Binding a part of a SETA protein.

-Erin McLaughlin

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Abstract:

Since brain cancer is extremely difficult to treat, due to the location and function, only limited methods have been researched in the published scientific literature. A growing method of treatment or prevention is gene therapy protocol. My experiment will assist in the research of developing a role of the SETA gene. By understanding SETA's characteristics it will help develop a method to attack the brain tumor, therefore a successful treatment.

Introduction:

Since the cause of brain tumors is still unknown, they cannot yet be prevented. To discover a cure, researchers are dissecting each part of the tumor in order to completely understand it as a whole. Gene therapy is thought to play a role in understanding the tumor completely. (Rosenfield & Ziff 1984). Understanding the different novel genes, such as SETA, is part of what must be done to understand brain tumors. SETA stands for SH3 containing, Expressed in, Tumorigenic, Astrocytes. (Bogler 1995). SETA is significant because it has an expression pattern correlating with tumorgencity in p53. When DNA is damaged p53 protein causes the cell cycle to stop. The SH3 domains of SETA are modular protein- protein binding domains, which may point to the function of the SETA protein. If the SETA gene is replicated through a transformation, and the coil-coil is cut off will it bind to form a dimer? Researchers hope that a cure or type of preventive medicine may be established.

Hypothesis:

Does binding a part of a SETA protein, a novel gene, form a dimer?

Materials & Methods:

1. The purpose of this experiment is to replicate the coil- coil in the SETA gene and chop off its tail, in order to bind it with the dimer. PCR, (Polymerase Chain Reaction,) is used to replicate the coil- coil. It is an in vitro method of DNA synthesis that allows a particular segment of DNA to be copied and amplified. Five tubes were labeled numbers 1, 2, 3, 4, and 5. Tube #4 was the control, it included water as well.

Tube #	Primer #34	Primer #52	Template #188	Template #191
1	X		X	
2		X	X	
3	X	X	X	
4	X	X		
5				X

Figure 1: The four tubes and their contents.

2. The results showed that template #188 proved to be acceptable, where as #191, failed. and the others were just controls.

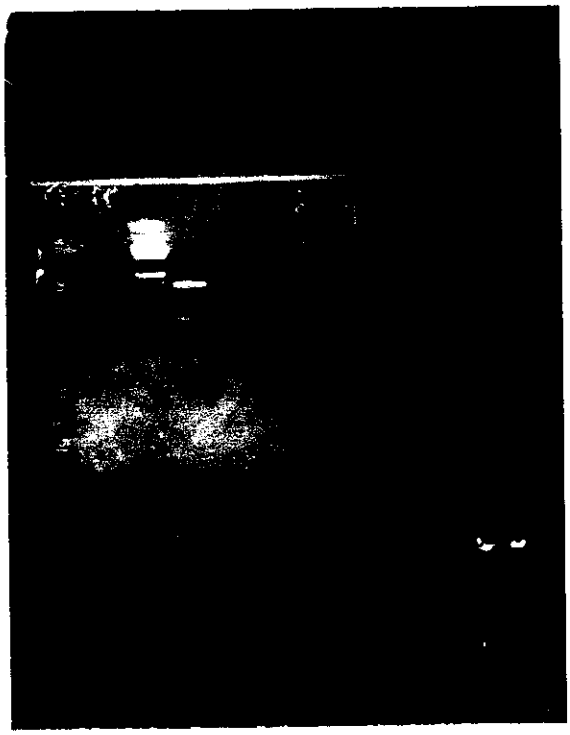

Figure 2: The first PCR picture showing results of the primers and template.

3. The next step was to do the transformation protocol, which copies the PCR gel sample to a thinner gel that can be stored.
4. The DNA samples that proved to be successful, we spread throughout a petry dish and stored in the incubator to grow the colonies over night.
5. The next step was then to digest the mini preps,

10 µl	**DNA**
2 µl	**10x**
6 µl	**water**
2 µl	**Enzyme (glycerol 1/10 vol.)**
= 20 µl	Total

Figure 3: These are the ingredients and measurements needed to perform a mini prep.

A cocktail is then needed, for five tubes.

