OptKnock Tutorial

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INTRODUCTION:

In this tutorial we will run optKnock. For a detailed description of the procedure, please see [1]. Briefly, the problem is to find a set of reactions of size "numDel" such that when these reactions are deleted from the network, the mutant created will produce a particular target of interest in a higher rate than the wild-type strain.

For example, imagine that we would like to increase the production of succinate or lactate in Escherichia coli. Which are the knock-outs needed to increase the production of these products? We will approach these problems in this tutorial.

MATERIALS

EQUIPMENT

1. MATLAB
2. A solver for Mixed Integer Linear Programming (MILP) problems. For example, Gurobi.

EQUIPMENT SETUP

Use changeCobraSolver to choose the solver for MILP problems.

PROCEDURE

The proceduce consists on the following steps

1) Define contraints.

2) Define a set of reactions to search knockouts. Only reactions in this set will be deleted.

3) Define the number of reactions to be deleted, the target reaction and some constraints to be accomplish.

4) Run optKnock.

TIMING: This task should take from a few seconds to a few hours depending on the size of your reconstruction.

We verify that cobratoolbox has been initialized and that the solver has been set.

```
global TUTORIAL_INIT_CB;
if ~isempty(TUTORIAL_INIT_CB) && TUTORIAL_INIT_CB==1
    initCobraToolbox
end
```

```
changeCobraSolver('gurobi','all');
```

```
> Gurobi interface added to MATLAB path.
> Solver for LP problems has been set to gurobi.

> Gurobi interface added to MATLAB path.
> Solver for MILP problems has been set to gurobi.

> Gurobi interface added to MATLAB path.
> Solver for QP problems has been set to gurobi.

> Gurobi interface added to MATLAB path.
> Solver for MIQP problems has been set to gurobi.
> Solver gurobi not supported for problems of type NLP. No solver set for this problemtype
```

```
fullPath = which('tutorial_optKnock');
folder = fileparts(fullPath);
currectDirectory = pwd;
cd(folder);
```

We load the model of E. coli [2].

```
model = readCbModel('iJO1366.mat')
```

```
model = 
           rxns: {2583×1 cell}
           mets: {1805×1 cell}
              S: [1805×2583 double]
             lb: [2583×1 double]
             ub: [2583×1 double]
              c: [2583×1 double]
       rxnNames: {2583×1 cell}
     subSystems: {2583×1 cell}
        grRules: {2583×1 cell}
       metNames: {1805×1 cell}
    metFormulas: {1805×1 cell}
          genes: {1367×1 cell}
    description: 'iJO1366.mat'
     rxnGeneMat: [2583×1367 double]
         csense: [1805×1 char]
              b: [1805×1 double]
         osense: -1
          rules: {2583×1 cell}
```

```
biomass = 'BIOMASS_Ec_iJO1366_core_53p95M';
```

```
Warning: A value of class "com.mathworks.mde.cmdwin.XCmdWndView" was indexed with no
subscripts specified. Currently the result of this operation is the indexed value itself,
but in a future release, it will be an error.
Warning: A value of class "com.mathworks.mde.cmdwin.XCmdWndView" was indexed with no
subscripts specified. Currently the result of this operation is the indexed value itself,
but in a future release, it will be an error.
```

We define the maximum number of solutions to find

```
threshold = 5;
```

First, we define the set for reactions which could be deleted from the network. Reactions not in this list are not going to be deleted.

```
selectedRxnList = {'GLCabcpp'; 'GLCptspp'; 'HEX1'; 'PGI'; 'PFK'; 'FBA'; 'TPI'; 'GAPD'; ...
                   'PGK'; 'PGM'; 'ENO'; 'PYK'; 'LDH_D'; 'PFL'; 'ALCD2x'; 'PTAr'; 'ACKr'; ...
                   'G6PDH2r'; 'PGL'; 'GND'; 'RPI'; 'RPE'; 'TKT1'; 'TALA'; 'TKT2'; 'FUM'; ...
                   'FRD2'; 'SUCOAS'; 'AKGDH'; 'ACONTa'; 'ACONTb'; 'ICDHyr'; 'CS'; 'MDH'; ...
                   'MDH2'; 'MDH3'; 'ACALD'};
```

Then, we define some constraints

```
% prespecified amount of glucose uptake 10 mmol/grDW*hr
model = changeRxnBounds(model, 'EX_glc__D_e', -10, 'b');

% Unconstrained uptake routes for inorganic phosphate, sulfate and
% ammonia
model = changeRxnBounds(model, 'EX_o2_e', 0, 'l');
model = changeRxnBounds(model, 'EX_pi_e', -1000, 'l');
model = changeRxnBounds(model, 'EX_so4_e', -1000, 'l');
model = changeRxnBounds(model, 'EX_nh4_e', -1000, 'l');

% The optimization step could opt for or against the phosphotransferase
% system, glucokinase, or both mechanisms for the uptake of glucose
model = changeRxnBounds(model, 'GLCabcpp', -1000, 'l');
model = changeRxnBounds(model, 'GLCptspp', -1000, 'l');
model = changeRxnBounds(model, 'GLCabcpp', 1000, 'u');
model = changeRxnBounds(model, 'GLCptspp', 1000, 'u');
model = changeRxnBounds(model, 'GLCt2pp', 0, 'b');

% Secretion routes  for acetate, carbon dioxide, ethanol, formate, lactate
% and succinate are enabled
model = changeRxnBounds(model, 'EX_ac_e', 1000, 'u');
model = changeRxnBounds(model, 'EX_co2_e', 1000, 'u');
model = changeRxnBounds(model, 'EX_etoh_e', 1000, 'u');
model = changeRxnBounds(model, 'EX_for_e', 1000, 'u');
model = changeRxnBounds(model, 'EX_lac__D_e', 1000, 'u');
model = changeRxnBounds(model, 'EX_succ_e', 1000, 'u');
```

Then, we calculates the production of metabolites before running optKnock.

```
% determine succinate production and growth rate before optimizacion
fbaWT = optimizeCbModel(model);
succFluxWT = fbaWT.x(strcmp(model.rxns, 'EX_succ_e'));
etohFluxWT = fbaWT.x(strcmp(model.rxns, 'EX_etoh_e'));
formFluxWT = fbaWT.x(strcmp(model.rxns, 'EX_for_e'));
lactFluxWT = fbaWT.x(strcmp(model.rxns, 'EX_lac__D_e'));
acetFluxWT = fbaWT.x(strcmp(model.rxns, 'EX_ac_e'));
growthRateWT = fbaWT.f;
fprintf('The production of succinate before optimization is %.1f \n', succFluxWT);
```

```
The production of succinate before optimization is 0.1
```

```
fprintf('The growth rate before optimization is %.1f \n', growthRateWT);
```

```
The growth rate before optimization is 0.2
```

```
fprintf(['The production of other products such as ethanol, formate, lactate and'...
         'acetate are %.1f, %.1f, %.1f and %.1f, respectively. \n'], ...
        etohFluxWT, formFluxWT, lactFluxWT, acetFluxWT);
```

```
The production of other products such as ethanol, formate, lactate andacetate are 8.1, 17.3, 0.0 and 8.2
```

