

# DNA-RNA hybridization -

Gillegie & Spiegelman J Mol Biol. 12, 829, 1965.

## 1. Immobilization of DNA

B-D S+S filters, coarse

Denat of DNA: alkali to pH 13.  $\bar{p}$  10' neutralize

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

## 2. Hybridization

DNA filter immersed in sol'n. vol in 5 ml  $^{32}P$  RNA in either 2X SSC or 6X SSC. 66° 5 shaking then chilled in ice bath. Vol. can be  $\bar{c}$  0.5 ml by using small tubes + rolling up the filter.

## 3. RNase

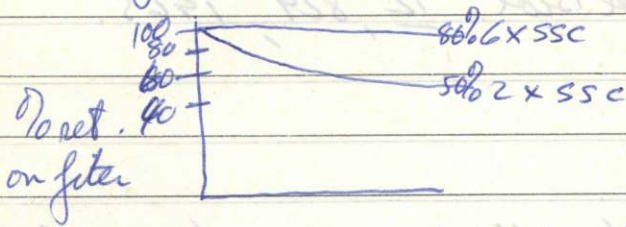
Filter removed + each side washed  $\bar{c}$  50 ml 2X SSC by suction filtration. Filter immersed in 5 ml 2X SSC  $\bar{c}$  200  $\mu$ g/ml <sup>boiled</sup> RNase for 1 hr. at room temp. Chilled + filters re-washed  $\bar{c}$  on each side. Filter dried + counted.

RNase step must be done in 2X SSC, not 6X.

$Mg^{++}$   $\bar{c}$  non-sp. binding

RNase treatment in sol'n  $\bar{c}$  complexing to filters  $\bar{c}$   $\bar{c}$  noise level, prob due to RNA bound to RNase sticking on filter.

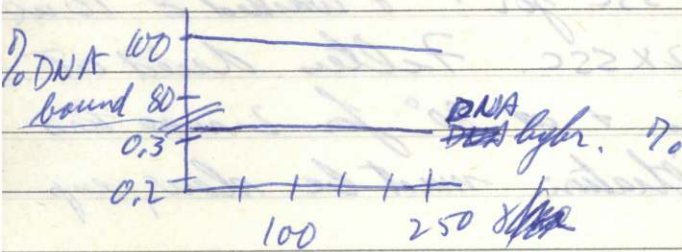
## Size of DNA vs. retention -



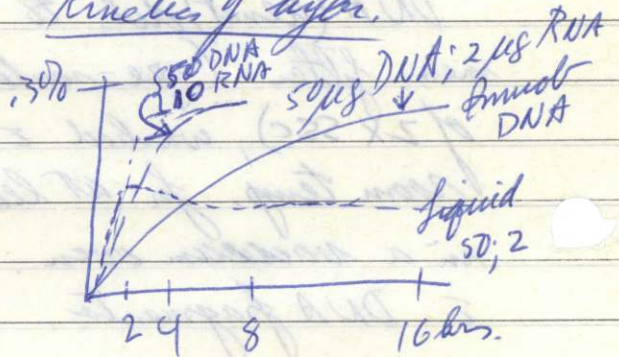
Each filter carried thru wash procedure; no loss with any size class.

mer ge. ft of denat DNA

## Capacity of filter for DNA



## Kinetics of hydr.



These exp use ml. 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 contamin RNase in DNA prep. - MAK a pronase

Remove DNA fr. RNA prep'ns.

Destroy DNase in RNase - 10' at 90°C pH 5

Remove RNase in DNase - DEAE cell. (Analyst Biochem 2, 161, 1961)

RNase treatment: > 0.25 M salt + < 37°

10x/ml, 30° 0.3 M NaCl - good.

# RNA polymerase purification

C. Balinet 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  $\mu$ l reac. vol.) Collect ppt on millipore HA 25 0.45  $\mu$ , wash  $\bar{c}$  5 ml cold 5% TCA, then 5 ml cold 0.5% TCA.

250 g frozen A19 ground for 20'  $\bar{c}$  500 g alumina.  
Extr.  $\bar{c}$  1 L buffer I (0.01 M TrisCl 8.4, 0.005 M ME, 0.01 M  $MgCl_2$ , 0.001 M EDTA) + cent for 30' at 9000 rpm in servall. Sup = Crude extr.

Crude extr. (920 ml)

+ PEG 6000 30% w/w 296 ml } Phase system I  
+ Dextran 500 20% w/w 106 ml }

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

upper  
PEG I  
discard

lower 160 ml D<sub>1</sub>  
+ 157 ml PEG 30% w/w  
+ 383 ml buffer I  
+ 82.5 g NaCl ( $\rightarrow$  2M final)

Ph. sys. II

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

upper  
PEG II  
discard

lower, D<sub>2</sub> 160 ml  
+ 157 ml PEG 30% w/w  
+ 383 ml Buffer I  
+ 146 g NaCl ( $\rightarrow$  4M)

Ph. sys III

45' stirring at 4°  
Cent 10' 14000g  $\rightarrow$  D<sub>3</sub>, 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 Swell SW HB-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)

ASI (80% of initial activity)

DEAE sephadex 18 cm x 5 cm<sup>2</sup>: equil in Buffer II

ASI, dial. for 60' ag. 2 changes of 4L of buffer II &  
dil to 120 ml in buffer II to give final prot conc. of 5 mg/ml.

Ads. to column at rate of 1 ml/min. Col. washed in  
50 ml buffer II & eluted stepwise:

a. Buffer II cont. 0.25 M KCl, 50 ml fr  
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 fr combined (150 ml) & evap. ppted in 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 fr (AS2) has

57% of original activity. (90 mg pure enz fr 250 g bacteria)

S<sub>0</sub> activ = 1400 U/mg

Stored in liquid N<sub>2</sub>.