C. elegans mapping 10/83 - 4/87

Publication/Creation

1983-1987

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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

'Birnboim' protocol.

Pellet ~~1ml~~ 2ml bugs - 15 secs. in 1.5 ml Epp. tube
Aspirate off super.
Resuspend in 200 μl lysozyme sol^n (2mg/ml in
- vortex ~ 10 secs.
0° 5'.

50 mM glucose
10 mM EDTA
25 mM Tris-Cl 8.0)

Add 200 μl 0.2N NaOH / 1% SDS
Mix by inversions.
0° 5' (H₂O/ice bath)
Add 150 μl 3M NaAc pH 4.8.
Mix by inversions.
60min 0° 60'+ (H₂O/ice bath)
Mix by inversion periodically.
3' spin.
Remove 400 μl super. with 'pipetman'.
Add 0.9 ml ethanol;
-20° 30'+.
Spin 3'.
Resuspend pellet in 50 μl 0.3M NaAc ~pH5 (200?)
Add 100 μl EtOH (400?)
-20° 15'+.
Spin 2'.
EtOH wash
Dry
Add 20 μl T.E..

1μl / agarose gel
1μl / labelled gel

610831.

Comparison of various gel systems for restriction mapping.

a) 20x40 6% TBU pH 8.3. (wackered)
b) " " " 8.8 (")
c) " 5% TBE (")
d) 20x50 " (origin wackered).

Samples for gels.

RCOS 1-12 RI/Taq 35S labelled.

Mix
H_2O 23
10xH 7.5
35S d-AP 2
10mg/ml RNase 0.5
RI 1
AMV pol. 1.

2 mix
2 DNA (s.s.)
(This should give sufficient material for 4 gels).

Mixed in drawn capillary & expelled into H&H longform tube.

37° 1.5 hr.
Ice 15' (forgot to switch on 70° water bath)
70° 10'.

Mix H_2O 10
10xH 0.25
Taq 3.

+ 1 µl mix to reacns. (Spin in)

67° 45'.

2.5 + 0.5 non-denat. dye mix

2.5 + 1.5 formamide/dye mi[illegible]

Non-denat. gels:

31 ml H_2O
4 10x TBE
5 38/2 acrylamide
(Degas)
0.28 10% Amps
40 TEMED.

PNAS 81 983

TCGA TCGA

↓ M.TaqI

TCG$\overset{m}{A}$ TCG$\overset{m}{A}$
^{m}AGCT^{m}AGCT

⇑

DpnI

ATCGATCGAT

↓ M.ClaI

ATCG$\overset{m}{A}$TCG$\overset{m}{A}$T
T$\overset{m}{A}$GCT$\overset{m}{A}$GCTA

⇑

DpnI

N.B. DpnI cleaves at GATC methylated on both strands.

610831 cont.

Denaturing gels run at 30mA (~1200 V) 3.15 – 5.00

3.30 – 40 cm non-denaturing gel 30mA (~ 850 V)

3.45 – 50 cm " " " (~1000 V).
to 1100 at 5.00 pm.

BPB ~ 4cm from bottom.

Denaturing gels got v. hot – too hot? (may cause fuzziness)

50 cm gel dried on paper.
Others (washed) ~~dried~~ heat-dried on plate.

1 ml culture stored in screw-cap culture vial with 6 drops glycerol.

1010831.

Restriction analysis of 710831 cosmid DNA preps.
HindIII/Taq and HindIII/Sau3A.

Mix H_2O 34
10 × H 7
35S dATP 4
RNase 1
HindIII 3
AMV pol 2.

1 DNA 2X.
1 mix

1.25 hr 37°
10' 70°

Mixes H_2O 9.5 | H_2O 4
10 × H 0.5 | 10 × H 0.5
Taq 3 | Sau3A 4.2

1-12 J. Karn Sau3A
13-24 commercial ..
(Biolabs (#13) 2u/μl.)

+ 0.5μl. | + 0.5μl.
68° 60' | 37° 1.5h.

+ 2μl formamide/dye/EDTA.
3' 100°.

½-⅔ samples loaded on 6% TBU 20×40 gels.
(Markers every 8 slots 4 λ/Sau3A CGT
4 form./dye.
~1μl loaded.)

30 mA (≃ 1.1 kV)

710831.

Isolation of cosmid DNA from O/N cultures grown by J-S. (2.5 ml).

NB 121 - 144

DNA isolated as per Ish-Horowicz / Grosveld alkali procedure.

121, 128, 134 had rather large amounts of ground cap aluminium.

~~Made up in final.~~

First spin of SDS ppt in 40 place Eppendorf left some gunk in super. Repeat spin in 12 place angle head removed this.

Made up in final vol. of 50 µl TE (May be too large).

0.5% TBE agarose minigels

NB 121 - 144   1 µl DNA / 4 µl dye mix   (200 H2O 50 5x dye)

RCOS 11, 12   0.5 µl .. / 4.5 .. ..

NB 97, 99   ~~0.5 µl ..~~
0.5 µl + 2 TE
0.5 µl / 4.5 dye mix.

121   144 RCOS NB
11 12 97 99

Running buffer for Sau3A gel may have been ~2/3 correct concn.
(Run slowly & not v. hot).

1110831.

Reppt^n of 110831 cosmid DNA preps (NB121-144)

- end-label restriction analysis gave virtual total blank.

+ 5 μl 3M NaAc.
100 μl EtOH. ; EtOH wash ; dry ;
+ 10 μl TE

HindIII digest of above:

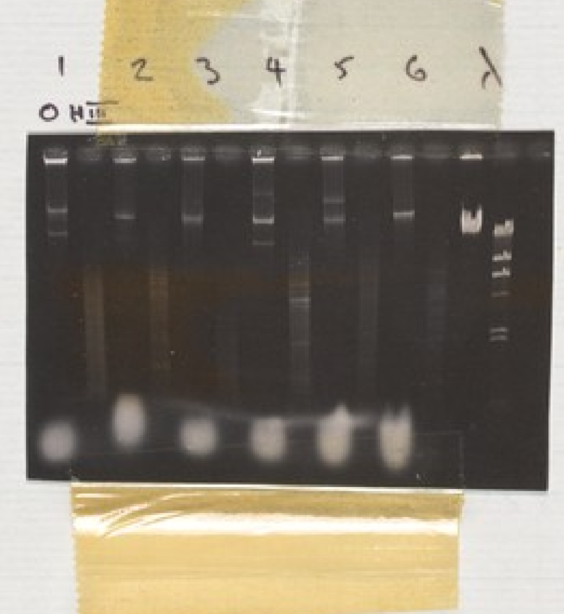
Mix: 20 H_2O
3.5 10xH
0.5 RNase
1.5 HindIII.

0.5 DNA (1-6) (Also parallel digest of
1 min. 150 ng λ DNA).

37° 1.25 hr.

+ 4 μl dye mix.

0.5% TBE ag. gel with undigested samples (0.5 μl)



Bad degradation.
Phenol DNA's ?

1110832.

DE52 column work-up of NA225-248

~ 12 ml lysates grown by JWD.

225-236 + ~0.5 ml $CHCl_3$
30' 37° shake.
30' ~~4°C~~ 4°

236-248 No $CHCl_3$.

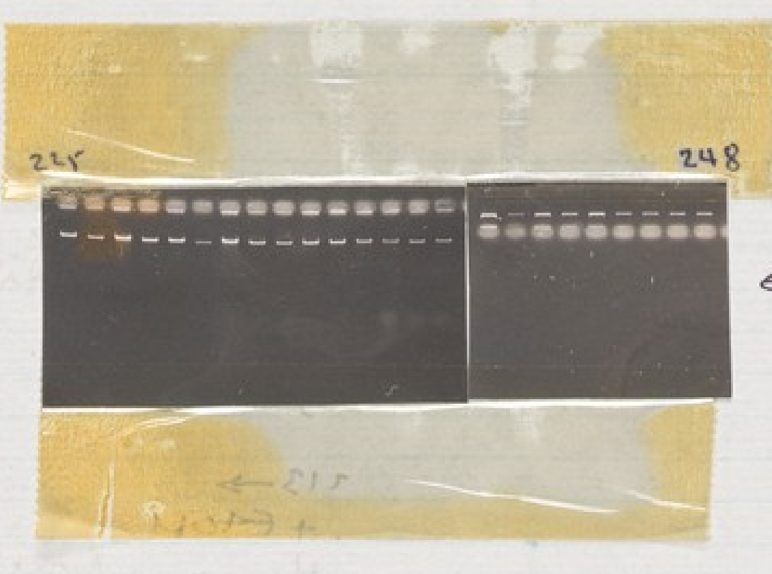
1 ml + 2 drops $CHCl_3$ for 4° storage.

10 ml + 5 ml 10 mM Tris-Cl pH 8.0.
2 ml column.

Simplified Olson work-up. (iso-prop. pptn).

+ 20 µl TE.

4 dye
20 H_2O
1 DNA
} 5 µl on 0.5% TBE agarose gel.



← ran backwards!

- Look good.

Fixing: 201 – 212 etc
No EtOH in fix.
5' H_2O
change H_2O
5'

213 →
+ EtOH
then as above

121083 2.

ϕol extrⁿ & reptⁿ of 110831 NB121-126 cosmid. DNA's.

+40 TE (→ 50 μl)
25 phenol.

No ether extⁿ.

EtOH ppt. & wash

+ 10 TE.

lysozyme buffer:

5 ml 20% glucose
10 ml 0.1M EDTA
2.5 ml M Tris pH 8.0 / 100 ml

Soln II   0.2 ml 10N NaOH
1 ml 10% SDS / 10 ml.

131085#1

Cosmid DNA preps. by method of Birnboim
(NAR 7 ('79) 1513)

1-18, A-F (grown from colonies by SGS)

0.5 ml spun 15".
Super removed by aspiration.

+ 100 μl soln I — 2 mg/ml lysozyme, 50 mM glucose, 10 mM EDTA, 25 mM Tris-Cl pH 8.0.

30' 0°.

+ 200 μl soln II — 0.2 N NaOH, 1% SDS. (fresh)

Gentle vortex

5' 0°

+ 150 μl soln III — 3M NaAc pH 4.8.

Gentle mix.

0° 60'.

Spin 5'.

400 μl removed

~~+ 2~~
+ 1 ml EtOH ; -20°C 30'.

EtOH wash ; dry ;

+ 10 TE.

125,126 not loaded.

131083 2.

Hind III digest of 131083 1 cosmid DNA preps for agarose gel analysis.

Mix
20 H_2O
3.5 10xH
0.5 RNase
1.5 Hind III.

0.5 DNA even no's 2–18, B, D, F.
1 mix

60' 37°.

Same mix used for digestion of 121083 2 phenol-extracted NB121–126 cosmid DNA's.

JES has photos.

Phenoled ~~samples~~ preps from 111083 1 look much better.

141083I.

ATP32 20 (dry)
H_2O 38
10x H 7

2x 20 μl

a) + 1.5 H~~~
1 AMV (10u/μl).

b) 1.5 H~~~
0.5 kl. pol. (30u/μl)

i) 0.5 NA 225 - NA 230 + 1 μl a) mix
ii) " " " " "
iii) " " " " b) "
iv) " " " " " "

At 1 hour, + 0.5 μl 0.5mM dATP to ii & iv.

At 1.25 hour 70° 10'.

Mix 8 H_2O
1 10xH
4 Sau3A (Karn)

0.5 μl mix to i - iv.

1.5 hr 37°

~~+3 form./dye/EDTA~~ + 0.5 μl ficoll/dye.

~~3' 100°~~ Spun in.
Load ½ on non. denat. gel

~~½ denat. gel (27mA)~~ + 2 μl formamide/dye

~~½ non-denat.~~ 100° 3'
Load denat. gel. 27mA

OVER

Band doubling still bad
Gel lanes better (ie. no narrowing)
Klenow hopeless.

1810831.

Various restriction / labelling cond^{ns} NA225-228

MIX ATP^{32} 20 (dry)
H_2O 38
10 x H 7
HindIII 3.5.

4 x 10 μl b) and d) + 1 μl 0.5 mM TTP.
a) and b) + 0.5 μl AMV pol.

6 x 0.5 μl samples NA225-228.

i) + 1 μl a) mix (AMV, no TTP – standard cond^{ns})

ii) + 1 μl b) mix (AMV + simultaneous TTP chase)

iii) + 1 μl c) mix (no TTP, AMV added at 30')

iv) + 1 μl d) mix (+TTP, ...)

v) + 1 μl c) mix (no TTP, klenow added at 30')

vi) + 1 μl d) mix (+TTP .. .. ..)

Mixes for subsequent add^n of AMV / klenow:

a) AMV diluted 1:10 in 1xH ; 0.5 μl added.

b) klenow (35 u/μl) diluted 1:20 ; .. ..

Reactions continued for 30' after add^{ns} of polymerase – AMV at 37°, klenow at r.t.

At t = 1.25 hr, 70° 10'.

MIX 8 H_2O
1 10xH
4 Sau3A (Karn) } 0.5 μl / reac^n. 1.5 hr 37°.

+ 2 μl formamide / dye ; 100° 3'.
~ 1/2 loaded 6% TBU.

OVER

Still double banding.

T chase seems to stimulate production of more longer fragment of doubles.

Addition of AMV pol after some restriction makes little difference to banding pattern except large partial products seem to be fewer.

(Could AMV be nibbling back before introduction of 1st lot A?
Could dATP32 be contaminated with GTP?)

Klenow gave a weird banding pattern for NA225, but only blank lanes for others. In NA225 it seems to give rise to products 1-3 nucleotides shorter than those produced by AMV.

Actually chase should be with G!

Try chasing with G and also GTC mixture. Retain addition of AMV after restriction.

191081.

More various restriction / labelling conditions.

MIX ATP32 20 (dry)
H_2O 38
10×H 7
Hind III 3.5

3 × 12 µl

a) no additions
b) + 0.5 µl 10mM dGTP
c) + 1 µl GTC (3mM) mix. (5ES)

3 × 0.5 µl NA 225-228 (λ clones)
+ 1 µl a, b, c.

1 × 0.5 µl NB 156-166 (even no's) (cosmid)
+ 1 µl b.

30' 37°

+ 0.5 µl AMV pol/10 in 1×H.

45' 37°

10' 70°.

Mix 8 H_2O
1 10×H
4 Sau3A (kara) } 0.5 µl / reacn.
1.5 hr 37°.

+ 2 µl form./dye./EDTA.

100° 3'.

~½ loaded 6% TBU 2h 27mA.

OVER

In most cases, double banding was cured by G chase. (GCT chases also, but these had v. high backgrounds).

But in some cases, double banding was apparently not 'cured' by G chase (assuming double banding to be genuine and not just a weakly-labelled fragment adjacent to a more strongly labelled one).

All fragments in 227 + 228 show relatively little double banding compared to previous expts e.g. 1810831.

(N.B. Double banding also seen in 610831 but this was R1 cut/35 label).

Cosmid labellings look pretty awful - realise I forgot to include RNase in reactions - this could well account for crumby result. (JES has checked this)

2010831

Restriction / labelling of cosmid DNA's
(including RNase in reac^n).

Mix dATP^{32} 10 (dry)
+ H_2O 19
10xH 3.5
RNase 0.5
Hind III 1.5

0.5 μl NB 121-126 (phenol-extracted)
NB 156-166 (even no's).

1 μl mix

30' 37°

+0.5 μl AMV/10 = 1xH.

30' 37°

10' 70°.

Mix 8 H_2O } 0.5 μl / reac^n
1 10xH
4 Sau3A (Karn) } 1.5 h 37°.

— Much better.

156-166 v. good apart from double banding
i.e. minor bands fewer & labelling stronger
than in 1910831 (due to RNase treatment).

121-126 not so good — only 2 gave
reasonable signal S/N. RA.

241083I.

Attempt to cure double banding by incorpⁿ. of terminal ddG.

Mix: dATP^{32} 20 (dry)
H_2O 38
10xH 7
RNase 1
Hind III 3.5

4 x 10 µl.

a no additions
b + 0.5 µl 10 mM dGTP
c + 1 µl 100 mM ddGTP
d + 1 µl 10 mM ddGTP.

4 x 0.5 µl NA 225-227, NB156, ~~B~~158, 160.
(NB 156-160 order probably reversed).

+ 1 µl a-d.

30' 37°

+ 0.5 µl AMV/10 in 1xH.

45 ~~[illegible]~~' 37°

10' 70°

Mix 8 H_2O
1 10xH
4 Sau3A (Karn) } 0.5 µl/reacⁿ. 1.5 h 37°.

+ 2 µl formamide/dye.

100° 3'.

1/2 6% TBU ; 1/2 5% TBU.

6% 225a, 226a, 227a etc
5% 226a, 225b, 225c etc.

OVER.

10 mM ddGTP in mix looks v. effective on λ DNA's.
100 mM ddGTP not so good.

All cosmid reactions pretty crumby; no ~~ddG~~ G chase reactions look best; anything chased seems to be wipe-out.

Try a) reprecipitating DNA's
b) lower ddG concns.

241083Z.

^{35}S λ Sau3A marker prep^n

H_2O 7
10 × H 1
λ S7 (800γ/λ) 0.75
Sau3A 0.5

37° 60'.

+ 0.5μl 35S dATP.
0.5 5mM dGTP
0.2 10mM ddTTP.
0.2 AMV.

37° 45'.

5μl ficol/dye 70° 10'.

- v. weak.

25.10.83.

Mix dATP³² (dry) 20
H_2O 38
10 x H 7
RNase 1
Hind III 3.5

a) 10 μl mix, no additions
b) 15 μl mix + 1.5 μl 10 mM ddGTP
c) 10 μl mix + 1 μl 1 mM ddGTP
d) 10 μl mix + 1 μl 0.1 mM ddGTP.

i)	NB 156, 158, 160 (not reppted)	0.5 μl +	1 μl	b
ii)	″ (reppted)	″	1	b
iii)	″ ″	″	1	a
iv)	NA 225 - 227 (λ)	0.5 +	1	b
v)	″	″	1	c
vi)	″	″	1	d
vii)	″	″	1	b.

45' 37° except vii) 15' 37° (-20° 30')

70° 10'.

Mix H_2O 8
10 x H 1
Sau3A 4
} 0.5/reacⁿ.
} 1.5 hr 37°.

Omitted to spin in Sau3A!
After 1.5 hr, further 0.5 μl Sau3A aliquot added and spun in. 1.5 hr 37°.

+ 2 μl formamide/dye; 100° 3'. 5% gel.

OVER

Reppt^n of cosmids probably hasn't made any significant difference.

Although ~~rec.~~ labelling of unreppted. material with ddG is weak (compared with no ddG sample) it does appear to be working. (Is ddG being misincorptd in 'A' posn, preventing labelling?).
- Try lower ddG concn with cosmid DNA's.

All λ reactions look good, even with addition of only 0.1mM ddGTP to mix. - try lower ddG concns (→ 0.001M to mix?)

15' incubation ~~[illegible]~~s shows signs of beginning to produce undesirable bands.

(In general, Sau3A didn't work as well as 241083? - this could be due to excess glycerol in reaction caused by having to add second aliquot of enzyme after forgetting to 'spin-in').

Marker looks O.K.

2510832.

32P Sau3A λ marker.

H_2O 7
10xH 1
λS7 (800γ/λ) 0.75
Sau3A Kern 0.5.

37° 60'.

+ 2.5 32P dATP (dry)
0.5 5mM dGTP.
0.5 10mM ddTTP.
0.2 AMV.

37° 30'.

1 μl + 2 μl form/dye ; 100° 3'.

Run on 5% TBO gel with 2510831.

261083 1.

Test of cosmid labelling, using lower ddG concn's.

Mix dATP32 (dry) 20
H_2O 40
10 x Hin 7
RNase 1
Klen 3.5

a 10 mix, no addns.
b .. + 1 μl 1 mM ddGTP
c .. .. 0.1 .. ..
d .. .. 0.01 .. ..
e .. .. 0.001 .. .. .

3 x 0.5 μl NB 156, 158, 160 cosmids

i) + 1 a
ii) + 1 b
iii) + 1 c

5 x 0.5 μl NA 225 - 227 λ.

iv) + 1 a
v) 1 b
vi) 1 c
vii) 1 d
viii) 1 e.

30' 37°
+ 0.5 μl AMV/10 in 1xH.

30' 37°
10' 70°

Mix H_2O 8
10xH 2
Sau3A 4
} 0.5 / sample
1.5 h 37°.

5% gel.

over.

Cosmids total wipe-out, even in no ddG control.

ddG effective down to 0.1mM (added bmix)

Bands get progressively weaker towards top of gel - can't think of any good reason.

Also, a few rather bad partials are cropping up in some lanes.

261083 2.

Hind III test digests of JES cosmid DNA preps.

Mix 20 H_2O
4 10× Hin
0·5 RNase
3 Hind III

1 DNA
1 mix

60' 37°.

+ 4μl 1/5× sucrose/dye.

0·5% ag. gel.

– Pretty awful.

2710831.

Repeat of NA 225-227 restriction and labelling using ~~expt~~ 0.1 mM ddG added to mix.
Also 228-230 (in case progressive weakening of bands is due to deterioration of DNA's because of being taken in & out of fridge.).

Mix 10 dATP32 (dry) (different batch from 2610831)
19 H_2O
3.5 10xH
0.5 RNase
2 Hind III
2.5 0.1 mM ddGTP

0.5 NA 225-230.
1 mix

30' 37°

0.5 AMV/10 in 1xH —

45' 37° (2610831 30').

10' 70°

0.5 8 H_2O
1 Hx10
4 Sau3A

1.5 h 37°.

2 form./dye/EDTA 100° 3'. 5% gel.

— No progressive weakening of larger fragments. But Sau3A digest looks rather partial.
Try various Sau3A conditions?

271082.

Cosmid preps.

'EMBO course' protocol v. Birnboim

Cosmids 13-18 x 2 grown O/N by SES.

1 ml spun 15"	1 ml spun 15".
500 µl lysozyme soln (2mg/ml)	200 µl lysozyme solⁿ.
Vortex 0° 5'	Vortex 0° 5'.
250 µl 0.3N alkali/SDS	200 µl 0.2N alkali/SDS.
Mixed by inversion	Mixed by inversion.
10' 70°	5' 0°. (Mod. protocol. (Meth. Enzym.)
80 µl phenol/$CHCl_3$	13-15 150 µl formic salt
Mixed (not vortexed).	16-18 150 µl acetic salt.
2' spin.	0° 60'.
— supers looked v. mucky	2' spin.
So 5' spin (supers clearer).	400 µl super
13-15 reextracted with further 400 µl phenol/$CHCl_3$	+ 0.9 ml EtOH ; -20° 30'.
	Spin. ; Heavy pellets.
	+ 50 µl 0.3M NaAc
+ 70 µl 3M NaAc to supers.	100 µl EtOH ; -20° 10'.
— 16-18 gave v. bad ppt. on addition of NaAc. This dissapeared on addition of isopropanol).	Spin
	EtOH wash.
	Dry
	+ 20 TE.
+ 700 µl isopropanol.	
Mixed by inversion.	
20' r.t.	
Spun ; EtOH reprtn & wash	
Dry ; + 20 TE.	
— no obvious ppts. except v. light ones in 14, 15.	

OVER

Hind III digests of cosmid preps:

Mix 20 H_2O
4 10×H
0.5 RNase
3 Hind III.

1 DNA
1 mix

60' 37°.

+ 4 sucrose / dye.

0.5% ag. TBE gel. (2 gels i EMBO 13°, 13H, 14°, 14H etc.
ii Birnboim 13°, 13H etc.

with 1 µl undigested material.

Birnboim / acetate salt looks good.

271082.

Cosmid preps.

'EMBO course' protocol v. Birnboim

Cosmids 13-18 x 2 grown o/N by JES.

1 ml spun 15"	1 ml spun 15".
500 µl lysozyme soln (2mg/ml)	200 µl lysozyme solⁿ.
Vortex 0° 5'	Vortex 0° 5'.
250 µl 0.3N alkali/SDS	200 µl 0.2N alkali/SDS.
Mixed by inversion	Mixed by inversion.
10' 70°	5' 0°. (Mod. protocol. Meth. Enzym)
80 µl phenol/$CHCl_3$	13-15 150 µl formic salt
Mixed (not vortexed).	16-18 150 µl acetic salt.
2' spin.	0° 60'.
– supers looked v. mucky	2' spin.
So 5' spin (supers clearer).	400 µl super
13-15 reextracted with	+0.9 ml EtOH ; -20° 30'.
further 400 µl phenol/$CHCl_3$	Spin. ; Heavy pellets.
	+ 50 µl 0.3M NaAc
+ 70 µl 3M NaAc to supers.	100 µl EtOH ; -20° 10'.
– 16-18 gave v. bad ppt. on addition of NaAc. This dissapeared on addition of isopropanol).	Spin
	EtOH wash.
	Dry
	+ 20 TE.
+ 700 µl isopropanol.	
Mixed by inversion.	
20' r.t.	
Spun; EtOH repptⁿ & wash	
Dry ; + 20 TE.	
– no obvious ppts. except v. light ones in 14, 15.	

OVER

281083l.

Restriction + labelling of good looking cosmids from 2710832.
Also, various Sau3A conditions on λ DNA's.

Mix 10 dATP32 (dry)
19 H_2O
3.5 10×H
0.5 RNase
2 HindIII.
2.5 0.1mM dGTP.

0.5 16-18B (cosmids)
3 X NA 231,232 (λ's)
1 mix
30' 37°
0.5 AMV/10 in 1XH.
45' 37°
10' 70°.

Mix 8 H_2O
1 H×10
4 Sau3A

Cosmids + 0.5 mix 1.5 h 37°

231,232 a) + 0.5 mix 1.5 h 37°.
b) + 1.5 mix "
c) + 0.5 mix at 0' and 30'. 1.5 hr total

Cosmids and λ a) + 2 μl formamide/dye.
λ b,c) + 3 μl formamide/dye.

100° 3'.

5% gel.

Cosmids look pretty good.

Addition of 1.5 μl Sau3A mix to reactions gives much improved digest. 2 additions of 0.5 μl doesn't make much difference.

281083?.

More cosmid DNA preps. using method successful in 271083?.

Cosmids 13-18 grown O/N (S.E.S.)

a) small diameter 10 ml tubes.

