Extraction of context-specific models via XomicsToModel

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INTRODUCTION

The `XomicsToModel` pipeline [1] of the COBRA Toolbox v3.4 [2], facilitates the generation a thermodynamic-flux-consistent, context-specific, genome-scale metabolic model in a single command by combining a generic model with bibliomic, transcriptomic, proteomic, and metabolomic data. To ensure the network's quality, several thermodynamic consistency checks are implemented within the function. To generate a thermodynamic-flux-consistent, context-specific, genome-scale metabolic model, the function requires three inputs: a generic COBRA model and two variables containing the context-specific data and technical information defined by the user.

This tutorial shows how to extract a context-specific genome-scale model of a dopaminergic neuron (*iDopaNeuroC* [3]) from the human generic model Recon3D [4] . The *iDopaNeuroC* [3] model is extracted using data from manual curation of a dopaminergic neuron to identify active and inactive genes, reactions, and metabolites, as well as information from in vitro experiments such as exometabolomic quantification and transcriptomic sequencing of a cell culture of pluripotent stem cell-derived dopaminergic neurons.

PROCEDURE

Install MATLAB and then the COBRA Toolbox as described here: https://opencobra.github.io/cobratoolbox/stable/installation.html

Select a solver suitable for solving linear (LP) and quadratic (QP) optimisation problems, e.g., mosek, gurobi, ibm_cplex, etc.

```
[~, ~] = changeCobraSolver('mosek', 'all', 0);
```

Generic model

The COBRA model Recon3D [4], representing human metabolic reconstruction, a can be found in a file with the extension ".mat". Recon3D [1] , which is found in the VMH database [2], can be used as a generic model for human metabolism. The thermodynamic consistent human metabolic reconstruction Recon3D [4].

```
inputFolder = ['~' filesep 'work' filesep 'sbgCloud' filesep
'programExperimental' ...
    filesep 'projects' filesep 'xomics' filesep 'data' filesep
'Recon3D_301'];
genericModelName = 'Recon3DModel_301_xomics_input.mat';
load([inputFolder filesep genericModelName])
```

Context-specific data

This type of information represents the biological system's phenotype and can be obtained through a review of the literature or experimental data derived from the biological system. The context-specific data can be loaded from a spreadsheet or added manually.

Automated data integration

Tables or multiple data sets can be inserted in an external worksheet document so that the `preprocessingOmicsModel` function can include them in the `options` variable. The name of the sheet corresponding to the options field must be the same as those specified above and in the manuscript, or they will be omitted.

Bibliomic data. It is derived from manual reconstruction following a review of the literature. This includes data on the activation or inactivation of genes, reactions, or metabolites. Another example is the addition of coupled reactions or the constraints of different reactions based on phenotypic observations.

- **`specificData.activeGenes:`** List of Entrez ID of genes that are known to be active based on the bibliomic data (Default: empty).
- **`specificData.addCoupledRxns:`** Logical, should the coupled constraints be added (Default: `true`).
- **`specificData.coupledRxns:`** Logical, indicates whether curated data should take priority over omics data (Default: false).
- **`specificData.essentialAA:`** List exchange reactions of essential amino acid (Default: empty).
- **`specificData.inactiveGenes:`** List of Entrez ID of genes known to be inactive based on the bibliomics data (Default: empty).
- **`specificData.presentMetabolites:`** List of metabolites known to be active based on the bibliomics data (Default: empty).
- **`specificData.rxns2add:`** Table containing the identifier of the reaction to be added, its name, the reaction formula, the metabolic pathway to which it belongs, the gene rules to which the reaction is subject, and the references. (Default: empty).
- **`specificData.rxns2constrain:`** Table containing the reaction identifier, the updated lower bound, the updated upper bound, a description for the constraint and notes such as references or special cases (Default: empty).

Manually curated data. To read the table and prepare the variable `specificData` it is used the function `preprocessingOmicsModel`. In this tutorial the bibliomic data is contained in the file *'bibliomicData.xlsx'*.

```
dataFolder = [fileparts(which('tutorial_XomicsToModel.mlx')) filesep
'iDopaNeuro' filesep 'data' filesep];
bibliomicData = 'bibliomicData.xlsx';
specificData = preprocessingOmicsModel([dataFolder bibliomicData], 1, 1);
```

```
Reading inputData from : /home/rfleming/work/sbgCloud/programExperimental/projects/xomics/code/tutorials/i
Reading sheet: activeGenes
Reading sheet: activeReactions
Reading sheet: cellCultureData
Reading sheet: coupledRxns
Reading sheet: essentialAA
Reading sheet: inactiveGenes
Reading sheet: mediaData
Reading sheet: presentMetabolites
```

```
Reading sheet: rxns2add
Reading sheet: rxns2constrain
Reading sheet: rxnsHypothesis
Reading sheet: rxns2remove
Reading sheet: sinkDemand
```

Metabolomic data. Differences in measured concentrations of metabolites within cells, biofluids, tissues, or organisms are translated into flux units of flux (μmol/gDW/h).

- **`specificData.cellCultureData:`** Table containing the cell culture data used to calculate the uptake flux. Includes well volume (L), time interval cultured (hr), average protein concentration (g/L), assay volume (L), protein fraction (g/g dry weight) , and the sign for uptakes (Default: -1).
- **`specificData.exoMet:`** Table with the fluxes obtained from exometabolomics experiments. It includes the reaction identifier, the reaction name, the measured mean flux, standard deviation of the measured flux, the flux units, and the platform used to measure it.
- **`specificData.mediaData:`** Table containing the initial media concentrations. Contains the reaction identifier, the maxiumum uptake (μmol/gDW/h) based on the concentration of the metabolite and the concentration (μmol; Default: empty).

In this tutorial the exometabolomic data is saved in the table 'exoMet'.

```
specificData.exoMet = readtable([dataFolder 'exometabolomicData.txt']);
```

Proteomic data. This information indicates the level of expression of the proteome.

- **`specificData.proteomics:`** Table with a column with Entrez ID's and a column for the corresponding protein levels (Default: empty).

For this tutorial no proteomic data was used.

Transcriptomic data. Indicates the level of transcriptome expression and can also be used to calculate reaction expression. Transcriptomic data can be analysed in FPKM.

- **`specificData.transcriptomicData:`** Table with a column with Entrez ID's and a column for the corresponding transcriptomics expresion value (Default: empty).

In this tutorial the transcriptomic analysis is saved in the table 'transcriptomicData'.

```
specificData.transcriptomicData = readtable([dataFolder
'transcriptomicData.txt']);
specificData.transcriptomicData.genes =
string(specificData.transcriptomicData.genes);
```

Technical parameters

With these options, technical constraints can be added to the model, as well as setting the parameters for model extraction or debugging.

Bounds. They are the instructions that will be set in the boundaries.

