Ms loose notes, (6/7), D88-D108(3), testing enzymes and polymerase on DNA.

Publication/Creation

1973 - 1974

Persistent URL

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TG (+dd) reverse transcriptase for dd (AF file)
(6-7-)
T4 pol for (dd)

[illegible](4) microscale dd

Pd mix

100 µl M Tris 7.4
50 M Mg
5 ME
350 H_2O

¼ Mix

Tris pH 8	·67 M	100 M
$MgCl_2$	·067 M	10 M
ME	0·1 M	20 M ?
		70 H_2O

Hin buffer (x1)

6·6 mM - $MgCl_2$ 1 M
6·6 mM Tris-HCl pH 7·4 1 M
6·6 mM ME 1 M
0·05 M - KCl or NaCl 7·5 M / 150

100 µl M $MgCl_2$
100 µl M Tris
100 µl M ME
750 M NaCl

} 1500

~~340~~ + ~~50 NaCl~~ ~~NaCl~~

450 H_2O

600
5.70
180
5
1800 - 180
10

4
8
32
62

's ~ 2.

5×3 µl.

3 µl mix, 2 µl – mixes, 1 µl pol (kl) 30' 0°

D 88   Restriction enzyme fragments from ØX

I got some RF1 and RF2 from John Sedat and some good restriction enzyme (Hin) from Tom Maniatis which is exonuclease-free. Set up three digests as follows:

1. 10 µl (20 µg) RF1 in ·01M tris ·001M EDTA

   10 µl Hin buffer (·06M tris ·6M NaCl, ·06M mercaptoethanol, ·06M Mg)

   50 µl water

   30 µl Hin enzyme

2. As above but with 10 µl RF2

3. As in 2. but using 10 µl of an older preparation of the Hin enzyme, which probably contained a small amount of exonuclease.

Incubation was at 37° overnight. This was probably unnecessarily long and 2 hr would be sufficient. Three samples were then run on a 5% acrylamide gel made up as follows:

25 ml 30% acrylamide (29 g acrylamide 1 g bis/100 ml)

15 ml of 10 x TBE (108 g tris 9·3 g EDTA, 55 g boric acid pH 8·3 /litre)

5 ml 1·6% ammonium persulphate

This was brought to 150 ml with water and degassed. 50 ml was used to make a seal at the bottom of the trough after addition of 50 µl TEMED. When this had set 50 µl TEMED was added to the remaining 100 ml and it was poured into the apparatus (20 x 20 cm). It was run for 3 hr at 300 v.

It was stained with methylene blue for 15 min (5 min would probably have been better as it was very slow destaining). It gave a good pattern of bands as follows:

The patterns for the three samples were essentially the same though (3) looked slightly less sharp, suggesting some exonuclease action. In (2) 7a and 7b could be seen ~~starting~~ separating, but not in the other ones. There was no separation of the three components of band 6.
The bands were cut out, chopped into small pieces and soaked in 1 ml of the elution buffer (0.5 M NaCl, 0.1 M tris Cl 8.5 0.005 EDTA) at 37° overnight. Bands 1-5 were eluted from all three samples, 6, 8 and 9 from (1) and (2) only, and 7a and 7b from (2) only.
ØX DNA was added to the preparation to act as carrier for precipitation and also as template for the subsequent reactions (12 μg/sample except for 7a and 7b which had 8 μg). The eluates were precipitated with ethanol, the precipitate washed with ethanol and dried. They were then dissolved in 10 μl ·1N NaCl ·01M tris (5 μl to 7a and 7b; the

blank experiment - i.e. without primer - was prepared from 4 µg ØX DNA and 5 µl NaCl tris). These were sealed in capillary tubes and heated at 95° for 5 min then left 20 min at 67° and put in a bath at 60°, which was allowed to cool slowly to anneal the products. Samples of these solutions were then used in the following experiments:

1) 1 µl samples were treated with DNA polymerase as follows and run on the gel. A mixture was made as follows:

10 µl ^{32}P dGTP was dried down and to this was added

10 µl S mix

10 µl rCTP

10 µl $\frac{\text{dTTP}}{20}$

10 µl $\frac{\text{dATP}}{20}$

~~50~~ 15 µl water

In each tube took 5 µl of the above mixture, 1 µl of the ØX and primer mixture, 3 µl water and 1 µl DNA polymerase (Klenow). Incubation was for 15 min at 4° and the reaction was stopped with 1 µl ·1M EDTA. In this experiment the samples were run on a 12% urea gel but not split with the Hin enzyme, which was a mistake as, under these conditions, many of the products would not be expected to move out from the origin. The results were rather inconclusive with most of the stuff sticking at the origin though there were faint bands moving out whose nature is not exactly clear. There is a very high blank (sample 10) which makes in~~corporation~~ terpretation rather difficult.

D 88 (cont.)

2) This was an experiment with larger amounts of bands 1, 2, 4 and 7b carried out as in 3) below, but in this case I forgot to denature the products: however the results looked much the same as in 3).

3) In this case incubated 5 µl samples of 1, 3, 5 and 8 with 25 µl of the mixture described in 1), 15 µl water and 2 µl polymerase (J.E.D.). Incubation was for 10 min at 0°. The products were phenolled and precipitated with ethanol, washed with ethanol and then dried down. They were then digested with 50 µl Hin buffer and 5 µl of the Hin enzyme for 1 hr at 37°. They were then heated at 95° for 3 min and rapidly cooled. 5 µl dye solution in 50% glycerol was added and they were applied to the gel, which was made up from

60 ml 30% acrylamide
15 ml 10 x TBE
5 ml 1·6% ammonium persulphate
63 g urea brought to 150 ml with water

It was run at 250 v until the faster dye (bromphenol blue) was at the front again. Most of the material is stuck at the origin. One would expect to have bands in the lower part of the gel but the only one that shows any is 5 and these are rather faint. The others do show specific bands, which may suggest that synthesis has gone rather farther than one would have expected. There are also a number of bands which seem to be common to all of them, suggesting they are derived from blank.

4. Ran incubations on 5% formamide gel. Gave better picture with nothing at origin & bands which seem to be related to sizes expected.

88 (4)

10 µl ^{3}H GTP . dry .

20 S mix ✓
20 rC ✓
20 T/20 ✓
20 A/20 ✓
20 H_2O ✓

2 µl 5, 6, 7a, 8, 9, 10 (blank) ✓
10 µl above mix ✓
7 H_2O ✓
1 pol (30)

10' 0°

→ 2 µl .1 M EDTA .

dried down add 25 µl formamide heat 90° 3'
1 µl dye in formamide

D 89 Priming with restriction enzyme fragments (D 88 ref.)

1) This was another somewhat larger scale preparation of the restriction enzyme fragments using two different concentrations of the Hin enzyme and two different preparation as in protocol. The products were run on a 40 cm long gel (5% acrylamide). a and b gave complete digests with good separations of 7a and 7b. There was some sign of resolution in band 6 but it was not possible to cut it out separately. b was only partially digested and gave a large number of very well resolved bands, which were also eluted. The various bands were eluted and precipitated with ethanol and dissolved up in water.

2) This was an experiment using band r2 and trying different concentrations of r2 and ØX. The products were run on a formamide gel but there is no sign of any intermediate. The experiment was repeated using band 6a and a different triphosphate. It seemed to work this time, giving a clear band that was not present in the control. The different concentrations of reagent did not seem to affect the reaction much. The yields more or less corresponded to the amount of the primer.

3) This was a larger scale experiment with the various primers. About $^1_4$ samples of the restriction fragments 1, 2, 4, 5, 7b, 8 and 9 were dried down and dissolved in 5 µl water
2 µl ØX (OD 75)
This was sealed in a capillary tube and heated in a boiling water bath for 3 min. After cooling, 1 µl NaCl tris (80 µl N-NaCl, 10 µl N-tris and 10 µl water) was added. It was incubated at 67° for 4 hrs.

D 89 (cont.)

(3) cont.

The following mixture was then made up for incubation with DNA polymerase:

50 µl ^{32}P dATP (6 Nov 1973) was dried down and to this was added 100 µl S mix:

100 µl rCTP

100 µl $\frac{TTP}{20}$

100 µl $\frac{GTP}{20}$

100 µl water

50 µl of the mix were used with the pre-annealed primer and 35 µl water and 5 µl DNA polymerase (JED). Incubation was for 30 min at 0°. The reaction was stopped by addition of 5 µl ·2M-EDTA and precipitated with 10 µl sodium acetate and 250 µl ethanol. After centrifuging down and drying, the residue was dissolved up in 25 µl formamide and run on a formamide 5% acrylamide gel. Quite good bands were obtained, the smaller ones being much stronger than the larger ones. These were eluted by electrophoresis and precipitated with ethanol. They were then digested with panc and run on the 2D system. The small ones, 8 and 9, are extremely complex and probably indicate the presence of a lot of blank material. 4 and 5 seem to be the best though they are still somewhat complex. It may be that the pancreatic digest is somewhat incomplete.

D 89 (1)

a) 30 μl (60 μg) RF II , 30 Hin buffer x 10 , 45 μl Hin 200 H_2O } ON 37°

b) 20 μl (40 μg) RF II 20 " 20 μl Hin 140 H_2O

c) 30 μl RF II 30 10 μl new Hin 200 1 hr

ppt EtOH
dissolve in 15 μl buffer 5 μl dye mix
4.00

20 hr 210 v
column at 17 , 33

b has beautiful ppt bands.
a & c cut out : 7a + b well sep'd.
6's beginning to separate could probably see
3 bands but cut arbitrarily to 6a + 6b

couldn't see 10

6a
6b
7a
7b

om

partials in b mostly big here about 30 bands

1 9
2 11
3 12
4 15
5 17.5
6 19.2
7a 20
7b 20.5
8 24
9 26

Extracted 1 ml + 5 EtOH ppt.
in 10 μl 40 . (2 4 6a 8 in 20)
1, 5, 3, 6a, 6b, 7a, 7b in 40

89(2)

a	2	1	
b	1 μl r2 ($\frac{1}{20}$ ≡ ·5 μg)	1 μl φx (·6 μg)	
c	1	2	1 H_2O
d	1	5	4 H_2O
e	~~4~~ –	~~P~~ 4 1	1 H_2O
f	1	1 μl ←	

Seal. heat & anneal.

5 φx (100 00)
~~30~~
8 N NaCl
1 N tris
20 H_2O

ditto – φx

~~10 mix~~ 10 ~~A~~TTP* dry

20 S mix.
20 rC
20 ~~P~~/20
20 4/20
20 H_2O

above sol^ns + a) 10 mix 6 H_2O 1 pol

a)	10 mix	6 H_2O	1 pol
b)	10	7	1
c)	10	~~6~~ 5	1
d)	20	8	2
e)	10	7	1
f)	10	7	1

remainder of e 20 5 H_2O

D 89(2)

	Φx (in NaCl/tris)	6A	H_2O
a)	2	—	2
b)	2	1	1
c)	2	2	—
d)	2	5 (dried	2
e)	5	2	3

Seal, heat & annealed.

10 new (Nov 1st) dATP dry.

20 S mix ✓
20 rC ✓
20 T/20 ✓
20 G/20 ✓
20 H_2O ✓

	mix	stuff	H_2O	pol
a)	10 mix	4 stuff	5 H_2O	1 pol
b)	"	"	"	"
c)	"	"	"	"
d) 1	20	4	15	2
e) 1	10	4	5 [illegible]	"
e) 2	20	4	15	"
f)	10	2 old 6	)	"

30' @ 0°

1 μl .1 M EDTA + 5 μl H_2O to wash out cap.'y

dry

25 μl formamide ^heat ; 1 μl dye

D 89(2)
GA runs. pure A^* labels.

	n. ns	dep.
1	A, G	-
2	A trG	-
3	G ~T	-
4	T	T
5	C	C (is old spot, probably C)
6	C	C
7	A. trC	trC

D 89(3)

c4.

n.ns of pame. (to A)

[illegible]	1.	G A T
	2.	C A T
	3.	C A T (but ?)
	4	C A T
	5	A T
	6	A T
	7	G & trc
	8	C
	9	C
	10	G
	11	C

D 89(3)
n.n. (to A)

1

1. C A T weak.
2. C A T (~~seems stretched~~) high C suggests may be pt[illegible]
3. C A T (C a bit strong)
4 A ~~[illegible]~~ T
5. C A T
6. ~~G~~ C + spot ahead of T maybe not digested.
7. T (seems a bit slower)
8. G

2a

1. C A G T } probably mixtures
2 C A G T
3. A G
4 G (+T.
5 G

2b 6. C A G T = 2a 2
7. A G 3

5 defn

1. C A G T pt[illegible]
2 C A G T T
3 ditto
4 C A G T
5 C T
6 C A G T T . CC' (diff spot in blue)
7 C A G T T . C
8 C A . C . CC
9 C A G T C T
10 C T C T
11 C T C T

76

1. C A G T
2. C A G T
3. C A G T
4. C A G T?
5. C A G T
6. A G (slightly more G)
7. A G (")
8. C A
9 C
10 C

} maybe mixture
all seem rather similar (partials?)

dep:

76
7. nothing.
8 (TC) TC(A)
9 double spot just behind TC could be something like no TTC
10 C (old spot)

D 89(3)

sple s

All v faint & unsatisfac

5.3 T x x A —

5.1 A A —

5.3 looks similar to 5.1.

5.4 A - C - A - T - A. If this really is a C (& it does look like it) it must be a partial. It is a strong spot, but might be mixture.

5.5 G - T - A (A) - T

7.3 could be G - A

7.4 A - T

7.5 A - T

could be same more both look similar.

