NAME _____

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Carolina™ Electrophoresis and Simulated Genetic Screen for AP Biology

Guided Inquiry Lab

Background

In many cases, the quality of experimental results is only as good as the equipment and techniques used for the experiment. For this reason, scientists continually improve and refine their techniques. In this Guided Inquiry, you will optimize the electrophoresis procedure that you will later use for a simulated genetic screen. The simulated genetic screen will determine if fraternal twins inherited a cystic fibrosis-associated mutation from either of their parents, both of whom are known carriers.

Cystic fibrosis is an autosomal recessive genetic disease that occurs in the United States with an approximate frequency of 1 in 3500 births. The disease results from mutation of the cystic fibrosis transmembrane conductor gene (CFTR) which codes for a protein (CFTR protein) that regulates the flow of chloride ions across membranes. As of 2012, at least 1906 different mutations had been identified in the CFTR gene. Different mutations confer different degrees and sometimes different types of disease. Some of the mutations do not cause disease at all.

Typically, the disease is characterized by thickened secretions throughout the body, which most notably affect the lungs, pancreas, and hepatobiliary system (liver, gallbladder, and bile ducts). The thickened secretions in the lungs lead to difficulty in clearing mucus from the lungs, thus increasing the chance of infection. Treatment includes clearing the lungs by gently pounding on the chest and back to loosen the mucus and dead cells, the use of antibiotics, and the inhalation of a drug to help thin the secretions. Thickened secretions in the pancreas affect the distribution of vital digestive enzymes. To alleviate this problem, people take capsules containing the needed enzymes.

In the 1970s, people with classic cases of cystic fibrosis lived only into their mid-teens. Since then, medical treatment of the disease has improved, and the lifespan of affected people has greatly increased. However, achieving optimal length and quality of life depends upon beginning medical treatment early. Detecting the disease at birth provides the greatest opportunity for treatment.

If symptoms are classic (typical) and severe enough early in a child's life, diagnosis is straightforward. If symptoms are mild, diagnosing cystic fibrosis can be a challenge. A sweat chloride test (which measures the amount of chloride ions in a person's sweat—most CF patients have elevated levels) is the classic diagnostic test for the disease, but it is not always definitive. In cases where neither the sweat chloride test nor symptoms allow for a definitive diagnosis, genetic testing can help.

Some methods of genetic testing use electrophoresis to separate the DNA fragments that are generated during the analysis (for more specifics see the Background to the Guided Lab). To accomplish this separation, the mixture of DNA fragments is drawn through an agarose gel or other matrix by applying an electric field to the gel. Since DNA is negatively charged, it will run toward the positive electrode (the anode, which in gel electrophoresis chambers is typically colored red). The gel is made with and sits in buffer containing ions which make it possible to create a uniform electric field across the gel.

The gel the fragments are drawn through is formed by first melting powdered agarose in buffer to make an agarose solution. The agarose solution is poured into a mold called a gel tray. While the agarose is still

liquid, a removable comb is placed at one end of the gel tray, either immediately after or before pouring the gel. Once the agarose solution has gelled, the comb is removed and the DNA samples are loaded into the wells created by the teeth of the comb.

Different-sized DNA fragments travel through the gel at different rates, with smaller fragments of DNA working their way through the gel matrix more quickly than larger fragments. Since DNA fragments of the same size move through the gel at the same rate, same-sized fragments collect in the same location and form a distinct band in the gel.

Depending upon the size of the fragments being separated, some aspects of how a gel is run may be altered. Some of the variables that can be altered are the percentage of agarose in the solution used to make the gel, the type of buffer used, the voltage at which the gel is run, and the length of running time. In this Guided Inquiry lab, you will explore and compare how effectively gels made at different percentages of agarose separate different-sized fragments. You will use your conclusions from the experiment to optimize the procedure for the simulated genetic screen performed in the Guided Lab.

Welsh, M.J., Smith, A.E., Cystic fibrosis. 1995. *Scientific American* Vol. 273(6): 52–9. (Although much information has been added to the field since 1995, this article is a good introduction to the subject and is written at an easily accessible level.)

Farrell, P.M., Rosenstein, B.J., White, T.B., Accurso, F.J., Castellani, C., Cutting, G.R., Durie, P.R., Legrys, V.A., Massie, J., Parad, R.B., Rock, M.J., Campbell, P.W. III. 2008. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *Journal of Pediatrics* Vol. 153 (2): S4–14.

This is a listing of identified CF mutations: http://www.genet.sickkids.on.ca/cftr/app

Pre-laboratory Questions

1. You read a poster from a student electrophoresis project comparing the effectiveness of two different concentrations of agarose gels at separating specific DNA fragments. In the experiment, the students ran the two different gels side by side at 120 volts. One gel ran for 30 minutes, and the other for 45 minutes. Do you think the group's results are valid? Why or why not?

