Dideoxy nucleotide syntheses, phi X 174 dideoxy sequencing, thin acrylamide gels, 2D frag prep

Publication/Creation

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Persistent URL

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Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

Q7 (-) (J.D. - strand)

A* label.

-'s : 1/4 µl klenow 30' 0°. Standard - mixes

+'s : 1/4 µl klenow 12' 37° pH 9.2 10µM + mixes.

Q7 (+).

A* for ddTTP and + + - T's.

10X pH 9.2 Pol mix.

100 µl M Tris - glycine pH 9.2.
50 µl M $MgCl_2$
5 µl M DTT
350 µl H_2O

10µl of 10µM + mix / sample
1 µl klenow/4

12* 37°.

⇒

100 M5 Alu
310/70 M6 Hinf
180. M8 HindIII

} + 10µl H_2O, 1µl 10x Hin buff, 1µl enzyme 45' 37°

Run 5% non-denat. gel.

Q6 (-) 10 µl annealed

Q7 (+) 5 µl ..

ddTTP of Q6(-), Q7(+).

1.5 µl annealed frag.
1 µl A+ (dry)
2 µl A mix (~~pol~~)
0.5 µl ddTTP. (mM?)
1 µl Klenow/5'
15' r.t.
+ 1 µl A/2O
15' r.t.
Run.

Rest of Q6(-) & Q7(+) A+.

Q7(+) pol tests.

a)	2 µl ~~#~~Q7(+) A*	2 µl std. +T mix	1 µl T4	30'	37°		
b)	.. ..	10 µl 10 µM pH9.2 +T	.5 Klenow	12'	37°		
c)	.. ..	.. .. ..	.25 ..	..	..		
d)	.. ..	2 µl std. +T mix	.5 Klenow	30'	37°.		
e)	.. ..	.. .. -T	2 µl H pol	30'	0°		
f)	.. ..	.. .. -T	1 µl Klenow	..	..		
g)	.. ..	.. .. ..	.25 ..				
h)	.. ..	.. .. ..	1 µl T4	30'	37°		

i) Run with ddT Q7(+).

j) 0

Ted Friedmann - suggestions for 11/9

20% gel.

Eppendorf tubes & fuge. (2' spin).
(1 ml).

G.
As recipe.

A. (NaOH). (Carbonate free).
with calf thymus.
90° 15'. (shorter than suggested)
? 10 mins (CGH)

CXT's.
As recipe. (~~possible step Calcium at half time~~ cold)
Hydrazine vac. off.,
(+250 · 3M NaAc
750 µl EtOH..).

Gels. 20% 750 V. (<20mA)
~~50 units/ml~~.
9 hours (= 900 V 12mA)
Fast blue just on for first residues.

R1, A9, Z2, A7 a+b.

Kinase labelled ≈ fresh γATP.

Cut R1 HHa
A9 (-7c) Hin f } 3 µl 3 hrs 37° 7c? 110/90, 40.
Z2 Hin f
A7.1b Taq 3 µl 3 hrs 58°. 240, 60.
a 45, 210.
b 150, 95

5% ~~stacking~~ fast Hae ½ way? (4 hrs 30 mA.)

a, b, c eluted from A7a/b.

M-G as recipes except a) hydrazine vac'd off.
b) A's 90° 15'.

20% gels ½ x TBE.

G4 Hae 1 & 3 incorp^n.

~~2 μl ...~~

2.5 μl Z1 — 2.5 μl Z3

6 μl − std. — 6 μl + std.

100° 3'.

\+ 1 μl 10x Hin buffer.

67° 30'.

(Remove 1.5 μl for ddT.)

Mix.

5 μl C/20

.. G/20

.. T/20

2 Hx10

3 H_2O

7 μl fragment

10 μl mix.

10 μl A*(dry)

2 μl Bo pol.

1-5' 5°.

phenol, ether, column

Z3*(+) low counts but enough, Z1(−) zero counts.

ddT: (Z3+ only)

As previous page, but chase 15' c 1 μl A/40, T/40.

\+ 1 μl Hae 30' 37°.

* −'s of Z3(+) 2 μl H pol 0°

Also 2 +G's 1 μl & 2 μl T4 pol from N_2 (no glycerol).

G4 Hind ~~B x A~~ A x B.

Annealing.

A 2 µl fragment 5 + strand (1 H x 10 / 1·5 H x 10)
B 10 " 5 " "

C *.

0.2 γ

Nick translate 2 γ ϕx RF in 100 λ

Digest with Hae III

Add 3λ 0.5 m $MgCl_2$

Incubate 10 λ aliquots with 1 λ of Ava I

for 1 hr, 2 hrs, 4 hrs and O/N

5% gel - fast blue to bottom

Nick. Trans:

2 μg RF I
10 μl ogata buffer
3 μl 0.1 mM dC
3 μl 0.1 mM dG
3 μl 0.1 mM dT

20 μl dATP* (dry)
1 μl Bo pol.

H_2O → 100 μl. (+80 μl)

1 hr r.t.

70° 10'.

+ 3 μl Hae III 60' 37°

+ 3 μl 0.5 M $MgCl_2$

10 aliquots + 1 μl Ava I 1h, 2h, 4h, ~~O/N~~ 37°.

also one without Ava I cutting

Purification of cellulose for DNA-cellulose.

250 g Whatman CF11 cellulose strained in
1l boiling ethanol and filtered 3X.
Then suspended in 0.1M NaOH
1mM EDTA
and filtered.
Then suspended in 10mM HCl
and filtered.
Then washed ∞ dist. H_2O to neutrality.
Dried (37° O/N).

200 ml c.t. DNA 3mg/ml in 10mM Tris-Cl 7.4
1mM EDTA

80 g cellulose added with stirring to produce a thick paste, spread in xtal. dish to dry.
2 weeks in dessiccator.
200 ml dry DNA/cellulose after powdering.
Suspended in 4l 10mM Tris pH 7.4.
1mM EDTA
4°C O/N.
Filtered, washed 2x in same buffer.
Made to slurry in TE buffer + 0.15M NaCl.

Assay.
1.g boiled 20' in 5 ml buffer.
Spun.
$O.D.^{260}$ super 0.54

O.D. unboiled 1 gm in 5 ml buffer
= 0.33.

Say 10x dil^{n}. O.D. = 5.4
= 200 mg/ml.
(Rather Low)

DNA unwinding protein (DUP) on T6(+).

Mix
T/20 1
C/20 20
G/20 20
Hx10 15

Take 10µl + 10µl mM ddTTP.

a). 2µl φxT6, 2µl mix, 1µl (dry) A*, 1µl K1/5 15' r.t.
b) " " " , ·1µg DUP, 1 K1/5, " "
c) " " " , ·2µg " , " "
d) " " " , ·5µg DUP, " "

(Add DUP and leave 5' r.t. before adding K1/5)

Then add 1µl chase mix (T/20 + A/20) 15' r.t.

+ 1µl Taq 60' 58°.

DUP on T6(+) - standard extension.

5 λ T6, unwind.
1 λ (+)

Mix.
5 · T/20
5 C/20
5 G/20
2 Hx10
3 H_2O

a) 2 T6(+), 3 mix, ·5 klenow 1', 3', 5' 0°.
b) 2 T6(+), 3 mix, ~~5 klenow~~ ·1µg DUP, ·5 klenow, ".
c) 2 T6(+), 3 mix, ·2µg DUP, ·5 klenow, ".

~~Taq out, gel~~. Run direct, unwt, v. long run.

(DUP added before pol. 5' r.t.).

George says riboflavin runs @ 1/c speed of star blue

Trial assay for T4 prep.

Assay mixes as for previous T4 prep.

But 50 μl Assay mix

		cpm
a)	1 μl Bo pol	428
b)	1 μl " " /5	565
c)	" " " /10	459
d)	" " " /20	349
e)	" " " /50	214
f)	blank.	77
	Blank	47

30' 37°

Take 10 μl and squirt onto 1 cm² #1 paper.
Wash 10' 5% TCA 1% p.p. (occasional swirl.)
Count by monitor or in H_2O on H^3 channel of S.C.

b). Reduce cold GTP by 10×
Increase dGTP* 5×

		cpm
a)	1 μl Bo pol	742
b)	0.1 μl	860
c)	0.01 μl	393
d	0	119.

c) Same mixes as b).

					c.p.m.
·1 units	a)	1 μl Bo pol /20	1 cm² 3MM 25 μl	15' wash	1909
·04 u	b	" " /50			2171
·02 u	c	" " /100			1100
·01 u	d	" " /200			732
·005 u	e	" " /400			719
	f	0			420
	g	0	#1 10 μl		(≡ 190 f) 76
	h	/20	#1 10 μl		782. (≡ 1950 a)

dd TTP. long / short

Z2(-) cut Z + M
Z2(+) Z cut long + short
Z4(-) " " " "

Run long + short on same gel.

24.83

Wt + flask ~~202.89~~ 24.6826 gr (topless)
.15 g

~~[illegible] A~~

A

C	g		
·1	·5	·9 g	$NaHCO_3$ (weigh into beaker under ice & pour stuff on.)
10	50	80 g	ice

extract with 4 30 ml portions $CHCl_3$

$CHCl_3$ washed with water & dried over sodium sulphate

filter

vac down (near to dryness, no heat)

3 12 20 ml acetic pyridine water.
14 : 1 : 3

leave 24 hr r.t.

Silica column.

C

same with half as much.

Hydrolyses started at 1 p.m.

(The A formed a v. heavy ppt; no sign of this in the C).

A work-up:

Vac down

+ ~ 50 ml chloroform

50g Load ~~on~~ column.

Wash c̄ chloroform.

Develop c̄ 5% ~~[illegible]~~ EtOH in $CHCl_3$.

5 100g

A (main).
Dried from pyridine
+ ~10 ml ether. - white ppt but mainly
sticky brown gunge.
Ether blown off, and whole resuspended
in pyridine.
Vac'd ix, + pyridine - froze.

9 (57%).
Vac down >x from pyridine.

3 ml + .3g tosyl Cl,
1 week (2.2.77 → 9.2.77).

GGB 10x T4 buffer:

0.5M Tris pH 8.0
0.05M $MgCl_2$
0.1M βM.E.

T4 pol assay. (F8 −, Hin f cut)

+C
- .1 µl T4 B
- .5 µl T4 B
- 1 µl T4 B
- 1 µl T4 A
- 1 µl Kan T4 (60' 37°)

} 30' 37°.

−C
- .1 µl T4 B
- 1 µl T4 B
- .1 µl T4 A
- .1 µl Kan T4

o

− 's o.k.
+ 's not quite worked.

Further prep. of F8.

Cut Hin f, ½ with stacked (DTT) Hin buffer.
½ with 10 mM BME. " "

Dialyze ½ ml T4 B against 0.1 M Tris. cl 7.5
DTT
50% glycerol.
O/N

a	2 F8 (DTT),	2 +C (S.F.)	1 K T4	60' 37°
b	"	" "	1 T4 B(P)	30' 37°
c	"	" "	1 T4 B(P)	60' 37°
d	"	" "	2 T4 B(P)	60' 37°
e	2 F8 (BME),	2 +C (10 mM BME)	1 T4 B(P)	30' 37°
f	" " .	" " (" ")	2 T4 B(P)	60' 37°
g	2 F8 (DTT)	" +C (S.F.)	1 T4 B Tris	30' 37°
h	2 F8 (BME)	" +C (BME)	1 T4 B Tris	30' "
* i	" "	" " "	2 " "	60' "
j	2 F8 (BME)	" "	1 K T4	60' 37°.

*+1 µl 0.025 M $MgCl_2$.

Concentration of sample of T4 pol. (Peak B)

~~5 µl diluted to 15 ml w 0.2M pho. plate.~~
~~Vol. reduced by dialysis against sephadex~~
~~Dialyse against 0.1M Tris, DTT, 50% glycerol~~

10 ml dial. against 0.02M K phos pH ~~6~~ 7.5 and run on 40 ml DE52 column poured in same buffer. Eluted ~ 0.1 → 0.5M KCl in phos. 50% of Sample recovered in 6 ml.
Dial. against 0.1M K phos, 0.01M βME, 50% glycerol. (≡ ~~T4B~~ T4C)

Assay. (on F8(-)).
+C.

a) 1 µl Kamen 60' 37°.
b) 1 µl undial. T4C 30' 37°
c) 2 µl " " 60' 37°
d) 1 µl dialysed T4C 30' 37°
e) 2 µl " " 60' 37° + 1 µl for further 30'
f) 2 µl " " 60' 37° + 1 µl Tris-Cl pH ~~8.0~~ 9.5
g) 1 µl T4B ~~dialysed, no~~, no XTP's, ~~60~~ 37°
h) zero.

T4 pol assay (4) F8(-)

2 µl F8(-) A* / expt.

a) 2 µl +C mix, 1 Kamen T4 60' 37°
b) 2 µl special* +C mix, 1 µl 50 mM p.p., 2 pol C 60' 37°
c) 1 " " " " " " " " 1 " " 30' "
d) " " " " " " " " 2 pol B 60' "
e) 2 µl +C mix, 2 pol B 60' 37°
f) " " , 1 pol B 15' 37°
g) " " , 1 pol C 15' 37°
h) " " , .25 pol C 15' 37°
i) " -C mix, 1 pol B 30' 37°.
j) 1 µl 50 mM p.p., 1 µl H_2O, 1 µl B 60' 37°.

* 1 10mM CTP
5 10x Hin buffer
4 H_2O.

(Gel left off for some time just after starting - may be v good)

C

Dry from pyridine

+ 0·5 ml dimethyl formamide
Dry (dessicator)

+ 2·5 ml DMF (soluble).
0·5 ml sodium methoxide (CH_3ONa) (red-brown).

45' r.t. (shake)

+ 20 ml dry methanol.
0·75 ml amberlite (IRC-50(H))

Stir 2 hrs.
Filter.
Run sample on silica (10% conc. NH).

~~Extract c hot acetone & filter.~~
Extract ~~5x~~ 4x 5 ml boiling acetone.
Filter
Vac. to small vol. (should produce xtals).

Sample
Dry, + 2 drops conc. ammonia.
c/M 45°.

Bands 1-3 eluted from silica / $CHCl_3$, 10% EtOH test of this.
Eluted c pyridine.
Vac.
+ NH_3 to each
Run silica with starting material.

T4 pol test (5).
F8(L)
a-d Complete set of +'s ± pyrophosphate, (2 µl T4B 30') (CTAG)
~~" " " -'s (1 µl T4B 30')~~
~~+T +1 µl M Tris pH8.0 (no pyrop.)~~
e +T " " (1 µl T4B 30')
f +T " " (1 " " B 60')
i +T (no pyrop.) + 1 µl M Tris pH8.0 (1 µl T4B 30')
j −T (1 µl T4B 30')
+T ± pyrop." (1 T4A 60')
g, k +T (no pyrop). (1 µl kamen 60')

+ mixes for p.p.

10 µl dXTP
50 µl 10xH
40 µl H_2O.

a–g (i.e. those ± pyrophosphate) seem to have slight ppt.

T4 pol ~~test~~ (6)
2 µl c8(-)/exp.
a) 2 µl + C mix, 1 B 60'
b) " C " , 1 µl M Tris pH8.0, 1 B 60' 37°
c) " C " , " 50mM p.p. pH7.0, " " "
d) " T " , Tris
e) " T " , pp
f) " A " , Tris
g) " A " , pp
k) " G " , Tris
i) " G " , pp

C phosphorylation.

Dry 1/10.

+ 0.1 ml cyanoethyl phosphate
Dry several times ± pyridine.
+ carbodiamide.*
2 days.

* 100 mg in 0.5 ml pyridine
(~~cares~~ wash implements in alkali).

+ 0.1 ml H_2O
30' r.t.
Vac. down near to dryness
+ 1 ml H_2O
(~~test~~). (cellulose T.L., iso-propanol/ammonia.).
Vac. down
+ 1 ml 0.4N LiOH
30' r.t.
(~~test~~ [v. small sample]) (should be slower product).
Dowex-50 (pyridinium) in pasteur p.p. (~2cm)
Rinse through ± H_2O.
(~~test~~).

