Extract a thermodynamically consistent subnetwork from a given model

1. Identify the largest subset of a model that admits a thermodynamically consistent flux
2. Specify a random subset of active/inactive reactions and present/absent metabolites
3. Remove absent metabolites and inactive reactions, then recalculate the largest subset of a model that admits a thermodynamically consistent flux
4. Compute the smallest thermodynamically consistent subnetwork containing a list of present metabolites and active reactions

```
[solverOK,solverInstalled]=changeCobraSolver('ibm_cplex','all');
```

```
> changeCobraSolver: IBM ILOG CPLEX interface added to MATLAB path.
> ibm_cplex (version 1210) is compatible and fully tested with MATLAB R2019a on your operating system.
> changeCobraSolver: Solver for LP problems has been set to ibm_cplex.

> changeCobraSolver: IBM ILOG CPLEX interface added to MATLAB path.
> ibm_cplex (version 1210) is compatible and fully tested with MATLAB R2019a on your operating system.
> changeCobraSolver: Solver for MILP problems has been set to ibm_cplex.

> changeCobraSolver: IBM ILOG CPLEX interface added to MATLAB path.
> ibm_cplex (version 1210) is compatible and fully tested with MATLAB R2019a on your operating system.
> changeCobraSolver: Solver for QP problems has been set to ibm_cplex.

> changeCobraSolver: IBM ILOG CPLEX interface added to MATLAB path.
> ibm_cplex (version 1210) is compatible and fully tested with MATLAB R2019a on your operating system.
> changeCobraSolver: Solver for MIQP problems has been set to ibm_cplex.
> changeCobraSolver: Solver ibm_cplex not supported for problems of type NLP. Currently used: matlab
```

```
%[solverOK,solverInstalled]=changeCobraSolver('gurobi','all');
%[solverOK,solverInstalled]=changeCobraSolver('ibm_cplex','QP');
```

Load model

```
modelToLoad='circularToy';
modelToLoad='ecoli_core';
modelToLoad='modelRecon3MitoOpen';
modelToLoad='Recon3DModel';
%modelToLoad='iDopa';
```

Load a model

```
driver_thermoModelLoad
```

```
Model loaded: Recon3DModel
lower bounds greater than zero
Internal stochiometric nullspace computed in 1.5352 seconds.
```

Remove forced reaction rates

```
forcedRxnBool = model.lb>0 | model.ub<0;
nForcedRxn = nnz(forcedRxnBool)
```

```
nForcedRxn =
     1
```

```
printConstraints(model,[],[],forcedRxnBool)
```

Forward_Reaction	Name	lb	ub	
'biomass_reaction'	'Generic Human Biomass Reaction'	1	1000	'20.6508 h2o[c] + 20.7045 atp[

```
model.lb(strcmp(model.rxns,'biomass_reaction'))=0;
```

Stoichiometric consistency

```
if ~isfield(model,'SConsistentRxnBool')  ||
~isfield(model,'SConsistentMetBool')
    massBalanceCheck=0;
    %massBalanceCheck=1;
    printLevel=2;
    [SConsistentMetBool, SConsistentRxnBool,
SInConsistentMetBool, SInConsistentRxnBool, unknownSConsistencyMetBool,
unknownSConsistencyRxnBool, model,stoichConsistModel]...
        = findStoichConsistentSubset(model, massBalanceCheck, printLevel);
else
    %Extract stoich consistent submodel
    if any(~model.SConsistentMetBool)
        rxnRemoveMethod='inclusive';%maintains stoichiometric consistency
        [stoichConsistModel, rxnRemoveList] = removeMetabolites(model,
model.mets(~model.SConsistentMetBool),rxnRemoveMethod);
        SConsistentRxnBool2=~ismember(model.rxns,rxnRemoveList);
        if ~all(model.SConsistentRxnBool==SConsistentRxnBool2)
            error('inconsistent reaction removal')
        end
        try
            stoichConsistModel = removeUnusedGenes(stoichConsistModel);
        catch ME
            disp(ME.message)
        end
    else
        stoichConsistModel = model;
    end
end

[nMet,nRxn]=size(stoichConsistModel.S)
```

```
nMet =
        5835
nRxn =
       10600
```

Flux consistency

```
fluxConsistentParam.method='fastcc';%can handle additional constraints
fluxConsistentParam.printLevel=1;
[~,~,~,~,stoichConsistModel]=
findFluxConsistentSubset(stoichConsistModel,fluxConsistentParam);
```

Extract flux consistent submodel

```
if any(~stoichConsistModel.fluxConsistentRxnBool)
    rxnRemoveList =
stoichConsistModel.rxns(~stoichConsistModel.fluxConsistentRxnBool);
    stoichFluxConsistModel = removeRxns(stoichConsistModel,
rxnRemoveList,'metRemoveMethod','exclusive','ctrsRemoveMethod','inclusive');
    try
        stoichFluxConsistModel = removeUnusedGenes(stoichFluxConsistModel);
        catch ME
        disp(ME.message)
    end
else
    stoichFluxConsistModel = stoichConsistModel;
end
[nMet,nRxn]=size(stoichFluxConsistModel.S)
```

```
nMet =
        5835
nRxn =
       10600
```

