Hybridization of Northern Blot Probes

20 mL Prehybe/Hybe
10 mL formamide
5 mL 20x SSPE
3.8 H2O
1 mL Denhardts

Northern Blot Wash Buffer		
20x SPEE	50	mL
10% SDS	50	mL
H2O	900	mL

**Heat the prehybe and hybe to 42 C in the gyro water bath prior to the addition of SSDNA or Probe
**Heat the salmon sperm to 75 C before addition to the prehybe and hybe solutions

Prepare a plastic prehyb/hybe bag by sealing the bottom of a plastic sleeve
Place thenorthern blot in the center of the closed sleeve and seal two sides of the bag
Aliquot 10 mL of the preheated prehybe solution into a 15 mL conical
Add 85 uL of boiled salmon sperm to the prehybe
Add the 10 mL prehybe solution to the plastic bag
Squeeze out any **LARGE** (bigger than a dime) air bubbles
Seal the final side but leave about 2 cm so it can be opened and resealed after the addition of the hybe solution
Incubate the nylon membrane in the prehybe solution for 3 hr in the 42 C Gyro water bath. Place orange lead weight on top of the bag, but not directly on the membrane blot. Be sure that the bag is completely submerged.
Heat the anti-7SK in vitro reaction to 75 C and add 25 uL (half) to the hybe solution.
Heat the salmon sperm to 75 C and add 25 uL of it to the hybe solution
Cut a corner off of the bag and squeeze out the prehybe solution
Add the remaining 10 mLs of hybe solution (containing the radio probe and the salmon sperm) to the bag. It may help to squeeze out all of the air bubbles and only insert a small portion of the pipette tip during the addition of the hybe solution. This will help prevent the formation of large air bubbles.
Incubate the nylon membrane in the hybe solution for 24 hr (or overnight) in the gyro water bath at 42 C. Submerge the blot bag.
Remove the hybe bag and place it on top of saran wrap on the bech top
Cut open the bag and carefully remove the nylon membrane and place it in a Tupperware container with 200 mLs wash buffer (1x SPEE and 0.5% SDS)
Throw the bag containing the radioactive hybe solution into the radioactive waste container
Wash the membrane for 15 minutes in the gyro water bath with the lead weight on top of the Tupperware container
Place the washed membrane in saran wrap and fold over the edges to prevent leakage of radioactive buffer
Visualize membrane with the instant imager
Repeat the wash steps 3 times and take an image each time

If the blot is overly fuzzy after the second was, increase the gyro bath temperature to 55 C
Take saran wrapped blot into the dark room, place in a film cassette with the radioactive side facing the film, and incubate in the -80 freezer for 3 hours