Name:_____

Date:_____ Period:_____

Biology 10
Bacterial ID Lab

"Intro" Tab Questions

1) What is the overall purpose of this lab?

2) What piece of DNA are we using in this experiment?

3) What does 16S rRNA mean?

When you've answered these three questions, click on either the lab area, or "1. Sample Prep" at the bottom left of the screen.

"1. Sample Prep" Questions
Read through the text that appears, and answer these questions.

4) What is the first step of this experiment?

5) How do we get the bacterial DNA out of the cell?

Now follow the directions in the window and answer these questions.

6) Why must the microcentrifuge tube sit in a hot water bath?

7) Where does the microcentrifuge tube go after the hot water bath?

8) After centrifuging, where is the DNA?

"2. PCR Amplification"
Read the text on the right and answer these questions

9) What does a PCR accomplish?

10) What is in the green capped-bottle? What is it used for?

11) What happens in each of the thirty cycles of the PCR? Fill in this chart

Melt: _____

Anneal: _____

Extend: _____

12) After 30 cycles, how many copies of the initial DNA can be produced?

Now follow the directions in the window and answer these questions:

13) What is in the red capped bottle you initially click?

14) What do you add to the blue PCR tube that is unique to the other tubes?

15) What is the purpose of the green and blue PCR tubes?

"3. Purify PCR Product"
Read through the text on the right, and answer these questions.

16) If you know that your PCR reaction worked, you can skip running a gel, and use what to purify your PCR product?

17) You will be centrifuging your PCR product in the microcentrifuge tube with the microconcentrator column in it. Where does the PCR product become trapped in this process?

18) How long do you spin the INVERTED column in the final step of this part?

Now click on the animation window and answer these questions.

19) What is in the yellow capped jar?

20) How long do you centrifuge the column after putting the DNA samples on ice?

21) What do you add to the new collection tube with the inverted column?

"Part 4, Prepare for Sequencing"
Click on the blue "Learn about Cycle Sequencing", and answer these questions

22) What are dideoxynucleotides used for? What do these nucleotides force DNA to do while it is replicating?

23) If you have the sequence 3' A-C-G-T-T-G 5', what are the six fragments of DNA that will result using cycle sequencing?

a) _____

b) _____

c) _____

d) _____

e) _____

f) _____

Now click on the "Back to part 4" link and answer these questions.

24) How many different primers are we using for our DNA sequencing?

25) What are some of the molecules found in the green and blue tubes? List 3.

a) _____ b) _____

c) _____

Now click on the animation and answer these questions as you go.

26) What's in the blue-capped bottle?

27) After adding the purified PCR to the strip tubes, what do you do next?

28) During the PCR reaction, what do the little colored “T”’s stand for?

Part 5: DNA sequencing
Read the text in the right window and answer these questions.

29) What does the automatic sequencer perform on the 12 tubes in order to sequence the DNA?

30) Which fragment of DNA in our lab is the smallest?

Now click on the animation window and answer these questions.

31) What is the first DNA sequence that you determine? Watch Closely! You may have to replay the animation a few times to get it.... (evil chuckle)

Part 6: Sequence Analysis
Read the text on the right and answer these questions.

32) How do we determine which species of bacteria this DNA came from?

33) What does BLAST stand for?

34) Once you’ve copied your DNA sequence, go to the BLAST page. Use these settings:

1) Paste your sequence into the box labeled “Enter accession number, gi, or FASTA sequence”

2) In the “Choose Search Set” section, select “Database: Others (nr etc)

3) “Nucleotide collection (nr/nt) should already be selected

4) click on the blue BLAST button. Let the computer search the database, which may take a few minutes.

What is the likely name of the bacteria from this sample? _____

Click on the computer in the animation window, and select your bacteria.

35) What is the common name of an infection from this bacteria?

YOUR TURN!

Choose three of the other samples (B through F) and see if you can identify the bacteria!

All you have to do is select your sample, and then on the Part 6 text window, click on "Step 1" to see your results. You don't have to go through all of the animations again.

Fill in the chart below for your results.

Sample	Name of Bacteria