Hybridization of RNA Blots

Reference:

- Hybridization buffer for RNA from the GeneScreen *Plus* manual.

Materials:

- 2 X SSC (Need: 50 mls per blot)
- Prehybridization buffer, pre-warmed to 42°C. (Need: 50 µl Prehybridization buffer per cm^2 of membrane)
  5 X SSPE
  50 % (v/v) deionized formamide
  5 X Denhardt's Solution
  1 % SDS

- Hybridization buffer, pre-warmed to 42°C. (Need: 50 µl hybridization buffer per cm^2 of membrane)
  5 X SSPE
  50 % (v/v) deionized formamide
  5 X Denhardt's Solution
  1 % SDS
  100 µg/ ml Salmon sperm DNA

- ^{32}P-labeled probe
- 2 X SSPE wash solution (Need 100 mls per blot)
- 2 X SSPE, 2 % SDS wash solution, pre-warmed to 65°C. (Need: 50 mls per blot)
- Saran wrap

Procedure:

1. Place the membrane into a hybridization bottle.
2. Wet the membrane in 50 mls of 2 X SSC.
3. Prehybridize the membrane in 50 µl pre-warmed prehybridization buffer per cm^2 of membrane at 42°C for 2 - 4 hours.
4. Remove the prehybridization buffer.
   Note: The prehybridization buffer and the hybridization buffer contain formamide. They must be collected into the "used formamide-containing hybridization buffer" container.
5. Add 50 µl pre-warmed hybridization buffer per cm^2 of membrane.
6. To a 1.5 ml microcentrifuge tube add:
   - 1 ml of hybridization solution.
   - 5 X 10^5 CPM of the ^{32}P-labeled probe for every ml hybridization buffer added to the hybridization bottle in Step 5.

Seal the lid of the microcentrifuge tube with a remote handling device (Phenix Research Products). Boil the hybridization buffer and probe in the temperature block for 5 minutes.

7. Add the denatured ^{32}P-labeled probe to the hybridization reaction.
8. Hybridize 16-24 hours at 42°C with gentle agitation.
9. Remove the hybridization buffer.
10. Wash the membrane in 50 mls 2 X SSPE at room temperature for 15 minutes.
11. Repeat Step 10.
12. Wash the membrane in 50 mls 2 X SSPE, 2 % SDS at 65°C for 15 minutes.
13. Wrap the membrane in Saran Wrap.
    Note: Do not allow the membrane to dry out after this point or the probe will become irreversibly bound to the blot.
14. The membrane is now ready to be PhosphorImaged or autoradiographed.