D 90 Restriction enzyme partial digests (D 89 ref.)

Attempts were made to redigest some of the fragments from the partial digestion obtained in D 89 (1) b.

1) Some experiments were done with the Gradipore system to see if this could be used for separating the fragments, as this would seem to be a more sensitive method for detecting the products. Fairly sharp bands were in fact obtained but in certain cases they seem to have streaked rather badly and moved faster than they should. It seems that this is largely a concentration effect but it looks as if it would be difficult to identify the products unequivocally.

2) The better system seems to be the 5% gel using a small slot former. The whole of bands 2, 6, 9, 13, 17, 22 and 26 were dissolved in 5 µl water and treated with 5 µl of the following mixture:

10 µl Hin enzyme (preparation A)
20 µl Hin buffer x 10
70 µl water

Incubation was for 3 hr at 37°. After staining the bands could be seen quite clearly but they were all undigested. Another experiment was done using smaller amounts of some of the bands (2/5ths or 1/5th) but these could not be seen on staining.

3) Another experiment was done as in 2) above but using about twice as much material and incubating a rather large amount of enzyme. The gel was stained up with ·05 µg/ml of ethidium bromide. The controls gave fairly good bright bands but nothing in the digests of the fragments, though one of these looked somewhat streaky, suggesting exonuclease action. It does not appear that the ethidium bromide is appreciably more sensitive than the methylene blue.

D 91 Priming with restriction enzyme fragment 8

The idea of this experiment was to try cutting the product with the Hin enzyme with a view to doing the one-dimensional sequencing, and also to do a blank run with only ØX and only primer. In view of the very complex pattern obtained with band 8 in D89 (3), this may not have been a very suitable choice.

1) Three separate tubes were set up - a, b and c - as in protocol, and these were denatured. Samples were removed from each one and treated with NaCl-tris to anneal. This gave three samples:

    a) 8 + ØX
    b) 8 only
    c) ØX only

Each of these was then incubated with polymerase in the normal way and treated with phenol, ethanol and acid. + fpme'd c. gives enormous blank v complex. b a weak specific pattern & a v complex but has some strong spots that stand out

2) The remainder of the main batch of 8 and ØX was treated with polymerase for various times and the product isolated on an Agarose column with approximately 9 x 10^5 counts. $1/10$th samples of this were treated with the Hin enzyme using two different preparations: a, which is exonuclease-free, and b, which probably has exonuclease in it. The product was run on a 7% homomix. Both gave reasonable bands but it looked as if b contained mononucleotide, suggesting the exonuclease action. Bands cut out & dephosphorylated.

D 91 (cont.)

3) ^{1}20th samples of the above material were treated with some more Hin enzyme as in (2). a was equivalent to $^{2}/_{3}$ µl, b $^{1}/_{3}$ µl, c 2 µl. c has digested considerably further, indicating that 2 µl is probably necessary.

4) A 2D run of some of the material treated with Hin as in (5). This looks a bit too complex to be really nice, though seem to be a number of main bands that belong

5) An experiment missing out different triphosphates and then treating the product with Hin and running on a 1D system with 5% homomix hydrolysed 10 min. Doesn't look too hopeless, though is difficult to relate O one to 4(above). A possible sequence could be something like C(A)T G(G) A G G A A G T C A G(C) A(A)
~~doesn't really look~~

6) The remainder (about half of the material prepared in (5)) was treated with more Hin and run in parallel. These are rather difficult to interpret, especially as several bands seem to go right across. Some bands were cut out and depurinated. Doesn't really look as good as in 5 though similar except for smaller ones!

D 91 (1)

a) 5 µl 8 ($\frac{5}{20}$)
2 µx (12 conc φx (100 OD), 4 H_2O) (6 µ)
b) 1 µl 8
c) 1 µl φx + 2 H_2O.
100° 3'
add NaCl tris (80 µl N NaCl, 10 N tris, 10 H_2O)
a 1 µl
b 1 µl of $\frac{1}{5}$ dil
c 1 .. .. $\frac{1}{2}$ ..
4 hr 67°

to a c add 2 1 µl H_2O

take 2 µl a, all b, 2 c, ✓
~~8~~ ~~10~~ 5 dATP* dry ✓
mix 10 S mix ✓
10 rC ✓
10 T 20 ✓
10 G 20 ✓
10 H_2O ✓
take 10 µl. ✓
7 H_2O ✓
1 pol/10

1 hr 0°
phenol, EtOH etc.

2)
remains of 8 (a)
5 dATP* dry
10 S mix ✓
10 C/20 ✓
10 T/20 ✓
10 G/20 ✓
50 H_2O ✓
5 pol

0° 1, 2, 4, 8, 15, ~~30~~, mins. (20 µl)
5 µl 2 m EDTA.
agarose column, hi sample.
approx 350 µl. 2600 / µl 9×10^5 cts total
a) $\frac{1}{10}$ dry 10 H_2O 1 10x buffer 2 thin exo – (2 µl/µl)
b) remainder in 20 µl take 2 + 10 H_2O 1 buffer 1 thin A (8/22) 1hr 37°

D 91(5)

	-C	-T	-A	-G		O
8	2	2	2	2	✓	2 μl. (looks like 5?)
pol mix	1	1	1	1	✓	
C/100	—	1	1	1	✓	
T/100	1	—	1	1	✓	
A/100	1	1	—	1	✓	
G/100	1	1	1	—	✓	
H_2O	~~4~~ 3	~~4~~ 3	~~4~~ 3	~~4~~ 3	✓	
pol (1-20)	1	1	1	1		

20' 0°

2 μl conc + 4 H_2O phenol ×2 + EtOH + Hin. (control 2 μl)

1 10x buffer. ✓ — O ditto
7 H_2O ✓ — 5 H_2O ✓
2 Hin exo⁻ (2 μg/λ)

1 hr 37°

½ onto TLC. C T O A G. 5% 10'

D 91(6)
rest add 2 μl Hin 10.00 + run

D 91(2)
Dep^n^ (A n.m.)

a	9	faint spot ahead of (TC) ?		
	8	C , T ,	tr (CT) .	slower (T, ")
	7	nothing!		
	6	TC	, C	, T
	5	TC	C	T
	6	TC	C	T
	3	TC	T	tr C
	2	TC	T	"
	1	TC	T	"
b	3	TC	C	T
	2	ditto .		
	1	TC	T	tr C .

Not too clear but rather suggests not a single series.

D 91(6)
dep^{-5}.

3.	T	, (TC)	
2	T	(TC)	
4	T	(TC)	C
5	T	TC	C
1	T	TC	C

} (TC) stronger than T

quant^n a bit difficult but could fit with

$\{T, (TC)_2\}\{T, C\}T, C$

Doesn't really fit too well with seq given in D 91(5):

C A T G (G) A G G A A G : T C A G (C) A (A)

Bone 3 ×
2
4
?

ie the TC given is probably not a TC(A), looks pretty like a CA so is probably TGCA
T & TC in 3 & 2 probably come before this seq.

seq: CA

... (TC, T) ... T C A T C (G) A G G A A G T G C A G C A A .

D 92 Formamide gel fractionation of products primed with restriction enzyme fragments

This was an experiment similar to 89 (3). 1/8th samples of various fractions were used as in protocol, some with rC and some with rG. The products were run on a formamide gel and gave quite nice bands, which were eluted.

1) Bands from 1, 4, 5 and 7b, which were labelled with CTP using rG, were digested with T1. These gave quite good and rather simple patterns.

2) 3 and half of 7a were digested with panc but gave rather complicated patterns suggesting probably that digestion is not complete, or else that synthesis has gone a very long way.

3) Half of 7a and the whole of band 10 were treated with Hin after annealing with some fresh ØX. The products were run on the 2D system. 7a gives quite a nice picture and spots were cut out for depurination. 10 looks very messy. 7a dep^n. spots 1-4 give I, C. (double spots) rest nothing so not v helpful. Fits reasonably with 93(2) but don't know how sizes match up.

D 92

approx $\frac{1}{8}$ - $\frac{1}{10}$ of 1, 3, 4, 5, 7a, 7b, 10 dried down
1 φx (75 OD)
5 H₂O
heat
1 NaCl tris (8.1.)
67° 12.00

3, 7a, 10
10 ATP dry
20 μl S mix ✓
20 rC ✓
20 T/20 ✓
20 G/20 ✓
20 H₂O ✓

take 25 15 H₂O . 5 $\frac{pol}{20}$

(1) 1, 4, 5, 7b.
10 CTP ✓
20 S mix ✓
20 rG ✓
20 T/20 ✓
20 A/20 ✓
20 H₂O ✓

10' 0°.

5 .1 M EDTA
5 NaAc
130 EtOH

formamide gel

1, 4, 5, 7b, T1
(2) 3, ½7a, 4, Panc
½7a, 10 Hin ~~20 hours~~ 7%
(3) ½ 7a, 10. more φx + Hin.
1 μl φx 3 μl H₂O heat
50 1 μl 10x Hin anneal. 1.00 – 5.00 67°
exo⁻ (2x/y) 4 μl Hin 10 H₂O + 1 10x buffer 2 hr
1 cover 30 H₂O 5 NaAc 120 EtOH

D 92. (rG C)

n.n's

4
1. C A G
2. C A G
3. C A (double spotting!)
4. C A
5 A
6 A
7 T

spot ahead of G.
dep^n (difficult as all seem to have several are several spots, quite slow nice but don't fit slow with n.n's
TC ✓
C.
110 C
–

dep^n

7b
1 T 110 TC
2 T 110 TC
3 G G
4 T 110 TC
5 T 110 TC

spde suggests starts A-

rGC

dep^n

1
1 C A G T (weak)
2 C A T? "
3 A
4 T
5 T
6 G

TC? (slower than G)

D 92

5

	n.n.			
1	C	, A		
2	G			
3	C	A	(or T)	
4	C	A	G	
5	C	A		
6	C	A		
7	C	A	G	
8	C	A		
9	C	A		

dep.

dbl spot in T posit^n ~~TG~~ : ..CC ?

G

spde T T T T / G G G G

D 93 One-dimensional sequencing with restriction enzyme primed products (D 91 (5) and D 87 ref.)

This was a fairly ambitious experiment taking restriction enzyme fragments and extending them with all four deoxys, isolating the products on Agarose, then taking samples and extending them in the absence of the different triphosphates. All of these were done in parallel and the final products dissolved up in 8 μl water.

1) The products from 3 and 8 were treated with Hin enzyme as in protocol and run on the 1D system. Unfortunately tubes were spilt* and to some extent mixed up. However the results with 8 looked considerably better than those with 3, though it is difficult to match up the bands here with those in 91 (5). Again there seem to be quite a lot of bands going right across the plate.

3 doesn't look too bad on further development but tubes too mixed up (ie don't know which is which)

8 C O A + G were identifiable, others not so were lettered U – Z. (= 8T + all the 3's)
U – Z run acrylamide 20% 20×40 cm. looks not too bad. Z must be 8T as is different from rest. 3's look OK.* 8's (remains) run on acrylamide c̄ 7a (see below)

2) 5 + 7a Hin d. run acrylamide. + sample of 5 on homo.
Gel dried + autographed. 7a doesn't look too bad.
Sort of seq A T A A T A(G) A G C A (A) T (G) G C A C A/G C/G A/G T T_n
then gets v fuzzy.

5 could go something like:

T_n A G A A A C_n A A C A G C_n A T/(ATG/AA,C+G)
(strong spots underlined)

Homo run on this could give T_n A G C_{1-2} A_{1-2} C A T preceding gel seq

* Seq for 3 would be like:
V W (W)(V,W) U V W V W U W V Y W (V V) W W V Y ~ U
this assumes X is control since it has most bands. W corresponds closest to X suggesting it is A
W + Y seem to give big jumps suggesting A + G resp.
U + V = T + C . ?

D 93

3(5 µl), 5(5 µl), 7a(5 µl) 8(2 µl) dried.

~~1 µl φx~~ 5 µl φx (OD 15) (3 µg)

seal boil 3'

1 NaCl - tris . 67°. 3.30

10 ATP* dry

20 S mix
20 dC/20 ✓
20 dG/20 ✓
20 T/20 ✓
20 H_2O ✓

take 25, 5 shift, 15 H_2O, 5 pol/20

10 µl at ·5, 1, 2, 5, 10 mins

5 µl ·1 M EDTA
agarose.

3-7, 8 ·75, 1½, 3, 7 15

3 cts up to ccu
5, 7 30-40
8 70

vac^d down dissolved in 50 µl.

D 93

	-C mix	-T mix	-A mix	-G mix	
Pol mix	10	10	10	10	✓
C/100	–	10	10	10	✓
T/100	10	–	10	10	✓
A/100	10	10	–	10	✓
G/100	10	10	10	–	✓
H_2O	10	10	10	10	✓

10 µl 3, 5, 7a, 8
10 µl -x mix
1 pol/10

20' 0°

8 µl H_2O
40 µl carrier.
take ·4
+ ·2 phenol
EtOH ppt. all in 8 µl H_2O.

① 8's
1 Hin buffer (x10)
8 H_2O
1 Hin (8/22)

3's
1 Hin buffer
8 H_2O 6.15. 2 hr
1 Hin exo⁻ 1µλ/1µg

Let on ice 5% ·0'

② 5's + ~~7~~ as for 3

7a
1 Hin buffer
8 H_2O
5 λDNA (2/5 µg) for carrier to prevent exo
1 Hin A (New Prep) 6.15

D 94   Priming with restriction enzyme fragments (D 92 etc. ref.)

