InSilicoKO – A MATLAB Application for Metabolite Overproduction Strain Design

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	1 - 1	Select Model					4 - Define D	eletion Candidates			6 - Perfe	orm Manual Design			
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Example – Determine a set of gene knockouts to maximize the microaerobic synthesis of R-1,2-propanediol production in *Escherichia coli*

1. Select the metabolic model for the wild-type strain. Strain design optimization requires a LP solver with MILP capability.

2. Define the culture medium in terms of supplied nutrients and their maximum uptake rates. The nutrients can be extracted from the model, specified manually, or loaded from a saved file.

3. Define the secreted product to be maximized. FVA calculates ranges of nutrient uptake rates and product secretion rates of the wild-type strain.



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Model Selecte

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O2 O2 Phosph: Potassii Selenat Selenite Sodium

Save

Target

Wild-Ty

(R)-Prop Acetate

CO2 CO Ethanol Formate

4

File Cas Case P

4. Define the subsystems containing the desired reaction deletion candidates. Specific subsystem reactions selected by the optimizer can be excluded from subsequent designs.

5. Perform optimized strain design by specifying the number of deletions and allowable growth rate. FVA calculates the strain growth rate and product secretion range for the identified reaction deletions.

		InSilicoKO	- Knock					_	and October 1	A - 4 - 1 1			
			- KIIOCK	out Strai	n Design fo	r Metabolite Overpi	oduction using	g Geno	me-Scale I	vietabol	ic Models		
	1 -	Select Model				4 - Define Deletion Candidates							
Folder	C:/Users/micha/OneDrive/Documents/Consulting/ISF/Matlab/GSI				/GSM/BIGG	Subsystem List	Pentose Phospha	te Pathwa	ay				
d Model	(iML1515				▼)	Add Subsyst	em Re	move Su	bsystem	Ву	pass Optimization		
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ane-1,2-diol			0		0.1811	design4-25	0.0658	-	0.0000		9.3059		
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roduct	Panel 3 Status	Target Product	Set			6-phosphogluconate	e denydratase	EDE		Pento	se Phosphate Pathy		
	0	Management				D lactate debudrance	arogenase (acetylatii	ig) ACA		Pyruva			
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Folder						Triose phosphate is	omeraça			Glycol	vsis/Gluconeogenes		
Folder Name				Sa	ve Case	Triose-phosphate is	omerase	TPI		Glycol	ysis/Gluconeogenes		

Metabolite Overproduction using Genome-Scale Metabolic Models 4 - Define Deletion Candidates • Subsystem List Pentose Phosphate Pathway Add Subsystem **Remove Subsystem** Reactions Included Candidate Subsystem 8 Anaplerotic Reactions Citric Acid Cycle 14 23 Glycolysis/Gluconeogenesis Oxidative Phosphorylation 52 12 Pentose Phosphate Pathway Disallowed Reaction Reaction ID Subsystem Panel 4 Status Reaction Deletion Candidates Set 5 - Perform Optimized Design Generate Design Maximum Deletions Minimum Growth Design Name desian5-28 Remove Design Design List design5-25 • Clear Designs Design Growth Rate Minimize Target Maximize Target 0.1969 0 0.0000 design1-25 design2-25 0.0714 -0.0000 9.0365 0.0668 9 1189 design3-25 -0.0000 design4-25 0.0658 -0.0000 9.3059 design5-25 0.0598 7.7625 9.5240 Deleted Reaction ID Allow Deletion **Block Deletion** Reaction ID **Deleted Reaction** Subsystem 6-phosphogluconate dehydratase EDD Pentose Phosphate Pathway Acetaldehyde dehydrogenase (acetylating) ACALD Pvruvate Metabolism D-lactate dehydrogenase LDH D Pyruvate Metabolism

F6PA

TPI

÷ In Silico Fermentation 6 - Perform Manual Design 6-phosphogluconate dehydratase; EDD Reaction List Filter Reactions Reaction ID EDD Add Deletion **Remove Deletion Clear Deletions** Deleted Reaction Reaction ID Gene Rule 6-phosphogluconate dehydratase EDD x(251) Acetaldehyde dehydrogenase (acetylating) ACALD x(103) | x(101) LDH D D-lactate dehydrogenase x(1136) Fructose 6-phosphate aldolase F6PA x(421) | x(416) TPI x(1004) Triose-phosphate isomerase Design Name design5-25 Add Design design5-25 Design List **v**] Remove Design Design Growth Rate Minimize Target Maximize Target design5-25 0.0598 7.7625 9.5240 Panel 6 Status Strain Design Transferred for Gene Analysis Ivze Genes

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7 - Gene Knockout Analysis Triose-phosphate isomerase; TPI Deleted Reaction List 1004 Gene List ▼ Add Knockout **Remove Knockout** Mutant Gene Knockout Gene ID **Reactions Affected** 1004 b3919 1 3 101 b1241 103 b0351 1 1136 b1380 1 Mutant FVA Simulate Mutant Export Mutant Model

Mutant Metabolite	MinimizeTarget	Maximize	
E. coli biomass objective function (iML1515) - core - with	0.0598	0.0598	4
(R)-Propane-1,2-diol	7.7625	9.5240	
(S)-Propane-1,2-diol	1.7614	0	
Acetate	9.1599	9.1599	-
•		•	
Panel 7 Status Knockout Strain Model File Created			/

6. Modify the optimized designs or perform manual strain design in the absence of a MILP solver.
Candidate reactions contained in the defined subsystems can be deleted or added.