I) SUCCINATE OVERPRODUCTION

EXAMPLE 1: finding optKnock reactions sets of size 2 for increasing production of succinate

```
fprintf('\n...EXAMPLE 1: Finding optKnock sets of size 2 or less...\n\n')
```

```
...EXAMPLE 1: Finding optKnock sets of size 2 or less...
```

```
% Set optKnock options
% The exchange of succinate will be the objective of the outer problem
options = struct('targetRxn', 'EX_succ_e', 'numDel', 2);
% We will impose that biomass be at least 50% of the biomass of wild-type
constrOpt = struct('rxnList', {{biomass}},'values', 0.5*fbaWT.f, 'sense', 'G');
% We will try to find 10 optKnock sets of a maximun length of 2
previousSolutions = cell(10, 1);
contPreviousSolutions = 1;
nIter = 1;
while nIter < threshold
    fprintf('...Performing optKnock analysis...\n')
    if isempty(previousSolutions{1})
        optKnockSol = OptKnock(model, selectedRxnList, options, constrOpt);
    else
        optKnockSol = OptKnock(model, selectedRxnList, options, constrOpt, previousSolutions,
    end

    % determine succinate production and growth rate after optimizacion
    succFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_succ_e'));
    growthRateM1 = optKnockSol.fluxes(strcmp(model.rxns, biomass));
    etohFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_etoh_e'));
    formFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_for_e'));
    lactFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_lac__D_e'));
    acetFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_ac_e'));
    setM1 = optKnockSol.rxnList;

    if ~isempty(setM1)
        previousSolutions{contPreviousSolutions} = setM1;
        contPreviousSolutions = contPreviousSolutions + 1;
        %printing results
        fprintf('optKnock found a optKnock set of large %d composed by ', length(setM1));
        for j = 1:length(setM1)
            if j == 1
                fprintf('%s', setM1{j});
            elseif j == length(setM1)
                fprintf(' and %s', setM1{j});
            else
                fprintf(', %s', setM1{j});
            end
        end
        fprintf('\n');
        fprintf('The production of succinate after optimization is %.2f \n', succFluxM1);
        fprintf('The growth rate after optimization is %.2f \n', growthRateM1);
        fprintf(['The production of other products such as ethanol, formate, lactate and aceta
               '%.1f, %.1f, %.1f and %.1f, respectively. \n'], etohFluxM1, formFluxM1, lactF
        fprintf('...Performing coupling analysis...\n');
        [type, maxGrowth, maxProd, minProd] = analyzeOptKnock(model, setM1, 'EX_succ_e');
        fprintf('The solution is of type: %s\n', type);
        fprintf('The maximun growth rate given the optKnock set is %.2f\n', maxGrowth);
        fprintf(['The maximun and minimun production of succinate given the optKnock set is '
               '%.2f and %.2f, respectively \n\n'], minProd, maxProd);
        if strcmp(type, 'growth coupled')
            singleProductionEnvelope(model, setM1, 'EX_succ_e', biomass, 'savePlot', 1, 'showP
                                    'fileName', ['succ_ex1_' num2str(nIter)], 'outputFolder',
        end,
    else
        if nIter == 1
            fprintf('optKnock was not able to found an optKnock set\n');
        else
            fprintf('optKnock was not able to found additional optKnock sets\n');
        end
        break;
    end
    nIter = nIter + 1;
end
```

```
...Performing optKnock analysis...
optKnock found a optKnock set of large 2 composed by
PFL
 and RPI
The production of succinate after optimization is 2.75
The growth rate after optimization is 0.14
The production of other products such as ethanol, formate, lactate and acetate are9.4, 0.0, -0.0 and 0.0
...Performing coupling analysis...
The solution is of type: growth coupled non unique
The maximun growth rate given the optKnock set is 0.12
The maximun and minimun production of succinate given the optKnock set is 0.04 and 2.31, respectively
...Performing optKnock analysis...
```

```
MILP problem with 8398 constraints 63 integer variables and 8370 continuous variables
Optimize a model with 8398 rows, 8370 columns and 34671 nonzeros
Variable types: 8307 continuous, 63 integer (63 binary)
Coefficient statistics:
  Matrix range     [2e-06, 1e+03]
  Objective range  [1e+00, 1e+00]
  Bounds range     [1e+00, 1e+03]
  RHS range        [1e-02, 1e+03]
Presolve removed 5079 rows and 3321 columns
Presolve time: 0.09s
Presolved: 3319 rows, 5049 columns, 21617 nonzeros
Variable types: 5012 continuous, 37 integer (37 binary)

Root relaxation: objective 1.230321e+01, 4944 iterations, 0.64 seconds

    Nodes    |    Current Node    |     Objective Bounds      |     Work
 Expl Unexpl |  Obj  Depth IntInf | Incumbent    BestBd   Gap | It/Node Time

     0     0   12.30321    0    2          -   12.30321      -      -    1s
H    0     0                       0.0915893   12.30321      -      -    1s
     0     0   12.30321    0    2    0.09159   12.30321      -      -    1s
     0     2   12.30321    0    2    0.09159   12.30321      -      -    1s
*   79    63              25       0.2411614   12.30321  5002%  60.1    1s
*   90    70              24       0.2411614   12.30321  5002%  59.3    2s
*  175    78              21       0.2856919   12.30321  4206%  58.0    2s
*  193    81              26       0.2864668   12.30321  4195%  54.6    2s
*  194    81              26       0.2864668   12.30321  4195%  54.3    2s
*  240   104              21       1.2019176   12.29845   923%  55.5    2s
*  291    87              19       1.4425937   12.28821   752%  58.5    2s
*  303    65              19       1.6792803   12.27798   631%  60.7    3s

Explored 379 nodes (35252 simplex iterations) in 3.71 seconds
Thread count was 8 (of 8 available processors)

Solution count 9: 1.67928 1.44259 1.20192 ... 0.0915893
Pool objective bound 1.67928

Optimal solution found (tolerance 1.00e-12)
Best objective 1.679280339834e+00, best bound 1.679280339834e+00, gap 0.0000%
```

```
optKnock found a optKnock set of large 2 composed by
PFL
 and TKT2
The production of succinate after optimization is 1.68
The growth rate after optimization is 0.22
The production of other products such as ethanol, formate, lactate and acetate are14.9, 0.0, 0.0 and -0.
...Performing coupling analysis...
The solution is of type: growth coupled non unique
The maximun growth rate given the optKnock set is 0.18
The maximun and minimun production of succinate given the optKnock set is 0.06 and 1.41, respectively
...Performing optKnock analysis...
```

```
MILP problem with 8399 constraints 63 integer variables and 8370 continuous variables
Optimize a model with 8399 rows, 8370 columns and 34674 nonzeros
Variable types: 8307 continuous, 63 integer (63 binary)
Coefficient statistics:
  Matrix range     [2e-06, 1e+03]
  Objective range  [1e+00, 1e+00]
  Bounds range     [1e+00, 1e+03]
  RHS range        [1e-02, 1e+03]
Presolve removed 5079 rows and 3321 columns
Presolve time: 0.10s
Presolved: 3320 rows, 5049 columns, 21619 nonzeros
Variable types: 5012 continuous, 37 integer (37 binary)

Root relaxation: objective 1.230321e+01, 5180 iterations, 0.69 seconds

    Nodes    |    Current Node    |     Objective Bounds      |     Work
 Expl Unexpl |  Obj  Depth IntInf | Incumbent    BestBd   Gap | It/Node Time

     0     0   12.30321    0    2          -   12.30321      -     -    1s
H    0     0                       0.0915893   12.30321      -     -    1s
     0     0   12.30321    0    2    0.09159   12.30321      -     -    1s
     0     2   12.30321    0    2    0.09159   12.30321      -     -    1s
*  174    80              16       0.2864668   12.30321  4195%  53.5    2s
*  245    91              21       1.2019176   12.30321   924%  57.3    2s
*  258    86              22       1.2019176   12.29845   923%  61.1    2s
*  291    53              21       1.4425937   12.27798   751%  68.9    3s

Explored 373 nodes (33379 simplex iterations) in 3.60 seconds
Thread count was 8 (of 8 available processors)

Solution count 5: 1.44259 1.20192 1.20192 ... 0.0915893
Pool objective bound 1.44259

Optimal solution found (tolerance 1.00e-12)
Best objective 1.442593733917e+00, best bound 1.442593733917e+00, gap 0.0000%
```

```
optKnock found a optKnock set of large 2 composed by
PFL
 and RPE
The production of succinate after optimization is 1.44
The growth rate after optimization is 0.22
The production of other products such as ethanol, formate, lactate and acetate are0.0, 0.0, 15.2 and 0.0
...Performing coupling analysis...
The solution is of type: growth coupled non unique
The maximun growth rate given the optKnock set is 0.18
The maximun and minimun production of succinate given the optKnock set is 0.06 and 1.22, respectively
...Performing optKnock analysis...
```

```
MILP problem with 8400 constraints 63 integer variables and 8370 continuous variables
Optimize a model with 8400 rows, 8370 columns and 34677 nonzeros
Variable types: 8307 continuous, 63 integer (63 binary)
Coefficient statistics:
  Matrix range     [2e-06, 1e+03]
  Objective range  [1e+00, 1e+00]
  Bounds range     [1e+00, 1e+03]
  RHS range        [1e-02, 1e+03]
Presolve removed 5079 rows and 3321 columns
Presolve time: 0.09s
Presolved: 3321 rows, 5049 columns, 21621 nonzeros
Variable types: 5012 continuous, 37 integer (37 binary)

Root relaxation: objective 1.230321e+01, 4774 iterations, 0.63 seconds

    Nodes    |    Current Node    |     Objective Bounds      |     Work
 Expl Unexpl |  Obj  Depth IntInf | Incumbent    BestBd   Gap | It/Node Time

     0     0   12.30321    0    2          -   12.30321      -     -    1s
H    0     0                       0.0915893   12.30321      -     -    1s
     0     0   12.30321    0    2    0.09159   12.30321      -     -    1s
     0     2   12.30321    0    2    0.09159   12.30321      -     -    1s
*   82    63              25       0.2411614   12.30321  5002%  40.6    1s
*   94    71              17       0.2864668   12.30321  4195%  41.6    1s
*  291    75              21       1.2019176   12.29845   923%  55.8    3s

Explored 390 nodes (32387 simplex iterations) in 3.94 seconds
Thread count was 8 (of 8 available processors)

Solution count 4: 1.20192 0.286467 0.241161 0.0915893
Pool objective bound 1.20192

Optimal solution found (tolerance 1.00e-12)
Warning: max constraint violation (3.1562e-09) exceeds tolerance
Best objective 1.201917633447e+00, best bound 1.201917633447e+00, gap 0.0000%
```

```
optKnock found a optKnock set of large 2 composed by
PFL
 and TALA
The production of succinate after optimization is 1.20
The growth rate after optimization is 0.22
The production of other products such as ethanol, formate, lactate and acetate are15.4, 0.0, -0.0 and 0.
...Performing coupling analysis...
The solution is of type: growth coupled non unique
The maximun growth rate given the optKnock set is 0.19
The maximun and minimun production of succinate given the optKnock set is 0.06 and 1.01, respectively
```