This was an experiment using some freshly specially prepared ^{32}P-GTP and adding various incorporations, as in protocol, with rC or with all four deoxys. a, b, c and d were with fragments prepared in 89 (1), and e, f, g and h with the remainder of the material from D88. a, b and c were with all four deoxys. The products were purified with Agarose and used for the experiments in D95. The remainder were purified on acrylamide formamide. c, d and g were digested with panc and run on 2Ds. f and h were re-annealed with 5 µl ØX (OD 15) and digested with Hin enzyme. put on 2D's (homo) pretty ghastly

D 94

a) 20 μl 3 (1/10) 5 μl φx (OD15)
b) 20 7a (1/10) 10 heat, Nacl-his. anneal 67° 5hr
c) all 5 (1/5) 10
d) all 7b (1/5) 10

	G* rC	G* 4 decoxy
hot G	20	10
S mix	50	20 ✓
rC	50	– ✓
dC/20	—	20 ✓
dA/20	50	20 ✓
dT/20	50	20 ✓
H_2O	50	20 ✓

a) 5 μl, 25 G* 4dc, 15 H_2O, 5 Pol/20 .5, 1, 2, 5, 10' 0°
b) 10 25 " , 10 " "
c) 10 50 , 30 , 10 "

d) 10 , 50 G* rC , 30 H_2O. 10 Pol/20 .
of remains of old prep (0'58)
e) 10 μl 5 " " " 10' 0°
f) 6 " " "
g) 7a " " "
h) 9 " " "

a, b + c through agarose a + b raced c ppt ETOH

d – h on formamide. eluted. pptd ETOH.

b, 9 5 μl φx (OD 15) (3 μg) anneal. 1.08 67° 4 hr
c, f, h

f(6) 3 H_2O 2 min (8/22) 1 hr buffer (– NaCl) sample on 20 home
h ditto but wait

d, e, g. 5 μl 10 mg/ml panc mix

Nov 27th

This is an unrecorded expt that didn't work

<10 μl 2, (the lot)
1/6 5 μl 3 10 μl (15) } actually inadvertently added d/s H_2O.
1/6 5 μl 7a 10
1/2 15-20 5 30

dry down

3, 7a 1 μl φx (7500)
5 H_2O — 5 μl φx 1500
2, 5 3 μl φx
15 H_2O — 15 " " 67° 11·45

mixes. 2x 5/10 new hot T* + G*

	G* rC	T* rC	T* rG	G* 4deoxy	
S mix	10	10	10	10	✓
rC	10	10	–	–	✓
rG	–	–	10	–	✓
dC/20	–	–	10	10	✓
dG/20	~~10~~	10	–	–	✓
dA/20	10	10	10	10	✓
dT/20	10	–	–	10	✓
H_2O	10	10	10	10	✓

take 25 μl ✓ 15 H_2O ✓ 5 Pol/20

a 3 G* 4 deoxy } ·5, 1, 2, 5 10' 0°
b 7a " "
c 2 5 μl G* rC
d " T* rC
e " T* rG } 10' 0°.
f 5 " G* rC
g T* rC
h T* rG

5μ ·1 M EDTA
5 NaAc
130 EtOH
wash EtOH. dry. 50 μl formamide. heat 3' 90° 1 μl colour
Load a – h (space)

wash-out. triphosphates lousy.

94(2)

10 μl 4 dry 10 μl φx (OD 15)
30 7a (5') anneal.

10 μl GTP* dry ✓
mix 20 S mix ✓
20 dC/20 ✓
20 T/20 ✓
20 A/20 ✓
20 H_2O ✓

for each 50 μl mix ✓ 20 μl
10 sample ✓ ·5 1 2 5 10'
30 H_2O ✓
10 pol/20

10 μl ·1 M EDTA.
agarose
dry a bit.

4 5 μl stuff 3 H_2O, 10 -x, 2 pol/20 (SED.)

7 10 " 10 -x "

20' ice bath.
5 μl ·2 M EDTA.
·4 (4 ml H_2O 20 μl carrier) + ·2 phenol.
ether extract
ethanol pptn.

8 μl H_2O
?
then: trk. 5 μl stuff 1 10x buffer 1 thm (2 μl/μg)
O.N.
samples on 5% 10' hyd.
4 ? thick on acrylamide.
ghastly.

D 94.

d (7b. G*C)

	m.n. (TCA)		dep^n
1.	C A G T?	(weak)	–
2.	C A G T		T C
3.	A G T	(weak)	most TT
4.	C T		C but presumably as Tp (C's are dud spots)

e (5. G*C)

	m.n.	
1.		A G
2.		A
3		A tr T
4	weak	A G T
5.		A tr T
6.		A
7		A T	tr T
8		C	C

f (7a G*C) rather weak.

	m.n.
1	A, G, T
2	C A G T
3	C A T
4	C A
5	A
6	C T

spots on d.

2. can see ~T T A~ / ~G G

↑ this is same rate as 4

4 was overdigested but undigested goes just behind blue suggesting not v large. TGC(G)or TGAC(G) ?

D 95 One dimensional sequencing (D 93 ref.)

The idea was to vary the conditions of preparing the material to ensure greater purification.

a) The material from the Agarose column was divided in four and extended in the absence of different triphosphates, as in protocol, and then run on a formamide gel. There was a relatively faint band in each case in the expected position and a good deal of background material. The band was cut out from each one, eluted and re-annealed and treated with Hin.

b) The product was extended and then treated directly with Hin. (i.e. This was as in D93.)

c) The product here was run directly on the formamide gel but there was very little material in the expected position and a lot of background streaking. Thus it seems that the Agarose column is not very effective in purifying the desired product. There did not seem to be enough to continue with.

D 95

a 50
ϕ_{X}, b, ϕ → 40 μl. } ex agarose colm (D 94).
take 10

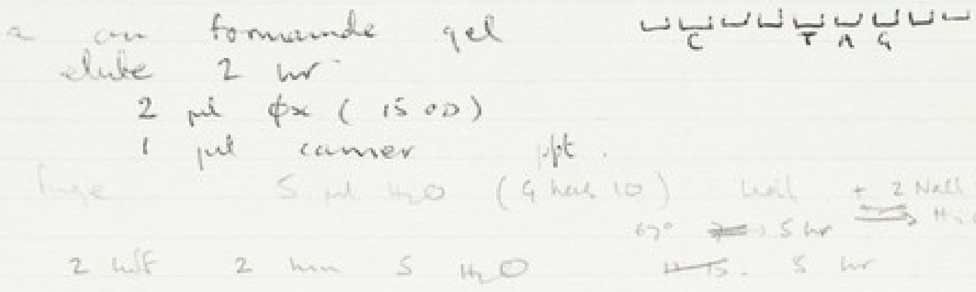
	- C mix	- T	- A	- G		
Pol mix	10	10	10	10	✓	✓
C/100	—	10	10	10	✓	✓
T/100	10	—	10	10	✓	✓
A/100	10	10	—	10	✓	✓
G/100	10	10	10	—	✓	✓
H_2O	10	10	10	10	✓	✓

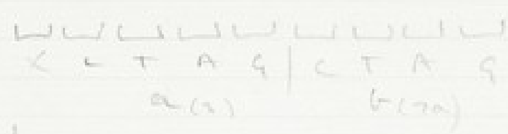
a 10. take 10 + 2 pol/20 20' 0°
b 20, 20 4
EDTA

a. 2 μl ·2 M EDTA 25 μl formamide dry
b. 2 μl ·1 M EDTA 0·4 (2 ml H_2O 10 μl carrier) + 2 phenol
ethOH ppt. H_2O 1 ml ether 2 min

c formamide (v. load) eluted. 5 μl ϕx (OD 15) ppt EtOH

a on formamide gel
elute 2 hr
2 μl ϕx (15 OD)
1 μl carrier ppt.
fuge 5 μl H_2O (9 hrs 10) boil + 2 NaOH
67° → 5 hr → H_2O.
2 hlf 2 min 5 H_2O TS. 5 hr





← b here shows good resolution of bands but all are the same, suggesting extension haven't worked.

HOPELESS!

−70
Stine cells

| Cells 50 | 32
24
6
62 g/ litre | M [illegible] g/l
·01 M |

Biochem 12, 3056.

12 3056

10 g lungs 17 ml ·01 M tris (pH 7·4) · ·01 M ME in stainless steel beaker in ice bath. Sonicate for 10 periods of 30 sec at max. output (c. 60 W) temp remains below 4°). Fuge Spinco angle 50 Ti rotor at 40,000 rpm 60 min at 0°. (to remove debris) supernatant (19 ml) adjusted to 1M with solid NaCl layered on agarose A - 0.5 colum 2·6 × 55 cm. equilibrate in 1·0 M NaCl 0·02 M tris (pH 7·4) 0·01 M ME, flow rate 15 ml / hr. 6 ml fractions assay 2 μl

60 g NaCl
·6 g ME
(·75 ml)
20 ml M tris

T. ÅGAAAC. AACAGC. AT

D96 Prep. of Hin. Lig Dec 1973.

Haemophilus influenzae Restriction Enzyme Prep 15-16/12/73.

Sharp, Sugden and Sambrook (1973) Biochemistry 12, 3055

C. Thomas exd(-) H. influenzae strain

9.8 g cells thawed in 27 ml 0.01M Tris pH 7.4, 0.01M β-ME.

Sonicated 30-60 sec bursts in ice/water bath 10X.
Added 5 more ml buffer Final conc = 9.8 g cells in 32 ml buffer
Centrifuged.

SN — TO 1M by adding solid NaCl.

Calculate - 0.0598 g NaCl / ml → 1M final conc =

27 ml SN
Added 1.62 g NaCl.

Loaded on 80 x 2 cm agarose column. Column vol. ≈ 480 ml.
Collected 6-7 ml fractions, starting from when all sample had entered gel.
Fractions assayed.

mix 375 μl λ DNA
125 HX 10x Hin buffer — NaCl
725 H_2O
1225

took 50 μl + 3 μl sample
digest in ~~~~ tubes 6-12
37 ml.

Hin

Pooled Fractions from Phosphocellulose Column -

Fractions
26 - 28

5 μl - partially digested 2.3 γ λ DNA

29 - 34 PEAK

1 μl - incompletely digested
3 μl completely digested
2.3 γ λ DNA

35 - 37 2 μl incompletely digested
5 μl Completely digested
2.3 γ λ DNA

D 97 One-dimensional sequencing with decamer (Ref. D 87)

1) This was a synthesis using the decamer and ØX and all four deoxy-triphosphates. Samples of the minus ones were put on homochromatography system, which looked O.K. though resolution of pyrimidines is still a problem. The rest of the material was all put on a gel which was shattered on drying.

2) Another similar experiment using the minus approach with coli polymerase and the plus approach with T4 polymerase. Samples were put on homochromo and 20% gel which was radioautographed without drying down for two days. This gave a good pattern and there appears to be resolution of pyrimidines whereas there is not on the homochromo.
Gel first radioautographed 1-2 days then dried & autographed 10 days. drying doesn't seem worth it. gives broader bands & lot of trouble with cracking.

3) The DNA polymerase used here is a batch from Boehringer which is less active than previous ones. Using 20 μl/100 μl incubation it gave quite good incorporation as judged from the Agarose column. A preliminary experiment was done with some of this to see if one could "spike" the sequence using exonuclease III. Various concentrations were used and the products run one-dimensionally on homochromo. There is definitely digestion but there still seems to be some specificity though the sites at which the product piles up are different from before.

4) The material obtained in (3) was used in another experiment. In this case more violent conditions were used for the T4 polymerase. The products were run on 5% unhydrolysed homomix. The plus ones look quite good here though minus ones are not entirely clear. The + and -G ones do not seem to line up with the others: maybe this is due to some

D 97 (cont.)

(4) cont.

different running effect, again suggesting that the acrylamide is better than the homo. This homomix (5% unhyd) obviously not good pyrimidines often don't give a jump.

5) Another experiment, essentially as above, running all the products on acrylamide.

6) Half of the material synthesised was treated with exonuclease to give different products and combined with the untreated material before running through the Agarose column. The pluses showed essentially nothing, suggesting that the exonuclease was still present, so the remaining material was extracted with phenol and then another experiment done (b). This gives a very clean picture with the minuses but pluses are still very faint and seem to have too many bands in them. Perhaps the trouble is due to treating for too long a time with the T4 polymerase (2 hours).

7) This is an experiment with increasing times of synthesis and also testing out the action of exonuclease using A labelling. There is clearly further synthesis under these conditions but only up to about 70 residues. The bands seem to pile up at A residues. In this case there was very little activity of the exonuclease.

D 97 (1) (see D 87)

S ~~10~~	G ~~A~~TP*	or new
H_2O	10	✓ ✓
S mix	5	✓
dA/20	5	✓ ✓
dC/20	5	✓ ✓
T/20	5	✓ ✓
φx	5	✓ ✓
deca	5	✓
new Kl	~~#~~10	

0° .5?, 1, 2, 4, ~~16~~ 8

counts up to about 60 on agarose

concentrate a bit.

take ~~10~~ 5 μl + ~~10~~ 5 μl −X mix ~~1~~ 2 μl (Kl) 0° 20' ✓

sample on [illegible] (this ok.)

rest on gel

T4 ~~5 5 5 5~~ 5 μl stuff 1 X/5, 1 T4 mix, 1 H_2O, 2 T4 pol ✓ ✓

~~sample~~ all on gel.

gel shattered on drying.

