

# 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
```

### 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 detailed information, refer to The Cobra Toolbox [solver installation guide](#).

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

$$\text{DM\_atp\_c\_h2o[c]} + \text{atp[c]} \rightarrow \text{adp[c]} + \text{h[c]} + \text{pi[c]}$$

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] = testATPYieldFromCsources(model);
    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

```

Now we will ensure that the reversibility of each reaction is in accordance to the defined lower bound.

```

model.rev(find(model.lb < 0)) = 1;
model.rev(find(model.lb >= 0)) = 0;

```

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] = fastcc(model, param);

```

```

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  
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New gene 55268.1 added to model  
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New gene 883.1 added to model  
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New gene 1644.1 added to model  
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New gene 8604.1 added to model  
New gene 51380.1 added to model  
New gene 2571.1 added to model  
New gene 2572.1 added to model  
New gene 2571.2 added to model  
New gene 2805.1 added to model  
New gene 2806.1 added to model  
New gene 4128.1 added to model  
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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  
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New gene 2352.1 added to model  
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New gene 205.1 added to model  
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New gene 205.2 added to model  
New gene 50808.1 added to model  
New gene 262.1 added to model  
New gene 112.1 added to model  
New gene 55811.1 added to model  
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New gene 112.2 added to model  
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New gene 9245.1 added to model  
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New gene 51301.1 added to model  
New gene 192134.1 added to model  
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New gene 56474.2 added to model  
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New gene 1666.1 added to model

New gene 8529.1 added to model  
New gene 4051.1 added to model  
New gene 126410.1 added to model  
New gene 64834.1 added to model  
New gene 8897.1 added to model  
New gene 8897.3 added to model  
New gene 8897.2 added to model  
New gene 2880.1 added to model  
New gene 2882.1 added to model  
New gene 284111.1 added to model  
New gene 9152.1 added to model  
New gene 9497.1 added to model  
New gene 115584.1 added to model  
New gene 6574.1 added to model  
New gene 6575.1 added to model  
New gene 142680.1 added to model  
New gene 6534.1 added to model  
New gene 3293.1 added to model

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New gene 1351.1 added to model  
New gene 1347.1 added to model  
New gene 1329.1 added to model  
New gene 1327.1 added to model  
New gene 341947.1 added to model  
New gene 9167.1 added to model  
New gene 1350.1 added to model  
New gene 4512.1 added to model  
New gene 1349.1 added to model  
New gene 1339.1 added to model  
New gene 1345.1 added to model  
New gene 4513.1 added to model  
New gene 9377.1 added to model  
New gene 125965.1 added to model  
New gene 4514.1 added to model  
New gene 170712.1 added to model  
New gene 1346.1 added to model  
New gene 1340.1 added to model  
New gene 84701.1 added to model  
New gene 1337.1 added to model

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New gene 1622.1 added to model  
New gene 225.1 added to model  
New gene 28965.1 added to model  
New gene 23205.1 added to model  
New gene 81616.1 added to model  
New gene 26061.1 added to model  
New gene 5825.1 added to model  
New gene 54716.1 added to model  
New gene 57393.1 added to model  
New gene 55748.1 added to model  
New gene 6564.1 added to model  
New gene 2694.1 added to model  
New gene 8029.1 added to model  
New gene 81693.1 added to model  
New gene 1800.1 added to model  
New gene 5184.1 added to model  
New gene 1200.1 added to model  
New gene 2168.1 added to model  
New gene 51056.1 added to model  
New gene 9390.1 added to model  
New gene 55065.1 added to model

```
New gene 1594.1 added to model
New gene 8435.1 added to model
New gene 6646.1 added to model
New gene 3988.1 added to model
New gene 4891.1 added to model
New gene 30061.1 added to model
New gene 9843.1 added to model
New gene 4524.1 added to model
```

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 s
```

```
#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.

## References

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- [4] Sahoo S, Haraldsdóttir HS, Fleming RM, Thiele I. Modeling the effects of commonly used drugs on human metabolism. *FEBS J.* 2015 Jan;282(2):297-317.
- [5] Vlassis N, Pacheco MP, Sauter T. Fast reconstruction of compact context-specific metabolic network models. *PLoS Comput Biol.* 2014 Jan;10(1).