

INTRODUCTION TO CELLULAR SYSTEM MODELING  
FINAL PROJECT 2020 SUMMER  
Due date: 30.6.2023

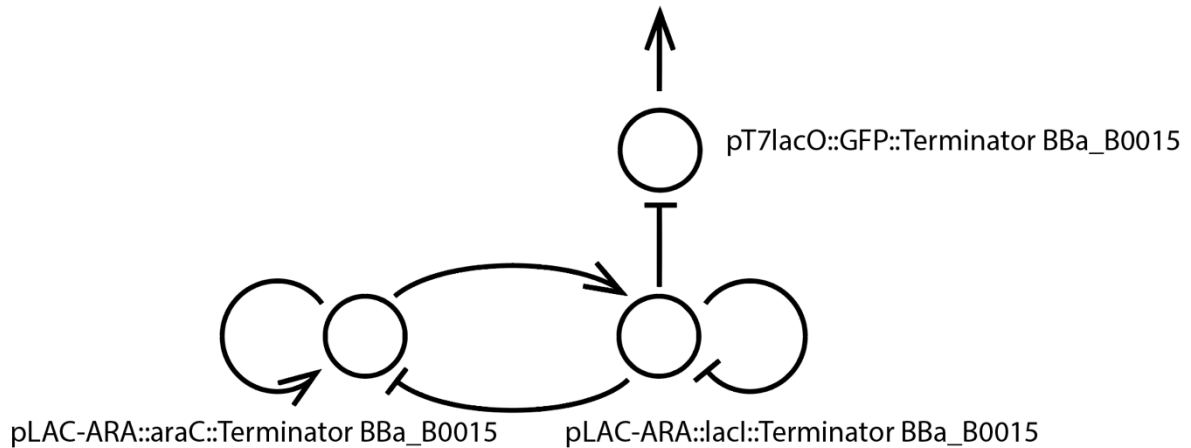
In this project you will complete the second and third steps of any bioengineering project, where step 1 is conceptual design, step 2 is DNA design, step 3 is mathematical validation, step 4 is strain modification, step 5 is experiment, and step 6 is analysis. In this project you will design the DNA constructs and model the behavior for the oscillator system published in Nature by Stricker et al. in 2008.

**PART I: DNA synthesis and assembly**

You are given the following library of DNA sequences:

- vectors
  - Vector-AmpR: bacterial expression vector with Ampicillin selection marker (benchling link: <https://benchling.com/s/seq-8664UI94qpdIG8z57ldv?m=slm-aozhGxHkIACJqz6H3uED>)
- promoters
  - pLAC-ARA: promoter repressed by lacI and activated by araC (benchling link: <https://benchling.com/s/seq-RWJeD9j4uv7yuSu5HLzr?m=slm-tSXvexYz0uuVixkFF0b7>)
  - pT7lacO: promoter repressed by lacI (benchling link: <https://benchling.com/s/seq-6mJoUaiUEpNNr28vV8Ve?m=slm-utEEkT4cPjWcjIQoXseh>)
- terminators
  - Terminator BBa\_B0015: generic terminator (benchling link: <https://benchling.com/s/seq-i4XIW5M9w3SnkZeBfLtm?m=slm-qeya6iycXxEICn13jeda>)
- open reading frames
  - araC: arabinose operon regulator protein that activates the arabinose enzyme genes (pdb link: <https://www.rcsb.org/structure/2ARC>, uniprot link: <https://www.uniprot.org/uniprot/P0A9E0>, benchling link: <https://benchling.com/s/seq-KXISNzX0KpGrhF70p1kV?m=slm-se5cEMMqIL8BokeiPi2a>)
  - lacI: lactose operon repressor (pdb link: <https://www.rcsb.org/structure/2PUB>, uniprot link: <https://www.uniprot.org/uniprot/P03023>, benchling link: <https://benchling.com/s/seq-bIPEka77OKo3IbBgT9IK?m=slm-vjNOF98484r82BdGS8YW>)

From these sources you will construct the following gene circuit that mirrors the system described by Stricker et al. in 2008.



Your circuit will comprise a single plasmid wherein the insert has 9 parts.

Break down the assembly of the plasmid into the following steps and submit results from each step.

- 1) Order your sequences 1 to 10, where 1 should be your vector. Select proper overhangs for Golden Gate assembly. Note, the vector already has BsaI restriction sites. It is therefore convenient to design your inserts so that the 5' most insert shares the 3' vector overhang and the 3' most insert shares the 5' vector overhang. To make overhang selection easier, use the validated overhangs introduced in Dueber et al. 2015 (Vector-AmpR uses these overhangs).
- 2) Primer design: Design extension PCR primers for your insert sequences. All inserts are given to you as fragments with the exception of GFP. You can PCR the GFP coding sequence from Vector-AmpR.
- 3) Attach all PCR primers to the respective library sequences.
- 4) Perform virtual PCR reaction to get the Golden Gate ready fragments.
- 5) Perform Golden Gate Assembly to build the final plasmid.

## PART II: Mathematical modelling

The system built in Part I is a gene regulatory network comprising two interconnected single node motifs, a PAR connected to an NAR. If correctly tuned, the system generates oscillations in GFP. In Part II you will derive the differential equation model, construct a Matlab simulation, and find parameter values that result in oscillatory behavior. Note, the mathematical model introduced by Stricker et al. in 2008 is very detailed. In this section it is ok to use the simplified models introduced in lectures.

Break down modelling into the following steps and submit results from each part.

- 1) Describe the underlying chemical reaction network. Note, this chemical reaction network will indicate how activation and repression in the first gene jointly affect gene 1 expression (AND/OR gate).
- 2) Write down the system of differential equations using the mass action law.
- 3) Select physically relevant values for all reaction rates.
- 4) Simulate your system using a numerical solver of your choice (e.g. MATLAB or SIMULINK).
- 5) Identify reaction rates that yield oscillations in GFP. Oscillation period should be approximately 40min and the magnitudes of lacI and araC oscillation should be within an order of magnitude of each other.
- 6) Rank your parameter search by laboratory time required to arrive at this design. Assume 1 week for each sequential step. (Hint: tuning steps can also be performed in parallel)
- 7) (BONUS, not mandatory) Simulate the same system using SSA and compare the trajectories. Do you see the same oscillatory behavior?