Model manipulation

Author(s): Vanja Vlasov, Systems Biochemistry Group, LCSB, University of Luxembourg,

Thomas Pfau, Systems Biology Group, LSRU, University of Luxembourg.

Reviewer(s): Ines Thiele, Systems Biochemistry Group, LCSB, University of Luxembourg.

Catherine Clancy, Systems Biochemistry Group, LCSB, University of Luxembourg.

INTRODUCTION

In this tutorial, we will do a manipulation with a simple model of the first few reactions of the glycolysis metabolic pathway as created in the "Model Creation" tutorial.

Glycolysis is the metabolic pathway that occurs in most organisms in the cytosol of the cell. First, we will use the beginning of that pathway to create a simple constraint-based metabolic network (Figure 1).

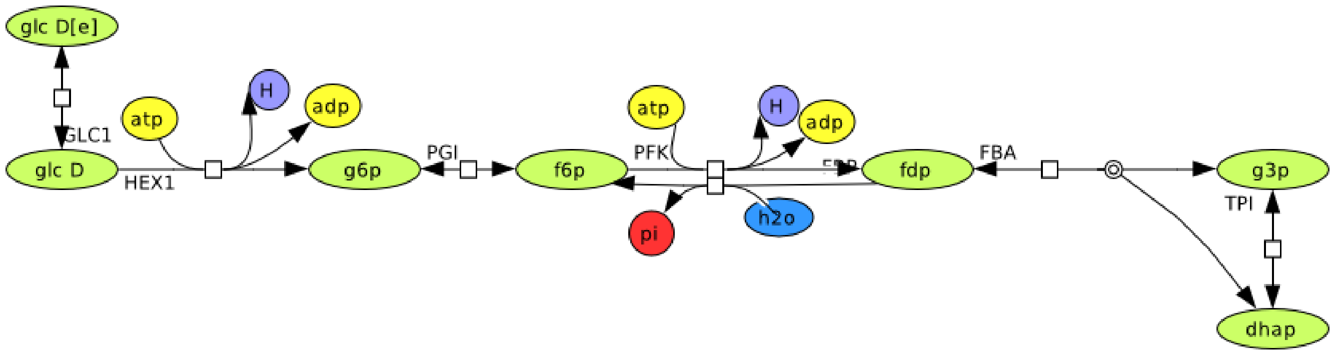


Figure 1: A small metabolic network consisting of the seven reactions in the glycolysis pathway.

At the beginning of the reconstruction, the initial step is to assess the integrity of the draft reconstruction. Furthermore, an evaluation of accuracy is needed: check necessity of each reaction and metabolite, the accuracy of the stoichiometry, and direction and reversibility of the reactions.

The metabolites structures and reactions are from the Virtual Metabolic Human database (VMH, http://vmh.life).

After creating or loading the model and to simulate different model conditions, the model can be modified, such as:

- Creating, adding and handling reactions;
- Adding exchange, sink and demand reactions;
- Altering reaction bounds;
- Altering reactions;
- Remove reactions and metabolites;
- Search for duplicates and comparison of two models;
- Changing the model objective;
- Changing the direction of reaction(s);
- Create gene-reaction-associations ('GPRs');

EQUIPMENT SETUP

Start CobraToolbox

```
initCobraToolbox;
```

```
      _____   _____   _____   _____     _____     |
     / ___| / _   \ |  _  \ |  _  \   / ___ \    |   COnstraint-Based Reconstruction and Analysis
    | |     | | | | | |_| | | |_| |  | |___| |   |   The COBRA Toolbox - 2017
    | |     | | | | |  _  { |  _  /  |  ___  |   |
    | |___  | |_| | | |_| | | | \ \  | |   | |   |   Documentation:
     \_____| \_____/ |_____/ |_|  \_\ |_|   |_|   |   http://opencobra.github.io/cobratoolbox
                                                  |

 > Checking if git is installed ...  Done.
 > Checking if the repository is tracked using git ...  Done.
 > Checking if curl is installed ...  Done.
 > Checking if remote can be reached ...  Done.
 > Initializing and updating submodules ... Done.
 > Adding all the files of The COBRA Toolbox ...  Done.
 > Define CB map output... set to svg.
 > Retrieving models ...   Done.
 > TranslateSBML is installed and working properly.
 > Configuring solver environment variables ...
   - [*---] ILOG_CPLEX_PATH: /opt/ibm/ILOG/CPLEX_Studio1271/cplex/matlab/x86-64_linux
   - [*---] GUROBI_PATH: /opt/gurobi702/linux64/matlab
   - [----] TOMLAB_PATH :  --> set this path manually after installing the solver ( see instructions )
   - [----] MOSEK_PATH :  --> set this path manually after installing the solver ( see instructions )
   Done.
 > Checking available solvers and solver interfaces ... Done.
 > Setting default solvers ... Done.
 > Saving the MATLAB path ... Done.
   - The MATLAB path was saved as ~/pathdef.m.

 > Summary of available solvers and solver interfaces

   Support     LP   MILP     QP   MIQP    NLP
 ----------------------------------------------------------------------
 cplex_direct  full               0      0      0      0      -
 dqqMinos      full               1      -      -      -      -
 glpk          full               1      1      -      -      -
 gurobi        full               1      1      1      1      -
 ibm_cplex     full               1      1      1      -      -
 matlab        full               1      -      -      -      1
 mosek         full               0      0      0      -      -
 pdco          full               1      -      1      -      -
 quadMinos     full               1      -      -      -      1
 tomlab_cplex  full               0      0      0      0      -
 qpng          experimental       -      -      1      -      -
 tomlab_snopt  experimental       -      -      -      -      0
 gurobi_mex    legacy             0      0      0      0      -
 lindo_old     legacy             0      -      -      -      -
 lindo_legacy  legacy             0      -      -      -      -
 lp_solve      legacy             1      -      -      -      -
 opti          legacy             0      0      0      0      0
 ----------------------------------------------------------------------
 Total         -                  8      3      4      1      2

 + Legend: - = not applicable, 0 = solver not compatible or not installed, 1 = solver installed.


 > You can solve LP problems using: 'dqqMinos' - 'glpk' - 'gurobi' - 'ibm_cplex' - 'matlab' - 'pdco' - '
 > You can solve MILP problems using: 'glpk' - 'gurobi' - 'ibm_cplex'
 > You can solve QP problems using: 'gurobi' - 'ibm_cplex' - 'pdco' - 'qpng'
 > You can solve MIQP problems using: 'gurobi'
 > You can solve NLP problems using: 'matlab' - 'quadMinos'

 > Checking for available updates ...
```