Wor T

D 97

(2)

10 GTP* (Dec 22 T.M. 1973) dry.
20 H_2O ✓
10 S mix ✓
10 dA/20 ✓
10 dC/20 ✓
10 T/20 ✓
10 φx (.6) ✓
10 deca ✓

10 KCl (should have been 20)

.5, 1, 2, 4 min.
10 μl .1 M EDTA.
to 70 on agarose.

Did expt as in (1) doing T4 pol 30' at RT (I think) ran samples
homo on acrylamide. (2 day radioautograph of wet gel

(3) repeat 5 μl GTP*
20 KCl

.75, 1.25 2 5 10 mins.
+ EDTA not run (ones 75)
agarose 1/1/74 to 170

down to about 50 μl.
PDK 20 μl ppt EtOH
dissolve 20 H_2O, take 10 μl 5 μl 10x buffer, ~~35 H_2O~~ 10 H_2O
(.075 M tris-HCl pH 8.
75×10^{-4} M $MgCl_2$ = buffer.
1 mM DTT (cleland's reagent)

10x = 100 μl M tris
1 μl M $MgCl_2$
15 M DTT
30 H_2O.)

take 5 μl samples a) –
b) 1 1/100 exo III 5' r.t.
c) 1 1/1000 exo III 20' r.t.
d) 1 1/100 " 5' "
e) 1 1/100 " 20 "
on homo 5' unhyd.

+ .1M EDTA.

D 97(4)
(−)

2 stuff D97(3)
2 − mix
1 pol KL 0° 20'

(+)

2 stuff
2 + mix
1 T4 pol 2 hr 37° 5.45 2¼ hr

+ mix's 20 S ✓
20 T4 mix ✓
40 H_2O ✓

run homo (5% unk)

D 97(5)

Combine remains of D 74(2) + (3)

+) 5 μl of 5 + mix (D 97(4)) 2 T4 pol.
37° 30'

−) 5 μl + 5 − mix 1 pol kl Jan 74.
0° 20'

25 μl formamide
dry.
heat
1 μl dye

20 % acrylamide

↙ perhaps in this length.

$C_+ \; C_- \; T_+ \; T_- \; A_+ \; A_- \; G_+ \; G_-$ [illegible]

[illegible] 300 V

D 987 (6)

5 μl ATP* dry.

pol mix is:
·2 M tris
·1 M Mg.
·01 M ME

10 pol mix ✓
10 G/20 ✓
10 C/20 ✓
10 T/20 ✓
10 φx ✓
10 deca ✓
30 H_2O ✓

10 Kl. (Jan 8)

2
20 μl. ·5, 1, 2, 4', 0° + 5 r.t.

into 100μl 1:1 phenol. wash ether
EtOH dissolve 30 μl H_2O
take ½ (15 μl) 2 μl 10x buffer ~~7 H_2O~~
1 μl 1/10 exo 10' r.t.

1 μl ·1 M EDTA.

combine agarose.
a)
5 μl for +'s & −'s
+ T4 2 hr 37°
− pol 30' 0°
C+T+A+G+ C− T− A− G−

5 μl untreated run 20 5% 10'
+'s nothing shown. All broken down presumably
exo is still active & not removed on agarose.
−'s ok but are bands that shouldn't be there suggesting
exo activity.

phenol remove, 3x ether.
5 μl + 5 μl +&− mix.
+ 1 μl T4 pol 7 hr 37° 3-30
− 2 pol (Kl) 30' 0°

b)
C+ T4 ... C− ... G−.

D 97(7)

5 μl ATP* dry.

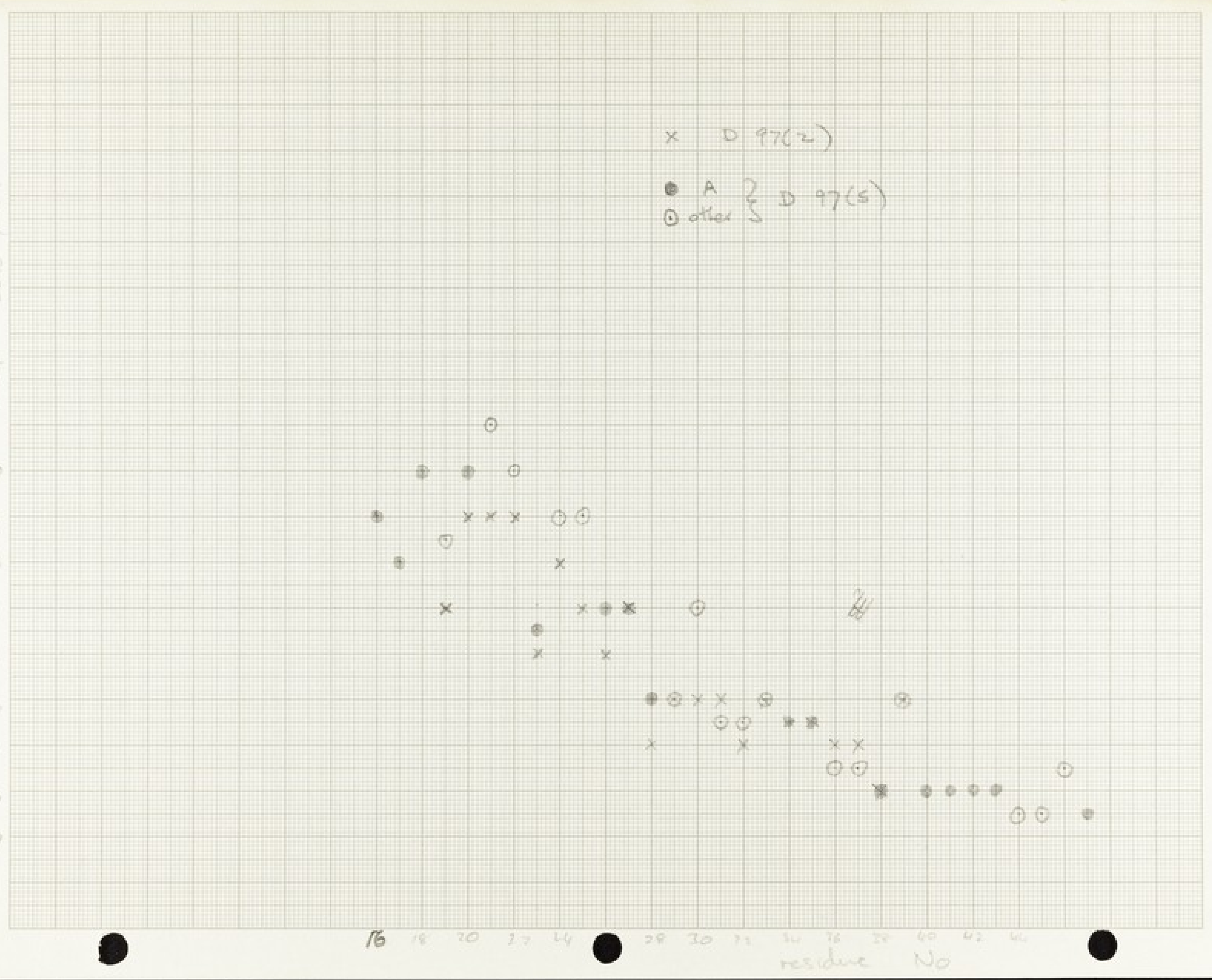
Pol mix
100 μl M tris. ✓
50 μl M Mg. ✓
5 μl M ME ✓
350 H_2O ✓

pol mix	5	✓
G/20	5	✓
C/20	5	✓
T/20	5	✓
φx	5	✓
decm	5	✓
H_2O	15	✓
klenow	5	

25 μl 0° 30'
phenol EtOH ✓ in 20 μl
a) 10 μl + formamide. ✓
b) " + 1 ✓exo buffer + $\frac{1}{10}$ exo 5' rt.

25 μl rt 20'
c) 10 μl + 25 μl formamide ✓
d) 1 μl $\frac{1}{10}$ ✓exo III 5' formamide ✓

		97(5)	97(2)	97(6)
16	A	·9		
	A	·8		
	A	1·0		1·6 (A, C)
	C	·4, ·8	·7	
20	A	1·0	·9	·9
	T	1·1	·9	2·0 (T, G)
	G	1·0	·9	
	A	·65	·6	·7
	T	·9	·8	·8
	T	·9	·7	·8
	A	·7	·6	·6
	A	·7	·7	·6
	A	·5	·4	·5
	C	·5	·5	·4
30	T	·7	·5	·5
	C	·9 (C, C)	·5	·9 (C, C)
	C		·4	
	T	·5	·5	·5
	A	·9 (A, A)	·9 (A, A)	·85 (A, A)
	A			
	G	·7 (G, C)	·4	1·1 (G, C, A)
	C		·7 (C, A) ·3	
	A	·3		
	G	·5		
40	A	1·2 (A, A, A, A)		
	A			
	A			
	A			
	C	·5 (C, C)		
	C			
	T	·35		
	A	·25		



× D 97(2)
A } D 97(5)
other
Jump 20% gel
1·0
·9
·8
·7
·6
·5
·4
·3
·2
·1
16
18
20
22
24
28
30
32
34
36
38
40
42
44
residue No

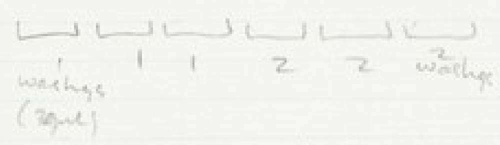
D 98 Preparation of Hin fragments (D 85, 89)

(1) 60 μl RF II 100 μl Hin (8/12), 50 Hin buffer, 300 H_2O

EtOH pptn dissolved 12·30

50 μl buffer 20 dye sucrose

+ 25 μl for washings



underdigested

50 μl RF II 50 Hin buffer 300 H_2O 50 Hin 1 hr + 50 Hin 3 hr

a is v partial.

b about OK but is hard above 1

added another 50 μl Hin 12-45 — 2½ hr

Hin 2 as above. 50 Hin 12-45. 2½ + 50 Hin 3½ hr

EtOH pptn 30 μl H_2O (+ buffer) + 20 buffer

washings (30 μl) 1 1 2 2 washings

OK but overloaded

one has 2 bands behind 1

6's not resolved

7's probably OK. but looked not so good on one of them.

9 hardly visible but cut rather wide

eluted + dissolved in 50 μl.

-2, -1, 1, 2, 3

D 99 One-dimensional sequencing with restriction enzyme fragments (ref. D 93)

Restriction enzyme fragments from D98 were used for extension using labelled A and purified on Agarose. Samples of these were digested with Hin and run on a 2D system, but essentially nothing was visible so the remaining material was precipitated with ethanol and dissolved in water and treated with Hin. The product was then run on a 20% acrylamide gel. Most of the material seemed to be near the top, suggesting it was linked with the primer except in the case of 8, which had moved down. Possible explanations of this are that the Hin has not worked, or else that the products formed are too short to be present on the gel, or that the labelling is simply onto the double-stranded Hin fragment.

2. Samples of 8 & 5 subjected to the ~~[illegible]~~ — treatment followed by Hin. Run gel. Nothing in right place. Main band is where unchanged 5 or 8 would be expected (Actually are several bands close together). Then lots of bands moving out in front of it which seem to vary with different —'s. Can't make it out.

3. Expt with samples of 7b & control with deca. Best one is d which is with G labelling. A does not seem to have worked well. Blank with 7b alone higher than blank with φx. Samples from front of b, c, e run on homo & all run v. fast: no small ones suggesting A label really hasn't worked

4. Similar expt using hot G. Main thing is v much better with Klenow enz, though do get some bands with JD enz. No Hin control (g) looks OK. Doing phenol ethanol treatment at either stage does ~~not~~ not help, so simplest procedure is best.

5 With proper blanks & using Klenow enz. φx blank has got stronger but get good bands in e which is standard procedure. Using A or less G seems bad (though probably something has gone wrong with h. JD enz bad. Could be this due to 5' exo during Hin cutting stage

D 99

Jan 74.

5 µl (1/10) 3, 5, 7a + 8 (D 98).
1 µl φx (OD 75) (3 µg)
boil 3'
1 µl NaCl-tris (D 89(3))
67° 11-15 -

10 µl *ATP dry

20 dC/20 ✓
20 dG/20 ✓
20 T/20 ✓
40 H_2O ✓

5 µl	3	5	7a	8	
mix	25	25	25	25	✓
S mix	5	-	5	-	✓
pol mix	-	5	-	5	✓
stuff	5	5	5	5	✓
H_2O	10	10	10	10	✓
pol (K)	5	5	-	-	
pol T4/20	-	-	5	5	

0° 10 µl 1 . 2 . 5 . 10 . ~~20~~ + 5' rt.

5, 8 10 µl ·2 M EDTA
3, 7a 5 ·1

agarose vol approx ·2 ml
20 µl 2 10x buffer 1 Hin (E.B.) 9.45. 4 hr.
40's
wait EtOH ppt.
25 µl H_2O ✓ ✓
take 5 + 1 buffer. 2 H_2O. 2 Hin 9.30 ← Hae (D 97(?))
run 20% gel

(2)
8 add 10 µl H_2O take 5 µl for -'s 1 µl Jan pol 0' 30' ½ µl
5 .. 30 5 .. 30' ?
4 ml H_2O 20 µl conc. take ·4 + ·2 phenol wash 3x ether EtOH ppt
Sugar 4 hr! reppt^d. !!!
20 buffer → 200, take 20 2 Hin

D 99(3)

10 µl 76 (12/12)
1 φx (00 75)
2 10x Hin buffer
7 H_2O Heat . anneal 9.00 4 hr
add 3 µl 10X Hin buffer

10 hot A
1 · G

A mix
10 hot A dry .

