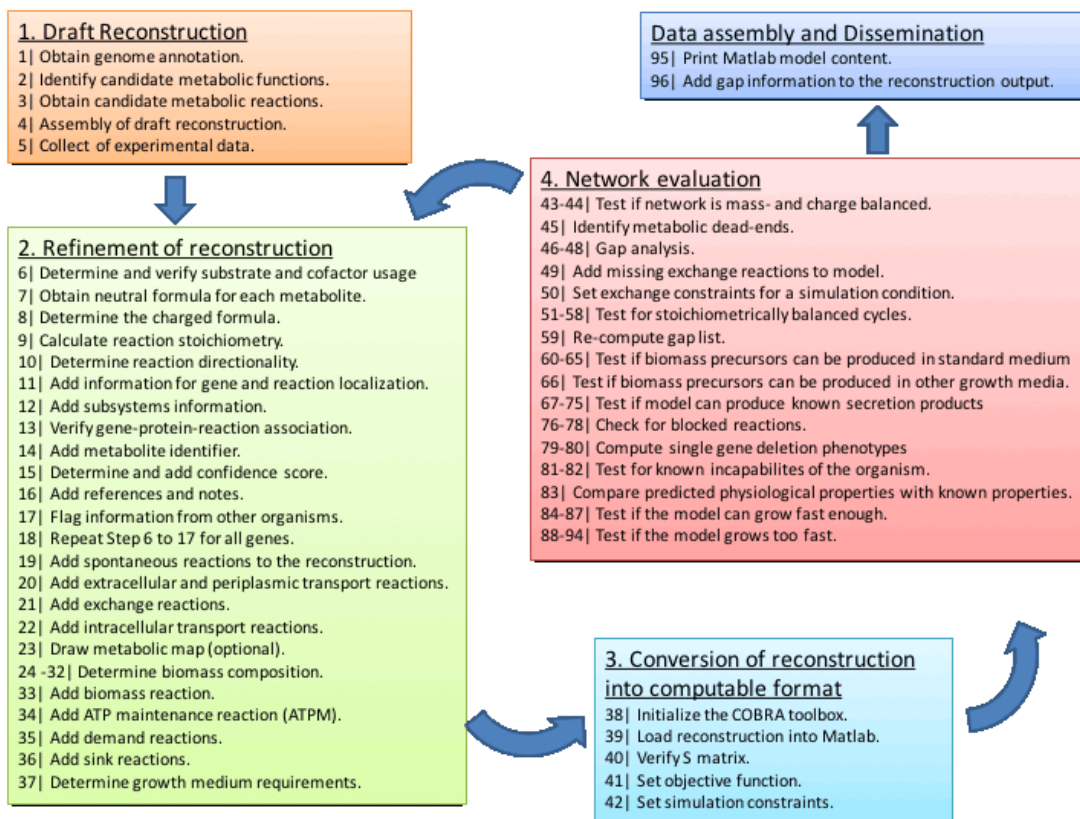# Example use of functions listed in the Standard operating procedure for metabolic reconstruction.

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INTRODUCTION

This tutorial has been adapted and expanded from the protocol for generating metabolic network reconstruction [1].

1. Draft Reconstruction
1| Obtain genome annotation.
2| Identify candidate metabolic functions.
3| Obtain candidate metabolic reactions.
4| Assembly of draft reconstruction.
5| Collect of experimental data.

2. Refinement of reconstruction
6| Determine and verify substrate and cofactor usage
7| Obtain neutral formula for each metabolite.
8| Determine the charged formula.
9| Calculate reaction stoichiometry.
10| Determine reaction directionality.
11| Add information for gene and reaction localization.
12| Add subsystems information.
13| Verify gene-protein-reaction association.
14| Add metabolite identifier.
15| Determine and add confidence score.
16| Add references and notes.
17| Flag information from other organisms.
18| Repeat Step 6 to 17 for all genes.
19| Add spontaneous reactions to the reconstruction.
20| Add extracellular and periplasmic transport reactions.
21| Add exchange reactions.
22| Add intracellular transport reactions.
23| Draw metabolic map (optional).
24 -32| Determine biomass composition.
33| Add biomass reaction.
34| Add ATP maintenance reaction (ATPM).
35| Add demand reactions.
36| Add sink reactions.
37| Determine growth medium requirements.

3. Conversion of reconstruction into computable format
38| Initialize the COBRA toolbox.
39| Load reconstruction into Matlab.
40| Verify S matrix.
41| Set objective function.
42| Set simulation constraints.

4. Network evaluation
43-44| Test if network is mass- and charge balanced.
45| Identify metabolic dead-ends.
46-48| Gap analysis.
49| Add missing exchange reactions to model.
50| Set exchange constraints for a simulation condition.
51-58| Test for stoichiometrically balanced cycles.
59| Re-compute gap list.
60-65| Test if biomass precursors can be produced in standard medium
66| Test if biomass precursors can be produced in other growth media.
67-75| Test if model can produce known secretion products
76-78| Check for blocked reactions.
79-80| Compute single gene deletion phenotypes
81-82| Test for known incapabilites of the organism.
83| Compare predicted physiological properties with known properties.
84-87| Test if the model can grow fast enough.
88-94| Test if the model grows too fast.

Data assembly and Dissemination
95| Print Matlab model content.
96| Add gap information to the reconstruction output.

This tutorial will illustrate the example input for The COBRA Toolbox functions applied in the <u>steps 38 to 96</u> of the protocol workflow, using the E. coli core reconstruction [2] as the model of choice.

When Matlab/Toolbox commands are following by a ';' the output is not printed. Omitting the ';' invokes printing of the variable content.

EQUIPMENT SETUP

Step 38. 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' package does not require additional instalation and configuration.

```
solverName = 'glpk';
solverType = 'LP';
changeCobraSolver(solverName, solverType);
```

However, for the analysis of large models, such as Recon 3, it is not recommended to use the 'glpk' package but rather an industrial strength solver, such as the 'gurobi' package. For detailed information, refer to The COBRA Toolbox solver instalation guide.

A solver package may offer different types of optimization programmes to solve a problem. The above example used a LP optimization, other types of optimization programmes include; mixed-integer linear programming ('MILP'), quadratic programming ('QP'), and mixed-integer quadratic programming ('MIQP').

```
warning off MATLAB:subscripting:noSubscriptsSpecified
```

PROCEDURE

Step 39. Load reconstruction into Matlab.

The metabolic network reconstruction, containing a reaction and metabolite list, is contained by the file 'Ecoli_core_model.mat'.

```
load('Ecoli_core_model.mat');
modelEcore = model; % TODO model does not load into workspace a 'modelEcore' but as 'model'.
```

The reconstruction is contained in the resulting model structure.
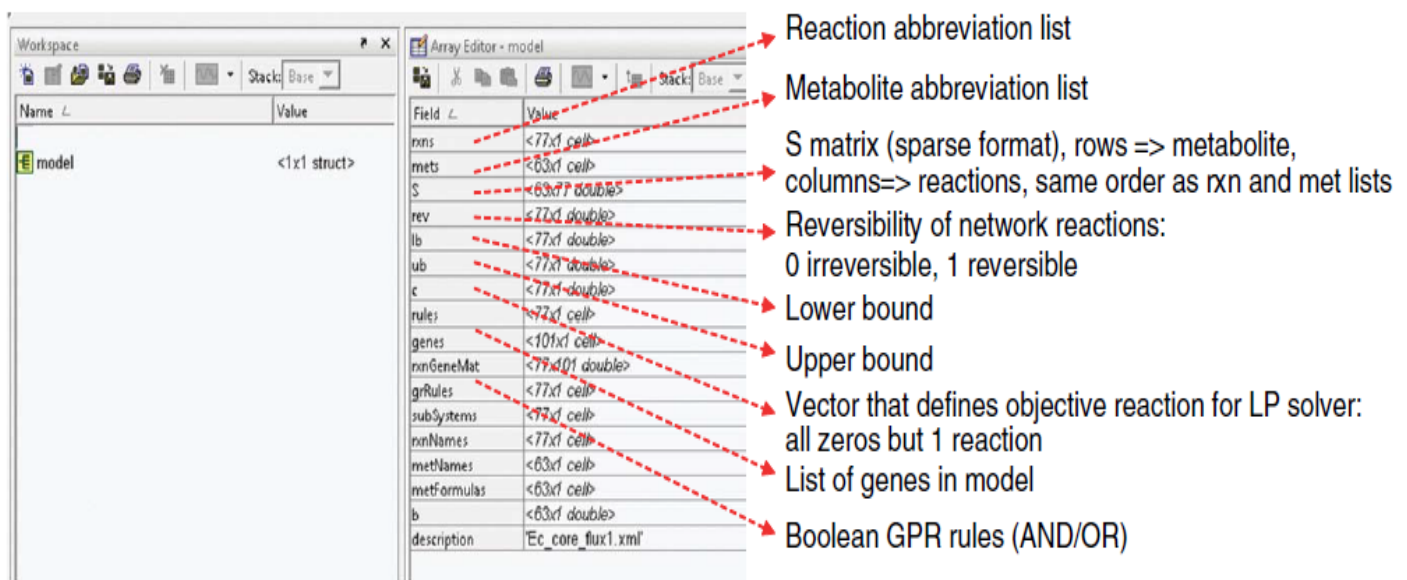
The figure above shows the data contained in the different structure fields. We will use this model structure for all consequent computation if not noted differently.

