Lecture 37

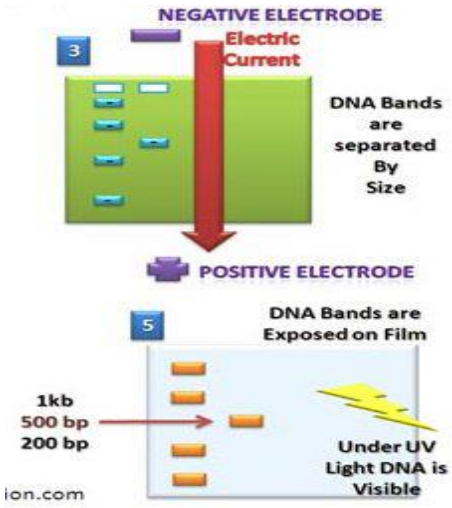
Tools in Molecular Biology

Gel electrophoresis

1. Gel electrophoresis is a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size. In gel electrophoresis, the molecules to be separated are pushed by an electrical field through a gel that contains small pores. The molecules travel through the pores in the gel at a speed that is inversely related to their lengths. This means that a small DNA molecule will travel a greater distance through the gel than will a larger DNA molecule.

2. Gel electrophoresis involves an electrical field; in particular, this field is applied such that one end of the gel has a positive charge and the other end has a negative charge. Because DNA and RNA are negatively charged molecules, they will be pulled toward the positively charged end of the gel.

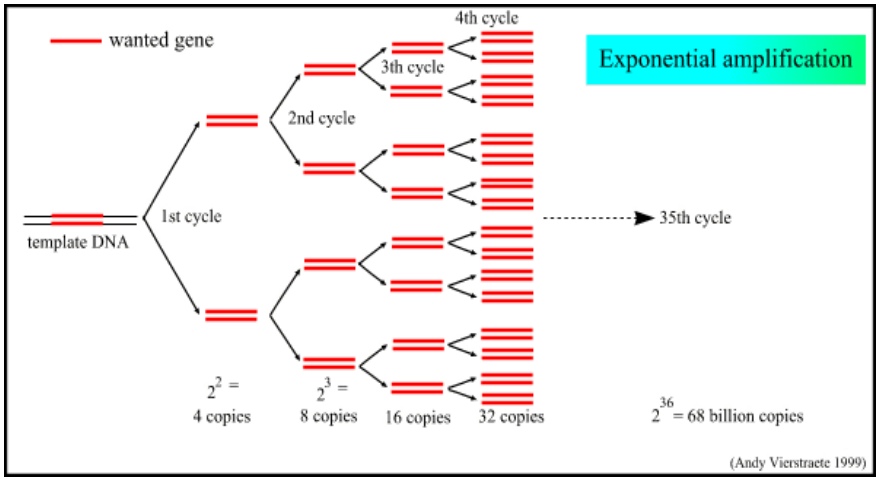
3. Proteins, however, are not negatively charged; thus, when researchers want to separate proteins using gel electrophoresis, they must first mix the proteins with a detergent called sodium dodecyl sulfate. Finally, after the DNA, RNA, or protein molecules have been separated using gel electrophoresis, bands representing molecules of different sizes can be detected.



Polymerase chain reaction (PCR)

Polymerase chain reaction, or PCR, is a laboratory technique used to make multiple copies of a segment of DNA. PCR is very precise and can be used to amplify, or copy, a specific DNA target from a mixture of DNA molecules. First, two short DNA sequences called primers are designed to bind to the start and end of the DNA target. Then, to perform PCR, the DNA template that contains the target is added to a tube that contains primers, free nucleotides, and an enzyme called DNA polymerase, and the mixture is placed in a PCR machine. The PCR machine

increases and decreases the temperature of the sample in automatic, programmed steps. Initially, the mixture is heated to denature, or separate, the double-stranded DNA template into single strands. The mixture is then cooled so that the primers anneal, or bind, to the DNA template. At this point, the DNA polymerase begins to synthesize new strands of DNA starting from the primers. Following synthesis and at the end of the first cycle, each double-stranded DNA molecule consists of one new and one old DNA strand. PCR then continues with additional cycles that repeat the mentioned steps. The newly synthesized DNA segments serve as templates in later cycles, which allow the DNA target to be exponentially amplified millions of times.