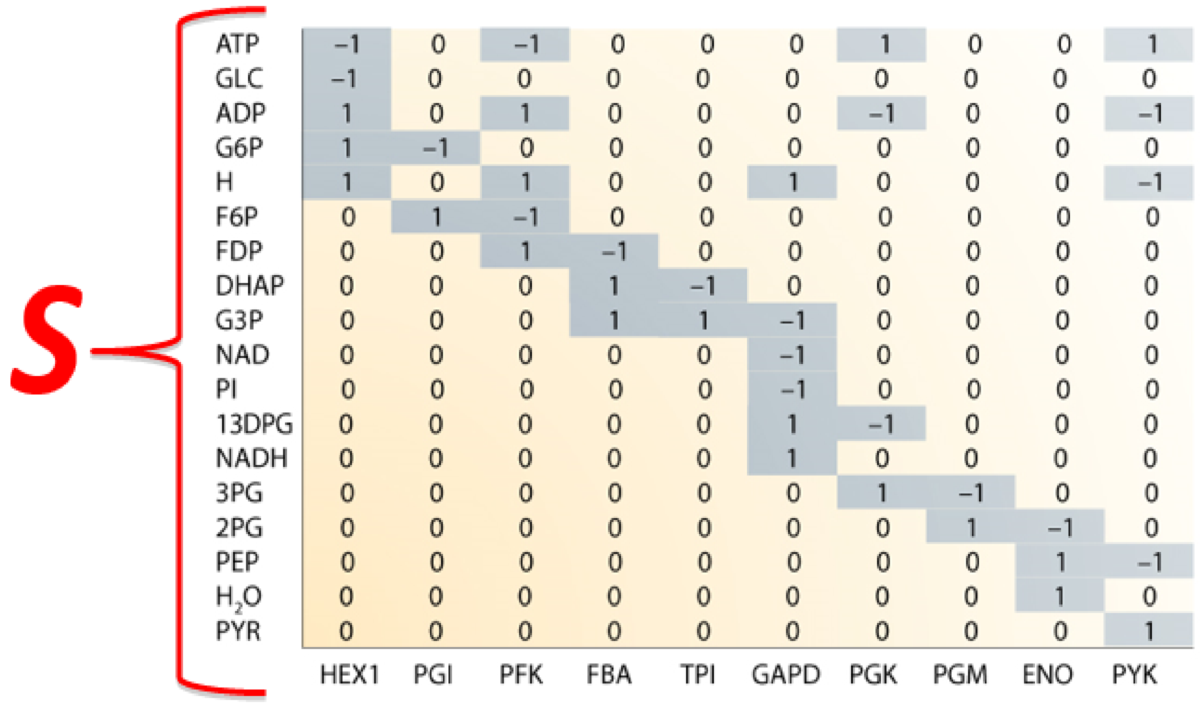
```
> The COBRA Toolbox is up-to-date.
```

PROCEDURE

Generate a network

A constraint-based metabolic model contains the stoichiometric matrix (S) with reactions and metabolites[1].

S is a stoichiometric representation of metabolic networks corresponding to the reactions in the biochemical pathway. In each column of the S is a biochemical reaction (n) and in each row is a precise metabolite (m). There is a stoichiometric coefficient of zero, which means that metabolite does not participate in that distinct reaction. The coefficient also can be positive when the appropriate metabolite is produced, or negative for every metabolite consumed[1].