Use the command `open` to view the model structure in Matlab.

```
open modelEcore
```

The content of the structure can be assessed as follows:

- You wish to see the abbreviation of the 8th metabolite in the model:

```
modelEcore.mets{8}
```

```
ans = acald[c]
```

- You wish to see the abbreviation of the 1st reaction in the model:

```
modelEcore.rxns{1}
```

```
ans = ACALD
```

- You wish to see the entry of the stoichiometric matrix of the 1st reaction (column) and 8th metabolite (row):

```
modelEcore.S(8,1)
```

```
ans =
   (1,1)        -1
```

- Print the reaction formula:

```
printRxnFormula(modelEcore,modelEcore.rxns(1))
```

```
ACALD acald[c] + coa[c] + nad[c]   <=> accoa[c] + h[c] + nadh[c]
ans =
    'acald[c] + coa[c] + nad[c]   <=> accoa[c] + h[c] + nadh[c] '
```

- You want to change the lower bound (lb) of the 5th reaction to 10 mmol/gDW/h (without using any COBRA Toolbox functions):

```
modelEcore.lb(5) = 10
```

```
modelEcore =
          rxns: {95×1 cell}
          mets: {72×1 cell}
             S: [72×95 double]
           rev: [95×1 double]
            lb: [95×1 double]
            ub: [95×1 double]
             c: [95×1 double]
         rules: {95×1 cell}
         genes: {137×1 cell}
```

```
    rxnGeneMat: [95×137 double]
        grRules: {95×1 cell}
     subSystems: {95×1 cell}
       rxnNames: {95×1 cell}
       metNames: {72×1 cell}
    metFormulas: {72×1 cell}
              b: [72×1 double]
    description: 'Ecoli_core_model'
```

- You want to add a field to the model structure.

A note:

```
modelEcore.newField = 'ABC — a note'
```

```
modelEcore =
           rxns: {95×1 cell}
           mets: {72×1 cell}
              S: [72×95 double]
            rev: [95×1 double]
             lb: [95×1 double]
             ub: [95×1 double]
              c: [95×1 double]
          rules: {95×1 cell}
          genes: {137×1 cell}
     rxnGeneMat: [95×137 double]
        grRules: {95×1 cell}
     subSystems: {95×1 cell}
       rxnNames: {95×1 cell}
       metNames: {72×1 cell}
    metFormulas: {72×1 cell}
              b: [72×1 double]
    description: 'Ecoli_core_model'
       newField: 'ABC — a note'
```

An array B = [1 2 3]

```
B = [1 2 3];
modelEcore.newField = B
```

```
modelEcore =
           rxns: {95×1 cell}
           mets: {72×1 cell}
              S: [72×95 double]
            rev: [95×1 double]
             lb: [95×1 double]
             ub: [95×1 double]
              c: [95×1 double]
          rules: {95×1 cell}
          genes: {137×1 cell}
     rxnGeneMat: [95×137 double]
        grRules: {95×1 cell}
     subSystems: {95×1 cell}
       rxnNames: {95×1 cell}
       metNames: {72×1 cell}
    metFormulas: {72×1 cell}
              b: [72×1 double]
    description: 'Ecoli_core_model'
       newField: [1 2 3]
```

- Create a list of strings:

```
ListStrings = {'A' 'B' 'C'}
```

```
ListStrings =
     'A'      'B'       'C'
```

- Create a list of numbers:

```
ListNumbers = [1 2 3]
```

```
ListNumbers =

     1     2     3
```

- Transpose a list:

```
ListTranspose = ListNumbers'
```

```
ListTranspose =

     1
     2
     3
```

- Find the index of a reaction, e.g., 'ATPM', in the model

```
rxnList='ATPM';
rxnID = findRxnIDs(modelEcore, rxnList)
```

```
rxnID = 11
```

Step 40. Verify S matrix.

Remember that in the S matrix the rows and the columns correspond to the metabolites and the reactions of the model, respectively. The number of non-zero entries in the S matrix is visualized graphically below using a spy image.

```
spy(modelEcore.S)
```
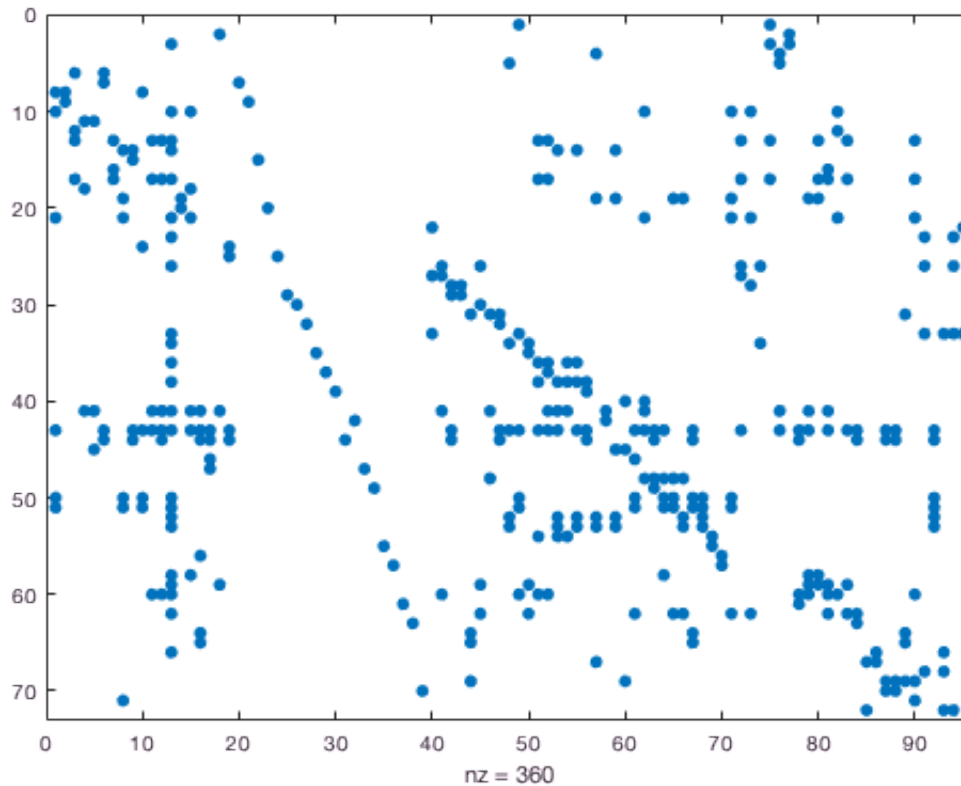
To put this number into perspective, we can calculate the percentage of non-zero entries of S.

```
[a,b] = size(modelEcore.S);
nz = nnz(modelEcore.S);
Perc_nz = nz*100/(a*b)
```

```
Perc_nz = 5.2632
```

- Many large scale models have less than 1% of non-zero entries in the S matrix.
- Looking at the S matrix is a quick way to see whether there is something obviously wrong with the model and the S matrix.

Here are two further examples of S matrices visualized using a spy image:

E. coli - AF1260 [3]
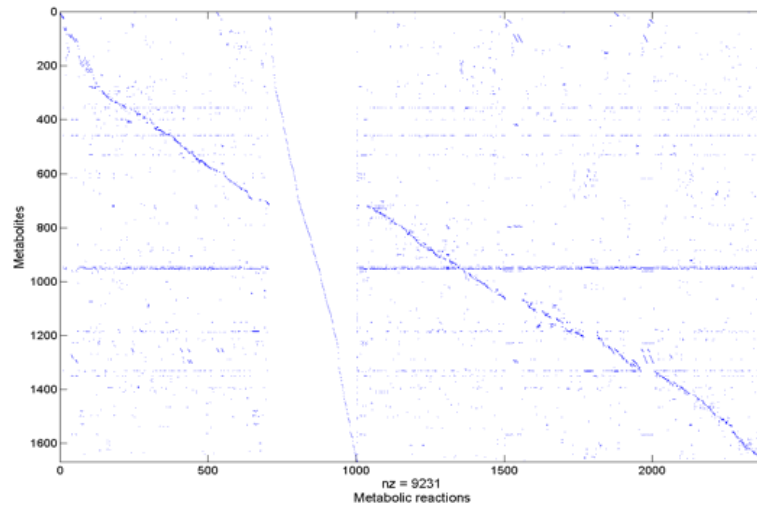
Geobacter sulfurreducans [4]
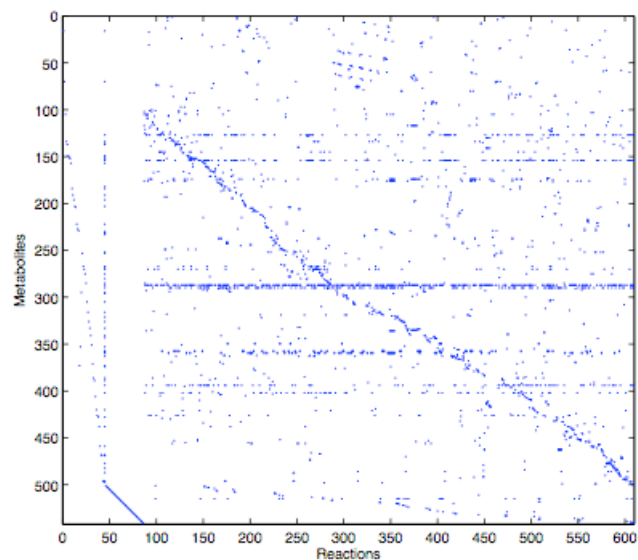
Consider to also use the tutorial on 'Numerical properties of a reconstruction' to investigate the propeties of the S matrix.

Step 41. Set objective function.

