Revised N.B. protocol --- Use of Hybridization Oven

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Buffer preparation:

50mM NaOH/10mM NaCl:

1N NaOH	25ml
5M NaCl	1ml
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Q.S. to	500ml

10x Sodium Phosphate Buffer:

Na_2HPO_4	160.8gm
NaH_2PO_4	55.2gm
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Q.S. to	4 L.

Pretreatment of gel:

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

Transfer:

----- Set up transfer device (from bottom to top):
glass > bridge filter paper (two pieces) > filter paper (two pieces) > gel (face down)
> membrane > filter paper (two pieces) > paper towels > glass > heavy thing
----- Transfer in 1x Sodium Phosphate Buffer for 24 hr.
----- Harvest the membrane, mark lane position on the top of it
----- Wash the membrane with 1x Sodium Phosphate Buffer, let air dry briefly
----- Autolink (1200uJ/cm^2) in Stratalink with RNA side facing up

Prehybridization, Hybridization and Washes:

----- Turn on the hybridization oven. Set up the temperature by press the “menu” and the “Δ” or “∇” button
----- Put the membranes into hybridization tubes

----- Add 8-12ml pre-warmed (at 65^0C) QuikHyb (Stratagene, shake well!) into the tubes, according to the tube's size
----- Prehyb at 68°C for 20min, or longer
----- Add labeled probe directly into the tubes and mix well by shaking
----- Hyb at 68°C for 1-2 hrs.
----- Wash with 0.3x SSC/0.1% SDS at 65°C for 5-40min in water bath
----- Wash twice with 0.1x SSC/0.1% SDS at 65°C briefly

Wrap the membrane and expose film or expose to a phosphoimager screen.