Genetic Diagnosis of Disease Using Restriction Enzyme Fraction Polymorphism Analysis

Sirah Bah

Partners: Devin Kuhn and Sheridan McNeil

November 17th, 2017

Abstract

In the medical field, physicians are constantly searching for ways to detect and properly diagnose genetic disorders. The purpose of this experiment was to check for a genetic mutation through the use of restriction enzymes. This experiment used restriction enzyme fraction polymorphism analysis as a method to diagnose a genetic disorder, CF. Restriction Enzyme Fraction Polymorphism Analyses or RFLPs work by employing the use of restriction endonucleases to cut a DNA sample from an individual suspected to have cystic fibrosis. After the addition of these restriction endonucleases the samples were then ran through a gel electrophoresis, and the banding patterns produced were used to make a diagnosis. In conclusion, this lab found that the DNA sample from Jeff showed the genetic mutation for cystic fibrosis due to the results produced in the gel electrophoresis.

Introduction

The detection of genetic disorders is a growing focus in medicine in order to diagnose and aid doctors in the treatment of certain symptoms present in patients. A genetic disorder is simply a mutation that can be present in the sequence of someone's DNA. The types of mutations that can occur include; missense, nonsense, insertion, deletion, duplication, frameshift, and repeat expansion ("What kinds of gene mutations are possible?", 2017). Often times these mutations can be fixed by cells through a series of different repair mechanisms, however, not all of these mutations can be repaired or caught which can lead to illness in the representative individual (Lage et al., 2007).

One disease that is a result of a genetic mutation is Cystic Fibrosis. Cystic Fibrosis or CF is a frameshift mutation in the genome. A frameshift mutation is the shifting of the bases in one's genome which ultimately leads to the altering of the code for amino acids. In the case of CF, the CFTR gene is defective in that it no longer functions as it does in a healthy individual. In an individual with CF the CFTR does not effectively regulate the movement of salt and water in and outside of the cells (Gadsby et al., 2006). The dysfunctionality in this gene leads to sweat and mucus of someone with cystic fibrosis to be very sticky and thick. This causes the plugging of tubes, ducts, and other passageways in the body. The most commonly affected places are the lungs and the pancreas. Individuals who have CF commonly have a persistent cough that creates thick mucus, wheezing problems, and breathlessness ("Cystic Fibrosis", 2016).

This lab was completed in order to diagnose an individual who presented several of the main symptoms attributed to cystic fibrosis. The individual, Jeff, is a 3-year-old boy who exhibited a mostly clear scan other than issues pertaining to his lungs. With the information collected, it was decided to run a detection of Jeff's DNA sequence in order to test for cystic fibrosis. There are many different ways to test for CF such as; Sweat Tests, Immunoreactivity Trypsinogen Tests, Chest X-Rays, Lung Infection Tests, and Restriction Enzyme Fraction Polymorphism Analyses. For the purpose of discovering if Jeff had CF or not the latter exam, restriction enzyme fraction polymorphism analysis, was completed. An RFLP works by using specific restriction endonucleases to cleave at specific sequences in the DNA sample (Carreel et al., 2002). These cuts create specific fragments that can only be done by using that specific restriction endonuclease. Once the fragments had been made, they can then be separated according to size by running a gel electrophoresis.

The question answered in this experiment was whether Jeff would test positive or negative for CF if the test used was an RFLP. The purpose of this study was to use two restriction endonucleases, EcoRI and HindIII, to test against Jeff's DNA sample to make a genetic diagnosis. The analysis of Jeff's DNA in the gel produced by gel electrophoresis would give the answer due to the banding patterns produced and the sizes of the bands. It was hypothesized that since Jeff's medical chart had already shown many symptoms attributed to CF that if an RFLP was completed then he would have similar banding patterns to the restriction endonucleases that make cuts for a CFTR defect.

Materials and Methods

A sample of negative control DNA, Jeff's DNA, and four microcentrifuge tubes were collected and labeled. Then 10µl of reaction buffer were added to the four microcentrifuge tubes. After the addition, 15µl of Jeff's DNA were moved to tube Jeff 1 and another 15 µl were added to tube Jeff 2. After this, 15 µl of negative control DNA were added to the tube labeled "neg 1" and another 15 µl of the negative control DNA were added to the tube labeled "neg 2". Then 15 µl of enzyme 1, EcoRI, were added to tubes Jeff 1 and neg 1. Enzyme 2, HindIII, was added to tubes Jeff 2 and neg 2 in the same quantity as enzyme 1. Following the several additions, the tubes were left to incubate for 30 minutes at 37˚C.

