

**ARULMIGU PALANIANDAVAR ARTS COLLEGE FOR
WOMEN**

(AUTONOMOUS)

P.G DEPARTMENT OF ZOOLOGY

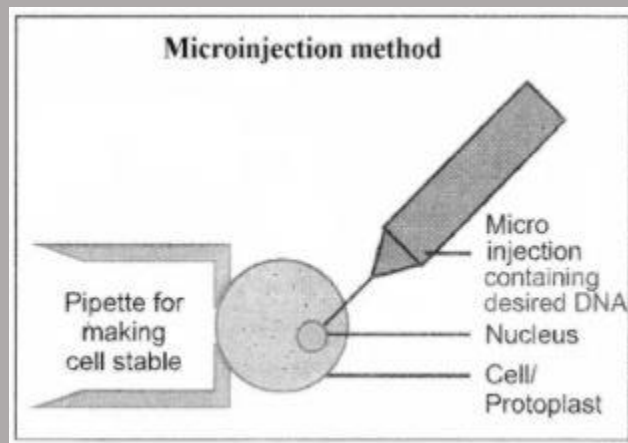
LEARNING RESOURCES

BIOTECHNOLOGY

Molecular Biology Techniques

1. Microinjection:

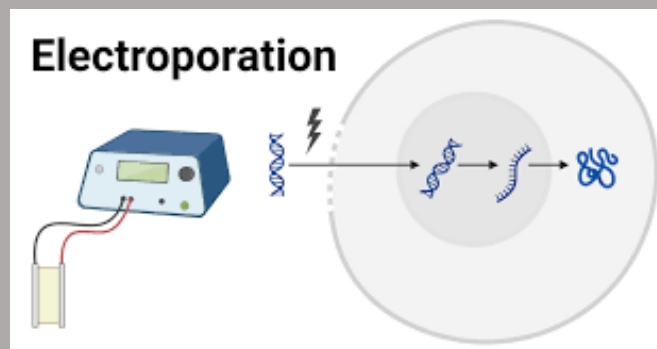
- Definition: Microinjection is a technique used to deliver substances directly into a cell using a fine needle or micropipette.
- Procedure:
 - A glass micropipette is used to penetrate the cell membrane.
 - The substance (e.g., DNA, RNA, proteins) is injected into the cytoplasm or nucleus.
 - It is commonly used in embryology, genetic engineering, and developmental biology.
- Applications:
 - Gene editing (e.g., CRISPR/Cas9).
 - Studying gene function and expression.
 - Creating transgenic organisms.



2. Electroporation:

- Definition: Electroporation involves using an electrical field to increase the permeability of the cell membrane, allowing the introduction of molecules like DNA, RNA, or drugs.

