NAME _____

DATE _____

Carolina™ Electrophoresis and Simulated Genetic Screen for AP Biology

Guided Lab: Simulated Genetic Screen

The Scenario

You are a technician in a medical lab sent blood samples from newborn fraternal twins. The parents of the twins both have a history of cystic fibrosis in their families.

The mother and father were both born and raised in a small community where, for many generations, there has been very little immigration and a great deal of intermarriage. Many of the genes present in the founding families predominate in the population. Two unique cystic fibrosis mutations are commonly found in members of the community. Because of their family history, both the husband and the wife have been tested to see if they carry either of these two CF mutations. They were both found to be heterozygous for the same mutation. The mutation is associated with varying degrees of disease (probably due to the influence of other genes co-inherited with the mutation). Because of the parents' genotypes and the family history, doctors perform several tests on the children to determine if they have, or are likely to develop, cystic fibrosis. As a lab tech, you will perform part of a genetic screen to determine if the children are homozygous for the mutation carried by both of the parents. Because the results of other tests were ambiguous, the results of the genetic test will be especially helpful in determining how to monitor and treat the children.

Before you arrived at work, your coworker isolated DNA from the blood samples and used the DNA to set up PCR (polymerase chain reaction) for the genetic screen. PCR is a technique that allows you to selectively amplify a specific region of DNA. In this case, the region containing the cystic fibrosis mutation is being amplified. In addition to setting up reactions using the children's DNA, your coworker set up three additional control reactions—one using DNA known to contain the mutation, one using DNA in which the mutation is known to be absent (wild type), and one with no template DNA at all. The reaction with no template DNA is a control to demonstrate that the DNA product obtained from the PCR reactions is not a result of contamination of the reaction with stray DNA. (PCR is so sensitive that very small amounts of extraneous DNA from the environment can be amplified and result in inaccurate results.) The controls set up using DNA with known wild-type and mutant sequences provide a known DNA banding pattern to compare with the results. The controls also help in troubleshooting should the experiment not work. Once the reactions, including all the appropriate controls were set up, they were placed into a thermal cycler.

After the PCR reactions were completed, the PCR products were digested with a restriction enzyme. Restriction enzymes cut DNA. Because specific enzymes cut at specific sequences, they can be used to distinguish between two sequences of DNA that are alike except for minor base-pair changes. In this case, the mutation of interest changes the DNA sequence so that the restriction enzyme used in the assay no longer recognizes one of its cut sites. As a result, the enzyme cuts the PCR fragment (also called a PCR product) generated from the wild-type sequence into four fragments, but cuts the PCR product from the mutant sequence into only three fragments. Patterns of DNA bands resulting from restriction digests such as these are often referred to as "DNA fingerprints."

When you arrive at lab, you are asked to use electrophoresis to separate the fragments of DNA created by restriction digestion of the PCR products and to determine the genotype of the children with respect to the cystic fibrosis mutation carried by both of the parents.

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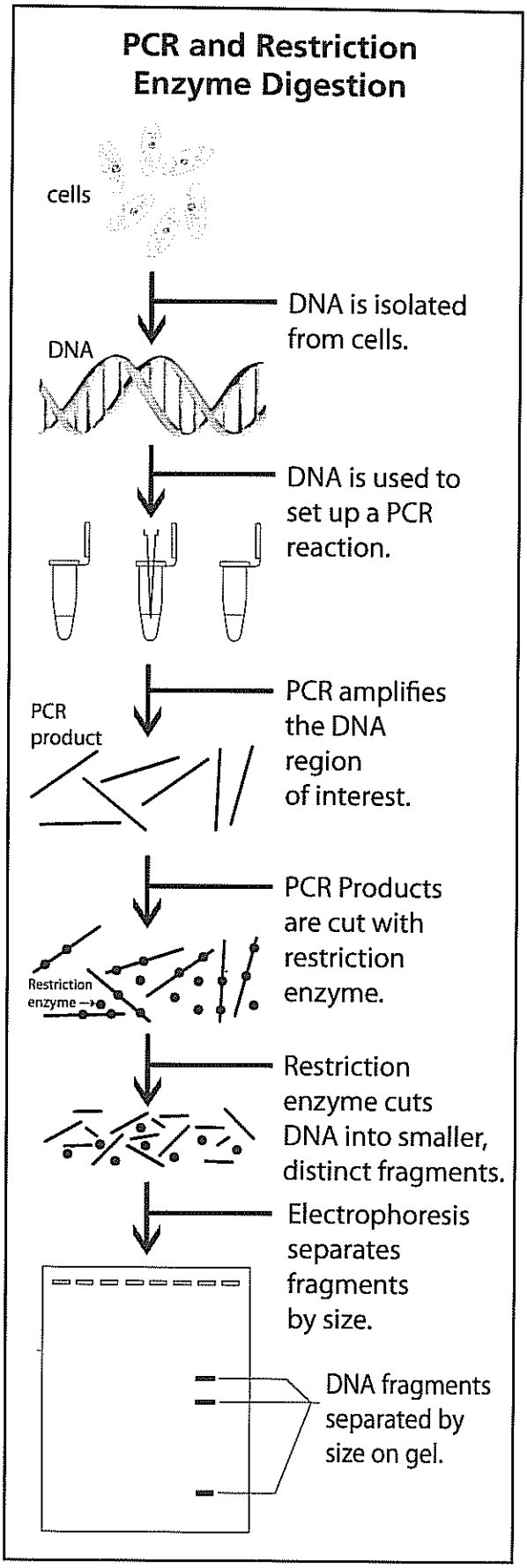
Pre-laboratory Questions

1. Explain how electrophoresis can be used as a part of genetic testing.

2. Why do you think it would be necessary to use PCR to amplify the region of DNA you wish to analyze?

3. What do restriction enzymes do? Describe how a restriction enzyme can be used to determine if there is a single base-pair change between two otherwise identical pieces of DNA.

4. Identify the three controls included in the simulated genetic screen and explain why they are necessary.



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