Vac to small vol.
Streak onto 3MM (~9"). & run isopropanol/NH_3.

Elute bands 1-5. (slow → fast; 4 bright U.V., rest dark).
Test ± ph'ase and alkali.

Ph'ase:
2 µl sample (from ~1 ml)
µl 0.1M Tris pH 8.
µl ph'ase (10 µl pc, purged, + 100 µl H_2O.
2 hrs 37°.
Run ± ~~starting~~ material samples on cellulose isoprop/NH_3

Kinase protocol.

T.F's. procedure for A4:

20 λ A4 + 20 λ pH 9.5 glycine buffer
5 λ BAP (dialysed)
37° 30'

+ 20 λ H_2O.
~~Phol extract~~
Reextract 1X ≈ 10 λ H_2O
Ether.
+ 20 λ pH 9.5 glycine buffer.
100 λ dried ~~PATP~~
5 λ PNK.
37° 30'.

Glycine buffer (2x):
100 mM glycine pH 9.5
20 mM $MgCl_2$
10 mM DTT
50% glycerol.

PMB 9,
Bam & R1 plasmid
Phase 2 kinase
Small samples for Z & Hha.
5% non-denat gel.
Take most nibble for whole prep.
M./S.

[Bam ~~test~~.
1 μg PMB 9
BAM
1% agarose]

linear runs faster than circle

R1 activity: 0.1 μl/μg/2 hrs.

Bam " : 0.1 μl/μg/30'.

10x R1 buffer: 0.9 M Tris pH 8
0.1 M $MgCl_2$.

Bam use Hin buffer.

A

TP (2)
Tues morning. filter + wash well with DMF
Add equal volume methanol, vac down.
If DMF doesn't come off without heating
add more MeOH + vac down.
~~combine with TP (1) (in deep freeze)~~

dissolve in 1 ml H_2O + run sample on PEI. .4 M ammonium bic (with AMP + ATP ~~and~~ markers.)

If something near ATP position combine with TP(1) (in deep freeze) + run on DEAE column. (0.1 → 1M TEC.)

Pyrophosphate
Vac'd down
+ pyridine & vac'd 3X
+ 10 ml DMF & vac'd 2X
+ 20 ml DMF.

DEAE-sephadex.

Column ≈ 1cm x 30. in 0·1M TEC pH 8·4.

(30% TEC = 2M).

0·1M TEC = 1.5% (30%/20)

Say 10 ml/g. Swell ~10g.

PMB 9.

a) 10µl mg/ml pMB9, 1µl 10xH, 2µl Bam (Miles).

b) " " " " , 1µl 0·9M Tris pH8·0, 2µl R1.
0·1M $MgCl_2$
(= 10x R1 buffer)

2hrs 37°.

+ 7µl H_2O
20µl 2x glycine buffer
5µl 5mg/ml BAP.

30' 37°

+ 20µl H_2O.
+ 50µl φol.
extract.
Reextract φol ≈ 10µl H_2O.
Ether 3x.

+ 20µl 2x glycine buffer
100µl dried γATP
5µl PNK
37° 30'.

+ 0·3ml 2M NH_4OAc
50µg tRNA
1ml EtOH ; freeze.
Spin (5' Eppendorf)
Wash 0·5ml EtOH
Dry.
+ 20µl H_2O.

1 µl of each for Hae III & Hha.

4 µl sample
3 µl 2x Hin buffer
1 µl enzyme

90' 37°.

+ 1 µl 50% sucrose, 20 mM EDTA, 1/50 ∈ buffer

5% gel. (20×40)

7.5 g acryl
0.4 g bis
15 ml 10×TBE
H_2O → 150 ml
0.8 ml 10% Amps
80 µl TEMED

B2 BH B2 BH (messy)

Run 35 mA, 3 Hrs
(Fast blue ~ ½ way)

- Rubbish.

=

B.S.G. T4 pol test (F8(-))

a	+C	1 µl	T4
b	+T	..	..
c	+A	..	..
d	+G	..	..
e	-T	..	..
f	-T	.1	T4
g	+T	.5	..
h	+T	.1	..
i	+C	.1	..
j	-C	.05	..

45' 37°.

- looks good.

2 (Eric. says A.C. stuff v. much better than B.S.G. T4 for end-label. Intact, B.S.G. stuff melts - this seems v. odd).

End-fill label of pMB9 (linear, 1.5 mg/ml, W.B.).

a) 7 µl pMB9, 1 H×10, 2 Bam
b) " " " " 2 Hind III
c) " " 1 R1 buffer, 2 R1

2 hrs 37°.

Bam: 5 µl of all 4 TP*'s (dry)
Hind III: " " " " " (")
R1 10 µl of dATP*, dTTP* (") (0.03 µg mols)

+ 1 µl 2 × Hin
+ 2 µl Klenow
20' 37°.

70° 10'.

Bam G↓GATCC
CTAG↑C
R1 G↓AATTC
Hind III A↓AGCTT

(Bam - Miles
R1 - Giovanni).

½ µl samples.

+ 4 µl 1.5 × H
1 µl Hae III or Hha.

90' 37°.

5% gel. B/2, B/H, H/2, H/H, R/2, R/H.

- Bam / Hae & Hha and Hind III / Hha v. good.
Hind III / Hae possible.

R1 not good.

Hae & Hha ½ of Bam & Hind III.

Elutions: B/2 1&2, B/H 1&2, H/2 1&2, H/H 1&2.

MaG's of these frags no good (C&T identical and other spurious bands).
B/2 & H and H/2 & H preps repeated as above.
- Same pattern - Ted F. trying M/G's.

C (main batch) phosphorylation.

As trial 1/10, but 10x quantities throughout.
(Glass wool in column for Dowex seems not get bunged up.
Also, put thru Dowex 2x).

Heated ~~at~~ to 30 cm 3mM for 15m prop/NH_3.

dd ATP.

Fractions 11-15 dried down a washed 3x ≅ H_2O.

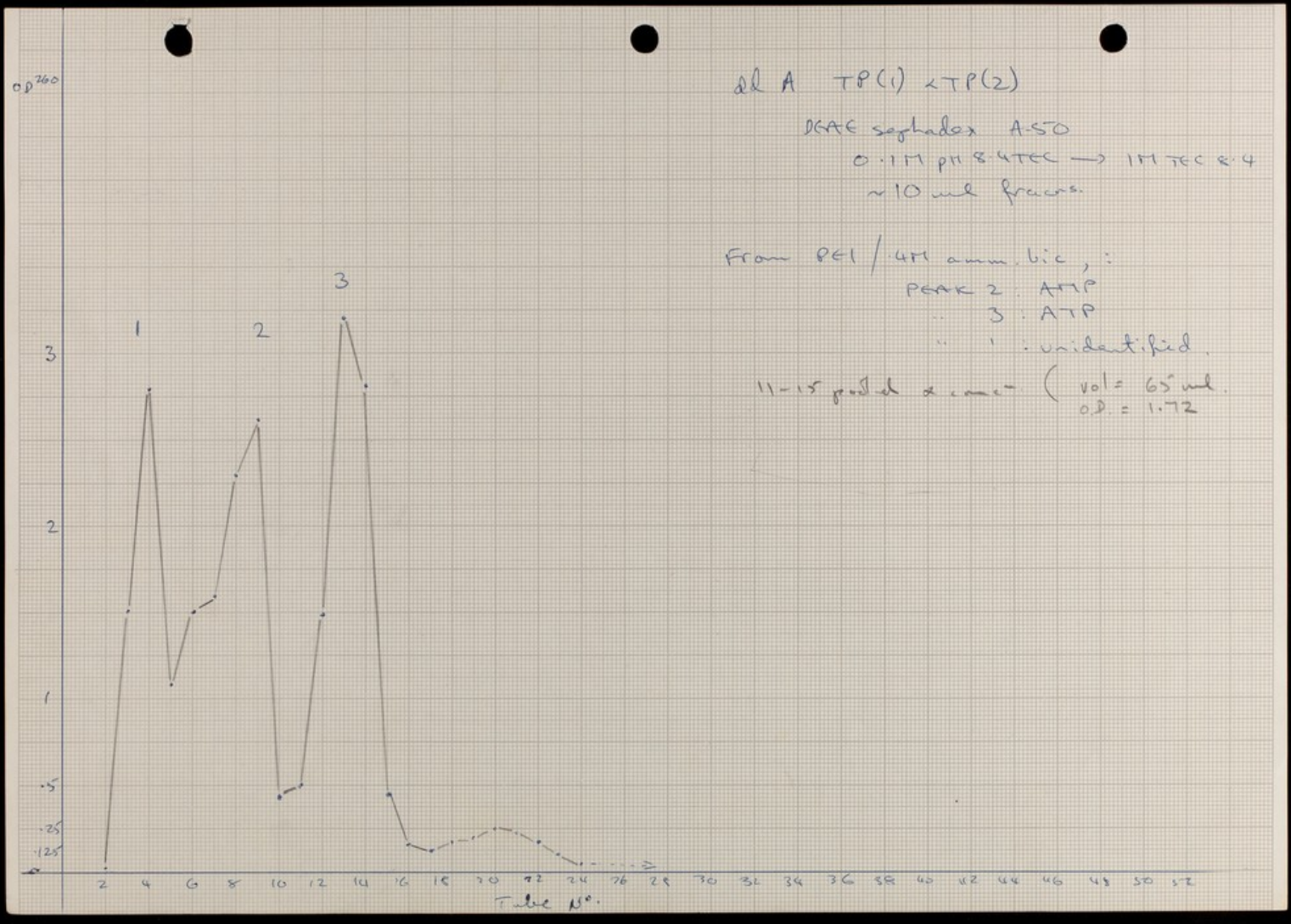
+ 1ml H_2O. $\frac{O.D.}{500} = 0.2$.

= 100 $O.D.^{260}$ total

~~≏ 2g (didn't look like 2g)~~.

≏ 4 mg.

≏ 0.65 ml 10mM.



dA TP(1) & TP(2)

DEAE sephadex A-50

0·1 M pH 8·4 TEC → 1 M TEC & 4

~10 ml fracns.

From 8el / 4M amm. bic, :

PEAK 2 : ATP

" 3 : ATP

" 1 : unidentified.

11-15 pooled & conc- (vol = 65 ml.
OD = 1.72

19/4/77

ddATP ~~test~~. (ddA 1).

Mix A/20 1 µl
T/20 20 µl
G/20 20 µl
Hx10 15 µl. (= C° ddA mix).

a 2 T10/φx(-), 1 mix, 1 10mM ddA, 1 dry C*, 1 kl/s, 15' r.t.
b " " " 5mM " " " "
c " " " 1mM " " " "
d " " " .5mM " " " "
e " " " .1 " " " "
f " " " H_2O " " " "

+ 1 µl chase mix (A/20 + G/20) 15' r.t.

Mix T/20 1
A/20 20
G/20 20 (= C° ddT mix)
Hx10 15

g 2 T10/φx, 1 mix, 1 mM ddT, 1 C*, 1 kl/s, 15' r.t.
h 2 F17/φx, " " " " " " " "

+ 1 µl chase mix (T/20 + G/20) 15' r.t.

+ 1 µl 0.2M EDTA
+ dye, formamide etc. 12% TBE gel.

dde - identification of bands from 3mM / iso-prop / NH_3

A) Bands 1, 2, 3, 4 80° 10'

B) " 3, 4, 5, 6 conc NH_3 20 hrs 67°

Run cellulose / iso-prop / NH_3 ~ o's.

(1/0, 1/A, 2/0, 2/A, 3/0, 3/A, 3/B, 4/0, 4/A, 4/B, 5/0, 5/B, 6/0, 6/B.)

Band 2 $\frac{O.D.^{260}}{100} = 0.13$

= 13 O.D. / ml

≃ 22 O.D. ~~total~~. (vol = 1.7 ml) (~1 mg)

Vac. down band 2.

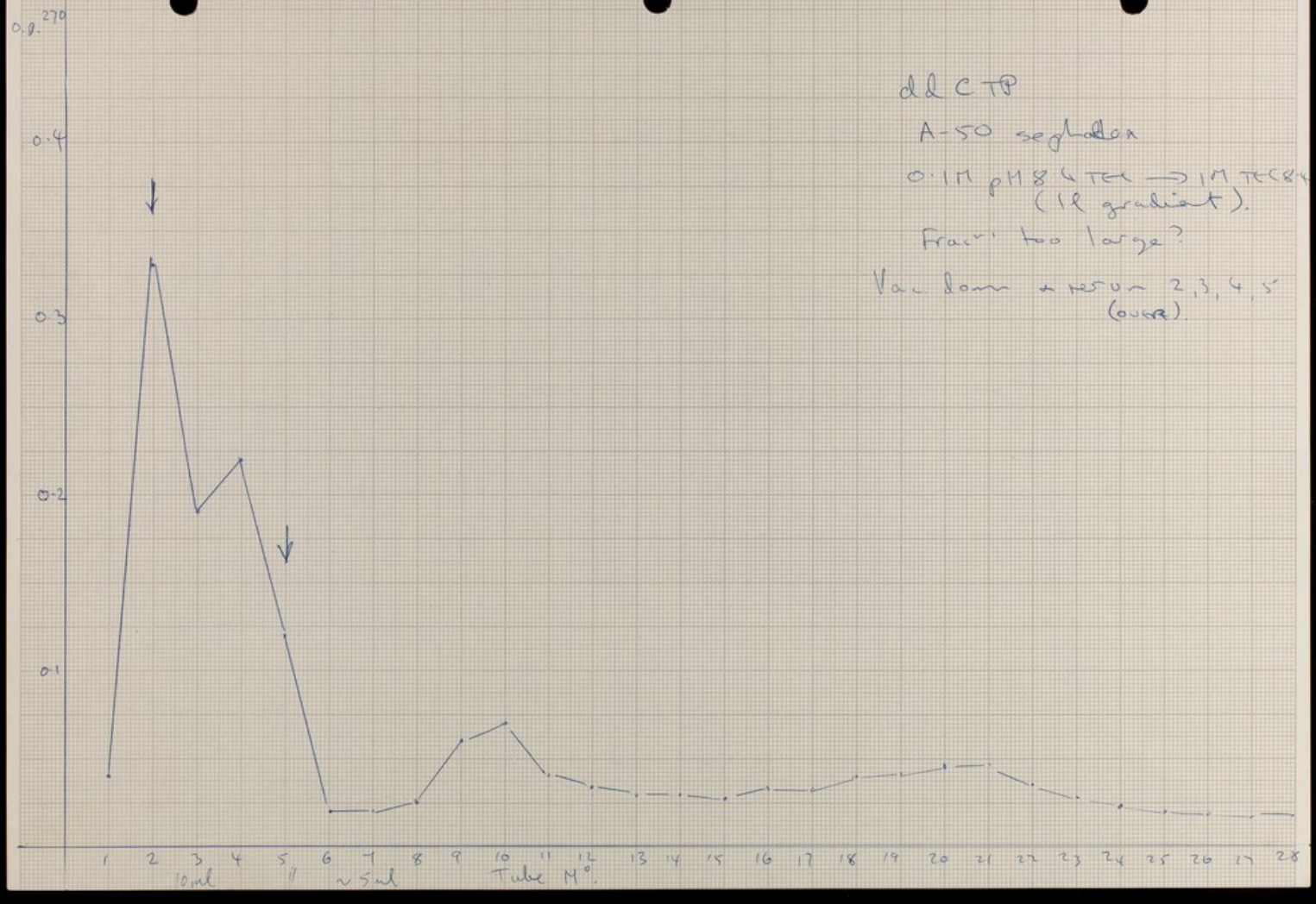
+ Pyridine
+ 1 µl tributylamine + 2nd µl.
Vac down several times (from pyridine and 1x from DMF (warm)