Thermodynamic consistency

```
%save('debug_prior_to_findThermoConsistentFluxSubset.mat')
%return
param.printLevel = 1;
[thermoFluxConsistentMetBool,thermoFluxConsistentRxnBool,stoichFluxConsistMod
el,stoichFluxThermoConsistModel] =
findThermoConsistentFluxSubset(stoichFluxConsistModel,param);
```

```
--- findThermoFluxConsistentSubset START ----
                 printLevel: 1
                          n: 200
    normalizeZeroNormWeights: 0
                     epsilon: 1e-06
                 formulation: 'pqzw'
             iterationMethod: 'random'
                        nMax: 20
                 relaxBounds: 1
           acceptRepairedFlux: 1
              warmStartMethod: 'random'
              thetaMultiplier: 1.5
                       theta: 0.5
              regularizeOuter: 0
     thermoConsistencyMethod: 'cycleFreeFlux'
                      bigNum: 10000
                       debug: 0

optCardThermo objective data:
```

```
        0.1 = beta, the global weight on one-norm of internal reaction rate.
         -5 = min(g0), the local weight on zero-norm of internal reaction rate.
         -0 = max(g0), the local weight on zero-norm of internal reaction rate.
          0 = min(h0), the local weight on zero-norm of metabolite production rate.
          0 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

0 min cardinality variables:
        NaN mean(c(p))            NaN min(c(p))            NaN max(c(p))
          1 lambda0             NaN min(k)             NaN max(k)
          1 lambda1             NaN min(o(p))             NaN max(o(p))

5358 max cardinality variables:
         -0 mean(c(q))              -0 min(c(q))              -0 max(c(q))
          1 delta0                5 min(d)                5 max(d)
          0 delta1                0 min(o(q))               0 max(o(q))

22824 cardinality free variables:
      0.077 mean(c(r))              -0 min(c(r))             0.1 max(c(r))
          0 alpha1                0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1      0.50  7.6672e+05    -8.8e+06    -2.2e+04     1.6e+03           0           0           0      -237
   2      0.75      161.49    -7.9e+02    -2.3e+04     1.1e+03           0           0           0      -238
   3      1.12      90.057    -4.3e+02    -2.3e+04     7.3e+02           0           0           0      -238
   4      1.69      59.684    -2.6e+02    -2.3e+04     4.9e+02           0           0           0      -238
   5      2.53      38.202    -1.6e+02    -2.4e+04     3.2e+02           0           0           0      -238
   6      3.80       25.75    -1.1e+02    -2.4e+04     2.2e+02           0           0           0      -238
   7      5.70      17.391         -72    -2.4e+04     1.4e+02           0           0           0      -238
   8      8.54      12.147         -48    -2.4e+04          97           0           0           0      -238
   9     12.81      13.536         -53    -2.4e+04          67           0           0           0      -238
  10     19.22      7.1044         -23    -2.4e+04          45           0           0           0      -238
  11     28.83      5.0586         -15    -2.4e+04          30           0           0           0      -238
  12     43.25      3.3766         -10    -2.4e+04          20           0           0           0      -238
  13     64.87      2.2681         -17    -2.4e+04          13           0           0           0      -238
  14     97.31        1.59        -4.4    -2.4e+04         8.9           0           0           0      -238
  15    145.96      1.0555          -3    -2.4e+04         5.9           0           0           0      -238
  16    218.95      0.6324          -2    -2.4e+04         3.9           0           0           0      -238
  17    328.42     0.46083        -1.3    -2.4e+04         2.6           0           0           0      -238
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
    Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
               iter            card(y)              nz      %feas int.nz.   tot %feas int.nz.   tot
                  1               5358            7593               1.00                0.67
                  2               1467            5506               1.00                0.85
                  3                706            5006               1.00                0.92
                  4                352            3403               1.00                0.95
                  5                218            4480               1.00                0.97
                  6                126            2147               1.00                0.97
                  7                118            1301               1.00                0.98
                  8                110            1095               1.00                0.98
                  9                113             639               1.00                0.98
                 10                 94               2                NaN                0.98
                 11                 93            1739               1.00                0.98
                 12                 87               2                NaN                0.98
                 13                 92               7               1.00                0.98
                 14                107               2                NaN                0.98
                 15                101               2                NaN                0.98
                 16                 93             342               1.00                0.98
                 17                 91            1014               1.00                0.98
                 18                 76               2                NaN                0.98
               iter            card(y)              nz      %feas int.nz.   tot %feas int.nz.   tot
```

```
findThermoConsistentFluxSubset terminating early: no progress on % internal reactions thermodynamically fl
--- findThermoFluxConsistentSubset END ----
```

Size of the largest flux, stoich and thermo consistent submodel

```
[nMet,nRxn]=size(stoichFluxThermoConsistModel.S)
```

```
nMet =
        5756
nRxn =
       10447
```

Nullspace

Nullspace is necessary for backup check of thermodynamic consistency using thermoFlux2QNty

```
[stoichFluxThermoConsistModel,rankK,nnzK,timeTaken] =
internalNullspace(stoichFluxThermoConsistModel);
rankK
```

```
rankK =
        5522
```