	HEX1	PGI	PFK	FBA	TPI	GAPD	PGK	PGM	ENO	PYK
ATP	−1	0	−1	0	0	0	1	0	0	1
GLC	−1	0	0	0	0	0	0	0	0	0
ADP	1	0	1	0	0	0	−1	0	0	−1
G6P	1	−1	0	0	0	0	0	0	0	0
H	1	0	1	0	0	1	0	0	0	−1
F6P	0	1	−1	0	0	0	0	0	0	0
FDP	0	0	1	−1	0	0	0	0	0	0
DHAP	0	0	0	1	−1	0	0	0	0	0
G3P	0	0	0	1	1	−1	0	0	0	0
NAD	0	0	0	0	0	−1	0	0	0	0
PI	0	0	0	0	0	−1	0	0	0	0
13DPG	0	0	0	0	0	1	−1	0	0	0
NADH	0	0	0	0	0	1	0	0	0	0
3PG	0	0	0	0	0	0	1	−1	0	0
2PG	0	0	0	0	0	0	0	1	−1	0
PEP	0	0	0	0	0	0	0	0	1	−1
H_2O	0	0	0	0	0	0	0	0	1	0
PYR	0	0	0	0	0	0	0	0	0	1

Generate a model using the `createModel()` function:

```
ReactionFormulas = {'glc_D[e]  -> glc_D[c]',...
    'glc_D[c] + atp[c]  -> h[c] + adp[c] + g6p[c]',...
    'g6p[c]  <=> f6p[c]',...
    'atp[c] + f6p[c]  -> h[c] + adp[c] + fdp[c]',...
    'fdp[c] + h2o[c]  -> f6p[c] + pi[c]',...
    'fdp[c]  -> g3p[c] + dhap[c]',...
    'dhap[c]  -> g3p[c]'};
ReactionNames = {'GLCt1r', 'HEX1', 'PGI', 'PFK', 'FBP', 'FBA', 'TPI'};
lowerbounds = [-20, 0, -20, 0, 0, -20, -20];
upperbounds = [20, 20, 20, 20, 20, 20, 20];
model = createModel(ReactionNames, ReactionNames, ReactionFormulas,...
                   'lowerBoundList', lowerbounds, 'upperBoundList', upperbounds);
```

```
Warning: Metabolite glc_D[e] not in model - added to the model
Warning: Metabolite glc_D[c] not in model - added to the model
```

```
GLCt1r glc_D[e]  <=> glc_D[c]
Warning: Metabolite atp[c] not in model - added to the model
Warning: Metabolite h[c] not in model - added to the model
Warning: Metabolite adp[c] not in model - added to the model
Warning: Metabolite g6p[c] not in model - added to the model
HEX1 glc_D[c] + atp[c]  -> h[c] + adp[c] + g6p[c]
Warning: Metabolite f6p[c] not in model - added to the model
PGI g6p[c]  <=> f6p[c]
Warning: Metabolite fdp[c] not in model - added to the model
PFK atp[c] + f6p[c]  -> h[c] + adp[c] + fdp[c]
Warning: Metabolite h2o[c] not in model - added to the model
Warning: Metabolite pi[c] not in model - added to the model
FBP fdp[c] + h2o[c]  -> f6p[c] + pi[c]
Warning: Metabolite g3p[c] not in model - added to the model
Warning: Metabolite dhap[c] not in model - added to the model
FBA fdp[c]  <=> g3p[c] + dhap[c]
TPI dhap[c]  <=> g3p[c]
```

We can now have a look at the different model fields created. The stoichiometry is stored in the S field of the model, which was described above. Since this is commonly a sparse matrix (i.e. it contains a lot of zeros), it may be useful for your understanding to display the full representation:

```
full(model.S)
```

```
ans =

    -1     0     0     0     0     0     0
     1    -1     0     0     0     0     0
     0    -1     0    -1     0     0     0
     0     1     0     1     0     0     0
     0     1     0     1     0     0     0
     0     1    -1     0     0     0     0
     0     0     1    -1     1     0     0
     0     0     0     1    -1    -1     0
     0     0     0     0    -1     0     0
     0     0     0     0     1     0     0
```

It is required for a model to consist of the descriptive fields: `model.mets` and `model.rxns`, which represent the metabolites and the reactions respectively.

```
model.mets
```

```
ans =
    'glc_D[e]'
    'glc_D[c]'
    'atp[c]'
    'h[c]'
    'adp[c]'
    'g6p[c]'
    'f6p[c]'
    'fdp[c]'
    'h2o[c]'
    'pi[c]'
    'g3p[c]'
    'dhap[c]'
```

```
model.rxns
```

```
ans =
    'GLCt1r'
    'HEX1'
    'PGI'
    'PFK'
    'FBP'
    'FBA'
    'TPI'
```

The fields in a COBRA model are commonly column vectors, which is important to note when writing functions manipulating these fields.

