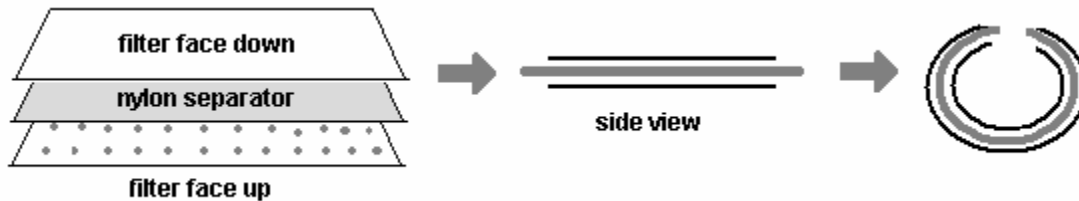


## HYBRIDIZATION WITH TWO FILTERS IN ONE BOTTLE

- 1) Heat 0.5% SDS to ~90°C. Prewash filters by immersion in solution, and agitate at room temperature for 5 min.
- 2) Place one filter face-up, then a nylon separator on top of the filter, and finally, the second filter facedown. The cDNA sides of the filters should be facing each other.
- 3) Roll this filter “sandwich” as shown below, and place carefully into a 35mm x 100mm hybridization bottle. Do not worry if the edges of the separator overlap each other; there should be minimal overlap of the filter edges, if possible.



- 4) Add **4 ml** of **prehyb solution 1** to each bottle; incubate for 1 hr at 42°C, with a rotation speed of 7-10 rpm.
- 5) Remove prehyb solution 1 and replace with **4 ml** of **prehyb solution 2**. Incubate for 2 hr at 42°C, same speed.
- 6) <sup>33</sup>P(α-dCTP)-labeled probes may be added directly to the second pre-hybridizing solution. They must be denatured first by boiling for 3 min. Hybridization time is 18 hours with rotation.

### ***Prehyb solution 1 (per sample):***

4 mL MicroHyb Buffer

4 µl Salmon Sperm (final concentration 1ug/mL)- denatured- boiled 3'.

### ***Prehyb solution 2 (per sample):***

4 mL MicroHyb Buffer

Poly dA (final concentration 1ug/mL)

Cot-1 DNA (final concentration 1ug/mL)- denatured- boiled 3'.

### REAGENTS:

Invitrogen Micro-Hyb solution (Cat # HYB250.GF)

Mouse Cot-1: (BRL- Lifetech cat # 18440-016)

Human Cot-1: Cell Center (BRL Lifetech cat # 15279-011). Make sure that Cot-1 DNA and Salmon Sperm have been denatured by boiling for 3min. before adding to prehyb buffer.

## WASHING FILTERS IN BATCH

- 1) ***Preliminary wash:*** Remove each filter and rinse in small Tupperware container with 2x SSC/1% SDS at room temperature.
- 2) Transfer filter to larger plastic container with 2X SSC/1% SDS, also at room temperature, and set up on a tilt table. When all filters have been transferred to larger room temp wash, remove room-temperature solution and add 2X SSC/1% SDS preheated to 50°C (use ~40 ml per filter, 1.5 L max volume in 2.8L Rubbermaid container).
- 3) Wash filters for 30 min. at 50°C in a shaking water bath (keep covered to maintain constant temperature). Speed of shaker should be fast enough that solution moves well to ensure good wash, but not so quickly that filters stick to the sides of the container. In a PRECISION Reciprocal Shaking Bath, optimal speed is 55 rpm.
- 4) Remove solution; repeat step 3.
- 5) Replace 2X/1% solution with 0.5X SSC/1% SDS solution preheated to 55°C. Wash filters for 30 min. at 55°C. Remove solution. If total RNA was used in hybridization, continue to wrapping step. If aRNA was used, continue to step 6.
- 6) Remove 0.5X/1% solution and add 0.1XSSC/0.5%SDS. Incubate in shaking bath at 55C for 20 minutes.

***\*\*Do not let filters dry out during any of the steps, or radioactivity will remain permanently on filter!***