

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

User manual

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GEMSiRV

Table of contents

Reconstruction4
Model importing and editing4
Reference database construction7
Draft reconstruction generation
Model refinement
Simulation
Dead-end metabolite identification
Objective optimization
Flux variability analysis
Robustness analysis
Essentiality analysis
Gene deletion analysis
Visualization
Metabolic map creation
KEGG map loading
Map replacement
Information extraction
Flux visualization
Gene expression visualization104

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 <u>Gene-protein-reaction associations are necessary</u> 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.

Create D	latabase	
?	Please choose a home directory: D:1	Browse
	Please create/open a project:	
	MyBioSysDB	
	Gene-protein-reaction associations are necessary. Create Cancel	

Reconstruction

Click on <u>Reconstruction</u> in the menu bar to open **Model databases** and **Reference databases**.

Model importing and editing

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

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Reconstruction Simu	lation	Visualization	Setting
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— — 🗋 Metabolite Ind	Impor	rt spreadsheet	s (.xis)
Reaction Inde	Close	models	

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

In table of Reaction Index:

李 Reconstruction Simulation Visualization Setting Project Gene Index 🖂 Reaction Index 🗵 Exchange Reaction InnerCell Reaction Mc . 🔶 🗂 Model databases . Abbreviation Confidence Equation Gene Name 🔶 🗂 SBML export E.coli textbook out.xml 0 ACALD [c] : acald + coa +... (b0351).(b1241) acetaldehvde de. Gene Index Update ACALDt 1 acald[e] <==> ac... (\$0001) acetaldehvde rev. Reaction Index ACKr 2 [c] : ac + atp <==... (b2296),(b1849)... acetate kinase Insert 3 ACONTa [c] : cit <==> acon... (b0118),(b1276) aconitase (half-r.. Exchange Reaction Delete ACONTD (b0118),(b1276) 4 [c] : acon-C + h2... aconitase (half-r.. InnerCell Reaction 5 ACt2r ac[e] + h[e] <==> .. acetate reversibl. Metabolite [c] : amp + atp <= ... (b0474) 6 ADK1 adenylate kinase 🕈 🗂 Reference database 7 AKGDH [c] : akg + coa + n... (b0727)+(b0116... 2-Oxogluterate d. 8 AKGt2r akq[e] + h[e] <==... (b2587) 2-oxoglutarate re.. Metabolite Index ALCD2x 9 [c] : etoh + nad <... (b0356),(b1241)... alcohol dehydrog. Reaction Index 10 ATPM [c] : atp + h2o --> ... ATP maintenanc. adp[c] + (4.0) h[e]... (b3739)+(b3737... ATP synthase (fo. 11 ATPS4r 12 Biomass Ecoli . [c] : (1.496) 3pg + ... core E. coli biom. co2[e] <==> co2[c] (s0001) 13 CO2t 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. FORt2 21 for[e] + h[e] --> fo... (b0904),(b2492) formate transport. 22 FORti for[c] --> for[e] (b0904),(b2492) formate transport. [c] : fum + q8h2 --... (b4151+b4153+... 23 FRD7 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. G6PDH2r 27 [c] : g6p + nadp <...] (b1852) glucose 6-phosp. GAPD 28 [c]: q3p + nad + . (b1779) glyceraldehyde-3. 111 4 1

In table of Gene Index:

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Reconstrucion Simulation Visualization Settin	ıg							
🗂 Project	Gene	Index 🗵						
🕈 🚍 Model databases		5'Coordinate	Locus Tag	Reaction	EC Number	Gene		
Gene Index			b4031	XYLt2pp				-
			b3189	UAGOVT	Update		Î	
- 🗋 Reaction Index	2		b4032	14GLUCANabc	Insert		+	
Evchange Reaction	3		b2813	MLTGY3pp,MLT	Delete			
	4		b4033	14GLUCANabc	Delete			
- D _InnerCell Reaction	5		b4034	14GLUCANabc				
— 🗋 _Metabolite	6		b2817	AGM4PApp,AG				
e- 🔚 Reference database	7		b2818	ACGS				
- 🗅 Metabolite Index	8		b3397	ADPRDP				
D Peaction Index	9		b2810	CYSSADS				
	10		b2429	ACMUMptspp,S				
L Maps	11		b3599	MNLptspp			1	
	12		b2421	CYSS			1	
	13		b2423	SULabcpp,TSU				
	14		b4025	PGI			1	
	15		b2422	SULabcpp,TSU			1	
	16		b3196	CAt6pp				
	17		b4024	ASPK				
	18		b3197	A5PISO				
	19		b2425	SULabcpp,TSU				
	20		b3198	KDOPP				
	21		b2424	SULabcpp,TSU			1	
	22		b2697	ALATRS				
	23		b2827	TMDS				
	24		b3389	DHQS				
	25		b3386	RPE				
	26		b3591	SELCYSS				
	27		b2418	PYDXNK,PYDX				
	28		b2417	ACMUMptspp,G				
		4	Л					

Reference database construction

Right click on **Reference database** to <u>Import database (.xls)</u>, 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 <u>http://sb.nhri.org.tw/GEMSiRV/en/Reference_Databases</u>.



Or, you can right click on the model you imported to <u>Add rxn&met to the Ref. DB</u>, 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 <u>Reaction Index</u> of **Reference database** to evaluate charge/mass balance of equation.

Reconstruction Simulation Visu	ializati	on Setting					
Project		abolite Index 🗵	Reac	Reaction Index 🗵			
P- ☐ Model databases		ABBREVIA	TION	EQUA	TION		
P SBML_export_E.coli tex	0	10FTHF5GLUt		10fthf5glu[c]	> 10fthf5		
— 🗋 Gene Index	1	1 10FTHF5GLUtm 2 10FTHF6GLUtl 3 10FTHF6GLUtm		10fthf5glu[m]> 10fthf5 10fthf6glu[c]> 10fthf6 10fthf6glu[m]> 10fthf6			
- 🗋 Reaction Index	2						
Evchange Reactin	3						
	4	10FTHF7GLUt		10fthf7alu[c]> 10fthf7			
	5	10FTHF7GLUtm	î.	10fthf7glu[m]> 10fthf7			
🗕 🗋 _Metabolite	6	10FTHFtl		10fthf[c] <==>	10fthf[l]		
🛉 🗂 Reference database	7	10FTHFtm		10fthf[c] <==>	10fthf[m]		
Metabolite Index	8	11DOCRTSLtm		11docrtsl[c] «	==> 11do		
	9	11DOCRTSLtr		11docrtsl[c] «	==> 11do		
Evaluate	charge	Mormula halanco	10	11docrtstrn[c] <==> 11d		
e Maps	charge	shormala parance		11docrtstrn[c] <==> 11d		

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.

1) 3pg[c] 1) atp[c]	() <==>	(1) 13dpg[c] (1) adp[c]	
Stoichiometry: 1 Compartment: c] <mark>0-></mark>]	Stoichiometry: 1 Compartment: c	
eactant: 📃 👻		Product: Add Delete	•

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 (<u>http://sb.nhri.org.tw/GEMSiRV/en/GBKParser</u>). 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 (<u>http://www.ncbi.nlm.nih.gov/RefSeq</u>). 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 (<u>http://bigg.ucsd.edu/</u>) and modify it with TextReplacer (<u>http://sb.nhri.org.tw/GEMSiRV/en/TextReplacer</u>). The ready-to-use model can be found and downloaded from <u>http://sb.nhri.org.tw/GEMSiRV/en/Metabolic_Models</u>.

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 (<u>http://sb.nhri.org.tw/MrBac</u>) 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 <u>Import spreadsheet (.xls)</u>.

Original spreadsheet file:

	dicrosoft Ex	ccel - NC_O	103197.gbk-blas	t.xls									
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-	F1		▼ fx	Ref-BLA	ST								
	A	B	C	D	E		G	I H		J	K	1	
1	Locus Tag	Gene	5'Coordinate	Product	EC Number	Ref-BLAST		5	-		1000 100		
2	STMUUU1	thrL	190	thr operon									
3	STM0002	thrA	337	bifunctiona	2.7.2.4;;1.1.	160002							
4	STM0003	thrB	2801	homoserin	2.7.1.39	b0003							
5	STM0004	thrC	3734	threonine	4.2.3.1	b0004							
6	STM0005	yaaA	5887	hypothetic	-	60006	2						
7	STM0006	yaaJ	7396	putative al	ŧ	60007							
8	STM0007	talB	7665	transaldola	2.2.1.2	60008	2						
9	STM0008	mogA	8729	molybden	a tenis	b0009							
10	STM0009	yaaH	9942	hypothetic	3-+3	b0010							Required fields
11	STM0010	htgA	10805	hypothetic	449	b0011							
12	STM0011	yaal	11245	hypothetic	- 44	b0013							
13	STM0012	dnaK	11593	molecular	<u>19</u> 7	b0014							
14	STM0013	dnaJ	13595	chaperone		60015							
15	STM0014	8258	15014	putative tra	170								
16	STM0015	1000	16088	putative ba	1	1	2						
17	STM0016	199	17026	hypothetic	9 8	1							
18	STM0017	(e+2)	17486	hypothetic	9 -	1							
19	STM0018	144	17867	putative ex	(1							
20	STM0019	3223	20058	putative hy	/								
21	STM0020		23335	putative cy	/	1							
22	STM0021	bcfA	24469	fimbrial su	1 <u></u>		0					12101	
33	STMODOO	ne Index	05110	fimbrial ch	10) (1)								
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Gene Index table of the imported blank reconstruction (NC_003197.gbk-blast.xls):