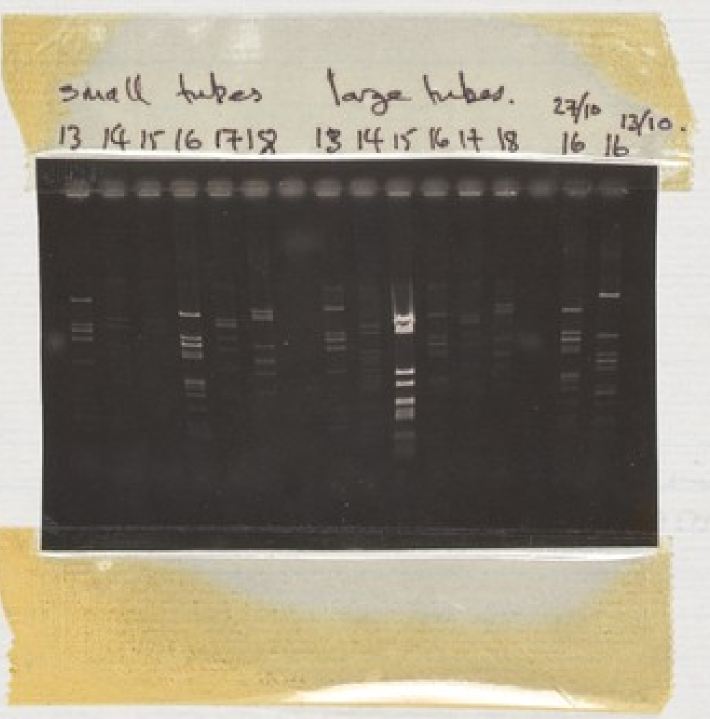
b) large diameter tubes.

Also worked up 1 ml samples from previous efforts (27/10 and 13/10). (These were stored at 4°C)

Work-up as 271083? (Birnboim) 16-18.

~~Ae~~ + 20 T.E. final.

1 μl Hind III restricted.



- Looks pretty good, although yields v. variable.
Note 1 day old and 10 day old bugs gave good yields.

30 6% TBU gel mix
5 8M urea / 1 x TBE
30 µl TEMED
250 µl 10% AMPS.

3110832

Restriction & labelling of cosmid DNA's 13-18S
from 2810832

Procedure as 3110831.

Also, λ DNA's NA 201-206, entirely by 'spinning-in'.

DNA's diluted x2 (i.e. 3μl + 3μl H_2O).

1μl DNA pipetted onto side of tube (pipetteman).

1μl mix .. .. (~~Si tip~~ pipetteman + Si tip).

Spun.
etc.
AMV mix added with drawn micropipette.
1.5μl Sau3A mix.
3μl formamide / dyes.

5% gel.

| 13-18S | 201-206 ~1μl | 201-206 ~3μl full well. |

—— .

Intensities of cosmids related to agarose gel intensities, as might be expected.

Pipette method samples look O.K.
Loading large sample doesn't appear to affect resolution.

111

~~2832.~~ 2832.

^{32}P marker.

H_2O	35
10×H	5
λ57 (800γ/ml)	3.5
Sau 3A (kan)	2.5

37° 60'.

+	12.5	^{32}P-dATP (dry)
	2.0	10mM dC_7TP
	2.5	10 mM ddTTP
	1	AMV.

39° 30'.

For gels, take 5 marker
8 dyes.

100° 3'

load ~ 1μl/lane.

– O.K.

711832

Varying HindIII digestion conditions.

(New tube HindIII, lot no. 32 ; previously 26).

Mix 20 dATP32 (dry)
35 H_2O
10 10xH
1 RNase
5 0.1mM ddGTP.

15 µl TE added to NA220-224

a) 30 mix 2.5 HindIII. (vs. standard cond^ns)
b) 10 mix 0.2 HindIII.

std. i) 1µl NA220-224 ; 1µl a, 30' ; then + 1 AMV/10 45'

no preincub^n. ii) .. ; 1µl a + 0.5 AMV/10 60'.

no preincub^n shorter time iii) .. ; 1µl a + 0.5 AMV/10 30'.

less HindIII. iv) .. ; 1µl b 30'; then + 1 AMV/10 45'

std. v) as i) for different Sau3A cond^ns.

After 10' 70°

i – iv) normal Sau3A (Mix 36 H_2O, 4.5 10xH, 12 Sau3A + 2µl)

v) less Sau3A, larger vol. (Mix 8 H_2O, 1 10xH, 1 Sau3A + 2µl)

1.5 hr 37°.

O/N -20°C

All i-iv reaction essentially indentical, but v's (less Sau3A in same vol.) are much cleaner – most minor bands are at least weaker, and many are obliterated.

811831.

^{35}S labelling attempt and denat v. non-denat gels.

Mix
17 H_2O
5 10xH
0.5 RNasin
2.5 0.1mM ddGTP.
2 ^{35}S dATP.
2 Hind III.

2x 1μl NA 220-224 (see also 711832
1μl mix. & 3110831)

30' 37°.

0.5μl AMV/10.

45' 37°
10' 70°.

Mix
18 H_2O
2 10xH
6 Sau3A
} 2μl / sample
1.5 hr 37°.

Denaturing: + 3μl formamide/dye
100° 3'.

non-denaturing: + 1μl ficol/dye.

Also, samples of denatured 220-224 loaded on non-denaturing gel.

Also, NA 220 ii & iii denatured samples. (loaded ~5' after others started.)

S35 labelling looks good.
Denaturing gel clearly better than non-denaturing.
Loading denatured material on non-denaturing gel probably best forgotten.

10.11.82.

Restriction & labelling reactions in microtitre plates.

NA 220-224.

Mix 10 dATP³² (dry)

15 H_2O
5 10×H
0.5 RNase
2.5 0.5 mM ddGTP
2 Hind III
2 AMV.

1 µl NA 220-224
1 µl mix — onto sides of wells (1/4") — conical bottom. Spun in. Sticky film sealed.

30' 37°
15' 68° (oven)

Mix 18 H_2O
1 10×H
2 Sau3A
(to allow for vol. reduction by evaporation).

2 µl / sample. (Also, 1 µl H_2O added to 4 & 5).

Spun in, sticky film sealed.

1¼ hr 37°

+ 3 µl formamide / dye spun-in.

5' 68° oven. (After a few minutes on 80° hot block).

4% gel.

– Promising – reactions probably not as good as tubes because of dehydration – try smaller wells.

'Amping' plates.

0.1 ml 25 mg/ml Amp. / plate.
Leave a few minutes to absorb into agar.
Invert 4°C for at least 12 hr.

Prep. amp. stock.

20 ml of 25 mg/ml

Neutralize with N NaOH while dissolving. Follow with pH meter.

Store -20°C

16 11 832.

λ Sau3A × β marker prep. as 11 1832

Looks as though may not be quite complete digest. (see 16 11831).

+ 1 μl Sau3A (Karn)

30' 37°.

121283L.

^{35}S marker in ^{32}P reactions & 2 films.

Marker made as 111832. (2.5 ^{35}S dAMP).
~~[illegible]~~). 5μl marker + 5μl H_2O.
1μl / sample (N3 505-510 leftovers).
N3 505-510 rather weak
Amount of marker bit too large (1/3 - 1/4 would probably be about right).
^{32}P bands of top film lose rather a lot of resolution.

31842.

Size estimation of N2 inserts.

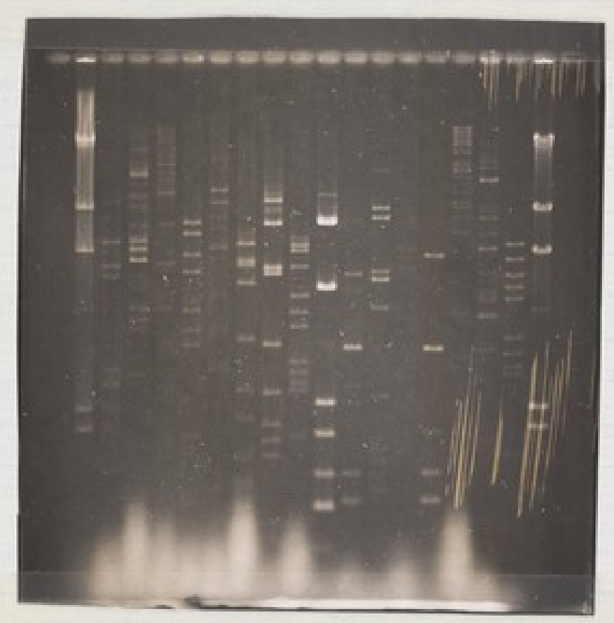
NB 289 - 296
NC02 A1 - B2

180 H_2O
22 10xH
2 Hind III

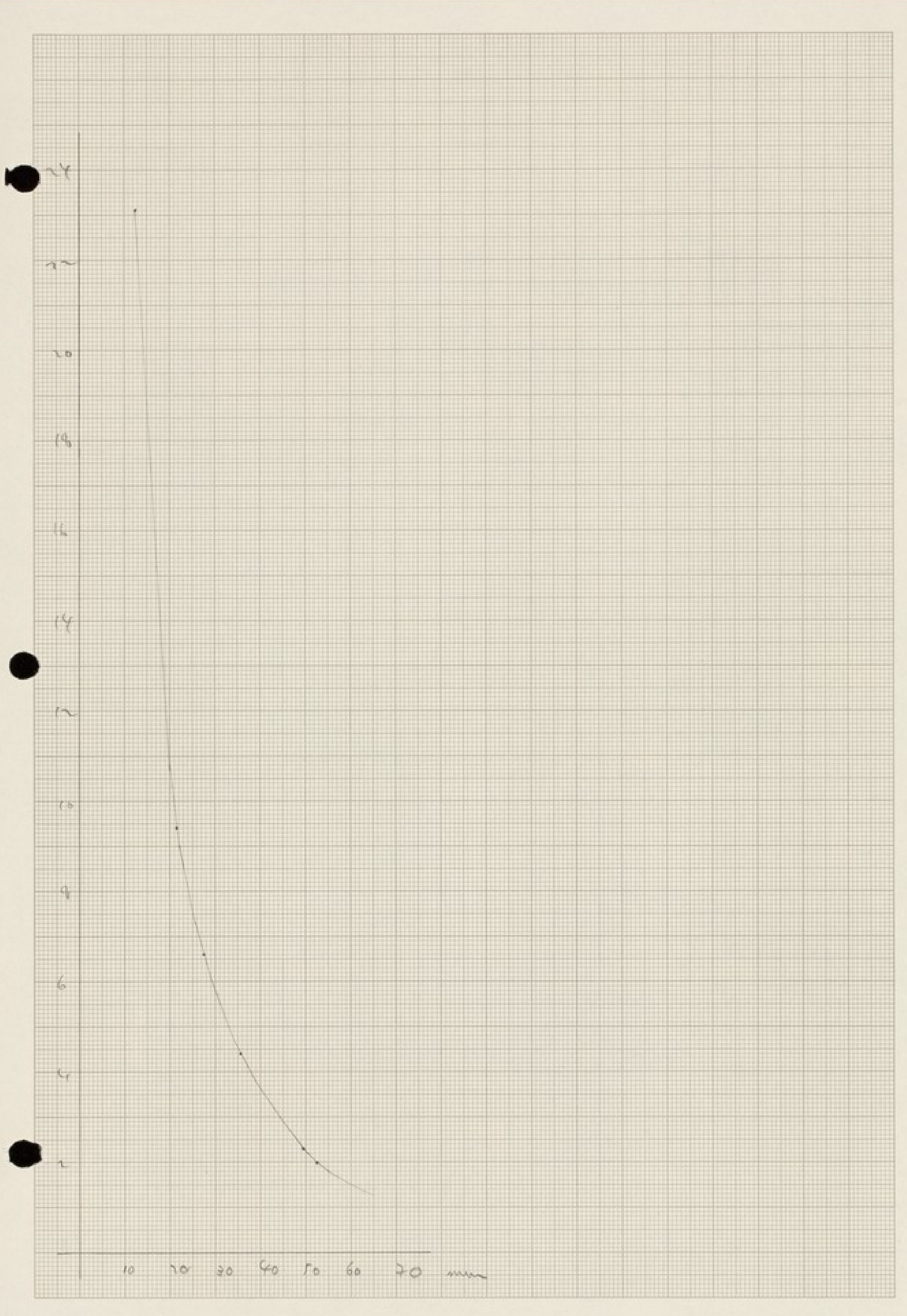
} 10 mix
1 DNA.

90' 37°.

0.5% 1xTBE 150 ml agarose gel. 18 mA 20hr.



OVER


24
22
20
18
16
14
12
10
8
6
4
2
10
20
30
40
50
60
70
mm

289	290	291	292	293	294	295	296
7			8.5	12	7	10	7
6			7.5	10	6	8	7
5.5			6.5	7	6	6	6
3			6	4	5	6	5
3			(5)	3	4	3.5	4
2			4		3	2.5	4
2			4		2	2	4x3
1.5			3		1	1.5	2
1.5			2		1	1.5	
31.5			41.5	36	35	41	47

Mean = 38.6
5
33.6

2A1	2A2	2A3	2A4	2A5	2A6	2B1	2B2
8	6	10		6.5			7.5
5	3.5	9		3.5			6
2.5	3	6		2			6
2	2.5	5.5		1.5			5
1.5	1.5	4.5		1			5
1	1	2.5					4
		4x1.5					3x3
							1.5
20	17.5 (rib)	43.5		14.5 (rib)			44

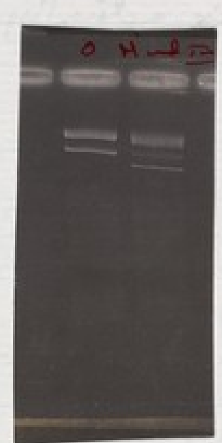
13/1/84.

Shotgun of HindIII fragments of pCE7 into mp9.

Vector

HindIII Digests of my & Gerrats mp9 most unsatisfactory.

Gerrats mp9, 2hr



Gerrats, further 1hr, 1/2 + RNase

my stuff.

1% TBE agarose.

Gave up. Scrounged some (20 ng/µl) off Graham Hudson - says it's good stuff. (phosphatased).

Inserts. Total shotgun, including pBR322 (Greg says I might expect about 10% of recombinants to be pBR).

4 µl pCE7 (≅ 1 µg)
2 10×H
1 HindIII (20 u)
13 H₂O
20

60' 37°

2 µl for 0.5% ag. TBE gel → o.k.

Phenol¹ˣ, ether¹ˣ, EtOH ppt.

+ 20 TE

1 µl for gel

· Good.

OVER.

Ligation

a) 1 20 ng/μl mp9 Hind III, phiazed.
 1 pCE7 Hind III (≃ 50 ng)
 1 10 x C
 1 10 mM rATP
 1 0.1 M DTT
 4 H_2O
 1 D.B. green label ligase (This actually blunt ligase).

b) no insert

c) vector, insert, no ligase.

(not impossible that I put insert in b instead of c.)

15° ~~o/n~~ 17 hr.

2 μl to transfect into TG1 by usual procedure.

a	3 W	1 B
b		2 B
c	1 W	
o	—	

not good!

Try transfecting rest a) 6 μl into same bugs as above
b) 2 " " Phil Jennings' bugs (rather old).
c) P.J. bugs control.

a) ~ 50 v. small whites ; no blues.
b) 1 w
c) 0.

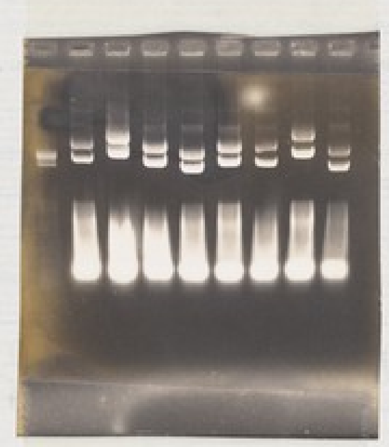
small whites may be o.k. (old plates?)

Mini RF preps. on 20. 1.5ml Growth 12.30 pm → 8.30 pm

RF preps (161842) by 'Birnboim' cosmid prep.
(Also, in parallel, NB48
NC5F1
NC17A6
NC13H7
cosmid preps for Iva Greenwald).

RF preps + 10 μl TE final.

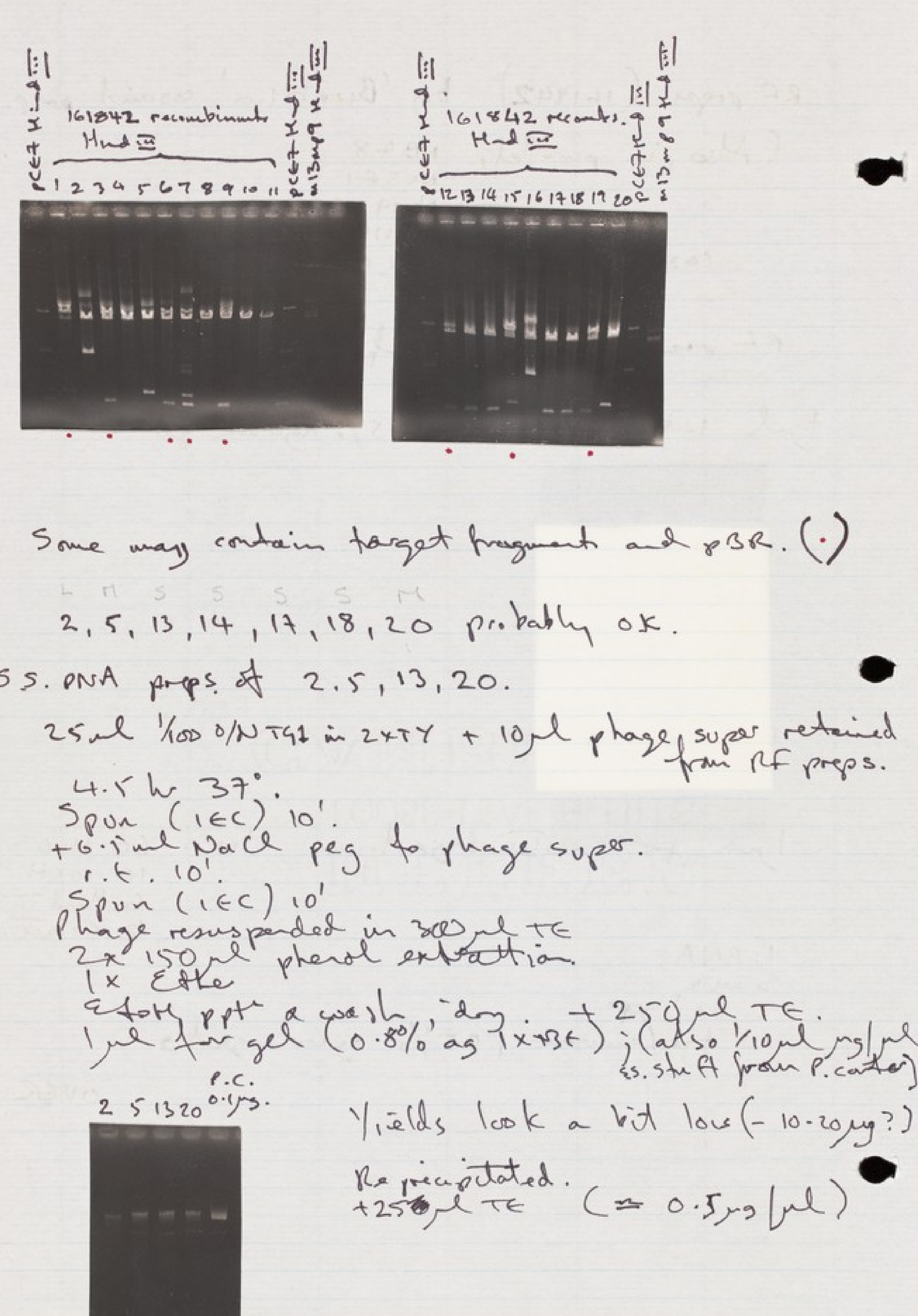
1 μl 1-8 taken for 0.5% agarose gel:



1 μl for HindIII digest. Mix: 60 H_2O
10 10×H
6 HindIII
2 RNase.

1 DNA
3 mix.
60' 37°
+1 dye/sucrose ; 0.5% agarose gel.

OVER



Some may contain target fragment and pBR. (·)

2, 5, 13, 14, 17, 18, 20 probably OK.

S.S. DNA preps of 2, 5, 13, 20.

25 ml 1/100 O/N TG1 in 2xTY + 10 μl phage super retained from RF preps.

4.5 hr 37°.
Spun (IEC) 10'.
+ 6.5 ml NaCl peg to phage super.
r.t. 10'.
Spun (IEC) 10'
Phage resuspended in 300 μl TE
2x 150 μl phenol extraction.
1x Ether
EtOH ppt + wash, dry. + 250 μl TE.
1 μl for gel (0.8% ag 1xTBE); (also 1/10 μl μg/μl s.s. stuff from P. Carter)

Yields look a bit low (~10-20 μg?)

Re precipitated.
+ 250 μl TE (≈ 0.5 μg/μl)

161841.

Restriction (HindIII / SauIIIB A) analyses various plasmids and λ recombinants.

	Dilution.
QP70	1:30
pCGA16	100
pCGA4	30
WK 4	15
pCSK 10 B	30
pCE 2002	10
pBR322	10
RW2070-4	20
RW2070-2	20
λ 2-3	–
λ 8-8	–
18-2	–
58-3	–
66	–

925 / 24 31 36 26 42 35 29 49 22 53.

Analysis (of 1μl) as per C.E. cosmids.

22284

Test of Anglian Biotechnology Sau3A (60 u/μl)

NC08 A1-4

In parallel with normal reactions using John Karn Sau3A (1 u/μl?) and BioLab " " (2 u/μl)

20 dATP32 (dry)
80 H_2O
20 10xH
2 RNase
5 0.5 mM ddGTP
2 Hind III
2 AMV.

2 mix
1 DNA (microtitre wells)

45' 37°
30' 67°.

Mix a) 22.5 H_2O
2.5 10xH
0.5 AST Sau3A

b) 22.5 H_2O
2.5 10xH
2 BioLab Sau3A.

c) 90 H_2O
10 10xH
12.5 Sau3A (J.K.)

2 μl mix/reaction.

2.5 hr 37°.

+ 4 μl formamide/dye.

90° 7'. gel.

282841.

Sau3A/Xba digest & labelling of Iva's 'Xba deletion' plasmids.

pSa2
Xba del. of 5F1

p11c1
Xba del. of 01E7

p14b1
Xba del. of C10G9

p15b2
Xba del. of 18H12

p21b1 15H7

Mix H2O 7
10xH 1.5
Sau3A (5 u/l) 0.5
Xba (2u/l) 0.5

0.5 DNA (50 μg/ml)
1 mix (microtitre)

37° 60'.

+ 1 mix → 10 dATP32 (dry)
2 10 uM dGTP
2.5 10 uM ddTTP
1 10xH
3.5 H2O
1 AMV.

37° 30'.
+ 4 formamide/dyes.

4% denat. gel.

21b1/11c1/5a2/14b1/15b2. (with marker λSau3A)

1 C18H12
2 C10G9
3 C05F1
4 C01E7
5 C15H7
6 C17A6
7 + 6a1, which is a Sac deletion of C05F1.

Digest all with Sau3a.

a1	C18H12
b4	15b2
a2	C10G9
b1	5a2
a4	C01E7
b5	21b1
	marker
a7	6a1
b2	11c1
a3	C05F1
b3	14b1
a7	6a1
a5	C15H7
	marker
a6	C17A6

8 H_2O
2 10xH
1 ~~[illegible]~~ Sau3A (5K.)

1 DNA
1 min

60' 37°

Mix
10 d A^{32}P (8 μg)
2 10 mM dGTP
2.5 10 mM dATTP
1 10xHi
3.5 H_2O
1 AMV.

+ 1 mix

30' 37°

263841.

Agarose gel clean up of cosmid DNA's for Donna.
(first attempt a dismal failure - discarded).

As John's protocol.

250 ml 0.4% TAE LGT gel (on glass plate).

20µl samples (whole prep) + 10µl dyes.

C01E7
C05F1
C07A9
B0220.

O/N 18 mA. r.t.

15µl 10mg/ml Eth Br into buffer ~~between~~ barriers.
20' r.t.

Slow and fast bands ~~[illegible]~~ cut from each sample

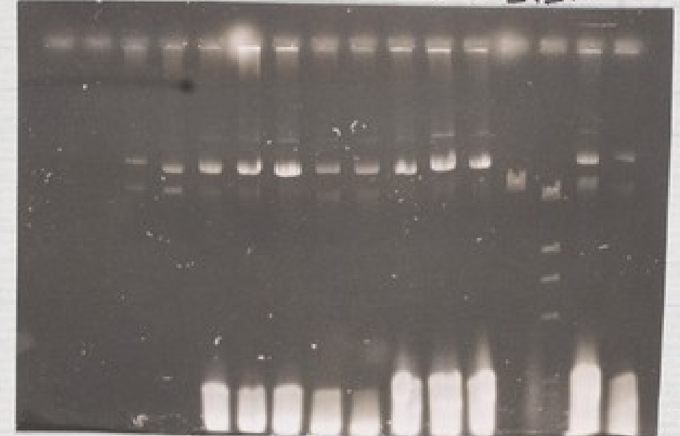
10' 70°
+ 400µl phenol.
" " /CHCl3

iso Bu-OH → 100µl
Ether.

54841.

0.4% HGT TBE gel of various cosmid DNA preps.

C14G1 ([illegible])
C02B11 ([illegible])
B0220
B0220
B0220
D2078
D2078
C01E7
C05F1
C07A9
1μl λST 1:100
1μl λH3 328/1
NC15 A7
" A8



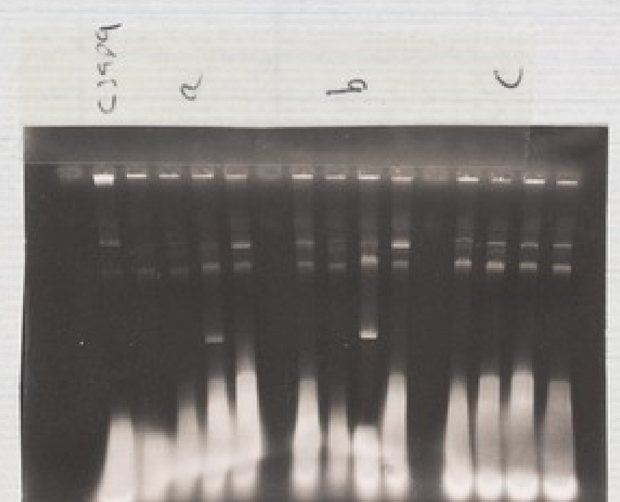
249841.

