IPRO 302

Synthetic Biology

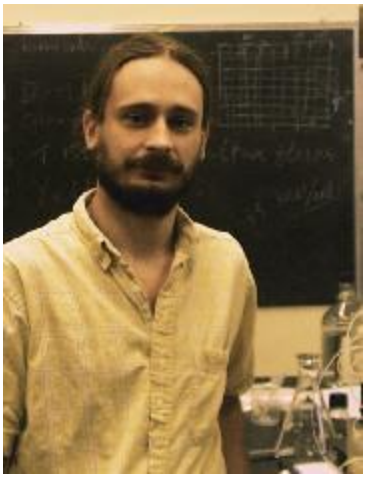
Engineering Novel Organisms

Synthetic biology is the discipline of engineering life. Not engineering things that interact with life, but life itself. But can we in fact design and construct synthetic living organisms? The idea may seem outrageous, but in fact the simplest living organisms have already been synthesized. Rapid progress has been made in the area of molecular biology, biochemistry and genetics, so that we now understand a great deal about the things that make up life. We now know the entire genetic sequence of several organisms - including humans - can thus in principle construct a synthetic human. The barriers, although large, are largely technical, not theoretical.

If indeed, we can now manipulate natural genetic sequences, this raises the possibility of constructing not only copies of existing organisms, but unnatural, designed organisms. Here we are in muddier water - the barriers are not merely technical. Although we can slavishly copy an existing organism, we do not yet have sufficient understanding to construct one de novo. Thus, synthetic biology: the science of how to put all the little bits together, and get them to work together in a living organism. 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.

Sponsor:



Nick Menhart

Assistant Professor of Biology

B.Sc.: University of Waterloo

Ph.D.: University of Waterloo

Office: 358 Life Sciences

Phone: 312.567.3123 **Fax:** 312.567.3494

e-mail: menhart@iit.edu

Professional Interests

My major research interest concern the use of spectroscopic techniques to study multi-domain proteins. In many cases high resolutions structural data is available for only individual domains of proteins, while their arrangement within the context of the entire molecule is unknown. This arrangement is crucial to the function in certain cases.

One of the systems currently under study, plasminogen, is such a case. This is a key enzyme in the haemostatic system, being responsible for the dissolution of blood clots. Treatments for heart attack and stroke, the first and third leading causes of mortality in affluent societies, involve activating plasminogen to its active form, plasmin. However, plasminogen normally circulates in a compact from that is not easily activated. It is known that it can adopt a more open form, for instance on the surface of a fibrin clot, that is much more readily activated.

This open form - closed form transition has been studies by a wide variety of techniques, however detailed structural information has not been forthcoming, since the intact molecule is too large for current NMR instruments, and has so far been refractory to crystallization. However, plasminogen is composed of several constituent units; in addition to its protease domains (~25kDa), plasminogen contains in five other compact domains known as kringles (~10kDa). The structures of most of these domains are known in isolation by either X-ray or NMR studies, or in some cases both. We are attempting to study this transition by placing spectroscopic probes (paramagnetic or fluorescent) on the various domains of plasminogen to try to localize these specific domains and determine their proximity to each other during this transition. By combining distance measurement between these domains, solvent accessibility data for the various surfaces, with the known high resolution structures of each

isolated domain, we hope be able to assemble a model of the structure of the intact molecule in each of its conformational states.

Representative Publications

- "Flexibility of the a-Spectrin N-Terminus by EPR and Fluorescence Polarization", Cherry L., Fung, L. and Menhart N., Biophys. J. 79:526-535 2000.

- "The Roles of Individual Kringle Domains in the Functioning of Positive and Negative Effectors of Human Plasminogen Activation by Urokinase-type Plasminogen Activator", Menhart N., Hoover G.J., McCance S. and Castellino F.J., Biochemistry, 34:1482, 1995.

Math Modeling Sub-Team Final Report

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

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. 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 understanding the circuit progression characteristics and the synchronization characteristics so that one might potentially tweaking the genetic system in order to encourage the cells to express it at the same time.