2. Explain why the DNA fragments run toward the positive, red electrode (the anode).

3. In general, what percentage gel do you think would separate large fragments of DNA more effectively—a gel with a low percentage of agarose, or a gel with a high percentage of agarose? Explain your answer.

Introduction

As mentioned in the Background, different percentage-agarose gels are used to separate different-sized fragments. In this Guided Inquiry Lab, you will determine the optimal percentage of agarose to use in preparing a gel to separate the DNA fragments in the simulated genetic screen. The DNA fragments used in the simulated genetic screen range in size from 849 bp (base pairs) to 2586 bp. The two bands that are the closest in size are between 800 and 950 bp in size. Make sure you pay attention to the separation of the marker bands in this size range. Commonly used agarose gel percentages range from 0.4 to 2.0 percent.

In this part of the lab, each group will have only enough materials to run one gel with one lane of marker on each gel. The marker is a mixture of DNA fragments of known sizes. When performing electrophoresis, a marker is run on the gel alongside the other samples as a way to confirm that the gel has run correctly and to determine or confirm the size of the other DNA fragments on the gel.

The marker you will use in both lab activities has a broad range of DNA band sizes, including 21,226 bp, 5148 bp, 4973 bp, 4268 bp, 3530 bp, 2027 bp, 1904 bp, 1584 bp, 1375 bp, 947 bp, 831 bp, 564 bp, and 125 bp. Depending upon the percentage of the gel run, bands that are close in size may appear as a doublet, two bands merged into one. Also, depending upon gel percentage, the 125-bp band will be only faintly visible, or not at all.

With your group, discuss what experimental approach to use to determine the best percentage gel for separating the DNA fragments in the samples used in the simulated genetic screen. To accomplish your goal, you may have to collaborate with other groups. Following, is a list of the materials available to you and the procedure for pouring and loading a gel.

Materials

Each group will have access to the following:

agarose
1× TAE buffer
1 tube of marker (lambda DNA cut with HindIII/EcoRI; see the preceding Introduction section for the DNA band sizes)
power supply
gel electrophoresis chamber
*Carolina*BLU™ DNA stain
distilled water
gel-loading devices

Procedure

1. Pre-lab Write-up

Write up your proposed experiment. In the proposal, discuss the following:

- **Question** - What are you testing in your experiment? What are you trying to find out? Be specific.
- **Hypothesis** - What percentage gel do you think will work best for the size fragments that you are using? Why do you think so? What do you already know or what reasoning helps support your hypothesis? If you have no previous experience or any reasoning on which to base a hypothesis, how does this affect how you set up the experiment?
- **Procedure** - Below is a basic procedure for how to pour and run a gel. However, you need to fill in some details. What percentage gel(s) will you run? How will you make the gel(s) (i.e., what amount

of agarose will you mix with what volume of buffer?) What safety practices do you need to use? You may not need to rewrite the procedure, but add notes where needed (e.g., record quantities, times, and variations in procedure).

- **Data Collection** - What data will you record, and how will you collect and present it?

Have your teacher approve your group's experimental plan before you begin the experiment.

2. Pouring an Agarose Gel

a. Follow your instructor's directions for determining how much agarose solution to make for one gel. To ensure that you have enough agarose solution to pour the gel to the proper depth, make 5 mL more than you determine you need.

b. Weigh out the agarose and place it in an appropriate container.

c. Add the appropriate volume of 1× TAE buffer and melt the agarose. Determine the equipment available and use one of the following three methods:

Methods for Melting Agarose

- Heat the agarose solution in a microwave for 2–4 minutes, or until no particulate matter can be seen in solution or stuck to the bottom of the flask. To prevent boiling over during heating, watch carefully and swirl the flask every minute or at the first sign of vigorous boiling.
- Alternatively, the agarose can be melted using a hot plate. If use of a magnetic stir bar is not possible, swirl the solution frequently to prevent boiling over or scorching and to help disperse the agarose. Heat until no particulate matter can be seen in solution or stuck to the bottom of the flask. This usually takes 5–10 minutes.
- A boiling water bath can be used instead; make sure the water in the water bath is at least level with the agarose solution in the flask. Heat until no particulate matter can be seen in solution or stuck to the bottom of the flask. This usually takes 5–10 minutes. Allow additional time for the water bath to come to boiling.

d. Allow the agarose to cool to the point at which you can barely touch the flask comfortably before pouring; too-hot agarose may cause gel trays to warp or crack.

e. While the agarose is cooling, seal the ends of the gel-casting tray with tape, dams, or any other method appropriate for the gel tray you are using. Insert the well-forming comb.

f. Pour enough agarose solution into the gel tray to fill it to the appropriate depth. Immediately, while the gel is still liquid, use the end of a paper clip or an extra disposable pipet tip to move large bubbles or solid debris to the sides or end of the gel tray.

g. The gel will become cloudy as it solidifies (15–20 minutes). Do not move or jar the gel tray while the agarose is solidifying.

h. When the agarose has set, unseal the ends of the gel tray and place the tray in the electrophoresis chamber so that the comb is at the negative (black/cathode) end.