TROUBLESHOOTING 1: "The algorithm takes a long time to find a solution"

TROUBLESHOOTING 2: "The algorithm finds a set of knockouts too big"

TROUBLESHOOTING 3: "The algorithm found a solution that is not useful for me"

EXAMPLE 2: finding optKnock reactions sets of size 3 for increasing production of succinate

```
fprintf('\n...EXAMPLE 1: Finding optKnock sets of size 3...\n\n')
```

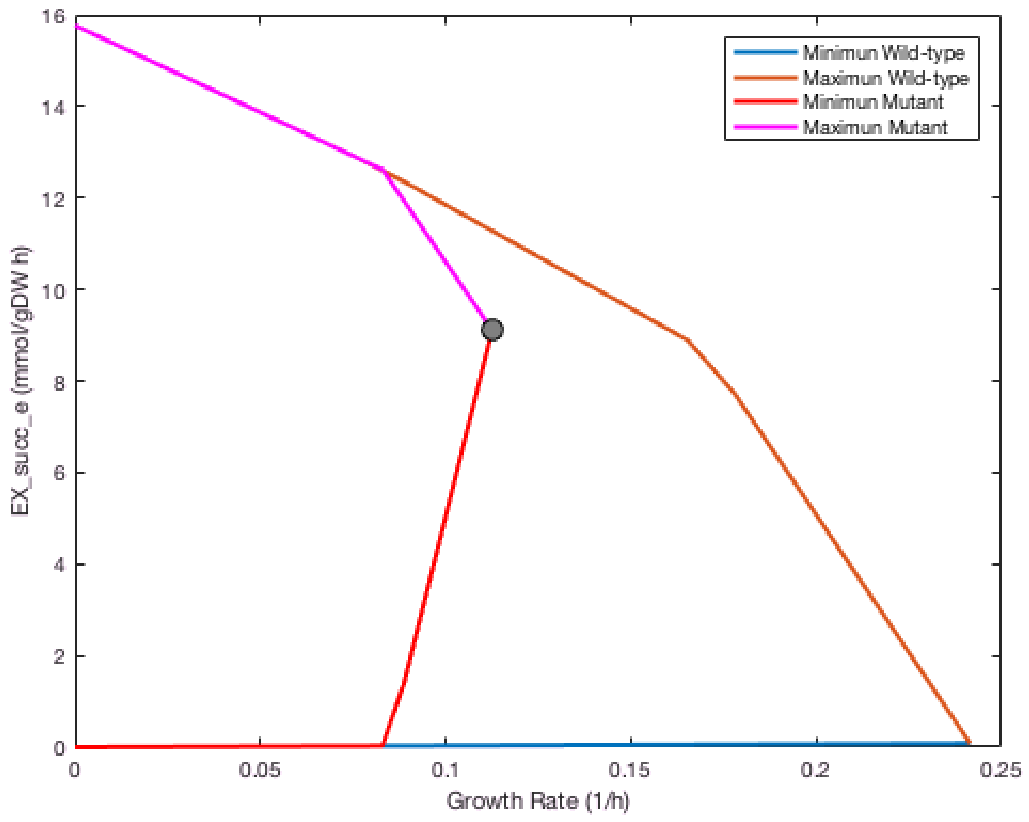
```
...EXAMPLE 1: Finding optKnock sets of size 3...
```

```
% Set optKnock options
% The exchange of succinate will be the objective of the outer problem
options = struct('targetRxn', 'EX_succ_e', 'numDel', 3);
% We will impose that biomass be at least 50% of the biomass of wild-type
constrOpt = struct('rxnList', {{biomass}}, 'values', 0.5*fbaWT.f, 'sense', 'G');
% We will try to find 10 optKnock sets of a maximun length of 3
nIter = 1;
while nIter < threshold
    fprintf('...Performing optKnock analysis...')
    if isempty(previousSolutions{1})
        optKnockSol = OptKnock(model, selectedRxnList, options, constrOpt);
    else
        optKnockSol = OptKnock(model, selectedRxnList, options, constrOpt, previousSolutions);
    end

    % determine succinate production and growth rate after optimizacion
    succFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_succ_e'));
    growthRateM1 = optKnockSol.fluxes(strcmp(model.rxns, biomass));
    etohFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_etoh_e'));
    formFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_for_e'));
    lactFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_lac__D_e'));
    acetFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_ac_e'));
    setM1 = optKnockSol.rxnList;

    if ~isempty(setM1)
        previousSolutions{contPreviousSolutions} = setM1;
        contPreviousSolutions=contPreviousSolutions + 1;
        %printing results
        fprintf('optKnock found a optKnock set of large %d composed by ',length(setM1));
        for j = 1:length(setM1)
            if j == 1
                fprintf('%s',setM1{j});
            elseif j == length(setM1)
                fprintf(' and %s',setM1{j});
            else
                fprintf(', %s',setM1{j});
            end
        end
        fprintf('\n');
        fprintf('The production of succinate after optimization is %.2f \n', succFluxM1);
        fprintf('The growth rate after optimization is %.2f \n', growthRateM1);
        fprintf(['The production of other products such as ethanol, formate, lactate and aceta
                 '%.1f, %.1f, %.1f and %.1f, respectively. \n'], etohFluxM1, formFluxM1, lactF
        fprintf('...Performing coupling analysis...\n');
        [type, maxGrowth, maxProd, minProd] = analyzeOptKnock(model, setM1, 'EX_succ_e');
        fprintf('The solution is of type: %s\n', type);
        fprintf('The maximun growth rate given the optKnock set is %.2f\n', maxGrowth);
        fprintf(['The maximun and minimun production of succinate given the optKnock set is '
                 '%.2f and %.2f, respectively \n\n'], minProd, maxProd);
        if strcmp(type,'growth coupled')
```

```
            singleProductionEnvelope(model, setM1, 'EX_succ_e', biomass, 'savePlot', 1, 'showP
                                         'fileName', ['succ_ex2_' num2str(nIter)], 'outputFolder',
        end
    else
        if nIter == 1
            fprintf('optKnock was not able to found an optKnock set\n');
        else
            fprintf('optKnock was not able to found additional optKnock sets\n');
        end
        break;
    end
    nIter = nIter + 1;
end
```

```
...Performing optKnock analysis...
optKnock found a optKnock set of large 3 composed by
ALCD2x
, LDH_D
 and PFL

The production of succinate after optimization is 8.81
The growth rate after optimization is 0.15
The production of other products such as ethanol, formate, lactate and acetate are 0.0, 0.0, -0.0 and 1.
...Performing coupling analysis...
The solution is of type: growth coupled
The maximun growth rate given the optKnock set is 0.11
The maximun and minimun production of succinate given the optKnock set is 9.11 and 9.11, respectively
...Performing optKnock analysis...
optKnock found a optKnock set of large 3 composed by
ACALD
, LDH_D
 and PFL

The production of succinate after optimization is 8.81
The growth rate after optimization is 0.15
The production of other products such as ethanol, formate, lactate and acetate are 0.0, 0.0, -0.0 and 4.
...Performing coupling analysis...
The solution is of type: growth coupled non unique
The maximun growth rate given the optKnock set is 0.11
The maximun and minimun production of succinate given the optKnock set is 4.23 and 9.11, respectively
...Performing optKnock analysis...
optKnock found a optKnock set of large 2 composed by
PFL
 and TKT1

The production of succinate after optimization is 1.20
The growth rate after optimization is 0.22
The production of other products such as ethanol, formate, lactate and acetate are 0.0, 0.0, 15.4 and 0.
...Performing coupling analysis...
The solution is of type: growth coupled non unique
The maximun growth rate given the optKnock set is 0.19
The maximun and minimun production of succinate given the optKnock set is 0.06 and 1.01, respectively
...Performing optKnock analysis...
optKnock found a optKnock set of large 3 composed by
ACALD
, ALCD2x
 and PFL
```

```
The production of succinate after optimization is 0.95
The growth rate after optimization is 0.22
The production of other products such as ethanol, formate, lactate and acetate are 0.0, 0.0, 15.7 and -0
...Performing coupling analysis...
The solution is of type: growth coupled non unique
The maximun growth rate given the optKnock set is 0.19
The maximun and minimun production of succinate given the optKnock set is 0.06 and 0.80, respectively
```



