NAME: M.ASIF KHAN

ID : 14710

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SUB TO: ZAHIR MIAN

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QUESTION: WHAT IS 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 develop 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 aggressively 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.

The majority of PCR methods rely on thermal cycling. Thermal cycling exposes reactants to repeated cycles of heating and cooling to permit different temperature-dependent reactions – specifically, DNA melting and enzyme-driven DNA replication. PCR employs two main reagents – primers (which are short single strand DNA fragments known as oligonucleotides that are a complementary sequence to the target DNA region) and a DNA polymerase. In the first step of PCR, the two strands of the DNA double helix are physically separated at a high temperature in a process called Nucleic acid denaturation. In the second step, the temperature is lowered and the primers bind to the complementary sequences of DNA. The two DNA strands then become arrangement for DNA polymerase to enzymatically assemble a new DNA strand from free nucleotides, the building blocks of DNA. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the original DNA template is exponentially amplified.
Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the thermophilic bacterium Thermus aquaticus. If the polymerase used was heat-susceptible, it would denature under the high temperatures of the denaturation step. Before the use of Taq polymerase, DNA polymerase had to be manually added every cycle, which was a tedious and costly process.

Applications of the technique include DNA cloning for sequencing, gene cloning and manipulation, gene mutagenesis; construction of DNA-based phylogenies, or functional analysis
of genes; diagnosis and monitoring of hereditary diseases; amplification of ancient DNA; analysis of genetic fingerprints for DNA profiling (for example, in forensic science and parentage testing); and detection of pathogens in nucleic acid tests for the diagnosis of infectious diseases.

USES OF PCR:

Dignosis of diseases:

The use of PCR in diagnosing genetic disease, whether inherited genetic changes or as a result of a spontaneous genetic mutations, is becoming more common. Diseases can be diagnosed even before birth. Examples include:

Genetic counselling – screening the parents for genetic disease before deciding on having children

Preimplantation diagnosis – screening for genetic disease before implantation of an embryo in IVF (in vitro fertilisation)

Screening for genetic disease before birth using tissue samples from the chorionic villus (the membranes found between the mother and unborn baby); foetal tissue from the amniotic fluid (the fluid around the unborn baby); or the small quantities of foetal DNA (DNA from the unborn baby) found in the mother's bloodstream

Diagnosing inherited or spontaneous diseases, either as a result of symptoms, or because of family history (e.g. Duchenne muscular dystrophy)

Genetic fingerprints:

One of the most famous uses for PCR is in the creation of a genetic fingerprint (also known as DNA profiling) from a sample of blood or semen, or from a hair root. Much beloved by writers of detective fiction, genetic fingerprints are profiles of specific stretches of DNA (loci – commonly, 13 loci are compared) that vary from person to person. PCR also plays a role in mitochondrial DNA analysis, used for samples from hair shafts and bones when other samples are not available. The UK police has a National DNA table.

Genetic analysis based on PCR is also used in paternity testing, and in tissue typing for organ transplantation.

Detection and diagnosis of infectious diseases:

PCR can detect infectious disease before standard serological laboratory tests (tests to detect the presence of antibodies), so allowing treatment to start much earlier. Because of this, PCR is also useful for screening donated blood for infections, and is especially useful for infections that are difficult to culture in the laboratory, such as tuberculosis.

Detection of infection in the environment:

PCR is used to monitor and track the spread of infectious disease within an animal or human population. PCR can also be used to detect bacterial and viral DNA in the environment, for example looking at pathogens in water supplies.

Personalised medicine:

PCR is used in Personalised medicine to select patients for certain treatments, for example in cancer when patients have a genetic change that makes a patient more or less likely to respond to a certain treatment.

PCR can be used to create copies of DNA for introduction into host organisms such as Escherichia coli in genetic engineering, and to amplify stretches of genetic material for Sanger sequencing – the Human Genome Project used PCR.

PCR can be used in analysis of gene expression, for example looking at levels of expression and when genes are switched on and off in physiological processes, including in health and disease.

Other uses:

PCR is used in archaeology, to identify human or animal remains, including insects trapped in amber, and to track human migration patterns; degraded DNA samples may be able to be reconstructed during the early cycles of PCR. PCR can be used to differentiate between similar organisms such as ticks, or work out relationships between different species.

QUESTION: Explain the agarose gel electrophoresis:

Gel Electrophoresis Definition

Gel electrophoresis is a procedure used to separate biological molecules by size. The separation of these molecules is achieved by placing them in a gel with small pores and creating an electric field across the gel. The molecules will move faster or slower based on their size and electric charge.