Prep^n of cosmid DNA direct from colonies.

a) NC19 A1-6 agar growth
b) NC19 A1-6 filter growth
c) NC19 A7-11, B7,8 filter growth + chloramphenicol (CAO) 1ml.

Toothpicked into 0.1ml lysozyme buffer. (much easier
d) filters than agar)
Vortexed.
5' 0°C
+ 0.1 ml NaOH/SDS.
5' 0°C
+ 0.075 ml 3M NaAc pH 4.8.
60' 0°.
Nearly all super removed
+ ~0.9 ml EtOH.
-20° 60'.
Spin;
resuspended in 0.3M NaAc (100 µl)
+ 200 µl EtOH
-20° 20'.
Spin, EtOH wash, dry.

+ 4 µl TE/dye 1µl ; 0.5% agarose gel
(i.e. whole prep. loaded).
1st 4 samples of each a-c only, + 1µl (1/10) C34D9



Looks promising.

CAO possibly more consistent than others!

26/9/841.

More direct colony work-ups.

a) filter NC19 A6-C4

b) filter /CAO NC19 B9-C12.

Work up as 24984l. (+2nd TE final).

For fingerprinting.

- no good.

29.11.84 I.

Prep. 3' ^{32}P labelled fragments for Maxam-Gilbert trial.

(Idea to match putative identical bands from fingerprint gel by M-G of eluted fragments.)

1μl (0.8μg) λ57 DNA
0.5μl 20u/μl Hind III
1μl 10x Hin
7.5μl H_2O.

30' 37°
2μl (2μCi) ^{32}P dATP
0.2μl Klenow (~1.0 unit)
10' r.t.
10' 68°.

5u Sau3A (~0.1μl ABT 60u/μl).

37° 60'.

+ 10μl formamide/dyes/EDTA

6% TBU gel. (6 3mm slots).

1.5 hr exposure.
6 bands cut out (1-6 increasing size)
+ 200μl gel elution buffer. 0.5M NH_4Ac
o/n 37°. (Si glass tubes). 0.01M $MgAc_2$
0.1% SDS
0.1mM EDTA

+20μg tRNA carrier to eluant
ethanol ppt. & wash.
Dry; + 10μl H_2O.

Meth. in Enzym.
1980 65 (I) 497

5 μg/μl
500.
2.5 μg (5 μl) DNA

Zaller & Smith

45° 20'.?

std/
20 μl 4 M NaCl
1 μg carrier DNA
3 μl (?) ^{32}P DNA
→ 0°C
25 μl hydrazine
20° 15'. (r.t. = 23°).

a) 20° 15'
b) 45° 20'
c) 45° 60'.

200 μl HZ stop

100 μg/ml tRNA

500 μl EtOH
-70° 10'.
5' huge
70% EtOH wash
repp t.

20 μl M piperidine (fresh dil) (redist/10)
90°C 30'. (capillary)

C

+ 20 μl H_2O (in new Eppendorf)
Dry.
+ 20 μl H_2O
Dry
+ 20 μl H_2O
Dry.
+ Formamide etc.

2 ml HZ stop
200 μl NaAc 3M
2 μl 0.1 M EDTA
2.5 μl 20 μg/μl tRNA
1.8 ml H_2O

50 + 200

HZ stop 2ml:

200 3M NaAc
2 0.1M EDTA
2.5 20mg/ml tRNA

3 12 84 1

Maxam-Gilbert 'C-track' analysis of 2911841 frags.

20µl 4M NaCl
1µg carrier DNA (salmon sperm, sonicated)
3µl ^{32}P Hind III / Sau3A λ frags.
→ 0°C.
25µl hydrazine

a) ~~20°~~ 22° 15' (standard)
b) 45° 20'
c) 45° 60'.

+ 200µl HZ stop.
500µl EtOH.
-70° 10'.
5' fuge.
EtOH wash.
Reppt. from 100µl 0.3M NaAc & wash.
Dry

+ 20µl 1M piperidine (redist./10, fresh dil^td)

90°C 30' (sealed cap.)

+ 20µl H_2O
Dried 3x from 20µl. H_2O
+ ~~3~~ 2.5µl formamide / dyes.

20% acryl 20x20 gel.

14mA (5.00 → 7.00.)

Gel buggered up after fixing - didn't Wacker properly.
Autoradiographed 'wet'. - no flashed film or -70° as intended.

- Looks hopeful after 2 day exposure

6128411.
Prep. of fragments from S313 and C10D6 for C track Maxam - Gilbert analysis.

2.5 µl 800 ci/mmol $dATP^{32}$. (aqueous)
16 H_2O
5 10×H
0.5 RNase
1 0.5 mM ddGTP
0.5 Hind III
1 AMV.

5 µl DNA (standard cosmid mini prep; so ~0.25 µg)
10 µl mix

45' 37°
30' 68°.

Mix: 18 H_2O
2 10×H
1 Sau3A (60 u/µl)

10 µl mix / reacⁿ.

2 hr 37° (capillary, sealed).

EtOH ppt & wash.
Dry. + 3 µl formamide / dyes.
100°C 1'.

Loaded onto 2 slots / sample 4% ~~[illegible]~~ gel.
1.75 hr 30 mA (BPB 1" from bottom).

2 hr autoradiography.

4 'matching' bands / sample eluted O/N (M-G elution buffer)
(1-3 Si glass tubes
4 Epp. tube (not Si).

EtOH ppt & wash
Dry.
Maxam - Gilbert 'C' track as 312841 (b)
i.e. 20' 45°C hydrazine; ~~short~~ 20 cm 20% gel.

OVER.

2 h 14 mA.
No fixation; Flatted film, -70° o/N.
Nice strong picture o/N.
Bands a–c gave identical patterns.
C1096 d has extra bands.
Presumably these could be single base sequence changes, but more likely that C1096 d is a mixture, although this is not apparent from finger print.

8/8/85

Prep. of partial Alu frags. of N2 DNA (for ligation into Brownlee blunt-end cosmid vector pDV1)

Digestion condition assay.

60 µl (20 µg) N2 DNA (19/9 (2))
30 µl 10x Hin
210 µl H_2O.

16 tubes on ice.

30 µl mix aliquoted to tube 1, 15 µl to remainder.

(1 µl) 1 u Alu I (diluted in 1x Hin from 8 u/µl AC 1/85) added to tube 1; mixed; 15 µl → tube 2; mixed; 15 µl tube 3 etc.

30' 37°.

+ 3 µl 5x agarose gel dyes.
All loaded onto 150 ml 0.4% HGT agarose.

1 µg λ / 1 µg λ Hind III / assay 1-16 / 1 µg λ Hind III / 1 µg λ.

28V (20 mA) (4.30 → 9.00) 16.5 hr room temp.

Even most severe digest looks slightly underdone (0.5 u/µg) but will take this as stringent digest for fragment prep. (over).

pH Alu digest of N2 19/9 for preparative gel

3x (20μg) 60μl N2 19/9 DNA
30μl 10x Hin
210μl H2O

a) 10μl 1u/μl Alu (AC1(85) in 1x Hin
b) 5 " "
c) 1 " "

30' 37°; Freeze

Aliquot ~~test~~.

15μl digests + 3μl dyes (5x)
1.5μl of this + 15μl 1x dyes

Markers 0.5μg λ ; 0.5μg λ Hind III.

on 200 ml 0.4% HGT agarose gel.

λ a b c λ Hd III

Forgot undigested N2 DNA!
Looks O.K.

EtOH ppt^n of digests
+ 30μl 3M NaAc
700μl EtOH.
-20°C 72hr ; 5' Epp.
EtOH wash ; Dry.
+ 30μl TE/sample.

Loaded on 2 x 0.5cm slots of
250ml 0.4% LGT gel. (No et Br)

(Markers: 0.5μg λ
0.5μg λ Hind III
~0.5μg uncut N2 DNA)

20mA/24V (4.00 pm → 11.30am)

Stained (40μl 10mg/ml EthBr/50ml
30')

Looks O.K., although ~~......~~ intensity
may be rather low.

4 slices (1-4, small-large) taken between
23K λ and above intact N2. (visualis^n
by long-wave U.V)

0.4% LGT
6μg
6 lanes
2 lanes 20μg
(don't need bigger)

Extraction of DNA from LGT (1st gel)

2063 (falcon) tubes
70° (H_2O bath) 10'. (no dil^n)
→ 35° with mixing.
+ equal vol. phenol
Spun 5' 6K IEC

- v. heavy interface.
aqueous layer removed
+ 1 ml TE to phenol/interface.
↳ combined with 1st aqueous.
2 more phenols.
iso-butanol → 0.3 ml
+ 15 μl 1M NaCl
+ 0.75 ml EtOH
-20°C O/N.
EtOH wash; Dry; + 20 TE.

1 μl of each for 0.5% HGT minigel:

1 2 3 4

Looks pretty good (4μg+?)

Cuts 3 and 4 (largest) given 2nd pass on 200ml 0.4% LGT gel with λ & λ HIII markers. (Some of markers looked a bit buggered up in loading - may not be very reliable).
15mA ≡ 26V 18hr
(Samples on 4 slots total)

Nice compact bands ~ 1cm depth
3 slices taken. Eluted and worked up as above. (4x phenols)

OVER

+ 20μl TE.

0.3% HGT minigel ~0.2μg λ
0.3μg λ Hind III

1μl samples 1-3

Prep-gel cuts look too small. (Last film)
Run 150 0.25% ag. gel. O/N.

Largest cut from 2nd gel looks to be only ~30K max

Not clear why this has happened. Size looked good on analytical gel of original digest, and cuts included largest material on preparative gel.
Possibly some digestion between analytical gel and running of preparative gel? (Mobilities on prep gel not to be relied upon)

Repeat but a) Phenol at end of digest
b) Run accurate (O/N 150ml) analytical gel of 1st gel fractions.
c) More starting material?

181851

2nd attempt at sizing N2 (19/9) pAlu frags.

Initial digest and analytical gel as 81851

Digests frozen -20° O/N.

Then 1x phenol
1x ether
+1/10 vol 3M NaAc
EtOH ppt^n.
20' -20°
EtOH wash. (70%)
Dry. ; +30 µl TE

300ml 0.4% LGT gel 24V 18mA (5.00 → 10.30am)
λ markers only.
Should have run further but o.k.
4 bands (a-d, small-large) cut out.
Worked up as previous prep.

+20 TE final (181851 1a-1[illegible])

.3 μg N2
N2/10
0.4 μg λ
λ/10
0.6 μg λH3
λH3/10
a
a/10
b
b/10
c
c/10
d
d/10

0.3% HGT
28V 19mA 16 hr.

c looks O.K.
Run 0.4% LGT

Eluted from 2nd gel as 2 bands.
Work-up as before, except gel vol. small enough to use Eppendorf tubes rather than Falcon/IEC.
+ 20 TE. final. = 181851 2a + 2b

1/20 + 1/200 on 150 ml 0.3% ag. gel O/N with markers

.3 N2
N2/10
.4λ
λ/10
2a
2a/10
2b
2b/10
λH3/10
.6λH3

2b size looks OK.
Yield say ~ 0.5-1 μg.

81852

Prepⁿ pDV1 (Brownlee's blunt-end cosmid vector)

Streaked from stab on amp. plate.
Colonies not too well separated, but probable single colony taken for O/N growth in 10 ml 2xTY / 75 μg/ml amp.
Also, secondary plate streak made from 1st plate.

(1 ml + 0.5 ml glycerol for −70° storage)

1 l 2xTY / 75 μg/ml Amp inoculated with 5 ml O/N culture.
37° shaking (5.00 → 9.00am) 16 hr.

Usual work-up ('40x scale-up' protocol).
Extremely gloopy lysate − + 12 ml solⁿ II
9 ml solⁿ III.

To dried EtOH ppt, add 13 ml TE
250 μl 0.1 M EDTA

TE → 14 g
+ 15.5 g CsCl
1.5 ml 10 mg/ml Eth. Br.
R.I. = 1.385
+ ~~0.7~~ 1.0 g CsCl
R.I. = 1.392

40K 20°C 40 hr. (2xTi:60)

Looks O.K. but prob. low yield.
Bands pumped out. ~ 7 ml total vol.
+ 7 ml TE
+ 8 g CsCl
+ 1.5 ml Eth Br (10 mg/ml) (overdose?)
+ 1 ml TE → R.I.₀ = 1392

40K 20°C 40 hr (1xTi:60)
O.K.; band just vis. in daylight.
Pumped out ~3 ml; isopropanol extracted 5X.
Dialysed v. 3x 1l TE 4°. 24 hr.

OVER

EtOH ppt, wash, dry, + 150 μl TE
≃ 1 μg/μl
(O.D.260 of dialysate = 0.52
≃ 2.86 O.D. total
≃ 148 μg)

Preliminary ~~test~~ of vector.

1 μl pDV1/5 (≃ 0.2 μg)
0.5 μl H×10
2.5 μl H_2O

a) 0.5 BamHI
b) 0.5 PvuII
c) 0.5 Bam + 0.5 PvuII

Pvu = 10 u/μl
Bam = 16 u/μl

30' 37°

0.3 μg λHind | 0.2 μg pDV1 | Bam | Pvu | Bam + Pvu

0.8% ag. gel.

Looks O.K.

Prepn of pDV1 for cloning.

20 μl (10 μg) pDV1
6 μl 10×H
28 μl H_2O
6 μl 10 u/μl PvuII

2 h 37°.

o PvuII.

← 1 μl for 0.8% MGT gel

Looks cut.

1x phenol
1x ether
EtOH pptn & wash.

+ 40 μl H_2O
5 μl 10x CIP buffer (Maria)
4 μl CIP

15' 37°C (CSH p.133)
15' 56° C

+ 4 μl CIP

15' 37°
15' 56°. – accidently left 24 hrs!

+ 1 μl 0.1M EDTA
3x phenol.

Despite overdigestion, looks ~~intact~~ on minigel so continue work-up and assay.

Prep. λ Alu frags for ligation assay.

6 μl (5 μg) λ DNA
37 μl H_2O
5 10x H
2 (16 u) Alu

2 hr 37°
2x phenol
1x ether
EtOH wash; dry; + 20 μl TE (= 0.25 μg/μl)

← Also, 20 μl (20 μg) pDV1 PvuII digested as, because forgot to retain ~~[illegible]~~ unphosphatased aliquot for assay.

Ph'ased and unphased pDV1 PvuII preps made to 20 μl in TE = 1 μg/μl.

0.8% gel of vector & fragments for assay:

0.25μg λAlu
0.25μg pDV1 unphased
0.25μg pDV1 phased

Ligation assay:

a) 0.5 μl phased pDV1 PvuII (1μg/μl)
6.5 μl H_2O

b) 0.5 μl " "
2 μl λAlu 0.25 μg/μl
4.5 μl H_2O

c) 0.5 μl unphased pDV1 PvuII
6.5 μl H_2O

3 μl 10x ligⁿ buffer (10 μl 10xC
mixed. 10 μl 10mM rATP
2 μl aliquot removed from 10 μl 0.1M DTT)
each & frozen

Remainder: + 1 μl Bentley green label blunt ligase
O/N 14°C

2 μl/reaction run with 0 aliquots on 0.7% gel

phosed 24hr
phosed lig^n
phosed + Alu frags
phosed + Alu frags lig^n
unphosed
unphosed lig^n

Smear behind supposedly phosed sample (no insert) rather odd. Otherwise looks O.K.

Further 10 mg of cut vector dephosphorylated for 1hr (4×15'), then work up as 24hr 56° dephosphorylated material.
Assay over.

231851

Attempted ligation of 181851 2 b sized N2 pAlu fragments to dephosphorylated pOV1 PvuII.

a) 2 μl (~ 0.1 μg) inserts
   0.5 μl (0.5 μg) 24 h ph'ased vector
   3 lig^n mix
   3.5 H_2O
   1 Bentley 'green label' ligase

b) 2 μl inserts
   0.5 μl 1 h phased vector.
   3 lig^n mix
   3.5 H_2O
   1 lig

c) (for gel test of) 1 h phased vector lig^n)
   6.5 H_2O
   0.5 1 h phased vector
   3 lig mix
   (2 μl removed and frozen for 0)
   1 lig

o/n 14°.

Packaging.

		with λ^{57} assay 1 μl 0.1 μg/μl λ57
Reac^n +	12 H_2O	10 H_2O
	3 10×A (SES)	1.5
	5 Ql (fresh thawed aliquot SES)	2.5
	20 Sc (SES)	10
	10×10 FTL over 60'	10×5
	+ 0.3 ml λ dil	"
	Drop $CHCl_3$	"
	Spin 2'	"
	S/N + drop $CHCl_3$; 4°	—

10 μl S/N + 0.1 ml λ dil + 0.2 ml o/n 1046
   20' 37°
   + 0.5 ml CY.
   30' 37°.

3 × 150 μl aliquots on TYE/Amp plates.

231851 result:

No colonies.

Also, plated λ57 assay on wrong bugs!! Try again with C282 (see over)

281852.

Repeat plating of 231851 a & b.

a) 100 λ a + 0.2 ml 1046 20'37° (4 day o/n) + 0.5 T(E40'. Plate ~~20~~ 200 μl on 50μg/ml Amp plates.

b) 100 λ b + 0.2 ml 1046 + 0.5 TYE 40'. Plate 200 μl on 50μg/ml Amp plate.

c) 50 λ b + 0.1 ml 1046 - plate on non-amp plates to check viability of bugs.

No colonies on amp plates, but bugs grew on non-amp plates, so not killed by $CHCl_3$.

25/8/85

Transformation of 1046 by vector ~~test~~ (ligation of λ Alu frags) samples.

		col's
a)	1µl a. (50ng ~~=~~ ligated phased^{246} pDV, PvuII)	2
b)	1µl b. (50ng ligated phased^{246} pDV + Alu frags)	31
c)	1µl c (50ng ligated unphased pDV, PvuII)	183
d)	1µl (50ng) phased^{246} ligated pDV PvuII	1
e)	1µl (50ng) unligated unphased pDV PvuII	3
f)	1µl (50ng) unligated phased^{246} pDV PvuII	0
g)	1µl (50ng) uncut pDV	~1000
g/100)	1µl (0.5ng) " -	14
h)	0	0

Vector, phasing and ligation look OK, although (g) is a bit low (5-10x?). This may be because bugs were not stored in CaCl after prep^n, but used directly. This can account for ~5x reduction in transformation efficiency.

28185 1

Repeat of λ57 packaging efficiency assay — plated on wrong bugs before. This time, plated on EQ82 / MgSO4 bugs.

·1μg/400μl

p λ $^{-1}$ $^{-1}$ $^{-1}$ $^{-1}$

10 10 10 10

a b c d

~400 4

0·1 ml plated.

1·6x10^{5} 4x10^{4} 400 tot.

1·6x10^{6}/μg.

So ~ 1·5 x 10^{6}/μg a bit low

29/8/84

Cloning of JS7A1 (WT ribo and frag?) into mp8 Hind III.

a) 1 μl 20 ng/μl mp8 Hind III C.I.P (N. Gay)
2 μl 10 ng/μl (?) fragment
3 μl H_2O
3 lig mix
1 D.B. green label ligase.

b) no frag. control

90' 15°.

Transfect 2 μl into TG1

Plenty whites (0 on control), ~~few~~ 3 blues.

6 plaques grown up for T-tracking.

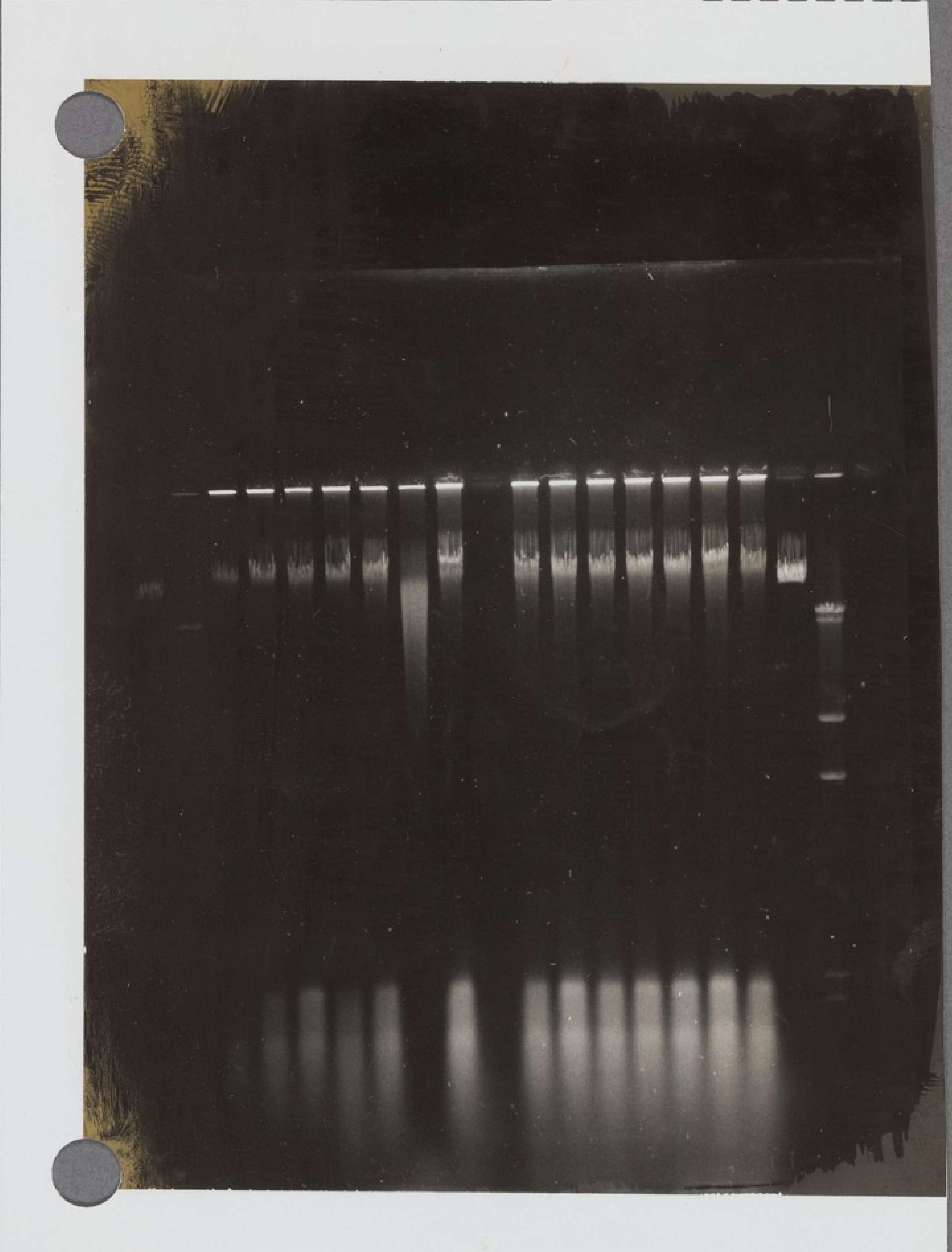
T tracked on ancient gel mix.

– wrong fragment.

Repeat c̄ JS7A2

Plenty whites but v. small plaques.

5/2/85
6 x JS7A2 grown 4.5 hr.



301851

Partial digestion condition assay of 30/1/85 N2 DNA
- prepd. by JCS - not EtOH'd or CsCl'd.
- dialysed O/N. after phenol

1/ Alu.

600µl ~ 10µg/ml 30/1/85 N2 DNA
66µl 10×Hin

Aliquoted
100µl tube 1, 80µl × 5 tubes; on ice

+ 4 u (4µl) AluI (A.S.)/8 (8u/µl) in 1×H — to 1.
Mix
20µl → tube 2
etc.
30' 37° H_2O bath
+ 10µl 3M NaAc
250µl EtOH.

2/ RI

600µl ~ 10µg/ml 30/1/85 N2 DNA
66µl 10×RI buffer

Aliquoted as Alu.

+ 4 u (4µl) 25u/µl RI/25 in 1× RI buff.; on ice
Mix
As Alu.

- No apparent digestion (except ~~on~~ Alu 6, which looks buggered-up).

Try a) EtOH ppt^n of DNA prior to digest^n with and without RNase
b) Dig^n ~~with RNase~~ in presence of RNase
c) ~~[illegible]~~ Repeat above c̄ more enzyme.

Digestion of 30/1/85 N2 – various conditions.

0.5 ml 30/1 N2 DNA
+ 25 µl 1M NaCl
1 ml EtOH.
-20° 20'
Spun, washed, dry; resuspended in 50 µl TE

EtOH pptd
a1 1 µl (0.1 µg) EtOH pptd N2 | R1
3 1.5x Hin | 1.5x R1 buffer
1 8u/µl Alu/10 | 1 25u/µl R1/10
a2 " + 1 Alu/100 | 1 R1/100
a3 " + 1 Alu/1000 | 1 R1/1000

EtOH pptd + RNase
a4 1 EtOH pptd DNA
3 1.5x Hin | 1.5x R1 buffer
1 mg/ml RNase
1 Alu/10 | 1 R1/10
a5 " + 1 Alu/100 | 1 R1/100
a6 " + 1 Alu/1000 | 1 R1/1000

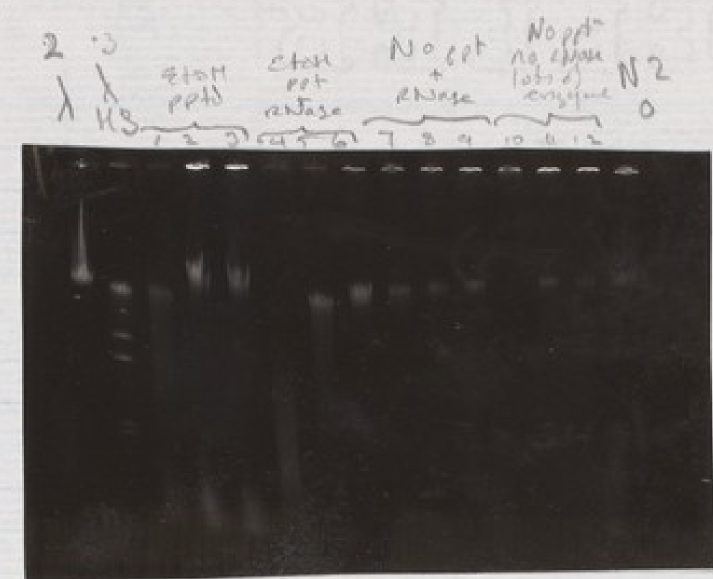
No ppt + RNase
a7 10 µl unpptd DNA (0.1 µg)
1.3 10x Hin | 1.3 10x R1 buffer
1 mg/ml RNase
1 Alu/10 | 1 R1/10
a8 + 1 Alu/100 | 1 R1/100
a9 + 1 Alu/1000 | 1 R1/1000

as before but more enzyme (no ppt no RNase)
a10 10 µl unpptd DNA
1.2 10x Hin | 1.2 10x R1 buffer
2 8u/µl Alu | 2 R1 (25u)
a11 2 Alu/10 | 2 R1/10
a12 2 Alu/100 | 2 R1/100.