- **`param.boundPrecisionLimit:`** Precision of flux estimate, if the absolute value of the lower bound (`model.lb`) or the upper bound (`model.ub`) are lower than `options.boundPrecisionLimit` but higher than 0 the value will be set to the boundPrecisionLimit (Default: primal feasibility tolerance).
- **`param.TolMaxBoundary:`** The reaction boundary's maximum value (Default: $1e3$).
- **`param.TolMinBoundary:`** The reaction boundary's minimum value (Default: $-1e3$).
- **`param.relaxOptions:`** A structure array with the relaxation options (Default: `param.relaxOptions.steadyStateRelax = 0`).

```
param.TolMinBoundary = -1e4;
param.TolMaxBoundary =  1e4;
feasTol = getCobraSolverParams('LP', 'feasTol');
param.boundPrecisionLimit = feasTol * 10;
```

Exchange reactions. They are the instructions for the exchange, demand, and sink reactions.

- **`param.addSinksexoMet:`** Logical, should sink reactions be added for metabolites measured in the media but without existing exchange reaction (Default: false).
- **`param.closeIons:`** Logical, it determines whether or not ion exchange reactions are closed. (Default: false).
- **`param.closeUptakes:`** Logical, decide whether or not all of the uptakes in the generic model will be closed (Default: false).
- **`param.nonCoreSinksDemands:`** The type of sink or demand reaction to close is indicated by a string (Possible options: `'closeReversible'`, `'closeForward'`, `'closeReverse'`, `'closeAll'` and `'closeNone'`; Default: `'closeNone'`).

```
param.closeIons = true;
param.closeUptakes = true;
param.nonCoreSinksDemands = 'closeAll';
param.sinkDMinactive = true;
```

Extraction options. The solver and parameters for extracting the context-specific model.

- **`param.activeGenesApproach:`** String with the name of the active genes approach will be used (Possible options: `'oneRxnsPerActiveGene'` or `'deletModelGenes'`; Default: `'oneRxnsPerActiveGene'`).
- **`param.fluxCCmethod:`** String with thee name of the algorithm to be used for the flux consistency check (Possible options: `'swiftcc'`, `'fastcc'` or `'dc'`, Default: `'fastcc'`).
- **`param.fluxEpsilon:`** Minimum non-zero flux value accepted for tolerance (Default: Primal feasibility tolerance).
- **`param.thermoFluxEpsilon:`** Flux epsilon used in `'thermoKernel'` (Default: feasibility tolerance).
- **`param.tissueSpecificSolver:`** The name of the solver to be used to extract the context-specific model (Possible options: `'thermoKernel'` and `'fastcore'`; Default: `'thermoKernel'`).

```
param.activeGenesApproach = 'oneRxnPerActiveGene';
```

```
param.tissueSpecificSolver = 'thermoKernel';
param.fluxEpsilon = feasTol * 10;
param.fluxCCmethod = 'fastcc';
```

Data-specific parameters. Parameters that define the minimum level of transcript/protein to be considered as present in the network (treshold) and weather the transcripts below the set treshold should be removed from the model.

- **`param.addCoupledRxns:`** Logical, should the coupled constraints be added (Default: false). **! CAUTION** If it is TRUE and the table coupledRxns is empty, the step is not performed.
- **`param.curationOverOmics:`** Logical, indicates whether curated data should take priority over omics data (Default: false).
- **`param.inactiveGenesTranscriptomics:`** Logical, indicate if inactive genes in the transcriptomic analysis should be added to the list of inactive genes (Default: true).
- **`param.metabolomicWeights:`** String indicating the type of weights to be applied for metabolomics fitting (Possible options: `'SD'`, `'mean'` and `'RSD'`; Default: `'SD'`).
- **`param.setObjective:`** Linear objective function to optimise (Default: empty).
- **`param.tresholdP:`** The proteomic cutoff threshold (in linear scale) for determining whether or not a gene is active (Default: 0).
- **`param.transcriptomicThreshold:`** The transcriptomic cutoff threshold (in logarithmic scale) for determining whether or not a gene is active (Default: 0)
- **`param.weightsFromOmics:`** Should gene weights be assigned based on the omics data (Default: 0).

```
param.addCoupledRxns = 1;
param.curationOverOmics = false;
param.inactiveGenesTranscriptomics = true;
param.metabolomicWeights='mean';
param.transcriptomicThreshold = 2;
param.weightsFromOmics = true;
```

Debugging options. The user can specify the function's verbosity level as well as save the results of the various blocks of the function for debugging.

- **`param.debug:`** Logical, should the function save its progress for debugging (Default: false).
- **`param.diaryFilename:`** Location where the output be printed in a diary file (Default: 0).
- **`param.printLevel:`** Level of verbose that should be printed (Default: 0).

```
param.printLevel = 1;
param.debug = true;
if isunix()
    name = getenv('USER');
else
    name = getenv('username');
end
param.diaryFilename = [pwd filesep datestr(now,30) '_' name '_diary.txt'];
```

