NORTHERN BLOTTING GEL TRANSFER AND HYBRIDIZATION

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I-Buffer preparation:

1. 50mM NaOH/10mM NaCl:
   1N NaOH 25ml
   5M NaCl 1ml

   Q.S. to 500ml
2. 10x Sodium Phosphate Buffer:
   Na_2HPO_4 160.8gm
   NaH_2PO_4 55.2gm

   Q.S. to 4 L.

II-Pretreatment of gel:

-----Wash with ddH_2O x 5-10min.
----- Wash with 50mM NaOH/10mM NaCl x 20-30min.
----- Wash with 1x Sodium Phosphate Buffer x 10-20min.

III-Gel Transfer:

----- cut proper size of filter paper, membrane (Immobilon-Ny, Millipore) and paper towels.
----- Set up transfer device (from bottom to top):
glass plate> bridge filter paper (two pieces) > filter paper(two pieces) > gel (face down) > membrane > filter paper(two pieces) > paper towels > glass plate > weight.
----- Transfer in 1x Sodium Phosphate Buffer for 24hrs.
----- Remove paper towels and the gel (**you can check on UV box for the presence of residual RNA**). Mark the top of each lane position on the membrane (**NOTE**: **Always handle the membrane with tweezers or forceps thereafter**).
----- Wash the membrane with 1x Sodium Phosphate Buffer, let air dry briefly on a piece of filter paper (**RNA side facing up**).
----- Crosslink at energy = 2000uj/cm2 for 30 seconds (UVP CL-1000) with RNA side facing up (**NOTE:** You'd better record an image of the RNA gel under UV. But you need to have the RNA side facing down this time!).

IV-Prehybridization, Hybridization and Washes:

----- Put membrane into a plastic bag (**using forceps or tweezers**).
----- Add 8-10ml pre-warmed QuikHyb (**prewarm at 68°C, & shake well**) into the bag (**make sure the membrane is all wet and well soaked, of course, no bubbles**).
----- Seal the bag after expelling the bubbles.
----- Prehybridize at 68°C for 20-30min.
----- Add labeled probe directly into the prehyb bag. Carefully seal the outmost opening of the bag, then expel bubbles, and seal close to the edge of the blot membrane.
------Mix well by massaging the bag (**also check any leaks**).

----- Hybridize at 68°C for 1-2 hrs.
----- Wash with 0.3x SSC/0.1% SDS at 65°C for 5-30min (**Note: Monitor the retained radioactivity every 5-10min**).
----- Wash twice with 0.1x SSC/0.1% SDS at 65°C briefly (Again, **monitor the retained radioactivity every 5-10min**).

Wrap the membrane, and expose to the x-ray film or a phospho Imager.