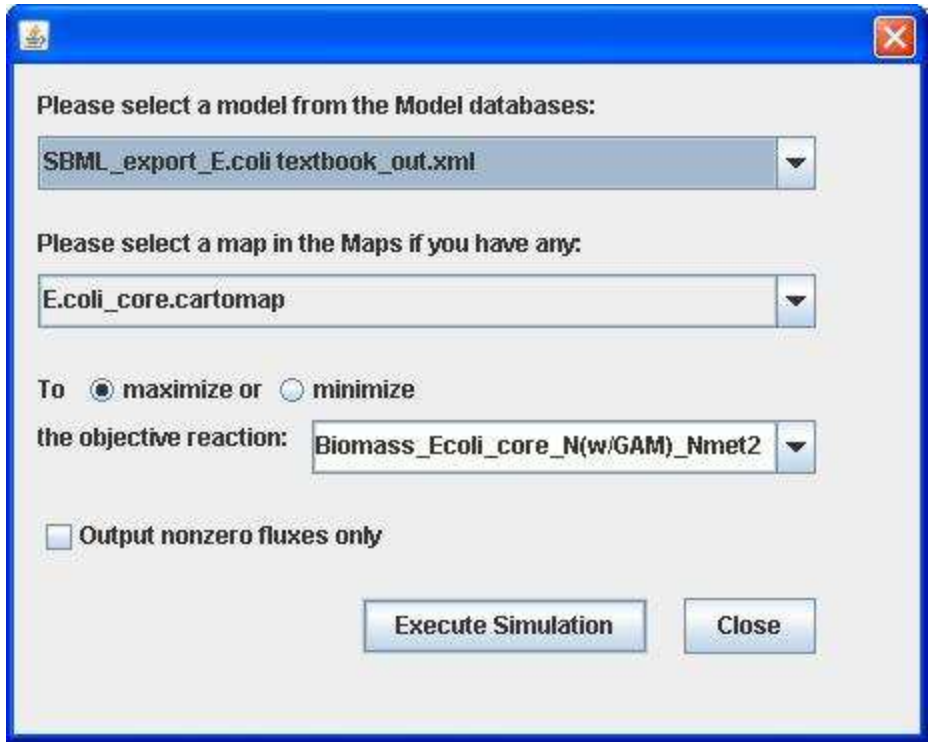

Reconstrucion
Simulation
Visualization
Setting
Project
Model databases
SBML_export_E.coli
Gene Index
Reaction Index
_Exchange Reac
_InnerCell Reac
_Metabolite
Reference database
Metabolite Index
Reaction Index
Maps
E.coli_core.cartoma
E.coli_core.cartomap
Dead-end list
co2[e]
CO2t
co2
mal-L[e]
MALt2_2
fum[e]
FUMt2_2
mal-L
MDH
FUM
ME1
o2[e]
O2t
o2
q8h2
fum
MALS
Glyoxylate and dicarboxylate metabolism
ME2
CYTBD
FRD7
SUCDi
h[e]
h2o
q8
glx
succ[e]
SUCCt3
succ
SUCCt2_2
ICL

Objective optimization

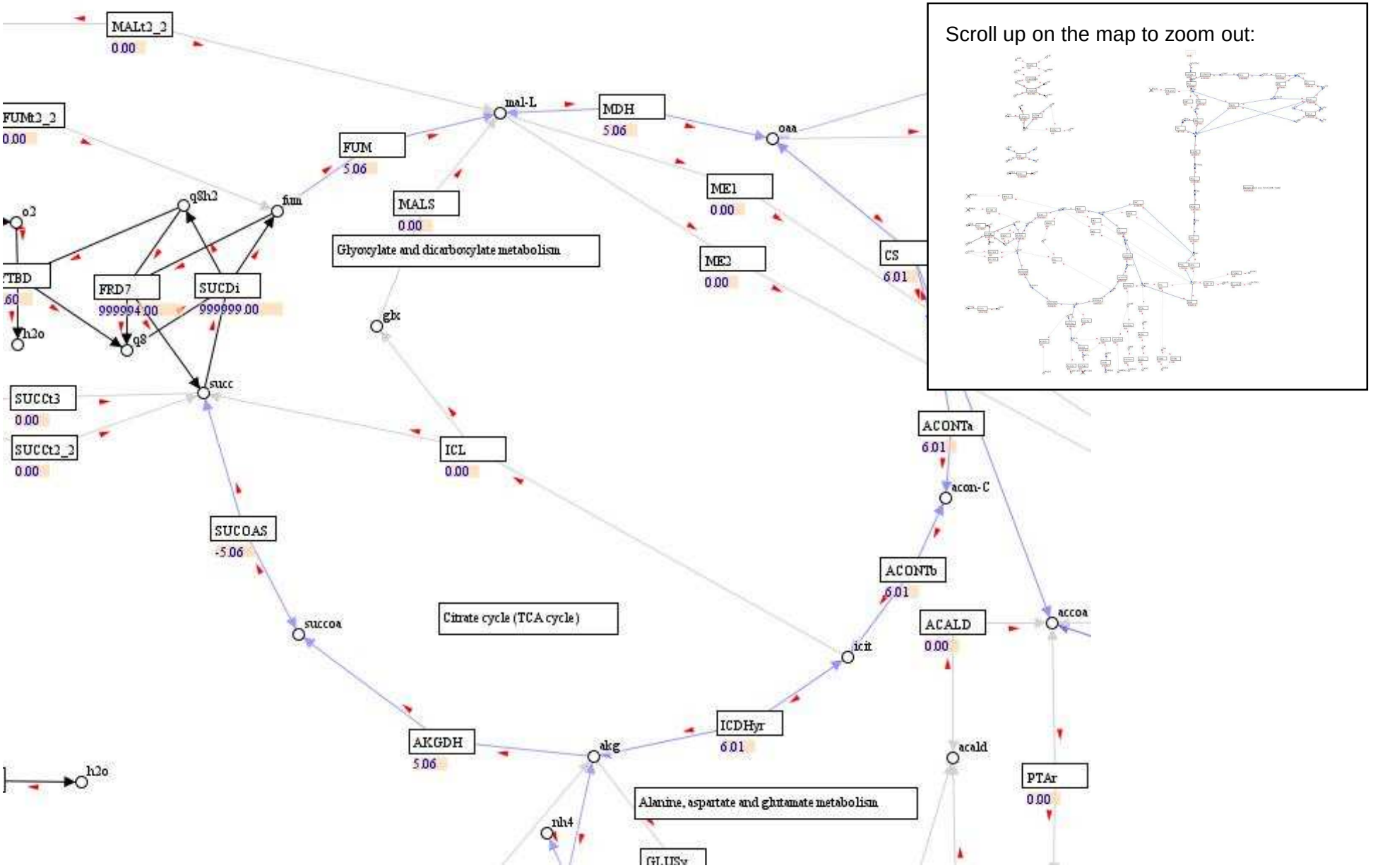
With a set linear programming solver (e.g. glpk), GEMSiRV can be used to simulate the imported metabolic network model. Please refer to http://sb.nhri.org.tw/GEMSiRV/en/Installation for setting up GEMSiRV. Given proper constraints and objective function, the flux results of all reactions in the model will be estimated.

You can select a model and a map (if you have) for objective optimization. The flux results can be visualized in the map.

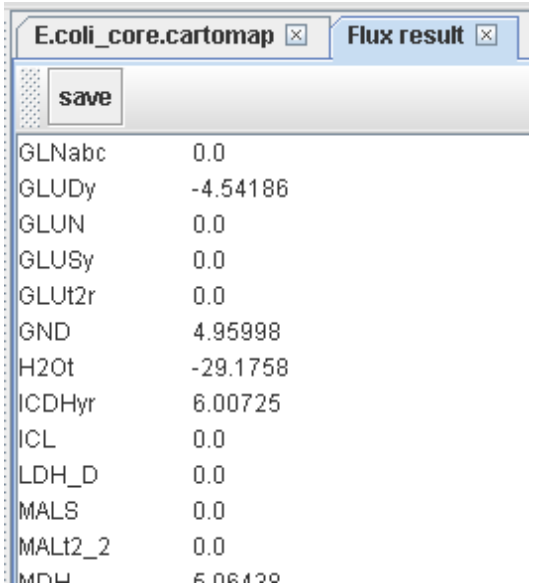




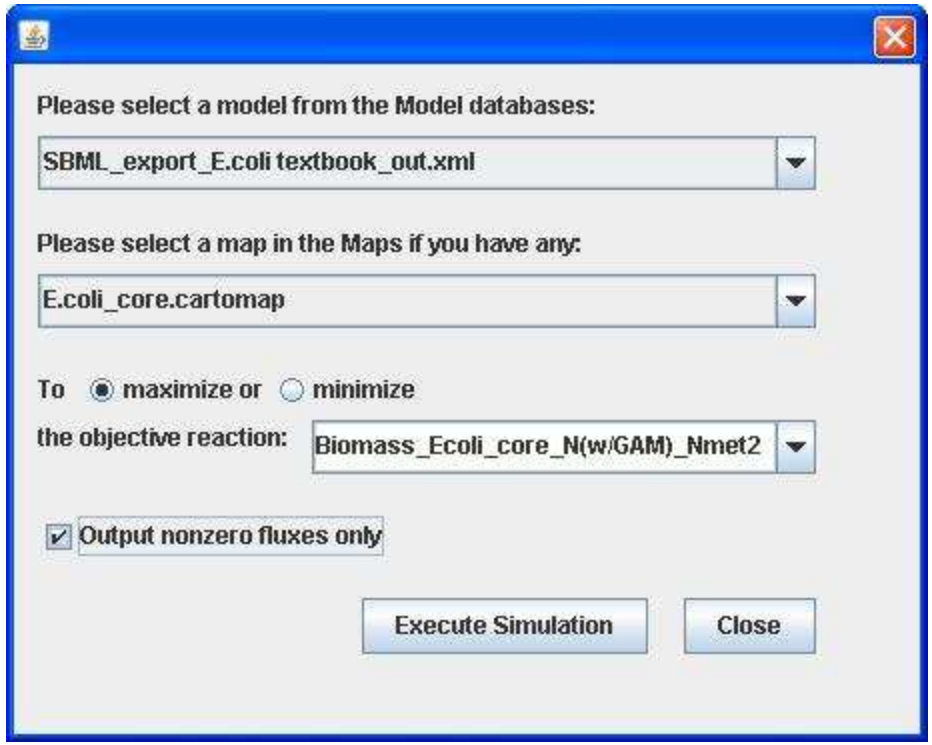
A visualization map with reaction fluxes:

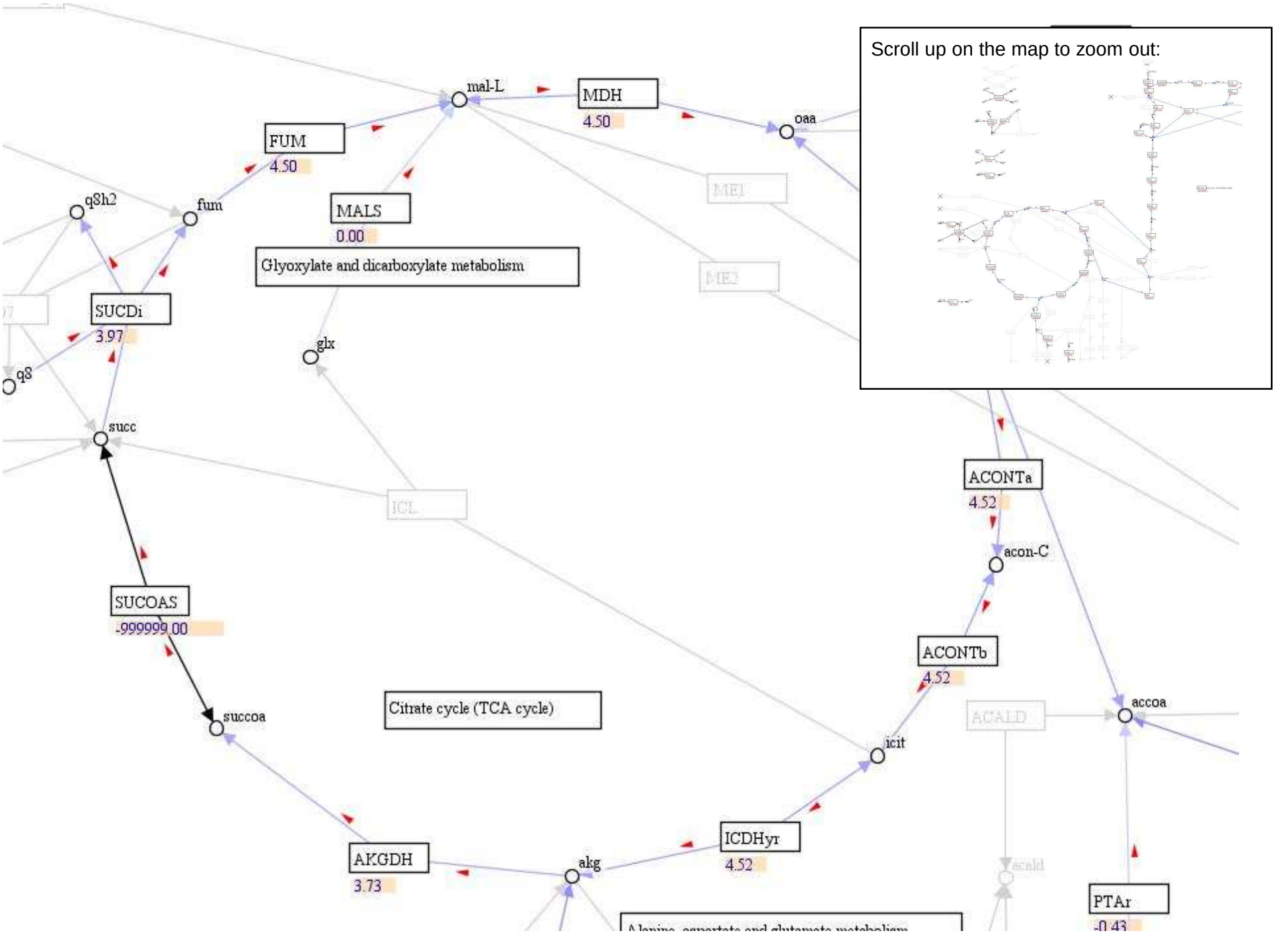

Scroll up on the map to zoom out:
MALt2_2
0.00
FUMt2_2
0.00
o2
TBD
.60
h2o
q8h2
fum
FRD7
999994.00
SUCDi
999999.00
q8
succ
FUM
5.06
mal-L
MDH
5.06
oaa
MALS
0.00
ME1
0.00
ME2
0.00
CS
6.01
Glyoxylate and dicarboxylate metabolism
glx
SUCCt3
0.00
SUCCt2_3
0.00
ICL
0.00
ACONTa
6.01
acon-C
SUCOAS
-5.06
ACONTb
6.01
Citrate cycle (TCA cycle)
succoa
icit
ACALD
0.00
accoa
AKGDH
5.06
akg
ICDHyr
6.01
acald
PTAr
0.00
h2o
Alanine, aspartate and glutamate metabolism
nh4

Flux result:



To check the checkbox for outputting nonzero fluxes only.

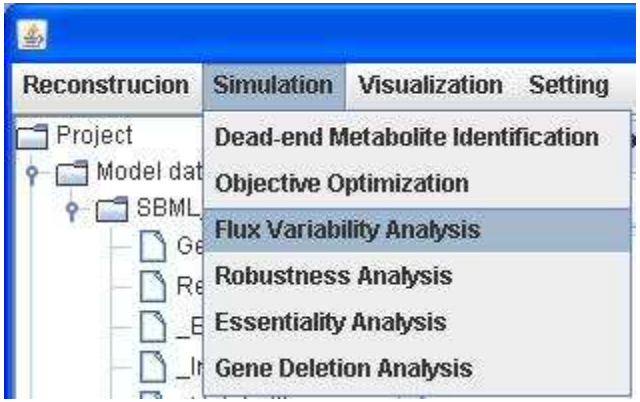

Please select a model from the Model databases:
SBML_export_E.coli textbook_out.xml
Please select a map in the Maps if you have any:
E.coli_core.cartomap
To maximize or minimize
the objective reaction:
Biomass_Ecoli_core_N(w/GAM)_Nmet2
Output nonzero fluxes only
Execute Simulation
Close

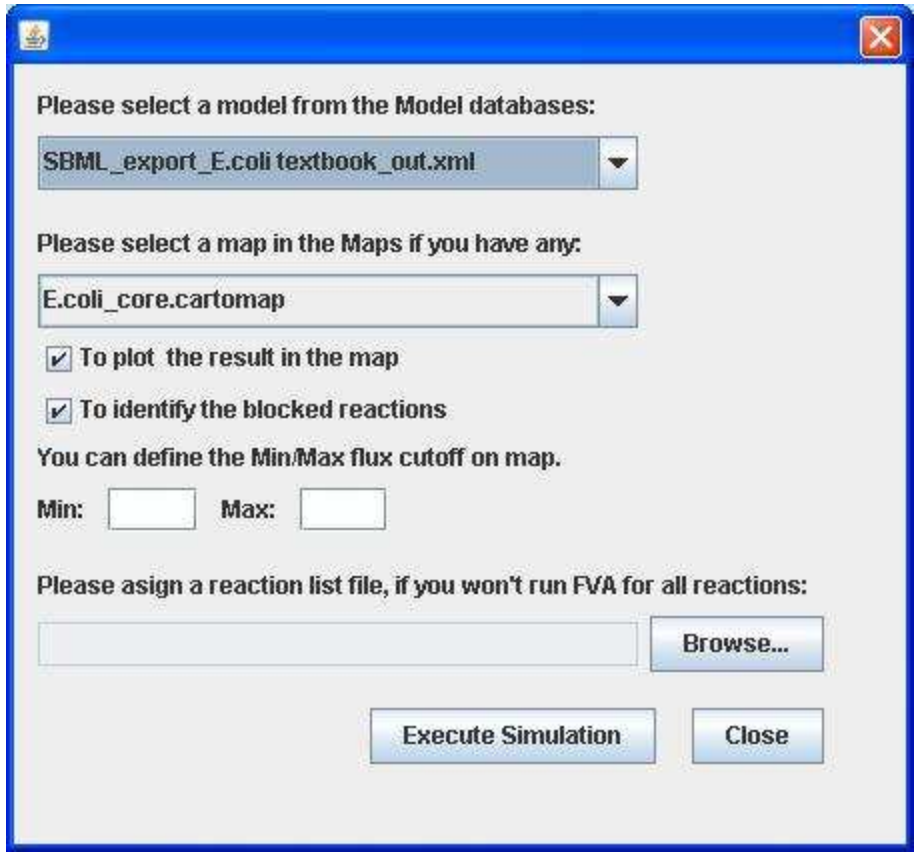

Scroll up on the map to zoom out:
mal-L
MDH
4.50
oaa
FUM
4.50
fum
MALS
0.00
q8h2
Glyoxylate and dicarboxylate metabolism
SUCDi
3.97
q8
glx
succ
ICL
ACONTa
4.52
acon-C
SUCOAS
-999999.00
ACONTb
4.52
accoa
succoa
Citrate cycle (TCA cycle)
ACALD
icit
AKGDH
3.73
akg
ICDHyr
4.52
PTAr

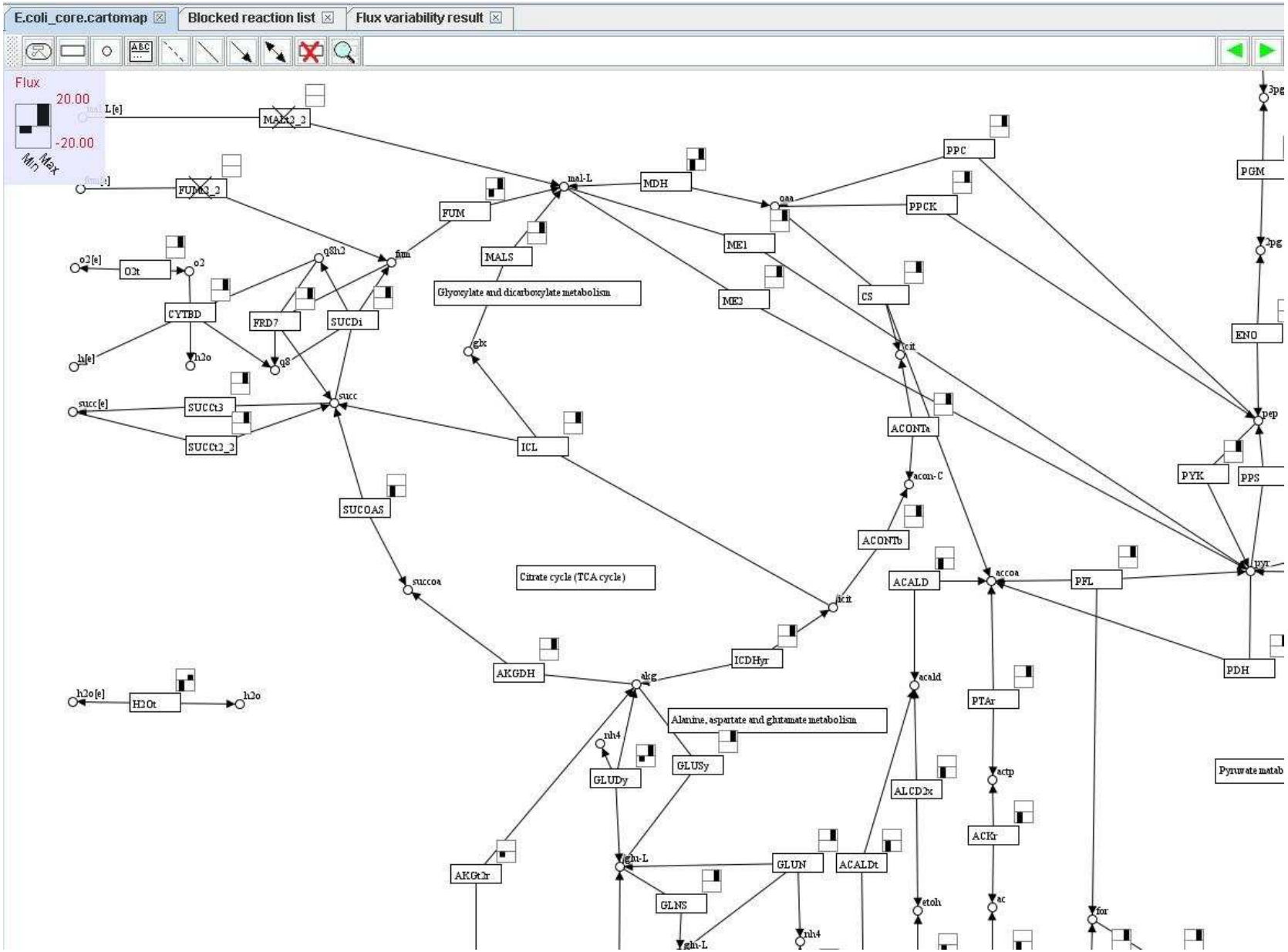
Flux variability analysis

Flux variability analysis can be used to study the redundancy of reactions in a network. GEMSiRV can determine the minimum and maximum flux values for each reaction in the model and thus identify the blocked reactions which carry zero fluxes for the both conditions and tag them with crosses in a map as well.

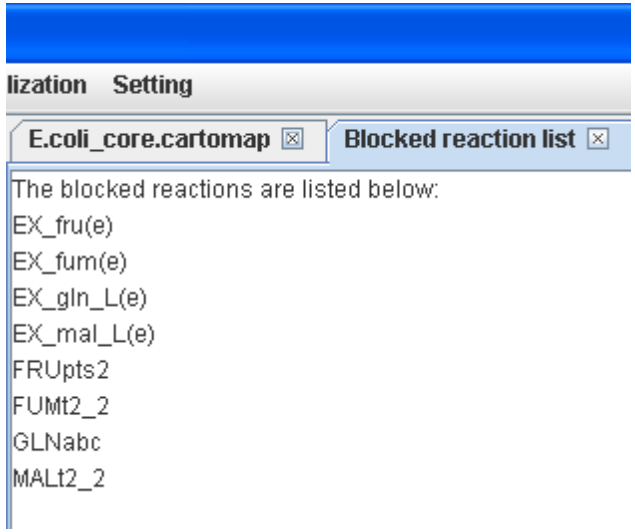
You can select a model and a map (if you have) for flux variability analysis. The min and max fluxes of reaction are plotted in the map and the blocked reaction are tagged with crosses.



