a.Procedure or Steps of Northern Blot b. Transfer of RNA from gel to the nylon membrane 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.The nylon membrane prepared is wetted with distilled water on an RNase-free dish for about 5 minutes.Separation of RNA on a denaturing gel The RNA gel solution is prepared by adding formaldehyde to the agarose solution.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.d. Hybridization The DNA or RNA probes to be used are to be labeled to a specific activity of >108 dpm/ug, and unincorporated nucleotides are to be removed.The desired volume of the probe is pipette into the hybridization tube and further incubated at 42?C.The membrane is placed in a hybridization tube with the RNA-side-up, and 1 ml of formaldehyde solution is added.Three ug of RNA markers are added in the same volume of RNA loading buffer.The samples are loaded to the equilibrated gel, and the first row of wells is filled with RNA markers.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 wetted membrane is placed on the surface of the gel while avoiding any air bubbles formation.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 membrane carrying the immobilized RNA is wetted with SSC.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 cast is assembled, and the prepared denaturing gel is poured into the cast. 15 ug RNA sample is mixed with an equal volume of RNA loading buffer.Once the electrophoresis process is complete, the RNA gel is removed from the tank and rinsed with water.A few pieces of Whatman 3mm papers are placed on top of the sponge and are wetted with SSC buffer.The surface is further flooded with SSC, and a few more filter papers are placed on top of the membrane.c. Immobilization 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.Once the gel is set, the comb is removed, and the gel is equilibrated with a running buffer for 30 minutes before running.The tube is placed in the hybridization oven and incubated at 42?C for 3 hours.The membrane is then observed under autoradiography.As the gel begins to set, a comb with appropriate teeth is added to form wells.The samples are incubated at 65?C on a heating block for about 12-15 minutes.The solution is poured off, and the membrane is washed with a wash solution.The gel is then run at 125V for about 3 hours.The structure is left overnight to obtain an effective transfer.A glass plate is placed on top of the structure in order to hold everything in place.

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