II) LACTATE OVERPRODUCTION

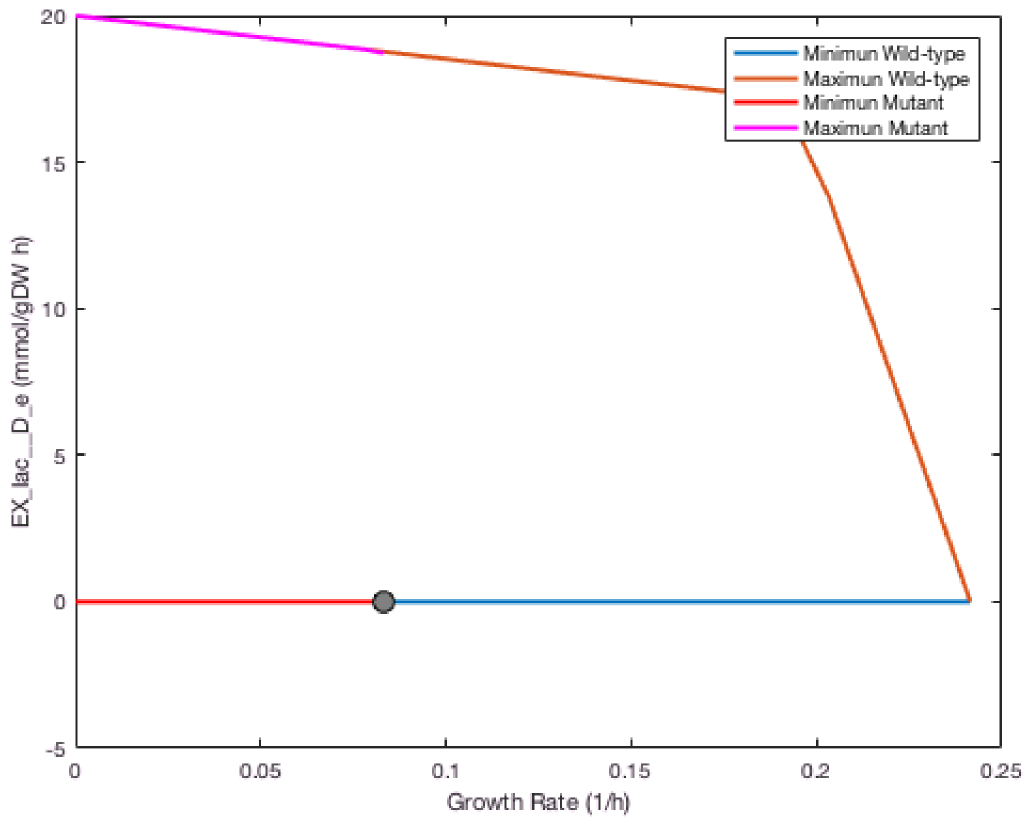
EXAMPLE 1: finding optKnock reactions sets of size 3 for increasing production of lactate

```
fprintf('\n...EXAMPLE 1: Finding optKnock sets of size 3...\n\n')
```

