Final Report

December 3, 2005

Ipro 302

Synthetic Biology: Engineering a Novel Organism

Project Team

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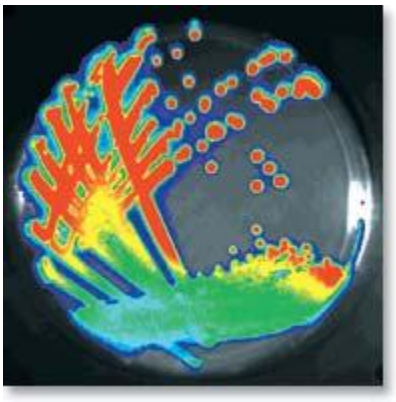
Project Website: http://omega.cs.iit.edu/~ipro302/



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Introduction

If you could understand what makes a living organism function and utilize that knowledge to help you change that organism for the benefit of mankind, would you do it? The members of this inter-professional project seized this opportunity. We are seeking to implement new, observable traits into bacteria, with the hopes of one day branching out to higher organisms. Such ability will have great ramifications upon the field of medicine and the areas of bio-sensing, bio-remediation, and the field of genetic engineering as a whole. In short, we are using the incredible wealth of genetic knowledge that has arisen in the last few years to create a new thing under the sun.

Background

All living things are composed of cells. Proteins control the main structure and function of cells. Proteins are encoded for by information containing compounds known as nucleic acids. Of particular importance is the polymer Deoxyribonucleic acid (DNA), which codes for messenger ribonucleic acids (mRNA's). DNA is organized into structural units, called genes, which tell the cell how to put together amino acids to form proteins. Thus, the "genetic code" is transferred from the DNA to the protein via the translation of mRNA. It is this process (the central dogma of molecular biology) that we are seeking to manipulate. If we can learn to speak the language of the genetic code fluently, then the possibilities of advancement in medicine, gene therapy and the pharmaceutical industry will be endless. At this point however, we are only in the stage of writing paragraphs. It is the dream of the members of this Ipro to one write entire novels (create entirely new and useful organisms from scratch).

Purpose

The primary objective of this project is to master techniques of genetic manipulation and utilize that mastery to create new and exciting organisms. Integrating cross-disciplinary knowledge, we seek to delve deeper into the mysteries of gene regulation and protein expression. We therefore dedicated most of our resources towards the development of novel *E. coli* bacteria would perform a certain programmed function. In this sense, we are treating metabolic pathways much like electronic circuits, in that we are rearranging certain "devices" to execute a previously unseen function. Specifically, we are expressing three different proteins in an oscillatory manner. Also, we are incorporating a "reporting" system that will enable us to see these oscillations via fluorescent proteins. Concurrently, we are modeling a system that will coordinate these oscillations, and hence, create entire bacterial colonies to flash pretty colors. This is one more step towards our ultimate goal of synthesizing a brand new organism. Thus, once we perfect this coordinated oscillation, we can apply similar systems to eukaryotic (multi-cellular) creatures. And so, we will seek to modify Zebrafish (*Danio rerio*) to flash pretty colors next semester.

Research Methodology

This project, in true inter-professional fashion, utilized strategic planning based on mathematical modeling. The strategic plan was then carried out using extensive laboratory techniques. The work in prior semesters has been the development of this model based on differential equations and the isolation of the genes that we wish to use. The bulk of the work this semester has been to construct the actual plasmids that will tell the *E. coli* cells when to oscillate, insert them into the cells, and create a new mathematical model in order to coordinate entire colonies to oscillate in unison. In order to construct the plasmids, we had to abandon our previous way of doing things. This was certainly a paradigm shift from standard operating procedures of modern genetic engineering. Last semester, the group attempted the standard "cut and paste" methodology, which used restriction enzymes (genetic scissors) and ligase (genetic paste) in order to put the desired plasmids together (see Fig. 1). Yet, we borrowed a modified form of the technology used in DNA fingerprinting for criminal investigation. DNA fingerprinting is made possible through the Polymerase Chain Reaction (PCR) technique. Essentially, billions of copies of the DNA of interest are made using this technique (see fig. 2). This process in commonly referred to as amplification.

