

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 = 2.2×10^{12} dpm

1mCi = 2.2×10^9 dpm

1 μ Ci = 2.2×10^6 dpm

4. Plasmid amplification

Grow until OD₆₀₀ = 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 H₂O

6. Phosphate buffer

To make 50 ml 1M phosphate buffer:

pH	6.4	6.6	6.8	7	7.2	7.4
H ₂	36.7	31.2	25.5	19.5	14	9.6
Na ₂	13.2	18.7	24.5	30.5	36	40.5

7. Tris buffer

To make 100 ml 2M Tris buffer:

pH	7.2	7.5	8.0	9.0
acid	89.2	80.7	56.4	9.7
base	10.8	19.3	43.6	90.3

8. Dialysis tubing preparation

Add 100 g anhydrous Na_2CO_3 to 250 ml dH_2O

Put up to 3 metres dialysis tubing into the solution & boil for 15'

Rinse 5 x with dH_2O

Boil in dH_2O

Boil 3 x in 10mM EDTA washing well with dH_2O 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 dH_2O

10. Chlorophyll assays (Arnon, D.L. 1949 Plant Phys. 24, 1-15)

Grind premeasured leaf disc in liquid nitrogen (about 1 cm diameter).

Thaw in 80% acetone in eppendorf tube

Spin out debris

Read OD_{645} & OD_{663} of supernate

Chlorophyll concentration = $\text{OD}_{645} \times 20.2 + \text{OD}_{663} \times 8.02 \mu\text{gml}^{-1}$

$\text{Chla} = 0.0127 \times \text{OD}_{663} - 0.00269 \times \text{OD}_{645}$

$\text{Chlb} = 0.0229 \times \text{OD}_{645} - 0.00468 \times \text{OD}_{663}$

11. 5-azacytidine

Either: germinate on filter paper in dark then after 2-3 days add $30 \mu\text{M}$ 5azaC. Leave for 3d then change for dH_2O .

Or: germinate in $30 \mu\text{M}$ 5azaC for 2 days and then transfer to dH_2O