SAE Information - Exploring the mutation D373V on RBM10

Name:	Exploring the mutation D373V on RBM10
SAE Type:	Research/Experimentation
AFNR Pathway:	Biotechnology Systems
SAE Subcategory:	Genetic Engineering
Date Range:	3/18/2017 - 3/19/2018

Scope - Exploring the mutation D373V on RBM10

Year	Description
2017	This year I planned to learn how to properly utilize all the equipment in our bio-med lab. I planned on learning how to properly dispose of bio-hazards, sharps, and materials in order to keep the lab safe.
2018	As the year progressed I successfully learned how to dispose of bio-hazards in the proper bag without it overflowing, how to safely dispense sharps and glass in there respective containers, and how to properly dispose of possible carcinogens such as ethidum bromide. Furthermore I have also learned different lab experiments such as miniprepping to extract DNA from E.coli colonies and gel electrophoresis following a digest to show bands of DNA after cutting a sequence with enzymes.

SAE Plan - Exploring the mutation D373V on RBM10

Description

RBM10 has been prone to missense mutations that affect its RNA binding protein and splicing. The effects of these missense mutations are associated with lung Adenocarcinoma (LUAD), the cancer with the leading amount of deaths, due to a lowered frequency of RNA expression. RBM10 is located on the x chromosome, and has been proven to have a higher frequency of mutations in men than in women. RBM10 has also been linked to cell apoptosis, stopping LUAD expansion, and exon skipping. D373V is a missense mutation on RBM10 located on the RRM2 complex (RNA Recognition Motif) This mutation changes from a glutamic acid, to a valine which changes from a charged R group to a nonpolar R group. This mutation is predicted to affect RNA expression as it is in a RRM protein domain. This domain regulates RNA binding and therefore a mutation here would remove RNA regulations.. To research the effects of the D373V mutation, the mutation will be recreated in a cDNA in a plasmid vector with quick change mutagenesis. The protein will then have a small protein tag added to the end of its amino acid sequence, so that the protein tag will be detectable under fluorescent lighting after being stained with fluorescent antibodies. This will allow for the results of the mutagenesis to be seen, as it will become visible where in the cell the mutated proteins have taken form.

The purpose of this study is to determine the effects of the mutation D373V and whether it leads to cancer, apoptosis, or undesirable cell affects due to the change in polarity of the R group.

Time Investment

This project will be extensive, and take multiple hours. I have reviewed other similar research done by previous students at Bowie High School and this will typically span between 1 and 2 school years. I plan to spend 2 hours every other day to develop my project in our Schools Bio Safety Level 1 lab. I will also plan on coming before school or after school when it is needed for time consuming experiments such as transformations or picking colonies or restriction digests. Finally I may have to travel to the University of Texas occasionally to use their lab equipment as well.

Financial Investment

Potential funding for my project is paid for through the Student Research Initiative at UT and tax payer dollars. All of the materials I am using are made available to me through my research class and lab at school.

Learning Objectives (SAE Skills)

From my project, I hope to gain knowledge and skills in BS.02.01.01.a Maintain a biotechnology laboratory notebook, BS.02.04.01.b Prepare buffers, reagents, solutions and media, BS.02.05.03.a Extract and purify DNA and RNA, and BS.02.05.03.b Perform electrophoretic techniques and interpret electrophoresis fragmentation patterns.

To obtain these learning outcomes I will be expected to document all of my research daily into a laboratory notebook. This will contain protocols, expected outcomes, reagants, outcomes, and data analysis. I will also be handling bufferes and enzymes such as CutSmart, DPN1, XBA1, MgCl, Loading Dye, and LB and SOC bacteria media. I will learn the different ways to handle each of thes buffers, reagants, and solutions/media. I will also miniprep to extract DNA from transformed e.coli clones. Finally I will perform multiple electrophoretic gels after pouring agarose gels to view the results of my restriction digests and gel purifications.

