USEFUL INFORMATION

1. Recycling Nytran
0.1M NaOH at room temperature for 30’ or 0.4M NaOH for 10’. Neutralize with 1M Tris pH7.5, 2 x 20’ at room temperature.

2. Ribosomal RNA sizes in maize
3.20 kb
1.85 kb
1.60 kb
1.15 kb

3. Radioactivity conversions
1Ci = 2.2 x 10^{12} dpm
1mCi = 2.2 x 10^9 dpm
1µCi = 2.2 x 10^6 dpm

4. Plasmid amplification
Grow until OD_{600} = 0.9. Add 100 mg chloramphenicol to 500 ml broth and continue to incubate at 37°C overnight.

5. Best FAA fixative
50 ml ethanol
5 ml acetic acid
10 ml 37% formaldehyde
35 ml H_2O

6. Phosphate buffer
To make 50 ml 1M phosphate buffer:

<table>
<thead>
<tr>
<th>pH</th>
<th>6.4</th>
<th>6.6</th>
<th>6.8</th>
<th>7</th>
<th>7.2</th>
<th>7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>H_2</td>
<td>36.7</td>
<td>31.2</td>
<td>25.5</td>
<td>19.5</td>
<td>14</td>
<td>9.6</td>
</tr>
<tr>
<td>Na_2</td>
<td>13.2</td>
<td>18.7</td>
<td>24.5</td>
<td>30.5</td>
<td>36</td>
<td>40.5</td>
</tr>
</tbody>
</table>

7. Tris buffer
To make 100 ml 2M Tris buffer:

<table>
<thead>
<tr>
<th>pH</th>
<th>7.2</th>
<th>7.5</th>
<th>8.0</th>
<th>9.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>acid</td>
<td>89.2</td>
<td>80.7</td>
<td>56.4</td>
<td>9.7</td>
</tr>
<tr>
<td>base</td>
<td>10.8</td>
<td>19.3</td>
<td>43.6</td>
<td>90.3</td>
</tr>
</tbody>
</table>
8. **Dialysis tubing preparation**
   Add 100 g anhydrous Na₂CO₃ to 250 ml dH₂O
   Put up to 3 metres dialysis tubing into the solution & boil for 15'
   Rinse 5 x with dH₂O
   Boil in dH₂O
   Boil 3 x in 10mM EDTA washing well with dH₂O in between
   Store in 10 mM EDTA

9. **Clearing leaves** *(Crookston & Moss, 1974)*
   Place in 95% ethanol until chlorophyll is extracted
   Place in 10% NaOH for 12-14 hr
   Rinse with dH₂O

    Grind premeasured leaf disc in liquid nitrogen (about 1 cm diameter).
    Thaw in 80% acetone in eppendorf tube
    Spin out debris
    Read OD₆₄₅ & OD₆₆₃ of supernate

    Chlorophyll concentration = OD₆₄₅ × 20.2 + OD₆₆₃ × 8.02 µgml⁻¹
    Chla = 0.0127 × OD₆₆₃ - 0.00269 × OD₆₄₅
    Chlb = 0.0229 × OD₆₄₅ - 0.00468 × OD₆₆₃

11. **5-azacytidine**
    Either: germinate on filter paper in dark then after 2-3 days add 30 µM 5azaC. Leave for 3d then change for dH₂O.
    Or: germinate in 30 µM 5azaC for 2 days and then transfer to dH₂O