Gel Electrophoresis Overview

The process of gel electrophoresis works because negatively charged molecules move away from the negative pole of the electric current and smaller molecules will move faster than larger molecules. Thus, a size separation is achieved within the pool of molecules running through the gel. The gel works in a similar manner to a sieve separating particles by size. The electrophoresis works to move the particles, using their inherent electric charge, through the sieve.

When researchers are trying to distinguish between different segments of DNA, for example, the process is simple. The samples are loaded into channels at the start of the gel. Each DNA molecule has the same charge (-1), because DNA is formed by the same 4 nucleotides and always carries a slightly negative charge regardless of its size. Therefore, each DNA molecule will have the same force pulling it through the gel.

However, the size of each molecule hinders its progress through the gel. Large molecules hit parts of the gel matrix, and are slowed down. Small DNA molecules can slip between the various components of the gel matrix, and quickly make their way to the other side of the gel. After a certain amount of time, the dyed DNA molecules can be seen aggregating in different areas of the gel, based on how far they moved during gel electrophoresis. This allows researchers to identify the segments, and compare the DNA of different organisms.

What is Gel Electrophoreses Used For?

The purpose of gel electrophoresis is to visualize, identify and distinguish molecules that have been processed by a previous method such as PCR, enzymatic digestion or an experimental condition. Often, mixtures of nucleic acids or proteins that are collected from a previous experiment/method are run through gel electrophoresis to determine the identity or differentiate between molecules.

Gel Electrophoresis Steps

The broad steps involved in a common DNA gel electrophoresis protocol:

1. Preparing the samples for running

The DNA is isolated and preprocessed (e.g. PCR, enzymatic digestion) and made up in solution with some basic blue dye to help visualize the movement of the sample through the gel.

2. An agarose TAE gel solution is prepared

TAE buffer provides a source of ions for setting up the electric field during electrophoresis. The weight-to-volume concentration of agarose in TAE buffer is used to prepare the solution. For example, if a 1% agarose gel is required, 1g of agarose is added to 100mL of TAE. The agarose percentage used is determined by how big or small the DNA is expected to be. If one is looking at separating a pool of smaller size DNA bands (<500bp), a higher percentage agarose gel (>1%) is prepared. The higher percentage of agarose creates a denser sieve to increase the separation of small DNA length differences. The agarose-TAE solution is heated to dissolve the agarose.

3. Casting the gel

The agarose TAE solution is poured into a casting tray that, once the gel solution has cooled down and solidified, creates a gel slab with a row of wells at the top.

4. Setting up the electrophoresis chamber

The solid gel is placed into a chamber filled with **TAE buffer.** The gel is positioned so that the chamber wells are closest to the negative electrode of the chamber.

5. Loading the gel

The gel chamber wells are loaded with the DNA samples and usually, a DNA ladder is also loaded as reference for sizes.

6. Electrophoresis

The negative and positive leads are connected to the chamber and to a power supply where the voltage is set. Turning on the power supply sets up the electric field and the negatively charged DNA samples will start to migrate through the gel and away from the negative electrode towards the positive.

7. Stopping electrophoresis and visualizing the DNA

Once the blue dye in the DNA samples has migrated through the gel far enough, the power supply is turned off and the gel is removed and placed into an ethidium bromide solution. Ethidium bromide intercalates between DNA and is visible in UV light. Sometimes ethidium bromide is added directly to the agarose gel solution in step 2. The ethidium bromide stained gel is then exposed to UV light and a picture is taken. DNA bands are visualized in from each lane corresponding to a chamber well. The DNA ladder that was loaded is also visualized and the length of the DNA bands can be estimated. An example is given in the figure below.

Types of Gel Electrophoresis

There are two types of gel electrophoresis: native and denaturing. Native gel electrophoresis usually attempts to keep RNA or protein in its native structure while running it through the gel. Denaturing gel electrophoresis attempts to reduce the RNA or protein into its most linear structure before or during gel electrophoresis.

The denaturation of the RNA or protein is accomplished by adding a reducing agent to the sample, gel and/or buffer. The reducing agent separates bonds within the RNA or protein molecule and thereby reduces its secondary structure. The secondary structure of a protein or RNA will influence, in a non-linear manner, how fast it migrates through a gel. A changed, linear form of RNA or protein, however, will migrate proportionally to its linear size (base pairs or kilo Daltons). Denaturing gel electrophoresis is often more accurate for size identification, whereas native gel electrophoresis is usually used to identify larger protein complexes.

Examples of Gel Electrophoresis

TAE Agarose Gel Electrophoresis is most commonly used for DNA.

TBE and Denaturing PAGE (polyacrylamide gel electrophoresis) are common for RNA separation.

SDS PAGE is a denaturing gel electrophoresis commonly used for protein identification and separation.

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