There are a few more fields present in each COBRA model:

`model.lb`, indicating the lower bounds of each reaction, and `model.ub` indicating the upper bound of a reaction.

```
% this displays an array with reaction names and flux bounds.
[{'Reaction ID', 'Lower Bound', 'Upper Bound'};...
 model.rxns, num2cell(model.lb), num2cell(model.ub)]
```

```
ans =
    'Reaction ID'    'Lower Bound'    'Upper Bound'
    'GLCt1r'         [        -20]    [         20]
    'HEX1'           [          0]    [         20]
    'PGI'            [        -20]    [         20]
    'PFK'            [          0]    [         20]
    'FBP'            [          0]    [         20]
    'FBA'            [        -20]    [         20]
    'TPI'            [        -20]    [         20]
```

```
% This is a convenience function which does pretty much the same as the line above.
printFluxBounds(model);
```

```
Reaction ID    Lower Bound    Upper Bound
     GLCt1r        -20.000         20.000
       HEX1          0.000         20.000
        PGI        -20.000         20.000
        PFK          0.000         20.000
        FBP          0.000         20.000
        FBA        -20.000         20.000
        TPI        -20.000         20.000
```

Before we start to modify the model, it might be useful to store in the workspace some of the current properties of the model:

```
mets_length = length(model.mets)
```

```
mets_length = 12
```

```
rxns_length = length(model.rxns)
rxns_length = 7
```

Creating, adding and handling reactions

If we want to add a reaction to the model or modify an existing reaction use the function `addReaction()`.

We will add to the model some more reactions from glycolysis. There are two different approaches to adding reactions to a model:

1. The formula approach
2. The list appraoch

The formula approach

```
model = addReaction(model, 'GAPDH',...
       'reactionFormula', 'g3p[c] + nad[c] + 2 pi[c] -> nadh[c] + h[c] + 13bpg[c]');
```

```
Warning: Metabolite nad[c] not in model - added to the model
Warning: Metabolite nadh[c] not in model - added to the model
Warning: Metabolite 13bpg[c] not in model - added to the model
GAPDH 2 pi[c] + g3p[c] + nad[c]  -> h[c] + nadh[c] + 13bpg[c]
```

```
model = addReaction(model, 'PGK',...
       'reactionFormula', '13bpg[c] + adp[c] -> atp[c] + 3pg[c]');
```

```
Warning: Metabolite 3pg[c] not in model - added to the model
PGK adp[c] + 13bpg[c]  -> atp[c] + 3pg[c]
```

```
model = addReaction(model, 'PGM', 'reactionFormula', '3pg[c] <=> 2pg[c]' );
```

```
Warning: Metabolite 2pg[c] not in model - added to the model
PGM 3pg[c]  <=> 2pg[c]
```

Display the stoichiometric matrix after adding reactions (note the enlarge link when you move your mouse over the output to display the full matrix):

```
full(model.S)
```

```
ans =

    -1     0     0     0     0     0     0     0     0     0
     1    -1     0     0     0     0     0     0     0     0
     0    -1     0    -1     0     0     0     0     1     0
     0     1     0     1     0     0     0     1     0     0
     0     1     0     1     0     0     0     0    -1     0
     0     1    -1     0     0     0     0     0     0     0
     0     0     1    -1     1     0     0     0     0     0
     0     0     0     1    -1    -1     0     0     0     0
     0     0     0     0    -1     0     0     0     0     0
     0     0     0     0     1     0     0    -2     0     0
```

- one extra column is added (for added reaction) and 5 new rows(for nadh, nad, 13bpg, 2pg and 3pg metabolites)

If you want to search for the indecies of reactions in the model, and change the order of the select reactions, use the following functions:

```
rxnID = findRxnIDs(model, model.rxns)
```

```
rxnID =

     1
     2
     3
     4
     5
     6
     7
     8
     9
    10
```

```
model.rxns
```

```
ans =
    'GLCt1r'
    'HEX1'
    'PGI'
    'PFK'
    'FBP'
    'FBA'
    'TPI'
    'GAPDH'
    'PGK'
    'PGM'
```

```
model = moveRxn(model, 8, 1);
model.rxns
```

```
ans =
    'GAPDH'
    'GLCt1r'
    'HEX1'
    'PGI'
    'PFK'
    'FBP'
    'FBA'
    'TPI'
    'PGK'
    'PGM'
```

While the function `moveRxn` does not modify the network structure it can be useful in keeping a model tidy.

The list approach

```
model = addReaction(model, 'GAPDH2',...
    'metaboliteList', {'g3p[c]', 'nad[c]', 'pi[c]', '13bpg[c]', 'nadh[c]', 'h[c]' },...
    'stoichCoeffList', [-1; -1; -2; 1; 1; 1], 'reversible', false);
```

```
Warning: Model already has the same reaction you tried to add: GAPDH
```

- The `addReaction` function has the ability to recognize duplicate reactions. No reaction added here since the reaction is recognised to already exist in the model.

Since the fourth reaction we attempted to add to the model was a duplicate, the number of the reactions in the model should only of increased by three and the number of metabolites in the model should of only increaed by five (13bpg, nad, nadh, 23bpg and 2pg).

```
assert(length(model.rxns) == rxns_length + 3)
assert(length(model.mets) == mets_length + 5)
```

Adding exchange, sink and demand reactions

The are three specific types of reactions in a COBRA model that use and recycle accumulated metabolites, or produce the required metabolites:

1. *Exchange reactions* - are reactions that move metabolites across *in silico* compartments. These *in silico* compartments are representive of intra- and inter- cellular membranes.
2. *Sink reactions* - The metabolites, produced in reactions that are outside of an ambit of the system or in unknown reactions, are supplied to the network with reversible sink reactions.
3. *Demand reactions* - Irreversible reactions added to the model to consume metabolites that are deposited in the system.