Minimal thermodynamically consistent submodel

Compute the minimal thermodynamically consistent submodel

```
[minimalModel, modelThermoMetBool, modelThermoRxnBool] =
thermoKernel(stoichFluxThermoConsistModel);
```

```
--- thermoKernel START ----
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 3
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0               1 min(k)                1 max(k)
           0 lambda1               0 min(o(p))                0 max(o(p))

0 max cardinality variables:
```

```
        NaN mean(c(q))             NaN min(c(q))             NaN max(c(q))
          1 delta0             NaN min(d)              NaN max(d)
          0 delta1             NaN min(o(q))              NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
          0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8642e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
    Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
    1         2               NaN              NaN         0               NaN               NaN      gre
           warmStartMethod: 'random'
               formulation: 'pqzwrs'
           thetaMultiplier: 1.5
                     theta: 0.5
           regularizeOuter: 1
                   epsilon: 1e-06
                printLevel: 2
               relaxBounds: 0
         acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                    bigNum: 10000
                     debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0              1 min(k)              1 max(k)
           0 lambda1              0 min(o(p))               0 max(o(p))

0 max cardinality variables:
         NaN mean(c(q))             NaN min(c(q))             NaN max(c(q))
           1 delta0             NaN min(d)              NaN max(d)
           0 delta1             NaN min(o(q))              NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
          0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8153e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
    Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
    2         2               NaN              NaN         0               NaN               NaN      gre
           warmStartMethod: 'random'
               formulation: 'pqzwrs'
           thetaMultiplier: 1.5
                     theta: 0.5
           regularizeOuter: 1
```

```
                  epsilon: 1e-06
                printLevel: 2
               relaxBounds: 0
         acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                    bigNum: 10000
                     debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
          1 = min(g0), the local weight on zero-norm of internal reaction rate.
          1 = max(g0), the local weight on zero-norm of internal reaction rate.
          1 = min(h0), the local weight on zero-norm of metabolite production rate.
          1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
          0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
          1 lambda0               1 min(k)                1 max(k)
          0 lambda1               0 min(o(p))                0 max(o(p))

0 max cardinality variables:
         NaN mean(c(q))             NaN min(c(q))             NaN max(c(q))
          1 delta0             NaN min(d)              NaN max(d)
          0 delta1             NaN min(o(q))             NaN max(o(q))

23034 cardinality free variables:
        0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
          0 alpha1              0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8337e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
    3         2               NaN               NaN         0               NaN               NaN      gre
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                printLevel: 2
               relaxBounds: 0
         acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                    bigNum: 10000
                     debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
          1 = min(g0), the local weight on zero-norm of internal reaction rate.
          1 = max(g0), the local weight on zero-norm of internal reaction rate.
          1 = min(h0), the local weight on zero-norm of metabolite production rate.
          1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
          0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
          1 lambda0               1 min(k)                1 max(k)
          0 lambda1               0 min(o(p))                0 max(o(p))
```

```
0 max cardinality variables:
         NaN mean(c(q))             NaN min(c(q))             NaN max(c(q))
           1 delta0             NaN min(d)             NaN max(d)
           0 delta1             NaN min(o(q))             NaN max(o(q))

23034 cardinality free variables:
        0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
           0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.7839e+05    -8.6e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
    4         2               NaN              NaN         0              NaN              NaN      gre
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
     thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0              1 min(k)              1 max(k)
           0 lambda1              0 min(o(p))              0 max(o(p))

0 max cardinality variables:
         NaN mean(c(q))             NaN min(c(q))             NaN max(c(q))
           1 delta0             NaN min(d)             NaN max(d)
           0 delta1             NaN min(o(q))             NaN max(o(q))

23034 cardinality free variables:
        0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
           0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8617e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
    5         2               NaN              NaN         0              NaN              NaN      gre
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
```

```
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0               1 min(k)               1 max(k)
           0 lambda1               0 min(o(p))               0 max(o(p))

0 max cardinality variables:
         NaN mean(c(q))             NaN min(c(q))             NaN max(c(q))
           1 delta0             NaN min(d)             NaN max(d)
           0 delta1             NaN min(o(q))             NaN max(o(q))

23034 cardinality free variables:
        0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
           0 alpha1              0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8379e+05    -8.6e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
    6         2               NaN               NaN         0               NaN               NaN      gre
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0               1 min(k)               1 max(k)
```

```
          0 lambda1              0 min(o(p))              0 max(o(p))

0 max cardinality variables:
         NaN mean(c(q))            NaN min(c(q))            NaN max(c(q))
           1 delta0            NaN min(d)            NaN max(d)
           0 delta1            NaN min(o(q))            NaN max(o(q))

23034 cardinality free variables:
        0.75 mean(c(r))              0 min(c(r))              1 max(c(r))
           0 alpha1              0 min(o(r))              0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8185e+05    -8.6e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
    7         2               NaN              NaN         0              NaN              NaN      gre
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
     thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0              1 min(k)              1 max(k)
           0 lambda1              0 min(o(p))              0 max(o(p))

0 max cardinality variables:
         NaN mean(c(q))            NaN min(c(q))            NaN max(c(q))
           1 delta0            NaN min(d)            NaN max(d)
           0 delta1            NaN min(o(q))            NaN max(o(q))

23034 cardinality free variables:
        0.75 mean(c(r))              0 min(c(r))              1 max(c(r))
           0 alpha1              0 min(o(r))              0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8498e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
    8         2               NaN              NaN         0              NaN              NaN      gre
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
```

```
                    theta: 0.5
          regularizeOuter: 1
                  epsilon: 1e-06
               printLevel: 2
              relaxBounds: 0
       acceptRepairedFlux: 1
   thermoConsistencyMethod: 'cycleFreeFlux'
                   bigNum: 10000
                    debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
          1 = min(g0), the local weight on zero-norm of internal reaction rate.
          1 = max(g0), the local weight on zero-norm of internal reaction rate.
          1 = min(h0), the local weight on zero-norm of metabolite production rate.
          1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
          0 mean(c(p))             -0 min(c(p))             -0 max(c(p))
          1 lambda0              1 min(k)              1 max(k)
          0 lambda1              0 min(o(p))              0 max(o(p))

0 max cardinality variables:
        NaN mean(c(q))            NaN min(c(q))            NaN max(c(q))
          1 delta0           NaN min(d)           NaN max(d)
          0 delta1           NaN min(o(q))            NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))              0 min(c(r))              1 max(c(r))
          0 alpha1             0 min(o(r))             0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8335e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
    9         2              NaN              NaN         0              NaN              NaN      gre
          warmStartMethod: 'random'
              formulation: 'pqzwrs'
          thetaMultiplier: 1.5
                    theta: 0.5
          regularizeOuter: 1
                  epsilon: 1e-06
               printLevel: 2
              relaxBounds: 0
       acceptRepairedFlux: 1
   thermoConsistencyMethod: 'cycleFreeFlux'
                   bigNum: 10000
                    debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
          1 = min(g0), the local weight on zero-norm of internal reaction rate.
          1 = max(g0), the local weight on zero-norm of internal reaction rate.
          1 = min(h0), the local weight on zero-norm of metabolite production rate.
          1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
          0 mean(c(p))             -0 min(c(p))             -0 max(c(p))
```

```
          1 lambda0              1 min(k)              1 max(k)
          0 lambda1              0 min(o(p))              0 max(o(p))

0 max cardinality variables:
        NaN mean(c(q))            NaN min(c(q))            NaN max(c(q))
          1 delta0            NaN min(d)            NaN max(d)
          0 delta1            NaN min(o(q))            NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))              0 min(c(r))              1 max(c(r))
          0 alpha1              0 min(o(r))              0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8281e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
