Midterm Progress Report

Ipro 302-Synthetic Biology

Revised Objectives

As stated in the Project Plan, the overall aim of this IPRO is to create a novel, flashy and aesthetically innovative metabolic pathway engineered organism. The overall goal can be accomplished by specifically concentrating on achieving the following primary aims:

1. Development of gene manipulation skills to achieve the major goal
2. Assembly of gene pieces and insertion in bacteria
3. Development of a design plan for synchronization of bacterial behavior
4. General IPRO project management aims to facilitate project administration.

And once again, the IPRO team along with faculty, staff and resources from the BCPS department at IIT plans to modify a genetic pathway in Escherichia Coli such that it would flash three separate colors with a predictable oscillation. This initial work has advanced quite rapidly thus, we have already begun to theorize and develop a plan that will be the basis for performing more elaborate experiments such that the behavior is coordinated amongst all bacterial cells in a population and so becomes macroscopically observable. We have continued to utilize modeling and preparation of individual gene pieces that was completed last semester and will carry on the manufacture of customized gene modules. Therefore, the primary focus of the IPRO for this semester is still the biological lab work needed to assemble all gene modules and insert them in bacteria.

However, the IPRO also aims to develop a design plan for behavior synchronization so that it can be observed on a macroscopic level.

After these objectives have been achieved, we will seek to manipulate eukaryotic genes in a similar fashion. Essentially, the IPRO team will seek to display the same oscillatory behavior in a living eukaryotic system, probably in aquatic animals such as Zebra fish (*Danio rerio*). Therefore, the perfection of genetic manipulation using various methods this semester is vital to this next leap in gene control for following semesters.

Results to Date

Currently, we are completing all of the gene modules. The team is roughly 60% completed with this project, thanks to the revolutionary speed in experimental techniques provided by the fusion PCR (Polymerase Chain Reaction). Only one pair of genes remains to be fused together and the rest just need a terminator sequence fused onto them. Some of these newly constructed genes have already been implemented into competent *E. coli* cells in order to make sure that they work. This was accomplished via the standard procedures for transformation of bacterial cells.

Revised Task / Event Schedule

The next step is to re-isolate each gene by means of alkaline Mini-Prep procedure. After this isolation of the DNA, we plan on digesting each module with various restriction enzymes. This is done in order to prepare each DNA fragment for sequencing, which will serve as a test to see if the gene modules have been constructed properly thus far. Hence, once we are certain that the modules are correct, the final steps will be to fuse the modules together into their respective plasmids, get those plasmids into our *E. coli* and also figure out a way to apply a coordinated timing mechanisms that will allow entire colonies to light up like Christmas trees (just in time for the holiday season).

In order to keep the flow of this project from stagnating, an intensive scheduling effort was incorporated in order to maximize results and prevent individual burn out. Each member was given a day of the week to complete the necessary procedures in lab and will hand off their assignments to the next team member the next day. However, this relay style approach was difficult to maintain, due to excessive strains on communication. Confusion ensued and progress was slowed. As a result, we are returning to the partner system and will continue to utilize the efficiency and accountability of working in a group. In order to complete our objectives, we will require much more individual communications, as well as a lot more feedback to and from the team as a whole. Hopefully, a return to this partner system will aid us in keeping on track and accomplishing our overall goals.

Updated Assignments

Due to the overwhelming miscommunication caused by the individual workload system, we are maintaining the partner system outlined in the project plan. Many of the partners are now transforming their completed pieces into the bacteria and are adhering to the following schedule:

1. Enzyme Analysis
    a. Transform
2. Miniprep and replicate
    a. pVuii/double Restriction Enzyme digest
3. Break for sequencing testing
4. Fusion of everything
    a. Construction of pREPbis, ptet,cFP,t7 fuse pLac, 7FP_t7
    b. Construction of pOSC
        i. CAM fuse High Ori
        ii. ptet_ci_t7 fuse with pR_LacI_t7
        iii. plac_tetR_t7 fuse CAM_Hig Ori
        iv. (ii) fuse with (iii)
        v. Sit back and enjoy the show under the microscope

Barriers and Obstacles

Genetic engineering, as an emerging field, is filled with new complications that few people have ever dealt with before. Because of this, we have run into a number of setbacks with the fusion PCR, promoter genes with extra gene fragments attached, and also any number of mistakes that have come from inexperience with laboratory techniques. In particular, the construction of the pLlac/tetR/T7 module has been extraordinarily difficult. On top of this, misunderstandings and lack of communication have added to confusion, which has often led to faulty results. If our team is going to be successful, we will need to step up and be diligent, even when things go wrong. Communication must be increased in order to prevent small mishaps. Yet, I am confident that we will overcome these obstacles and succeed. In fact, I doubt that few Ipro groups are as committed to making this project a success as our team.