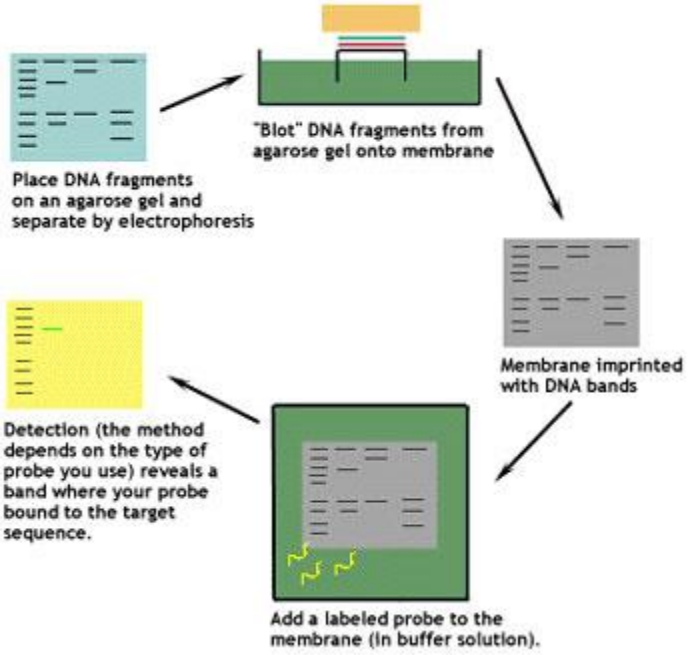
Applications of PCR

1. The first application of PCR was for genetic testing, where a sample of DNA is analyzed for the presence of genetic disease mutations. Prospective parents can be tested for being genetic carriers, or their children might be tested for actually being affected by a disease. DNA samples for prenatal testing can be obtained by amniocentesis, chorionic villus sampling, or even by the analysis of rare fetal cells circulating in the mother's bloodstream. PCR analysis is also essential to preimplantation genetic diagnosis, where individual cells of a developing embryo are tested for mutations.
2. PCR can also be used as part of a sensitive test for tissue typing, vital to organ transplantation. As of 2008, there is even a proposal to replace the traditional antibody-based tests for blood type with PCR-based tests.
3. Many forms of cancer involve alterations to oncogenes. By using PCR-based tests to study these mutations, therapy regimens can sometimes be individually customized to a patient.

Southern blotting

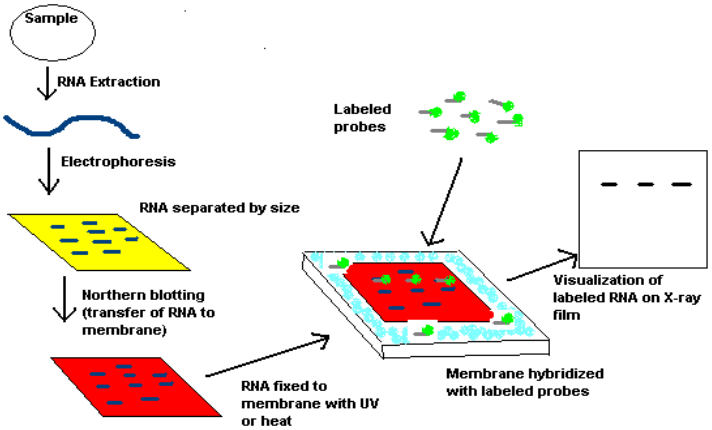
A Southern blot is a method used in molecular biology for detection of a specific DNA sequence in DNA samples. Southern blotting combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization. The membrane is then exposed to a labeled DNA probe. The amount of DNA needed for this technique is dependent on the size and specific activity of the probe. Short probes tend to be

more specific. Under optimal conditions, you can expect to detect 0.1 pg of the DNA for which you are probing.



Northern blotting

A northern blot is a laboratory method used to detect specific RNA molecules among a mixture of RNA. Northern blotting can be used to analyze a sample of RNA from a particular tissue or cell type in order to measure the RNA expression of particular genes. RNA is separated based on size and transferred to a membrane .It is probed with a labeled complement of a sequence of Interest. The results may be visualized depending on the label used. Most result in the revelation of bands representing the sizes of the RNA detected in sample. The band intensity is related to the amount of the target RNA.



Western blotting

Western blot is often used in research to separate and identify proteins. In this technique a mixture of proteins is separated based on molecular weight, and thus by type, through gel electrophoresis. These results are then transferred to a membrane producing a band for each protein. The membrane is then incubated with labels antibodies specific to the protein of interest. The unbound antibody is washed off leaving only the bound antibody to the protein of interest. The bound antibodies are then detected by developing the film. As the antibodies only bind to the protein of interest, only one band should be visible. The thickness of the band corresponds to the amount of protein present; thus doing a standard can indicate the amount of protein present.

