

Solution to Exercise 8

Task 1

First, we read the *E. coli* core model into the Matlab workspace:

```
clear
model = readCbModel('e_coli_core.mat');
```

Task 2

Next, we want to determine a wild-type flux distribution w . To this end, we solve a FBA problem using the COBRA toolbox function `optimizeCbModel`.

```
% first, we can check the objective:
fprintf('Current objective: %s\n', model.rxnNames{model.c==1})
```

Current objective: Biomass Objective Function with GAM

```
% FBA
wt_sol = optimizeCbModel(model);
w = wt_sol.v;
```

Task 3

Now, we come to the part, where we want to determine a flux for the mutant. Therefore, we first determine the significance thresholds for changes in flux for each reaction:

$$w_i^u = w_i + \delta |w_i| + \varepsilon \quad // \text{"significantly" higher}$$

$$w_i^l = w_i - \delta |w_i| - \varepsilon \quad // \text{"significantly" lower,}$$

where $\delta = 0.05$ and $\varepsilon = 0.001$.

```
delta = 0.05;
epsilon = 0.001;
% calculate thresholds
t_upper = w + delta * abs(w) + epsilon;
t_lower = w - delta * abs(w) - epsilon;
```

We need ε to account for possible numerical issues. For example, this means that even if the mutant flux exceeds the $w_i + \delta |w_i|$ by a small values due to some numerical instability, it will not be recognized as significantly changed because we accounted for this error by adding a small value ε to the threshold.

Task 4

Now, we start constructing the mixed-integer linear problem (MILP) by forming the inequality constraints

$$v_i - y_i(v_{\max,i} - w_i^u) \leq w_i^u, \text{ and}$$

$$v_i - y_i(v_{\min,i} - w_i^l) \geq w_i^l.$$

Here, we introduce binary variables y_i associated with each of the reactions. If $y_i = 1$, it means that reaction i is significantly changed either above or below the previously defined thresholds w_i^u and w_i^l .

We further have to transform the second inequality constraint into a lower-than-or-equal constraint by multiplying by -1:

$$-v_i + y_i(v_{\min,i} - w_i^l) \leq -w_i^l.$$

The result inequality constraint matrix will thus have the dimensions $2r \times 2r$, where r is the number of reactions in the model. The matrix and associated right hand side of the of constraints will look like this:

$$\begin{bmatrix} 1 & 0 & \cdots & 0 & -v_{\max,1} + w_1^u & 0 & \cdots & 0 \\ 0 & 1 & & \vdots & 0 & -v_{\max,2} + w_2^u & & \vdots \\ \vdots & & \ddots & 0 & \vdots & & \ddots & 0 \\ 0 & \cdots & 0 & 1 & 0 & \cdots & 0 & -v_{\max,r} + w_r^u \\ -1 & 0 & \cdots & 0 & v_{\min,1} - w_1^l & 0 & \cdots & 0 \\ 0 & -1 & & \vdots & 0 & v_{\min,2} - w_2^l & & \vdots \\ \vdots & & \ddots & 0 & \vdots & & \ddots & 0 \\ 0 & \cdots & 0 & -1 & 0 & & 0 & v_{\min,r} - w_r^l \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_r \\ y_1 \\ y_2 \\ \vdots \\ y_r \end{bmatrix} \leq \begin{bmatrix} w_1^u \\ w_2^u \\ \vdots \\ w_r^u \\ -w_1^l \\ -w_2^l \\ \vdots \\ -w_r^l \end{bmatrix}$$

It follows the implementation:

```
r = size(model.S,2);
% left-hand side
A = [
%      v      y
      +eye(r) diag(t_upper - model.ub)
      -eye(r) diag(model.lb - t_lower)
];
% right-hand side
b = [t_upper; -t_lower];
```

Task 5

We proceed with the remaining parts of the MILP and the the knock-out part.

To knock-out reaction NAD transhydrogenase we knockout genes b3962 and b1602 or b1603.

```
% knock-out genes of reaction 'NAD transhydrogenase'
% rule: 'b3962 or (b1602 and b1603)'
idx_b3962 = find(strcmp(model.genes, 'b3962'));
idx_b1602 = find(strcmp(model.genes, 'b1602'));
idx_b1603 = find(strcmp(model.genes, 'b1603'));
```

We also check which other reaction(s) may be affected by the knock-out.

```
rxns_related_to_b3962 = find(model.rxnGeneMat(:,idx_b3962)~=0);
```

```

rxns_related_to_b1602 = find(model.rxnGeneMat(:,idx_b1602)~=0);
rxns_related_to_b1603 = find(model.rxnGeneMat(:,idx_b1603)~=0);

model.grRules(unique([rxns_related_to_b3962; rxns_related_to_b1602; rxns_related_to_b1603]))

ans = 2x1 cell
'b1602 and b1603'
'b3962 or (b1602 and b1603)'

```

We find that these genes relate to one other reaction, checking the rules we find that this reaction will be blocked as well.

```

% using the COBRA function
ko_gene = {'b3962' 'b1602'};

ko_rxns = struct2cell(findRxnsFromGenes(model,ko_gene));
ko_rxns = find(ismember(model.rxns,cellfun(@(x) x(1),ko_rxns)));

% Equality constraints(all-zero for y-part)
Aeq = [
%      v              y
      model.S zeros(size(model.S));
];
beq = model.b;

% lower and upper bounds
%      v              y
lb = [model.lb; zeros(r,1)];
ub = [model.ub; ones(r,1)];

% block reactions that are no longer active in the mutant
lb(ko_rxns) = 0;
ub(ko_rxns) = 0;

% objective
%      v              y
f = [zeros(r,1), ones(r,1)];

% finally, we have to specify that the y variables are binary and not
% continuous (indices are "number of reactions until 2 times number of
% reactions)
intcon = r+1:2*r;

% solve the MILP to obtain the mutant flux distribution
options = optimoptions('intlinprog');
options.Display = 'off';
v = intlinprog(f,intcon,A,b,Aeq,beq,lb,ub,[],options);

% find the reactions that are significantly changed
changed_rxns = model.rxnNames(logical(v(intcon)));
disp('The following reaction changed significantly with KO of');...
disp(ko_gene);

```

The following reaction changed significantly with KO of

```
{'b3962'} {'b1602'}
```

```
arrayfun(@(i)fprintf('%d\t\t%s\n',i,changed_rxns{i}),1:numel(changed_rxns))
```

```
#1 Glucose-6-phosphate isomerase
#2 Acetaldehyde dehydrogenase (acetylating)
#3 Alcohol dehydrogenase (ethanol)
#4 ATP maintenance requirement
#5 Pyruvate transport in via proton symport
#6 D lactate transport via proton symport
#7 NAD(P) transhydrogenase
#8 Formate exchange
#9 Isocitrate lyase
#10 D-lactate dehydrogenase
#11 Malic enzyme (NADP)
#12 NAD transhydrogenase
```

Task 6

Finally, we calculate the Euclidean distance between the wild-type and the mutant flux vectors.

```
dist = sqrt(sum((w-v(1:r)).^2));
fprintf('The Euclidean distance between wild-type and mutant flux vectors is %.2f\n',dist)
```

```
The Euclidean distance between wild-type and mutant flux vectors is 9.70
```