So, what we did this semester was kill to metaphorical birds with one hypothetical stone. Phusion PCR (see Fig. 3) is a variation of normal PCR which uses tags (sticky ends) of DNA to latch onto billions of copy of each gene of interest. Then the two (or three) genes are amplified together and each complementary tag recognizes its mate and sticks to each other, effectively fusing the genes together.

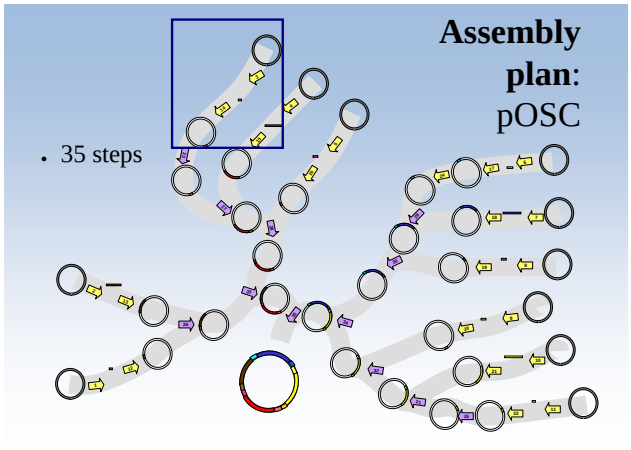

Fig 1: Last Semester's cockamamie plan

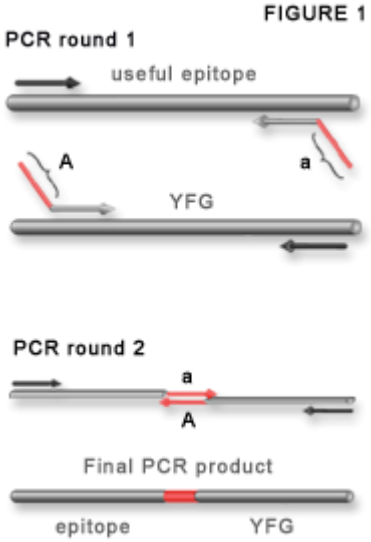

Fig.3: This semester's plan

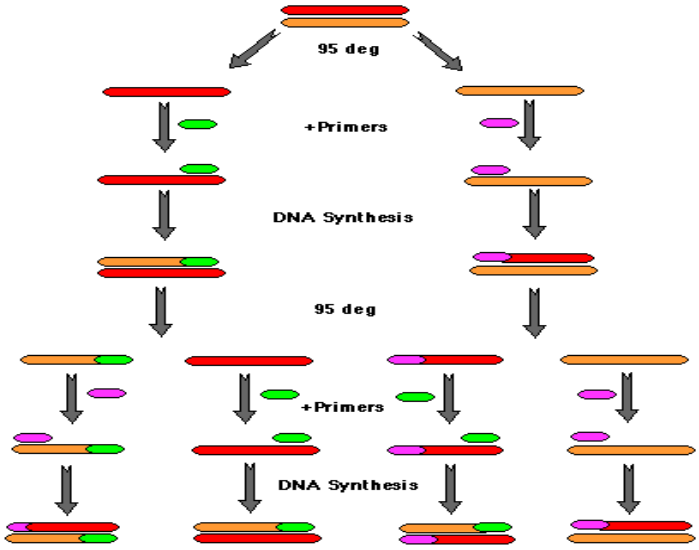

Fig. 2: Normal PCR

Assignments

The methods of assignments changed halfway through the semester. At first we split the team up into partners. Each pair of partners was given a gene module that they were responsible for assembling. A copy of our gene module assignments and associated reactions need to build each one is given below.