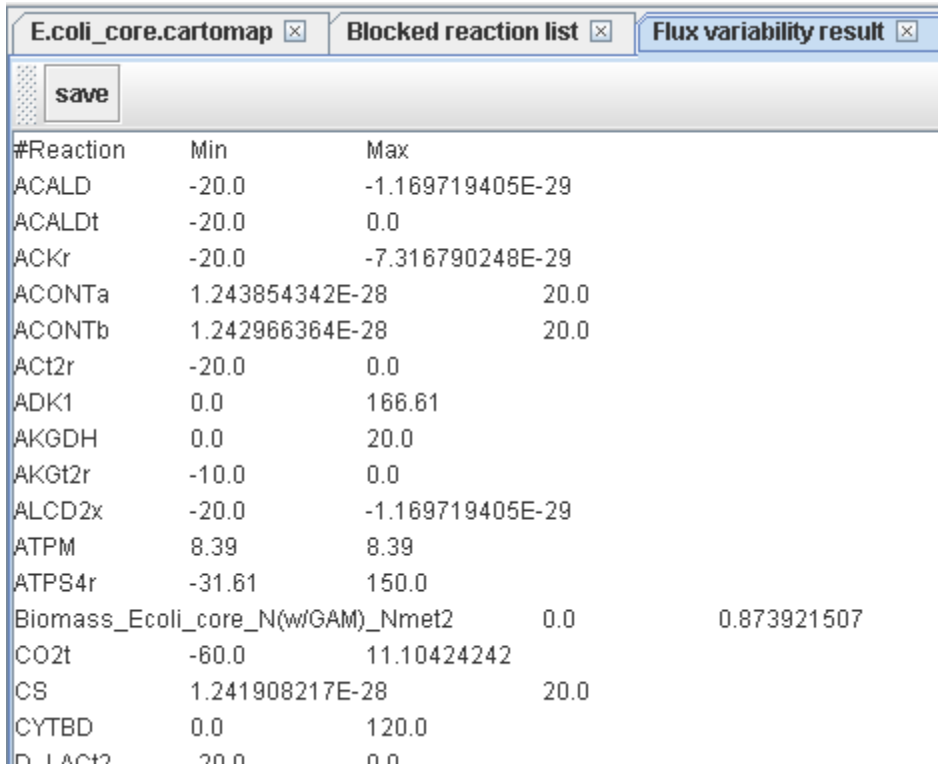

Please select a model from the Model databases:
SBML_export_E.coli textbook_out.xml
Please select a map in the Maps if you have any:
E.coli_core.cartomap
To plot the result in the map
To identify the blocked reactions
You can define the Min/Max flux cutoff on map.
Min:
Max:
Please asign a reaction list file, if you won't run FVA for all reactions:
Browse...
Execute Simulation
Close


E.coli_core.cartomap
Blocked reaction list
Flux variability result
Flux
20.00
-20.00
Min
Max
MALS2_2
FUMK2_2
FUM
mal-L
MDH
oaa
PPC
PPCK
PGM
3pg
2pg
ENO
pep
ME1
ME2
CS
cit
ACONTa
ACONTb
acon-C
ICDHyr
icit
AKGDH
akg
SUCOAS
succoa
succ
SUCCt3
SUCCt2_2
succ[e]
ICL
glx
Glyoxylate and dicarboxylate metabolism
Citrate cycle (TCA cycle)
Alanine, aspartate and glutamate metabolism
MALS
FRD7
SUCDi
CYTBD
O2t
o2[e]
o2
q8h2
q8
fum
h[e]
h2o
H2Ot
h2o[e]
nh4
GLUDy
GLUSy
gln-L
GLUN
GLNS
AKGt2r
ACALD
ACALDt
acald
PFL
accoa
PTAr
actp
ACKr
ac
ALCD2x
etoh
for
PDH
PYK
PPS
pyr
Pyruvate metab

A list for the blocked reactions:



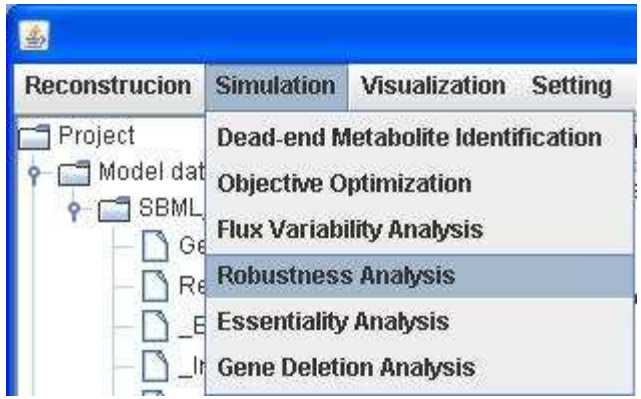
Flux variability result:



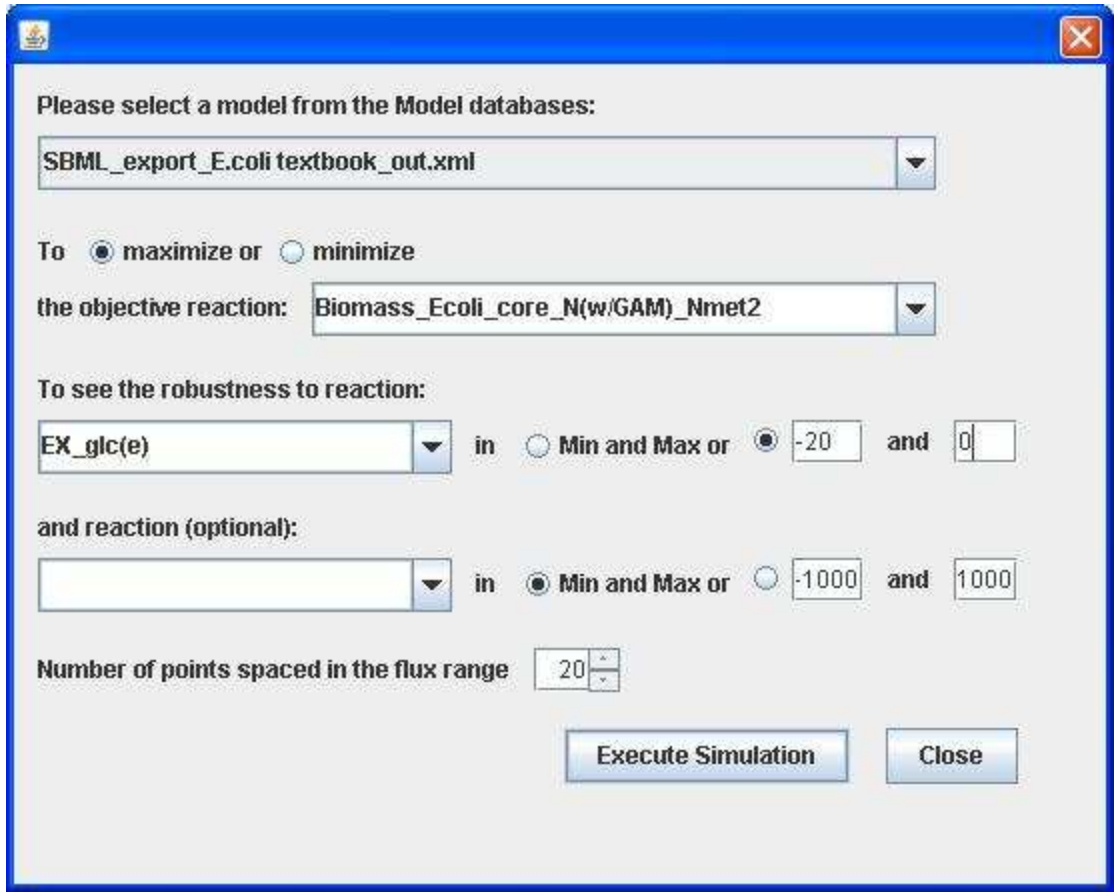
#Reaction	Min	Max		
ACALD	-20.0	-1.169719405E-29		
ACALDt	-20.0	0.0		
ACKr	-20.0	-7.316790248E-29		
ACONTa	1.243854342E-28		20.0	
ACONTb	1.242966364E-28		20.0	
ACt2r	-20.0	0.0		
ADK1	0.0	166.61		
AKGDH	0.0	20.0		
AKGt2r	-10.0	0.0		
ALCD2x	-20.0	-1.169719405E-29		
ATPM	8.39	8.39		
ATPS4r	-31.61	150.0		
Biomass_Ecoli_core_N(w/GAM)_Nmet2			0.0	0.873921507
CO2t	-60.0	11.10424242		
CS	1.241908217E-28		20.0	
CYTBD	0.0	120.0		
D_LACt2	-20.0	0.0		

Robustness analysis

Robustness analysis can be used to study the effect of changing a reaction flux on the other reaction flux, especially on the objective of interest (e.g. growth rate). Therefore, you can select the reactions of interest in the model for robustness analysis.

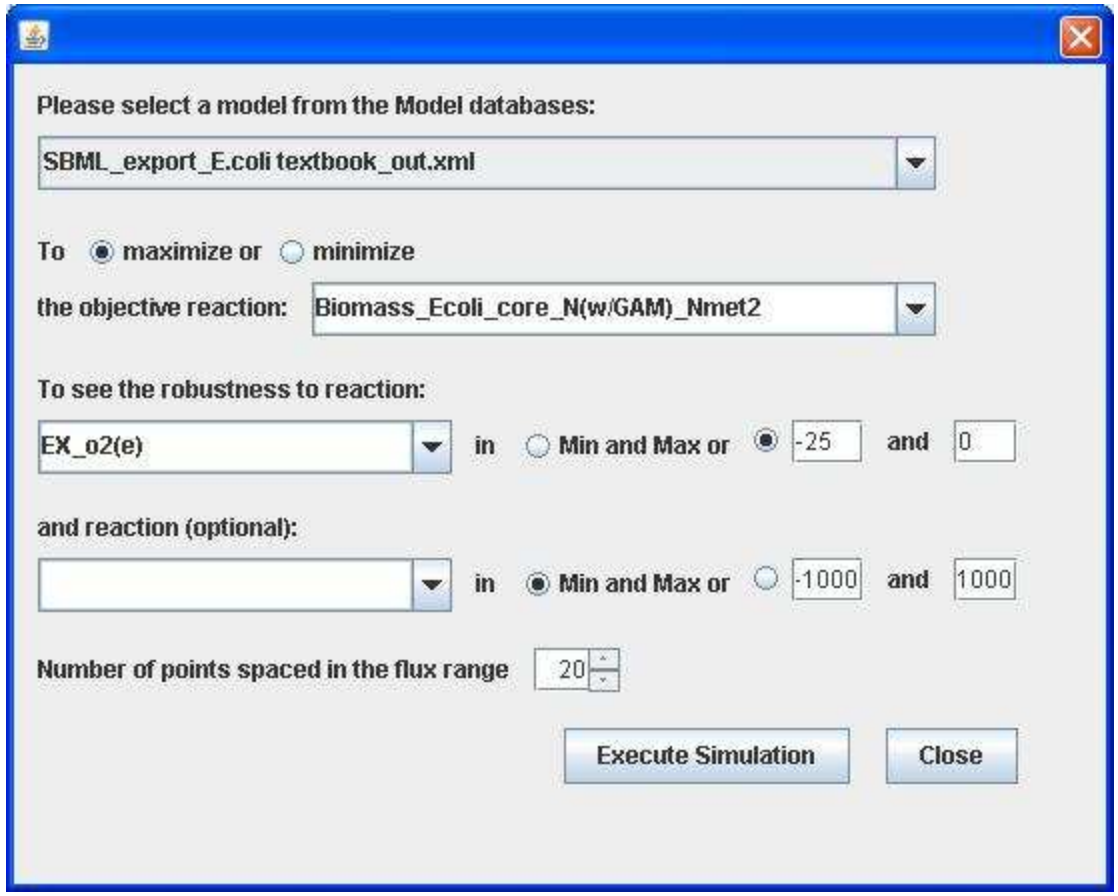


To see how sensitive of the objective reaction (Biomass) is to the glucose uptake rate in the range of -20 to 0 mmol/gDW/h.

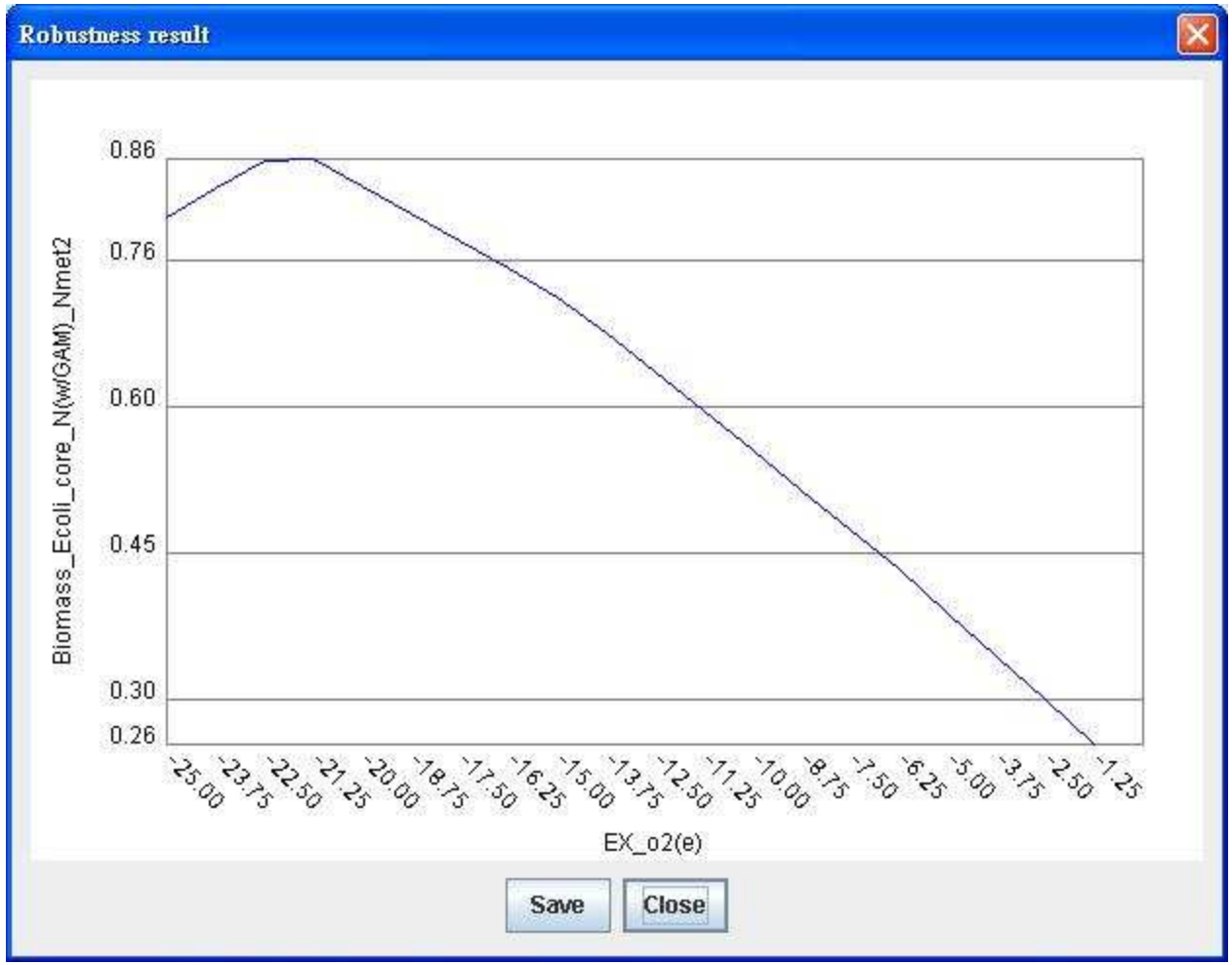


Robustness analysis for growth rate maximization while changing glucose uptake rate (uptaking 0-20 mmol/gDW/h) with oxygen uptake fixed at 17 mmol/gDW/h (set LB to -17)

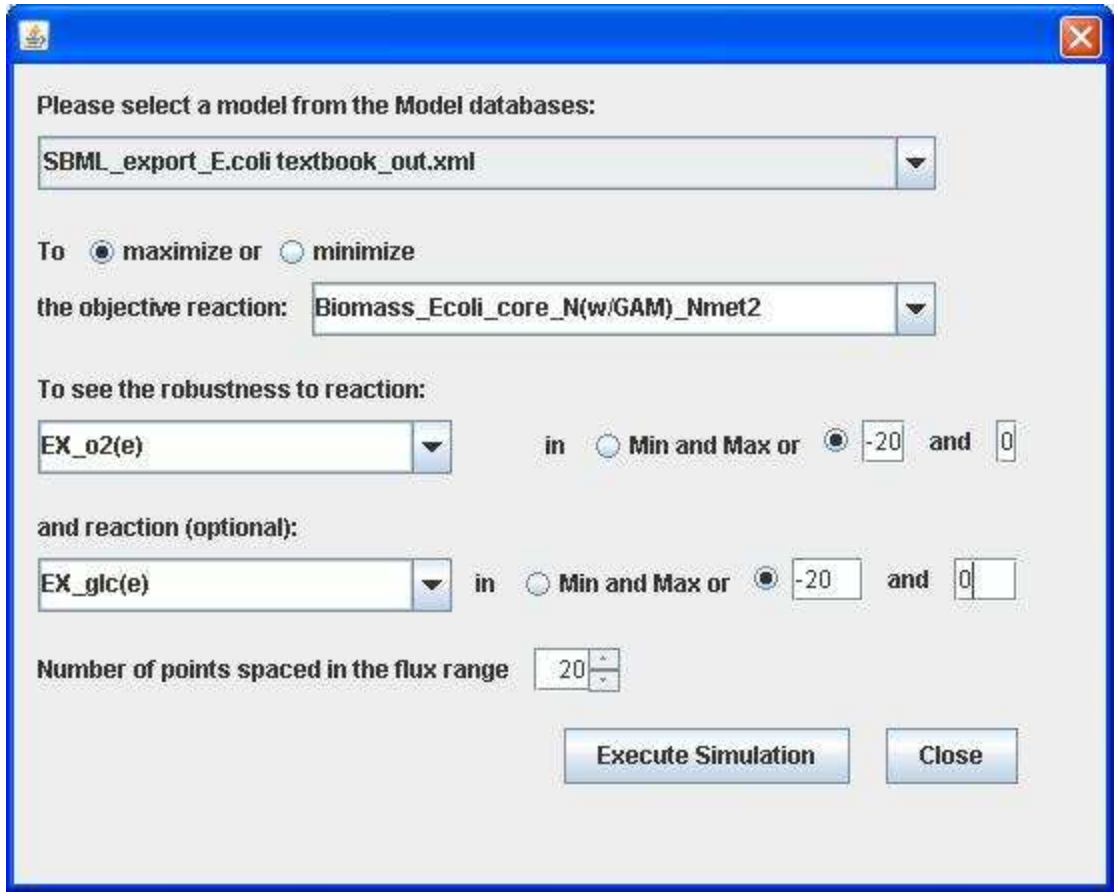
To see how sensitive of the objective reaction (Biomass) is to the oxygen uptake rate in the range of -25 to 0 mmol/gDW/h.



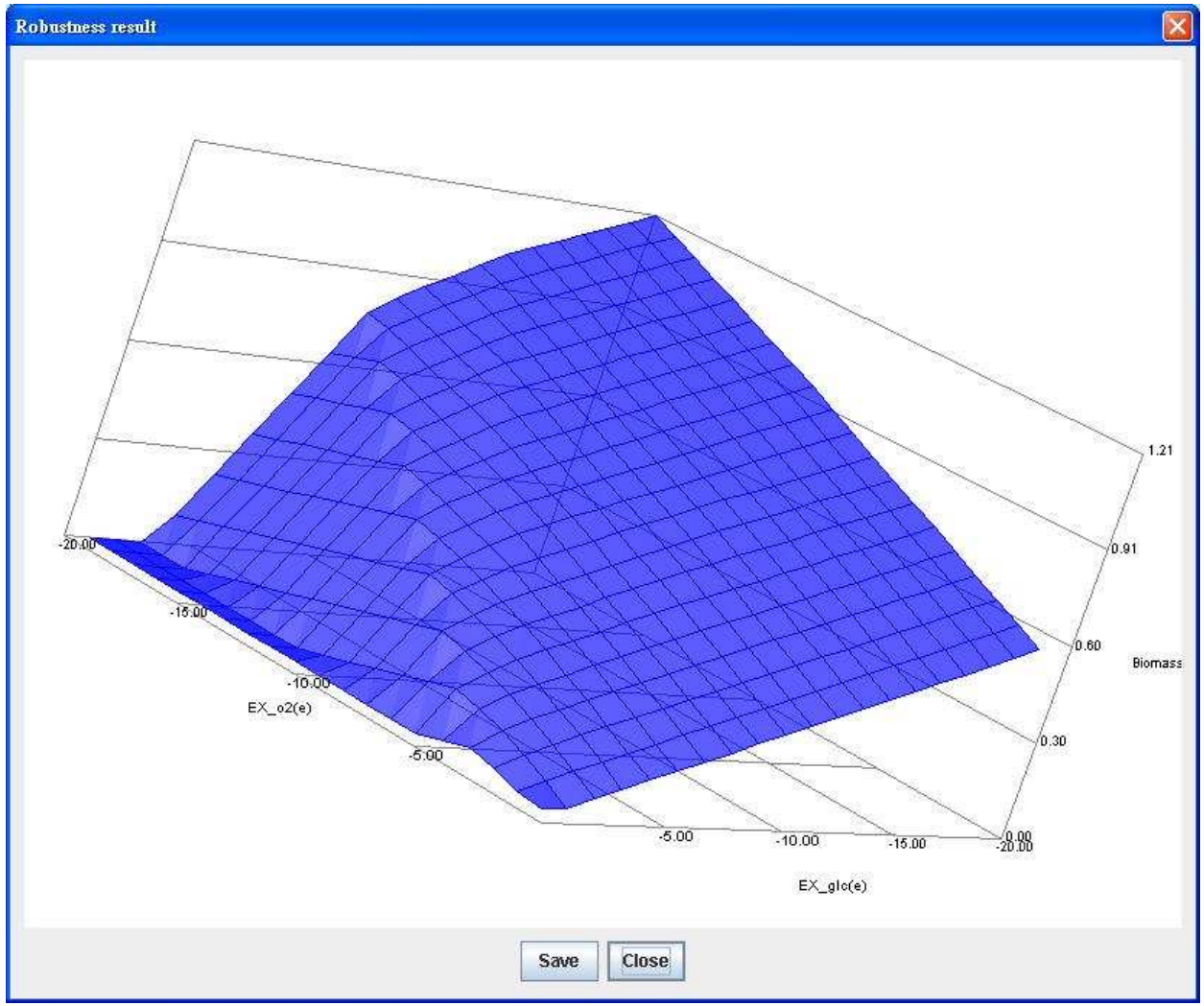
Robustness analysis for growth rate maximization while changing oxygen uptake rate (uptaking 0-25 mmol/gDW/h) with glucose uptake fixed at 10 mmol/gDW/h (set LB to -10).



To change two reactions simultaneously. GEMSiRV can plot the results as a phenotypic phase plane.



The phenotypic phase plane for growth rate maximization while changing glucose and oxygen uptake rates in the range of -20 to 0 mmol/ gDW/h.