We will set the biomass reaction (Biomass_Ecoli_core_w_GAM) of the *E. coli* core model as objective function:

```
modelEcore = changeObjective(modelEcore, 'Biomass_Ecoli_core_w_GAM');
```

If you wish to check which reaction(s) make up the objective function and its components, use the following function:

```
objectiveAbbr = checkObjective(modelEcore)
```

```
summaryT =
    Coefficient    Metabolite    metID        Reaction              RxnID

    _____    _____    _____    _____    _____

      -1.496        3pg[c]         3      Biomass_Ecoli_core_w_GAM      13
      -3.7478       accoa[c]      10      Biomass_Ecoli_core_w_GAM      13
       59.81        adp[c]        13      Biomass_Ecoli_core_w_GAM      13
       4.1182       akg[c]        14      Biomass_Ecoli_core_w_GAM      13
      -59.81        atp[c]        17      Biomass_Ecoli_core_w_GAM      13
       3.7478       coa[c]        21      Biomass_Ecoli_core_w_GAM      13
      -0.361        e4p[c]        23      Biomass_Ecoli_core_w_GAM      13
      -0.0709       f6p[c]        26      Biomass_Ecoli_core_w_GAM      13
      -0.129        g3p[c]        33      Biomass_Ecoli_core_w_GAM      13
      -0.205        g6p[c]        34      Biomass_Ecoli_core_w_GAM      13
      -0.2557       gln-L[c]      36      Biomass_Ecoli_core_w_GAM      13
      -4.9414       glu-L[c]      38      Biomass_Ecoli_core_w_GAM      13
      -59.81        h2o[c]        41      Biomass_Ecoli_core_w_GAM      13
       59.81        h[c]          43      Biomass_Ecoli_core_w_GAM      13
      -3.547        nad[c]        50      Biomass_Ecoli_core_w_GAM      13
       3.547        nadh[c]       51      Biomass_Ecoli_core_w_GAM      13
      13.028        nadp[c]       52      Biomass_Ecoli_core_w_GAM      13
     -13.028        nadph[c]      53      Biomass_Ecoli_core_w_GAM      13
      -1.7867       oaa[c]        58      Biomass_Ecoli_core_w_GAM      13
      -0.5191       pep[c]        59      Biomass_Ecoli_core_w_GAM      13
       59.81        pi[c]         60      Biomass_Ecoli_core_w_GAM      13
      -2.8328       pyr[c]        62      Biomass_Ecoli_core_w_GAM      13
      -0.8977       r5p[c]        66      Biomass_Ecoli_core_w_GAM      13

objectiveAbbr =
    'Biomass_Ecoli_core_w_GAM'
```

Step 42. Set simulation constraints.

A first step when using and debugging a model should be to identify the constraints that have been applied.

```
minInf = -1000;
maxInf = 1000;
printConstraints(modelEcore, minInf,maxInf)
```

```
MinConstraints:
ACONTb 10
ATPM 8.39
EX_glc(e) -10
maxConstraints:
```

- As you can see, only three reactions are constrained in this model. The glucose exchange reaction (EX_glc(e)), the aconitase reaction (ACONTb) and the ATP non-growth associated maintenance reaction (ATPM). Note that in all three cases, a lower bound has been set only but no upper bound.
- The 'minInf' and 'maxInf' were set to -1000 and 1000, respectively, as these values represent the infinity of the E. coli core model. Other models may have different infinities.
- Note also that the `printConstraints` function returns only those constraints that are greater than -1000 but are smaller than 1000.

To know which medium constraints are applied to the model, we can use the following function:

```
printUptakeBound(modelEcore);
```

```
EX_co2(e) -1000
EX_glc(e) -10
EX_h(e) -1000
EX_h2o(e) -1000
EX_nh4(e) -1000
EX_o2(e) -1000
EX_pi(e) -1000
```

- As you can see, the model is set to a minimal medium with the presence of oxygen.

Let's assume that you would like to set the lower bound of the ATP maintenance reaction ('ATPM') to 8.39 mmol/gDW/h:

```
modelEcore = changeRxnBounds(modelEcore,'ATPM',8.39,'l');
```

and the upper bound of the 'ATPM' reaction to 8.39 mmol/gDW/h:

```
modelEcore = changeRxnBounds(modelEcore,'ATPM',8.39,'u');
```

To change both the lower and upper bound in the same command use:

```
modelEcore = changeRxnBounds(modelEcore,'ATPM',8.39,'b');
```

Let's assume that you would like to set the lower bound of the 'ATPM' reaction to 8.39 mmol/gDW/h and the ATP synthetase ('ATPS4r') to an upper bound of 100 mmol/gDW/h:

```
modelEcore = changeRxnBounds(modelEcore,'ATPM' ,8.39,'l' );
modelEcore = changeRxnBounds(modelEcore, 'ATPS4r',100, 'u');
```

The set constraints can be checked using the following function:

```
printConstraints(modelEcore, -1000,1000)
```

```
MinConstraints:
ACONTb 10
ATPM 8.39
EX_glc(e) -10
maxConstraints:
ATPM 8.39
ATPS4r 100
```

Steps 43 - 44. Test if network is mass- and charge balanced.

Check mass- and charge balance for the entire network of the model:

```
[massImbalance, imBalancedMass, imBalancedCharge, imBalancedRxnBool, Elements, missingFormulae
```

Step 45. Identify metabolic dead-ends

Detect deadend metabolites:

```
outputMets = detectDeadEnds(modelEcore)
```

```
outputMets =

    30
    32
    37
    49
```

Print the corresponding metabolite names:

```
DeadEnds = modelEcore.mets(outputMets)
```

```
DeadEnds =
    'fru[e]'
    'fum[e]'
    'gln-L[e]'
    'mal-L[e]'
```

- These metabolites are only produced or consumed in the network and the associated reactions are blocked reactions.

Identify associated reactions:

```
[rxnList, rxnFormulaList] = findRxnsFromMets(modelEcore, DeadEnds)
```

```
rxnList =
    'EX_fru(e)'
    'EX_fum(e)'
    'EX_gln_L(e)'
    'EX_mal_L(e)'
    'FRUpts2'
    'FUMt2_2'
    'GLNabc'
    'MALt2_2'

rxnFormulaList =
    'fru[e]   -> '
    'fum[e]   -> '
    'gln-L[e]   -> '
    'mal-L[e]   -> '
    'fru[e] + pep[c]   -> f6p[c] + pyr[c] '
    'fum[e] + 2 h[e]   -> fum[c] + 2 h[c] '
    'atp[c] + gln-L[e] + h2o[c]   -> adp[c] + gln-L[c] + h[c] + pi[c] '
    '2 h[e] + mal-L[e]   -> 2 h[c] + mal-L[c] '
```

- As you can see, these metabolites have each two reactions associated. Why are they then detected as deadend metabolites?

Let's have a look at the lower and upper bounds of these reactions:

```
modelEcore.lb(find(ismember(modelEcore.rxns,rxnList)))
```

```
ans =

     0
```

```
          0
          0
          0
          0
          0
          0
          0
```

```
modelEcore.ub(find(ismember(modelEcore.rxns,rxnList)))
```

```
ans =

        1000
        1000
        1000
        1000
        1000
        1000
        1000
        1000
```

- In this particular case, these four metabolites are deadend metabolites as the associated exchange reactions are set to lb=0 (i.e., no uptake is permitted). As we are interested in dead-end metabolites that are generally only consumed or produced by the network, irrespective of the applied constraints, we will set the lower bound of all exchange reaction to -1000 and the upper bound to 1000.

```
modelEcore_New = modelEcore;
modelEcore_New.lb(strmatch('EX_',modelEcore_New.rxns)) = -1000;
modelEcore_New.ub(strmatch('EX_',modelEcore_New.rxns)) = 1000;
```

Now we repeat the identification of deadend metabolites:

```
outputMets = detectDeadEnds(modelEcore_New)
```

```
outputMets =

  0×1 empty double column vector
```

```
DeadEnds = modelEcore_New.mets(outputMets)
```

```
DeadEnds =

  0×1 empty cell array
```

```
[rxnList, rxnFormulaList] = findRxnsFromMets(modelEcore_New, DeadEnds)
```

```
rxnList =

  0×1 empty cell array
rxnFormulaList =

  0×0 empty cell array
```

- And indeed no deadend metabolites remain. This example also illustrates how such issues can be fixed - one option is to revert the directionality of the associated reactions.
- Note that changing the directionality must be carefully evaluated in each case, to ensure that the resulting model is biologically accurate. For instance, changing the directionality of the 'FRUpts_2'

reaction would have not been biologically meaningful, as the Phosphotransferase system is known to be unidirectional under physiologically relevant conditions.

Steps 46 - 49. Refer to 'gap analysis' tutorial.

Step 50. Set exchange constraints for a simulation condition.

The Cobra Toolbox funtion, `changeRxnBounds`, is used to set constraints on a reaction which is demonstrated in step 67 of this tutorial.

Steps 51 - 59. Test for stoichiometrically balanced cycles (SBCs).

SBCs or Type III pathways, are formed by internal network reactions and can carry fluxes despite closed exchange reactions (closed system). Therefore, use the following `testForTypeIIIPathways` function:

- The indices of the exchange reactions ('EX_') are input as a list.
- The output filename can be specified with 'test', it receives automatically the extension '.expa'. The filename is optional, the default name is: 'ModelTestTypeIII'

```
listExch = strmatch('EX_', modelEcore.rxns);
try
    testForTypeIIIPathways(modelEcore, listExch, 'test');
catch ME
    getReport(ME)
end
```

```
ans =
Error using fprintf
Function is not defined for sparse inputs.

Error in convertModelToEX (line 54)
            fprintf(fid, '%i\t%s\t', model.S(reactionPlace(j), i), model.mets{reactionPlace(j)});

Error in testForTypeIIIPathways (line 48)
convertModelToEX(model,strcat(filename, '.expa'),rxnzero,model.rxns(ListExch));

Error in LiveEditorEvaluationHelper (line 52)
    testForTypeIIIPathways(modelEcore, listExch, 'test');

Error in matlab.internal.editor.Evaluator/evalFile

Error in matlab.internal.editor.Evaluator/evaluateFile

Error in matlab.internal.editor.Evaluator/evaluateCode

Error in matlab.internal.editor.RegionEvaluator/evaluateRegions

Error in matlab.internal.editor.evaluateRegions

Error in matlab.internal.editor.EvaluationOutputsService.evalRegions
```

- *This error message is returned from X3.exe since there are no SBCs in the E. coli core network.*

Steps 60 - 66. Test if biomass precursors can be produced in standard medium

%TODO steps 64 to 66 are not referenced in this section.

