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PCR (Polymerase Chain Reaction)

* PCR
  + 1983 first discovered by Kary Mullis
  + used to amplify a single copy or a few copies of DNA in order to create 1000s or millions of copies of a particular DNA sequence
  + OVERVIEW
    - relies on thermal cycling, consisting of repeated heated and cooling reactions
  + COMPONENTS
    - Heat Stable DNA Polymerase (usually Taq Polymerase)
    - DNA template
      * should contain DNA region that is to be amplified
    - DNA primers
    - dNTPS (deoxynucleotide triphosphates)
      * nucleotides with triphosphate groups
      * building blocks that DNA polymerase uses to synthesize new strand
    - Buffer Solution
      * provides best chem environment for best activity and stability of DNA polymerase
    - Thermal Cycler→ heats and cools tubes (with the DNA) to performs each step
  + consists of 20-40 repeated cycles→ each cycle has 3 steps
  + STEPS
    - 1. Denaturation
      * reaction heated to 94-95 C for 30 seconds
      * causes breakage of Hydrogen bonds between two strands
      * result: single stranded DNA template
    - 2. Annealing Step
      * 50 C for 30 seconds
      * allows primers to attach to single stranded DNA template
        + if temp too low primers could bond imperfectly, so temperature has to be correct
    - 3. Elongation Step
      * 72 C
      * DNA polymerase (taq polymerase) synthesizes a new DNA strand complementary to the DNA template using dNTPS
        + add dNTPS in 5’-3’ direction
  + amplified by the power of 2 for each PCR cycle→ 2^n
  + RELEVANT USES OF PCR:
    - DNA cloning for sequencing
    - DNA based phylogeny
    - Diagnosis of hereditary disease
      * ex: Huntington's Disease
    - Identification of fingerprints
      * ex: Forensic Science
    - Detection and diagnosis of infectious diseases
      * ex: AIDS

MULTIPLE CHOICE QUESTIONS :

1. Which of the following best describes the steps that occur during every cycle of PCR?

1. The primers bond to the target DNA.

2. The mixture is heated to a high temperature to denature the double-stranded template DNA.

3. Heat-stable DNA polymerase is added.

4. DNA polymerase extends the primers to make a copy of the target DNA.

**A) 2, 1, 3, 4**

B) 1, 3, 2, 4

C) 3, 4, 1, 2

D) 2, 4, 3, 1

1. If temperature is too low during the Annealing Step:
2. The primers will be able for perfectly with the template strand
3. The primers will still be able to bond, but will be shorter
4. **The primers will bond imperfectly and will not work properly**
5. The primers will bond imperfectly, but the reaction will still work as it should

1. PCR is used for all EXCEPT:
2. Forensic Science
3. Creating Phylogenetic Trees
4. **Maternity testing**
5. Diagnosis of hereditary diseases
6. The reason for using Taq Polymerase is:
7. **It is heat stable and can withstand the change in temperature throughout the cycle**
8. Only a small amount is needed
9. It is the best at adding dNTPs when creating the complementary strand
10. It binds the best to the primers compared to other polymerases
11. All of the above are correct
12. WITHOUT the use of Buffer Solution:
13. The chemical environment would remain the same and the DNA polymerase would work just as well
14. The stability of the DNA polymerase would increase and the reaction would occur more efficiently
15. **Both the chemical environment and stability of DNA polymerase would be affected negatively**
16. A & B only

ESSAY QUESTIONS:

1. You have just learned of your deceased mother’s Huntington’s Disease condition and you are concerned with the potential likelihood that you too have inherited this disease. After talking with your spouse, you are still unsure of whether or not to be tested. If you decide to be tested; go through the process of who you talk to and then go through the scientific process. What is done scientifically to confirm whether or not you have Huntington's or not. Be specific.

KEY:

* The answer should include: the mention and definition of a genetic counselor, sending your DNA to a company like 23&me who will go through the PCR process and Sanger Sequencing (which should be described), the results that will come back to you and then what to do with those results (send them to another company who will go through something like BLAST and translate your results so you can read them)

1. Four specimens are place in front of you and you are asked to determine which are the most closely related and your evidence behind your determination. Using your scientific knowledge describe all aspects of the processes you would go through.

KEY:

* The answer should include detailed description of Isolating the DNA, PCR method, Sanger sequencing and BLAST, and it should be described in the correct order. It should also include the description of DNA Subway and why it is important to use this in order to differentiate between the specimen. When discussing BLAST, the significance and description of the E value should mentioned. Lastly, the essay should include a description of what the tree will show; i.e will it show homologies or analogies or both