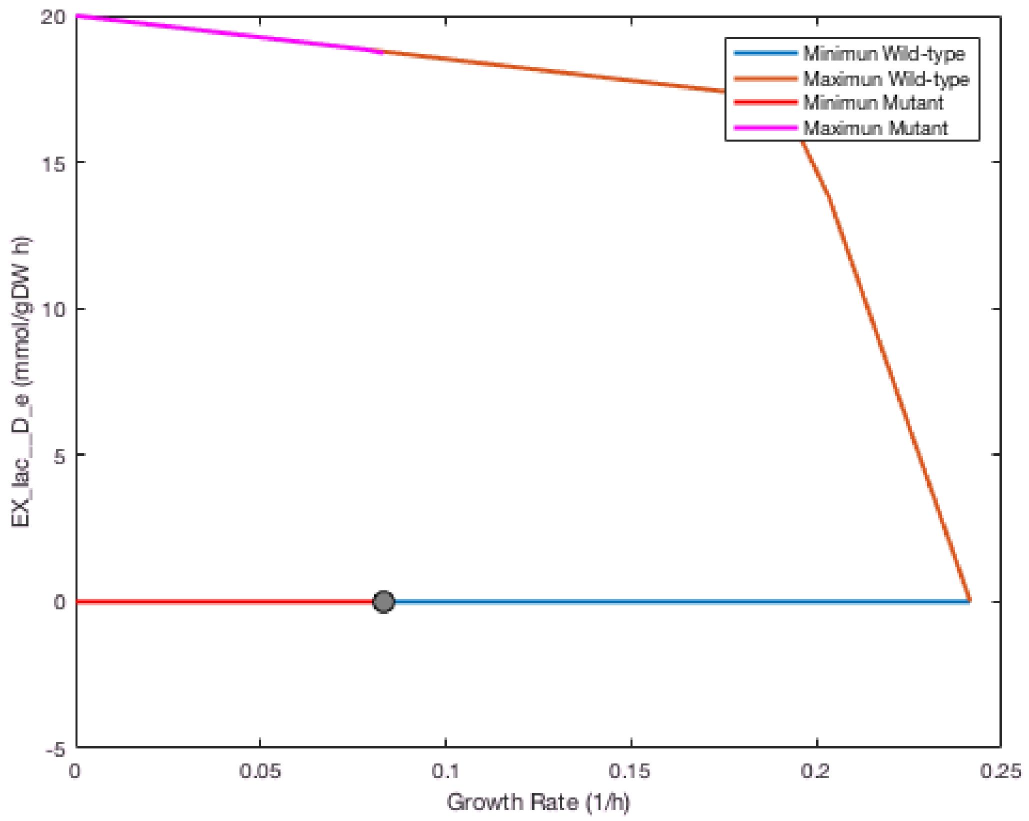
```
...EXAMPLE 1: Finding optKnock sets of size 3...
```

```
% Set optKnock options
% The exchange of lactate will be the objective of the outer problem
options = struct('targetRxn', 'EX_lac__D_e', 'numDel', 3);
% We will impose that biomass be at least 50% of the biomass of wild-type
constrOpt = struct('rxnList', {{biomass}}, 'values', 0.5*fbaWT.f, 'sense', 'G');
% We will try to find 10 optKnock sets of a maximun length of 6
previousSolutions = cell(100, 1);
contPreviousSolutions = 1;
nIter = 1;
while nIter < threshold
    fprintf('...Performing optKnock analysis...')
    if isempty(previousSolutions{1})
        optKnockSol = OptKnock(model, selectedRxnList, options, constrOpt);
    else
        optKnockSol = OptKnock(model, selectedRxnList, options, constrOpt, previousSolutions);
    end

    % determine lactate production and growth rate after optimizacion
    lactFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_lac__D_e'));
    growthRateM1 = optKnockSol.fluxes(strcmp(model.rxns, biomass));
    etohFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_etoh_e'));
    formFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_for_e'));
    succFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_succ_e'));
    acetFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_ac_e'));
    setM1 = optKnockSol.rxnList;

    if ~isempty(setM1)
        previousSolutions{contPreviousSolutions} = setM1;
        contPreviousSolutions=contPreviousSolutions + 1;
        %printing results
        fprintf('optKnock found a optKnock set of large %d composed by ',length(setM1));
        for j = 1:length(setM1)
            if j == 1
                fprintf('%s', setM1{j});
            elseif j == length(setM1)
                fprintf(' and %s', setM1{j});
            else
                fprintf(', %s', setM1{j});
            end
        end
        fprintf('\n');
        fprintf('The production of lactate after optimization is %.2f \n', lactFluxM1);
        fprintf('The growth rate after optimization is %.2f \n', growthRateM1);
        fprintf(['The production of other products such as ethanol, formate, succinate and ace
                 '%.1f, %.1f, %.1f and %.1f, respectively. \n'], etohFluxM1, formFluxM1, succF
        fprintf('...Performing coupling analysis...\n');
        [type, maxGrowth, maxProd, minProd] = analyzeOptKnock(model, setM1, 'EX_lac__D_e');
        fprintf('The solution is of type: %s\n', type);
        fprintf('The maximun growth rate given the optKnock set is %.2f\n', maxGrowth);
        fprintf(['The maximun and minimun production of lactate given the optKnock set is ' ..
                 '%.2f and %.2f, respectively \n\n'], minProd, maxProd);
        singleProductionEnvelope(model, setM1, 'EX_lac__D_e', biomass, 'savePlot', 1, 'showPlo
                                 'fileName', ['lact_ex1_' num2str(nIter)], 'outputFolder', 'Op
    else
        if nIter == 1
            fprintf('optKnock was not able to found an optKnock set\n');
        else
            fprintf('optKnock was not able to found additional optKnock sets\n');
        end
        break;
    end
    nIter = nIter + 1;
end
```

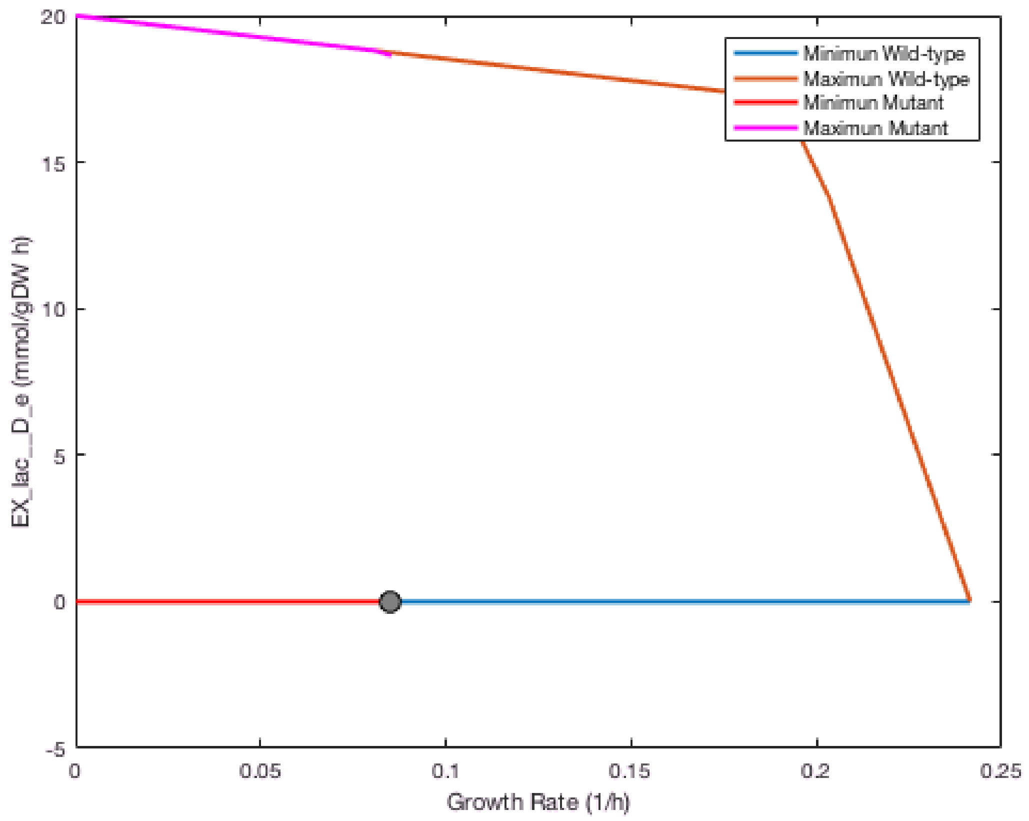
```
...Performing optKnock analysis...
optKnock found a optKnock set of large 3 composed by
GLCabcpp
, PFL
 and PGI
The production of lactate after optimization is 18.13
The growth rate after optimization is 0.12
The production of other products such as ethanol, formate, succinate and acetate are 0.0, 0.0, 0.0 and 0
...Performing coupling analysis...
The solution is of type: non unique
The maximun growth rate given the optKnock set is 0.08
The maximun and minimun production of lactate given the optKnock set is 0.00 and 18.72, respectively
...Performing optKnock analysis...
optKnock found a optKnock set of large 2 composed by
PFL
 and PGI

The production of lactate after optimization is 18.13
The growth rate after optimization is 0.12
The production of other products such as ethanol, formate, succinate and acetate are 0.0, 0.0, 0.0 and 0
...Performing coupling analysis...
The solution is of type: non unique
The maximun growth rate given the optKnock set is 0.08
The maximun and minimun production of lactate given the optKnock set is 0.00 and 18.72, respectively
...Performing optKnock analysis...
optKnock found a optKnock set of large 3 composed by
GND
, PGI
 and PTAr

The production of lactate after optimization is 18.01
The growth rate after optimization is 0.12
The production of other products such as ethanol, formate, succinate and acetate are 0.0, 0.6, 0.0 and 0
...Performing coupling analysis...
The solution is of type: non unique
The maximun growth rate given the optKnock set is 0.09
The maximun and minimun production of lactate given the optKnock set is 0.00 and 18.64, respectively
...Performing optKnock analysis...
optKnock found a optKnock set of large 3 composed by
ACKr
, GND
 and PGI

The production of lactate after optimization is 18.01
The growth rate after optimization is 0.12
The production of other products such as ethanol, formate, succinate and acetate are 0.0, 0.6, 0.0 and 0
...Performing coupling analysis...
The solution is of type: non unique
The maximun growth rate given the optKnock set is 0.09
The maximun and minimun production of lactate given the optKnock set is 0.00 and 18.64, respectively
```



