SOUTHERN & NORTHERN BLOTTING

The transfer of macromolecules such as nucleic acids and proteins to solid-phase membranous support is known as blotting. Fragments of DNA and RNA molecules separated by gel electrophoresis are transferred to a nylon or nitrocellulose membrane in a process termed as Southern and Northern blotting, respectively. Southern blotting was introduced by Edwin Southern in 1975 as a method to detect specific sequences of DNA in DNA samples. The other blotting techniques emerged from this method have been termed as Northern (for RNA), Western (for proteins), Eastern (for post-translational protein modifications) and Southwestern (for DNA-protein interactions) blotting.

Southern and Northern blotting protocols involve the following major steps:

Purification of DNA/RNA: Extract and purify the DNA/RNA from either cells or tissue sources.

Digestion of DNA: Digest the DNA into fragments with restriction enzymes. This step is not required for RNA.

Gel electrophoresis: Separate the DNA fragments on agarose gel. The RNA samples can be separated on agarose gel with formaldehyde as the denaturing agent that limits secondary structures of RNA molecules.

Transfer: Transfer the DNA/RNA fragments from the gel onto a nylon membrane.

Prehybridization (Blocking): Wash the nylon membrane with a prehybridization solution containing salmon sperm DNA to block non-specific DNA interactions and reduce background noise. Alternatively, use the PerfectHyb™ Plus buffer, which doesn't require salmon sperm DNA for blocking.

Preparation of probe: Prepare fresh probe DNA and label with 32P alpha-labeled dCTP.

Hybridization: Incubate the blot with labeled probe.

Detection of probe: Detect the probe and the DNA/RNA sequence of interest by exposure to film at -80 °C.

Steps involved in DNA/RNA blotting procedure

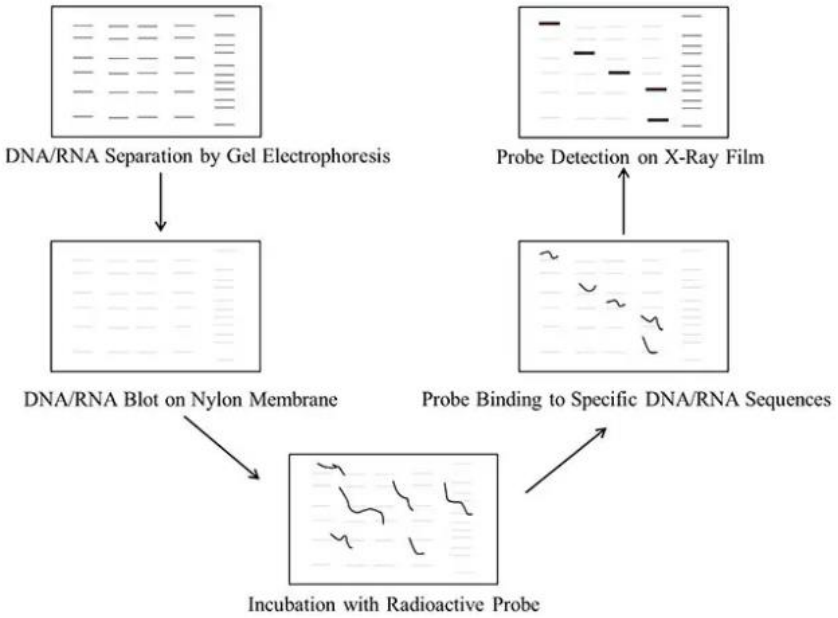

Figure 1. Steps involved in DNA/RNA blotting procedure

SOUTHERN BLOTTING PROTOCOL

Isolation of DNA

Restriction Digestion

Digest the DNA sample with appropriate restriction enzyme for 2-24 h at 37 °C. If the DNA sample is clonally derived a digestion time of 1-2 h is sufficient. For genomic DNA, overnight incubation is generally required with excess enzyme (5-10X).

If necessary, concentrate the digested DNA using ethanol precipitation. The traces of ethanol must be completely removed before separation on the gel.

Gel Electrophoresis

Resolve the digested DNA on agarose gel. TAE should be used for shorter runs (<4hrs) and larger DNA fragments, while TBE should be used for longer runs (>4hrs) and smaller DNA fragments (<1kb). Alternatively, use Bionic™ Buffer (B6185) for sharper band resolution in less time than TAE or TBE (for more information see Bionic™ Buffer Data). Stain the gel with ethidium bromide and acquire the gel image using UV transilluminator. If ethidium bromide has been incorporated into the agarose prior to electrophoresis, the gel image can be acquired immediately after the run.

Transfer

During the transfer step the DNA (or RNA) from the electrophoresis gel will be transferred onto a nylon membrane so it may be accessible to a probe for hybridization and detection.

Transfer the gel into a tray containing denaturing solution enough to cover the gel completely. Wash the gel twice with this solution, each wash lasting for 25 min on a shaker.

Rinse the gel with water twice.

Wash the gel twice with neutralizing solution, each wash lasting for 15 min on a shaker.

Rinse the gel with water twice.

Wash the gel with 20X SSPE on a shaker for 30 min.

During the above step, prepare the Whatman paper and membrane for the transfer procedure.

Cut a strip of the nylon membrane (15356) and soak it in water. Cut a strip of Whatman paper, wider than the gel and soak it in 10X SSPE buffer (transfer buffer).

Cut three strips of Whatman paper almost the same size of the gel.

Cut multiple paper towels almost the same size of the gel.

Place a stable platform on a tray containing 10X SSPE.

Place a saran wrap on the platform. Place a Whatman paper soaked with 10X SSPE on the saran wrap. Ensure no air bubbles are trapped by rolling them off using a glass rod. Ensure that the edges of the Whatman paper are touching the SSPE buffer in the tray. This piece of Whatman paper will act as a wick to pull the SSPE buffer up and through the gel, depositing the DNA bands onto the nylon membrane.