14 µl	**10x**
42 µl	**water**
7 µl	**EcoR1**
7 µl	**Sal II**
=70 µl	**Total**

Figure 4: These are the ingredients and measurements needed for a buffer cocktail to balance the mini preps.

6. Then the cells need to be put in the sample, and chilled in ice for 30 minutes. While the ice is chilling, it is best to warm the ampacilin plates, in the incubator, (37 °C). An agarose gel needs to be made in order to run the samples through. The samples need to be shocked by putting them in boiling water for 1 minute and then directly back in the ice. The samples then need to be dyed with 10 µl of dye. And then loaded into the agarose gel.
7. After the gel runs, it should have a picture taken and studied. The gel showed that only column #3 was successful. The gel was a failure, which means the preserved samples of the control, 2, 4, 6, and 8 in the –20 °C box needed to be digested again. This time the amount of DNA was doubled from 5 µl to 10 µl. The same process completed previously was repeated.
8. After another failed attempt, after the PCR was studied, the DNA was just not showing any protein. In another attempt, the bacteria, 50 ml of ampicillin, and 450 ml of the LB were put in the Roto- Incubator, (30.0 °C). The cycle was again repeated with another protein gel. A new bottle of ampicilian was used because the other was five years old. Overall it can be assumed that this was why the protein was not working.
9. This gel proved to be a success. The protein was there.

10. The protein was then taken out and added to five test tubes.

	1	2	3	4	5
GST- SETA- CC	50 µl	-	-	50 µl	50 µl
SETA- FI (NCcc) nc cc	-	50 µl	-	50 µl	-
Seta- Nc	-	-	50 µl	-	50 µl

**Also added to the tubes was 200 µl of TNEGN.

Figure 5: This chart shows what the five tubes contained besides the protein.

11. Three washes were then performed and another gel was run. This gel showed that we did not have enough protein, so more was made.
12. The growing bacteria, was monitored within an allotted amount of time. Then the bacteria samples along with added 500 µl of IPTG were put in the centrifuge with a speed of 4300 rotations per minute, for twenty minutes. The bacteria than grew on plates overnight in the incubator.
13. The samples were then resuspended washed and ran through another protein gel. The bacteria was then measured to +0.8, which was the goal, then added 100 µl of IPTG put back into the rotator for two hours, then were spun down in the centrifuge and stored in the –20 °C overnight.

14. Although a slight problem was encountered when one, (FYN) plate did not grow bacteria as quick as the others did. Finally the solution did become cloudy and were refrigerated over night. They solutions again had IPTG added to them and were spun down.
15. The four samples GST, YES, YRK, FYN again had their bacteria levels monitored. After three and a half hours of growing, they were again resuspended in frozen cultures with 5 ml of PBS, then sonicated, to make a smooth liquid and spun down in the centrifuge for thirty minutes. After a wash and pouring out the supernatant three times the protein was sure to be there, and another gel was ran.

Results and Discussion:

The results of my experiment could be a small step in a very large area of research. Much work has been done about brain tumors but a lot of research is still being performed. Researchers have felt that gene therapy is just one of many areas of research. In the area of my work, it is hoped that this will lead to a type of cure that can be used as preventive medicine throughout the world.

Replicating the coil- coil in the SETA gene a polymerase chain reaction, PCR, using different samples in an in vitro method. After a PCR proved template #188 successful while template #191 failed in producing protein. Using the successful PCR result (#188), we preformed a transformation protocol that made it easy to compare later on. Template #188 was colonized over night to form bacteria. Pipeting a sample of the

colonized bacteria the goal was to perform a new PCR. Using an enzyme and buffer cocktail my PCR results failed.