IPRO 302, Math Modeling Team:

Members:

Kyle J Carlton

4th Year Biomedical Engineer

Individual Focus:

- Project Management
- Visualization

Sunil Vasireddi

3rd Year Biomedical Engineer

Individual Focus:

- GUI Development
- Matlab Development

Ben Ingvoldstad

4th Year Electrical Engineer

Individual Focus:

- C++ and Matlab Integration
- C++ optimization

Group Tasks:

1. Develop standard operating environment (software, add-ins, compilers, etc.)

DUE: Febuary 16th, 2007

Done: Matlab 7.1, Visual Studio 2003 C++ Compiler

Problems Encountered and Solved: Obtaining the correct version and compatibility issues from within Matlab to Visual Studio and between Windows OS and Visual Studio on some systems. Solved by experimenting and testing versions from those already installed in the computer lab to be used as the common ground regardless of issues on other computers.

2. Integrate synch.cpp with matlab mex function -- i.e. we should be able to do everything we need from within matlab, including changing matrix size/ # of frames simulated/ type of simulation/etc.
DUE: Febuary 28th, 2007

Done: The mex function was setup to the selected standard environment and used for interfacing between Matlab and C++. Then the main focus was shifted to using this function to call C++ to compile the synch function which was previously created for modeling the oscillation behavior. Also some select parameters like the modeling time, number of frames and the number of cells, type of simulation were passed from Matlab to the C++ compiler to control the simulation from Matlab. Further coding was needed to return the results of the compilation as cells into Matlab for further analysis in the future. The purpose to use C++ to compile was to able to more efficiently analyze a large number of cells whose effects have been observed after the completion of this phase due to faster processing times.

Problems Encountered and Solved: Although the synch function was assumed to be ready to be used, it had to debugged to eliminate warnings for compilations. Also some of the inefficiencies in coding were rectified; places where there is unnecessary processing. Since the original synch function was crude and simple with many constants that needed to be variables that could be controlled for analysis, the appropriate modifications were made to introduce variables into coding.

3. Create a matlab gui so we can change the parameters of the synch function easily and run the simulation.
DUE: March 23rd, 2007

Working, Final Stages: The initial task for this was to explore and understand the creation and working of GUIs in matlab. Also the GUI developed previously was explored and debugged to gain a better understanding of the GUI itself and the plans of the previous group. After some comfort was gained, a simple GUI was created to take in the four parameters described and plot the results in time with changing frames as an intensity distribution for a defined array size that represents the cells. After discussion it was decided that the visual aspects of presentation of data in the previous GUI would be used in the new GUI and the rest will be discarded as it was an initial simple representation. The format of

the GUI was then discussed and finalized to include tabs for improved visual representation of data within the screen size and also help organization of parameters.

Immediate Goals:

Pass all the parameters, not just the few already included, from the GUI. The coding for this has been done but the control from GUI is still underway.

Include Tabs for changing parameters. The study and process to do this has been mapped out; a crude example with tabs has also been created. The actual creation of tabs with the panels with all the parameters within the GUI needs to be done. This delayed the GUI completion from this week.

Import the data visualization style from the previous GUI and improve on it.

4. Create optimal operating environment to quickly analyze the data.

DUE: March 31st, 2007

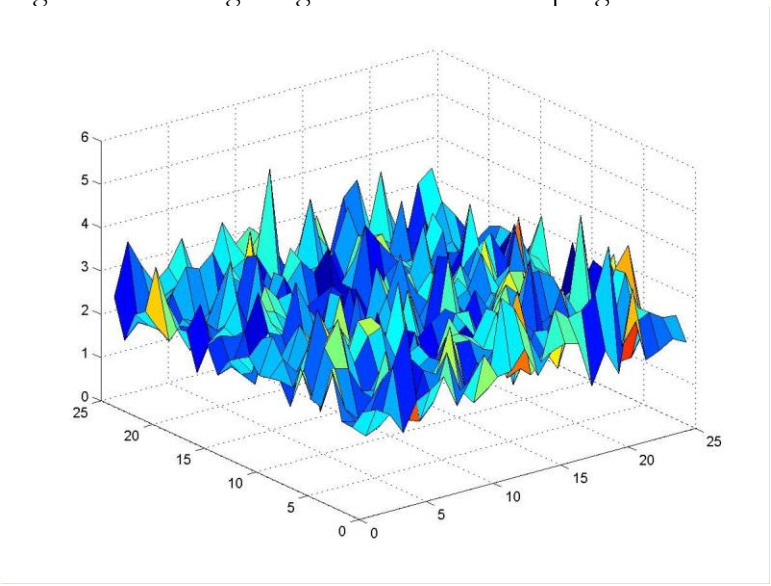
Future Plan: To improve upon the GUI within itself and the processing code to make it more efficient. Improve the environment as a whole and start analysis of data.

