Northern blotting

By: Norsham Bt Shamsuddin
P45107
Introduction

Northern Blot-RNA blotting technique—developed in 1977 by Alwine et al. at Stanford University.

Southern blot – technique which blots for DNA, invented by Edwin M. Southern in 1975.

Western blot – a method for blotting for proteins and was named in 1981.
Applications of the Northern blot

- Detection of mRNA transcript size
- Study RNA degradation
- Study RNA splicing-can detect alternatively spliced transcripts
- Study RNA half-life
- Often use to confirm and check transgenic/ knockout mice (animals)
Advantages

- Gold standard, widely accepted and well regarded method
- Straight-forward method
- Used as a confirmation or check
- It can allow the usage of many types of probes
- Sequences with even partial homology, can be used as hybridization probes (i.e sequence from different species for homology analysis, or even genomic fragments can be used)
Disadvantages

- The whole process takes a long time
- If RNA samples are even slightly degraded by RNAses, the quality of the data and quantitation of expression is quite negatively affected
- The standard northern blot method is relatively less sensitive than nuclease protection assays and RT-PCR.
- Detection with multiple probes is problem. Often, the membranes must be stripped before hybridization and detection with a second probe.
Northern blot protocol

- RNA isolation
- Gel electrophoresis of RNA for separation
- Transfer to membrane
- Cross-linking of RNA to membrane (usually by UV-cross linking or chemical means)
- Hybridization
- Detection
NORTHERN BLOT METHOD

1. RNA
2. Separate RNA on an Agarose Gel
3. Transfer or BLOT RNA from GEL to Nylon Membrane
4. Membrane with RNA bands transferred to it
5. Radiolabeled probe incubated with Nylon Membrane
6. Bound RNA Bands are exposed on film
Northern - up close

2 different mRNAs are bound to a membrane.

The membrane is incubated with a solution containing a probe which is homologous to one of the 2 mRNAs on the membrane.

The probe has an indicator attached to it.

The binding is very specific and requires the base pairs of the probe and the bound mRNA to be perfectly complementary.

Step removes any probe which is not tightly bound to the mRNA on the membrane.

Only probe that matches exactly will remain bound.

mRNA homologous to the probe and therefore a band will develop where this mRNA is bound to the membrane.

No band will show where "B" is bound.
Detection

- If a **radiolabeled** probe – the blot can be wrapped in plastic wrap (avoid dried), then immediately exposed to film or autoradiography.

- If a **nonisotopic** probe – the blot treat with nonisotopic detection reagents prior to film exposure.
Northern transfer technique

- Capillary transfer
- Turbo blotter
- Vacuum blotting
- Electro blotting (use nylon or nitrocellulose)
- Alkaline blotting (SSC or SSPE vs NaOH)
Capillary transfer

- Requiring the fewest accessories
- The least expensive and most time consuming technique
- Stack of absorbent (paper towel @ blotting paper) :
  - is used to draw (wick) the transfer buffer from reservoir through the gel and
  - finally into the dry stack of paper towel
- RNA sample is essentially eluted from the gel and is trapped on a filter membrane
Capillary transfer technique

A typical setup for capillary transfer of RNA from denaturing agarose gels to a filter membrane. (a) Side view. Invert the gel to reduce the blotting time and achieve greater resolution, because the RNA does not have to travel as far through the gel matrix to reach the membrane. (b) Top view. Place overlapping strips of Parafilm directly on the wick and up against the gel (not on top of the gel). Set Parafilm in place around the sides of the gel first and then overlay filter membrane and other blotting materials.
Turbo blotter

- Take advantage of gravity
- Offer enhanced transfer efficiency in a much shorter time (less than 4 hours)
- Eliminates the need for heavy weights on top of the capillary stack: *minimizing compression* & *concomitant collapsing* of the pores of the gel
- Does not require the use of any expensive equipment
- The record for assembling: 58 seconds!
TurboBlotter transfer method. The principle is essentially that of an inverted capillary transfer. By taking advantage of gravity, rapid downward movement of the high-salt transfer buffer accelerates the transfer and dramatically improves transfer efficiency. The absorbent blotting paper is at the bottom of the stack and the buffer reservoir on top, precisely the opposite of the classical capillary blot setup shown in Figure 12.1. Courtesy of Schleicher & Schuell.
Vacuum blotting
- Negative pressure is applied to accelerate the transfer process (vacuum assisted capillary transfer)
- Advantage: The transfer occurs far more rapidly (30-60 min vs. overnight)

Electro blotting
- Widely used for transferring proteins from polyacrylamide gels
- Standard transfer methodology for Western Blot
- Advantage: Low voltage & current are need for transfer

