Note: There is the last assignment for BioE.144 this term. Your lowest assignment grade will still be dropped providing a total for the "assignment component" of your final grade as 56%.

Note: Please adhere to the stated expectations: "I expect that you'll interact with your colleagues throughout the course, in discussing any readings, during class, or in considering any assignments. However, your submitted assignments should reflect and be of your own individual work." ***For this particular assignment, please use the scheduled class time on Thursday 12 March to review the question as a class, and outline approaches to a solution to the problem.***

Note: This assignment are both by midnight Friday, 13 March. Please submit your assignment by email to: endy@stanford.edu

Note: Please send any questions about this assignment by email to endy@stanford.edu. I'll try to respond in real time.

Assignment #4 Abstraction (Devices, Devices, Devices!!!)

BACKGROUND: Ron Weiss and his team at Princeton have figured out how to program bacteria to respond to environmental signals, so as to form patterns in space:

"Spatiotemporal control of gene expression with pulse-generating networks" by Basu et al. (PMID: 15096621)

"A synthetic multicellular system for programmed pattern formation" by Basu et al. (PMID: 15858574)

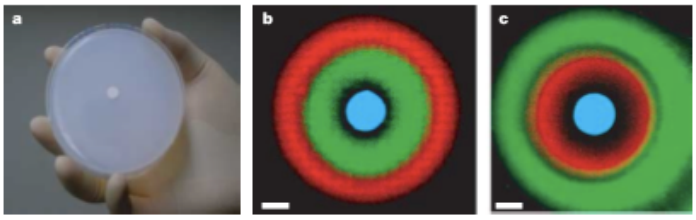

Figure 3 Experimental solid-phase behaviour of band-detect networks. **a**, Picture of the Petri dish used in the BD2-Red/BD3 experiment showing the sender disk in the middle. **b**, Bullseye pattern as captured with a fluorescence microscope after incubation overnight with senders in the middle of an initially undifferentiated 'lawn' of BD2-Red and BD3 cells. Surface maps depicting red and green fluorescence intensities are included in Supplementary Information. The senders in the middle are expressing CFP. **c**, Another bullseye pattern, this time with a mixture of BD1 and BD2-Red cells. Scale bar, 5 mm.

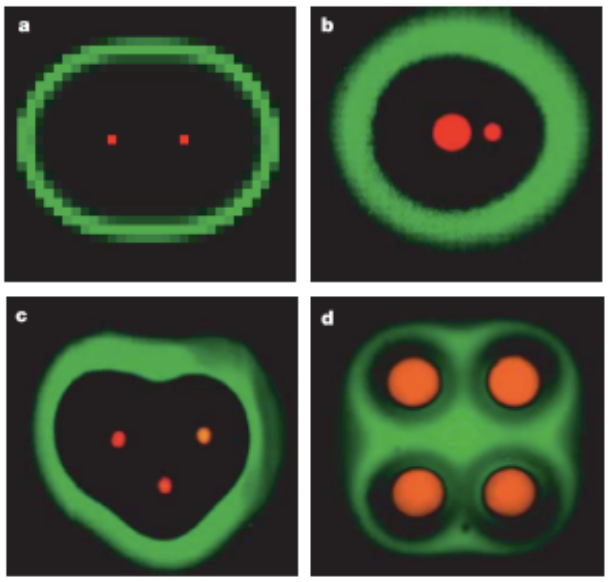

Figure 5 Formation of various patterns. **a**, Simulation of band-detect behaviour on solid media with two senders that results in the formation of an ellipse. **b**–**d**, Experimental results showing various GFP patterns formed based on the placement and initial concentrations of sender cells expressing DsRed-Express: **b**, ellipse, two sender disks; **c**, heart, three sender disks; and **d**, clover, four sender disks.

The genetic architecture of these bacterial pattern forming systems is somewhat complicated, sometimes involving "sender" cells that produce and release a chemical (AHL) and "receiver" cells (called "pulse-generating cells" in the figure legend below) that have a transcription factor (R) that is activated when AHL levels are high.