5. Create visualizations and presentation format for data.

DUE: April 20th, 2007

Future Plan: Present the data in a appealing and informative manner, through control from GUI, to make analysis easier and visually insightful.

All the planned tasks were completed. The program is working well. Still has a few minor bugs to it that are getting sovled out but the program is well made and easy to use.



This is a graphical image produced from the program. It displays the oscillations in a multi cellular system.

Futures and Ethics Final Report

Members: Greg Kisiel, Shubhi Sharma, Chris Ruszczak, Karol Sobczyk, Soo Lim, Matthew Heller (Leader)

The Futures and Ethics team was created in order to research and discuss the future possibilities of synthetic biology, as well as the ethical issues that currently have evolved or may come up in the future. Through this our goal is to gain a greater understanding of what impact our current research may have on society's future. 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.

IPRO 302, Futures and Ethics Team:

Members:

Greg Kisiel

3rd Year Biomedical Engineering major

Individual Focus:

- Researches topics

Shubhi Sharma

3rd Year Biomedical Engineering major

Individual Focus:

- Researches topics

Chris Ruszczak

3rd Year Molecular Biochemistry and Biophysics major

Individual Focus:

- Researches topics

Karol Sobczyk

3rd Year Molecular Biochemistry and Biophysics major

Individual Focus:

- Researches Topics

Soo Lim

3rd Year Biomedical Engineering major

Individual Focus:

- Researches topics

Matthew Heller

3rd Year Molecular Biochemistry and Biophysics major

Individual Focus:

- Group Leader
- Assigns tasks
- Take minutes
- Researches topics

Group Tasks:

1. The purpose of this group is to discuss future possibilities of synthetic biology, and the ethical implications thereof. This information will then be gathered and presented in a final report.

Done: Several members of the team have conducted research into what has been done in the field of synthetic biology, as well as what is currently being done and what may be done in the future. Then, as a group, we discussed current ethical debates in different areas in biology and how synthetic biology may affect them in the future.

Goals: Our immediate goals are to discuss what was done at the Asilomar Conference on Recombinant DNA and to then compile a rough draft of our final report. After that, our topics of research and discussion will be as follows: genetic engineering in agriculture, genetic engineering in healthcare, and compiling our final copy of the Futures and Ethics report.

Construction Team Final Report

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.

1.0. Revised Objectives

The construction team's objectives have not changed

2.0. Results to Date

1. The oscillator fragment denoted "Scissors" has been completed.
2. The oscillator fragments denoted "Rock" and "Paper" are both two successful fusion PCRs from completion.
3. More GFP has been produced to allow the fish team to practice microinjection.

3.0. Revised Task / Event Schedule

Due to failed procedures the completion of the oscillator fragments has been delayed, this subsequently has caused a delay in the eventual assembly of the oscillator. In addition, the research into the synchronizing gene has been slowed due to the focused effort in obtaining successful laboratory results.

Due to these delays the schedule has been completely changed.

Task	Hours*	Start	Finish
Finish "Rock"	9	3/21	3/25
Finish "Scissors"	9	3/21	3/25
Grow Competent Cells	3	3/25	3/25
Ligation 1	10	3/26	3/30
Ligation 2	10	4/2	4/6
Ligation 3	10	4/6	4/8

*** These times are assuming positive results; reaction failures may delay increase these times radically.**

4.0. Updated Task Assignments and Designation of Roles

Team member Christopher Ruszczak has been designated chief team leader, in order to increase efficiency through the consolidation of responsibility. The designated roles of each team member remains laboratory worked based on a weekly schedule where the work to be done is dictated by the progress of each procedure.

