



Blotting Techniques: Southern, Northern and Western Blotting and Their Applications in Agriculture

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Agriculture is increasingly dependent on biotechnology and molecular biology for improving crop productivity and sustainability. Traditional breeding methods are often slow and may not provide detailed information about the genetic basis of desirable traits. Molecular techniques help researchers understand the structure, expression and regulation of genes responsible for important agricultural characteristics. Blotting techniques are among the most widely used molecular tools for understanding the molecular basis of plant growth, stress tolerance and disease resistance. The term “blotting” refers to the transfer of biological molecules from a gel to a membrane, followed by their detection using labelled probes or antibodies. These methods provide highly specific information regarding DNA, RNA and proteins. Southern blotting was developed for DNA analysis. Northern blotting was subsequently developed for RNA detection, while Western blotting became a standard method for protein analysis. Together, these techniques form the foundation of molecular diagnostics and biotechnology research. They are widely applied in crop improvement programs, genetic engineering projects, pathogen detection, stress-response studies and molecular breeding.

General Principle of Blotting Technique

All blotting methods follow a similar workflow:

1. Electrophoresis separates biomolecules by size.
2. Molecules are transferred onto a solid support (nylon, nitrocellulose or PVDF membranes).
3. A probe (DNA, RNA or antibody) binds specifically to the target molecule.
4. Detection is achieved through autoradiography, fluorescence, chemiluminescence or colorimetric methods.

Requirements for Blotting Technique

1. **Sample Material:** DNA, RNA or Protein
2. **Extraction Reagents:** Buffers
3. **Gel Electrophoresis System:** Agarose gel electrophoresis (for DNA and RNA), polyacrylamide gel electrophoresis (PAGE/SDS-PAGE for proteins), electrophoresis apparatus with power supply and molecular weight markers (ladders)
4. **Transfer Membrane:** Nitrocellulose membrane, nylon membrane (for nucleic acid blotting) and PVDF membrane (for protein blotting)
5. **Transfer System:** Blotting apparatus (capillary, vacuum or electroblotting setup), filter papers and sponges

6. **Probes or Antibodies:** Labelled DNA/RNA probes (Southern and Northern blotting) and primary and secondary antibodies (Western blotting)
7. **Blocking Agents:** BSA (Bovine Serum Albumin) and blocking buffers to reduce non-specific binding
8. **Detection Reagents:** Chemiluminescent substrates, radioactive or fluorescent labels, colorimetric substrates and X-ray film
9. **Washing Solutions:** Buffers for removing non-specific binding and washing solutions specific to probes or antibodies
10. **Equipment Required:** Micropipettes and tips, incubator or hybridization oven, UV transilluminator, imaging system or densitometer and shaker
11. **Environmental Conditions:** RNase-free conditions for RNA work with appropriate temperature and pH control

Southern Blotting

Southern blotting is a DNA–DNA hybridization technique used to identify specific DNA sequences in a genome and to determine the presence and size of a gene within chromosomal DNA.

This technique is named after its inventor **Edwin Southern** (1975).

Procedure

- a) High molecular weight DNA is first digested using restriction endonucleases which cuts the DNA into smaller fragments. These fragments are then separated by agarose gel electrophoresis based on size.
- b) Fragment Treatment for Efficient Transfer - If fragments are larger than 15 kb, the gel may be treated with dilute HCl, which depurinates the DNA by breaking it into smaller fragments, thereby improving transfer efficiency to the membrane.
- c) In alkaline transfer methods, the gel is soaked in NaOH solution. This denatures double-stranded DNA into single strands enhancing probe binding and simultaneously removing residual RNA contamination.
- d) Membrane Placement and Contact - A sheet of nitrocellulose or nylon membrane is placed on or beneath the gel. Uniform pressure is applied either by suction or by stacking paper towels and a weight to ensure tight contact between gel and membrane.
- e) DNA Transfer by Capillary Action - Buffer moves through the gel and membrane by capillary action carrying DNA fragments onto the membrane. The DNA binds to the membrane through electrostatic (ionic) interactions with negatively charged DNA adhering to the positively charged membrane surface (nylon membrane).
- f) The membrane is stabilized by either baking at 80 °C for 2 hours in a vacuum oven or exposure to UV radiation. This step ensures DNA fragments are permanently attached.
- g) Hybridization with Probe - The membrane is incubated with a labelled DNA probe which is a fragment complementary to the target sequence. Probes may be tagged with radioactive isotopes, fluorescent dyes or chromogenic markers for detection.
- h) Excess probe is removed by washing and the hybridization pattern is then visualized by autoradiography (for radioactive) using X-ray film or colour development directly on the membrane (for chromogenic probes).