60. Obtain a list of biomass components:

```
BiomassComponents = modelEcore.mets(find(modelEcore.S(:,strmatch('Biomass',modelEcore.rxns))))
```

```
BiomassComponents =
    '3pg[c]'
    'accoa[c]'
    'adp[c]'
    'akg[c]'
    'atp[c]'
    'coa[c]'
    'e4p[c]'
    'f6p[c]'
    'g3p[c]'
    'g6p[c]'
    'gln-L[c]'
    'glu-L[c]'
    'h2o[c]'
    'h[c]'
    'nad[c]'
    'nadh[c]'
    'nadp[c]'
    'nadph[c]'
    'oaa[c]'
    'pep[c]'
    'pi[c]'
    'pyr[c]'
    'r5p[c]'
```

61. Add a demand function for each biomass precursor:

```
[modelEcore_NEW ,rxnNames] = addDemandReaction(modelEcore, BiomassComponents);
```

```
DM_3pg[c] 3pg[c]   ->
DM_accoa[c] accoa[c]   ->
DM_adp[c] adp[c]   ->
DM_akg[c] akg[c]   ->
DM_atp[c] atp[c]   ->
DM_coa[c] coa[c]   ->
DM_e4p[c] e4p[c]   ->
DM_f6p[c] f6p[c]   ->
DM_g3p[c] g3p[c]   ->
DM_g6p[c] g6p[c]   ->
DM_gln-L[c] gln-L[c]   ->
DM_glu-L[c] glu-L[c]   ->
DM_h2o[c] h2o[c]   ->
DM_h[c] h[c]   ->
DM_nad[c] nad[c]   ->
DM_nadh[c] nadh[c]   ->
DM_nadp[c] nadp[c]   ->
```

```
DM_nadph[c] nadph[c]  ->
DM_oaa[c] oaa[c]   ->
DM_pep[c] pep[c]   ->
DM_pi[c] pi[c]   ->
DM_pyr[c] pyr[c]   ->
DM_r5p[c] r5p[c]   ->
```

For each biomass component i, perform the following test:

```
for i = 1 : length(BiomassComponents)
```

62. Change objective function to the demand function:

```
    modelEcore_NEW = changeObjective(modelEcore_NEW, rxnNames(i));
```

63. Maximize ('max') for new objective function (Demand function)

```
    FBAsolution = optimizeCbModel (modelEcore_NEW,'max');
```

- FBAsolution is a structure containing the result of the optimization. FBAsolution.f gives the maximal value of the objective reaction (i.e., 'DM_pep[c]'), which is greater than 0 mmol/gDW/h. This means that our *E. coli* core model can produce pep[c].
- Store each solution in a vector:

```
    BiomassComponentsValue(i,1) = FBAsolution.f;
end
```

- Print each BiomassComponent and the corresponding value:

```
[BiomassComponents  num2cell(BiomassComponentsValue)]
```

```
ans =
    '3pg[c]'      [    10.0000]
    'accoa[c]'    [ 1.7764e-15]
    'adp[c]'      [-3.5527e-15]
    'akg[c]'      [    10.0000]
    'atp[c]'      [         0]
    'coa[c]'      [         0]
    'e4p[c]'      [    7.5000]
    'f6p[c]'      [         5]
    'g3p[c]'      [    10.0000]
    'g6p[c]'      [    5.0000]
    'gln-L[c]'    [    10.0000]
    'glu-L[c]'    [    10.0000]
    'h2o[c]'      [      1000]
    'h[c]'        [      1000]
    'nad[c]'      [-7.1054e-15]
    'nadh[c]'     [ 7.1054e-15]
    'nadp[c]'     [         0]
    'nadph[c]'    [         0]
    'oaa[c]'      [    10.0000]
    'pep[c]'      [    10.0000]
    'pi[c]'       [      1000]
    'pyr[c]'      [    10.0000]
    'r5p[c]'      [    6.0000]
```

- As we can see, not all biomass components (or rather their corresponding demand reaction) can have a non-zero flux. Why is that?
- Just to remember us, the model constraints are:

```
printConstraints(modelEcore_NEW, -1000,1000)
```

```
MinConstraints:
ACONTb 10
ATPM 8.39
EX_glc(e) -10
maxConstraints:
ATPM 8.39
ATPS4r 100
```

- Note that only those constraints will be printed that are smaller greater than -1000 and smaller than 1000.
- Let's revisit how the biomass reaction is formulated in this model:

```
modelEcore = changeObjective(modelEcore,modelEcore.rxns(strmatch('Biomass',modelEcore.rxns)));
[objectiveAbbr] = checkObjective(modelEcore)
```

```
summaryT =
    Coefficient     Metabolite     metID             Reaction             RxnID

    _____     _____     _____     _____     _____

     -1.496         3pg[c]           3       Biomass_Ecoli_core_w_GAM       13
     -3.7478        accoa[c]        10       Biomass_Ecoli_core_w_GAM       13
     59.81          adp[c]          13       Biomass_Ecoli_core_w_GAM       13
      4.1182        akg[c]          14       Biomass_Ecoli_core_w_GAM       13
    -59.81          atp[c]          17       Biomass_Ecoli_core_w_GAM       13
      3.7478        coa[c]          21       Biomass_Ecoli_core_w_GAM       13
     -0.361         e4p[c]          23       Biomass_Ecoli_core_w_GAM       13
     -0.0709        f6p[c]          26       Biomass_Ecoli_core_w_GAM       13
     -0.129         g3p[c]          33       Biomass_Ecoli_core_w_GAM       13
     -0.205         g6p[c]          34       Biomass_Ecoli_core_w_GAM       13
     -0.2557        gln-L[c]        36       Biomass_Ecoli_core_w_GAM       13
     -4.9414        glu-L[c]        38       Biomass_Ecoli_core_w_GAM       13
    -59.81          h2o[c]          41       Biomass_Ecoli_core_w_GAM       13
     59.81          h[c]            43       Biomass_Ecoli_core_w_GAM       13
     -3.547         nad[c]          50       Biomass_Ecoli_core_w_GAM       13
      3.547         nadh[c]         51       Biomass_Ecoli_core_w_GAM       13
     13.028         nadp[c]         52       Biomass_Ecoli_core_w_GAM       13
    -13.028         nadph[c]        53       Biomass_Ecoli_core_w_GAM       13
     -1.7867        oaa[c]          58       Biomass_Ecoli_core_w_GAM       13
     -0.5191        pep[c]          59       Biomass_Ecoli_core_w_GAM       13
     59.81          pi[c]           60       Biomass_Ecoli_core_w_GAM       13
     -2.8328        pyr[c]          62       Biomass_Ecoli_core_w_GAM       13
     -0.8977        r5p[c]          66       Biomass_Ecoli_core_w_GAM       13


objectiveAbbr =
    'Biomass_Ecoli_core_w_GAM'
```

- As you can see, there are numerous metabolites that have a positive coefficient in the biomass equation, meaning that they are produced by the biomass reaction and thus we need to test whether these metabolites can be removed, rather than produced by the model. Hence, we need to add sink reactions (rather than demand reactions) and minimize them:

- Let's get all biomass components with a positive coefficient in the biomass reaction:

```
BiomassComponentsPos = modelEcore.mets(find(modelEcore.S(:,strmatch('Biomass',modelEcore.rxns)
```

```
BiomassComponentsPos =
    'adp[c]'
    'akg[c]'
    'coa[c]'
    'h[c]'
    'nadh[c]'
    'nadp[c]'
    'pi[c]'
```

```
BiomassComponentsNeg = modelEcore.mets(find(modelEcore.S(:,strmatch('Biomass',modelEcore.rxns)
```

```
BiomassComponentsNeg =
    '3pg[c]'
    'accoa[c]'
    'atp[c]'
    'e4p[c]'
    'f6p[c]'
    'g3p[c]'
    'g6p[c]'
    'gln-L[c]'
    'glu-L[c]'
    'h2o[c]'
    'nad[c]'
    'nadph[c]'
    'oaa[c]'
    'pep[c]'
    'pyr[c]'
    'r5p[c]'
```

```
[modelEcore_NEW] = addSinkReactions(modelEcore, BiomassComponentsPos);
```

```
sink_adp[c] adp[c]   <=>
sink_akg[c] akg[c]   <=>
sink_coa[c] coa[c]   <=>
sink_h[c] h[c]   <=>
sink_nadh[c] nadh[c]   <=>
sink_nadp[c] nadp[c]   <=>
sink_pi[c] pi[c]   <=>
```

```
[modelEcore_NEW ,rxnNames] = addDemandReaction(modelEcore_NEW, BiomassComponentsNeg);
```

```
DM_3pg[c] 3pg[c]   ->
DM_accoa[c] accoa[c]   ->
DM_atp[c] atp[c]   ->
DM_e4p[c] e4p[c]   ->
DM_f6p[c] f6p[c]   ->
DM_g3p[c] g3p[c]   ->
DM_g6p[c] g6p[c]   ->
DM_gln-L[c] gln-L[c]   ->
DM_glu-L[c] glu-L[c]   ->
DM_h2o[c] h2o[c]   ->
DM_nad[c] nad[c]   ->
DM_nadph[c] nadph[c]   ->
```

```
DM_oaa[c] oaa[c]  ->
DM_pep[c] pep[c]  ->
DM_pyr[c] pyr[c]  ->
DM_r5p[c] r5p[c]  ->
```

- Note that we added both the sink and the demand reactions to the model simultanously. The reason for this is that metabolites such as coa and accoa, or nadh and nad, are not produced or consumed in this model but only recycled. Hence, for obtaining a non-zero flux for any of the associated sink or demand reactions one needs to add the reaction pair. In larger networks (than the E. coli core model) this will be less of a problem as they capture the biosynthetic pathways for these metabolites.