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	(Stationer)	1 Same
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Reconstrucion Simulation Visualization Setting								
🗂 Project	Gene	index 🗵						
🛉 🚍 Model databases	15	5'Coordinate	Locus Tag	Gene	EC Number	Product	Rof BLAST	1.
SBML_export_E.coli textbook_out.xml	0	1190	STM0001	thri	Londiniber	thr oneron lead	Repution	
- 🗋 Gene Index	1	337	STM0002	thrA	2724-11	hifunctional asn	b0002	
- Reaction Index	2	2801	STM0003	thrB	27139	homoserine kin	h0003	-
	3	3734	STM0004	thrC	4231	threonine synth	b00004	-
	4	5887	STM0005	vaaA		hypothetical pro	60006	-
- D _InnerCell Reaction	5	7396	STM0006	vaa.l		nutative alanine	b0007	-
— 🗋 _Metabolite	6	7665	STM0007	talB	2212	transaldolase B	b0008	-
• 🗂 SBML export E. coli iAF1260 out xml	7	8729	STM0008	Anom		molyhdenum co	60009	-
	8	9942	STM0009	vaaH		hypothetical pro	b0010	-
	9	10805	STM0010	htaA		hypothetical pro	b0011	-
- D Reaction Index	10	11245	STM0011	vaal		hypothetical pro	b0013	
- 🗋 _Exchange Reaction	11	11593	STM0012	dnaK		molecular chap	b0014	
- Call Reaction	12	13595	STM0013	dnaJ		chaperone prot	b0015	
	13	15014	STM0014			putative transcri	2000/05	
 Imetabolito Imetabolito Imetabolito Imetabolito Imetabolito 	14	16088	STM0015		- Contraction of the Contraction	putative bacteri		
PNC_003197.gbk-blast.xis	15	17026	STM0016	44	- Contraction of the Contraction	hypothetical pro		
Gene Index	16	17486	STM0017	44		hypothetical pro		
— 🗋 Reaction Index	17	17867	STM0018	44		putative exochiti		
🕈 🗂 Reference database	18	20058	STM0019		-	putative hydroxy		
T Metabolite Index	19	23335	STM0020		and the second s	putative cytopla		
	20	24469	STM0021	bcfA	and the second s	fimbrial subunit		
	21	25112	STM0022	bcfB	in .	fimbrial chapar		
🕈 🛄 Maps	22	25803	STM0023	bcfC	in .	fimbrial usher	b0940	
— 🗋 E.coli_core.cartomap	23	28425	STM0024	bcfD		fimbrial subunit		
-0	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		
		•		II				

Empty Reaction Index table of the imported blank reconstruction:

Reconstrucion Simulation Visualization Setting						
Project	Gene Index 🗵	Reaction	n Index 🗵			
 Project Model databases SBML_export_E.coli textbook_out.xml Gene Index Exchange Reaction InnerCell Reaction Metabolite SBML_export_E. coli iAF1260_out.xml Gene Index Exchange Reaction InnerCell Reaction Exchange Reaction InnerCell Reaction InnerCell Reaction InnerCell Reaction Metabolite Metabolite Reaction Index Gene Index Reference database Metabolite Index Reaction Index Reaction Index E.coli_core.cartomap 	Gene Index Abbrev 0 1 2 3 4 5 6 7 8 9 10 11 12 3 4 5 6 7 8 9 100 11 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 27	Reaction	Confidence	Equation	Gene	Name
						• •

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

<u>å</u>	
Reconstrucion Simulation V	fisualization Setting
Project Project Gene Index Control Contro Control Control Control Co	AF1260_out.xml
— 🗋 Gene Index	
 Construction P □ Reference database P □ Reference database P □ Metabolite Index P □ Reaction Index 	Generate simulation tables Define environmental conditions Export model (.xml) Download model (.xls) Filter reaction by a map Delete 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 (<u>http://seed-viewer.theseed.org/seedviewer.cgi?page=ModelView</u>) 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 <u>Load and update gene's info.</u>, and upload the spreadsheet file generated by GBKPaser (<u>http://sb.nhri.org.tw/GEMSiRV/en/GBKParser</u>), e.g. NC_000913.gbk.xls for ECO.



Gene information can be loaded and updated accordingly:

					_
Index 🗵					
5'Coordinate	EC Number	Gene	Locus Tag	Reaction	T
		1	s0001	GLYCtpp,FEEN	Г
190		thrL	b0001		tł
337	2.7.2.4;;1.1.1.3	thrA	b0002	ASPK, HSDy	fu
2801	2.7.1.39	thrB	b0003	HSK	h
3734	4.2.3.1	thrC	b0004	THRS,4HTHRS	tł
5234		yaaX	b0005		p
6459		yaaA	b0006		c
7959		yaaJ	b0007	GLYt4pp,ALAt4pp	p
8238	2.2.1.2	talB	b0008	TALA	tr
9306		mog	b0009		p
10494		yaaH	b0010		c
11356		yaa₩	b0011		c
11786	Sec.	vaal	h0013		In



Reconstruction Simulation Visualization Setting

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Project	Gene	Index 🗵						
🕈 🔚 Model databases		5'Coordinate	EC Number	Gene	Locus Tag	Reaction		
📍 🗂 SBML_export_E. coli iAF1260_out.xml	0		2011000		s0001	GLYCtop FEEN	-	1
- 🗋 Gene Index	1	190		thri	60001		thr	
- Reaction Index	2	337	2724:1113	thrA	b0002	ASPK HSDV	fus	
Sychange Reaction	3	2801	2.7.1.39	thrB	b0003	HSK	ho	
	4	3734	4.2.3.1	thrC	b0004	THRS,4HTHRS	thr	
- D_InnerCell Reaction	5	5234		уааХ	b0005		pre	
— 🗋 _Metabolite	6	6459	1	yaaA	b0006		col	
🗣 🗂 NC_003197.gbk-blast.xls	7	7959		yaaJ	b0007	GLYt4pp,ALAt4pp	pre	
Gene Index	8	8238	2.2.1.2	talB	b0008	TALA	tra	
	9	9306		mog	b0009		pre	
	10	10494	200	yaaH	b0010		cor	
P Reference database	11	11356	-	yaaVV	b0011		col	
— 🗋 Metabolite Index	12	11786		yaal	b0013		pre	
🗕 🔄 Reaction Index	13	12163		dnaK	b0014		cha	
- D Maps	14	14168		dnaJ	b0015		cha	
	15	15445		insL	b0016		IS1	
	16	16903		hokC	b4412		tox	4
	17	17489		nhaA	b0019	NAt3_2pp	SO	
	18	18715		nhaR	b0020		DN	1
	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	bifi	
	24	22391	6.1.1.5	ileS	b0026	ILETRS	isc	
	25	25207	3.4.23.36	IspA	b0027		pro	
	26	25826	5.2.1.8	fkpB	b0028		FK	
	27	26277	1.17.1.2	ispH	b0029	DMPPS, IPDPS	1-1	
	28	27293	3.2.2	rihC	b0030	ADNUC, URIH, C	rib	
B		4		11/2010 (2)				

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 <u>Generate simulation tables</u> to generate a model containing a stoichiometric matrix as well as default systems boundaries.

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Reconstrucion Simulation Vis	ualization Sett	ing			
🗂 Project		Rea	ction Index 🗵 🎽		
• C Model databases	1000	1	Abbreviation		
P SBML_export_E. coll IAP	1260_out.xmi	0	12PPDRtex		
— 🗋 Gene Index		1	12PPDStex		
📃 📃 🦳 Reaction Index		2	14GLUCANabcp		
Exchange Reaction	i li	3	14GLUCANtexi		
		4	23CAMPtex		
	5	23CCMPtex			
- Metabolite		6	23CGMPtex		
Gene Index	Draft a recons Add rxn&met f	to the Ref. DB			
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←	Define environ Export model (Download mod Filter reaction Delete model	menta (.xml) lel (.xl: by a m	s) s) ap		

After clicking on <u>Generate simulation tables</u>, 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:

· · · · · · · · · · · · · · · · · · ·						
Reconstrucion Simulation Visualization Settin	ng					
Project	_Exc	hange Reaction 🗵	_InnerCell Read	tion 🗵 🚺 _Metabo	olite 🗵	
🕈 🔚 Model databases		Abbreviation	Association	Equation	LB	Name 🔺
P SBML_export_E. coli IAF1260_out.xml	0	12PPDRtex	STM2267,STM03	12ppd-R[e] <==>	-1000.0	(R)-Propane-1,2
— 🗋 Gene Index	1	12PPDStex	STM2267,STM03	12ppd-S[e] <==>	1000.0	(S)-Propane-1,2
- 🗋 Reaction Index	2	14GLUCANabcpp	STM4230+STM4	14glucan[p] + atp	.0	1,4-alpha-D-gluc.
Evchange Reaction	3	14GLUCANtexi	STM4231	14glucan[e]> 1	0	1,4-alpha-D-gluc.
	4	23CAMPtex	STM2267,STM03	23camp[e] <==>	-1000.0	23cAMP transpor.
InnerCell Reaction	5	23CCMPtex	STM2267,STM03	23ccmp[e] <==>	-1000.0	23cCMP transpo
— 🗋 _Metabolite	6	23CGMPtex	STM2267,STM03	23cgmp[e] <==>	-1000.0	23cGMP transpo
🕈 🗂 NC_003197.gbk-blast.xls	7	23CUMPtex	STM2267,STM03	23cump[e] <==>	-1000.0	23cUMP transpo
Gene Index	8	23DAPPAtex	STM2267,STM03	23dappa[e] <==>	-1000.0	2,3-diaminopropi.
Departian Index	9	23PDE2pp	STM4403	[p] : 23cump + h2	.0	2',3'-cyclic-nucle
Reaction muex	10	23PDE4pp	STM4403	[p] : 23ccmp + h2	0	2',3'-cyclic-nucle
Exchange Reaction	11	23PDE7pp	STM4403	[p] : 23camp + h2	.0	2',3'-cyclic-nucle
- 🗋 InnerCell Reaction	12	23PDE9pp	STM4403	[p] : 23cgmp + h2	.0	2',3'-cyclic-nucle
Invalid Reaction Index	13	26DAHtex	STM1473,STM22	26dap-M[e] <==>	-1000.0	meso-2,6-Diami
□ - □ Matabalita	14	2AGPA120tipp	STM3009	2ddecg3p[p]>	0	2-Acyl-sn-glycero.
	15	2AGPA140tipp	STM3009	2tdecg3p[p]> 2	0	2-Acyl-sn-glycero.
P C Reference database	16	2AGPA141tipp	STM3009	2tdec7eg3p[p]	0	2-Acyl-sn-glycero.
— 🗋 Metabolite Index	17	2AGPA160tipp	STM3009	2hdecg3p[p]>	0	2-Acyl-sn-glycero.
- 🗅 Reaction Index	18	2AGPA161tipp	STM3009	2hdec9eg3p[p]	0	2-Acyl-sn-glycero.
	19	2AGPA180tipp	STM3009	20deca3p[a]>	0	2-Acvl-sn-alvcero.

