Protocol

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RNA Gel and Northern Blotting Protocol

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Introduction

Northern blotting is a classic and common molecular biology technique used in the study of gene expression through identification of specific RNAs in a sample. This technique was derived from the first blotting technique, the Southern blot, developed by Dr. Edwin Southern, which can be used to identify specific DNAs in a sample. Through the Northern blotting process, we can determine both the levels and size of specific RNA in a sample providing insight into novel genes and their function. Variances in blots also allow for the discovery of transcription errors. Briefly, RNA is separated according to size through gel electrophoresis, then transferred to a nylon membrane and cross-linked. Then, a labeled probe complementary to the transcript of interest is hybridized to the RNA. Here, we provide a general protocol describing the steps required to perform a Northern blotting procedure, as well as the preparation of solutions.

Materials

- Agarose LE
  (GoldBio Catalog # A-201)
- DEPC Treated H_2O
- MOPS (GoldBio Catalog # M-790)
- Formaldehyde
- Sodium Acetate
- EDTA (GoldBio Catalog # E-210)
- Formamide
  (GoldBio Catalog # F-035)
- ETBr
- 6x Stopper loading buffer
- Sodium Citrate
- NaCl
- 20x SSC (Citric Acid Buffer)
- 100x Denhardt's Solution
- SDS

Solutions
For Agarose Gel

- 140 ml DEPC Treated H_2O
- 20 ml 10x MOPS
- 40 ml Formaldehyde
- Total 200 ml

For 10x MOPS

- 41.9 g MOPS
- 4.1 g Sodium Acetate
- 3.7 g EDTA

- Fill to 1 liter with DEPC Treated H_2O and adjust to pH 7.0

For 20x SSC

- 440 g Sodium Citrate
- 875 g NaCl
- Fill to 5 liters with DEPC Treated H_2O and adjust to pH 7.0

For Wash Solutions

- 20 ml 20x SCC
- 1 ml 100x Denhardt's Solution
- 0.5 g SDS
- Fill to 100 ml with DEPC Treated H_2O

For Low Stringency Buffer

- 20 ml 20x SCC
- 0.5 g SDS
- Fill to 100 ml with DEPC Treated H_2O

For High Stringency Buffer

- 10 ml 20x SCC
- 0.5 g SDS
- Fill to 100 ml with DEPC Treated H_2O

For Loading Buffer

- 1000 µl Formamide
- 300 µl 37% Formaldehyde
- 200 µl 10x MOPS
- 460 µl DEPC Treated H_2O
- 10 µl ETBr (10 µg/µl)
- 300 µl Stopper (6x loading buffer for agarose gels)
- Total volume should be 2300 µl

Method

Gel Preparation

1. Boil 2 g of Agarose LE in 140 ml DEPC Treated H_2O until dissolved. Let cool to 50°C.

2. Pour agarose gel containing MOPS and formaldehyde in fume hood.

3. Let solidify (gel may be wrapped in plastic wrap and stored at 4°C overnight, if desired).

RNA Preparation

1. Dry 20 µg total RNA in a speedvac (~15-30 minutes for 1 µg/µl RNA).

2. Add 20 µl of Loading Buffer to each RNA sample, vortex for 15 minutes in a cold room then store at -20°C overnight.

3. Dilute RNA markers in same volume (20 µl) of loading buffer.

4. Incubate at 60°C for 15 minutes in a heating block, place in ice bath for 1 minute, spin for 15 seconds in a micro-centrifuge and put on ice immediately afterwards.

RNA Gel

Separate RNA based on size using denaturing agarose gel and formaldehyde.

1. Equilibrate the gel with 1x MOPS buffer for at least 30 minutes before running.

2. Load prepared RNA samples and RNA markers on the gel.

   Note: Sample volume should not exceed the well volume. If the RNA sample is too diluted, salt-precipitate the RNA and resuspend in a smaller volume of DEPC Treated H_2O.

   Note: Leave space between the RNA marker and the first sample.

3. Run the gel in 1x MOPS buffer under a fume hood at 80 V for around 4 hours.

   Note: The gel can be run faster by increasing the voltage and decreasing the time. For example, run the gel at 125 V for about 3 hours.

4. Take a picture of the gel while sitting in the gel tray.

5. Soak the gel for 10 minutes in DEPC Treated H_2O to remove formaldehyde. Discard DEPC Treated H_2O and repeat wash for an additional 10 minutes.