The 0.8% agarose gel was prepared by setting a gel holder into the preparation rack, and adding a comb to one of the ends of the gel space. Then 0.4 g of agarose was added to 50 ml of 1x TAE buffer to then be mixed and heated in a microwave for 2 minutes. After the removal of the flask from the microwave, 5 µl of 10,000x Sybr Safe DNA gel stain were put into the flask. The contents were then poured into the gel rack, and left to solidify. Once the gel had solidified, the comb was removed and the resultant gel was placed in an electrophoresis chamber. More 1x TAE buffer was placed on top of the gel to completely inundate it.

After the 30-minute incubation period had elapsed, 5 µl of loading dye were added to each of the 4 tubes and these tubes were immediately placed on ice. Then tubes of marker DNA, positive control DNA cut with enzyme 1, and positive control DNA cut with enzyme 2 were obtained. When all the tubes were collected, the samples could then be loaded into the gel. The gel was loaded with 30 µl of each sample into their designated well. The loading order went as follows by reading the gel from left to right; Negative Control Enzyme 1, Positive Control Enzyme 1, Jeff Enzyme 1, Negative Control Enzyme 2, Positive Control Enzyme 2, Jeff Enzyme 2, and Marker. Once the gel was loaded, the cover was put on top of the apparatus with the loaded wells close to the black electrode and the apparatus was plugged in. The gel was running at 100V for 30-45 minutes. When the gel was finished running, the power supply was turned off and the leads were disconnected from the apparatus. While wearing gloves, the gel was picked up and put onto a UV transiluminator for analysis. The distance traveled by the bands recorded were used to create a standard curve.

Results

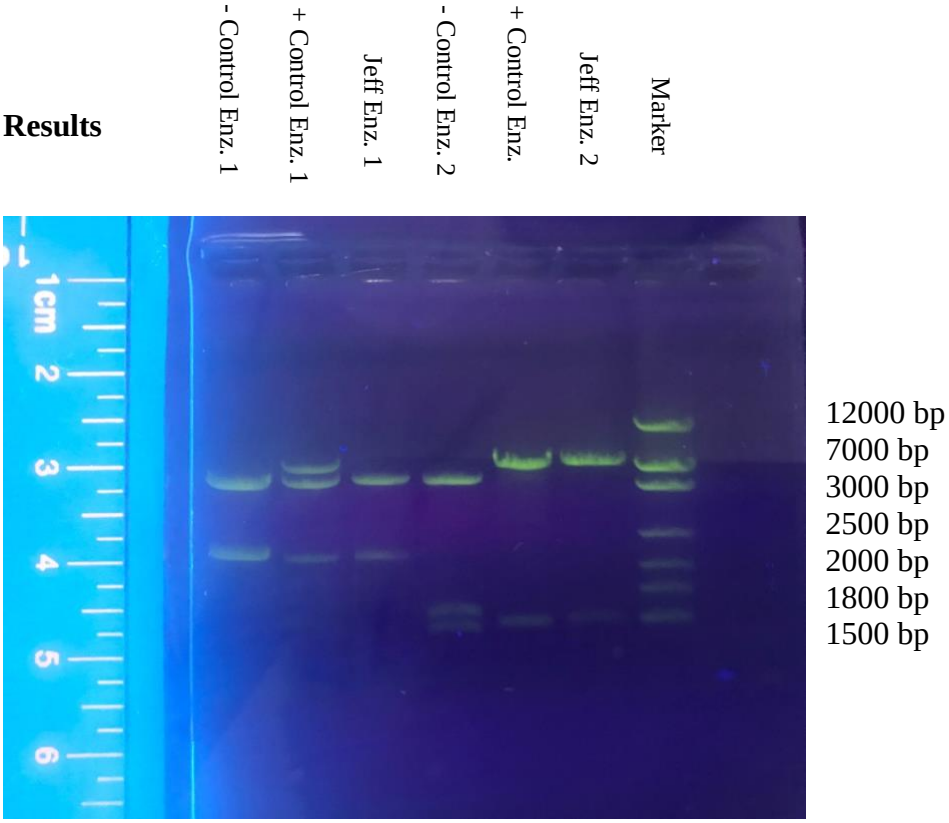

Figure 1: Results of the gel electrophoresis. The image shows the well contents on the horizontal axis, and the vertical axis shows the marker sizes.