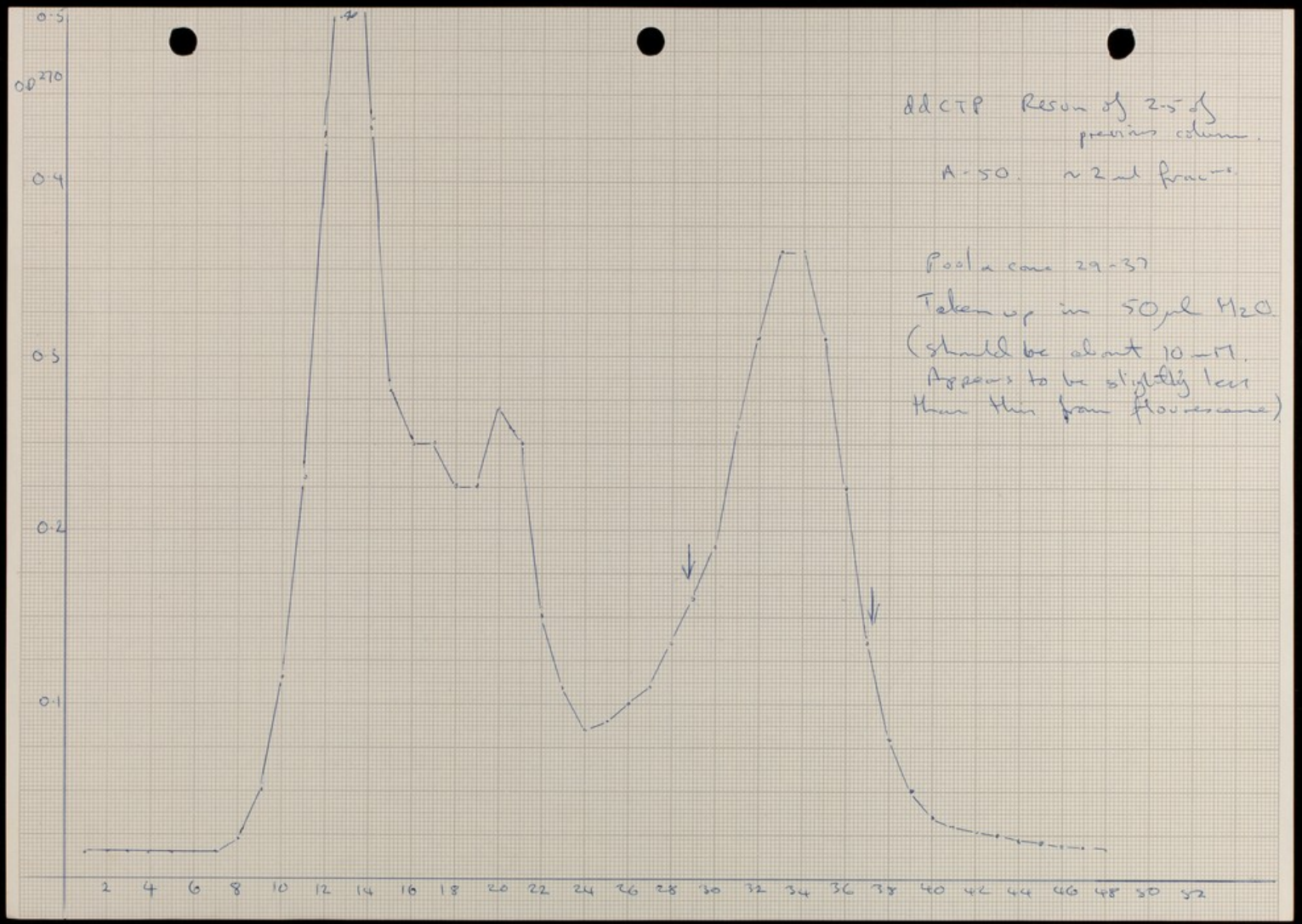
+ 3 mg carbonyldiamidazole in 0.1 ml DMF
4 hr r.t. shake occais.

+ 1 µl $MeOH$
30' r.t.

+ 0.15 ml pyrophosphate in DMF

20 hrs.
Filter & wash ~ DMF.
+ equal vol methanol.
Vac to dryness
+ H_2O. (250 µl).
Test (pet / am. bi).


O.D. 270
0.4
0.3
0.2
0.1
dLCTP
A-50 sephadex
0·1M pH 8 4 TEA → 1M TEC8
(1ℓ gradient).
Frac: too large?
Vac dom + rerun 2, 3, 4, 5
(outer).
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
10ml
Tube N°.


0·5
OD 270
0·4
0·3
0·2
0·1
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52
ddCTP Resin of 2·5 ml of previous column.
A-50. ~2 ml fract°
Pool a conc 29-37
Taken up in 50 µl H2O
(should be about 10 mM.
Appears to be slightly less
than this from fluorescence)

pM39 sequences.

R/Z 1
3'→5'
GAGTACAAACCGTCG

R/Z3. 3'→5'
(TAA)GATACGAGGATATAACTA/TTCTTATTTGAA-
— TTATGCATTACCAACAT
?

A?

7-87
Shuttle

B/Z1 $3' \rightarrow 5'$

GGG (T) TGTCCTG CCCACACCAGCGGTACT –

– AGCGCATCAGC(?)ACACGAGT

B/Z2. & B/H2.

G (G) AGATGCGGACCTGCGTAGCAGC.

B/H1.

GGG (G) TGTCCTGCCCACACCAGCGGTACT –

– AGCGCATCAGCA (T ?) CACGAGTCA.

prob.

pMB9 fragment preps. (PMB9 (3))

R1/Hae A+T label. (~~Hae 2~~) (3.5% gel)

Bam/Hae + HLa G+A label. (5% gel).

(Try priming / ddT ext^n ± portion of Bam frags.).

Elution: B/Z 1+2
B/H 1+2
R1/Z 1-3.
(+ 50 µg tRNA carrier).

(Keep 1/4 B/H 1+2 for priming expt.).

B/H 1+2 priming:

Dry samples.
+ 2 µl H_2O
+ 1 µl 1/6 pMB9 (1.5 ng/µl).
100° 3'
+ 1 µl 5× Hin buffer H.
30' 67°.

Mix:

T/20	1
A/20	20
G/20	20
C/20	20
Hx10	15

4 annealed frag, 2 mix, 1 mM ddT, 1 K/S 15' r.t.
+ 1 µl T/20 15' r.t.
+ 1 µl Bam 60' 37°.
+ 1 µl 0.25 TBM, dye, gel.

ddCTP ~~test~~.

Appeared from T.L. to be almost completely degraded to cMP.

Mix

C/20 1
T/20 20
G/20 20
H x10 15 (= A° ddC mix)

a 2 T10/fx, 1 mix, 1 ~10mM ddC, 1 dry A*, 1 K/5, 15' r.t.
b " - 5mM " " "
c " - 1mM " " "
d " - .5mM " " "
e " - .1mM " " "
f - - H_2O " " "

+ 1 µl chase mix (A/20 + C/20) 15' r.t.

g 2 T10/fx, 1 C° ddT mix, 1 mM ddT, 1 dry C*, 1 K/5 15' r.t.

+ 1 µl chase mix (T/20 + A/20) 15' r.t.

+ 1 µl EDTA
dye, gel.

=

Wt dideoxy dihydro guanosine = 0.134 g

Reduction:
Dissolve in ~ 12 ml aqueous (~80%) EtOH.
Bubble N_2
+ Pd catalyst (~ 2 spatula points)
Bubble H_2 60'
" N_2
Filter (paper) (#1)
Wash residue ~ hot EtOH.
[Test ~ ~~starting~~ material on cell/isopropyl/NH_3.
Looks good (quantitative conversion)]
Dry (+ 1 drop pyridine)
+ 10 ml chloroform, 1 ml EtOH.

Comparison of R1 digest of pTB9 monomer & dimer.

1ug monomer/dimer in 10µl H_2O
1µl R1 buffer.
1µl R1
90' 37°
+ 1µl dATP* (Dry)
+ 1µl 10x Hin buffer.
+ 0.5µl Klenow
20' 37°
10' 70°

½ sample + 1µl dye onto 8% TBE gel.

(This to see if dimer is giving rise to small fragment i.e. 2 R1 sites
Monomer from E. de Robertis).

Rest of samples Hae III cut (1µl 8u/µl 30' 37°)
Run on 4% gel - v. blurry picture so inconclusive.

Repeat label as above (only ½µl dATP* available)
Hae III ½.
Run with uncut stuff on 3.5% gel.

5µg pTB9 monomer R1 cut and A* labelled.
2 cut
3.5% gel.
Two main bands - secondary bands appear v. weak compared with dimer prep.
There 2 bands eluted for n-c.

d2GMP.

Run A-50 column to purify product from LiOH/Dowex.

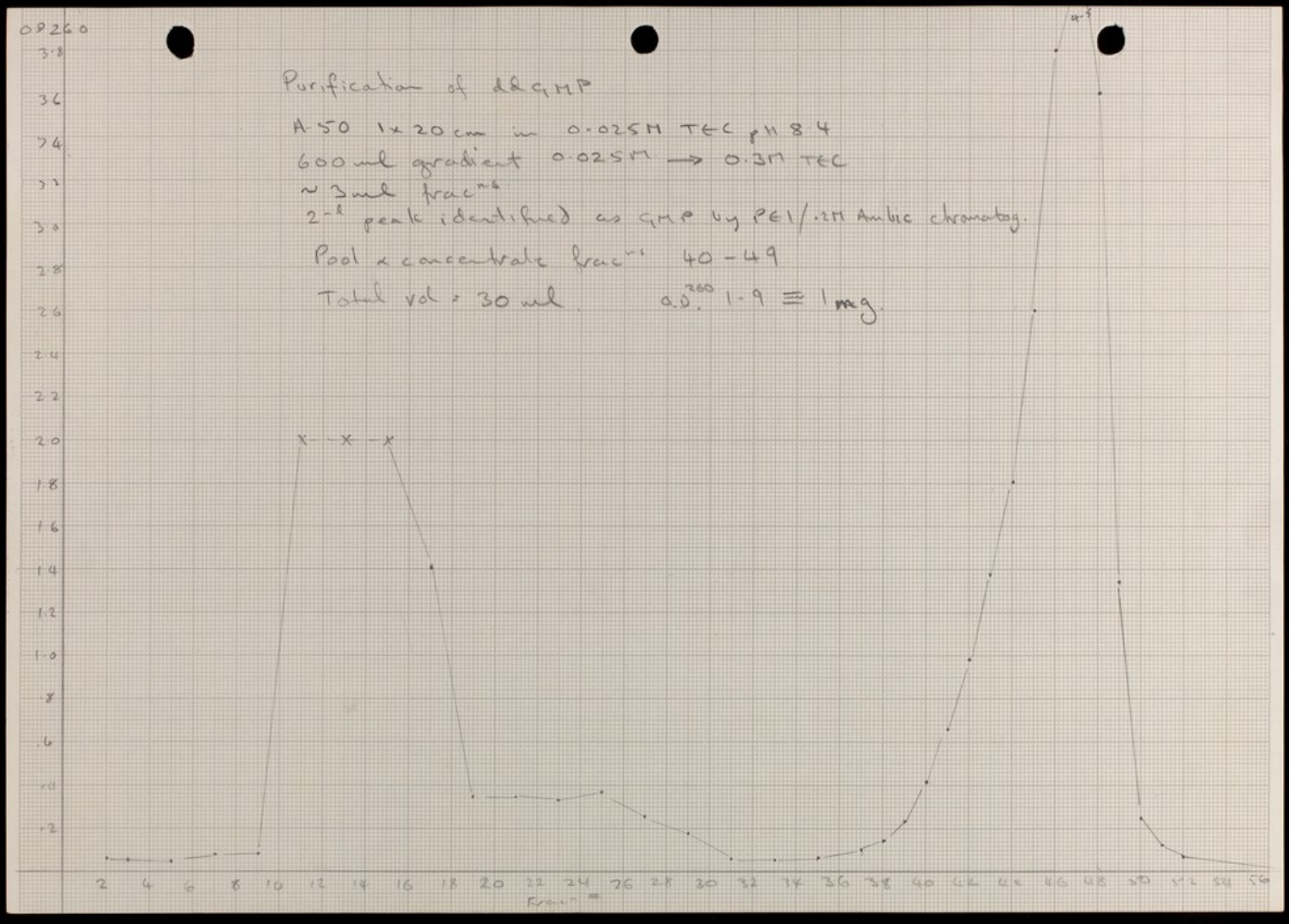
1x25 A-50 in 0.025M TEC pH 8.4

Gradient 0.025M → 0.3M TEC. 600 ml.
~3ml fracs.

Fracs 40-49 dried down, resuspended in pyridine + 1µl tributylamine. ~~Didn't dissolve~~ looked as though most didn't dissolve.
Dried down 3x from pyridine
+ ~0.5 ml DMF.
Still large amount insoluble.
+ 1µl tributylamine
Still didn't dissolve.
Dried down.
+ 3mg carbonyldiimidazole in 0.1ml DMF.
Shaked 4hr r.t.
+ 2µl methanol (dry)
30' r.t.
+ 0.15 ml pyrophosphate in DMF.
20hrs r.t.

Filter (glass sinter)
Washed well ⊂ DMF
+ equal vol. methanol.
Vac'd to dryness.
+ 200µl H_2O. Freeze.

Test with GMP & GTP on PEI/.4M Ambic.
Looks good - mostly stuff running = GTP marker.


OD260
Purification of dd GMP
A-50 1 x 20 cm in 0.025M TEC pH 8.4
600 ml gradient 0.025M → 0.3M TEC
~ 3 ml fracⁿˢ
2⁻ᵈ peak identified as GMP by PEI/.2M Amine chromatog.
Pool & concentrate fracⁿˢ 40-49
Total vol = 30 ml
O.D.²⁶⁰ 1.9 ≡ 1 mg.
Fracⁿ =

ddGTP - test prior to final column purification.

Anneal T10/ϕx(-).

Mix: G/20 1
A° ddG: T/20 20
G/20 20.
Hx10 15

A° ddT mix: T/20 1
G/20 20
C/20 20
Mx10 15'
(= ~~A° ddG mix~~)

a) 2 T10/ϕ3c, 1 A° ddG mix, 1~10 mM ddG, 1 A* dry, 1 K/S 15 r.t.
b) " " 1~1 mM " , " " "
c) " 1 A° ddT mix. 1 mM ddT, " " "

+ 1 µl chase mix (A/20 + G/20 or T/20) 15 r.t.

+ EDTA, gel etc.

Looks good at ~~1~~ 1 µl of 1 mM level.

Try complete set of ddT, ddA, ddG and araC on T10.

ddT : 0.1 mM (final conc").
ddA : 0.1 mM (" ").
ddG : 0.2 mM (" ").

(Aras:
Final conc" i) (short) 5 mM — Ratio dxTP. 5x10³
ii) (long) 1 mM. 1x10³.
r.t. 15' as dd's.
(100 mM stocks of ara's in S.N.'s freezer).
Chase ara's ± label TP only.)

						chase
a	2µl T10/ϕx,	1 0.5 mM ddT,	1 A° ddT mix,	1 A* dry,	1 K/S,	15' r.t., 1µl AT/20 15' r.t.
b	"	1 0.5 mM ddA,	1 C° ddA " ,	C*	"	" A/C
c	"	1 1 mM ddG,	1 A° ddG " ,	A*	"	- A/G
d	short -	1 25 mM AraC,	1 A° ddC mix,	A*	"	" A
e	long "	1 5 mM " ,	" " ,	A*	"	" A
f	short "	1 25 mM AraA,	1 C° ddA " ,	C*	"	" C
g	long "	1 5 mM " ,	" "	C*	"	" C

½ a,b,c + d,f long run. (10 hrs)
½ a b c + d,f short run (5 hrs).

G.

dddG purific^n.
10g silica column in 5% MeOH/CH_2Cl_2
Eluted ā 20% meOH.
Relevant fract^ns (3-7 [~40ml each]) pooled + dried

C.

+ .5 ml C.C.P.
Dry from pyridine 3x
+ 0.5g DCC in 2.5 ml pyridine
2 days.

[Prep^n cyanoethyl phosphate

0.8g Ba cyanoethyl phosphate
10ml H_2O.
+ a little Dowex 50 (H^+) to dissolve.

