Flux Balance Analysis Lab

Objective:
Create models that represents the lycopene-producing strains of *E. coli* described in the paper by Alper et al. (2005) *Nature Biotechnology*. Simulate the model to answer the following questions.

Questions:
What is the theoretical maximum yield of lycopene (mol lycopene/mol glucose)?

How much lycopene is produced by the wild type strain (plus lycopene pathway)?

How much lycopene is produced in mutant strains with gene knockouts (see the paper)?

How much lycopene is produced in mutant strains with genes (see the paper) overexpressed?

How much lycopene is produced in mutant strains with overexpression and knockouts (described in the paper)?

Suggested workflow:

1) Set up a model for the wild type *E. coli* strain used in the paper
    What is the genotype for the base strain (before any engineering)? Do you need to modify the E. coli iJO1366 metabolic model in any way to represent this strain?
2) Add the lycopene pathway to the model
    What genes were added? What reactions do those genes catalyze (Hint: see Alper et al 2005 Metabolic Engineering)? Do you need to add any new metabolites?
3) Simulate the model from Step 2
    What objective function should you choose to answer the questions above?
4) Add gene knockouts from the paper to the lycopene model
    Which gene knockouts can we simulate?
5) Simulate the model from Step 4
6) Add gene overexpression to the lycopene model from Step 2
7) Simulate the model from Step 7
8) Create a lycopene-producing model with overexpression + knockouts
9) Simulate model from Step 8

See slides for basic FBA script and useful commands.

Helpful references:

E. coli database:
https://ecocyc.org

Description of all COBRA functions:
https://cobrapy.readthedocs.io/en/latest/index.html

BiGG database entry for iJO1366:
http://bigg.ucsd.edu/models/iJO1366

FBA tutorial from Orth, Thiele, & Palsson:
http://www.nature.com/nbt/journal/v28/n3/extref/nbt.1614-S1.pdf