

# DNA Hybridization, Southern Blotting & Western Blotting

Molecular Biology — Study Notes

## A. DNA / Nucleic Acid Hybridization

### 1. Definition

Hybridization is the process by which two complementary single strands of nucleic acid (DNA–DNA, DNA–RNA, or RNA–RNA) base-pair to form a stable double-stranded hybrid. It is the fundamental principle underlying probe-based detection techniques such as Southern blotting, Northern blotting, in situ hybridization, and microarray analysis.

### 2. Principle

Double-stranded DNA can be denatured (melted) into single strands by heat or alkali treatment, breaking the hydrogen bonds between base pairs without breaking the sugar-phosphate backbone. When conditions are reversed (cooling, neutral pH), complementary single strands re-associate, or anneal, through specific Watson–Crick base pairing (A–T and G–C). A labelled single-stranded probe added to a mixture will seek out and hybridize only with its complementary target sequence, allowing that specific sequence to be identified even within a complex mixture of nucleic acids.

#### Principle of Nucleic Acid Hybridization

##### Step 1 — Denaturation (heat/alkali separates strands)



##### Step 2 — Labelled probe anneals to its complementary sequence



Fig. A1 — Denaturation of double-stranded DNA followed by annealing of a labelled probe to its complementary single strand.

### 3. Factors Affecting Hybridization (Stringency)

“Stringency” refers to the reaction conditions that determine how precisely a probe must match its target before a stable hybrid forms.

- Temperature — higher temperatures require closer sequence matches (higher stringency)
- Salt (ionic strength) of the buffer — lower salt concentration increases stringency
- Length and GC content of the probe — GC-rich or longer probes form more stable hybrids
- Formamide concentration — destabilizes hybrids, allowing hybridization at lower temperatures
- Duration of hybridization and washing steps

## 4. Types of Probes

| Probe type            | Description                                                          |
|-----------------------|----------------------------------------------------------------------|
| Genomic DNA probe     | Fragment of genomic DNA complementary to the target sequence         |
| cDNA probe            | Derived from mRNA via reverse transcription; contains no introns     |
| Oligonucleotide probe | Short, chemically synthesized single-stranded sequence (15–30 bases) |
| Riboprobe (RNA probe) | Single-stranded RNA probe, often used in in situ hybridization       |

Probes are commonly labelled with radioisotopes ( $^{32}\text{P}$ ), fluorescent dyes, or enzyme/chemiluminescent tags (e.g. digoxigenin) for non-radioactive detection.

## B. Southern Blotting

### 1. Overview

Southern blotting, developed by Edwin Southern (1975), is a technique used to detect a specific DNA sequence within a complex mixture of digested genomic DNA. DNA fragments are separated by agarose gel electrophoresis, transferred (“blotted”) onto a solid membrane, and probed with a labelled complementary sequence.

### 2. Procedure

1. **Restriction digestion:** Genomic DNA is cut into fragments using one or more restriction enzymes.
2. **Gel electrophoresis:** Fragments are separated by size on an agarose gel.
3. **Denaturation:** The gel is soaked in alkali (NaOH) to convert double-stranded DNA fragments into single strands in situ.
4. **Neutralization:** The gel is neutralized to a suitable pH for transfer.
5. **Transfer (blotting):** DNA is transferred from the gel onto a nitrocellulose or nylon membrane, most commonly by capillary (buffer-wicking) transfer, preserving the relative band positions.
6. **Fixation:** The transferred DNA is permanently fixed to the membrane by baking or UV cross-linking.
7. **Hybridization:** The membrane is incubated with a labelled probe complementary to the sequence of interest.
8. **Washing:** Unbound/non-specifically bound probe is washed off under controlled stringency.
9. **Detection:** Bound probe is visualized by autoradiography (radioactive probe) or chemiluminescent/colorimetric imaging (non-radioactive probe).