7. Select gene knockouts to functionally eliminate the deleted reactions. FVA calculates ranges of nutrient uptake rates and product secretion rates of the knockout mutant strain.

Refine Design Panel 5 Status Optim

Fructose 6-phosphate aldolase

Triose-phosphate isomerase

Optimized Strain Design Transferred

Glycolysis/Gluconeogenesis

Glycolysis/Gluconeogenesis

The current state of the App can be saved and later reloaded to allow all work to be preserved. The gene knockout design can be saved as a COBRA metabolic model for use outside the App.

承 MATLAB App

		InSilicoKO	- Knockout	Strain	Design for	Metabolite Overpr	oductio	n using G	enome-S	cale M	etabolio	Models	
	1 - S	4 - Define Deletion Candidates											
Model Folder	C:/Users/micha/OneE	GSM/BIGG	Subsystem List	Pentose	Phosphate P	athway			•				
Selected Model	(IML1515				▼)	Add Subsyst	em	Remo	ve Subsyste	m		ss Optimization	
LP Solver	gurobi	,	 Solver M 	ILP Capa	able								
Set Model	Panel 1 Status	Selected Model	Loaded				o						
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	2 - De	fine Medium				Glycolysis/Gluconec	aenesis		23				
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Nutrent List	02 02			Add	Nutrient	Pentose Phosphate	Pathway		12				
Filter Nutrients				Remo	ve Nutrient								
Medium List			▼	Clear	r Nutrients	Disallowed React	ion	Reaction	ID		Subsyste	bsystem	
Nutrient	Uptake Bound	Minimize Ta	rget Max	imize Ta	arget								
O2 O2	-1	-1.0000	-1.00	00									
Phosphate	-1000	-0.1880	-0.18	80									
Potassium	-1000	-0.0381	-0.0381										
Selenate	-1000	0	0			Optimize Design	Panel	4 Status	Reaction De	eletion Ca	Indidates	Set	
Selenite	Selenite -1000 0							L					
Sodium	-1000	0	0		-			5 - Perform	Optimized	Design			
Save Medium	Medium File					Maximum Dele	tions	5	Minimum G	rowth	25%	Generate Design	
Set Medium	Panel 2 Status	Medium List Cre	ated			Design Name	design5	-25				Remove Design	
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	3-De	anne Product	_			Destina	0	th Data	D.d. in the state	- T			
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Wild-Type Produc	:t		Minimize Ta	rget I	Maximiz	design1-25	0.196	9	0		0.	0000	
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						Deleted Reaction			Reaction	ID	Subsys	tem	
Set Product	Panel 3 Status	Target Product S	Set			6-phosphogluconate	e dehydrata	ise	EDD		Pentose	Phosphate Pathway	
	Case	Mananamant				D lactate debydroge	nase	(acelylatility)	IDH D		Pyruvate	Metabolism	
[Case	wanagement				Fructose 6-phospha	te aldolase	2	EGDA		Chronwsie/Chronopaganasia		
File Folder	C:/Users/micha/Or	neDrive/Documen	ts/Consulting/I	SF/Matla	b/ME/Files	Triose-phosphate is	omerase		TPI		Glycolys	is/Gluconeogenesis	
Case Name	iML1515-R-1,2-pro	panediol_comple	te_case	Save	e Case						3., 00.,0		
Case Panel Status	Case File Create	d		Load	d Case	Refine Design	Panel	5 Status	Optimized 8	Strain Des	ign Trans	ferred	

Gene Knockout Design for R-1,2-propanediol Overproduction in Escherichia coli

Wild-type Metabolic Model

- BIGG model iML1515
- Escherichia coli K-12 MG1655 •
- 2712 reactions, 1877 metabolites, 1516 genes

Specified Culture Medium

- Glucose minimal medium
- Microaerobic growth
- O2 uptake limited

Target Product

- R-1,2-propanediol
- Not secreted by wild-type strain at maximum growth rate

Candidate Reaction Deletions Optimized Design Refinement

- 6 subsystems
- 0 disallowed reactions
- 119 candidate deletions

Strain Design Optimization

- Maximum 5 reaction deletions
- Minimum 25% wild-type growth rate
- Best design: 5 deletions in glycolysis, pentose phosphate pathway & pyruvate metabolism

• None

Gene Knockout Mutant

- 5 reaction deletions achieved with 7 gene knockouts
- Mutant growth rate 30% of wild-type growth rate
- R-1,2-propanediol secretion rate: 7.8-9.5 mmol/gDW/h
- Other major products are S-1,2-propanediol, acetate & CO₂