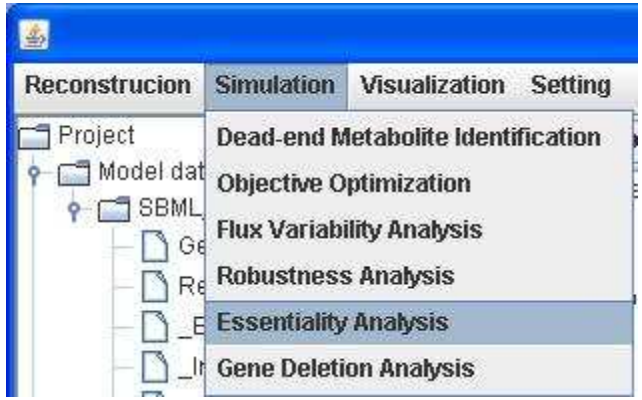


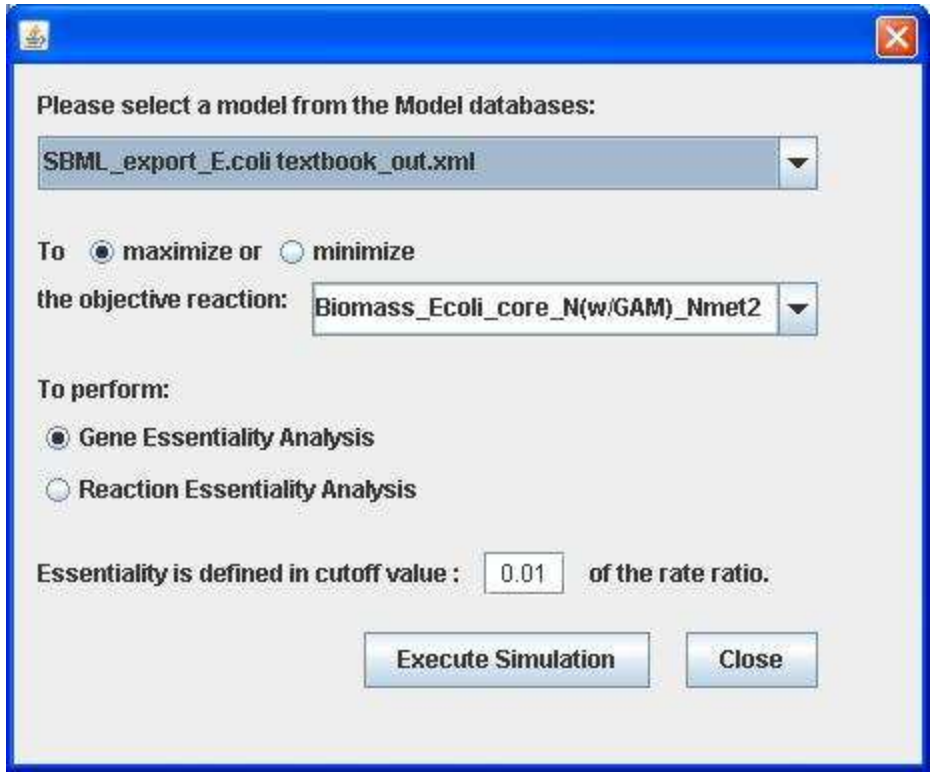
Essentiality analysis

To constrain a reaction in a zero flux can simulate the reaction deletion. Likewise, to constrain the reaction corresponding to a deleted gene can simulate the gene deletion. GEMSiRV performs essentiality analysis for gene and reaction separately and determines the rate

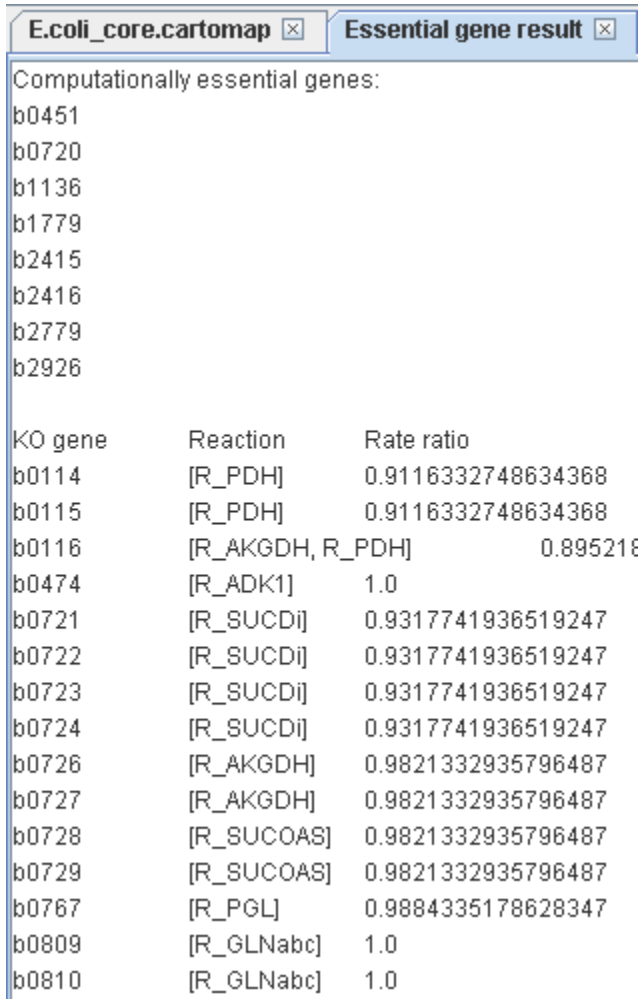
ratio (objective flux of deleted model to that of wild-type model) for every single-knockout condition.

You can select a metabolic model for essentiality analysis, the computational essential genes or reactions can be identified.

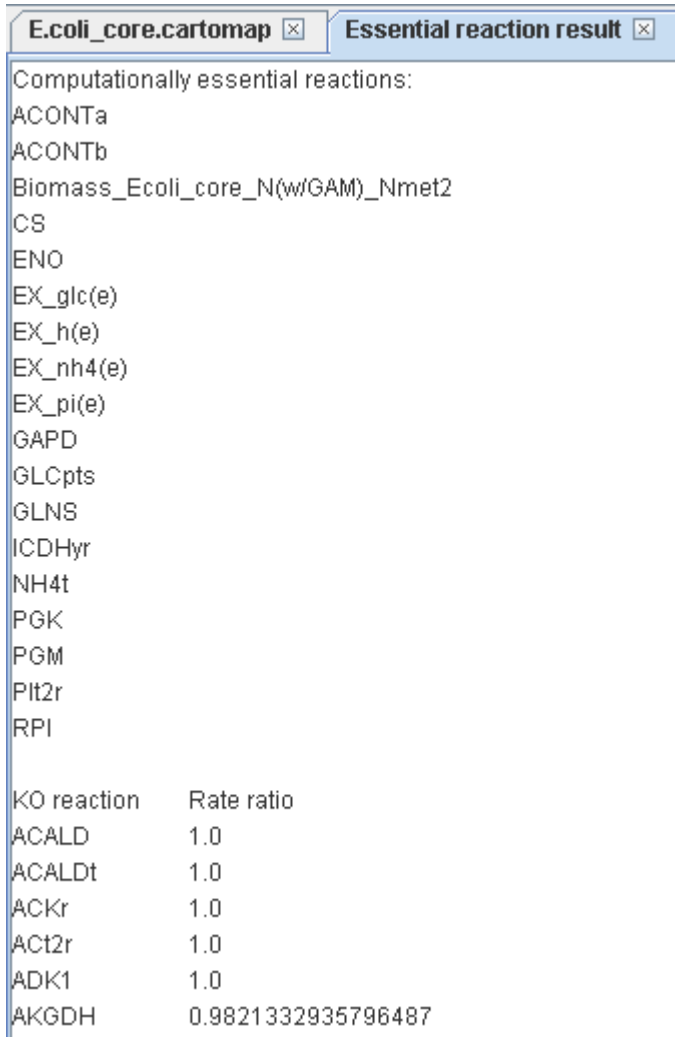




Results of gene essentiality analysis:



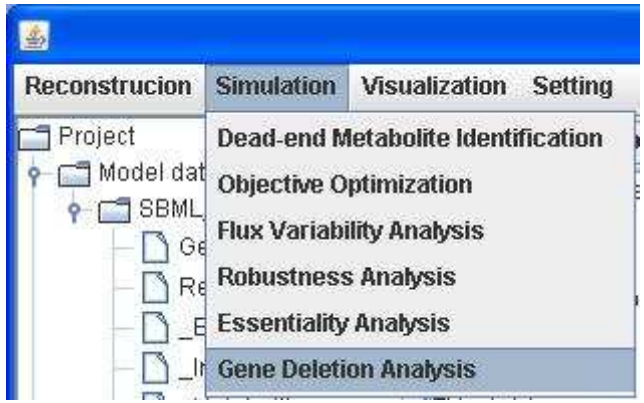
Results of reaction essentiality analysis:



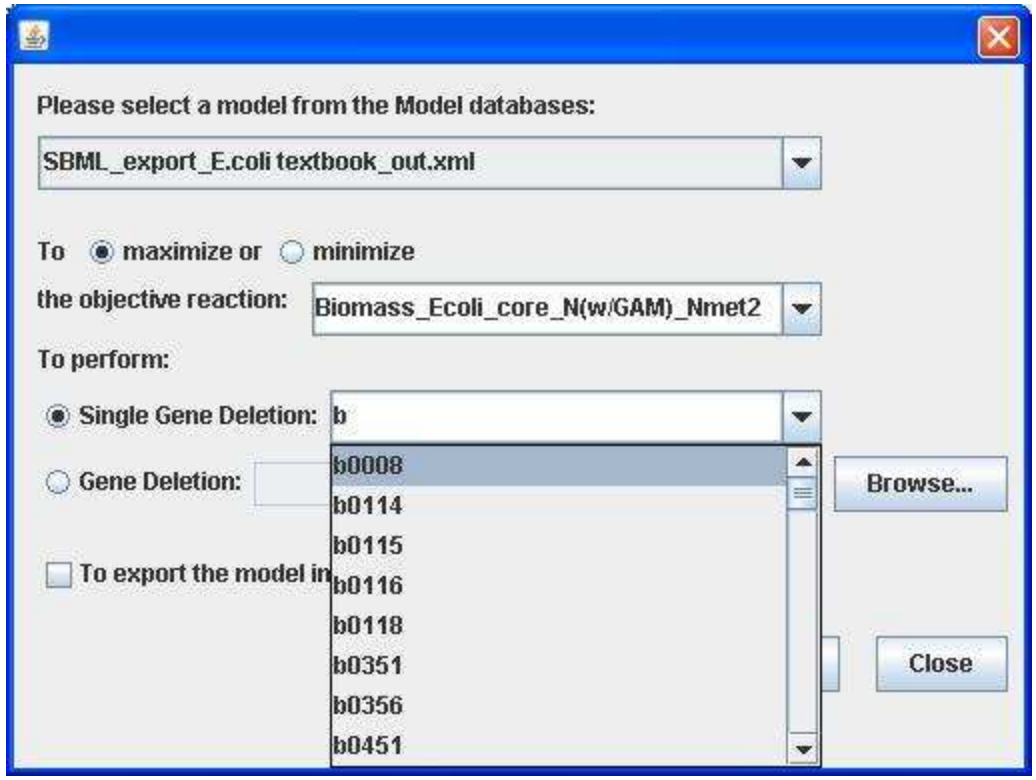
Gene deletion analysis

Gene deletion analysis is carried out by imposing a single-gene deletion or a set of gene deletions at a time, which simulates biological knockout mutant or transcriptional regulatory constraints. GEMSiRV performs the gene deletion analysis to generate the flux result and a

SBML model for the specified condition.

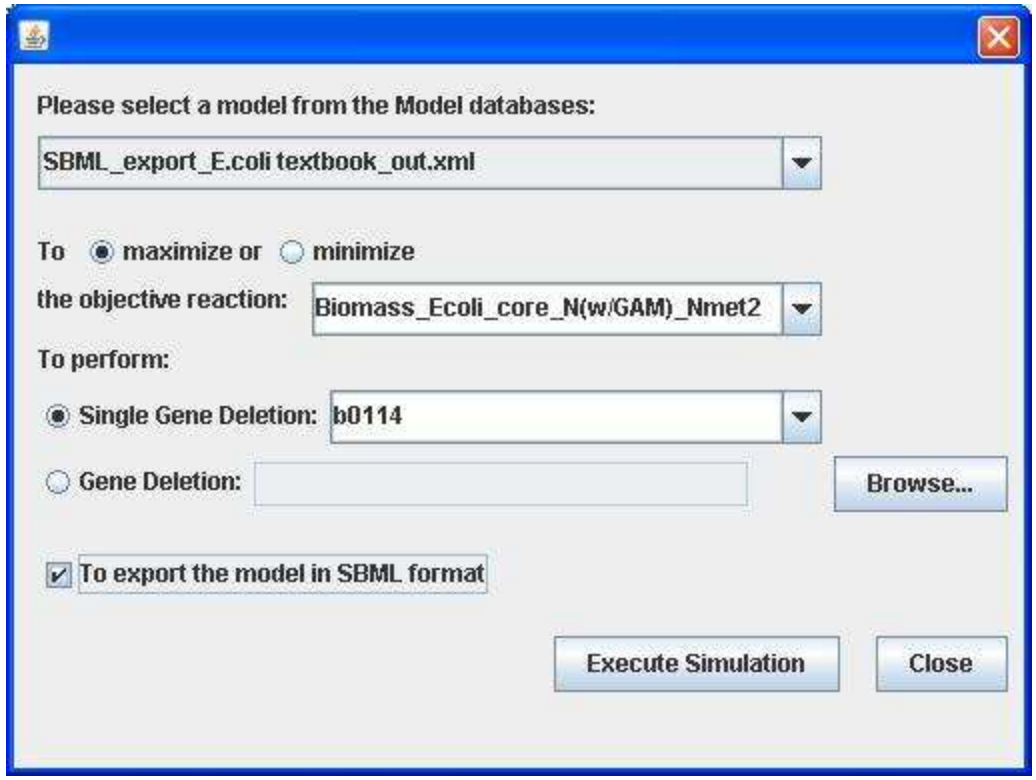


To delete a single gene:

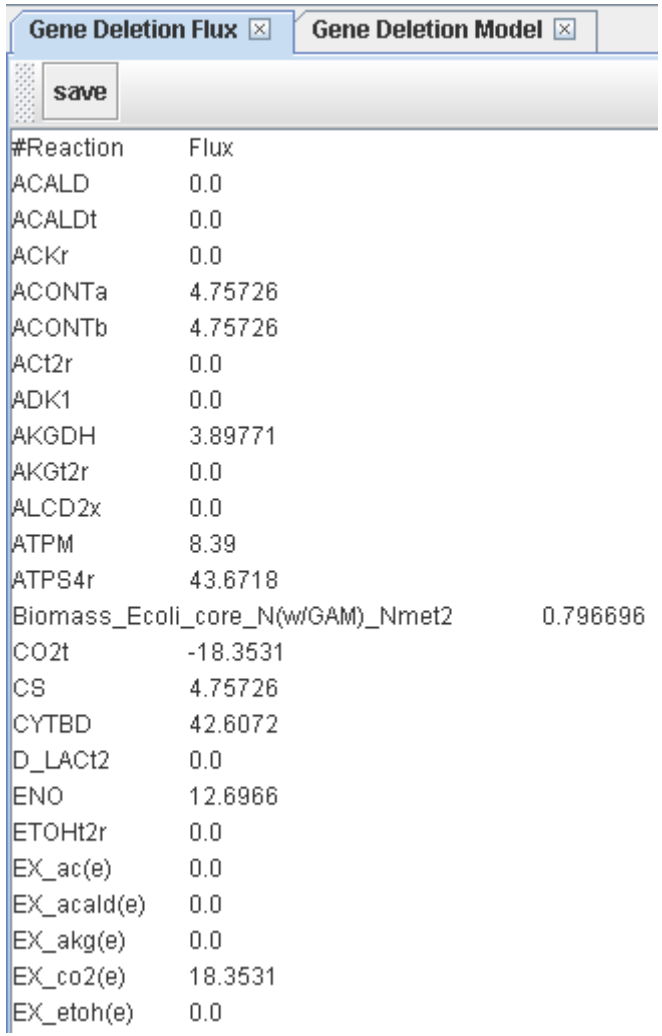


Or you can upload a list of genes for multiple-gene deletion.

To export the SBML file with single gene deletion by checking the checkbox.

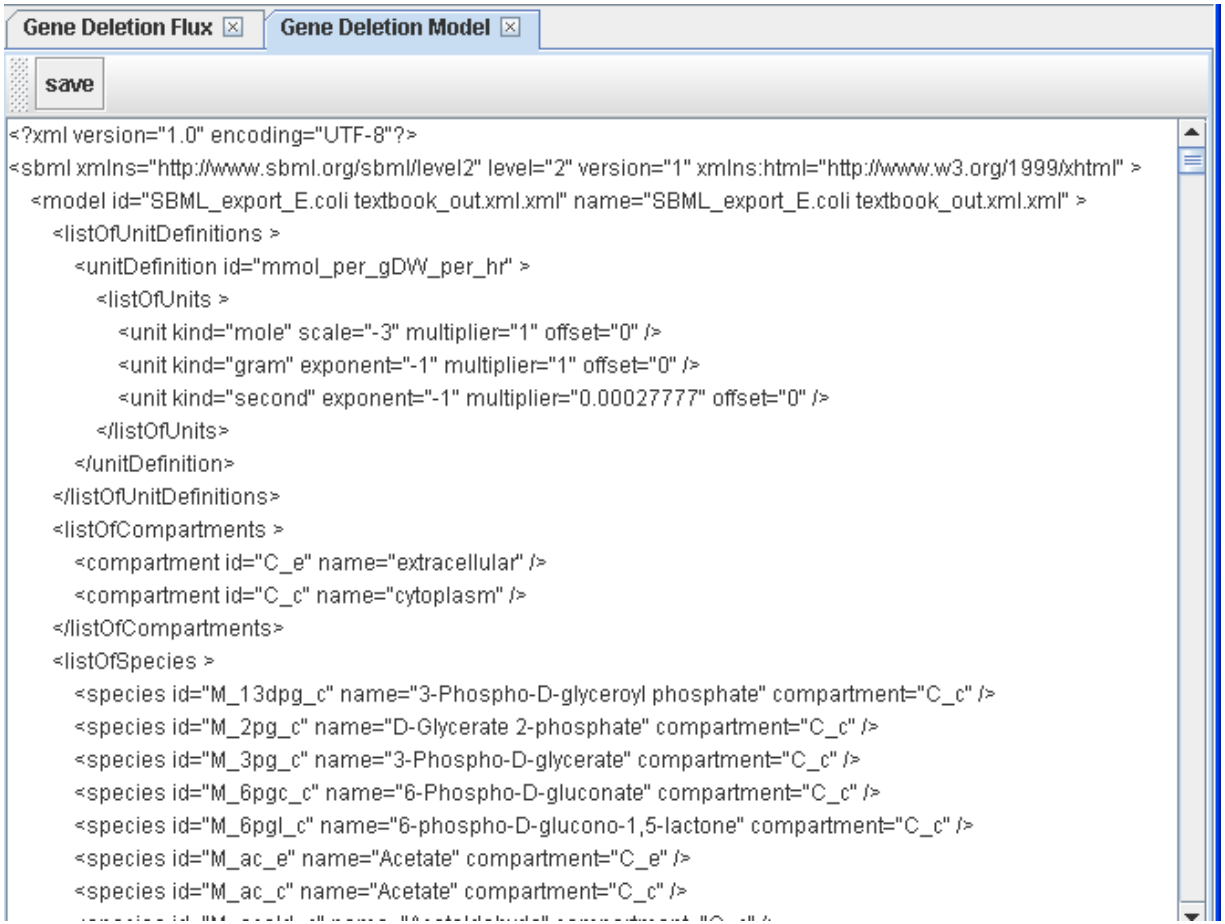


The flux result for the single gene deletion model:



#Reaction	Flux	
ACALD	0.0	
ACALDt	0.0	
ACKr	0.0	
ACONTa	4.75726	
ACONTb	4.75726	
ACt2r	0.0	
ADK1	0.0	
AKGDH	3.89771	
AKGt2r	0.0	
ALCD2x	0.0	
ATPM	8.39	
ATPS4r	43.6718	
Biomass_Ecoli_core_N(w/GAM)_Nmet2		0.796696
CO2t	-18.3531	
CS	4.75726	
CYTBD	42.6072	
D_LACt2	0.0	
ENO	12.6966	
ETOHt2r	0.0	
EX_ac(e)	0.0	
EX_acald(e)	0.0	
EX_akg(e)	0.0	
EX_co2(e)	18.3531	
EX_etoh(e)	0.0	

The gene-deletion model in SBML format:

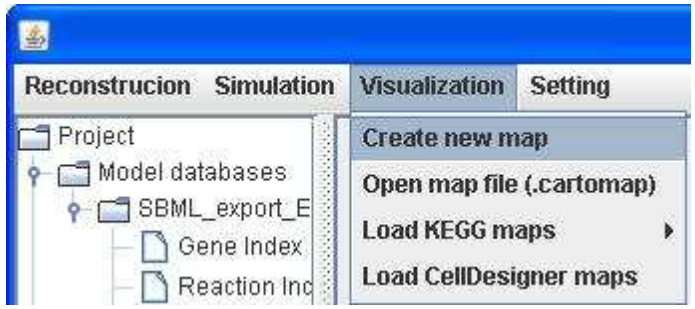


Such a model can be further imported into GEMSiRV for the other network evaluations as described early.

Visualization

Metabolic map creation

Click on Visualization in the menu bar to Create new map. You can create a metabolic map by clicking and moving network objects from the toolbar onto the main network view window.



The toolbar for creating/editing a map:

Add a map

Add a reaction

Add a metabolite

Add a label

Add a dotted line

Add an undirected line

Add a directed line

Add a bidirected line

Delete the selected item(s)

Search

Pan: Yon can pan a map by dragging and dropping left-click button over an empty point.

Zoom: You can zoom out or zoom in a map by scrolling up or down, respectively.

Select: You can click on an object to select it or you can hold right-click button to drag the mouse to select groups of objects.

Move: You can move any selected object by dragging and dropping it.

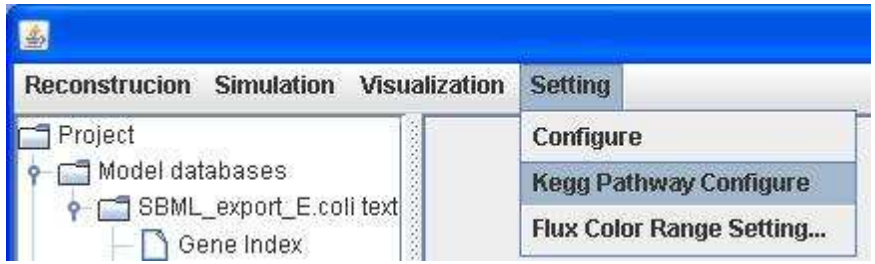
Delete: After selecting objects, you can right click over the selected items or click the Delete button in the toolbar for deletion.

Merge: After selecting identical objects, you can right click over the selected items to merge.