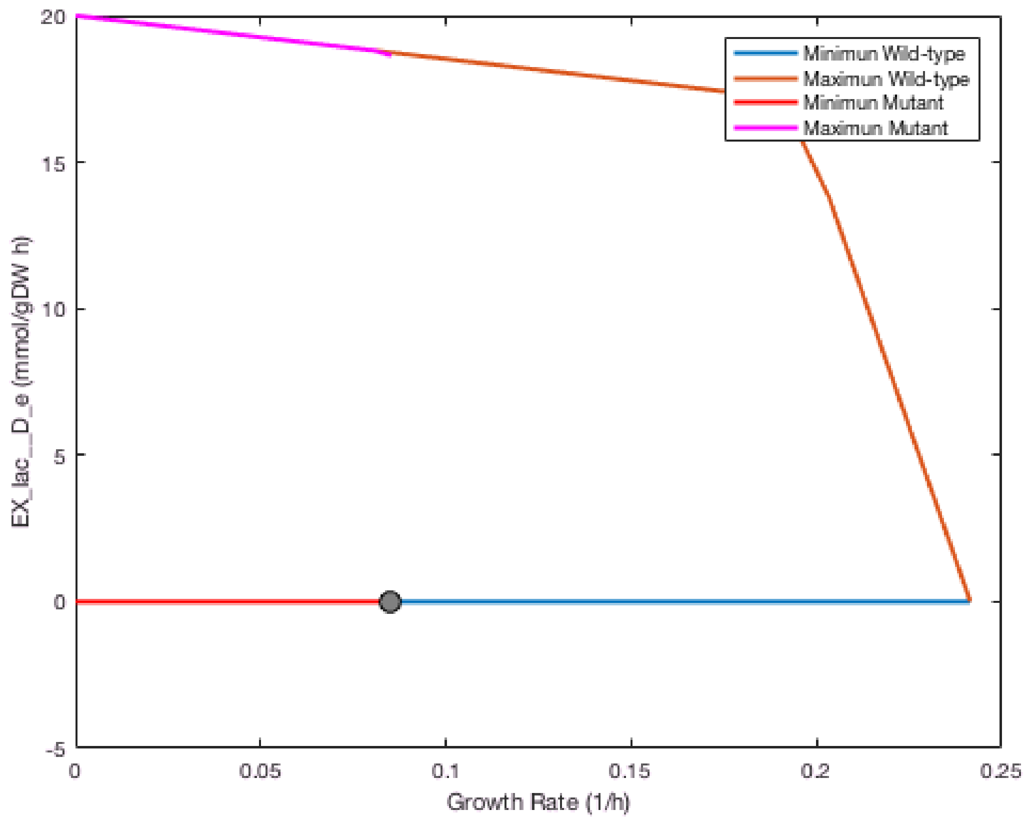
Minimun Wild-type
Maximun Wild-type
Minimun Mutant
Maximun Mutant
EX_lac__D_e (mmol/gDW h)
Growth Rate (1/h)
20
15
10
5
0
-5
0
0.05
0.1
0.15
0.2
0.25



Minimun Wild-type
Maximun Wild-type
Minimun Mutant
Maximun Mutant
EX_lac__D_e (mmol/gDW h)
Growth Rate (1/h)
20
15
10
5
0
-5
0
0.05
0.1
0.15
0.2
0.25



Minimun Wild-type
Maximun Wild-type
Minimun Mutant
Maximun Mutant
EX_lac__D_e (mmol/gDW h)
Growth Rate (1/h)



Minimun Wild-type
Maximun Wild-type
Minimun Mutant
Maximun Mutant
EX_lac__D_e (mmol/gDW h)
Growth Rate (1/h)

EXAMPLE 2: finding optKnock reactions sets of size 6 for increasing production of lactate

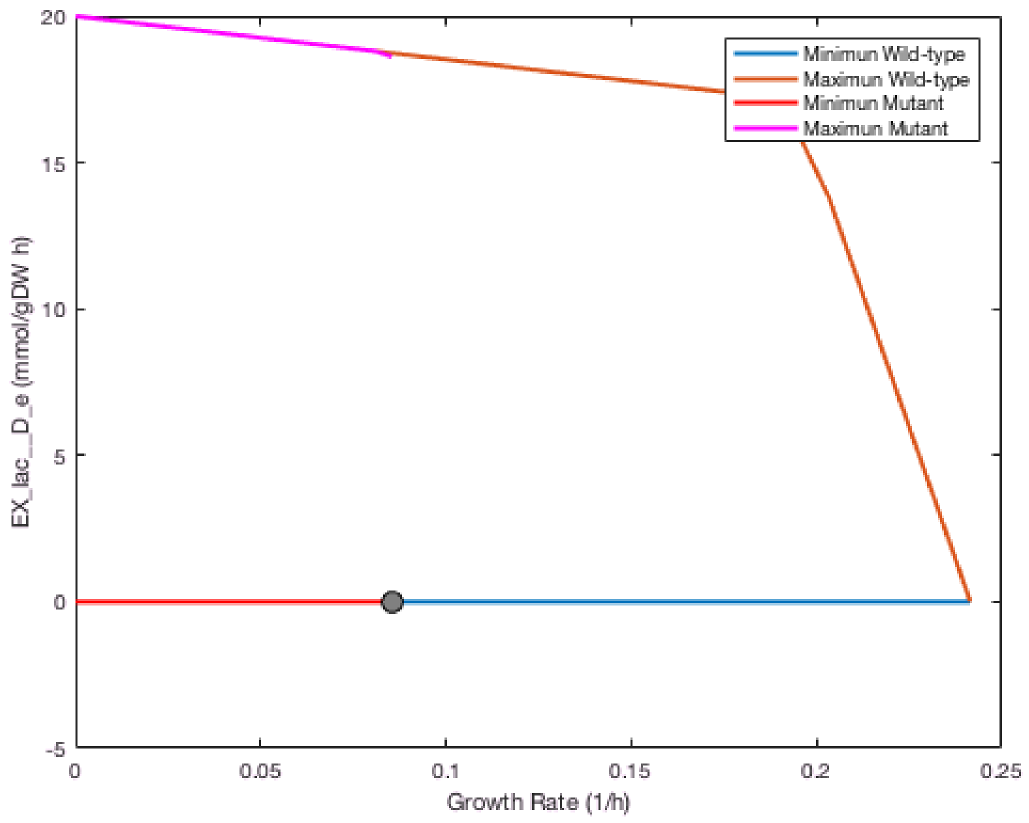
```
fprintf('...EXAMPLE 3: Finding optKnock sets of size 6...\n')
```