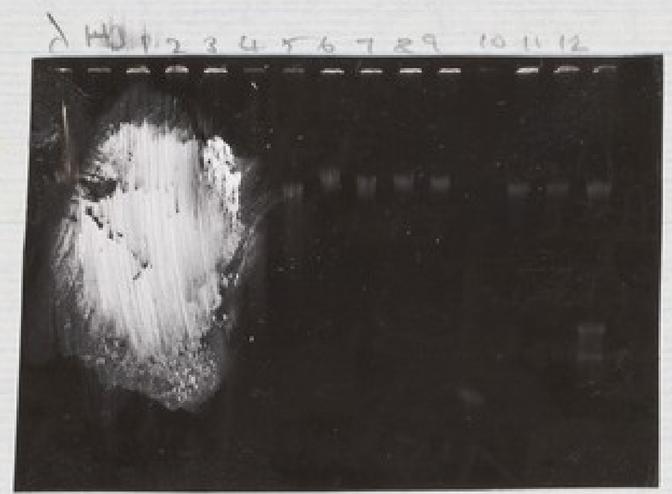
30' 37°.

+ dyes ; 0.4% minigels (not all 7–12 loaded)

Alu:



R1:



Film buggered up.

Both RNase and EtOH look desirable.

In both R1 & Alu, conditions round about 5 look correct.
i.e. ~ 1 µl enzyme/100 / 0.1 µg
+ 1 mg/ml RNase.
30' 37°

12851

To establish partial dign^n conditions for 30/1 N2 DNA

30/1 N2 DNA EtOH pptd (JES)
+ TE → 0.3 mg/ml.

Alu:
30 µl (10 µg) 30/1 N2
16 10 x Hin
105 H_2O
10 10 mg/ml RNase
Aliquot
1x 30 µl, 7 x 15 µl
↓
+ 4 u Alu (2 µl AC Alu/8) → 15 µl 2 → 15 µl 3 etc. 8 = 0

37° 30'. (H_2O bath)

R1:
30 µl (10 µg) 30/1 N2
16 µl 10 x R1 buffer
105 H_2O
10 10 mg/ml RNase
Aliquot.
1x 30 µl, 7 x 15 µl
↓
+ 5 u R1 (2.5 µl R1/12.5) → 15 2 etc 8 = 0

37° 30'.
-20°C stop.
0.4% 150 ml HGT gel. o/N.

λ/H3 N2 Alu R1 λ/H3 N2

Prep. digests 3-6 Alu and 2-5 R1.

Alu 4 x 20 μg 60 μl N2 DNA 30/1
32 10 x Hin
210 H_2O
20 10 mg/ml RNase

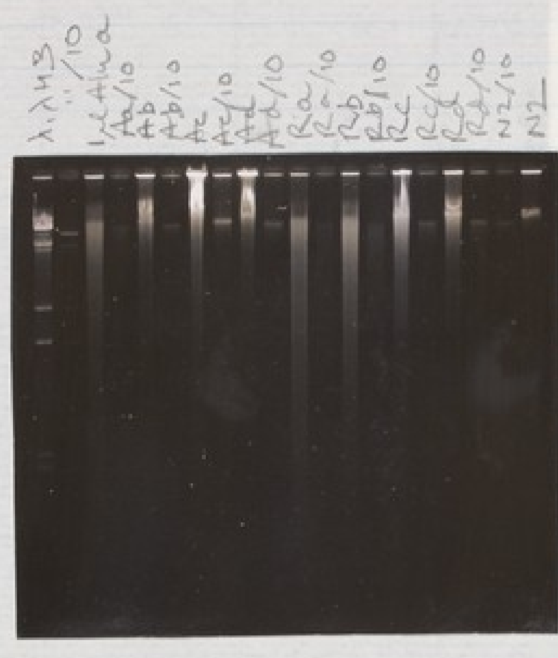
a) 5 u Alu 10 μl Alu/16
b) 2.5 u 5 ..
c) 1.25 u 2.5 ..
d) 0.6 u 1.25 .

R1 4 x 20 μg 60 μl N2 DNA 30/1
32 10x R1 buffer
210 H_2O
20 10 mg/ml RNase

a) 25 u R1 10 μl R1/10
b) 12.5 u 5 R/10
c) 6 u .. 2.5 ..
d) 3 u .. 1.25 ...

30' 37°
-20°C.

150 ml 0.4% ag. gel of pAlu & R1 samples



Alu c and d look a bit underdone
R1's good. (Phenol & EtOH)
Redigest Alu c & d

C + 8 u Alu 30' 37°
d + 4 u Alu " "

λ λ/10 c c/10 d d/10 a a/10 b b/10 N2/10 N2

O.K.

Phenol extrac^n of p. digests.

Equal vol. of phenol added.
Behaved oddly - DNA, esp. of Alu samples, appeared to 'coagulate'. (Dilution x2 didn't help).
(This phenol (BDH chromatog. grade) had given ~~an~~ odd-looking interface on addition of water).

Phenol removed.
~~Eth~~ 1x Ether extrac^n.
EtOH ppt., wash 70% EtOH, dry.
+ 50 μl TE.
Difficult to dissolve up ∈

+ 1 μl 10% SDS.
Dissolved OK eventually.
R1 c & d hardest to dissolve and behaved oddly when gel loaded - shot out of well.

Gel looked O.K.

4 slices from each (Alu & R1) cut out & melt eluted (A1-4, R1-4 7/2).
+ 30 μl TE final.

1.5 μl and 1.5/10 μl on 150 ml 0.4% HGT gel

N2 : 3 μg
N2
N2/10
Alu a
Alu a/10
A b
A c
A d
R a
R b
R c
R d
λ/λ H3/10
λ/λ H3

Size frac^n looks O.K., but intensities reverse of expected, since ½ R1 samples lost on loading. Possible that samples got reversed.
Try ligating Ad and Rd to both R1 and ~~Al~~ blunt vectors. Package and plate, and also run gel to assess ligation of vector to fragments.

5-10 μg R's?
2-5 " A's?

82851

Ligation of 4 (overleaf) Ad and Rd single gel purified N2 pAlu and pR1 fragments.
(pAlu frags should be ligated into pDVPvu and pR1 frags into pJB8 HindIII/R1 and Sal/R1 arms, but because of possible inversion of sample order, decided to ligate each into both)

Ad and Rd given extra phenol and EtOH pptn, + 20 μl H_2O final.

Ad1
2.5 μl Ad frags (~0.5 μg?)
2 HindIII/R1 arm/10 (~0.1 μg)
2 Sal/R1 arm/10 (")
2 10x C
2 rATP 10 mM
2 0.1M DTT
6.5 H_2O
1 ligase (DB green label)

Rd1
1 frags (0.5 μg?)
"
"
"
"
"
8 H_2O
"

(Arms from JCS)

Ad2
2.5 μl Ad frags (~0.5 μg)
0.5 μl pDVI pvu phosed
1 10xC
1 rATP
1 DTT
1 ligase

Rd2
1 frags
"
"
"
"
1.5 H_2O
1 ligase

16 h 14°C

Packaging as 271851 - 10 μl Ad1 + Rd1 packaged
3 μl Ad2, Rd2 "

25 μl S/N + 0.1 ml λ dil + 0.2 ml O/N 1046
20' 37°
+ 0.5 ml CY
30' 37°
1x 200 μl plated

Plating result

	200μl	tot. transfec^n	tot. pack.
Ad 1	2	8	128
Rd 1	9	36	576
Ad 2	0		
Rd 2	0		
0	0		

u.v. assay showed bugs ok

0.6% LGT minigel

Looks as though I may have omitted to dilute arms /10. i.e. used 10x excess of arms in ligation.

Makes sense that R1 frags. gave most recombinants with HindIII arms, but Alu fragments obviously should have given nothing with arms. (could colonies be concatamers of undephosphorylated arms, seen because of use of 10x excess of arms?)

11/2/85

Plating of 50μl Rd1 to provide ~50 colonies for work-up and f/p analysis

Also, 2nd LGT gel of Ac, Ad and Rc, Rd.

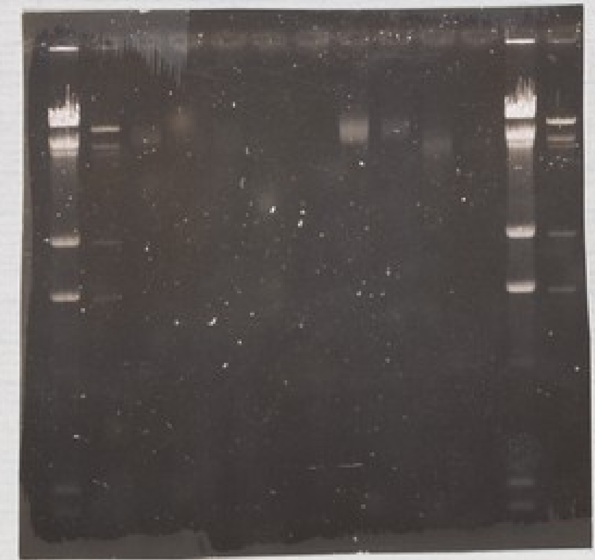
--- | λ HIII | - | Ac | Ac | Ad | Ad | - | Rc | Rc | Rd | Rd | - | λ | ---

1 band from Ac, Ad, Rc, Rd cut out & eluted

= Ac2, Ad2, Rc2, Rd2 12/2/85
+ 20μl TE final

0.4% HGT ag. gel

·4μg λ / lane
·5μg λ HindIII / lane



Rc2 looks ok. (~ 1μg ~~total~~?)

Rd2 too small (took wrong gel slice?)

(These are only probable R1 fragments - see possible balls-up re ~~$~~ analytical gel of 1st cuts)

13/2/85
Ligation of Rc2 (2x gel purified) to pJB8 arm

a) 4 μl Rc2 (0.2μg?)
1 H3/R1 arm/10 (0.1μg)
1 Sal/R1 arm/10 (0.1μg)
1 10xC
1 rATP 10mM
1 DTT 0.1M
1 ligase

b) no insert, 4 H₂O

o/N 14°C.
Packaged as 23/1/85
25μl ~~absorbed~~ (0.8ml) and 150μl plated.

Gel of ligation reac^n rather puzzling. - why streak in control, apparently supressed in presence of fragments.
Arms probably not incorporated into recombinant.

Plating result:

150μl RC2   30 colonies = 30 × 5 × 14 ≈ 2000 total

No colonies in no-fragment control, so looks hopeful.

(Plate out of 0.8 ml absorbtion and streak out colonies for growth.)

48 grown - see clone log.

2/12/88

C01C7 no repeat remarks
C08E1 o/l WOLG1 : Rem'd strong repeat RcS5, Rc34, RcB1
C14H8 (TCLIN14A) o/l ZK1102 : Rem'd RcC9?
C16A6 no repeat remarks
C41D9 (NOTES) no repeat remarks
C35F12 (lin.11) no repeat remarks
R4 (NOTES) no repeat remarks
M9 no repeat remarks.

27/2/85

Hind III digest / agarose gel of ~~4~~ cosmids showing 'heavy bands' in f/p's.

Mix 22 H_2O
6 10×H
2 Hind III (20u/μl)
0.5 RNase

60' 37°

+1μl sucrose/dyes.
0.5% agarose gel.

C01K7° | " Hind III | C08F1° | " Hind III | C14H8° | " Hind III | C16A6° | " Hind III | C41D9° | " Hind III | C35FVR° | " Hind III | NR4° | " Hind III | λ° | λ Hind III

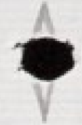
C16A6 prob. pJB8 only.

C01K7, C08F1, C14H8 may have a doublet in common.

C41D9, NR4 have ladder.

C35FVR may be normal. ('heavy bands' not as extreme as others anyway).

Try more partial digest of C01K7, C08F1, C14H8 and more extreme digest of C41D9, and NR4



OVER

More partial digest of C01C7
C08C1
C14H8

Mix HH_2O
3 10xH—
0.25 RNase
·1 Hind III (20u/μl)

(i.e 1/10 x Hind III of previous digests)

2 mix
2 DNA
30' 37°.

More complete digest of C41D9
NR4

1 DNA
2 2xHin/RNase (10 2xHin, 0.25μl RNase)
1 Hind III (20u/μl)

2 h 37°

C01C7 C08C1 C14H8 C41D9 NR4

5/3/85.

Further plating 25μl Rc2 (13/2)
5 plates.

13/3/85

Further platings (for dish gridding) of Rc2

200μl super
+ 1μl λ dil
20' 37° + 1μl 1046 (o/n, 1 week old)
+ 1μl C7/Mg
30' 37°

20 x 150μl on Amp plates.

15/3

^{35}S / ^{32}P labelling comparison NM02 H7 → H12

2 ^{35}S dATP
20 H_2O
5 10x H
0.5 RNase
1.25 0.5mM ddG or
0.5 Hd ω
0.5 AMV.
etc.

Run next to 13/3/85 NM02 ^{32}P reacns.
+ ^{32}P marker.

10/4/85

^{35}S labelling ZL 80 - 91 for comparison with ^{32}P labelled samples (4/4/85 c.l.)

Reaction on 15/3

23/4/85

Comparative minipreps C37 H11
N3332
C12E8 (my growths from plate streaks)

1-6 (leftovers from JE8 prep)

28/5/85.

Various growth cond^ns.

Normally 0.3 ml ~~=~~ 25 mg/ml / 100 ml = 80 µg/ml Amp

actually A2 – B1 (A1 poor growth).

↙

	Clone	Inoc	Amp	Vol.	37° Incub	Tube	
Fresh plating	NC49A1A6	Av.	80 µg/ml	4 ml	H_2O	large	1-6
	"	"	120	"	"	"	7-12
	"	"	200 µg/ml	"	"	"	13-18
	"	~1/4 x	120	"	"	"	19.24
	"	~~~~~~1/2 x	80	2.5	Air	small	25-30
	"	~1/2 x	160	2.5	"	"	31-36
old plating	MC47A2	Av	80	4	H_2O	large	37-42
	"	"	160	"	"	"	43-48.

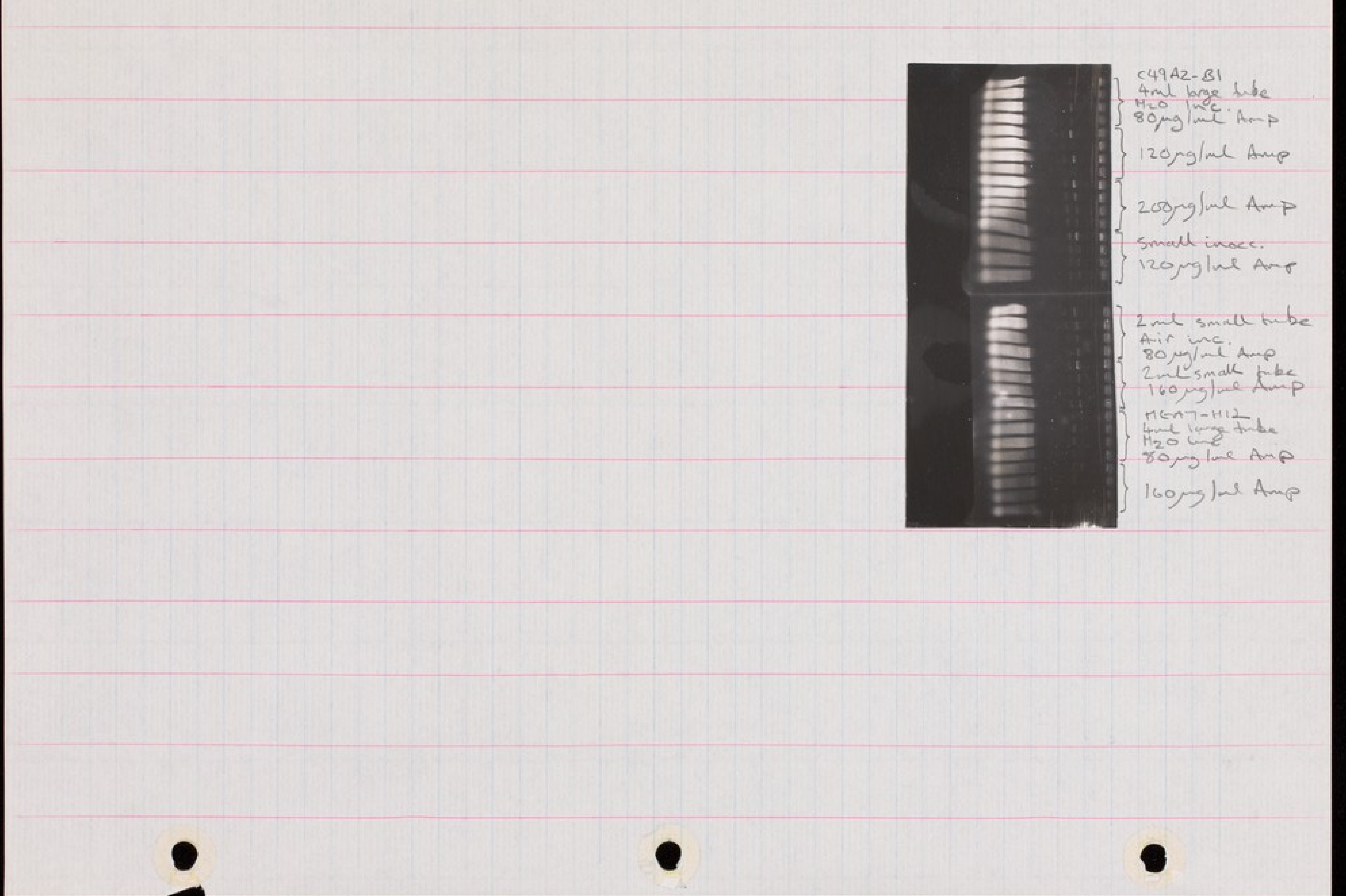
5.30pm ⟹ 9.00 am

Small tubes had big pellet (or debris?) in bottom of tubes – not shaken hard enough? Vortexed prior to work-up.
All others appeared to have equal growth

8/6/4.96 + 25 µl TE final.

1 µl 1st 4 of each set taken for 0.5 ag. gel

OVER



C49A2-B1
4ml large tube
H2O inc.
80µg/ml Amp
120µg/ml Amp
200µg/ml Amp
small inocc.
120µg/ml Amp
2ml small tube
Air inc.
80µg/ml Amp
2ml small tube
160µg/ml Amp
MG47-H12
4ml large tube
H2O inc
80µg/ml Amp
160µg/ml Amp

29/5/85.

Lower temp. cosmid growth 32°.

1-6 C49 A2-B1 80 μg/ml Amp

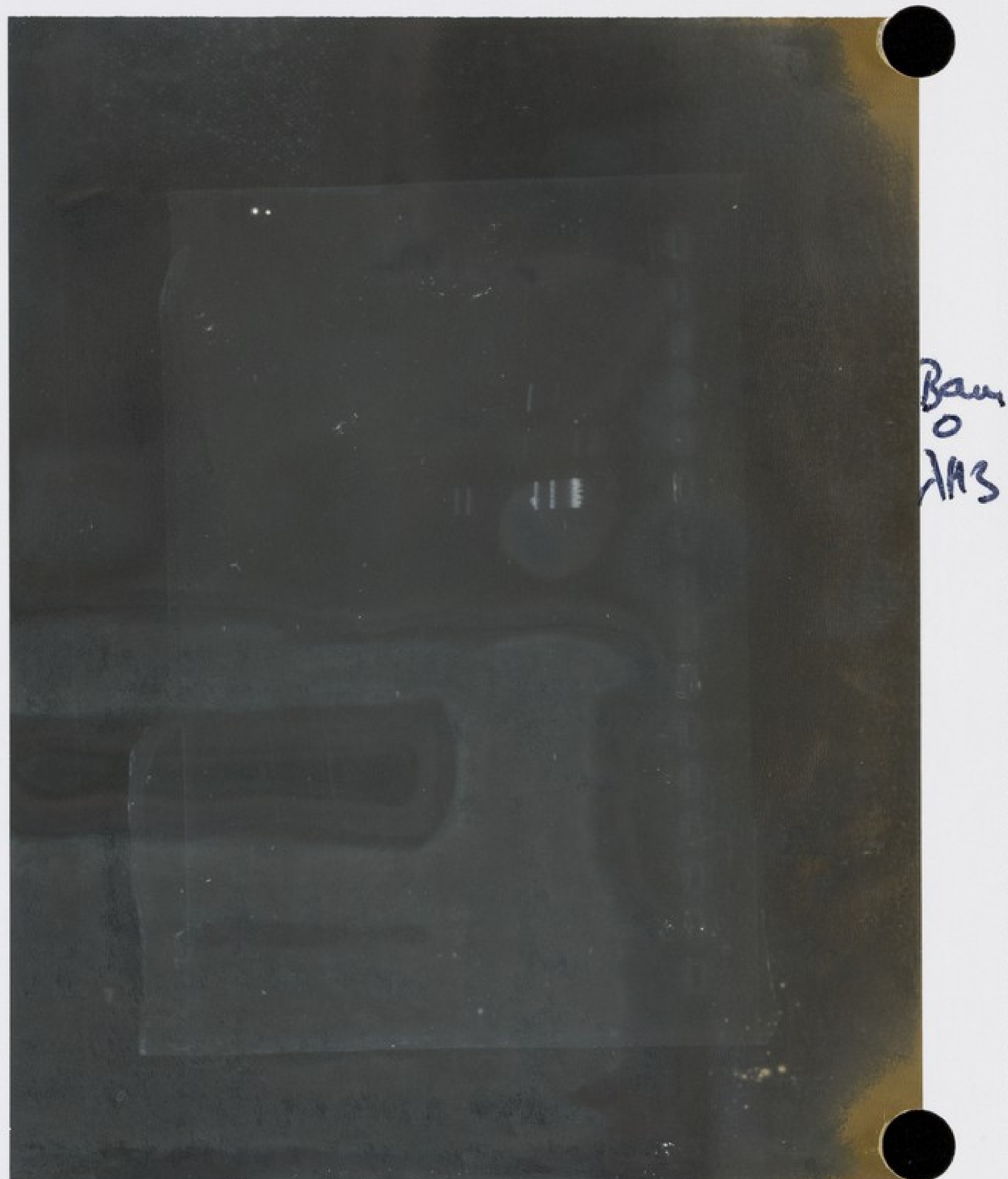
7-12 C49 A2-B1 160 μg/ml Amp.

4 ml H_2O incubator.

+25 TE final

1 μl 1+4 of each set, also 28/5 13-16, on 0.5% agarose gel.

29/5 1-4 32° (80 μg/ml Amp) | 29/5 7-10 32° (160 μg/ml) | 28/5 13-16 37° (200 μg/ml)



Bam
o
[illegible]

PEEL QUICKLY

POLAROID L41917A57559A

30/6/85

LORIST B prep.

5 ml 2 week old O/N (SE3, from Little plate)
→ 1 l L Broth (Sanger) + 30 μg/ml Kanamycin.

37° shaking (5.00 pm → 9 am.) 16 hr.

Deininger 1 l work-up.

40 K 40 hr 20° Ti60. 2x.

Yield looks v. poor. Barely visible under U.V.

4.25 ml (3.75 + 0.5 TE)

2nd grad.

+10.75 TE
+0.5 ml EtBr. 10 mg/ml.
+12.9 g CsCl
+0.5 g CsCl. RI = 1.39

Vol after 3x dialysis = 3.7 ml

O.D. = 0.24 = 0.9 O.D. ~~total~~
≃ 45 μg DNA.

+40 μl T.E. after EtOH pptn, wash, dry

4 μl H_2O
0.5 μl 10x Hin
0.5 μl 1 μg/μl LORIST B
0.25 μl 16 u/μl BamHI
60' 37°

1 μl run on 0.8% gel with undigested material and 0.1 μg λHS
- Looks O.K. but probably only ~ 5 μg DNA.

10 mM Tris — 10 ml 1M/l
0.1 mM EDTA — 1 ml .1M/l.

.01

M/100

.0001

M/10,000 — .1M/1000

10/7/85.

Repeat of LOR1STRB prep.

1l 2XTY + Kan inocc. 5 ml fresh o/N.

37° shaking 4.30pm →

Work-up as 30/6/85 prep. except LiCl pptⁿ.

(Bug pellet looked considerably larger than previous prep - maybe 2xTY better than L broth).

Again apparently v. low yield - looks about same as 30/6 prepⁿ.

1x CsCl only.

9/7/85.

Packaging mix prepⁿ NS428/NS433 Mullins protocol.

Stocks from R. Baer.
Checked 30° and 42°. - O.K.
Growth 30° ~ 3.5 hrs 2X 433 OD^{600} 0.36
428 OD^{600} 0.30

Induction at 42° 15' - should have been 45°!

50 μl aliquots -70°. ~~tubes~~ (1.5 ml Epps.)

10/7/85.

Packaging mix assays.

a). 2 μl 0.5 μg/μl λ2001 DNA (T.G.)
1 μl 100 mM rATP
20 μl CH+ATP
added to 50 μl 9/7 packaging mix aliquot.
60' 37°
+ 20 μl DNased extract (see Mullins protocol)
30' 37°
+ 0.5 ml λ dil.

b) As a), no λ DNA
+ 0.5 ml λ dil

c) 15 μl buffer A
2 μl 0.5 μg/μl λ2001 DNA (T.G.)
2.5 μl M1
10 μl JES S.E.
10 μl JES F.T.L.
10' r.t.
+ 10 μl F.T.L.
etc → 60'.

