Metabotools tutorial I

Authors:

Reviewer(s): Anne Richelle

INTRODUCTION

In this tutorial, we generate contextualized models of two lymphoblastic leukemia cell lines, CCRF-CEM and Molt- 4 cells. They will be generated by integrating semi-quantitative metabolomic data, transcriptomic data, and growth rates. We will afterwards analyze the solution space of these models by using a sampling analysis.

Before running a section in the tutorial, read the corresponding sections in the MetaboTools protocol and supplemental tutorial (Data sheet 2, http://journal.frontiersin.org/article/10.3389/fphys.2016.00327/full).

PROCEDURE

Clear workspace and initialize the COBRA Toolbox

```
clear
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: /home/syarra/Dropbox/software/gurobi/gurobi652/linux64/matlab
   - [----] TOMLAB_PATH :  --> set this path manually after installing the solver ( see instructions )
   - [----] MOSEK_PATH: /home/syarra/Dropbox/software/mosek/linux/8/
   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                1      1      1      -      -
 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         -                   9      4      5      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' - 'mosek' -
 > You can solve MILP problems using: 'glpk' - 'gurobi' - 'ibm_cplex' - 'mosek'
 > You can solve QP problems using: 'gurobi' - 'ibm_cplex' - 'mosek' - 'pdco' - 'qpng'
 > You can solve MIQP problems using: 'gurobi'
 > You can solve NLP problems using: 'matlab' - 'quadMinos'

 > Checking for available updates ...
ssh: /usr/local/MATLAB/R2016a/bin/glnxa64/libcrypto.so.1.0.0: no version information available (required
ssh: /usr/local/MATLAB/R2016a/bin/glnxa64/libcrypto.so.1.0.0: no version information available (required
OpenSSL version mismatch. Built against 1000207f, you have 100010bf
fatal: Could not read from remote repository.

Please make sure you have the correct access rights
and the repository exists.
 > The changes of The COBRA Toolbox could not be fetched. > There are 169 new commit(s) on <master> and
 > You can update The COBRA Toolbox by running updateCobraToolbox() (from within MATLAB).
```

Step 0 - Define the output location and set the LP solver

Define the output path and set the solver for LP problem

```
global CBTDIR % set path to cobratoolbox (pathToCOBRA)
outputPath = pwd;% ouputPath = 'ADD YOUR PATH TO YOUR OUTPUT FOLDER'
solver = 'glpk'; % solver = 'ADD YOUR SOLVER'; %, e.g., 'cplex_direct' for ILOG
solverOK = changeCobraSolver(solver, 'LP');
```

Check the solver setup

```
if solverOK == 1
    fprintf('Solver %s is set.\n', solver);
else
    error('Solver %s could not be used. Check if %s is in the matlab path (set path) or check
end
```

```
Solver glpk is set.
```

Load and check that the input model is correclty loaded

```
tutorialPath = [CBTDIR filesep 'tutorials' filesep 'metabotools' filesep 'tutorial_I'];
```

```
if isequal(exist([tutorialPath filesep 'starting_model.mat'], 'file'), 2)
    load([tutorialPath filesep 'starting_model.mat']);
    fprintf('The model is loaded.\n');
else
    error('The model ''starting_model'' could not be loaded.');
end
```

```
The model is loaded.
```

Check output path and writing permission

```
if ~exist(outputPath, 'dir') == 7
    error('Output directory in ''outputPath'' does not exist. Verify that you type it correctl
end

% Make and save a dummy file to test the writing to output directory
A = rand(1);
try
    save([outputPath filesep 'A']);
catch ME
    error('Files cannot be saved to the provided location: %s\nObtain rights to write into %s
end
```