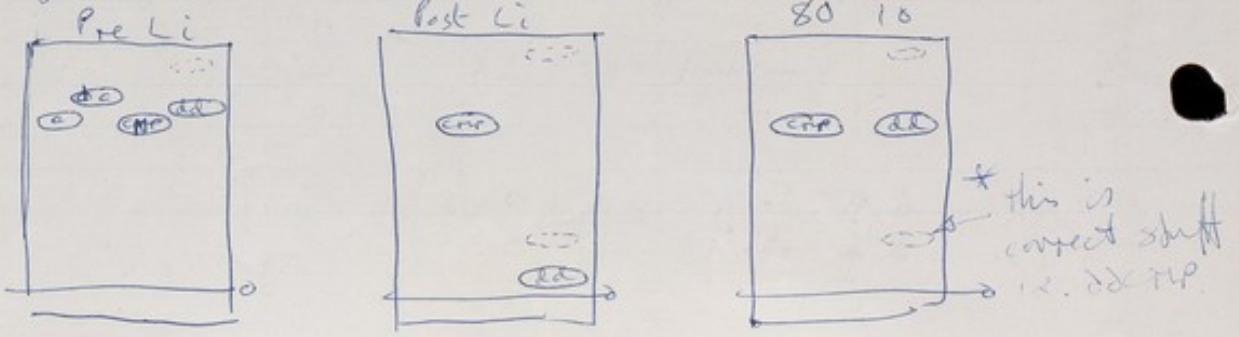
Wash thro' 1x 10cm D50 (H^+) column.
+ 1 ml pyridine

Concentrate → 1 ml.

+ pyridine → 2.5 ml.

Keep ~ 1 month at 0°.]

+ 0.5 ml H_2O
30' r.t.
Vac'd to near dryness.
+ 1 ml H_2O. (~~tested~~). (cellulose/iso-prop)/NH_3/H_2O)
Vac'd down.
+ 5 ml 0.4N LiOH.
30' r.t.
Dowex (pyridinium) column. * this turns out not to be CMP.
- v. slow product.
Sample 80° 10'
- most runs ± CMP marker.
whole 80° 10'.



T6(+)

							chase
a	2 T6/+	1 0.5mM ddT	1 A° ddT.	1A*	1K/5 15' r.t.		A/T /20 10' r.t.
b	..	1 0.4mM ddA	1 C° ddA	1C*	..		A/C
c	..	1 1.0mM ddG	1 A° ddG	1A*	-		A/G
d	..	1 25mM AraC	1 A° ddC	1A*	-		A
e.	-	1 25mM AraA	1 C° ddA	1C*	-		C.

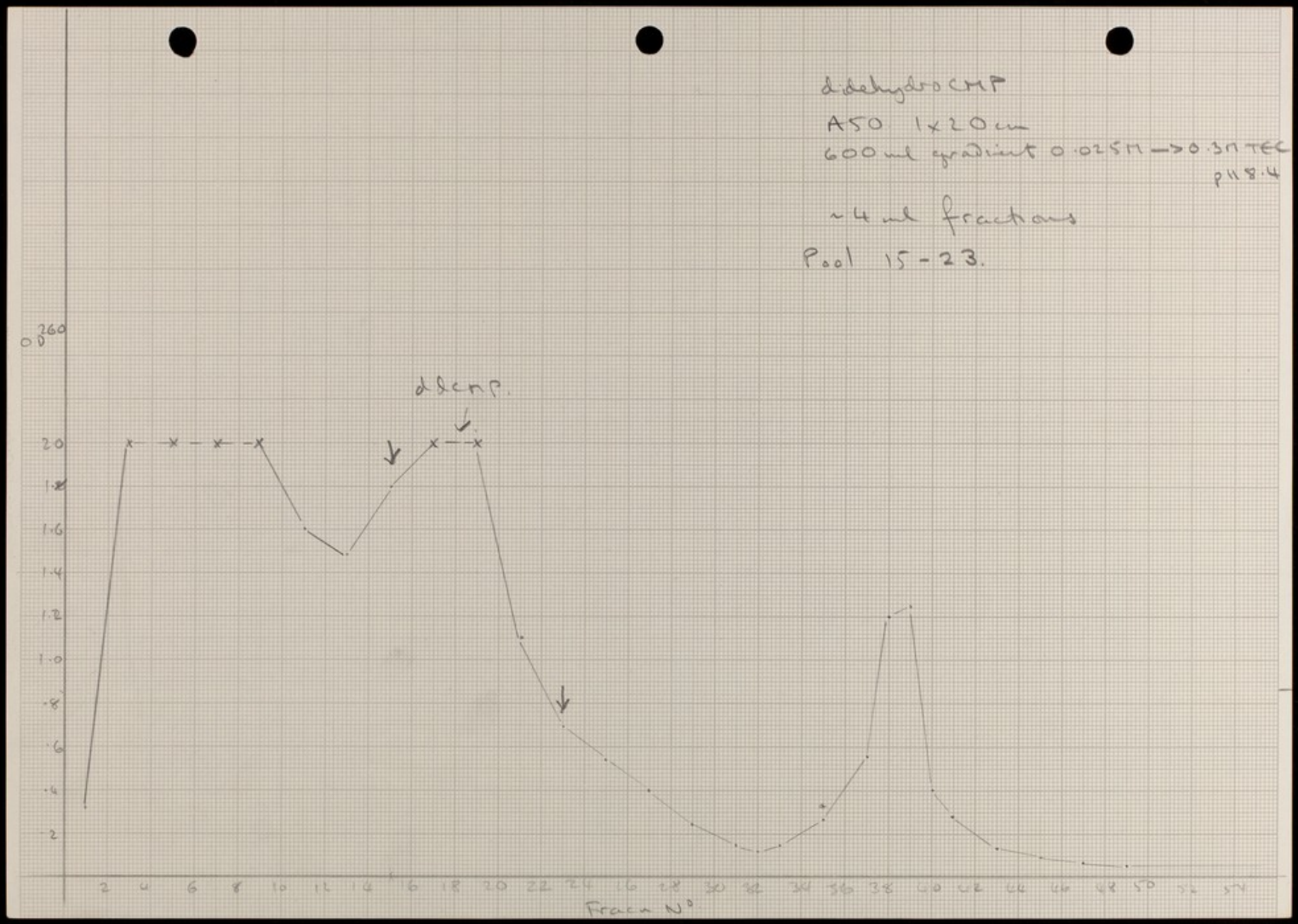
+ 1.5 Taq 60' 60°. + 1μl co M.

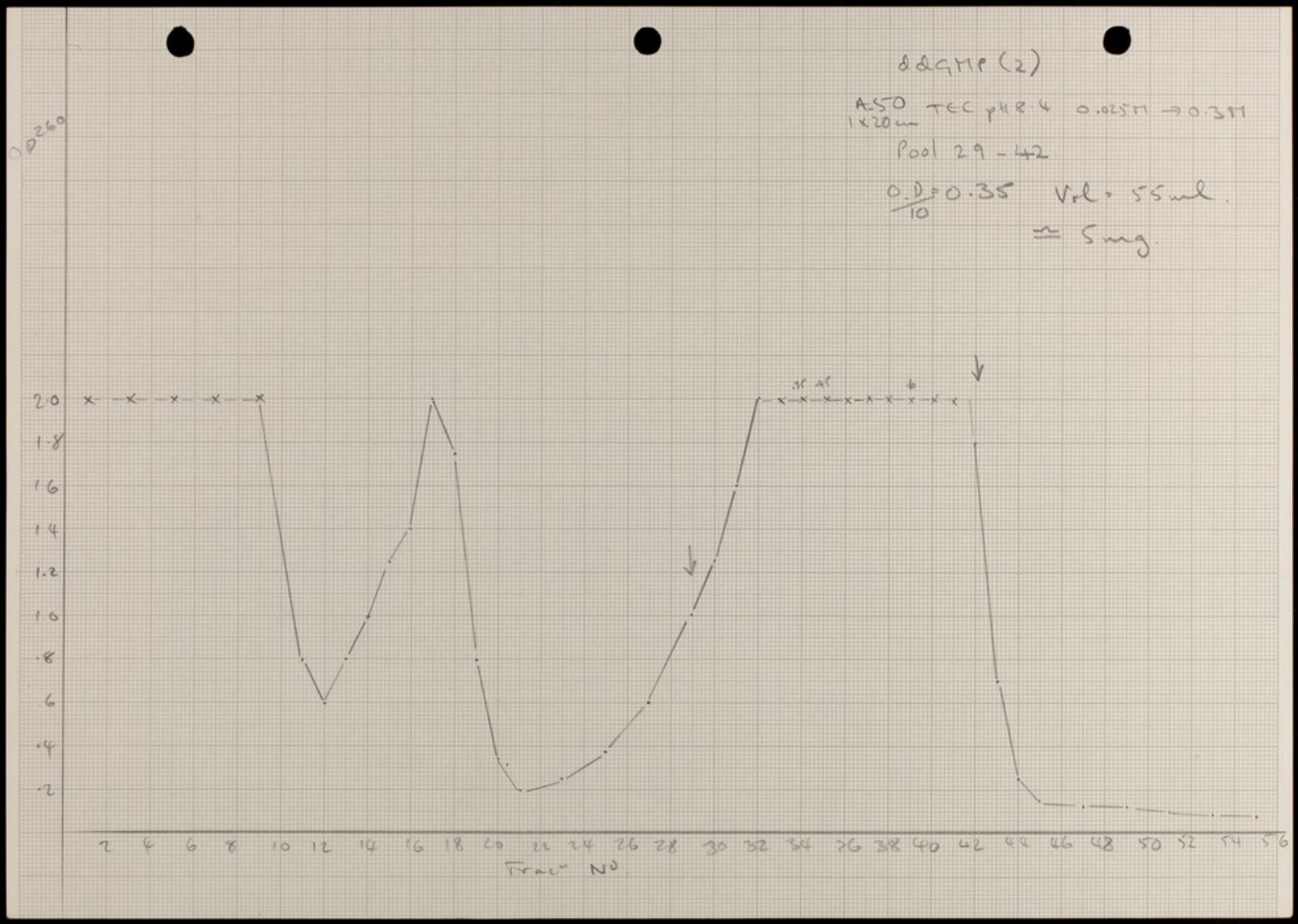
Divide samples in 2. i) 24 hrs H.V.
ii) 12 hrs H.V. (slow blue just off)
(800v)

e b a c d.

didehydro CMP
Run on A-50 column as for ddGMP.

CMP peak pooled, dried and resuspended in pyridine (dissolved easily.) (1/5 spilled).
+ 1μl tributylamine.
Dried from pyridine 3x
.. .. DMF 1x
+ 3mg carbonyldiimidazole in 0.1ml DMF
4hrs.

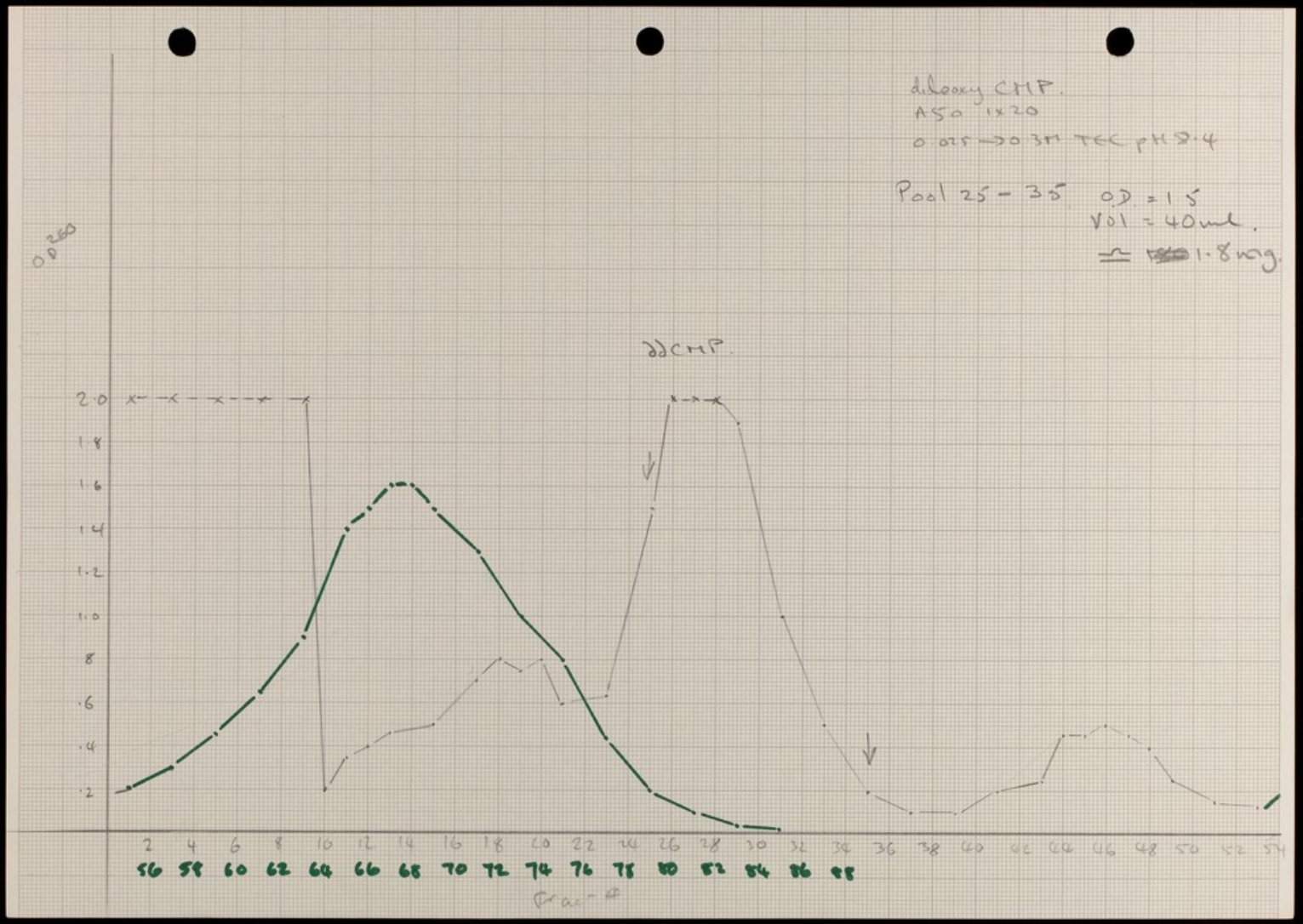

d dehydro CMP
A50 1×20 cm
600 ml gradient 0.025M → 0.3M TEC
pH 8.4
~4 ml fractions
Pool 15-23.
OD 260
d dcmp
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
Fract N°


dAdGMP (2)
A-50
1×20cm TEC pH 8·4 0.025M → 0.3M
Pool 29 - 42
0.D/10 = 0.35 Vol = 55ml.
≃ 5mg.
O.D 260
2·0
1·8
1·6
1·4
1·2
1·0
·8
·6
·4
·2
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56
Frac N°

4. (5mg GMP(II))
29-42 pooled & vac'd down.
Taken up in pyridine
Dried.
+ pyridine
+ 5 µl tributylamine.
Dried 3x pyridine and 1x DMF
+ 16 mg carbonyldiimidazole in 0.2 ml DMF.
12-4.00. 4 hrs r.t.
+ 6 µl methanol 30'.

+ 0.75 ml pyrophosphate in DMF.

20 hr r.t. (5pm - 1pm).


dAcmp.
A50 1x20
0.025 → 0.3M TEC pH 9.4
Pool 25 - 35
OD = 1.5
Vol = 40ml.
1.8mg.
OD 260
ddCMP
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54
56 58 60 62 64 66 68 70 72 74 76 78 80 82 84 86 88
Frac #

ddCTP ~ GTP (II) ~~test~~.

							chase
a	2 TIO(-),	1 0.5mM ddT,	1 A° ddT,	1 A*,	1 K/S	15' + ↓.	A,T/20
b	"	1 ddC/1	1 A° ddC	"	"	"	A,C/20
c	"	1 ddC/5	"	"	"	"	"
d	"	1 ddC/10	"	"	"	"	"
e	"	1 25mM AraC,	"	"	"	"	A/20
f	"	1 1mM ddG(I)	1 A° ddG	"	"	"	A,G/20
g	"	1 ddG(II)/5	"	"	"	"	"
h	"	1 ddG(II)/10.	"	"	"	"	"

15' chase, + GPA gel etc.