+ 0.5 ml λ dil.

a) ~ 6 × 10^{6}/μg.
b) 0
c) ~ 3 × 10^{6}/μg.

OVER

9/7 packaging mix ~ 2x old mixes, but rather low titre.
Try again – 45° induction

(Try induction by heating to 45° in v. hot water bath (boiling?) and measuring temperature by thermometer before transfer to 45° bath for remaining time).

16/7.

Repeat packaging mix prep. as 9/7/85

But Induced by swirling in 90°C H_2O bath
with thermometer → 45°C.
Then 15' 45°C.

(NS428 0.08 → 0.41 4.75 hr

2xNS433 0.06 → 0.44 and 0.39 5.25 hr).

50 µl aliquots, small tubes.

17/7

Packaging mix assay.

a). 50µl 16/7 PM
20µl CH + ATP
2µl 0.1M rATP
2~~5~~ µl (1µg) λ2001 DNA.

b) 50µl 16/7 PM
50µl CH + ATP
2µl 0.1M rATP
2µl λ2001 DNA

c) 50µl 9/7 PM
20µl CH + ATP
2µl 0.1M rATP
2µl λ2001 DNA

~~d) as a, no DNA.~~

Accidently added 25µg DNase instead of 0.5µg (as per recipe) after 60' incub^n.

a) ~10^7 total
b) ~4×10^5 ..
c) ~2×10^5 .. (i.e. ~30x down on 10/7/85 assay).

Why (c) so low c.f. 10/7/85? DNase excess?
Repeat assay (also cadge Baer pack. mix for comp).

Discrepancy between a and b also seems surprisingly large.

20/7/85

a) 50 μl 16/7 PM

b) 50 μl 9/7 PM

c) 50 μl Base PM.

2 μl 0.5 μg/μl λ 2001 DNA
20 μl CH MGP
2 μl 0.1 M MGP

a) 2.5×10^7 /μg

b) 6×10^7 /μg

c) 4×10^7 /μg.

5 μg/μl
0.1 μg/μl
stock 1/50

24/7/85

Prepⁿ of Bam cut phased LORISTB.

25 µl LORISTB 30/6/85 (~2.5µg?)
3 µl 10×Hin
2 µl 16 u/µl Bam
2 h 37°.

Apparently hardly any digestion

+2 µl Toby Bam
60' 37°

Gel.
a) 0.5 µl 0.3µg/µl λ Hind III
b) 0.5 µl LORISTB Bam
c) 0.5 µl LORISTB 30/6
d) 0.5 µl LORISTB 10/7

a b c d

Still v. partial (LORISTB = 5.4kb)

+2 µl Bam (Toby)

2 h 37°

a) as b above
b) extra dige⁻.

a b

Cut o.k., but smearing as if degrading.

Carry on anyway.

2x phenol
1x ether
EtOH pptd.

+ 42 μl H_2O
+ 5 μl 10x CIP buffer
+ 2 μl Bohringer CIP
30' 37°
+ 2 μl – –
30' 37°
2x phenol
EtOH
Gel
Lost!

Repeated above. After phase, + phenol & pptn, + 10 μl TE

a b

a) 1 μl 0.1 μg/μl λ H3
b) 0.5 μl LORIST8 Bam/phase

~ 0.05 μg ~~total~~?

U.V. RecA⁻ assay of GD8767 looks o.k. v. EQ82, 0550

28/4/85

Ligation LORIST6 / N2 Mbo

4 μl LORIST6 Bam/phase (24/7)
2 μl N2 Mbo (JES 10/2)
2 10xC
2 rATP 10mM
2 0.1mM DTT
7 H_2O
1 ligase (D.B. green label)
(0 : no fragments - bit of a balls-up in that concn of vector in control was ⅕ that in fragment ligation).
12 hr 14°C

50 μl 9/7 pack. mix
2 μl 100mM rATP
20 CH+ATP
10 ligns. ← (10 μl in freezer)

60' 37°.

$MgCl_2$ and DNase added directly to this reacn by mistake, rather than to 2nd mix before addition.
20 μl 2nd pack. mix added to reacns.

30' 37°

+ 0.5 ml λ dil
drop $CHCl_3$
Quick spin
4°.

10 μl ligations + 0.1 ml λ dil + 0.2 ml ED8767
(pretty dense but prob. not saturated)
20' 37°
+ 0.5 ml C-Y
30' 37°
4 x 150 μl plated TYE/Kan (50 μg/ml) (Kan)

OVER

4x 150µl plates = 134 colonies ~~total~~
(No frags. and bugs/dil only = 0)

134 colonies + ~100µl not plated ≐ 150 colonies

10µl phage stock = 150 cosmids
~~total~~ " " = 150 x 50 (~500µl ~~total~~)
= 7500 ~~total~~.

(Also 10µl. ligated unpackaged DNA in freezer
– potentially another 7500 cosmids)

these colonies streaked and grown as KO1A1 →
KO1A12

21/8 Further 10µl of phage stock plated (608767 absorp^n)
(U.V. assay OK)

94 colonies total

34000
60,000

172000
25900

20/8

Further ligation RC2 (12/2/85) N2 R1 fragments to pJB8 arms.

Ligation as 13/2/85.

All ligation (10μl)
+ 50μl 9/7 packaging mix.
+ 2μl 100mM rATP
+ 20μl CH + ATP
60' 37°

To 2nd pack. mix + 2.5μl M $MgCl_2$
2μl 5mg/ml DNAse / 10

1046

20μl this DNased mix to reaction.

37° 30'.

+ 500μl λ dil
2 drops $CHCl_3$
Spin
4°C.

22/8 25μl + 0.1ml λ dil + 0.2ml O/N 1046 (U.V. assay OK)
20' 37°
+ 0.5ml C-L
30' 37°
150μl plated.

!?

= 3000+ colonies. = 250,000 complete package.

c.f. previous RC2 ligation which gave 30 colonies (same amount ligated, packaged, plated.)

2.5×10^5 / 0.2μg (approx) ≃ 10^6/μg

plates off?

Must be something wrong - much too high efficiency.

24/8 Spread 150μl of above absorption on fresh amp plate. - hundreds
Also 150μl of bugs-only control. - 0
Also streak 40 colonies from 22/8 plating
- all grew.

OVER

So high titre not due to plates being off. Maybe O.K.
Grow some of streaked colonies for f/p analysis.
(see clone log.)

(f/ps look O.K.)

Plating of bank for dishing (~2000 clones?)

10 µl package
0.1 ml λ dil
0.2 ml o/n 1046

20' 37°

0.5 ml CY

30' 37°

8 × 50 µl plated.

1500

~300/plate

Dishing: ~0.17 ml 2×TY/75 µg/ml amp dispensed into 96-well corning 25860 flat-bottom plates from 2 ml syringe in Skatron AS dispenser. Yellow tip with end cut off on syringe. In hood.
Colonies toothpicked into wells, plate at ~45° angle. Sticks per row left in place, stirred on removal.
37° 24 hr in slightly open plastic bag.
In hood, 1 drop glycerol/well (2 ml syringe, Skatron dispenser, set ~50).
Stirred with flamed hedgehog. (Wash in dish H_2O, then EtOH, then flame)

Freezing stack: 2 H_2O containing plates on top.
1 frozen plate at bottom.

Plating of clones for probing.

150 µl 20/8 R1 package.
0.4 ml λ dil
0.8 ml U.V. tested 1046 bugs o/n.

20' 37°
1.0 ml CY
30' 37°

6 x 0.375 ml plated on nylon membranes on 130 mm T-Y/amp plates.

15 hr 37°

Also, 25 µl of absorption plated on standard amp plate.
= 550 colonies.

Should be ~ 8250 colonies/filter. (= R1 20/8 1-6)

JES made nitrocellulose copies.

10/10/85.

Plating of cosmids from 28/7/85 LORIST6/Mbo stock.

50μl phage stock (should give ~ 500 colonies if no reduction in viability).

0.1ml λ dil
0.2ml o/N GD8767.

20' 37°

0.5ml CY

30' 37°.

5×150μl plated (KAN/TYE plates).

20 hr. 37°.

~ 5 × 70 colonies == 350 total.

Streak ~ 200 for growth & f.p.

24/10.
Further Plating of cosmids from 28/7/85 LORIST/Mbo. stock.

25μl phage stock.
50μl λdil
100μl o/N GD8767

20' 37°
0.25 ml CY

DNA SEQUENCE
VERSION 1.01

SEQUENCE NAME: SY-89
SEQUENCE LENGTH: 15
DATE: Sep 30, 1985
TIME: 9:25
COMMENT: COULSON.PBR322

5'- GAT AAG CTT GTC AAC -3'

↑ 150 µl H_2O

Spin out cnid.

~ 2/3 A_{260} / base coupling.

40 OD ≡ 2 mg

Wallace et. al.

0.3 pmol DNA (≈ 1μg)
in 10μl 60mM NaCl
50mM Tris-Cl 7.5
7mM $Mg(OAc)_2$
7mM DTT

7u Bam (or Pst)
30' 37°

+ 6 pmol primer (≈ 50ng)
100° 3'
Ice water
Sequence

I would like one xerox copy of:-

Journal ______________________________

Vol. __________ pages ______________ year __________

Title ______________________________

Author(s) ______________________________

Size __________ (if suitable for reduction).

I confirm that the material is required solely for research or private study.

[illegible]nature ______________________________

Date ______________________

Room no. ______________________

21/10/85.

Concⁿ pBR322
100µl pBR322 50µg/ml (rather old stuff)
10µl 3M NaAc
250µl EtOH
O/N -20°
EtOH wash, dry etc
+ 10µl TE (≃ 500µg/ml).

Test of SY-89 pBR322 counter clockwise 15-mer
Delivered as ~2mg in 150µl H_2O. Crud spun out.
Otherwise unpurified. (Chen & Seeburg protocol)

a) 2.5µl (~1µg) pBR322
15.5 H_2O
1 5mM EDTA
1 4N NaOH

b) 2.5µl (~1µg) pJD8 (?es)
15.5 H_2O
1 5mM EDTA
1 4N NaOH

c) 5µl C48A1
13 H_2O
1 5mM EDTA
1 4N NaOH.

5' r.t.
+ 30µl 1.5M NaAc pH 4.5
150µl 95% EtOH.
15', dry ice/ethanol
7' spin; wash; dry; + 25µl TE/10 (just prior to aliquoting for sequencing)

a) 2µl template
1µl ng/µl primer (stock/10,000)
1 TM
14 H_2O

b) as a.

c) 12.5 template
1 primer
1 TM
4 H_2O.

15' 45°

4µl / sequencing reaction
(Sequencing mixes a bit old.
#20 klenow (5u/µl) 1µl / 8 reactions. ³²P)

6% gel; undeionised mix.

Further tests of SY-89 by Ish-Horowitz method

d) 2 µl pBR322 (~1 µg)
1 µl 10 x H in
7 H_2O
1 50 ng/µl SY-89

e) 2 µl p538 (~1 µg)
1 10 x H
7 H_2O
1 50 ng/µl SY-89

f) 5 µl C48A1
1 10 x H
4 H_2O
1 50 ng/µl SY-89

Sealed in cap.
3' 100°C H_2O bath
Straight into ice / H_2O; left till aliquoted for sequencing

2 µl aliquots for sequencing as a-c.

(Ish says this works on CsCl purified plasmids.
C48A1 as used above is straight from standard minipred i.e. not LiCl pptd or CsCl purified.
Iva says she purified primers by dilution (after spinning dirt, crud) and passing through a NACS column.)

- Awful - mainly streaks - some bands visible amongst muck of d and a few bands in a narrow region of f. No bands in a-c.

(Test primer on linearised pBR322?
- Try protocol of Wallace et al Gene 16, 21, 1981
0.3 pmol DNA (≈ 1 µg)
in 10 µl 60 mM NaCl
50 mM Tris-Cl 7.5
7 mM Mg (OAc)$_2$
7 mM DTT
7 u BamHI (or Pst?)
30' 37°
+ 6 pmol primer (≈ 50 ng)
100° 3'
Ice water
Sequence

Also try a (above) using more primer.)

SY89 is wrong sequence! (Taken from BioLab catalog)
- Order new 20-mer GATGATAAGCTGTCAAACAT

4/10/85

Nick translation

	μl	
Water	13	
2×NT buff	20	
GTC (1mM each)	1	← cold triphosphates
Probe DNA	3	← ~0.3 μg?
^{32}P - 800	2	
DNase 5×10^{-6} mg/ml	0.5	← start with 5mg/ml glycerinated frozen
Pol I	0.8	1μl → 100μl ← DNase
		1μl → 100μl ← dilution buffer

15° 1-2 hrs

Water	50
0.1M EDTA	10
SS DNA (10mg/ml)	10

G50 spin column

This gives plenty of probe for e.g. the entire gridded bank.

DNase buffer: 10mM NaCl
10mM MgCl$_2$
10mM Tris-Cl 7.4
100mg/ml gelatin

(0.5% gelatin = 5mg/ml)
+ 50% glycerol for DNase stock

Hybridisation

I don't filter dextran solution now, but stir initial solution well for 1-2 hrs.

6X
30g
90ml
9ml
48ml

20x SCP 15ml
+ NL30 1.5ml
+ water 8g

dextran sulphate 5g (added last) → suddenly start on stirrer at full speed.

Best to have plenty of volume in bag, but for cosmids have bag not much bigger than filters. One bag for 6 filters is O.K.

No need to discard any cold solution.

In plastic, capped, 50 ml tubes

Tube A	ml		Tube B	ml	
Water	12	9	Water	12	9
SSDNA	0·1	75 μl	SSDNA	0·1	75 μl
			✓ + probe		

Heat in boiling water, say 10 min.
Cool in ice.

Tube A + ~ equal volume of (SCP, N(30, dextran)
Mix, add to bag, let filters absorb liquid (be gentle!)
Discard some of liquid.

[Can add half volume to see how wetting goes at first].

Tube B + ~ equal volume (———)
Mix, add to bag, seal.
~~Rock~~
Rock 65-68° O/N.

Washing

N.B. Cosmid colonies can be knocked off at any stage.

I mostly wash with ~1 lit ~1 x SCP +1% SDS, heated to 50° and allowed to cool during wash.
With big nylons, I end up with ~ 4 rather minimal washes. Vigorous rocking seems to be unnecessary, and will displace colonies.
Final quick rinse (pass filter through with forceps) in ~3 mM tris 8 ~~[illegible]~~
Dry filter thoroughly in air, expose (colonies are sticky!).

Minimalist hybridization/washing protocol.

Mix: 120 ml 20×SCP
12 ml NL30
64 ml H_2O
40 g dextran sulphate
(Tip dextran sulphate into rapidly stirring solution or it will clump).

20×SCP: 116.88 g NaCl
214 g $Na_2HPO_4 \cdot 12H_2O$
7.44 g EDTA
HCl → pH 6.2. (~15 ml conc HCl)

For 3 filters in a bag:
5 ml H_2O
~~4~~0 µl 10 mg/ml sonicated salmon sperm DNA (see Maniatis)
100°C water bath 10'
Cool in ice
Add 5 ml above mix (dextran)
Add to filters in bag (to wet; no actual prehybridization)

Probe: ~~5 ml~~ 4 ml H_2O
30 µl SSSDNA
probe
100°C 10'
Cool in ice
Add 4 ml mix (dextran)
Add to bag
Seal
O/N ~68°C. (we rock v. gently)

OVER

Washing.

We generally wash in 4 x 500ml 1 x SCP, 1% SDS heated to 50°C and allowed to cool during the wash (~30'). Rock gently occaisionally.

Finally, pass the filters through 3MM Tris pH8.

Dry (in air) ~ 3 hours. +.

We put them in wallets of tissue paper taped to 3MM sheets for exposure.

ie. literally pull through Tris with forceps – no soaking.

I. Greenwald from Kim Nasmyth

Probe

Nick translation mix. (25λ)

Extract 1x with ØOH/$CHCl_3$

Then 1x spin dialysis after adding 1/40 vol of stop mix

5% SDS
0.25% BpB = stop mix
0.25M EDTA

55λ DW
10λ 0.1M EDTA
10λ 10mg/ml carrier DNA

Σ 8.7 ml / 100 cm^2.

Filters: I use Sartorius nitrocellulose with which I have no difficulty in "wetting" blots.

Hybridization

Prepare two tubes (± probe) as follows:

→0.46 vols. of gDw
→0.0046 vols. of 10 mg/ml Sp DNA (± probe)

heat 100°C. for 10'

then rapid chill on ice.

Add 0.54 vols. of Scp/Sarc/DS Mix.

20 x Scp = 2 M NaCl
0.6 M Na_2HPO_4
0.02 M EDTA
pH → 6.2 with Hcl.
sterilize by autoclaving

Scp/Sarc/DS
= 30 ml 20 x Scp
4 ml 25% N-lauroyl sarcosine
20 ml 50% Dextran sulphate
Mix and filter through millipore.

Mix:

Add. (-) probe solution to filter(s) in bag.
Wet filter thoroughly, then squeeze out excess liquid.
Then add (+) probe solution and seal the bag avoiding air bubbles.
Incubate at 65°C. gently rocking.

Washing:

Prepare 1-2 L of 2x Scp. 1% SDS
Heat to 50°C.

Remove filter from bag into some of this solution in a glass casserole dish.
Rinse briefly with 2 changes, then rinse more thoroughly 2-3x for > 10'.
On adding each batch of fresh solution, its temperature should be 50°C, but let it cool in dish while shaking. Note, this is not a stringent wash.
Finally rinse 1-2 x with 3 mm Tris Hcl 8.0 at r.t. (>10'). I usually rinse the filter briefly 2-3 x then leave shaking in more liquid for ≥ 10'.

Dry the filter thoroughly before covering in saran wrap and exposing to film using a cronex lightning plus screen $\overline{M}$ -70°C.

Hybridization (JH)

(1) Prepare two tubes, each with
11.5 ml dDW
115 λ 10 mg/ml carrier DNA
use orange-top plastic tube for one + glass for the other

(2) Add probe to plastic tube

(3) Put both tubes in boiling water bath for 10'. Then put on ice.

(4) When cool, add 13.5 ml SSDM to each (see Xerox for recipe)
Mix with 25 ml pipet

(5) Put nitrocellulose filter into poly bag, rinse with cold soln.
Should seal side edge first (side)

(6) Remove cold soln. + add hot soln. Try not to get air bubbles.

(7) Put sealed poly bag into another bag w/ wet paper towel +
seal.

(8) Hybridize at 67°

~~M~~ IT High Hopper Uncs

Unc-49

Unc-63

Unc-58 recessive

Unc-35 (?)

at least 2 more,

1 maternal effect of*

Unc-9 (Gian Garriga)

Biodyne (also Kodak)

Autoclave on 3MM

Temp up to 121°

~~then~~ immediately slow exhaust.

(then baked as well).

soaked for luck

colonies rubbed off with glove. ~~(β-lactamase inhibitor~~

~~sulbactam added to growth plates with ampicillin @ 5 μg/ml (50 ng/ml) Pfizer, Inc, Groton, Conn~~

Must use for liquid culture
10-1000 × yield

p 6

Problem may be that extra high nucleotide concns needed.

---

washing — 90% det SDS worked fairly well after UV

Mike: OH gave high bgnd

Victor is making jumping library

~~Sulbactam: filter sterilize~~

4/11/85.

Nick translation of ZL53 (sup. 5) for probing of R1 bank random filters.

	μl	
H_2O	13	
2×NT buffer	20	
ZL53/3 sup	3	← 0.3μg?
dATP^{32}P 800Ci/mmol	2	
GTC (1mM)	1	
DNase 5×10^{-4} mg/ml	0.5	← 5mg/ml /100/100 in dil^n buffer
Pol I	0.8	

15° 1.5 hr.

H_2O	50
0.1M EDTA	10
SS DNA (10mg/ml)	10

G-50 spin column. (~1ml G-50 in holed Epp. over glass beads; Spin out TE. Replace bottom tube Add reac^n Spin ~1' 1200 rpm)

Filters from sup 5 (R1 20/8 1C11 – 6C11)

Hybridisation as 4/10/85 Nick transl^n protocol except ½ vol solns used. (Filters sealed in bag with ~1cm margins).
o/N 68°C

4×10' 500ml 1×SCP/1%SDS 50°C → r.t
No agitation.
Final wash 1X pass through 3mM Tris-Cl pH8
Air dried.

	+	++	+++	
1	5	7	39	51
2	2	4	12	18
3	2	5	14	21
4	2	5	19	26
5	1	2	11	14
6	4	6	21	31.

24hr exposure showed +'ves and many bkgnd. colonies.
72hr better for bkgrnd ident.

Colonies picked from masters

– daughter filters pricked through film with hypodermic needle into centers of strong 4' iv's.

at 4°C, visual identification of master filter colonies by reference to mirror image daughters?

Colonies streaked ; o/N 37°c. Amp plate

Single colonies (1/streak) subbed onto amp plate streak.
o/N 37°

Filter 1 1-6 (1 & 4 didn't grow good streaks)
2 1-3
3 1-2
4 1-2
5 1-3

= ZC1-16.

5/10/85

Further ligation for packaging of Rc2/pUC8 arms

As 13/2/85

Packaging as 20/8 (cold room)

+ 500 μl λ dil, $CHCl_3$ etc.

Assay.

0.1 ml λ dil — Also no phage control.
10 μl package
0.2 ml O/N 1046
20' absorb.
0.5 ml CY
30' 37°

Plate 150 μl
50 μl — 118
10 μl. — 22

≃ 100,000 total

50 000 sent to Phil Anderson.

~~150 μl package~~
~~0.4 ml λ dil~~
~~0.8 ml 1046~~
~~20' 37°~~
~~1.0 ml CY~~
~~30'~~
~~Plate 6 x 0.375 on millipore. BA85~~

13/11/85
Gel purification of 1/2 of SY-123. (——————→)

12% acryl: bis 19:1 TBE 20 x 40 x 1.5 mm gel.
(not deionised).

200 ml mix
200 μl TEMED
1.75 ml 10% Amps

30 μl SY-123 (0.15 mg?)
(smelt strongly of thiophenol.
Also v. cruddy; could spin out)

+ 10 μl sucrose / dye

Loaded on 1 2.5 cm slot.

300 V 30 mA 10.30 → 1.30

U.V. shadowing (gel transferred to Saranwrap
Place on Kodak intensifying screen)
Hand held U.V.)

Strong band just ahead of BPB.

Cut out; + 1 ml M-G gel elution buffer.
37° o/N [illegible]

6 OD ~~total~~ after elⁿ.

= 120 μg

Balls up! Added iso-propanol to precipitate
oligo, but probably didn't
Dried down in rotavac (2 x 1.5 Epp tubes)
to ~100 μl
+H2O → 1 ml.
SEP-PAK as next page

SEP-PAK columns for oligo purification.

For 17-mer, use C18 cartridge.

Pass 10ml HPLC grade acetonitrile.
- 10 ml sterile H_2O
- load sample (max 5 A_{260} units) in 1 ml aqueous soln. (Load v. slowly from syringe)
- wash column ~5 ml dist H_2O - collect effl.
- elute with 60% Methanol/H_2O - 3ml
- collect 1 ml fractions - check OD_{260}

16/11/85

$O.D.^{260}$ SY-123 SEPPACK fractions (1ml +)

LOAD		0.06	
WASH	1	0.09	
"	2	0.05	
"	3	0.03	
"	4	0.015	
ELUT^n	1	2.86	≃ 50μg/ml
	2	0.65	≃ 13μg/ml
	3	0.05	
	4	0.04	

(Gel purification probably unnecessary anyway - SEPPAK column sufficient.)

18/11/85.

Test of SY123 pBR primer.

a) 0.25 µl (0.5 µg) pBR322
18 H_2O
1 4N NaOH
1 5mM EDTA

b) 1 µl pS38 (0.5 µg)
17 H_2O
1 NaOH
1 EDTA

c) 5 C48A1 cos
13 H_2O
1 EDTA
1 NaOH

5' r.t.

+ 30 1.5M NaAc pH 4.8
150 95% EtOH
dry ice/EtOH 10'
Spin 8', wash 95% EtOH, dry.

+ 5 TE/10 ; remove ½ (= a1 - c1)

+ 10 TE/10 to remainder (2 µl of this = a2 - c2)

a1-c1 2.5 template
1 50 ng/µl SY123
1 TM
14 H_2O

a2-c2 2 template
10 1 ng/µl SY123
1 TM
5 H_2O

45° 15'.

4 µl / seq. reaction

– overdid Klenow by 5x (?).

pBR & pS38 ½-template reactions gave rather crumby but recognisable sequence on o/N exposure. (T tracks better than others - would be pretty good sequence if all tracks as good as T's).
C48A1 cosmid gave primarily non-specific streaks with a few bands near top.
'1/10-template' reac^ns gave similar but proportionately weaker results.

20/11/85
Attempt to make primed probe on untreated miniprep of cosmid C4834

5 µl cos C4834 (ep7)
13 H_2O
1 G0.2A
1 NaOH

5' r.t.

30 1.5 M NaAc
150 95% EtOH
Dry ice / [illegible] 10'

Spin wash dry

+ 10 µl TE/10

a) 9 µl temp
2 µl 50 ng/µl 5'/123
1 TM
6 H_2O

15' 45°

b) 1 template
~~1 µl 10 ng/µl 5'/123~~
~~1 TM~~ 1 ~~2~~ TM/5
~~16 H_2O~~

Mix 10 dATP* 400 Ci/mmol
20 ddT mix
0.8 ~~25~~ 5 u/µl klenow #20

a) + 18 A*/T mix.
15' rt
(No chase)

b). + 2 A*/T mix
15' rt

[Take 3.5] a] for 6% gel.
all] b

+ H_2O 50
·1 M EDTA 10
SS DNA 10

Spin column.

Gave uniform streak in b and much weaker streak in a — i.e. reverse of what might be expected — did I get samples muddled? Repeat.

OVER

Some apparent +'ives after o/n -70° + screen
but these mostly turned out to be from known
repetitive sequence (i.e. heavy band) clones.

[illegible]

19/11/85.
Various conditions to try and obtain recognisable sequence (i.e. specific priming) from cosmids.

