

MIT9904-10

Biological Computing Technologies

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Much of the work in our laboratory during the last year focussed on acquiring and bringing up equipment and debugging protocols. We have succeeded in developing much of the required technology, but important pieces remain undeveloped.

Our initial work focussed on some simple bacterial reporter and communication mechanisms.

We have successfully cloned the bioluminescence system out of two separate bacterial species, *Vibrio fischeri* and *Photobacterium luminescens*. We sequenced both of these constructs, depositing the sequence of the *Vibrio fischeri* lux operon in Genbank as sequence AF170104, and verifying (with small mutations) the pre-existing sequence of the *Photobacterium* operon (M90093).

Building on our sequencing of the Lux operon, we have isolated the intercellular communications mechanisms of the bacterium, and used it to communicate from a genetic regulatory network in one bacterial cell to a second genetic regulatory network in another cell. The signalling relies on the creation of a specific signal molecule, an autoinducer,

One specific project for the next year is to isolate several more such autoinducer/responder systems. We already have under way the isolation of a similar system from *Pseudomonas aeruginosa*. It is important to have more than one such system because, at least with our current techniques, only a single signal can be sent with each such chemical. Even having two such signalling chemicals will allow us to construct some compelling genetic networks, which may, for the first time, allow us to demonstrate engineered patterning of a bacterial culture, implementing some of the pattern formation ideas of Turing (1952).

A second theme of our proposed work for the next year is to start looking at very simple bacterial cells. One of the simplest such cells, *Mycoplasma genitalium*, was the second

organism to be completely sequenced, and has been the subject of an intriguing paper in which the individual gene knockouts were performed, leading to an absolute requirement of only approximately 290 genes (Hutchinson, 1999).

Unfortunately, *Mycoplasma genitalium* is a human pathogen, and is unsuitable for research work in our facility. We have located a plant pathogen, *Mesoplasma lactucae*, which also has a relatively small genome, but has a biosafety level of 1. We plan to begin a set of experiments with this organism to determine its suitability for the longer term project of reducing the complexity of living cells, and creating an understandable and predictable engineering infrastructure.

We have also learned a lot during the past year about the types of tools necessary to effectively work in this field. We have been surprised, for example, at the remarkable efficiency of sequencing DNA, but equally at the remarkable difficulty of de-novo synthesizing long strands of DNA. It is as if we can read the documents very well, but can print them out only by cutting and pasting words or paragraphs we happen to find in the newspaper.

Efficient "printing" of our engineered constructs would have a profound effect not only on our work, but on the work of thousands of researchers who spend much of their time painstakingly cutting and pasting DNA sequences.

We have some ideas on how to dramatically speed up this process, using enzymatic reactions based on terminal deoxynucleotide transferase (TdT) which we would like to try. A more traditional approach of high throughput chemical synthesis, driven by careful sequence compilation software is a backup approach.

Finally, we believe that to be an effective laboratory we must begin to perform genetic network analysis through the use of high throughput techniques such as gene arrays or gene chips. We are still analyzing the correct protocols and equipment in this area, but this is an essential part of augmenting our data analysis tool set.

References

Turing AM; The Chemical Basis of Morphogenesis, Proc. Royal Soc. (London) B 237:37-72, (1952).

Hutchison CA, Peterson SN, Gill SR, Cline RT, White O, Fraser CM, Smith, HO, Venter JC; Global Transposon Mutagenesis and a Minimal Mycoplasma Genome, *Science*, 286: 2165-69 (1999).