XomicsToModel function

```
[iDopaNeuro1, modelGenerationReport] = XomicsToModel(model, specificData,
param);
```

```
XomicsToModel run, beginning at:16-Dec-2022 08:56:14
------------------------------------------------------------

XomicsToModel input specificData:

             inputData: '/home/rfleming/work/sbgCloud/programExperimental/projects/xomics/code/tutorials/i
           activeGenes: {239×1 cell}
       activeReactions: {334×1 cell}
       cellCultureData: [1×6 table]
           coupledRxns: [11×5 table]
           essentialAA: [9×1 table]
         inactiveGenes: {61×1 cell}
             mediaData: [56×3 table]
    presentMetabolites: [45×4 table]
              rxns2add: [21×9 table]
        rxns2constrain: [48×5 table]
        rxnsHypothesis: [33×5 table]
           rxns2remove: [233×5 table]
            sinkDemand: [49×8 table]
                exoMet: [49×13 table]
    transcriptomicData: [18530×2 table]

XomicsToModel input param:

                      TolMinBoundary: -10000
                      TolMaxBoundary: 10000
                  boundPrecisionLimit: 1e-05
                            closeIons: 1
                          closeUptakes: 1
                  nonCoreSinksDemands: 'closeAll'
                       sinkDMinactive: 1
                  activeGenesApproach: 'oneRxnPerActiveGene'
                 tissueSpecificSolver: 'thermoKernel'
                           fluxEpsilon: 1e-05
                         fluxCCmethod: 'fastcc'
                       addCoupledRxns: 1
                   curationOverOmics: 0
        inactiveGenesTranscriptomics: 1
                  metabolomicWeights: 'mean'
              transcriptomicThreshold: 2
                     weightsFromOmics: 1
                           printLevel: 1
                                debug: 1
                       diaryFilename: '/home/rfleming/20221216T085613_rfleming_diary.txt'
                  inactiveReactions: []
                           thresholdP: 0
                           uptakeSign: -1
                  thermoFluxEpsilon: 1e-05
    growthMediaBeforeReactionRemoval: 1
        metabolomicsBeforeExtraction: 1
                    workingDirectory: '/home/rfleming'
      findThermoConsistentFluxSubset: 1
               plotThermoKernelStats: 0
              plotThermoKernelWeights: 0
                 finalFluxConsistency: 0
                        relaxOptions: [1×1 struct]
                  boundsToRelaxExoMet: 'both'
```

```
Replacing reaction name DM_atp_c_ with ATPM, because it is not strictly a demand reaction.

Old reaction formulas
ATPS4mi    adp[m] + pi[m] + 4 h[i]     ->    h2o[m] + 3 h[m] + atp[m]
CYOOm2i    o2[m] + 8 h[m] + 4 focytC[m]     ->    2 h2o[m] + 4 ficytC[m] + 4 h[i]
CYOOm3i    o2[m] + 7.92 h[m] + 4 focytC[m]     ->    1.96 h2o[m] + 4 ficytC[m] + 0.02 o2s[m] + 4 h[i]
CYOR_u10mi    2 h[m] + 2 ficytC[m] + q10h2[m]     ->    q10[m] + 2 focytC[m] + 4 h[i]
NADH2_u10mi    5 h[m] + nadh[m] + q10[m]     ->    nad[m] + q10h2[m] + 4 h[i]
  0×0 empty char array

New reaction formulas
ATPS4minew    4 h[c] + adp[m] + pi[m]     ->    h2o[m] + 3 h[m] + atp[m]
CYOOm2inew    o2[m] + 8 h[m] + 4 focytC[m]     ->    2 h2o[m] + 4 h[c] + 4 ficytC[m]
CYOOm3inew    o2[m] + 7.92 h[m] + 4 focytC[m]     ->    1.96 h2o[m] + 4 h[c] + 4 ficytC[m] + 0.02 o2s[m]
CYOR_u10minew    2 h[m] + 2 ficytC[m] + q10h2[m]     ->    4 h[c] + q10[m] + 2 focytC[m]
NADH2_u10minew    5 h[m] + nadh[m] + q10[m]     ->    4 h[c] + nad[m] + q10h2[m]

Feasible generic input model.

------------------------------------------------------------

Generating model without an objective function.


------------------------------------------------------------
Adding 21 reactions ...

Reaction boundaries not provided. Default (min and max) values will be used based on the reaction formula.
acleua    h2o[c] + acleu_L[c]     ->    ac[c] + leu_L[c]
acthra    h2o[c] + acthr_L[c]     ->    ac[c] + thr_L[c]
acileua    h2o[c] + acile_L[c]     ->    ac[c] + ile_L[c]
acglua    h2o[c] + acglu[c]     ->    glu_L[c] + ac[c]
CE1554tm    CE1554[c]     <=>    CE1554[m]
RE2031M    accoa[m] + ala_L[m]     <=>    h[m] + coa[m] + CE1554[m]
RE2642C    h2o[c] + CE1554[c]     <=>    ac[c] + ala_L[c]
CE1554t    CE1554[c]     <=>    CE1554[e]
EX_CE1554[e]    CE1554[e]     <=>
DM_ps_hs[c]    ps_hs[c]     <=>
CYSTS_H2S    cys_L[c] + hcys_L[c]     <=>    HC00250[c] + cyst_L[c]
DHBOX    h2o2[c] + quinonemethide[c]     ->    3,4-dihydroxybenzaldehyde[c] + methanimine[c]
DM_clpn_hs[c]    clpn_hs[c]     ->
EX_adocbl[e]    adocbl[e]     <=>
EX_ca2[e]    ca2[e]     <=>
EX_cl[e]    cl[e]     <=>
EX_mg2[e]    mg2[e]     <=>
EX_selni[c]    selni[c]     <=>
EX_zn2[e]    zn2[e]     <=>
NORCON    dopa[c] + fald[c] + 3,4-dihydroxybenzaldehyde[c]     ->    CE2172[c]
Q-METHRED    fe2[c] + CE5276[c]     ->    quinonemethide[c]
0 deleted non-core metabolites,  corresponding to generic model.
0 deleted core metabolites,  corresponding to generic model.
Old model does not contain these core reactions (now removed from core reaction set):
    {'DM_ca2[c]'    }
    {'EX_HC01944[e]'}
    {'EX_adpcbl[e]' }
    {'Htmi'         }
    {'RE1917C'      }

0 deleted non-core reactions,  corresponding to generic model.
0 deleted core reactions,  corresponding to generic model.
------------------------------------------------------------

Identifying the stoichiometrically consistent subset...
```

```
--- findStoichConsistentSubset START ----
--- Summary of stoichiometric consistency ----
  5843     10620     totals.
     3      1818     heuristically external.
  5840      8802     heuristically internal:
  5840      8800     ... of which are stoichiometrically consistent.
     0         2     ... of which are stoichiometrically inconsistent.
     0         0     ... of which are of unknown consistency.
  5840      8800     Confirmed stoichiometrically consistent by leak/siphon testing.
--- findStoichConsistentSubset END ----
3 deleted non-core metabolites,  corresponding to stoichiometric inconsistency.
0 deleted core metabolites,  corresponding to stoichiometric inconsistency.
Old model does not contain these core reactions (now removed from core reaction set):
    {'DM_ca2[c]'    }
    {'EX_HC01944[e]'}
    {'EX_adpcbl[e]' }
    {'Htmi'         }
    {'RE1917C'      }

0 deleted non-core reactions,  corresponding to stoichiometric inconsistency.
5 deleted core reactions,  corresponding to stoichiometric inconsistency.
```

Reversible_Reaction	Name	lb	ub	
{'RE2130C' }	{'RE2130C' }	-1000	1000	{'dopa[
{'CYSTS_H2S' }	{'Cystathionine Beta-Synthase (sulfide-forming)'}	-10000	10000	{'cys_L
{'EX_adocbl[e]'}	{'Exchange of adenosylcobalamin' }	-10000	10000	{'adocb
{'EX_mg2[e]' }	{'Exchange of magnesium' }	-10000	10000	{'mg2[e
{'EX_selni[c]' }	{'Exchange of selenite' }	-10000	10000	{'selni

```
3 stoichiometrically inconsistent metabolites removed.
5 stoichiometrically inconsistent reactions removed.

Feasible stoichiometrically consistent model with new reactions.

Feasible model with default bounds.

-------------------------------------------------------------

Assuming gene expression is NaN for 160 genes where no transcriptomic data is provided.

Model statistics:

5840 x 10615 stoichiometric matrix.
1566 exchange reactions.
109 exchange reactions in the core reaction set.
7 exchange reactions in the rxns2Constrain set.

1456 exchange reactions with uptake closed

239 closed non-core sink/demand reactions via param.nonCoreSinksDemands = closeAll
10 core sink/demand reactions.
10 open core sink/demand reactions.

Feasible after closing non-core sink/demand reactions.

-------------------------------------------------------------

Adding growth media information...
The following reactions could not be constrained since they are not present in the model:
    {'EX_HC01944[e]'}
    {'EX_adpcbl[e]' }
```

```
    {'EX_mg2[e]'    }
    {'EX_selni[c]'  }

Adding constraints on 52 reactions
These reactions have bounds larger than the recommended value = abs(10000)
The bounds for the following reactions have been adjusted:
Number of corrected bounds (to min/max boundary):
     2
```

