Northern Blot - Hiroki Kuroda

Sometimes useful to check amount and size of RNA

(RNA extraction with RNA-STAT)

1. Mix with 800 µl of RNA-Stat.
   Note: 10 animal caps or five ventral marginal zone explants provide you almost same amount of total RNA as one whole embryo.
   Note: You can keep sample in -80ºC forever.
2. Add 0.2 vol choloroform and vortex.
3. Centrifuge at maximum speed (e.g. 15k rpm) for 10 min at 4ºC.
4. Recover the supernatant (usually 200 µl) and do another chloroform extraction.
5. Save 180 µl of supernatant and add 180 µl of isopropanol.
6. Mix well and incubate in dry ice until frozen or in -80ºC for 30 min.
   Note: You can store sample in -20ºC forever.
7. Centrifuge at maximum speed for 15 min at 4ºC.
8. Wash the pellet with 70% EtOH and dissolve the pellet into 15 µl of water.
   Note: Use 4 µl for the following room temperature reaction.

(Loading Sample)

Components	20 µl final	Master Mix. (x20)
Total RNA Soln	5 µl	----
0.4 mg/ml EtBr	1 µl	20 µl
20 x MOPS	0.5 µl	10 µl
formaldehyde	3.5 µl	70 µl
formamide	10 µl	200 µl

1. Mix 15 µl of master mix and 5 µl of total RNA solution.
2. Store at 60 ºC for 30 min.
3. Store sample on ice.
4. Add 2 µl of Nuclease free 10 x Loading buffer.
5. Electrophoresis using a formaldehyde gel.

(Blotting)

1. Transfer overnight using the following system (From top to bottom)
   Weight (5g/cm_2)
   Glass plate
   5 cm of Kim Towel
   3 wet paper (11 x 6 cm)
   wet Hybrid N+
   Gel (gel surface-bottom)
   3 wet paper (11 x 20 cm)
   glass, 20 x SSC
2. Write a origin loading point on blotted paper.
3. Check gel and membrane using UV to confirm whether blotting process is fine.
4. Wash membrane with 2 x SSC for 1 min.
5. UV crosslink and dry.

(Probe)

1. Make DNA probe by PCR with & alpha-^{32}P-dCTP
2. Purify with a PCR purification kit.

(20 x MOPS) 250 ml:
20.9 g of MOPS, 3.4 g $CH_3COONa(3H_2O)$, 1.86 g of EDTA(2Na/$2H_20$), pH 7.0

(20 x SSC) 500 ml
87.6 g of NaCl, 50 g of Sodium Citrate ($2H_2O$), pH 7.0

(Gel for Northern Blot)
-Mix 1 g of agarose, 5 ml of 20 x MOPS, and 77 ml of water using microwave
-Store at 60ºC for 30 min.
-Add 18 ml of Formaldehyde

(Hybridization and detection) Note: Use Hybribag for hybridization. 1. Wash the membrane with 5 x SSC (Note. RNA-side-up). 2. Move membrane into hybribag. 3. Add 15 ml pre-hybridization solution, then place the hybri-bag in the hybridization oven and incubate with rotation 6 hr at 42ºC for DNA probe. 4. Pipet the desired volume of probe into hybribag. Note. Double-stranded probe was denatured by heating in a water bath for 10 min at 100ºC, then transferred to ice. 5. Continue to incubate with rotation overnight at 42ºC for DNA probe. 6. Wash membrane twice for 10 min with wash-buffer at room temperature. 7. Wash membrane twice for 15 min with wash-buffer at 65ºC. 8. Remove final wash solution and rinse membrane in 5 x SSC at room temperature. 9. Cover membrane in UV-transparent plastic wrap. 10. Do not allow membrane to dry out if it is to be probed again. 11. Blot was exposed at -80ºC using Kodak XAR film and x-ray intensifying screens.	