

(A) checked for size on agarose gel  
not too large pieces  $\approx 3.8$  Kb. size.

(B) checked for glucosyl residues by  
con A sepharose column chromatography.  
binds well at pH 6.5-  
is eluted off at pH 8.2.

(C) calculated that there are approx.  
3

Nick Translation of Ad-2 and  $\lambda$ Fel V DNAs 6.24.82  
with malto-triose d UTP

Ad-2 DNA 1  $\mu$ g/ $\mu$ l From Henry.  
 $\lambda$  Fel-V DNA 1  $\mu$ g/ $\mu$ l " "  
 malto-triose d UTP 0.3 mM From Stan.

	A	B	C	D
$^3$ H d ATP	12 $\lambda$	12 $\lambda$	12 $\lambda$	12 $\lambda$
10 x NICK T. buffer.	12.5	12.5	12.5	12.5
d NTP mix	12.5	12.5	12.5	12.5
TTP	12.5	-	12.5	-
M.T d UTP	-	12.5	-	12.5
Ad-2	12 $\lambda$	14 $\lambda$	-	-
$\lambda$ Fel V	-	-	50 $\lambda$	50 $\lambda$
DNase 0.1 $\mu$ g/ $\mu$ l	10 $\lambda$	10 $\lambda$	10 $\lambda$	10 $\lambda$
DNA-P	10 $\lambda$ ( <del>30 <math>\mu</math></del> )	10 $\lambda$	10 $\lambda$	10 $\lambda$ (30 $\mu$ )
d. H <sub>2</sub> O	55.5	53.5	17.5	17.5

14<sup>o</sup> 2 hrs.

2  $\mu$ l aliquots at 30' intervals for end for checking incorporation.

terminated with 10  $\mu$ l of 0.5 M EDTA.

Dollie  
6/24/82

1 68  
010.00  
015.00  
000020.20  
1 69  
010.00  
001.50  
003071.30  
1 70  
010.00  
003.00  
002911.30  
1 71  
010.00  
001.90  
006771.30  
1 72  
010.00  
005.00  
006330.00

Removal of radioactive  
unreacted nucleotides  
from nick-translated  
probes.

+ - 1 ml syringes  $\bar{c}$   
G-50 columns  
washed  $\bar{e}$  5 mM Tris pH 7.5

Centrifuged 2 K for 5'  
all liquid removed

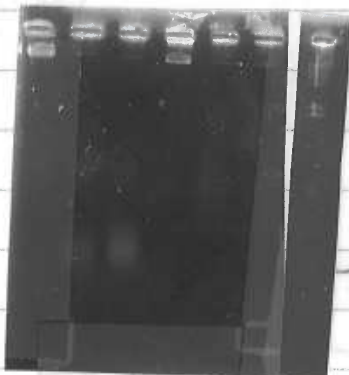
Samples applied.  
Centrifuged 2 K for 5'  
liquid collected in sterile  
ependorf tubes.

The tubes containing samples original  
washed 1 x  $\bar{e}$  5 mM Tris pH 7.5  
same volume as samples.

Applied to syringe columns.  
liquid collected in ependorf tubes.

Total volume 258 microliters.

checked on agarose gel for size.



Ad. A B C D  
x Fel  
v HindIII

~~ALI~~

6/23/82

Phage  $\lambda$  Fel V Titration

Phage  $\lambda$  Fel V  $\sim 10^7$  PFU/wl  
(From Henry.)

RW 262 Permissive host for phage  $\lambda$

L. agar plates Bottom agar.

0.7% L. agar Top Agar.

Phage dilution broth L Broth with  $Hg^{++}$   $Ca^{++}$

Serial dilution ten fold series.

0.1 wl  $10^{-5}$  dilution

0.1 wl  $10^{-6}$  dilution.

Mixed with equal volume 4 hour host culture.

Added 3.0 wl soft agar

Poured on plates

... .. repeat.

6/23/82.

## Nick translation using malto-triose dUTP

### Reagents.

<u>DNA's.</u>	Ad-2 DNA	1 mg/ul
	$\lambda$ FelV DNA	1 mg/ul
	Pst DNA	1.4 mg/ul
	$\lambda$ plac DNA	1.6 mg/ul.

### dNTP's

dNTP mix	0.3 mM dATP	} in 50 mM Tris.HCl (7.5)
	0.3 mM dGTP	
	0.15 mM dCTP	
TTP	0.3 mM	in 50 mM Tris (7.5)
malto-Triose dUTP	0.3 mM	" "
$^3\text{H}$ dATP	1 $\mu\text{Ci}$ /ul	

### ENZYMES.

DNase I	0.5 mg/ul	in
		10 mM Tris.Cl (7.5)
		50 % glycerol
DNA pol I	3 units / microliter	in
		0.1 M NaPO <sub>4</sub> (pH 7.2)
		50 % Glycerol
		1 mM DTT

### Buffers

① 10 x Nick Translation buffer  
10 mM Tris.Cl 7.5, ~~10 mM BSA~~  
50 mM MgCl<sub>2</sub>

② Nick Translation stop buffer

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