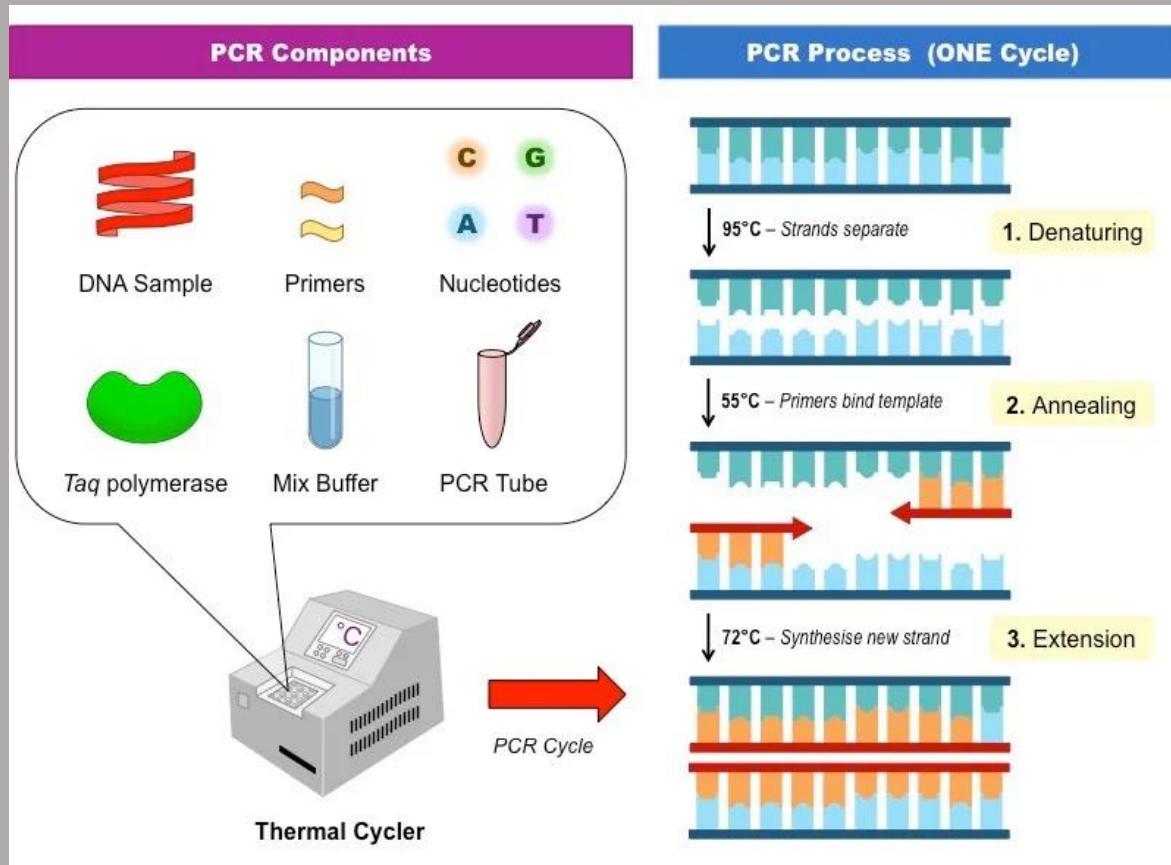
- Procedure:
- Cells and the substance to be introduced are suspended in a conductive solution.
- A brief electric pulse is applied, creating temporary pores in the cell membrane.
- Molecules enter the cell through these pores before the membrane reseals.
- Applications:
- Genetic transformation in bacteria, yeast, plant, and animal cells.
- Gene therapy research.
- Transfection of cells with plasmid DNA.



3. Polymerase Chain Reaction (PCR):

- Definition: PCR is a technique used to amplify a specific segment of DNA, generating millions of copies from a small initial sample.
- Procedure:
- Denaturation: Heating the DNA sample to separate the strands.
- Annealing: Cooling the sample to allow primers to bind to the target DNA sequences.
- Extension: DNA polymerase extends the primers, synthesizing new DNA strands.
- The cycle is repeated multiple times to amplify the DNA.

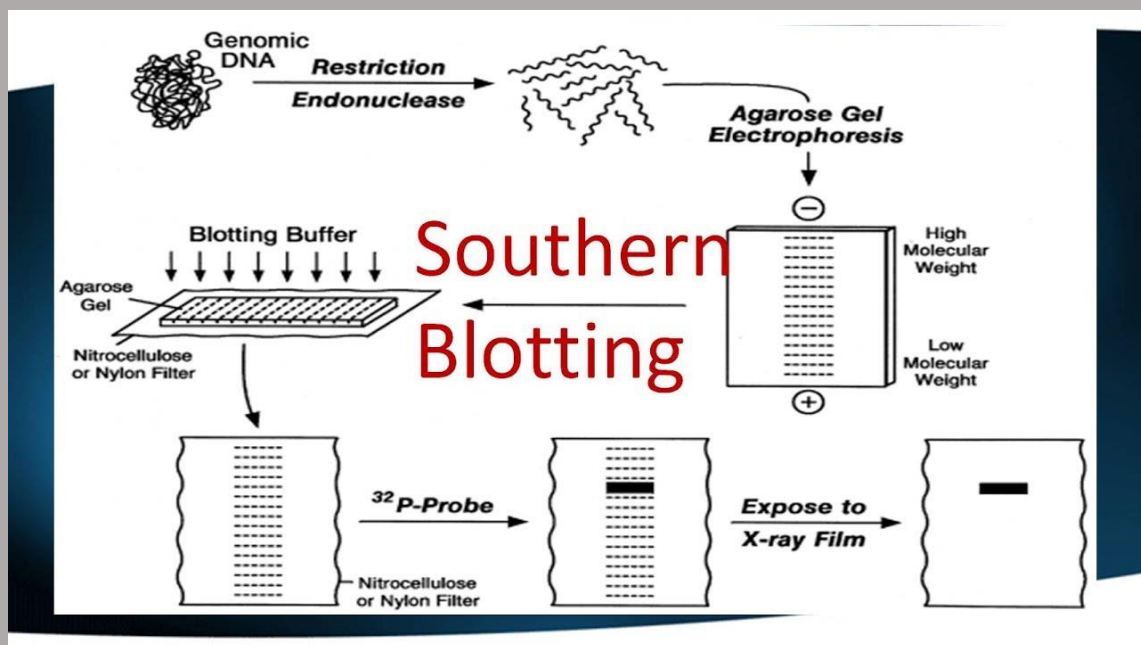
- Applications:
- Diagnosing genetic disorders.
- Forensic analysis and DNA fingerprinting.
- Cloning and sequencing genes.



