Final Report

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Ipro 302

Synthetic Biology: Engineering a Novel Organism

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Project Website: www.iit.edu/~ipro302s06

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Introduction

Perhaps one of the greatest mysteries left to unravel in the scientific world is the secret of what makes something live. What is life? If we can start to understand the edges of this mystery, then we can also start to do something that will benefit the greater good of society at large. If life can be understood at a molecular level so well that human beings can create it from scratch, then such an understanding would enable medical science to obliterate genetic diseases like cancer or heart disease. If such a thing is possible, then it would mark a great transition in biology. We would no longer be just studying life, but we would be engineering it. Such a huge paradigm shift requires that the scientific and engineering communities be actively involved in ensuring that this world-transforming technological advance does not become abused. It is the responsibility of those who seek to ride the Pegasus to not fly in the face of Olympus. And so it is with this Ipro. We totter on the cutting edge (perhaps the bleeding edge?) of a new era, with the ambition of not only seeking to pursue genetic engineering for its own sake, but for the greater good of the scientific community, the medical field, and for humanity as a whole.

Background

We are indeed indebted to that brave group of people who have donned the title "genetic engineers" that were before us. This stalwart community of our forbears, were very much the logical continuation of the work of Rosalind Franklin, Jacob and Monod, and, of course, Watson and Crick. The structure and function DNA have been determined in the first half of the 20th century, the scientific progeny of McCarty and McCleod began to tinker with the genetic make-up of organisms. The generation prior to them had showed the world that the hereditary molecule of life was DNA and that its structure was the double helix. They proved that Gregor Mendel had been right and showed us that the physical quantities that controlled the construction of our bodies were "genes". The subsequent work of the geneticists and genetic engineers, working with eloquent experiments and brilliant science, showed us how genes were organized, what their sequences were, and how the information contained in them was unlocked to create proteins.

And yet, for all their work, the genetic engineers and gene scientists of the past decades have brought us only to the brink of innovation. The enormous potential of living genomes (the entire genetic sequence inside an organism) has yet to be unlocked. And so, we are witnessing the creation of many different field related to genetics that will delve deeper into the mysteries of life than ever before. And that's where our Ipro comes into play.

Purpose

The point of this Ipro is to create a genetic circuit. A genetic circuit is a system of about 20 or so genes that interact with each other in a certain way. Genes regulate each other in

nature the way that parts of a circuit regulate each other (hence the name "genetic circuit"). Also, we would like to do this in such a way that is ethically and scientifically responsible, while exploring other projects to pursue in the future. Specifically, were are creating a genetic circuit that will instruct *E. coli* to oscillate the concentration of foreign proteins within its cell. Also, we are seeking to create an ancillary "reporter" system that will allow these oscillations to be visualized by fluorescent proteins. Hence, our desired products are bacteria that will flash three different colors. How sweet is that? After we construct the proper genetic circuit to accomplish this, we are also seeking to implement a synchronization technique that will allow entire colonies of bacteria to flash the same color at the same time. This is important because our penultimate desire is to move our system into higher organism, such as Zebra fish (*Danio rerio*). Since higher organisms are multi-cellular and contain genetic systems that communicate across cells, porting our simple genetic circuit would be a critical step in understanding cellular communication, eukaryotic genetic diseases, and the operation of genetic systems inside higher life forms.

Research Methodology

In order to create the genetic system that we aspire to build, our group first had to design our system. This was accomplished in the first semester via mathematical modeling and computer simulation. With this plan in hand we then proceeded to isolate genes from various organisms within nature and to create our own gene bank. With this stockpile of genes in hand, we then started to construct our artificial plasmids (circular, double-stranded molecules of bacterial DNA). This was accomplished by using fusion PCR (Polymerase Chain Reaction), which is a modified technique from the DNA fingerprinting technology used by forensics laboratories the world over. Essentially, each gene is copied many times by in vitro, thermo-tolerant DNA polymerase found in thermophilic bacteria that reside in hot springs. The polymerase/DNA milieu is placed in a PCR machine, along with certain primers, which acts as "sticky ends". These primers are single stranded homologous DNA pieces. Once the individual genes are amplified with their respective primers, several genes are placed *in situ* at the same time and the genes are fused together and copied simultaneously. This protocol is repeated in order to create several gene "modules", which are a three gene system of a promoter (genetic switch), the structural gene (one that produces proteins), and a terminator (one that stops the process of mRNA transcription). Methods such as agarose gel electrophoresis, alkaline mini-prep, and competent cell transformation were also used over the course of this project.