Now lets repeat the analysis (note that we minimize the objective):

```
for i = 1 : length(BiomassComponentsPos)
    modelEcore_NEW = changeObjective(modelEcore_NEW, strcat('sink_',BiomassComponentsPos(i)));
    FBAsolution = optimizeCbModel (modelEcore_NEW,'min');
    BiomassComponentsValuePos(i,1) = FBAsolution.f;
end
[BiomassComponentsPos  num2cell(BiomassComponentsValuePos)]
```

```
ans =
    'adp[c]'     [     -1000]
    'akg[c]'     [     -1000]
    'coa[c]'     [-663.3333]
    'h[c]'       [     -1000]
    'nadh[c]'    [     -1000]
    'nadp[c]'    [     -1000]
    'pi[c]'      [     -1000]
```

- All these metabolites can be removed by the model.
- We repeat the same analysis for all negative biomass components.

Now lets repeat the analysis (note that we maximize the objective):

```
for i = 1 : length(BiomassComponentsNeg)
    modelEcore_NEW = changeObjective(modelEcore_NEW, strcat('DM_',BiomassComponentsNeg(i)));
    FBAsolution = optimizeCbModel (modelEcore_NEW,'max');
    BiomassComponentsValueNeg(i,1) = FBAsolution.f;
end
[BiomassComponentsNeg  num2cell(BiomassComponentsValueNeg)]
```

```
ans =
    '3pg[c]'      [   555.8050]
    'accoa[c]'    [   663.3333]
    'atp[c]'      [1.0000e+03]
    'e4p[c]'      [   280.4025]
    'f6p[c]'      [   186.9350]
    'g3p[c]'      [   370.5367]
    'g6p[c]'      [   186.9350]
    'gln-L[c]'    [       500]
    'glu-L[c]'    [1.0000e+03]
    'h2o[c]'      [      1000]
    'nad[c]'      [1.0000e+03]
    'nadph[c]'    [      1000]
    'oaa[c]'      [   990.1464]
    'pep[c]'      [   555.8050]
    'pyr[c]'      [   992.5000]
    'r5p[c]'      [   224.3220]
```

- All these biomass precursors can be produced by the model. Hence a non-zero, positive flux through the biomass reaction should be possible:

```
FBAsolution = optimizeCbModel(modelEcore,'max');
FBAsolution.f
```

```
ans = 0.7082
```

FBAsolution.x contains the flux value for each reaction in the network. To view the flux values use:

```
printFluxVector(modelEcore,FBAsolution.x,'true')
```

```
ACONTa 10
ACONTb 10
AKGDH 9.23588
ATPM 8.39
ATPS4r 28.2124
Biomass_Ecoli_core_w_GAM 0.708235
CO2t -29.8607
CS 10
CYTBD 58.0838
ENO 17.0578
EX_co2(e) 29.8607
EX_glc(e) -10
EX_h(e) 14.2072
EX_h2o(e) 35.0198
EX_nh4(e) -3.86187
EX_o2(e) -29.0419
EX_pi(e) -2.60539
FBA 9.29552
FORt2 127.234
FORti 127.234
FUM 9.23588
GAPD 18.1173
GLCpts 10
GLNS 0.181096
GLUDy -3.68077
H2Ot -35.0198
ICDHyr 10
MDH 9.23588
NADH16 48.8479
NH4t 3.86187
O2t 29.0419
PDH 12.6543
PFK 9.29552
PGI 9.85481
PGK -18.1173
PGM -17.0578
PIt2r 2.60539
PPC 2.02952
PYK 4.66061
RPE -0.50908
RPI -0.50908
SUCDi 9.23588
SUCOAS -9.23588
TALA -0.126703
THD2 2.90759
TKT1 -0.126703
TKT2 -0.382376
TPI 9.29552
```

- To see which network reactions participate in the optimal solution. Keep in mind that there may be more than one optimal solution (so-called alternate optimal solutions, which have the same optimal value for the objective function but the internal flux distribution may be different) .
- Compare this solution with a sparse FBA solution, which returns an optimal flux distribution with the least number of active model reactions. Note that the underlying algorithm is an approximation to the exact sparsest solution and also that there may be alternative optimal solutions with equal numbers of active reactions.

```
FBAsolution = optimizeCbModel(modelEcore,'max','zero');
FBAsolution.f
```

```
ans = 0.7082
```

```
printFluxVector(modelEcore,FBAsolution.x,'true')
```

```
ACONTa 10
ACONTb 10
AKGDH 3.69917
ATPM 8.39
ATPS4r 31.7196
Biomass_Ecoli_core_w_GAM 0.708235
CO2t -11.6696
CS 10
CYTBD 39.8928
ENO 17.0578
EX_co2(e) 11.6696
EX_for(e) 18.191
EX_glc(e) -10
EX_h(e) 32.3982
EX_h2o(e) 16.8287
EX_nh4(e) -3.86187
EX_o2(e) -19.9464
EX_pi(e) -2.60539
FBA 9.29552
FORti 18.191
FUM 9.23588
GAPD 18.1173
GLCpts 10
GLNS 0.181096
GLUDy -3.68077
H2Ot -16.8287
ICDHyr 4.46328
ICL 5.53672
MALS 5.53672
MDH 11.2654
ME2 3.5072
NADH16 30.6569
NH4t 3.86187
O2t 19.9464
PFK 9.29552
PFL 18.191
PGI 9.85481
PGK -18.1173
PGM -17.0578
PIt2r 2.60539
PYK 6.69013
RPE -0.50908
RPI -0.50908
SUCDi 9.23588
SUCOAS -3.69917
TALA -0.126703
THD2 4.93711
```

```
TKT1 -0.126703
TKT2 -0.382376
TPI 9.29552
```

Steps 67 - 70. Test if model can produce known secretion products.

67. Collect a list of known secretion products and medium conditions.

For this example, acetate secretion ('EX_ac(e)') was chosen and let's require that secretion flux is at least 2 mmol/gDW/h (i.e. the lower bound is constrained to 2).

```
modelEcore_New = changeRxnBounds(modelEcore, 'EX_ac(e)',2,'l');
```

68. Set the constraints to the desired medium condition (e.g., minimal medium + carbon source). For changing the constraints use the following function:

```
modelEcore_New = changeRxnBounds(modelEcore, {'EX_glc(e)' 'EX_o2(e)'},[-10 -18.5],'l');
```

- If the model is required to grow in addition to producing the by-product, set the lower bound of the biomass reaction to the corresponding value required for growth.

Note: If the model is required to grow in addition to producing the by-product, set the lower bound of the biomass reaction to the corresponding value required for growth. We determine the value based on the following FBA solution.

- Optimize for growth:

```
modelEcore_New = changeObjective(modelEcore_New,'Biomass_Ecoli_core_w_GAM');
FBAsolution = optimizeCbModel(modelEcore_New,'max')
```

```
FBAsolution =
        full: [95×1 double]
         obj: 0.6411
       rcost: [95×1 double]
        dual: [72×1 double]
      solver: 'glpk'
   algorithm: 'default'
        stat: 1
    origStat: 5
        time: 0.0165
       basis: []
           x: [95×1 double]
           f: 0.6411
           y: [72×1 double]
           w: [95×1 double]
           v: [95×1 double]
```

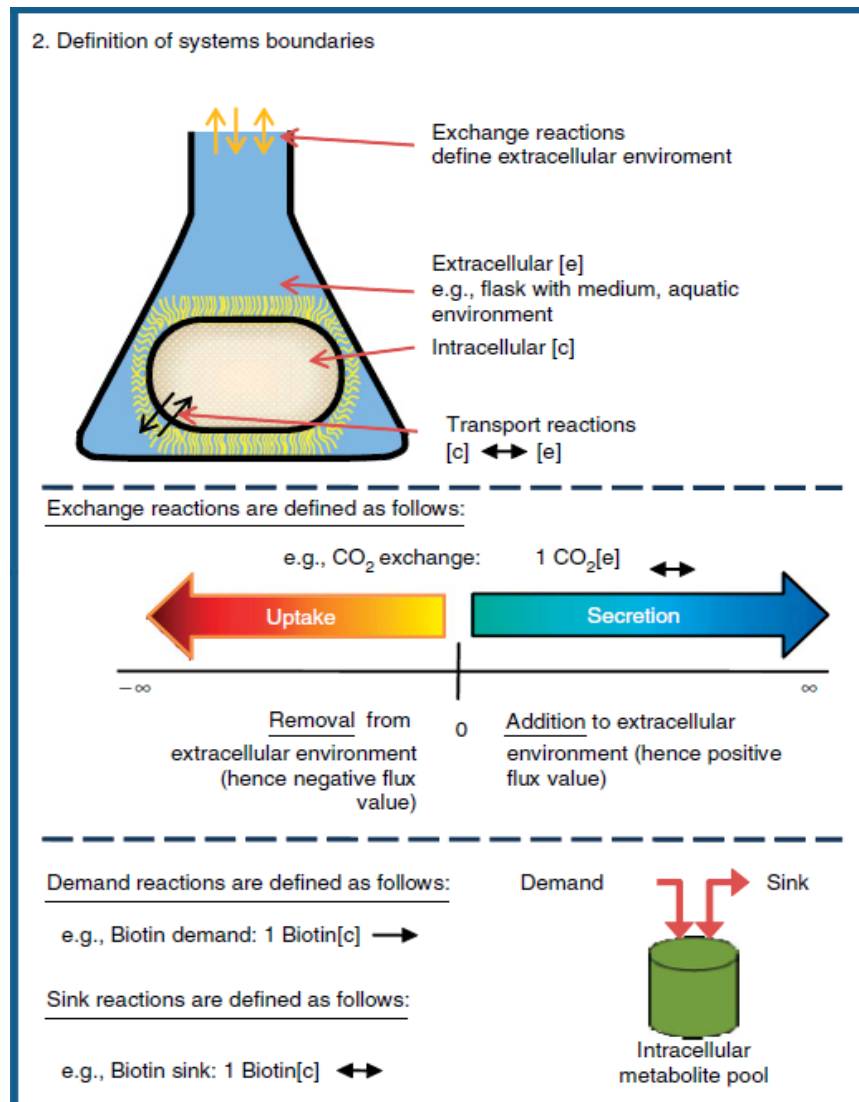- Set the lower bound of the biomass reaction to the value of the FBA solution:

```
modelEcore_New = changeRxnBounds(modelEcore_New, 'Biomass_Ecoli_core_w_GAM',0.64,'l');
```

