Study guide for PCR

Polymerase Chain Reaction (PCR)

What is PCR: PCR is a means to amplify (make copies of) a piece of DNA, PCR can make billions of copies out of one piece of DNA in just a few hours

Key components of PCR:

-A small amount of DNA that serves as the initial template/target sequences

-A pair of primers designed to bind to each end of the target sequence

-A DNA polymerase

-Four dNTPs (dATP, dCTP, dGTP,dTTP)

-A machine called a thermocycler that jump-starts each stage of the PCR reaction by raising and lowering the temperature of specific chemical components in cycles

3 Main steps of PCR:

1. Denaturation of DNA

-DNA sample is heated in order to break the hydrogen bonds between the double strand

-At 90 to 100 degrees Celsius this is the hottest part of the PCR process

-Using the heat to denature the DNA is used in place of the enzyme Helicase that ordinarily unravels DNA in replication

1. Primers anneal

-The mixture is cooled between 30-65 degrees Celsius, this gives the primers that are present in the mixture an opportunity to anneal (bind) to their complimentary nucleotide sequences on the single strands of DNA

1. Extension

-Sample is heated again, this time between 60 and 75 degrees Celsius

-DNA polymerase begins making a new DNA strand by attaching to the primers and then adding the four different dNTPs to the template strand

Additional information:

-The number of copies of DNA based on the template strand doubles with each 3 step cycle, this can be represented by the equation 2n

-Violations of conventional PCR include real-time PCR, which is a technique that makes it possible to measure the amount of DNA produced during each PCR cycle as the PCR is underway by marking the DNA with fluorescent markers

-By combining real time PCR with reverse transcription, it is possible to determine the amount of kinds of genetic material besides DNA like RNA that PCR is producing

Multiple choice questions:

1. What are the 3 main steps of the Polymerase chain reaction?
2. Sequencing, Binding, and Consolidation
3. Separation, Connection, and Quantification
4. Denaturation, Annealing, and Extension
5. Priming, Denaturation, Extension
6. What is a DNA barcode?
7. A region of the DNA that contains nucleotides
8. A sequence of DNA that uniquely identifies each species of living things
9. A key ingredient in the Polymerase Chain Reaction
10. A sequence used in bioinformatics to search for specific types of DNA
11. What are paralogues?
12. The same gene shared between two different species
13. Phylogenic trees that share similar species
14. Variations on a DNA sequences within an organism’s genome
15. A gene shared between members of the same species that may be different in sequence composition and function
16. What enzyme is used to covalently join DNA segments?
17. Helicase
18. dNTP
19. Ligase
20. DNA polymerase
21. What physical features are the best indications of relatedness between species?
22. Homologous features
23. Convergent features
24. Phylogenic features
25. Analogous features

Sample essay questions

1. Describe the major molecular events of DNA replication of Eukaryotic cells, making sure to note the significant function each step plays in the overall process of replication. Be sure to make note of at least 4 of the significant enzymes involved in the replication process

Point distribution:

(4)- Listing each of the 4 steps of DNA replication; initiation, unwinding, primer synthesis and elongation

(3)- Accurate description of initiation, making note of the initiator proteins binding to the replication origin, and how this leads into the unwinding step

(3)- Accurate description of unwinding, noting the breakdown of the hydrogen bonds between the double strand of DNA by Helicase

(3)- Description of how primer synthesis marks the beginning of the actual synthesis of the DNA molecule, with the addition of deoxyribonucleotides to the 3-OH group of the leading strand of DNA

(3)- Description of elongation, noting how this step involves the addition of nucleotides to the newly synthesized DNA strand

(4)- Mention of any of the following enzymes with an acceptable description of their function earns 1 point, up to a max of 4 points:

-primase

-DNA helicase

-Single Strand binding protein

-DNA ligase

-DNA polymerase 1

-DNA polymerase 3

-topoisomerase

1. Define the term “operon”. Describe the operation of the LAC operon in E.Coli, specifically making note of its intended function, at least 3 of the genes that compose it, the way it is induced, and two ways that it can be “turned off”.

Point distribution:

(4) Definition of operon along the lines of how it is a series of linked genes under control of a single operator (regulatory gene)

(2) Note the function of the LAC operon in E. Coli, intended to make enzymes that will process lactose

(6) Listing at least 3 the genes that compose the LAC operon, this could include lacI, lacO, lacB (B-galactosidase), and lacP (lactose permase)

(2) Note the method of induction: derepression

(2) Describe how derepression works: the repressor enzyme that is normally active to repress the production of the enzymes of the LAC operon is inactivated by the presence of lactose

(4) Description of how the LAC operon can be “turned off” by both:

-The removal of all the lactose immediately surrounding it

-The presence of glucose, whose processing is prioritized over the processing of lactose