MediumFBA – A MATLAB Application for Integrated Medium Design and Flux Balance Analysis

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Example – Determine an anaerobic glucose minimal medium for a 4-species community consisting of the gut pathogen *Clostridioides difficile* and three Enterobacteriaceae species

1. Select metabolic models for the strains of interest. The designed medium will ensure monoculture growth of all selected strains.

2. Define core nutrients contained in the desired medium. The core nutrients are common media components such as inorganic salt ions.

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ostridium_difficile_CD196		1	899	1170					
iterobacter_cloacae_EcW	SU1	2	1325	1414					
cherichia_coli_str_K_12_s	substr_MG1655	3	1190	1348					
bsiella_pneumoniae_pne:	umoniae_MGH78578	4	1393	1379					
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3. Impose constraints on the medium design solution. Nutrients can be included or excluded according to their suitability as medium components.

4. Design the growth medium by ensuring monoculture growth. The constraints can be altered in an iterative manner to generate an acceptable set of growth nutrients.

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codium -1000 1 2 3 4 L-valine -15.2275 -0.2856 -5.1135 -0.1238 Save Core Medium Core File Save Growth Medium Growth File	Vedium Folder C: Core Medium List Core Nutrient List Filter Core Nutrient Add Core Nutrient ore Nutrient mmonium hloride 02+ u2+ 22+ 23+	/Users/micha/OneDrive/Do Basic_ions_agora Ammonium s C Uptake Bound -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000	Nutrient	Clear Cc Strains Tran 1234 1234 1234 1234 1234 1234 1234 1234	ittab/GrowthMedii	LP Solver Mininum Allowabl Design Strains Add Design Res Growth Nutrient proton sulfate L-leucine (R)-Pantothenate Pyridoxine	Faints F gurobi e le Growth Rati Clostridium_of uit	Panel 3 Status Design Growth M te te Strain 2 -992.5885 -0.1829 -0.3734 -0.0050 -0.0025	Medium Constrain Medium Force Full Mec 0.1 1 esult Cl Strain 3 -31.6608 -90.0652 -5.4497 -0.1940 -0.0970	tis Set dium Design Design Medium ear Design Tabl Strain 4 13.4887 -0.0811 -0.1319 -0.0047 -0.0023
Save Core Medium Core File Save Growth Medium Growth File	Medium Folder C: Core Medium List Core Nutrient List Filter Core Nutrient: Add Core Nutrient core Nutrient mmonium hloride o2+ u2+ e2+ e3+ e3+ in2+	/Users/micha/OneDrive/Do Basic_ions_agora Ammonium s C Uptake Bound -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000	Nutrient	Sulting/ISF/Ma Clear Cc Strains Tran 1234 1234 1234 1234 1234 1234 1234 1234	itlab/GrowthMedii	LP Solver Mininum Allowabl Design Strains Add Design Res Growth Nutrient proton sulfate L-leucine (R)-Pantothenate Pyridoxine L-tryptophan	Faints F gurobi de Growth Rate clostridium_o de Growth Rate x de Growth Rate Clostridium_o de Growth Rate x de Growth Rate Clostridium_o de Growth Rate x de Growth R	Panel 3 Status Design Growth M te te Strain 2 -992.5885 -0.1829 -0.3734 -0.0050 -0.0025 -997.9106	Medium Constrain Medium Force Full Mec 0.1 1 esult Cl Strain 3 -31.6608 -90.0652 -5.4497 -0.1940 -0.0970 -1000	tis Set dium Design Design Medium ear Design Tabl Strain 4 13.4887 -0.0811 -0.1319 -0.0047 -0.0023 -0.0166
Save Core Medium Core File Save Growth Medium Growth File	Wedium Folder C: Core Medium List Core Nutrient List Filter Core Nutrient: Add Core Nutrient ore Nutrient mmonium hloride o2+ u2+ 22+ 13+ n2+ 13+ n2+	/Users/micha/OneDrive/Do Basic_ions_agora Ammonium s C Uptake Bound -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000	Nutrient	Sulting/ISF/Ma Clear Co Strains Tran 1234 1234 1234 1234 1234 1234 1234 1234	itlab/GrowthMedi:	LP Solver Mininum Allowabl Design Strains Add Design Res Growth Nutrient proton sulfate L-leucine (R)-Pantothenate Pyridoxine L-tryptophan L-valine	Faints F gurobi de Growth Rati clostridium_c dit the Growth Rati dit Clostridium_c dit strain 1 dit 12.4190 -0.4521 -1000 -0.0329 -0.0165 -0.1130 -15.2275 -0.1130	Panel 3 Status Design Growth N	Medium Constrain ✓ Force Full Mec 0.1 1 esult Cl Strain 3 -31.6608 -90.0652 -5.4497 -0.1940 -0.0970 -1000 -5.1135	tis Set dium Design Design Medium ear Design Tabl Strain 4 13.4887 -0.0811 -0.0811 -0.047 -0.0023 -0.0166 -0.1238
	Aedium Folder C: Core Medium List Core Nutrient List Filter Core Nutrient: Add Core Nutrient ore Nutrient nmonium Noride 22+ 12+ 12+ 12+ 12+ 12+ 12+	/Users/micha/OneDrive/Do Basic_ions_agora Ammonium s Remove Core Uptake Bound -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000	Nutrient	Clear Cc Strains Tran 1234 1234 1234 1234 1234 1234 1234 1234	itlab/GrowthMedi:	LP Solver Mininum Allowabl Design Strains Add Design Res Growth Nutrient proton sulfate L-leucine (R)-Pantothenate Pyridoxine L-tryptophan L-valine	Faints F gurobi de Growth Rati clostridium_o de Growth Rati 12.4190 -0.4521 -1000 -0.329 -0.0165 -0.1130 -15.2275 -0.1130	Panel 3 Status Design Growth N	Medium Constrain Medium ✓ Force Full Mec 0.1 1 esult Cl Strain 3 -31.6608 -90.0652 -5.4497 -0.1940 -0.0970 -1000 -5.1135	Set dium Design Design Medium ear Design Tabl 13.4887 -0.0811 -0.1319 -0.0047 -0.0023 -0.11238
Set Core Medium Panel 2 Status Core Medium Transferred Set Crowth Medium Panel 4 Statue Medium Design Complete	Medium Folder C: Core Medium List Core Nutrient List Filter Core Nutrients Add Core Nutrient mmonium hloride 02+ u2+ 2+ 3+ n2+ >dium	/Users/micha/OneDrive/De Basic_ions_agora Ammonium s Uptake Bound -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000 -1000	A A A A A A A A A A A A A A A A A A A	Clear Co Strains Tran 1234 1234 1234 1234 1234 1234 1234 1234	ttlab/GrowthMedi	LP Solver Mininum Allowabl Design Strains Add Design Res Growth Nutrient proton sulfate L-leucine (R)-Pantothenate Pyridoxine L-tryptophan L-valine 4 Save Growth Med	Faints F gurobi Ide Growth Rat Clostridium_c Ide Growth Rat 2.100 Ide Growth Rat Strain 1 12.4190 12.4190 Ide Growth Rat -0.4521 Ide Growth Rat -0.0165 Ide Growth Rat -0.0165 Ide Growth Rat Itium Ide Growth Rat	Panel 3 Status Design Growth N	Medium Constrain ✓ Force Full Mec 0.1 [1] esuit [C] Strain 3 -31.6608 -90.0652 -5.4497 -0.1940 -0.0970 -1000 -5.1135	tis Set dium Design Design Medium ear Design Tabl Strain 4 13.4887 -0.0811 -0.0811 -0.0047 -0.0023 -0.0166 -0.1238

