

Create a generic subnetwork from Recon 3D

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In this tutorial, we show how to create a generic subnetwork from Recon 3D that can still perform all metabolic test functions as well as has physiologically defined ATP yield from defined carbon sources. The resulting model does not contain a specified list of reactions, except if they are still needed for the aforementioned tasks, and that is flux consistent.

EQUIPMENT SETUP

Initialize the COBRA Toolbox

Initialize the Cobra Toolbox using the `initCobraToolbox` function.

```
initCobraToolbox(false) % false, as we don't want to update
```

Setting the optimization solver

This tutorial will be run with a 'glpk' package, which is a linear programming ('LP') solver. The 'glpk' solver does not require additional installation or configuration.

```
% solverName='glpk';
```

However, for the analysis of large models such as Recon 3D, it is not recommended to use the 'glpk' package, but rather a commercial-grade solver, such as 'gurobi'.

For the analysis of a Recon model, change the solver to 'gurobi':

```
solverName = 'gurobi';  
changeCobraSolver(solverName, 'LP');
```

```
> Gurobi interface added to MATLAB path.
```

PROCEDURE

Load the model

In this tutorial, the used model is the generic model of human metabolism, Recon 3D [1]. If Recon 3D is not available, use Recon 2 [2] provided in The COBRA Toolbox. Other COBRA models may be downloaded from the [Virtual Metabolic Human](#) website and saved to your preferred directory.

Before proceeding with the simulations, the path for the model needs to be defined.

```
global CBTDIR  
  
fileName= 'Recon2.0model.mat'; % if using Recon 3 model, amend filename.
```

```
model = readCbModel([CBTDIR filesep 'test' filesep 'models' filesep
fileName]);
model.csense(1:size(model.S,1),1) = 'E';
```

Set the lower bounds on all biomass reactions and sink/demand reactions to zero.

```
model.lb(find(ismember(model.rxns, 'biomass_reaction')) = 0;
model.lb(find(ismember(model.rxns, 'biomass_maintenance_noTrTr')) = 0;
model.lb(find(ismember(model.rxns, 'biomass_maintenance')) = 0;
DMs = (strmatch('DM_', model.rxns));
model.lb(DMs) = 0;
Sinks = (strmatch('sink_', model.rxns));
model.lb(Sinks) = 0;
model.ub(Sinks) = 1000;
```

Identify the model reactions that are needed to ensure that all carbon sources result in a physiologically relevant ATP yield. (Note that this function uses sparseFBA, i.e., alternative solutions may exist but are not considered here.)

```
[Table_csourcesOri, TestedRxnsC, Perc] = testATPYieldFromCsources(model);
```

Warning: Reaction with the same name already exists in the model, updating the reaction
DM_atp_c_ h2o[c] + atp[c] -> adp[c] + h[c] + pi[c]

Identify the model reactions that are needed to ensure that all metabolic functions can have a non-zero flux. (Note that this function uses sparseFBA, i.e., alternative solutions may exist but are not considered here.)
Applicable to Recon3 only.

```
if ~isempty(strfind(fileName, 'Recon3'))
    [TestSolutionOri, TestSolutionNameClosedSinks, TestedRxnsClosedSinks,
PercClosedSinks] = test4HumanFctExt(model, 'all', 0);
    TestedRxns = unique([TestedRxnsC; TestedRxnsClosedSinks]);
    TestedRxnsX = intersect(model.rxns, TestedRxns);
end
```

Next we remove all human metabolic reactions (HMRs) (i.e., those reactions originating from HMR 2.0 [3] and that start with 'HMR_') that are not needed for the aforementioned tasks. Applicable to Recon 3 only.

```
if ~isempty(strfind(fileName, 'Recon3'))
    HMR = model.rxns(strmatch('HMR_', model.rxns));
    HMR_NE = setdiff(HMR, TestedRxnsX);
    model.lb(find(ismember(model.rxns, HMR_NE))) = 0;
    model.ub(find(ismember(model.rxns, HMR_NE))) = 0;
end
```

