DNA-RNA hybridization. -
Gillespie & Spiegelman J Mol Biol. 12, 829, 1965.

1. Immob. of DNA
B-D S&S filters, coarse.
Denat of DNA: alkali to pH 13. @ 10' neutralize

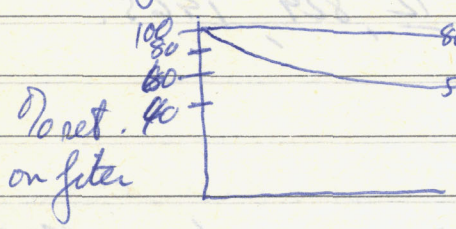
DNA (denat) dil. to 5 ml ē 2X SSC & passed thru mem. filter (pre-soaked in 2X SSC for 1' & washed ē 10 ml of 2X SSC), washed ē 100 ml 2X SSC. Filters dried at room temp. for at least 4 hrs & at 80° for 2 more hrs in a vacuum oven. Filtration must be slow, esp ē DNA fragments.

2. Hybridization
DNA filters immersed in scint. vial in 5 ml ^{32}P-RNA in either 2X SSC or 6X SSC. 66° ō shaking then chilled in ice bath. Vol. can be ↓ to 0.5 ml by using small tubes & rolling up the filter.

3. RNase
Filter removed & each side washed ē 50 ml 2X SSC by suction filtration. Filter immersed in 5 ml 2X SSC ē 20 µg/ml boiled RNase for 1 hr. at rm temp. Chilled & filters re-washed on each side.
Filters dried & counted.
RNAse step must be done in 2X SSC, not 6X.

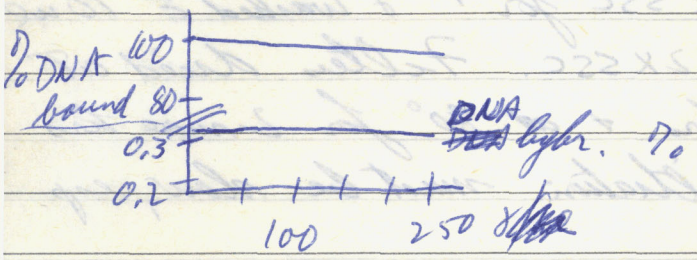
Mg^{++} ↑ non-sp. binding
RNase treatment in sol'n ā complexing to filters → ↑ noise level, prob due to RNA bound to RNase sticking on filter.

Size of DNA vs. retention –

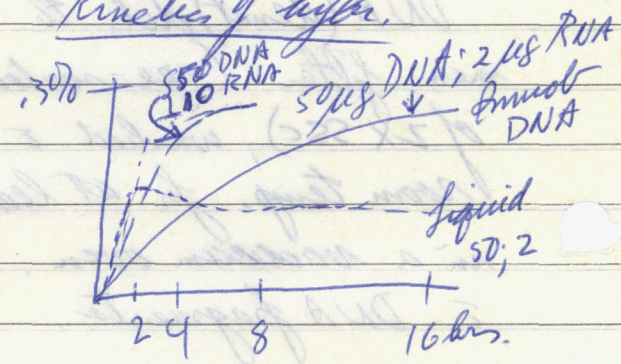


Each filter carried thru wash procedure; no loss with any size class.

Capacity of filter for DNA



Kinetics of layer.



These exp use mix. RNA + E. coli DNA.

i.e. ↑ RNA → ↑ rate of achieving plateau value
Amt DNA on filter has little effect on rate.

Precautions:

Basic proteins → high blanks (incl methylated alb.)
Remove contam RNase in DNA prep. – MAK a pronase
Remove DNA fr. RNA preps.
Destroy DNase in RNase – 10' at 90°C pH 5
Remove RNase in DNase – DEAE cell. (Analyt Biochem 2, 161, 1961)
RNase treatment: > 0.25 M salt + < 37°.
10⁸/ml, 30° 0.3 M NaCl – good.

RNA polymerase purification

C. Babinet BBRC 26, 639, 1967

E. coli A19

Assay: Furth et al JBC 237, 2611, 1962
except only Mn^{++} added. 10' at 37° then add 4 ml cold 5% TCA (to 250 µL reac. vol.) Collect ppt on millipore HA 25 0.45µ, wash ē 5 ml cold 5% TCA, then 5 ml cold 0.5% TCA.

250 g frozen A19 ground for 20' ē 500 g alumina. Extr. ē 1 L buffer I (0.01 M Tris Cl 8.4, 0.005 M ME, 0.01 M $MgCl_2$, 0.001 M EDTA) + cent for 30' at 9000 rpm in Sorvall. Sup = Crude extr.

Crude extr. (920 ml)
+ PEG 6000 30% w/w 296 ml
+ Dextran 500 20% w/w 106 ml
} Phase system I.

30' stirring at 4°
Cent. 10' at 14000 g

upper PEG I discard

lower 160 ml D_I
+ 157 ml PEG 30% w/w
+ 383 ml buffer I
+ 82.5 g NaCl (→ 2M final)
} Ph. sys. II

30' stirring at 4°
Cent 10' at 14,000 g

upper PEG II discard

lower, D_2 160 ml
+ 157 ml PEG 30% w/w
+ 383 ml Buffer I
+ 146 g NaCl (→ 4M)
} Ph. sys III

45' stirring at 4°
Cent 10' 14000 g
→ D_3, discard

PEG 3 530 ml

dial. for 60', ag. 3 changes of 2L of buffer I

[Note: need to stir contents of bag to avoid ppt; use rocker]

Dialyzed PEG 3 697 ml
+ 114 g solid AS
5' cent at 9000 rpm in Sorvall SW 18-4
upper PEG discard
lower phase, 600 ml
+ 42 g solid AS
centrifuge
ppt. → super discard
diss in ↓ 40 ml Buffer II (0.01 M Tris Cl 8.4; 0.005 M ME; 0.001 M EDTA)
AS I (80% of initial activity)

DEAE sephadex 18 cm x 5 cm^2 : equil ~ Buffer II
AS I, dial. for 60' ag. 2 changes of 4 L of buffer II &
dil to 120 ml ~ buffer II to give final prot conc. of 5 mg/ml.
Ads. to column at rate of 1 ml/min. Col. washed ~
50 ml buffer II & eluted stepwise:
a. Buffer II + cont. 0.25 M KCl, 50 ml fx
collected until A_{280} < 0.05/ml.
b. Buffer II cont. 0.33 M KCl til A_{280} < 0.05
c. Buffer II cont. 0.40 M KCl elutes activity.
Active fx combined (150 ml) & enz. ppted ~ 44 g solid
AS. ppt diss in 35 ml buffer cont. 0.05 M Tris 8.0,
0.005 M ME, 0.001 M EDTA. This fx (AS2) has
57% of original activity. (90 mg pure enz fr 250 g bacteria)
Sp activ = 1400 U/mg

Stored in liquid N_2.