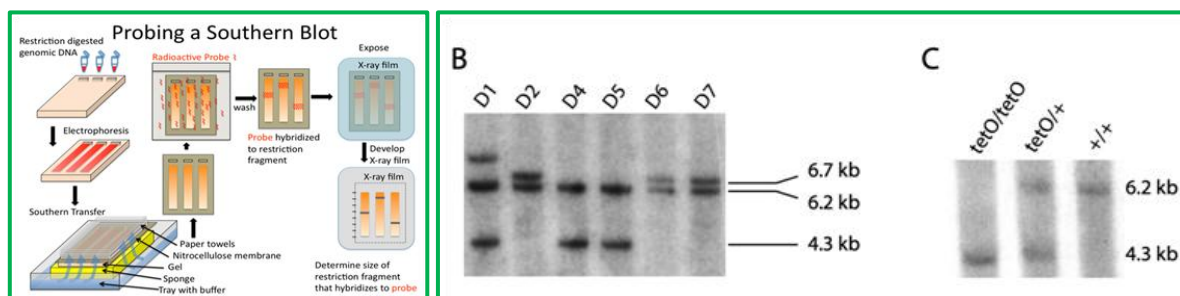


Figure 1: Procedure and results for Southern blotting

Agricultural Applications

- It is widely used for gene mapping, identification of transgenic plants, mutation analysis and genome organization studies.
- Detecting disease resistance genes in crops.
- Mapping restriction sites for RFLP analysis.
- Southern blotting helps scientists verify whether a desired gene has been successfully incorporated into a crop genome.
- Studying genetic variation for plant breeding programs.
- Identifying phylogenetic relationships among plant species.

Northern Blotting

Northern blotting is a technique which uses DNA probes that hybridize with complementary RNA sequences. It is an ideal tool to study the products of gene transcription and gene expression by detecting RNA/mRNA.

This method was developed by **James Alwine**, **David Kemp** and **George Stark** in 1977 at Stanford University.

Procedure

- RNA Extraction** - Total RNA is extracted from homogenized tissue samples.
- Isolation of mRNA** - mRNA is separated using oligo (dT) cellulose chromatography, which selectively retains RNAs possessing a poly(A) tail.
- Gel Electrophoresis** - The RNA samples are separated according to size through gel electrophoresis.
- Transfer to Membrane (Blotting)** - RNA is transferred onto a positively charged nylon membrane which efficiently binds negatively charged nucleic acids due to high affinity.
- Use of Transfer Buffer** - The transfer buffer facilitates the efficient transfer of RNA from the gel to the membrane while maintaining RNA integrity. It provides suitable ionic conditions for RNA binding to the membrane and helps prevent RNA degradation during the transfer process.
- Immobilization of RNA** - After transfer RNA molecules are fixed onto the membrane through covalent linkage using UV irradiation or heat treatment.
- Probe Labelling and Hybridization** - A labelled probe is prepared and hybridized with complementary RNA sequences present on the membrane.
- Membrane Washing** - The membrane is washed to remove non-specifically bound probes and ensure specific hybridization.
- Detection and Quantification** - Hybridization signals are detected using X-ray film and the results can be quantified using densitometry.

