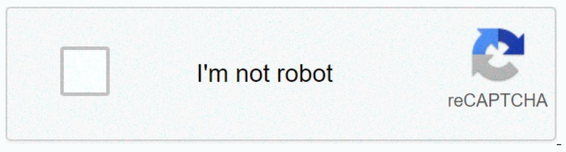

I am not robot!

Northern blot analysis steps

Reagents for DNA isolation and purification Reagents for restriction digestion of DNA Reagents and buffers for agarose gel electrophoresis Apparatus for agarose gel electrophoresis Whatman filter papers Paper towels Positively charged nylon membrane Salmon sperm DNA Hybridization tube X-Ray films 10X TBE (93290), used during gel electrophoresis.
TAE buffer (65497) may be used in place of TBE for larger DNA fragments. Alternatively, use Bionic™ Buffer (B6185) in place of TAE or TBE for sharper bands in less time.
You may prepare the 10X TBE buffer with the following reagents: TRIS 1.3 M Boric acid 450 mM EDTA 25 mM 20X SSPE (S2015), used as a prehybridization and transfer buffer. 20X SSC (93017) may be used in place of SSPE in similar blotting protocols. Alternatively, you may prepare the 20X SSPE solution with the following reagents: NaCl 2.98 M EDTA 0.02 M Phosphate buffer (pH 7.4) 0.2M Denaturing solution (N1531), used to denature dsDNA, making it accessible to the probe. Alternatively, you may prepare the 1X denaturing solution with the following reagents: NaCl 1.5 M NaOH (S5881) 0.5 N Adjust the pH to ~13 Neutralizing solution (N6019), used to neutralize the gel after denaturing dsDNA. Alternatively, you may prepare the 1X neutralizing solution with the following reagents: NaCl 1.5 M TRIS HCl 1 M Adjust the pH to 7.5 *Denhardt’s solution (D2532), used during prehybridization to block non-specific DNA hybridizations. Alternatively, you may prepare the 50X Denhardt’s solution with the following reagents: Bovine serum albumin (A7906) 1% Ficoll (F2637) 1% Polyvinylpyrrolidone (PVP40) 1% Dissolve the components in water to a final volume of 50 mL. Sterilize by filtration. davako *2X Prehybridization solution (P1415), used to prepare the membrane for probe hybridization. Alternatively, you may prepare the 1X prehybridization solution (100 mL) with the following reagents: 20X SSPE 30 mL 100X Denhardt’s solution 10 mL 10% SDS 10 mL Water 50 mL *Hybridization solution (H7140), used during the hybridization step.
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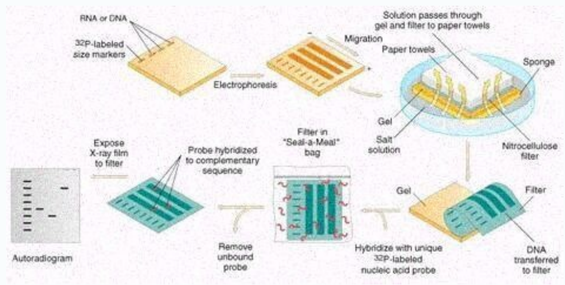

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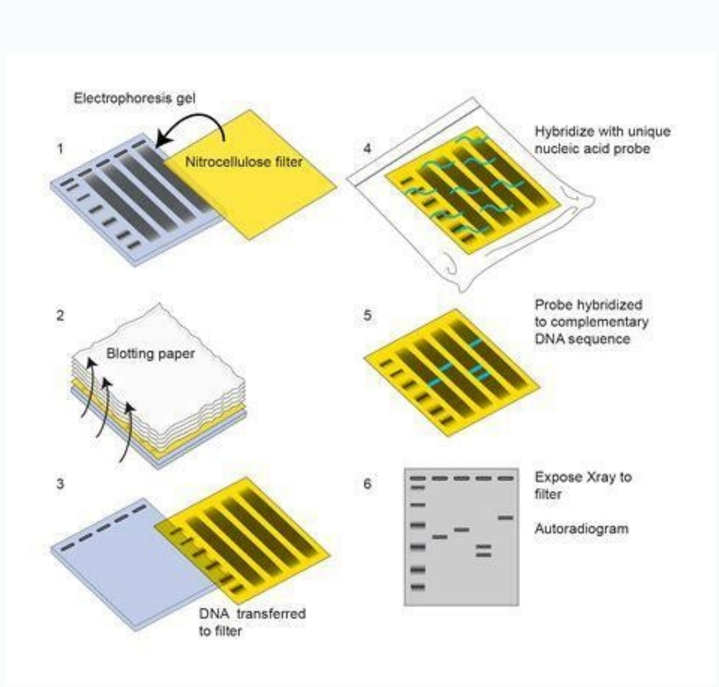
Place a saran wrap on the platform. Place a Whatman paper soaked with 10X SSPE on the saran wrap. Ensure no air bubbles are trapped by rolling them off using a glass rod. Ensure that the edges of the Whatman paper are touching the SSPE buffer in the tray. This piece of Whatman paper will act as a wick 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.



