Colony Hybridization

- Label 10 cm diameter Millipore HATF nitrocellulose membranes using a marker pen.
- Place the membrane DNA side down on the agar plates (including background negative plate) and allow it to become completely wet. Using a hypodermic needle, poke 5 holes around the circumference of the membrane in an assymetric pattern. Turn the plate over and mark the holes on the plate bottom using a felt pen.
- Place the membrane colony side up on a piece of whatman 3MM paper soaked with denaturing solution. Set a timer for 5 min.
- Carefully transfer to whatman soaked with neutralization solution, wait 5 minutes.
- Carefully transfer to whatman soaked with 2X SSC for 5 min.
- Stratalink lifts (one pulse)
- Place membranes in a hybridization bag. Add pre-hyb solution (20 ml, in which denatured (10 @ 95C) sheared salmon sperm DNA (ssDNA) is added to a final conc. of 100 ug/ml. Seal bag and incubate at 42 oC while preparing the probe.

PROBE PREPARATION

Thaw the Prime it -II (Stratagene) kit components. Thaw the P32 dCTP. Get the heat block to 95oC.Melt the insert gel slice at 70oC.

2-3 ul insert gel
10 ul random primers
QH20 to a volume of 23 ul.
Boil for 10 minutes. Place the tube at 37oC.
Add 10 ul 5X dCTP buffer, add 5 ul P32 dCTP and making sure the tube has cooled add the klenow 1 ul.
Fick mix incubate at 37oC for 20-30 minutes.
Add. 2 ul stop solution and 20 ul STE.
Wet a nunc push column with 70 ul STE
Add probe to the column push thru and collect
Add 70 ul STE push and collect
push air to get the rest of probe.
**caution always hold on to the tube and the column tightly so you don't have a radioactive spill. Work behind shielding!

- Squeeze out pre-hyb. Add 10 ml hyb solution.
- Boil probe(either ½ or all of the label rxn*** save a little-2 ul to use in orienting at the end**) with ss DNA.
- Add to the hyb cocktail in the bag. Away from the membrane. Never pipet anything directly on the membrane.
- Incubate at 42oC 3 hours or O/N.
- Drain hyb into radioactive waste. Wash membrane in 2X SSC RT 2 x 10 min.
- Wash in 2X SSC 15 minutes x 2 at 55oC
- Evaluate w/ the geiger counter.
- Arrange on an old film orienting with hot filter paper spots (the 2 ul probe mixed with 50 ul QH20 and some blue gel loading dye) taped to the old film.

SOLUTIONS

Pre-Hyb

6X SSC
0.5% SDS
50% Formamide
5X Denhardt's

For 100 ml:
30 ml 20X SSC
5 ml 10% SDS
50 ml deionized formamide (aliquoted at -20 walk-in)
10 ml 50X denhardt's (in -20)
QH20 to 100 ml. Store at 4oC.***Note due to the high salt in the presence of SDS the SDS drops out of solution in the cold simply prewarm to 42oC before use.

Hyb Cocktail

For 100 ml:
30 ml 20X SSC
5 ml 10% SDS
50 ml deionized formamide
QH20 to 100 ml. Store at 4oC

ssDNA
ss DNA from testes(Sigma) Solution made at 10mg/ml in QH20 and sheared thru a syringe with a 20 gauge needle. Aliquoted and frozen at -20.

STE/SSC/Denhard'ts/neutralization solution/denaturing solution/---see lab reagent prep list.