SILICOLIFE
Computational Biology Solutions for the Life Sciences

Metabolic Engineering with OptFlux

Exercise Workbook

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Exercise 1

Consider the simplified *E. coli* core model (Orth et al, 2009).

1. Find and load the model directly from the OptFlux model repository *(File → Model → New Project)*
2. Export the model to SBML *(File → Export → Model to SBML)*
3. Analyse the SBML file. What kind of information is stored in the SBML file for each reaction and for each metabolite?
4. Explore model properties inside OptFlux: Number of reactions, metabolites, genes, stoichiometric matrix and steady-state equations *(Clipboard → Metabolic Model)*
5. Analyse gene-protein-reaction rules (GPRs) *(Clipboard → Metabolic Model)*
6. Analyze the degree of underdetermination of this model. How many measurements would be necessary in order to have a determined system? (Simulation → Flux Analysis → Flux Analysis)
7. Load the layout file (*E.coli_central_carbon.xgmml*) and explore the layout capabilities.
8. Find a genome-scale metabolic model (GSMM) for *E. coli K12* in the OptFlux model repository (iJR904, iAF1260 or iJO1366) and repeat steps 4, 5 and 6. Discuss you findings.

Exercise 2

Consider the simplified *E. coli* core model (Orth et al, 2009).

1. Perform a wild-type phenotype prediction - analyse results *(Simulation → Wild type)*
2. Define anaerobic medium conditions and repeat previous step *(File → Create → Environmental conditions)*
3. Analyse main differences in the layout visualizer (using the layout loaded in exercise 2) – create a comparison to help *(Analysis → Simulation comparison)*
4. Calculate the maximum theoretical flux yield and maximum theoretical carbon yield of Succinate having the following information in consideration: *(Analysis → Flux Variability Analysis → Determine Flux Limits)*

- Glucose → C6H12O6
- Succinate → C4H6O4
- $YIELD(V_i) = \frac{v_{target}}{v_{substrate}}$
- $CYIELD(V_i) = \frac{v_{target} \times C_{target}}{v_{substrate} \times C_{substrate}}$

5. Evaluate the maximum theoretical succinate production for the various levels of minimum desired biomass. .

Analysis → Flux Variability Analysis → Flux-variation plot

6. Perform mutant phenotype predictions (one deletion at a time - incremental)

Simulation → Knockout

- 1st - R_SUCDi – succinate dehydrogenase (b0721 & b0722 & b0723 & b0724)
- 2nd - R_G6PDH2r - glucose 6-phosphate dehydrogenase (b1852)
- 3rd - R_ACKr - acetate kinase (b3115 | b2296 | b1849)
- Analyse and interpret the results
- Perform this exercise using both reaction and gene deletions, checking the effects of the latter in the set of inactivated reactions.

Hint: Selecting a previous simulation in the clipboard will pre-fill future simulation operations

7. Evaluate the results of the previous mutant, using different phenotype prediction methods.

8. Evaluate the robustness of the solution using Flux Variability Analysis (FVA)

Hint: Use the flux variation plot

9. Evaluate the mutant against one of the previously loaded GSMMs (iJR904, iAF1260 or iJO1366).

Exercise 3

Consider the *E. coli* core model (Orth et al, 2009).

1. Compute the set of critical genes and critical reactions.

2. Develop a metabolic engineering strategy for the production of succinate, taking the following premises into account:
   - The ME strategy can take either gene or reaction information into account
   - The larger the number of gene deletions, the more expensive the process will be to implement in the lab.
   - Biomass formation is to be expected.
   - You can use knockouts as well as over/under expressions.

3. Justify the selected strategy, verifying the validity of your selected modifications using appropriate databases.

Hint: Use Ecocyc

4. Create a comparison between your strategy and the wild-type flux distribution and analyze the differences in the layout visualizer.