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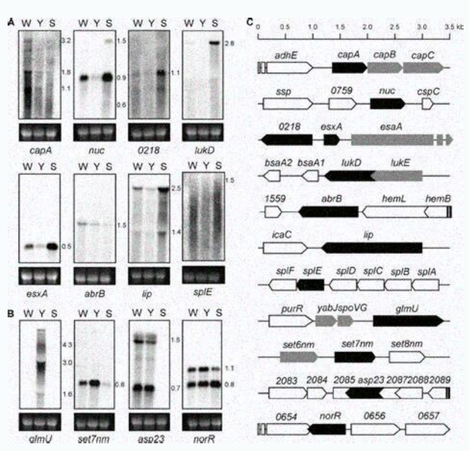

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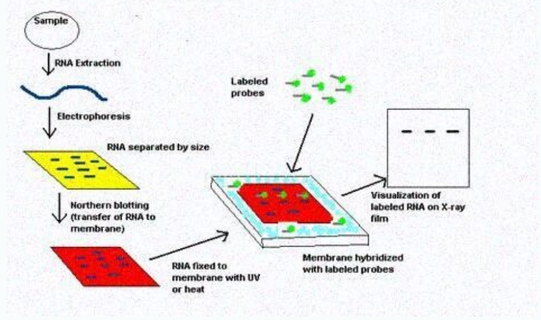

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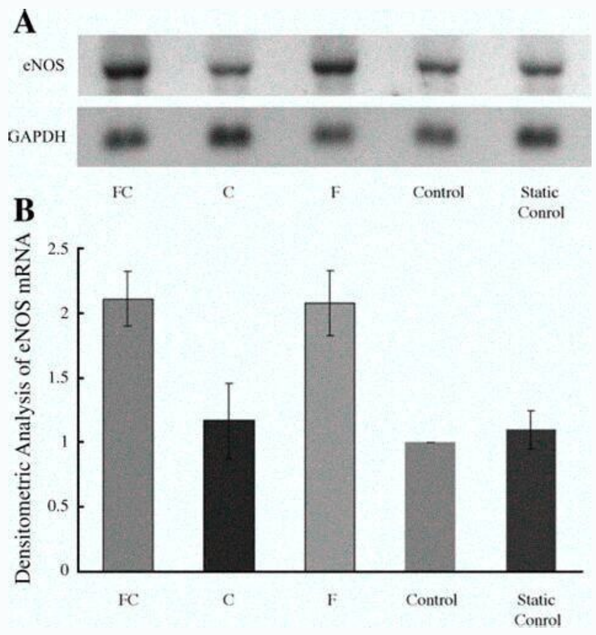