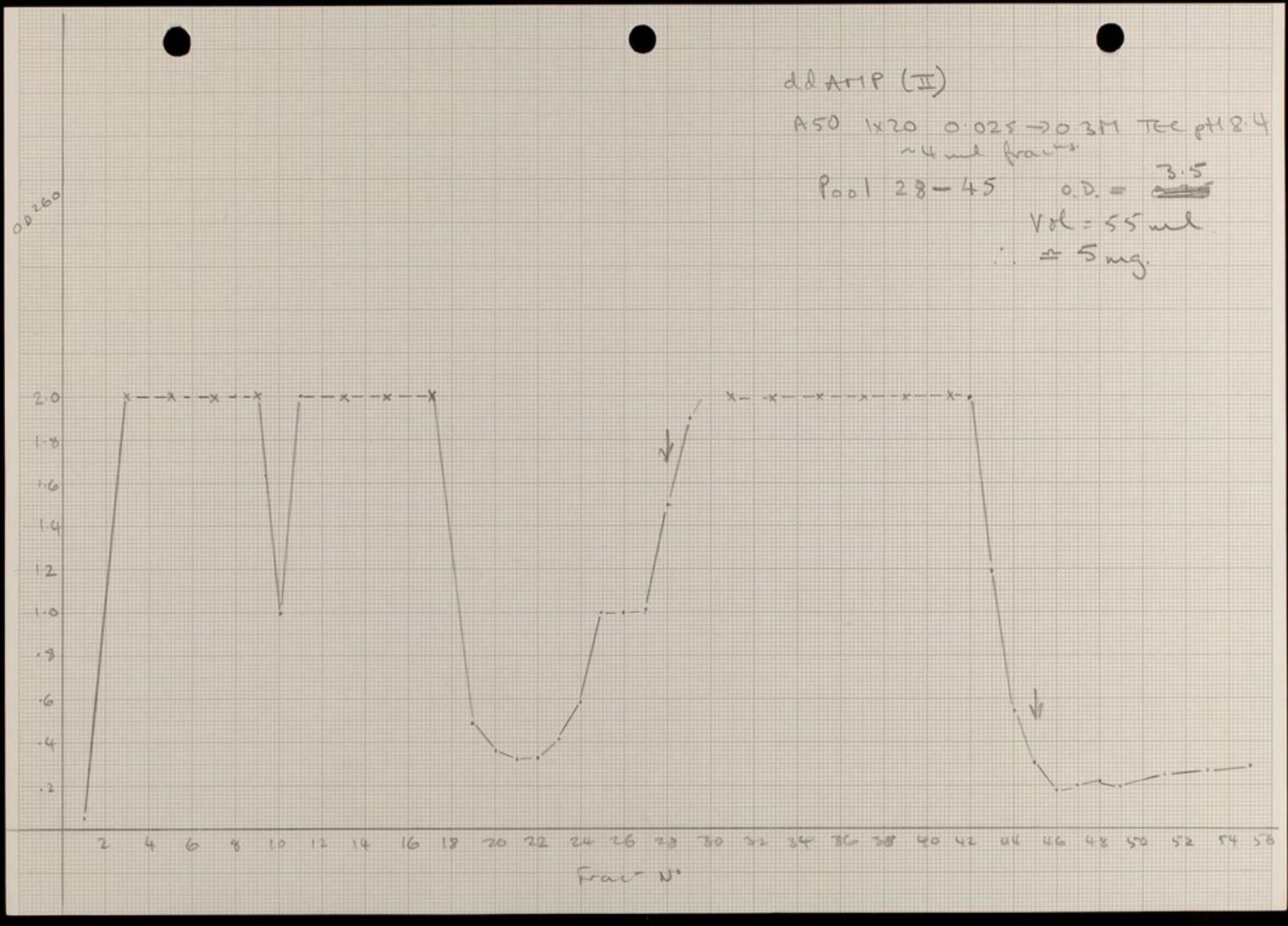
ddAMP (II).

1x20 A-50, 0.025 → 0.3M TEC pH 8.4.
From 28-45 pooled and concentrated.
Vac'd from pyridine
Then triphosphate work-up as for 9 (II).

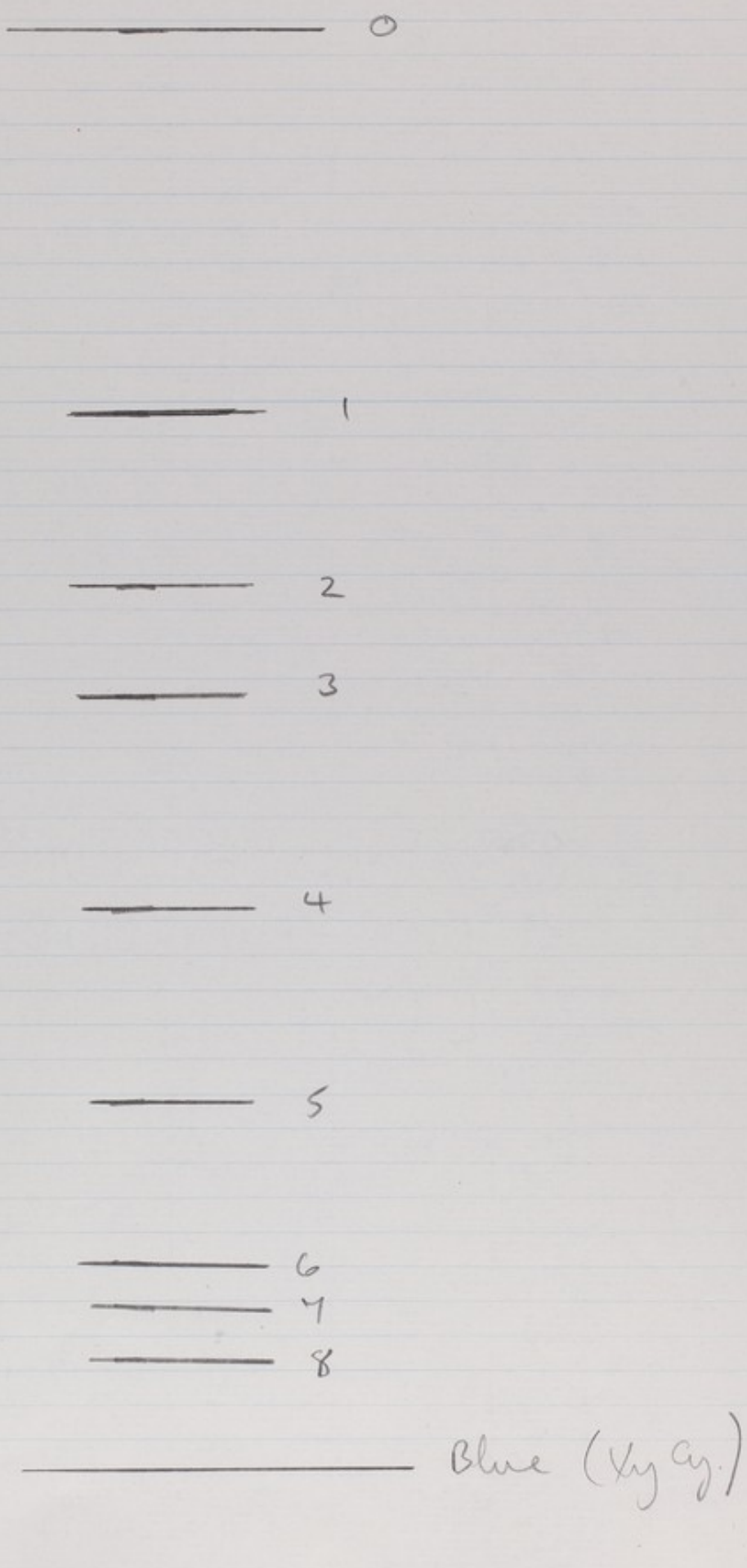
~~(Initial solid residue on addition of pyrophosphate to redissolved overnight)~~.

Finaly dissolved in 0.75 ml H_2O. (= ddATP (II))

Periodic. ~~Test~~ - good proportion of ~~ATP~~ATP
looks roughly 10 mM?


dd AMP (II)
A50 1x20 0·025→0·3M TEC pH 8·4
~4 ml fract.
Pool 28–45 O.D. = 3.5
Vol = 55 ml
∴ ≏ 5 mg.
O.D 260
2·0
1·8
1·6
1·4
1·2
1·0
·8
·6
·4
·2
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56
Frac N°

Alu frag prep 10% gel.



Prep^{n}. Alw frags 14-17.

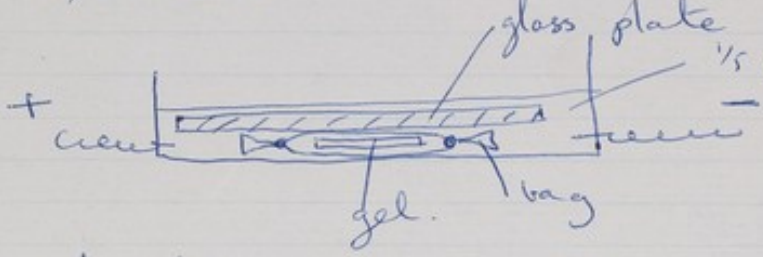
50 µg dx RF in 150 µl H_2O (S.D.)
18 µl 10x Hin buffer
10 µl Alw. (Miles).
2 hrs 37°.

Pol extracted 2x.
Pol H_2O extracted.
Ether 5X
EtOH ppt'd.
+ 40 µl H_2O, 10 µl 50% sucrose / dye sol^{n}.
10% gel. (Most blue near bottom).

(Dried out during run).

Stained Et. Br. (1 ml of 5 mg/ml / litre). 10'.

Bands 1-8 eluted by Vaynes method.
(large - small)
(Intact gel band put in dialysis bag sealed at each end containing c. 2 ml of 1/5 TBE + 0.1% SDS.



40 mA o/N.

+ NaAc, EtOH.

Spun · 40' 40K (SW50.1).
Decanted; + 50 µl H_2O; Dry; + 50 µl H_2O.

(Labelled A1-A8 6/77).

T6(+).

					chase
a	2T6/ϕx,	1 0.5mM ddT,	1A° ddT,	1 A*,	1 K/5 15' c.t. A,T/20 15'
b	"	1 1mM ddG,	1A° ddG,	"	"
c	"	1 25mM AraC,	1A° ddC,	"	"
d	"	1 ddC/10,	1A° ddC,	"	"
e	"	1 0.4mM ddA,	1A° ddA,	"	A/10 [redacted] 15'
f	"	"	"	"	" 5'
g	"	"	"	"	NONE
h	"	"	"	2A*	" A/20 15'
i	"	1 ddA(II)/10,	"	1A*	" "
j	"	1 ddA(II)/20,	"	"	" "

- Forgot about Taq cutting before adding EDTA to f and g. These 2 samples scrapped.

Gel dried out sometime after both blues had run off. (800V 17hrs).

A16 rCT*.

10µl A16
2µl + ϕx
3' 100°
2µl Smix
60' 67°
10µl T* (dry).
2.5µl 10mM rCTP
2.5µl Smix
5µl H_2O
2µl Klenow.

30' 0°
1µl 0.2M EDTA
G-100 column.

Q7(+).
4x dd.
Uncut run at v. high current on 12% TBE gel made in heat resistant (pyrex?) plates.
Lag edges of plate (lagging wrapped in polythene)

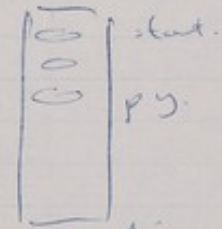
Beginning of run (80mA 1.2kV).
Began to bend wrong way (i.e. ⌒).
Lagging moved further towards edge.
Voltage dropped to 1kV after ~ 30'.
Lagging removed after ~ 4hrs.

DDC (III).

After acid hydrolysis. (200mg starting material).

~~15g silica column.~~

Vac. down and take up in $CHCl_3$. (20 ml?)
Load onto 15g silica column.
Wash well with $CHCl_3$.
Elute with 5% EtOH/$CHCl_3$.



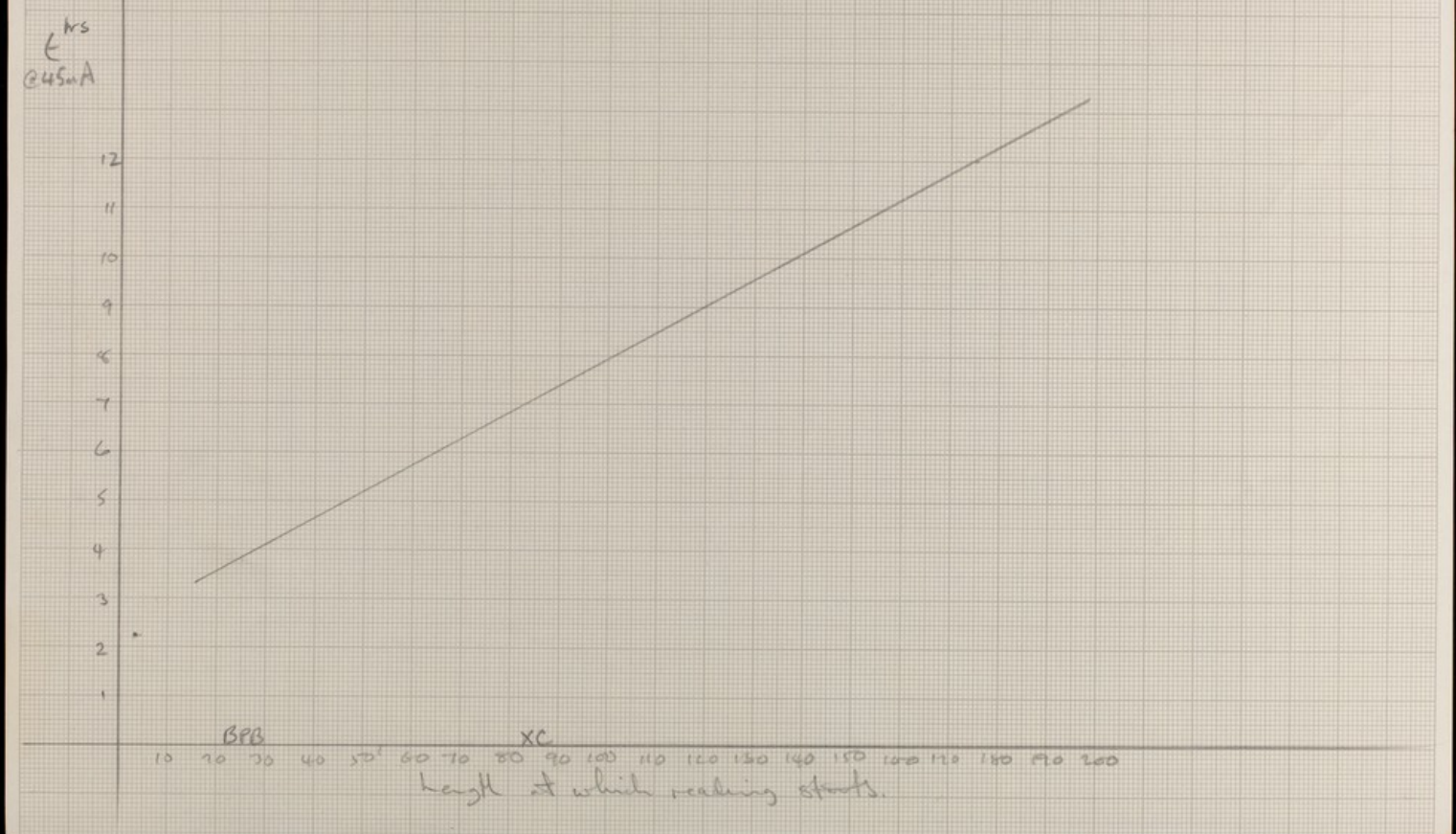
Test run on Si / 10% EtOH/$CHCl_3$.