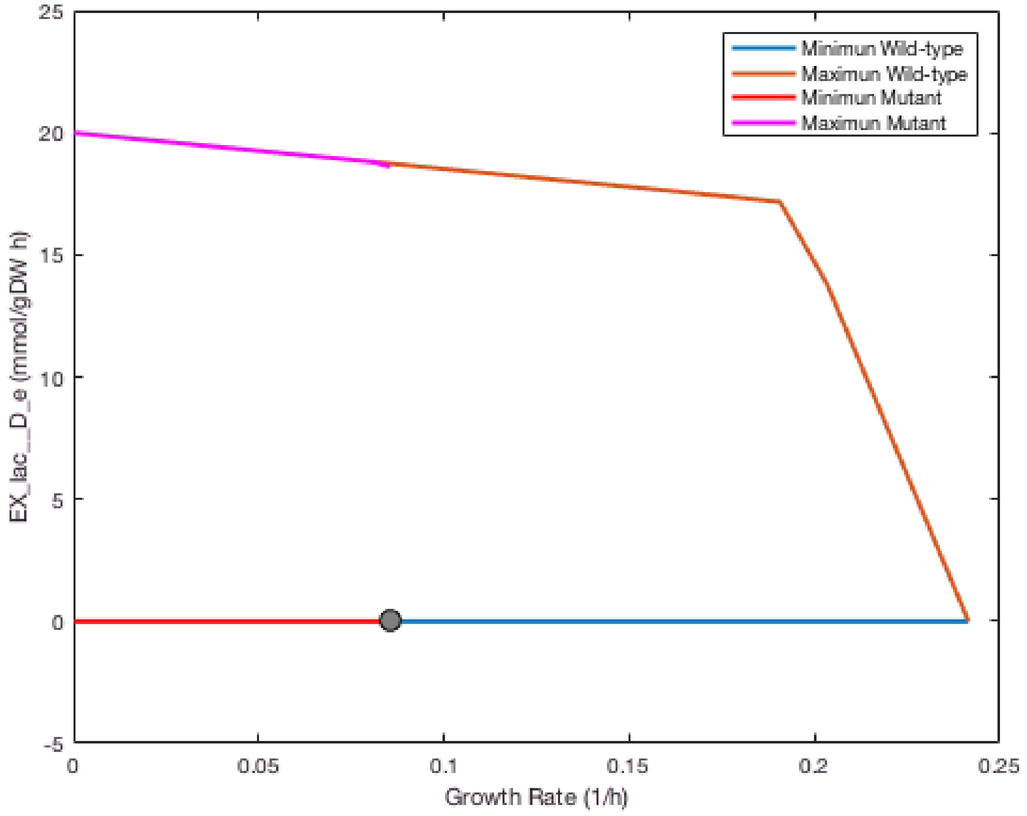
```
...EXAMPLE 3: Finding optKnock sets of size 6...
```

```
% Set optKnock options
% The exchange of lactate will be the objective of the outer problem
options = struct('targetRxn', 'EX_lac__D_e', 'numDel', 6);
% We will impose that biomass be at least 50% of the biomass of wild-type
constrOpt = struct('rxnList', {{biomass}}, 'values', 0.5*fbaWT.f, 'sense', 'G');
% We will try to find 10 optKnock sets of a maximun length of 2
nIter = 1;
while nIter < threshold
    fprintf('...Performing optKnock analysis...')
    if isempty(previousSolutions{1})
        optKnockSol = OptKnock(model, selectedRxnList, options, constrOpt);
    else
        optKnockSol = OptKnock(model, selectedRxnList, options, constrOpt, previousSolutions);
    end

    % determine lactate production and growth rate after optimizacion
    lactFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_lac__D_e'));
    growthRateM1 = optKnockSol.fluxes(strcmp(model.rxns,biomass));
    etohFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_etoh_e'));
    formFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_for_e'));
    succFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_succ_e'));
    acetFluxM1 = optKnockSol.fluxes(strcmp(model.rxns, 'EX_ac_e'));
    setM1 = optKnockSol.rxnList;

    if ~isempty(setM1)
        previousSolutions{contPreviousSolutions} = setM1;
        contPreviousSolutions = contPreviousSolutions + 1;
        %printing results
        fprintf('optKnock found a optKnock set of large %d composed by ', length(setM1));
        for j = 1:length(setM1)
            if j == 1
                fprintf('%s', setM1{j});
            elseif j == length(setM1)
                fprintf(' and %s', setM1{j});
            else
                fprintf(', %s', setM1{j});
            end
        end
        fprintf('\n');
        fprintf('The production of lactate after optimization is %.2f \n', lactFluxM1);
        fprintf('The growth rate after optimization is %.2f \n', growthRateM1);
        fprintf(['The production of other products such as ethanol, formate, succinate and ace
                 '%.1f, %.1f, %.1f and %.1f, respectively. \n'], etohFluxM1, formFluxM1, succF
        fprintf('...Performing coupling analysis...\n');
        [type, maxGrowth, maxProd, minProd] = analyzeOptKnock(model, setM1, 'EX_lac__D_e');
        fprintf('The solution is of type: %s\n', type);
        fprintf('The maximun growth rate given the optKnock set is %.2f\n', maxGrowth);
        fprintf(['The maximun and minimun production of lactate given the optKnock set is ' ..
                 '%.2f and %.2f, respectively \n\n'], minProd, maxProd);
        singleProductionEnvelope(model, setM1, 'EX_lac__D_e', biomass, 'savePlot', 1, 'showPlo
                                 'fileName', ['lact_ex2_' num2str(nIter)], 'outputFolder', 'Op
    else
        if nIter == 1
            fprintf('optKnock was not able to found an optKnock set\n');
        else
            fprintf('optKnock was not able to found additional optKnock sets\n');
        end
        break;
    end
    nIter = nIter + 1;
end
```