KEGG map loading

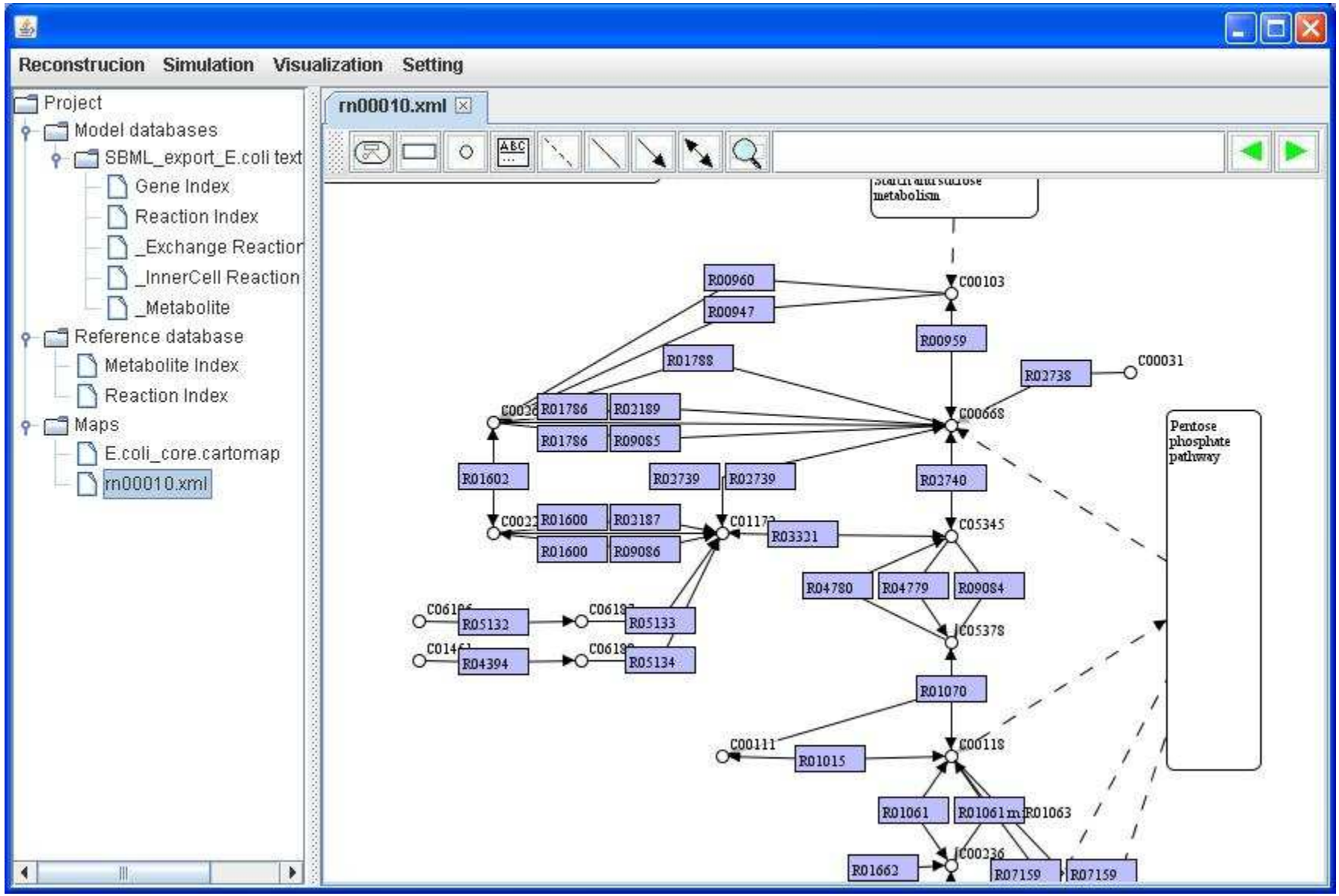
Click on Visualization in the menu bar to Load KEGG maps by either Import KEGG map (.xml) or Retrieve KEGG map depending on whether you have KGEE maps in hand.

If not, you can click on Setting in the menu bar to KEGG pathway Configure and set the link to where the KEGG pathway maps can be retrieved, e.g. http://www.genome.jp/kegg-bin/download.



Then you can retrieve KEGG pathway by choosing from the KEGG Pathway List.

The KEGG pathway map of rn00010:


Reconstrucion
Simulation
Visualization
Setting
Project
Model databases
SBML_export_E.coli text
Gene Index
Reaction Index
_Exchange Reaction
_InnerCell Reaction
_Metabolite
Reference database
Metabolite Index
Reaction Index
Maps
E.coli_core.cartomap
rn00010.xml
rn00010.xml
Starch and sucrose metabolism
R00960
R00947
C00103
R00959
R01788
R02738
C00031
C00668
R01786
R02189
R01786
R09085
R01602
R02739
R02739
R02740
Pentose phosphate pathway
R01600
R02187
R01600
R09086
R03321
C05345
R04780
R04779
R09084
R05132
R05133
R04394
R05134
C05378
R01070
C00111
R01015
C00118
R01061
R01061
R01063
C00236
R01662
R07159
R07159

We set rectangular nodes to represent reactions and define node name and node caption for each reaction. We directly use the entry name and reaction in KEGG maps as the node name and node caption respectively. Therefore, you can decide to Show node name or Show node caption by right clicking on a map.

Content of KEGG pathway map (rn00010.xml):

rn00010.xml

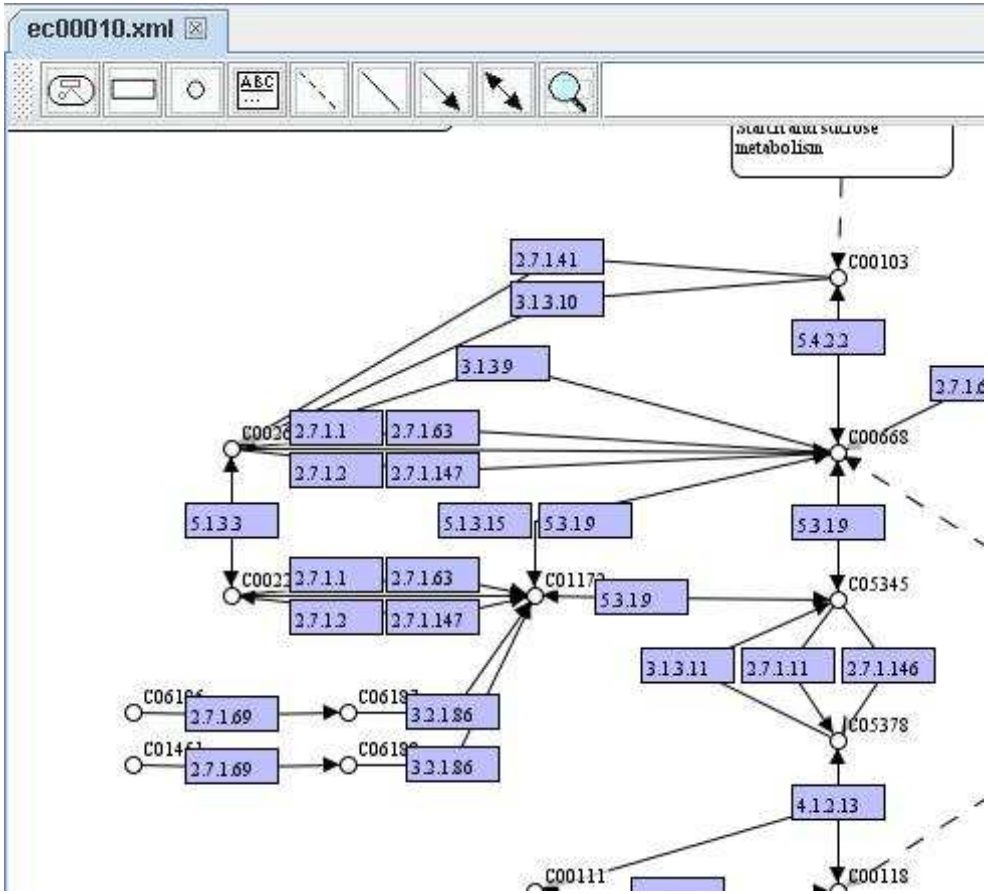
```
1  <?xml version="1.0"?>
2  <!DOCTYPE pathway SYSTEM "http://www.genome.jp/kegg/xml/KGML_v0.7.1_.dtd">
3  <!-- Creation date: Nov 16, 2010 13:49:39 +0900 (GMT+09:00) -->
4  <pathway name="path:rn00010" org="rn" number="00010"
5           title="Glycolysis / Gluconeogenesis"
6           image="http://www.genome.jp/kegg/pathway/rn/rn00010.png"
7           link="http://www.genome.jp/kegg-bin/show_pathway?rn00010">
8      <entry id="13" name="rn:R01070 rp:RP01274 rp:RP01275 rc:RC00438 rc:RC00439" type="reaction" reaction="rn:R01070"
9          link="http://www.kegg.jp/dbget-bin/www_bget?R01070+RP01274+RP01275+RC00438+RC00439">
10         <graphics name="R01070..." fgcolor="#000000" bgcolor="#BFBFFF"
11             type="rectangle" x="483" y="404" width="46" height="17"/>
12     </entry>
13     <entry id="37" name="rn:R00710 rp:RP00128 rc:RC00047" type="reaction" reaction="rn:R00710"
14         link="http://www.kegg.jp/dbget-bin/www_bget?R00710+RP00128+RC00047">
15         <graphics name="R00710..." fgcolor="#000000" bgcolor="#BFBFFF"
16             type="rectangle" x="289" y="943" width="46" height="17"/>
17     </entry>
```

Content of KEGG pathway map (ec00010.xml):

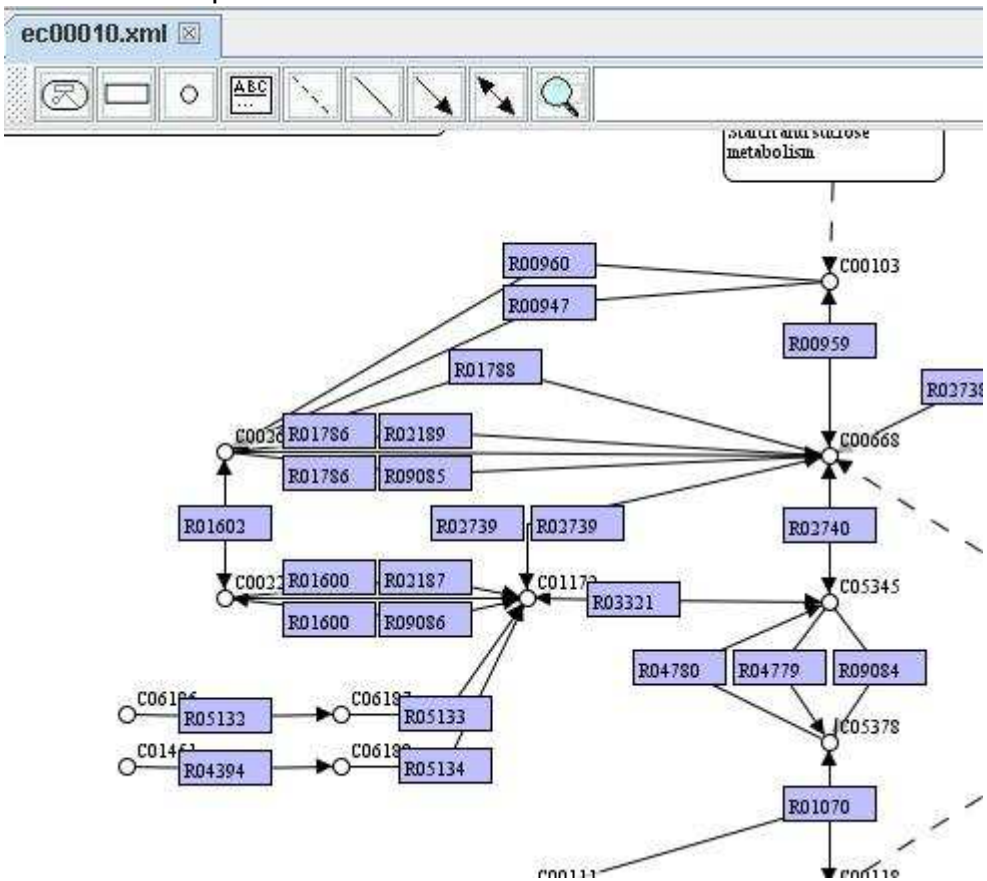
ec00010.xml

```
1  <?xml version="1.0"?>
2  <!DOCTYPE pathway SYSTEM "http://www.genome.jp/kegg/xml/KGML_v0.7.1_.dtd">
3  <!-- Creation date: Nov 16, 2010 13:49:39 +0900 (GMT+09:00) -->
4  <pathway name="path:ec00010" org="ec" number="00010"
5           title="Glycolysis / Gluconeogenesis"
6           image="http://www.genome.jp/kegg/pathway/ec/ec00010.png"
7           link="http://www.genome.jp/kegg-bin/show_pathway?ec00010">
8      <entry id="13" name="ec:4.1.2.13" type="enzyme" reaction="rn:R01070"
9          link="http://www.kegg.jp/dbget-bin/www_bget?4.1.2.13">
10         <graphics name="4.1.2.13" fgcolor="#000000" bgcolor="#BFBFFF"
11             type="rectangle" x="483" y="404" width="46" height="17"/>
12     </entry>
13     <entry id="37" name="ec:1.2.1.3" type="enzyme" reaction="rn:R00710"
14         link="http://www.kegg.jp/dbget-bin/www_bget?1.2.1.3">
15         <graphics name="1.2.1.3" fgcolor="#000000" bgcolor="#BFBFFF"
16             type="rectangle" x="289" y="943" width="46" height="17"/>
17     </entry>
```

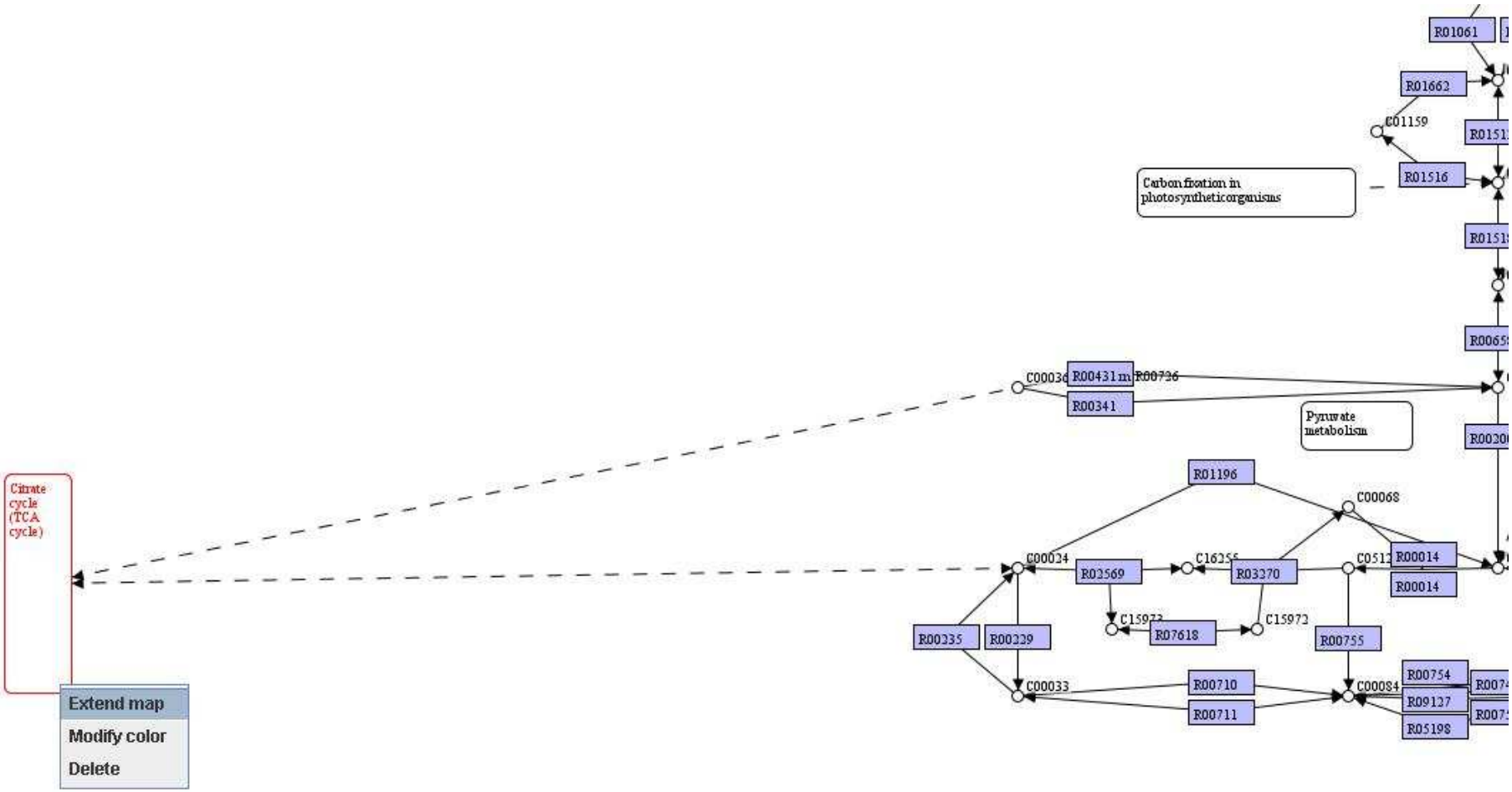
Show node name:



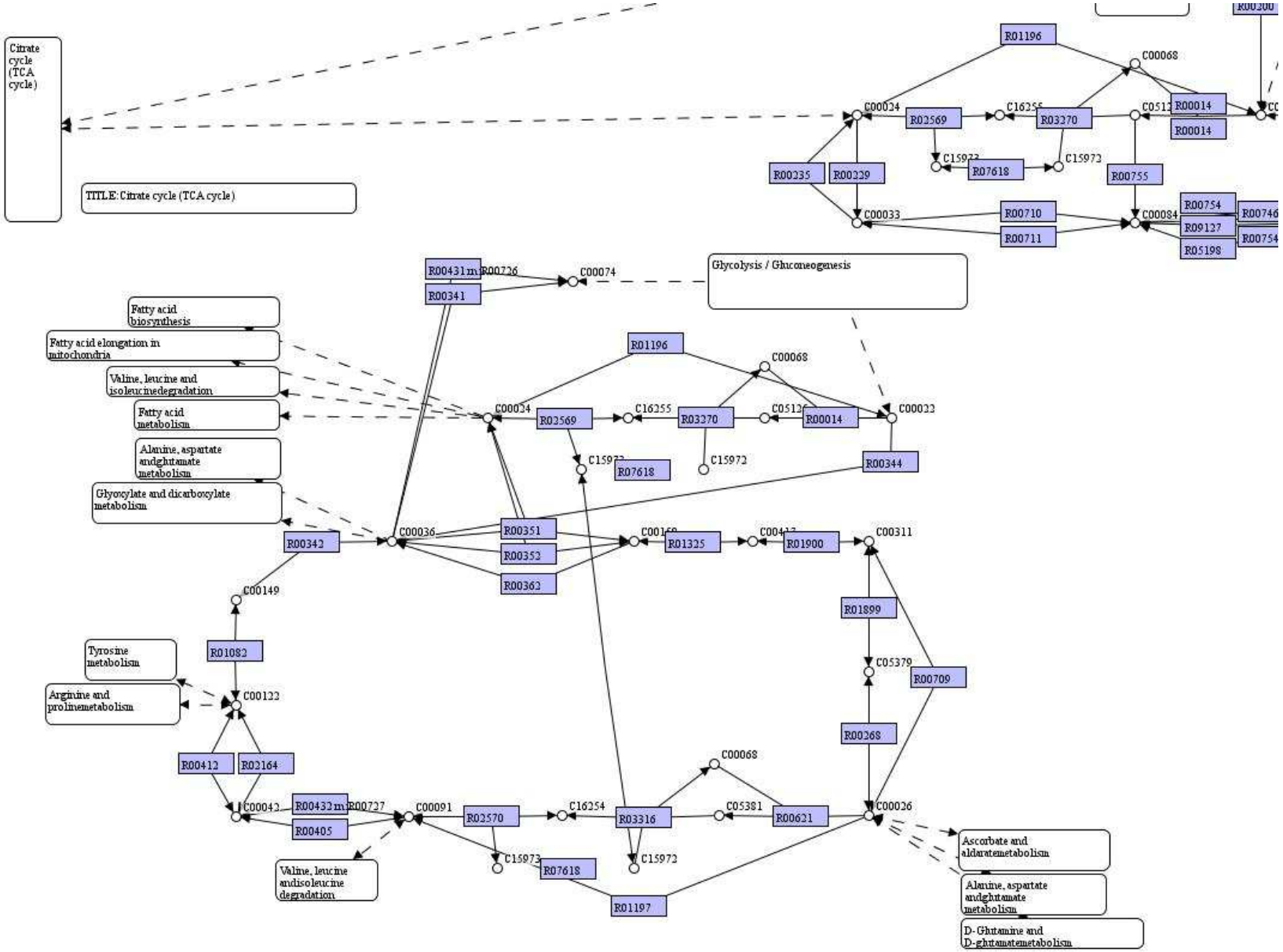
Show node caption:



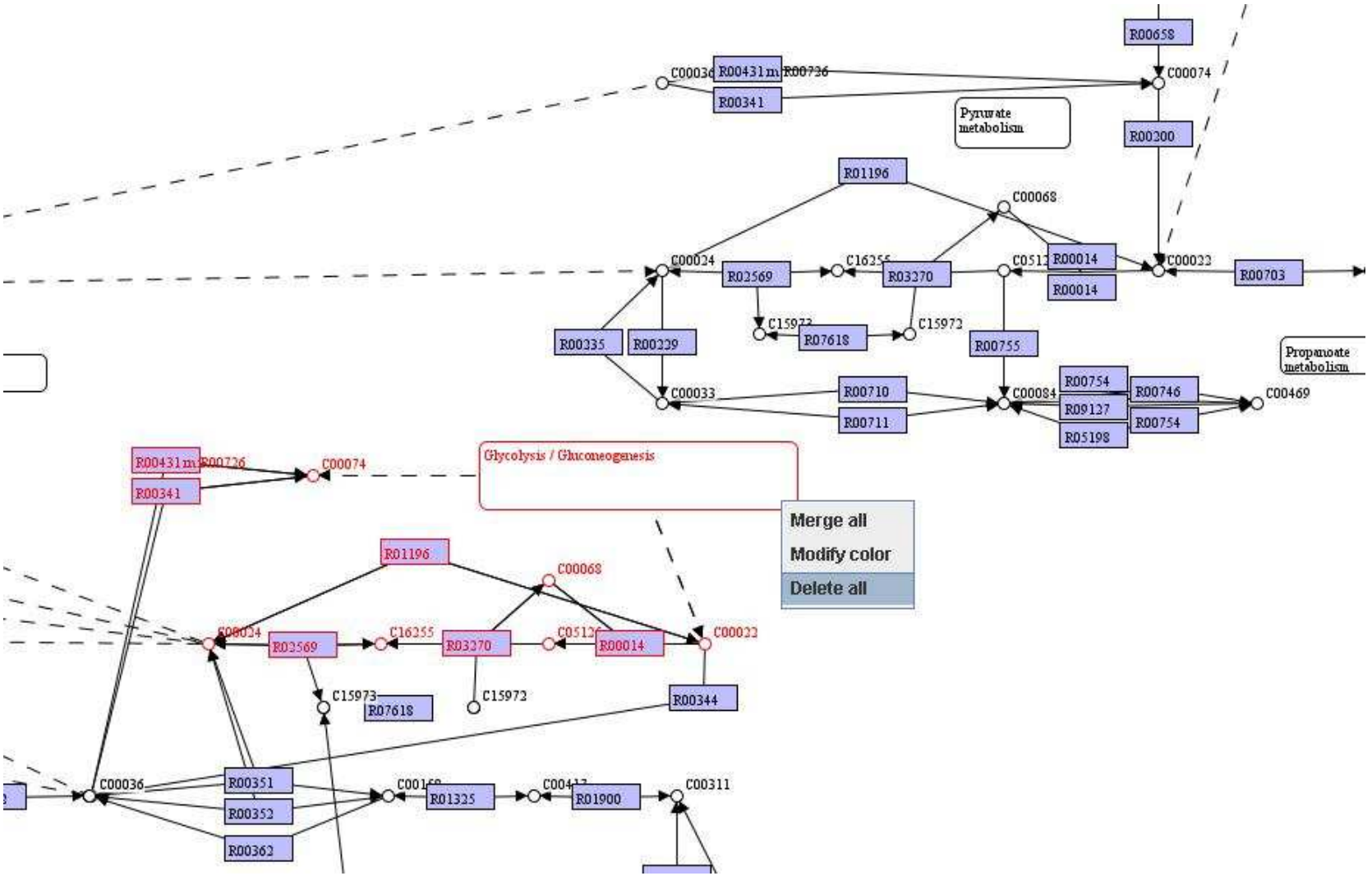
You can extend other pathway maps in the map you have in the main network view window. A pathway map is represented in a rounded rectangle. We can move the pathway that you would like to extend to an empty region and right click on it to Extend map.