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lysis for Genome-Scale Metabolic Mode

3 - D	efine Medium Design Const	raints	
ained Nutrient List	D-glucose		•
nstrained Nutrients			
lude Nutrient	Exclude Nutrient	Clear Nutrient	
d Nutrient	Uptake Bound	Strains Transporter	
im	-1000	1234	
	-1000	1234	
	-1000	1234	
	-1000	1234	
e	-5	1234	-
		۱.	
d Nutrient	Uptake Bound	Strains Transporter	
	0	1234	

	4 - De	sign Growth M	ledium		
olver	gurobi	•	Force Full Med	ium Design	
um Allowa	ble Growth Rate		0.1	Design Medium	
Strains	Clostridium_diffic	ile_CD196			۲
Design Re	esult Rer	move Design Re	esult Cl	ear Design Table	
Nutrient	Strain 1	Strain 2	Strain 3	Strain 4	Т
	12.4190	-992.5885	-31.6608	13.4887	
	-0.4521	-0.1829	-90.0652	-0.0811	
	-1000	-0.3734	-5.4497	-0.1319	
thenate	-0.0329	-0.0050	-0.1940	-0.0047	
e	-0.0165	-0.0025	-0.0970	-0.0023	1
han	-0.1130	-997.9106	-1000	-0.0166	
	-15.2275	-0.2856	-5.1135	-0.1238	-
				•	

