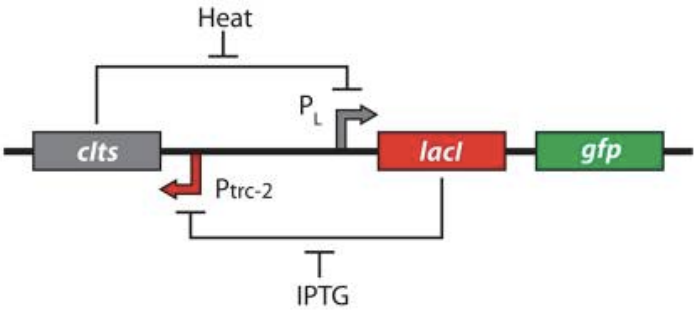
Principles of Synthetic Biology – PSET 1
Due 9/30/2013

1. Genetic Logic

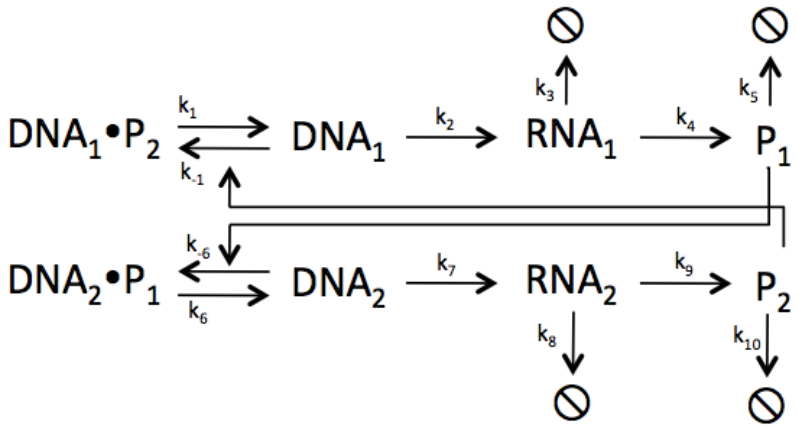


(Khalil, Collins 2010)

a. For all input combinations of the above circuit, complete and explain the truth table for steady-state outputs.
b. If you induce the system with a pulse of IPTG, what would you expect the output to be over time (may be helpful to draw a timecourse)?
c. What useful properties does the circuit have?
d. Say you add a pulse of heat to the circuit and see GFP expression peak then gradually decay. Is this the expected output? What is a potential mechanism for this behavior? How could you revise the circuit to give sustained GFP expression?
e. Say you apply pulses of heat to the system (GFP is off initially). Draw qualitatively what the GFP fluorescence would look like over time in the case of constant a) low IPTG, b) intermediate IPTG, and c) very high IPTG concentrations.

2. Modeling

Say you want to model the circuit described above using the following simplifications: the P_L-LacI DNA (DNA_1) can be reversibly bound by λ Cl repressor (P_2) to form an inactive DNA1•P2 complex in a second order reaction. Free DNA_1 is transcribed in a first order reaction to instantaneously form RNA_1 (DNA_1 is not consumed) and RNA1 undergoes first order degradation. RNA_1 is transcribed in a first order reaction to LacI (P_1) and P_1 is undergoes first order degradation. The same process occurs for DNA_2 which is reversibly inactivated by P_1 while RNA_2 and P_2 undergo first order synthesis and degradation. For the sake of simplicity, assume that protein-DNA binding does not significantly alter the concentration of protein.



a. Write differential equations for all relevant species in the model
b. Find an expression for the steady state level of DNA_1 (and thus P_1) in terms of the relevant constants. You can assume rate constants for RNA and proteins are similar between lacI and cl-ts such that $k_2=k_7$, $k_3=k_8$, $k_4=k_9$, $k_5=k_{10}$. What does the steady state level of DNA_1 approach as you add a large number of repeats of DNA_2? What if you add many repeats of DNA_1 instead?
c. Is this model relevant for circuits that have been integrated into the genome? What about circuits on plasmids transformed into e. coli?

3. Circuit Design and DNA Assembly

a. If you want to extend the circuit to include 3 states in mammalian cells (ie. GFP on, BFP off, mKate off; GFP off, BFP on, mKate off; GFP off, BFP off, mKate on) how would you design the new circuit given the parts below:

Hef1a promoter – Human elongation factor. A constitutive promoter
CAG promoter – Synthetic constitutive promoter
CMV promoter – Cytomegalovirus promoter. A constitutive promoter
TetO – Operator which can be appended to a promoter to make a TetR-repressible promoter
LacO1 – Operator which can be appended to a promoter to make a promoter that can be repressed by LacI
FF4 shRNA – Short hairpin RNA that can be coded in the intron of a gene or commonly driven by the U6 or H1 promoters. When the gene is expressed or the U6 drives transcription of the shRNA, the intron is spliced out, the shRNA is folded into a hairpin, and is processed by RNAi machinery to promote degradation of mRNA molecules bearing the FF4 target site (usually the 3' UTR is used).
FF4 target site – Complementary sequence to FF4 shRNA of siRNA that can be inserted into the 3' UTR following a gene.
FF4 siRNA – Small interfering RNA that acts in a similar manner as FF4 shRNA. The major differences are that siRNA is double stranded RNA and is usually exogenously introduced to the cell/circuit usually using liposomes or polymers.