Blotting Techniques

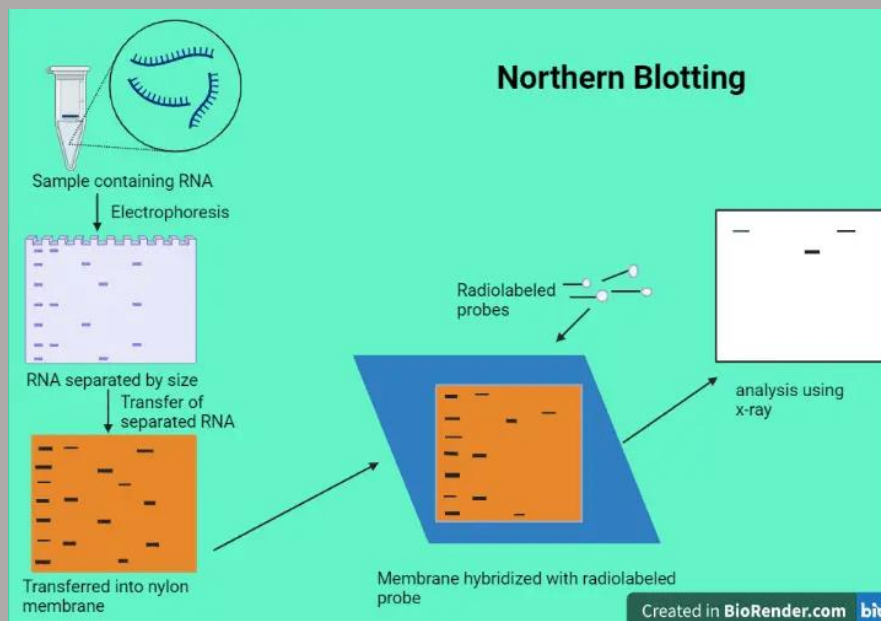
1. Southern Blot:

- Definition: Southern blotting is used to detect specific DNA sequences within a complex DNA sample.
- Procedure:
 - DNA is extracted and digested with restriction enzymes.
 - The fragments are separated by agarose gel electrophoresis.
 - The DNA is transferred to a nylon or nitrocellulose membrane.
 - The membrane is probed with a labeled DNA probe complementary to the target sequence.
 - Detection is done using autoradiography or chemiluminescence.
- Applications:
 - Gene mapping and identification.
 - Diagnosing genetic disorders.
 - Detecting mutations and polymorphisms.



2. Northern Blot:

- Definition: Northern blotting is used to detect specific RNA sequences within a sample, providing information about gene expression.
- Procedure:
 - RNA is extracted from cells or tissues.
 - The RNA is separated by gel electrophoresis.
 - The RNA is transferred to a nylon or nitrocellulose membrane.
 - The membrane is probed with a labeled DNA or RNA probe complementary to the target RNA sequence.
 - Detection is done using autoradiography or chemiluminescence.
- Applications:
 - Studying gene expression patterns.
 - Identifying mRNA sizes and abundance.
 - Investigating alternative splicing and RNA processing.



Molecular biology techniques such as microinjection, electroporation, and PCR are fundamental tools for manipulating and analyzing genetic material. Blotting

techniques like Southern and Northern blots are essential for detecting specific DNA and RNA sequences, respectively, providing crucial insights into gene function and regulation. Together, these techniques drive advances in genetics, biotechnology, and medical research.