Step 1: Shaping the model's environment using setMediumConstraints

Constrain the model using the data related to RPMI medium composition. To this end, define the set of exchange reactions for which exometabolomic data are available

```
medium_composition = {'EX_ala_L(e)';'EX_arg_L(e)';'EX_asn_L(e)';'EX_asp_L(e)';'EX_cys_L(e)';'E
    'EX_glu_L(e)';'EX_gly(e)';'EX_his_L(e)';'EX_ile_L(e)';'EX_leu_L(e)';'EX_lys_L(e)';'EX_met_
    'EX_phe_L(e)';'EX_4HPRO(e)';'EX_pro_L(e)';'EX_ser_L(e)';'EX_thr_L(e)';'EX_trp_L(e)';'EX_ty
    'EX_val_L(e)';'EX_ascb_L(e)';'EX_btn(e)';'EX_chol(e)';'EX_pnto_R(e)';'EX_fol(e)';'EX_ncam(
    'EX_pydxn(e)';'EX_ribflv(e)';'EX_thm(e)';'EX_inost(e)';'EX_ca2(e)';'EX_fe3(e)';'EX_k(e)';'
    'EX_na1(e)';'EX_pi(e)';'EX_glc(e)';'EX_hxan(e)';'EX_lnlc(e)';'EX_lipoate(e)';'EX_pyr(e)';'
    'EX_gthrd(e)';'EX_anth(e)'};

% Medium concentrations
met_Conc_mM = [0.1;1.15;0.15;0.379;0.208;2;0.136;0.133;0.0968;0.382;0.382;0.274;0.101;0.0909;0
    0.286;0.168;0.0245;0.129;0.171;0.00863;0.00082;0.0214;0.000524;0.00227;0.082;0.00485;0.000
    0.194;0.424;0;5.33;23.81;127.26;5.63;11.11;0;0;0;1;0;0.00326;0.0073];
```

Define constraints on basic medium components (i.e., metabolites that are uptake from the medium but not captured by the measured data)

```
mediumCompounds = {'EX_co2(e)';'EX_h(e)';'EX_h2o(e)';'EX_hco3(e)';'EX_nh4(e)';'EX_o2(e)';'EX_p
mediumCompounds_lb = -100;
```

Define also additional constraints to limit the model behaviour (e.g., secretion of oxygen, essential amino acids that need to be taken up)

```
customizedConstraints = {'EX_o2(e)';'EX_strch1(e)';'EX_acetone(e)';'EX_glc(e)';'EX_his_L(e)';'
customizedConstraints_lb = [-2.3460;0;0;-500;-100;-100;-100];
```

```
customizedConstraints_ub = [500;0;0;500;500;500;500];
```

Apply the medium constraints previously defined using *setMediumConstraints.* Note that this function also require the definition of the cell concentration (*cellConc*), the cell weight (*cellWeight*), the time (*t*), the current value and the new value for infinite constraints (respectively *current_inf* and *set_inf*).

```
cellConc = 2.17 * 1e6;
cellWeight = 3.645e-12;
t = 48;
current_inf = 1000;
set_inf = 500;
[modelMedium, ~] = setMediumConstraints(starting_model, set_inf, current_inf, medium_compositi
    t, cellWeight, mediumCompounds, mediumCompounds_lb, customizedConstraints, customizedConst
```

