

# Engineering Microbial Computational Systems

## MIT9904-10

**Progress Report: January 1, 2000 — June 30, 2000**

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### **Project Overview**

Genetic regulatory networks form one of the basic computational infrastructures of life. The science of such systems has been well established over the past twenty years through pioneering work of Monod and Ptashne, among others.

This project undertakes to transfer that scientific knowledge into engineering practice, by starting the serious work of characterizing components, engineering interfaces, simplifying the technology, and educating a set of students who can easily cross the boundaries between biological science and computational engineering. Stated more directly, the project is to learn how to engineer life.

Our approach has been to start our efforts with very simple structures, using well characterized organisms and genetic regulatory elements. In particular, we are working with standard laboratory strains of *E. coli* and standard promoter and reporter gene constructs.

An important initial goal was the construction and outfitting of a microbiology laboratory within the computer science building at MIT. This effort was largely complete by September, 1999. Another important initial goal was attracting and educating a core of motivated and educated graduate students and staff to perform the research. Early on we decided that it was easier to educate researchers with a CS background about biology than the other way around. Today, we have two full time graduate students, a post doctoral student, and two staff, all of whom are trained as computer scientists, but, equally, are trained in molecular biology theory and practice.

Our initial experiments primarily centered around the development of our skills in gene transfer, plasmid construction, and bacterial transformation. In these experiments, we focussed on simple gene promoter and reporter systems, such as the LacZ promoter and Green Fluorescent Protein (GFP).

Our previous work included the cloning, sequencing (genbank AF170104), and transfer of the bioluminescence and quorum sensing system from the marine bacterium *Vibrio fischeri* into laboratory strains of *E. coli*. This work was presented in June, 2000 at the DNA VI meeting in Leiden, Netherlands.

## Progress January 1 through June 30, 2000

During this spring, we upgraded our microscopic imaging equipment in the laboratory with a Hamamatsu ORCA I cooled CCD camera which allows us to quantitate individual cell fluorescence, and to take time lapse images of cell growth. We also acquired a gradient PCR machine to enable quicker optimization of PCR conditions.

Much of our effort went into a careful analysis of simple inverter transfer curves. We routinely are able to make constructs which exhibit switching behavior in bulk, while colonies grow in agar plate form. We continue to have difficulty in controlling single cell behavior in liquid culture. When measuring single cell behavior, we see large, as yet unexplained statistical variation, which we are attempting to more fully understand.

Our approach has been to backtrack to a simpler set of experiments, to develop our probe technology more effectively, and to understand these simpler systems more completely.

The reporter genes we are using are Green Fluorescent Protein, and its variants, YFP and CFP, with slightly different fluorescent emission peaks. We investigated an alternative protein, DsRed during the spring, and found the protein slow to form a chromophore in our laboratory strains. Other researchers have reported similar difficulties in yeast, and it appears make the red fluorescent proteins unusable for our purposes.

Our interest in multicellular systems and cell to cell communications continues. We have started preliminary work with two interesting bacteria which exhibit cooperative behavior, *Myxococcus xanthus* and *Paenibacillus dendritiformis*. Each of these exhibit swarming behavior and long term shifts in behavior depending on their location within a large culture. Although these systems are far more complex than we envision building in the near term, general principles and signalling pathways may be available through their further study.

We continue our work on expanding the repertoire of available intercellular signals through the cloning of the LasI/LasR system from *Pseudomonas aeruginosa*. Evaluation of the crosstalk between its signalling system and the LuxI/LuxR system is underway.

## Research Plan for the Next Six Months

While we continue to work on many of the efforts described above, two important new projects are under way.

First, we are evaluating new technology for fully automated plasmid synthesis. An irony is that we can sequence millions of bases per day, yet it takes weeks for a postdoc to create novel, 10,000 base plasmids. We are excellent at reading the bits, but pathetically bad at writing them. We believe that some high speed automated approaches can solve this problem. In collaboration with the Whitehead Genome Sequencing Center, we have started looking at technology alternatives, including new enzymatic synthesis pathways and better approaches to oligonucleotide purification which will allow us to create 10 kb plasmid constructs in bulk, and with little human intervention. We believe this will revolutionize biology in a way similar to the change brought about by automated sequencing.

Secondly, we have begun our efforts to understand and create simpler living cells. We are culturing two species of non-pathogenic Mycoplasmas, *Mesoplasma florum* and *Mesoplasma lactucae*, and doing preliminary work which will lead to choosing one of those for large scale automatic sequencing, using the Whitehead facilities. Discussions with Whitehead staff lead us to believe that using their resources for a weekend will result in a complete genome for this simple, non-pathogenic, free living bacteria. The hard work will then begin, of developing plasmids, genetic tools, and methods for removing and inserting chromosomal genetic information to transform this cell into the simple “power supply and chassis” we need for engineering living systems.