Project Plan: IPRO 302, Fall 2005

Background:

This is a continuing IPRO from last semester, Spring 2005 and involved creating a synthetic metabolic pathway in *Escherichia Coli.* There have been a growing number of research projects outside IIT investigating engineered organisms whose processes have been modified towards performing a specific task.

The scientific inspiration for this project is provided in a demonstration of the production of "flashing" bacteria (*Nature* **403:**335 2000) as well as theoretical analysis suggesting that coordinated flashing may make this behavior observable on a macroscopic scale (*PNAS* **99:**5988 2002).

Research Methodology

The specific problem we need to address this semester is the assembly of all three plasmids that make up the oscillator pathway. In order to complete this task, we need to combine all individual gene pieces obtained last semester into modules and then combine the modules to form the different plasmids. Each single module will also be incorporated into an E. coli bacterium and archived for future use. To accomplish this we are using a technique called fusion PCR that was previously developed in order to work more efficiently within the time allotted. The success of each fusion will be confirmed by the agarose gel electrophoresis, which will be documented throughout the project in order to in the future create reproducible results. Part of the team effort will be spent on the synchronization design plan that will enable us to synchronize the timing of a colony of bacteria exhibiting a specific color. The final results should be confirmed by the evidence of synchronized blinking E.coli that can be visible without the aid of a microscope.

Objectives:

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 primary aim
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.

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. Once this initial work has

progressed sufficiently, we will begin the groundwork, theorize and evolve a plan which will be the basis for performing more elaborate experiments such that the behavior is coordinated amongst all cells in a population and so becomes macroscopically observable. The modeling and preparation of individual gene pieces was completed last semester and indeed those tools and other preexisting tools will be used as an aid to design biological modules and predict their behavior. Therefore, the primary focus of the IPRO for this semester will be on biological lab work 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.

Expected Results:

Since this is a continuing IPRO, the project plan takes into account the work done during previous semesters and has incorporated certain changes in the approach and process of achieving the aim. The success will be directly measured in terms of whether or not the pathway is indeed produced in the organism and if indeed the bacterium in which the pathway exists exhibits oscillatory display of three different colors. The modeling will be fine tuned to accommodate increasing needs of the project as it develops.

Budget:

The largest costs incurred are in the procurement of the various pieces required. Most of the elements and lab equipment required to assemble the pathway has been found within IIT. However it has been necessary in the past to order some elements and a detailed breakup is given on the PRS database.

The largest single expense will be for biological supplies needed to implement the modified assembly plan detailed below. Total expenditures of ~$3000 are planned. Initial procurements and anticipated budget are as follows:

company		ordered	received	
New England Biolabs	$833.00	3-Sep-05	9-Sep-05	enzymes
Stratagene	$609.00	3-Sep-05	--	vecotrs and enzymes
Biologix Research Co	$394.00	3-Sep-05	--	lab supplies
IDT DNA	$500.00	3-Sep-05	--	oligonucleotides
U Chicago	$300.00		--	DNA sequenceing
N/A	$300.00			new genes for synchonization
TOTAL	**$2,936.00**			

Scheduling and Milestones

The project will continue to run in three parallel tracks: lab work to implement the plan, development to elaborate our objectives and documentation to communicate within the team and to the world at large.

I. Lab Work

Last semester's IPRO team finished isolating the individual gene pieces required for assembly. This semester's lab work will focus on putting all the modules together in order to complete the metabolic pathway. A major bottleneck identified by last semester's team was the RE mediated assembly, which was time consuming and hierarchical, preventing parallel progress. Over the summer a modified assembly protocol (fusion PCR) was identified and will be implemented this semester

II. Documentation and Inventory

The overall goal of this phase is to ensure that every step of the project is well recorded as this will not only help an outsider to gain an understanding of the undertaken but it will also serve as a means for the team to constantly self evaluate and assess its progress. Inventory will ensure that the project runs smoothly and delays due to lack of material can be avoided.

This will proceed by:

1. Production of documents describing the key aspects of the teams objectives, work products, and methodology. Specific documents include:
    a. Databases documents (primers, plasmids and modules)
    b. Protocol documents
    c. Project plan (objectives and assembly) documents
2. Continuing development of the official team website as a repository of completed documents, and a working website (blackboard or yahoo, or some other such environment) as a work-in-progress site

Individual Assignments

All the team members are required to assist both in the actual completion of the project in terms of lab-work, design and administrative tasks. These administrative tasks have been divided among the different group members and include: secretary, liaison officer, webmaster, and database/archive planner. The job of assembling of the different modules has been divided into five different groups of two, who each must complete their assigned module and work with the other groups to combine their modules into a plasmid. The synchronization design plan and the website are also being simultaneously developed.