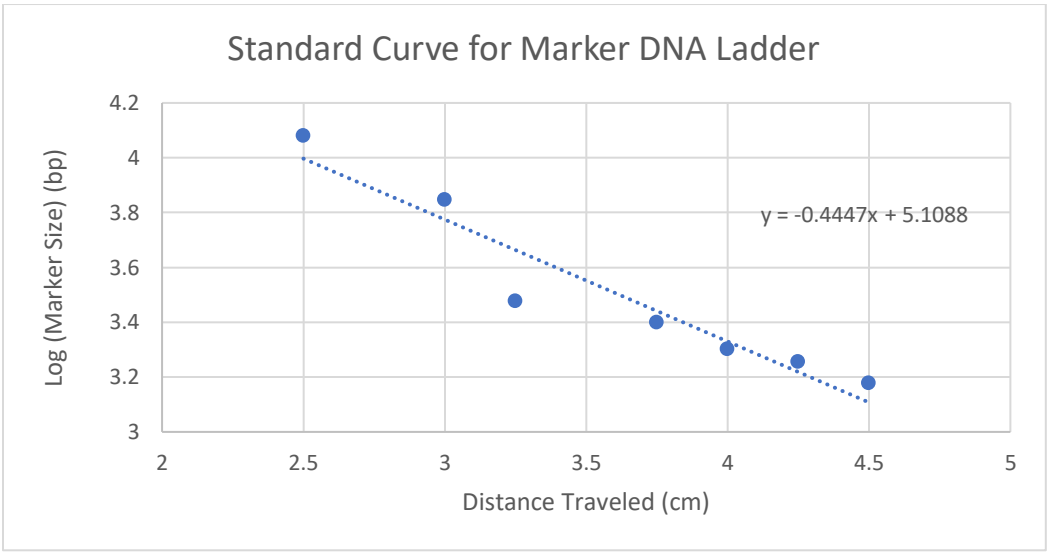

Figure 2: Graph of the logarithmic molecular weight of the base pairs on the y-axis and the distance that these bands traveled in the gel on the x-axis. The equation for the standard curve is y = -0.4447x + 5.1088, and this equation was used to fill out the table below

log(markersize)	Lane Number	Distance traveled (cm)	Marker Size (bp)
3.99705	7	2.5	9932.303917
3.7747	7	3	5952.508168
3.663525	7	3.25	4608.132945
3.441175	7	3.75	2761.690462
3.33	7	4	2137.96209
3.218825	7	4.25	1655.102901
3.10765	7	4.5	1281.297563
3.7747	6	3	5952.508168
2.996475	6	4.75	991.9162383
3.7747	5	3	5952.508168
2.996475	5	4.75	991.9162383
3.663525	4	3.25	4608.132945
3.10765	4	4.5	1281.297563
2.996475	4	4.75	991.9162383
3.663525	3	3.25	4608.132945
3.33	3	4	2137.96209
3.7747	2	3	5952.508168
3.663525	2	3.25	4608.132945
3.663525	1	3.25	4608.132945
3.33	1	4	2137.96209

Table 1: Identifies the distances traveled for the samples in the numbered wells in cm, marker sizes, and the log of the marker sizes. These values were calculated by using the equation of the standard curve (Figure 2).

Figure 1 shows that Jeff's DNA and the positive control enzyme (HindIII) show similar banding patterns in their individual loading wells. This similarity can help to conclude that Jeff

does have Cystic Fibrosis, because HindIII was manufactured so that it will only make specific cuts for specific fragment sizes. Jeff's DNA shows to have made the same cuts as the positive control enzyme HindIII, which has the ΔF508 mutation. The ΔF508 mutation shows only in patients with cystic fibrosis. HindIII makes 2 cuts if CFTRΔF508 is present, and 3 cuts if it's not. Jeff's DNA showed two cuts which means that he has Cystic Fibrosis. When looking at enzyme 1, EcoRI, it is harder to tell if CF is present or not. So, the banding patterns from enzyme 2 were used to conclude on Jeff's cystic fibrosis status. Figure 2 was important in order to determine the fragment sizes, and this is important because of the specific cuts that HindIII makes due to the ΔF508 mutation. Table 1 shows the marker sizes that were able to be calculated by the equation of the line of the standard curve, which again is helpful in the fragment sizes for the different samples.

