Gel Electrophoresis Lab

	Points Possible	Points Earned
Pre-lab		
- Agarose concentration equation	2	
- Part 1 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 gel for 45 minutes. Do you think the group's results are valid? Why or why not?	4	
2. Explain why the DNA fragments run toward the positive, red electrode (the anode).	2	
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	3	
- Part 2 questions		
1. Explain how electrophoresis can be used as a part of genetic testing. (Think about what you will be seeing at the end of the lab on your gel)	3	
2. Why do you think it would be necessary to use PCR to amplify the region of DNA you wish to analyze?	2	
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	
4. Identify the three controls included in the simulated genetic screen and explain why they are necessary. (Which vials of DNA are your comparison groups?)	6	
Results		
- Complete the data table with the distance traveled and Rf values for Marker DNA, Child 1 and Child 2 with Interpolated bp lengths for the Child 1 and 2	16	
- Graph the known marker DNA Rf values on the graph paper provided and use the line to interpolate the bp amounts for each child's DNA	5	
- Calculate percent error each band for each child • % error = (actual – interpolated / actual) x 100	4	
Analysis Questions		
1. Compare the DNA fingerprints produced by restriction digestion of the PCR products generated from the children's DNA and the DNA fingerprints created by digestion of the PCR products from the control CF mutant and wild-type DNA.		
a. Which pattern does the DNA fingerprint from each child's DNA match – the pattern from the mutant DNA, the pattern from the wild-type DNA, or a combination of both?	2	
b. What does this tell you about the genotype of the children with respect to this CF mutation?	2	
c. Remember that this particular mutation in the cystic fibrosis gene destroys a restriction enzyme site. If you did not know about the destroyed restriction site, what about the fragment patterns on your gel would indicate to you that a restriction site had been destroyed?	3	

2. Discuss how each of the following factors would affect the results of electrophoresis:		
a. Voltage used	2	
b. Running time	2	
c. Amount of DNA used	2	
d. Reversal of polarity	2	
3. What is the source of restriction enzymes? What is their function in nature?	2	
4. Describe the function of electricity and the agarose gel in electrophoresis.	4	
5. A certain restriction enzyme digest results in DNA fragments of the following sizes: 4,000 base pairs, 2,500 base pairs, 2,000 base pairs, and 400 base pairs. Sketch the resulting separation by electrophoresis. Show starting point in the wells, positive and negative electrodes, and the resulting bands **under one well**.	6	
6. What are the functions of the loading dye in electrophoresis? How can DNA be prepared for visualization?	4	
Extension Questions		
7. If two parents are heterozygous for a cystic fibrosis mutation, what is the probability that their child is homozygous for the cystic fibrosis mutation? Show how you determined the answer with either a Punnett square or used probability rules.	4	
8. How can possessing a mutation in both alleles of the CFTR gene cause the disease?	4	
9. A couple plans to have a child. The mother is homozygous for an autosomal recessive mutation. The father is heterozygous for the same mutation. With respect to this mutation, what are the possible genotypes of any children they have? Calculate the probability of each genotype's occurrence. Show how you got your answer.	4	
10. Both members of a couple come from families with a history of a severe disease (i.e. affected children do not live beyond a year or two after birth) caused by an autosomal recessive mutation. The disease appears in all people homozygous for the mutation and is present from birth on. People heterozygous for the mutation have, as they get older and to varying degrees, a higher risk for specific health problems that require medical care. The couple is deciding whether, before having children, they should be genetically tested for the mutation. **List 3 pros and 3 cons of their being genetically tested.** Consider the question from all perspectives (i.e. with respect to privacy, medical care, insurance, emotional effects and ethical implications)	6	
Total	**100**	