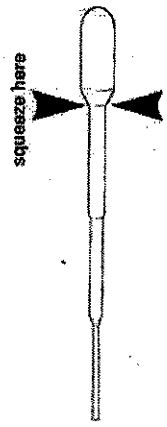
i. Use 1× buffer to fill the electrophoresis chamber to the level that just covers the entire gel.

j. Remove the comb by gently pulling it straight up. Take care not to rip the wells.

k. Make sure that the sample wells left by the comb are completely submerged. If "dimples" are observed around the wells, slowly add buffer until the dimples disappear.

l. The gel is now ready to load.

3. Loading the Gel



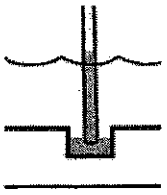
These instructions apply to the disposable plastic needle-point pipets supplied with the kit. If you are using a different device, follow the proper procedure for that device.

a. Gently squeeze the conical area below the bulb of the pipet and maintain that pressure.

b. Place the pipet tip into the DNA sample and partially release the pressure to aspirate some DNA into the pipet tip. Do not allow the sample to go all the way up into the pipet, and do not fully release the pressure.

c. Ready the pipet over the well you are going to load and use your other hand to aim the tip precisely.

d. Expel any air in the very end of the pipet tip before loading the DNA sample. (If an air bubble caps the well, the sample will instead flow into the buffer around the edges of the well. If a bubble is forced into the well before the sample, it may affect the clarity of the bands that form during electrophoresis.)



e. Dip the pipet tip through the surface of the buffer, position it just inside the well, and slowly expel the sample. Do not punch the tip of the pipet through the bottom of the gel. To avoid re-aspirating your sample, do not release pressure until you have pulled the pipet tip completely out of the buffer.

f. Repeat the same procedure for each sample you load (using a new pipet each time).

4. Running the Gel

a. Close the top of the electrophoresis chamber and then connect the electrical leads to an appropriate power supply, anode to anode (red to red) and cathode to cathode (black to black). Make sure both electrodes are connected to the same channel of the power supply.

b. Turn on the power supply and set the voltage as directed by your instructor. Shortly after the current is applied, you should see the loading dye moving through the gel toward the positive, red terminal of the electrophoresis apparatus.

c. The purplish-blue band is the loading dye, bromphenol blue, which migrates through the gel at the same rate as a DNA fragment approximately 300 base pairs long.

d. Allow the DNA to electrophorese until the bromphenol blue band is about 1 cm from the end of the gel. If *Carolina*BLU Gel and Buffer Stain was used in the gel and buffer, some of the DNA bands will be faintly visible during electrophoresis.

e. Once the gel has run, turn off the power supply, disconnect the leads from it, and remove the lid from the electrophoresis chamber.

f. Transfer the gel to the staining tray.

5. Staining the Gel

a. Pour enough *Carolina*BLU Final stain into the staining tray to just cover the gel.

b. Keep the gel in the stain for 20 minutes. Do not allow the gel to sit in the stain for longer than 45 minutes, or it will be hard to destain.

c. Pour the *Carolina*BLU stain back into the bottle, rinse the gel with distilled or deionized water, and fill the tray with just enough distilled or deionized water to submerge the gel. Further destain the gel with a couple more changes of distilled or deionized water at intervals of 15–30 minutes (again, just

deep enough to submerge the gel). Alternatively, the gel can be left overnight in the refrigerator to destain. The DNA bands will reach maximum visibility after destaining for several hours. Do not destain with tap water or with too much water, or the bands will fade (the chloride ions in tap water cause fading).

Laboratory Questions

1. Based on what you have seen in this experiment, would a lower- or higher-percentage gel be better for separating large fragments of DNA? What did you see from your experiment to make you think this?

2. Analyze your data and any necessary data from other groups in your class.
   a. Given what you observed, what percentage gel will best separate the DNA fragments present in the simulated genetic screen?

   b. Did you observe anything that surprised you? Identify some possible sources of error in your experiment. If given the opportunity, would you, and, if so, how might you conduct the experiment differently?

   c. Be prepared to present the findings of your experiment to the class according to your teacher's instructions.

3. You load your samples on a gel, connect the electrophoresis apparatus to the power supply, and turn on the power. When you look at the gel 5 minutes later, the samples have run opposite the direction you expected. What mistake did you make in setting up the gel and in connecting it to the power supply?