

(A) checked for size on agarose gel
not too large pieces ≈ 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 λ Fel V DNAs 6.24.82
with malto-triose d UTP

Ad-2 DNA 1 μ g/ μ l From Henry.
 λ Fel-V DNA 1 μ g/ μ l " "
 malto-triose d UTP 0.3 mM From Stan.

	A	B	C	D
3 H d ATP	12 λ	12 λ	12 λ	12 λ
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 λ	14 λ	-	-
λ Fel V	-	-	50 λ	50 λ
DNase 0.1 μ g/ μ l	10 λ	10 λ	10 λ	10 λ
DNA-P	10 λ (30 μ)	10 λ	10 λ	10 λ (30 μ)
d. H ₂ O	55.5	53.5	17.5	17.5

14^o 2 hrs.

2 μ l aliquots at 30' intervals for end for checking incorporation.

terminated with 10 μ 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
001.00
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

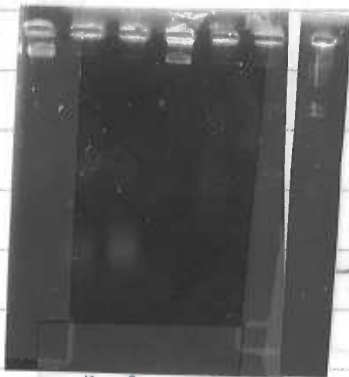
Samples applied.
Centrifuged 2 K for 5'
liquid collected in sterile
epiendorf tubes.

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 λ Fel V Titration

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

RW 262 Permissive host for phage λ

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</u> s.	Ad-2 DNA	1 mg/ul
	λ FelV DNA	1 mg/ul
	Pst DNA	1.4 mg/ul
	λ plac DNA	1.6 mg/ul.

dNTPs.

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 HdATP	1 mCi/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 ₄ (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 MgCl₂

② Nick Translation stop buffer

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