Alkaline blotting
Alkaline blotting vs. capillary transfer
- Key difference: method of denaturation and nucleic acid transfer
- Capillary transfer – Use of high-salt buffer (15-20 x SSC or SSPE)
- Alkaline blotting- Use of 0.4 N NaOH
Choice of filter membrane

- **Nitrocellulose**
  - original RNA blotting technique: diazobenzyloxyethylmethyl paper – nucleic acid immobilization
  - so-called classical membrane of molecular biology

- **Nylon**
  - circumvent many of difficulties in inherent nitrocellulose
  - Advantage : exhibit great tensile strength
  - exhibit an enhanced nucleic acid binding capacity
  - show particular affinity for smaller nucleic acid molecules (500 bases)

- **Polyvinylidene Difluoride (PVDF)**
  - Fluorocarbon polymer (Western Blot)
  - Highly durable, chemically stable membrane
About filter membrane:

- Avoid touching the filter with ungloved fingers
- Stored in a cool dry location
  (out of direct sunlight, not be refrigerated, frozen or heated above RT)
- Stored correctly – stable for many months
Buffers:

10 x MOPS:
0.4 M Morpholinopropanesulfonic acid (free acid); 0.1 M Na-acetate-3 x H2O; 10 mM EDTA; adjust to pH 7.2 with NaOH; store dark in fridge:
[500 ml: 41.9 g MOPS, 6.8 g NaAc, 10 ml 0.5 M EDTA]

Loading Buffer:
1 x MOPS; 18.5 % Formaldehyde; 50 % Formamide; 4 % Ficoll400; Bromophenolblue; store at -20 °C:
[1 ml: 100 µl 10 x MOPS, 500 µl Formamide, 185 µl Formaldehyde, 40 mg Ficoll400, Bromophenolblue, 215 µl H2O]

Prehybridization-buffer:
5 x SSC; 50 % Formamide; 5 x Denhardt‘s-solution; 1 % SDS; 100 µg/ml heat-denatured sheared non- homologous DNA (Salmon sperm DNA or yeast tRNA)
[100 ml: 25 ml 20 x SSC, 50 ml Formamide, 5 ml 100 x Denhardt’s, 1 g SDS, 1 ml 10 mg/ml DNA]
Hybridization-buffer:
Prehybridization buffer with 5 % Dextran sulfate (Na-salt, MW 500,000, 50 % stock-solution) and without non-homologous DNA

- **100 x Denhardt's solution:**
  [for 500 ml: 10 g Ficoll 400; 10 g polyvinylpyrrolidone MW 360000; 10 g BSA fraction V; H₂O]
  store at -20 °C.

20 x SSC:
3 M NaCl; 0.3 M Na-citrate
[1 l: 175.3 g NaCl, 88.2 g NaCitrate]

Strip-solution:
5 mM Tris pH 8; 0.2 mM EDTA; 0.05 % Na-pyrophosphate; 0.1 x Denhardt's solution
[500 ml: 2.5 ml 1 M Tris, 200 µl 0.5 M EDTA, 5 ml 5 % NaPP, 1 ml 50 x Denhardt's]
# Comparison of different methods of mRNA detection

<table>
<thead>
<tr>
<th>Feature</th>
<th>Nuclease Protection Assays</th>
<th>Northern Blotting</th>
<th>RT-PCR</th>
<th>In Situ Hybridization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative and Absolute Quantitation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Detection Limit (copies of mRNA)</td>
<td>4,000-5,000</td>
<td>10,000</td>
<td>1 (theoretical)</td>
<td>Medium</td>
</tr>
<tr>
<td>Level of Optimization Required</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Sample Size</td>
<td>100 μg max</td>
<td>30 μg max</td>
<td>Not critical</td>
<td>Tissue sections</td>
</tr>
<tr>
<td>Detect Multiple Transcripts</td>
<td>Yes, in same tube</td>
<td>Yes, generally requires stripping and reprobing</td>
<td>Yes, in same tube</td>
<td>No</td>
</tr>
<tr>
<td>Localizes mRNA Expression Within Tissue/Cells</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Mapping Studies</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Resolve Comigrating mRNAs</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Sizing mRNAs</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Detection of Alternatively Spliced Transcripts</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Distinguish Between Members of Multi-Gene Families</td>
<td>Yes</td>
<td>Only if they are different sizes</td>
<td>Only if they are characterized</td>
<td>No</td>
</tr>
<tr>
<td>Tolerates Partially Degraded RNA</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Advantages</td>
<td>• Can use up to 100 μg of sample RNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Ease of multiprobing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Tolerates partially degraded RNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• RNA undergoes minimal processing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Technique can be easily evaluated at various points in procedure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Most sensitive technique available</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Localizes mRNA within tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Tolerates partially degraded RNA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Reference


- http://g1.ion.ac.cn/methods.htm
THANK YOU