

Dot Blot Protocol

1. Denaturing of Samples

8 DNA
 8 dH2O
4 1N NaOH (final 200mM)
 20

- Room Temp 10 minutes (not over)
- Add 8 5M NaOAc- finger mix well
- Add 800 of 6X SSC

Stock 20X SSC 30mls
 DH2O 70mls
 100mls

2. Preparing Nitrocellulose Membrane

- Cut 2 membranes 9X12 cm with new razor - mark edge with # and date
- Wet in dH2O for 5 min. slide in on edge
- Soak in 6X SSC for 10 min.

3. Dot Blot

- set up apparatus - see procedure sheets
- wash each lane with 100 of 6X SSC
- apply sample 100
- wash with 150 of 6X SSC
- remove blot, dry -> crosslink C-L program

4. Prehyb - 30 min

LONGER OLIGO'S	Stock	25mls
6X SSC	20X SSC	7.5 mls
5X Denhardt's	50X Denh.	2.5 mls
0.05% NaPyrophosphate	10% NaPyr	125
100 g/ml boiled sal sp DNA	10mg/ml	250
0.5% SDS	10% SDS	1.25 mls
dH2O	dH2O	8.5 mls

ssperm DNA - 600 100mg/ml -> boil 5 min then quench on ice

TMAC SHORT OLIGO'S

use hyb juice for 1-2 hours, do not change after prehyb

5. Kinase - do this reaction while prehybing

HOT HOT HOT HOT - do in hood

1 diluted oligo (do for G and A)
 2 10X buffer
 1 P - ATP
 15 dH₂O - clean
1 T4 PNKinase
 20

45-60 minutes 37 C
 RT 5 minutes
 column purify
 70 STE
 sample + 50 STE
 70 STE

6. Hyb - do overnight

LONGER OLIGO'S	Stock	30 mls
6X SSC	20X SSC	9 mls
100 g/ml ssDNA	10mg/ml	300
0.05% NaPyrophosphate	10%	150
dH ₂ O	dH ₂ O	20.55 mls
SHORTER OLIGO'S	Stock	30 mls
3M TMAC	6M TMAC	15 MLS
0.1M NAPO ₄ pH 6.8	1.4mls Na ₂ PO ₄ 1M / 1.6mls NaH ₂ PO ₄ 1M	
1mM EDTA pH 8.0	0.5M EDTA	60
5X Denhardt's	50X Den	3 mls
0.6% SDS	10% SDS	1.8 mls
100 g/ml denat ssDNA	10 mg/ml	300
dH ₂ O	dH ₂ O	6.84mls