- Note that the maximally possible biomass reaction flux decreased substantially, with these additional constraints.

69. Change the objective function to the exchange reaction of your secretion product (i.e., acetate):

```
modelEcore_New = changeObjective(modelEcore_New, 'EX_ac(e)');
```

70. Maximize ('max') for the new objective function (as a secretion is expected to have a positive flux value, see Figure):

2. Definition of systems boundaries

Exchange reactions define extracellular enviroment

Extracellular [e] e.g., flask with medium, aquatic environment

Intracellular [c]

Transport reactions [c] ⟷ [e]

Exchange reactions are defined as follows:

e.g., CO_2 exchange: 1 CO_2[e] ⟷

Uptake   Secretion

$-\infty$ ∞

Removal from extracellular environment (hence negative flux value)   0   Addition to extracellular environment (hence positive flux value)

Demand reactions are defined as follows: Demand   Sink

e.g., Biotin demand: 1 Biotin[c] ⟶

Sink reactions are defined as follows:

e.g., Biotin sink: 1 Biotin[c] ⟷   Intracellular metabolite pool

```
FBAsolution = optimizeCbModel (modelEcore_New,'max')
```

```
FBAsolution =
        full: [95×1 double]
         obj: 0.0448
       rcost: [95×1 double]
        dual: [72×1 double]
      solver: 'glpk'
   algorithm: 'default'
        stat: 1
    origStat: 5
        time: 0.0103
       basis: []
```

```
        x: [95×1 double]
        f: 0.0448
        y: [72×1 double]
        w: [95×1 double]
        v: [95×1 double]
```

- It seems that the model can produce 3.5x mmol/gDW/h of acetate with the following constraints:

```
printConstraints(modelEcore_New, -1000,1000)
```

```
MinConstraints:
ACONTb 10
ATPM 8.39
Biomass_Ecoli_core_w_GAM 0.64
EX_glc(e) -10
EX_o2(e) -18.5
maxConstraints:
ATPM 8.39
ATPS4r 100
```

Steps 71 - 75. Test if model can produce a certain ratio of two secretion products.

Acetate and Formate secretion are the two secretion products used in this example.

71. Set the constraints to the desired medium condition (e.g., minimal medium + carbon source). As shown above in step 68.

72. Let's verify that both metabolites can be secreted independently. Repeat steps 69 and 70.

```
modelEcoreAc = changeObjective(modelEcore,'EX_ac(e)');
FBAsolution = optimizeCbModel(modelEcoreAc,'max')
```

```
FBAsolution =
        full: [95×1 double]
         obj: 10.0000
       rcost: [95×1 double]
        dual: [72×1 double]
      solver: 'glpk'
   algorithm: 'default'
        stat: 1
    origStat: 5
        time: 0.0098
       basis: []
           x: [95×1 double]
           f: 10.0000
           y: [72×1 double]
           w: [95×1 double]
           v: [95×1 double]
```

```
modelEcoreFor = changeObjective(modelEcore,'EX_for(e)');
FBAsolution = optimizeCbModel(modelEcoreFor,'max')
```

```
FBAsolution =
        full: [95×1 double]
         obj: 40
```

```
        rcost: [95×1 double]
         dual: [72×1 double]
       solver: 'glpk'
    algorithm: 'default'
         stat: 1
     origStat: 5
         time: 0.0082
        basis: []
            x: [95×1 double]
            f: 40
            y: [72×1 double]
            w: [95×1 double]
            v: [95×1 double]
```

73. Add a row to the S matrix to couple the by-product secretion reactions:

```
modelEcore_NEW = addRatioReaction(modelEcore, {'EX_ac(e)' 'EX_for(e)'},[1 1]);
```

- Acetate and Formate secretion are coupled to a ratio of 1:1.

Also, let's require that the acetate secretion flux is at least 1 mmol/gDW/h (i.e. the lower bound is constrained to 1).

```
modelEcore_NEW = changeRxnBounds(modelEcore_NEW,'EX_ac(e)',1,'l');
```

Note: If the model is required to grow in addition to producing the by-product, set the lower bound of the biomass reaction to the corresponding value required for growth. We determine the value based on the following FBA solution.

- Optimize for growth:

```
modelEcore_NEW = changeObjective(modelEcore_NEW,'Biomass_Ecoli_core_w_GAM');
FBAsolution = optimizeCbModel(modelEcore_NEW,'max')
```

```
FBAsolution =
         full: [95×1 double]
          obj: 0.6374
        rcost: [95×1 double]
         dual: [73×1 double]
       solver: 'glpk'
    algorithm: 'default'
         stat: 1
     origStat: 5
         time: 0.0090
        basis: []
            x: [95×1 double]
            f: 0.6374
            y: [73×1 double]
            w: [95×1 double]
            v: [95×1 double]
```

- Note that the maximally possible biomass reaction flux decreased due to the additional constraints.
- Set the lower bound of the biomass reaction to the value of the FBA solution:

```
modelEcore_NEW = changeRxnBounds(modelEcore_NEW, 'Biomass_Ecoli_core_w_GAM',0.63,'l');
```

What is the flux through the two secretion reactions:

```
FBAsolution.x(find(ismember(modelEcore_NEW.rxns,'EX_for(e)')))
```

```
ans = 1
```

```
FBAsolution.x(find(ismember(modelEcore_NEW.rxns,'EX_ac(e)')))
```

```
ans = 1
```

- Keep in mind that the `optimizeCbModel` only returns one of the possible flux distributions with maximal biomass yield.

74. Change the objective function to the exchange reaction of one of your secretion products:

```
modelEcore_NEW = changeObjective(modelEcore_NEW, 'EX_ac(e)');
```

75. Maximize for the new objective function (as a secretion is expected to have a positive flux value):

```
FBAsolution = optimizeCbModel (modelEcore_NEW,'max')
```

```
FBAsolution =
           full: [95×1 double]
            obj: 1.1047
          rcost: [95×1 double]
           dual: [73×1 double]
         solver: 'glpk'
      algorithm: 'default'
           stat: 1
       origStat: 5
           time: 0.0120
          basis: []
              x: [95×1 double]
              f: 1.1047
              y: [73×1 double]
              w: [95×1 double]
              v: [95×1 double]
```

- Check that the second secretion product can be produced in the defined ratio:

```
FBAsolution.x(find(ismember(modelEcore_NEW.rxns,'EX_for(e)')))
```

```
ans = 1.1047
```

Steps 76 - 77. Check for blocked reactions.

76. Change simulation conditions to rich medium or open all exchange reactions.

- Identify the exchange reactions and set the reaction values to − infinity (e.g., − 1,000) and + infinity (e.g., + 1,000):

```
ExR = modelEcore.rxns(strmatch('EX_',modelEcore.rxns));
modelEcore_Open =  changeRxnBounds(modelEcore, ExR , -1000,'l');
modelEcore_Open =  changeRxnBounds(modelEcore_Open, ExR, 1000,'u');
```

- Verify the constraints on the model:

```
printConstraints(modelEcore_Open,-1000,1000)
```

```
MinConstraints:
ACONTb 10
ATPM 8.39
maxConstraints:
ATPM 8.39
ATPS4r 100
```

```
printUptakeBound(modelEcore_Open);
```

```
EX_ac(e) -1000
EX_acald(e) -1000
EX_akg(e) -1000
EX_co2(e) -1000
EX_etoh(e) -1000
EX_for(e) -1000
EX_fru(e) -1000
EX_fum(e) -1000
EX_glc(e) -1000
EX_gln_L(e) -1000
EX_glu_L(e) -1000
EX_h(e) -1000
EX_h2o(e) -1000
EX_lac_D(e) -1000
EX_mal_L(e) -1000
EX_nh4(e) -1000
EX_o2(e) -1000
EX_pi(e) -1000
EX_pyr(e) -1000
EX_succ(e) -1000
```

77. Run analysis for blocked reactions. The `findBlockedReaction` function returns a list of blocked reactions ('BlockedReactions').

```
BlockedReactions = findBlockedReaction(modelEcore_Open)
```

```
BlockedReactions =
     {''}
```

- The answer is an empty array since the *E. coli* core network has no blocked reactions.
- If the model contains blocked reactions, please refer to the tutorial for 'gap filling' on how to proceed.