Reverse_Reaction, 0 bound	Name	lb_before	lb_after	ub_befor
{'EX_na1[e]'}	{'Exchange of Sodium' }	-17903.1402873266	-10000	0
{'EX_cl[e]' }	{'Exchange of chloride ion'}	-14916.2499880923	-10000	0

```
Feasible after application of media constraints.

------------------------------------------------------------

Adding quantitative metabolomics constraints ...

        -0.621631949021564


Feasible after application of metabolomic constraints

------------------------------------------------------------

Checking for mismatches ...

------------------------------------------------------------

Adding custom constraints ...

tissueSpecificSolver = thermoKernel. Ignoring specificData.rxns2constrain for demand reactions, i.e. with
Adding constraints on 38 reactions

Feasible after application of custom constraints.


------------------------------------------------------------

Adding 11 sets of coupled reactions ...
```

coupledRxnId	constraints
{'Phosphatidylcholine' }	{'PCHOLP_hs + PLA2_2 + SMS >= 2.025'
{'Adenosine Monophosphate'}	{'AMPDA + NTD7 >= 0.2265'
{'Glutamate' }	{'- ALATA_L + GLUCYS + GLUDxm + GLUDym - ASPTA - ILETA - LEUTA - VALTA
{'Aspartate' }	{'ARGSS + ASPTA >= 1.1925'
{'Serine' }	{'GHMT2r + r0060 >= 0.8625'
{'Arginine' }	{'GLYAMDTRc + r0145 + ARGN >= 0.7245'
{'Tyrosine' }	{'TYR3MO2 + TYRTA + HMR_6728 + HMR_6874 >= 0.55875'
{'Histidine' }	{'HISDC + HISD >= 1.095'
{'Leucine + Isoleucine' }	{'ILETA + LEUTA >= 1.305'
{'Valine + Methionine' }	{'METAT + VALTA >= 0.705'
{'Glycine' }	{'GTHS - GHMT2r >= 0.7725'

coupledRxnId	constraints

```
    {'Phosphatidylcholine'    }    {'PCHOLP_hs + PLA2_2 + SMS >= 2.025'
    {'Adenosine Monophosphate'}    {'AMPDA + NTD7 >= 0.2265'
    {'Glutamate'              }    {'- ALATA_L + GLUCYS + GLUDxm + GLUDym - ASPTA - ILETA - LEUTA - VALTA
    {'Aspartate'              }    {'ARGSS + ASPTA >= 1.1925'
    {'Serine'                 }    {'GHMT2r + r0060 >= 0.8625'
    {'Arginine'               }    {'GLYAMDTRc + r0145 + ARGN >= 0.7245'
    {'Tyrosine'               }    {'TYR3MO2 + TYRTA + HMR_6728 + HMR_6874 >= 0.55875'
    {'Histidine'              }    {'HISDC + HISD >= 1.095'
    {'Leucine + Isoleucine'   }    {'ILETA + LEUTA >= 1.305'
    {'Valine + Methionine'    }    {'METAT + VALTA >= 0.705'
    {'Glycine'                }    {'GTHS - GHMT2r >= 0.7725'


Feasible model after adding coupling constraints.

------------------------------------------------------------------

Removing 233 reactions ...

The following reaction(s) to be removed is(are) not in the model:
    {'HMR_biomass_Renalcancer'   }
    {'DM_HMR_biomass_renalcancer'}
    {'biomass_components'        }
    {'EX_ser_D[e]'               }
    {'EX_pro_D[e]'               }
    {'HMR_1708'                  }
    {'HMR_1934'                  }
    {'r0947'                     }
    {'r1431'                     }
    {'r1432'                     }
    {'RE1096R'                   }
    {'RE1134R'                   }
    {'RE2117M'                   }
    {'RE2768R'                   }
    {'RE2782C'                   }
    {'RE3111M'                   }
    {'RE3338C'                   }
    {'RE3340C'                   }
    {'RE3448C'                   }
    {'RE3564C'                   }
    {'RE3627C'                   }
    {'DM_adchac[c]'              }
    {'DM_alchac[c]'              }


Feasible model after removing inactive reactions.

2 deleted non-core metabolites,  corresponding to bibliomic inactive reactions.
0 deleted core metabolites,  corresponding to bibliomic inactive reactions.
210 deleted non-core reactions,  corresponding to bibliomic inactive reactions.
0 deleted core reactions,  corresponding to bibliomic inactive reactions.
------------------------------------------------------------------

Removing 973 inactive genes...
57 manually selected inactive genes have been marked as active by omics data and will be discarded:
    {'5834'     }
    {'10165'    }
    {'246213'   }
    {'2571'     }
    {'26227'    }
    {'100137049'}
    {'100526794'}
```

```
    {'2752'     }
    {'26227'    }
    {'10060'    }
    {'10858'    }
    {'10873'    }
    {'1160'     }
    {'125965'   }
    {'130752'   }
    {'1346'     }
    {'1468'     }
    {'1583'     }
    {'1588'     }
    {'1607'     }
    {'170712'   }
    {'206358'   }
    {'2110'     }
    {'240'      }
    {'2645'     }
    {'27165'    }
    {'2747'     }
    {'2820'     }
    {'3099'     }
    {'3101'     }
    {'341947'   }
    {'349565'   }
    {'366'      }
    {'374291'   }
    {'3767'     }
    {'412'      }
    {'43'       }
    {'5053'     }
    {'5106'     }
    {'548596'   }
    {'57084'    }
    {'622'      }
    {'64802'    }
    {'6505'     }
    {'6529'     }
    {'6531'     }
    {'6538'     }
    {'6571'     }
    {'6581'     }
    {'6582'     }
    {'6818'     }
    {'6833'     }
    {'7054'     }
    {'79751'    }
    {'83733'    }
    {'84889'    }
    {'8659'     }

11 inactive genes are not in the model to be removed.
Infeasible model after temporarily closing reactions corresponding to inactive genes, relaxing...

  itn          obj     obj_old    err(obj)      err(x)   card(v)   card(r)   card(p)   card(q)
    0       8.4066      1332.5      1324.1  2.8021e+05      1272         0         1         4
    1       8.2951      8.4066     0.11146  5.1996e+05      1242         0         1         4
    2       8.4569      8.2951     0.16175   5.362e+05      1283         0         1         4
    3       8.4867      8.4569    0.029796  5.3872e+05      1281         0         1         4
    4       8.6194      8.4867     0.13272  5.3926e+05      1384         0         1         4
    5        8.285      8.6194     0.33442  5.3742e+05      1246         0         1         4
    6       8.7531       8.285     0.46819   5.398e+05      1322         0         1         4
    7        8.264      8.7531     0.48915  5.4022e+05      1295         0         1         4
    8       8.6802       8.264      0.4162  5.3896e+05      1326         0         1         4
```

```
    9      8.4196      8.6802     0.26057  5.4105e+05      1277         0         1         4
   10      8.5304      8.4196     0.11073  5.3927e+05      1277         0         1         4
   11      8.3826      8.5304     0.14776   5.397e+05      1263         0         1         4
   12      8.6824      8.3826     0.29983  5.4096e+05      1335         0         1         4
   13      8.4864      8.6824     0.19599  5.4213e+05      1273         0         1         4
   14      8.4448      8.4864    0.041662  5.4075e+05      1273         0         1         4
   15      8.3701      8.4448    0.074667  5.4132e+05      1282         0         1         4
   16      8.7422      8.3701     0.37209  5.4318e+05      1311         0         1         4
   17      8.5835      8.7422      0.1587  5.4349e+05      1293         0         1         4
   18      8.6017      8.5835    0.018147  5.4035e+05      1310         0         1         4
   19      8.4429      8.6017     0.15872  5.3978e+05      1300         0         1         4
  itn         obj     obj_old    err(obj)      err(x)   card(v)   card(r)   card(p)   card(q)
Relaxed model is feasible.
Statistics:
1 lower bound relaxation(s)
4 upper bound relaxation(s)
0 steady state relaxation(s)
The lower bound of these reactions had to be relaxed:
    Closed_Reaction                      Name                                lb_before         lb_after
    _______________    ____________________________________________________    _________    ____________________

       {'URAt'}        {'Uracil Transport via Faciliated Diffusion'}        0        -0.00701902520631847


The upper bound of these reactions had to be relaxed:
    Closed_Reaction                       Name                                lb_before    lb_after    ub_befo
    _______________    ____________________________________________________    _________    ________    _______

      {'KHK'   }       {'Ketohexokinase'                                 }       0            0           0
      {'MMEm'  }       {'Methylmalonyl Coenzyme A Epimerase/Racemase'}       0            0           0
      {'OIVD1m'}       {'2-Oxoisovalerate Dehydrogenase (Acylating'  }       0            0           0
      {'SERPT' }       {'Serine C-Palmitoyltransferase'              }       0            0           0


... done.
5 reaction(s) were not deleted based on inactive genes as their removal would cause the model to be infeas
480 genes were specified as inactive but not removed as they are involved in reactions that may be catalys
123 deleted non-core metabolites,  corresponding to inactive genes.
0 deleted core metabolites,  corresponding to inactive genes.
1747 deleted non-core reactions,  corresponding to inactive genes.
0 deleted core reactions,  corresponding to inactive genes.

Feasible model after removing inactive genes (that do not affect core reactions).

------------------------------------------------------------

Identifying flux consistent reactions ...

5715 x 8658 stoichiometric matrix, before flux consistency.
--- findFluxConsistentSubset START ----
2965 flux consistent metabolites
2750 flux inconsistent metabolites
4983 flux consistent reactions
3675 flux inconsistent reactions
--- findFluxConsistentSubset END ----
2739 deleted non-core metabolites,  corresponding to flux inconsistency.
11 deleted core metabolites,  corresponding to flux inconsistency.
          mets                                        metNames
    _______________    ____________________________________________________________________

    {'Tyr_ggn[c]'   }    {'Tyr-194 Of Apo-Glycogenin Protein (Primer For Glycogen Synthesis)'}
    {'pre_prot[r]'  }    {'Glycophosphatidylinositol (Gpi)-Anchored Protein Precursor'       }
    {'retfa[c]'     }    {'Fatty Acid Retinol'                                               }
    {'thm[m]'       }    {'Thiamin'                                                          }
```