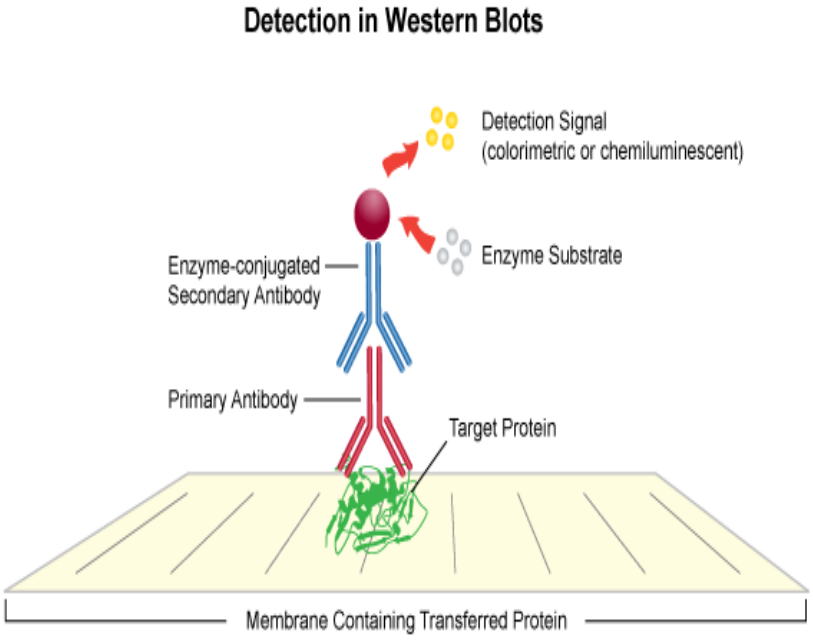


Diagram 2: *Illustration of detection in Western Blots.*

Principles of DNA Isolation and Purification
Sources of DNA include
• Blood
• Buccal cells
• Cultured cells (plant and animal)
• Bacteria
• Biopsies
• Forensic samples i.e. body fluids, hair follicles bone and teeth roots.

Basic steps in DNA Extraction

There are three four steps involved in a DNA extraction.

1. Breaking cells open to release the DNA

The cells in a sample are separated from each other, often by a physical means such as grinding put into a solution containing salt. The positively charged sodium ions in the salt help protect the negatively charged phosphate groups that run along the backbone of the DNA.

2. Separating DNA from proteins and other cellular debris

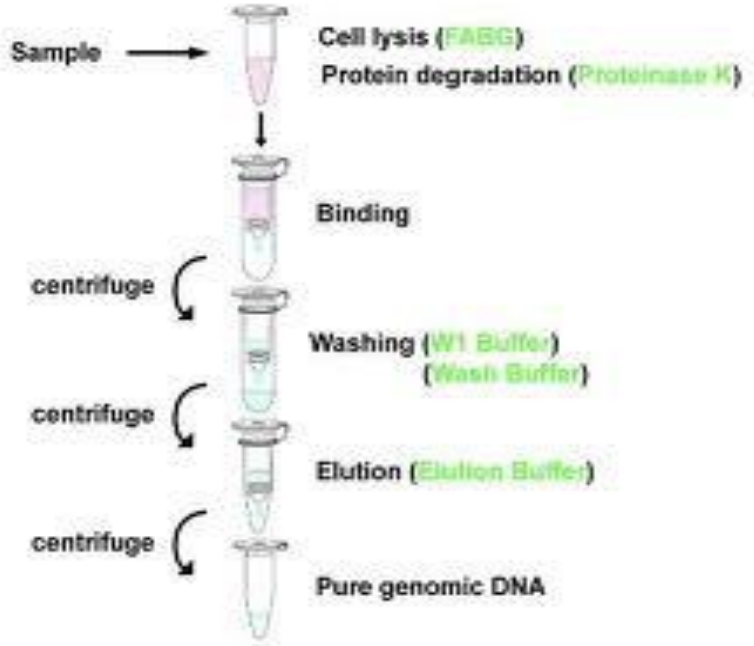
To get a clean sample of DNA, it's necessary to remove as much of the cellular debris as possible. This can be done by a variety of methods. Often a protease (protein enzyme) is added to degrade DNA-associated proteins and other cellular proteins. Alternatively, some of the cellular debris can be removed by filtering the sample.

3. Precipitating the DNA with an alcohol

Finally, ice-cold alcohol (either ethanol or isopropanol) is carefully added to the DNA sample. DNA is soluble in water but insoluble in the presence of salt and alcohol. By gently stirring the alcohol layer with a sterile pipette, a precipitate becomes visible and can be spooled out. If there is lots of DNA, you may see a stringy, white precipitate.

4. Cleaning the DNA

The DNA sample can now be further purified (cleaned). It is then resuspended in a slightly alkaline buffer and ready to use. A pellet is formed upon centrifugation. This step also removes alcohol soluble salt.



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