   10         2              NaN              NaN         0              NaN              NaN      gre
           warmStartMethod: 'random'
               formulation: 'pqzwrs'
           thetaMultiplier: 1.5
                     theta: 0.5
           regularizeOuter: 1
                   epsilon: 1e-06
                printLevel: 2
               relaxBounds: 0
         acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                    bigNum: 10000
                     debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
          1 = min(g0), the local weight on zero-norm of internal reaction rate.
          1 = max(g0), the local weight on zero-norm of internal reaction rate.
          1 = min(h0), the local weight on zero-norm of metabolite production rate.
          1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
          0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
          1 lambda0              1 min(k)              1 max(k)
          0 lambda1              0 min(o(p))              0 max(o(p))

0 max cardinality variables:
        NaN mean(c(q))            NaN min(c(q))            NaN max(c(q))
          1 delta0            NaN min(d)            NaN max(d)
          0 delta1            NaN min(o(q))            NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))              0 min(c(r))              1 max(c(r))
          0 alpha1              0 min(o(r))              0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.7854e+05    -8.6e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
   11         2              NaN              NaN         0              NaN              NaN      gre
           warmStartMethod: 'random'
               formulation: 'pqzwrs'
```

```
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0               1 min(k)               1 max(k)
           0 lambda1               0 min(o(p))               0 max(o(p))

0 max cardinality variables:
         NaN mean(c(q))             NaN min(c(q))             NaN max(c(q))
           1 delta0             NaN min(d)             NaN max(d)
           0 delta1             NaN min(o(q))             NaN max(o(q))

23034 cardinality free variables:
        0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
           0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8416e+05    -8.6e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
   12         2               NaN               NaN         0               NaN               NaN      gre
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
```

```
          0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
          1 lambda0              1 min(k)              1 max(k)
          0 lambda1              0 min(o(p))              0 max(o(p))

0 max cardinality variables:
        NaN mean(c(q))             NaN min(c(q))             NaN max(c(q))
          1 delta0             NaN min(d)             NaN max(d)
          0 delta1             NaN min(o(q))             NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
          0 alpha1              0 min(o(r))              0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8362e+05    -8.6e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
   13         2               NaN              NaN         0              NaN              NaN      gre
           warmStartMethod: 'random'
               formulation: 'pqzwrs'
           thetaMultiplier: 1.5
                     theta: 0.5
           regularizeOuter: 1
                   epsilon: 1e-06
                printLevel: 2
               relaxBounds: 0
        acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                    bigNum: 10000
                     debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
          1 = min(g0), the local weight on zero-norm of internal reaction rate.
          1 = max(g0), the local weight on zero-norm of internal reaction rate.
          1 = min(h0), the local weight on zero-norm of metabolite production rate.
          1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
          0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
          1 lambda0              1 min(k)              1 max(k)
          0 lambda1              0 min(o(p))              0 max(o(p))

0 max cardinality variables:
        NaN mean(c(q))             NaN min(c(q))             NaN max(c(q))
          1 delta0             NaN min(d)             NaN max(d)
          0 delta1             NaN min(o(q))             NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
          0 alpha1              0 min(o(r))              0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8003e+05    -8.6e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
   14         2               NaN              NaN         0              NaN              NaN      gre
           warmStartMethod: 'random'
```

```
               formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0               1 min(k)                1 max(k)
           0 lambda1               0 min(o(p))                0 max(o(p))

0 max cardinality variables:
         NaN mean(c(q))              NaN min(c(q))              NaN max(c(q))
           1 delta0             NaN min(d)              NaN max(d)
           0 delta1             NaN min(o(q))              NaN max(o(q))

23034 cardinality free variables:
        0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
           0 alpha1              0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8108e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
   15         2               NaN               NaN         0               NaN               NaN      gre
            warmStartMethod: 'random'
               formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:
```

```
16203 min cardinality variables:
          0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
          1 lambda0               1 min(k)                1 max(k)
          0 lambda1               0 min(o(p))                0 max(o(p))

0 max cardinality variables:
        NaN mean(c(q))              NaN min(c(q))              NaN max(c(q))
          1 delta0              NaN min(d)              NaN max(d)
          0 delta1              NaN min(o(q))              NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
          0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8477e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
   16         2               NaN               NaN         0               NaN               NaN      gre
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
     thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
          1 = min(g0), the local weight on zero-norm of internal reaction rate.
          1 = max(g0), the local weight on zero-norm of internal reaction rate.
          1 = min(h0), the local weight on zero-norm of metabolite production rate.
          1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
          0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
          1 lambda0               1 min(k)                1 max(k)
          0 lambda1               0 min(o(p))                0 max(o(p))

0 max cardinality variables:
        NaN mean(c(q))              NaN min(c(q))              NaN max(c(q))
          1 delta0              NaN min(d)              NaN max(d)
          0 delta1              NaN min(o(q))              NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
          0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.7946e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
   17         2               NaN               NaN         0               NaN               NaN      gre
```

```
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0               1 min(k)               1 max(k)
           0 lambda1               0 min(o(p))               0 max(o(p))

0 max cardinality variables:
         NaN mean(c(q))             NaN min(c(q))             NaN max(c(q))
           1 delta0             NaN min(d)             NaN max(d)
           0 delta1             NaN min(o(q))             NaN max(o(q))

23034 cardinality free variables:
        0.75 mean(c(r))               0 min(c(r))               1 max(c(r))
           0 alpha1              0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.7824e+05    -8.6e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
   18         2               NaN               NaN         0               NaN               NaN      gre
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 2
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
           1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:
```

```
16203 min cardinality variables:
          0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
          1 lambda0                1 min(k)                1 max(k)
          0 lambda1                0 min(o(p))                0 max(o(p))

0 max cardinality variables:
        NaN mean(c(q))              NaN min(c(q))              NaN max(c(q))
          1 delta0               NaN min(d)               NaN max(d)
          0 delta1               NaN min(o(q))               NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))                0 min(c(r))                1 max(c(r))
          0 alpha1                0 min(o(r))                0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8468e+05    -8.7e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
   19         2               NaN               NaN         0               NaN               NaN      gre
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                printLevel: 2
               relaxBounds: 0
          acceptRepairedFlux: 1
     thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
          1 = min(g0), the local weight on zero-norm of internal reaction rate.
          1 = max(g0), the local weight on zero-norm of internal reaction rate.
          1 = min(h0), the local weight on zero-norm of metabolite production rate.
          1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

16203 min cardinality variables:
          0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
          1 lambda0                1 min(k)                1 max(k)
          0 lambda1                0 min(o(p))                0 max(o(p))

0 max cardinality variables:
        NaN mean(c(q))              NaN min(c(q))              NaN max(c(q))
          1 delta0               NaN min(d)               NaN max(d)
          0 delta1               NaN min(o(q))               NaN max(o(q))

23034 cardinality free variables:
       0.75 mean(c(r))                0 min(c(r))                1 max(c(r))
          0 alpha1                0 min(o(r))                0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.8464e+05    -8.6e+07           0           0           0           0           0
   2    0.75           0           0           0           0           0           0           0
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
```

```
   20         2               NaN               NaN         0               NaN               NaN      gre
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
thermoKernel terminating early: n = nMax = 20
--- thermoKernel END ----
```