We will also remove all drug module reactions, i.e., those ones with the term 'Xeno' in the subsystem, mostly originating from [4]. Applicable to Recon 3 only.

```

if ~isempty(strfind(fileName, 'Recon3'))
    DM = model.rxns(strmatch('Xeno', model.subSystems));
    model.lb(find(ismember(model.rxns, DM))) = 0;
    model.ub(find(ismember(model.rxns, DM))) = 0;
    DMt = (strmatch('Transport of Xenobiotic', model.rxnNames));
    model.lb(DMt) = 0;
    model.ub(DMt) = 0;
end

```

We will also remove all reactions from the 'Peptide metabolism' subsystem. Applicable to Recon 3 only.

```

if ~isempty(strfind(fileName, 'Recon3'))
    DM = model.rxns(strmatch('Peptide metabolism', model.subSystems));
    model.lb(find(ismember(model.rxns, DM))) = 0;
    model.ub(find(ismember(model.rxns, DM))) = 0;
end

```

We will use the method FASTCORE, 'fastcc', to ensure a flux-consistent subnetwork [5].

```

param.epsilon = 1e-4;
param.modeFlag = 0;
param.method = 'fastcc'; %'null_fastcc'
printLevel = 2;
[fluxConsistentMetBool, fluxConsistentRxnBool, fluxInConsistentMetBool,
fluxInConsistentRxnBool, modelOut] = findFluxConsistentSubset(model, param,
printLevel);

```

```

7440    Total reactions
3260    Reversible reactions.
4180    Irreversible reactions.
5096    Flux consistent reactions, without flipping.
1266    Flux inconsistent irreversible reactions, without flipping.
1078    Flux inconsistent reactions, without flipping.
5237    Flux consistent reactions.
  937    Flux inconsistent reversible reactions left to flip.
5239    Flux consistent reactions.
  935    Flux inconsistent reversible reactions left to flip.
5241    Flux consistent reactions.
  933    Flux inconsistent reversible reactions left to flip.
5245    Flux consistent reactions.
  880    Flux inconsistent reversible reactions left to flip.

```

And remove the flux inconsistent reactions from the model.

```

modelConsistent =
removeRxns(model, model.rxns(find(fluxInConsistentRxnBool)));

```

We will now update the GPR associations.

```

modelConsistent.genes = [];
modelConsistent.rxnGeneMat = [];
modelgrRule = modelConsistent.grRules;

```

```

for i = 1 : length(modelgrRule)
    if ~isempty(modelgrRule{i})
        modelConsistent = changeGeneAssociation(modelConsistent,
modelConsistent.rxns{i}, modelgrRule{i});
    end
end

```

```

New gene 8639.1 added to model
New gene 26.1 added to model
New gene 314.2 added to model
New gene 314.1 added to model
New gene 1591.1 added to model
New gene 89874.1 added to model
New gene 92483.1 added to model
New gene 3948.2 added to model
New gene 55293.1 added to model
New gene 3945.1 added to model
New gene 3939.1 added to model
New gene 160287.1 added to model
New gene 3948.1 added to model
New gene 9123.1 added to model
New gene 9194.1 added to model
New gene 6566.1 added to model
New gene 57835.3 added to model
New gene 8671.1 added to model
New gene 57835.2 added to model
New gene 57835.1 added to model
New gene 57835.4 added to model
New gene 4967.1 added to model
New gene 1738.1 added to model
New gene 8050.1 added to model
New gene 1743.1 added to model
New gene 4967.2 added to model
New gene 130.1 added to model
New gene 127.1 added to model
New gene 125.1 added to model
New gene 124.1 added to model
New gene 131.1 added to model
New gene 126.1 added to model
New gene 128.1 added to model
New gene 137872.1 added to model
New gene 117247.1 added to model
New gene 218.1 added to model
New gene 222.1 added to model
New gene 220.1 added to model
New gene 221.1 added to model
New gene 3242.1 added to model
New gene 18.1 added to model
New gene 18.2 added to model
New gene 2531.1 added to model
New gene 23498.1 added to model
New gene 55268.1 added to model
New gene 51166.1 added to model
New gene 883.1 added to model
New gene 51166.2 added to model
New gene 1644.1 added to model
New gene 10165.1 added to model
New gene 8604.1 added to model
New gene 51380.1 added to model
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New gene 2805.1 added to model

