

InSilicoKO – A MATLAB Application for Metabolite Overproduction Strain Design

The screenshot displays the InSilicoKO MATLAB application interface, which is divided into several panels for different stages of the strain design process. The application is titled "InSilicoKO - Knockout Strain Design for Metabolite Overproduction using Genome-Scale Metabolic Models" and is running in a MATLAB App environment.

1 - Select Model

Model Folder: C:/Users/micha/OneDrive/Documents/Consulting/ISF/Matlab/GSM/BIGG
Selected Model: IML1515
LP Solver: gurobi (Solver MILP Capable)
Panel 1 Status: Selected Model Loaded

2 - Define Medium

Nutrient List: O2 O2
Filter Nutrients: (empty)
Medium List: (empty)
Panel 2 Status: Medium List Created

Nutrient	Uptake Bound	Minimize Target	Maximize Target
O2 O2	-1	-1.0000	-1.0000
Phosphate	-1000	-0.1880	-0.1880
Potassium	-1000	-0.0381	-0.0381
Selenate	-1000	0	0
Selenite	-1000	0	0
Sodium	-1000	0	0

3 - Define Product

Target Product: (R)-Propane-1,2-diol
Wild-Type FVA: 99%

Wild-Type Product	Minimize Target	Maximize Target
E. coli biomass objective function (IML1515) - core - with ...	0.1949	0.1949
(R)-Propane-1,2-diol	0	0.1811
Acetate	9.1005	9.6439
CO2 CO2	0.8991	-0.0065
Ethanol	7.9660	7.2415
Formate	16.9665	17.6910

4 - Define Deletion Candidates

Subsystem List: Pentose Phosphate Pathway

Candidate Subsystem	Reactions Included
Anaplerotic Reactions	8
Citric Acid Cycle	14
Glycolysis/Gluconeogenesis	23
Oxidative Phosphorylation	52
Pentose Phosphate Pathway	12

5 - Perform Optimized Design

Maximum Deletions: 5
Minimum Growth: 25%

Design	Growth Rate	Minimize Target	Maximize Target
design1-25	0.1969	0	0.0000
design2-25	0.0714	-0.0000	9.0365
design3-25	0.0668	-0.0000	9.1189
design4-25	0.0658	-0.0000	9.3059
design5-25	0.0598	7.7625	9.5240

6 - Perform Manual Design

Reaction List: 6-phosphogluconate dehydratase; EDD
Filter Reactions: (empty)
Reaction ID: EDD

Deleted Reaction	Reaction ID	Gene Rule
6-phosphogluconate dehydratase	EDD	x(251)
Acetaldehyde dehydrogenase (acetylating)	ACALD	x(103) x(101)
D-lactate dehydrogenase	LDH_D	x(1136)
Fructose 6-phosphate aldolase	F6PA	x(421) x(416)
Triose-phosphate isomerase	TPI	x(1004)

7 - Gene Knockout Analysis

Deleted Reaction List: Triose-phosphate isomerase; TPI
Gene List: 1004

Mutant Gene Knockout	Gene ID	Reactions Affected
1004	b3919	1
101	b1241	3
103	b0351	1
1136	b1380	1

Case Management

File Folder: C:/Users/micha/OneDrive/Documents/Consulting/ISF/Matlab/ME/Files
Case Name: IML1515-R-1,2-propanediol_complete_case
Case Panel Status: Case File Created

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.

InSilicoKO - Knockout Strain Design for Metabolite Overproduction using Genome-Scale Metabolic Models

1 - Select Model

Model Folder:
Selected Model:
LP Solver: [Solver MILP Capable](#)
[Set Model](#) Panel 1 Status: [Selected Model Loaded](#)

2 - Define Medium

Nutrient List: [Add Nutrient](#)
Filter Nutrients: [Remove Nutrient](#)
Medium List: [Clear Nutrients](#)

Nutrient	Uptake Bound	Minimize Target	Maximize Target
O2 O2	-1	-1.0000	-1.0000
Phosphate	-1000	-0.1880	-0.1880
Potassium	-1000	-0.0381	-0.0381
Selenate	-1000	0	0
Selenite	-1000	0	0
Sodium	-1000	0	0

[Save Medium](#) Medium File:
[Set Medium](#) Panel 2 Status: [Nutrient Uptake Bounds Set](#)

3 - Define Product

Target Product: Wild-Type FVA:

Wild-Type Product	Minimize Target	Maximize Target
E. coli biomass objective function (iML1515) - core - with ...	0.1949	0.1949
(R)-Propane-1,2-diol	0	0.1811
Acetate	9.1005	9.6439
CO2 CO2	0.8991	-0.0065
Ethanol	7.9660	7.2415
Formate	16.9665	17.6910

[Set Product](#) Panel 3 Status: [Wild-Type Simulation Complete](#)

4 - Define Deletion Candidates

Subsystem List:
[Add Subsystem](#) [Remove Subsystem](#) [Bypass Optimization](#)