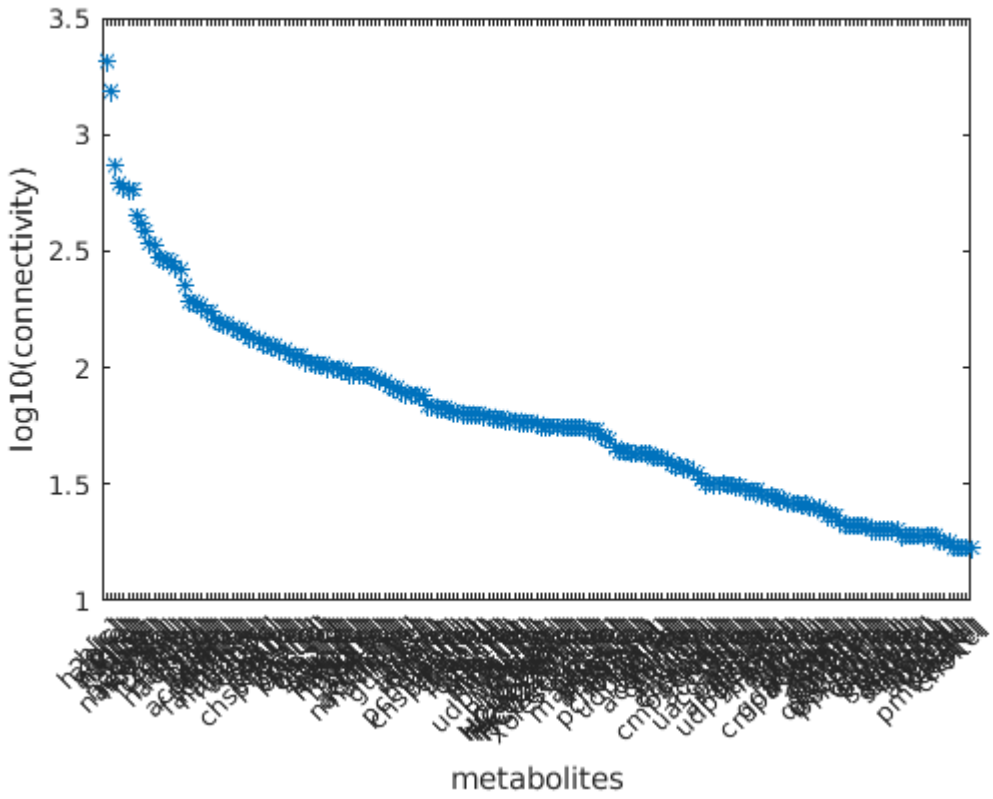
```
[nMet,nRxn]=size(minimalModel.S)
```

```
nMet =
    41
nRxn =
     2
```

Data to define a thermodynamically consistent subnetwork

Setup random data to select a random subset

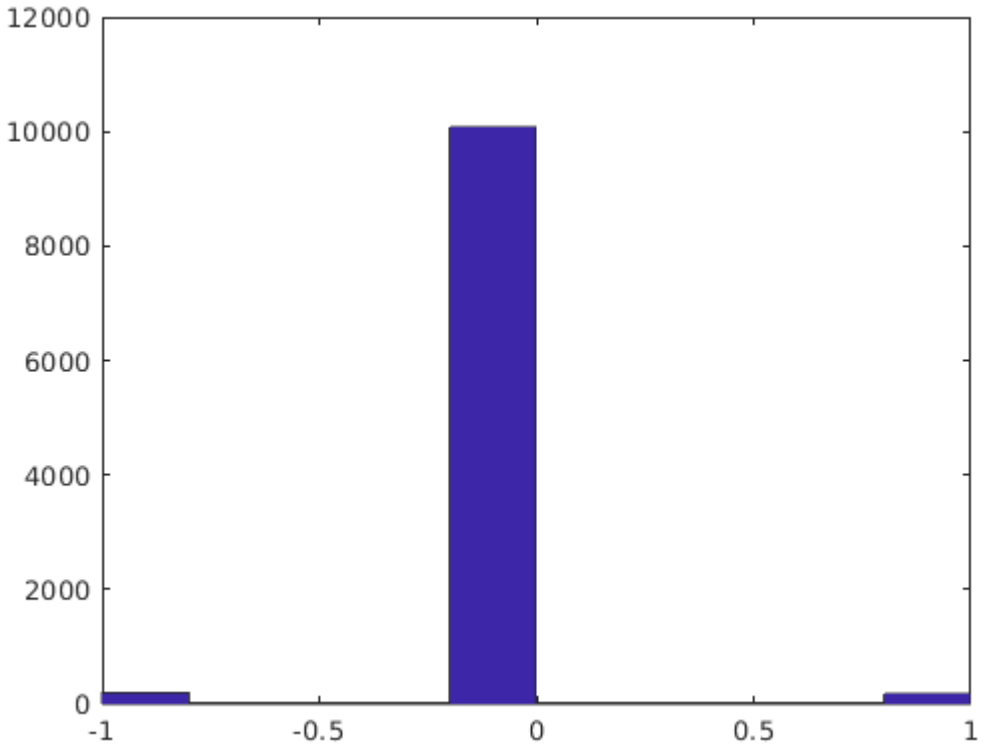
```
param.n=200;
[rankMetConnectivity,rankMetInd,rankConnectivity] =
rankMetabolicConnectivity(stoichFluxThermoConsistModel,param);
```



