# Southern blotting

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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 method is named after its inventor, the British biologist Edwin Southern.

**Method:**

1. Restriction endonucleases are used to cut high-molecular-weight DNA strands into smaller fragments.
2. The DNA fragments are then electrophoresed on an agarose gel to separate them by size.
3. If some of the DNA fragments are larger than 15 kb, then prior to blotting, the gel may be treated with an acid, such as dilute HCl. This depurinates the DNA fragments, breaking the DNA into smaller pieces, thus allowing more efficient transfer from the gel to membrane.
4. If alkaline transfer methods are used, the DNA gel is placed into an alkaline solution to denature the double-stranded DNA. The denaturation in an alkaline environment may improve binding of the negatively charged thymine residues of DNA to a positively charged amino group of membrane, separating it into single DNA strands for later hybridization to the probe, and destroys any residual RNA that may still be present in the DNA.
5. A sheet of nitrocellulose membrane is placed on top of the gel. Pressure is applied evenly to the gel to ensure good and even contact between gel and membrane. Buffer transfer by capillary action from a region of high water potential to a region of low water potential is then used to move the DNA from the gel on to the membrane.
6. The membrane is then baked in a vacuum to permanently attach the transferred DNA to the membrane.
7. The membrane is then exposed to a hybridization probe—a single DNA fragment with a specific sequence whose presence in the target DNA is to be determined. The probe DNA is labelled so that it can be detected, usually by incorporating radioactivity or tagging the molecule with a fluorescent or chromogenic dye.
8. After hybridization, excess probe is washed from the membrane and the pattern of hybridization is visualized on X-ray film by autoradiography.

**OR**

**1. Digestion of the DNA** with an appropriate restriction enzyme.

**2. Run the digested** on an agarose gel.

**3. Denaturation of the DNA** usually while it is still on the gel. For example, soaking it in about 0.5M NaOH, which would separate double-stranded DNA into single-stranded DNA.

**4. Transfer of the denatured DNA to the membrane.** Traditionally, a nitrocellulose membrane is used, although nylon or a positively charged nylon membrane may be used. Transfer is usually done by capillary action, which takes several hours. Capillary action transfer draws the buffer up by capillary action through the gel and into the membrane, which will bind ssDNA.

**5. Probing the membrane with labeled ssDNA.** This is also known as hybridization. This process relies on the ssDNA hybridizing (annealing) to the DNA on the membrane due to the binding of complementary strands. Probing is often done with $^{32}$P labeled ATP, biotin/streptavidin or a bioluminescent probe.
**6. Visualization:** If radiolabeled $^{32}$P probe is used, then it can be visualize by autoradiograph.

**OR**

**Step 1. Digestion of the DNA** with an appropriate restriction enzyme.

**Step 2: Gel electrophoresis**

Fragmented DNA is typically electrophoresed on an agarose gel to separate the fragments according to their molecular weights. Acrylamide gels can alternatively be used for good resolution of smaller DNA fragments<800 bp).

**Step 3: Blotting**

After electrophoresis, DNA is transferred to a positively charged nylon membrane. Traditional transfer of DNA is done overnight using an upward-transfer method. The membranes are ideal for use with radiolabeled and nonisotopic probes to achieve maximum hybridization signal.

**Step 4: Probe labeling**

A nucleic acid probe with sequence homologous to the target sequence under study is labeled with radioactivity, fluorescent dye, or an enzyme that can generate a chemiluminescent signal when incubated with the appropriate substrate.

**Step 5: Hybridization & washing**

During hybridization, the labeled probe is incubated with the DNA fragments that are immobilized on the blot under conditions that promote hybridization of complementary sequences.
After hybridization, the unhybridized probe is removed by washing in buffer. The result is that only fully hybridized labeled probe molecules, with complementary sequence to the region of interest, remain bound.

**Step 6: Detection**

In the detection step, the bound, labeled probe is detected using the method required for the particular label used. For example, radiolabeled probes may be detected using X-ray film or a phosphorimaging instrument, and enzymatically labeled probes are typicallly detected by incubating with a chemiluminescent substrate and exposing the blot to X-ray film.

**Result:**

Hybridization of the probe to a specific DNA fragment on the filter membrane indicates that this fragment contains DNA sequence that is complementary to the probe.

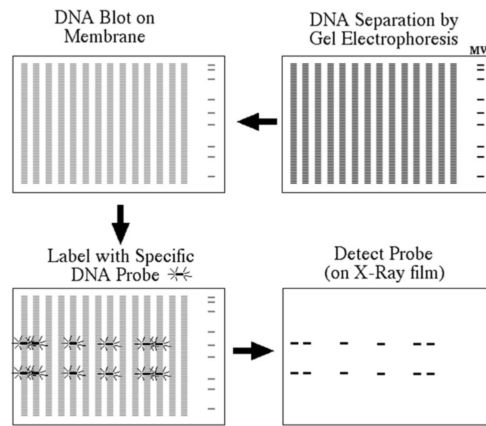


Fig: Southern Blot Technique