Experiment 7

The Polymerase Chain Reaction (PCR) of human mtDNA

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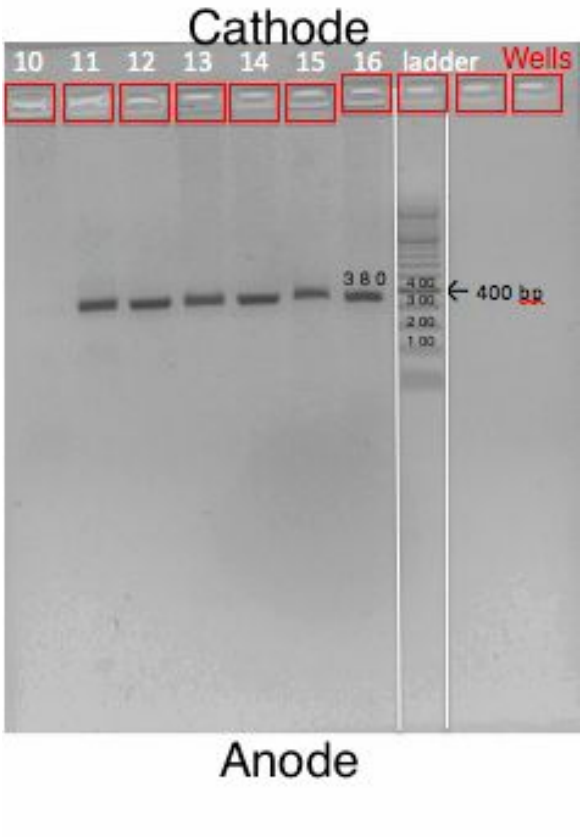
ABSTRACT

1. Understand the concepts and principles of polymerase chain reaction.
2. Understand and describe the major characteristics of mitochondrial DNA and single nucleotide polymorphism of the control region sequences.
3. Demonstrate laboratory techniques used in DNA isolation, PCR reaction and visualization of PCR product by DNA agarose gel electrophoresis.

Part I: Answer the following questions. Include questions 1-10 in your report.

1. **Presentation of the picture of the gel, with proper labeling of each lane, wells, the sizes of the marker bands, and the anode and cathode. (2 pts)**



My lane is #16.

2. **How did you predict the size of the PCR product (Human mitochondrial genome map on page 3)? What is the estimated size of your PCR product based on the DNA size marker loaded on your gel when you compare it to the ladder? Why was the 100 base-pair ladder used instead of 500 bp ladder for this exercise? (3 points)**

   The size of the PCR product was predicted using the difference between the left and right primer on the human mitochondrial genome map on page 3. Compared to the DNA ladder, The size of the PCR product is estimated to be 450 base pairs. The 100 base pair ladder was used instead of 500 base pair ladder for this exercise because the human mitochondrial DNA is very small, thus smaller increments are necessary for a more precise and accurate measurement.

3. **Each round of PCR doubles the number of DNA molecules present in the reaction. How many molecules can be generated from one of your DNA template molecules after 30 cycles? (1 points)**

   2^30 = 1,073,741,824 molecules

4. **You amplified a segment of your own mitochondrial DNA. Do you expect the sequences of your PCR product vary from those of your classmates? Why or Why not? (2 points)**

   While the sequences vary from student to student because mitochondrial DNA is maternally inherited, the sequences of the PCR product will be the same for everyone because the same region is being amplified.

5. **What does the term "denaturation" mean in relation to DNA? (2 pts)**
   The denaturation of nucleic acids such as DNA due to high temperatures is the separation of a double strand into two single strands, which occurs when the hydrogen bonds between the strands are broken.

6. **What does the term "annealing" mean in relation to DNA? What kinds of chemical bonds are formed when two strands of DNA anneal? (2 points)**
   Annealing means for complementary sequences of single-stranded DNA to pair by hydrogen bonds to form a double-stranded polynucleotide.

7. **The process of PCR is dependent upon the use of certain DNA polymerases.**

   a) Why are they special and where do they come from? (2 points)
   They're special because they are stable at high temperatures, which allows for the millions of copies of a single DNA fragment to be produced within hours. They come from a bacterium hanging out in a hot spring in the Yellowstone National Park.

   b) Why does the PCR reaction catalyzed by the Taq DNA polymerase have a higher mutation rate than the normal DNA replication process found in living cells?
   The Taq DNA polymerase has no proof-reading function, which leads to a higher mutation rate than the normal DNA replication process found in living cells.

8. **What is the basis of separation of DNA molecules in the agarose gel that you run in this experiment? What are the electrical charges of the DNA molecules? (2 pts)**

   DNA migration down the gel depends on the molecular size of DNA, the conformation of the DNA, the agarose gel concentration, the buffer and the applied voltage. Specifically, the mobility of linear DNA fragments is inversely proportional to the log10 of their molecular weight. DNA is negatively charged.

9. **What reagent is added to the 0.5X TBE buffer to allow visualization of the DNA bands in the agarose gel? Describe how you can see the DNA after the gel running is stopped. (2 pts)**

   Ethidium Bromide is added to the 0.5X TBE buffer to allow visualization of the DNA bands in the agarose gel. It fluoresces under UV light.