_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.

s Andreas and a second							×
Reconstrucion Simulation Visualization Sett	ing				(i) -		
Project	_Exc	change Reaction 🗵 🚺 In	nerCell Reaction 🗵 🎽 Metab	olite 🗵			
🕈 🔚 Model databases		Abbreviation	Equation	1	LB		
P SBML_export_E. coli iAF1260_out.xml	0	EX 12ppd-R(e)	[e]:12ppd-R <==>	0		0	
— 🗋 Gene Index	1	EX_12ppd-S(e)	[e]:12ppd-S <==>	0		0	
Reaction Index	2	EX_14glucan(e)	[e]:14glucan <==>	0		0	
- Fychange Reaction	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	
🛉 🗂 NC_003197.gbk-blast.xls	7	EX_23cump(e)	[e] : 23cump <==>	0		0	
- 🗅 Gene Index	8	EX_23dappa(e)	[e] : 23dappa <==>	0		0	
Reaction Index	9	EX_26dap-M(e)	[e] : 26dap-M <==>	0		0	
	10	EX_2ddglcn(e)	[e] : 2ddglcn <==>	0		0	
- D Lexchange Reaction	11	EX_34dhpac(e)	[e] : 34dhpac <==>	0		0	
- 🗋 _InnerCell Reaction	12	EX_3amp(e)	[e] : 3amp <==>	0		0	
- 🗋 _Invalid Reaction Index	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	
— 🗋 Metabolite Index	17	EX_3ump(e)	[e] : 3ump <==>	0		0	
🗕 🗋 Reaction Index	18	EX_4abut(e)	[e] : 4abut <==>	0		0	
	4.0	EXC. Also as we are also at	The state of the second s	0		0	

_Metabolite:

The rest of	1000

Reconstruction Simulation Visualization Set	ting					
🗂 Project	Exe	change Reaction 🗵	_InnerCell Reac	tion 🗵 🔪 Metabolite 🗵		
🕈 🚍 Model databases	7	Abbreviation	Formula	Name	Compartment	
P SBML_export_E. coli IAF1260_out.xml	0	10fthf	C20H21N707	10-Formyltetrahydrofolate	cytoplasm	
🚽 🗋 Gene Index	1	12dgr120	C27H52O5	1,2-Diacyl-sn-glycerol (didod	cytoplasm	Γ
🗕 📄 Reaction Index	2	12dgr120	C27H52O5	1,2-Diacyl-sn-glycerol (didod	periplasm	
- T Exchange Reaction	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	
- D_Metabolite	6	12dgr141	C31H56O5	1,2-Diacyl-sn-glycerol (ditetr	periplasm	
🛉 🔚 NC_003197.gbk-blast.xls	7	12dgr160	C35H68O5	1,2-Diacyl-sn-glycerol (dihex	cytoplasm	
— 🗋 Gene Index	8	12dgr160	C35H68O5	1,2-Diacyl-sn-glycerol (dihex	periplasm	
- Reaction Index	9	12dgr161	C35H64O5	1,2-Diacyl-sn-glycerol (dihex	cytoplasm	
	10	12dgr161	C35H64O5	1,2-Diacyl-sn-glycerol (dihex	periplasm	
- D _Exchange Reaction	11	12dgr180	C39H76O5	1,2-Diacyl-sn-glycerol (diocta	cytoplasm	
— 🗋 _InnerCell Reaction	12	12dgr180	C39H76O5	1,2-Diacyl-sn-glycerol (diocta	periplasm	
- 🗋 Invalid Reaction Index	13	12dgr181	C39H72O5	1,2-Diacyl-sn-glycerol (diocta	cytoplasm	
Metabolite	14	12dgr181	C39H72O5	1,2-Diacyl-sn-glycerol (diocta	periplasm	
	15	12ppd-R	C3H8O2	(R)-Propane-1,2-diol	cytoplasm	
e Catabase	16	12ppd-R	C3H8O2	(R)-Propane-1,2-diol	extracellular	
— 🗋 Metabolite Index	17	12ppd-R	C3H8O2	(R)-Propane-1,2-diol	periplasm	
- 🗋 Reaction Index	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 <u>Define environmental conditions</u>.

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

Reconstrucion Simulation Visualizati	on Setting					
T Project						
🛉 🗂 Model databases						
SBML_export_E. coli iAF1260_0	out.xpst					
Gene Index	Draft a reconstruction					
- 🗍 Reaction Index	Add rxn&met to the Ref. DB					
— <u> </u>	Generate simulation tables					
- 🗋 _InnerCell Reaction	Define environmental conditions					
Metabolite	Funant model (umb					
P T NC_003197.gbk-blast.xls	Export moder (.xmi)					
- C Gene Index	Download model (.xls)					
- Reaction Index	Filter reaction by a map					
- K Exchange Reaction	Delete model					

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 <u>Define environmental conditions</u>.

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 x 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 5 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- \overline{D} (e):L \overline{B} =-8

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

complete medium.TXT
complete medium.TXT
l [ALL EXCHANGE REACTIONS]
2 LB=-1000
3 UB=1000
4 Objective Coefficient=0
5

<u>\$</u>			
Reconstrucion Simulation Visualizatio	on Setting		
Project Project Model databases SBML_export_E. coli iAF1260_o	put.xm ¹		
Gene Index G	Draft a reconstruction Add rxn&met to the Ref. DB Generate simulation tables		
 	Define environmental conditions Export model (.xml) Download model (.xls)		
Reaction Index Section Index Description	Filter reaction by a map Delete model		

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

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1.00		Contraction of the local distribution of the
		\sim

Project	_Exchange R	eaction 🗵				
Project Model databases SBML_export_E. coli iAF1260_out.xml Gene Index Reaction Index Exchange Reaction Metabolite Colo3197.gbk-blast.xls Gene Index Reaction Index Exchange Reaction Colo3197.gbk-blast.xls Colo	Exchange R Abbr 0 EX_zn2 1 EX_tung 2 EX_so4 3 EX_pi(e 4 EX_nh4 5 EX_na1 6 EX_moi 7 EX_moi 9 EX_k(e) 10 EX_h2o 11 EX_h(e)	eviation Equation (e) [e]: zn2 <==> gs(e) [e]: tungs <==> (e) [e]: so4 <==> (e) [e]: pi <==> (e) [e]: nh4 <==> (e) [e]: na1 <==> bd(e) [e]: mobd <==> 2(e) [e]: mg2 <==> (e) [e]: h20 <==> (e) [e]: h20 <==>	LB -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000	UB 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0	Objective 0	≥ C ▲
InnerCell Reaction Invalid Reaction Index	12 EX_fe3(13 EX_fe2(14 EX_cu2 15 EX_cob 16 EX_co2 17 EX_cl(e 18 EX_ca2 19 EX_o2(20 EX_glc- 21 EX_xylu	(e) [e]: fe3 <==> (e) [e]: fe2 <==> (e) [e]: cu2 <==> alt2(e) [e]: co2 <==> (e) [e]: cl <==>	-1000 -1000 -1000 -1000 -1000 -1000 -1000 -18.5 -8 U	1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0 1000.0	0 0 0 0 0 0 0 0 0 0 0 0	

1.

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

and the second second	ut 🖾 Reaction inu	ex 🗵 🛛 _Exchange F	leaction 🗵			
	Abbreviation	Equation	LB	UB	Objective C	
58	EX_butso3(e)	[e] : butso3 <==>	0	9999999	0	1
59	EX_ca2(e)	[e]:ca2 <==>	-999999	9999999	0	
60	EX_cbi(e)	[e] : cbi <==>	0	9999999	0	
61	EX_cbl1(e)	[e] : cbl1 <==>	01	9999999	0	
62	EX_cd2(e)	[e] : cd2 <==>	0	9999999	0	
63	EX_cgly(e)	[e] : cgly <==>	0	9999999	0	
64	EX_chol(e)	[e] : chol <==>	0	9999999	0	
65	EX_cit(e)	[e] : cit <==>	0	9999999	0	
66	EX cl(e)	[e] : c] <==>	-999999	9999999	0	
	* Abbrevi	ation: EX_ca2(e) LB:-9999999	Objec	Equa tive Coeffic	ition: [e] : ca2 <==> cient: 0	
	* Abbrevi	ation: EX_ca2(e) LB: -9999999 UB: 999999	Objec	Equa tive Coeffic	ntion: [e] : ca2 <==> cient: 0	
	* Abbrevi	ation: EX_ca2(e) LB: -9999999 UB: 9999999 Submit	Objec	Equa	ntion: [e] : ca2 <==> :ient: 0	
78	EX cynt(e)	ation: EX_ca2(e) LB: -999999 UB: 999999 Submit	Object	Equa	ntion: [e] : ca2 <==>	
78 79	EX_cynt(e) EX_cys D(e)	ation: EX_ca2(e) LB: -999999 UB: 999999 Submit [e] : cynt <==> [e] : cys-D <==>	Cancel	Equa tive Coeffic 999999 999999	otion: [e] : ca2 <==>	
78 79 80	EX_cynt(e) EX_cys_D(e) EX_cys_L(e)	ation: EX_ca2(e) LB: -999999 UB: 999999 Submit [e] : cynt <==> [e] : cys-D <==> [e] : cys-L <==>	Object	Equa tive Coeffic 999999 999999 999999	otion: [e] : ca2 <==>	
78 79 80 81	EX_cynt(e) EX_cys_D(e) EX_cys_L(e) EX_cytd(e)	ation: EX_ca2(e) LB: -999999 UB: 999999 Submit [e] : cynt <==> [e] : cys-D <==> [e] : cys-L <==> [e] : cytd <==>	Object Object Cancel 0 0 0 0 0 0 0 0	Equa tive Coeffic 999999 999999 999999 999999	tion: [e] : ca2 <==> cient: 0	
78 79 80 81 82	Abbrevia EX_cynt(e) EX_cys_D(e) EX_cys_L(e) EX_cytd(e) EX_dad_2(e)	ation: EX_ca2(e) LB: -999999 UB: 999999 Submit [e] : cynt <==> [e] : cys-D <==> [e] : cys-L <==> [e] : cytd <==> [e] : cytd <==> [e] : dad-2 <==>	Cancel Cancel Co	Equa ctive Coeffic 999999 999999 999999 999999 999999 9999	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	
78 79 80 81 82 83	EX_cynt(e) EX_cys_D(e) EX_cys_L(e) EX_cytd(e) EX_dad_2(e) EX_dad_2(e) EX_damp(e)	ation: EX_ca2(e) LB: 999999 UB: 999999 Submit [e] : cynt <==> [e] : cys-D <==> [e] : cys-L <==> [e] : cytd <==> [e] : dad-2 <==> [e] : damp <==>	Cancel Cancel Cancel Co	Equa stive Coeffic 999999 999999 999999 999999 999999 9999	ation: [e] : ca2 <==> cient: 0 0 0 0 0 0 0 0 0	
78 79 80 81 82 83 84	EX_cynt(e) EX_cys_D(e) EX_cys_L(e) EX_cytd(e) EX_dad_2(e) EX_damp(e) EX_dca(e)	ation: EX_ca2(e) LB: 999999 UB: 999999 Submit [e] : cynt <==> [e] : cys-D <==> [e] : cys-L <==> [e] : cytd <==> [e] : dad-2 <==> [e] : damp <==> [e] : daca <==>	Cancel Cancel Co	Equa tive Coeffic 999999 999999 999999 999999 999999 9999	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	
78 79 80 81 82 83 83 84 85	Abbrevia EX_cynt(e) EX_cys_D(e) EX_cys_L(e) EX_cytd(e) EX_dad_2(e) EX_dad_2(e) EX_damp(e) EX_dca(e) EX_dca(e)	ation: EX_ca2(e) LB: -9999999 UB: 9999999 Submit [e]: cynt <==> [e]: cys-L <==> [e]: cytd <==> [e]: cda-2 <==> [e]: damp <==> [e]: damp <==> [e]: dca <==> [e]: dcmp <==>	Cancel Cancel Cancel C Cancel C C C C C C C C C C C C C C C C C C C	Equa tive Coeffic 999999 999999 999999 999999 999999 9999	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	
78 79 80 81 82 83 84 85 86	Abbrevi EX_cynt(e) EX_cys_D(e) EX_cys_L(e) EX_cytd(e) EX_dad_2(e) EX_damp(e) EX_dca(e) EX_dcmp(e) EX_dcmp(e) EX_dcmp(e) EX_dcmp(e) EX_dcmp(e)	ation: EX_ca2(e) LB: -9999999 UB: 9999999 Submit [e]: cynt <==> [e]: cys-L <==> [e]: cys-L <==> [e]: cytd <==> [e]: dad-2 <==> [e]: dad-2 <==> [e]: damp <==> [e]: dcmp <==> [e]: dcmp <==> [e]: dcmp <==>	Cancel Ca	Equa tive Coeffic 9999999 999999 999999 999999 999999 9999	on: [e] : ca2 <==> cient: 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	