Skill Area	Planned Activities	Results or Outcome

Journal - Experience-related Activity - Exploring the mutation D373V on RBM10

Date	Activity / Description	Hours
3/19/2018	**Conducting activities in Biotechnology** Today I poured an analytical gel in order to run my miniprepped ligations on a gel to screen them for the correct RB10 cDNA size. I left my gel running at 50V in order for them to continue for a few hours. I will analyze the results of this gel later.	3.00
3/17/2018	**Performing laboratory skills** Today I miniprepped the 6 colonies some friends picked for me yesterday from my transformed plate. I got high concentrations, one was 500ng/ul. This is awesome. My next step will be to do an analytical digest on all six concentrations in order to see if each of them has the write DNA size. If they do not I will throw them out and only work with the ones that do. Hopefully some will...	2.50
3/16/2018	**Activities in the Biomed Industry** Today I attended a research symposium at the University of Texas to present my research this far on Creating myc tagged mutants of RBM10 to study changes in cellular localization and interactoins.	10.75
3/15/2018	**Performing laboratory skills** Today I transformed my ligation into e.Coli cells. I successfully had white and blue colonies grow. Just like last time, I will miniprep colonies, this time I will pick 6.	4.50
3/14/2018	**Performing laboratory skills** Today I completed a ligation of my Ethanol Precipitated Gel Purification. I followed the same protocol as last time however I used 1.64ng/ul of insert and 14.338 ul of PCR water that way I would have some insert left over if this ligation does not work.	4.50
3/13/2018	**Performing laboratory skills** Today my teacher helped me run a gel purification while I made fresh TAE buffer. She got a concentration of 13.1 ng/ul. While this is not high, it is better than my last negative DNA concentration. She proceeded to doing an ethanol precipitation which raised my concentration to 300.2. This is amazing. After having 2 failed gel purification, my changes to the protocol this third time seem to have paid off. My next step will be to perform a ligation of my gel purification that has been ethanol precipitated.	8.00
3/12/2018	**Performing laboratory skills** Today I set up another prep digest following my failed gel purification. However I am changing my protocol and using 40ul of DNA and 37 ul of PCR water to see if a higher concentration of DNA will help my gel purification. The results of this prep digest will not be seen until it is run on a gel.	4.50
3/11/2018	**Performing laboratory skills** Today I ran my prep digest on a gel and the results are pictured below. I went before school and poured a fresh agarose gel. I have one band at 3000bp which represents my cDNA like I should. Therefore I transitioned directly into a gel purification to remove and purify the cDNA from the gel. This ended terribly as my concentration was -22.1 ng/ul. I will once again have to set up a prep digest for another gel purification. After I ran the gel I properly disposed of it, for it contains the possible carcinogen ethidium bromide.	6.00
3/10/2018	**Performing laboratory skills** Since my analytical digest showed my DNA was the wrong size. I am once again setting up a prep digest following the same procedure. I will not see the results of this digest until after I run it on a gel. The purpose of this prep digest is to prepare the DNA for a gel purification to get the correct band size.	3.67
3/9/2018	**Performing laboratory skills** Today I ran my analytical digest on a gel. I have no band at 7000 bp as I am in well 2 and 3. Therefore I will have to start over and get a new prep digest for a new gel purification. I then properly disposed of the gel containing ethidum bromide.	4.67
3/8/2018	**Performing laboratory skills** Today I set up an analytical digest the same way I did the last one in order to run the digest on the gel and assure the ligation transfered the correct vector into pbng.	3.00
3/6/2018	**Performing laboratory skills** Today I finished extracting and purifying the DNA. My DNA concentration from my two colonies is seen below. I have one concentration at 409.3ng/ul and another concetration at 72.4ng/ul. My expected outcome was about 100 ng/ul so both concentrations are use able. My next step will be to perform a prep digest in order to prepare my ligation for another gel purification.	4.00
3/5/2018	**Performing laboratory skills** Today I picked my two white colonies from my ligation transformation. Tomorrow I will miniprep them and extract their DNA.	3.00
3/5/2018	**Performing laboratory skills** Today I began miniprepping in order to extract the DNA of the white colonies from my ligation transformation. I only picked colonies today to replicate the bacteria. I will purify the DNA tomorrow.	3.00
3/2/2018	**Performing laboratory skills** Today I transformed my Ligations into E.coli cells. Colonies will grow blue if my DNA sequence did not successfully transfer from a Pgem vector to a PBNG vector. However, if the vector is not empty, colonies will grow white. The picture represents the outcome of my transformation. I have white and blue colonies. My next step will be to pick two white colonies and miniprep them.	4.00
3/1/2018	**Applying biotech or genetic engineering processes** Today I completed a transformation on my ligation. If colonies grow blue, they will not have my cDNA sequence. If colonies grow white, they will have my cDNA sequence as they will have broken down the sugar on the agarose.I ended up having two white colonies. My next step will be to miniprep them.	3.00
2/27/2018	**Performing laboratory skills** Today I performed a ligation on my ethynol precipitated DNA concentration. The hopes is that after my ligation, my PBNG vecotr will have my cDNA insert. My next step will be to complete at transformation to see if my ligation worked.	4.00
2/22/2018	**Performing laboratory skills** Today I performed an ethanol precipitation of my DNA concentration from the gel purification. I did not add yeast that way I could still nanodrop my concentration free from the effects of the yeast's tRNA. The resulted DNA concentration was no where near expected. I no longer have the contamination spike in my graph, however the shape of the graph is still wrong and my concentration is -5 ng/ul. I will move on to a ligation next.	4.00

