

Hybridization of Northern Blot Probes Using UltraHybe (Ambion)

Prewarm UltraHybe Solution at 50-60°C for 20 minutes (or until it becomes fluid)

Add 50mL UltraHybe solution to your membrane (best done in a Tupperware container, if your blot is too large to fit in a small Tupperware, use a larger container and 100mL. You want to be sure that the UltraHybe covers the entire blot. You can attempt to use a blot bag, but it's extremely messy)

Block membrane in UltraHybe for 1 hr shaking at 60°C

After 1 hr, pipette UltraHybe into a 50 mL conical tube

Add 15 uL of your radio-labeled oligo prep

Pour UltraHybe solution back on blot and probe overnight shaking and at 60°C

(I'm pretty sure you can probe for 2 hrs, but I always do mine overnight.)

Remove the UltraHybe/Probe solution

Wash 2x for 5 minutes each with 2x SSC 0.1% SDS

Wash 2x for 5 minutes each with 0.1x SSC 0.1% SDS

Detect with an instant imager or expose to film

20X SSC

3M NaCl

0.3M Sodium Citrate

Dissolve the following in 800ml of distilled H₂O.

175.3g of NaCl

88.2g of sodium citrate

Adjust the pH to 7.0 with a few drops of 1M HCl.

Adjust the volume to 1L with additional distilled H₂O.