Preliminary:

a) C48A6 and C48B1 lithium chloride pptd.

minipreps + 20 TE (→ 50 μl)
+ 50 5M LiCl
-20° 5'
3' spin
+ 5 μl 5M NaCl to super
480 μl EtOH (mistake)
Spin 7'
95% EtOH washed; dry; + 25 TE

(5 μl C48D1 kept before LiCl, 4 μl for reac^n, 1 μl for agarose gel)

b) RNase of C48A1.

5 μl C48A1 miniprep
10 H_2O
1.5 10×H
0.5 RNase 10 mg/ml
15' 37°

a	b	c	d	e	f
2.5 C12E8 CsCl (Rita) 120 γ/ml	5 B020B CsCl (Rita) 80 γ/ml	4 C48B1 No LiCl	5 C48D1 LiCl	5 C48A6 LiCl	16 C48A1 RNase).
15.5 H_2O	13 H_2O	14 H_2O	13 H_2O	13 H_2O	3 H_2O
1 5mM EDTA	1 EDTA	1 EDTA	1 EDTA	1 EDTA	1 EDTA
1 4N NaOH	1 NaOH	1 NaOH	1 NaOH	1 NaOH	1 NaOH

5' r.t.

+ 30 1.5M NaAc
150 95% EtOH
Dry ice / ethOH 10'
Spin; wash 95%; dry.

+ 5 TE/10

over

2.5 template
1 50ng/μl M13O SY123 } 15' 45°
1 TM
14 H_2O

ddT mixes: 1) 6 dATP* dry
12 ddT mix
3 5u/μl klenow
(as 18/11)

2) 6 dATP* dry
12 ddT mix
0.5 5u/μl klenow
(as should be)

2 μl template/primer
2 μl ddT mix/pol
15' r.t.
2 μl 4x dNTP·5mM chase
15' r.t.
6 μl formamide/dyes.
3' boil
6% gel

0.4% agarose gel of templates:

1 λ C1278 CsCl
1 λ B2028 CsCl
" C4881
" C4881 LiCl
" C4876 "
" C4881
1 λ 100/λ H3

20/11/85

Pst cleavage of cosmids prior to 'Kwofmj-type annealing & extension of SY123

a) 5 μl CsCl CRE8 (~0.5 μg) 2 10xH 12 H_2O 1 Pst sample

b) 8 μl CsCl 60408 (~0.5 μg) 2 10xH 9 H_2O 1 Pst

c) 5 C4 8B1 (Li) 2 10xH 12 H_2O 1 Pst 1 RNase/10

60' 37°

(−20° ; Ag. gel sample
If ok, pol, EtOH, dry
+ 10 H_2O
x primer — various primer conc.?
100° 3' → dry ice/ethanol
→ r.t
+ 1 10xH
r.t 20'
dNT.

2 μl (1/10) for 0.4% agarose gel.

Digests look ok, except CsCl purified samples look 'streaky' (R.W. also noted this — protein in samples?)
1x pol.
EtOH, wash, dry + 10 μl H_2O

1	2.5a,	1 SY123	25 ng/μl,	0.5 H_2O
2	"	1	" 2.5 ng/μl	"
3	"	—		1.5 "
4	2.5b	1 SY123	25 ng/μl	0.5 "
5	"	1	" 2.5 "	"
6	"	—		1.5 "
7	2.5c	1 SY123	25 ng/μl	0.5 "
8	"	1	" 2.5 "	"
9	"	—		1.5

100° 3'
dry ice/etoH 1'
0.5 10xR (100 mM Tris pH 8.0, 100 mM $MgCl_2$)
(by mistake, add 1 μl 10xR to 1-5)
50° 15'

OVER

2 μl annealing
+ 2 μl ddT mix / A* / Klenow

15' r.t , stop

Mix 12 dATP* dry
24 T mix
1 5u/μl Klenow

10 2 μl #7 annealing , + 2 μl mix , 15 r.t then 10' 50°
11 2 μl #8 " " "

~1/3 on 6% gel.

26/11/85

'Kwo Fung' type annealing of 5Y123 to uncut cosmid DNA. c.f. 20/11 pst cut DNA.

C48E2 and C48E5 RNased

5 µl cos miniprep
1 µl RNase/10 (mg/ml)
12 µl H_2O
2 µl 10×H

60' 37°

phenol / EtOH / wash dry +10 µl H_2O

5 µl C12E8 (Rita, (yCl prep) (~0.5 µg)
phenoled, EtOH, wash, dry; +10 H_2O

1) 2.5 C12E8, 1 5Y123 2.5 ng/µl, 0.5 H_2O
2) " " 1.5 "
3) " C48E2, 1 5Y123 2.5 ng/µl, 0.5 "
4) " " 1.5 "
5) " C48E5 1 5Y123 2.5 ng/µl, 0.5 H_2O
6) " " 1.5 H_2O

3' 100° (capillary)
1' dry ice / EtOH

? buggered up
pos. also 3.
(water dropped in tube)

+0.5 TM
30' 55°

+Tris / A* / klenow

20' r.t.; 4% gel after usual denaturation.

14/11/85

Nick translation for probing of R1 20/8 1C11-6C11 by BO464 (1-3).

As 4/11 protocol, except 10 µl 300 Ci/mmol ^{32}P dATP (dry) instead of 2 µl 800 Ci/mol dATP.

20/8 1C11-6C11 reprobed without washing off ZL53/3 probing.

Bag sol^{ns} 3/4 x vol of protocol i.e.

	A	B
H_2O	9	9
SS DNA	75λ	75λ
		200λ probe

100° 10'
Ice 10'

DS/SCP/NC30 9.1 9.3.

This seems about right for 13 cm filters with small-margin bag.

1x SCP/SDS washes. 4 hr r.t. dry.

Looks pretty good. Hotter than ZL53 probe (2x?)
JES picked:
2 from 2C11
4 .. 4C11 = ZC 23-29
2 .. 5C11

Also 5 .. 6C11 ZL53 probing not picked previously
= ZC 17-22

Streaked.
Grown f/p'd.

21/11/85

Prep. nick. trans. probe from C33C3 (onc-86).
for probing R1 20/8 1C11-6C11.

As 14/11 B0464 prepn. i.e. 300 ci/mmol d ATP^{32}

Sounded pretty hot - about same as B0464

Good A/R o/N

Picked by transfering +'s to strong 'background' A/R (from B0464 probing) and aligning this film over master plate. 4°C.

A/R Master

	#
1 x	1
3 x	2
4 x	3
1 x	4
2 x	5
5 x	6

= CZ 30-45

Streaked, colonies streaked for o/N's.

18/11/85.

'Blueing' of filter-bound colonies for probing.

1046 streaked on TYE plates previously spread with 50μl each of BCIG & IPTG (cf. as used for M13 plaque analysis).

O/N growth - colonies not v. blue (and rather patchy - spreading with glass rod of BCIG & IPTG probably not best way).

After 2 days at 4°C, ~~[illegible]~~ some colonies v. blue.

Small region replicated onto a piece of PALL BIODYNE.

Colonies denatured (inc. SDS) as in CSH manual (method I).

Mock probing & washes as 4/10/85 protocol.

Blue coloration at end of procedure looks little diminished (if at all), but may have been some spreading away from colonies (esp. larger ones) during denaturation!

Problems: ~~[illegible]~~ Does 4°C 3 day storage lead to loss of or damage to cosmids?

Can 4°C storage be avoided?

More BCIG / IPTG? (expensive)

Incorpt " - into agar. (H tox?)

McKonkey?

Loss of colour on repeated probings?

2/12/85

Assay of 5/11/85 R1/pJB8 cosmid prep.

Assay as 5/11

50 μl 121
10 μl 19

R1 3/12 1-6

i.e. no change

Plating of master filters to give ~ 5000 colonies / 14 cm filter.

0.4 ml λ dil
150 μl 5/11/85 R1/pJB8 package
0.8 ml 1046 (O/N stored at 4°C)
20' 37°
1.0 ml CY

Spread 6 x 0.375 ml on sterile BA85 filters on TYE/75 μg/ml plates, with minimal spreading about (to prevent previous problem of high density of colonies near edges of filters.)

O/N 37° 5.30 → 9.00 - titre looks v. low.

After 20 hr, titre still v. low - could be due to deleterious effect of BA85 filters - try small scale assay on detergent-free filters v. BA85 v. nylon v. agar.

3/12/85

Prep of mixed probes from unattached clones.

f/p in situ: +++ above av.
++ average
+ below av.

	B0001	+ 5 μl TE	+++
	B0019		+
	41		++
	47		++
✓	257		++
	264		++
✓	274		++
✓	294		++
	310		++
	353		++

5 μl of each, mixed (= MP1)

Also, preps of probes from C49E1 (LIN12) R1

MFL3317 (λ2001) (UAC86) Xba

a) 50 μl MP1
6 10x R1 buffer
0.5 10 mg/ml RNase
2 μl EcoR1 20u/μl

b) 2 μl pJB8 600 ng/ml
1 10x R1 buffer
6.5 H₂O
0.5 EcoR1 20u/μl

c) 10 μl C49E1 (0.5-1 μg?)
2 μl 10x R1 buffer
1 μl 10 mg/ml RNase/10
6.5 μl H₂O

d) 10 μl MFL3317 (1 μg?)
2 μl 10x R1 buffer
1 μl Xba 10u/μl (9.w.)
7 μl H₂O

60' 37°; 1 μl of each for 0.6% HGT minigel.

λH3 (0.5 μg)

Xba digest of MKC3317 no good - abandoned.
Others look OK.

MP1 + 6μl 3M NaAc
150μl EtOH
Spin, wash, dry;

+ 20 TE

~~0.5%~~ 0.6% LGT / 200ml TBE   1cm slots

track
a) All MP1 R1 + 10μl dyes
b) 1μl p538/R1, + 19 H_2O + 10 dyes
c) All C49E1 R1, + 10μl dyes
d) 2μl λ Hind III 0.3μg/μl. + 18 ddH_2O + 1 dyes.

13 hr 25 mA

50μl 10mg/ml EthBr / 150ml TBE
30' stain

MP1 p538 C49E1 λHindIII

1, 2
3
4

Indicated bands cut out.
MP1 block lost (used phenol-sensitive tubes - should use polyprop.)

C49E1 1+2, 3, 4
2x phenol after 10' 70°C
(in Eppendorf tubes).
EtOH ppt, wash, dry
+ 10μl TE.

C49E1 / 1,2

Probe prepared as B0464 (14/11/85)
5µl (1/2 band prep) labelled.
Volumes in bag also as "

RI 20/8 1C11 - 6C11 probed.

(Rather a lot of air in bags)

No new +'s re. previous (C33C3) probing.
- a bit odd, since +'s prior to C33C3 had disappeared.

[Spin column seemed to indicate presence of labelled probe. Run samples of C49E1 bands on minigel, to assay for presence of DNA.
- probably should have added glycogen to EtOH pptn. (10µg).]

2µl of each (from 10µl). 0.5µl 38/λ λHind III

1,2 3 4 λH3

~~1,2 ≈ 0.03µg~~
1,2 probably 0.03µg total

8/12
Xho I digest of MFL3316 & MFL3317
(To prepare probe from 3317 vrc 86)

10 MFL3316 / 7
2 µl 10x R1 buffer
1 µl Xho 17 u/µl (rat)
7 µl H_2O

60' 37°

Repeat of mixed probe prep as 3/12/85
- same mixture of unattached clones = MP2

MFL3316 Xho
MFL3317 Xho
MP2 R1

MP2 R1 looks OK.
but MFL's look underdigested.

+ 2 µl Xho
2 hr 37°.

- Looks better.

0.6% LGT 250 ml TBE gel, 1cm slots
15 hr 27.5 mA;
50 µl 10 mg/ml EthBr / 150 ml TBE
30' stain

MP2
MFL3317 Xho

MP2
1
2

Bands eluted as shown
ppt. + 10 µg glycogen
iso-propanol
EtOH wash.

+ 10 µl TE final.

MP2 results.

18 +'s analysed.
5 new contigs
1 extensⁿ (correction).
1 con-con join
1 no match
3 probes hit — B0274 x3
B0257 x2
B0294 x3

4 hits on R01E12 (internals) (but no match to probe)

MP3 results.

9/10 hits (not C01B10) from 27 clones

Also, from 12 other clones analysed,: 8 int (1x2)
2 ext
1 n m.

MP3

B0363 lit (but actually was internal)
365 no lit con-con join
394 lit con-con join
524 lit but not extended
574 lit cos-con join + exten.
C01A1 lit cos-con join
C01B6 lit con-con join
C01G1 lit
C01H4 lit
C01B10 no lit.

9/10 lit. by 23 clones ;
16/39

~~2C114 (,110) int cont 541~~
~~2C116 lit~~
~~2C117 lit~~
~~2C118, 126, 156 int. cont 364~~
~~2C149 lit~~

114 int 541
124, 150 int 81
131 int 451
135 ext (?) 478
137 int 9
138 int 516

140 int 79
141 n.m.
144 ext 842
153 int 633
154 int 150.

2C
127
136
151
121
118 146
126
156
139 132
134 147
142 111
152 125
143
128
117
145
129
155
148
130
147
116
123
21

MP4

ZC157 604 hits (but probe supposedly omitted)
CO294
190
178
170
180
175
176
181
196
169

ZC160 49 - hit - should have been picked up.
CO262.
186
164
177
168
193

ZC162 452 int

ZC166 864 int

ZC 167 / 185 new contig - hit CO296

ZC171 80 int } no obvious probe match
172 " "

ZC174 160 int
ZC 186 49 int
ZC194 254 int
ZC195 100 int.
ZC197 cos-cm join (352) but should have been picked up. (bad dig.)
ZC 201 47 int
not from probe?

---

overall - only 3 probes hit (18x) - 1 should have been picked up.
1 probe should have been omitted (deleted)

Of rest, 9 ints (1x2), 1 cos-cm join.

```
TSLIST  4  2  0  0  3


MINSCO,DB,OFFSET -194    15    10
   -78   -78 -129 -129 -178 -178   -47   -31 -460 -486
  -405  -405 -213 -448 -194     0     0     0    0    0
TSLIST  4  2  3  0  1


MINSCO,DB,OFFSET-1036    16     1
   -78   -78 -129 -129 -178 -178   -47   -31 -460 -486
  -405  -405 -213 -448 -194-1036  -500  -488 -194    0
TSLIST  0  2  3  1  4

MESH ORDER        4    2    3    1
FINAL SCORES-1036 -500 -488 -194 TOTAL-2218
FINAL POS     1    1   -4    0 RMS     2.1

LANE OFFSETS     -6      5    -5      4

LANE OFFSETS     -6      4    -1      3

MINSCO,DB,OFFSET-1019    16    -1
   -78   -78 -129 -129 -178 -178   -47   -31 -460 -486
  -405  -405 -213 -448 -194-1019  -539  -473 -266    0
TSLIST  0  2  3  1  4

MESH ORDER        4    2    3    1
FINAL SCORES-1019 -539 -473 -266 TOTAL-2297
FINAL POS    -1    1    0    1 RMS     0.9

LANE OFFSETS     -6      4    -1      3

LANE OFFSETS     -7      3    -1      4

MINSCO,DB,OFFSET-1004    16     1
   -78   -78 -129 -129 -178 -178   -47   -31 -460 -486
  -405  -405 -213 -448 -194-1004  -518  -499 -268    0
TSLIST  0  2  3  1  4

MESH ORDER        4    2    3    1
FINAL SCORES-1004 -518 -499 -268 TOTAL-2289
FINAL POS     1    0    0   -1 RMS     0.7

LANE OFFSETS     -7      3    -1      4

LANE OFFSETS     -6      4    -1      3

     66     56  -9870.000          54
  ZONEA,B,P       9    10       8

   ZA    B    WMINK WMAX  FITTOL
```

C1022

MP7 analysis

6 hits

C09C9	749	X8	new contig
C09A2	551	X11	con-con join
C07E3	844	X 1	new contig
C07G2	304	X3	con-cos join
C08D8	294	X3.	contig extens.
C08E3	241	X1	new contig

Rest — B.4.m.
1 new contig.
inits.

MP8 analysis

5 hits

C10F9	618	X13	cos-con join
C12C10	877	X5	new contig
C13F10	68	X2	cos-con join
C16C3	651	X5	cos-con join
C16F2	472	X3	con-con join

MP5 analysis 1 hit! C03F7 x 7 cont 290.

Also cont 511 x5 no hit
" 32 x2 " " (new contig)

MP6 analysis 2 hits C06D1 X1
C06H10 X1

Also C30B3 etc (235) x 10 (not hit).

MP9-12 analysis

9 hits: C32A12 x9 — C24F7 x1
C33F6 x 5 — C29B12 x1
C18G2 x 7 — C33C12 x 2
C30E7 x 3
C25H7 x4
C30G8 x1

over

MP13

C35C9 x1
C37A3 x11
C37B6 x3

1600/5μl

320/μl

16

6

~~800~~/5μl

160

4000
/ 126000

9/12

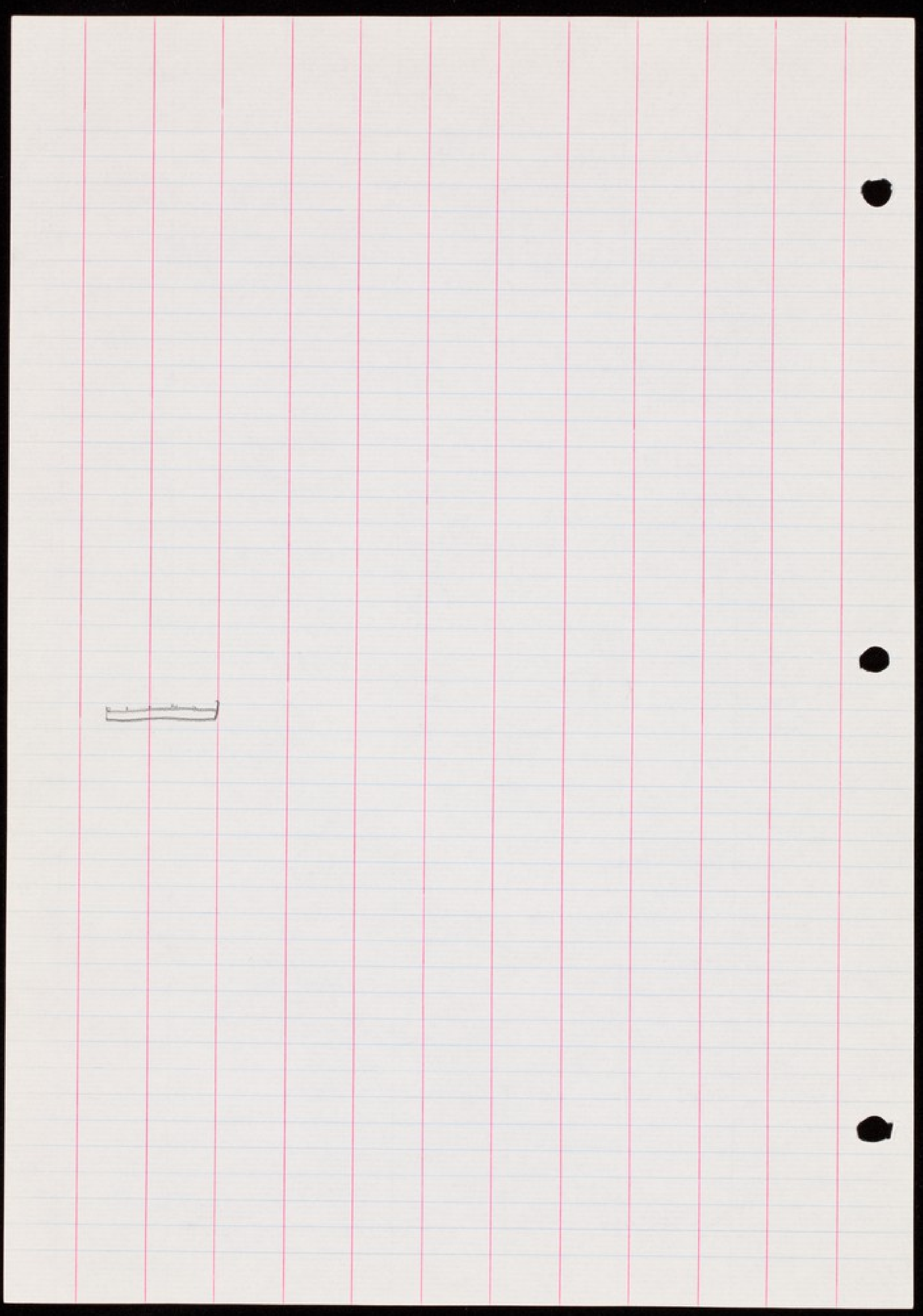
R1 back assays on various surfaces.

a) 5 μl 5/11 R1/pSS8 library
0.1 ml λ dil
0.2 ml O/N 1046
20' 37°
0.5 ml L-Y
30' 37°

b) 5 μl 21/8 R1 library
etc

		colonies
1)	50 μl a) on 75 μg/ml amp plate	85
2)	50 μl a) on (unsterilised) BA85 on amp plate	29
3)	50 μl a) on HATF " " "	15
4)	50 μl a) on Biodyne A (Pall) " "	41
5)	50 μl b) on amp plate	118
6)	50 μl b) on (unsterilised) BA85 " "	42
7)	50 μl b) on HATF " "	44
8)	0.	0

Reduction in viability on BA85 and HATF = ~2/3
" " " on Biodyne A = ~50%.



11/12/85

More plating of N2 R1 (21/8) library for masters.

Because filters lead to reduced viability, decided to plate on agar and transfer colonies.

Aiming for 6000 colonies / 13 cm plate (T-Y/E/amp 75)

Based on 9/12 assay:

0.4 ml λ dil
80 μl 21/8 library
0.8 ml 1046 (grown 9 hrs from 1/100 dil^n).
20' 37°
1.0 ml CY
30' 37°.

6 x 0.375 ml, minimal spreading.

13 hr 37°

Look O.K.

2 hr 4°C

SCS ~~[illegible]~~ did pick-up onto BA85

midline marked

N2 R1 11/12 1-6

Filter left in contact with colonies for ~2'
Peeled off and placed on fresh plate (colonies up).
37° 2 hr.
2^nary agar plates should probably have been incubated at 37° for a while prior to transfer, because 2 filters (6 v. bad and 1 less bad) became v. wet.
Removed from incubator; r.t. with lids ~~open~~ ajar ~60'

John made 2 slaves sets on nylons.

13/12/85

Probing of RI 20/8 K11 - 6C11 with MP2 (8/12)

N.Trans of MP2

H₂O	26	
2×NT	40	
MP2	5	(½)
dATP³²	20	(dry)
GTC	2	
DNase/100/100	1	
Pol I	1·6	

15° 1·5 hr

0.1M EDTA	20
SS DNA	10.

Then as 14/11 probing. 65°

15/12

- Difficult to distinguish +'s from background of previous probing; 18 +'s picked.

1×1
2×2
7×3
4×4
2×5
2×6

(Try washing background off filters & reprobing)

14/12.

Nick trans. of ~~[illegible]~~ MFL 3317/1 (8/12) ~~for~~
probing of R1 ~~20/8 1c11 – 6c11~~. 11/12 1/1 → 6/1

3μl (from 10) NT'd as 4/10 protocol (10μl $^{400 \, Ci/mM}$ [illegible])

15/12

NT material used to probe R1 11/12 1/1 → 6/1 (65°)

Probing spent some time (1 hr?) at
a temp. approaching 80°C (oven not equilib.)
then o/N 65°C.

16/12/85

Washing of RI 20/8 1C11-6C11 to reduce background.

As Maniatis p314

1-4 with pre-SDS ; 1&2 final wash in 3mM Tris
5, 6 no SDS.

After drying, decided to try different procedure (counts still detectable by monitor on filters, possibly less on 1 and 2 (which had Tris wash)

Boiled 10' in 10mM Tris pH8.0 500ml

(Steve Powell)

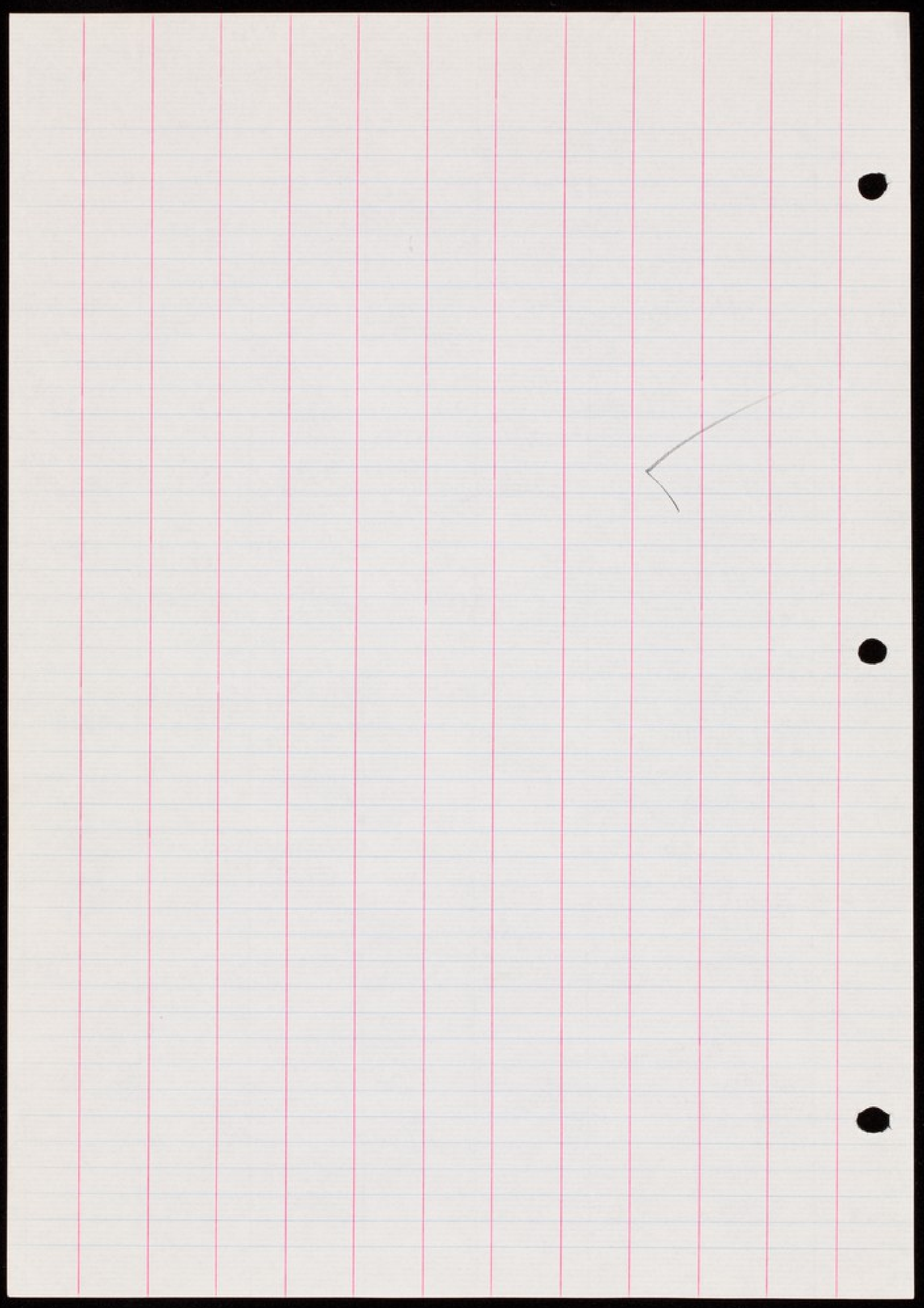
Counts not detectable by monitor.
(Reprobe these filters with c49E1 1,2).