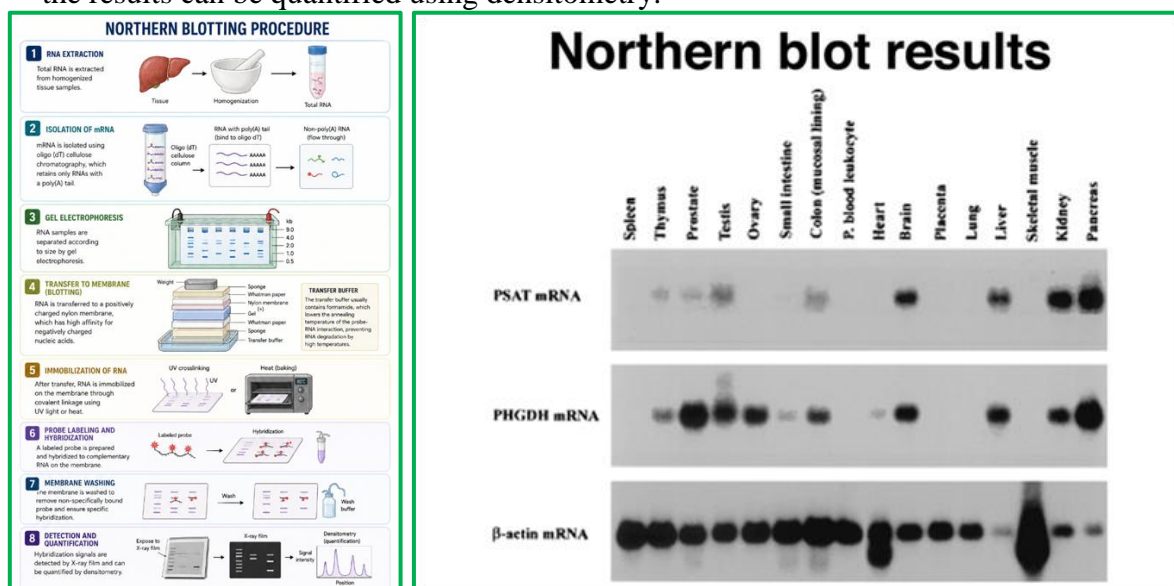


Figure 2: Procedure and results of Northern blotting

Agricultural Significance

- The technique plays a major role in understanding how crops respond to drought, salinity, heat stress, cold stress and pathogen attack. By analyzing gene expression patterns scientists can identify stress-responsive genes and develop improved crop varieties capable of tolerating adverse environmental conditions.
- Northern blotting is also used to evaluate transgene expression levels in genetically engineered plants, ensuring that introduced genes are functioning as intended.
- Studying alternative splicing in plant transcripts.
- Detecting errors in transcript processing.
- Comparing expression patterns between healthy and diseased tissues.

Western Blotting

Western blotting is a highly sensitive technique used for detecting specific proteins in biological samples. Since proteins perform most cellular functions, their analysis is essential for understanding plant growth, development and stress responses.

The term Western blot was coined by **W. Neal Burnette** in 1981.

Principle

Proteins are separated by size using SDS-PAGE and then transferred to a membrane. Specific antibodies bind to the target protein, allowing visualization and quantification.

Procedure:

- a) Proteins are extracted and isolated from the selected biological sample.
- b) The isolated protein suspension is then treated with beta-mercaptoethanol (BME) and sodium dodecyl sulphate (SDS). These reagents denature the proteins and impart a uniform negative charge to them.
- c) The resulting protein-SDS complexes are loaded into the wells of the gel. A molecular weight marker is placed in one well to serve as a reference for estimating protein sizes while the remaining wells are filled with the prepared samples.
- d) An electric current is applied across the gel during electrophoresis causing negatively charged protein molecules to migrate toward the positive electrode. Smaller proteins move more rapidly through the gel matrix compared to larger proteins allowing separation based on molecular weight.
- e) Following electrophoresis, the gel is positioned adjacent to a membrane and an electric current is passed to transfer the separated proteins from the gel onto the membrane surface.
- f) The membrane is then subjected to immunoblotting. Initially, blocking is performed using non-specific proteins to prevent unwanted binding of antibodies to unoccupied membrane sites.
- g) A primary antibody is subsequently added which selectively binds to the target protein by recognizing a specific epitope on the target protein.
- h) Excess primary antibody is removed through washing after which a secondary antibody linked to an enzyme is introduced. This secondary antibody specifically binds to the primary antibody.
- i) Another washing step is carried out to eliminate any unbound secondary antibodies.
- j) Finally, detection is achieved using a chemiluminescent substrate. Upon reaction with the enzyme light is produced which can then be captured and visualized using an imaging system or film detector.

Agricultural Applications

- Identifying stress-responsive proteins in crops.
- Detecting pathogen-related proteins in infected plants.
- Studying enzyme isoforms and their role in metabolism.
- Validating transgenic plants by confirming protein expression.

