HYBRID SELECTION

Hybrid selection is one step in proving the identity of a clone. The DNA clone will "select" (anneal) its complementary mRNA from the total messenger population. The DNA is usually immobilized on a nitrocellulose filter and hybridized to the mRNA in solution. The filter is then washed and the mRNA released by methylmercury, precipitated and translated in a cell-free translation system. With slight modification, this procedure can also be used for Hybrid Arrest Translation. In this case the RNA which does not anneal to the DNA clone is precipitated (multiple times to remove the formamide) and translated in the cell-free translation system. The protein coded by the clone of interest will be missing from the translation products.

Materials:

Nitrocellulose
Blotting manifold
4x SSC
2x Denhart's solution
2 M NH_4OAc, 1 M NH_4OAc
10 M NaOH

For the hybridization buffer:
- Formamide
- Pipes
- NaCl
- SDS
- tRNA
- EDTA

NET: 0.1 M NaCl
1m M EDTA
10 mM Tris pH7.4

NETS: NET + 0.5% SDS

Preparation of DNA: (Modification of Kafatos et al., (1979) Nuc. Acid Res. 7: 1541-1551)

Prepare as for cold DNA probes for Nuclei Run off transcription--use l µg DNA/filter

1. Linearize DNA, extract, EtOH ppt, resuspend in TE to 750 µl.

or

2. Sonicate DNA in a total vol. of 750 µl setting "2" for 15 seconds-- use vector DNA as negative control.
3. Add 30 µl of 10 N NaOH; chill to denature (final 0.3-0.4 N NaOH).
4. Dilute 1:1 with 2 M NH_4OAc.
5. Filter onto water-soaked nitrocellulose.

6. Wash w/1M NH_4OAc.
7. Swirl NC in 4x SSC. Air dry.
8. Swirl in 2x Denhardts, air dry.
9. Bake at 80°C under vacuum for 2 hours.

Pretreatment of filters before hybridization:

1. Wash excised dots
2. Place filters of like DNA against the side of sterile eppendorf tube.
3. Add 1 ml sterile dH_2O, boil 1 min. discard water.
4. Add 1 ml sterile dH_2O, vortex, discard dH_2O.
5. Plate each filter in tube in which hybridization is to be done.

Hybridization:

I. Hybridization Solution: Use about 3x the RNA that you normally use for in vitrotranslation. Spin down NA into eppendorf. Make sure RNA gets resuspended well in Hybridization buffer.

I find that the best way to do this is to make up the hybridization buffer without the water. Resuspend the RNA pellet in the water, then add the remaining components.

65% Formamide
40mM PIPES pH6.4
0.4M NaCl
0.2% SDS
100-150µg/ml tRNA
2mM EDTA

1. Heat to 70°C to denature RNA ~10 min.
2. Add 80µl to each filter in tube.
3. Incubate 53°C 3 hours - O/N.

II. Wash filters
1. Heat NET and NETS to 65°C.
2. Remove hybridization solin.
3. Wash 10x w/NETS (add buffer, vortex, remove).
4. Wash 3x w/NET.

Elution of RNA from filter:

1. To each filter add 80µl 10 mM CH_3HgOH containing 10 mg tRNA.

CAUTION: MeHg is highly toxic and volatile. USE IN HOOD

and dispose of toxic waste properly. MeHg can be neutralized with sulphydral compound.

2. Incubate 15 min at 20°C.
3. Vortex gently, remove RNA-containing supernatant.
4. Add 8 µl of 220 mM DTT sulphydral compound.
5. EtOH (2 vols.)

Prepare for translation:

1. Spindown.
2. Wash 2x w/70% EtOH ~dry.
3. Resuspend in vol. for 2 translations.

References:

Goldberg, et. al, (1979) Meth. Enzymol. 68: 206
(Ricciardi, R.P. et al., (1979) PNAS 76: 4927-4931)