IPRO-302: Synthetic Biology

Introduction:

Synthetic biology is a relatively new approach to genetic research where the focus is to build a simple genetic system with known processes and test it in a biological setting. This is in contrast to the reductionist approach where a complex pre-existing biological system is analyzed in an attempt to discover the purpose of each of the parts. In this IPRO, a very simple genetic system is being designed, built, and tested in a biological setting. Previous semesters have already accomplished such in bacteria. This semester's focus will be on building the system designed last semester and inserting it into an eukaryotic organism; the zebrafish.

The main purpose of this IPRO is to advance the knowledge of biological systems and their manipulation. This is being done through the design and creation of a simple, but novel, genetic circuit, and the introduction of this circuit into an organism to observe its properties *in vivo*. In order to accomplish this goal, we have split up into 4 different sub-teams: Fish, Math Modeling, Futures and Design, and Construction.

Objectives:

Construction:

Members: Corina Sandulescu (co-leader), Christopher Ruszczak (co-leader), Natalia Ervin, Soo Lim, Matthew Heller (co-leader), Greggory Kisiel, Shubhi Sharma, Sunil Vasireddi

The purpose for the Construction sub-team is to actually build the genetic circuit (Oscillator) that was designed last semester. This circuit is the product that this IPRO is built around. It is a 3-part genetic system. The 3 parts have been nicknamed Rock, Paper, and Scissors, in reference to the simple children's game. This reference is due to the systems similarity to the concept of the game. Each part of the system is turned off by the part previous to it, because it contains an inhibitor that is activated by the protein produced by the part before it. So when the Rock gene is on, the Scissors gene is turned off, which allows the Paper gene to be on. This turns off the Rock gene, which then allows the Scissors gene to be expressed, turning off the Paper gene. This cycle will theoretically continue indefinitely, and hopefully follow the pattern predicted by the Math Model, which will be discussed later. In order to observe the oscillations between Rock, Paper, and Scissors, each gene was linked to another gene that produces a fluorescent protein. So, every time one of the genes is on, the cell will glow a corresponding color in UV light, which makes it easier to observe the cycles.

This system will be constructed using genes taken from other systems that are already in existence. So the genes that produce protein Rock or protein Paper already exist, but combined with different combinations of genes. The first task is

to isolate all of the necessary genes. This is done by taking a plasmid (circular section of DNA) with numerous genes and using Polymerase Chain Reaction (PCR) to create millions of copies of only the gene we want. After all the necessary genes have been separated and amplified in large quantities, they will then be put together in the desired sequence. This will be done using Fusion PCR, which basically links the ends of 2 specific genes and amplifies the resulting combination. This will be done for Rock, Paper, and Scissors, at which point a ligation procedure will be done to link all 3 of the segments into one system.

In addition to creating the Oscillator, the Construction team will also be researching a way to synchronize the fluctuations of the system in multiple cells. Without such a system, it is likely that once the Oscillator is in the zebrafish, the cells will oscillate at different rates, expressing different colors for Rock, Paper, and Scissors. This makes it very difficult to actually observe the changes as the circuit progresses. Once a way to synchronize the cells has been found, the necessary components will be inserted into the system.

Fish:

Members: Shubhi Sharma, Jeffrey Lin (leader), Natalia Ervin, Bennett Ingvoldstad

The Fish sub-team exists to maintain and breed zebrafish (*Danio rerio*). The zebrafish are vital to the project because they will be used as the hosts for the genetic circuit once it has been constructed. In order to further the IPRO's objective, the fish team has 4 main goals: maintenance of adult fish, fostering of larval fish, microinjection of eggs, and standardization of care.

To maintain the adult fish, which were obtained from the University of Chicago, there are both daily and weekly tasks that must be performed. The daily tasks include feeding and making brine shrimp, while the weekly tasks are changing the tank water and breeding the adults.

Feeding is done twice a day, once in the morning and once in the evening. A new batch of brine shrimp, which are used to feed the fish, is made once every morning. Once weekly, a portion of the water is changed out. This is done in order keep the water relatively clean but to also allow for the maintenance of an stable microorganism environment in the tank to prevent proliferation of harmful bacteria in the tank water. The tank being used, hereafter referred to as the fish hotel, is actually a structure containing numerous separate removable compartments for the fish. It also has a multi-level filtration system and a UV sterilizer. Finally, the fish are put together to breed once a week in the evening, and the eggs are collected in the morning.

In order to foster the growth of the larval fish hatched from the eggs, there are also daily and weekly tasks that must be done. The daily task is feeding, and the weekly tasks are changing the water, growing paramecium, and visually inspecting the larvae for growth.

Feeding for the larvae is done once daily. They are fed paramecium, a culture of which must be made weekly to ensure a steady supply. The water also

must be changed weekly to prevent accumulation of waste products and proliferation of harmful microorganisms, since the tank that the larvae are kept in is not circulated or filtered due to the delicate nature of the young fish. Also, they are weekly inspected for growth under a microscope, in order to ascertain both their level of development and their general health. After 21 days, the larvae are ready to be moved to the fish hotel, and soon after are large enough to be fed brine shrimp. They become breeding adults at around 10-12 weeks.