There are two ways to implement these type of reactions:

1. **Use the `addReaction` function, detailing the stoichiometric coefficient:**

```
model = addReaction(model, 'EX_glc_D[e]', 'metaboliteList', {'glc_D[e]'} ,...
                    'stoichCoeffList', [-1]);
```

```
EX_glc_D[e] glc_D[e]  <=>
```

To find exchange reactions in the model use the `findExcRxns` function:

```
% determines whether a reaction is a general exchange reaction and
% whether its an uptake.
[selExc, selUpt] = findExcRxns(model, 0, 1)
```

```
selExc =
   0
   0
   0
   0
   0
   0
   0
   0
   0
   0
   1

selUpt =
   0
   0
   0
   0
   0
   0
   0
   0
   0
   0
   0
```

2. Use a utility function to create the specific type of reaction: `addExchangeRxn`, `addSinkReactions`, `addDemandReaction`.

```
model = addExchangeRxn(model, {'glc_D[e]', 'glc_D[c]'})
```

```
Warning: Reaction with the same name already exists in the model, updating the reaction
EX_glc_D[e] glc_D[e]  <=>
EX_glc_D[c] glc_D[c]  <=>
model =
          rxns: {12×1 cell}
             S: [17×12 double]
            lb: [12×1 double]
            ub: [12×1 double]
             c: [12×1 double]
          mets: {17×1 cell}
             b: [17×1 double]
         rules: {12×1 cell}
         genes: {0×1 cell}
        osense: -1
        csense: [17×1 char]
    rxnGeneMat: [12×0 double]
      rxnNames: {12×1 cell}
    subSystems: {12×1 cell}
      metNames: {17×1 cell}
       grRules: {12×1 cell}
```

```
model = addSinkReactions(model, {'13bpg[c]', 'nad[c]'})
```

```
sink_13bpg[c] 13bpg[c]  <=>
sink_nad[c] nad[c]  <=>
model =
          rxns: {14×1 cell}
             S: [17×14 double]
            lb: [14×1 double]
            ub: [14×1 double]
             c: [14×1 double]
          mets: {17×1 cell}
             b: [17×1 double]
         rules: {14×1 cell}
         genes: {0×1 cell}
        osense: -1
        csense: [17×1 char]
    rxnGeneMat: [14×0 double]
      rxnNames: {14×1 cell}
    subSystems: {14×1 cell}
      metNames: {17×1 cell}
       grRules: {14×1 cell}
```

```
model = addDemandReaction(model, {'dhap[c]', 'g3p[c]'})
```

```
DM_dhap[c] dhap[c]  ->
DM_g3p[c] g3p[c]  ->
model =
          rxns: {16×1 cell}
             S: [17×16 double]
            lb: [16×1 double]
            ub: [16×1 double]
             c: [16×1 double]
          mets: {17×1 cell}
             b: [17×1 double]
         rules: {16×1 cell}
         genes: {0×1 cell}
        osense: -1
        csense: [17×1 char]
    rxnGeneMat: [16×0 double]
      rxnNames: {16×1 cell}
    subSystems: {16×1 cell}
      metNames: {17×1 cell}
       grRules: {16×1 cell}
```

Setting a ratio between the reactions

It is important to emphasise that previous knowledge base information should be taken into account when generating a model. If this information is ommited, the analysis of a model could be adversely altered and consequent results not representative of the network.

For instance, if it is known that the flux of one reaction is *X* times the flux of another reaction, it is recommended to 'couple' (i.e., set a ratio) the reactions in the model.

E.g. $1\ v\,\mathrm{EX\_glc\_}D[c] = 2\ v\,\mathrm{EX\_glc\_}D[e]$

```
model = addRatioReaction (model, {'EX_glc_D[c]', 'EX_glc_D[e]'}, [1; 2])
```

```
model =
          rxns: {16×1 cell}
             S: [18×16 double]
            lb: [16×1 double]
            ub: [16×1 double]
             c: [16×1 double]
          mets: {18×1 cell}
             b: [18×1 double]
         rules: {16×1 cell}
         genes: {0×1 cell}
        osense: -1
        csense: [18×1 char]
    rxnGeneMat: [16×0 double]
      rxnNames: {16×1 cell}
    subSystems: {16×1 cell}
      metNames: {18×1 cell}
       grRules: {16×1 cell}
```

```
note: 'EX_glc_D[c] andEX_glc_D[e]are set to have a ratio of1:2.'
```

Constraining the flux boundaries of a reaction

In order to respect the transport and exchange potential of a particular metabolite, or to resemble the different conditions in the model, we frequently need to set appropriate limits of the reactions.

```
model = changeRxnBounds(model, 'EX_glc_D[e]', -18.5, 'l');
```

Modifying reactions

The `addReaction` function is also a good choice to modify reactions. By supplying to the function a new stoichiometry, the old will be overwritten.