FF5 shRNA, siRNA, and target sites – Same RNAi mechanism as FF4 but the sequence is different, making FF4 and FF5 mostly orthogonal.
TetR – Repressor against TetO
LacI – Repressor against LacO1
IRES – Internal ribosome entry site which can be placed between two genes following a single promoter. This allows translation of two proteins from a single promoter.
2A linker – "self cleaving" small peptide. When placed between two proteins, the proteins are translated then the linker is cleaved, again resulting in expression of two proteins from a single promoter.
GFP – Green fluorescent protein – a reporter
BFP – Blue fluorescent protein – a reporter
mKate – Red fluorescent protein – a reporter

b. What do you predict the main challenges will be in implementing the circuit you designed?
c. How does the total number of elements (promoters, repressors etc) needed for the circuit scale with the number of states desired?
d. Describe how you would assembe your circuit given that you have access to BioBricks and Gateway entry vectors for all promoter/operator combinations and for all genes (can be linked, have specified introns, or defined UTRs), and also any primers that you need up to 60 bp. You can also design your own BioBrick backbones or Gateway destination vectors if that speeds up your assembly. (Note: assume the cell line you are studying is hard to transfect)
e. Say you have problems assembling the Tet repressor BioBrick after a promoter. You decide to sequence the BioBrick and find the following mutations (the TetR sequence is at the end of the assignment):
   A383T
   A18G
   A9G
   G576C
   Which mutation(s) is/are likely causing problems with your cloning? How would you proceed after determining that the mutation is causing your assembly woes? Which mutations may cause further problems in future experiments and why?
f. When you test the circuit, the TetR/TetO pair shows much less repression than the other repressor pairs. Do you expect this to be a problem for overall behavior of the circuit? Just in case, you decide to use 4 repeats of TetO to increase repression. Which of these method(s) would allow you to make the TetO repeats: oligo synthesis, scarless Gibson, Golden Gate, BioBricks. Which would you choose and why?

TetR Sequence:

5’-
ATGTCCAGATTAGATAAAAGTAAAGTGATTAACAGCGCATTAGAGCTGCTTAATGAGGT
CGGAATCGAAGGTTTAACAACCCGTAAACTCGCCCAGAAGCTAGGTGTAGAGCAGCCTAC
ATTGTATTGGCATGTAAAAAATAAGCGGGCTTTGCTCGACGCCTTAGCCATTGAGATGT
TAGATAGGCACCATACTCACTTTTGCCCTTTAGAAGGGGAAAGCTGGCAAGATTTTTTAC
GTAATAACGCTAAAAGTTTTAGATGTGCTTTACTAAGTCATCGCGATGGAGCAAAAGTA
CATTTAGGTACACGGCCTACAGAAAAACAGTATGAAACTCTCGAAAATCAATTAGCCTT
TTTATGCCAACAAGGTTTTTCACTAGAGAATGCATTATATGCACTCAGCGCTGTGGGGCA
TTTTACTTTAGGTTGCGTATTGGAAGATCAAGAGCATCAAGTCGCTAAAGAAGAAAGGG
AAACACCTACTACTGATAGTATGCCGCCATTATTACGACAAGCTATCGAATTATTTGATC
ACCAAGGTGCAGAGCCAGCCTTCTTATTCGGCCTTGAATTGATCATATGCGGATTAGAAA
AACAACTTAAATGTGAAAGTGGGTCCTAA
-3’

Hef1a Sequence
5’-
TTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGG
TGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA
AGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGT
GCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGT
GCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGA
ATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGG
TGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCC
TGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCT
GCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTC
TGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGG
GCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTG
CGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTG
CCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCAC
CAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGA
GGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTC
CGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCG
ATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCG
ATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATG
TAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAG
ACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGT
-3’

4. Heat Dissipation in Bacteria

Sometimes it's interesting to take stock of the real physical numbers that come from cellular processes when scaled up to large reactors. One thing that is often overlooked when thinking about the dynamics of the cell is heat transport.

a. When dividing, cells require about 32 mmol of ATP as energy input to create 1g of new cell matter. Most of that energy is stored in the chemical bonds that comprise the cell, but as you know, no process is 100% efficient. Assume that the cell is able to convert the energy at 90% efficiency and the rest is dissipated as heat. An *E. coli* contains about 1.0e-12g of biomass and doubles its biomass about every 20 minutes. On average, how much heat is produced by a cell every second. (Hydrolysis of ATP in physiological conditions is about 48kJ/mol.
b. A cell is only about a 1e-15L in volume. How much does the temperature increase inside the cell every second if there is no heat transfer out of the cell. Assume that the cell is made of water. (Hint: the definition of a calorie might be useful)
c. Fortunately, the cells are conductive such that the energy does not increase the internal temperature a lot. In fact, the surface area of a cell is significantly larger than that required for efficient heat transfer. Why do you think cell surface area is not minimized to this constraint? On the other hand, why are cells not usually maximizing their surface area by becoming very long flat sheets?
d. Back to the heat question. A single cell will not boil itself, but a lot, working rubbing together might. Imagine a 10,000 L cylindrical reactor of 1m radius filled to the brim with cells making biofuels. Cells in such a system can reach an OD_{600} of 50 ($1OD_{600}$ = 1e9/mL cells). Let's say that each cell is still growing well and is generating the same amount of heat as in part a.

   A reactor that is not cooled will lose most of its heat through conduction with the outside air. A very simple model for this phenomenon is

$$\dot{q} = -kA\frac{\Delta T}{\Delta x}$$

   Where $\dot{q}$ is the rate of heat transfer (watts). k is the thermal conductivity of the material (~20 W m^{-1} K^{-1} for stainless steel), A is the area of the material, Δx is the thickness of the material and ΔT is the difference in temperature on each side of the material (ignore top and bottom of tank surface). Given that the thickness of the reactor would be about 10cm, find out the temperature difference between the inside and outside of the tank if cell propagation is the only source of heat.