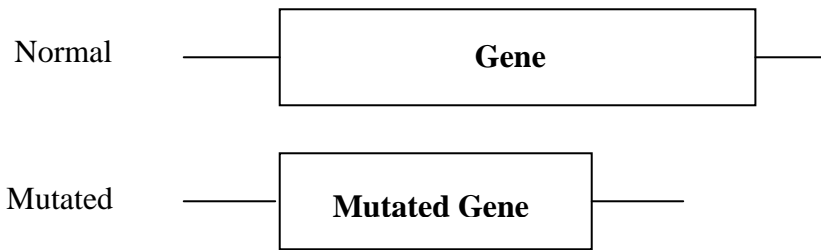
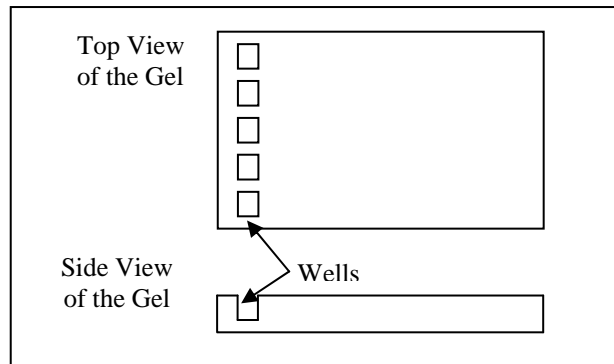




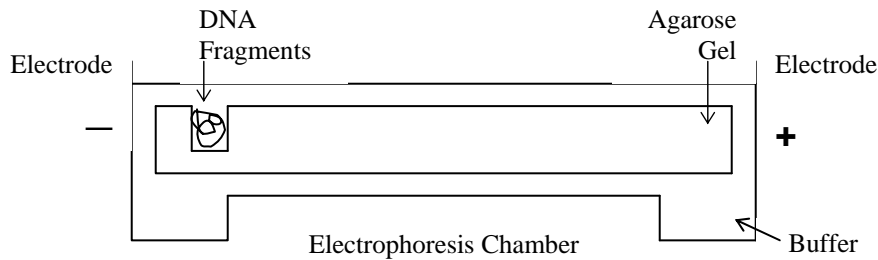
In this lab, you will perform gel electrophoresis in order to determine the genotype of a mother, father and their fetus. In order to be affected by cystic fibrosis, a person must have two copies of the mutated gene. The mutated gene is a result of a portion of the normal gene being deleted. The diagram below illustrates the relative sizes of a normal gene and a mutated gene.



The gel electrophoresis technique consists of an agarose gel, a buffer solution and a gel electrophoresis chamber. The gel has small holes, or wells, in it where DNA is placed.



The gel is placed in the electrophoresis chamber and a small sample of DNA is placed in each well. Once the chamber is hooked up to electricity and turned on, the electricity will drag the DNA molecules through the gel. Because it will be easier to move small pieces of DNA< shorter fragments will be moved further down the gel. Larger pieces of DNA will remain closer to the wells.



Do you expect to see a difference between the DNA from the mutated gene and the normal gene? Why?

Today, you will use gel electrophoresis to determine if Jim, Jane and their fetus carry the genes for cystic fibrosis. Jim and Jane both do not have cystic fibrosis. However, there is a history of the disease in Jane's family. They have come to the doctor to test if their fetus will have cystic fibrosis and also to determine if Jim or Jane is carrying the mutated gene. This information can be used to calculate the probability of their next child having the disease.

Five samples of DNA will be given to you: CF, N, 1, 2, and 3. Sample CF has cystic fibrosis. Sample N does not have it. Sample 1 is Jim's DNA; Sample 2 is Jane's DNA; Sample 3 is their fetus' DNA. You will run samples of each patient's DNA on a gel as described above. By comparing the patients' DNA samples to the cystic fibrosis and normal DNA samples, you will be able to determine whether or not they have cystic fibrosis or whether they are carrying the mutated gene.

Procedure

Make an Agarose Gel

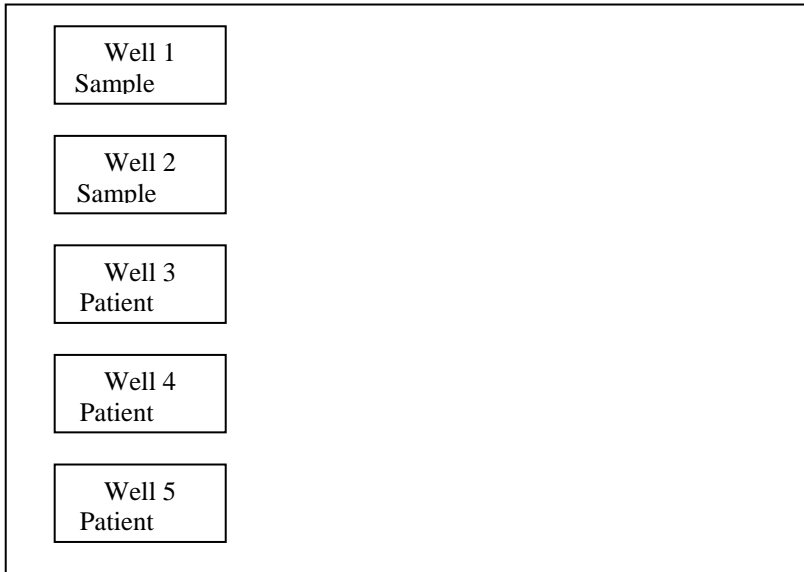
1. Place 0.8g of powdered agarose in 100 mL of 1X TEA buffer in a clean 250-mL Erlenmeyer flask.
2. Microwave to dissolve the agarose. CAUTION – THE FLASK WILL BE EXTREMELY HOT! Remove with a glove, swirl gently to mix and try to avoid bubbles. Let it cool with occasional swirling to keep a uniform temperature.
3. When flask is cool to the touch but before the solution gels, pour into a prepared casting tray. Place the combs in the casting tray for well formation.
4. Wait for the gel to form. It looks almost cloudy when it has solidified.
5. GENTLY remove the comb and take the gel from the casting tray and place in electrophoresis chamber.

Load and Run the Gel

1. Place the two gels and backing plates on the "shelf" of the gel box with the wells at the negative (black) end of the box. Completely cover the gels with about 500 mL of 1X TEA buffer.
2. Use a micropipette to place 10uL of the sample into each well by placing the pipette tip IN the buffer and just ABOVE the well and releasing the sample.
3. Repeat Step 2 for all of the samples. Make sure you use a DIFFERENT TIP for each sample. Record the order you load the samples in the diagram below.
4. When both gels are ready, put the top on the gel electrophoresis box, connect the wires, and GET THE INSTRUCTOR to show you how to turn on the power. DO NOT TOUCH ONCE TURNED ON – HIGH VOLTAGE!

Results

Sketch the results of the gel on the diagram below. Draw exactly what you see on your gel.



Diagnosis		
	Phenotype	Genotype
Jim		
Jane		
Fetus		