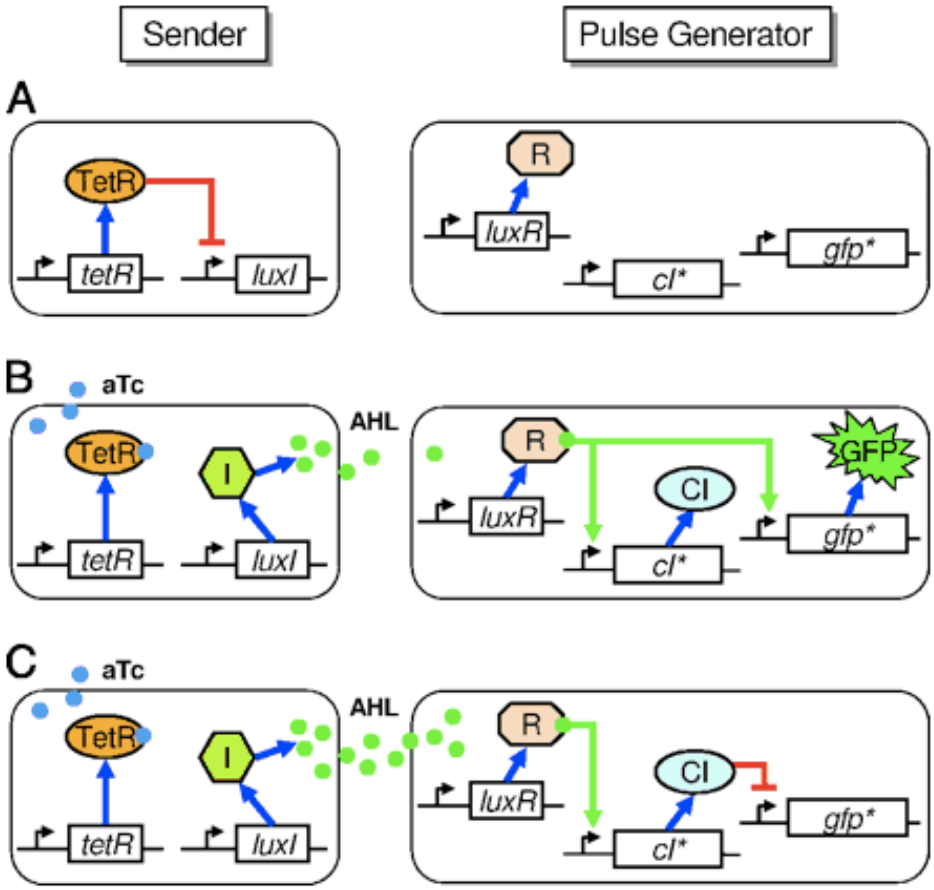

Figure Legend from PNAS Paper (PMID: 15096621): *Engineered sender cells are instructed to communicate a signal to the pulse-generating cells, which respond with transient expression of a fluorescent protein. TetR, I, R, CI, and GFP represent the protein products of the tetR, luxI, luxR, cI *, and gfp * genes, respectively, where * denotes a destabilized version of the protein. (A) Initially, no communication is taking place between the sender and pulse-generating cells. (B) Addition of anhydrotetracycline (aTc) instructs the sender cells to transmit the AHL signal to the pulse-generating cells, which in turn respond by expressing GFP and CI. (C) Continuous transmission of the AHL signal ultimately results in CI concentrations above the threshold required to repress GFP. The fluorescence disappears as GFP decays quickly.*

[NOTE: In the figure above the rightward facing arrows depict transcriptional promoters (elements on DNA that initiate transcription, and the resulting production of mRNA). Not all of these promoters are the same. Some of these promoters are regulated by proteins. For example, the TetR protein is a repressor protein that turns OFF its cognate operator. As a second example, the R protein is encoded by the LuxR gene, turning ON gene expression at its cognate operator. Ribosome binding sites (RBS) and transcription terminators are now shown. For the work of this assignment (below), you might want to redraw the above labeling each specific promoter, as well as RBSs and transcription terminators.]

Part 1: (Devices, Devices, Devices!)

Ron's team moved directly from a system-level specification (e.g., make a bull's eye pattern) directly to a parts-level implementation. Their designs do not define any intermediate-level genetic devices that might be readily reused in other systems.

Starting with the parts-level depiction above, identify the best set of genetic devices that could be readily reused in other systems. Use a PoPS-based signal carrier for device boundaries so that your resulting devices can be reconnected with any other device that could accept or receive a PoPS signal. Recall that PoPS = POlymerase Per Second.

Remember that (i) engineered genetic Devices contain of one or more genetic Parts, and (ii) produce a human-defined function.

For each Device sketch out and label (i) all the Parts, and (ii) the Device boundaries (hopefully including PoPS signals).