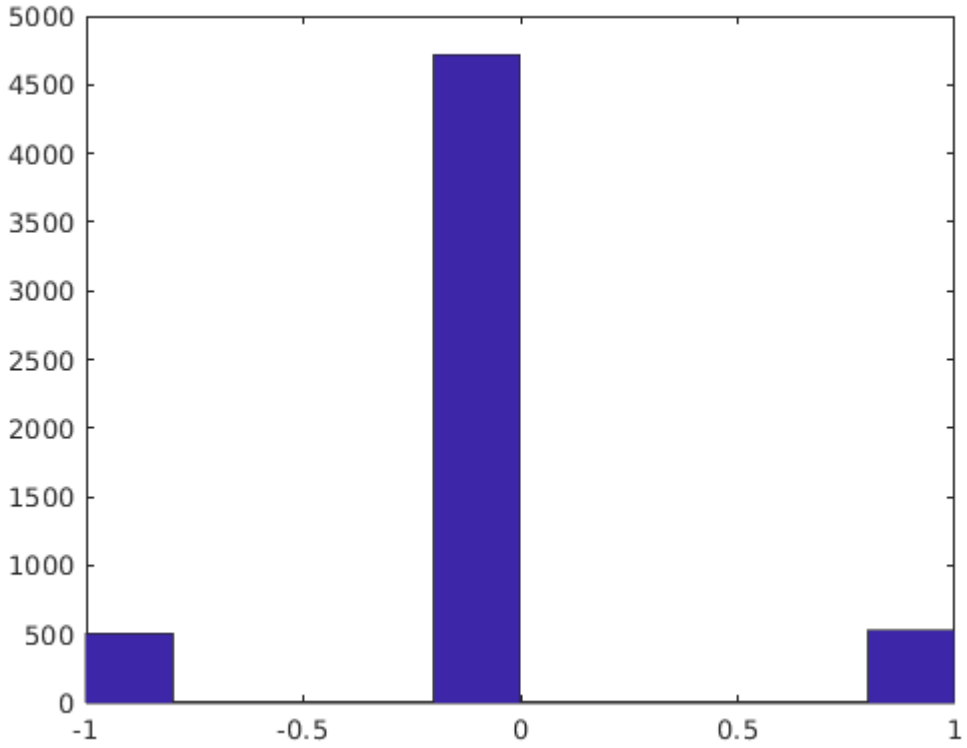

```
[nMet,nRxn]=size(stoichFluxThermoConsistModel.S);
rxnWeights=rand(nRxn,1)-0.5;
rxnWeights(stoichFluxThermoConsistModel.SConsistentRxnBool)=0;

coreRxnBool=rxnWeights<0.45;
removeRxnBool=rxnWeights>0.48;
rxnWeights(rxnWeights>0.4)=1;
```

```
rxnWeights(rxnWeights<-0.4)=-1;
rxnWeights(rxnWeights>=-0.4 & rxnWeights<=0.4)=0;
hist(rxnWeights)
```




```
metWeights=rand(nMet,1)-0.5;
metWeights(rankMetInd(1:200))=0;
coreMetBool=metWeights<0.45;
removeMetBool=metWeights>0.5;
metWeights(metWeights>0.4)=1;
metWeights(metWeights<-0.4)=-1;
metWeights(metWeights>=-0.4 & metWeights<=0.4)=0;
hist(metWeights)
```



