Name_____

FS 362 PCR Instruction Evaluation

1. What is polymerase chain reaction (PCR) and what is the purpose of this reaction?

2. Diagram the Central Dogma and identify which aspect is exploited by polymerase chain reaction.

3. What role does temperature play in PCR?

4. What role do primers play in PCR?

5. List 3 characteristics of well-designed primers.

6. Describe 5 ways to optimize a PCR reaction.

7. Why are PCR detection methods preferred over classic microbiological methods?

8. Describe two advantages and disadvantages of PCR-based pathogen detection methods.

9. Describe two general PCR-based methods that could be used to differentiate *Salmonella* and *Listeria* spp.

10. List 3 characteristics of a well-designed PCR assay for detection of foodborne pathogens

Name_____

FS 363 Lab Evaluation

1. What is the initial step required to extract DNA from bacterial cells?

2. Identify two methods commonly used to prepare DNA template for PCR

3. List 3 characteristics of well-designed primers which will enhance the likelihood of a successful PCR reaction

4. What enzyme is can be used to degrade proteins in a DNA preparation?

5. Describe how agarose gel electrophoresis can be used to differentiate DNA fragments and its role in PCR-based detection applications

6. Explain the purpose of positive, negative, and internal controls in PCR-based detections systems.

7. How does ethidium bromide allow us to visualize DNA embedded in an agarose gel?

8. What is the purpose of a DNA ladder when running PCR products on an agarose gel?

9. How do you determine if primers designed to detect *Salmonlla* spp. were unique to *Salmonella* and would not detect other species such as *Listeria*?

10. What class of enzymes will degrade DNA?

Name_____

FS 362 PFGE Instruction Evaluation

1. What is pulse field gel electrophoresis (PFGE) and what is the purpose of this technique?

2. What is the main governmental agency that utilizes PFGE?

3. What are the 4 main components of PFGE equipment system?

4. What is different between the electrophoresis in PFGE and that of traditional PCR electrophoresis?

5. What role do restriction enzymes play in PFGE?

6. What is the purpose of suspending the bacterial DNA in plugs prior to treatment with enzymes?

7. How specific is the band profile from PFGE in recognizing a bacterium? (Hint: Genera, species, strain level?)

8. Describe 3 ways to optimize and retain precision in band separation for PFGE.
   Any of the following will work:

9. If you were investigating an outbreak incidence, what database would you compare your PFGE profile to in order to determine other occurrences sourced from the same bacteria?

10. Describe two advantages and disadvantages of PFGE-based pathogen tracking methods.

Name_____

FS 363 PFGE Lab Evaluation

1. What does the acronym PFGE stand for?

2. What is the initial step required to extract DNA from bacterial cells?

3. Explain what a casting plug is.

4. Why is it important to have a reference plug slice in multiple wells?

5. Explain why one must use the two restriction enzymes specified by the CDC for the bacteria in question.

6. What are the two restriction enzymes used for PFGE analysis of *Listeria monocytogenes*?

7. Why is it important to have a cooling module while running electrophoresis in PFGE?

8. Describe how using multiple pulse angles allow for increased band separation.

9. What are 3 possible reasons for poor resolution and smearing of bands on the gel?

10. How does ethidium bromide allow us to visualize DNA embedded in a gel?