You can freely export or save a metabolic model in SBML format or in spreadsheet format by right clicking on a model to <u>Export model</u> (.xml) or to <u>Download model (.xls)</u>. 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

D	atabase	
	Please choose a home directory: D:\dist	Browse.
	Please create/open a project: MyBioSysDB_BiGG.GPR	
	☑ Gene-protein-reaction associations are necessary.	
	Create Cancel	

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

Reconstrucion Simulation Visualiza	tion Setting	ì							
🗂 Project	Gen	e Index 🗵	Protein	n Index 🗵	Rea	ction Index 🗵			
Y Model databases		Abbrev	riation	Confide	nce	Equation	Name	Note	
P [AF1260_GPR.XIS]	Š 0	ALAR				[c] : ala-L <==> al.	alanine racemase		
— 🗋 Gene Index	÷ 1	ALATA_L				[c]; akg + ala-L <	. L-alanine transa		
– 🗋 Protein Index	2	ASNN		[[c] : asn-L + h2o	L-asparaginase		
- B Reaction Index	3	ASNNpp		[[p] : asn-L + h2o	L-asparaginase		
	4	ASNS1				[c] : asp-L + atp +.	. asparagine synt		
	5	ASNS2				[c]: asp-L + atp +.	asparagine synt		
 Metabolite Index 	6	ASPT				[c] ; asp-L> fu	L-aspartase		
— 🗋 Protein Index	8 7	ASPTA				[c] : akg + asp-L	aspartate transa		
Reaction Index	8	DAAD				[c] : ala-D + fad + .	D-Amino acid de		
	a	VPAMT				Icl: 3moh + ala-l	Valina-nuruvata a		

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

<u>a</u>					
Reconstrucion Simulation Visualiza	tion Setting				
🗂 Project	Gene Ind	ex 🗵 🛛 Protein Ind	ex 🗵 🛛 Reaction Index 🗵	Protein Index 🗵	Metabolite Index 🗵 📊 🖡
♀ ☐ Model databases		ABBREVIATION	EQUATION	KEGGID	NAME
P I IAF1260_GPR.XIS	0 10	FTHF5GLUti	10fthf5glu[c]> 10fthf5		5-glutamyl-10FTHF tr
— 🗋 Gene Index	1 10	FTHF5GLUtm	10fthf5glu[m]> 10fthf5		5-glutamyl-10FTHF tr
— 📉 Protein Index	2 10	FTHF6GLUti	10fthf6glu[c]> 10fthf6		6-glutamyl-10FTHF tr
Beaction Index	3 10	FTHF6GLUtm	10fthf6glu[m]> 10fthf6		6-glutamyl-10FTHF tr
	4 10	FTHF7GLUti	10fthf7glu[c]> 10fthf7		7-glutamyl-10FTHF tr
	5 10	FTHF7GLUtm	10fthf7glu[m]> 10fthf7		7-glutamyl-10FTHF tr
 Metabolite Index 	6 10	FTHFtl	10fthf[c] <==> 10fthf[l]		10-Formyltetrahydrofd
— 🗋 Protein Index	7 10	FTHFtm	10fthf[c] <==> 10fthf[m]		10-Formyltetrahydrofd
- 🗋 Reaction Index	8 11/	DOCRTSLtm	11docrtsl[c] <==> 11do		11-deoxycortisol intra
	9 11/	DOCRTSLtr	11docrtsi[c] <==> 11do		11-deoxycortisol intra

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 <u>Insert</u>. 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.

4						
Reconstrucion Simulation Visualizati	ion Setting					
Project	Reaction Index 🗵	Protein Index 🗵	Metabolite Index 🗵	Reaction Index 🗵		4
Model databases	Abbrevia	tion Confidence	e Equation	Name	Note	
	0 ALAR	19	[c]∶ala-L <==> al	alanine racemase		
	1 ALATA_L		[c]∶akg + ala-L <	L-alanine transa	-	
🗕 🗋 Protein Index	2 ASNN		[c] : asn-L + h2o	. L-asparaginase		
- C Reaction Index	3 ASNNpp		[p] : asn-L + h2o	L-asparaginase		
a CE Poforonco databaco	4 ASNS1		[c] : asp-L + atp +	. asparagine synt		
	5 ASNS2		[c] ; asp-L + atp +	. asparagine synt		
- 🗋 Metabolite Index	6 ASPT		[c] : asp-L> fu	L-aspartase		
— 🗋 Protein Index	7 ASPTA		[c] : akg + asp-L	aspartate transa		
Reaction Index	8 DAAD	Search	[c] : ala-D + fad +	.D-Amino acid de		
	9 VPAMT	Iludate	[c] : 3mob + ala-L	.Valine-pyruvate a		
	10 2DGLCNRx	Update	[c] : 2dhglcn + h	2-dehydro-D-glu		
	11 2DGLCNRy	Insert	[c] : 2dhglcn + h	2-dehydro-D-glu		
	12 2DGULRx	Doloto	[c] : 2dhguin + h	2-dehydro-L-gulo		
	13 2DGULRV	Delete	[c] : 2dhquln + h	2-dehydro-L-gulo		
						-1

Insert				X
?	Click Submit to insert this Abbreviation: Bio Note: Protein:	s record, or Cancel mass_Ecoli_core Submit Cancel	Confidence:	

<u>s</u>								
Reconstrucion Simulation Visualization	Setting	i						
Project	Rea	ction Index 🗵	Protein Index 🗵	Metaboli	te Index 🗵	Reaction Index 🗵		a
Model databases		Abbreviatio	n Equat	ion	1.	Name	Confidence	
P III IAF1260_GPR.XIS	0	ALAR	[c] : ala-L <==>	ala-D	alanine race	mase		
— 🗋 Gene Index	1	ALATA_L	[c] : akg + ala-L	<==> glu	L-alanine tra	ansaminase		
- 🗋 Protein Index	2	ASNN	[c] : asn-L + h2	o> asp	L-asparagin	ase		
- Reaction Index	3	ASNNpp	[p] : asn-L + h2	o> asp	L-asparagin	ase		
C Deference detebace	4	ASNS1	[c] : asp-L + atp) + gln-L +	asparagine	synthase (glutam		
	5	ASNS2	[c] : asp-L + atp) + nh4	asparagine	synthetase		
 Metabolite Index 	6	ASPT	[c] : asp-L> fu	um + nh4	L-aspartase			
— 🗋 Protein Index	7	Biomass_Eco	li [c] : (1.496) 3pg) + (3.747	core E. coli I	piomass equatio		
Reaction Index	8	ASPTA	[c] : akg + asp-	_ <==> gl	aspartate tra	insaminase		
	9	DAAD	[c] : ala-D + fad	+ h2o>	D-Amino aci	d dehydrogenase		

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

<u>≗</u>				
Reconstrucion Simulation Visualizat	ion Setting			
🗂 Project	Reaction Index 🗵 🎽 Protein Index 🖂 🕇 Metabo	olite Index 🗵	Reaction Index 🗵	4
P- □ Model databases P- □ iAF1260_GPR.xls	ABBREVIATION 1221 Biomass Ecoli core N (w/ GAM)-Nmet2	[c] : (1.4	EQUATION 96) 3pg + (3.7478) accoa + (59.81)	
- 🗋 Gene Index - 🗋 Protein Index	1222 biomass_SA_2a 1223 biomass_SA_2b	Search	2) 12dgr_EC + (1.27) 26dap-LL + (0 2) 12dgr_EC + (1.27) 26dap-LL + (0	
Reaction Index	1224 biomass_SA_3a	Update i	2) 12dgr_EC + (1.27) 26dap-LL + (0	
	1225 biomass_6A_30 1226 biomass_8A_4a	Insert	2) 12dgr_SA + (1.27) 26dap-LL + (0 2) 12dgr_SA + (1.27) 26dap-LL + (0	-3
	1227 biomass_SA_5a	Delete	2) 12dgr_SA + (1.27) 26dap-LL + (0	

The reaction of Ec_biomass_iAF1260_core_59p81M can be added into the reference database. Abbreviation: Ec_biomass_iAF1260_core_59p81M Equation: $\begin{array}{l} (0.000223) \ 10fthf[c] + (0.000223) \ 20hph[c] + (0.5137) \ ala-L[c] + (0.000223) \ amet[c] + (0.2958) \ arg-L[c] + (0.2411) \ asn-L[c] + (0.2411) \ asn-L[c] + (0.2411) \ asn-L[c] + (0.004737) \ ca2[c] + (0.004737) \ cl2[c] + (0.000576) \ coa[c] + (0.003158) \ cobalt2[c] + (0.1335) \ ctp[c] + (0.003158) \ cu2[c] + (0.09158) \ cys-L[c] + (0.02617) \ dtp[c] + (0.02702) \ dtp[c] + (0.02702) \ dtp[c] + (0.02617) \ dttp[c] + (0.000223) \ fad[c] + (0.007106) \ fe2[c] + (0.007106) \ fe3[c] + (0.2632) \ gln-L[c] + (0.2632) \ glu-L[c] + (0.6126) \ gly[c] + (0.2151) \ gtp[c] + (54.462) \ h20[c] + (0.09474) \ his-L[c] + (0.2905) \ ile-L[c] + (0.1776) \ k[c] + (0.01945) \ kdo2lipid4[e] + (0.4505) \ leu-L[c] + (0.3432) \ lys-L[c] + (0.1537) \ met-L[c] + (0.007895) \ mg2[c] + (0.000223) \ mlthf[c] + (0.003158) \ mn2[c] + (0.003158) \ mobd[c] + (0.01389) \ murein5px4p[p] + (0.001831) \ nad[c] + (0.000447) \ nadp[c] + (0.011843) \ nh4[c] + (0.04148) \ pe160[p] + (0.02233) \ pe160[c] + (0.02632) \ pe161[c] + (0.04889) \ pe161[p] + (0.1759) \ phe-L[c] + (0.000223) \ pheme[c] + (0.2211) \ pro-L[c] + (0.000223) \ pydx5p[c] + (0.000223) \ ribflv[c] + (0.2158) \ ser-L[c] + (0.000223) \ sheme[c] + (0.003948) \ so4[c] + (0.000223) \ ribflv[c] + (0.000223) \ ribflv[c] + (0.0000253) \ sheme[c] + (0.0003948) \ so4[c] + (0.000223) \ ribflv[c] + (0.000223) \ ribflv[c] + (0.000055) \ udcpdp[c] + (0.1441) \ utp[c] + (0.4232) \ val-L[c] + (0.003158) \ zn2[c] --> (59.81) \ adp[c] + (59.801) \ h[c] + (59.806) \ pi[c] + (0.7739) \ ppi[c] \end{array}$

