

(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.

4 - 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

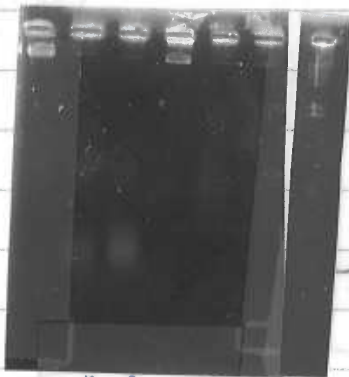
Samples applied.  
Centrifuged 2 K for 5'  
liquid collected in sterile  
epiendorf tubes.

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 epiendorf 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  $\approx 10^7$  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<br>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       | " "                          |
| $^3$ HdATP        | 1 mCi/ul     |                              |

### ENZYMES.

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