RNA BIO-DOT ('SLOT BLOT')

Adapted from existing protocols by Vinh Pham.
Last modified: July 8, 2003

MATERIALS:

DRY
Bio-Rad Bio-Dot SF Microfiltration Apparatus
Nytran membrane
Bio-Dot SF filter paper 60
3mm Whatman paper

SOLUTIONS
10mM EDTA
20X SSC
10X SSC
37% Formaldehyde
dH_2O

PROCEDURE:

Part I – Sample Extraction

1. Extract total RNA by standard methods.
2. Measure [RNA] by spectrophotometry at 260nm. One A_{260} unit equals 40μg/ml.
3. Store at -80ºC until needed.

Part II – Sample Preparation

1. Set water bath to 60ºC.
2. Resuspend 5-100μg of total RNA in 1mM EDTA, pH 8.0 to a final volume of 50μl. The amount of RNA used depends on the abundance of the message. A dilution series may be required to determine the optimal amount of RNA for the experiment.

   Positive control: Use a dilution series of chromosomal DNA to serve as both a positive control and as a way to determine when the signal intensity as detected by the STORM machine is in the linear range.

   Negative control: Use 50μg of yeast RNA.

3. Add 30μl 20X SSC.
4. Add 20μl 37% Formaldehyde.
5. Incubate at 60ºC for 30'.
6. Keep on ice until needed.

Part III – Apparatus Preparation

1. While samples are incubating, heat 100ml water for 60" and pour through apparatus slots while on vacuum. This cleans the apparatus.
2. (Optional) Rinse with ethanol and allow to air dry.

Part IV – Slot Blotting

1. Cut Nytran (charged nylon) membrane and 3 pieces of Whatman paper (use more if bleeding of samples is a problem) to size of Bio-Dot SF filter paper 60.
2. Soak membrane in dH_2O (if using nitrocellulose membranes, soak in 6X SSC).
3. Transfer to 10X SSC.
4. Touch membrane on dry filter paper to wick off excess liquid.
5. Place membrane on 1 piece of Bio-Dot SF filter paper 60.
6. Place into Bio-Dot SF apparatus:

TOP	Sample template with screws
	Nitrocellulose membrane
	Filter paper 60
	Whatman paper
	Whatman paper
	Whatman paper
	Gasket
	Gasket support plate
BOTTOM	Vacuum manifold

7. Press out bubbles with glass pipet.
8. Turn on vacuum and tighten screws in diagonal pattern.
9. Apply atmosphere pressure. A good seal is quiet.
10. Rehydrate membrane with ~200 µl 10X SSC per well.
11. Apply vacuum/atmosphere pressure.
12. Repeat rehydration with ~200 µl 10X SSC per well.
13. Apply samples while maintaining vacuum/atmosphere pressure to avoid bleeding.
14. Wash wells with ~200 µl 10X SSC per well.
15. Apply vacuum/atmosphere pressure.
16. Remove membrane (remove sample template while vacuum/atmosphere pressure is applied, turn off vacuum, remove membrane).
17. Air dry membrane.
18. UV crosslink for 3'.