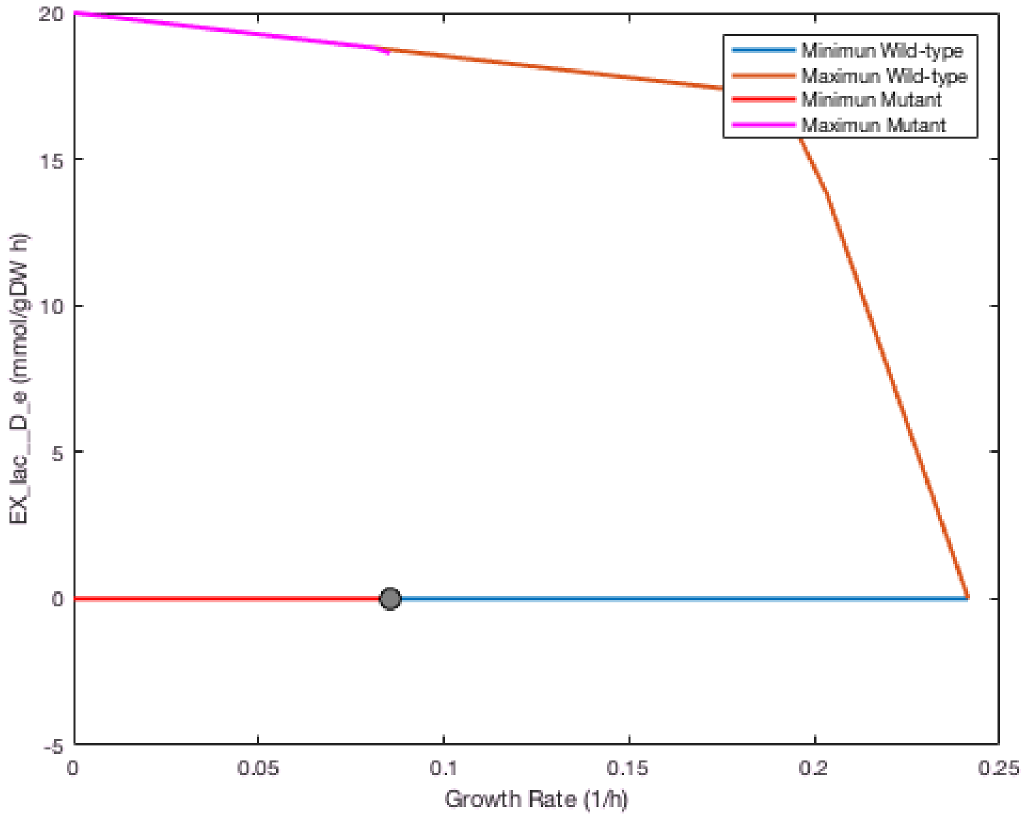
```
...Performing optKnock analysis...
optKnock found a optKnock set of large 4 composed by
HEX1
, PGI
, PTAr
 and TKT1
The production of lactate after optimization is 17.99
The growth rate after optimization is 0.12
The production of other products such as ethanol, formate, succinate and acetate are 0.0, 0.7, 0.0 and 0
...Performing coupling analysis...
The solution is of type: non unique
The maximun growth rate given the optKnock set is 0.09
The maximun and minimun production of lactate given the optKnock set is 0.00 and 18.62, respectively
...Performing optKnock analysis...
optKnock found a optKnock set of large 5 composed by
GLCabcpp
, PGI
, PTAr
, PYK
 and TKT1
The production of lactate after optimization is 17.99
The growth rate after optimization is 0.12
The production of other products such as ethanol, formate, succinate and acetate are 0.0, 0.7, 0.0 and 0
...Performing coupling analysis...
The solution is of type: non unique
The maximun growth rate given the optKnock set is 0.09
The maximun and minimun production of lactate given the optKnock set is 0.00 and 18.62, respectively
...Performing optKnock analysis...
optKnock found a optKnock set of large 4 composed by
GLCabcpp
, PGI
, PTAr
 and TKT1
The production of lactate after optimization is 17.99
The growth rate after optimization is 0.12
The production of other products such as ethanol, formate, succinate and acetate are 0.0, 0.7, 0.0 and 0
...Performing coupling analysis...
The solution is of type: non unique
The maximun growth rate given the optKnock set is 0.09
The maximun and minimun production of lactate given the optKnock set is 0.00 and 18.62, respectively
...Performing optKnock analysis...
```

```
optKnock found a optKnock set of large 6 composed by
ACKr
, ALCD2x
, GLCabcpp
, PGI
, PYK
 and TKT1
The production of lactate after optimization is 17.99
The growth rate after optimization is 0.12
The production of other products such as ethanol, formate, succinate and acetate are 0.0, 0.7, 0.0 and 0
...Performing coupling analysis...
The solution is of type: growth coupled non unique
The maximun growth rate given the optKnock set is 0.09
The maximun and minimun production of lactate given the optKnock set is 10.38 and 18.62, respectively
```

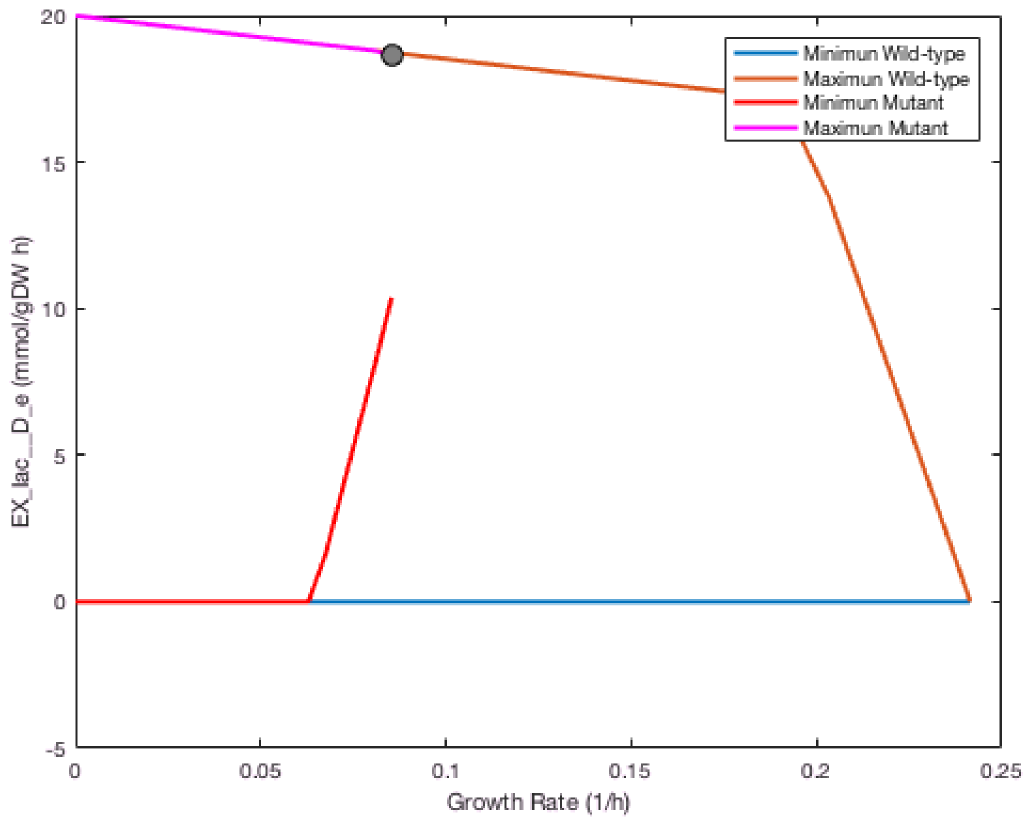




Minimun Wild-type
Maximun Wild-type
Minimun Mutant
Maximun Mutant
EX_lac__D_e (mmol/gDW h)
Growth Rate (1/h)



Minimun Wild-type
Maximun Wild-type
Minimun Mutant
Maximun Mutant
EX_lac__D_e (mmol/gDW h)
Growth Rate (1/h)



```
cd(currectDirectory);
```

TIMING

1. Example 1 ~ 1-2 minutes
2. Example 2 ~ 1-2 minutes
3. Example 3 ~ 1-2 minutes
4. Example 4 ~ 1-2 minutes
5. Example 5 ~ 1-2 minutes
6. Example 6 ~ 1-2 minutes

TROUBLESHOOTING

1) If the algorithm takes a long time to find a solution, it is possible that the seach space is too large. You can reduce the search space using a smaller set of reactions in the input variable "selectedRxnList."

2) The default number of deletions used by optKnock is 5. If the algorithm is returning more deletions than what you want, you can change the number of deletions using the input variable "numDel."

3) optKnock could find a solution that it is not useful for you. For example, you may think that a solution is very obvious or that it breaks some important biological contraints. If optKnock found a solution that you don't want to find, use the input variable "prevSolutions" to prevent that solution to be found.

ANTICIPATED RESULTS

The optKnock algorithm will find sets of reactions that, when removed from the model, will improve the production of succinate and lactate respectively. In this tutorial, once optKnock finds a solution, then the type of solution is determined (if the product is coupled with biomass formation or not). Some of the sets will generate a coupled solution, i.e., the production rate will increase as biomass formation increases. For these kind of reactions a plot will be generated using the function singleProductionEnvelope and will be saved in the folder tutoriales/optKnock/optKnockResults

When you find a solution with OptKnock, you should always verify the minumum and maximum production rate using the function analizeOptKnock.

References

[1] Burgard, A. P., Pharkya, P. & Maranas, C. D. (2003). OptKnock: A Bilevel Programming Framework for Identifying Gene Knockout Strategies for Microbial Strain Optimization. Biotechnology and Bioengineering, 84(6), 647–657. http://doi.org/10.1002/bit.10803.

[2] Orth, J. D., Conrad, T. M., Na, J., Lerman, J. A., Nam, H., Feist, A. M., & Palsson, B. Ø. (2011). A comprehensive genome - scale reconstruction of Escherichia coli metabolism—2011. *Molecular systems biology*, *7*(1), 535.