

## Southern Blotting:

Named after Edwin M. Southern.

It is a DNA hybridization technique to locate a specific DNA sequence in a sample or test DNA.

**Principle:** Hybridization of a labelled complimentary oligonucleotide probe to the denatured target DNA which contains the sequence of interest for complimentary probe to bind on.

The binding is detected using an autoradiogram, which helps to identify the presence of the DNA having the sequence of interest.

### Procedure

- The high molecular weight DNA is digested using restriction endonucleases.
- The DNA fragments are electrophoresed on an agarose gel to separate fragments of different lengths.
- If the DNA fragments are still larger than 15kb, then it is treated with dil. HCl. (HCl depurinates the DNA, thus reducing its size).
- The double stranded DNA is then denatured by keeping it in an alkaline medium.
- DNA is transferred to a positively charged nylon or nitrocellulose membrane. (By capillary action or current mediated transfer on the membrane from the gel).
- Since, the nucleotides are negatively charged, they bind to the membrane.
- The nitrocellulose membrane is baked at 800°C to immobilize the DNA.
- The immobilized DNA is exposed to the hybridization probe or sequence complementary to the DNA of interest, which has been labelled.
- The hybridization probe will bind to any complementary sequence present in the test DNA.
- The membrane is washed with appropriate buffer to remove any unbound probe.
- Radioactivity is detected by autoradiography.
- The site of positive signal represents the complementary DNA sequences present in the test DNA.

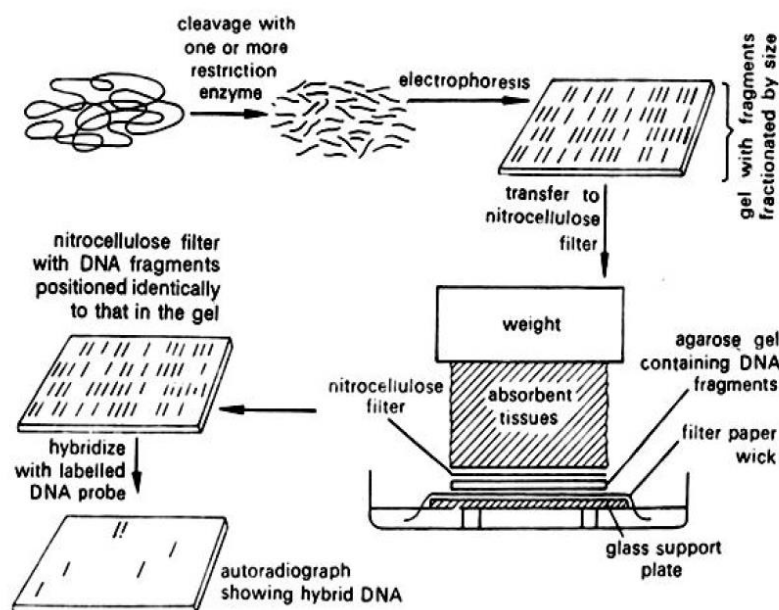


Figure: Southern Blotting workflow

**Applications:**

- DNA libraries can be analyzed using an oligonucleotide sequence of a particular gene to find that gene in other organisms.
- It can be used to identify methylated sites in a DNA sequence using methylation sensitive restriction enzymes such as MspI.
- It can be used to find mutations in a gene.
- It can be used to identify infectious microorganisms related to a particular disease.