#### Southern Blotting — Capillary Transfer Set-up

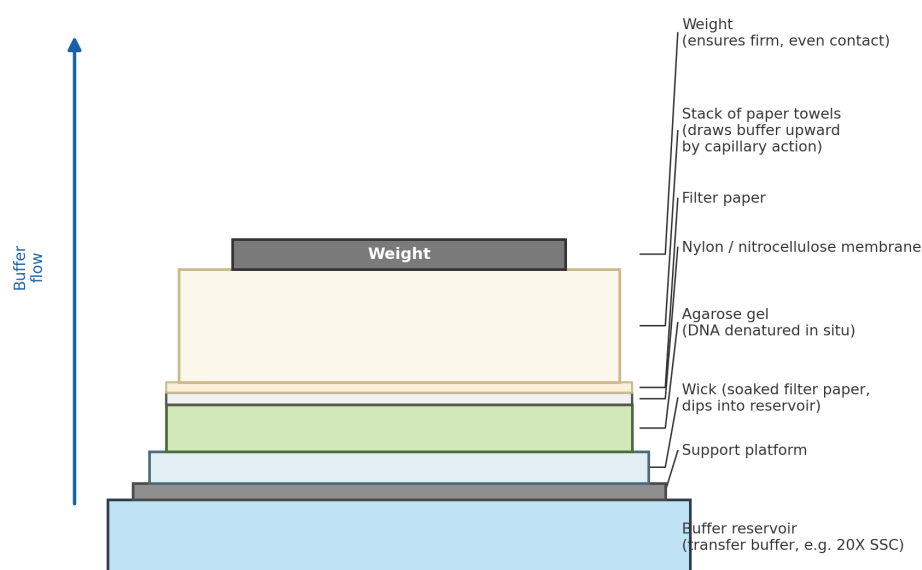


Fig. B1 — Classic capillary (Southern) transfer stack: buffer is drawn upward from the reservoir, through the gel and membrane, into the paper towels, carrying DNA onto the membrane.

### 3. Applications

- Detecting specific genes or mutations within genomic DNA
- Restriction fragment length polymorphism (RFLP) analysis
- Gene copy number and genomic organization studies
- Confirming transgene integration in genetically modified organisms
- Paternity testing and forensic DNA fingerprinting (historically)

## C. Western Blotting

### 1. Overview

Western blotting (immunoblotting) is used to detect and identify a specific protein within a complex protein mixture using antibodies. Unlike Southern/Northern blotting (nucleic acids), Western blotting relies on antigen–antibody recognition rather than base-pair hybridization.

### 2. Procedure

1. **Sample preparation:** Cells/tissues are lysed and total protein is extracted.
2. **SDS-PAGE:** Proteins are denatured (with SDS and heat) and separated by size on a polyacrylamide gel.
3. **Transfer (electroblotting):** Proteins are transferred from the gel onto a PVDF or nitrocellulose membrane using an electric current.
4. **Blocking:** The membrane is incubated in a blocking buffer (e.g. BSA or non-fat milk) to prevent non-specific antibody binding.
5. **Primary antibody incubation:** The membrane is probed with an antibody specific to the target protein (antigen).
6. **Secondary antibody incubation:** An enzyme-linked secondary antibody (e.g. HRP- or AP-conjugated) that recognizes the primary antibody is applied.
7. **Substrate/detection:** A chemiluminescent or colorimetric substrate is added; the enzyme produces a detectable signal at the position of the target protein.
8. **Imaging:** The band is visualized and recorded using X-ray film or a digital imaging system.

#### Western Blotting — Overall Workflow

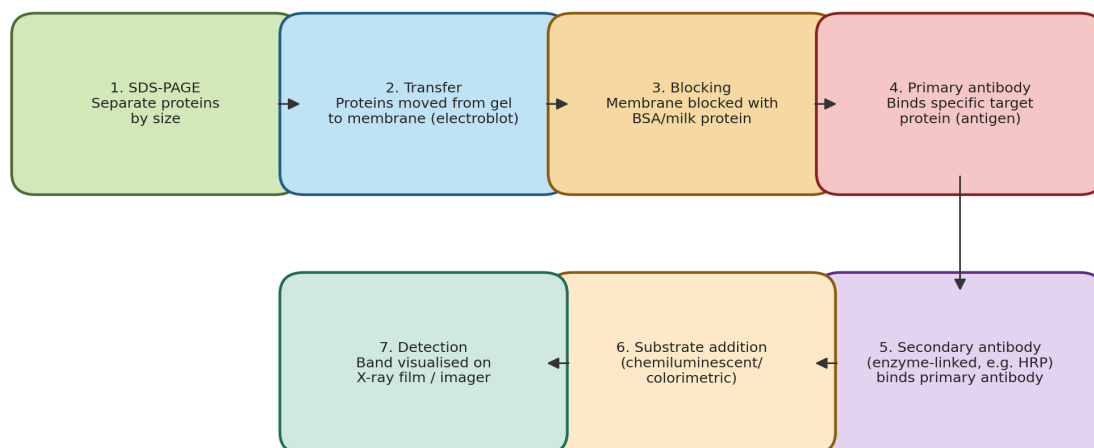


Fig. C1 — Overall Western blotting workflow, from protein separation through to antibody-based detection.

### 3. Applications

- Confirming protein expression (e.g. after transfection or induction)
- Detecting post-translational modifications (with modification-specific antibodies)
- Estimating relative protein abundance between samples
- Diagnostic confirmatory testing (e.g. historically used to confirm HIV infection)

- Validating knockdown/knockout or overexpression experiments

## 4. Comparison of Blotting Techniques

| Technique     | Molecule detected | Probe used                     | Transfer basis           |
|---------------|-------------------|--------------------------------|--------------------------|
| Southern blot | DNA               | Labelled DNA/RNA probe         | Base-pair hybridization  |
| Northern blot | RNA               | Labelled DNA/RNA probe         | Base-pair hybridization  |
| Western blot  | Protein           | Antibody (primary + secondary) | Antigen–antibody binding |

**Note:** The naming is a play on words — “Southern” blot is named after its inventor Edwin Southern; “Northern” and “Western” blots (for RNA and protein respectively) were named later by analogy, not after real people or directions.