Microbiological equipment

A. Polymerase chain reaction (PCR):

is a laboratory technique used to **amplify a specific segment of DNA**. This method allows researchers to obtain **multiple copies of a DNA sequence** from a small sample, which can be used for various applications such as genetic testing, forensics, and research.

Principle:

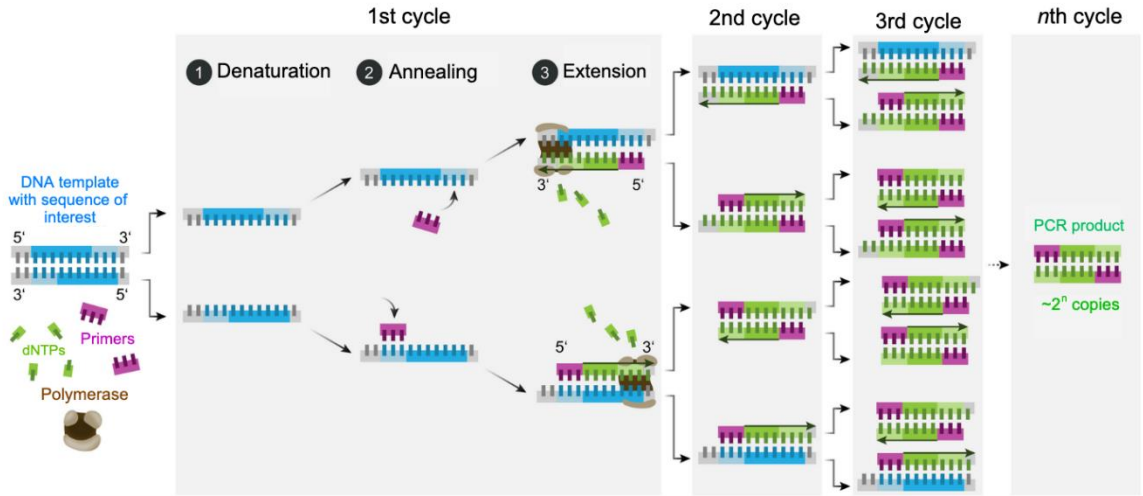
PCR works by using a special enzyme called **Taq polymerase**, which can extend and replicate DNA strands. This enzyme, along with primers and nucleotides, is mixed with the DNA sample in a reaction tube. The sample is then subjected to a series of temperature changes that cause the DNA strands to separate and allow the primers to bind to their complementary sequences. The Taq polymerase then **extends the primers, creating new copies of the DNA sequence.**

The most commonly used temperature cycles in PCR include:

1) **Denaturation:** This is typically done at a temperature of 94-98°C, where the double-stranded DNA template is separated into two single strands. This is necessary to allow the primers to bind to the target DNA.

2) **Annealing:** This is typically done at a temperature of 50-65°C, where the primers anneal or bind to their complementary sequences on the template DNA. This step is necessary for the amplification of the target DNA.

3) **Extension:** This is typically done at a temperature of 72°C, where the DNA polymerase enzyme extends the primers to generate new DNA strands. This step is necessary for the amplification of the target DNA.



Steps for using the PCR:

1. Prepare the DNA sample, primers, Taq polymerase, and nucleotides for the reaction.
2. Mix the components in a reaction tube and place it in a **thermal cycler.**
3. Run the thermal cycler program, which typically includes cycles of denaturation, annealing, and extension.
4. Analyze the PCR products using gel electrophoresis or other techniques.

Safety and care:

1. Follow safety guidelines and wear appropriate PPE, such as gloves and lab coat, when handling DNA samples and reagents.
2. Use clean and sterile techniques to avoid contamination of the samples.
3. Properly dispose of biohazardous waste and contaminated materials.
4. Regularly clean and maintain the thermal cycler to ensure proper function and prevent contamination.

B. Thermal cycler:

A thermal cycler, also known as a PCR machine, is a laboratory instrument used to amplify DNA samples through the polymerase chain reaction (PCR). It uses a precise temperature control system to carry out the temperature cycles required for PCR.
Principle of work:

The thermal cycler works by cycling through a series of **temperature changes** to facilitate the different stages of the PCR reaction. This involves **heating and cooling** the sample to specific temperatures to denature the DNA, anneal the primers, and extend the DNA strands.

Steps for using the Thermal cycler:

1. Prepare the PCR reaction mix according to the protocol for the experiment.
2. Set up the thermal cycler by inserting the PCR tubes or plate and programming the cycling parameters.

3. Load the PCR reaction mix into the PCR tubes or wells of the plate.
4. Place the PCR tubes or plate into the thermal cycler and start the program.
5. After the program is complete, remove the PCR tubes or plate from the thermal cycler and store the PCR products as required.

Safety and care:

1. Always wear gloves and lab coats when handling PCR samples and using the thermal cycler.
2. Keep the thermal cycler clean and free of any spills or contamination.
3. Follow the manufacturer's instructions for maintenance and cleaning of the thermal cycler.
4. Regularly check the calibration of the thermal cycler to ensure accurate temperature control.
5. Turn off the thermal cycler when not in use to save energy and prevent overheating.

C. VITEK II system:

Is an automated microbial identification and antimicrobial susceptibility testing system developed by bioMérieux. It is widely used in clinical microbiology laboratories to identify microorganisms and determine their susceptibility to various antibiotics.

Principle of work:

The VITEK II system uses a combination of technologies including **fluorescence, spectrophotometry,** and **turbidimetry** to identify microorganisms and determine their susceptibility to antimicrobial agents. The system works by incubating microbial isolates with different antimicrobial agents in a series of wells on a **specialized card**. The growth and **metabolic activity** of the microorganisms in each well are monitored using various sensors and probes. The resulting data is analyzed by the **VITEK II software**, which compares the growth patterns of the microorganisms in each well to a database of known microbial species and their corresponding antimicrobial susceptibility patterns.

Steps for using the VITEK II system:

1. Inoculation: The first step in using the VITEK II system is to inoculate the microbial isolate onto a specialized card containing various wells filled with different antimicrobial agents.
2. Loading the card: The card is then loaded into the VITEK II instrument and the appropriate test protocol is selected.
3. Incubation: The instrument incubates the card at the appropriate temperature and monitors the growth and metabolic activity of the microorganisms in each well.
4. Data analysis: The resulting growth patterns and metabolic data are analyzed by the VITEK II software, which compares them to a database of known microbial species and their corresponding antimicrobial susceptibility patterns.
5. Results interpretation: The final step is to interpret the results generated by the VITEK II system and use them to guide appropriate antimicrobial therapy.

Self-assessment 😊 :

1) What is the principle behind Polymerase Chain Reaction (PCR)?

2) What is the purpose of the Taq polymerase enzyme in PCR?

3) What are the three temperature cycles commonly used in PCR?

4) How should you dispose of biohazardous waste and contaminated materials when working with PCR?

5) What is the principle behind Thermal Cycler?

6) What is the function of the temperature control system in a Thermal Cycler?

7) How should you properly maintain the Thermal Cycler to ensure proper function and prevent contamination?

8) What is the purpose of the VITEK II system in clinical microbiology laboratories?