{'no2[c]' }	{'Nitrite' }
{'CE1273[c]' }	{'5Beta-Cholestane-3Alpha,7Alpha,12Alpha,24S,25-Pentol' }
{'pail35p_hs[n]'}	{'1-Phosphatidyl-1D-Myo-Inositol 3,5-Bisphosphate' }
{'c101coa[c]' }	{'Decenoyl Coenzyme A' }
{'fe3[c]' }	{'Iron (Fe3+)' }
{'6hddopaqn[c]' }	{'6-Hydroxydopamine-Quinone' }
{'gm1_hs[n]' }	{'Ganglioside Gm1' }

3630 deleted non-core reactions, corresponding to flux inconsistency.
45 deleted core reactions, corresponding to flux inconsistency.

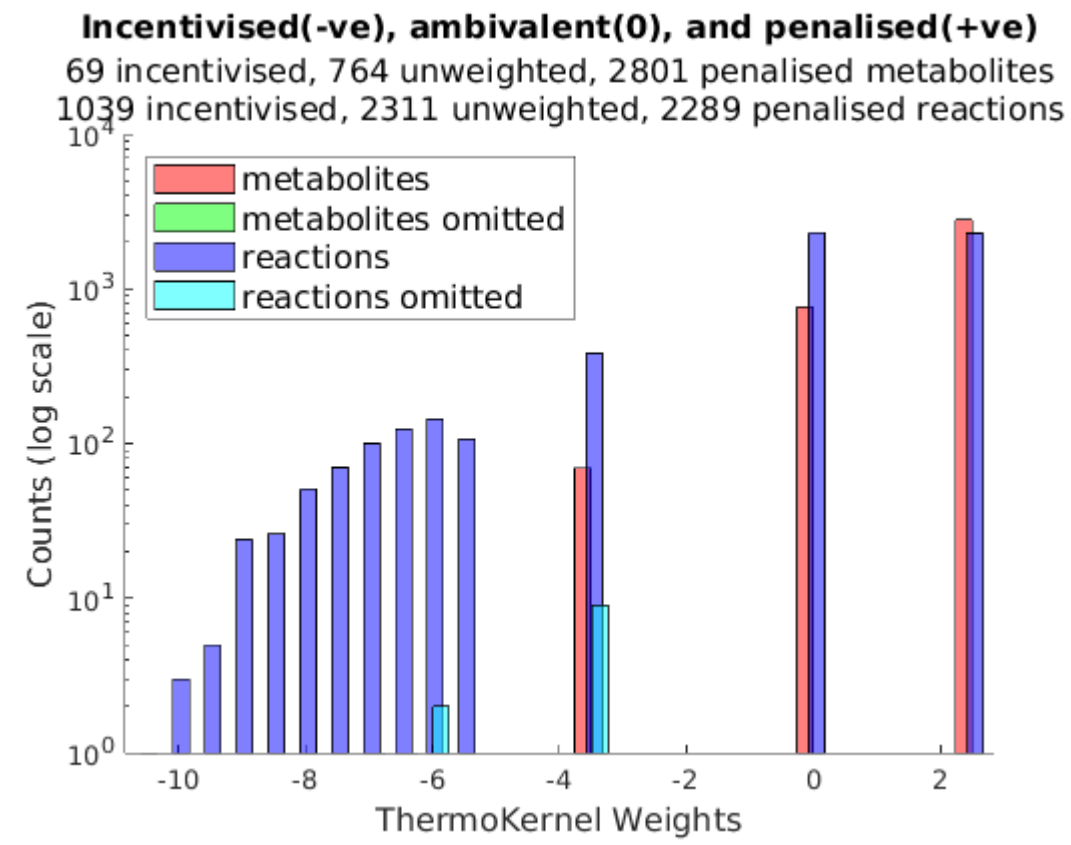
Forward_Reaction, 0 bound	Name	lb
{'ACHEe' }	{'Acetylcholinesterase' }	0
{'APOC_LYS_BTNPm'}	{'Proteolysis of ApoC-Lys-Biotin, Mitochondrial' }	0
{'ARGNm' }	{'Arginase, Mitochondrial' }	0
{'CLS_hs' }	{'Cardiolipin Synthase (Homo Sapiens)' }	0
{'DURIK1m' }	{'Deoxyuridine Kinase (ATP:Deoxyuridine), Mitochondrial' }	0
{'EX_co[e]' }	{'Exchange of Carbon Monoxide ' }	0
{'G3PD2m' }	{'Glycerol-3-Phosphate Dehydrogenase (FAD), Mitochondrial' }	0
{'GLYKm' }	{'Glycerol Kinase' }	0
{'OCOAT1m' }	{'3-Oxoacid Coa-Transferase' }	0
{'P45011A1m' }	{'Cytochrome P450 11A1, Mitochondrial [Precursor]' }	0
{'P45027A11m' }	{'5-Beta-Cholestane-3-Alpha, 7-Alpha, 12-Alpha-Triol 27-Hydrox'}	0
{'P45027A14m' }	{'5-Beta-Cytochrome P450, Family 27, Subfamily A, Polypeptide '}	0
{'RBK_D' }	{'D-Ribulokinase' }	0
{'SARDHm' }	{'Sarcosine Dehydrogenase, Mitochondrial' }	0
{'STS1' }	{'Steryl-Sulfatase' }	0
{'r0321' }	{'Acetoacetate:Coa Ligase (AMP-Forming)' }	0
{'FE2DMT1' }	{'Uptake of Food Iron by Dmt1 Transporter' }	0
{'ARGN' }	{'Arginase' }	0
{'RBK' }	{'Ribokinase' }	0
{'DOPAOQNOX' }	{'Dopamine-O-Quinone Oxidase' }	0
{'HMR_9726' }	{'5-Formyltetrahydrofolate:L-Glutamate N-Formiminotransferase' }	0
{'DHBOX' }	{'3,4-dihydroxybenzaldehyde oxidase' }	0
{'DM_clpn_hs[c]' }	{'Demand of cardiolipin' }	0
{'NORCON' }	{'Norsalsolinol consendation' }	0
{'Q-METHRED' }	{'Quinonemethide reductase' }	0