Step 2: calculate the limit of detection (LODs) for each metabolites

Use the function *calculateLODs* to converts detection limits of unit *ng/mL* to *mM* using the theoretical mass (g/mol)

```
ex_RXNS = {'EX_5mta(e)';'EX_uri(e)';'EX_chol(e)';'EX_ncam(e)';'EX_3mop(e)';'EX_succ(e)';'EX_pr
    'EX_5oxpro(e)';'EX_thm(e)';'EX_anth(e)';'EX_4HPRO(e)';'EX_lac_L(e)';'EX_3mob(e)';'EX_his_L
    'EX_trp_L(e)';'EX_orn(e)';'EX_arg_L(e)';'EX_thr_L(e)';'EX_fol(e)';'EX_gln_L(e)';'EX_4pyrdx
    'EX_ser_L(e)';'EX_glc(e)';'EX_ribflv(e)';'EX_glu_L(e)';'EX_tyr_L(e)';'EX_phe_L(e)';'EX_inc
    'EX_Lcystin(e)';'EX_leu_L(e)';'EX_met_L(e)';'EX_cys_L(e)';'EX_asn_L(e)';'EX_mal_L(e)';'EX_
    'EX_pyr(e)';'EX_lys_L(e)';'EX_ala_L(e)';'EX_cit(e)';'EX_pro_L(e)';'EX_gly(e)';'EX_asp_L(e)
    'EX_octa(e)';'EX_4mop(e)';'EX_glyb(e)';'EX_val_L(e)';'EX_ade(e)';'EX_hxan(e)';'EX_gua(e)';
    'EX_orot(e)';'EX_ura(e)';'EX_ahcys(e)';'EX_cbasp(e)';'EX_Lcystin(e)';'EX_ser_L(e)';'EX_cys
    'EX_thm(e)';'EX_arg_L(e)';'EX_ncam(e)'};

theo_mass = [298.0974;243.0617;104.1075;123.0558;129.0552;117.0188;220.1185;128.0348;265.1123;
    132.0661;89.0239;115.0395;156.0773;205.0977;133.0977;175.1195;120.0661;440.1319;147.077;18
    106.0504;179.0556;377.1461;148.061;182.0817;166.0868;179.0556;241.0317;132.1025;150.0589;1
    133.0613;133.0137;132.1025;87.0082;147.1134;90.0555;191.0192;116.0712;74.0242;134.0453;180
    172.265;130.142;118.0868;118.0868;136.0623;137.0463;152.0572;267.0729;155.0093;111.0195;38
    175.0355;241.0317;106.0504;122.0276;265.1123;175.1195;123.0558];

lod_ngmL = [0.3;1.7;2.8;3;3.5;3.9;4;4.8;6.1;7.7;8.1;10.9;11.2;13.6;15.7;16.9;24.8;25.6;25.7;28
    37.5;44;45;45;47.4;48.4;59;59.7;68.9;74.1;77;82.1;99.2;112.9;121.3;131.7;133.5;150.8;169.2
    229.5;537.3;10.9;3.5;2.8;28.2;1.6;0.8;48.9;8.8;37.1;52.4;50;229.5;59.7;37.5;77;6.1;24.8;3]

[lod_mM] = calculateLODs(theo_mass, lod_ngmL);
```

Step 3: define the uptake and secretion profiles

Exclude metabolites with uncertain experimental data from the list of metabolites for which uptake and secretion profiles need to be computed

```
exclude_upt = {'EX_gln_L(e)'; 'EX_cys_L(e)'; 'EX_ala_L(e)'; 'EX_mal_L(e)'; 'EX_fol(e)'};
exclude_secr = {'EX_gln_L(e)'; 'EX_cys_L(e)'; 'EX_ala_L(e)'};
```

Define metabolites with missing experimental points but for which uptake and secretion profiles need to be computed

```
add_secr = {'EX_mal_L(e)'};
add_upt = {};
```

The essential amino acids should be excluded from the secretion profile

```
essAA_excl = {'EX_his_L(e)'; 'EX_ile_L(e)'; 'EX_leu_L(e)'; 'EX_lys_L(e)'; 'EX_met_L(e)';...
    'EX_phe_L(e)'; 'EX_thr_L(e)'; 'EX_trp_L(e)'; 'EX_val_L(e)'};
```

Define the list of metabolites for which experimental data are available

```
data_RXNS = {'EX_orn(e)';'EX_mal_L(e)';'EX_lac_L(e)';'EX_gly(e)';'EX_glu_L(e)';'EX_cit(e)';...
    'EX_5oxpro(e)';'EX_4mop(e)';'EX_3mop(e)';'EX_3mob(e)';'EX_tyr_L(e)';'EX_trp_L(e)';...
    'EX_thr_L(e)';'EX_pyr(e)';'EX_phe_L(e)';'EX_lys_L(e)';'EX_leu_L(e)';'EX_ile_L(e)';...
    'EX_glc(e)';'EX_chol(e)';'EX_anth(e)';'EX_val_L(e)';'EX_met_L(e)';'EX_his_L(e)';...
    'EX_gln_L(e)';'EX_cys_L(e)';'EX_ala_L(e)';'EX_pi(e)';'EX_asp_L(e)';'EX_4HPRO(e)';...
    'EX_pnto_R(e)';'EX_pro_L(e)';'EX_fol(e)'};
```