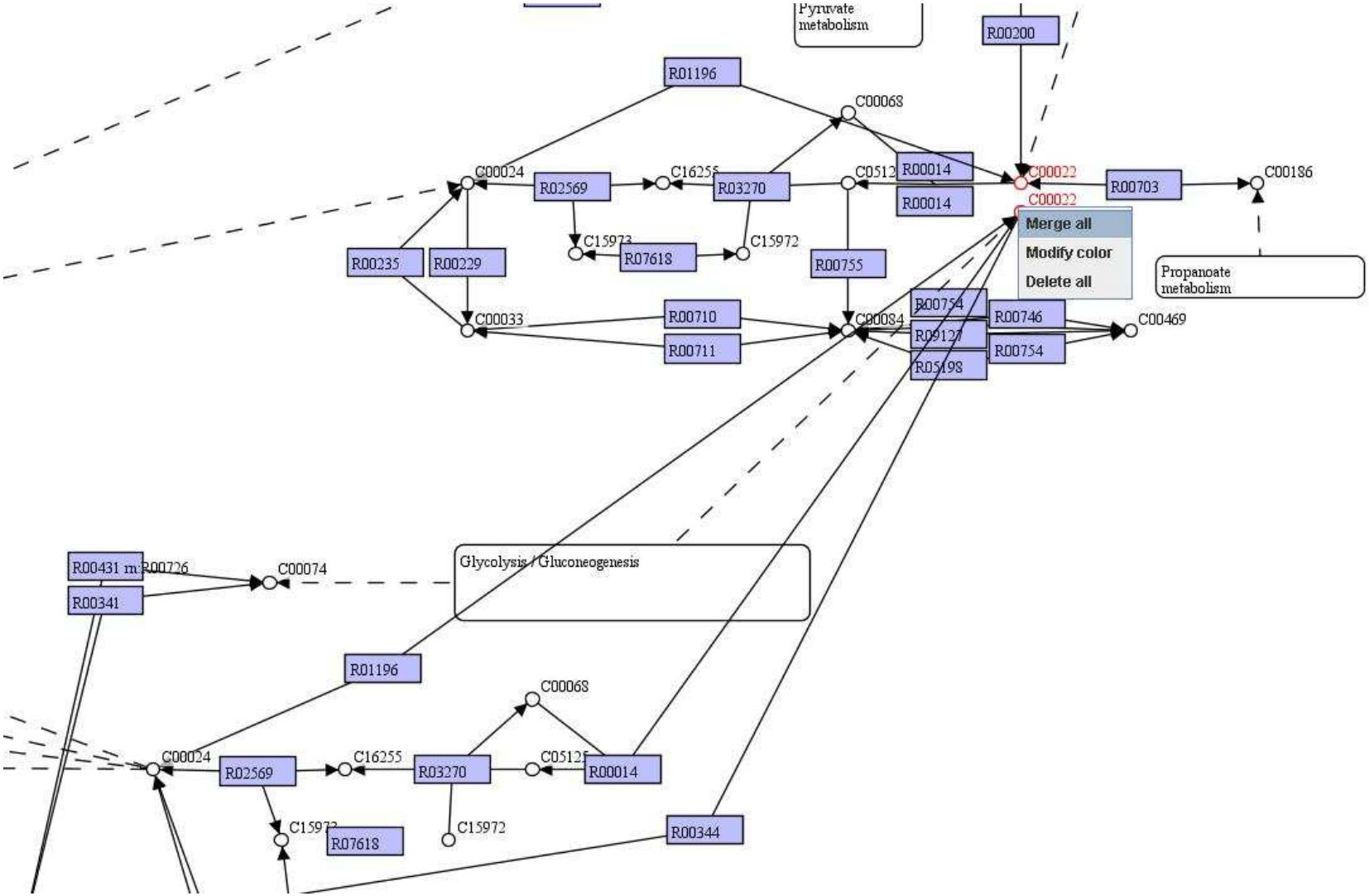
Map of Citrate cycle (TCA cycle) is extended in the map:

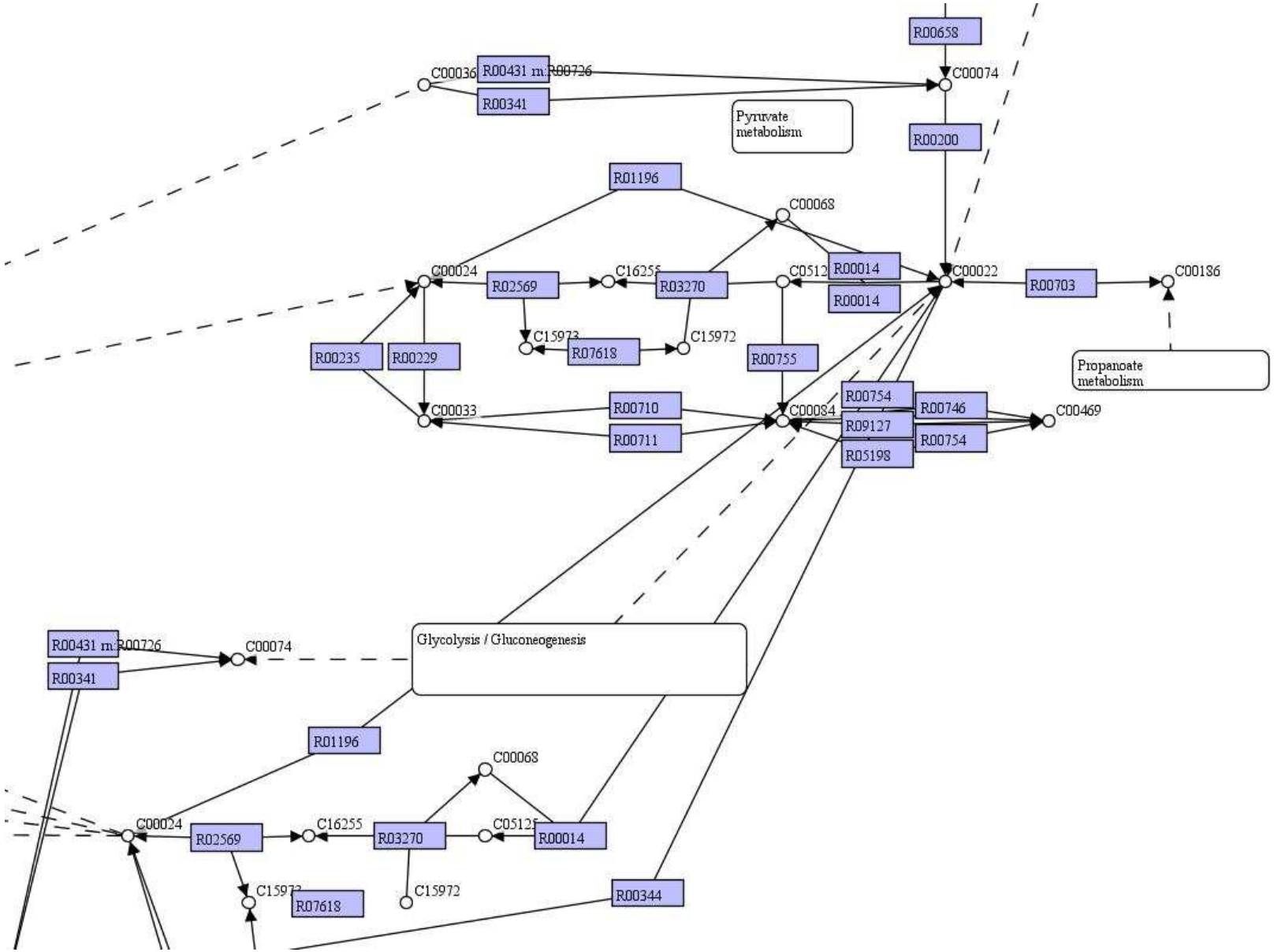

Citrate cycle (TCA cycle)
TITLE: Citrate cycle (TCA cycle)
R01196
C00068
C00024
R02569
C16255
R03270
C05125
R00014
R00014
C15973
R07618
C15972
R00235
R00239
R00755
C00033
R00710
R00711
C00084
R00754
R09127
R05198
R00746
R00754
Glycolysis / Gluconeogenesis
R00431 m
R00726
C00074
R00341
Fatty acid biosynthesis
Fatty acid elongation in mitochondria
Valine, leucine and isoleucinedegradation
Fatty acid metabolism
Alanine, aspartate andglutamate metabolism
Glyoxylate and dicarboxylate metabolism
R01196
C00068
C00024
R02569
C16255
R03270
C05125
R00014
C00022
C15973
R07618
C15972
R00344
R00342
C00036
R00351
R00352
R00362
C00169
R01325
C00417
R01900
C00311
C00149
R01899
C05379
R00709
R00268
Tyrosine metabolism
Arginine and prolinemetabolism
R01082
C00122
R00412
R02164
C00068
C00043
R00432 m
R00727
R00405
C00091
R02570
C16254
R03316
C05381
R00621
C00026
C15973
R07618
C15972
R01197
Valine, leucine andisoleucine degradation
Ascorbate and aldaratemetabolism
Alanine, aspartate andglutamate metabolism
D-Glutamine and D-glutamatemetabolism

You can hold right-click button on the map and drag a rectangular region for selecting groups of objects, then right click over the selected objects to delete them all.

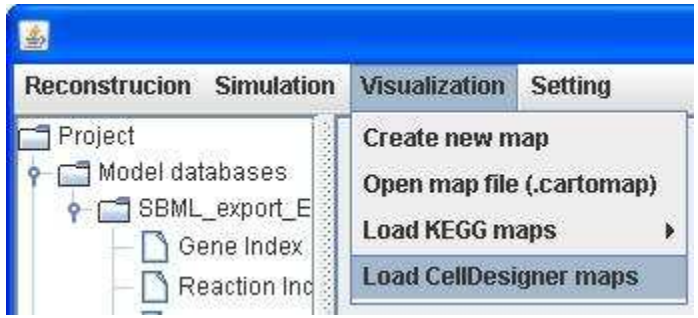


You can move identical objects close to each other. Select the identical objects and then right click on them to Merge all.

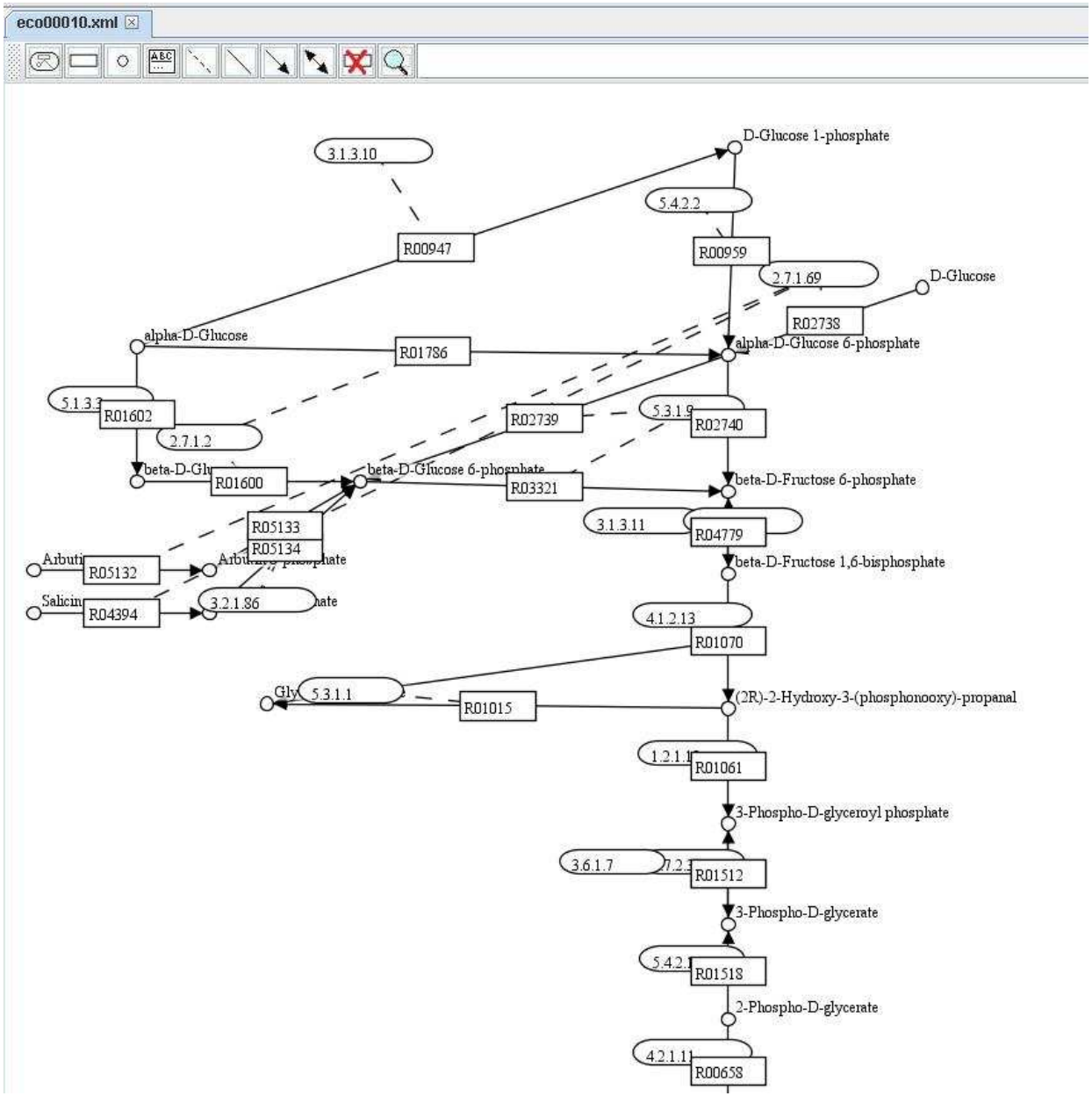



R00658
C00036
R00431 m:R00726
C00074
R00341
Pyruvate metabolism
R00200
R01196
C00068
C00024
R02569
C16255
R03270
C05125
R00014
R00014
C00022
R00703
C00186
C15973
R07618
C15972
R00235
R00229
R00755
Propanoate metabolism
C00033
R00710
R00711
C00084
R00754
R00746
C00469
R09127
R00754
R05198
R00431 m:R00726
C00074
Glycolysis / Gluconeogenesis
R00341
R01196
C00068
C00024
R02569
C16255
R03270
C05125
R00014
C15973
R07618
C15972
R00344

You can also load SBML models compatible to CellDesigner (http://www.celldesigner.org/index.html) to GEMSiRV. The SBML models for KEGG can be found and downloaded in http://www.systems-biology.org/001/001.html. You can click on Visualization in the menu bar to Load CellDesigner maps.

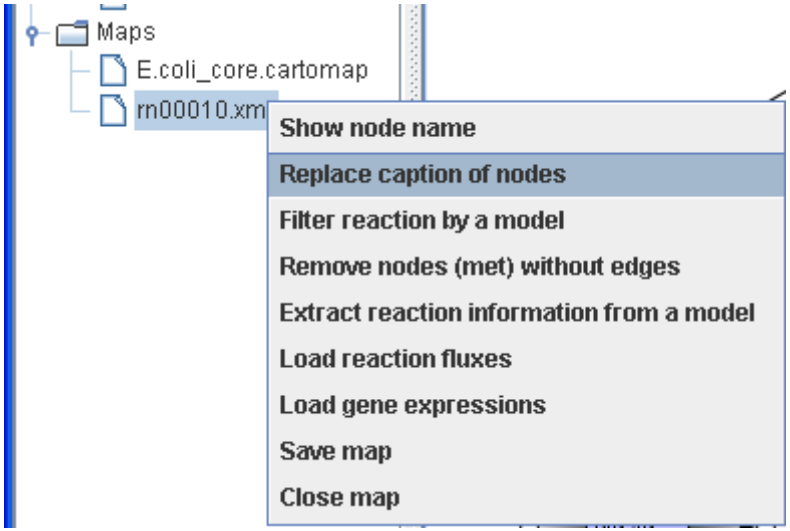


A SBML file eco00010.xml provided in http://sb.nhri.org.tw/GEMSiRV/en/Metabolic_Maps can be downloaded for demonstration.


eco00010.xml
D-Glucose 1-phosphate
3.1.3.10
5.4.2.2
R00947
R00959
2.7.1.69
D-Glucose
R02738
alpha-D-Glucose
R01786
alpha-D-Glucose 6-phosphate
R01602
2.7.1.2
R02739
5.3.1.9
R02740
beta-D-Glu
R01600
beta-D-Glucose 6-phosphate
R03321
beta-D-Fructose 6-phosphate
R05133
R05134
3.1.3.11
R04779
R05132
beta-D-Fructose 1,6-bisphosphate
R04394
3.2.1.86
4.1.2.13
R01070
5.3.1.1
R01015
(2R)-2-Hydroxy-3-(phosphonooxy)-propanal
R01061
3-Phospho-D-glyceroyl phosphate
3.6.1.7
R01512
3-Phospho-D-glycerate
R01518
2-Phospho-D-glycerate
4.2.1.11
R00658

Map replacement

In order to ease the creation of customized maps, GEMSiRV provide a function in map replacement. You can right click on a map to Replace caption of nodes to convert the map to a customized map.



For example, we replace a KEGG map (e.g. rn00010.xml) to a Model SEED-based map by providing two separate lists for metabolite and reaction mapping. The KEEG to Model SEED mapping lists can be found and downloaded in http://sb.nhri.org.tw/GEMSiRV/en/Manual.

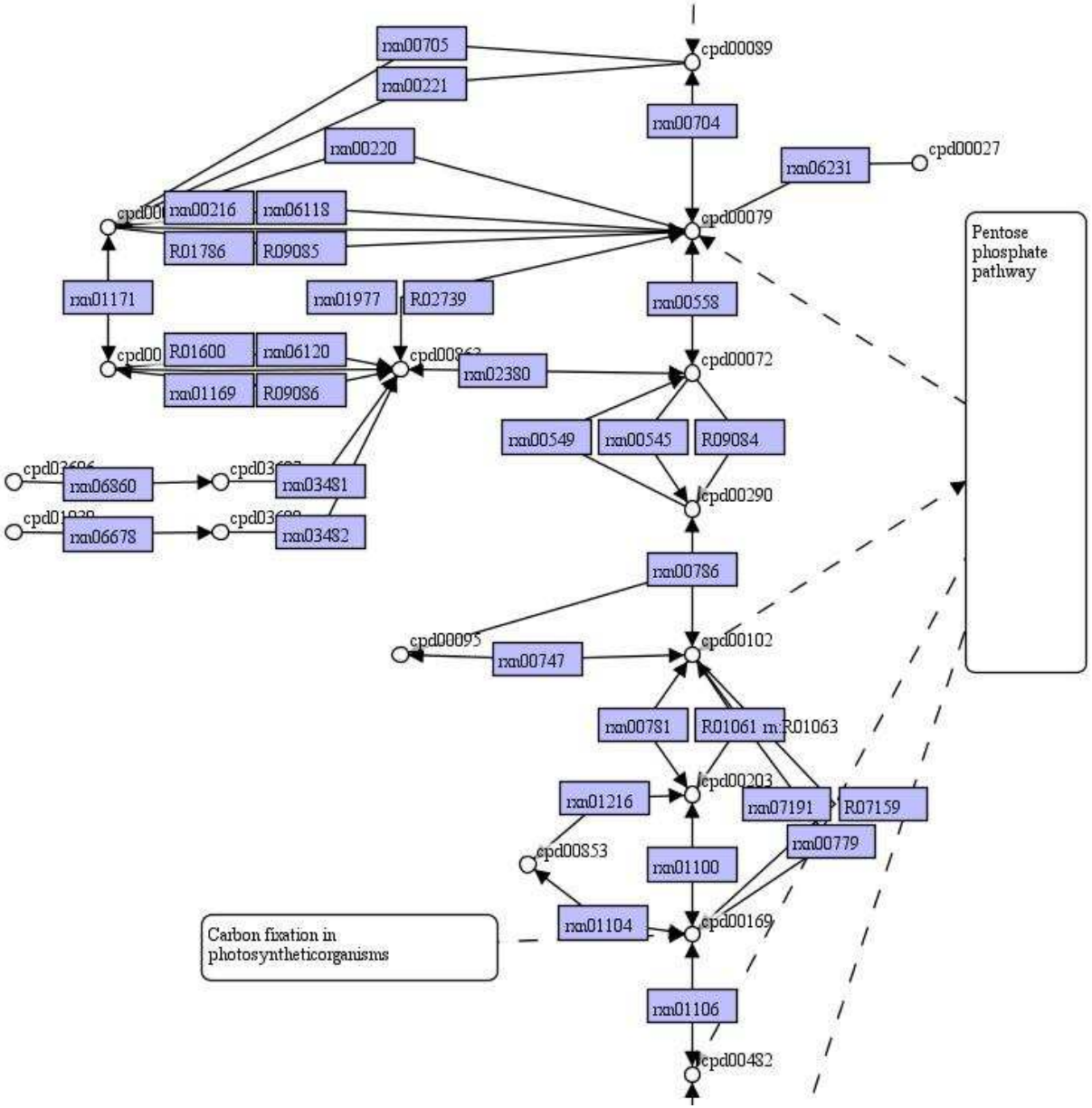
met_KEGGtoSEED.TXT

1	C16254	cpd00860
2	C00149	cpd00130
3	C00002	cpd00002
4	C00003	cpd00003
5	C00004	cpd00004
6	C00005	cpd00005
7	C00006	cpd00006
8	C00007	cpd00007
9	C00008	cpd00008
10	C00009	cpd00009

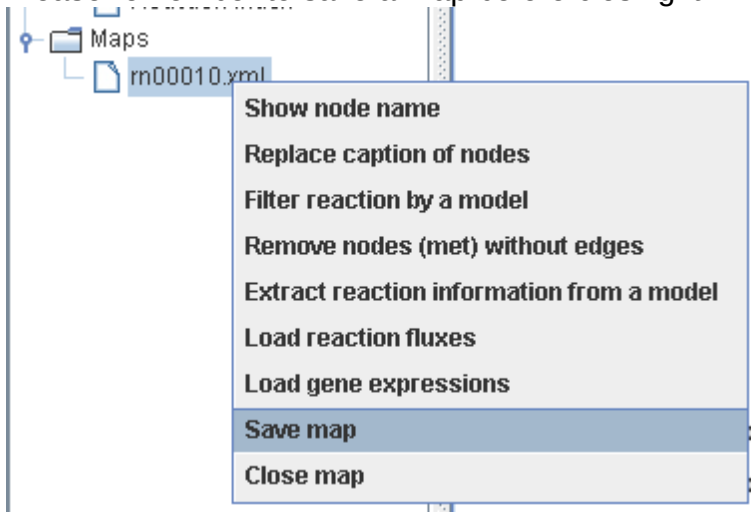
rxn_KEGGtoSEED.TXT

1	R00432	rxn00306
2	R07618	rxn01241
3	R00001	rxn05757
4	R00002	rxn11947
5	R00004	rxn00001
6	R00005	rxn00002
7	R00008	rxn00004
8	R00009	rxn00006
9	R00010	rxn00007
10	R00011	rxn00008

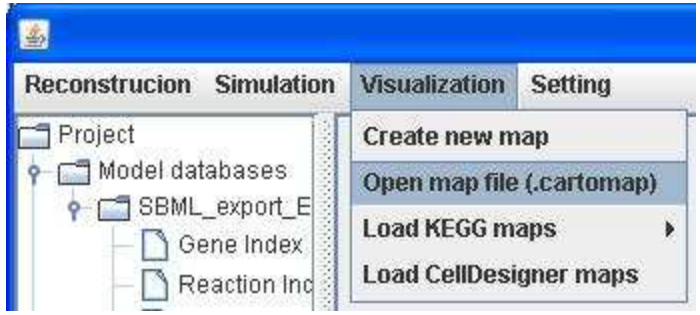
Therefore, some nodes of metabolite and reaction can be replaced to form a Model SEED-based map.


rxn00705
cpd00089
rxn00221
rxn00704
rxn00220
rxn06231
cpd00027
rxn00216
rxn06118
cpd00079
R01786
R09085
Pentose phosphate pathway
rxn01171
rxn01977
R02739
rxn00558
R01600
rxn06120
rxn02380
cpd00072
rxn01169
R09086
rxn00549
rxn00545
R09084
rxn06860
rxn03481
cpd00290
rxn06678
rxn03482
rxn00786
cpd00095
rxn00747
cpd00102
rxn00781
R01061
R01063
cpd00203
rxn01216
rxn07191
R07159
rxn00779
cpd00853
rxn01100
Carbon fixation in photosyntheticorganisms
rxn01104
cpd00169
rxn01106
cpd00482

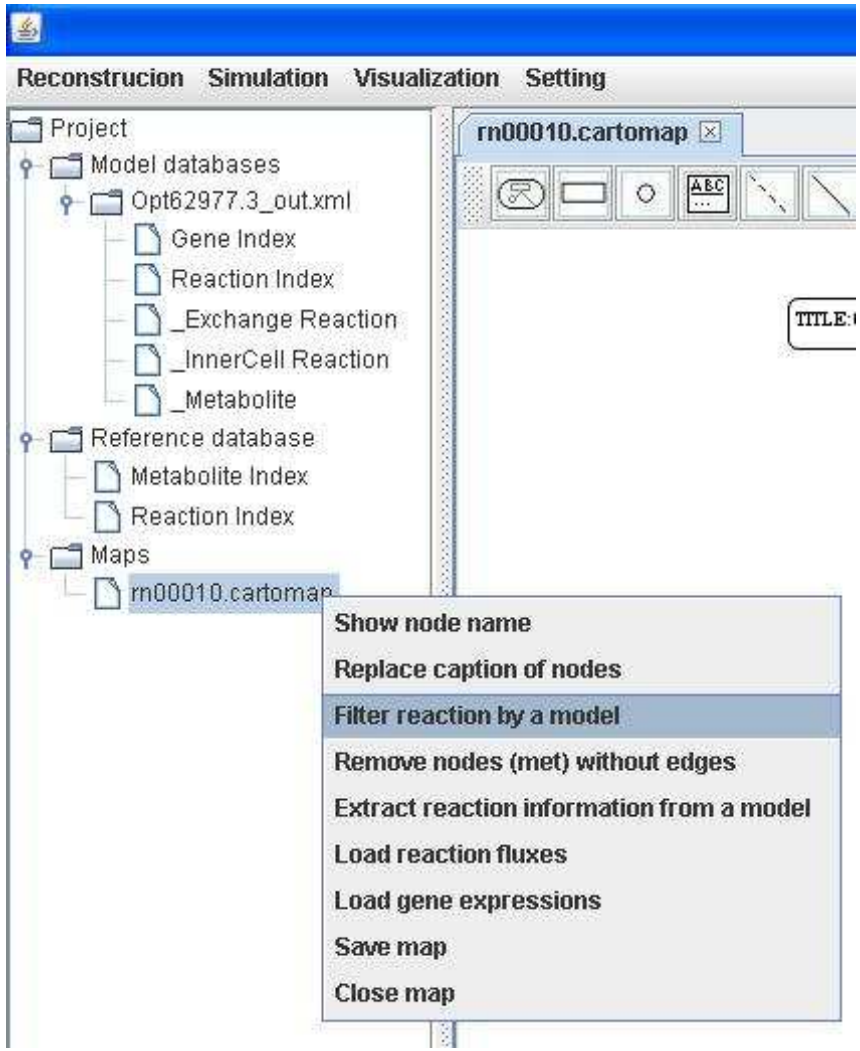
Please remember to save a map before closing it.