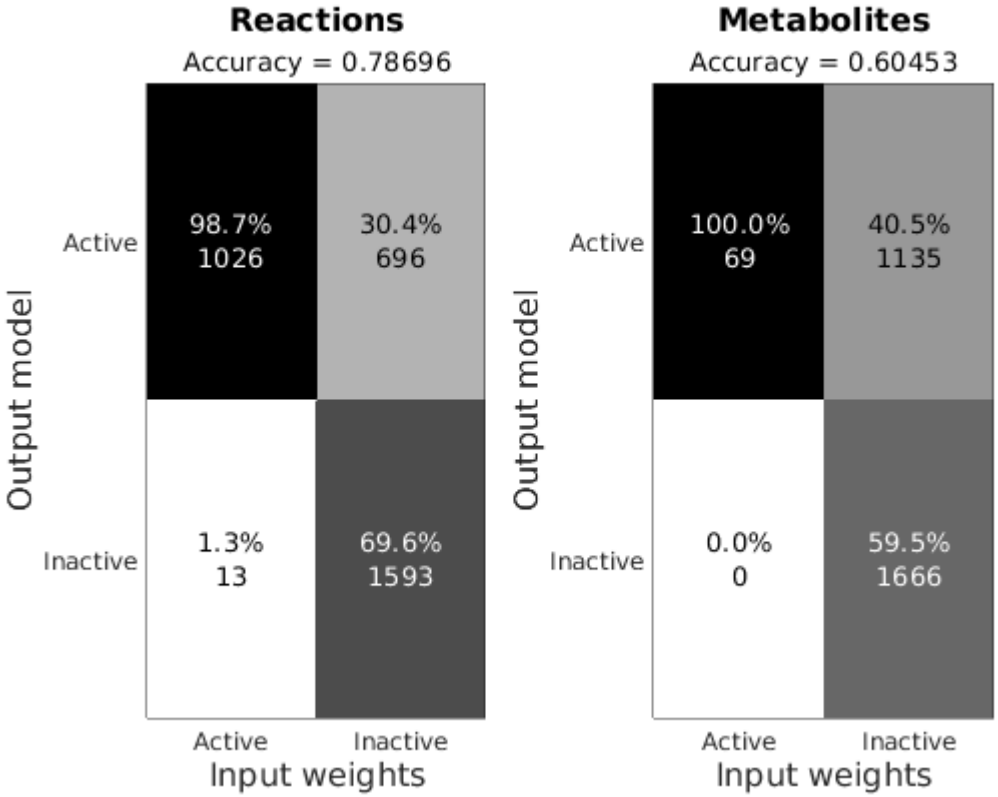
Reverse_Reaction, 0 bound	Name	lb	ub	
{'r0245' }	{'Glycerol:NADP+ Oxidoreductase'}	-10000	0	{'nadp[c
{'EX_fe3[e]'}	{'Exchange of Iron (Fe3+) ' }	-1.63928248314438	0	{'fe3[e]
{'EX_k[e]' }	{'Exchange of Kalium' }	-733.702544787044	0	{'k[e]
{'EX_na1[e]'}	{'Exchange of Sodium' }	-10000	0	{'na1[e]
{'EX_ca2[e]'}	{'Exchange of calcium' }	-220.501332473743	0	{'ca2[e]
{'EX_cl[e]' }	{'Exchange of chloride ion' }	-10000	0	{'cl[e]
{'EX_zn2[e]'}	{'Exchange of zinc (II) ion' }	-0.283998779119298	0	{'zn2[e]

