Northern
03/30/06

1. Determine RNA concentration by diluting 1/100 in TE and heating before takeing A260. 260/280 ratio should be around 1.8. Multiply A260 by 4 to get ug/ul of RNA.
2. Run equal ratios of RNA blue juice to sample in every lane and calculate ul of RNA needed to get 10 or 20 ug per lane. (500ml large gel can hold total of 40ul).
3. Rinse everything with RNase Away.
4. Heat samples to 50C for 5 minutes to denature.
5. Run samples on a 1.5% agarose gel.
   *0.015 = grams of agarose
   /10 = ml of 10x MOPS needed
   /50 = ml of formaldehyde needed
   -(amt of 10x MOPS+amt fomaldehyde)= amt of DEPC treated water
   /20 = ul of EtBr
6. Heat agarose, MOPS and water together and then add the formaldehyde and EtBr when cool to the touch.
7. Load samples and run 120-175V.
8. Take picture with ruler.
9. Let blot transfer to Gene Screen membrane overnight.
10. Prehyb blot in 18 ml Prehybe and 2 ml 10% SDS at 42C. Set up 9 ml Hybe and 1 ml 10% SDS in conical tube to preheat.
11. Set up 5 tubes for each probe labeled in the following order: name, name and date, name 1/10, name 1/500 and name.
12. Dilute 2.5 ul cDNA in 45.5 ul TE buffer in tube 1. Denature 5 minutes at 95C.
13. Place on ice 5 minutes.
14. Spin down and add to rediprime tube.
15. Add 2.5 ul of radioactive dCTP and pipette up and down several times to mix.
16. Place in water bath for at least 20 minutes at 37C.
17. Prepare G50 column by vortexing briefly, loosening cap, and snapping off bottom tip. Place in eppie and spin 735 x g for 1 minute.
18. Transfer column to tube 2 and add probe to column. Spin 735 x g for 2 minutes.
19. Discard column. Add 1 ul of probe to tube 3, mix, and add 1 ul of this to tube 4 and mix.
20. Count radioactivity of 1/500 dilution. >1000 split before use, 800<cts<1000 then use whole thing, <800 don't use.
21. Poke hole in tube 5 and denature 30ul of herring sperm DNA with probe (25 ul if split) at 95C for 5 minutes. Save remainder in tube 2 in refrigerator.
22. Add denatured probe to hyb buffer, discardy prehyb, and put hyb mixture in tube at 42C overnight.
23. Wash blots:

2x SSC	2x at 42C
	2x at 50C
	1-2x at 60C
0.2xSSC	1-2x at 60C
0.1xSSC	0-2x at 60C

24. Expose to film overnight in –80C.