16/12
NT of c49E1 (3/12) frags

H_2O	10
2×NT	20
c49E1 1,2	3
" 3	5
dA&G p32	10 (dry)
GTC	1
DNase/10K	0.5
Pol 1	0.8

90' 15°C.

All used to probe RI 20/8 1C11-6C11 boiled filters.



16/12 unattached clones.

BO 363
365
394
524
550
554

C01 A1
B6
G1
10 H4
B10
B11
G7

C02 A1
B5
E2
G4
G6
H3
20 E7
G10

C03 G1
F7
H12

D 1030
1033
1042
1063
1069

30 D 2028
2063
2078

C04 B3
F3

C05 D3
D6
G4
E9

C06 B2
40 D1
D2
E4
C10
E12
45 H10.

17/12/85

MP3

(Omit B0550, C0294).

	μl
B0363	7.5
365	7.5
394	5
524	5
554	5
C01A1	5
B6	7.5
G1	7.5
H4	5
B10	5
	60

MP4

C01B11	5
G7	5
C02A1	2.5
B5	10
E2	10
G6	5
H3	7.5
E7	5
G10	7.5
C03G1	5
	62.5

(Start next 16 at C03F7).

R1 digests:

MP3/4	60 μl
10x R1 Buff	7
10mg/ml RNase	0.5
EcoR1 20u/μl	2

120' ~~60'~~ 37°

Gel as MP2 8/12

After extraction (2x phol) + 20 TE.

MP3 pS38(2) MP4

MP3 MP4 λH3

1μl MP3, MP4
0.1μg λH3

20/12/85

NT of MP3 and 4
- as NT of MP2 NT 13/12/85

10μl (½) of each. (½ MPS buggered up, so none left)

To probe N2 R1 11/12 3, 4

(Monitoring of incorpn (across '6 side' of 4x6 rack)
MP3 180
MP4 200) O/N exposure (no flash, or screen etc)

22/12

46 colonies picked from MP4 +'s
- all +'s picked from filters 1-3.

23/12

53 colonies picked from MP3 +'s - filters 3-6

23/12

16 colonies picked from C49E1 probe (16/12/85) of washed
20/8 1911-6911 filters.

8/1/86

0.5% HGT TBE minigels of unattached clones

Eliminate: D1030
1033
C06B2
C09F11

Eliminate: C11H5
C14D1
C17E6
C18D2
C18D8
C23F11
C26G1

Eliminate: C33G5

Eliminate: C37D11
C38B1

unattached 7/1/86 (continued from 16/12; D1030 – C09H10 to be inc. in prelim. assay; C03F7 & C03H12 for inclus^n in probe).

C07E3
G2
G4

C08B3
E3
D8

C09A2
C12
E9
F11

C10D4
F9

C11H5

C12E2
C10

C13F7
F10

C14D1 ?

C15C6

C16B2
C3
F2
G5
G11

C17E6

C18A4
D2
G2
A9
D8

C23F11
H4

C24C2
B12
D10
F7
F8

C25H5
H7

C26B3
F6
G1
C9
D11

C27H2

C28A3
B4
E2
E10
G10

C29A8
B12
C10

C30F2
E7
G8

C31F5
F7
H7

C32A12
C12

C33E1
F6
G1
G5
A8

C34F1
A8
H11

C35D6
C7
F11

C36C9

C37A3
B6
C5
F3
G2
B8
D11
E10

C38B1
D7
D10
F11

C39A4
E3
F2
G3
E7
H7

C40D2
A10
B11
C7

8/1

C41E4
F6
B9
B10
D11

C42B1
E3
B10
E9
E11
H8

C43H2
A10
B9

C44B2
D2
D5
B9
B10
H12

C46B1
E1
A7
B8

Start next agarose gel analysis at C41E4!

Start MP15 at ~~C40A10~~ C40D2

[initials]

8/1/86

Mixed probe constructions

MP5	C03 F7	5	
	C03 H12	5	
	D1042	5	
	1063	3	
	1069	5	
	D2028	3	
	2063	7.5	
	2078	3	
	C04 B3	5	
	F3	7.5	
MP6	C05 D3	5	
	D6	5	
	G4	5	
	E9	5	
	C06 D1	5	
	D2	5	
	E4	5	
	C10	8	
	E12	8	
	H10	8	
MP7	C07 E3	5	
	G2	3	
	G4	5	
	C08 B3	5	
	E3	5	
	D8	5	
	C09 A2	3	
	C12	7.5	
	E9	5	
	C10 D4	7.5	

MP8	C10	F9	5
	C12	E2	5
		C10	3
	C13	F1	7.5
		F10	5
	C15	F6	7.5
	C16	B2	7.5
		C3	5
		F2	5
		G5	10
MP9	C16	G11	7.5
	18	A4	5
		G2	5
		A9	5
	C23	H4	5
	C24	C2	5
		B12	5
		D10	5
		F7	5
		F8	5
MP10	C25	H5	5
		H7	7.5
	C26	B3	5
		F6	5
		C9	5
		D11	5
	C27	H2	5
	C28	A3	2.5
		B4	3
		E2	5

OVER

MP11	C28	E10	5
		G10	7.5
	C29	A8	5
		B12	5
		C10	5
	C30	F2	5
		E7	3
		G8	5
	C31	F5	5
		F7	7.5
MP12	C31	H7	10
	C32	A12	3
		C12	5
	C33	E1	5
		F6	5
		G1	5
		A8	10
	C34	F1	5
	C34	A8	5
		H11	5
MP13	C35	D6	5
		C7	5
		F11	3
		C9	5
	C37	A3	5
		B6	5
		C5	5
		F3	5
		G2	5
		B8	5

	C37	E10	5
MP14	C38	D7	5
		D10	5
		F11	5
	C39	A4	5
		E3	5
		F2	5
		G3	5
		E7	5
		H7	10
	C40	D2	5

9/1/86

Preps. MP5 - 14

Mixed as 8/1

+ 6 µl 10 x R1 buff.
0.5 µl 10 mg/ml RNase

2 hr 37°. ; + 15 µl 5 x loading buffer

0.6% LGT 250 ml TBE gel.

16 hr 25 mA

50 µl Eth Br + 150 ml TBE
30' stain

pBSBR1 MP 5 6 7 8 9 10 11 12 13 14

2 x phenol extr^n of LGT.
Iso-prop. ppt^n.
+ 20 µl TE final.

11/1/86

Nick trans. of MP5-8

H_2O	16
2xNT	40
MP	10
dATP	20 (dry)
GTC	2
DNase/100/100	1
pol I	1.6

90' 15°

0.1mM EDTA	20
SS DNA	10

Spin column. (Counts (along long side of 6x4 rack:

5	350
6	400
7	350
8	400

)

N2 R1 11/12 1-4 filters boiled 10' 10mM Tris pH 8.0
Dried 2hr.
Then as 4/10 protocol, 2/3 vols.

14/1/86

50 +'s picked from MP8 filters 1-5.

50 +'s " " MP7 " 1-4

15/1

54 +'s " " MP5 " 1-5

50 " " " MP6 " 1-4

16/1/86

NT of MP 9-12 as 11/1/86

for probing of boiled N2 R1 11/12 1-4 filters

Incorp - seemed a bit low re. MP5-8 i.e. 150-300

Result: Not v. good - few obvious +'s; some possible +'s same as MP5-8 weak '+'s'.

12 +'s from MP9 (all 6 filters in each case)
9 " " MP10
17 + " MP11
28 + " MP12 (not too bad)

66 in total, picked as MP9-12.

21/1

Assay of 21/8 R1 library prior to large scale plating.

Assay as of 9/12/85

59 colonies i.e. down by 1/2 on 9/12 assay.

- Repeat.

Repeat = 126 colonies

23/1 plating.

As 11/12, except 100 μl 21/8 library.

Look good.

JCS did lifting & printing.

29/1/86.

Nick trans. of MP 13.
As 11/1/86.

For probing of 23/1 filters (new plating of 21/8 library - see 21/1. etc.)

(MP 13 looks rather low yield from 9/1 prep. gel)

3/2/86

Rewashing of 29/1 MP13 probing after A/R to try and improve +/bkgrnd ratio.

a) filters 1,2 1×SCP, 1% SDS 50° 30'
b) " 3,4 1×SCP, 1% SDS 65° 20'
c) " 5,6 0.3×SCP, 1% SDS 65° 20'.

Quick rinse 3mM Tris pH8.0; dry; A/R

— No apparent change.

8/2/86
36 +'s picked from MP13

4/2

'Pointed slot' gel

2mm spacing

1st 20 ~4mm deep
2nd 26 ~2mm deep.

Bottoms of wells seem to become a bit irregular after setting.
Samples (ZC298 →, not strictly in order) seem more prone to diffusion

1-17 : 4mm slots, almost full.

18-28 : 2mm slots, " "

29-34 : 2mm slots, small volume.

Some double-wackering over small slot lanes.

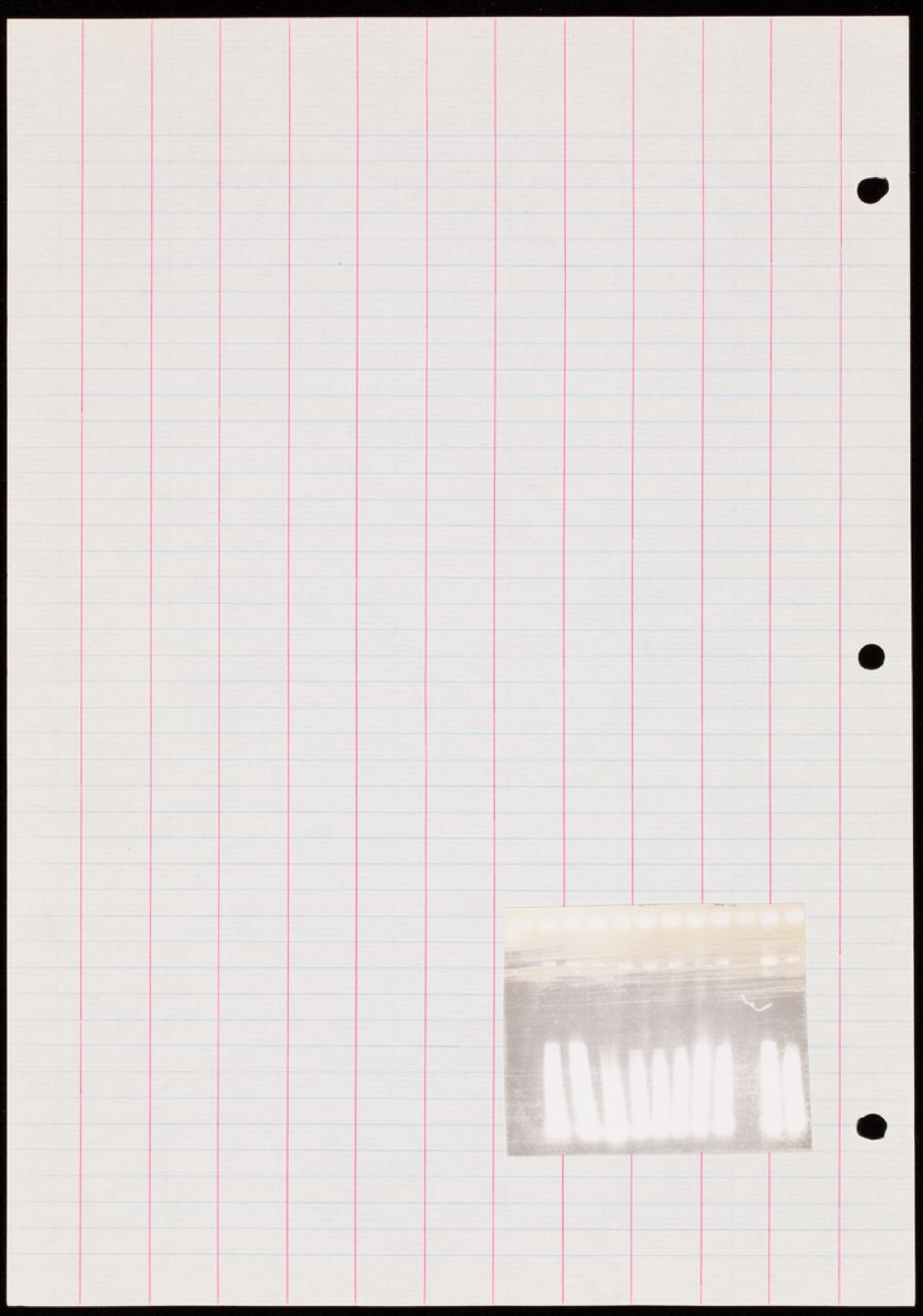
(VVVV may be better)

10/2.

71 +'s picked from Ralph Schmaked differential probing of 23/1 R1 plating, 2nd lift.

11 failed to grow.

Rest streaked after o/N



13/2/85.

Trial of cosmid prepⁿ in Millititer-SV plate (5.gun)

8 wells filled with ~200 μl mixture of residues of o/N cosmid growths, after melting off of lugs from bottom of plate to allow close contact of filter plate with collection plate.

Medium spun through 5' 2K.

Bugs resuspended (sucking + scraping with Eppendorf) in 50 μl lysozyme buffer

+ 50 μl SDS/NaOH. Mixed with tip.

+ 40 μl 3M NaAc pH 4.5

5' r.t.

3K 5'
then 3.6K 5'. (Plate began to crack up)

Varying amounts of super spun through, from none to complete.
50 μl taken from all that spun through.
Into round bottom microtitre plate.

+ 125 μl EtOH
15' -20°C
3K 5'
Inverted onto tissue
EtOH wash, dry.
+ 8 μl TE

← 4 μl + 1 μl dyes for 0.6% gel. Also 1 μl of 2 cosmids from normal prep for comparison (2C385, 386)

Promising.

14/2

(Very preliminary) growth of bugs in Millititer-SV.

200µl TYE / 125µg/ml amp. / well.

Bugs Toothpicked from Schnabel differential screening streaks.

12 wells, 5-8 smallest inoculum.

All stirred in fairly well.

Plate supported to allow air to circulate underneath.

Static incubation 37° 4.00pm → 2pm (22hrs)

Dropped! – didn't appear to be much growth.

16/2

More Millititer bug growth 5µm filter.

350µl TYE / 125µg/ml Amp / well

Bugs toothpicked from MP13 streaks (decreasing inoculum (→r)).

12 wells.

Plate supported to allow air circulation beneath filter.

18 hr 37° static.

Volume reduced to c ½ – ⅓.

Doesn't appear to be much growth.

2K 5' to remove medium.

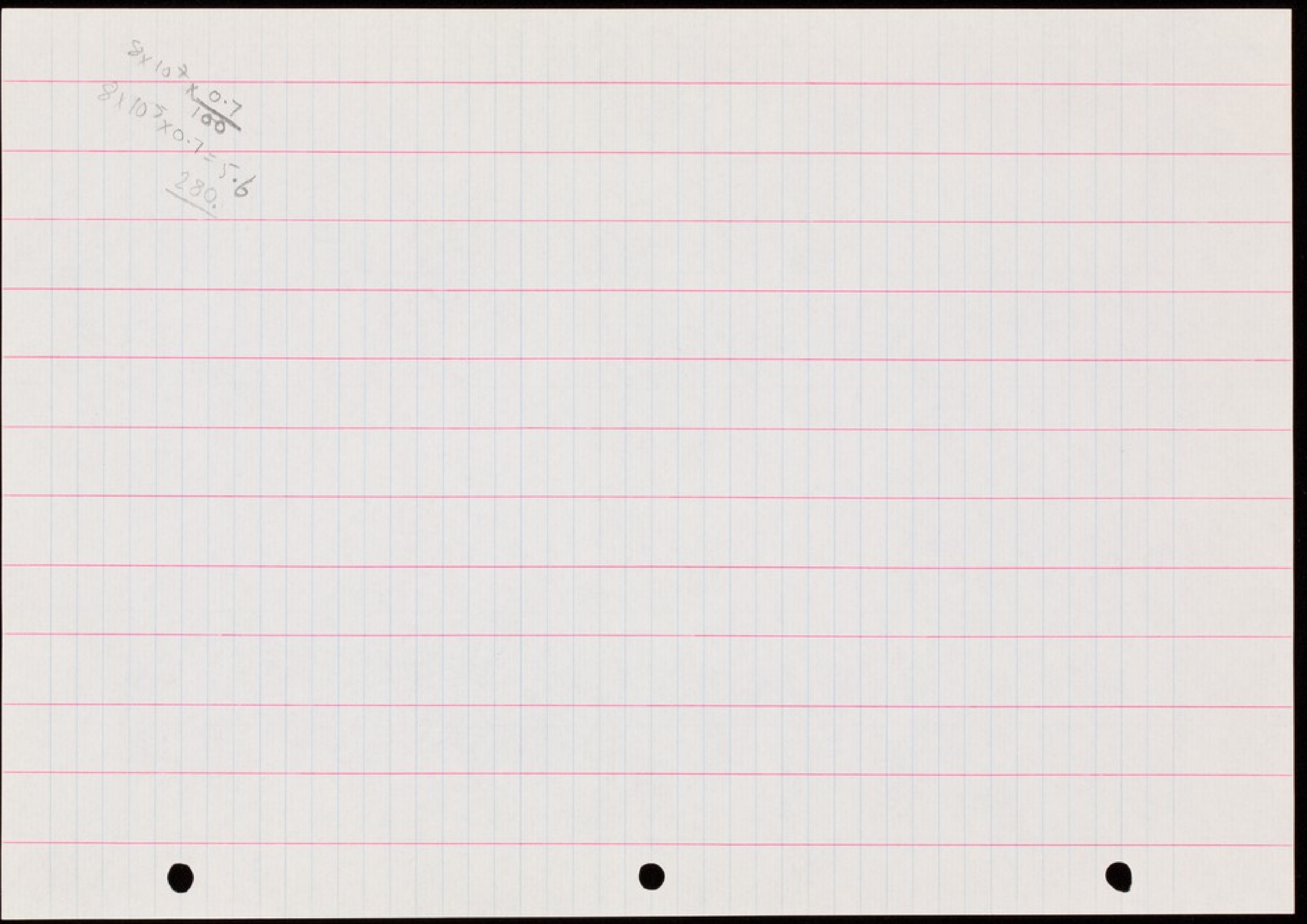
+ 50 lysozyme buffer
+ 50 SDS / NaOH
+ 40 3M NaAc
5' r.t.
2K 5'

Couple of wells missed target wells.

Appears to be considerable crud in eluates.

– Could be because extraction performed at r.t., but filtration at 4° i.e. something precipitated in eluate after filtration.

Abandoned.


8x10⁷ x 0.7 / 100
8x10⁵x0.7 = 5.6
280:

17/2

^{35}S labelings of ZC 461 - 464

Mix

4 µl ^{35}S-dATP
80 H_2O
2 RNase 10mg/ml
5 ddGTP 1mM
10 10x Hin
2 Hind III 20u/µl
2 AMV.

a) 2 mix	1 DNA	1	1x Hin	4
b) 2 mix	2 DNA	1	2x Hin	5
c) 2 mix	4 DNA	1	4x Hin	7
d) 4 mix	4 DNA	1	4x Hin	9

In round-bottom Corning 25850
Sealed by flexible microtit. plate pushed into wells.

45' 37°
25' 68°
Quick spin

Mix 36 H_2O
2 10x Hin
1.5 Sau3A 60u/µl.

2 mix/well spun in

Sealed as above. 90' 37°.

+ 4 µl formamide/dye to ZC461, 462
+ 2 µl " " " ZC463, 464

15' 80°.

→ ~1/2 loaded

→ all loaded. (looked badly retarded at beginning of run relative to ^{32}P controls
(Clone log 14/2/86)

17/2

10 t's picked from Jim Priess probing of 23/1 filters (1st lift). — not very hopeful (t's dubiously above bkgrd.)

19/2/86

Prep. 'C' packaging mix from SMR10

O/N grown in 2xTY from colony from 32°/42° selection.

1/100 O/N → 500ml 2xTY

O.D at 3 hr = 0.63

Work up as Mullins protocol.

(Fresh ATP pH 7.0; spermidine/putrescine for CH mix from Baer).

Final bug pellet + 2ml CH/ATP
50µl aliquots → liq. N2 → -70°.

(This work up is different from that of Rosenberg).

Assay

a) 20λ CH+ATP
1 100mM rATP
2 0.5µg/µl λ2001
50λ 9/7 Pack.mix.
60' 37°
20λ DNased 9/7 mix
30' 37°

b) 2λ CH+ATP
1 100mM rATP
2 0.5µg/µl λ2001
50λ 19/2 pack mix
60' 37°
20λ 19/2 DNased mix
30' 37°

c) 20λ CH+ATP
1 100mM rATP
50λ 19/2 Pack mix
60' 37°
20µl DNased 19/2
30' 37°

+0.5 ml λ dil, 25µl $CHCl_3$. Spun.

10µl → 10ml → (1ml) → 9ml
(1) (2)

·1ml a, b + 0.2ml TG1 (Toby)
20' 37°
Plated.

OVER

Packaging mix assay.

	1	2		
a		~1200	≡	7×10^7 pfu/μg (cf. 20/7 assay = 6×10^7)
b		300	≡	2×10^7 " "
c	0	0		
d	0			

20/2/86.

Prepⁿ of λ JB8 / N2 Mbo recombinant for packaging with SMR10 packaging mix.

2 H_2O
0.5 μl each arm λ JB8 (JES)
2 N2 Mbo frags (~0.3 μg) (JES)
1 10×C
1 rA
1 DTT
2 ligase (D.B. green label)

O/N 15°.

Packaging

20 CH+ATP
2 100mM rATP
5 ligation (½ in −20°C)
50 μl 19/2/86 packaging mix

60' 37°

RNAase ~~treatment~~ as Mullins protocol

+ 0.5 ml λ dil
drop $CHCl_3$; 4° C.

Assay

10 μl library
0.1 ml λ dil
0.2 ml O/N 1046 (recA ~~test~~ not too convincing – ~~need~~ reisolate for major prep)
20' 37°
0.5 ml CY
30' 37°

Plate 150, 50, 10 μl

– NOTHING.

OVER

a b c

a) 1μl 20/2 ligⁿ
b) 1μl reacⁿ as 20/2 no ligⁿ
c) 1μl 10μg/ml λ H3

20/2 ligaⁿ o.k., assuming both arms initially present.

Ligate b) (+ 1.5μl DB ligase) o/N 14°C.

⇇?

25/2 Packaging.

a) 4μl above ligation 'C' mix
b) 2.5μl " " 9/7 pack. mix
10μl absorbed to 1046 (0.8ml)
150μl of each plated

a) 0
b) 27

30
150/10μl
60
1500
9,000

25/2

Plating of Lorist/Mbo library (28/7/85)

50 μl library
0.1 ml λ dil
0.2 ml 1046
20' 37°
0.5 ml CY
30' 37°

Plate 4 x 150 μl (Kan T.G. plates).

~50 colonies/plate.

28/2/86.

Attempt to obtain colonies from 'C' packaging mix (19/2) using pJ38/R1 ligation

2 μl RC2 (0.1 μg?)
0.5 μl H3/R1 arm /10 (0.1 μg)
0.5 μl Sal/R1 " " (")
0.5 10xC
0.5 rATP (100mM — should be 10mM?) - prob ok.
0.5 DTT 1M
1 ligase.

O/N 14°C

a) 5 μl lig^n / 'C' pack. mix → 600 μl
b) 0.5 μl " / 9/7 pack. mix → 600 μl

0.1 ml λ dil
10 μl libraries
0.2 ml 1046
20' 37°
0.5 ml LY
30' 37°

150 μl plated.

a) 0
b) ~50 (15,000 total, so should be ~150,000 in (a))

4/3/86

Repeat assay of λ packaging by 'C' mix

a) 20 CH + ATP
 1 0.1M ATP
 2 0.5μg/μl λ 2001 } C mix 60' 37°

b) as a)
 no λ

Usual DNase ~~treatment~~.

+500μl λ dil, $CHCl_3$.

10μl → 1ml → 10ml 9ml

↓ 0.1ml
+ 0.2ml TG1 o/n (stephen)
20' 37°
Plate.

a) 420 ≡ 2.4 × 10^7 pfu/μg
b) 0

5/3
Repeat plating of Mbo (20/2) & RI (28/2) 'C' packages

50μl each package
100μl λ dil
200μl 1046 (2 weeks 4°).
20' 37°
500 CY
30' 37°
Plate 200μl each.

7/3/86

TG lorist (+ terminators) / N2 Mbo library.

(LORIST2)

a) 1 μl TG lorist (≈ 0.3 μg?) Bam phased (TG)
2 N2 Mbo (JCS) (0.3 μg?)
2 10 x C (0.5M Tris-Cl, 0.1 M $MgCl_2$)
2 rATP 10 mM
2 0.1M DTT
9 H_2O
2 D.B. ligase (JCS)

b) no fragments

16 h 14°C

10 μl (½) Packaged 9/7 packaging mix
(½ in -20°C)

↓ 600 μl.