For example, further up, we added the wrong stoichiometry for the GAP-Dehydrogenase with a coefficient of 2 for phosphate. Print the reaction to visulize:

```
printRxnFormula(model, 'rxnAbbrList', 'GAPDH');
```

```
GAPDH 2 pi[c] + g3p[c] + nad[c]  -> h[c] + nadh[c] + 13bpg[c]
```

Correct the reaction using `addReaction:` with the corrected stoichiometry:

```
model = addReaction(model, 'GAPDH',...
    'metaboliteList', {'g3p[c]', 'nad[c]', 'pi[c]', '13bpg[c]', 'nadh[c]', 'h[c]' },...
    'stoichCoeffList', [-1; -1; -1; 1; 1; 1]);
```

```
Warning: Reaction with the same name already exists in the model, updating the reaction
GAPDH pi[c] + g3p[c] + nad[c]  -> h[c] + nadh[c] + 13bpg[c]
```

We can add a gene rule to the reaction using the `changeGeneAssociation` function:

```
model = changeGeneAssociation(model, 'GAPDH', 'G1 and G2');
```

```
New gene G1 added to model
New gene G2 added to model
```

```
printRxnFormula(model, 'rxnAbbrList', {'GAPDH'}, 'gprFlag', true);
```

```
GAPDH pi[c] + g3p[c] + nad[c]  -> h[c] + nadh[c] + 13bpg[c]  G1 and G2
```

Alternatively, one can add a gene rule to a reaction using the `addReaction` function, and within this function applying the `geneRule` input option.

```
model = addReaction(model, 'PGK', 'geneRule', 'G2 or G3', 'printLevel', 0);
```

```
Warning: Reaction with the same name already exists in the model, updating the reaction
New gene G3 added to model
```

```
printRxnFormula(model, 'gprFlag', true);
```

```
GAPDH pi[c] + g3p[c] + nad[c]  -> h[c] + nadh[c] + 13bpg[c]  G1 and G2
GLCt1r glc_D[e]  <=> glc_D[c]
HEX1 glc_D[c] + atp[c]  -> h[c] + adp[c] + g6p[c]
PGI g6p[c]  <=> f6p[c]
PFK atp[c] + f6p[c]  -> h[c] + adp[c] + fdp[c]
FBP fdp[c] + h2o[c]  -> f6p[c] + pi[c]
FBA fdp[c]  <=> g3p[c] + dhap[c]
TPI dhap[c]  <=> g3p[c]
PGK adp[c] + 13bpg[c]  -> atp[c] + 3pg[c]  G2 or G3
PGM 3pg[c]  <=> 2pg[c]
EX_glc_D[e] glc_D[e]  <=> 2 Ratio_EX_glc_D[c]_EX_glc_D[e]
EX_glc_D[c] glc_D[c] + Ratio_EX_glc_D[c]_EX_glc_D[e]  <=>
sink_13bpg[c] 13bpg[c]  <=>
sink_nad[c] nad[c]  <=>
DM_dhap[c] dhap[c]  ->
DM_g3p[c] g3p[c]  ->
```

Remove reactions and metabolites

To delete reactions from the model, use the `removeRxns` function:

```
model = removeRxns(model, {'EX_glc_D[c]', 'EX_glc_D[e]', 'sink_13bpg[c]', ...
                              'sink_nad[c]', 'DM_dhap[c]', 'DM_g3p[c]'});

assert(rxns_length + 3 == length(model.rxns));
```

- The reaction length was updated since a number of reactions were removed from the model.

To remove metabolites from the model, use the `removeMetabolites` function:

```
  model = removeMetabolites(model, {'3pg[c]', '2pg[c]'}, false);
  printRxnFormula(model, 'rxnAbbrList', {'GAPDH'}, 'gprFlag', true);
```

```
GAPDH pi[c] + g3p[c] + nad[c]  -> h[c] + nadh[c] + 13bpg[c]  G1 and G2
```

- The 'GAPDH' reaction is still present in the model since there are other metabolites in the reaction, not just the metabolites we tried to delete. The 'false' input option of the `removeMetabolites` function indictes that only empty reactions should be removed.

To delete metabolites and reactions with zero rows and columns, the `removeTrivialStoichiometry` function can be used:

```
  model = removeTrivialStoichiometry(model)
```

```
model = 
          rxns: {9×1 cell}
             S: [15×9 double]
            lb: [9×1 double]
            ub: [9×1 double]
             c: [9×1 double]
          mets: {15×1 cell}
             b: [15×1 double]
         rules: {9×1 cell}
         genes: {3×1 cell}
        osense: -1
        csense: [15×1 char]
    rxnGeneMat: [9×3 double]
      rxnNames: {9×1 cell}
    subSystems: {9×1 cell}
      metNames: {15×1 cell}
       grRules: {9×1 cell}
          note: 'EX_glc_D[c] andEX_glc_D[e]are set to have a ratio of1:2.'
```

```
model = removeRxns(model, {'GAPDH', 'PGK'});
```

Search for duplicate reactions and comparison of two models

Since genome-scale metabolic models are expanding every day[2], the need to compare models is also growing. The elementary functions in The Corba Toolbox can support simultaneous structural analysis and comparison.

Checking for reaction duplicates with the `checkDuplicateRxn()` function (i.e. by reaction abbreviation), using either the method:

- 'S' (does not detect reverse reactions), or
- 'FR' (neglects reactions direction).