4									X
Reconstrucion Simulation V	isualizat	ion Setting							
Project	Reac	tion Index 🗵	Protein Index 🖂	Metabolite	Index 🗵	Reaction Index 🗵			n d
Model databases		Į.	ABBREVIATION		1	EQUATION		KEGGID	
P AF1260_GPR.XIS	1221	Biomass_Eco	oli_core_N (w/ GAM)-	Nmet2	[c] : (1.496) 3pg + (3.7478) accoa + (59.81)			10000100000000	
- D Gene Index	1222	Ec_biomass_	iAF1260_core_59p8	1 M	(0.000223) 10fthf[c] + (0.000223) 20hph[c] +				
— 🗋 Protein Index	1223	biomass_SA_	_2a		[c]: (0.42)				
Beaction Index	1224	biomass_SA_	_2b		[c]: (0.42)	12dgr_EC + (1.27) 26	dap-LL + (0		
	1225	biomass_SA_	_3a		[c]: (0.42)	dap-LL + (0			
	1226	biomass_SA_	_3b		[c]: (0.42)) 12dgr_EC + (1.27) 26	dap-LL + (0		
- Metabolite Index	1227	biomass_SA_	_4a		[c]: (0.42)) 12dgr_SA + (1.27) 26(dap-LL + (0		
— 🗋 Protein Index	1228	biomass_SA_	5a		[c]: (0.42)	dap-LL + (0			
- C Reaction Index	1229	biomass_SA_	6a		[c]: (0.42)	12dgr_SA + (1.27) 260	dap-LL + (0		
	1230	biomass_SA_	_6b		[c]: (0.42)	12dgr_SA + (1.27) 260	lap-LL + (0		

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



ualization Setting
Protein Index 🗵 🕇 Metabolite
ABBREV
a reconstruction
environmental conditions
t model (.xml) oad model (.xls) eaction by a map 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.

Reconstrucion Simulation Visualization	in Set	ting					6.7°		
Project	Prot	ein Index 🗵	Met	abolite Index 🗵 🎽	Reaction Index 🗵	Flux result 🗵	Gene Index 🗵	Prot	
♀ ☐ Model databases ♀ ☐ iAF1260_GPR.xls	0	Abbreviat	ion	Equation	Name (R)-Propage-1 2-	Protein OmnC PhoE 0	Note Draft from iAE126	0.6	
- Cene Index	1	12PPDStex	henn	12ppd-S[e] <==>	(S)-Propane-1,2	OmpN, PhoE, O	Draft from iAF126	0_G	
- C Reaction Index	3	14GLUCANt	exi	14glucan[e]> 1	. 1,4-alpha-D-gluc	LamB	Draft from iAF126	0_G	
Exchange Reaction	4	23CAMPlex 23CCMPtex		23ccmp[e] <==>	. 23cAMP transpor	OmpC, PhoE, O OmpC, OmpN, P	Draft from iAF126	0_G	
	б 7	23CGMPtex 23CUMPtex		23cgmp[e] <==> 23cump[e] <==>	. 23cGMP transpo 23cUMP transpo	PhoE, OmpC, O OmpN, PhoE, O	Draft from IAF126 Draft from IAF126	J_G 0_G	
P C NC_003197.gbk-blast.xls	8	23DAPPAtex 23PDE2pp		23dappa[e] <==> [p] : 23cump + h2.	. 2,3-diaminopropi 2',3'-cyclic-nucle	OmpC, PhoE, O CpdB	Draft from iAF126 Draft from iAF126	0_G 0_G	
- D Protein Index	10	23PDE4pp 23PDE7pp		[p] : 23ccmp + h2 [p] : 23camp + h2	. 2',3'-cyclic-nucle 2',3'-cyclic-nucle	CpdB CpdB	Draft from iAF126 Draft from iAF126	0_G 0_G	
Reaction Index Invalid Reaction Index	12 13	23PDE9pp 26DAHtex		[p] : 23cgmp + h2 26dap-M[e] <==>	2',3'-cyclic-nucle meso-2,6-Diami	CpdB OmpC, OmpN, P	Draft from iAF126 Draft from iAF126	0_G 0 G	
	4.4	200004200		2ddoog2phal s	2 foul on alugara	LAT	Dyoff from 10 E4 28	0.0	

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.

Insert		
?	Click Submit to insert this record, or Ca	mcel Gene: STM2024
	Name: Other:	Note: synthesize cobalamin
	Submit	Cancel

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

<u>.</u>								×
Reconstrucion Simulation Visua	lization	Setting						
🗂 Project 📃		Gene Index 🗵 🎽	Gene Index 🗵	Protein Index 🗵	Protein Index 🗵	Gene Index 🗵	Reaction In	1
- C Model databases		5'Coordinate	Gene	Locus Tag	Protein	Note	EC Number	
P AF1260_GPR.XIS	1937	2097225	entK	STM2015				
- 🗋 Gene Index	1938	2098373	cobT	STM2016	CobT		2.4.2.21	1
— 🗋 Protein Index	1939	2099143	cobS	STM2017	CobS		2	
- Reaction Index	1940	2099685	cobU	STM2018	CobU		?	1
	1941	2101202	cbiP	STM2019				
	1942	2102014	cbiO	STM2020				1
— 🗋 _InnerCell Reaction	1943	2102700	cbiQ	STM2021				1
— 🗋 _Metabolite	1944	2102968	cbiN	STM2022				
• 🗂 NC 003197.gbk-blast	1945	2103617	cbiM	STM2023				1
Gene Index	1946	2104417	cbiL	STM2024	CbiL		2.1.1.130	
	1947	2105208	cbiK	STM2025				

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.

2	Click Submit to insert this record, or Cancel		
•	Abbreviation: CPC2MT	Confidence: 3	
	Note: synthesize cobalamin	Other:	
	Protein: CbiL		

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

Reconstrucion Simulation Visualization Setting										
Project	X F	leaction Index 🗵 🎽	Metabolite Index 🗵 🎽 Reaction Index 🗵 🎽 Protein Ind		ex 🗵 🛛 Reaction Index 🗵 🗃 👘					
• C Model databases		Abbreviation	Equation	Name	Protein	Note	Confide 🔺			
P AF1260_GPR.XIS	0	12PPDRtex	12ppd-R[e] <==> (R)-Propane-1,2	OmpC, PhoE, O	Draft from iAF12				
— 🗋 Gene Index	1	12PPDStex	12ppd-S[e] <==> ((S)-Propane-1,2	OmpN, PhoE, O	Draft from iAF12				
— 🗋 Protein Index	2	14GLUCANabcpp	14glucan[p] + atp 1	l,4-alpha-D-gluc	MalG+MalE+Mal	Draft from iAF12				
- 🗋 Reaction Index	3	14GLUCANtexi	14glucan[e]> 1 /	1,4-alpha-D-gluc	LamB	Draft from iAF12				
	4	23CAMPtex	23camp[e] <==> 2	23cAMP transpor	OmpC, PhoE, O	Draft from iAF12				
	5	23CCMPtex	23ccmp[e] <==> 2	23cCMP transpo	OmpC, OmpN, P	Draft from iAF12				
- 🗋 _InnerCell Reaction	6	23CGMPtex	23cgmp[e] <==> 2	23cGMP transpo	PhoE, OmpC, O	Draft from iAF12				
🗕 🗋 _Metabolite	7	23CUMPtex	23cump[e] <==> 2	23cUMP transpo	OmpN, PhoE, O	Draft from iAF12				
• 🗂 NC 003197.gbk-blast.	8	23DAPPAtex	23dappa[e] <==> 3	2,3-diaminopropi	OmpC, PhoE, O	Draft from iAF12				
Gene Index	9	23PDE2pp	[p] : 23cump + h2 :	2',3'-cyclic-nucle	CpdB	Draft from iAF12				
	10	23PDE4pp	[p] : 23ccmp + h2 1	2',3'-cyclic-nucle	CpdB	Draft from iAF12				
- D Protein Index	11	23PDE7pp	[p] : 23camp + h2 :	2',3'-cyclic-nucle	CpdB	Draft from iAF12				
— 🗋 Reaction Index 📃	12	23PDE9pp	[p] : 23cgmp + h2 :	2',3'-cyclic-nucle	CpdB	Draft from iAF12				
Invalid Reaction In	13	26DAHtex	26dap-M[e] <==> 1	meso-2,6-Diami	OmpC, OmpN, P	Draft from iAF12				
• FILLIZZE GPR xis	14	CPC2MT	[c] : amet + copre [precorrin-2 C20	ObiL	Added to synthes	3			
	15	2AGPA120tipp	2ddecg3p(p)>	z-Acyl-sn-glycero	LpIT	Draft from iAF12				

Likewise, the protein-reaction association will be automatically brought into the Reaction Index table.
- Project	5V					
		Reaction index 🗵 M	etabolite index 🗵 🛛 React	tion index 🗵 🔤 Protein ind	Reaction index	
		Abbreviation	Gene	Reaction	Name	
P AF1260_GPRXIS	0	Aas	STM3010	AACPS1,AACPS2,AACP	. acyl-ACp synthase	Draft
— 🗋 Gene Index	1	Acc	STM0232+STM2366+S	ACCOAC	AcetylCoA carboxylase	Draft
– 🗋 Protein Index	2	AceA	STM4184	ICL	isocitrate lyase	Draft
- Beaction Index	3	AceB	STM4183	MALS	malate synthase A	Draft
	4	AceEec	STM0152	PDH	Pyruvate Dehdrogenas	Draft
	5	AceFec	STM0153	PDH	Pyruvate dehydrogenas	Draft
— 🗋 _InnerCell Reaction	6	AckA	STM2337	ACKr	Acetate kinase	Draft
- Metabolite	7	AcnA	STM1712	ACONTa,ACONTb	aconitase A	Draft
CINC 003197 gbk-blast	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
Protein Index	11	CbiL	STM2024	CPC2MT	precorrin-2 C20-methylt	Adde
🗕 🗋 Reaction Index 🛛 🛓	12	2 AcpS	STM2577	ACPS1	Holo-acp synthase	Draft
			OTHOROO	40004		5

Simulation

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

To download GNU Linear Programming Kit (GLPK). <u>http://sourceforge.net/projects/winglpk/</u> (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 <u>Simulation</u> in the menu bar to choose which analysis you want to perform.