Time	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Morning (8-11am)					Gregg		Matt 10-1
Noon (11-3pm)	Shubhi 11:20-3:00, Gregg, Corina 11:30-7	Shubhi 12:45-3:30	Shubhi 11:20-3:00, Corina 11:30-6:25		Gregg		Chris

Evening (3-9pm)	Matt 3-5, Natalia, Soo 3-6	Gregg	Matt, Natalia, Soo 3-6	Soo		Chris	Chris
Maybes		Chris Nights		Chris Nights		Corina Mornings, Chris at Noon	

5.0. Barriers and Obstacles

1. The planned fusion PCRs for the "Rock" have repeatedly failed; this problem is believed to be due to an error in the purchased DNA sequence dsRED. To circumvent this problem this gene is being replace with CFP, which required that new primers be ordered, and that the GFP component of "Paper" be switched to YFP.

2. The planned fusion PCRs for "Paper" have repeatedly failed; the cause of this problem remains unknown. To combat this problem the assembly order for the fragment was changed in hopes that the error was in part due to the disproportional sizes of the pieces being combined to form the fragment.

3. Not all members of the construction team have been fully briefed on the needed procedures; this is further exasperated by the constraints on the work schedule imposed by the class schedules of each member. The original plan was to have experienced members scheduled to work along side the new members, but this has only been partially successful. Therefore, a more direct scheduling of the "training" procedures will be proposed to the team members who remain unfamiliar this the laboratory techniques.

Fish Team Final Report

Members: Jeffrey Lin (leader), Shubhi Sharma, Gregg Kisiel, Natalia Ervin, Soo Lim, Karol Sobczck

The Fish sub-team was implemented to take care of the fish, breed the fish, and raise the babies to make sure that we have next generations of fish for breeding and injecting. The eggs are the most important part of the fish team towards the project because they will be used to microinject the DNA created by the construction team and the results will speak for themselves.

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, 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.

Members:

Jeffrey Lin

3rd Year Biomedical Engineer

Individual Focus:

- Overseeing the care of the fish and the fish tank
- Planning and scheduling tasks

Shubhi Sharma

3rd Year Biomedical Engineer

Individual Focus:

- Research of methods and fish care
- Fish feeding
- Cleaning fish tank

Gregg Kisiel

3rd Year Biomedical Engineer

Individual Focus:

- Co-Leader of team which makes decisions while Jeff is away
- Research of methods and fish care
- Fish feeding
- Cleaning fish tank

Soo Lim

4th Year Biomedical Engineer

Individual Focus:

- Fish feedings

Natalia Ervin

5th Year Biomedical Engineer

Individual Focus:

- Overall caretaker of the baby fish
- Research for new methods and protocols for fish husbandry
- Fish feeding and brine shrimp making

Karol Sobczck

3rd Year Molecular Biochemistry and Biophysics

Individual Focus:

- Documentation of egg development and photography
- Displaying techniques of fish husbandry to new members
- Overall leadership

Group Tasks:

1. Fish Feeding Schedule

Due: January 31st, 2007

Done: Three times a day everyday feeding schedule has been setup and a process where the group members report the feedings to Jeff, Prof. Menhart, and Gregg is in place.

Problems Encountered: Getting people's schedules in order and going from a two a day feeding schedule to a three a day feeding schedule. These problems arose at the early stages of the process and where simple scheduling issues that was handled quickly.

2. Breeding

Due: February 14th, 2007

Done: There was one successful breeding that produced several eggs and a few babies that are currently being taken care of. Hopefully there will be several more successful breedings that produce many healthy eggs and babies.