~~[illegible]~~ 10 H_2O ✓
T/20 10 ✓
C/20 10 ✓
G/20 10 ✓

	a	b	c	d	e	f	g	h	i	
76 φx mix			4	4	4	4	4	–	–	✓
76		2								✓
φx (00 15)	1									✓
A mix	4	4	4	–	4	4	4	4	4	✓
10x Hin buff / ~~H~~	1	1		1				1	1	✓
T/20				1						✓
C/20				1						✓
A/20				1						✓
hot G				dry+						✓
φx								1	1	✓
deca								1	1	✓
H_2O	3	2	1	1	–	1	1	2	1	✓
pol JD	1	1	1	1		1	1	1	–	✓
pol (Kl)					2				2	✓

30' 0°
freeze

g, phenol, ·1 ·1 H_2O, EtOH ppt.
10 µl H_2O 1 Hin buffer

all → 1 hin 1 hr , a – g + 1 hin
(f added tRNA H_2O + vac'd down) 10 µl H_2O.
all except f ph EtOH
20 µl carrier in 5 ml H_2O (20k .5 ml + .2 phenol
EtOH ppt .
10 µl H_2O EDTA ?
25 formamide

D 99(4)

10 76
5 φx (on 15)
3 H_2O

3' 100°

2 μl Hin x10 buffer
67° 11-21

G mix
10 μl hot G dry.
T/20 10 ✓
A/20 10 ✓
C/20 10 ✓
H_2O 4 ✓
Hin x 10 bf 6 ✓

then add 20 G mix ✓
[illegible] d ✓
not (c, e, f, g)
4 H_2O ✓ 4 70

	a	b	c	d	e	f	g
φx (15)	1	–	–		✓		
76	–	2	–	✓			
76 φx			4	4	4	4	4
G mix	4	4 ✓	4	4	4	4	4
H_2O	4	3 ✓	1	~~[illegible]~~ –	1	1	1
pol 7A/20	1	1	1		1	1	1
pol kl	✓	✓		2 ✓			

10' 12

f. 0·1 ml H_2O 0·1 phenol ether, ethanol. 10 H_2O 1 buffer.
a, b, g. ·1 ·2 M EDTA ✓
c d e f 2 hin. 8·35 2 hr 37°
a, b, c, d, f, g. ·5 ml H_2O(f) – E DNA. ·2 phenol. EtOH
~~[illegible]~~ 10 H_2O
all ~~[illegible]~~ 1 μl ·2 M EDTA, 25 μl formamide.
15% short gel

D 99(5)

10 μl 7b, 3, 5, 7a, 8 (12/12)
5 φx
3 H_2O

heat.

2 Hin x10 buffer

67° G-45

7b + 5 ~~H_2O~~ hin buffer. ✓

mix		
T/20	10	
A/20	10	✓
C/20	10	✓
buffer	6	✓
H_2O	4	✓

	a	b	c	d	e	f	g	h	i	
hot G =	·5	·5	·5	·5	·5	·5	·2	·1		
hot A =									2	
φx	1									✓
7b		2	2							✓
7bφx	–	–	–	4	4	4	4	4	2 4	✓
mix	4	4	4	4	4	4	4	4	–	
4x Hin	1	1	1				~~Hin~~		1	✓
T/20									1	✓
G/20									1	✓
C/20									1	✓
H_2O	2	1	1	–	–	1	–	–	–	✓
pol (kl)	2	2	2	2	2	–	2	2	2	
h JO						1				
			0	+5		·5				

30' 0°

b, d (– Hin) ... 1 μl 2 M EDTA
rest 2 μl Hin. ~~600~~ 2 hr
1 μl ·2 M EDTA

all 25 μl formamide.

D 99(6)

take 3, 5, 7a 8 (D 99(5))
+ 70 mix
10 pol (kl)

30' 0°

mix
10 hot G dry
20 5 T/20 ✓
20 5 A/20
20 5 C/20 ✓
15 buffer ✓
10 H_2O ✓

(6) take 5 µl + 1 Hin 3.30 2 hr
1 EDTA.

~~rest put through~~ 20 formamide 15% gel
(On gel with D 105) Look quite decent simple picture.

(7) rest put through agarose. dry to about 20 µl
→ 50 µl

mix's with Hin buffer.

	−C	−T	−A	−G	+C	+T	+A	+G	
Hin x10	10	10	10	10	10	10	10	10	
C/100	−	10	10	10	✓				
T/100	10	−	10	10	✓				
A/100	10	10	−	10	✓				
G/100	10	10	10	−	✓				
C/S					10				
T/S						10			
A/S							10		
G/S								10	✓
H_2O					25	25	25	25	✓

take 5 / 12 or 11

5 Ø 1 Hin x10
4 H_2O

5+10
5 + 8 −'s. 5 µl − mix 5 µl 5 or 8, 1 µl (kl) 30' 0
8 + + 8 1 T4 30 37
2 µl Hin ~~4 o.s.~~ 2 hr 37°
20% gel this-gly all the way.
80 8+C → +G 8−C → −G, 5−C → −G.

6 x 5 362
8 x 5 400

(9) 7a from this was + & −ed, & run on a 20% gel with borate. Not too bad.

D 99(8)

Annealed.

(<)10 μl	8	5	φx	3 H_2O
10	7a	"		—
>10 μl	5	"		?
≠10	1	"		"

heat 2 μl Hin x10 buffer anneal

8.

G mix / hin buff — T mix

	G	T	
label dy	$\frac{1}{20}$ G	$\frac{1}{20}$ T	
φx 8	2	2	✓
- G h mix	2	-	✓
- T -	-	2	✓
≠ kl	1	1	✓
	30'	0°	
hin	1 μl		✓

N.B. This has 1½ times Hin buffer

run haste 20% gel 4-45

	G	T	A	C	
	$\frac{1}{10}$ G	$\frac{1}{5}$ T	1 G	1 G	✓
φx 8	4	4	4	4	X
buffer 6x	1	1	1	1	X
C/20	1	1	1	—	X
T/20	1	—	1	1	X
A/20	1	1	—	1	X
G/20	—	1	—	—	X
A/10,000			1		X
C/10,000				1	X
pol (Kl)	2	2	2	2	

mucked up 8 so repeat on 7a

30' 0° remove 1-2 μl, 2 μl hin mix (7½ H_2O, 1 buffer 2½ hin)

~~take about 2 μl~~ ~~1-2 M EDTA~~ ~~hot~~

rest 1 μl 2 M EDTA combine agarose.

to 50 μl

5 μl + 5 μl H — mix 1 pol (Kl) rt 30'

5 μl H + mix 1 T4 (new batch) 37 30'

2 hrs 500

8 D 99(7)

+ −

T (G) C T
] 1·5] 2·5] 1·8
C G
~~G~~
G
C A] 1·3
? A A ·7] 1·1
T] ·7] 2] 1·4

A G

~~GGT~~] 1
A (T) G?
G C] ·4] 1·2
C
C G
G A] ·6
(A)] ·7 ·8] ·4 } v close
(C)
T
G A
~~A~~] ·7
G A
] ·8
A T
T G A G] ·3 TGA
] ·7
A G

~~C~~ C
C G
G

A
T

D 99(8) 7a

−	+
C	
T	
	A
G	(C)
(C)	G
C	C
A	A(C)
T	T A
G	G (T)
A	(T) A
C	C (A)
G	
T	
G	

barrier.

A bit unsatisfactory
maybe 2 sequences

D 99(9)

7a

−	+
	C
	G
A	
	T
	C T?
	G
A	A
G	A
G	
C	C
C A	A
A	
G	
C	G
A	A
C	C
G T	G T
C	C
T A	A
	T
G (C)	G
C	C
A	A
G	G
T	T
A	
G	G
A	A
(A)	
T	T
	C
A_n	
	A
C	
G	G A
	A
T	G
A C	C T
	A
G	
	G (T)
A	A

−	+
G	G
A C	C
T	T
C	
G	
R T	
C	
T	

D 100 Sequencing with decamer (ref. D 97)

1) In order to vary the complexity of the product for the one-dimensional sequencing, tried preparing on material with different hot triphosphates using considerably longer times of incubation than previously. The products were run through agarose and samples put on 15% acrylamide. Samples of the G labelled one were also treated with exonuclease III. There is a fantastic difference in the size of the products formed, the G labelled being very much the largest. This also appeared to be the hottest from the yield off the agarose. The size of the C labelled material is intermediate and seems to be about what is required.

2) 40 µl A, 20 µl C, 10 µl G from the previous experiment were combined, dried down, dissolved in 50 µl water and 5 µl taken for the plus and minus treatment. For the plus took 1 µl T4 polymerase for 30 min at 37°: for the minus 2 µl polymerase (Klenow - December) for 30 min at 0°.

2. Expts with low concs of triphosphates to get conditions & a & b were same amount of A but diff times, no difference showing incorpn is probably complete in 30 mins.

3. On the basis of 2 chose amnts of TP's that would give similarly length shift. Each one run through agarose separately. Samples of these were A_0 G_0 C_0. equal amnts (40 µl) combined & + & − ed (20 µl lots for − & ¼ for direct running in a. The remainder combined put on ~~acryla~~ agarose & redone i + & −. These have got v big presumably when I mixed them to put on agarose they had all 4 TPs & were extended. + s from this look good but uninterpretable. Unfortunately all the −'s here are mainly stuck at top!!. +'s look pretty good

D 100 (1) (see D 97)

5 ATP* .. ing .
5 GTP*
5 CTP*

	A	G	C	
~~pol~~ mix	25	25	25	✓
A/20	–	5	5	✓
G/20	5	–?	5	✓
C/20	5	5	–	✓
~~T/20~~				
H_2O	5	5	5	✓
pol KRe	10	10	10	

~~pol mix~~
~~φx~~
~~deca~~

pol mix	10-30' 20	✓
T/20	20	✓
φx	20	✓
deca	20	✓
H_2O	20	✓

10 μl ~~1, 2, 5, 10, 10~~ 5 ~~10~~ 15 30 60 0° + ~~30 rt~~ ~~rt~~ 15' 37°.

10 μl 2 M EDTA
agarose
A to about 100
~~G~~ C 200
G 300

10 μl A
5 μl C
5 μl G

a)	5 μl G	1 exo buffer	~~3~~ $3H_2O$	1	exo/100	10'	rt
b)	5	1	2	2			
c)	5	1	–	4			
d)	5	1	3	1	exo/10		

* – A C G a b c d 20% gel

Exo does not seem to do much, but a bit hard to say as G gone so far.
Is big difference between labels G > C > A. Piling up seems to be at radioactive TP (certainly for A). This could be simplest way of doing sequence.

D 100

C
T .8
A 1.1
A 1.3
G .6 1.1
C .9
A .6
G .5
A .4
A .5 1.8
A .5
A
C
C 1.2 .8
C
T .5 .9
A .4 1.1 2.8 2.4
C cc .7
G .5
C G 1.0
G
C .3
T .3
T 1.5 - 1.2
T C
T
G .1
G
G
A .9
A
C
C
C
T
A
G

CTTCXGT
CGC

CTT G
CTT GTG
CTTCXGTG

CTTCTG

CTTC GT

CCGCGCTTCGTCCAA - -

D 100 (2)

2 μl dATP* dry a
1 μl dGTP* → 20 take 5 (b) 2 (c) 1 (d)
1 CTP 20 5 (e) 10 (f) } 5 μl.
1 g (g)

C mix

A/20 5 ✓
G/20 5 ✓

G mix

~~mix 8 16~~
~~dG dA CTP 20 4~~
A/20 5 ✓
G/20 5 ✓

mix

pol mix 5 ✓
T/20 5 ✓
φx 5 ✓
deca 5 ✓

~~ix for a~~

~~G mix~~

	a	b	c	d	e	f	g	
	2 ATP*	·25 G	·1 G	·05 G	·25 C	·5 C	1 C	
mix	4	2	2	2	2	2	2	✓
G/mix	–	1	1	1	–	–	–	✓
C mix	–	–	–	–	1	1	1	✓
G/20	1							✓
C/20	1							✓
H_2O	2	1	1	1	1	1	1	✓
pol (ke)	2	1	1	1	1	1	1	

45' 0° 4-15.

a1 5 μl 45'
a2 2½ hr

3P
a1 → g

D 100(3)

10 A /50
2 G /100
5 C /50

Pol mix	20 ✓
T/20	20 ✓
ϕx	20 ✓
deca	10 ✓
H_2O	30 ✓

	A	G	C	
mix	25	50	25	✓
A/20	–	10	5	✓
G/20	5	–	5	✓
C/20	5	10	–	✓
H_2O	5	10✓	5	✓
pol (Dec)	10	20	10	✓

30' 0° — ~~[illegible]~~

~~10 μl~~ → No EDTA. Agarose → 200 [illegible].

vac a little.

about 100 μl

take ~~45~~ 40 each. = 120 0 = 5 μl

– 20	+ 20	– mix	5	pol (Dec)	30'	0°
+ 5	5	+ mix	1	T4 pol	30'	37° (A spilt & picked up.)

a) take 10 μl – 's.

remainder of –'s mixed, agarose redone

b) 5 μl samples.