Collect 5% fracs 3x4 (=20 ml).
Concentrate wash (=100 ml) which seem to contain some stuff (column overloaded?).
+2 ml acetic/pyr./H_2O for 2nd hydrolysis; 4 days
Vac.; + 5 ml $CHCl_3$
15g Si column.
Pool 5% fracs 3x4. and pool with 3x4 from first acid hydrolysis
Vac'd down.
+2 ml pyridine
Vac'd down 3x from pyridine
1x from DMF.
1.0 ml DMF
+0.3 ml CH_3ONa.
45' r.t.
+20 ml MeOH
0.1g Amberlite (IRC-50 H+)
2 hrs stirring.
Test Si/10% EtOH/$CHCl_3$
Gave rather complex picture
Tested again after 24 hrs at -20°.
Looks pretty much the same.

NH_3.

Vac. down
+2 ml conc. ammonia 1 hr 45°.
Si column (10g) (Elute 20% EtOH.).

8% gel 'Raven type' gel apparatus.



A16 (+)

				chase
a	2 µl A16(+),	1 µl 5 mM ddA,	1 µl A°ddA mix, 1 µl K/S, 15' rt,	1 µl A/20
b	-	1 µl mM ddG,	1 µl A°ddG " " "	A+G
c	-	1 µl ddC/10,	A°ddC " " "	A+C
d	-	1 µl 25 mM AraC,	A°ddC " " "	A+C*
e	-	1 µl mM ddT,	A°ddT " " "	A+T

*Both - should have been A only.

Run 8% gel.

8% gel calibration:

			Time 45 mA Rowe type
A	BPB to bottom	reading at 25	3.75 hrs
B	XC " " " " "	85	7.25
C	1.5 x B " " "	135	10
D	3 x A " " "	175.	12

For required region of this expt, should be about 9 hrs at 45 mA in Rowe type apparatus.

8% gel. 72 g urea. TEMACAC
12 g acryl.
0.4 g bis
15 ml 10 x TBE
H_2O → 150 ml.
0.5 ml 10% Amps
50 µl TEMED.

rCT* on A9(+), A10(+), A11(+) and A11(-)

Annealed by Fred. Presumably they contain Hin buffer. Anyway add S mix and hope for the best. (I think this has worked before).

6µl annealed fragment.
1µl 10mM rCTP
1µl S mix
2µl H_2O
5µl T* (dry)
1µl Klenow
30' 0°
1µl 0.2M EDTA
G-100.

- all seem o.K. (~ 20 cps.)
In fact, incorpn seems somewhat higher than expected.
Dried down, + ~~10µl H_2O~~ 30µl 1x Hin buffer.

Usual dideoxy's. (fresh mixes).
Chased 15' dideoxy ~~TR~~ + label /20. 15'
+ 1µl 0.1M EDTA
+ 1µl 10mg/ml Panc mix
60' 37°

A9(+)	XC	to bottom
A10(+)	XC	to bottom.
A11(+)	BPB	just off
A11(-)	XC	to bottom.

[Test new Alu frags.
Small scale dd T]

Identification of small Alw fragments (6/77 prep).
Single site rCT* on fragments 1-8. (- strand)
- all gave usual sort of incorporation (i.e. 10-20 c.p.s. coming off column).
Dried down and + 30µl 1x Hin.
ddT on each.

⌈ Q1 (-) Hin cut ~~long run~~ Long run (to read at about 100).
Lower A+C (½ normal) ⌋.

⌈ A10 (+) v. short ⌋
A11 (+) " " ⌋ ✓

A9 seems contaminated. - scrap further runs.

Long runs: A10 (+) 130 to bottom 9.5 hrs 45 mA
A11 (+) " " "
A11 (-) 120 " " " ✓

A13(+) rC.

Testing of G° mixes. (To test with dGTP* from Amersham about 3 weeks old).

	G° ddA	G° ddC	G° ddT	G° ddG
0.5mM dATP	1	20	20.	15
0.5mM dCTP	20	1	20	15
0.5mM dTTP	20	20	1	15
Hin x10	15	15	15	15

Looks as though following changes are desirable for this system:-

½ ddG

½ dATP in G° ddA mix.

⌈A10⁺, ~~A11~~ rC's⌋

⌈A17 - rC⌋ (long).

⌈A11 - rcshort⌋ (~~[illegible]~~

⌈R4(-) Z cut 5' 37° (1μl 8u/E)

Z1(-

Z4(-) ..

Q3G Hha 1μl 15'

Q3c(-) Hinf 1μl 15'

} +10μl 1xH.

ddC instead of araC in all.

1μl 0.25mM ddA instead of 0.5mM

3c 2-R, r.– slow blue 2/3 down.

(nd).

A17rCC(-) 8% gel
A13rCC(+) "

(6 bis
11.4 acryl.).

A11(-) rC from 65 - 12% x 5 to bottom

A4+ } rCT*
A2+
(annealed without Mn).

A2(vac) short and 170+ on 8%.
R4(vac) 8% 180

A15rc + reagent

Q3c 8% 140+
Run: A17(-).

A2(+).
V. long run lower dd concns. (1/2x)
(250+? 24hrs 42mA)

8%: A13 v. long (see A2)
A17+ 110→ (8 hrs).
A18 110→
190→ (13 hrs).

- Q4 (+) Hin f cut (~2840 30→).
- A13 (+) rc v. long lower d.d. conc^ns. } same 8%
- A18 (+) " " " " "
- A13 (+) Hin f cut 135→ 8%
- R4 (+) v. short (3670 12% 80) & long (3540 8% 100 3600)
- A10 (+) Hha cut (3370) 200→ 8%
- Q3c (-) Hha (2170 - 2330 99→) 8%.
- Q1 (-) Hin f cut (1300 - 1320) (100→) 8%

Q4, A13 (f cut), Q1 - 2 µl dATP* / 1 µl 0.5 mM dd ATP-
f cutting : 1 µl 15' 37°.

8% :
A13 rc, A18 rc.
R4 long, Q3c r
~~A13 Hin f, A3c (-)~~
A10

12%
R4 short.

Hha : 1 µl 15'
Hind III : 1 µl 15'

as used for failures

a 2 A17 -, 1 A°ddT, 1 mM ddT, 1 k/5, 1 NEW A* 15' rt. AT chase 15' rt
b 2 A17 + " " " " " "
c 2 A17 + " " " 1 new Amer A* " "
d 2 A17 + " " 1 other k/5 " "
e 2 A17 - " " " " "
f 2 A17 - 1 fresh A°ddT, " 1 k/5 1 NEW A* "

some lost before gel added

Kornmann.

Prepⁿ of stock solⁿ of DNase A'

+ 100µl 0.02M NaAc (pH 5.0), 2.5% PEG 400.
to ~~str~~ dry enzyme/PEG 20,000 in tube. Stored -20°.

A13 rc (+)
A21 no cut (-). (v. low incorpⁿ). } 8hrs 8%.

Ammended dideoxy concⁿˢ: ddC/10.
0.5mM T.
0.25mM A.
1mM G.

0.4mM thick 8% gel. (vol: ~35ml)

Side strips & template made from 2 thicknesses of polythene. (= 0.4mM).
Standard 8% TBU gel poured (difficult to pour without bubbles).
A21 (-) uncut (as above) aC aC T A G²/3 G¹/3.
(cap. broke)

1.5kV = 20mA.
Turned up to **25**mA after 30'. ~~Not [illegible]~~. 11.30-1.30
Fort Hoe off

Problems:
~~Diff~~ Bubbles on pouring
Interface with template not absolutely smooth,
Loading with v. narrow capⁿ tricky.
Firing etc. tricky.

/

Large prep. A21(-) ddA for testing thin gels.
Total final vol = 35 µl.

12 µl A21(-) ddA / 40 µl formamide/dye.

8% 0.4 mM gel.

	std. slot		~½ width			~¼ width		
10 µl	5	3	5	3	2	5	2	1

there 2 slots pretty rough.

Loaded ≈ Hamilton syringe + v. fine needle.

2 hr 25 mA (1.5 kV). BPB off.

A16 (-) long

A16 (+) v. long - 1

Narrow divider. —

A	A	T	—	T	T	A	A	A	T	T	A	A	A
3	3	5		2	3	1	2	5	3	5	2	3	5

narrow

redried in formamide 24 hrs -20°

Loading pretty awful.

Narrow slots v. difficult to work out.

8% thin with shallower slots & narrow dividing strips.
Wells formed v. nicely & easy to wash.
Loading with Hamilton still tricky and takes too long (first loadings diffused before others ready).

	·5cm				·25cm				
A21(-) dd	A	A	T	T	A	A	A	T	T
vol. λ	5	3	3	5	5	5	2	5	>

bubble.

⌜ R9(+) (2240 → 2200 100 → 120)
R10(-) (~2800. 200+) ⌟

R9(+) & R10(-) 8% thin (0.6mM). (vol ~ 50ml).

R10(-) 7W 45-A
R9(+) 4W 45-A.

⌜ Repeat A13 rc(+). 8W 8% ⌟
v. long A21(-). (360+)

V. long A21: ½ x n dd concns. except A (0.25nM)
Run 0.6mM 8% (30mA 16W?).

⌜ Longer A13 rc(+) ⌟

8% non-denat. gel for R10 & RF T4 ligated
Should be R10 ~ 80, R9 ~ 120.

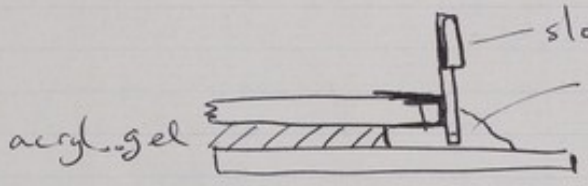
⌜ A21(-) ~ 2600 ⌟ (140+)

2.30.
1.45

Q3c	(-)	f cut	2170 - 2230	140	6 hr
A13	(+)	f cut	~ 4610	80.	3.5 hr.

(P3c - most of DC lost after add^{n} of enzyme!.

Loading of samples onto 0.4mM gel using agarose slots.



5 µl A13(G) (as above) loaded into slots & sealed with agarose / buffer
- Slots too large
- v. streaky at beginning of run.
But basically quite easy.

A/r pretty poor but not hopeless

Try 0.7% agarose / 2.5% acrylamide.

{ 50 ml 1.4% agarose / 1 x TBE / 7M Urea
{ 50 ml 5% acrylamide / bis / Urea / TBE / .25 ml 10% amp^{n} 25 µl TEMED.

- This won't work - urea prevents setting of agarose!

ddC (II) prep.

2-d silica column after acid ~~treatment~~.
~2 l ddC/$CHCl_3$ dried down from 1st column.
Silica (200 g) made up in $CHCl_3$ from this.
ddC loaded & washed in thin $CHCl_3$ fray conc^n.
Yellow band eluted v. slowly with wash.
Eluted after ~1.5 l
Fracs 14-17 in (2 Gradd) (100 ml fracs) pooled and concentrated to ~20 ml. (-20°C).

Elutions from R9/R10 (?) preps.

5 µg RF; so each made up in 5 µl H_2O after spinning (sw 60 Ti) & drying down.

2.5 µl each
0.5 µl 0x s.s. (-).
0.5 µl 1 x H
anneal
H_2O → 10 µl for dd's.

After dd reaction (6 µl final, after cutting ± HindIII)
3 µl added to 10 µl formamide / EDTA.
3 µl of this loaded onto 4% gel.
Loaded ± glass caps. 1 broke :- 2C.
Result :- 1 is R8, so 2 and 3 presumably R9 & 10 respect.

25 mA
12.15 → 2.15

⌈ A21 (+).

Q3c ddT - rerun with others. ~140. ⌋

A21 2 hr, 4 hr, 7 hr, 12 hr. 0.6 mM 25 mA

6.00 3.15 10.15.

⌈ R9 (+) cut HindIII (2230 →) 100+
R10 (-) " " (~2800) 200+ ⌋

Peterward GG477

⌈ ~~R8 (+) Tag~~

TG (+) XC to bottom.
R8 (-) " "

Q3c 8 hr.

12.15 3.45 6 8
A21 (+) (0.2 mM 25 mA). 15 µl Formdye; 3 µl/slot / 10k 9, 7, 4, 2
T6 (+) repeat A (0.2 mM 25 mA). Taq 1 µl 60° 10.

To see if thin gels more efficient at eradicating pile-ups.

A19(-) uncut XC 3/4 (~3920) 3hr?

Q36(-) Hha cut BPB 3/4. (~3000). 1.5 hr?

+ 15 µl form/dye; load 5 µl.

Q36 loading a bit messy.

---

a-l (various).

1 µl sample
3 µl formamide/dye
All loaded 0.4 mM gel 25 mA 1.45 pm –

2.5 — 20% gradient gel (λ digests)

A) 2.5%: 6.25 g acryl.
0.2 g bis-ac.
25 g sucrose
25 ml 10× buffer
H_2O 250 ml.

720 µl 10% TEMED
360 µl 10% amps / 150 ml.

B) 20%: 50 g acryl.
1.25 g bis-ac.
50 g sucrose
25 ml 10× GGB
H_2O 250 ml.

180 µl 10% TEMED
360 µl 10% amps. / 150 ml.

C) Sucrose: 35 g sucrose
10 ml 10× GGB.
H_2O 100 ml. + BPB.

D) 1× GGB
+ BPB.

E) 10× GGB
48.4 g Tris base
27.2 g $NaAc.3H_2O$
7.44 g EDTA
HAc pH 8.3
H_2O 1 l.

F) Starting gel

2.5 g acryl.
0.125 g bis-ac
2 ml 10× GGB
H_2O 100 ml
100 µl TEMED
1 ml 10% Amps

Stain
Eth. Br. 1 mg/l 15'.

Az1 (+)
7 x 12 hr. 0.4 mA 25 mA
j b e f l h m n o
7hr.
↑ RE 90° 3'
(2) 5 x 10 hr. .4 25 mA
c' c² c³ c+ A G e f l h

10 x Terminal transferase buffer.

4.83 g cacodylic acid 1.4M
0.91 g Tris base 0.3M
H_2O 15 ml
KOH → pH 7.6
0.25 ml 0.1M DTT 1mM
2.5 ml 0.1M fresh $CoCl_2$ 10mM.
H_2O 25 ml
(last added slowly with stirring)

1.1

Sharples centrifuge.

(1). Insert cylinder of film (± small overlap) into metal cylinder.
(2). Put top on cylinder and tighten by banging spanner on wall.
(3) Place loaded cylinder in conical cup.
(4). Put spouts in place.
(5). Put lid on spouts.
(6) Tighten cylinder to top spindle with spanners.
(7) Insert bottom plug (opposite thread) and tighten with spanners.
(8) Insert inlet pipe into bottom
(9) Insert 3/4-cylindrical safety piece at top and tighten
(10). Attach inlet to pump, and attach piping from fermentor to pump.
(11). Open tap (speed must not go past red line on dial). Small distance.
(12) Turn pump on (May be necessary to run fast to begin with before decreasing to mid-point [vertical]).

Yeast mitochondrial DNA prep.

A364A (A. Travers)
10 ml inoculum in YEP + 0.5% glucose.

YEP: 20 g peptone
10 g yeast extract /l.

Aerated 30° (water-bath) o/N. 00[00] 11.00 0.057
17.00 —

[Hollenberg et al BBA 209 1.]

Didn't grow terribly well.

Try with 0.5% glucose (0.25 ml 20%/10 ml).
0.05% $MgSO_4$ (0.05 ml/10 ml)

S288C (should have fewer requirements)
Plate from slant (A. Travers).

grew up after about 24 hr
.1 ml put in 20 ml same medium Fri evening

1st ... three all bug contaminated

2nd efforts all OK
288 + Mg looks best

10.00 10 ml of this o/N culture added to 1 l YEP + 25 ml 20% glucose, 5 ml M $MgSO_4$.
12.00 0.075
2.00 0.2 24 hour O.D. = 2.
3.00 0.3
4.30 0.65 300 ml o/N culture added to 2 x 10 l fermentors of YEP + 250 ml 20% glucose and 50 ml M $MgSO_4$.
start 11.30. Contaminated.
Start again.