piece	reaction #	forward primer	reverse primer	template 1	template 2	team
pLtet/cI/t7	rxn1	ipro302**036**	ipro3021**10**	**pLtet**	**None**	Rickardo & Lily
pLtet/cI/t7	rxn2	ipro3021**09**	irpo3021**12**	**cI**	**None**	Rickardo & Lily
pLtet/cI/t7	rxn3	ipro302**036**	irpo3021**12**	**rxn1**	**Rxn2**	Rickardo & Lily
pLtet/cI/t7	rxn4	ipro3021**11**	ipro302**021**	**t7 (Maggie)**	**None**	Rickardo & Lily
pLtet/cI/t7	rxn5	ipro302**036**	ipro302**021**	**rxn3**	**Rxn4**	Rickardo & Lily
pR/lacI/t7	rxn1	ipro302**004**	ipro3021**14**	**pR**	**None**	Ricot & Heather
pR/lacI/t7	rxn2	ipro3021**13**	irpo3021**16**	**lacI**	**None**	Ricot & Heather
pR/lacI/t7	rxn3	ipro302**004**	irpo3021**16**	**rxn1**	**Rxn2**	Ricot & Heather
pR/lacI/t7	rxn4	ipro3021**15**	ipro302**024**	**t7 (6/24)**	**None**	Ricot & Heather
pR/lacI/t7	rxn5	ipro302**004**	ipro302**024**	**rxn3**	**Rxn4**	Ricot & Heather
pLlac/Rtet/t7	rxn1	ipro302**007**	ipro3021**18**	**pLlac (T)**	**None**	Kaylyn & Bryan
pLlac/Rtet/t7	rxn2	ipro3021**17**	ipro3021**20**	**Rtet**	**None**	Kaylyn & Bryan
pLlac/Rtet/t7	rxn3	ipro302**007**	ipro3021**20**	**rxn1**	**Rxn2**	Kaylyn & Bryan
pLlac/Rtet/t7	rxn4	ipro3021**19**	ipro302**027**	**t7 (9/27)**	**None**	Kaylyn & Bryan
pLlac/Rtet/t7	rxn5	ipro302**007**	ipro302**027**	**rxn3**	**Rxn4**	Kaylyn & Bryan
pLtet/CFP/t7	rxn1	ipro302**036**	ipro30210**2**	**pLtet**	**None**	Zdravka & Emad
pLtet/CFP/t7	rxn2	ipro30210**1**	ipro30210**6**	**CFP**	**None**	Zdravka & Emad
pLtet/CFP/t7	rxn3	ipro302**036**	ipro30210**6**	**rxn1**	**Rxn2**	Zdravka & Emad
pLtet/CFP/t7	rxn4	ipro30210**5**	ipro302**024**	**t7 (6/24)**	**None**	Zdravka & Emad
pLtet/CFP/t7	rxn5	ipro302**036**	ipro302**024**	**rxn3**	**Rxn4**	Zdravka & Emad
pLlac/YFP/AspA	rxn1	ipro302**007**	ipro30210**4**	**pLlac (T)**	**None**	Hoa & Emily
pLlac/YFP/AspA	rxn2	ipro30210**3**	ipro30210**8**	**YFP**	**None**	Hoa & Emily

pLlac/YFP/AspA	rxn3	ipro302**007**	ipro30210**8**	**rxn1**	**Rxn2**	Hoa & Emily
pLlac/YFP/AspA	rxn4	ipro30210**7**	ipro302**032**	**AspA**	**None**	Hoa & Emily
pLlac/YFP/AspA	rxn5	ipro302**007**	ipro302**032**	**rxn3**	**Rxn4**	Hoa & Emily
pLlac/GFP	rxn1	ipro302**037**	ipro3021**22**	**pLlac (Hoa)**	**None**	
pLlac/GFP	rxn2	ipro3021**21**	ipro302**043**	**GFP**	**None**	
pLlac/GFP	rxn3	ipro302**037**	ipro302**043**	**rxn1**	**Rxn2**	

Yet, after these gene modules were constructed, the team found that it was much more useful and efficient to depart from this method of assignment. Instead, at the team meetings each week, our team leader (Hoa Nguyen) would type up an agenda and a list of things that needed to be done and each member of the team would volunteer for specific tasks. This method required a lot more open communication and technical savvy, but it proved to be extremely efficient. As a whole, it allowed us to become more cohesive and practice the fine art of teamwork.

