PREPARING AND RUNNING NORTHERN BLOTTING GEL (HORIZONTAL)

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1. Prepare MOPS/Formaldehyde Agarose Gel

Low EEO agarose (Sigma A6013)	1.05g
H_2O	43.4ml
Microwave to boil (melt agarose completely)	
5x MOPS	14.0ml
37% Formaldehyde	12.6ml

   Cool the gel mix down to approx. 50°C, and pour to an Owls medium size gel box, insert 12-well or 16-well 1.5 cm combs (**Note: you'd better run a two-tiered gel**).

2. Mix RNA samples with RNA sample buffer (**see below, ask TCH for your own stock**). (**Note**: Usually you load 5-10ug RNA/lane. If you the RNA volume is <10ul, you can use 15ul RNA sample buffer per sample. For RNA vol. >10ul, you may have to use 20ul of RNA buffer).
3. Heat RNA/sample buffer mixture at 65°C for 5 min., then chill tubes on ice until loading.
4. [Optional: Load samples and 2ug RNA ladder on the gel].
5. Run at 90V for 90 min. with 1x MOPS running buffer.

Buffer and Solution:

5x MOPS:

167.5g	MOPS free acid
27.2g	NaOAc-3 H_2O
100ml	0.2M EDTA
pH to 7.0 with NaOH	
Q.S. to 4.0 liters	

NOTE: It's important to keep the stock solution in dark at 4°C. Discard if it turns yellow.

Super RNA sample buffer:

2.0ml	5x MOPS buffer
3.5ml	37% Formaldehyde
10 ml	Formamide
4.0ml	50% glycerol
31 ul	Ethidium Bromide (10mg/ml)

Total volume = 20 ml. Aliquot and freeze @ -80°C