<u></u>			
Reconstrucion	Simulation	Visualization Setting	
Project P C Model dat P C SBML O Ge C Re C L L L L L L L L L L L L L	Dead-end M Objective O Flux Variabi Robustness Essentiality Gene Deleti	letabolite Identification ptimization ility Analysis s Analysis Analysis on Analysis	

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_Models and http://sb.nhri.org.tw/GEMSiRV/en/Metabolic_Models and 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.

Reconstrucion	Simulation	Visualization	Setting
Project	Dead-end M	letabolite Identi	fication
Model dat Carl SBML Carl SBML	Objective O Flux Variabi Robustness Essentiality Gene Deleti	ptimization ility Analysis s Analysis Analysis on Analysis	1128
Please select a SBML_export	a model from _E.coli textbo	the Model data	ibases:
Please select a SBML_export Please select a	a model from _E.coli textbo a map in the l	the Model data ok_out.xml Maps if you hav	ibases: e any:
Please select a SBML_export Please select a E.coli_core.ca	a model from _E.coli textbo a map in the l rtomap	the Model data ok_out.xml Maps if you hav	ibases: e any:
Please select a SBML_export Please select a E.coli_core.ca	a model from _E.coli textbo a map in the l Irtomap	the Model data ok_out.xml Maps if you hav	ibases: e any:

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

A list for dead-end metabolites:

<u>솔</u>				
Reconstrucion Simulation V	isualization Setting			
Project	E.coli_core.cartomap 🗵	Dead-end list 🗵		
🕈 🔚 Model databases	The dead-end metabolites are listed below:			
SBML_export_E.coli	fruíal			
— 🗋 Gene Index	fumfel			
📃 📄 Reaction Index	ala-l [o]			
- 🗋 _Exchange Read	mal-l [e]			
InnerCell Read				

A visualization map with dead-end metabolites:



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.



<u>4</u>		X
Please select a model f	rom the Model databases: xtbook_out.xml	•
Please select a map in t	the Maps if you have any:	
E.coli_core.cartomap		•
To () maximize or () the objective reaction:	minimize Biomass Ecoli core N(w/GAM) Nmet2	-
🔲 Output nonzero flux	es only	
	Execute Simulation Close	

A visualization map with reaction fluxes:



Flux result:

E.coli_cor	re.cartomap 🗵	Flux result 🗵
save		
GLNabc	0.0	
GLUDy	-4.54186	
GLUN	0.0	
GLUSy	0.0	
GLUt2r	0.0	
GND	4.95998	
H2Ot	-29.1758	
ICDHyr	6.00725	
ICL	0.0	
LDH_D	0.0	
MALS	0.0	
MALt2_2	0.0	
мпц	6 06 M 20	

To check the checkbox for outputting nonzero fluxes only.

<u>\$</u>		
Please select a model f	rom the Model databases:	
SBML_export_E.coli te	ktbook_out.xml 👻	
Please select a map in	the Maps if you have any:	
E.coli_core.cartomap	•	
To (e) maximize or () the objective reaction:	minimize Biomass_Ecoli_core_N(w/GAM)_Nmet2 💌	
Output nonzero flux	es only	
	Execute Simulation Close	
	Execute Simulation Close	



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 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
✓ 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



A list for the blocked reactions:

lization Setting

E.coli_core.cartomap 🗵 🛛 Blocked reaction list 🗵

The blocked reactions are listed below: EX_fru(e) EX_fum(e)

EX_gln_L(e)

EX_mal_L(e)

FRUpts2 FUMt2_2

GLNabc MALt2_2

MALIZ_Z

Flux variability result:

E.coli_core.c	artomap 🖂 🛛 E	Blocked reaction	i list 🗵 👔	Flux variability result 🗵
save				
#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_Ecol	i_core_N(w/GAM)_Nmet2	0.0	0.873921507
CO2t	-60.0	11.10424242		
CS	1.241908217E-	-28	20.0	
СҮТВД	0.0	120.0		
D 1 AC42	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.

iease select a model fi	om the Model databases:	
SBML_export_E.coli tex	tbook_out.xml	
o 💿 maximize or 🔾	minimize	
ne objective reaction:	Biomass_Ecoli_core_N(w/GAM)_Nmet2	
o see the robustness to	reaction:	
X_glc(e)	In ○ Min and Max or ● -20 and	0
nd reaction (optional):		
	in Min and Max or 1000 and	1000
	- 123	
lumber of points space	l in the flux range 20	
		232
	Execute Simulation Close	se

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.

Please select a model from the Model databases:	
SBML_export_E.coli textbook_out.xml	•
To 💿 maximize or 🔾 minimize	
the objective reaction: Biomass_Ecoli_core_N(w/GAM)_Nmet2	
To see the robustness to reaction:	and
IN O Min and Max or e 23	
and reaction (optional):	
in Min and Max or 10	00 and 1000
Number of points spaced in the flux range 20	
Execute Simulation	Close

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.

Discon colori a model from the Medal date	kanner
Please select a model nom the Model data	uases:
SBML_export_E.coli textbook_out.xml	
To 💿 maximize or 🔾 minimize	
the objective reaction: Biomass_Ecoli_co	ore_N(w/GAM)_Nmet2
To see the robustness to reaction:	
EX_02(e)	in 🔾 Min and Max or 🖲 -20 and 0
and reaction (optional):	
EX_glc(e) vin	○ Min and Max or ● [-20] and [0]
Number of points enseed in the flux range.	20.*
number of points spaced in the nux range	20.
	Execute Simulation Close

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.

4		
Reconstrucion	Simulation	Visualization Setting
Project P I Model dat P I SBML, P I SBML,	Dead-end M Objective O Flux Variabi Robustness Essentiality Gene Deleti	letabolite Identification ptimization ility Analysis Analysis Analysis on Analysis

e Model databases: _out.xml	•
_out.xml	-
ize	
120	
ss_Ecoli_core_N(w/GAM)_Nmet2	-
sis	
value : 0.01 of the rate ratio.	
Execute Simulation Close	e
	iss_Ecoli_core_N(w/GAM)_Nmet2 sis value : 0.01 of the rate ratio. Execute Simulation Clos

Results of gene essentiality analysis:

E.coli_core	e.cartomap 🗵	Essential gene result 🗵
Computation	nally essential ge	nes:
b0451		
b0720		
b1136		
b1779		
b2415		
b2416		
b2779		
b2926		
KO gene	Reaction	Rate ratio
b0114	[R_PDH]	0.9116332748634368
b0115	[R_PDH]	0.9116332748634368
b0116	[R_AKGDH, F	R_PDH] 0.895218
b0474	[R_ADK1]	1.0
b0721	[R_SUCDi]	0.9317741936519247
b0722	[R_SUCDi]	0.9317741936519247
b0723	[R_SUCDi]	0.9317741936519247
b0724	[R_SUCDi]	0.9317741936519247
b0726	[R_AKGDH]	0.9821332935796487
b0727	[R_AKGDH]	0.9821332935796487
b0728	[R_SUCOAS]	0.9821332935796487
b0729	[R_SUCOAS]	0.9821332935796487
b0767	[R_PGL]	0.9884335178628347
b0809	[R_GLNabc]	1.0
b0810	[R_GLNabc]	1.0

Results of reaction essentiality analysis:

E.coli_core.c	artomap 🖂	Essential reaction result 🗵
Computational	ly essential re	actions:
ACONTa		
ACONTE		
Biomass_Ecol	i_core_N(w/G/	AM)_Nmet2
cs		
ENO		
EX_glc(e)		
EX_h(e)		
EX_nh4(e)		
EX_pi(e)		
GAPD		
GLCpts		
GLNS		
ICDHyr		
NH4t		
PGK		
PGM		
Plt2r		
RPI		
VO reaction	Poto rotio	
	Nale Ialio 1 0	
ACALD	1.0	
ACK	1.0	
ACt2r	1.0	
	1.0	
AKGDH	0.982133293	35796487
1.0011	0.002100200	/0100401

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:

<u>4</u>			
Please select a model fro	om the Model databases:		
SBML_export_E.coli text	book_out.xml	-	
To 💿 maximize or 🔾 r	ninimize		
the objective reaction:	Biomass_Ecoli_core_N(w/GAM)_Nmet2	-	
To perform:			
Single Gene Deletion:	b	-	
○ Gene Deletion:	b0008	-	Browse
	b0114		DIGWSC
	b0115		
To export the model in	b0116		
	b0118		
	b0351		Close
	b0356		
	b0451	-	

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.