Place the gel, face down on the wet Whatman paper. Place the membrane on the top of the gel. Ensure no air bubbles are trapped by rolling them off using a glass rod.

Place three strips of Whatman paper on the membrane. Ensure no air bubbles are trapped by rolling them off using a glass rod.

Place a stack of paper towels on this, followed by weight (such as glass slab).

Let this assembly stand overnight for complete transfer of the DNA fragments. Transfer of DNA fragments up to 15kb takes about 18 hours.

After the transfer is complete, place the blot in a UV crosslinker on automatic setting. UV crosslinking is done to covalently bind DNA or RNA to a nylon membrane, which increases hybridization signals during detection. Another option is to bake the dry membrane after the transfer, which results in non-covalent but semi-permanent bonds of nucleic acids to the membrane. DNA cannot be UV crosslinked to a nitrocellulose membrane and must be baked.

Southern/northern blot transfer assembly

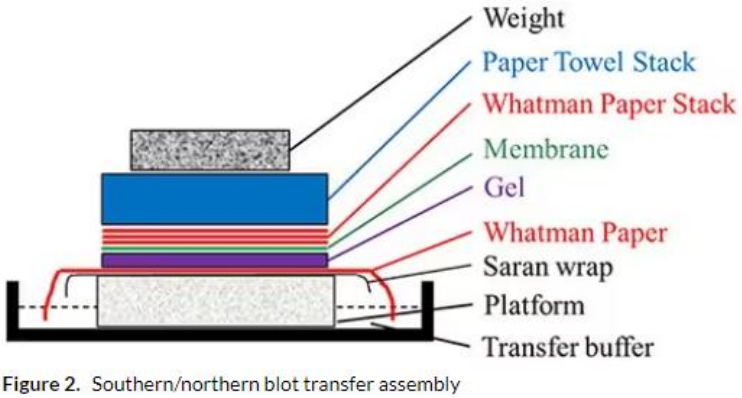

Figure 2. Southern/northern blot transfer assembly

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Prehybridization (Blocking)

The prehybridization step (also known as blocking) is done to minimize non-specific attachments of a probe to the nylon membrane. Salmon sperm DNA is commonly used as a blocking agent to prevent the probe from sticking to the membrane, ensuring that it will only interact with the desired DNA bands that have been transferred to the membrane.

Warm the prehybridization solution to 42 °C.

Heat a sample of salmon sperm DNA (D9156) to 95 °C for 5 min and immediately chill on ice.

Add salmon sperm DNA to the warm prehybridization solution so that the final concentration of DNA is 50 µg/mL.

Take the blot out of UV crosslinker and roll it carefully into a hybridization tube.

Add prehybridization solution with sperm DNA into the hybridization tube and place it at 42 °C in hybridization oven for 5 h.

Hybridization

A complementary strand of DNA (a probe) is used to detect the desired sequence that should be present on the membrane. DNA from two different sources combine to make a "hybrid" dsDNA, but only if the two strands are homologous.

Prepare 1X probe mix and incubate in water bath at 37 °C for 40 min.

The free label (unincorporated probe) can be removed by passing the probe mix through G-25 Sephadex column.

Determine the specific activity of the probe using liquid scintillation.

Warm 10 mL of hybridization solution to 49 °C, add 10-15 X 106 cpm of probe to it and mix well.

Discard the pre-hybridization solution from the blot; add the hybridization solution with the labeled probe. Incubate overnight at 49 °C.

Warm the 6X wash solution to 49 °C. Discard the hybridization solution from the blot and add 6X wash solution.

Warm the 6X low-stringency wash solution to 49oC. Discard the hybridization solution from the blot and add the 6X wash solution. The low-stringent wash removes low-homology hybridizations in order to leave high-homology interactions behind, refining the desired DNA sample.

Prepare the 1X high-stringency wash solution and warm it to 49oC. Wash the blot for about 30 seconds and discard the wash solution. This step uses a high-stringent solution to further refine the desired DNA by removing closely homologous hybridizations.

Check the blot for activity with a Geiger counter. Readings of 10-50 counts per second with bands of irradiation peaks are desirable. If the background is too high, wash the blot again with 1X wash solution.

Detection of probe

Place the blot in a film cassette lined with new saran wrap and carefully wrap the blot ensuring no air bubbles are trapped between the blot and the wrap.

Carry the cassette to the darkroom and place the X-ray film over the blot. Lock the cassette and place it at -80 °C overnight. Develop the film the following day.

Another sheet of X-ray may be placed over the blot and returned to -80 °C for another exposure.

NORTHERN BLOTTING PROTOCOL

The protocol for Northern blotting is similar to that of Southern blotting. However, RNA sequences are separated on denaturing agarose gel incorporated with formaldehyde.

Isolation of RNA

Isolate RNA from cells or tissue samples using the TRI Reagent® (T9424) or GenElute™ kit for mammalian cells or tissues (RTN70, RTN10 and RTN350).

To determine the quality and concentration of RNA, read the absorbance of the samples at 260 nm and 280 nm. The ratio of absorbance, A260/A280 of 1.8-2.1 indicates good quality RNA.

Gel electrophoresis

Load the samples carefully into the wells using pipettes. A suitable marker containing RNA fragments of various sizes (R7020, R7644) may also be loaded, if required.

For a detailed protocol on denaturing RNA in agarose gel electrophoresis, refer to the Introduction to Nucleic Acid Electrophoresis

If ethidium bromide has been incorporated into the agarose prior to electrophoresis, the gel image can be acquired immediately after the run.

Transfer

The transfer setup, prehybridization, hybridization, and detection protocols are the same as those for Southern blotting.