Problems Encountered: The fish unwilling to breed and lay eggs. The thought behind this problem was that they were not being fed enough that is why there is a three a day feeding schedule in place now instead of the two a day. Also the setting in which the fish are in did not provide a covered area for the females to lay their eggs, which is why there were plants placed in the tanks with the fish.

3. Microinjections

Due: March 5th, 2007

Behind Schedule: With the first batch of eggs we wanted to make sure we could raise the babies to adulthood without harming them before they were hatched. It was not expected that there would be only one batch of eggs at this point of the experiment. Once there is another batch of eggs microinjections will be done and then the successfulness can be determined.

3. 500 completed Microinjections

Due: April 27th, 2007

Problems Encountered: The fish are again nwilling to breed and lay eggs. The thought behind this problem was that they were not being fed enough that is why there is a three a day feeding schedule in place now instead of the two a day. Also the setting in which the fish are in did not provide a covered area for the females to lay their eggs, which is why there were plants placed in the tanks with the fish.

Additional Key Milestones for the group was completing the following procedures successfully:

Growing Baby Fish

Day 0-3

After eggs hatched, fish larvae are kept in petri dishes, 10 babies per dish. At this age, the larvae do not need to be fed because of the storage nutrients in amniotic fluid. Water is changed preferably every/every other day. HBSS buffer is added to the content of petri dish in ratio 1:10 of HBSS to water. At this age mortality is the highest, but it can be significantly minimized by regular water change. Make sure pink color from HBSS is never turning yellow, which indicates dangerous acidic environment (low pH) for the babies.

Day 4-13

Babies are fed with concentrated paramecia and kept in petri dishes. The water is changed every/every other day. HBSS is added with every water change in the same ratio. If water is not changed regularly the mortality rate can reach 80%. The most optimal amount of paramecia volume needed at a time is 30 drops of paramecia for twenty baby fish in one petri dish.

Day 13-20

Larvae are fed with powdered baby food and brine shrimp. Paramecium is taken out of the diet. One needs to observe under the microscope if the fish larva is big enough to eat the brine shrimp. Larvae are transferred from all petri dishes in a tank with not circulating water. Brine shrimp (~1 ml) and baby powdered are fed. The water is changed regularly preferably every day ten percent of the volume of water in the tank. HBSS is not added. At this age the mortality rate is near zero if water is changed often.

Day 21

Fish is transferred to the adult tanks.

Raising Paramecia

P. multimicronucleatum, very large strain of Paramecium, promotes rapid growth of baby fish (Fig. 1). Assuming we do not have this strain, we add more paramecia culture than described below for preparation of the seed cultures.

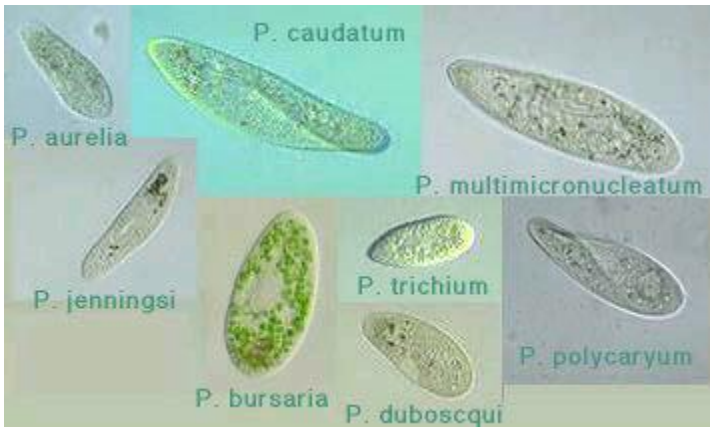


Figure1. Varies species of Paramecium.

Grow seed cultures in 150x25 mm plastic Petri dishes at 28.5 C in the fish hotel room. In addition, grow several small batches of seed cultures in other rooms at room temperatures as a back-up. The cultures in cooler conditions grow slowly and need care only every 4-5 weeks.

