Research Synopsis Exemplars

Exemplar #1:

Title: Starting the C. elegans DNA extraction protocol

Monday:

While I was preparing for the class, I decided to get my science fair forms signed off by you. Once I gave my forms out to you to sign, I wanted to make some more agarose, 0.7%, in case I might need some more in the future. After making the agarose, I checked over the OP50 E. Coli inoculated plates, that were left for over two weeks, to see if there is any contamination. The process of making an additional 0.7% agarose gel took about 20 minutes to finish and after doing that and checking over the plates, I wanted to culture some more NGM agar plates in order to make a fresh set for the C. elegans. Inoculating the NGM Agar plates took the majority of the class period all the way to the end of the class time. I also got my Science Fair Forms signed off by you.

Tuesday:

The first thing I did today was preparing my materials for my DNA extraction of the C. elegans. I was just informed that my C. elegans might be here today; if not, tomorrow would be the latest time for the delivery. My cultured NGM inoculated with E. Coli OP50 are considered successful and fresh to be used for the C. elegans either today or tomorrow. This lets me have an accumulated stack of 8 plates that are ready to have C. elegans "chunked" inside. After parafilming the two new inoculated plates, I checked over my 0.7% agarose and decided that I can cast two gel molds. This took about the majority of the class period to do which was about 48 minutes just to heat and fully cool off the molds. The rest of the class period was then spent on cleaning up my lab station from the gel casts I made.

Wednesday:

Today, my C. elegans should have arrived today which means that I can start "chunking" out the new stock into 4 of my inoculated plates. However, my package is not in the classroom yet so I will have to do that later during the class period. What I can do right now is to pick the plates I can use for the "chunks" of C. elegans as well as double checking materials to make sure I have them. After doing that, I helped out one of my classmates racking up some pipet tips in order to use my class time wisely. Apparently, I was informed by you that you couldn't find my package for my C. elegans so it will take time to find it. I also decided to make another set of NGM Agar because I am almost running out of NGM stock plates and this was spent for the majority of the class period. The remaining class time was spent on autoclave some more equipment to restock on some sterile supplies.

Thursday:

My C. elegans have officially arrived today which means I can chunk out four out of 8 of the inoculated E. Coli OP50 plates. This took about 15 minutes to remove the packaging, "chunk" out the four plates, and leave it at room temperature in order for the C. elegans to effectively thrive and prosper. As a reminder plates 91 and 96 are cultured two days ago while plates 85 and 100 were cultured two weeks ago. The lifespan of these four plates should last about 2 - 3 weeks before it is needed to be "chunked" out into another before, depending on the contamination and condition of the plate. After chunking out the plates, I forgot to turn on the autoclave yesterday so I decided to put some more material, inside before

turning it on, which took about 30 minutes to do. I decided to prepare two conical tubes for DNA extraction that will start next week in order for it to be put for convenience. This was spent for a few minutes, the majority of class all the way to the end of the period was spent on going over the STELA protocol in order for me to be prepared.

Friday:
My first priority for today is to check if the C. elegans growing in plates 85, 91, 96, and 100 and are also thriving. Plates #85 and #91 have small populations, plate #100 has a medium size population of C. elegans for the past 24 hours. This means that my plates still have E. Coli OP50 that are useful for the C. elegans from the past two weeks but with varying size of populations of C. elegans. The original plate or the stock plate still has a multitude of C. elegans and it will be expected to last for about 1 - 2 weeks depending on proper maintenance of limiting contaminants. I then continued making for NGM Agar plates for the majority of the class period which took about 40 minutes to do. The rest of class was spent on cleaning up, cooling down the NGM Agar plates, and parafilming the NGM plates for a few minutes.

Note: I did not contact any professionals that could help me with my research project.

The goal for next week: Start extracting the C. elegans DNA if ever possible for this week.
eDNA Metabarcdoing

Exemplar #2:

Title: eDNA Metabarcdoing

This week was spent finishing filtration and extraction, ensuring that DNA was present in all of the new samples, and starting new rounds of PCR and gel electrophoresis.

Monday: We set up the filtration equipment to filter the fourth and final water sample from Raccoon Creek. We followed the usual filtration protocol and we each took turns using the drill. Near the end of class, I made the lysing solution of 360 microliters of ATL buffer and 40 microliters of Proteinase K. The filter then went into this solution and was placed onto a heat block to sit for the next few hours. We also prepared all of our materials to be autoclaved.

Tuesday: We got out the Qiagen DNeasy materials and protocol to do extraction on the lysing solution that was made yesterday. We first spun out any liquid from the filter and then followed the protocol starting at step three. When the entire process was complete, the new DNA was placed into the fridge to sit until further use. We then made new 1X TAE buffer and 1.5% TAE gel. The gel was not able to be poured though, because we did not have enough time left in class.

Wednesday: I remelted the 1.5% TAE gel and then poured them into 7 molds. Once they were solid, we put one of them into a gel chamber along with 1X TAE buffer in order to do gel electrophoresis. We decided to run a DNA smear to ensure that the extraction was done correctly and there was actually DNA in the samples. The gel ran on 100 volts for 30 minutes. While my group member finished the set up for the DNA smear, I labeled the plastic bags and put away all of the finished gels. I then worked on my research notebook until the end of class. The gel was lastly put into 1X Fast Blast to rock overnight.

Thursday: We replaced the Fast Blast with water and then let it rock for around 15 minutes in order for it to destain. It was then imaged and bands could be seen, meaning that there is in fact DNA in the samples. The gel image is below. I then started doing PCR with the MOL16S primers. I used the usual PCR protocol and the DNA to primer ratio was 2.5 microliters of DNA to 1 microliter of each primer. The reactions were then run on the same Thermal Cycler setting that I had already programmed. While the PCR was going, I continued to work on my lab notebook. The PCR was then placed into the fridge to sit until the next day.

ER, MW 6 Dec DNA Smear.scn

Friday: I took out the PCR that was done on Thursday and used it to do gel electrophoresis. I used 1X TAE buffer and a 1.5% TAE gel. The gel ran on 100 volts for 50 minutes. While the gel was running, I continued to work on my lab notebook. After it was complete, it was placed into 1X Fast Blast to rock until the end of the day. I then came back once school ended and imaged the gel. No bands could be seen except for those belonging to the standard.

I do not have any new links or contacts.

Next week, I plan to continue to do more PCR and gel electrophoresis using the new Raccoon Creek and DNA samples and the primers that I ordered during this semester. I also plan to work on finishing my entries in my lab notebook and completing all of the required research materials for the final.