Observation Questions (PCR only) (cloning questions follow below)

1. There are 3 key temperature changes in a PCR reaction. What are these temperatures? What is occurring at each of these temperatures?

2. The thermocycler cycles through the 3 temperatures 25-30 times. Why?

3. PCR reactions are performed with Taq DNA polymerase. What is the key feature of this enzyme that makes it different from most other enzymes?

4. What is the role of the DNA primers in a PCR reaction?

5. It is possible to visualize ~1 ug of DNA on your stained agarose gel. Given that your DNA is ~ 1000bp, how many molecules is this?

6. In gel electrophoresis, to which pole does the DNA migrate? Why?

7. What is the function of the gel?

8. Why is ice used?

9. Describe 2 practical applications of using the PCR.

10. Describe three experimental errors that may have influenced the procedure.

QUESTIONS

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3. PCR reactions are performed with Taq DNA polymerase. What is the key feature of this enzyme that makes it different from most other enzymes?

4. What is the role of the DNA primers in a PCR reaction?

5. In which motion picture was PCR popularized?

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In gel electrophoresis, to which pole does the DNA migrate? Why?

7. Which migrates more quickly on an agarose gel, a big DNA fragment or a smaller one?

8. What are the features of a plasmid DNA that make it suitable for use as a cloning vector?

9. Why is it important to balance your samples prior to centrifugation?

10. Your centrifuge rotor is spinning at ~10,000 rpm. How much distance does the outside of the rotor travel in one minute?

11. A transformation reaction contains millions of E. coli cells yet very few grow on the agarose plate. Why?

12. Given that a bacterial cell divides ~ every 20 minutes, how many cells are there in each of your bacterial colonies.

13. What does lysozyme do to bacterial cells? Humans make a form of lysozyme. Where is it found?

14. What (other than DNA) is contained in the isopropanol pellet of your DNA preparation?

15. What is the recognition sequence for EcoRI? Why might this sequence be called a palindrome?

16. Given that the human genome is ~3billion base pairs long, how many times would it be cut by EcoRI

17. What would your DNA sample look like on an agarose gel if you forgot to add EcoRI to your digest?

18. Why is the EcoRI digestion done at 37^{o}C?

19. Not all of your transformed *E.coli* cells contain plasmids that have the KanR gene. Why?

20. Which colonies (red or white) grew on the kanamycin plate? Why?

21. Why is it important that sterile solutions be used in the cloning of DNA?

22. Name a drug that is made after its expression in bacteria.