

Name _____ Period _____ Date _____



RFLP

STEM Sims

Lesson 1: Identifying Sickle Cell

Increased risk of strokes, organ damage, and a general lack of energy are just a few of the conditions associated with sickle cell anemia. The early detection and treatment of symptoms is critical in reducing the risks of sickle cell. RFLP is one technique used to identify the presence of a sickle cell. Are you ready to determine a patient's risk of sickle cell anemia?

Doing the Science

Part I: Preparing and Running the Gel

1. Open the RFLP simulation.
2. Select the number on the rack in front of each patient sample to observe the ID code for each patient sample. Make sure to record these Patient ID# in table 1. Each patient sample contains 10 μL of patient's DNA.
3. Select the pipette from the shelf and move the pipette to the New Tips area.
4. Move the pipette to the 10x buffer on the shelf. Select the Fill button on the pipette.
5. Use the blue arrows to adjust the volume of the pipette to 4 μL . Select the fill button to add 10x buffer to the pipette.
6. Move the pipette to the first patient sample and use the button to dispense the buffer into the sample tube. Repeat this process adding 4 μL of 10x buffer to each of the five patient samples. After filling all five samples tubes, move the pipette to the Waste Area to discard the tip.
7. Select the pipette from the shelf and move the pipette to the New Tips area.
8. Move the pipette to the restriction enzyme container on the shelf. Select the Fill button on the pipette.
9. Move the pipette to the first patient sample and use the button to dispense the enzyme into the sample tube. Repeat this process adding 2 μL of restriction enzyme to each of the five patient samples. After filling all five samples tubes, move the pipette to the Waste Area to discard the tip.
10. Select the pipette from the shelf and move the pipette to the New Tips area.
11. Move the pipette to the distilled water beaker on the shelf. Select the Fill button on the pipette.
12. Use the blue arrows to adjust the volume of the pipette to 24 μL . Select the fill button to add distilled water to the pipette.
13. Move the pipette to the first patient sample and use the button to dispense the water into the sample tube. Repeat this process adding 24 μL of water to each of the five patient samples. After filling all five samples tubes, move the pipette to the Waste Area to discard the tip.
14. Select and move each patient sample to the 37°C water bath on the tabletop. Set the timer for 1 hour and select the Start button.
15. Select the pipette from the shelf and move the pipette to the New Tips area.
16. Move the pipette to the first patient sample. Select the Fill button on the pipette.

17. Use the blue arrows to adjust the volume of the pipette to 40 μ L. Select the fill button to add the sample to the pipette.
18. Move the first patient sample to the first well on the electrophoresis gel. Dispense the sample into the well.
19. Move the pipette to the Waste Area to discard the tip.
20. Repeat the gel loading process for the other four patient samples. Make sure to place a new tip on your pipette for each patient sample.
21. Select the pipette from the shelf and move the pipette to the New Tips area.
22. Move the pipette to the DNA 200 bp ladder marker. Select the Fill button on the pipette.
23. Use the blue arrows to adjust the volume of the pipette to 40 μ L. Select the fill button to add the DNA marker to the pipette.
24. Move the DNA marker to the last well on the electrophoresis gel. Dispense the marker into the well.
25. Move the pipette to the Waste Area to discard the tip.
26. Set the timer on the power supply to 30 minutes and then turn on the power supply.
27. Select the Analyze button at the bottom of the screen to view your results.

Part II: Analyzing Results

28. Observe the electrophoresis gel and note the results. Select the Evaluate button when ready to evaluate the samples. Enter your results in table 1.
29. Evaluate each of the five patient samples and if in the Practice Mode, then select the Check Answers button to have your response evaluated. Select the Return to Gel button if you need to relook at the electrophoresis gel. If you are in the Test Mode, select the Submit Answer button to have your responses evaluated.

Table 1: Analysis Results

Sample #	Patient ID#	Normal	Carrier	Sickle Cell
1				
2				
3				
4				
5				

Do You Understand?

1. What is the function of the DNA maker ladder?

2. RFLP analysis results for sickle cell using a 200 bp DNA marker show a patient sample after electrophoresis having two distinct bands, one at 1.4 kbp and the other band at 1.2

kbp. How would you classify this patient's sample; as normal, as a carrier, or as having sickle cell?