Define the data associated with Molt-4 cell cultures

```
input_A = [
    % control TP 1 control TP 2 Cond TP 1 Cond TP 2
    65245.09667 68680.93 54272.41667 65159.50333
    3000 30970.784 20292.406 27226.6555
    2038946.433 1917042.967 5654513.467 101768253
    163882.9467 186682.92 121762.3567 310547.7
    473539.8667 455197.4667 462903.8333 1024508.5
    8681.527333 8704.7345 9459.837 34177.945
    29168.15 21808.73 120655.9867 2060525.467
    3000 3000 34436.50433 113668.5123
    3000 3000 25108.829 121927.3673
    3000 3000 3000 14717.55667
    4142302 4063607.667 3934639.333 3075783.333
    2153692 2132723.667 2037735.333 1387754.333
    406102.2667 417512.6333 381085.2333 259555.2667
    465074.6 387569.1333 439148.0667 210407.8333
    8087955 8345511.333 8215168.333 5360276
    198435.8 195675.8 188473.1 112386.1667
    20823770.33 20801258.67 19725086.67 15148808
    21229254.67 21225778.33 20799761 17160163
    76555640.67 71459886.33 61697085.33 34981419.33
    876300.4333 905132.5 892182.2 541860.4667
    159124.46 178538.2167 162567.13 3000
    2857012.667 2900419.667 2853523.667 1793173.667
    2995910.333 3018536.333 3024630.333 2266832.333
    69077.16333 67843.12 69406.69 95624.28
    3000 3000 824549.3667 2283200.867
    45304.84667 52977.77333 56566.27667 60759.23
    1613345.1 1258710.1 3430342.067 25970024.1
    216828142.3 221118425 223518663 216863897.3
    632160.0333 612562.3 590881.7333 940705.6
    814465.8333 786011.5667 630513.4 622493.9
    84638.70667 86751.96 89717.10667 68882.68333
    5107317.333 5168599.333 5163708.333 5263614.333
    95419.73667 105904.7067 97550.78667 102678.49
];
```

Define the data associated with CCRF-CEM cell cultures

```
input_B = [
    % control 2 TP 1 control 2 TP 2 Cond 2 TP 1 Cond 2 TP 2
    65245.09667 68680.93 73850.77 98489.89
    3000 30970.784 3000 94181.77233
    2038946.433 1917042.967 5222377.933 134980059.9
    163882.9467 186682.92 219683.7 460476.5267
    473539.8667 455197.4667 437398.3667 630407.2667
    8681.527333 8704.7345 8317.144 86546.77933
    29168.15 21808.73 62146.47333 1012932.38
    3000 3000 9918.992 129433.4973
    3000 3000 7222.259333 145547.7347
    3000 3000 3000 17641.55667
    4142302 4063607.667 4023284.333 3489981.333
    2153692 2132723.667 2068977 1570648
    406102.2667 417512.6333 386495.2 303808.2
    465074.6 387569.1333 376779.1 249036.3333
    8087955 8345511.333 8237784.667 6540301.667
    198435.8 195675.8 196447.1 149861.6667
    20823770.33 20801258.67 21119935.67 16346765.67
    21229254.67 21225778.33 20790535.33 17219085
    76555640.67 71459886.33 65009057.67 24330565.33
    876300.4333 905132.5 884112.5667 259273.9333
    159124.46 178538.2167 158271.14 60631.19333
    2857012.667 2900419.667 2668140 2790196.333
    2995910.333 3018536.333 2890029.333 2538211
    69077.16333 67843.12 74035.24 86165.55
    3000 3000 323185.6667 2063962.067
    45304.84667 52977.77333 62076.23333 64524.22333
    1613345.1 1258710.1 2788313.567 30868376.53
    216828142.3 221118425 212276379 208623151.3
    632160.0333 612562.3 680373.4333 770903.9333
    814465.8333 786011.5667 679862.7 582257.4667
    84638.70667 86751.96 88002.12 99449.36667
    5107317.333 5168599.333 5134219 4445918.333
    95419.73667 105904.7067 100629.24 84807.62333
];
```