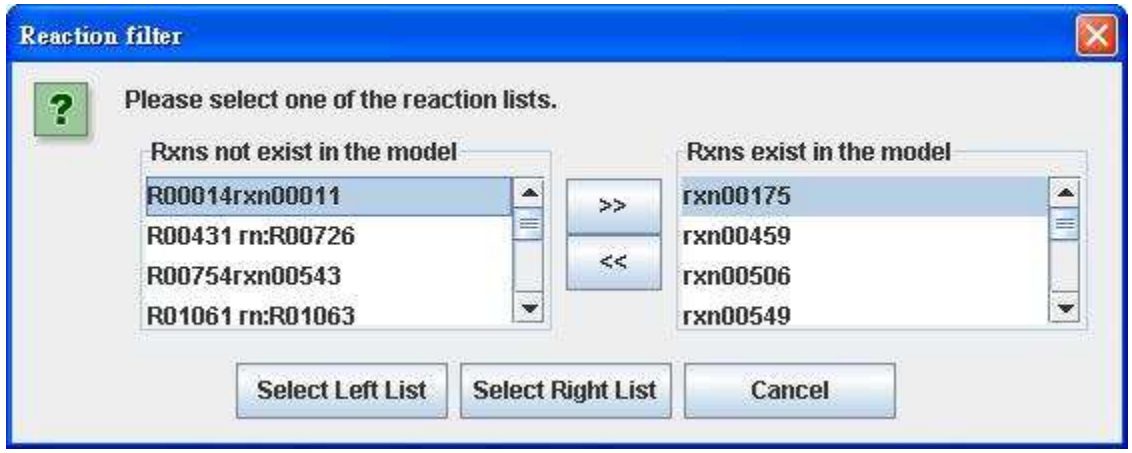
You can open a map saved in cartomap format.



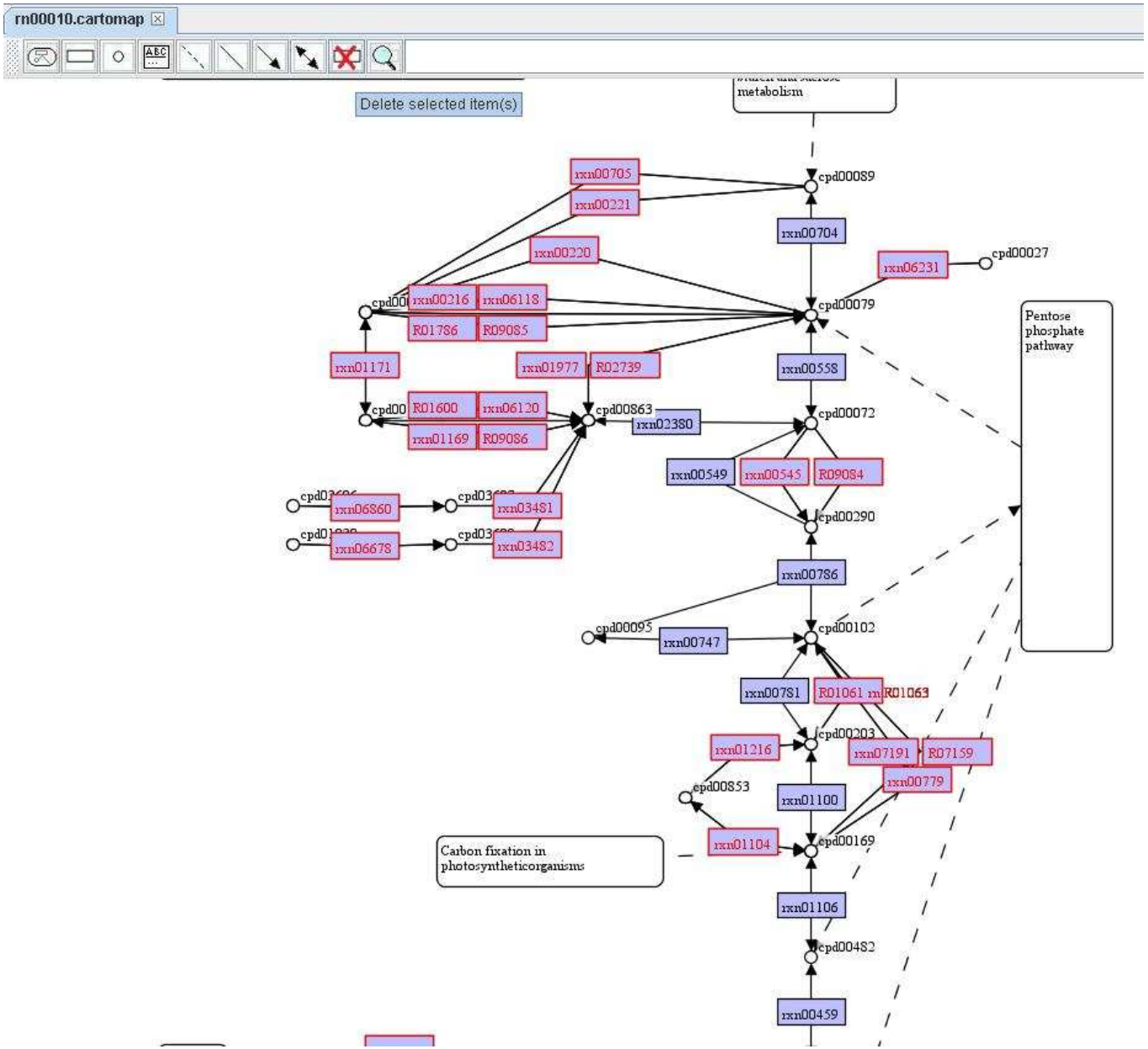
In order to create a useful map for visualization, an interactive function between model reconstruction and map visualization is implemented in GEMSiRV.
For demonstration, we import a Model SEED model Acinetobacter sp. ADP1 (Opt 62977.3.xml).

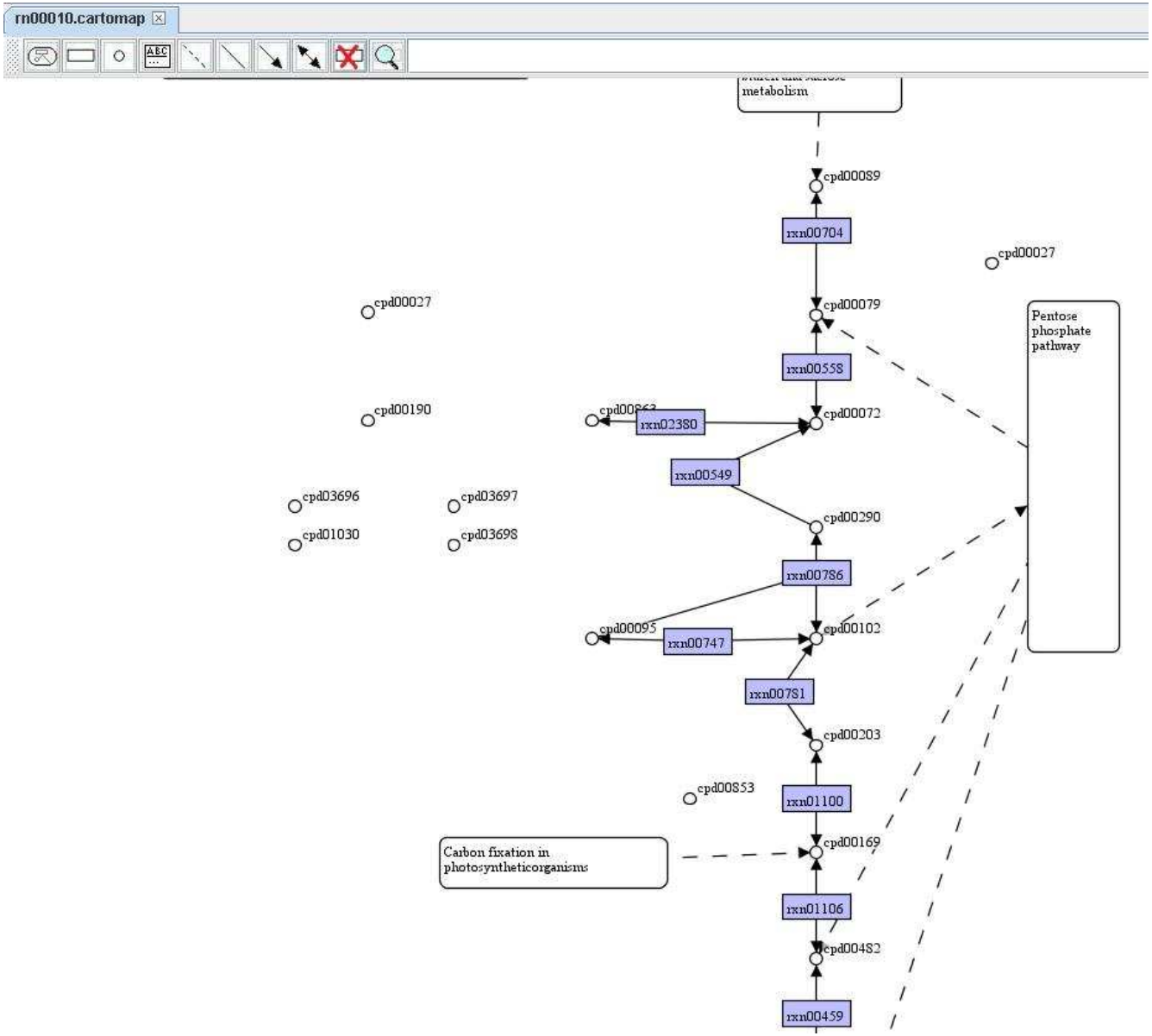


You can filter reactions by comparing with the metabolic model you select and you can get the reaction lists for reactions not existing or existing in the model.

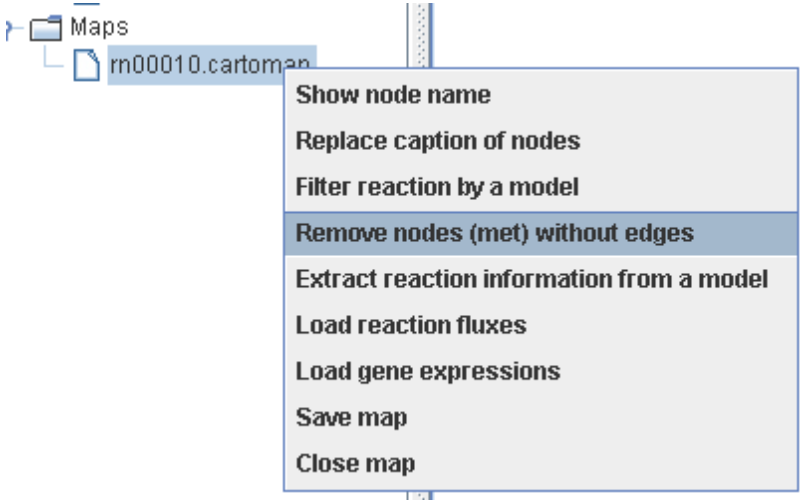


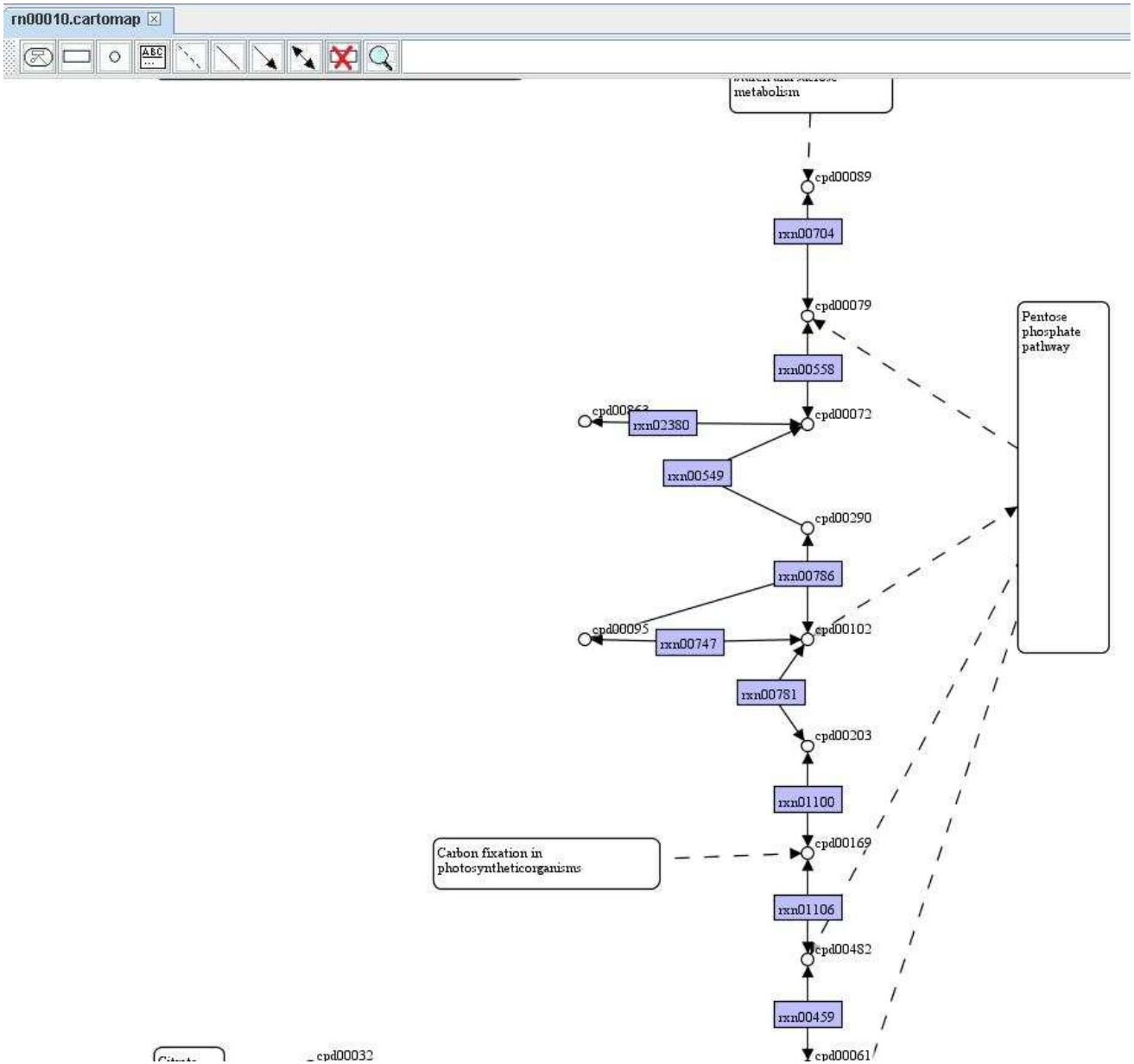
We select and delete the left list of reactions for creating a model-specific map.


rn00010.cartomap
Delete selected item(s)
metabolism
rxn00705
rxn00221
cpd00089
rxn00704
rxn00220
rxn06231
cpd00027
rxn00216
rxn06118
cpd00079
R01786
R09085
Pentose phosphate pathway
rxn01171
rxn01977
R02739
rxn00558
R01600
rxn06120
cpd00863
rxn02380
cpd00072
rxn01169
R09086
rxn00549
rxn00545
R09084
rxn06860
rxn03481
cpd00290
rxn06678
rxn03482
rxn00786
cpd00095
rxn00747
cpd00102
rxn00781
R01061
R01063
cpd00203
rxn01216
rxn07191
R07159
rxn00779
cpd00853
rxn01100
rxn01104
cpd00169
Carbon fixation in photosyntheticorganisms
rxn01106
cpd00482
rxn00459

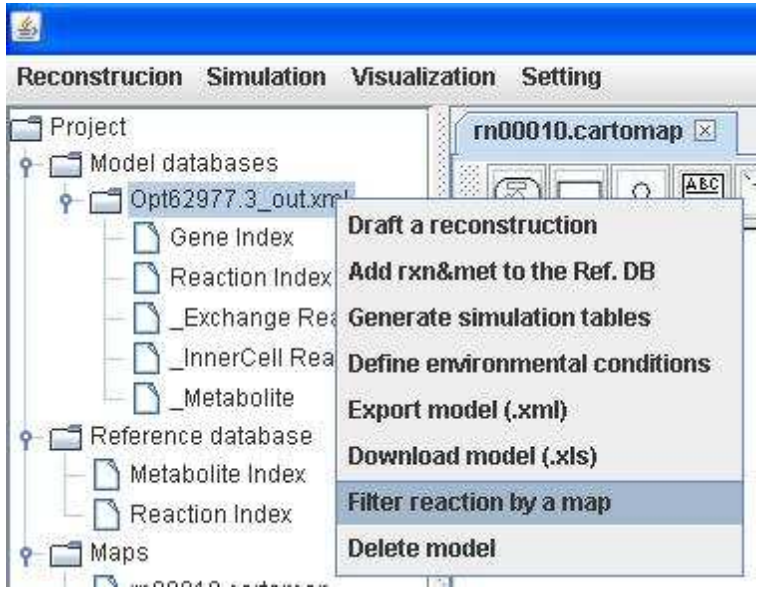

rn00010.cartomap
metabolism
cpd00089
rxn00704
cpd00027
cpd00027
cpd00079
Pentose phosphate pathway
rxn00558
cpd00190
cpd00863
rxn02380
cpd00072
rxn00549
cpd03696
cpd03697
cpd00290
cpd01030
cpd03698
rxn00786
cpd00095
rxn00747
cpd00102
rxn00781
cpd00203
cpd00853
rxn01100
Carbon fixation in photosyntheticorganisms
cpd00169
rxn01106
cpd00482
rxn00459

You can remove those nodes of metabolite without linking to reaction by right clicking on the map to Remove nodes (met) without edges.

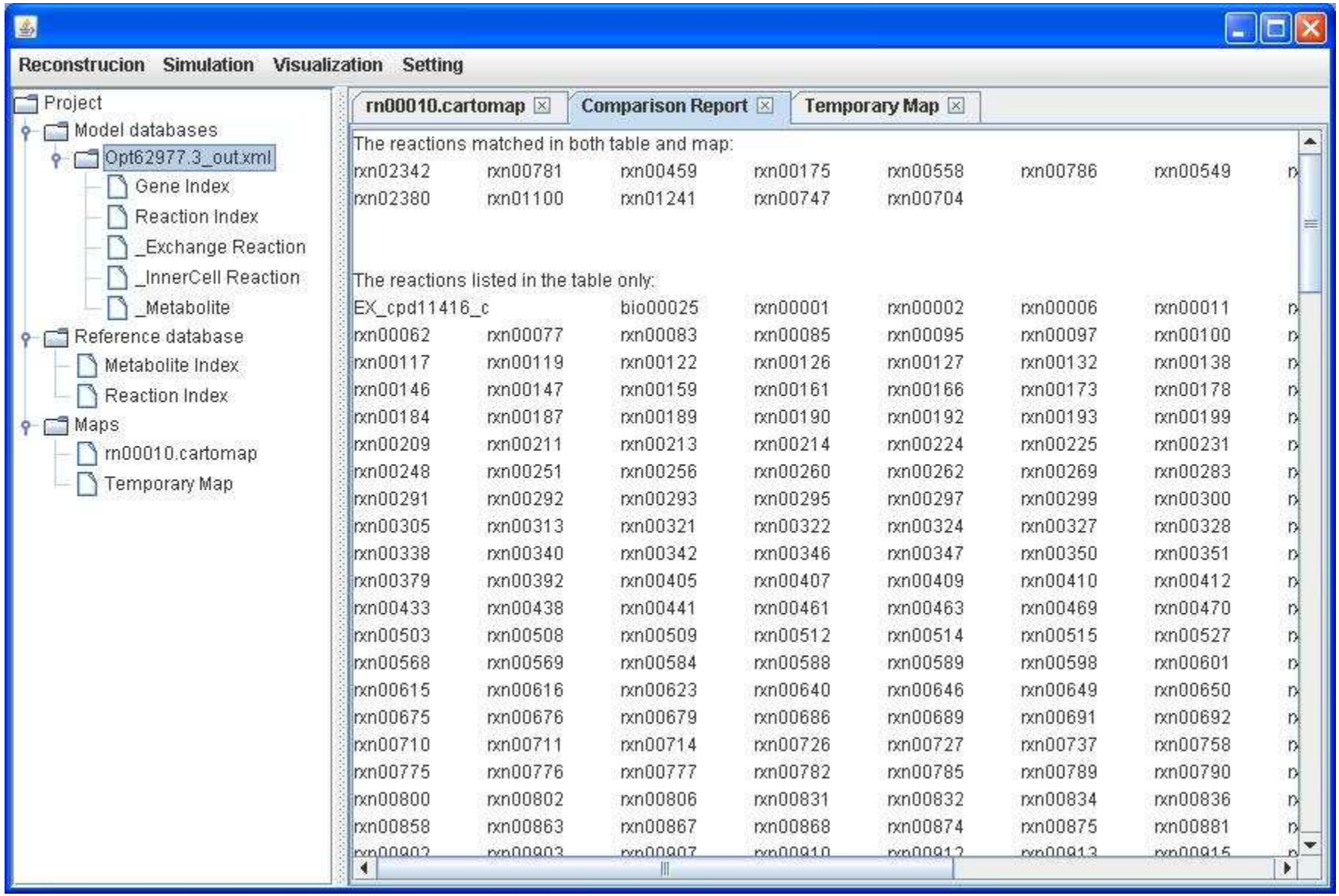



rn00010.cartomap
metabolism
cpd00089
rxn00704
cpd00079
rxn00558
Pentose phosphate pathway
cpd00072
rxn02380
rxn00549
cpd00290
rxn00786
cpd00102
rxn00747
rxn00781
cpd00203
rxn01100
cpd00169
Carbon fixation in photosyntheticorganisms
rxn01106
cpd00482
rxn00459
cpd00061