Mitochondria prep Tzagoloff JBC 246 3050

Suspend 50g yeast (wet wt) (A. Travers / Porton)
in 300ml MTE:

0.25 M mannitol
0.05 M Tris-acetate pH 7.5
1mM EDTA

Homog. ± 250 ml glass beads (0.45-0.5 mm)
45 secs
Braun MSK mech-cell homog?
Decant
Wash 2 × 100 MTE

Fuge 750 × g 20' (6X100) (3K)
Super 19,000 × g 20' (12.5 K).

Wash pellet ± 300 ml MTE

Suspend in 0.25 M sucrose
0.01 M Tris-acetate 7.5
at protein conc of 3-5 mg/ml (20ml)

(17 mg/ml

Bernardi, Pigeno & Fouty BBRC 65 173 (1975)

DNase treatment of mitochondria
5mM $MgCl_2$ (0.1ml M/20ml)
200 µg Pan. DNase (400µl of 10 mg/ml)
20' 0°
1200 × g 10'
Super 17000 g 20'
+ 10 ml 0.35 M Sucrose
0.1 M EDTA pH 8.0
17K 10'.
+ 1 ml 0.35 M Sucrose
0.1 M EDTA pH 8.0

Aves PNAS 58 (1967) 620

lysis of mitochondria.

2ml Lysis in 1:10 SSC + 1% SDS 60° 10'
cool → 37°
+ 100 µg/ml pronase o/N 37°
200 µg
10 µl of 20 mg/ml
+ equal vol CHCl3 - 1500 amyl alcohol 24:1
shake 30' (Marmur JMB 3 1961 208)

13 000 x g 5'

Remove aqueous ~~Dialyse o/N SSC~~. O.D. 500/ml 10 O.D. total = 500 µg.

EtOH ppt'd.
+ 5ml 0.27M Na(P) pH 6.8.

Absorbed o/N 4° onto 1ml HAP.
Washed 2x3ml 0.27M Na(P) pH 6.8.
Loaded above 4ml HAP column.
Washed 5ml 0.27M Na(P) 6.8
2x25ml 0.27M → 0.5M Na(P) gradient, 1ml fractions, 4°C. Buffer ~~stalled~~ crystallised o/N! OD_{260} stuff dribbled off in broad peak after refilling column

Gel apparatus.

M.S. Reid & R.L. Bieleski
Anal. Biochem 22 374-381 (1968)

to room temperature. Abandoned.

Try simple HAP column of whole yeast nucleic acid extract. (Bernardi et al ~~1966~~ 1972).

A21(+) 6%

a) ddC/20 — 1µl incub + 4µl form/dye
6mM araC
0.5mM ddT
0.25mM ddA (3A*) — 5.5 hr
0.25mM ddG.

b) ddC/10
12mM araC — 5.5 & 4 hrs.
1mM ddT
.5mM ddA (3A*)
.5mM ddG.

6%: 72 Urea
5.8g acryl
.4g bis
etc.
(25µl TEMED
.25ml 10% amps)

This is 4%!!

ddT tracks blank.
Make fresh mixes + repeat.

2nd gel (6%):

a) 1µl : 4µl formamide/dye.

Set of 1µl 5.5 hr. 25mA 220
" " 3µl }
" " 3µl 4 hr. " 3.50

b) ~~3µl 2.5 hr. 5.70.~~

T tracks O.K. but gel a bit fuggy.

3rd gel.

a) ~~1µl~~ 1µl : 4µl form/dye
:) Set of 1µl
:) " " 3µl } 20mA 7hr.
:) 1µl : 1µl

2x

Lithium buffer (Lids.)

	/10 ml
1·0M LiCl	5 ml 2M
20mM tris pH 7·4	0·2 ml 1M
0·2mM EDTA	10 µl ·2M
1% Li dodecyl sulphate.	0·1 gm.

R1 of term. trans. product.

Dried down after column.
+ 10µl 1x R1 buffer
+ 1µl Eco R1 (~~10~~ 10u/µl).

90' 37°

+ 1µl 0·2M EDTA
5' 100° quick cool.

+ 40µl H_2O Eppendorf tubes
+ 50µl Lids x2

+ 1mg oligo-d-T cellulose
2' r.t. with mixing

Spin = super 1
Wash 2 x 40µl 1x Lids = supers 2 & 3

Cellulose eluted 3x40µl 0·1mM EDTA
5' 40°.
= supers 4, 5, 6.

Super 1 reabsorbed to 1mg oligo d-T cell.
Spin (= super 16)
Wash 2x40µl 1x Lids (supers 26 & 36)

Eluted 2 x 40µl 0·1mM EDTA
= supers 46 & 56

4 and 5 combined: + 1/10 vol. NaAc, 2½ vol EtOH
4b " 5b " " "

16 : + 2½ vols EtOH.
Spun, washed EtOH, dried, + 5µl H_2O.

40 [8cm]

4a & 4b and whole prep (b) on 3% gel.

strand sep' b:

Dried down from column
+ 10µl 1x R1 buffer
+ 1µl R1

60' 37°.

2µl of this + 20µl ~~50~~ Formamide/dye/EDTA

4a & 4b (from 1st prep.):-

+20µl form/dye/EDTA.

Gel:

4.2 g acrylamide
0.3 g bis-acryl (i.e. 1:15)
15 ml TBE 10x
72g Urea ~~H2O~~ 150 ml
1.5 ml 10% Amps
150µl TEMED.

~~Each~~ 1.5 mm thick gel.
9 slot spacer.

Run 0/n 200V.

Fragments from 1st prep, annealed by F.S. and used in unsuccessful expt. with low conc^n dideoxy's & mixes, used for dd expt. using regular concentration mixes, dd TTP only.

(Result: no better).

Staining with Et. Br. showed nothing.
(Gel virtually impossible to handle)

q4 pst for term. trans.

10 µl 1.75 mg/ml RF1 q4 (17.5 µg)
100 µl buffer (12 mM Tris 7.4
12 mM $MgCl_2$
1 mM DTT)
80 µl H_2O
10 µl PSE (Porton 2 u/µl)
37° 2 hrs.

Dialyse / 1.4 M K-Cac
0.3 M Tris
1 mM DTT
10 mM $CoCl_2$
pH 7.6.

After phenol & ether.

V.l. after dialysis = 200 λ.

A21(+) fresh annealing

2.5 µl A21
.5 µl +
.5 µl 11x10.
anneal
+1xH ⟶ 15 µl.

Reaction mixes as a) previously.

Run 6% gel.
Deionised formamide for loading.

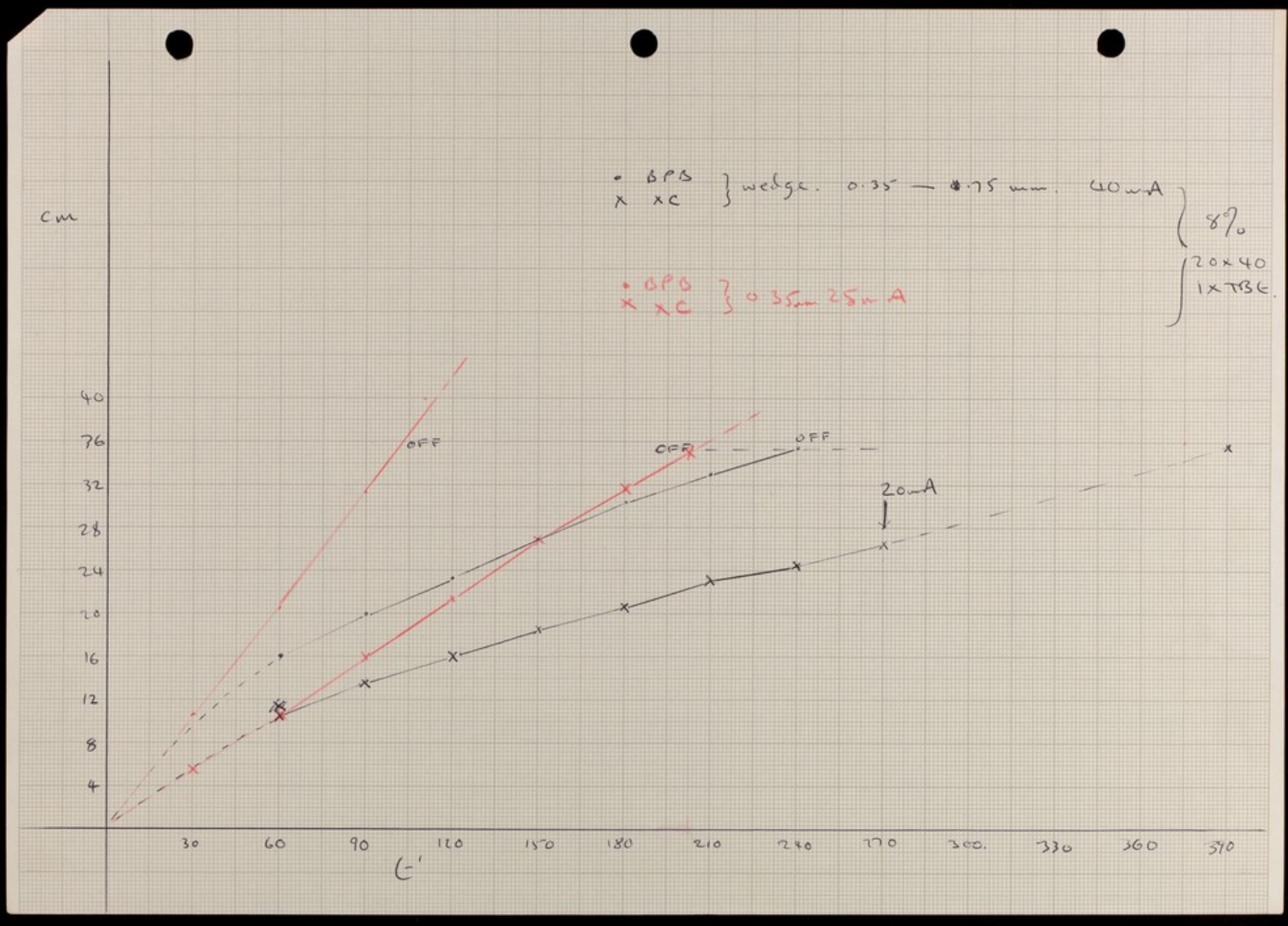
Tapered gel.
Andrews idea to get more even bands throughout length of gel.

1 layer - 5 layers of Plastikcard.
i.e. 0.35 mm ⟶ 1.75 mm
40x20 cm.
8% gel (~100 ml)

Run 1kV 50mA
Too hot at top - back plate cracked.
Reduced to 40mA after 1.5 hr.

2nd gel:
0.35 mm region lengthened (~⅓ of gel).

- Faster bands loose too much resolution because a) more squashed b) thicker gel.
So rather useless, but works in principle


• BPS
x XC
wedge. 0.35 — 0.75 mm. 40mA
8%
20x40
1xTBE.
• BPS
x XC
0.35mm 25mA
cm
40
36
32
28
24
20
16
12
8
4
OFF
OFF
OFF
20mA
30
60
90
120
150
180
210
240
270
300
330
360
390

Various 6% gels to optimise resolution.

a) undeionised, run after 60' setting.
b) deionised; " " " "
c) undeionised; " " O/N " .

6% mix : 72 g urea (SwetA3/Mann U.P.)
8.5 g acrylamide
0.5 g bis
15 ml 10 x TBE (fresh).

Deionisation of b):
Stirred with 5 g MB-1 30' before add^{n} of TBE.

Run A21(+) (as for wedgy gel etc.)
3.50-7.30. 4 hrs 25 mA.

2-D sep^{n} of restric^{n} frags. (Derynck & Fiers JMB (1977) 110, 387)

Stock solns:
HP buffer

	10x g/l
0.036M Tris	43.56
0.03M NaH_2PO_4	46.8
0.001M EDTA	3.7

~2 ml H_2PO_4 → pH 7.7

40% acryl/H_2O (100g/250ml)

40% acryl (100g) 2% bis- (5g/250ml)/H_2O

1st dimension (20 x 40 x 1.5mm) (Separation of small Alu frags of ϕx)
Bottom (5cm sealer gel)

6 ml 40% acryl/bis (20:1)
2.4 ml 10x HP buffer
15.6 ml H_2O
25 µl TEMED
250 µl 10% amps.

20% gel:
50 ml 40% acryl.
0.2 ml 10% amps.
50°C
+ 50 ml 2% agarose in 2x HP buffer at 50°C
+ 40 µl TEMED

12% gel:
30 ml 40% acryl
20 ml H_2O
0.2 ml 10% amps
50°C
+ 50 ml 2% agarose in 2x HP buffer at 50°C
+ 40 µl TEMED.

T4 end-labelled AluI frags of ΦX (~20 μg) loaded in 50% sucrose/BPB/XC. (1:5)
9/10 × 1/10 samples loaded.

Run o/N 350 V (≡ 50 mA) at 4°C.

Gel seemed v. sticky — v. difficult to extract slot former without damaging slots.

After 18 hr, Amps dropped to 10 mA volts to give c.v. to 800 V.

XC just eluted 20%.

2nd dimension (20×40 cm × 1.5 mm)

Faster fragments (all in 20% region) cut out. Placed at about 30° at bottom of former to counteract slope of bands. (Gel to be run bottom to top).

Cap gel.
7.5% acryl. in HP buffer.

7.5 ml 40% acryl/bis (20:1)
4 ml 10×HP
28.5 ml H_2O.
40 μl TEMED
0.4 ml 10% amps.

Poured to ~ 0.5 cm above 1st dimens. segment.
Butanol layered on top.
Gel set in ~ 5'.
Butanol poured off & interface washed with 8% soln before addition of amps.

8% TBE soln:-
30 ml 40% acryl/bis (20:1)
15 ml 10×TBE
H_2O → 150 ml.
75 μl TEMED
0.75 ml 10% amps.

1× TBE in reservoirs
Run 500 V ≡ 50 mA (fairly warm) 1:30 pm → o/n

Got abit overheated – cap gel appeared to separate from main gel.
Turned down after 2 hr. to 20 mA (250 V)
Finally reduced to 150 V O/N. (5 pm – 7.30 am)
BPB ran to 1 cm of bottom.

Elution comparison:

Fragments 12, 14, 16, 18 eluted by soaking in Maxam-Gilbert soaking buffer.

Fragments 13, 15, 17, 19 eluted by electrophoretic method.

2nd 2D gel.
ϕx Alu digest. (½ loaded.)

1st Dimension: 20% throughout. – slot former came out O.K.
Run O/N 350 V (50 mA.)
2nd Dim: 10% →

Frags from 1st 2D gel spun down, + 20 µl H_2O
1 µl counted

(120)	12	1452
	13	594
	14	1332
	15	223
	16	1563
	17	966
	18	1155
	19	901

Soaking appears to be the better method!

Testing of eluted fragments

1 µl ϕx (+)
3 µl H_2O
1 µl H x10

} 1 µl + 1 µl fragment
Anneal.
1 x H → 10 µl
dd T.

0.35 mm gradient gel.

Made in 1 gel machine 6% – 15%.

Mixes.

① Top buffer: 48 g urea
10 ml 10×TBE
H_2O → 100 ml + BPB.

② 6%.

48 g urea
5.7 g acryl.
0.3 g bis –
H_2O → 80 ml.
Deionise (30' MB-1)
+10 g sucrose
+10 ml 10×TBE.
H_2O → 100 ml.

③ 15%

48 g urea
14.25 g acryl.
0.75 g bis –
H_2O → 80 ml
Deionise (30' MB-1)
+20 g sucrose
10 ml 10×TBE
H_2O → 100 ml.

④ Bottom

120 g urea
125 g sucrose
25 ml 10×TBE
H_2O → 250 ml.

Catalysts:

6% (100 ml) 40 µl TEMED
0.4 ml 10% amps

15% (85 ml) 10 µl TEMED
0.2 ml 10% amps.

OVER.

Problems:
- Air bubbles between plates + gel on dismantling apparatus
- messed up interface before pouring slot-former gel (6% standard)
- Amps wrong - did I forget to put buffer in half of gradient?
(2 kV = 15 mA at start of run)
A21(+) loaded.