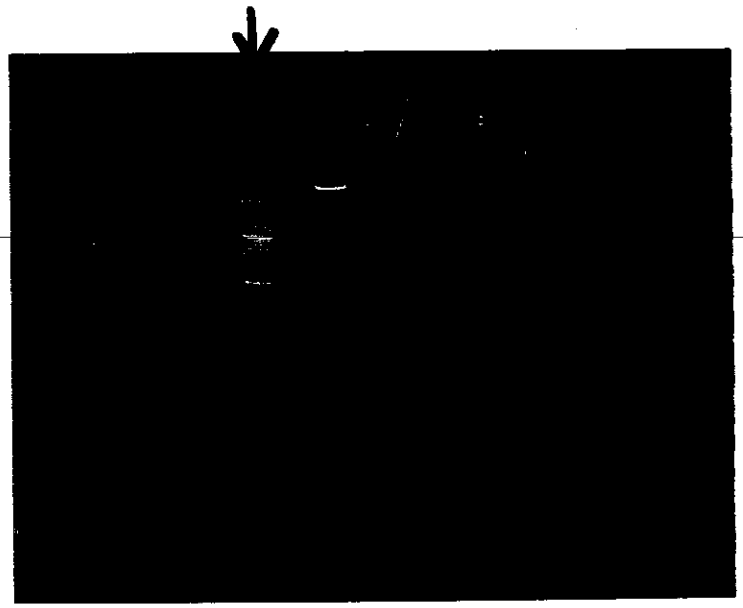

Figure 6: Column #1is the scale, and column #3 proved to be successful.

Unsuccessful		**Successful**	
5 µl	DNA	10 µl	DNA
1 µl	10x	02 µl	10x
3 µl	water	06 µl	water
1 µl	enzyme	02 µl	enzyme
=10 µl		=20 µl	

Cocktails

7 µl	10x	14 µl	10x
21 µl	water	42 µl	water
3.5 µl	EcoR1	7 µl	EcoR1
3.5 µl	Sal I	7 µl	Sal I
=35 µl		=70 µl	

Figure 7: My enzyme cocktail was changed (doubled), because it was not strong enough.

This cocktail produced a successful DNA of SETA. As a confirmation that we succeeded, another PCR was run and proved column #3 was acceptable.

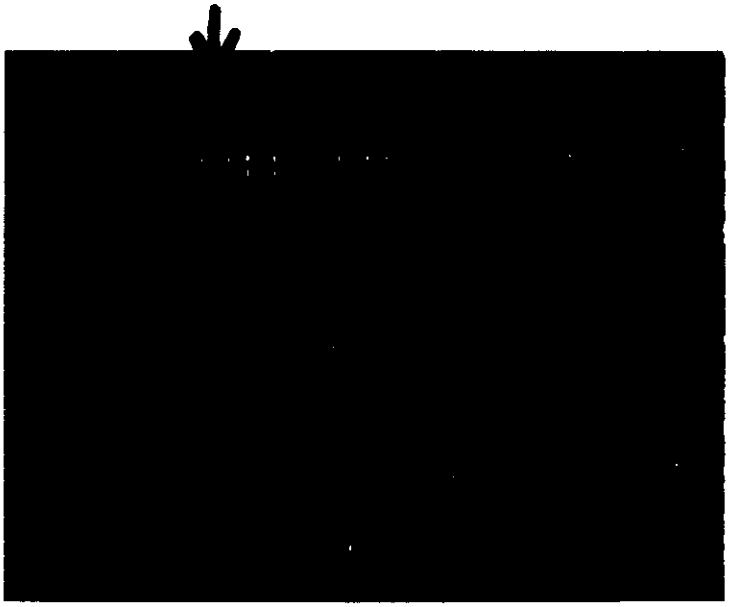

Figure 8: This PCR picture shows how column #3 possessed the protein needed.

After I grew the protein, 50 μl of the three different parts of the SETA gene, TNEGN was used to make a larger volume of the protein. Three washes in a centrifuge at speeds of 4300 RPM for 20 minutes was done. The bacteria colonized overnight in the incubator. One of the plates, the FYN needed more time to grow, to reach the other samples level, then I continued. The bacteria levels were monitored to see how much they grew. The thick bacteria solution was sonicated to turn into a smooth liquid.

Conclusion:

The four samples were looked at after the gel was ran, and it showed that the novel SETA gene did bind in order to form a dimer. Could this be a step in the future of curing or preventing brain tumors?

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Figure 9: This is myself working in the lab, thanks to everyone mentioned above.