Use the function *defineUptakeSecretionProfiles* to calculate the uptake and secretion rate over the time of the culture for both condition (e.g. CCRF-CEM and Molt- 4 cells)

```
tol = 0.05;
[cond1_uptake, cond2_uptake, cond1_secretion, cond2_secretion, slope_Ratio] = defineUptakeSecr
    (input_A, input_B, data_RXNS, tol, essAA_excl, exclude_upt, exclude_secr, add_secr, add_up
```

Step 4: Calculate the difference between the uptake and secretion profiles from the two conditions

Use *calculateQuantitativeDiffs* to calculate the sets of exchange reactions with higher uptake and secretion in condition 1 than in condition 2.

```
[cond1_upt_higher, cond2_upt_higher, cond2_secr_higher, cond1_secr_higher, cond1_uptake_LODs,.
    cond2_uptake_LODs, cond1_secretion_LODs, cond2_secretion_LODs] = calculateQuantitativeDiff
    slope_Ratio, ex_RXNS, lod_mM, cond1_uptake, cond2_uptake, cond1_secretion, cond2_secretion
```

NOTE: Sometimes, you will need to remove some metabolites from the uptake and secretion profiles, e.g. those for which you assume a different directionality as in the data or if the metabolites is not detected at a specific sampling time. Indeed, the inclusion of these extreme point could distort the results. Example of consumption slope ratio associated to *EX_anth(e)* is 1975% higher in Molt-4 compared to CCRF-CEM cells. Therefore, these metabolites need to be removed from the input for semi-quantitative adjustment unless such large differences are justified and make sense biologically.

```
remove = {'EX_anth(e)'; 'EX_ile_L(e)'};
A = [];
for i = 1:length(cond2_upt_higher)
    if find(ismember(remove, cond2_upt_higher{i, 1})) > 0
        A = [A; i];
    end
end
cond2_upt_higher(A, :) = [];
```

Step 5: Enforce uptake and secretion rate using qualitative constraints

Use the function *setQualitativeConstraints* to enforce minimal uptake or secretion based on individual detection limits (e.g., based on the uptake and secretion profile of metabolites measured through mass-spectrometry). If these values are not available, a very small value (e.g., 1.0E-06) can be used. Note that this value has to be below the concentrations defined in the medium, otherwise the model will be infeasible.

Definition of the qualitative constraints for Molt-4 cells

```
ambiguous_metabolites = {'EX_ala_L(e)'; 'EX_gln_L(e)'; 'EX_cys_L(e)'};

basisMedium = {'EX_o2(e)'; 'EX_strch1(e)'; 'EX_acetone(e)'; 'EX_glc(e)'; 'EX_his_L(e)'; 'EX_ca
    'EX_fe2(e)'; 'EX_fe3(e)'; 'EX_k(e)'; 'EX_na1(e)'; 'EX_i(e)'; 'EX_sel(e)'; 'EX_co2(e)'; 'EX
    'EX_nh4(e)'; 'EX_o2(e)'; 'EX_pi(e)'; 'EX_so4(e)'};

[model_A] = setQualitativeConstraints(modelMedium, cond1_uptake, cond1_uptake_LODs, cond1_secr
    cellConc, t, cellWeight, ambiguous_metabolites, basisMedium);
```

Definition of the qualitative constraints for CCRF-CEM cells

```
ambiguous_metabolites = {'EX_ala_L(e)'; 'EX_gln_L(e)'; 'EX_pydxn(e)'; 'EX_cys_L(e)'};

basisMedium = {'EX_ca2(e)'; 'EX_cl(e)'; 'EX_co(e)'; 'EX_fe2(e)'; 'EX_fe3(e)'; 'EX_k(e)'; 'EX_n
    'EX_co2(e)'; 'EX_h(e)'; 'EX_h2o(e)'; 'EX_hco3(e)'; 'EX_nh4(e)'; 'EX_o2(e)'; 'EX_pi(e)'; 'E
    'EX_o2(e)'; 'EX_strch1(e)'; 'EX_acetone(e)'; 'EX_glc(e)'; 'EX_val_L(e)'; 'EX_met_L(e)'};

[model_B] = setQualitativeConstraints(modelMedium, cond2_uptake, cond2_uptake_LODs, cond2_secr
    cellConc, t, cellWeight, ambiguous_metabolites, basisMedium);
```

