QUESTIONS

1. Qualitative PCR and quantitative PCR provide information on _____ and _____, respectively.

A. Presence/absence of specific DNA product; how much of a specific DNA product is present.

B. How much of a specific DNA product is present; presence/absence of DNA product.

C. RNA; DNA.

D. Gene by-products; RNA.

2. The most widely used method of analysis of the PCR product is:

A. Agarose gel electrophoresis.

B. Western blot.

C. ELISA.

D. FISH.

3. A major advantage of using PCR as compared with other molecular biology techniques is:

A. Low risk of contamination.

B. Rapidity.

C. Low sensitivity.

D. Low specificity.

4. The PCR process contains these three steps:

A. Denaturation, transcription, annealing.

B. Annealing, denaturation, transcription.

C. Denaturation, annealing, transcription.

D. Transcription, annealing, denaturation.

ANSWERS

1. A. Presence/absence of specific DNA product; how much of a specific DNA product is present. Qualitative PCR is used to detect the presence or absence of a specific DNA product. Qualitative PCR is a good technique to use when PCR is performed for cloning purposes or for identification of a pathogen. On the other hand, quantitative PCR provides more information beyond the mere detection of DNA. It is able to indicate how much of a specific DNA or gene is present in

the sample.

2. **A. Agarose gel electrophoresis.** The most widely used and easiest method for analyzing the PCR product involves agarose gel electrophoresis. It allows determination of the presence and size of the PCR product. A predetermined set of DNA products with known sizes is run on the gel as molecular markers to help determine the size of the product. The two other methods for visualizing PCR products are staining of the amplified DNA product using a chemical dye such as ethidium bromide, which intercalates between the two strands of the duplex, and labeling the PCR primers or nucleotides with fluorescent dyes (fluorophores) prior to PCR amplification.

3. **B. Rapidity.** PCR allows the creation of billions of copies of a specific DNA fragment or gene, which allows for detection and identification of gene sequences using visual techniques based on size and charge. It is a highly sensitive technique and can provide results within a shorter time frame than that required for most molecular techniques.

4. **C. Denaturation, annealing, transcription.** The PCR process can be divided into three main steps. In the denaturation process, the solution is heated above the melting point of the two complementary strands of the template DNA, which allows the strands to separate. Then the annealing process allows the primers to bind to the specific DNA segment. The annealing between the primers and the template DNA occurs only if they are complementary in sequence. The temperature is raised again, at which point the DNA polymerase is able to extend the primers by adding nucleotides to the developing DNA strand, known as transcription. With each repeat of these three steps, the number of copied DNA molecules doubles.