Candidate Subsystem	Reactions Included
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Disallowed Reaction	Reaction ID	Subsystem
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[Optimize Design](#) Panel 4 Status:

5 - Perform Optimized Design

Maximum Deletions: Minimum Growth: [Generate Design](#)
Design Name: [Remove Design](#)
Design List: [Clear Designs](#)

Design	Growth Rate	Minimize Target	Maximize Target
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Deleted Reaction ID: [Allow Deletion](#) [Block Deletion](#)

Deleted Reaction	Reaction ID	Subsystem
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[Refine Design](#) Panel 5 Status:

Case Management

File Folder:
Case Name: [Save Case](#)
Case Panel Status: File Folder Does Not Exist [Load Case](#)

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 - Knockout Strain Design for Metabolite Overproduction using Genome-Scale Metabolic Models

1 - Select Model

Model Folder: C:/Users/micha/OneDrive/Documents/Consulting/ISF/Matlab/GSM/BIGG
 Selected Model: iML1515
 LP Solver: gurobi (Solver MILP Capable)
 Panel 1 Status: Selected Model Loaded

2 - Define Medium

Nutrient List: O2 O2
 Filter Nutrients:
 Medium List:
 Panel 2 Status: Nutrient Uptake Bounds Set

Nutrient	Uptake Bound	Minimize Target	Maximize Target
O2 O2	-1	-1.0000	-1.0000
Phosphate	-1000	-0.1880	-0.1880
Potassium	-1000	-0.0381	-0.0381
Selenate	-1000	0	0
Selenite	-1000	0	0
Sodium	-1000	0	0

3 - Define Product

Target Product: (R)-Propane-1,2-diol
 Wild-Type FVA: 99%

Wild-Type Product	Minimize Target	Maximize Target
E. coli biomass objective function (iML1515) - core - with ...	0.1949	0.1949
(R)-Propane-1,2-diol	0	0.1811
Acetate	9.1005	9.6439
CO2 CO2	0.8991	-0.0065
Ethanol	7.9660	7.2415
Formate	16.9665	17.6910

Panel 3 Status: Target Product Set

4 - Define Deletion Candidates

Subsystem List: Pentose Phosphate Pathway

Candidate Subsystem	Reactions Included
Anaplerotic Reactions	8
Citric Acid Cycle	14
Glycolysis/Gluconeogenesis	23
Oxidative Phosphorylation	52
Pentose Phosphate Pathway	12

Disallowed Reaction	Reaction ID	Subsystem
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Panel 4 Status: Reaction Deletion Candidates Set

5 - Perform Optimized Design

Maximum Deletions: 5
 Minimum Growth: 25%
 Design Name: design5-25
 Design List: design5-25

Design	Growth Rate	Minimize Target	Maximize Target
design1-25	0.1969	0	0.0000
design2-25	0.0714	-0.0000	9.0365
design3-25	0.0668	-0.0000	9.1189
design4-25	0.0658	-0.0000	9.3059
design5-25	0.0598	7.7625	9.5240

Deleted Reaction ID:
 Allow Deletion | Block Deletion

Deleted Reaction	Reaction ID	Subsystem
6-phosphogluconate dehydratase	EDD	Pentose Phosphate Pathway
Acetaldehyde dehydrogenase (acetylating)	ACALD	Pyruvate Metabolism
D-lactate dehydrogenase	LDH_D	Pyruvate Metabolism
Fructose 6-phosphate aldolase	F6PA	Glycolysis/Gluconeogenesis
Triose-phosphate isomerase	TPI	Glycolysis/Gluconeogenesis

Panel 5 Status: Design Optimization Converged. Execution 164 sec

Case Management

File Folder:
 Case Name:
 Case Panel Status: File Folder Does Not Exist

4 - Define Deletion Candidates

Subsystem List Pentose Phosphate Pathway

Add Subsystem Remove Subsystem Bypass Optimization

Candidate Subsystem	Reactions Included
Anaplerotic Reactions	8
Citric Acid Cycle	14
Glycolysis/Gluconeogenesis	23
Oxidative Phosphorylation	52
Pentose Phosphate Pathway	12

Disallowed Reaction	Reaction ID	Subsystem

Optimize Design Panel 4 Status Reaction Deletion Candidates Set

5 - Perform Optimized Design

Maximum Deletions 5 Minimum Growth 25% Generate Design

Design Name design5-25 Remove Design

Design List design5-25 Clear Designs

Design	Growth Rate	Minimize Target	Maximize Target
design1-25	0.1969	0	0.0000
design2-25	0.0714	-0.0000	9.0365
design3-25	0.0668	-0.0000	9.1189
design4-25	0.0658	-0.0000	9.3059
design5-25	0.0598	7.7625	9.5240