For demonstration of the S method, first check for dupicates and then add the duplicate reaction to the model:

```
[model, removedRxn, rxnRelationship] = checkDuplicateRxn(model, 'S', 1, 1);
```

```
Checking for reaction duplicates by stoichiometry ...
 no duplicates found.
```

```
printRxnFormula(model, 'rxnAbbrList', {'GLCt1r'});
```

```
GLCt1r glc_D[e]  <=> glc_D[c]
```

```
model = addReaction(model, 'GLCt1r_duplicate_reverse',...
                    'metaboliteList', {'glc_D[e]', 'glc_D[c]'},...
                    'stoichCoeffList', [1 -1], 'lowerBound', 0, ...
                    'upperBound', 20, 'checkDuplicate', 0);
```

```
GLCt1r_duplicate_reverse glc_D[c]  -> glc_D[e]
```

Detecting duplicates using the S method:

```
method = 'S';
[model,removedRxn, rxnRelationship] = checkDuplicateRxn(model, method, 1, 1);
```

```
Checking for reaction duplicates by stoichiometry ...
 no duplicates found.
```

- The GLCt1r_duplicate_reverse reaction is not detected as a duplicate reaction therefore will not be removed as the S method does not detect a reverse reactions.
- Reevaluate the reaction length to show this:

```
assert(rxns_length + 1 == length(model.rxns));
```

Detecting duplicates using the FR method:

```
method = 'FR';
[model, removedRxn, rxnRelationship] = checkDuplicateRxn(model, method, 1, 1)
```

```
Checking for reaction duplicates by stoichiometry (up to orientation) ...
     Keep:  GLCt1r glc_D[e]  <=> glc_D[c]
Duplicate:  GLCt1r_duplicate_reverse glc_D[c]  -> glc_D[e]
```

```
model = 
          rxns: {7×1 cell}
             S: [12×7 double]
            lb: [7×1 double]
            ub: [7×1 double]
             c: [7×1 double]
          mets: {12×1 cell}
             b: [12×1 double]
         rules: {7×1 cell}
         genes: {3×1 cell}
        osense: -1
        csense: [12×1 char]
    rxnGeneMat: [7×3 double]
      rxnNames: {7×1 cell}
    subSystems: {7×1 cell}
      metNames: {12×1 cell}
       grRules: {7×1 cell}
          note: 'EX_glc_D[c] andEX_glc_D[e]are set to have a ratio of1:2.'
```

```
removedRxn = 8
rxnRelationship = 1
```

```
assert(rxns_length == length(model.rxns))
```

- The GLCt1r_duplicate_reverse reaction is detected as a duplicate reaction therefore will not be removed as the FR method does detect a reverse reactions.

Checking for non-unique reactions and metabolites in a model using the `checkCobraModelUnique` function:

```
model = checkCobraModelUnique(model, false)
```

```
model = 
          rxns: {7×1 cell}
             S: [12×7 double]
            lb: [7×1 double]
            ub: [7×1 double]
             c: [7×1 double]
          mets: {12×1 cell}
             b: [12×1 double]
         rules: {7×1 cell}
         genes: {3×1 cell}
        osense: -1
        csense: [12×1 char]
    rxnGeneMat: [7×3 double]
      rxnNames: {7×1 cell}
    subSystems: {7×1 cell}
      metNames: {12×1 cell}
       grRules: {7×1 cell}
          note: 'EX_glc_D[c] andEX_glc_D[e]are set to have a ratio of1:2.'
```

- Input option 'false' means the function will not renames non-unique reaction names and metabolites

Changing the model's objective

Simulating specific objectives of a model is often necessary in order to perform an investigation of different conditions. One of the fundamental objectives is optimal growth[3]. The model can be modified to get different conditions by changing the model objective.

One reaction is set as the objective, and has an objective coefficient of 0.5:

```
modelNew = changeObjective(model, 'GLCt1r', 0.5);
```

Multiple reactions are set collectively as the objective, and the default objective coefficient of 1 for each reaction:

```
modelNew = changeObjective(model, {'PGI'; 'PFK'; 'FBP'});
```

The direction of reactions

Sometimes it may be important to have all reactions in a model as irreversible reactions (i.e. only allow a forward reaction / positive flux in reactions). This can be important if, for example, the absolute flux values are of interest, and negative flux would reduce an objective while it should actually increase it. The COBRA Toolbox offers functionality to change all reactions in a model to an irreversible format. IT does this by splitting all reversible reactions and adjusting the respective lower and upper bounds, such that the model capacities stay the same.