Obstacles

This project faced several major challenges. First of all, due to the technical nature of this ipro, it took several weeks for all of the team members to become skilled in the laboratory techniques. Once this hurdle was overcome, however, there were other ominous threats to our team. Early in the semester, communication was an issue and continued to be one until mid-October. Each team member, however, developed a rhythm and a personal connection with the rest of the team and communication became less of an issue. Even though communications improved drastically, still other problems lurked. Some of the gene modules were particularly difficult to construct. Specifically, the pLlac/tetR/T7 module was a unique challenge. Also, various modules were stubborn, in that they were hard to fuse into the overall plasmids. It was this stubbornness, along with time constraints that prevented us from finishing this project. Also, a slew of minor setbacks, such as running out of restriction enzymes, faulty electrophoresis gels, and human errors had to be dealt with on a weekly basis. In spite of the difficulty, Ipro 302 team diligently remained dedicated to the project and each other, so much so that I would say that this is the best project team I have ever worked with.

Results

Our team, thanks to the effective leadership skills of our team leaders, the willingness of our members to communicate and work together, the implementation of a new plasmid construction plan and the continual persistence of our drive to take synthetic biology to a new level, we have completed roughly 80% of our project. The first two semester, I would estimate that the project only reached 20% completion, due to the time consuming nature of previous construction methods described above (see Research Methodology). So, in one semester, we have TRIPLED our productivity. I cannot emphasize how much phusion PCR and good team work help this project along. The bacteria do not oscillate colors, as of yet, but we have got different colonies of bacteria to glow different colors (see Fig. 4, 5, 6). Because of these encouraging results, we are planning on implementing a similar oscillatory system in eukaryotic systems. So, next semester we are planning on finishing our *E. coli* oscillator, synchronize the colony oscillations, and converting this system into Zebrafish (*Danio rerio*).

Fig. 4

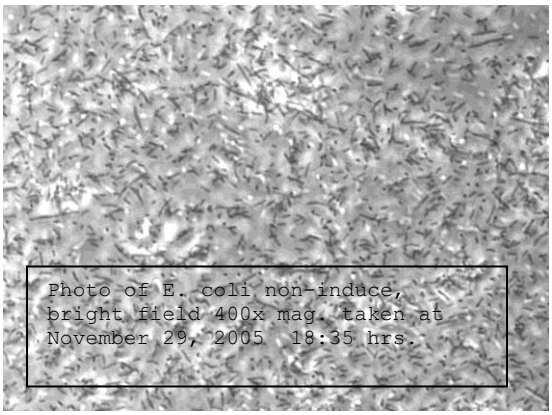

Fig. 5

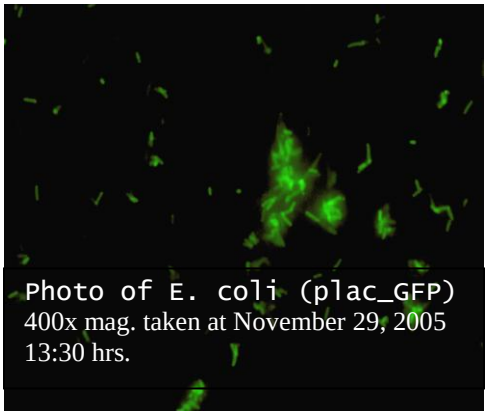

Fig. 6

Recommendations

Because of the problems we faced this semester it is recommended that all incoming team members next semester read up on the literature involving this Ipro. Also, it is recommended that the returning members would take about a week of time at the beginning of next semester and give the new members a sort of orientation. This orientation would include spending time in the lab showing them lab procedures, as well as elucidating protocols with them. Also, extreme care should be taken when labeling our frozen gene bank from this semester.

References

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

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