Deleted Reaction ID Allow Deletion Block Deletion

Deleted Reaction	Reaction ID	Subsystem
6-phosphogluconate dehydratase	EDD	Pentose Phosphate Pathway
Acetaldehyde dehydrogenase (acetylating)	ACALD	Pyruvate Metabolism
D-lactate dehydrogenase	LDH_D	Pyruvate Metabolism
Fructose 6-phosphate aldolase	F6PA	Glycolysis/Gluconeogenesis
Triose-phosphate isomerase	TPI	Glycolysis/Gluconeogenesis

Refine Design Panel 5 Status Optimized Strain Design Transferred

6 - Perform Manual Design

Reaction List 6-phosphogluconate dehydratase; EDD

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)
D-lactate dehydrogenase	LDH_D	x(1136)
Fructose 6-phosphate aldolase	F6PA	x(421) x(416)
Triose-phosphate isomerase	TPI	x(1004)

Design Name design5-25 Add Design

Design List design5-25 Remove Design

Design	Growth Rate	Minimize Target	Maximize Target
design5-25	0.0598	7.7625	9.5240

Analyze Genes Panel 6 Status Strain Design Transferred for Gene Analysis

7 - Gene Knockout Analysis

Deleted Reaction List Triose-phosphate isomerase; TPI

Gene List 1004 Add Knockout Remove Knockout

Mutant Gene Knockout	Gene ID	Reactions Affected
1004	b3919	1
101	b1241	3
103	b0351	1
1136	b1380	1

Mutant FVA 100 Simulate Mutant Export Mutant Model

Mutant Metabolite	Minimize Target	Maximize
E. coli biomass objective function (iML1515) - core - with ...	0.0598	0.0598
(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.

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.

1 - Select Model

Model Folder: C:/Users/micha/OneDrive/Documents/Consulting/ISF/Matlab/GSM/BIGG
Selected Model: iML1515
LP Solver: gurobi (Solver MILP Capable)
Set Model Panel 1 Status: Selected Model Loaded

2 - Define Medium

Nutrient List: O2 O2 (Add Nutrient)
Filter Nutrients: (Remove Nutrient)
Medium List: (Clear Nutrients)

Nutrient	Uptake Bound	Minimize Target	Maximize Target
O2 O2	-1	-1.0000	-1.0000
Phosphate	-1000	-0.1880	-0.1880
Potassium	-1000	-0.0381	-0.0381
Selenate	-1000	0	0
Selenite	-1000	0	0
Sodium	-1000	0	0

Save Medium Medium File: (Set Medium) Panel 2 Status: Medium List Created

3 - Define Product

Target Product: (R)-Propane-1,2-diol (Wild-Type FVA: 99%)

Wild-Type Product	Minimize Target	Maximize Target
E. coli biomass objective function (iML1515) - core - with ...	0.1949	0.1949
(R)-Propane-1,2-diol	0	0.1811
Acetate	9.1005	9.6439
CO2 CO2	0.8991	-0.0065
Ethanol	7.9660	7.2415
Formate	16.9665	17.6910

Set Product Panel 3 Status: Target Product Set

4 - Define Deletion Candidates

Subsystem List: Pentose Phosphate Pathway (Add Subsystem, Remove Subsystem, Bypass Optimization)

Candidate Subsystem	Reactions Included
Anaplerotic Reactions	8
Citric Acid Cycle	14
Glycolysis/Gluconeogenesis	23
Oxidative Phosphorylation	52
Pentose Phosphate Pathway	12

Disallowed Reaction	Reaction ID	Subsystem
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Optimize Design Panel 4 Status: Reaction Deletion Candidates Set

5 - Perform Optimized Design

Maximum Deletions: 5 (Minimum Growth: 25%) (Generate Design)
Design Name: design5-25 (Remove Design)
Design List: design5-25 (Clear Designs)

Design	Growth Rate	Minimize Target	Maximize Target
design1-25	0.1969	0	0.0000
design2-25	0.0714	-0.0000	9.0365
design3-25	0.0668	-0.0000	9.1189
design4-25	0.0658	-0.0000	9.3059
design5-25	0.0598	7.7625	9.5240

Deleted Reaction ID: (Allow Deletion, Block Deletion)

Deleted Reaction	Reaction ID	Subsystem
6-phosphogluconate dehydratase	EDD	Pentose Phosphate Pathway
Acetaldehyde dehydrogenase (acetylating)	ACALD	Pyruvate Metabolism
D-lactate dehydrogenase	LDH_D	Pyruvate Metabolism
Fructose 6-phosphate aldolase	F6PA	Glycolysis/Gluconeogenesis
Triose-phosphate isomerase	TPI	Glycolysis/Gluconeogenesis

Refine Design Panel 5 Status: Optimized Strain Design Transferred

Case Management

File Folder: C:/Users/micha/OneDrive/Documents/Consulting/ISF/Matlab/ME/Files
Case Name: iML1515-R-1,2-propanediol_complete_case (Save Case)
Case Panel Status: Case File Created (Load Case)

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

- 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

Optimized Design Refinement

- 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