Remove inactive reactions and absent metabolites

```
param.printLevel = 1;
[solverOK,solverInstalled]=changeCobraSolver('gurobi','QP');
```

```
 > changeCobraSolver: Gurobi interface added to MATLAB path.
 > gurobi (version 811) is compatible and fully tested with MATLAB R2019a on your operating system.
```

```
[thermoFluxConsistentMetBool,thermoFluxConsistentRxnBool,stoichFluxThermoCons
istModel,stoichFluxThermoConsistModelRed] =
findThermoConsistentFluxSubset(stoichFluxThermoConsistModel, param,
removeMetBool, removeRxnBool);
```

```
--- findThermoFluxConsistentSubset START ----
46 flux inconsistent metabolites
48 flux inconsistent reactions
                  printLevel: 1
                           n: 200
    normalizeZeroNormWeights: 0
                     epsilon: 1e-06
                 formulation: 'pqzw'
             iterationMethod: 'random'
                        nMax: 20
                 relaxBounds: 1
           acceptRepairedFlux: 1
              warmStartMethod: 'random'
              thetaMultiplier: 1.5
                       theta: 0.5
              regularizeOuter: 0
     thermoConsistencyMethod: 'cycleFreeFlux'
```

```
                    bigNum: 10000
                     debug: 0

optCardThermo objective data:
         0.1 = beta, the global weight on one-norm of internal reaction rate.
          -5 = min(g0), the local weight on zero-norm of internal reaction rate.
          -0 = max(g0), the local weight on zero-norm of internal reaction rate.
           0 = min(h0), the local weight on zero-norm of metabolite production rate.
           0 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

0 min cardinality variables:
         NaN mean(c(p))            NaN min(c(p))            NaN max(c(p))
           1 lambda0             NaN min(k)             NaN max(k)
           1 lambda1             NaN min(o(p))             NaN max(o(p))

5046 max cardinality variables:
          -0 mean(c(q))              -0 min(c(q))              -0 max(c(q))
           1 delta0                5 min(d)                5 max(d)
           0 delta1                0 min(o(q))                0 max(o(q))

22498 cardinality free variables:
       0.076 mean(c(r))              -0 min(c(r))             0.1 max(c(r))
           0 alpha1                0 min(o(r))                0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1      0.50  7.5581e+05    -8.5e+06      -2e+04     1.5e+03           0           0           0      -221
   2      0.75       159.51    -9.2e+02    -2.1e+04       1e+03           0           0           0      -223
   3      1.12       105.52    -4.4e+02    -2.2e+04       7e+02           0           0           0      -224
   4      1.69       65.094    -2.5e+02    -2.2e+04     4.7e+02           0           0           0      -224
   5      2.53       39.738    -1.7e+02    -2.2e+04     3.1e+02           0           0           0      -224
   6      3.80       26.358    -1.1e+02    -2.2e+04     2.1e+02           0           0           0      -224
   7      5.70       17.391         -69    -2.2e+04     1.4e+02           0           0           0      -224
   8      8.54       38.487         -73    -2.2e+04       1e+02           0           0           0      -225
   9     12.81       15.112         -48    -2.2e+04          67           0           0           0      -225
  10     19.22        10.09         -32    -2.2e+04          44           0           0           0      -225
  11     28.83       6.7343         -15    -2.3e+04          30           0           0           0      -225
  12     43.25       4.4555        -9.9    -2.3e+04          20           0           0           0      -225
  13     64.87       2.9919        -6.6    -2.3e+04          13           0           0           0      -225
  14     97.31       1.9934        -4.4    -2.3e+04         8.8           0           0           0      -225
  15    145.96       1.3302        -2.9    -2.3e+04         5.8           0           0           0      -225
  16    218.95      0.89203        -1.9    -2.3e+04         3.9           0           0           0      -225
  17    328.42      0.59077        -1.3    -2.3e+04         2.6           0           0           0      -225
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
                iter            card(y)                nz       %feas int.nz.   tot %feas int.nz.   tot
                   1               5046              7439              1.00              0.68
                   2               1459              5243              1.00              0.84
                   3                677              5398              1.00              0.93
                   4                310              3769              1.00              0.96
                   5                190              1675              1.00              0.97
                   6                150              2816              1.00              0.97
                   7                118              2522              1.00              0.98
                   8                 81              3234              1.00              0.98
                   9                 75               153              1.00              0.98
                  10                 77               275              1.00              0.98
                  11                 71               135              1.00              0.98
                  12                 68                 1               NaN              0.98
                  13                 69              1162              1.00              0.98
                  14                 85              1537              1.00              0.98
                  15                 81                 2               NaN              0.98
                  16                 66                 2               NaN              0.98
```

```
                17                72                 2                NaN               0.98
                18                63                 2                NaN               0.98
              iter            card(y)                nz       %feas int.nz.   tot %feas int.nz.   tot
findThermoConsistentFluxSubset terminating early: no progress on % internal reactions thermodynamically fl
--- findThermoFluxConsistentSubset END ----
```

```
[nMet,nRxn]=size(stoichFluxThermoConsistModelRed.S)
```

```
nMet =
        5633
nRxn =
       10225
```

Remove the corresponding entries from the weights

```
bool = coreRxnBool & ~thermoFluxConsistentRxnBool;
if any(bool)
    fprintf('%u%s\n',nnz(bool), ' core reactions inconsistent due to removed
reactions')
    if nnz(bool)<0
        stoichFluxThermoConsistModel.rxns{bool}
    end
end
```

```
183 core reactions inconsistent due to removed reactions
```

```
bool = coreMetBool & ~thermoFluxConsistentMetBool;
if any(bool)
    fprintf('%u%s\n',nnz(bool),' core metabolties inconsistent due to
removed metabolites')
    if nnz(bool)<10
        stoichFluxThermoConsistModel.mets{bool}
    end
end
```

```
117 core metabolties inconsistent due to removed metabolites
```

```
rxnWeightsRed = rxnWeights(thermoFluxConsistentRxnBool);
metWeightsRed = metWeights(thermoFluxConsistentMetBool);
coreRxnBoolRed = coreRxnBool(thermoFluxConsistentRxnBool);
coreMetBoolRed = coreMetBool(thermoFluxConsistentMetBool);
```