```

New gene 2806.1 added to model
New gene 4128.1 added to model
New gene 4129.1 added to model
New gene 8659.1 added to model
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New gene 6799.1 added to model
New gene 6817.3 added to model
New gene 6799.2 added to model
New gene 6818.2 added to model
New gene 6817.2 added to model
New gene 6818.1 added to model
New gene 6817.1 added to model
New gene 6817.4 added to model
New gene 6818.3 added to model
New gene 6817.5 added to model
New gene 4363.1 added to model
New gene 6822.1 added to model
New gene 6573.1 added to model
New gene 6573.2 added to model
New gene 316.1 added to model
New gene 6570.1 added to model
New gene 6571.1 added to model
New gene 2348.5 added to model
New gene 2348.3 added to model
New gene 2348.1 added to model
New gene 2348.6 added to model
New gene 2352.1 added to model
New gene 2348.4 added to model
New gene 2348.2 added to model
New gene 4125.1 added to model
New gene 53947.1 added to model
New gene 51146.1 added to model
New gene 65985.1 added to model
New gene 28.1 added to model
New gene 223.1 added to model
New gene 206358.1 added to model
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New gene 39.1 added to model
New gene 3030.1 added to model
New gene 3032.1 added to model
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New gene 31.1 added to model
New gene 32.1 added to model
New gene 9197.1 added to model
New gene 55902.1 added to model
New gene 55902.2 added to model
New gene 84532.1 added to model
New gene 5973.1 added to model
New gene 64841.1 added to model
New gene 55577.1 added to model
New gene 5238.1 added to model
New gene 43.1 added to model
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New gene 112611.1 added to model
New gene 98.1 added to model
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New gene 97.1 added to model
New gene 100.1 added to model
New gene 3177.1 added to model
New gene 122481.1 added to model
New gene 203.1 added to model
New gene 26289.1 added to model
New gene 26289.2 added to model
New gene 205.3 added to model
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New gene 146712.1 added to model
New gene 374907.1 added to model
New gene 93010.1 added to model
New gene 374907.2 added to model
New gene 10331.1 added to model
New gene 10678.2 added to model
New gene 51005.1 added to model
New gene 79814.1 added to model
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New gene 6520.1 added to model
New gene 11212.1 added to model
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New gene 3141.1 added to model
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New gene 56052.1 added to model
New gene 23743.1 added to model
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New gene 162466.1 added to model
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New gene 9468.1 added to model
New gene 6579.1 added to model
New gene 6554.1 added to model
New gene 6555.1 added to model
New gene 8714.3 added to model
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New gene 54675.1 added to model
New gene 10559.1 added to model
New gene 55907.1 added to model
New gene 8034.1 added to model
New gene 51805.1 added to model
New gene 51004.1 added to model
New gene 51004.2 added to model
New gene 10229.1 added to model
New gene 9245.1 added to model
New gene 2650.1 added to model
New gene 51301.1 added to model
New gene 192134.1 added to model
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New gene 55350.3 added to model
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New gene 8875.1 added to model
New gene 55350.1 added to model
New gene AA081045.1 added to model
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New gene 978.1 added to model
New gene 57379.1 added to model
New gene 7371.1 added to model
New gene 83549.1 added to model
New gene 51727.1 added to model
New gene 9162.1 added to model
New gene 1607.1 added to model
New gene 8525.2 added to model
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New gene 1633.1 added to model
New gene 1635.1 added to model
New gene 1638.1 added to model
New gene 6319.1 added to model
New gene 9415.1 added to model
New gene 3992.1 added to model
New gene 8694.1 added to model
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```

Save the resulting model.

```
save('SubNetworkRecon.mat', 'modelConsistent')
```

Size of the original Recon model:

```

[nMet,nRxn] = size(model.S);
fprintf('%6s\t%6s\n', '#mets', '#rxns');
fprintf('%6u\t%6u\t%s%s\n', nMet, nRxn, ' total in Recon')

```

#mets	#rxns	
5063	7440	total in Recon

Size of the resulting Recon subnetwork:

```

[nMet,nRxn] = size(modelConsistent.S);
fprintf('%6s\t%6s\n', '#mets', '#rxns');
fprintf('%6u\t%6u\t%s%s\n', nMet, nRxn, ' total in Recon subnetwork')

```

#mets	#rxns	
2910	5245	total in Recon subnetwork

Consider to evaluate the resulting model with the tutorial `modelProperties` and `modelSanityChecks` to ensure proper functioning of the generic subnetwork of Recon.

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