O, T+ G+, C+ A+, T– G– C– A–

– ones here gave lot at origin ∴ repeated.

c) A_0 C_0 ~~[illegible]~~ C– T– A– G– ~~G0~~ $\frac{A}{G}$0. G0

D 100(3)

+		−	
C	.6	C	.6
C		G	
G	.5	G	.5
C	.2	G	
G	.3	C	
C	.3	T .4	
(T)	1.2	T	1.3
(T)	1.5	(T)	
T		(C)	1.5
C		G	
(G)		G	
T	2.2		
G	2.3		
C			
T x			
A			
T			
C			

ones taken for depurination:

← T

← G

← C

Depurinations

3 strong bands from the T C + G + columns were eluted + depurinated. It is possible to suggest what diff ~~bans~~ bands are:

From known seqs would expect:

TCT, C, T, TT, CTCCT, C, CCT, CC

G + T bands there and a band that could be C_2T_2, would fit CTTC seq

C sample seems to have extra CC + (TC) suggesting there come between

D 100(3)
O's

Agrees with 100(4) roughly.
~~At v. strong C (probably at least CC)~~
A T A C gives a good C then
~~C A G~~ nothing till coms of C (v strong)
at least CC! no sign of G here
possible faint A

A T̊ A C - ~~G~~ C ~~T~~ A C T C perhaps.

then C A G C (Â) G A A

then? C's, A T T (A)$_n$ C T (G) T A C̲ G$_n$
(from 100(4))

from T's
strong TG then C T ... A ~ T ~ C̲ T ~ G ~ C

D 100(4)

Remains of AG from 100(3) + some of from 100(1)

20 μl + 20 μl - mixes 5 μl pol (Dec)
5 μl ·2 N EDTA in each
remove 10 μl for running. freeze. (1)
combine rest + agarose, dry
dissolve 50 μl

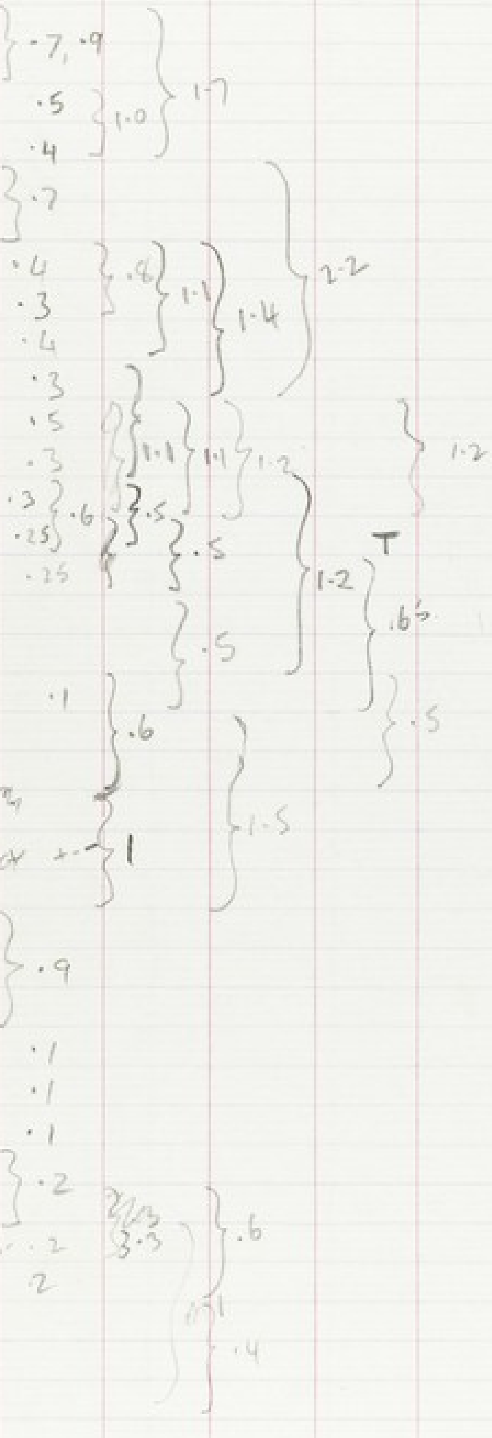
+ 5 + 5(4 mix) 1 T4 pol 30' 37°
− 5 + 5 - mix 2 pol (Dec) 30' 0°
1 μl ·2 N EDTA.
25 μl formamide. (2)

$\bar{A}1$ $\bar{A}$ $\overset{+}{A}$ $\bar{T}$ $\overset{+}{T}$ $\overset{+}{G}$ $\overset{2}{\bar{G}}$ $\overset{+}{C}$ $\bar{C}$

(Trough buffer pH 8.75)

Picture looks pretty good. But are a few wrong bands eg G⁺'s in AAAA part (40-43), so interpret- slightly doubtful. Problem is jumps get v small around the 55-60 region

D 102(4)



D 10/1. thin fragment primers rC. (92 94)

20 µl 3
20 µl 5
4 µl φx

4 NaCl-tris 5.45

	3A	3G	5A	5G	
3	1/4(?)	1/4	–	–	✓
5	–	–	1/4	1/4	✓
Int A	+	–	+	–	} 5 µl, dried
Int G	–	+	–	+	
A/20	–	10	–	10	✓
G/20	20	–	10	–	✓
T/20	10	10	10	10	✓
rC	10	10	10	10	← S mix 10 } √50 ←
H_2O	13.5 (10)	13.5 (10)	13.5 (10)	13.5 (10)	✓
pol K	10	10		–	
pol $\frac{TD}{20}$	–	–	10	10	

ie T/20,

mix: S mix 50 ✓, rC 50 ✓, T/20 50 ✓, H_2O 100 ✓

15' 0°

200 H_2O
200 phenol
2 x extn ether.
EtOH ppt.

80 H_2O . 10 thin x10 buff. 10 µl thin 12.30 – 3.30

ppt EtOH 20

These look pretty awful with most of counts on origin.

D 107.

Products run on 2D homo; stuff at origin was 1. This & other spots were cut out & pancd.

5A1 & 3G1 run 2D others 1D.

Some of panc spots n.r.ed.

~~3G1~~
5A1 (2D)

1. A, T
2. C A T
3. C A G T
4. A G (tr C, T)
5. A (tr C)
6. C
7. T (tr C & G)
8. T

5A2 (hm 1D)

1 A
2 A
3 C
4 A

Doesn't seem to correspond to 5A1 v well.

5G3

1. T
2. band ahead of T

may correspond to 5A2/1 or 2

3G1 (2D) looks rather complex

1. T, G
2. A
3. A, G (tr C + C)
4. G (tr C + T)
5 A.

3G2 (1D)

1. C (tr A)
2 T

3G3 1 C = 3G2/1)

3G4 1 C

3A1 1 T
2 C (tr A)

Some question that samples might have got mixed so all a bit difficult

D 102 Radioactive restriction enzyme fragments

This was an attempt to make radioactive Rf by extending the nicks with DNA polymerase. The product was digested with Hin and run on acrylamide. It was stained up and gave a normal pattern, but on radioautography certain of the bands seemed much stronger than the others. These are particularly band 3 and band 8, which are probably in fact near to the site of the specific nick responsible for replication. The actual counts are not very high so that this method does not seem of very general use.

D 102.

5 μg RF II (10 μg) ✓ 5 ~~[illegible]~~ wt ~~GTP~~ CTP.

10 Pol mix ✓
10 T/20 ✓
10 A/20 ✓
10 G/20 ✓
545 H_2O ✓

10 Pol/20 (S.D) ✓

20 mins / 0° rt.

agarose ^EtOH ppt Hin acrylamide sample

5 min buffer, 10 min ~~3H~~ 35 H_2O
~~H~~ " 9 hr

EtOH ppt.

Reverse transcriptase.

	a	b	
mix	5	5	✓
octa	—	1	✓
H_2O	3	2	✓
R.T	2	2	

r.t.

1 µl at 2', 5', 10', 20' ~~[illegible]~~

5 µl hot CTP*

25	buffer		✓
2	10 mM	dATP	✓
2	..	dGTP	✓
2	..	TTP	✓
10		φx	✓
10		H_2O	✓

got nothing

20 hot C dry.
10 above mix

	a	b	
mix	5	5	✓
deca	~~[illegible]~~ –	1	✓
H_2O	3	2	✓
R.T	2	2	~~[illegible]~~

(half 10 of 99B.)

37°

5' 10' 20' 40'

	a	b
0	95 ~~[illegible]~~	00
5'	3800	4200
10'	3600	2800
20'	3600	4300
40'	8040	8500

?

back in at 37° 1130 – 6 30
3544 3760

hopeless.

Unsuccessful experiment trying to see what happens with rev trans.

D 103 Low concentrations of triphosphates (see D 100(2))

(1)

1 μl A dry in 10 H_2O
1 G 20
1 C 10

	·2μM a	·4μM b	c	d	e	f
A*	1	2	–	–	–	–
G*	–	–	1	2	–	–
C*	–	–	–	–	1	2

dry.

pol mix	10 ✓
T/100	10 ✓
ΦX	10 ✓
deca	5 ✓
H_2O	5 ✓
take a → b.	
H_2O	10 ✓

	a	b	c	d	e	f
pp mix	2	2 ✓	10	10	10	10 ✓
A/100	–	–	2	2	2	2 ✓
C/100	1	1 ✓	2	2	–	– ✓
G/100	1	1	–	–	2	2 ✓
H_2O	–	–	2	2	2	2 ✓
pol	1	1	4	4	4	4

30'

From 100(2) seems at low concn of a hot TP piling up occurs at that residue, suggesting even simpler approach to sequencing. This expt done & run on 20% gel. Shows appropriate piling up though are some bands that should not be there. Bigger things are streaked. Trouble we are having with denaturation (see 100(2) + 104(2))

D. 103 (2)

C used here was some rescued from waste bin by GGB & believed to be ½ strength thus figs below are ½ what I actually took. In fact seems to be pretty weak judging from extent of synthesis

	−A mix	−C mix	−G mix	
~~d~~ C/100	5	–	5	✓
G/100	5	5	–	✓
A/100	–	5	5	✓
H_2O	10	10	10	✓

mix ~~d~~

φx	10	✓
T/100	10	✓
deca/2	10	✓
pol a mix ~~H_2O~~	10	✓
~~H_2O~~	~~5~~	take 2/5

	a	b	c	d	e	f	g	h	i	
hot A =	1	·5	1							
" C =				·1	·1	·2				
" G =							·1	·1	·1	
mix	10	2	2	4	2	2	10	4	2	✓
−A mix	10	2	2							✓
−C mix				4	2	2	~~10~~	~~4~~	~~2~~	✓
−G mix							10	4	2	
pol kl	5	1	1	2	1	1	5	2	1	

5 μl samples

1	9′
2	17′
3	30′
4	60′

EDTA. 1 ·1 / 5

sp. a1, a2, a3, a4, d, g, b, e, h, c, f, i

Seq looks pretty good with no sign of artefact spots. Extent of synthesis depends on TP concn not time. (a1 (7′) is same as a4 (60′))

Gel tho here was prerun for a bit. This lowers artificial front but may also lead to slightly fuzzier bands (though this could be due to smaller slots at top.

D 103(3)

~~1 old A → 10 lab 5 for b~~
1 old G → 10 2 d
1 lab C (J.W.S) → 10 2 g

	a	b	c	d	e	f	g	
hot	10 5/20 ✓	·5 old A/10	1 5/20 ✓	2 ·45/10	1 5/20 ✓	1 5/20 ✓	2 lab C/10	✓
mix	2	2	2	8	2	2	2	✓
C/100	1	1	1	4	1	1	—	✓
~~T~~ A/1000 ~~/100~~	1	—	—	—	—	—	—	✓
A/100	—	—	1 ✓	4	1	1	1	✓
G/100	—	1	—	—	—	—	1	✓
pol (K)	1	1	1	4	1	1	1	

mix (2)
5 φx
5 deca/2
5 pol mix
5 T/20

30′ 0° 1 hr 0° 30′ r.t.

New G & A systems have gone rather far but does appear that old ones may be a bit more specific though hard to be certain. Looks as if f (room temp) might be a bit more specific

Depurinations

Some bands depurinated
Expected fragments from G label to up to 50 GA are TCT
T
CC

these are present in 4 (this is a mixture piling up at the 2 Gs
— CCGC

3 has also C & one slower than TCT, rel rate $\frac{x}{TCT}$ = ·65. According to Ziff's film this could be T_2C_2 (ratio ·6) or T_3 (·7). Thus probably is ~~T~~ CTTC (which is expected). T_2C is v strong suggesting may be another residue
1 & 2 has extra C. Can't really account for this corresponds to G's in seq GCG (106(4)) but this shouldn't label C in 2

see quantitations.
2 T_2C's in 1 & 2
2 C's in 1

D103(3) - Depurination quants

	cpm	cpm -blank	
1 C_2T_2	282	200	1·2
TCT	428	346	2·0
CC	244	162	·9
T	258	176	1·0 -
C	399	317	1·8
2 C_2T_2	749	667	1·2
TCT	1286	1204	2·3
CC	630	548	1·0
T	607	525	1·0 -
C	649	567	1·1
3 C_2T_2	217	135	·4
TCT	483	401	1·3
CC	415	333	1·0
T	400	318	1·0
C	390	308	1·0
4 TCT	221	139	1·3
CC	150	68	·6
T	188	106	1·0

Blank. 82

D 104. Trouble with denatⁿ (100(3), 103)

mix the lot A C G (D 100) ran a bit

a) 5 μl + 20 formamide
b) 5 μl + 5 μl old T- mix 2 pol (Dec)
c) " " new " 6'
d) as c, terminate with 1 μl ·2 M EDTA
e) " " don't dry formamide
f) 5 5 new 1 μl (Jan) 3' ✓
g) " " " 1 " (JD) ✓ ✓
h) as C put on in SDS mix. ✓

(for c take 70' + 20 ? pol 10 μl aliquots)

30' 0° d 1 μl ·2 M EDTA
b - g 25 μl formamide.
h. 20 μl SDS mix 3% SDS, 10% ME, 20% glycerol

a, b, c, d, f, g dry.
all heat & put on.
20 cm 20% gel

Idea was to see which have garbage stuck at origin
a) no garbage bands diffuse.
b) garbage thus T-mix OK.
c) "
d) No garbage. This is the best
e) V ~~v~~ little garbage, thus drying off water seems to be harmful
f) garbage
g) Not much garbage but bands diffuse suggesting exo
h) garbage

Thus seems important to add EDTA presumably renaturation takes place if Mg present. Best not to dry off. Seems prot is also involved as pol JD gives no trouble & T4 was OK (D 100(3)

D 105. Sequential extensions with − mix's 29/1/74

10 A dry.

25 A⁻ mix ✓ (dTTP)
5 φx ✓
5 deca/2 ✓
5 H_2O ✓
10 pol (dec) ✓

5 µl ·2 M EDTA agarose → 100 ctz.