Step 6: Define semi quantitative constraints

Use the relative difference of signal intensities previously calculated for the two conditions (*calculateQuantitativeDiffs*) to define semi-quantitative constraints (setSemiQuantConstraints).

```
[modelA_QUANT, modelB_QUANT] = setSemiQuantConstraints(model_A, model_B, cond1_upt_higher, con
```

Step 7: Define growth constraints

Using the data related to the doubling time for each cell, constrain the growth reaction using *setConstraintsOnBiomassReaction*

```
GrowthRxn = 'biomass_reaction2';
tolerance = 20;
doublingTimeA = 19.6; %MOLT4 cells
[model_A_BM] = setConstraintsOnBiomassReaction(modelA_QUANT, GrowthRxn, doublingTimeA, toleran
doublingTimeB = 22; %CCRF-CEM
[model_B_BM] = setConstraintsOnBiomassReaction(modelB_QUANT, GrowthRxn, doublingTimeB, toleran
```

Step 8: Delete absent genes

Constrain to zero the set of absent genes, defined in *DataGenes*

```
dataGenes = [535;1548;2591;3037;4248;4709;6522;7167;7367;8399;23545;129807;221823]; % set of g
[model_A_GE] = integrateGeneExpressionData(model_A_BM, dataGenes);

dataGenes = [239;443;535;1548;2683;3037;4248;4709;5232;6522;7364;7367;8399;23545;54363;66002;1
[model_B_GE] = integrateGeneExpressionData(model_B_BM, dataGenes);
```

Step 9: Extract a condition specific FVA

Use extractConditionSpecificModel to prune the model based on a user-defined flux value threshold. This function a flux variability analyis to extract a subnetwork for which all reactions carry fluxes higher or equal to the defined threshold value

```
theshold = 1e-6;
model = model_A_GE;
[model_Molt] = extractConditionSpecificModel(model, theshold);%  MOLT4 condition specific mode
[model_CEM] = extractConditionSpecificModel(model_B_GE, theshold);%  CCRF-CEM condition specif
```

ANTICIPATED RESULTS

Compare the differents model generated previously by analysing the metabolite connectivity of the networks

```
[MetConn, RxnLength] = networkTopology(modelMedium); % model constrained by medium composition
[MetConnA, RxnLengthA] = networkTopology(model_Molt); %  MOLT4 condition specific model
[MetConnB, RxnLengthB] = networkTopology(model_CEM); %  CCRF-CEM condition specific model
MetConnCompare = sort(MetConn, 'descend');
MetConnCompareA = sort(MetConnA, 'descend');
MetConnCompareB = sort(MetConnB, 'descend');
```

Plot metabolite connectivity

```
figure
semilogy(sort(MetConnCompare, 'descend'), 'ro')
hold
semilogy(sort(MetConnCompareA, 'descend'), 'bo')
semilogy(sort(MetConnCompareB, 'descend'), 'go')
title('Metabolite connectivity')
```

The models can also be compared by performing a sampling analysis using *performSampling*