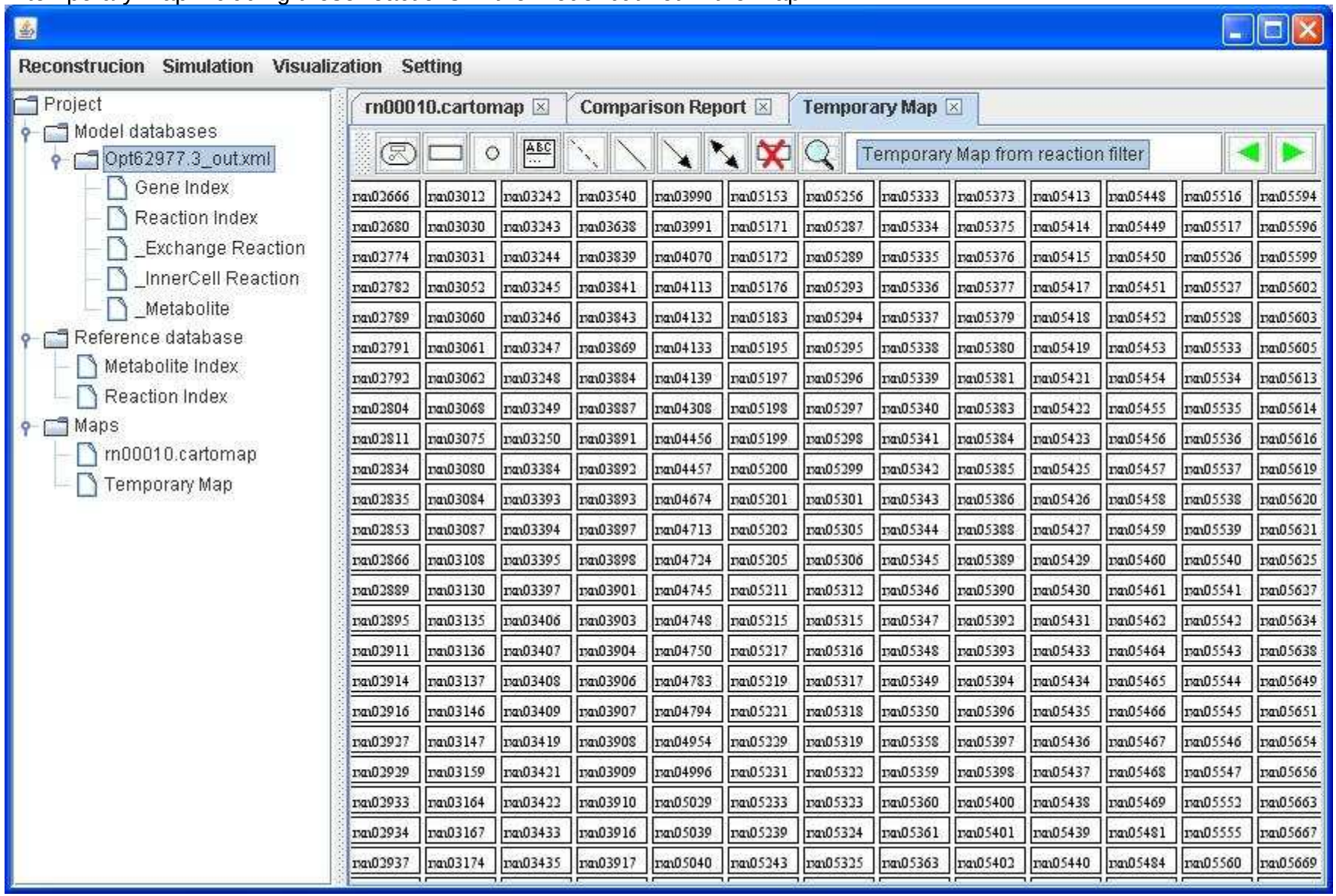
You can also filter reactions in a metabolic model by comparing with a map. Right click on the model to Filter reaction by a map and choose a map you want to compare with. Then you can get a comparison report as well as a temporary map including those reactions not present in the map you chosen.



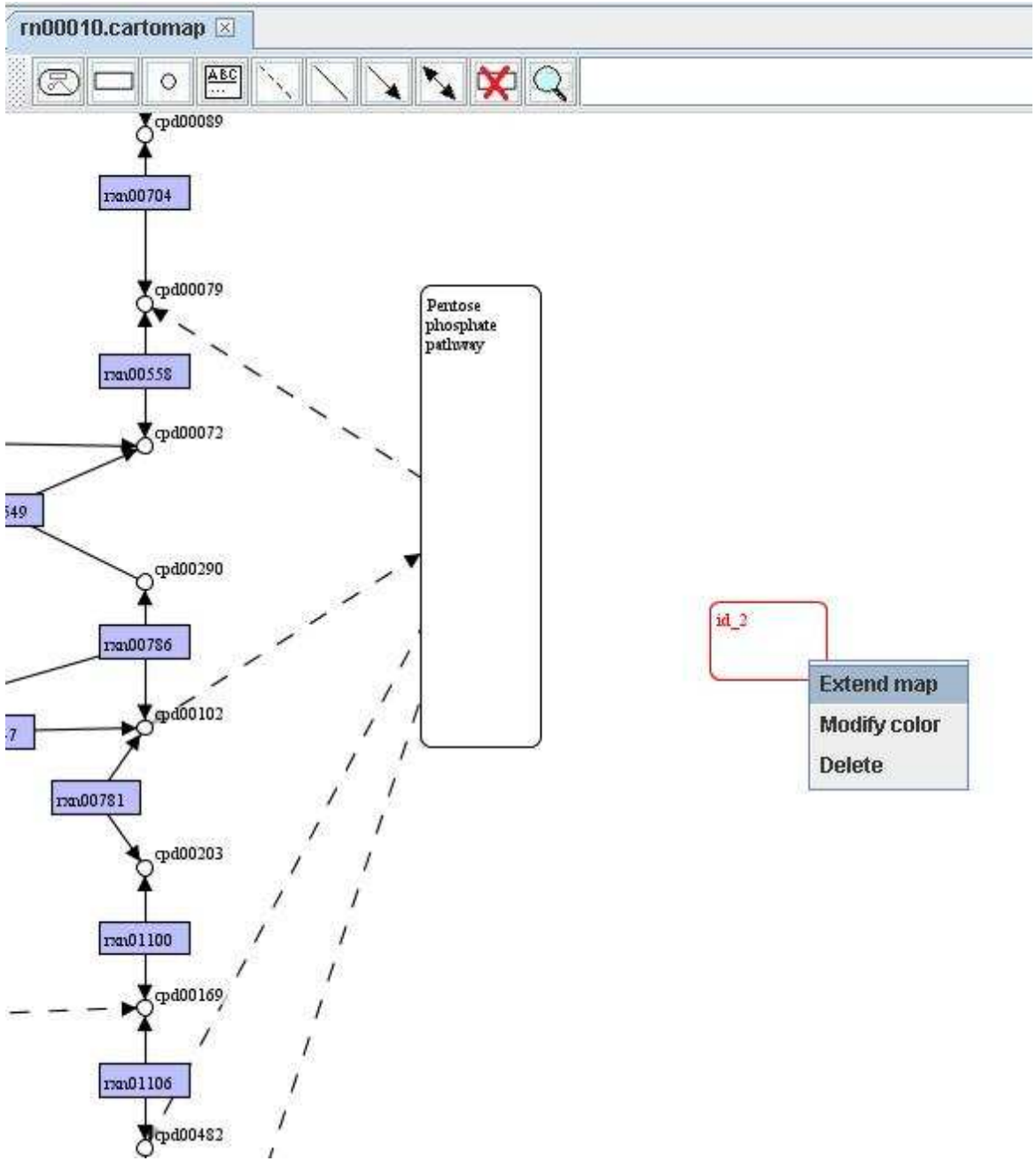
A comparison report showing what reactions are present in the model only, in the map only, and in the both.

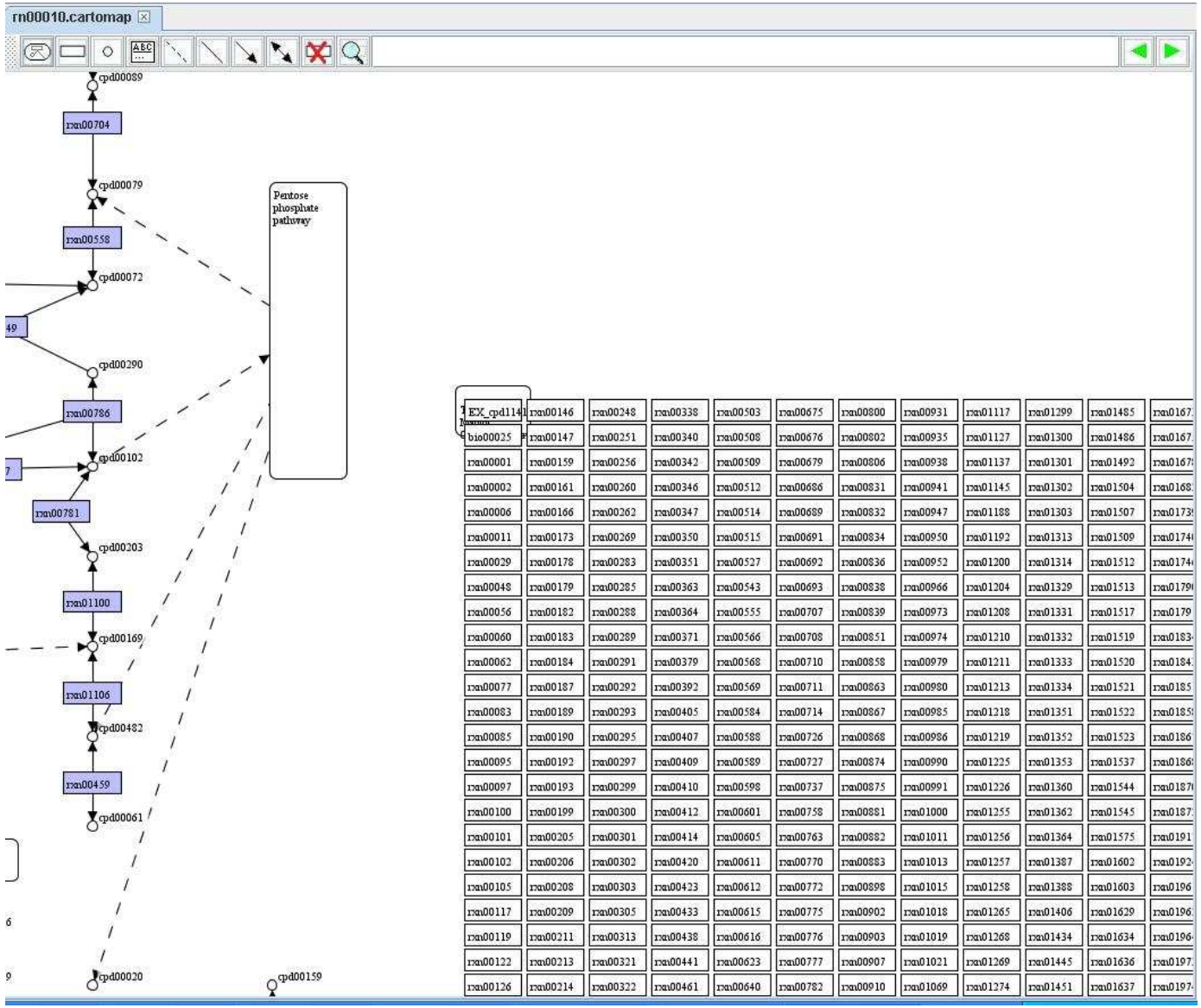

Reconstrucion
Simulation
Visualization
Setting
Project
Model databases
Opt62977.3_out.xml
Gene Index
Reaction Index
_Exchange Reaction
_InnerCell Reaction
_Metabolite
Reference database
Metabolite Index
Reaction Index
Maps
rn00010.cartomap
Temporary Map
rn00010.cartomap
Comparison Report
Temporary Map
The reactions matched in both table and map:
rxn02342 rxn00781 rxn00459 rxn00175 rxn00558 rxn00786 rxn00549
rxn02380 rxn01100 rxn01241 rxn00747 rxn00704
The reactions listed in the table only:
EX_cpd11416_c bio00025 rxn00001 rxn00002 rxn00006 rxn00011
rxn00062 rxn00077 rxn00083 rxn00085 rxn00095 rxn00097 rxn00100
rxn00117 rxn00119 rxn00122 rxn00126 rxn00127 rxn00132 rxn00138
rxn00146 rxn00147 rxn00159 rxn00161 rxn00166 rxn00173 rxn00178
rxn00184 rxn00187 rxn00189 rxn00190 rxn00192 rxn00193 rxn00199
rxn00209 rxn00211 rxn00213 rxn00214 rxn00224 rxn00225 rxn00231
rxn00248 rxn00251 rxn00256 rxn00260 rxn00262 rxn00269 rxn00283
rxn00291 rxn00292 rxn00293 rxn00295 rxn00297 rxn00299 rxn00300
rxn00305 rxn00313 rxn00321 rxn00322 rxn00324 rxn00327 rxn00328
rxn00338 rxn00340 rxn00342 rxn00346 rxn00347 rxn00350 rxn00351
rxn00379 rxn00392 rxn00405 rxn00407 rxn00409 rxn00410 rxn00412
rxn00433 rxn00438 rxn00441 rxn00461 rxn00463 rxn00469 rxn00470
rxn00503 rxn00508 rxn00509 rxn00512 rxn00514 rxn00515 rxn00527
rxn00568 rxn00569 rxn00584 rxn00588 rxn00589 rxn00598 rxn00601
rxn00615 rxn00616 rxn00623 rxn00640 rxn00646 rxn00649 rxn00650
rxn00675 rxn00676 rxn00679 rxn00686 rxn00689 rxn00691 rxn00692
rxn00710 rxn00711 rxn00714 rxn00726 rxn00727 rxn00737 rxn00758
rxn00775 rxn00776 rxn00777 rxn00782 rxn00785 rxn00789 rxn00790
rxn00800 rxn00802 rxn00806 rxn00831 rxn00832 rxn00834 rxn00836
rxn00858 rxn00863 rxn00867 rxn00868 rxn00874 rxn00875 rxn00881

A temporary map including those reactions in the model but not in the map



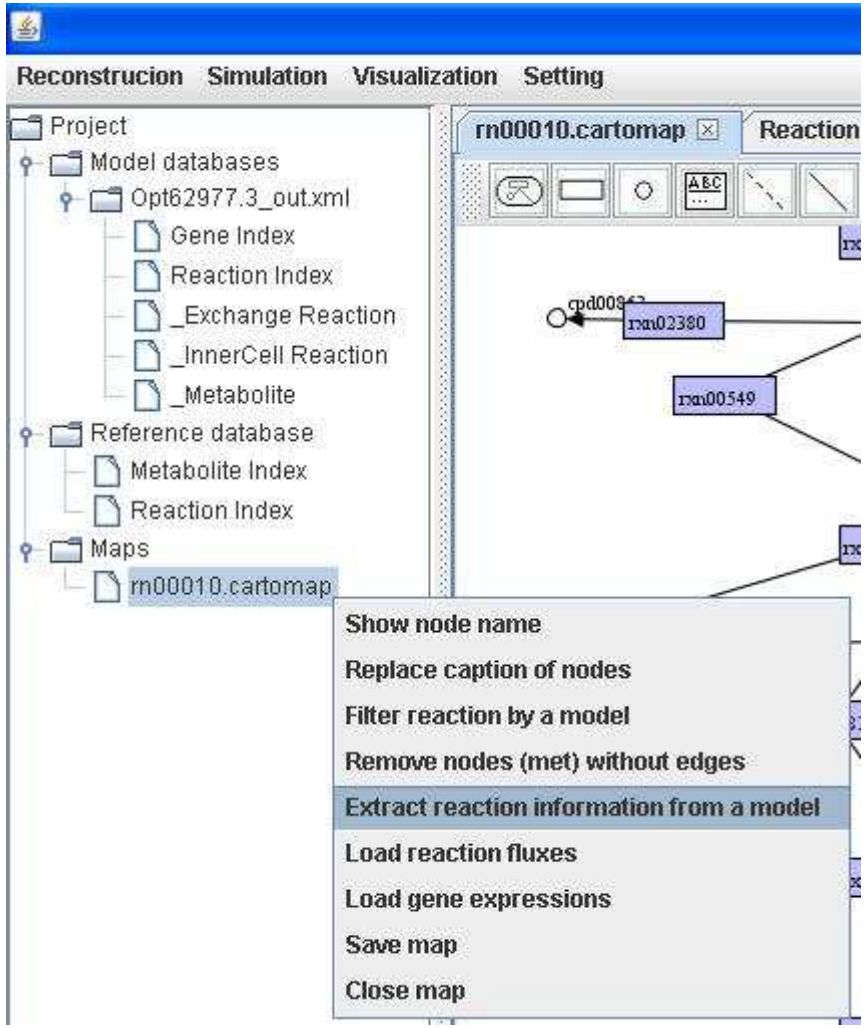
You can save the temporary map and add it into the map you are working with.
Add a map by clicking Add a map in the toolbar and dropping in an empty region of the map and extend the map by right clicking on the added map to Extend map.

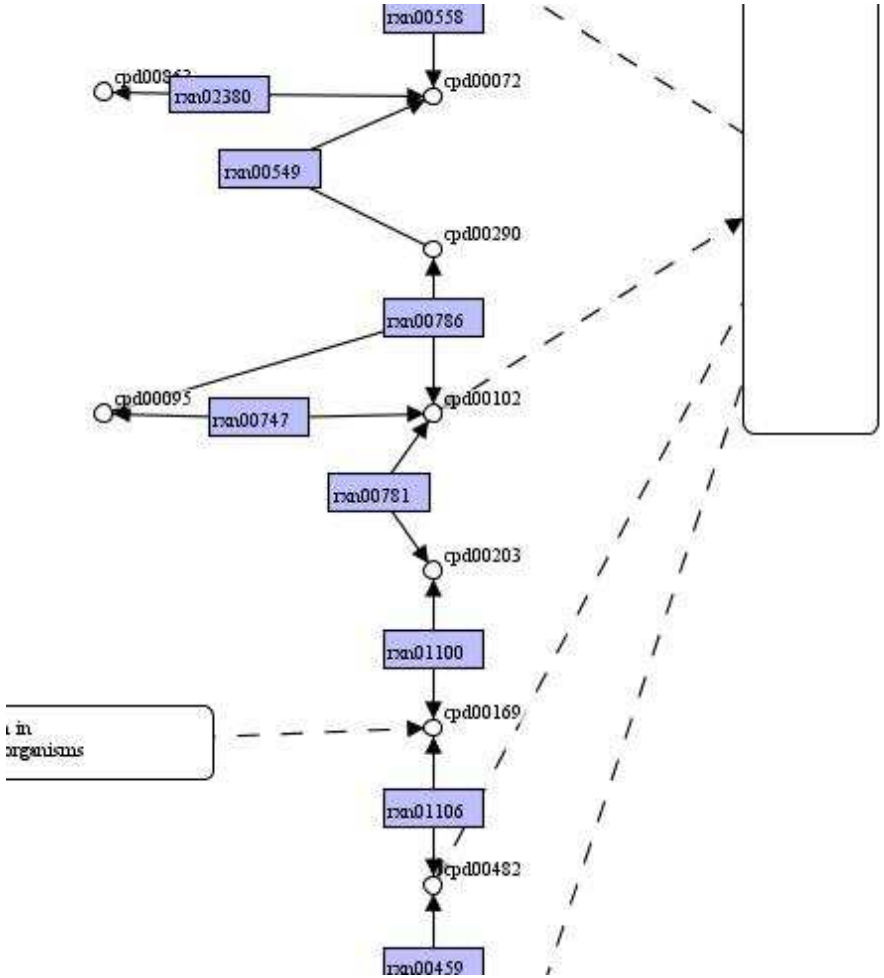

rn00010.cartomap
cpd00089
rxn00704
cpd00079
rxn00558
cpd00072
549
cpd00290
rxn00786
cpd00102
7
rxn00781
cpd00203
rxn01100
cpd00169
rxn01106
cpd00482
Pentose phosphate pathway
id_2
Extend map
Modify color
Delete


rn00010.cartomap
cpd00089
rxn00704
cpd00079
rxn00558
cpd00072
cpd00290
rxn00786
cpd00102
rxn00781
cpd00203
rxn01100
cpd00169
rxn01106
cpd00482
rxn00459
cpd00061
cpd00020
cpd00159
Pentose phosphate pathway
EX_cpd11416
bio00025
rxn00001
rxn00002
rxn00006
rxn00011
rxn00029
rxn00048
rxn00056
rxn00060
rxn00062
rxn00077
rxn00083
rxn00085
rxn00095
rxn00097
rxn00100
rxn00101
rxn00102
rxn00105
rxn00117
rxn00119
rxn00122
rxn00126
rxn00146
rxn00147
rxn00159
rxn00161
rxn00166
rxn00173
rxn00178
rxn00179
rxn00182
rxn00183
rxn00184
rxn00187
rxn00189
rxn00190
rxn00192
rxn00193
rxn00199
rxn00205
rxn00206
rxn00208
rxn00209
rxn00211
rxn00213
rxn00214
rxn00248
rxn00251
rxn00256
rxn00260
rxn00262
rxn00269
rxn00283
rxn00285
rxn00288
rxn00289
rxn00291
rxn00292
rxn00293
rxn00295
rxn00297
rxn00299
rxn00300
rxn00301
rxn00302
rxn00303
rxn00305
rxn00313
rxn00321
rxn00322
rxn00338
rxn00340
rxn00342
rxn00346
rxn00347
rxn00350
rxn00351
rxn00363
rxn00364
rxn00371
rxn00379
rxn00392
rxn00405
rxn00407
rxn00409
rxn00410
rxn00412
rxn00414
rxn00420
rxn00423
rxn00433
rxn00438
rxn00441
rxn00461
rxn00503
rxn00508
rxn00509
rxn00512
rxn00514
rxn00515
rxn00527
rxn00543
rxn00555
rxn00566
rxn00568
rxn00569
rxn00584
rxn00588
rxn00589
rxn00598
rxn00601
rxn00605
rxn00611
rxn00612
rxn00615
rxn00616
rxn00623
rxn00640
rxn00675
rxn00676
rxn00679
rxn00686
rxn00689
rxn00691
rxn00692
rxn00693
rxn00707
rxn00708
rxn00710
rxn00711
rxn00714
rxn00726
rxn00727
rxn00737
rxn00758
rxn00763
rxn00770
rxn00772
rxn00775
rxn00776
rxn00777
rxn00782
rxn00800
rxn00802
rxn00806
rxn00831
rxn00832
rxn00834
rxn00836
rxn00838
rxn00839
rxn00851
rxn00858
rxn00863
rxn00867
rxn00868
rxn00874
rxn00875
rxn00881
rxn00882
rxn00883
rxn00898
rxn00902
rxn00903
rxn00907
rxn00910
rxn00931
rxn00935
rxn00938
rxn00941
rxn00947
rxn00950
rxn00952
rxn00966
rxn00973
rxn00974
rxn00979
rxn00980
rxn00985
rxn00986
rxn00990
rxn00991
rxn01000
rxn01011
rxn01013
rxn01015
rxn01018
rxn01019
rxn01021
rxn01069
rxn01117
rxn01127
rxn01137
rxn01145
rxn01188
rxn01192
rxn01200
rxn01204
rxn01208
rxn01210
rxn01211
rxn01213
rxn01218
rxn01219
rxn01225
rxn01226
rxn01255
rxn01256
rxn01257
rxn01258
rxn01265
rxn01268
rxn01269
rxn01274
rxn01299
rxn01300
rxn01301
rxn01302
rxn01303
rxn01313
rxn01314
rxn01329
rxn01331
rxn01332
rxn01333
rxn01334
rxn01351
rxn01352
rxn01353
rxn01360
rxn01362
rxn01364
rxn01387
rxn01388
rxn01406
rxn01434
rxn01445
rxn01451
rxn01485
rxn01486
rxn01492
rxn01504
rxn01507
rxn01509
rxn01512
rxn01513
rxn01517
rxn01519
rxn01520
rxn01521
rxn01522
rxn01523
rxn01537
rxn01544
rxn01545
rxn01575
rxn01602
rxn01603
rxn01629
rxn01634
rxn01636
rxn01637

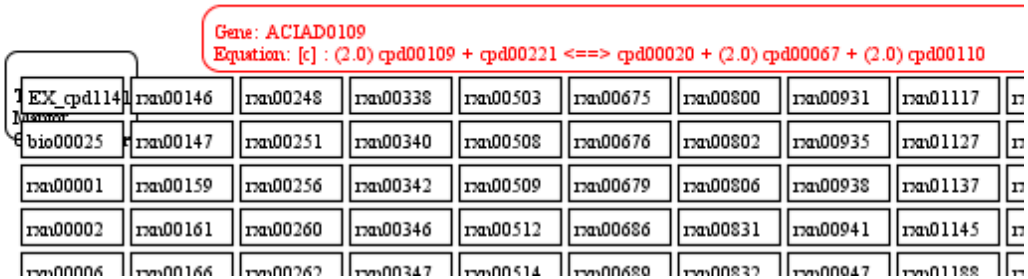
Information extraction

Right click on a map to Extract reaction information from a model and choose a model you want to extract information from. Then you can show the extra information of reaction in the map by right clicking a reaction to Show extra info..