Compute the smallest thermodynamically consistent subnetwork containing a list of present metabolites and active reactions

```
activeInactiveRxn=coreRxnBoolRed;
presentAbsentMet=coreMetBoolRed;
activeInactiveRxn(:)=0;
presentAbsentMet(:)=0;
activeInactiveRxn(~stoichFluxThermoConsistModelRed.SConsistentRxnBool)=0;
param.normalizeZeroNormWeights=0;
```

```
[tissueModel, thermoModelMetBool, thermoModelRxnBool] =
thermoKernel(stoichFluxThermoConsistModelRed, activeInactiveRxn,
rxnWeightsRed, presentAbsentMet, metWeightsRed,param);
```

```
--- thermoKernel START ----
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5
                      theta: 0.5
            regularizeOuter: 1
                    epsilon: 1e-06
                 printLevel: 1
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
          -1 = min(g0), the local weight on zero-norm of internal reaction rate.
           1 = max(g0), the local weight on zero-norm of internal reaction rate.
          -1 = min(h0), the local weight on zero-norm of metabolite production rate.
           1 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

649 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0               1 min(k)               1 max(k)
           0 lambda1               0 min(o(p))               0 max(o(p))

1188 max cardinality variables:
           0 mean(c(q))              -0 min(c(q))              -0 max(c(q))
           1 delta0               1 min(d)               1 max(d)
           0 delta1               0 min(o(q))               0 max(o(q))

36568 cardinality free variables:
        0.46 mean(c(r))              -0 min(c(r))               1 max(c(r))
           0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.6816e+05    -8.5e+07         -18          67          14         9.1           0        -1
   2    0.75      20.361         -25         -43          52          13         9.2           0        -1
   3    1.12      16.927         -19         -63          38          11         7.9           0        -1
   4    1.69      11.532         -13         -76          26          11         7.8           0        -1
   5    2.53      8.2997        -9.9         -86          19          10         7.3           0        -1
   6    3.80      5.7142        -7.1         -93          14           9         6.9           0        -1
   7    5.70        3.85        -4.5         -97         9.1           9         6.9           0        -1
   8    8.54      2.2986          -3      -1e+02         6.1           9         6.9           0        -1
   9   12.81      1.7613          -2      -1e+02           4           9         6.9           0        -1
  10   19.22      1.3692        -1.3      -1e+02         2.7           9         6.9           0        -1
  11   28.83     0.86665        -0.9      -1e+02         1.8           9         6.9           0        -1
  12   43.25     0.49273        -0.6    -1.1e+02         1.2           9         6.9           0        -1
  13   64.87     0.41444        -0.4    -1.1e+02         0.8           9         6.9           0        -1
  14   97.31     0.21692       -0.27    -1.1e+02        0.53           9         6.9           0        -1
  15  145.96     0.14927       -0.18    -1.1e+02        0.35           9         6.9           0        -1
  16  218.95    0.097833       -0.12    -1.1e+02        0.24           9         6.9           0        -1
  17  328.42    0.065178      -0.079    -1.1e+02        0.16           9         6.9           0        -1
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
```

```
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
    1      1578              1.00             0.23       893              1.38              0.22      gre
    2      3929              1.00             0.47      1936              1.32              0.58      gre
    3      1162              1.00             0.55       814              1.20              0.68      gre
    4      1108              1.00             0.69       810              1.18              0.75      gre
    5       879              1.00             0.79       538              1.42              0.79
```

```
[nMet,nRxn]=size(tissueModel.S)
```

Compare the target versus predicted model

```
plotThermoKernelExtractStats(stoichFluxThermoConsistModelRed,
activeInactiveRxn, rxnWeightsRed, presentAbsentMet, metWeightsRed,
thermoModelMetBool, thermoModelRxnBool);
```

Save weights

```
rxnWeightsRedTmp=rxnWeightsRed;
metWeightsRedTmp=metWeightsRed;
return
```

Submodel with just metabolites specified

```
metWeightsRed=metWeightsRedTmp;
rxnWeightsRed(:)=0;
[tissueModel, thermoModelMetBool, thermoModelRxnBool] =
thermoKernel(stoichFluxThermoConsistModelRed, activeInactiveRxn,
rxnWeightsRed, presentAbsentMet, metWeightsRed,param);
[nMet,nRxn]=size(tissueModel.S)
```

Compare the target versus predicted model

```
plotThermoKernelExtractStats(stoichFluxThermoConsistModelRed,
activeInactiveRxn, rxnWeightsRed, presentAbsentMet, metWeightsRed,
thermoModelMetBool, thermoModelRxnBool)
```

Submodel with just reactions specified

```
rxnWeightsRed=rxnWeightsRedTmp;
metWeightsRed(:)=0;
[tissueModel, thermoModelMetBool, thermoModelRxnBool] =
thermoKernel(stoichFluxThermoConsistModelRed, activeInactiveRxn,
rxnWeightsRed, presentAbsentMet, metWeightsRed,param);

[nMet,nRxn]=size(tissueModel.S)
```

Compare the target versus predicted model

```
plotThermoKernelExtractStats(stoichFluxThermoConsistModelRed,
activeInactiveRxn, rxnWeightsRed, presentAbsentMet, metWeightsRed,
thermoModelMetBool, thermoModelRxnBool)
```

Submodel with just active metabolites specified

```
metWeightsRed=metWeightsRedTmp;
rxnWeightsRed(:)=0;
metWeightsRed(metWeightsRed>=0)=0;
```

```
[tissueModel, thermoModelMetBool, thermoModelRxnBool] =
thermoKernel(stoichFluxThermoConsistModelRed, activeInactiveRxn,
rxnWeightsRed, presentAbsentMet, metWeightsRed,param);
[nMet,nRxn]=size(tissueModel.S)
```

Compare the target versus predicted model

```
plotThermoKernelExtractStats(stoichFluxThermoConsistModelRed,
activeInactiveRxn, rxnWeightsRed, presentAbsentMet, metWeightsRed,
thermoModelMetBool, thermoModelRxnBool)
```

Submodel with just active reactions specified

```
rxnWeightsRed=rxnWeightsRedTmp;
metWeightsRed(:)=0;
rxnWeightsRed(rxnWeightsRed>=0)=0;
[tissueModel, thermoModelMetBool, thermoModelRxnBool] =
thermoKernel(stoichFluxThermoConsistModelRed, activeInactiveRxn,
rxnWeightsRed, presentAbsentMet, metWeightsRed,param);

[nMet,nRxn]=size(tissueModel.S)
```

Compare the target versus predicted model

```
plotThermoKernelExtractStats(stoichFluxThermoConsistModelRed,
activeInactiveRxn, rxnWeightsRed, presentAbsentMet, metWeightsRed,
thermoModelMetBool, thermoModelRxnBool)
```