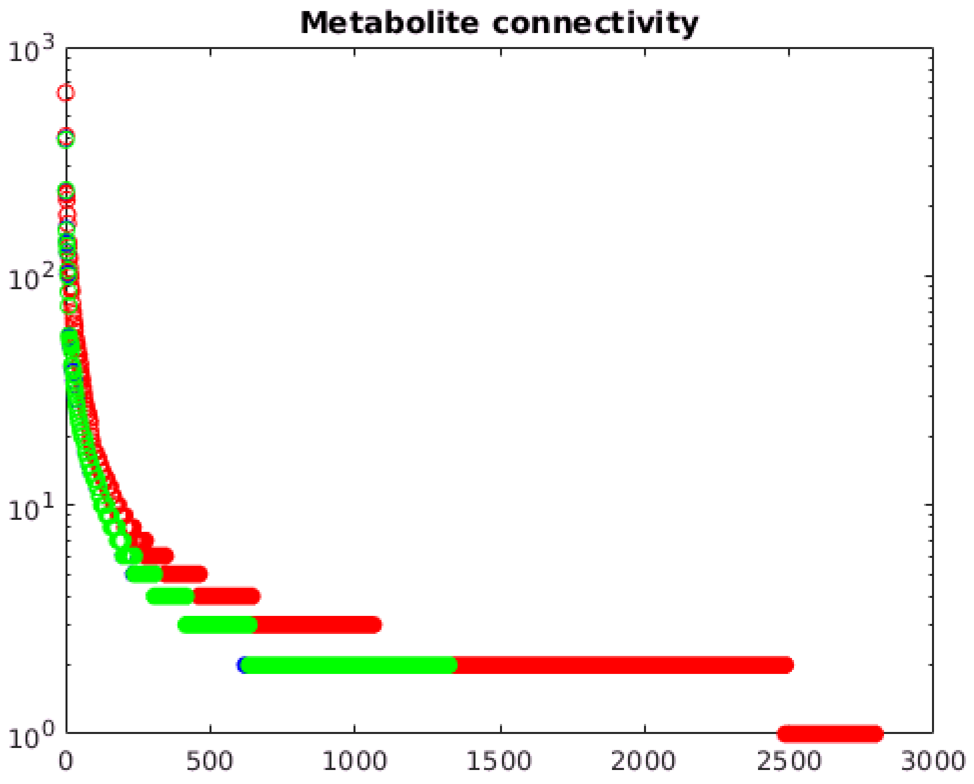
```
fprintf('Perform sampling analysis\n');
warmupn = 2000;
nFiles = 10;
pointsPerFile = 1000;
stepsPerPoint = 500;
fileBaseNo = 0;
maxTime = 3600000;

fileName = 'modelA';%  MOLT4 condition specific model
performSampling(model_Molt, warmupn, fileName, nFiles, pointsPerFile, stepsPerPoint, fileBaseN
fileName = 'modelB';%  CCRF-CEM condition specific model
performSampling(model_CEM, warmupn, fileName, nFiles, pointsPerFile, stepsPerPoint, fileBaseNo
```

Use the function *summarizeSamplingResults* to return the median of the flux values from the two sampled models. The analysis can be limited to a specific set of reaction defined in *show_rxns*. Moreover, reactions associated with genes of special interest (e.g. differentially expressed genes) can be defined in *dataGenes* to facilitate the analysis

```
fonts = 8;
nFiles = 10;
pointsPerFile = 1000;
starting_Model = modelMedium;
hist_per_page = 4;
bin = 30;
modelA = model_Molt;
modelB = model_CEM;
dataGenes = [32;205;411;412;1537;1608;1632;1645;1737;1757;2108;2184;2224;2539];
show_rxns = {'PYK';'SUCD1m';'ATPS4m';'ETF'};
[stats, statsR] = summarizeSamplingResults(modelA, modelB, outputPath, nFiles, pointsPerFile,
```

```
Current plot held
```



```
Perform sampling analysis
Warning: Need a minimum of 3828 warmup points
Creating warmup points ...
     ⋮
Warning: Need a minimum of 3876 warmup points
Creating warmup points ...
     ⋮
```

```
Loading samples ...
10%     [....                                    ]��������������������������������
Loading samples ...
10%     [....                                    ]��������������������������������
```

```
Warning: 3rd argument is numericFlag, currently redundant, will be depreciated
Starting parallel pool (parpool) using the 'local' profile ... connected to 12 workers.
Saving results to /home/syarra/Dropbox/uni.lu/github/opencobra/cobratoolbox/tutorials/metabotools/tutori
```