Reversible_Reaction	Name	lb
{'EX_i[e]' }	{'Exchange of Iodide ' }	
{'RE1530M' }	{'Thymidine Kinase' }	
{'C02712tm' }	{'Transport of N-Acetylmethionine, Intracellular' }	
{'ACGLUtm' }	{'Transport of N-Acetyl-L-Glutamate, Mitochondrial' }	
{'r2535m' }	{'Transport of L-Homoserine, Mitochondrial' }	
{'EX_fe2[e]' }	{'Exchange of Iron (Fe2+)' }	
{'EX_pnto_R[e]'}	{'Exchange of (R)-Pantothenate ' }	-1.01132
{'EX_hxan[e]' }	{'Exchange of Hypoxanthine ' }	-1.16204
{'EX_thm[e]' }	{'Exchange of Thiamin' }	-1.41539
{'EX_pydxn[e]' }	{'Exchange of Pyridoxine' }	-2.26640
{'EX_btn[e]' }	{'Exchange of Biotin ' }	-0.00110892

```
     {'CE2172t'     }      {'Transport of 6, 7-Dihydroxy-1, 2, 3, 4-Tetrahydroisoquinolin'}
     {'EX_CE2172[e]'}      {'Exchange of 6, 7-Dihydroxy-1, 2, 3, 4-Tetrahydroisoquinoline'}


2965 x 4983 stoichiometric matrix, after flux consistency.

------------------------------------------------------------

Identifying thermodynamically flux consistent subset ...

1 model.C constraints removed
          solver: 'mosek'
       algorithm: 'default'
            stat: 0
        origStat: 'PRIMAL_INFEASIBLE_CER'
    origStatText: []
            time: 0.00464499999999646
           basis: []
               f: NaN
               v: []
               y: []
               w: []
               s: []
         ctrs_y: []
         ctrs_s: []
               x: []
Warning: findThermoConsistentFluxSubset: thermoConsistModel is not feasible.
0 deleted non-core metabolites,  corresponding to thermodynamic flux inconsistency.
0 deleted core metabolites,  corresponding to thermodynamic flux inconsistency.
1 deleted non-core reactions,  corresponding to thermodynamic flux inconsistency.
12 deleted core reactions,  corresponding to thermodynamic flux inconsistency.
    Forward_Reaction, 0 bound          Name           lb          ub                          equat
    ___________________________    ________________    __    _________________    ____________________

            {'KHK'}             {'Ketohexokinase'}    0     0.287664725528884    {'atp[c] + fru[c]  -> h[

    Forward_Reaction, non-0 bound                              Name
    _____________________________    ____________________________________________________________    _

           {'ALATA_L' }              {'L-Alanine Transaminase'                                  }    1
           {'LYSOXp'  }              {'Transport of L-Lysine Oxidase, Peroxisomal'              }
           {'PIK4'    }              {'Phosphatidylinositol 4-Kinase'                           }
           {'ATPM'    }              {'Demand for ATP, Cytosolic'                               }
           {'GHMT2r'  }              {'Glycine Hydroxymethyltransferase, Reversible'            }    0
           {'ILETA'   }              {'Isoleucine Transaminase'                                 }
           {'LEUTA'   }              {'Leucine Transaminase'                                    }
           {'PROD2'   }              {'Proline Dehydrogenase'                                   }    0
           {'NTD2'    }              {'5'-Nucleotidase (UMP)'                                   }    0
           {'THRD_L'  }              {'L-Threonine Deaminase'                                   }
           {'HMR_0653'}              {'S-Adenosyl-L-Methionine:Phosphatidylethanolamine N-Methyltra'}


2965 x 4970 stoichiometric matrix, after thermodynamic flux consistency.
Infeasible after extraction of thermodynamically feasible subset, relaxing...
  itn         obj     obj_old    err(obj)      err(x)   card(v)   card(r)   card(p)   card(q)
    0      8.5431      651.14      642.59  2.8354e+05      1252         0         3         0
    1      7.9475      8.5431     0.59563  5.2136e+05      1172         0         3         0
    2      8.4058      7.9475     0.45835  5.3075e+05      1279         0         3         0
    3      8.5054      8.4058    0.099608  5.3921e+05      1213         0         3         0
    4      8.3797      8.5054     0.12571  5.3952e+05      1284         0         3         0
    5      8.6121      8.3797     0.23242  5.3995e+05      1276         0         3         0
    6      8.5337      8.6121    0.078416  5.4113e+05      1299         0         3         0
    7      8.5236      8.5337    0.010169   5.411e+05      1241         0         3         0
    8      8.5332      8.5236   0.0096319  5.4235e+05      1293         0         3         0
```

```
    9      8.5411      8.5332   0.0079158  5.4318e+05      1242         0         3         0
   10      8.6421      8.5411     0.10104  5.4241e+05      1243         0         3         0
   11      8.4761      8.6421     0.16606  5.4348e+05      1225         0         3         0
   12      8.7017      8.4761     0.22564  5.4381e+05      1295         0         3         0
   13      8.6743      8.7017    0.027477  5.4587e+05      1272         0         3         0
   14      8.6722      8.6743   0.0020358  5.4543e+05      1269         0         3         0
   15      8.4168      8.6722     0.25544  5.4252e+05      1224         0         3         0
   16      8.8788      8.4168     0.46204  5.4502e+05      1283         0         3         0
   17      8.3864      8.8788     0.49246  5.4402e+05      1240         0         3         0
   18      8.8933      8.3864     0.50697  5.4341e+05      1301         0         3         0
   19      8.5211      8.8933     0.37227  5.4603e+05      1221         0         3         0
  itn         obj     obj_old    err(obj)      err(x)   card(v)   card(r)   card(p)   card(q)
Relaxed model is feasible.
Statistics:
3 lower bound relaxation(s)
0 upper bound relaxation(s)
0 steady state relaxation(s)
The lower bound of these reactions had to be relaxed:
    Forward_Reaction, non-0 bound                    Name                          lb_before
    _____________________________    ____________________________________    ________________    _____

          {'EX_2hb[e]'   }           {'Exchange of 2-Hydroxybutyrate '   }    0.0327019285314542    -8.789
          {'EX_glyc_R[e]'}           {'Exchange of D-Glycerate'          }     0.287664725528066    -8.185
          {'EX_3hivac[e]'}           {'Exchange of 3-Hydroxy-Isovalerate'}     0.922557070436904


... done.
184 active genes not present in model.genes, so they are ignored.
------------------------------------------------------------

Extracting tissue specific model ...

Using real valued weights on metabolites and reactions as input to thermoKernel.
Using real valued weights from omics on dummy reactions as input to thermoKernel.
13 forced internal reactions and relaxation of bounds so cycleFreeFlux only determines thermodynamic feasi
[mosek] reports OPTIMAL but Primal optimality condition in solveCobraQP not satisfied, residual = 0.000803
[mosek] reports OPTIMAL but Primal optimality condition in solveCobraQP not satisfied, residual = 0.000265
[mosek] reports OPTIMAL but Primal optimality condition in solveCobraQP not satisfied, residual = 0.000379
[mosek] reports OPTIMAL but Primal optimality condition in solveCobraQP not satisfied, residual = 0.000216
[mosek] reports OPTIMAL but Primal optimality condition in solveCobraQP not satisfied, residual = 0.000430
[mosek] reports OPTIMAL but Primal optimality condition in solveCobraQP not satisfied, residual = 0.000396
[mosek] reports OPTIMAL but Primal optimality condition in solveCobraQP not satisfied, residual = 0.000359
[mosek] reports OPTIMAL but Primal optimality condition in solveCobraQP not satisfied, residual = 0.001583
[mosek] reports OPTIMAL but Primal optimality condition in solveCobraQP not satisfied, residual = 0.000423
All incentivised metabolites are produced.
```






```
2335 deleted non-core metabolites,  corresponding to removal by createTissueSpecificModel.
0 deleted core metabolites,  corresponding to removal by createTissueSpecificModel.
3520 deleted non-core reactions,  corresponding to removal by createTissueSpecificModel.
9 deleted core reactions,  corresponding to removal by createTissueSpecificModel.
```

Forward_Reaction, 0 bound	Name	lb	ub

{'OIVD1m' }	{'2-Oxoisovalerate Dehydrogenase (Acylating'}	0	0.922557070436596
{'EX_3hivac[e]'}	{'Exchange of 3-Hydroxy-Isovalerate' }	0	10000

Reverse_Reaction, 0 bound	Name	lb
{'URAt'}	{'Uracil Transport via Faciliated Diffusion'}	-0.00701902520631847

