RNA Gel Quantification/Northern Blot Analysis of RNA Extraction Process

Notes:

- Make sure the solutions used are RNase free. Treat with DEPC!!
- Use a chamber/comb dedicated to RNA use.

Solutions Needed:

- 10x MOPS-EDTA Buffer:
  - 0.2 M MOPS (41.86g)
  - 50 mM Sodium Acetate anhydrous (4.102g)
  - 10 mM EDTA (20 ml 0.5M solution pH8.0)
  - Fill to 800 ml with DEPC.H_2O and pH to 7.0 using 10M NaOH.
  - Finally fill to 1000 ml with DEPC.H_2O and add 200 µl DEPC. Mix and stand ON prior to autoclaving.
  - Store at 4°C in Dark.
- RNA Gel Loading Buffer:
  - 750 µl Deionised Formamide
  - 250 µl 37% Formaldehyde
  - 150 µl 10x MOPS-EDTA buffer
  - 116 µl 86% Glycerol
  - 122 µl 0.25% Bromphenol Blue
  - 122 µl 0.25% Xylene Cyanol
  - 6 µl Ethidium Bromide (10 mg/ml)
  - Aliquot into 250 µl tubes and store at -70°C.

Procedure:

1. Weigh out 0.6 g of agarose into a clean DEPC treated conical flask.
2. Add 5 ml of 10x MOPS-EDTA buffer and 43.25 ml DEPC treated H_2O.
3. Heat in the microwave to dissolve the agarose, **be careful not to reduce volume**.
4. Place on shaker to cool to temperature where it can be held (55°C).
5. In a fume hood add 2.56 ml of 37% formaldehyde, mix gently and pour into a level casting mould.
6. Leave covered loosely to set for 1 hour in fume hood.
7. Put a heat block on to 65°C.
8. Into a clean, sterile, DEPC treated 0.5 ml Eppendorf tube, take 5 µl of RNA sample to measure (if highly concentrated then take less and make volume up to 5 µl). Add 5 µl of loading buffer.
9. Into a new tube place 2 µl of RNA standards from Sigma and add 5 µl of loading buffer.
10. Place both tubes at 65°C for 15 minutes.
11. Chill immediately on ice.
12. Make up a 1x MOPS-EDTA buffer, and pour into gel chamber.
13. Load samples leaving one lane free between and run at 50 V for 240 mins.
14. Turn off and look under UV illumination at bands.