Discussion

The results confirmed the hypothesis in that Jeff does have cystic fibrosis. This statement can be confidently stated when consulting the data from figure 1. Figure 1 shows the gel after it had undergone electrophoresis. When Jeff's DNA was cut with enzyme 1, EcoRI, the banding pattern matched that of the negative control. However, when Jeff's DNA was cut with enzyme 2, HindIII, the banding pattern matched that of the positive control for that enzyme. The HindIII positive control was programmed to recognize CFTRΔF508, the mutation present for cystic fibrosis, and this was the banding pattern that Jeff was a match for. With the production of these banding patterns it could then be said that Jeff's DNA has a mutation of the CFTR gene, because of the presence of CFTRΔF508.

The diagnosis of Jeff would not have been possible without the implementation of both positive and negative controls of the restriction enzymes. These controls were put in place so that Jeff's DNA sample had something to be compared to in order to ascertain a mutation or its absence in his DNA. The positive and negative controls aided in the interpretation of the results as they provided the framework for what to compare or contrasts Jeff DNA sample to. For example, in the case of comparing Jeff's DNA to the EcoRI enzyme, the negative control would be creating a banding pattern to correlate the absence of the CF mutation and the positive control would create a banding pattern to depict the presence of the CF mutation. So, with those two controls in place when Jeff's DNA was cut with EcoRI, he showed to not have the specific banding pattern of the positive EcoRI control.

Cystic Fibrosis is an illness that is a result of a loss of a single amino acid in a protein. Normally if there is a single amino acid loss the body has repair mechanisms to counteract the negative effects of it, however, cystic fibrosis is a unique case. Cystic fibrosis is the result of a frameshift mutation, so if there is even one single change in the DNA sequence the codon will now code for a new amino acid (Gadsby et al, 2006). This ultimately results in a dysfunctional protein, and the imbalance of movements for salt and water within and outside of a cell. The now mutated CFTR gene can no longer properly regulate these movements, and is a misfolded protein. The misfolded protein causes a buildup of mucus and liquid most notably lining the lungs and pancreas. The impact of this for humans is wheezing, breathlessness, and a cough. These symptoms presented themselves in Jeff, however, a full diagnosis could not be confirmed until the analysis of the gel electrophoresis.

In a follow-up for this experiment it would be recommended to cut the DNA of the individual of interest with more than two known restriction enzymes. This would be useful in backing up a diagnosis with more evidence than the results from just two restriction enzymes. Another suggestion to surefire the diagnosis of the individual would be to use two different testing methods to see if the results of the genetic disorder in question would be yielded by using the two methods. Sources of error in this lab include the cross contamination of the wells during gel electrophoresis. It is possible that the amount of each sample loaded into each individual well could have been too much, and overflow would occur and flow into the well next over. This error would result in inaccurate results of the gel electrophoresis, and ultimately a mistaken diagnosis of the presence or absence of CFTRΔF508.

References

Carreel, F., Leon, D. G., Lagoda, P., Lanaud, C., Jenny, C., Horry, J. P., & Montcel, H. T. (2002). Ascertaining maternal and paternal lineage within Musa by chloroplast and mitochondrial DNA RFLP analyses. *Genome*,*45*(4), 679-692. doi:10.1139/g02-033

Cystic fibrosis. (2016, October 13). Retrieved November 30, 2017, from https://www.mayoclinic.org/diseases-conditions/cystic-fibrosis/symptoms-causes/syc-20353700

Gadsby, D. C., Vergani, P., & Csanády, L. (2006). The ABC protein turned chloride channel whose failure causes cystic fibrosis. *Nature,440*(7083), 477-483. doi:10.1038/nature04712

Lage, K., Karlberg, E. O., Størling, Z. M., Ólason, P. Í, Pedersen, A. G., Rigina, O., . . . Brunak, S. (2007). A human phenome-interactome network of protein complexes implicated in genetic disorders. *Nature Biotechnology,25*(3), 309-316. doi:10.1038/nbt1295

What kinds of gene mutations are possible? - Genetics Home Reference. (2017, November 28). https://ghr.nlm.nih.gov/primer/mutationsanddisorders/possiblemutations