Figure 1: The Oscillator Plasmid

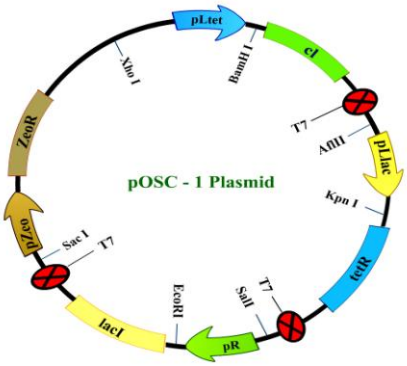
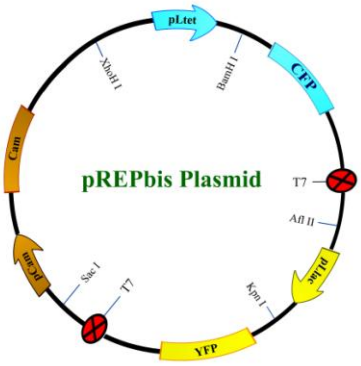
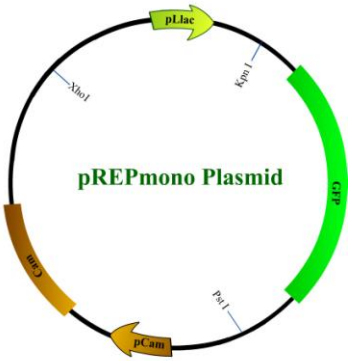


Figure 2: The Reporter Plasmids





Once these modules of DNA are constructed, we had to fuse several together to form the plasmids. These are given in the following figures (Figure 1 and 2). We used Restriction Enzymes (genetic scissors) in order to cut these genes at certain sites, and then "glued" them together using DNA ligase. Also, work was done to isolate and purify a gene that would allow for synchronization.

Aside from the DNA cloning work, a more complex math modeling system was developed in order to create a more realistic simulation. This simulation was created to be interactive and is on the URL listed above. Our group developed more thorough differential equations to realistically model the behavior of unsynchronized, as well as synchronized cells. And also, our group use the MatLab program in order to move away from a theoretical approach and then create a stochastic model.

Assignments

As we progressed through the semester, it became more useful to assign specific reactions in the lab by week. Below is an example Excel worksheet from a week in February. It became more useful to install and utilize a whiteboard in our lab for organizing the work for each module. In the latter part of the semester, teams consisting of three people were specifically devoted to one module.

Organizing the cloning work in such a manner allowed the team the freedom to set up other teams to work on specific projects within the Ipro. For instance, we had a team that worked on the synchronization, another that worked on the simulation, one team brainstormed future projects for the Ipro, etc.

Figure 3: Sample Assignment Sheet

Template	Primers	Instructions	Where	Due Date	Done By
004/027(pR_laci_t7)	004/027	reamplify with GC and HF Buffers in 2 60 uL Reactions	template: 004/27 in C2 Primers: Primer Box	2/23/2006	
004/108 & 007/027	004/027	Redo the phusion rxn in 1, 4-tube, 60 uL rxn	template: 004/108 in B3 007/027 in A3 Primers: Primer Box	2/23/2006	
pLlac01	007/104	Regular 60 uL rxn	Template: pL in F9 of templates box Primers: Primer Box	2/24/2006	
pEYFP-nuc	103/108	Regular 60 uL rxn	Template: ???? Primers: Primer Box	2/24/2006	
7/104 & 103/108	7/108	Regular 60 uL rxn	From previous 2 rxns	2/25/2006	
007/108&107/027	007/27	Regular 60 uL rxn	007/027 in A3 other from previous rxn	2/26/2006	

Obstacles

This Ipro had its share of challenges. Along with the normal challenges that come with the Ipro program, this Ipro also had the added obstacle of technical difficulty. Major setbacks, such as the failure of last semester's Ipro team to freeze all of the gene modules and the mutation of TWO of our gene promoters in TWO of the completed modules, did not let us fully complete the construction of the plasmids. However, real progress was made in the synchronization, simulation, and development teams. Their progress can be evidenced on our website. Yet, in spite of these annoying and trying obstacles, our team persevered.

Results

Our team has reached the point where a summer Ipro team could quickly and efficiently finish the original circuit. Also, with the advances in simulation and synchronization, a synchronized plasmid system could be created in no time. With the particular advances of the development team, our group is poised to make the leap into eukaryotic cell culture and could possibly partner with a corporation to make some useful or beneficial product. Overall, our team has either advanced significantly.

Recommendations

It is the recommendation of this Ipro member that a system of documenting cloning work be organized and implemented early on in the next semester of work in order to efficiently communicate results and speed everything up. Also, I feel that the next Ipro team must be diligent in updating procedures documents, organizing lab supplies, and endeavoring to push or MatLab modeling, synchronization schemes, Zebrafish tissue cultures, and other adventures in science to the next level.

References

Modeling a synthetic multicellular clock:
Repressilators coupled by quorum sensing
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http://www.nature.com/nature/comics/syntheticbiologycomic/index.html

Acknowledgements

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