Finally, there is the eventual goal of microinjection of the eggs gathered from the breeding adults. Microinjection is a technique that is used in order to incorporate the genetic circuit into the fishes' genome simply by injecting a DNA sample directly into the nucleus of the egg. Since microinjection is a rather difficult task and has a low success rate due to the small scale on which it is performed, practice is done on eggs with a standard gene for fluorescence, so that success is indicated by the fish cells glowing a certain color. Once the genetic circuit has been finished, it will be injected into numerous eggs.

Since the fish are a relatively recent addition to this IPRO, procedures for care must be standardized and optimized. Because zebrafish are a well known and documented organism, research is done by a member of the sub-team on a certain technique, and the knowledge is distributed to the rest of the sub-team. The technique is then incorporated into the system and evaluated to discover if it is necessary or an improvement on the current procedures.

Math Modeling:

Members: Kyle Carlton (leader), Sunil Vasireddi, Bennett Ingvoldstad

The Math Modeling sub-team was created in order to develop a computer model to simulate the progression of the genetic circuit in an organism. This semester, the objective is integrate the C++ and MatLab portions of the program developed previously into a purely MatLab format, then to make it work faster for multiple cells, and finally to develop an easily-read visual representation of the data. It is necessary for multiple cells to be able to be simulated simultaneously because in a zebrafish, there will be billions of cells with the genetic circuit in them at a time, and those cells will all express it at different rates. The eventual intent of this program is to aid the team in tweaking the genetic system in order to encourage the cells to express it at the same rate.

Futures and Ethics:

Members: Christopher Ruszczak (co-leader), Matthew Heller (co-leader), Shubhi Sharma, and Kyle Carlton

The purpose of the futures and ethics sub-team is to research the possibilities of synthetic biology in the future, and whether or not those avenues of research should be explored. Research will be done on what has already been done with genetic engineering to gain knowledge of previously debated ethical

issues. Then, what is being done and what could be done will be explored and debated. While there are only very minor ethical issues to be discussed about the current project, the possible future applications are both wonderful and frightening. The eventual goal is to find the point where the ends do not justify the means or the possible consequences.

Team:

The overall objective of this IPRO is to create the Oscillator, insert it into the zebrafish, and get it to express itself in a controlled manner.

Plan:

Currently compelted tasks in bold.

Construction:

- **02-16-07: New primers designed and purchased.**
- **02-23-07: 1st round PCRs screeing of genes, revised primers designed if necessary**.
- 03-09-07: individual arms, Rock, Paper, and Scissors assembled by fusion PCR.
- 04-06-07: ligation of arms Rock, Paper, and Scissors to Oscillator into completed.
- 04-13-07: design of triparatite oscillator completed
- 04-13-07: feasibility analysis of hormonal synchronization system completed

Fish:

- **02-04-07: fish breeding protocols developed, batch 1 of babies produced**
- 2-19-07: microinjection of XFP standards; 200 eggs.
- 3-01-07: batch 2 of babies produced.
- 3-30-07: microinjection of Rock and Paper arms; 200 eggs.
- 3-30-07: fish cell culture transformation of Rock and Paper arms.
- 4-15-07: microinjection of Oscillator.

Math Modeling:

- **02-16-07: Develop standard operating environment (software, add-ins, compilers, etc.)**
- 02-28-07: Integrate synch.cpp with MatLab mex function -- i.e. be able to do all necessary processes with MatLab, including changing matrix size/# of frames simulated/type of simulation/etc.
- 03-23-07: Create a MatLab gui so the parameters of the synch function can be changed easily and the simulation can be run.
- 03-31-07: Create an optimal operating environment to quickly analyze the data.
- 04-20-07: Create visualizations and presentation format for data.

Futures and Ethics:

- **02-19-07: Have researched past and current genetics applications.**
- 02-26-07: Have researched genetics in the near future.
- 03-05-07: Have researched predicted possibilities of genetics in the far future.
- 03-19-07: Have researched the Asilomar Conference.
- 03-23-07: Have prepared a rough draft of Futures and Ethics report.
- 04-02-07: Have researched ethics of genetic engineering in agriculture.
- 04-09-07: Have researched ethics of genetic engineering in healthcare.
- 04-16-07: Have prepared a final copy of Futures and Ethics report.

Team Members:

Kyle Carlton:	Math Modeling team - leader Futures and Ethics team Deliverables Officer
Natalia Ervin:	Fish team Construction team
Matthew Heller:	Construction team - co-leader Futures and Ethics team - co-leader
Bennett Ingvoldstad:	Math Modeling team Fish team
Greggory Kisiel:	Construction team Futures and Ethics team
Soo Lim:	Construction team Math Modeling team
Jeffrey Lin:	Fish team - leader Construction team
Christopher Ruszczak:	Construction team - co-leader Futures and Ethics team - co-leader
Corina Sandulescu:	Construction team - co-leader
Shubhi Sharma:	Construction team Fish team Futures and Ethics team
Karol Sobczck:	All Team Leader
Sunil Vasireddi:	Construction team Math Modeling team