Journal - Experience-related Activity - Exploring the mutation D373V on RBM10

Date	Activity / Description	Hours
2/20/2018	**Performing laboratory skills** Today I went before school and made fresh TE buffer and poured a gel. When I ran my prep digest on a gel there were three separated bands. I then proceeded to do a gel purification, by cutting the band that was approximately 2948 bp long out of the agarose and mixed with EB buffer. I then purified and extracted my cDNA from the gel. This gel purification extracted my cDNA sequence with my mutation. My DNA concentration was lower than expected. I will now have to perform an ethynol precipitation in hopes of raising my DNA concentration.	5.00
2/15/2018	**Performing laboratory skills** TOday I prepared the DNA for a gel purification by completing a prep digest to get the correct band size. I expected the DNA preparation to work and for there to be 3 separated bands. This prep digest used the enzymes Not1-HF, Xba1, and Pvul-Hf.	3.00
2/13/2018	**Applying biotech or genetic engineering processes** Today I created a high and low concentration of my DNA by combining my 8 minipreps into two different 1.5 ml tubes. THe concentrations are attached. My next step is to complete a prep digest to cut the DNA plasmid into 3 separate segments to run in a gel.	4.00
2/12/2018	**Performing laboratory skills** Today I picked 8 colonies from my re-transformation plate and got the following nano-drop concentrations in ng/ul.	3.00
2/9/2018	**Performing laboratory skills** Today I examined my plates from my transformation yesterday and I have colonies evenly spread on my plate. My next step will be to miniprep and pick new colonies.	2.00
2/8/2018	**Applying biotech or genetic engineering processes** Today I completed a retransformation of my miniprep into ecoli cells to introduce the D373V mutation into a ecoli. I expect for there to be colonies evenly spread on my plate.	4.00
2/6/2018	**Performing laboratory skills** Today I analyzed my sequence data from UT. THere were no mutations besides D373V or gaps. Therefore my next step will be to complete a re-transformation using my plasmid template.	3.50
1/11/2018	**Performing laboratory skills** Today I received my full clone RBM10 sequence data back from the Sequencing center at the University of Texas. There were many gaps in my sequencing, however my mutation is still there. However, I have two big gaps. One between 760-1131 base pairs, and another between 2074-2490 base pairs. I prepared 3 more samples of the same miniprep I sent off for sequencing, to send back off. Hopefully the primers used during the sequencing over the segments of the DNA that had gaps will be altered or used again, only this time hopefully there will be no gaps.	4.00
1/9/2018	**Safely manage biological materials, chemicals and** Today I am still awaiting my sequencing data from the UT sequencing lab, so I aided 4 other lab students set up an E.COli transformation following their Dpn1 digest. I had to inform them on safely handling bio hazards, and how to keep e.Coli cells alive throughout both a heat shock, and incubation.	3.50
1/5/2018	**Performing laboratory skills** After analyzing the mutation in my clone miniprep sequences yesterday, today I prepared 3 sequencing samples of my clone miniprep number 3. I will send this clone's miniprep to the UT sequence lab to receive the full DNA sequence of my tranformed clones cDNA sequence.	4.00
1/4/2018	**Performing laboratory skills** Today my sequencing Data has come back from the lab at UT. I analyzed my sequence Data and found that my mutation was found in all 4 clones. The data figure attached illustrates the results of the sequencing done on miniprep plasmid DNAs extracted from the transformed RBM10 clones. The top line and highlighted yellow line represent normal RBM10 cDNA. The four lines of sequence below represent 4 miniprep DNA samples taken from 4 colonies grown on my transformed clone plates following quik change mutagenesis. As shown in the figure, a mutation takes place in all 4 miniprep DNA samples at approximately 1515bp which changes an adenine to a thymine. This proves that the mutation D373V has taken place as glutamic acid has been replaced with valine. My next step is to send off more copies of one of the 4 miniprep DNAs in order to have the entire clone cDNA sequenced to verify that thereare no unwanted mutations throughout the rest of the DNA sequence. Then I can proceed into phase 2 of my experiment.	4.00
12/3/2017	**Performing laboratory skills** Today I toured the UT biological sciences lab. I learned about the types of cells I will be using next semester, how a nanodrop measures DNA concentrations, how to properly dispose of cells, how to make and order primers, and how to use the different equipment in the lab if I ever need to.	10.00
11/5/2017	**Conducting activities in Biotechnology** Today I poured an analytical gel in class and ran my digest. I found that my RBM10 clone is not the right size RBM10 cDNA should be 7000bp and mine is not.	4.00
11/3/2017	**Conducting activities in Biotechnology** Today I digested my RBM10 clone with the enzyme XBA1. Tomorrow I will run my digest on a gel in order to see the size of my RBM10 cDNA.	4.00
11/1/2017	**Conducting activities in Biotechnology** Today I began sequencing and RBM10 clone in order perform an analytical digest and see the size of its cDNA.	4.00
10/8/2017	**Conducting activities in Biotechnology** Today I poured an analytical gel in class. I loaded my extracted DNA into it and analyzed the band. I learned how to properly dispose of gels containing ethidium bromide since ethidium bromide is a possible carcinogen.	4.00
10/5/2017	**Performing laboratory skills** Today I continued to purify my DNA from my saliva by miniprepping it. I practiced nanodropping my DNA and got a concentration of 120 ng.ul Tomorrow I will run it on a gel.	4.00
10/4/2017	**Conducting activities in Biotechnology** In order to begin learning how to properly extract DNA from cells, We began an experiment to extract our DNA from our saliva. Today I spit in a tube and extracted my DNA, putting it into the shaking incubator.	4.00
9/12/2017	**Performing laboratory skills** Today I learned about the process of Gel electrophoresis as well as learned how to operate all of the machinery made available to me in our school lab. I learned how to operate our vortex, centrifuge, microcentrifuge, water bath, shaking water bath, incubator, freezer, dry ice freezer, nanodrop, PCR machine, flammable cabinet, gel casting trays, ice machines, and hot blocks.	4.00
9/7/2017	**Maintain and interpret biotechnology laboratory re** Today I was taught about common laboratory math that I shall need to know to successfully make dilutions and calculations during my research this semester. I practiced making calculations of how much loading dye to add to a miniprep prior to pipetting it into a well of a gel.	4.00
9/5/2017	**Demonstrate proper laboratory procedures using bio** Today I was tested over the process of PCR and learned how to operate a PCR machine. PCR machines are very expensive, and multiple settings must be put into place in order for the DNA to replicate properly. Now knowing this I chose to make a goal for myself to always take my time when setting up a PCR and take care of the PCR machine and relative PCR equipment.	4.00
8/31/2017	**Performing laboratory skills** Today we learned how a Polymerase Chain Reaction is set up, as well as the scientific process behind how it works. During our expiriments we will frequently have to set up a PCR in order to multiply plasmid DNA. Furthermore, we learned that when setting up a PCR, one must always use barrier tips on their micropipettors.	4.00
8/29/2017	**Performing laboratory skills** Today we watched videos learning about the central dogma of biology as well as DNA replication. This is to reinforce what we have learned in previous classes such as AP Biology and Chemistry prior to beginning our research on human genes.	4.00
8/25/2017	**Operate biotechnology laboratory equipment accordi** Today we learned how to properly use micropipettors. We also learned the difference between accuracy and precision. Accuracy being results that conform to an accepted value and precision being results that are relatively close in measurement and repeated. We practiced using micropipettors with water with green food dye, and learned about the different sizes and different tip sizes used while handling a micropipettor. We practiced pipetting different amounts of liquid (microlitters) onto a small disk of paper.	4.00