SBML_export_E.coli te	xtbook_out.xml	•	
ſo 💿 maximize or 🧲) minimize		
he objective reaction:	Biomass_Ecoli_core_N(w/GAM)_Nme	t2 💌	
lo perform:			
Single Gene Deletion	n: b0114		
🔾 Gene Deletion:		1	Browse
To export the mode	I in SBML format		

The flux result for the single gene deletion model:

Gene Deletion	n Flux 🗵 🛛 Gene Deletion Model 🗵
save	
#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_Ecol	i_core_N(w/GAM)_Nmet2 0.796696
CO2t	-18.3531
CS	4.75726
СҮТВД	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:

G	ene Deletion Flux 🗵 Gene Deletion Model 🗵
	save
x</td <td>ml version="1.0" encoding="UTF-8"?></td>	ml version="1.0" encoding="UTF-8"?>
≺sb	ml xmlns="http://www.sbml.org/sbml/level2" level="2" version="1" xmlns:html="http://www.w3.org/1999/xhtml" > 🛛 💻
<	model id="SBML_export_E.coli textbook_out.xml.xml" name="SBML_export_E.coli textbook_out.xml.xml" >
	stofUnitDefinitions >
	<unitdefinition id="mmol_per_gDW_per_hr"></unitdefinition>
	<listofunits></listofunits>
	<unit kind="mole" multiplier="1" offset="0" scale="-3"></unit>
	<unit exponent="-1" kind="gram" multiplier="1" offset="0"></unit>
	<unit exponent="-1" kind="second" multiplier="0.00027777" offset="0"></unit>
	stofCompartments >
	<compartment id="C_e" name="extracellular"></compartment>
	<compartment id="C_c" name="cytoplasm"></compartment>
	stofSpecies >
	<species compartment="C_c" id="M_13dpg_c" name="3-Phospho-D-glyceroyl phosphate"></species>
	<species compartment="C_c" id="M_2pg_c" name="D-Glycerate 2-phosphate"></species>
	<species compartment="C_c" id="M_3pg_c" name="3-Phospho-D-glycerate"></species>
	<species compartment="C_c" id="M_6pgc_c" name="6-Phospho-D-gluconate"></species>
	<species compartment="C_c" id="M_6pgl_c" name="6-phospho-D-glucono-1,5-lactone"></species>
	<species compartment="C_e" id="M_ac_e" name="Acetate"></species>
	<species compartment="C_c" id="M_ac_c" name="Acetate"></species>

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

Visualization

Metabolic map creation

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



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

<u>≗</u>	
Reconstrucion Simulation Visualization	Setting
🗂 Project	Configure
P→ I Model databases	Kegg Pathway Configure
- Gene Index	Flux Color Range Setting

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

Config	ulation	
?	Kegg Pathway List.	
-	rn00010.xml	-
	rn00010.xml	
	rn00020.xml	5

The KEGG pathway map of rn00010:


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 <u>Show node name</u> or <u>Show node caption</u> by right clicking on a map.

Content of KEGG pathway map (rn00010.xml):

× rn0	0010.xml
	0
1	xml version="1.0"?
2	pathway SYSTEM "http://www.genome.jp/kegg/xml/KGML_v0.7.1dtd"
3	Creation date: Nov 16, 2010 13:49:39 +0900 (GMT+09:00)
4 🗆	<pre><pathway <="" name="path:rn00010" number="00010" org="rn" pre=""></pathway></pre>
5	title="Glycolysis / Gluconeogenesis"
6	<pre>image="http://www.genome.jp/kegg/pathway/rn/rn00010.png"</pre>
7	<pre>link="http://www.genome.jp/kegg-bin/show_pathway?rn00010"></pre>
8 🗆	<pre><entry <="" id="13" name="rn:R01070 rp:RP01274 rp:RP01275 rc:RC00438 rc:RC00439" pre="" reaction="rn:R01070" type="reaction"></entry></pre>
9	<pre>link="http://www.kegg.jp/dbget-bin/www_bget?R01070+RP01274+RP01275+RC00438+RC00439"></pre>
10	<pre><graphics <="" bgcolor="#BFBFFF" fgcolor="#000000" name="R01070" pre=""></graphics></pre>
11	type="rectangle" x="483" y="404" width="46" height="17"/>
12	
13 🗆	<entry <="" id="37" name="rn:R00710 rp:RP00128 rc:RC00047" reaction="rn:R00710" th="" type="reaction"></entry>
14	<pre>link="http://www.kegg.jp/dbget-bin/www_bget?R00710+RP00128+RC00047"></pre>
15	<graphics <="" bgcolor="#BFBFFF" fgcolor="#000000" name="R00710" th=""></graphics>
16	type="rectangle" x="289" y="943" width="46" height="17"/>
17	

Content of KEGG pathway map (ec00010.xml):

ec0	0010.xml
	0, , , 10, , , , , , , , , , , , , , , ,
1	xml version="1.0"?
2	pathway SYSTEM "http://www.genome.jp/kegg/xml/KGML_v0.7.1dtd"
3	Creation date: Nov 16, 2010 13:49:39 +0900 (GMT+09:00)
4 -	<pre><pathway <="" name="path:ec00010" number="00010" org="ec" pre=""></pathway></pre>
5	title="Glycolysis / Gluconeogenesis"
6	<pre>image="http://www.genome.jp/kegg/pathway/ec/ec00010.png"</pre>
7	<pre>link="http://www.genome.jp/kegg-bin/show_pathway?ec00010"></pre>
80	<pre><entry <="" id="13" name="ec:4.1.2.13" pre="" reaction="rn:R01070" type="enzyme"></entry></pre>
9	link="http://www.kegg.jp/dbget-bin/www_bget?4.1.2.13">
10	<graphics <="" bgcolor="#BFBFFF" fgcolor="#000000" name="4.1.2.13" th=""></graphics>
11	type="rectangle" x="483" y="404" width="46" height="17"/>
12	
13 🗖	<pre><entry <="" id="37" name="ec:1.2.1.3" pre="" reaction="rn:R00710" type="enzyme"></entry></pre>
14	<pre>link="http://www.kegg.jp/dbget-bin/www_bget?1.2.1.3"></pre>
15	<graphics <="" bgcolor="#BFBFFF" fgcolor="#000000" name="1.2.1.3" td=""></graphics>
16	type="rectangle" x="289" y="943" width="46" height="17"/>
17	



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 <u>Extend map</u>.



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



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.



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



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



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 <u>Replace caption of nodes</u> 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 <u>http://sb.nhri.org.tw/GEMSiRV/en/Manual</u>.

	× me	et_KEGGtoSE	ED.TXT	× rx	n_KEGGtoSE	ED.TXT
[0	1,0,		Φ	1,0,
	1	016254	cpd00860	1	R00432	rxn00306
	2	CO0149	cpd00130	2	R07618	rxn01241
	з	C00002	cpd00002	3	R00001	rxn05757
	4	COOOO3	cpd00003	4	R00002	rxn11947
	5	C00004	cpd00004	5	R00004	rxn00001
	6	C00005	cpd00005	6	R00005	rxn00002
	7	COOOO6	cpd00006	7	R00008	rxn00004
	8	C00007	cpd00007	8	R00009	rxn00006
	9	C00008	cpd00008	9	R00010	rxn00007
	10	C00009	cpd00009	10	R00011	rxn00008
		000040	100040			

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



Please remember	er to save a map before closing it.
	Show node name
	Replace caption of nodes
	Filter reaction by a model
	Remove nodes (met) without edges
	Extract reaction information from a model
	Load reaction fluxes
	Load gene expressions
	Save map
	Close map
1 '	12

You can open a map saved in cartomap format.

Reconstrucion Simulation	Visualization	Setting				
Project	Create new map					
🕈 🚍 Model databases	Open map file (.cartomap)					
P SBML_export_E	Load KEGG maps					
	Load CellDesi	gner maps				

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.

Rxns not exist in the model	li internet i internet	Rxns exist in the model	
R00014rxn00011	A >>	rxn00175	
R00431 rn:R00726		- rxn00459	
R00754rxn00543		rxn00506	
R01061 rn:R01063		rxn00549	

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





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





You can also filter reactions in a metabolic model by comparing with a map. Right click on the model to <u>Filter reaction by a map</u> 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.

<mark></mark>	
Reconstrucion Simulation	Visualization Setting
Project P- 1 Model databases - 1 Opt62977 3 out yrr	rn00010.cartomap 🗵
Gene Index	Draft a reconstruction Add rxn&met to the Ref. DB Generate simulation tables Define environmental conditions Export model (.xml) Download model (.xls)
Reaction Index	Filter reaction by a map
e 🗖 Maps	Delete model

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

_	-	-
	1 mm	1.00
dans .		
10.00		

Reconstrucion	Simulation	Visualization	Setting	
acconstruction	Jimaladon	VISUAIIZACION	Security	

🗂 Project	rn00010.ca	artomap 🗵	Comparison Rep	ort 🗵 🕇 T	emporary Map 🗵]		
🛉 🔚 Model databases	The reaction	e matched in	hoth table and mar	n:		l)		
Copt62977.3_out.xml Gene Index	rxn02342	rxn00781 rxn01100	rxn00459 rxn01241	rxn0017 rxn0074	75 rxn00558 17 rxn00704	rxn00786	rxn00549	n
Comparison Index Comparison Index Comparison Index Comparison Index Comparison Index								-
	The reaction	s listed in the	table only:	10000000000		10000000000	10000000000	
- D _Metabolite	EX_cpd1141	6_c	bio00025	rxn0000)1 nxn00002	rxn00006	rxn00011	D
🕈 🚍 Reference database	rxn00062	rxn00077	rxn00083	rxn0008	35 nxn00095	nxn00097	rxn00100	୍ଷ
— 🗋 Metabolite Index	rxn00117	rxn00119	rxn00122	rxn0012	26 rxn00127	rxn00132	rxn00138	p
🗕 🗋 Reaction Index	rxn00146	rxn00147	rxn00159	rxn0016	31 nxn00166	rxn00173	rxn00178	p
• [¯] Maps	nxn00184	nxn00187	nxn00189	rxn0019	90 nxn00192	rxn00193	nxn00199	D
└── In m00010.cartomap	nxn00209	rxn00211	rxn00213	rxn0021	4 rxn00224	rxn00225	rxn00231	ಿರಿ
Tomporary Man	rxn00248	rxn00251	rxn00256	rxn0028	30 rxn00262	rxn00269	rxn00283	n
	rxn00291	rxn00292	rxn00293	rxn0029	95 rxn00297	rxn00299	rxn00300	p
	nxn00305	nxn00313	rxn00321	rxn0032	22 nxn00324	rxn00327	rxn00328	D
	nxn00338	rxn00340	rxn00342	rxn0034	16 rxn00347	rxn00350	rxn00351	ಂ
	nxn00379	rxn00392	rxn00405	rxn0040)7 rxn00409	rxn00410	rxn00412	n
	rxn00433	rxn00438	rxn00441	rxn0048	61 rxn00463	rxn00469	rxn00470	p
	nxn00503	rxn00508	rxn00509	rxn0051	2 rxn00514	rxn00515	rxn00527	p
	rxn00568	rxn00569	rxn00584	rxn0058	38 rxn00589	rxn00598	rxn00601	D
	rxn00615	rxn00616	rxn00623	nxn0064	10 rxn00646	rxn00649	rxn00650	n
	rxn00675	rxn00676	rxn00679	rxn0068	36 nxn00689	rxn00691	rxn00692	p
	nxn00710	pxn00711	rxn00714	rxn0072	26 nxn00727	rxn00737	nxn00758	p
	rxn00775	rxn00776	rxn00777	rxn0078	32 rxn00785	rxn00789	rxn00790	p
	rxn00800	rxn00802	rxn00806	_mn0083	nxn00832	rxn00834	rxn00836	p
	pxn00858	pxn00863	rxn00867	rxn008P	18 pxn00874	rxn00875	rxn00881	p
	mn00002	rvn00003	rvn00007	rvn0001	0 rvn00012	rvn00913	rvn00015	