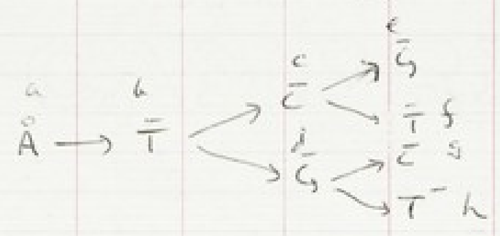
a run down dissolved 50 µl.

b Take 40 + 40 −T mix 5 pol (kl)
5 ·2 M EDTA
agarose dry.
in 22 µl Keep 2 µl (T1)

c −C 10 µl + 10 −C mix 3 µl pol
d −G −G
2 ·2 M EDTA
agarose run down. (agarose in −C tryed as ...)

15 µl H_2O 5 keep A-T-C
A-T-G

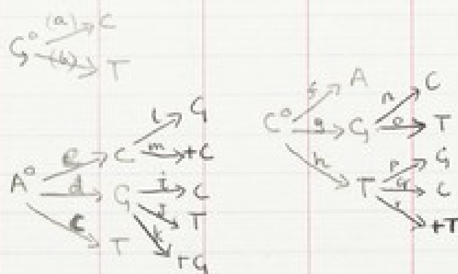
e C 5 µl + 5 µl −G mix 2 pol (k')
f −T
g
g G −C
h −T



105 (2)

4 μl G → dry, 10 take 5

hot	5	
C/10,000	10	✓
T/20	5	✓
A/20	5	✓
pol mix	5	✓
φx	5	✓
deca/2	5	✓
pol (kl)	10	



5 μl ·2 M EDTA agarose = C°
→ 40 μl

a)	5 μl	G°	(0 105(4))	5 μl	C⁻ mix	1 pol	G → C
b)	5	G°	"	"	T⁻	"	G → T
c)	5	A°	"		T⁻	"	A → T
d)	20	A°		20	G⁻ "	5	A → G
e)	20	A°		20	C⁻	5	A → C
f)	5	C°	(above)	5	A⁻	1 pol	C → A
g)	20	C°		20	G⁻	5	C → G
h)	10	C°		10	T⁻	2	C → T

d) (A → G) 1/4 (60 μl) 1/4 = d.

i)	5 d (+ add 5 μl to dry stuff)	5	C⁻ ✓	1		A → G → C
j)	5	5	T⁻ ✓	1		A → G → T
k)	5 d	5	G⁺ ✓	1	T4 pol	A → G + G

e (A → C) (trouble with agarose forged a track 50 μl) dry. 1/3 = e

l	5 e	5	G⁻ ✓	1	pol	A → C → G
m	5 e	5	C⁺ ✓		T4	A → C + C

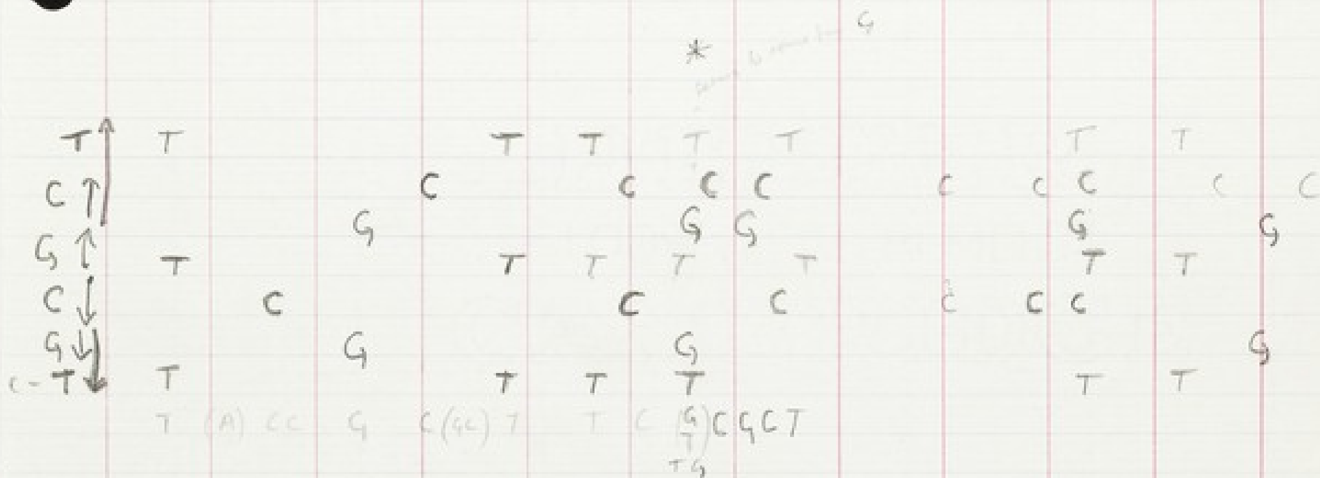
g (C → G) 1/4 = g.

n	1/2 10 μl	10	C⁻ ✓	2	pol	C → G → C – dry take 5 μl
o	1/4 5	5	T⁻ ✓	1	pol	C → G → T

h (C → T) 1/5 = h.

p	1/5	5	G⁻ ✓	1	pol	C → T → G
q	5	5	C⁻ ✓	1	pol	C → T → C
r	1/5	5	T⁺ ✓	1	T4	C → T + T

D 105

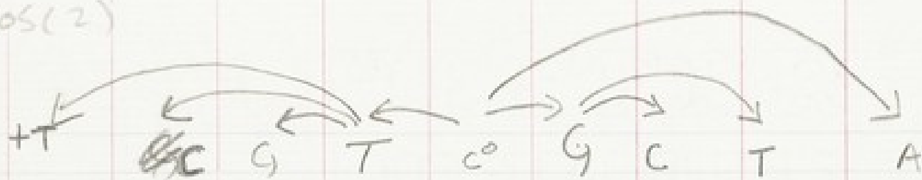


Fairly complex expt but seems to have worked o.k where seq known piling up as expected.
Note the second GC is not seen (this would be expected from seq. Still problem where the v small jumps occur.
* in the G→T it looks as if The T here comes from the G which is slightly ~~ahead~~ behind of it. A similar thing seems to occur with the ~~faster~~ & largest G band which with T- mix gives nothing higher up but a ~~some~~ strong one appears below.
Suggests possibility that larger ones may sometimes travel faster than slower. That's a problem. Presumably due to 2ry structure?
JF has good evidence for TTC(G) in a pane of rC suggesting seq.

T A C C G C G C T T C G T C G C T ?

though doubtful if we can't rely on order.

105(2)

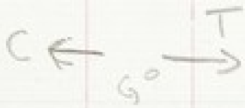


+T	C	G	T	C	G	C	T	A
	C			C				
	C			C				
				C				
			T					
+T								A
								(A)
		G	G		G			
	C			C		C		
								A
		G	G		G			
								A
	C			C		C		
				C				
			T				T	
+T								A
	C			C				
		G	~		G		T?	
				C		C		
					G			
	C			C		C		(A?)
			T				T	
			T				T	
+T	C							
		G			G		T	
						C	T?	
		G?			G			
							T	
								A

TTCGTCTGGGTCA

TTCGCTTGGTCA

105(2)



C	G	T
	G	
C		
	G	
C		
		T
	G	
C		
	G	
C		
		T
	G	T
C		
	G	
	G	
		T
C		
C	G	
		T
	G	
C		
	G	
C		
		T
		T

probably G(C T/G G T C

space

D 105 (3)

5 g G* dry

10 's (as D 107 (2))

15' 0° 5 μl ·2 M EDTA → 200

divided into 20, 20, 10 μls 1 for G° dry

C' . 20 μl G°, 20° C⁻ mix 5 μl pol. aq. → 25 μl
T' 20 G° 20° T⁻ 5 → 25
A' 10 10 A⁻ 3 → 15

a) 5 μl C' 5 μl G⁻ ✓ 1 pol C → G⁻
b) " G⁺ 1 T4 C → G⁺
c) " T⁺ ✓ 1 T4 C → T⁺
d) " T⁻ ✓ 1 pol C - T⁻

e) 5 μl T' 5 G⁻ ✓ 1 pol T → G⁻
f) " " C⁻ ✓ 1 pol . T → C⁻
g) " " C⁺ ✓ 1 T4 T → C⁺
h) " A⁺ ✓ 1 T4 T → A⁺

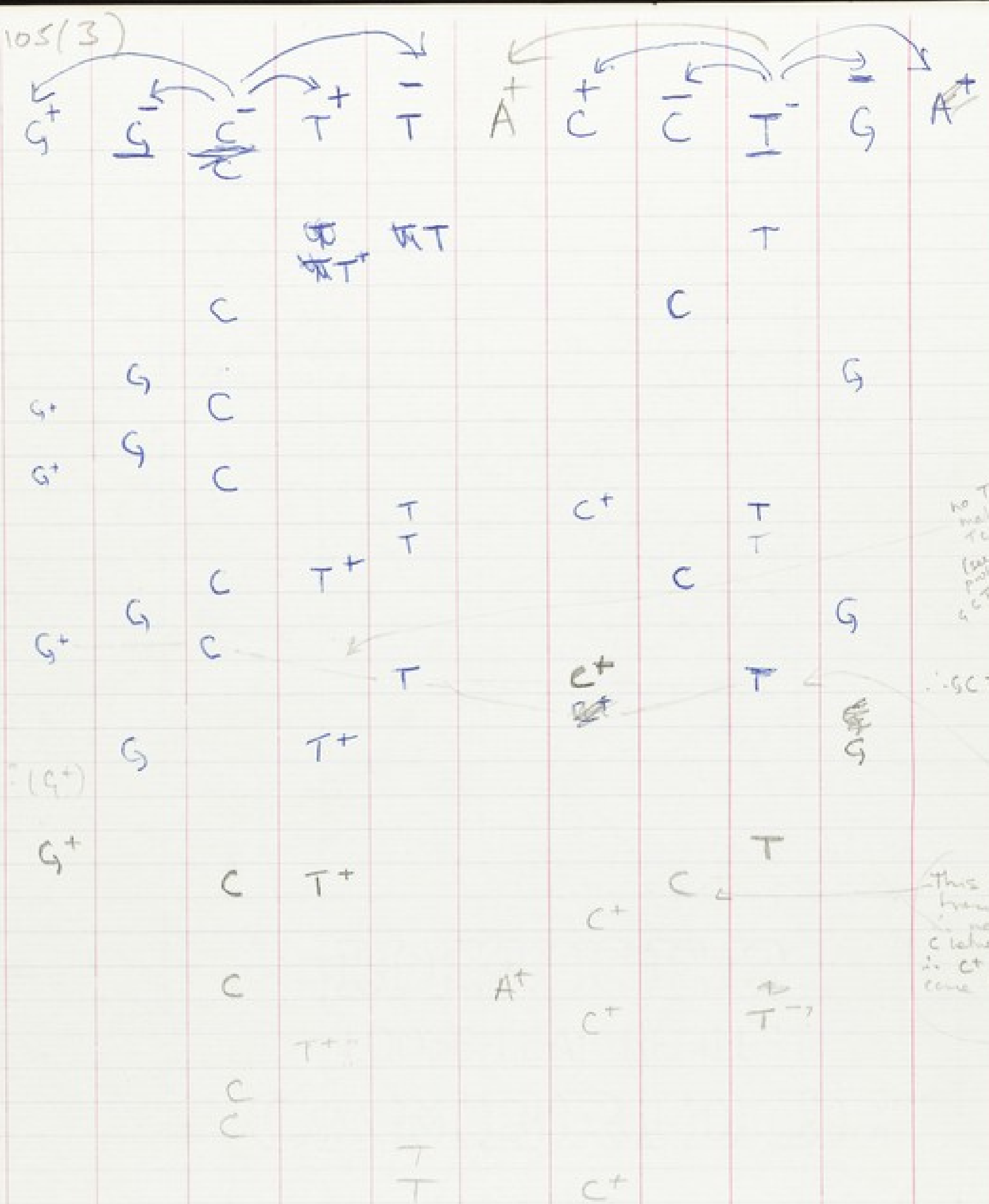
i) 10 A' 10 T⁻ ✓ 2 pol A → T⁻

took about ½ for running 12% gel. urea tris-gly.

G° C' a b c d T' e f g h A' i

5·30 450 v
~~6·30 32 mA~~ → 400
9·30 11 — ~~375~~ 400
9·30[10] blue at bottom

f, h, b, T', g, C' d A' run on formamide gel. looks awful.



Best seems to be TTCGCTTGGTCA
(problem is deaminations)

This was a 12% gel. This seems to have put the T up ahead of the G & C which was causing a problem before.

