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**Viva assignment: Molecular biology**

**Q1**

**PCR**

**Polymerase chain reaction (PCR) is a method widely used to rapidly make millions to billions of copies of a specific DNA sample, allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail. PCR was invented in 1984 by the American biochemist Kary Mullis at Cetus Corporation. It is fundamental to much of genetic testing including analysis of ancient samples of DNA and identification of infectious agents. Using PCR, copies of very small amounts of DNA sequences are exponentially amplified in a series of cycles of temperature changes. PCR is now a common and often indispensable technique used in medical laboratory and clinical laboratory research for a broad variety of applications including biomedical research and criminal forensics.**

**Procedure**

**Typically, PCR consists of a series of 20–40 repeated temperature changes, called thermal cycles, with each cycle commonly consisting of two or three discrete temperature steps (see figure below). The cycling is often preceded by a single temperature step at a very high temperature (>90 °C (194 °F)), and followed by one hold at the end for final product extension or brief storage. The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters, including the enzyme used for DNA synthesis, the concentration of bivalent ions and dNTPs in the reaction, and the melting temperature (Tm) of the primers. The individual steps common to most PCR methods are as follows:**

**Initialization:**

**This step is only required for DNA polymerases that require heat activation by hot-start PCR.[12] It consists of heating the reaction chamber to a temperature of 94–96 °C (201–205 °F), or 98 °C (208 °F) if extremely thermostable polymerases are used, which is then held for 1–10 minutes.**

**Denaturation:**

**This step is the first regular cycling event and consists of heating the reaction chamber to 94–98 °C (201–208 °F) for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.**

**Annealing:**

**In the next step, the reaction temperature is lowered to 50–65 °C (122–149 °F) for 20–40 seconds, allowing annealing of the primers to each of the single-stranded DNA templates. Two different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region. The primers are single-stranded sequences themselves, but are much shorter than the length of the target region, complementing only very short sequences at the 3’ end of each strand.**

**It is critical to determine a proper temperature for the annealing step because efficiency and specificity are strongly affected by the annealing temperature. This temperature must be low enough to allow for hybridization of the primer to the strand, but high enough for the hybridization to be specific, i.e., the primer should bind only to a perfectly complementary part of the strand, and nowhere else. If the temperature is too low, the primer may bind imperfectly. If it is too high, the primer may not bind at all. A typical annealing temperature is about 3–5 °C below the Tm of the primers used. Stable hydrogen bonds between complementary bases are formed only when the primer sequence very closely matches the template sequence. During this step, the polymerase binds to the primer-template hybrid and begins DNA formation.**

**Extension/elongation:**

**The temperature at this step depends on the DNA polymerase used; the optimum activity temperature for the thermostable DNA polymerase of Taq (Thermus aquaticus) polymerase is approximately 75–80 °C (167–176 °F),though a temperature of 72 °C (162 °F) is commonly used with this enzyme. In this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free dNTPs from the reaction mixture that are complementary to the template in the 5’-to-3’ direction, condensing the 5’-phosphate group of the dNTPs with the 3’-hydroxy group at the end of the nascent (elongating) DNA strand. The precise time required for elongation depends both on the DNA polymerase used and on the length of the DNA target region to amplify. As a rule of thumb, at their optimal temperature, most DNA polymerases polymerize a thousand bases per minute. Under optimal conditions (i.e., if there are no limitations due to limiting substrates or reagents), at each extension/elongation step, the number of DNA target sequences is doubled. With each successive cycle, the original template strands plus all newly generated strands become template strands for the next round of elongation, leading to exponential (geometric) amplification of the specific DNA target region.**

**The processes of denaturation, annealing and elongation constitute a single cycle. Multiple cycles are required to amplify the DNA target to millions of copies. The formula used to calculate the number of DNA copies formed after a given number of cycles is 2n, where n is the number of cycles. Thus, a reaction set for 30 cycles results in 230, or 1,073,741,824, copies of the original double-stranded DNA target region.**

**Final elongation:**

**This single step is optional, but is performed at a temperature of 70–74 °C (158–165 °F) (the temperature range required for optimal activity of most polymerases used in PCR) for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully elongated.**

**Final hold:**

**The final step cools the reaction chamber to 4–15 °C (39–59 °F) for an indefinite time, and may be employed for short-term storage of the PCR products**

**Uses**

**The polymerase chain reaction (PCR) is used to make millions of copies of a target piece of DNA. It is an indispensable tool in modern molecular biology and has transformed scientific research and diagnostic medicine.**

**Q2.**

**Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA or proteins in a matrix of agarose, one of the two main components of agar. The proteins may be separated by charge and/or size (isoelectric focusing agarose electrophoresis is essentially size independent), and the DNA and RNA fragments by length.Biomolecules are separated by applying an electric field to move the charged molecules through an agarose matrix, and the biomolecules are separated by size in the agarose gel matrix.**

**Agarose gel is easy to cast, has relatively fewer charged groups, and is particularly suitable for separating DNA of size range most often encountered in laboratories, which accounts for the popularity of its use. The separated DNA may be viewed with stain, most commonly under UV light, and the DNA fragments can be extracted from the gel with relative ease. Most agarose gels used are between 0.7–2% dissolved in a suitable electrophoresis buffer.**