

Computation and analysis of rescued lethal gene deletions in a host-microbe model

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Constraint-based modeling has useful applications for predicting the metabolic interactions between a mammalian host and its commensal gut microbes. For example, the potential of a human gut microbe to rescue lethal gene defects in the mouse has been predicted. Some of these rescued gene defects correspond to human inborn errors of metabolism (IEMs) (Heinken et al., Gut Microbes (2013) 4(1):28-40). A variety of IEMs are documented in human and can be browsed at <https://www.vmh.life/#diseases>.

This tutorial demonstrates how to predict the potential of a commensal gut microbe to rescue lethal gene deletions in a mammalian host. For this purpose, a microbe is joined with a mouse host.

We will use the AGORA resource (Magnusdottir et al., Nat Biotechnol. 2017 Jan;35(1):81-89) in this tutorial. AGORA can be downloaded from <https://www.vmh.life/#downloadview>.

As the host model, the global mouse reconstruction (Sigurdsson et al., BMC Systems Biology (2010) 4:140) will be used. The mouse reconstruction can be downloaded from https://wwwen.uni.lu/content/download/72950/917509/file/Mus_musculus_iSS1393.zip.

Initialize the COBRA Toolbox

```
initCobraToolbox(false) %don't update the toolbox
```

change directory to where the tutorial is located

```
tutorialPath = fileparts(which('tutorial_hostMicrobeInteractions'));  
cd(tutorialPath);
```

Prepare the models

Download the mouse reconstruction.

```
system('curl -O https://wwwen.uni.lu/content/download/72950/917509/file/Mus_musculus_iSS1393.zip');  
currentDir=pwd;  
unzip('Mus_musculus_iSS1393.zip',currentDir)  
iSS1393=readCbModel('iSS1393.mat');  
iSS1393=changeObjective(iSS1393,'biomass_mm_1_no_glygln');  
% Define an AGORA model that can grow on the reduced diet and will be joined  
% with the mouse.  
system('curl -O https://www.vmh.life/files/reconstructions/AGORA/1.02/reconstructions/Escherichia_coli_str_K_12_substr_MG1655.xml');  
model=readCbModel('Escherichia_coli_str_K_12_substr_MG1655.xml');
```

NOTE: Since dietary nutrients can also rescue many lethal gene defects, a diet reduced in nutrients will be used in this simulation to identify the effect of the microbes. Not all AGORA models are able to grow on the given diet. Due to this, only microbes that can grow on the reduced diet can be used.

Define the reduced diet.

```
reducedDietConstraints={'EX_12dgr180[u]','-1','1000';'EX_26dap_M[u]','-1','1000';'EX_20
```

```
models={};
nameTagsModels={};
bioID={};
models{1,1}=model;
bioID{1,1}=model.rxns(find(strcmp(model.rxns,'biomass',7)));
nameTagsModels{1,1}=strcat('Escherichia_coli_str_K_12_substr_MG1655_');
modelHost=iSS1393;
nameTagHost='Mouse_';
```

Join the microbe with the mouse.

```
[modelJoint] = createMultipleSpeciesModel(models,'nameTagsModels',nameTagsModels,'model
```

Define the coupling parameters.

```
c=400;
u=0;
[modelJoint]=coupleRxnList2Rxn(modelJoint,modelJoint.rxns(strmatch(nameTagsModels{1,1},
[modelJoint]=coupleRxnList2Rxn(modelJoint,modelJoint.rxns(strmatch('Mouse_',modelJoint.
```

Some changes need to be made to the host model to constrain the body fluids compartment and the simulated intestinal barrier. This code needs to be adapted to each host since the IDs of created body fluid reactions may differ.

```
modelJoint = changeRxnBounds(modelJoint,modelJoint.rxns(strmatch('Mouse_EX_',modelJoint
modelJoint=changeRxnBounds(modelJoint,'Mouse_EX_o2(e)b',-100,'1');
```

Make unidirectional transport lumen -> host extracellular space

```
modelJoint = changeRxnBounds(modelJoint,modelJoint.rxns(strmatch('Mouse_IEX',modelJoint
```

Exception for metabolites host secretes into mucus/ lumen

```
modelJoint=changeRxnBounds(modelJoint,{'Mouse_IEX_chol[u]tr';'Mouse_IEX_galam[u]tr';'Mo
modelJoint=changeRxnBounds(modelJoint,{'Mouse_IEX_no[u]tr';'Mouse_IEX_n2m2nmasn[u]tr';'
```

Implement the reduced diet.

```
modelJoint=useDiet(modelJoint,reducedDietConstraints);
```

Run the prediction of rescued genes. This will take some time.

```
[OptSolKO,OptSolWT,OptSolRatio,RescuedGenes,fluxesKO]=computeRescuedGenes('modelJoint',
```

Show the mouse genes that caused a lethal phenotype when deleted in germfree mouse but not in presence of the microbe:

```
RescuedGenes.Mouse_biomass_mm_1_no_glygln.RescuedLethalGenes
```

The gene identifiers are NCBI Gene IDs and can be looked up to find the corresponding human genes and associated inborn errors of metabolism (IEMs). The reactions associated with the IEMs can subsequently be browsed at <https://www.vmh.life/#diseases>. For example, the gene 22247.1 encodes UMP synthase and its deletion can be rescued by the presence of E. coli. The corresponding IEM in human is orotic aciduria (<https://www.vmh.life/#disease/OROA>).

We will now identify the mechanisms of rescued KO phenotypes. Metabolites secreted by each species into the lumen may be taken up by the joined species and provide the metabolites that are essential due to the gene defect. To find the lumen exchange reactions of E. coli:

```
microbeExchanges=find(strcmp(modelJoint.rxns, strcat(nameTagsModels{1,1}, 'IEX'), length
```

Now, let us find out which of the metabolites secreted by E.coli was essential for rescuing the defect in mouse UMP synthase.

```
[model,hasEffect,constrRxnNames,deletedGenes] = deleteModelGenes(modelHost,'22247.1');  
constrRxnNames = strcat(nameTagHost,constrRxnNames);  
modelJoint=changeRxnBounds(modelJoint,constrRxnNames,0,'b');  
modelJoint=changeObjective(modelJoint,'Mouse_biomass_mm_1_no_glygln');  
modelJoint=changeRxnBounds(modelJoint, strcat(nameTagHost,'ATPM'),0,'l');  
modelJoint=changeRxnBounds(modelJoint, strcat(nameTagsModels{1}, 'DM_atp_c_'),0,'l');
```

To print out the E. coli exchange that had to carry flux to rescue the orotic aciduria-like mouse phenotype, use the following code:

```
for i=1:length(microbeExchanges)  
    if isempty(strfind(modelJoint.rxns{microbeExchanges(i)}, 'biomass'))  
        % prevent secretion flux through the exchanges one by one while predicting mouse  
        modelJointDel=changeRxnBounds(modelJoint,modelJoint.rxns{microbeExchanges(i)},0,  
        solution=solveCobraLP(modelJointDel);  
        if solution.obj<0.0000000001  
            fprintf('%s \n',modelJoint.rxns{microbeExchanges(i)}, ' is essential for res  
        end  
    end  
end  
end
```

Can you explain what you observe? You can look up the metabolite ID of the respective exchange at <https://www.vmh.life>. Hint: Check the description of orotic aciduria at <https://www.vmh.life/#disease/OROA>. Follow the external link to OMIM (Online Mendelian Inheritance in Man) to find more information.