Project Plan: IPRO 302, Fall 2006

Background:

Within the exciting and groundbreaking world of molecular biology, there lies a nexus where engineering meets biology. This place is called synthetic biology. This emerging, cross-disciplinary field is the focus of this inter-professional project. The ideas underlying it are elegantly simple, allowing this project to epitomize the inter-professional ideal of the Ipro program. The basic notion is that the fundamental entities that control life through metabolic processes are genes and that if one can manipulate these functional units of life, then one can manipulate life itself. Several of our colleagues abroad have successfully synthesized simple organisms, and we seek to press the envelope at the frontier of technology. Thus, our motto at Ipro 302 is, changing the world one organism at a time.

Objectives:

Our objectives are fairly straightforward. If the genetic code can be called its own language, then we want to become fluent in it. The current technology does not allow us the ability to "write our own novel" (i.e. make a new organism from scratch), but what we can do is write our own pamphlet. This semester, we are trying to complete our test case, which is to create a colony of *Escherichia coli* that flash different colors all in unison. Once we complete this task, we hope to export this system into higher organisms, such as Zebrafish (*Danio rerio*). We are also seeking to move forward conceptually by planning long term goals for the Ipro, such as the development of not just test cases, but new, useful organisms.

Methodology:

How do we change the world, one organism at a time? Well, we first have to understand how genes function within an organism and use this knowledge to create wonderfully new and sensational critters. We then apply this theoretical knowledge to the lab. Essentially, the physical product of our labors will be completely new, artificial plasmids. Plasmids, in nature, are circular segments of DNA that are usually found in bacteria. These plasmids are generally separate from bacterial genomes, but get expressed by the bacterial expression machinery. This makes them exceptionally useful to genetic engineering, genetic manipulation, and other crazy exploits into the realm of molecular biology.

So, the idea that was conceived in prior semesters was to "cut and paste" individual genes out of their native organisms and place them into artificial operons (gene systems), called modules. They are called modules because the gene products they form modulate (repress) the expression of other genes. These modules can then be assembled to form

artificial plasmids and can be linked in order to control the expression of different genes. This was originally done using restriction enzymes, or more simply, genetic scissors. Then genes were "pasted" together via the enzyme ligase, which anneals the DNA. But this method was slow and time consuming. Thus, our team turned to phusion PCR, which is a variation of the genetic amplification techniques used by crime scene investigators. How it works is short "tags" of DNA are complimentarily hooked onto the ends of each amplified gene and then these genes are amplified together. The tags stick together and viola! The two genes become one. Yet, now that we have most of the gene modules constructed, we will most likely have to return to restriction enzyme methods, in order to finish plasmid assembly.

Administration and organization

To make the overall project goals of the project more tractable, we have organized into six sub-teams with defined goals and expectations.

Assembly/Cloning team:

Leader: Bryan Bridgeman

This team is responsible for repairing the pieces of DNA with structural defects and for finishing construction on the remaining plasmids. It consists of a majority of the students in the group and is also responsible for coming up with assembly plans and executing them with efficiency. It also involves transforming the gene pieces into the living bacteria. Communication is a key necessity for making sure that each gene piece is constructed quickly and accurately.

Simulation Team:

Leader: Emad Allam

The simulation team will be the go-to guys for hashing out the details for a mathematical model to describe in theory the actual oscillatory fluctuations of the protein concentrations. A new model for describing the synchronized oscillations is still being worked out. Also, new mathematical models will need to be created with each subsequent organism that we work on.

Synchronization/Development Team:

Leader: Emad Allam

This team is actually a tight coordination of two smaller teams. The synchronization team is a specialized subset of the Assembly/Cloning team that is focused upon coordinating the flashing oscillations of each bacterium to create entire colonies of macroscopically flashing bacteria. This is a crucial step in the export of this system into eukaryotic systems. In order to accomplish this, the synchronization team will have to

specifically devise a plan for implementing the genes that will enable synchronization to occur into the already constructed gene system. This will probably include the completion of another plasmid. Since this is such a huge step in our availability to create "gene circuits" inside higher organisms, the synchronizers will be working closely with the long term developers.

Website/Documentation Team:

Leader: Emad Allam/Siddartha

This team will be responsible for updating and renovating the project's website, and maintaining the team documentations. In this way, they will function as the "Pimp-my-Ride" crew for the website.

Imaging Team:

Leader: Heather King

This team will document our progress at different stages of plasmid construction. It will be a crucial step in determining whether or not our oscillations are actually functioning. They will take pictures via the fluorescent microscope in the Life Sciences building. As such, they will have to develop techniques for either time-lapse or cinematic photography of the final bacteria.

LongTerm Planning

Leader: Bryan Bridgeman

This team will focus on the direction this project will take in the future. It is our hope that this will lead from the simple test case to more advanced work with a group of new and useful targets. We will attempt to come up with new and exciting innovation that will change the face of science for the benefit of mankind. In order to accomplish this, the team will have to survey the progress of prior semesters and evaluate how to proceed within our means (and our budget). A panel of innovative targets will be developed , withy a preliminary biological feasibility analysis.

Individual Assignments:

Every member of the IPRO is enrolled in two subteams, as well a cloning group that will ensure that a specific gene piece gets made. Each cloning group is under the guidance of a student that has returned for a second semester to the project. Below is a list of each team member's affiliation.

Cloning/Assembly:

Heather- Hua, Hazel

Bryan- Siddhartha, Trillian

Emad- Faraz, Edward

Thien- Daniel, Saba

Lily- Tae-Young, Soo Yeun

Imaging- Lily, Daniel, Heather, Edward, Soo Yeun, Tae-Young, Hua

Synchronization- Lily, Faraz, Trillian, Emad, Heather, Hua, Soo Yeun, Tae-Young, Bryan

Simulation- Emad, Siddhartha, Daniel, Hazel

Website/Documentation- Emad, Trillian, Siddhartha

Longterm Development- Faraz, Bryan, Edward, Hazel

Budget:

These goals and objectives are accomplished within the constraints of a $3000 IPRO budget. These are estimated as follows

category	$
Oligonucleotides	500
Enzymes	500
Chemicals and reagents	700
Cell lines and biologicals	300
Growth media	600
Plastic ware	400

Expected Results:

An initial estimate of the semester goals of each team are.

Assembly/Cloning team:

Finish assembly of pOSC and pREP1 andpREP2

Simulation Team:

Development of an iterative kernel for the MATLAB simulation model

Incorporation of the lux-type sychronization

Make recommendations to synch team with regard to synchronization network topology

Synchronization/Development Team:

Characterization and cloning of the two pLux plasmids

Development of a specific cloning plan (primers and steps) to incorporate these genes within the existing unsynchronized network.

Website/Documentation Team:

Maintain and improve the website
Production of vision document in conjunction with long term development team.

Imaging Team:

Image mixtures of dark/yellow/blue bacteria and resolve them
Image oscillating bacteria

LongTerm Planning

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Goals:

Develop a synopsis of what has been done in synthetic biology
Develop a vision document in conjunction with WebDoc team