The gene and equation information for the reaction can be seen on the map.



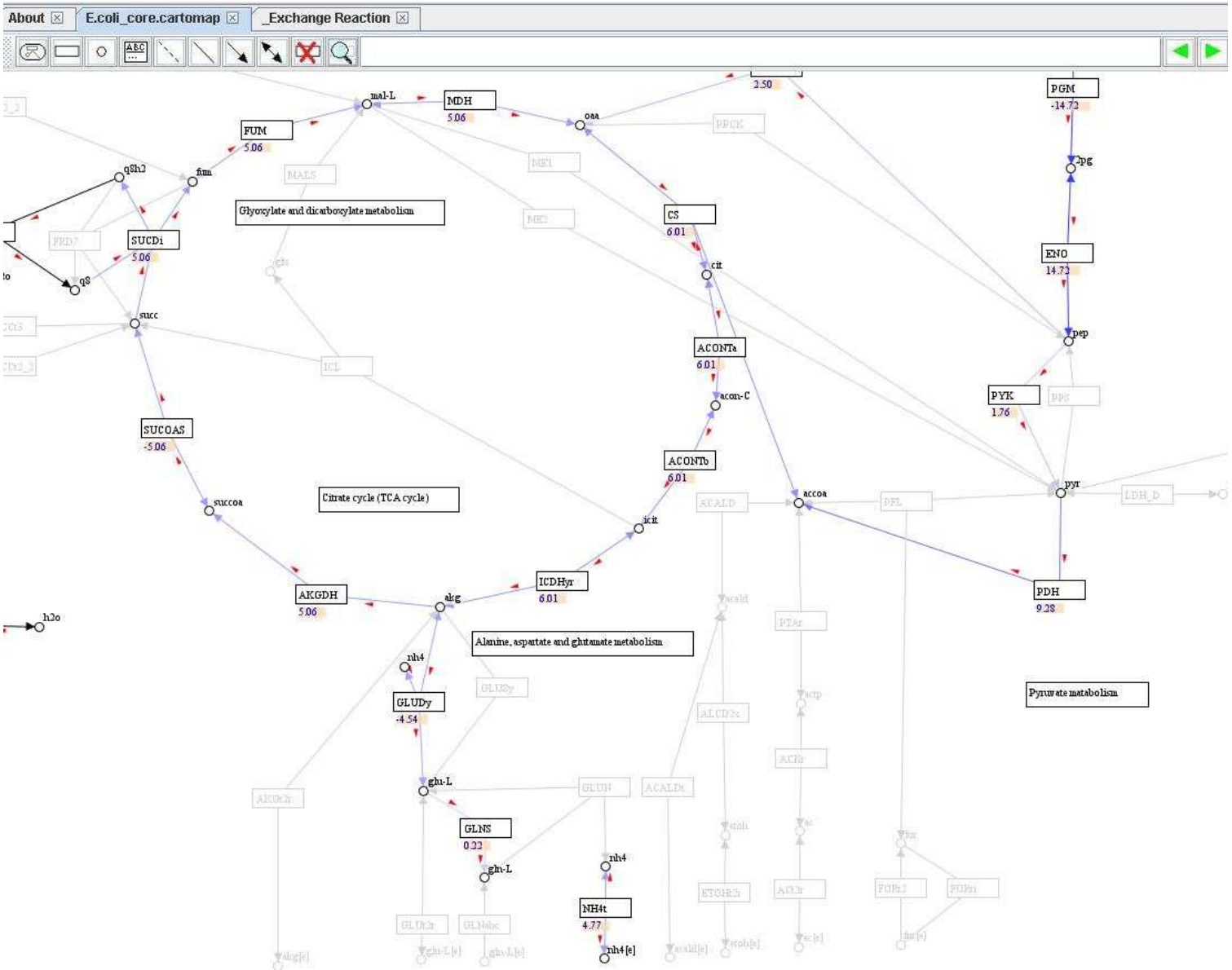
The function in information extraction can aid in metabolic map creation and providing associated genes for later loading gene expression.

Flux visualization

As mentioned early in Simulation, reaction fluxes can be loaded into a map for visualization. GEMSiRV provides a function in loading reaction fluxes by right clicking on a map to Load reaction fluxes.

A single run of simulation: (A header line beginning with "#" is optional)

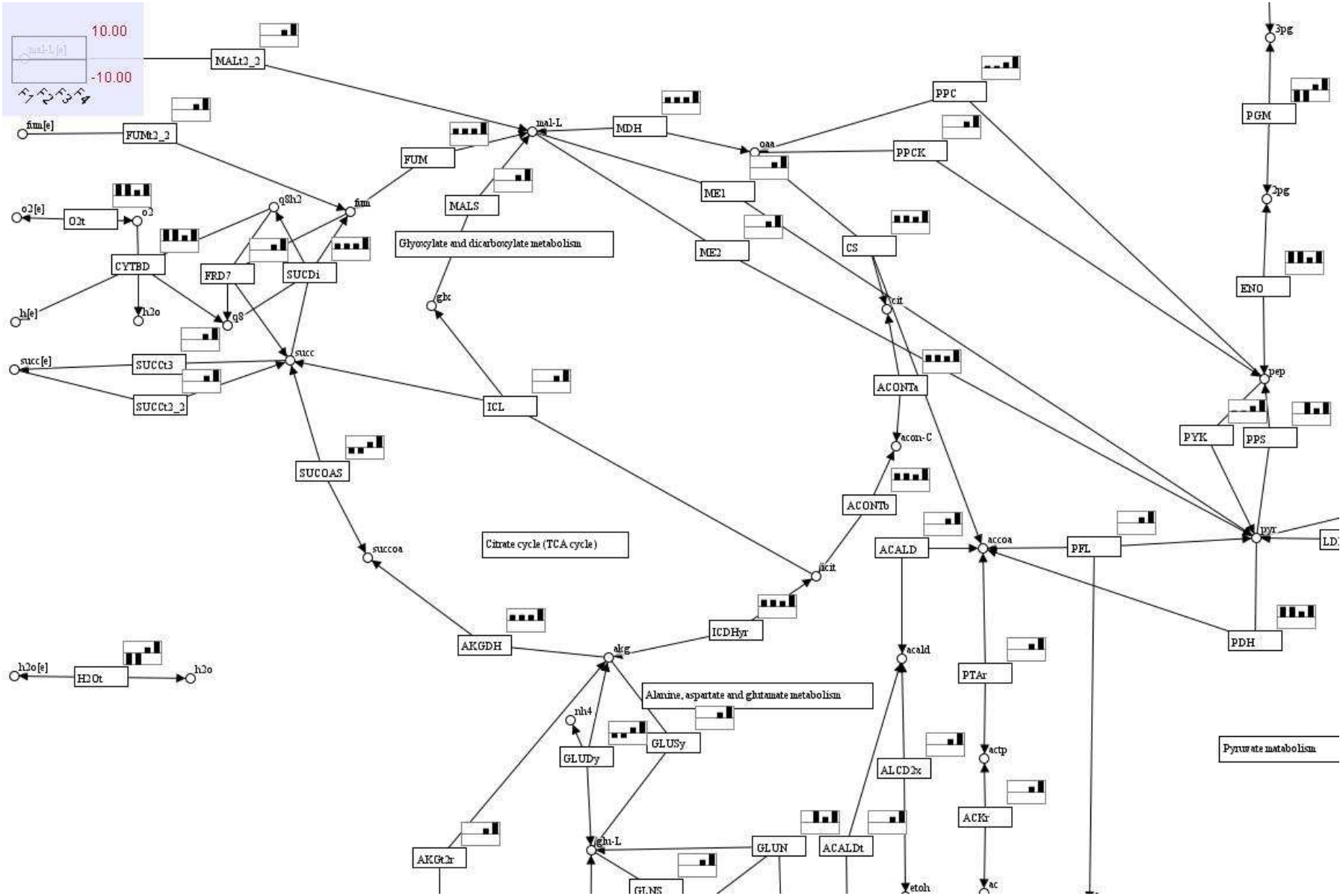
```
#Reaction Flux
ACONTa  6.00725
ACONTb  6.00725
AKGDH 5.06438
ATPM  8.39
ATPS4r  45.514
Biomass_Ecoli_core_N(w/GAM)_Nmet2 0.873922
CO2t  -22.8098
CS  6.00725
CYTBD 43.599
ENO 14.7161
EX_co2(e) 22.8098
EX_glc(e) -10.0
```


About
E.coli_core.cartomap
_Exchange Reaction
mal-L
MDH
5.06
oaa
FUM
5.06
fum
q8h2
q8
SUCDi
5.06
succ
Glyoxylate and dicarboxylate metabolism
CS
6.01
cit
ACONTa
6.01
acon-C
ACONTb
6.01
SUCOAS
-5.06
succoa
Citrate cycle (TCA cycle)
icit
ICDHyr
6.01
AKGDH
5.06
akg
h2o
Alanine, aspartate and glutamate metabolism
nh4
GLUDy
-4.54
glu-L
GLNS
0.22
gln-L
nh4
NH4t
4.77
nh4[e]
accoa
PGM
-14.72
2pg
ENO
14.72
pep
PYK
1.76
pyr
PDH
9.28
Pyruvate matabolism

Multiple runs of simulation: (A header line beginning with “#” is preferred for labeling in legend)

```
#Reaction F1  F2  F3  F4
ACALD 0 0 5 10
ACALDt  0 0 5 10
ACKr  0 0 5 10
ACONTa  6.00725 6.00725 5 10
ACONTb  6.00725 6.00725 5 10
ACt2r 0 10  5 10
ADK1  0 10  5 10
AKGDH 5.06438 5.06438 5 10
AKGt2r  0 0 5 10
ALCD2x  0 0 5 10
ATPM  8.39  8.39  5 10
ATPS4r  45.514  45.514  5 10
Biomass_Ecoli_core_N(w/GAM)_Nmet2 0.873922  0.873922  5 10
CO2t  -22.8098  -22.8098  5 10
CS  6.00725 6.00725 5 10
CYTBD 43.599  43.599  5 10
D_LACt2 0 0 5 10
ENO 14.7161 14.7161 5 10
ETOHt2r 0 0 5 10
EX_ac(e)  0 0 5 10
EX_acald(e) 0 0 5 10
```

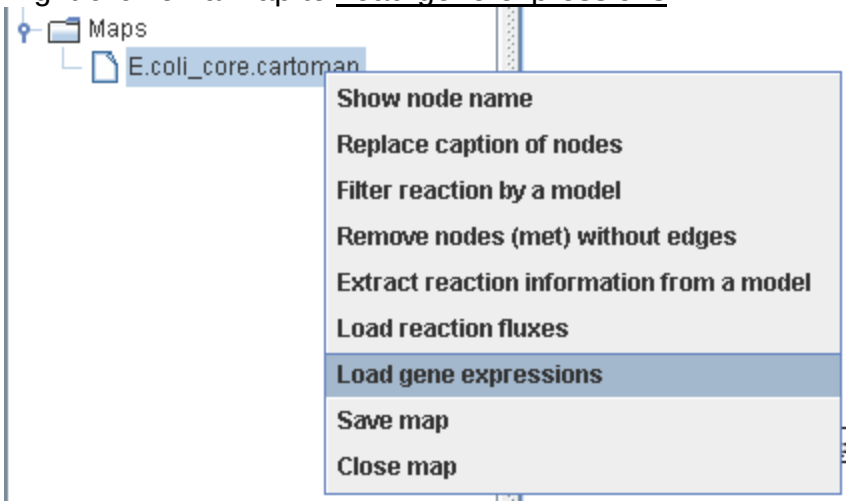
A visual flux range can be set:


10.00
-10.00
MALt2_2
fum[e]
FUMt2_2
o2[e]
O2t
o2
CYTBD
h[e]
h2o
q8
q8h2
FRD7
SUCDi
fum
FUM
mal-L
MDH
MALS
Glyoxylate and dicarboxylate metabolism
succ[e]
SUCCt3
SUCCt3_2
succ
ICL
SUCOAS
succoa
Citrate cycle (TCA cycle)
h2o[e]
H2Ot
h2o
AKGDH
akg
ICDHyr
icit
Alanine, aspartate and glutamate metabolism
nh4
GLUDy
GLUSy
AKGt2r
glu-L
GLUN
GLNS
oaa
PPC
PPCK
ME1
ME2
CS
glx
ACONTa
acon-C
ACONTb
ACALD
accoa
PFL
acald
ALCD2x
etoh
PTAr
actp
ACKr
ac
ACALDt
3pg
PGM
2pg
ENO
pep
PYK
PPS
pyr
PDH
Pyruvate metabolism

Gene expression visualization

Because GEMSiRV allows users to extract information from a model to a map, in addition to reaction fluxes, gene expressions can also be loaded into a map for visualization. In this circumstance, we can simultaneously compare the differences of reaction fluxes with that of gene expressions in two conditions (e.g. aerobic and anaerobic conditions).

Right click on a map to Load gene expressions.

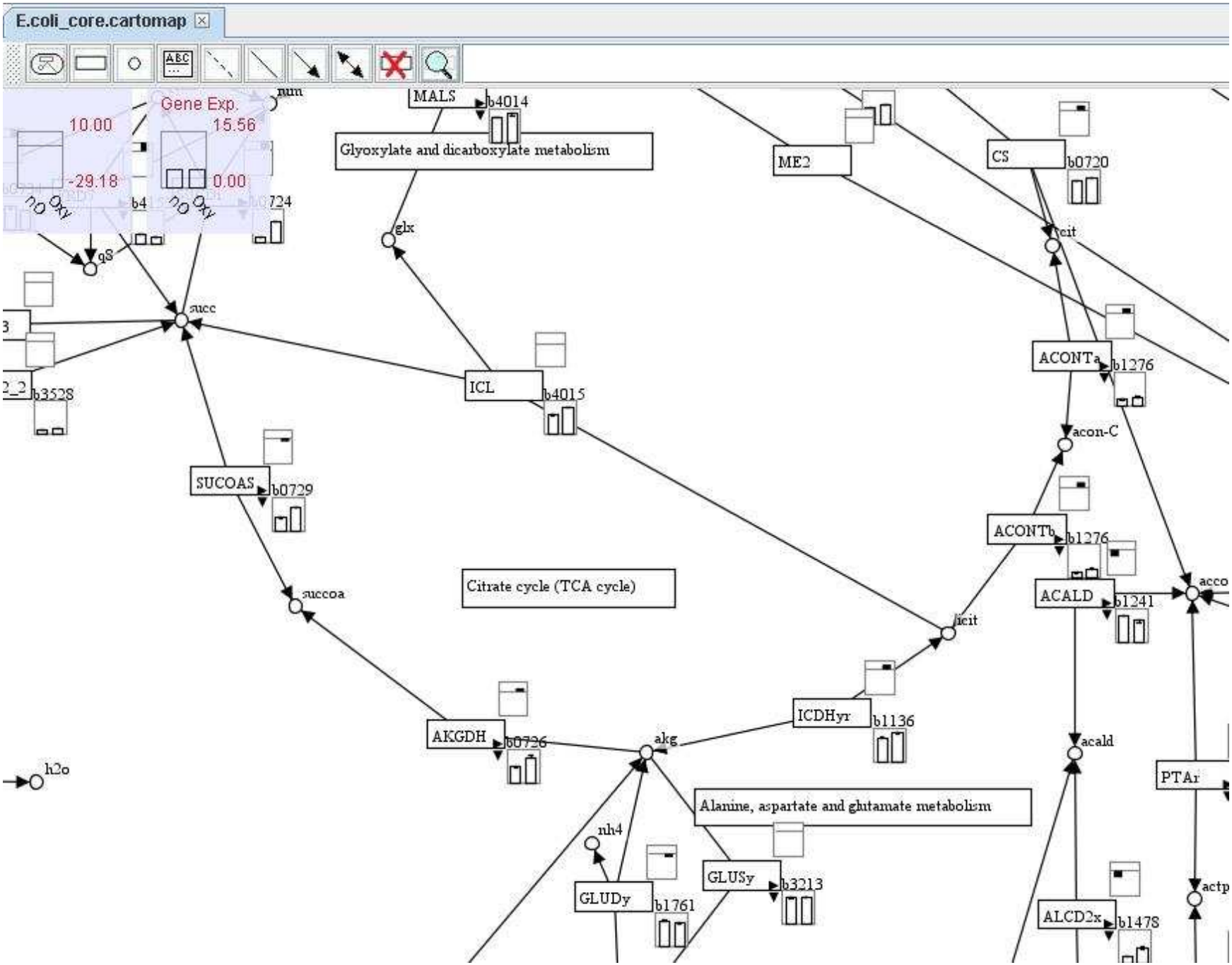


You can use identical header to represent the replicates of condition. Then the mean and standard deviation of gene expression for a specific gene will be shown in the map. Here, we used the expression data (array number 42-48) available in http://systemsbiology.ucsd.edu/In_Silico_Organisms/E_coli/E_coli_expression2.

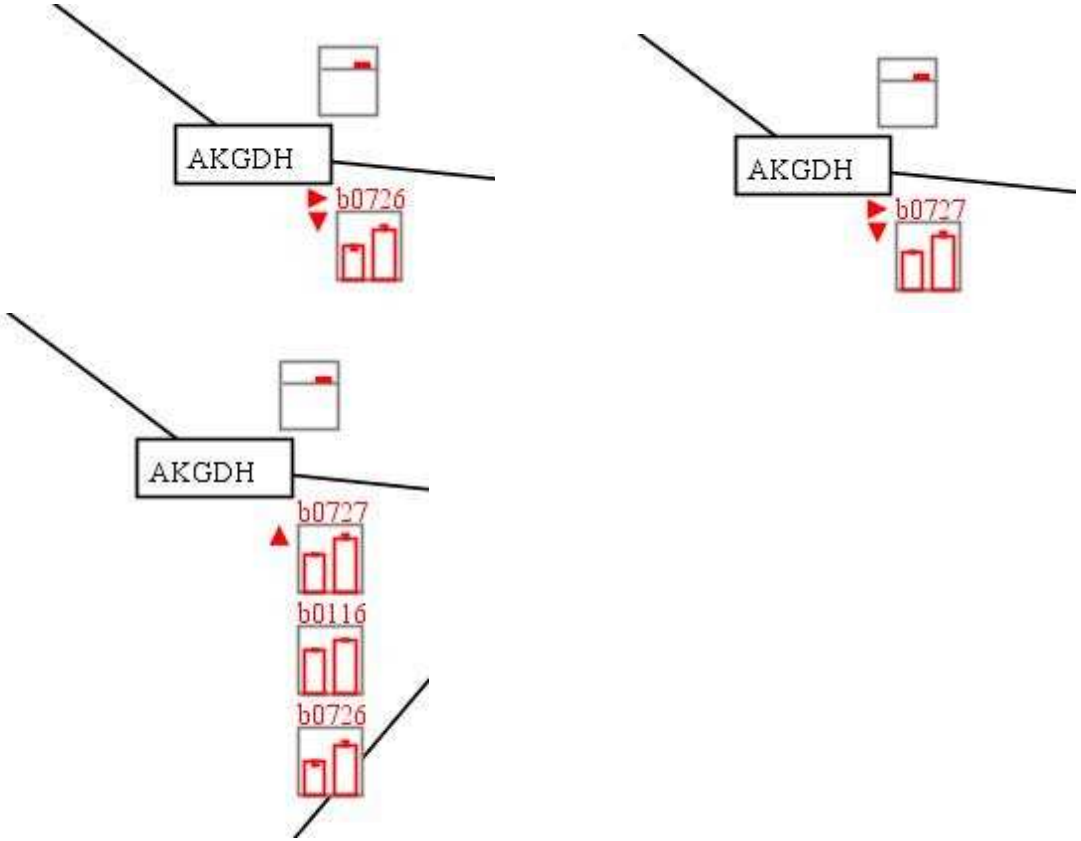
Expr_o2 effect on Ecoli_rep.TXT

1	#Gene	nO	nO	nO	nO	Oxy	Oxy	Oxy
2	b2836	5.769096757	5.879973845	5.889739712	5.867358643	5.760884085	5.873523604	5.957984412
3	b0885	3.535727661	3.469050784	4.049806246	3.645329898	3.879638515	3.597923384	3.593089884
4	b0199	9.804808142	10.40118062	10.57760507	10.25695657	9.301827015	9.898372638	10.09632395
5	b0715	2.565546658	2.567760685	2.536834823	2.513905138	2.533385516	2.555976111	2.552486193
6	b0185	6.661911901	6.094619316	7.696563672	6.963963632	7.476477903	7.20705541	7.645863323
7	b3255	12.24120191	12.17555935	12.10939238	12.14068385	11.88957437	11.98381957	11.53553843
8	b3256	8.626216539	8.9348643	8.754524155	9.051725652	8.586089409	8.749223826	8.216142737
9	b2316	10.30507868	10.30964272	10.70406165	10.96516547	10.13253158	10.50413996	9.648748283
10	b4015	9.463208813	9.972113349	8.9044422	9.819722455	12.72892109	12.51954356	12.59735882
11	b4014	11.97537857	12.07528508	11.84044276	11.72425199	13.23488622	13.29639349	13.19317442
12	b0114	11.76711824	11.58164936	11.88583332	11.57104461	12.49915551	12.55704041	12.55320524
13	b0115	11.02053116	10.81358943	11.14457251	11.36213807	11.755529	12.04086088	12.18448931
14	b4016	2.963904777	2.966189525	2.93471414	2.895148444	4.296326343	3.846552601	3.597761521

Because we want to visualize reaction fluxes and gene expressions on a map, we firstly load the reaction fluxes which were simulated by setting the LB and UB of EX_o2(e) to close and open bound for anaerobic and aerobic conditions (nO and Oxy), respectively. The right upper panel of reaction shows the reaction fluxes for the two conditions.


E.coli_core.cartomap
Gene Exp.
10.00
-29.18
15.56
0.00
MALS
b4014
Glyoxylate and dicarboxylate metabolism
ME2
CS
b0720
glx
cit
succ
ICL
b4015
ACONTa
b1276
acon-C
b3528
SUCOAS
b0729
ACONTb
b1276
Citrate cycle (TCA cycle)
ACALD
b1241
succoa
icit
AKGDH
b0726
akg
ICDHyr
b1136
acald
PTAr
h2o
Alanine, aspartate and glutamate metabolism
nh4
GLUDy
b1761
GLUSy
b3213
ALCD2x
b1478

The right lower panel of reaction shows the expressions of associated genes. In default, the gene with the largest expression among the associated genes will be present. You can click on the small right arrow to present other gene expressions of associated genes or you can click on the small down arrow to show all gene expressions of associated genes.



In this example, we can see that the AKGDH reaction-associated genes b0116, b0726 and b0727 were up-regulated in aerobic condition and the corresponding reaction flux was increased.