BACTERIAL IDENTIFICATION LAB HANDOUT

INTRODUCTION

Go to https://www.hhmi.org/biointeractive/explore-virtual-labs. Scroll down and click on "The Bacterial Identification Virtual Lab." Maximize the screen if you wish. Answer the following questions in the spaces provided.

1. What is the overall purpose of this virtual lab?

2. What are the four basic steps involved in this bacterial identification lab?

3. What is "16S rDNA," and how is it used to identify species of bacteria?

Click to Enter the Lab. (Click the window on the left-hand side of the screen to enter the lab.) As you enter the lab, follow the instructions in the lab (left-hand window). Using the information in the Notebook window on the right, answer the following questions.

PART 1: SAMPLE PREPARATION

4. As the pathology lab technician, what is your task in this virtual lab?

5. Extracting DNA involves which initial step?

6. What is the wire ring used for?

7. Why are the proteolytic enzymes necessary?

8. Why do you then need to inactivate the proteolytic enzymes and how do you do it?

9. After removing the enzymes, why do you spin the sample in the centrifuge?

10. a. What is the pellet?

    b. What is the supernatant?

    c. Where is the DNA?

PART 2: PCR AMPLIFICATION

Go on to Part 2 and work through the PCR steps. Be sure to read the information in the notebook, including "What is PCR?"

11. What does "PCR" stand for and what is the purpose of PCR?

12. Summarize the process of PCR in a diagram. Include all the steps, labeled and in the right order. *(If you are completing this handout online, draw the diagram on a piece of paper, take a photo, save the image as a PDF, and upload it in the space below.)*

Add the Master Mix and answer the following questions:

13. What does the Master Mix contain?

14. What are primers? Why is a primer added?

15. Once the primers bind, what occurs next?

16. What does "highly conserved" mean?

17. Why are highly conserved regions important in this lab?

18. What does "highly variable" mean?

19. Why are highly variable regions important in this lab?

20. What is missing in the negative control tube?

21. What is present in the positive control tube that is not in the negative control tube?

Now run the PCR. Be sure to watch the virtual lab animation before proceeding to the questions.

22. List each step of a PCR cycle, the temperature, and the duration (time).
    a.
    b.

c.

23. Describe what happens during each of the steps in one or two sentences.
    a.

    b.

    c.

24. After eight cycles, how many copies of the desired DNA have been synthesized?

25. After 30 cycles?

PART 3: PCR PURIFICATION

26. Approximately how long is the 16s rDNA (bp)?

27. Why would it be useful to run an electrophoresis gel at this point?

28. If you were to run a gel, it would have three lanes. What would each lane contain, and what would you see in each lane after running the gel?
    a.

    b.

    c.

29. The gel is not run in this virtual lab. In order to purify the PCR product, you use a microconcentrator column. *(Proceed through the virtual lab steps.)* What should the final collection tube contain?

PART 4: SEQUENCING PREPARATION

Click on "Learn about cycle sequencing before proceeding."

30. Read the first two paragraphs and list the steps in cycle sequencing in the space provided.

Click to go back to Part 4.

31. What do the green and blue tubes contain? Describe the "sequencing brew" to which you added your purified PCR.

32. The purpose of the second PCR is not to create identical copies like the first PCR you ran. What is the purpose of this PCR?

33. Where do scientists obtain primers to be used in PCR and in this technique?

Watch the virtual lab animation before proceeding to Part 5.

PART 5: DNA SEQUENCING

34. What is the final PCR product, the stuff contained in your 12 tubes?

35. What is the purpose of gel electrophoresis?

36. How do DNA molecules move in relation to charge? Why?

37. What is the purpose of the laser beam in determining a DNA sequence?

Be sure to watch the virtual lab animation before proceeding to Part 6.

PART 6: DNA SEQUENCE ANALYSIS

Click on "Learn about the science behind sequence matching."

38. What is the ultimate goal of the sequence matching analysis?

39. What is "homology"?

40. What is BLAST and how is it used?

41. What's a major assumption when drawing evolutionary relationships between organisms based on DNA sequences?

Click to go back to Part 6 and click on "Learn more about BLAST search results."

42. Explain what the "Score (bits)" means on an actual BLAST search result.

43. What does an E-value of 3 or less represent?

Click to go back to Part 6 and proceed through the instructions in the right-hand notebook window.

- *Hints: "Ctrl A" will select all the data in the pop-up window, "Ctrl C" will copy it, and "Ctrl V" will paste it into the NCBI website (large yellow box at the top of the BLAST search page).*
- *Follow the steps listed on the page and be patient. BLAST data can take a while to search.*
- *When the BLAST results appear, scroll down below the color key to the significant alignments, and then go back to the virtual lab window (left) and follow the instructions.*

44. What is the scientific name of the bacterium you sequenced?

45. Write a brief description of this bacterium in the space provided.

After completing Sample A, perform DNA sequence analysis on three of the other five samples.

46. Write in the letter of the samples you choose, the scientific name of the bacterium (after doing a BLAST search), and a brief description of each.

Sample Letter	Bacteria Scientific Name	Brief Description