## Steps 79 - 80. Compute single gene deletion phenotypes

79. Use The Cobra Toolbox function, `singleGeneDeletion`, to simulate gene deletion:

```
[grRatio,grRateKO,grRateWT, hasEffect] = singleGeneDeletion (modelEcore);
```

- The variable 'hasEffect' is returned, and an entry of 1 in the vector indicate that a gene deletion had an affect on the objective function (here, growth rate).

Let's visualize hasEffect:

```
bar(sort(hasEffect,'descend'))
xlabel('Model genes (rank ordered)');
ylabel('Lethality (1 = lethal, 0 = no effect)')
```
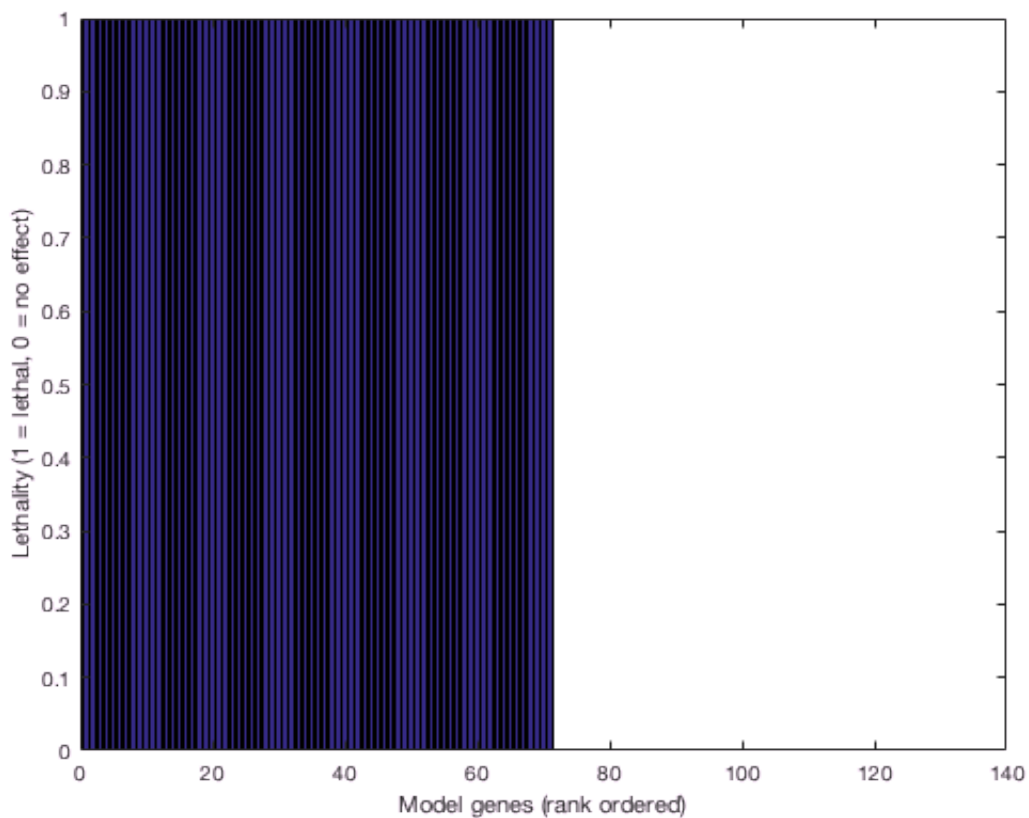

How many genes are in my model?

```
length(modelEcore.genes)
```

```
ans = 137
```

How many genes have an effect and which ones?

```
length(find(hasEffect))
```

```
ans = 71
```

Which gene deletions had an affect?

```
modelEcore.genes(find(hasEffect))
```

```
ans =
    'b0114'
    'b0115'
    'b0116'
    'b0474'
    'b0720'
    'b0721'
    'b0722'
    'b0723'
    'b0724'
    'b0726'
    'b0727'
    'b0728'
    'b0729'
    'b0767'
    'b0809'
    'b0810'
    'b0811'
    'b1136'
    'b1479'
    'b1602'
    'b1603'
    'b1702'
    'b1761'
    'b1779'
    'b1817'
    'b1818'
    'b1819'
    'b1852'
    'b2029'
    'b2276'
    'b2277'
    'b2278'
    'b2279'
    'b2280'
    'b2281'
    'b2282'
    'b2283'
    'b2284'
    'b2285'
    'b2286'
    'b2287'
    'b2288'
    'b2415'
    'b2416'
    'b2463'
    'b2587'
    'b2779'
    'b2926'
    'b3212'
    'b3213'
    'b3236'
    'b3403'
    'b3528'
    'b3731'
    'b3732'
    'b3733'
    'b3734'
    'b3735'
    'b3736'
    'b3737'
```

```
    'b3738'
    'b3919'
    'b3956'
    'b4015'
    'b4025'
    'b4077'
    'b4151'
    'b4152'
    'b4153'
    'b4154'
    's0001'
```

Which gene deletions are lethal?

- We define all growth rates lower than 0.0001 1/hr as no growth.

```
tol = 1e-3;
LethalGenes = modelEcore.genes(find(grRateKO< tol))
```

```
LethalGenes =
    'b0721'
    'b0722'
    'b0723'
    'b0724'
    'b1136'
    'b3919'
```
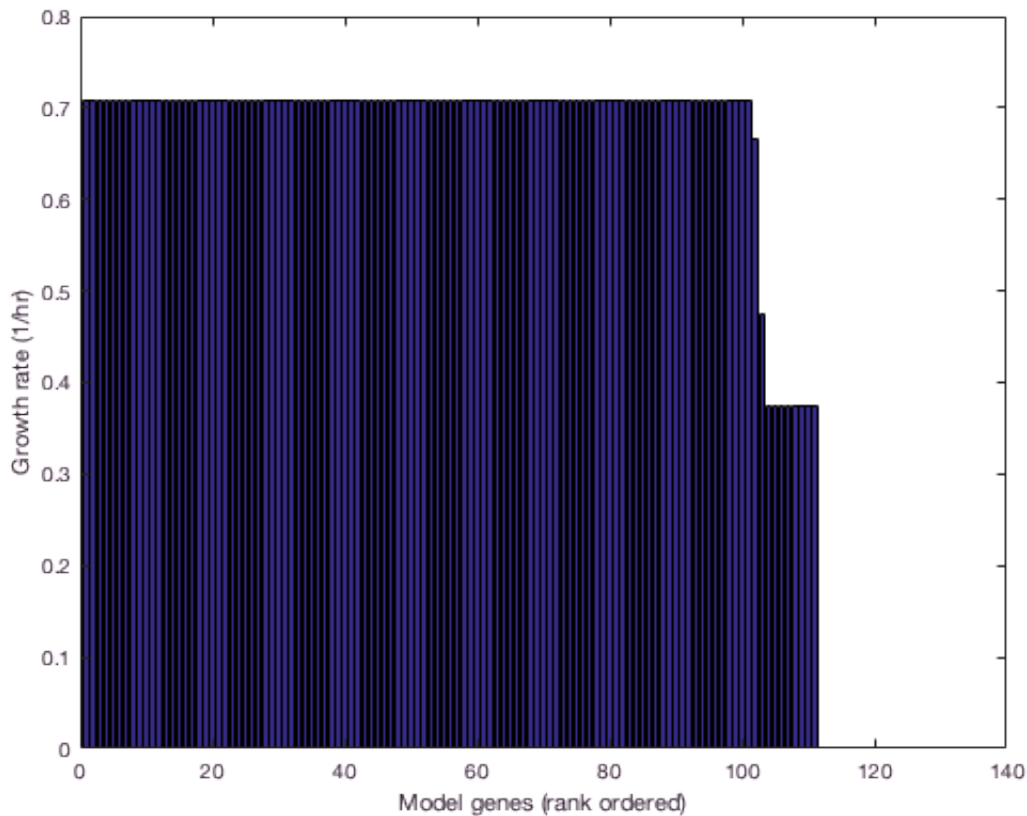
```
length(LethalGenes)
```

```
ans = 6
```

- Plot the effect of gene deletions on growth rate:

Some entries in 'grRateKO' maybe NaN. This is because the model is infeasible for those knockouts due to the lower bound on the 'ATPM'. We will replace those instances by zero.

```
grRateKO(isnan(grRateKO)) = 0;

bar(sort(grRateKO,'descend'));
xlabel('Model genes (rank ordered)');
ylabel('Growth rate (1/hr)')
```

80. Compare with experimental data.

- Are those genes known to be lethal in the organism (in vivo)?

%TODO Steps 81 - 87 ?

Steps 88 - 89. Test if the model grows too fast.

When optimization results for a biomass reaction in different medium conditions are compared with experimental data, one can evaluate if the model grows too fast. Analysis of modeling constraints, reduced cost and single gene deletion, are helpful in the evaluation of growth conditions.