	5 - R	efine Growth M	edium			
Refined Medium Li	ist					
Refined Nutrient L	ist L-leu	cine				
Filter Refined Nutrie	ents					
Add Refined Nutris	Pont	tove Refined Nu	trient	Clea	r Defined Nutrie	nte
Add Keimed Nutrie		love Relified Nu		Clea	r Kenneu Nuure	
Refined Nutrient	Uptal	ke Bound		Strains	Transporter	
L-leucine	-1			1234		
L-tryptophan	-1			1234		
L-valine	-1			1234		_
Mn2+	-1000			1234		
Pyridoxine	-1000			1234		
€ Sodium	1000			1001		F
Save Refined Medi	um Re	fined File				
oure Renned medi						
Set Refined Mediu	im Pan	el 5 Status	Refined I	Aedium (ici -	
Simulate Medium	6 - Sim	nulate Refined I	Medium Clostridiu	m_diffici	e_CD196	-
Simulate Medium Add Simulation Res	6 - Sin n Simul sult Rem	ation Strains	Medium Clostridiu Result	m_diffici Clea	e_CD196 Ir Simulation Tal	ble
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass	6 - Sin Simul suit Rem Strain 1	ation Strains () ove Simulation F Strain 2	Medium Clostridiu Result Stra	m_diffici Clea in 3	e_CD196 rr Simulation Tal	ble
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide	6 - Sin Simul suit Rem Strain 1 0.2739 1.6629	ove Simulation F Strain 2 0.2618 0.0110	Medium Clostridiu Result Stra 0.259	m_diffici Clea in 3	e_CD196 r Simulation Tal Strain 4 0.3008 5.1030	ble
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate	6 - Sin Simul sult Rem Strain 1 0.2739 1.6629 0	ove Simulation F strain 2 0.2618 0.0110 7.5933	Medium Clostridiu Result Stra 0.259 0 7,419	m_diffici Clea in 3 96	e_CD196 r Simulation Ta Strain 4 0.3008 5.1030 0	ble
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate Hydrogen	6 - Sin Simul Suit Rem Strain 1 0.2739 1.6629 0 0 0	Aulate Refined I ation Strains (ove Simulation F Strain 2 0.2618 0.0110 7.5933 0	Medium Clostridiu Result Stra 0.259 0 7.415 6.04/	m_diffici Clea in 3 96 51	e_CD196 Simulation Tai Strain 4 0.3008 5.1030 0 9.7603	ble
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate Hydrogen Hydrogen sulfide	6 - Sin Simul Sult Rem Strain 1 0.2739 1.6629 0 0 0 0 0 0	Aulate Refined I ation Strains (ove Simulation F Strain 2 0.2618 0.0110 7.5933 0 1.0653	Stra 0.259 0 7.415 0.044 0.044	m_diffici Clea in 3 96 51 48	E_CD196 Strain 4 0.3008 5.1030 0 9.7603 0	Die
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate Hydrogen Hydrogen sulfide Indole-3-propionate	6 - Sim Simul Suit Rem Strain 1 0.2739 1.6629 0 0 0 0 0 0 0 0 0 0 0 0 0	Autor Strains (ove Simulation F Strain 2 0.2618 0.0110 7.5933 0 1.0653 0	Stra 0.259 0 7.418 0.044 0 0	m_diffici Clea in 3 96 51 48	E_CD196 Strain 4 0.3008 5.1030 0 9.7603 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	ble
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate Hydrogen Hydrogen sulfide Indole-3-propionate Isocaproate	6 - Sin simul Strain 1 0.2739 1.6629 0 0 0 0 0 0 0 0 0 0 0 0 0	Aulate Refined I ation Strains (ove Simulation F Strain 2 0.2618 0.0110 7.5933 0 1.0653 0 0 0	Stra 0.255 0 7.415 0.044 0 0 0 0 0 0	m_diffici Clea in 3 96 51 48	e_CD196 r Simulation Tal Strain 4 0.3008 5.1030 0 9.7603 0 0 0 0 0 0 0 0 0	ble
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate Hydrogen Hydrogen sulfide Indole-3-propionate Isocaproate	6 - Sin Simul Suit Rem Strain 1 0.2739 1.6629 0 0 0 0 0 0 0 0 0 0 0 0 0	Aulate Refined I ation Strains (1) ove Simulation F Strain 2 0.2618 0.0110 7.5933 0 1.0653 0 0 0 0	Stra 0.259 0 7.418 6.044 0 0	m_diffici Clea in 3 96 51 48	e_CD196 T Simulation Tai Strain 4 0.3008 5.1030 0 9.7603 0 0 0 0 0 0 0 0 0 0 0 0	Die
