Detection of microRNAs by Northern blot hybridization using 5'-end labeled miRCURY™ LNA microRNA probes

Protocol prepared by Dr. Zoltan Havelda, Agricultural Biotechnology Center, Godollo, Hungary. (Nucleic Acids Research, 2004, vol.32 (22), e175)

- Extract total RNA by using the TRI Reagent (Sigma-Aldrich, USA) according to the manufacturer's instructions.

- Fractionate the total RNA sample on a denaturing 12% polyacrylamide gel containing 8 M urea, then transfer the fractionated RNAs to Nytran N membrane (Schleicher & Schuell, Germany) by capillary method and fix by UV cross-linking according to the manufacturer's instructions. For a standard small RNA gel, load 10 micrograms of total RNA on the gel (up to 100 micrograms of total RNA can be loaded per lane).

- For radiolabeling of the miRCURY™ probe, use 10 pmol of probe for the end-labeling reaction combined with one microliter of gamma-32P-ATP and T4 polynucleoitde kinase according to standard protocol (alternatively 20 pmol probe can be labeled using twice the amount of gamma-32P-ATP).

- Prehybridize the membrane in small RNA hybridization buffer. (50% formamide, 0.5% SDS, 5xSSPE, 5xDenhardt's solution, and 20 µg/mL sheared, denatured, salmon sperm DNA)

- Hybridize the membrane in the same solution at 34-45 °C depending on the desired stringency. Heat the labeled miRCURY™ probe were for one minute at 95°C before addition to the filters in the prehybridization solution.

- After hybridization the membranes are washed at low stringency in 2xSSC, 0.1% SDS at 34-45°C twice for five minutes or at high stringency in 0.1 SSC, 0.1% SDS at 65°C twice for five minutes. If you have several membranes with different probes wash them separately to prevent potential cross-contamination. It is recommendable to wash the membrane first using low stringency conditions followed by exposure of the membrane. Upon evaluation of the initial results, the membranes can then be washed further under increasing stringency to remove unspecific background.

Note! It is important to prevent the membranes from drying, by storage in plastic film, which is impermeable to moisture (e.g. Saranwrap).

For preparation of buffers please refer to:
Molecular cloning : a laboratory manual / Sambrook, Joseph; Russell, David W. --
3rd ed. -- New York: Cold Spring Harbor Laboratory, 2001.