Let us see, how the glycolysis model currently looks:

```
printRxnFormula(model);
```

```
GLCt1r glc_D[e]  <=> glc_D[c]
HEX1 glc_D[c] + atp[c]  -> h[c] + adp[c] + g6p[c]
PGI g6p[c]  <=> f6p[c]
PFK atp[c] + f6p[c]  -> h[c] + adp[c] + fdp[c]
FBP fdp[c] + h2o[c]  -> f6p[c] + pi[c]
FBA fdp[c]  <=> g3p[c] + dhap[c]
TPI dhap[c]  <=> g3p[c]
```

To convert a model to an irreversible model use the *convertToIrreversible* command:

```
[modelIrrev, matchRev, rev2irrev, irrev2rev] = convertToIrreversible(model);
```

Compare the irreversible model with the original model:

```
printRxnFormula(modelIrrev);
```

```
GLCt1r_f glc_D[e]  -> glc_D[c]
HEX1 glc_D[c] + atp[c]  -> h[c] + adp[c] + g6p[c]
PGI_f g6p[c]  -> f6p[c]
PFK atp[c] + f6p[c]  -> h[c] + adp[c] + fdp[c]
FBP fdp[c] + h2o[c]  -> f6p[c] + pi[c]
```

```
FBA_f fdp[c]  -> g3p[c] + dhap[c]
TPI_f dhap[c]  -> g3p[c]
GLCt1r_b glc_D[c]  -> glc_D[e]
PGI_b f6p[c]  -> g6p[c]
FBA_b g3p[c] + dhap[c]  -> fdp[c]
TPI_b g3p[c]  -> dhap[c]
```

- You will notice, that there are more reactions in this model and that all reactions which have a lower bound < 0 are split in two.

There is also a function to convert an irreversible model to a reversible model:

```
modelRev = convertToReversible(modelIrrev);
```

If we now compare the reactions of this model with those from the original model, they should look the same.

```
printRxnFormula(modelRev);
```

```
GLCt1r glc_D[e]  <=> glc_D[c]
HEX1 glc_D[c] + atp[c]  -> h[c] + adp[c] + g6p[c]
PGI g6p[c]  <=> f6p[c]
PFK atp[c] + f6p[c]  -> h[c] + adp[c] + fdp[c]
FBP fdp[c] + h2o[c]  -> f6p[c] + pi[c]
FBA fdp[c]  <=> g3p[c] + dhap[c]
TPI dhap[c]  <=> g3p[c]
```

Create gene-reaction-associations (GPRs) from scratch.

```
model.genes = [];
model.rxnGeneMat = [];
modelgrRule = model.grRules;
for i = 1 : length(modelgrRule)
    if ~isempty(modelgrRule{i})
        model = changeGeneAssociation(model, model.rxns{i}, modelgrRule{i});
    end
end
```

Check that there are no empy columns left.

```
find(sum(model.rxnGeneMat)==0)
```

```
ans = 1
```

Replace an existing GPRs with a new one.

Here, we will search for all instances of the existing GPR and replace it with the new one.

Define the old and the new one.

```
GPRsReplace = {'(126.1) or (137872.1) ' '126.1 or 137872.1'};
GPRexist = (model.grRules)
```

```
GPRexist =
        ''
        ''
        ''
        ''
        ''
        ''
        ''
```

```
a = 1;
for  i = 1 : size(GPRsReplace,1)
    tmp2=[];
    for j = 1 :length(GPRexist)
        tmp2 = strmatch(GPRsReplace{i,1}, GPRexist{j});
        % replace old GPR by new
        model.grRules{j} = GPRsReplace{i,2};
    end
end

GPRnew = model.grRules
```

```
GPRnew =
    '126.1 or 137872.1'
    '126.1 or 137872.1'
    '126.1 or 137872.1'
    '126.1 or 137872.1'
    '126.1 or 137872.1'
    '126.1 or 137872.1'
    '126.1 or 137872.1'
```

Remove issues with hyphens in the GPR definitions.

```
for i = 1 : length(model.grRules)
    model.grRules{i} = strrep(model.grRules{i}, '''', '');
end
```

Remove spaces from reaction abbreviations.

```
for i = 1 : length(model.rxns)
    model.rxns{i} = strrep(model.rxns{i}, ' ', '');
end
```

Remove unneccessary brackets from the GPR associations.

```
for i = 1 : length(model.grRules)
    model.grRules{i} = strrep(model.grRules{i}, '''', '');
    % remove unnecessary brackets
    if length(strfind(model.grRules{i},'and')) == 0 % no AND in gprs
        model.grRules{i} = strrep(model.grRules{i}, '(', '');
        model.grRules{i} = strrep(model.grRules{i}, ')', '');

    elseif length(strfind(model.grRules{i},'or')) == 0 % no OR in gprs
        model.grRules{i} = strrep(model.grRules{i}, '(', '');
        model.grRules{i} = strrep(model.grRules{i}, ')', '');
    elseif length(strfind(model.grRules{i},'(')) == 1 && length(strfind...
            (model.grRules{i},'or')) == 0 && length(strfind...
            (model.grRules{i},'and')) == 0
        model.grRules{i} = strrep(model.grRules{i}, '(', '');
        model.grRules{i} = strrep(model.grRules{i}, ')', '');
    end
end
```

REFERENCES

[1] Orth, J. D., Thiele I., and Palsson, B. Ø. What is flux balance analysis? *Nat. Biotechnol.,* 28(3), 245–248 (2010).

[2] Feist, A. M., Palsson, B. Ø. The growing scope of applications of genome-scale metabolic reconstructions: the case of *E. coli*. *Nature Biotechnology,* 26(6), 659–667 (2008).

[3] Feist, A. M., Palsson, B. Ø. The Biomass Objective Function. *Current Opinion in Microbiology,* 13(3), 344–349 (2010).