Repeat of above:
No air bubbles.
Interface o.k.
Amps as before but buffer definitely o.k.
A21(+) 9.5 hr + 4 hr.

=

2nd 20 gel (continued from overleaf)

Frags 19a, 19b, 20a, 20b, 21 and 22 eluted by soaking and EtOH ppte spun down in SW60 Ti. Taken up in 10 µl H_2O.

Strand sep^n agarose gel.

Hayward Virology 49 342.

1% agarose 20×40×.3 cm gel.

Buffer: 0.036 M Tris.
0.03 M NaH_2PO_4.
1mM EDTA pH 7.7 (HR buffer)

Load sample in 0.1 vol 1M NaOH.
0.1 vol 60% sucrose / 0.05% BPB.

Run at 4° 2.5 V/cm 15 hr?

2.

Prep^n of) yeast spheroplasts.

0.1g yeast cells.
1ml buffer (0.63M sorbitol
0.1M citrate-KPO_4 pH 5.8
0.03M β mercaptoethanol.
4×10^{-4} M EDTA)

a 100µl snail gut enzyme (HPJ)
b 10µl " "
c 1µl " "
d no enzyme.

60' r.t.

10µl into 100µl H_2O. (Spheroplasts should rupture)

No apparent change in number of whole cells.

Agarose strand sep^n gel (2)

As before but 1.5%.

40µl sample
+ 5µl .1M NaOH
+ 5µl 60% sucrose / dye.
30
Run N [redacted] O/N.

Dried out during night.
Blue dye not visible.

Post cutting gives 2 bands.

1. Eluted by:
Squeeze through 18 needle.
Into 1ml 150mM NaCl
10mM Tris pH 8.0
1mM EDTA
O/N 37°.
Should have been larger volume.

+ 4ml 150 NaCl
10mM Tris pH 8.0
1mM EDTA
O/N 37°.
Fuge SW40 39K 30'
(Tubes washed in 0.5% albumin and washed well with dist. H_2O.)
Super diluted ē 5ml H_2O
+ Punch HAP in 0.12M phosphate (equimolar)
(Punch = small spatula end)
Shake gently 30' r.t.
Spin down HAP.
Wash 2x ē 200µl .1M phos. (Eppendorf tubes)
Elute ē 3x50µl 0.15M phos.
10' 60° each elution.
Desalt on G-100 (Tris-60mM)
Treat glass column with albumin before pouring.

2. Eluted by:
Band squeezed thro' 18 needle into
1ml of sat. KI
1mM $Na_2S_2O_3$
(to final agarose conc^n of ~ 0.1–0.2%)

Pass over 0.6 x 1 cm HAP column equil.
in sat. KI, 1mM $Na_2S_2O_3$
Wash column = 4 ml KI/$Na_2S_2O_3$
" " " = 4 ml H_2O
Elute = 1 ml of 1M TEC pH 7.8 (2x TEC 1&2)
hydrolyze 3x.

Each sample made up in 2 µl H_2O.

Testing:
1 µl strand.
1 µl G4 Hin f 19 (~606 p)
1 µl 10x M
7 µl H_2O.
Anneal.

Standard digest.
1mM G made up from ddGTP (II) prep^n.
No chase.

KI elution gave total blank.
NaCl " " weak + fuzzy + strand pattern.

Q4 strand sep^{n}.

T4 end-label

5 µl Q4 RF
1 µl R1 buffer x10
2 µl H_2O
2 µl R1.

2hr 37°.

+ 1 µl 10 mM DTT

20 µl dATP* (dry)
2 µl T4 pol.
10' 37°
+ 2 µl 0.2 M EDTA.

+ 15 µl H_2O
25 µl ø.l.
Ether; Q100.

Dried to 35 µl.
3 samples:

µl	H_2O	1M NaOH	dye
20	20	5	5
10	30	5	5
5	35	5	5

Run on 1.5% 20x40x.3cm 1xHP agarose gel.
16 hrs 75 V.

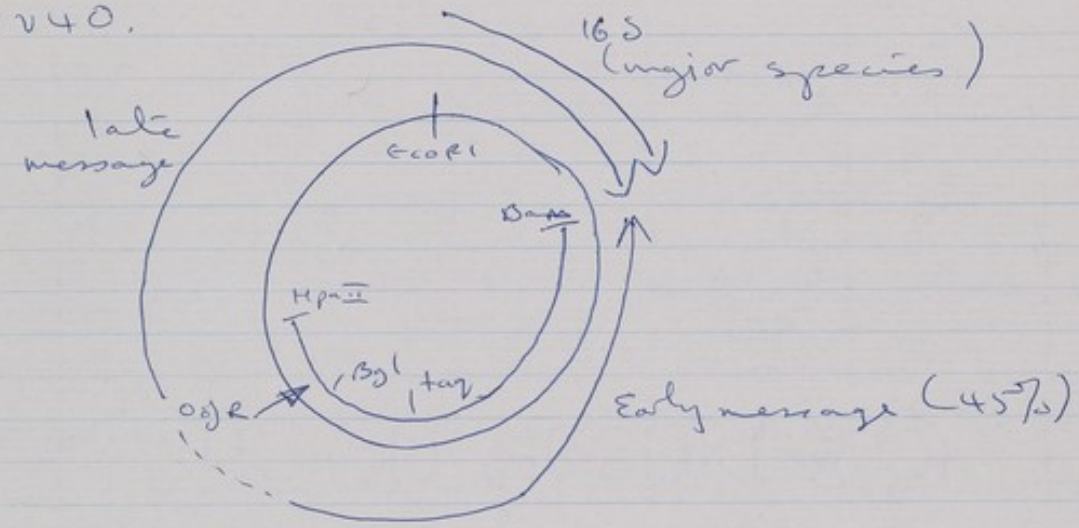
Bands had only migrated 5 cm, but gave good separation. (Just behind dye).
Bands (1&2) ~~eluted~~ cut out
O/N 4°.
Syringed into 5 ml 150 mM NaCl
10 mM Tris pH 8.0
1 mM EDTA.
(SW40 tubes, washed 0.5% bsa)
37° 36hr.
SW40 30' 35K.
← Not v. well eluted ~50%?
(Try eluting remainder by electrophoresis).

W. Fiers - SV40

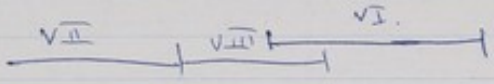
Polyadenylation of MS2, Qβ 5's.
Reverse transcription using $(pT)_{10}$ primer
how TF can.

AATAAA in SV40 as in other messengers
(globin, ovalbumin etc)

SV40.



HpaII, BglI and TaqI → Bam fragments all
(probably) transform.
(SacIII TTCGA)



~~AAAC.AAAA~~

GCCAGAAACAAAAACTAGGGGCGGCCTCATCAGGG-

-TTAGGAACATTAGAGCCTTGAATGGCAGATTTAA-

-TACCAGCATGC
GTC. AAGCn.

GACn.

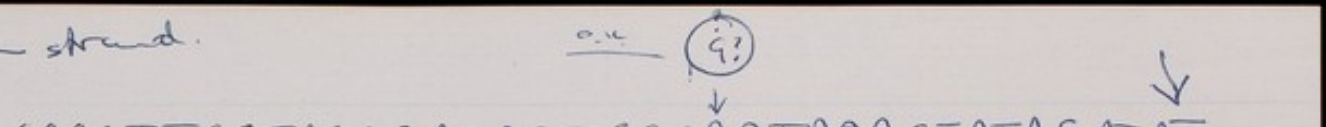
+ GCATGCTGGTATTAAATCTGCCATTCAAGGCTCTAA-

-TGTTCCTAACCCTGATGAGGCCGCCCCTAGTGTG$_3$-

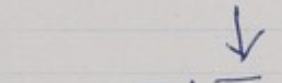
-CTGGC

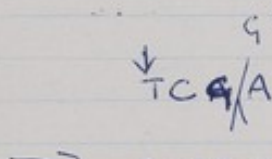
GnCTT

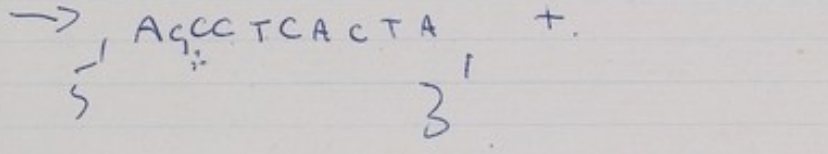
– strand.

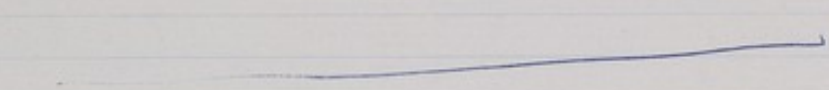


TATAGATATCAAATAACCCTGAAACAAATGCTTAGGG









dd's in A9 - A11, rC's Fresh solns.

30 µl start.

RdA(2)

1 0·05
3 2+
5 2+
7 2+
9 2+ - 1·0
11 2·0 -2+
13 2+
15 2+
17 2+
19 0·5 - 0·36
21 0·32 - 0·33
23 0·41 - 0·58
25 1·0 - 1·0
27 1·1 - 1·5
29 1·9
31 2+
33 2+
35 2+
37 2+
39 2+
41 2+ - 2·0
43 1·2 - 0·55
45 0·3 - 0·18
47 0·2 - 0·22
49 0·2
51 0·25
53 0·27
55 0·29
57 0·33
59 ..
61 0·24
63
65
67
69
71
73
75
77
79.

·80 ml A
0·08A
80 ml
5·20
3·60
5·65
4-05
3·60
45
70
9
11
12-20

5, 14, 36, M

100 = 25 mg/l
3·5
750
125
3·50l = 87·5 mg/l

-87·55 × 55/1000

5·5
·9
4·95

DCMP

1	2+		
3	"		
5	"		
7	"		
9	"	−10 0·2	
11	~~0·35~~ 0·35	−12 0·4	
13	~~0·4~~ 0·45		
15	~~1·50~~ 0·5		
17	0·7.	−19 0·8	
19	0·75	−20 0·8	
21	0·6		
23	0·625		
25	1·50	−26 2+	
27	2+	−28 2+	
29	1·9.		
31	1·0		
33	0·50		
35	0·2		
37	0·1		
39	0·11		
41	0·2		
43	0·25	−44 0·45	
45	0·45	−46 0·5	
47	0·45	−48 0·4	
49	0·25		
51	0·15		
53	0·13		
55	0·20		
57	0·3		
59	0·45		
61	0·65		
63	0·9		
65	1·4~~5~~.	−66 1·5	
67	1·6	−68 1·6	
69	1·5		
71	1·3		
73	1·0		
75	0·8		
77	0·45		
79	0·2		
81	0·09		
83	0·02		
85	0·01		
87			

CMP.

5
19
27
46
67.

1

9(II)

1	2+		
3	2+		
5	2+		
7	2+		
9	2+		
11	0·8	12	0·6
13	0·8	14	1·0
15	1·25	16	1·4
17	2·0	18	1·75
19	0·8	20	0·35
21	0·20		
23	0·25		
25	0·38		
27	0·6		
29	1·0	30	1·25
~~30~~	~~1·25~~		
31	1·6		
32	2·0		
33.	~~2·0~~ 2+		
34	2+		
35	2+.		
36	2+		
37	2+		
38	2+		
39	~~2+~~ 2+		
40	2+		
41	2+		
42	1·8		
43	0·7		
44	0·25		
45	0·15		
47	0·125		
49	0·12		
51	0·1		
53	0·09		
55	0·085		
57	0·075		
59	0·07		
61	0·065		

3	
5	
7	
9	
11	

A. COULSON.

10	
·35	
·45	
~~·6~~	
·6	

1 01 = 32 mg/l.
= 30 µg/ml.

3·5 0 D 55 ml.

3·5 0 1 = 100 µg/ml

55 ml = 5500 µg
= 5·5 mg.

100 mg/l.

)) CMP.

1	0.32
3	2 +
5	2 +
7	2 +
9	2 +
11	1.6
13	1.48
15	1.80
17	2 +
19	2 +
21	1.1
23	0.7
25	0.55
27	0.40
29	0.25
31	0.15 - 0.12
33	0.15
35	0.27
37	0.58 - 1.2
39	1.25 - 0.4
41	0.28
43	0.14
45	0.095
47	0.075
49	0.06
51	"
53	"
55	"
57	0.07
59	0.075
61	"
63	"
65	0.07
67	"
69	0.065
71	0.06
73	0055
75	"
77	"
79	
81	
83	

GMP

37 – 50

1	0.05
3	0.035
5	0.04
7	0.08
9	0.83.
11	~~0.83~~ 2+
13	2+
15	2+
17	1.4
19	0.35
21	0.34
23	0.325
25	0.37
27	0.26
29	0.18
31	0.06
33	0.05 35 0.065
37	0.10
39	0.24
41	0.65
41	1.35
5	2+
7	2+
9	1.35
51	0.15
3	0.07
5	0.05
7	0.05
9	0.05
61	"
3	0.055
5	0.065
7	0.06
9	0.055
71	0.05
3	0.045
5	"
7	0.04
9	0.035
81	"
3	"
5	0.03
7	"
9	"

37	0.10
38	0.14
39	0.24
40	0.41
41	0.65
42	0.98
43	~~1.38~~ 1.38
44	1.80
45	2+
46	2+
47	2+
48	2+
49	1.35
50	0.25
51	0.12
52	0.07

45/10	0.26 = 2.6
46/10	0.38 = 3.8
47/10	0.45 = 4.5
48/10	0.36 = 3.6

40-49

5 g $NaHCO_3$
50 ~~80~~ g Ice.

Extract 4 x 30 ml CH2Cl2
wash c H2O
~~Dr~~ dry c sod. sulph.
Filter

20+5 /8
12. T...

15 - 23.

84.360
84.226
00.134

pMB9 sequences.

CCGG R/21

3'→5'

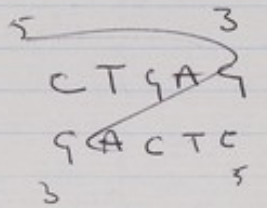
GAGTACAAACCGTCG

R/23

(TAA)GATACGAGGATATAACTAA?ATTCTTATTTGAA-

-TTA?TGCATTACCAACAT

B/21



(2)

1	0.015
2	"
3	"
4	"
5	"
6	"
7	"
8	0.022
9	0.05
10	0.115
11	0.23
12	0.42
13	0.56
14	0.43
15	0.28
16	0.25
17	0.25
18	0.225
19	"
20	0.27
21	0.225
22	0.15
23	~~0.225~~ 0.11
24	0.085
25	0.09
26	0.10
27	0.11
28	0.135
29	0.16
30	0.19
31	0.26
32	0.31
33	0.36
34	0.36
35	0.31
36	0.225
37	0.135
38	0.08
39	0.05
40	0.035
41	0.028
42	" 6
43	0.025
44	0.021
45	0.020
46	0.019
47	"
48	0.016
49	"
50	"
51	"

4 Z5
4 2.5 x RT

Z5.

~~4 μl Z5~~ 2 μl Z5. ~~2~~ ~~3 exo 5~~ 2 exo
~~4 μl~~ 2 μl exo 2 exo.
~~1 μl 10xRT~~ 1 μl 10xRT 42
~~5~~ ~~μl~~ ~~H_2O~~. 5 μl H_2O 4 exo.

✓ 2 mix
1 μl A° RTA*
✓ 1 μl dd.
1 μl RT/5
15' 37°

1 μl 0.5 mM dATP

15' 37°
1 μl 0.2M EDTA 7 μl.

1/2 dry, + form, min length run

Rest (2x2 μl) longer. runs.

Z1

~~Z~~ 1 exo
~~1~~ ~~2 μl~~ Z1
.5 ~~2~~ ~~5~~ μl 10 x RT
2.5 ~~2~~ μl H_2O.

1 mix
.5 ~~1~~ μl A° RTA*
.5 μl dd
.5 μl RT/5.

15' 37°
.5 ~~1~~ μl 0.5 mM dATP
15' 37°

.5 μl 0.2M EDTA. 3.5 μl.