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

teconstrucion Simulation Visualization Setting													
Project	rn000	10.carton	nap 🗵	Compar	ison Rep	ort 🗵	Tempor	ary Map [×				
Model databases P Opt62977.3_out xml	Ø		ABC			. 🗙	QT	emporary	Map fron	n reactior	ı filter		
- D Gene Index	1:03666	nan03012	ran03242	rxan03540	าหลา03990	na105153	ran05256	ran05333	าหลา05373	nan05413	17an05448	nan05516	rxar05594
- C Reaction Index	1xan03680	man03030	man03243	170103638	rxn03991	nan05171	nan05287	170105334	rxn05375	man05414	man05449	1xan05517	rxn05596
- D _Exchange Reaction	rxa102774	man03031	ran03244	ran03839	าหลา04070	man05172	ran05289	nan05335	าหลา05376	man05415	1xan05450	nan05526	rxa105599
- D _InnerCell Reaction	ran03783	man03052	man03245	17an03841	rxn04113	nan05176	nan05293	170105336	rxan05377	nan05417	man05451	1xan05527	ran05602
- Metabolite	rxa102789	na103060	ran03246	man03843	rxn04132	man05183	ran05294	nan05337	าหลา05379	man05418	man05452	nan05528	ra105603
P Reference database	rxan03791	nan03061	man03247	170103869	rxn04133	nan05195	nan05295	170105338	rxan05380	nan05419	man05453	1xax05533	ran05605
- Metabolite Index	rxa102792	man03062	1)an03248	ran03884	rxn04139	man05197	ran05296	ran05339	rxan05381	man05421	17an05454	man05534	rxax05613
Reaction Index	ran02804	na103068	man03349	170103887	rxan04308	nan05198	nan05297	17ar05340	rxan05383	nan05422	man05455	1xax05535	ran05614
P Maps	ran03811	man03075	ra103250	nan03891	rxan04456	na105199	ran05298	nan05341	rxan05384	man05423	17an05456	na105536	ra105616
	ran02834	man03080	man03384	170103892	rxan04457	na105200	na105299	17an05342	rxan05385	nan05425	man05457	1xax05537	ran05619
- D Temporary Map	ra103835	man03084	1×an03393	ran03893	rxan04674	man05201	ran05301	nan05343	rxan0.5386	man05426	17an05458	nan05538	ra105620
	ran02853	man03087	man03394	170103897	rxn04713	man05202	na105305	17ar05344	1xan0.5388	man05427	man05459	170105539	ran05631
	1:03866	man0310S	1×an03395	1×1103898	rxan04724	man05205	ra105306	ran05345	rxan05389	man05429	1xan05460	man05540	ra105625
	ran02889	man03130	ran03397	17an03901	rxan04745	nan05211	nan05312	17an05346	rxan05390	man05430	man05461	17an05541	1xan05637
	ran03895	na103135	1×an03406	rxn03903	rxn04748	man05215	ran05315	ran05347	rxn05392	man05431	1)an05462	ran05542	rxan05634
	ran02911	man03136	man03407	1%103904	rxn04750	nan05217	ran05316	17an05348	rxn05393	man05433	man05464	1xan05543	1xan0.5638
	rxan03914	man03137	12an03408	1×1103906	rxan04783	man05219	ran05317	ran05349	างลา05394	man05434	17an05465	man05544	11/21/05649
	ran02916	man03146	man03409	130103907	1xan04794	nan05221	nan05318	17an05350	rxn05396	nan05435	man05466	17an05545	ran05651
	ra103937	man03147	12an03419	1×1103908	rxan04954	man05339	ran05319	ran05358	างลา05397	man05436	1xan05467	ran05546	1xax05654
	ran02929	man03159	man03421	170103909	130104996	nan05231	na105322	17an05359	1xan05398	man05437	man05468	170105547	110105656
	ra103933	man03164	1)an03422	ran03910	ran05039	man05233	nan05323	ran05360	rxan05400	man05438	1)an05469	ran05552	1xax05663
	1xan02934	man03167	man03433	13an03916	rxan05039	man05239	man05324	13a105361	rxn05401	man05439	man05481	170105555	13005667
	rxn03937	nan03174	17an03435	ran03917	rxax05040	nan05243	man05325	man05363	rxn05402	na105440	17an05484	nav05560	ra105669

You can save the temporary map and add it into the map you are working with. Add a map by clicking <u>Add a map</u> 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.



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7	× '	$, \square$	170100002	man00161	170100260	mm00346	Tan00512	17an00686	17000831	17000941	170101145	170001302	170101504	17010168
1701007	81 /	1	170100006	170100166	170100262	170100347	170100514	170100689	17000832	130100947	17an01188	170101303	170101507	rmn0173
	∇ i	/	17an00011	17an00173	I7an00269	17a100350	ITAN00515	17000691	17an00834	17an00950	17an01192	170101313	man01509	17an0174
	₽ ^{qpd00203} / /		170100029	17an00178	170100283	max00351	170100527	170100692	170100836	17a100952	170101200	170101314	man01512	17an0174
			17a100048	17an00179	170100285	17a100363	17an00543	170100693	17a100838	170100966	170101204	17an01329	170101513	17an0179
2	man01100		17an00056	170100182	170100288	man00364	170100555	170100707	17an00839	130100973	ran01208	170101331	rxn01517	17an0179
	pd00169		17an00060	17an00183	17an00289	rxn00371	172100566	17an00708	17an00851	17an00974	170101210	17an01332	rxn01519	rxn0183
			170100062	17an00184	170100291	17x1\00379	174100568	rxn00710	170100858	170100979	17an01211	17an01333	rxn01520	rmn0184
4	man01106 /		17a100077	170100187	170100292	172100392	170100569	17xn00711	170100863	170100980	170101213	170101334	rxn01521	17xn0185
200	Trpd00482		17an00083	17an00189	170100293	ກຄ າ00405	170100584	170100714	17an00867	13an00985	rmn01218	170101351	rmn01522	rmn0185
	(/ /		17an00085	170100190	rman00295	170100407	170100588	17400726	170100868	170100986	170001219	170101352	rxn01523	17an0186
E.	/		170100095	170100192	170100297	max00409	170100589	rxax00727	170100874	170100990	170101225	170101353	man01537	ran0186
2	1200439		170100097	170100193	170100299	174100410	174100598	13000737	170100875	17000991	170101226	170101360	170101544	17an0187
	S [,]		ma00100	1100199	1300300	mm00412	1300001	13000758	1300881	1301000	man01255	13001302	11201545	natu187
	/		17200101	12000205	12000302	1200414	1200003	13200703	13000883	12001011	12001250	13001304	12001575	1200191
	į.		13000103	12000208	120100303	12000423	12000011	13000772	17000898	120001015	12001258	12001388	120001603	10192
			170100117	170100209	170100305	man00433	170100615	170100775	174100902	man01018	170101265	170101406	170101629	man0196
6	7		17000119	170100211	17an00313	170100438	170100616	17an00776	170100903	17an01019	170001268	170101434	17an01634	17an0196
	J.		170100122	rmn00213	17an00321	17a100441	າາສາ00623	rxn00777	17an00907	17an01021	170101269	17m01445	170101636	rxn0197
9	o ^{cp400020}	Q ^{qpd00159}	17an00126	17a100214	170100322	17an00461	170100640	1x1100782	170100910	170101069	17an01274	17an01451	170101637	17xn0197

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.





EX_qpd114	1 man00146	17000248	170100338	170
bio00025	17an00147	Show ext	tra info.	172
าวลา00001	man00159	Update c	170	
17an00002	man00161	Modify co	170	
170100006	170100166	Delete	170	
170100011	170100173	170100269	TM100350	170
rm100029	17a100178	17an00283	INI100351	170
17an00048	17an00179	17m00285	170100363	170
170100056	170100182	170100288	17/100364	170
174100060	170100183	170100289	rxax00371	170
rxn00062	170100184	17an00291	174100379	170
17an00077	17a100187	17an00292	170100392	170
170100083	170100189	170100293	17/100405	170
170100085	170100190	170100295	174100407	172
INANO0095	170100192	1700297	170100409	170
17000007	120000103	177000200	17000410	1

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

Gene: ACIAD0109 Equation: [c] : (2.0) qpd00109 + qpd00221 <==> qpd00020 + (2.0) qpd00067 + (2.0) qpd0011										
	EX_cpd114	17a100146	17a100248	17a100338	17a100503	170100675	170100800	171100931	rxax01117	17
E	bio00025	171100147	rmn00251	17an00340	17art00508	170100676	1700802	171100935	rxax01127	17
	17a100001	171100159	171100256	17a100342	170100509	170100679	170100806	171100938	17an01137	17
	170100002	170100161	170100260	170100346	17a100512	170100686	17a100831	17an00941	17an01145	17

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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 <u>Load reaction fluxes</u>.

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



#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 ACONTE 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:

?	Min/Max cutoff. Min:
	-10
	Max:
	10



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/ln_Silico_Organisms/E_coli/E_coli expression2.

Expr_o2 effect on Ecoli_rep.TXT

	Φ	1,0,		. 2,0,	3,0, , , , , , , , , , , , 4,0,				
1	#Gene	n0 n0	nO	nO Oxy Oxy	Оху				
2	b2836	5.7690	96757	5.879973845	5.889739712	5.867358643	5.760884085	5.873523604	5.957984412
3	b0885	3.5357	27661	3.469050784	4.049806246	3.645329898	3.879638515	3.597923384	3.593089884
4	b0199	9.8048	08142	10.40118062	10.57760507	10.25695657	9.301827015	9.898372638	10.09632395
5	b0715	2.5655	46658	2.567760685	2.536834823	2.513905138	3 2.533385516	2.555976111	2.552486193
6	b0185	6.6619	11901	6.094619316	7.696563672	6.963963632	7.476477903	7.20705541	7.645863323
7	b3255	12.241	20191	12.17555935	12.10939238	12.14068385	5 11.88957437	11.98381957	11.53553843
8	b3256	8.6262	16539	8.9348643 8	.754524155 9	.051725652 8	3.586089409 8	.749223826 8	.216142737
9	b2316	10.305	07868	10.30964272	10.70406165	10.96516547	10.13253158	10.50413996	9.648748283
10	b4015	9.4632	08813	9.972113349	8.9044422 9	.819722455 1	2.72892109 1	2.51954356 12	2.59735882
11	b4014	11.975	37857	12.07528508	11.84044276	11.72425199) 13.23488622	13.29639349	13.19317442
12	b0114	11.767	11824	11.58164936	11.88583332	11.57104461	12.49915551	12.55704041	12.55320524
13	b0115	11.020	53116	10.81358943	11.14457251	11.36213807	11.755529 1	2.04086088 12	2.18448931
14	b4016	2.9639	04777	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.



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.