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8/24/2017	**Conducting activities in Biotechnology** Today we created online google drive data folders. Throughout the process of this experiment we will be uploading data figures to the google drive cloud. This gives both our instructors and fellow peers access to our data figures so we can analyze them and print them to put them into our lab notebook.	4.00
8/22/2017	**Operate biotechnology laboratory equipment accordi** Today was an day devoted to an introduction to both the school's lab, as well as our project overview. I am expected to by a National Brand Computation NOtebook with the barcode ending in 43648.	4.50
3/18/2017	**Conducting activities in Biotechnology** Today I ran an analytical digest using the six minipreps I purified yesterday. I used the enzyme XBA1 in order to cut the RBM10 cDNA out of my PBNG vector. My expected outcome will be for there to be a bright band at about 7000bp. I will not see the expected outcome until I run the digests on a gel.	3.00
	Total Entries: 45	**189.58**

Profit/Loss Report - Exploring the mutation D373V on RBM10

Type	2017	2018	Total
1. Revenues from Operations			
Beginning Current Inventory			
Market Inventory Adjustments			
Ending Current Inventory			
Change in Current Inventory			
Gross Cash Revenues			
Gross Non-Cash Revenues			
Gross Revenues			
2. Expenses from Operations			
Contract/Custom			
Total Cash Expense			
Non-Cash Contract/Custom			
Total Non-Cash Expense			
Total Operating Expense			
3. Net Income from Operations			
Journaled time (hours)	69.5	120.1	189.6