(see 114(4))

P 106 Decamer sequencing (D 100)

NB 2mC/1ml !!

new dGTP + TTP (Feb 5 1974)

1 μl T dry - 20 H_2O
1 G ''

	a	b	c	d	e	f	g	h	
G^+ dry	10	4	2	1					
T^+					10	4	2	1	
mix	2	2	2	2	2	2	2	2	✓
-G mix	2	2	2	2					✓
-T mix					2	2	2	2	✓
pol(k)	1	1	1	1	1	1	1	1	

15' 0°
1 ·1 M EDTA
20 formamide

mix
5 φx
5 decam/2 ✓
5 pol mix ✓
5 A/100 ✓

-G mix
5 T/100 ✓
5 C/100 ✓
10 H_2O ✓

-T mix
5 G/100 ✓
5 C/100 ✓
10 H_2O ✓

20% acrylamide Tris borate 300 V 4-50 ·1.6 cm
400 6-10 1.5
10-10

In general it is difficult to identify seqs but seems to have synthesized quite a long way in all cases with average above 50

106 (2)

1 μl T dry → 100 ($\frac{T}{100}$)
3 G → 60 ($\frac{G}{20}$)
10 → 100 ($\frac{G}{200}$)

<u>mix</u>
10 φx ✓
10 $\frac{deca}{2}$ ✓
10 pol mix
10 H_2O ✓

− T mix (a−d)
5 G/100 ✓
5 C/100 ✓
5 A/100 ✓
5 H_2O ✓
✓

− G mix (e−h)
5 T/100 ✓
5 C/100 ✓
5 A/100 ✓
5 H_2O ✓
✓

	a	b	c	d	e	f	g	h	i	J	k	L
$T^{*}\frac{1}{10}$ dry	5	2	1	.5								✓
$G^{*}\frac{1}{20}$					1				10	10	10	10 ✓
$G^{*}\frac{1}{200}$						5	2	1				✓
$\frac{A}{10,000}$									5	2		✓
$\frac{C}{10,000}$											5	2 ✓
mix	2	2	2	2	2	2	2	2	2	2	2	2
−T mix	2	2	2	2								✓
−G mix					2	2	2	2				
T/100									1	1	1	1 ✓
A/100									−	−	1	1 ✓
C/100									1	1	−	− ✓
pol (k)	1		15′	0°		1 μl		·1 M EDTA				

<u>Urea</u> tris gly gel 20% 400 5-45
... moving up to 500

Has gone slow both running & migration relative to markers. Can read off right from first G (11) which is behind the front marker. Piling up is not v good here. Is only really possible at lowest concns. The A & C's (i−L) seem to have worked reasonably.

106(3)

4 µl G* dry → 40 µl.
1 T*
1 Iodo C

	a	b	c	d	e	f	g	h	i	k	l	m	n	o
√ hot G	5/100 [illegible]		5/10	5/10	2/100		5/10	5/10	5/10	1/100 √		5/10	5/10	1K
√ hot T		5/50				2/50					1/50 √			
√ A/50,000			5					2				1		5
√ G/50,000				5					2				1	
√ T/20,000					[illegible]		1							
√ G/20	–	1	–	–	–	1	–	–	–	–	1	–	–	1
√ A/20	1	1	–	1	1	1	1	–	1	1	1	–	1	–
C/20	1	1	1	–	1	1	1	1	–	1	1	1	–	–
T/20	1	–	1	1	1	–	–	1	1	1	–	1	1	1

dry

4 mix, 1 pol Kl.
20' r.t.

mix
10 ϕx
10 deca/2
10 pol mix
50 H_2O

e – n on 20% borate

a–d, o, T's on 15% borate.

1µl $\frac{A}{10,000}$ = ~~10 mµ mole~~ 1 µµ mole or in 5 µl = ·2 µM
hot $\frac{G}{20}$ in 5 µl = ·2 µM

These are not too good. Seem to be a lot of bands that should not be there, specially in A & C. T & G seem reasonable. Doesn't really seem this would be a reliable method as it stands. Probably + & – approach is better.

Iodo C (o) seems to have incorporated O.K. but bands are fuzzy & piles up mainly at A's as it should but also a bit at C's suggesting this doesn't go in too easy.

Jumps seem pretty irregular & seems to be an inversion at CAG (38). Perhaps this is due to using borate system.

D 106 (4/Cy)

1 T → 20 & take 5
1 G → " " "

mix ①
20 25 20 φx
20 25 20 dec./2
20 25 20 pol mix
20 25 20 H_2O

	T	G	A	C	
label =	·25	·25	25	25	
mix	20	20	20	<20	
G/20	5	–	–	–	✓
A/20	5	5	–	5	✓
C/20	5	5	5	–	✓
T/20	–	5	5	5	✓
A/1000	–	–	2	–	✓
C/1000	–	–	–	1	✓
H_2O	5	5	10	10	✓
pol (K)	10	10	10	5	

30' 0°
5 pl ·2 M EDTA
agarose.

T 25
G 100
A 300
C 100

dried.
T, C, dry rolled 50
A 100
G a bit over 50
take 5 T, 2 of the rest

20 A
30 G
30 C ✓
340 T ✓

5 pl + 5 + mix 1 T4
20 pl + 20 – mix(H) 5 Pol(kl)

5 + 1 thinx10 left 2 H_2O 2 thin. Not sure which one I took here but think it

5 A
10 G
10 C
5 for + 's

106(4) borate 20%

−	+	− no urea
	C	
G		G
C	C	C
G	G	G
C T	C (A)	C
		T
T	T	T
C T	C	C
G	G	(G)
C	T	T
	G	C
	C	
A		
A^n		A n
	A	
C		C
	T	
C		C
C	C	C
T		T
C		C
A		A
G	G	G
C	C	C
(G)		G
A 3		C
	A	A
		A:n
T	T	
A 3		
	3 A	
T	T	
G		
C		

106(4) unear. 15%

	−	+	
		A	
	C		.6
	C(A)	C(A,G)	
	T	T	.4
1	A	A .3	
	C		.9
	C	C	1
.6	G	G	.6
	C	C	
.6	(G)	G	.6
2.0	G		.6
	C	C	
.25	T	T	.5
	T	T	2.0 / 1.3
	(C)	C	
.8	G	G	.9 / .6
	T(C)	T	
1.2	G		1.0 / 1.1
		G	
	T	T	
.35	C	C .25	.9
	A		.6
1.1	A	A	1.1
	C		.75
	C		
	T		
	C?		
	A		
1.3	G		1.3
	C		
	G		
	C	C	
	A		.6
	A	A	
	(G?)		
.8	T	T(C) .4	.8
	A	A	
	T	(G?)	.6
		T	
	A		
	C	C	
	G		

this − G looks like a doublet but jump suggests only 1 G

looks like GG on − side

looks like AA

2-3 C's

A^+ corresponds to T^- so G unlikely

looks more like TT on − side

AA?

D 107(1)

2 μl *GTP dry.

20 μl mix → *GTP ✓
+ 4 μl C/20 ✓
~~10~~ 8 H_2O ✓

8 pol (kl)

5 μl mix ✓
5 φx ✓
5 deca/2 ✓
5 T/20 ✓
5 A/20 ✓

0°

samples at a 40 secs
b $1\frac{1}{4}$
c 2 mins
d 4
e 8
f 15
g 30

~~4~~ 5 μl mix
~~1 C/20~~

h.) 1 G*/20 ✓
1 C/20 ~~dry~~
$2\frac{1}{2}$ mix ✓
1 pol (kl)
15'

Definate differance now between diff times. at short times (up to 2 mins) can see pretty well all products to about 30. At longer times ~~two~~ & smaller ones fade out & seems to be more piling up.

Idea ~~is~~ was to ~~do~~ try and get heterogeneous mixture as possible by doing short time with excess triphosphate. Seems to work but probably no of counts a bit low.

D 107(2)

5 GTP* dry.

10 μl mix ✓
10 φx ✓
10 deca/2 ✓
10 T/20 ✓
10 A/20 ✓
10 C/20 ✓
20 H_2O ✓
20 μl (kl)
0°

2 mins.	50 μl	+ 5	·2 N EDTA
8 "	30	2	
30	20	2	

107 (2)

−	+
C	
C	C
G	
C	C
G(G)	G
C	C
T	
T	T
C	C.
G	
T	G
C	
	T
G	
✓ G	G
✓ T	T
C	C
A	
A	A
C	
	T?
C	
(C)	C
T	
(T)	
A	
G	(T)
C	
G	G
C	C
(A)n	
	A
T	T
A	
(A)	
(T)n	
A	
C	
G	

D 108.

	A	B	C	D	
hot GTP (dry)	1	1	$\frac{1}{4}$ (5)	2	✓
mix	10	10	25	10	✓
C/20	2	2	5	—	✓
C/10,000	—	—	—	5	✓
H_2O	4	4	10 (5) the rest	1	✓
pol (x)	4	4	8 → 40	4	

20 pol mix ✓
20 φx ✓
20 deca/2 ✓
20 T/20 ✓
20 A/20 ✓

2' ~~3~~ 15' ~~[illegible]~~ EDTA agarose.
30' — [illegible]

C a. remove 4 μl ~~5' [illegible]~~ 1 pol 5' 0° ✓
b. 4 ✓ " 10' ~~[illegible]~~ r.t ~~[illegible]~~
c. ✓ $\frac{1}{3}$ pol 30' 0° 12'
d. 5 μl as big C. 30' 0°

} run 15% gel [illegible] [illegible] 99(?)

rest of A,B,C,D dried.

A 5 μl H_2O 5 μl −G ~~2~~ 1 pol = e ✓
B 10 ~~20~~ μl H_2O ✓ + 10 −G
(~~[illegible]~~ gel) ~~5 μl~~ + 10 ~~[illegible]~~ μl 1 pol 0° = 10' f ✓.
30' g
5 μl 1 pol 0° 30' h
5 " $\frac{1}{2}$ " → 20° 30 i

C. 20 ~~30~~ μl ~~10~~
~~10 μl~~ < 5 μl = J ✓
5 μl + 5 μl + G (T4 k ✓
>10 μl + 10 −G agarose dry 5 [illegible]
8 μl H_2O 3 = l

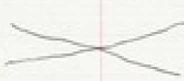
G° → G⁻ → G⁺
G° → G⁺

~~D 10 μl~~ ~~[illegible]~~ " 5 μl 5 μl + G⁺ ✓ ✓ = m ✓

D 10 μl 5 μl ✓ = n
5 μl + 5 μl C⁻ 20' rt. = o ✓

1 GTP dry. 5 mix ✓
2 C/10,000 ✓
1 H_2O ✓
2 pol

5 μl 10' rt
5 μl 10' 37°



p ✓
q.

108(1)

a – d. with limited GTP^* diff conditions of incubⁿ to test accuracy of method. b looks v much better than others suggesting it. better than C^o. c with less pol is v bad ∴ keep up polⁿ concⁿ.

e – ~~f~~ i various ways of doing a – G on shift labelled with xs G for 2′ (e) or 15′ (the rest). Doesn't really seem to make much difference. They all seem pretty good anyhow.

j – m. doing ~~a~~ +G on a G^o or G^- derived from it. Not much difference. both give trouble around 20 area ~~x~~ k has one false spot, m two ~~xx~~ m has more spots up ~~xxx~~ top so question is are these artefacts or not?

n – q. This is a C^o system
n = C^o, o the C^- arising from it not much difference. both have v strong spot at 22 (should be a G) otherwise perfect. C^o if anything better, shows doubles well.

108(2)

	a	b	c	d	e			
hot GTP	$\frac{1}{40}$	$\frac{1}{20}$	$\frac{1}{10}$	$\frac{1}{20}$	$\frac{1}{20}$	dry	20	108 mix
~~mix~~ mix	4	4	4	4	4		4	5/20
pol	1	1	1	2	1		8	H_2O
	<	30'	0°	> 10' r.t.				

1 2 7a 7b a b c d e (109) reused Saran 15% urea

Main problem seems to be extra spots down at bottom. The best is probably e. Thus room temp seems preferable to 0°. d also seems an improvement suggesting more enz may be needed. Presumably to have q as the only limiting thing everything else must be going well.

108(3)

2× 2 GTP. dig.

	a	b	c	d	e	f		g
GTP* dig	1			1/20	2	2	TTP*	2/40 1/4?
mix	20				10	10		10 ✓
A/20	5			8 mix	—	2	✓	2 ✓
C/20	5			from	2	—	✓	2 ✓
T/20	5			108(2)	2	2	✓	C/20 2 ✓
H_2O	5 ✓			C/10,000	—	~~4~~ 5		H_2O 4 ✓
take	5 for b			A/10,000	~~4~~ 5	—	✓	
pol	10	2		2	5	5		5
take	10 for c							
	10' rt		5' 37°	10' rt	10' rt	10' rt.		
	2	0	2	0	9	?		9
for running	~10 µl	all	all	all	~10	~10		~10

pol mix	20	✓
φx	20	✓
dew/2	20	✓
H_2O	20	✓

run 12% urea gel start pol on also 105(2) a
φx10 (no hu)
a — f ~~[illegible]~~, 10, 105(2) [illegible]

Run samples on 12%, urea gel 20 cm 500 v 5 hr most of it has run off bottom so difficult to tell what's happened. repeated with a longer gel (see 110,(1)). ~~but~~ a a b both look OK. probably b better. c a bit streaky. Thus 37° doesn't seem good. probably room temp the best.

On longer hot gel (110) these seem OK. though are a little fuzzy, probably largely edge effect.