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Let this assembly stand overnight for complete transfer of the DNA fragments. Transfer of DNA fragments up to 15kb takes about 18 hours. After the transfer is complete, place the blot in a UV crosslinker on automatic setting. UV crosslinking is done 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. Northern blot is a technique based on the principle of blotting for the analysis of specific RNA in a complex mixture. The technique is a modified version of the Southern Blotting, which was discovered for the analysis of DNA sequences. The detection of certain sequences of nucleic acids extracted from different types of biological samples is essential in molecular biology, which makes blotting techniques imperative in the field. The principle is identical to southern blotting except for the probes used for the detection as northern blotting detects RNA sequences. This technique provides information about the length of the RNA sequences and the presence of variations in the sequence. Even though the technique is primarily focused on the identification of RNA sequences, it has also been used for the quantification of RNA sequences. Since the discovery of the technique, several modifications have been made in the technique for the analysis of mRNAs, pre-mRNAs, and short RNAs. Northern blotting was employed as the primary technique for the analysis of RNA fragments for a long time; however, new, more convenient, and cost-effective techniques like RT-PCR have slowly replaced the technique. Northern Blot. Created with BioRender.com The principle of the northern blot is the same as all other blotting technique that is based on the transfer of biomolecules from one membrane to another. The RNA samples are separated on gels according to their size by gel electrophoresis.
Since RNAs are single-stranded, these can form secondary structures by intermolecular base pairing. The electrophoretic separation of the RNA segments is thus performed under denaturing conditions. The separated RNA fragments are then transferred to a nylon membrane. Nitrocellulose membrane is not used as RNA doesn't bind effectively to the membrane. The transferred segments are immobilized onto the membrane by fixing agents. The RNA fragments on the membrane are detected by the addition of a labeled probe complementary to the RNA sequences present on the membrane. The hybridization forms the basis of the detection of RNA as the specificity of hybridization between the probe, and the RNA allows the accurate identification of the segments. Northern blot utilizes size-dependent separation of RNA segments and thus can be used to determine the sizes of the transcripts. Agarose Gel cast Power Supply Microwave Centrifuge Heating block UV crosslinker Hybridization oven Hybridization vessels Vials Forceps Pipettes Glass tubes Agarose gel Sodium citrate Ethylenediaminetetraacetic acid disodium salt dehydrate NaOH HCl Formaldehyde Glycerol Ethidium bromide Bromophenol Blue RNA ladder MgCl2 NaCl Polyvinylpyrrolidone Bovine Serum Albumin SDS NaH2PO4 Tris-HCl Triton-X DTT Taq buffer Taq polymerase The RNA gel solution is prepared by adding formaldehyde to the agarose solution. The cast is assembled, and the prepared denaturing gel is poured into the cast. As the gel begins to set, a comb with appropriate teeth is added to form wells. Once the gel is set, the comb is removed, and the gel is equilibrated with a running buffer for 30 minutes before running. 15 µg RNA sample is mixed with an equal volume of RNA loading buffer. Three µg of RNA markers are added in the same volume of RNA loading buffer. The samples are incubated at 65°C on a heating block for about 12-15 minutes. The samples are loaded to the equilibrated gel, and the first row of wells is filled with RNA markers. The gel is then run at 125V for about 3 hours. Image Source: Ilewieszoośmiornicach (Wikimedia). A nylon membrane is cut that is larger than the size of the denaturing gel, and a filter paper with the same size as the nylon membrane is also prepared. Once the electrophoresis process is complete, the RNA gel is removed from the tank and rinsed with water. An oblong sponge that is slightly larger than the gel is placed on a glass dish, and the dish is filled with SSC to a point so as to leave the soaked sponge about half-submerged in the buffer. A few pieces of Whatman 3mm papers are placed on top of the sponge and are wetted with SSC buffer. The gel is then placed on top of the filter paper and squeezed out to remove air bubbles by rolling a glass pipette over the surface. The nylon membrane prepared is wetted with distilled water on an RNase-free dish for about 5 minutes. The wetted membrane is placed on the surface of the gel while avoiding any air bubbles formation. The surface is further flooded with SSC, and a few more filter papers are placed on top of the membrane. A glass plate is placed on top of the structure in order to hold everything in place. The structure is left overnight to obtain an effective transfer. Once the transfer is complete, the gel is removed and rinsed with SSC, and allowed to dry. The membrane is placed between two pieces of filter paper and baked in a vacuum oven at 80°C for 2 hours. In some cases, the membrane can be wrapped in a UV transparent plastic wrap and irradiates for an appropriate time on a UV transilluminator. The DNA or RNA probes to be used are to be labeled to a specific activity of >108 dpm/µg, and unincorporated nucleotides are to be removed. The membrane carrying the immobilized RNA is wetted with SSC. The membrane is placed in a hybridization tube with the RNA-side-up, and 1 ml of formaldehyde solution is added. The tube is placed in the hybridization oven and incubated at 42°C for 3 hours. If the probe used is double-stranded, it is denatured by heating in a water bath or incubator for 10 minutes at 100°C. The desired volume of the probe is pipette into the hybridization tube and further incubated at 42°C. The solution is poured off, and the membrane is washed with a wash solution. The membrane is then observed under autoradiography. The RNA bands are observed under radiography in the form of bands. The distance of the bands from the markers can be used to determine the length and semi quantification of the RNA fragments. The technique can be used for the identification and separation of RNA fragments collected from different biological sources. Northern blotting is used as a sensitive test for the detection of transcription of DNA fragments that are to be used as a probe in Southern Blotting. It also allows the detection and quantification of specific mRNAs from different tissues and different living organisms. Northern blotting is used as a tool for gene expression studies related to overexpression of cancer-causing genes, and gene expression during transplant rejects. Northern blotting has been used as a molecular tool for the diagnosis of diseases like Crohn's disease. The process is used as a method for the detection of viral microRNAs that play important roles in viral infection. Northern blotting has a lower sensitivity as compared to other modern techniques like RT-PCR and nuclease protection assays. The method requires a large amount of sample RNA, and these should be of high quality. The technique is time-consuming and complex, especially in cases where multiple probes are to be added. He, Shan L, and Rachel Green. 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PMID: 18428227. – 11% – 5% – 4% – 3% – 2% – 1% – 1% – 1% – 1% – 1% – <1% About Author