RNA blot analysis

RNA gel blot- Sample preparations

<table>
<thead>
<tr>
<th>Sample buffer stock 2x (store @ 20°C)</th>
<th>Compound</th>
<th>Stock concentration</th>
<th>Amount of stock (For 100µl)</th>
<th>Amount of stock (For 10ml)</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deionized formamide</td>
<td></td>
<td>50.0 µl</td>
<td>5 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium phosphate buffer</td>
<td>1M</td>
<td>4.0 µl</td>
<td>0.4 ml</td>
<td>40 mM</td>
</tr>
<tr>
<td></td>
<td>EDTA pH: 7.5</td>
<td>0.5M</td>
<td>2.0 µl</td>
<td>0.2 ml</td>
<td>10 mM</td>
</tr>
<tr>
<td></td>
<td>Formaldehyde pH: 7.0</td>
<td></td>
<td>16.5 µl</td>
<td>1.65 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EtBr</td>
<td>10mg/ml</td>
<td>2.0 µl</td>
<td>0.2 ml</td>
<td>0.2mg/ml</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td></td>
<td>25.5 µl</td>
<td>2.55 ml</td>
<td></td>
</tr>
</tbody>
</table>

Prepare sample to the desired concentration (around 6.5 µl).
Add same amount of sample buffer. Mix well by vortexing. Touch-spin.
Place in heat-block at 65°C for 15 min. cool rapidly on ice for 5 min. Touch-spin.
Add loading dye. Mix by tapping. Touch-spin and load.
Run gel at 90 Volt with peristaltic pump, on pumping from red electrode (+) compartment to the black electrode (-) compartment.
The large box needs 1700ml running buffer.

<table>
<thead>
<tr>
<th>Running buffer 1700ml</th>
<th>Compound</th>
<th>Stock concentration</th>
<th>Amount of stock</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium phosphate buffer pH: 6.8</td>
<td>1M</td>
<td>18.42 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td></td>
<td>1544.17 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formaldehyde</td>
<td></td>
<td>137.41 ml</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Loading dye</th>
<th>Compound</th>
<th>Stock concentration</th>
<th>Amount of stock</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycerol</td>
<td></td>
<td>50% v/v</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bromophenol blue</td>
<td></td>
<td>0.2% w/v</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium phosphate buffer pH: 6.8</td>
<td>1M</td>
<td>5mM</td>
<td></td>
</tr>
</tbody>
</table>
**Northern Hybridization**

Preheat at oven at 55°C for at least one hour before hybridization.

**Prehybridization**

We use church buffer:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Stock concentration</th>
<th>Amount of stock</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>20%</td>
<td>350ml</td>
<td>7%</td>
</tr>
<tr>
<td>Sodium phosphate</td>
<td>1M</td>
<td>300ml</td>
<td>0.3M</td>
</tr>
<tr>
<td>pH: 7.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td>0.5M</td>
<td>2ml</td>
<td>1mM</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>348ml</td>
<td></td>
</tr>
</tbody>
</table>

Place multiple membranes among screens.
Place membranes in hybridization tubes and add 10ml church buffer per membrane (large tubes) or 7 ml per membrane (small tubes).
Prehybridize for at least 30 min at 55°C. Use oven in radio room.

**G50 column preparation**

Prepare just before radiolabeling.
Fill old nucleospin columns with G50 in TE buffer. Pack the column by centrifugation for 1min at 1100rpm. Refill.

**DNA radiolabeling protocol**

According to Invitrogen Random primer labeling kit

1. Use 25-50 ng of DNA dissolved in 21µl of sterile H₂O.
2. Boil for 5 min to denature DNA. Rapidly cool on ice.
3. While on ice add:
   - 20 µl buffer 25x
   - 1 µl dGTP
   - 1 µl dATP
   - 1 µl dTTP
   - 5µl d*CTP (a-³²P)
   - and mix by pipeting up and down
4. Add 1 µl Klenow fragment enzyme. Final volume 50 µl. Mix and spin down.
5. Incubate at 37°C for 30 min.
6. Add 5 µl stop buffer (0.5M EDTA).
7. Clean probe with G50 column.
   - Centrifuge column at 1100 rpm for 1min
   - Place the probe on top
   - Centrifuge at 1100 rpm for 4min
Check with Geiger.

!!!Don’t let the column dry out.

8. Keep 2 µl of the mixture in scintillation vial (tip too) to use for specific activity determination. Use the scintillation counter.

9. Discard prehyb solution. Pour fresh church (pre-heated at 55°C) into tubes.

10. Boil probe for 5 min and pour into tube.

11. Hybridize for at least 20 hours.

!!! Remember

- Need glass beaker and plastic bag for waste. Place it in plexiglass.
- Pipette and filter tips for 1 µl and 5 µl.
- Check for hybridization tube lick.
- Check the area with Geiger.

**Wash**

1. Pour probe and church into falcon.
2. Keep falcon in beakers in 4°C.
3. Wash using 175ml of washing buffer per tube. Wash 3 times in the tube and once in a taper.

<table>
<thead>
<tr>
<th>Washing buffer (for 1 lt)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>SDS</td>
</tr>
<tr>
<td>SSC</td>
</tr>
</tbody>
</table>

Place membranes in saran membrane to avoid drying. Use tape to arrange them on white sheet. Place in cassette. Place on top the intensifying screen add the film. Place the cassette in -80°C.

**To develop the film**

Developer : Dilute 31.25 ml in 968.75 ml water.
Fixer : dilute 1:10 with water.

**To hybridize used membranes**

Boil stripping buffer.
Pour in taper and place the membrane in.
Let on shaker for 15min.

<table>
<thead>
<tr>
<th>Stripping buffer (for 1 lt)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>SDS</td>
</tr>
<tr>
<td>SSC</td>
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</table>