Reversible_Reaction	Name	lb	u
{'EX_2hb[e]' }	{'Exchange of 2-Hydroxybutyrate ' }	-8.78928874126217e-13	10
{'EX_lipoate[e]'}	{'Exchange of Lipoate ' }	-0.0394044564234044	10
{'EX_ncam[e]' }	{'Exchange of Nicotinamide ' }	-3.81469600757283	10
{'EX_prgstrn[e]'}	{'Exchange of Progesterone ' }	-0.00161329356250689	10
{'EX_CE2028[e]' }	{'Exchange of Beta-Hydroxy-Beta-Methylbutyrate'}	-10000	10
{'EX_glyc_R[e]' }	{'Exchange of D-Glycerate' }	-8.18511924904897e-13	10

Infeasible tissue specific model. Trying relaxation...

itn	obj	obj_old	err(obj)	err(x)	card(v)	card(r)	card(p)	card(q)
0	5.3659	276.96	271.6	2.2509e+05	888	0	1	0
1	5.5265	5.3659	0.16051	4.1938e+05	901	0	1	0
2	5.764	5.5265	0.23751	4.3585e+05	959	0	1	0
3	5.5763	5.764	0.18767	4.3948e+05	928	0	1	0
4	5.8715	5.5763	0.29517	4.4035e+05	932	0	1	0
5	5.7167	5.8715	0.15471	4.426e+05	944	0	1	0
6	5.8007	5.7167	0.083906	4.4116e+05	940	0	1	0
7	5.6403	5.8007	0.1604	4.4086e+05	927	0	1	0
8	5.8213	5.6403	0.18102	4.4043e+05	957	0	1	0
9	5.5644	5.8213	0.25686	4.3868e+05	929	0	1	0
10	5.7181	5.5644	0.15373	4.3992e+05	938	0	1	0
11	5.6189	5.7181	0.099209	4.408e+05	929	0	1	0
12	5.6687	5.6189	0.049738	4.3927e+05	931	0	1	0
13	5.6075	5.6687	0.061117	4.3911e+05	947	0	1	0
14	5.7732	5.6075	0.16563	4.3951e+05	957	0	1	0
15	5.7726	5.7732	0.00057859	4.4213e+05	939	0	1	0
16	5.8206	5.7726	0.048032	4.4014e+05	954	0	1	0
17	5.7132	5.8206	0.10742	4.3954e+05	939	0	1	0
18	5.6906	5.7132	0.022593	4.4018e+05	951	0	1	0
19	5.7724	5.6906	0.081751	4.4154e+05	933	0	1	0
itn	obj	obj_old	err(obj)	err(x)	card(v)	card(r)	card(p)	card(q)

Relaxed model is feasible.
Statistics:
1 lower bound relaxation(s)
0 upper bound relaxation(s)
0 steady state relaxation(s)
The lower bound of these reactions had to be relaxed:

Forward_Reaction, non-0 bound	Name	lb_before	lb_after
{'EX_ura[e]'}	{'Exchange of Uracil '}	0.00701902520608372	-2.34757525918727e-

... done.
... relaxation worked.
1299 x 2110 stoichiometric matrix after model extraction.

Feasible at end of XomicsToModel.

```
debugXomicsToModel:

#Active_genes               #Active_rxns              #Active_mets                Stage
          909                        432                        45                Active list
#Active_genes #Model_genes  #Active_rxns #Model_rxns  #Active_mets #Model_mets     lb_obj    Obj  Message
          885         2248           401       10600            45        5835         0  755.003 Feasible
          882         1892           422       10615            45        5840        NaN        0 Fea
          882         1892           422       10615            45        5840        NaN        0 Fea
          882         1892           422       10615            45        5840        NaN        0 Fea
          882         1892           422       10615            45        5840        NaN        0 Fea
          882         1892           422       10615            45        5840        NaN        0 Fea
          882         1892           422       10615            45        5840        NaN        0 Fea
          882         1892           422       10615            45        5840        NaN        0 Fea
          882         1892           422       10615            45        5840        NaN        0 Fea
          882         1892           422       10615            45        5840        NaN        0 Fea
          882         1892           422       10405            45        5838        NaN        0 Fea
          873         1620           422        8658            45        5715        NaN        0 Fea
          719         1307           377        4983            34        2965        NaN        0 Fea
          719         1305           366        4970            34        2965        NaN        0 Fea
          719         1305           366        5639            34        3634        NaN        0 Fea
          715         1224           359        2110            34        1299        NaN        0 Fea
------------------------------------------------------------
Diary written to: /home/rfleming/20221216T085613_rfleming_diary.txt
XomicsToModel run is complete at:16-Dec-2022 09:09:37
```

Examining when active metabolites, reactions and genes were added or removed during the model generation process

```
debugXomicsToModel(model, pwd, modelGenerationReport)
```

```
#Active_genes               #Active_rxns              #Active_mets                Stage
          909                        432                        43                Active list
#Active_genes #Model_genes  #Active_rxns #Model_rxns  #Active_mets #Model_mets     lb_obj    Obj  Message
          885         2248           401       10600            43        5835         0  755.003 Feasible
          882         1892           422       10615            43        5840        NaN        0 Fea
          882         1892           422       10615            43        5840        NaN        0 Fea
          882         1892           422       10615            43        5840        NaN        0 Fea
          882         1892           422       10615            43        5840        NaN        0 Fea
          882         1892           422       10615            43        5840        NaN        0 Fea
          882         1892           422       10615            43        5840        NaN        0 Fea
          882         1892           422       10615            43        5840        NaN        0 Fea
          882         1892           422       10615            43        5840        NaN        0 Fea
          882         1892           422       10615            43        5840        NaN        0 Fea
          882         1892           422       10405            43        5838        NaN        0 Fea
          873         1620           422        8658            43        5715        NaN        0 Fea
          719         1307           377        4983            32        2965        NaN        0 Fea
          719         1305           366        4970            32        2965        NaN        0 Fea
          719         1305           366        5639            32        3634        NaN        0 Fea
          715         1224           359        2110            32        1299        NaN        0 Fea
```

TIMING

TIMING: 15 minutes to hours (computation) - days (interpretation)

Bibliography

1. German Preciat, Agnieszka B. Wegrzyn, Ines Thiele, et al., "XomicsToModel: a COBRA Toolbox extension for generation of thermodynamic-flux-consistent, context-specific, genome-scale metabolic models", *bioRxiv* (**2021**)
2. Laurent Heirendt, Sylvain Arreckx, Thomas Pfau, et al., "Creation and analysis of biochemical constraint-based models using the COBRA Toolbox v. 3.0", *Nature protocols* (**2019**).
3. German Preciat, Edinson Lucumi Moreno, Agnieszka B. Wegrzyn, et al., "Mechanistic model-driven exometabolomic characterisation of human dopaminergic neuronal metabolism", *bioRxiv* (**2021**)
4. Elizabeth Brunk, Swagatika Sahoo, Daniel C. Zielinski, et al., "Recon3D enables a three-dimensional view of gene variation in human metabolism", *Nature biotechnology* (**2018**)