I. *Paramecium* Seed Culture:

1. In Petri dish, add 175 ml double distilled water, about 10 boiled wheat seeds, 1/5 of one 7.5 grain standard brewer's yeast tablet bought in GNC store, and approximately 15 ml from a good clean young paramecia culture.
2. Stack the Petri dishes 3 or 4 high and store at 28.5 C in a lighted place. The culture will be ready to use in 2 days and will last for several weeks. One seed culture will start 10 new seed cultures or six 2-liter pots for baby fish feed. One need no more than one liter of paramecia for the amount of babies below 100.

It is a good practice to look under the microscope to make sure there are paramecia in the drop of water from the seed culture when it is ready to use.

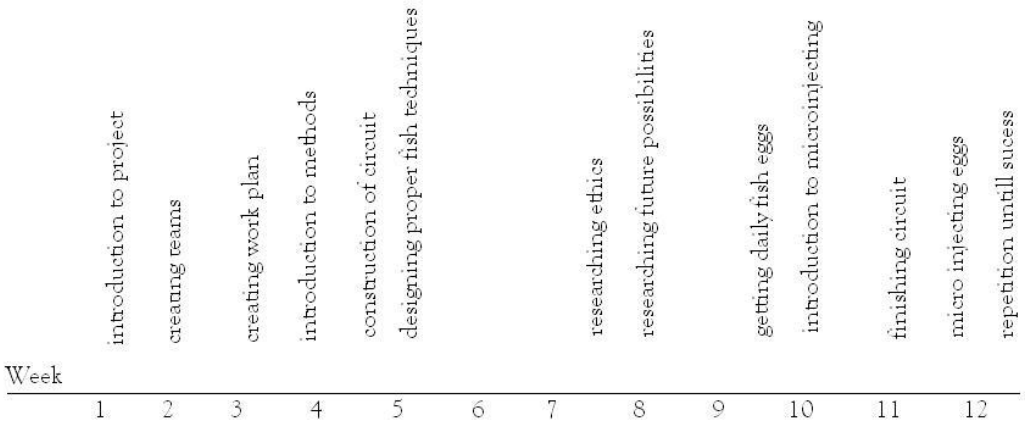
II. Growing Small Batches of *Paramecia:*

After we have a seed culture the batches of Paramecia should be prepared. They last longer and get enough Paramecia concentration only in several weeks.

1. Use glass container of a cube shape of 500 ml volume. Any glassy container of similar volume can be used for this purpose.
2. Add 350 ml of system water.

3. Boil 10 grains of weed for ten minutes. One can easily boil them in a microwave in appropriate container with enough volume of water in order to keep grains covered with water to make them soft and not dry if water evaporates.
4. To the glass container add 8-9 grains of boiled wheat, 1/5 of one 7.5 grain brewer's yeast tablet, and 8 ml of paramecia seed culture
5. Cover, and store at 28.5C on well-lit shelves. The batch is ready to use in two weeks from now.
6. After 14-days, the paramecia are ready to feed to the fish larvae. Cultures remain useable and healthy for 3 weeks or more. Under the light microscope, make sure you have paramecia before the larvae feeding.

Additional Information



Expected Results:

The only deliverables possible to be sent are peer evalvs, reflections, and team minutes. The rest of the project key milestones and achievements are work based.

Key Milestones:

Getting regular fish egg production, completing the genetic circuit, successfully modeling oscillations via computers, and successfully injecting fish eggs with a resulting genetically modified organism.

Budget:

We were given a smaller budget this year. Most of the money has gone to ordering DNA construction parts, new tools for the lab, fish, and to some team building activities. We did not deal with the budget directly, but went through our project sponsor, Prof. Menhart.

Performance Metrics Data Gathering & Documentation Tools/Processes

Meeting minutes:

Meeting minutes were assigned to a certain person each week. Too many separate positions had to be filled for one person to be completely in charge of uploading minutes.

Weekly time sheets were made and used, but not organized and summarized for the final report. Unfortunately all our time goes into work towards our project and we don't have

proper time to summarize. If we were working in a actual professional environment, we would have more work time to analyze this.

Weekly tasks list are not created as there is a complete overall task process already assigned to everyone.

The iGroup account was maintained each week by whoever was assigned to upload minutes for that week.