6. Equilibrate gel for 15 minutes in 20x SSC solution.

Northern Blot

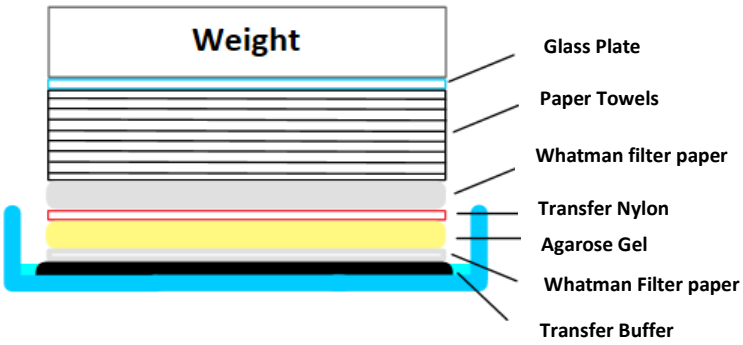
RNA is transferred to a nylon membrane from the gel using a transfer apparatus.

1. Cut a nylon membrane the size of the denaturing RNA gel (or a little bigger). Cut Whatman filter paper the same size as the nylon membrane.

2. Prewet the nylon membrane and 3 sheets of Whatman filter paper in DEPC Treated H_2O, followed by 20x SSC.

3. Set up the transfer apparatus inside a large glass dish filled with 20x SSC from bottom to top.

    a. Dish
    b. Glass plate
    c. Whatman filter paper as a wick hanging over the plate into 20x SSC (Transfer buffer)
    d. Gel
    e. Transfer Nylon membrane
    f. 3 sheets of Whatman filter paper
    g. Stack of paper towels
    h. Glass plate
    i. Weight



Note: The wick is larger than the gel, whereas the nylon, 3 sheets and paper towels are consistent with the gel size.

Note: Ensure the buffer level is above the gel.

4. Transfer overnight.

5. Take a picture to ensure the gel transfer was completed.

    Note: The gel can be illuminated with UV to determine if there is any remaining RNA.

    Note: Clean the transfer system with tap H_2O.

6. Air dry the nylon membrane for 30 minutes. Place under UV in tissue culture hood for 1 minute to cross-link. Use a fine marker to indicate the edge of the side with RNA.

Note: It is optional to bake the membrane for 2 hours at 80°C.

7. Prewash the membrane for 2 x 30 minutes at 68°C in Wash Solution. The volume is around 100-200 ml depending on the size of the membrane.

Hybridization

The radioactively labeled RNA probes complementary to RNA transcript of interest are hybridized to the membrane. The nonspecifically bound probes are washed away after hybridization.

1. Prehybridize for 1 hour at 68°C in 10 ml Wash Solution and 100 µg/ml of boiled ssDNA with RNA-side up.

2. Hybridize in 5 ml Wash Solution and 100 µg/ml of boiled ssDNA, plus boiled probe, and marker probe overnight at 68°C.

3. Prewarm the Low and High Stringency Buffer solutions (4x SSC, 0.5% SDS and 2x SSC, 0.5% SDS solutions).

4. Pour off the probe into a 15 ml conical tube for storage at -20°C.

5. Wash twice with 10 ml hybridization solution and pour wash into absorbent container.

6. Remove membrane and place in tray for washing.

7. Wash membrane
    a. Once in Low Stringency Buffer for 30 minutes at 68°C.
    b. Twice in High Stringency Buffer for 30 minutes at 68°C.
    c. Once in High Stringency Buffer for 30 minutes at 68°C.

8. Remove membrane, dry off all liquid, but do not dry completely. Wrap in plastic wrap and expose filter to film overnight or as necessary.

Tips

- RNAse-free conditions are important throughout the procedure.
- Disposable gloves must be worn at all times.

- All materials should be autoclaved or filter-sterilized.

Associated Products

- Agarose LE (GoldBio Catalog # A-201)
- MOPS (GoldBio Catalog # M-790)
- EDTA (GoldBio Catalog # E-210)
- Formamide (GoldBio Catalog # F-035)
- GelRed™ Nucleic Acid Gel Stain (GoldBio Catalog # G-720)

References

He, S. L. & Green R. (2013). Northern Blotting. *Methods Enzymol., 530*(75), 87th ser. Doi:10.1016/B978-0-12-420037-1.00003-8.

Wolf, D. D. (n.d.). *Protocols*. Retrieved from Wolf Lab @ Sanford Burnham Brebys Medical Discovery Institute.