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate Hydrogen Hydrogen sulfide Indole-3-propionate Isocaproate Panel 6 Status	6 - Sin simul suit Rem Strain 1 0.2739 1.6629 0 0 0 0 0 0 0 0 0 0 0 0 0	Aulate Refined I ation Strains (1) ove Simulation F 0.2618 0.0110 7.5933 0 1.0653 0 0 0 0 Simulation Comp	Stra 0.258 0 7.418 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	m_diffici Clea in 3 96 51 48	e_CD196 Strain 4 0.3008 5.1030 0 9.7603 0 0 0 0 0 0 0	ble
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate Hydrogen Hydrogen sulfide Indole-3-propionate Isocaproate Panel 6 Status	6 - Sin Simul Suit Rem Strain 1 0.2739 1.6629 0 0 0 0 0 0 0 0 0 0 0 0 0	Aulate Refined I ation Strains (1) ove Simulation F Strain 2 0.2618 0.0110 7.5933 0 1.0653 0 0 0 Simulation Comp 7 - Manage Cas	Stra 0.255 0 7.415 0.044 0 0 0 0 0 0	m_diffici Cles in 3 96 51 48	e_CD196 Strain 4 0.3008 5.1030 0 9.7603 0 0 0 0	
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Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate Hydrogen Hydrogen sulfide Indole-3-propionate Isocaproate Panel 6 Status Case Folder	6 - Sin Simul Sult Rem Strain 1 0.2739 1.6629 0 0 0 0 0 0 0 0 0 0 0 0 0	Aulate Refined I ation Strains (ove Simulation F Strain 2 0.2618 0.0110 7.5933 0 1.0653 0 0 Simulation Comp 7 - Manage Cas	Medium Clostridiu Result Stra 0.259 0 7.418 6.044 0 0 0 0 0 0 0 0 0 0 0 0	m_diffici Cles in 3 96 51 48 Consulti	e_CD196 r Simulation Tai Strain 4 0.3008 5.1030 0 9.7603 0 0 0 0 0 0 0 0 0 0 0 0	ble
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate Hydrogen sulfide Indole-3-propionate Isocaproate Panel 6 Status Case Folder Save Results	6 - Sin simul suit Rem Strain 1 0.2739 1.6629 0 0 0 0 0.9851 0.8820 Refined Medium C:/Users/micha	Aulate Refined I ation Strains (ove Simulation F Strain 2 0.2618 0.0110 7.5933 0 1.0653 0 0 Simulation Comp 7 - Manage Cas a/OneDrive/Docu	Medium Clostridiu Result \$tra 0.255 0 7.418 6.044 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	m_diffici Clea in 3 96 51 48 Consultin	e_CD196 r Simulation Ta Strain 4 0.3008 5.1030 0 9.7603 0 0 0 0 0 0 0 0 0 0 0 0	ble
Simulate Medium Add Simulation Res Nutrient or Product Produced biomass carbon dioxide Formate Hydrogen Hydrogen sulfide Indole-3-propionate Isocaproate Panel 6 Status Case Folder Save Results Save State	6 - Sin suit Rem Strain 1 0.2739 1.6629 0 0 0 0.9851 0.8820 Refined Medium C:/Users/micha Results File State File	Aulate Refined I ation Strains (ove Simulation F Strain 2 0.2618 0.0110 7.5933 0 1.0653 0 0 Simulation Comp 7 - Manage Cas a/OneDrive/Docu	Medium Clostridiu Result \$tra 0.259 0 7.419 6.044 0 </td <td>m_diffici Cles in 3 96 51 48 Consultin Species_</td> <td>e_CD196 r Simulation Tai Strain 4 0.3008 5.1030 0 9.7603 0 0 0 0 0 0 0 0 0 0 0 0</td> <td>ble irov</td>	m_diffici Cles in 3 96 51 48 Consultin Species_	e_CD196 r Simulation Tai Strain 4 0.3008 5.1030 0 9.7603 0 0 0 0 0 0 0 0 0 0 0 0	ble irov

5. Refine growth medium by modifying uptake bounds and adding non-essential nutrients such as other sugars and amino acids.

6. Simulate monoculture growth in the refined medium. FBA predicts the growth rate, nutrient uptake rates and product secretion rates of each strain.

7. Manage case by saving results and the current App state and by loading previously stored states.

Glucose Minimal Medium for Metabolic Model of 5-species *C. difficile*/Enterobacteriaceae Community

Core Nutrients Specified

AmmoniumWaterChlorideZincCo2+calcium(2+)Cu2+hydrogenphosphateFe2+magnesiumFe3+potassiumMn2+proton

Sodium sulfate

Growth Nutrients Included

D-glucose

Growth Nutrients Excluded O2 Amino Acids Identified

L-leucine L-tryptophan L-valine

Other Nutrients Identified

(R)-Pantothenate Pyridoxine