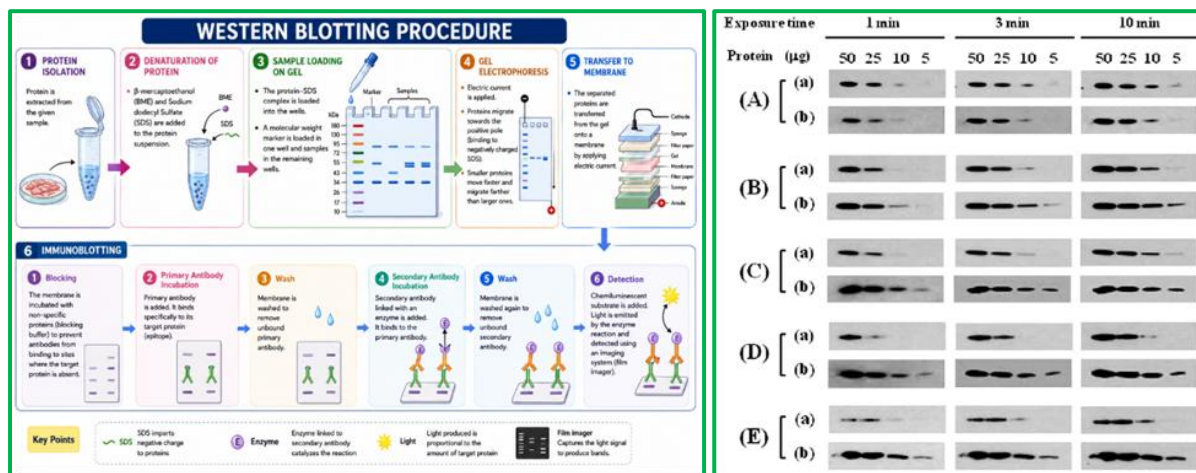


Figure 3: Procedure and results for Western blotting

Applications of Blotting Techniques in Agriculture

- Crop Improvement:** Researchers use these techniques to identify desirable genes associated with high yield, disease resistance and stress tolerance.
- Genetic Engineering:** Southern blotting confirms transgene integration, Northern blotting evaluates gene expression and Western blotting confirms protein expression ensuring that introduced genes in transgenic plants are functional.
- Plant Disease Diagnosis:** Pathogens can be detected by identifying their DNA, RNA or proteins enabling early disease management.
- Marker-Assisted Selection:** Blotting techniques support breeding programs by identifying genetic markers linked to important traits.
- Stress Biology:** Scientists investigate crop responses to drought, salinity, heat and cold by studying changes in RNA and protein expression.
- Seed Quality Assessment:** Molecular markers detected through blotting methods help assess genetic purity and seed quality.
- Biodiversity and Germplasm Characterization:** Southern blotting assists in evaluating genetic variation among crop species and germplasm collections.
- Food Security:** Development of improved crop varieties through molecular analysis contributes to sustainable agricultural production and food security.

Table 1: Comparison of Blotting Techniques

Technique	Target Molecule	Probe Type	Detection Method	Agricultural Applications
Southern blot	DNA	DNA probe	Autoradiography, fluorescence, chromogenic dyes	Identifying disease resistance genes, RFLP mapping, phylogenetic analysis and detecting genetic variation in crops
Northern blot	RNA (mRNA)	DNA or RNA probe	Autoradiography, densitometry	Studying gene expression under stress, pathogen infection responses, detecting alternative splicing and transcript errors
Western blot	Protein	Primary and secondary antibodies	Colorimetric, chemiluminescent, fluorescent detection	Identifying stress-responsive proteins, pathogen-related proteins, enzyme isoforms and validating transgenic plants

Future Prospects

- Modern molecular technologies such as quantitative PCR, microarrays and next-generation sequencing have expanded research capabilities. However blotting techniques continue to serve as gold-standard validation methods. Future developments are expected to improve sensitivity, reduce processing time and integrate automated detection systems.
- In agriculture, the combination of blotting techniques with genomics, proteomics and bioinformatics will enhance crop improvement programs and accelerate the development of climate-resilient crops. These advances will be critical for meeting the food demands of a growing global population.

Conclusion

Southern, Northern and Western blotting are fundamental tools of agricultural biotechnology that enable the analysis of DNA, RNA and proteins respectively. Southern blotting aids in gene mapping, marker-assisted selection and transgene verification. Northern blotting helps in understanding gene expression and plant responses to environmental stresses. Western blotting facilitates the study of protein expression and plant-pathogen interactions. Together, these techniques play a crucial role in crop improvement by supporting the development of high-yielding, disease-resistant and stress-tolerant varieties. Despite advances in genomics, transcriptomics and proteomics blotting techniques remain valuable for gene and protein validation, molecular diagnostics and functional studies thereby contributing significantly to sustainable agriculture and global food security.

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