88. Determine the reduced cost associated with network reactions when optimizing for an objective function:

```
FBAsolution = optimizeCbModel(modelEcore,'max',false)
```

```
FBAsolution =
        full: [95×1 double]
         obj: 0.7082
       rcost: [95×1 double]
        dual: [72×1 double]
      solver: 'glpk'
   algorithm: 'default'
        stat: 1
    origStat: 5
```

```
      time: 0.0176
     basis: []
         x: [95×1 double]
         f: 0.7082
         y: [72×1 double]
         w: [95×1 double]
         v: [95×1 double]
```

- FBAsolution.y contains the shadow price for each metabolite and FBAsolution.w contains the reduced cost for each network reaction.

$$\text{Shadow price:}\, \pi_i = -\frac{\partial Z}{\partial b_i}\left[\frac{\partial \text{ growth}}{\partial \text{ nutrient}}\right]$$

+ or -

1. $\Pi_i = 0$; not a governing constraint

2. $\Pi_i < 0$; more 'i' the higher Z becomes

3. $\Pi_i > 0$; more 'i' the lower Z becomes

$$\boxed{\text{Reduced cost:}\quad \rho_i = \frac{\partial Z}{\partial v_i}}$$

Amount by which the objective function will change with
the flux level through an internal flux that is not in the basis
solution

- We find the reduced cost particularly informative to identify constraints that limit the maximal value of the objective function.
- Note that by definition the reduced cost has a negative sign. Meaning that a reaction A with a reduced cost of 35 would lead to an increase in the objective value by 35 if the flux through this reaction would be increased by 1 flux unit.

Print those reactions that have the smallest reduced cost associated:

```
[a,b] = sort(FBAsolution.w,'descend');
modelEcore.rxns(b(1:10))
```

```
ans =
    'EX_fru(e)'
    'EX_glc(e)'
    'ACONTb'
    'EX_ac(e)'
    'EX_acald(e)'
    'EX_etoh(e)'
    'EX_fum(e)'
    'EX_lac_D(e)'
    'EX_pyr(e)'
    'EX_succ(e)'
```

```
a(1:10)
```

```
ans =

    0.1416
    0.1416
    0.0708
    0.0708
    0.0708
    0.0708
    0.0708
    0.0708
    0.0708
    0.0708
```

- Generally, we are looking for exchange reactions that may be limiting the objective value (e.g., growth yield) as those ones are easy to adjust *in silico* (and *in vitro*).
- The uptake of glucose is limiting and the associated reduced cost is 0.1416, i.e., if we were to increase the flux through this reaction by 1 unit we would increase the objective by 0.1416.
- Let's test this by repeating step 88 with an increase to the flux through EX_glc(e) by one unit.

89. Beforehand we proceed, let's verify the constraints are set as intended:

```
printConstraints(modelEcore,-1000,1000);
```

```
MinConstraints:
ACONTb 10
ATPM 8.39
EX_glc(e) -10
maxConstraints:
ATPM 8.39
ATPS4r 100
```

```
printUptakeBound(modelEcore);
```

```
EX_co2(e) -1000
EX_glc(e) -10
EX_h(e) -1000
EX_h2o(e) -1000
EX_nh4(e) -1000
EX_o2(e) -1000
EX_pi(e) -1000
```

Increase the flux through EX_glc(e) by one unit (keep in mind that uptake is defined as a negative flux through exchange reactions):

```
modelEcore_New = changeRxnBounds(modelEcore,'EX_glc(e)',-11,'l');
FBAsolution = optimizeCbModel(modelEcore_New,'max',false)
```

```
FBAsolution =
        full: [95×1 double]
         obj: 0.8499
       rcost: [95×1 double]
        dual: [72×1 double]
      solver: 'glpk'
```

```
    algorithm: 'default'
        stat: 1
    origStat: 5
        time: 0.0101
       basis: []
           x: [95×1 double]
           f: 0.8499
           y: [72×1 double]
           w: [95×1 double]
           v: [95×1 double]
```

- And indeed the biomass flux rate increased accordingly.
- For more systematic analysis of the effect of reduced costs and limiting variable, please also refer to the tutorial on robustness and phase plane analysis.

93. Use single-reaction deletion to identify single reactions that may enable the model to grow too fast.

```
[grRatio,grRateKO,grRateWT] = singleRxnDeletion(modelEcore);
```

- Which gene deletion would lead to a lower growth rate?

```
plot(grRateKO)
```
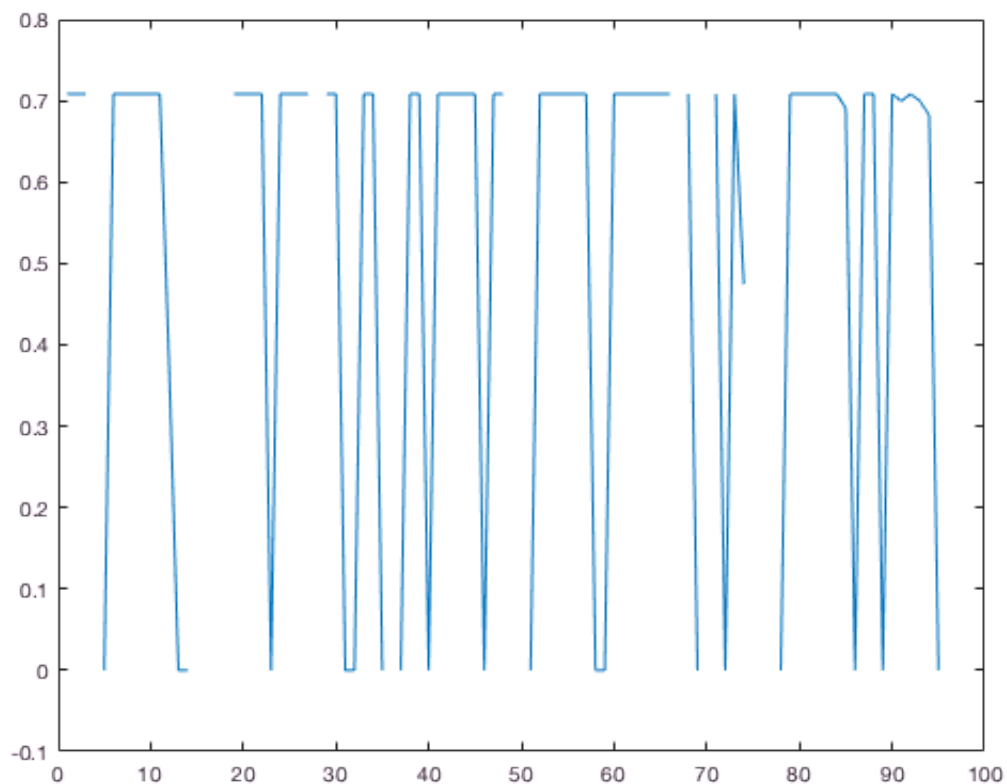
94. Reduced cost.

The reduced cost analysis can be used to identify those reactions that can reduce the growth rate (positive cost value). Reduced cost demonstrated as as part of step 89.

95. Print Matlab model content.

- Add a field if missing:

```
if ~isfield(modelEcore, 'osense')
    modelEcore.osense = -1;
end
if ~isfield(modelEcore, 'csense')
    modelEcore.csense(1:length(modelEcore.mets),1) = 'E';
end
```

- Write a SBML file:

```
writeCbModel(modelEcore,'sbml', 'EColiCore.xml')
```

```
Document written
ans =
            constraint: [1×0 struct]
    functionDefinition: [1×0 struct]
                 event: [1×0 struct]
                  rule: [1×0 struct]
        unitDefinition: [1×1 struct]
     initialAssignment: [1×0 struct]
            SBML_level: 3
          SBML_version: 1
            annotation: ''
             areaUnits: ''
       avogadro_symbol: ''
      conversionFactor: ''
         delay_symbol: ''
           extentUnits: ''
    fbc_activeObjective: 'obj'
           fbc_version: 2
                    id: 'COBRAModel'
           lengthUnits: ''
                metaid: 'COBRAModel'
                  name: ''
                 notes: ''
               sboTerm: -1
        substanceUnits: ''
             timeUnits: ''
           time_symbol: ''
              typecode: 'SBML_MODEL'
           volumeUnits: ''
               species: [1×72 struct]
           compartment: [1×2 struct]
             parameter: [1×7 struct]
              reaction: [1×95 struct]
         fbc_fluxBound: [1×2 struct]
       fbc_geneProduct: [1×137 struct]
         fbc_objective: [1×1 struct]
            namespaces: [1×2 struct]
            fbc_strict: 1
```

TIMING

The tutorial runs as given in a few seconds to minutes. However, if you use this tutorial for debugging and generating your own model, please consider the timing of the steps, as they have been given in the original protocol [1].

The timing of the entire reconstruction process depends on the properties of the target organism (prokaryote vs. eukaryote, genome size), the quality of the genome annotation, and the availability of experimental data.

The timing listed below represents an average and can be used to plan the different stages.

Step 1 - 4 (Stage 1, draft reconstruction): days to a week.

Step 5 (Stage 1, collection of experimental data): ongoing throughout the reconstruction process

Step 6 - 23 (Stage 2, reconstruction refinement): months to a year (if debugging and gap filling is done along the way)

Step 24 - 32 (Stage 2, biomass determination): days to weeks, depending on data availability

Step 34 - 36 (Stage 2, biomass determination): days to a week.

Step 37 (Stage 2, growth requirements): days to weeks, depending on data availability

Step 38 - 42 (Stage 3, conversion): days to a week.

Step 43 - 94 (Stage 4, network evaluation/debugging): week to months.

Step 95 - 96 (Data assembly): days to weeks, depending how much and in which format data was collected.

TROUBLESHOOTING

As given in original protocol [1].

Step 38 See installation instructions of the COBRA Toolbox for details on how to install and setup Matlab, SBML and COBRA Toolbox.

Step 51 Make sure that you are working in the directory were the X3.exe script was copied to. The .expa file produced by the function must be in the same directory as X3.exe.

ANTICIPATED RESULTS

As given in original protocol [1].

This protocol will result in a reconstruction that covers most of the known metabolic information of the target organism and represents a knowledge database. This reconstruction can be used as a resource for information (query tool), high-throughput data mapping (context for content), and a starting point for mathematical models.

References

[1] Thiele Nat Protoc

[2] EcoSal

[3] iAF1260

Geobacter sulfurreducans [4]