Detection of Biotinylated RNA

Either do "dot blot" – pipette biotinylated RNA directly to nylon membrane, followed by UV crosslink as below, or do gel transfer (*in italics below*)

1) Run biotinylated RNA on 0.8 – 1.0% gel in TAE with ethidium bromide.
   a. Run 2 - 5 μg total RNA to detect biotinylation
   b. Run at 200 volts for 15-20 minutes, monitor for heat, can place ice pack under gel box. Larger volume gel box is better for keeping heat low.
   c. Photograph gel to check RNA integrity. Drosophila large rRNA (28S) is heat-sensitive and normally cleaved into two fragments that migrate at same size as small rRNA (18S) – typically see faint large rRNA band and strong small rRNA band

2) Transfer to Nylon membrane
   a. Use Hybond-N+ membrane (Amersham)
   b. Set up transfer according to Northern protocol of choice
   c. Following transfer, UV cross-link at 1200 joules

3) Streptavidin-HRP detection
   d. Prepare the following solutions:
      i. Blocking Soln. 500 ml
         NaCl: 3.65 g
         Na_2HPO_4: 1.20 g
         NaH_2PO_4: 0.5 g
         SDS: 50.0 g
         *need to heat soln. to 37° C for SDS to dissolve
      ii. Wash I: 1:10 dilution of Blocking soln.
      iii. Wash II: 500 ml of <u>10X stock</u>
         Tris: 6.0 g
         NaCl: 2.9 g
         $MgCl_2$: 1.0 g
         pH to 9.5 w/ HCl, use at 1:10 dilution
   e. Block membrane in Blocking Soln,
      i. 30 min.
   f. Incubate with Streptavidin-HRP (Pierce) diluted 1:5000 in Blocking Soln
      i. 5 min.
   g. Wash in Wash I:
      i. 20 min., 2X
   h. Wash in Wash II:
      i. 5 min., 2X
   i. Add ECL reagent for 1 min. and expose to film / measure chemiluminescence on Chemidoc