10 μl of each
0.1 ml λ dil
0.2 ml 1046
20' 37°
0.5 ml ~~λ dil~~ CY

4 x 150 μl a) plated
1 x 150 μl b.)

(Also 50 μl 28/7 lorist library
/ 4 x 150 μl)

→ ~400 colonies / plate
= 2000 " / 10 μl package
= 2000 x 60 ≈ 120,000 total

10/3

2.5μl 7/3 ligation for 'C' mix package.

↓

~ 600μl

↓ 10μl absorbed

100μl λ dil

200μl 1046

20' 34°

500μl c-y

30' 37°

150μl plated.

— nothing.

13/3/86.

(Prep'n mp8 Hind III / Bam H1. vector
(for cloning of contig 'end frags'))

a) 2.5 µl (2.5 µg) mp8 RF (Sandra Satchwell)
2.5 µl 10x Hm
20 H₂O
1 µl 20u/µl Hind III
60' 37°

b) 2.5 µl mp8 RF
2.5 µl 10x Hi
20 µl H₂O
1 µl 25u/µl Bam H1.
60' 37°.

1 µl each, + 1 µl undigested ⓐ, on 0.7% gel
Crumby gel, but digests look o.k.

1 µl Hind III to Bam digest
1 µl Bam H1 to Hind III digest
60' 37°
Combined ; 1x phenol
1x ether

1 ml Sepharose 4B column in 10 mM Tris 7.4
1 mM EDTA
10 x 2 drop fractions.
4 µl + 1 µl dyes on 0.7% ag. gel

5 6 7 8 9 10

7.8 combined (=160 µl)
+ 16 µl 3M NaAc
400 µl EtOH
o/n -20°C.
ppt. looks rather large
EtOH wash, dry
+ 50 µl TE (well soluble).

1 µl 0.6% ag. gel. (T4) = ~0.3 µg

over

17/3
Prepⁿ λ Hind III / Bam H1 frags for vector test

2 μl 500ng/μl Biolab λ DNA
2 μl 10 x H
14 H_2O
1 16 u/μl Hind III
1 25 u/μl Bam H1

60' 37°

2 x phenol
1 x ethanol ; wash ; dry

+ 10 μl TE

1 μl on 0.7% gel
also 1 μl 100 ng/μl Hind III λ

H III / Bam
H III

Looks < 0.05 μg/μl

Vector test

a) 1 μl mp8 H3/Bam 10ng/μl
1 10 mM r ATP
1 10 x C
1 DTT
6 H_2O

b) + ligase (0.5)

c) + 1 μl λ H III / Bam frags + ligase

o/n 14°C

5 μl each transfected. (Ca Cl cells, overgrew a bit)
Also ~0.1 μl vector.

a) 0
b) 5 blue
c) ~ 200 white 2 blue
d) 0

Looks good

20/3.

Prepⁿ fragments for M13 cloning.

1. Sup5 object C5593
reference B0303

2. Lim12L obj. C07D12 (band just below comma)
ref B0027

3. cont 616L obj JH98503
ref. JH98514

4. cont616 R obj ZL 54
ref D2036

^{32}P labelled Hind III / Sau3A digests as per f/p.

Labelled digests : 2 µl
+ 1 µl dye / ficol

Preparative lanes : 2 µl cosmid / λ DNA Hind III / Sau3A
0.5 µl ^{35}S marker
1 µl dye

4% non-denaturing TBE gel
(1) lane 1 ^{35}S marker λ Sau3A
lane 2 object clone Hind III / Sau3A ^{32}P
lane 3 reference clone " " "
lane 4 preparative clone / λ marker
gap
etc
30 mA 2.5 hrs (1)

Gel turned out to weekend!
Daft putting ^{35}S label marker on gel - won't be visible through swamp.
R/A -70° O/N.
Gel went funny from freezing. Water squeezed out on thawing - may have become v. messy.
- film looks o.k., but not easy to interpret
re. denaturing gel? Abandoned.

22/3.

Repeat of 20/3 gel.
No markers.
Label digests sounded a bit weak - had dried up; + 2 μl H_2O
1 μl dyes.

25 mA (5.00 → 8.15 2.5 hr)

(R/A 4°) Looks ok, but realised I had forgotten to digest preparative samples!
Start again.

25/3/86

Repeat of 20/3 for object/reference digests

Preparative samples
Mix 10 H_2O
2.5 10xH
0.15 DNase
0.5 Hind III 20 u/μl
0.5 Sau 3A 16 u/μl

2 DNA
1 mix
60' 37°
+ 1 μl ficol/dye

4% gel (non denat) 30 mA 25 W
R/A o/n 4°C
Bands eluted as seen on film.
Gel removed; + 50 μl gel eln buffer; o/N 37°
(in Eppendorf)

Eluate glass-wool filtered
Gel washed c 20 μl TE
+ 20 μg tRNA carrier
+ 150 μl EtOH ; −20°C

7/4/86.

25/3 fragments >pun down (5' Epp)
EtOH washed; dried; + 10µl TE.

Ligations : a-d
1 mp8 HindIII/Bam 10ng/µl
3 frags 1-4 (see 20/3)
1 rATP 10mM
1 10×C
1 DTT 0.1M
2 H_2O
1 DB ligase.

e λ HindIII / Bam frags.
f no frags.

(n.b. unphosphatased vector - may be better to phosphatase to prevent formation of concatamers.)

O/N 14°C.

5µl a-d
0.1µl e
1µl e
5µl f
} TG1/Hanahan transfected

(Hanahan cells made competent using Tab j1 solutions).

(f accidentally mixed with d on plating).

a (C5593) ~1600 white ~250 blue
b (C8701.2) white near confluent; ~500 blue
c (511985?) ~200 white; ~250 blue.
d (2454) ~40 white ~400 blue.

f 75 Blue 5 white

OVER/

6 whites a-d
also 6 blues from d
picked and grown up. (4.75 hr growth)

11/9/86. T tracked

C5593 all the same
C07D12 .. .. -
JH98503 5 .. .., 1 different (#4)
ZL54 white all different (#1 looks to have correct sized insert)
" blue all no insert

(Best C5593 /2
C07D12 /4
JH98503 /3
ZL54 /1)

14/4 'Simplistic' attempt at primed probe labelling of above clones.

5 μl template DNA
1 μl DB1/10 (Dan Brown H+ a Messing primer - conc^n uncertain but this dil^n probably used previously by F.S.)
1 10xH.L
3 H_2O
100° 3' (capillary)
→ r.t. 2 hrs.

+ 10 μl dATP[32] (400 Ci/mmol) dry
1 μl 0.5 M GTC mix
1 μl Klenow/10 (~0.5 u)
/35' r.t.
+ 2 μl 0.1 M EDTA

C5593 and C07D12 - spin column
(should have diluted first - column washed through with 40 μl TE after 1st spin - some extra material recovered).
(No spin column = 200 cps) Both gave 40 cps along 6x4 ft. rack
∴ This seems a bit low - decided to probe with C5593 and JH98503 on unused filter sets - save (-20°) C07D12 and ZL54

36 ml H_2O
0.4 ml SS DNA

Boiled 10'; ice 10'

+ 36 ml SCP/NC30/dextran

18 ml → bag
18 ml + probe → bag

60°C O/N
14 hrs

C5593 :	23/1	3	} previously unused
SH98503 :	23/1	4	

(Perhaps should use 3000 Ci/mMol label
Also check out primer conc^n?)

Washed 4x 1xSCP/1% SDS 50°C → r.t. 10'
1x 0.3mM Tris pH8.0 r.t. brief

16/4
Above probings look promising, so probe with C07D12 and ZL54 probes, as above

C07D12 :	11/12	2	(used)	(previous used 16/1 MP10)
ZL54 :	23/1	1	(used)	(previous use 29/1 MP13) probably

17/4
C5593 and SH98503 have quite a lot of apparent +'s, but many look suspiciously non-specific i.e. small specks, not colony shaped, although restricted to colonies. This could be due to crappy dextran sulphate – try filtering hybridisation mix.

Repeat of C5593 probing, as above, but using filtered SCP/NC30/dextran sulphate and R1 23/1 B (3) (slave from regrowth of master plate; previously unprobed)

OVER

Filtration of hybdⁿ mix:
2x through HA 0.45μm filter in Swinnex 47 holder.

Result
Far fewer hot spots with filtered hybdⁿ mix. Spots that there are show no correlation with spots produced using unfiltered mix.
So abandon picking of colonies from these expts, but use filtered mix in future.

21/4
Making prime cut probe for hybdⁿ.

5 M13 DNA (C5593/2, C07012/4)
1.5 HMB2
1 TM
2.5 H_2O
100° 90"
→ r.t.

Mix: 10 dATP* (400 Ci/mmol)
5 0.5mM dGTC
1 TM
15 H_2O

10 mix
10 annealed primer/template
0.5 5u/μl Klenow.
10' r.t
4 0.5mM dATP
10' r.t
+ 0.5μl 20u/μl HindIII
0.5μl 4u/μl SauIIIA
60' ~~rt~~ 37°

+ 25μl formamide/dye (~ equal vol)
100°/5'
Each loaded on 3cm slot of 4% 0.35mm TBU gel
1 hr 30mA
15' A/R

C5593 appeared not to have obviously worked, but C07012 gave good band (+ 1 weaker band somewhat above general background level).
C07012 band cut out
Eluted o/N in 200μl M.g. el. buffer.
Good elution (gel not crushed), washed + 100μl
Filtered through Si glass wool

~35cps from tube held above monitor

22/4

All used to probe R1 11/12 1 filters
(last used MP9 16/1/86)
(- not boiled - 3 months decomp but have been previously boiled)
O/N 68° hybd (bag leaked a bit)
4x10' 50° → r.t 1xSCP, 1% SDS washes.
1x 3mM Tris pH8.0
6 days - 70° fogged film exposure
Some weak +'s, but no way to align a pick - probe needs to be hotter.

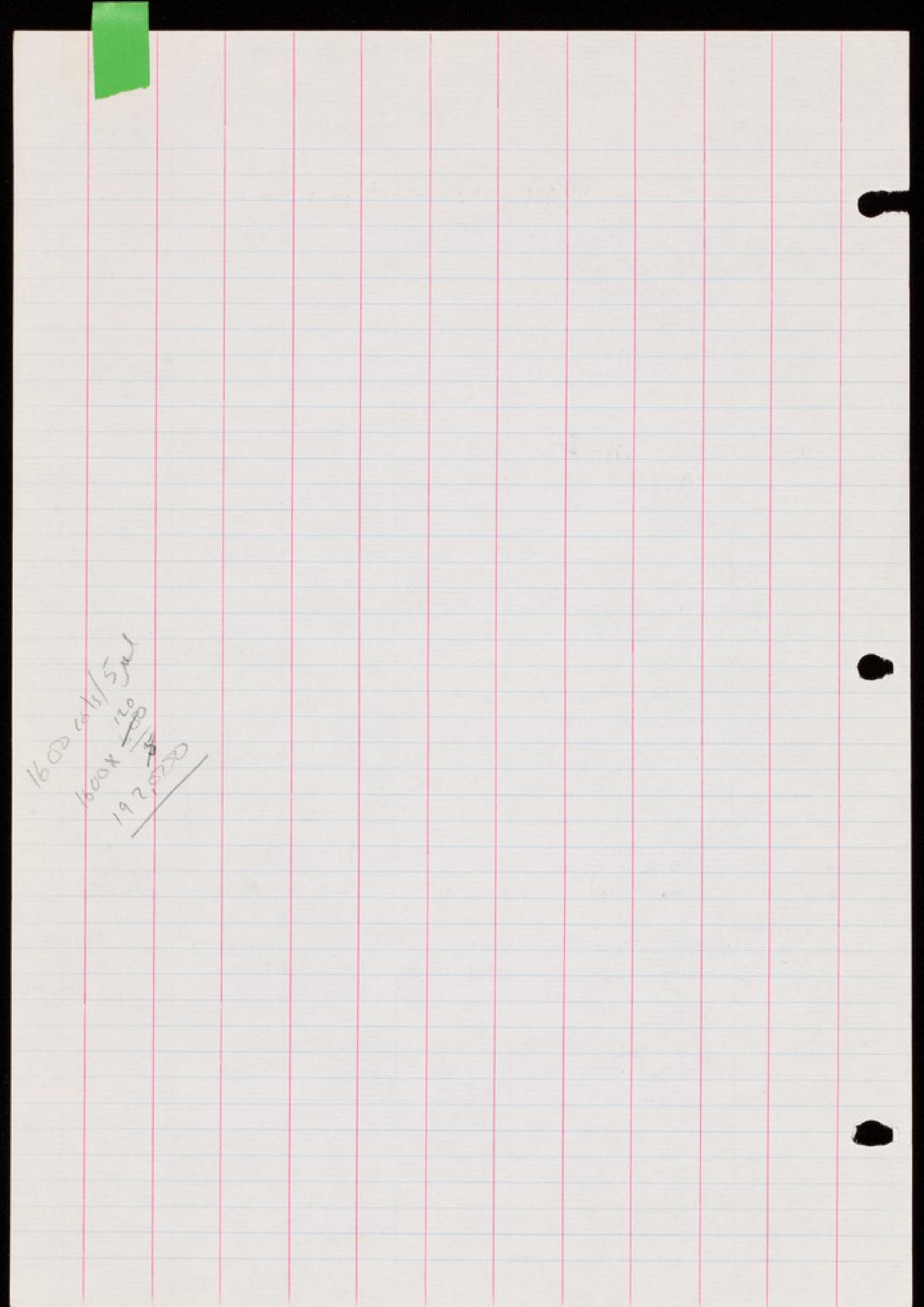
25/4

More gentle washing of filters (i.e. not boiling)
27/1 (1) (used for Z654 16/4 probe)
1l 10mM Tris pH8.0 80°C 10'. Slight sloshing.
72 hr exposure. Looks clean - some general background, but o.k.

27/3

More plating (lower density) of 7/3
TG Lorist library.

10 µl TGL
0.1 ml x dil
0.2 ml 1046
20' 37°
0.5 ml ~~[illegible]~~ LCY
30' 34°
12 x 50 µl plated (kan)


1600x
120
192,000

22/4

TGLori?T (4/3) library assay.

5ml TGL
0·1 ml λ dil
0·2 ml 1046 O/N
20' 37°
0·5 ml CY

a) 30' Plate 50μl
b) 30' Plate 50μl
c) 60' Plate 50μl

a) 30' Kan exposure 30 colonies
b) 60' " " 110 "
c) 2hr " " 0 (Amp plate!)

(110 colonies ≏ upper limit for selection for growth).

But
U.V assay of 1046 used for above showed total recovery, even at 30" exposure level (TG1 used as control).
Repeated assay on same O/N (21/4) in case fluorescent light used by mistake. OP50 also included. (Should be intermediate in growth).

Fresh streak of 1046 from -20° glycerol stock.
O/N
UV assay O.K.

25/4

Plating of 7/3 TGLovi1t/c1b. for f/p

5μl library
0.1ml × dil
0.2ml 1046
20'
0.5 ml CY

60' 37°

10 × 50μl plated ≃ 70 colonies/plate
(700 total)

60' 37°

5 × 25μl plated ≃ 50/plate
(250 total)

1/5

Assay of ABT Sau3A (Rec'd 29/4/86, Lot# BA1)
v. Biolabs Sau3A (4 u/µl)

Mix 15 (7.5µg) 560µg/ml Biolabs λ DNA
37.5 10x tri
320 H2O

25µl mix / reac^n (0.5µg λ DNA)

+ a)	1µl Biolabs Sau3A (4 u/µl)		4u
b)	" " "	/5	0.8u
c)	" " "	/10	0.4u
d)	" " "	/20	0.2u
e)	" " "	/100	0.04u
f)	1µl ABT 40 u/µl		40u (?)
g)	" "	/5	8u
h)	" "	/20	2u
i)	" "	/60	0.7u
j)	" "	/100	0.4u
k)	" "	/300	0.1u

l) no enzyme

30' 37°

4µl reac^n + 2µl dyes ; 1.3% ag. gel

l a ef k

ABT/20 looks slightly less active than Biolabs/5.
So ABT ~ 20u/µl or less (if Biolabs conc^n O.K.).

~ 8u ABT needed to digest 0.5µg λ DNA in 30'.

5/10/86

Further Sau3A assays

Mix 15 μl (7.5 μg) 560 μg/ml Biolabs λ
37.5 10 X Hi
320 H_2O

25 mix/reaction

Dil^n.

a) 1 μl Kam (Epp)
b) 1 μl " /5 — 1 stock + 4 dil
c) " " /10 — 2 b + 2 dil
d) " " /20 — 2 c + 2 dil

e) 1 μl Kam (culture tubes)
f) " " /5 — 1 stock + 4 dil
g) " " /10 — 2 f + 2 dil
h) " " /20 — 2 g + 2 dil

i) 1 μl ABT /10 — 1 stock + 9 dil
j) " " /20 — 2 i + 2 dil

k) 1 μl Boehringer
l) " " /5 — 1 stock + 4 dil
m) " " /10 — 2 l + 2 dil
n) " " /20 — 2 m + 2 dil

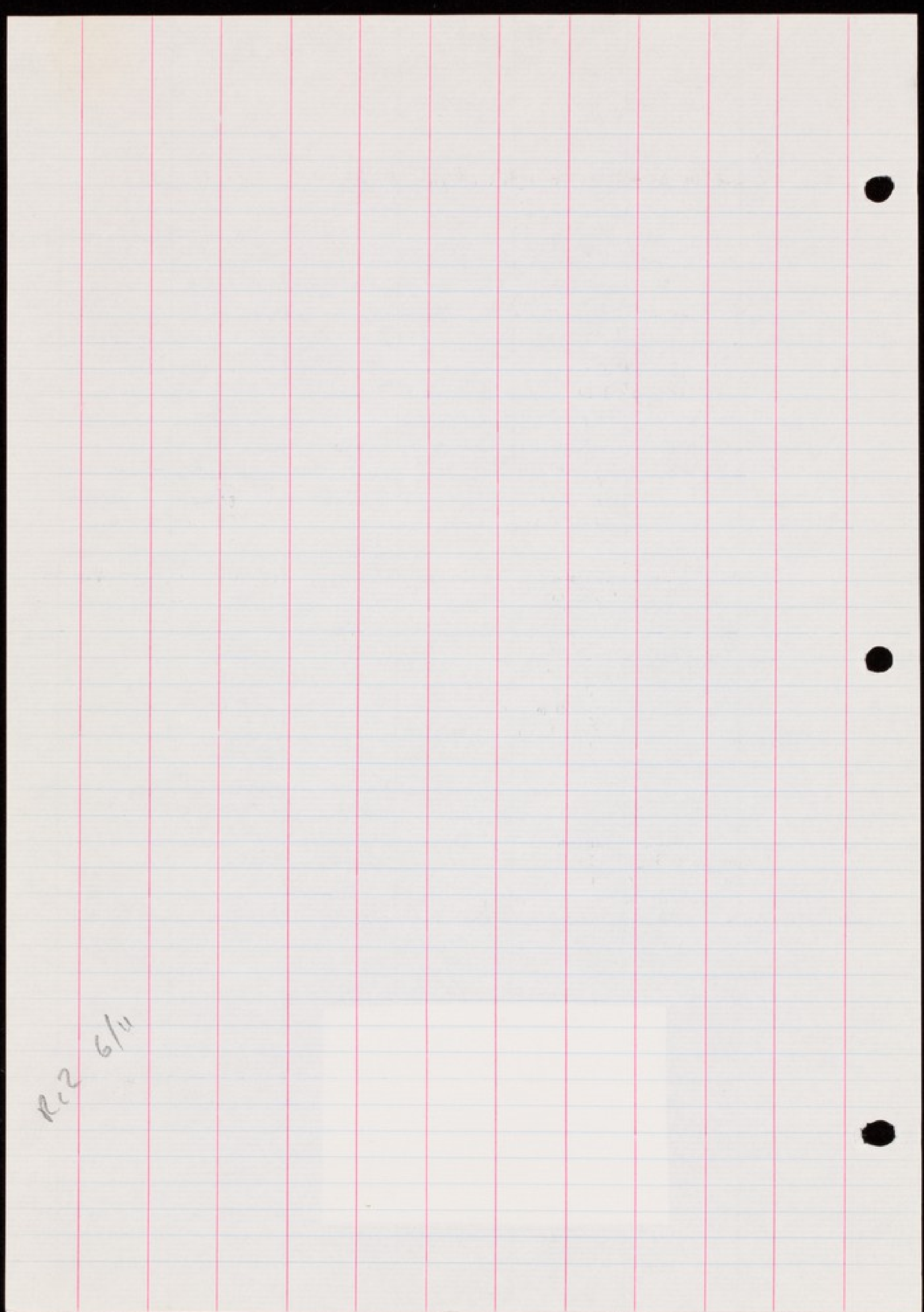
(14)

o) 0

60' 37°

4 μl reac^n. + 1 μl dyes ; 1.3% minigel

o a n



3/5/86

Packaging of rest of 7/3 TqLorist/NZMbo ligⁿ

10µl ligⁿ
20µl KH + ATP
2µl 100mM rATP
9/7 pack. mix

60' 37°
DNase / pack mix
30' 37°
↓
0.5ml λ dil, CHCl₃, spin.

4/5/86
Library assays

40µl λ dil
2µl library
80µl O/N 1046
20' absorpⁿ
200µl CY
30' expression for Amp library
60' " " Kan "

		/ml
pJB8/R1	21/8/85	265
pJB8/R1	6/11/85	275
pJB8/R1	28/2/86	14
pJB8/Mbo?	25/2/86	10
Lorist6/Mbo	28/7/85	12
TqL/Mbo	7/3/86	230
" "	3/5/86	104

(400µl sent to Marty Chalfie)

9/5/86

Sau3A assays.

C52A1 - A4 x 3 dil^ns each of
1) ABT Sau3A 40u/μl. rec'd 29/4/86 Lot # BA1
2) ABT Sau3A 50u/μl. rec'd 9/5/86 Lot # BA2
3) CBL Sau3A 35u/μl rec'd 9/5/86

(Fresh tube RT from -70°)

Dil^ns:

a)	b)	c)
12 H_2O	12 H_2O	12 H_2O
1.2 10xHi[illegible]	1.2 10xHi[illegible]	1.2 10xHi[illegible]
1 Sau3A	0.5 S3A	0.25 Sau3A

standard f/p reac^ns
ie 2μl Sau3A mix / reac^n, 2 hr 37°.

In terms of volume (disregarding fact that ~~prev~~ previous ABT Sau3A has been 70u/μl)
a = 4X Sau3A, b = 2X, c = same.

13/5

Assay of large batch of CBL Sau3A (70Ku, 35u/μl)

1) Various dil^n CBL Sau3A
2) Kara Sau3A 1 u/μl?
3) BioLab Sau3A 4 u/μl
4) AST Sau3A 140 u/μl (?)

CBL dil^ns:

a)	b)	c)	d)	e)
12 H_2O	12 H_2O	12 H_2O	25 H_2O	100 H_2O
1.2 10xH	1.2 10xH	1.2 10xH	2.5 10xH	11 10xH
1 Sau3A	0.5 Sau3A	0.25 Sau3A	0.25 Sau3A	0.25 Sau3A
= 5u/2μl	2.5u/2μl	1.25u/2μl	0.65u/2μl	0.16u/2μl

f) 5 H_2O
1 10xH
5 Kara Sau3A

g) 5 H_2O
1 10xH
3 BioLab Sau3A

h) 12 H_2O
1.2 10xH
0.5 Sau3A AST

Usual HindIII/RT label C52B4, C52B5
68° 25'
2μl Sau3A dil^n
2 hrs 37°.

Amersham International plc
Amersham Laboratories
White Lion Road Amersham
Buckinghamshire England HP7 9LL

Radiochemical batch analysis

Caution: The product is prepared for laboratory use only and not warranted for use in humans or for clinical diagnosis

DEOXYADENOSINE 5'-[α-^{32}P]TRIPHOSPHATE, TRIETHYLAMMONIUM SALT
in stabilized aqueous solution
Code PB.10384
Batch 8835

BATCH TECHNICAL DATA

Specific activity	:	29.6	TBq/mmol at 1200 GMT on 9th September 1988	800 Ci/mmol
Molecular weight	:	491	(free acid)	
Radioactive concentration	:	370	MBq/ml,	10 mCi/ml
Radionuclidic purity	:	<2% ^{33}P No radionuclidic impurity is detected by γ-spectrometry		

Radiochemical purity

by constant flow high performance liquid chromatography (H.P.L.C.) on a strong anion exchanger chemically bonded to 10μm silica gel, using a gradient from 0.15M (pH 3.5) to 1.0M (pH 4.15) potassium dihydrogen orthophosphate : 94%

Biological assay data - See Page 3

Analysed 30th August 1988

Chromatographic methods

The chemical concentration of the sample is adjusted to approximately 1 mg/ml by the addition of unlabelled material. 5-10μl samples are injected onto the column using a stop-flow technique. The column eluate is passed through a bremsstrahlung radiometric detector and an ultraviolet monitor in series. The radioactivity count rate, and optical absorbance at 265 nm, are displayed on a chart recorder.

The radiochemical purity of this material may also be determined by paper chromatography on Whatman No. 1 paper in

isobutyric acid:water:ammonia(d.0.880):EDTA (100:56:4.2:0.06)

and by thin-layer chromatography on precoated PEI cellulose plates in
(a) 0.2M ammonium bicarbonate
(b) 1.0M potassium dihydrogen phosphate at pH 3.4

Amersham Australia Pty Limited
PO Box 99
North Ryde
NSW 2113

Amersham France SA
Avenue du Canada
BP 144
91944 Les Ulis Cedex

Amersham Belgium SA/NV
Av. R Vandendriesschelaan 18 B.9
B-1150 Brussels

アマシャム・ジャパン株式会社

Amersham Buchler GmbH & Co. KG
Gieselweg 1
D-3300
Braunschweig

Amersham Nederland BV
Spoorhaag 134
Postbus 32 3990 DA Houten

Amersham Canada Ltd
505 Iroquois Shore Road
Oakville
Ontario L6H 2R3

Amersham Sweden AB
Hagalundsgatan 30
S-171 50 Solna

Amersham Corporation
2636 S. Clearbrook Drive
Arlington Heights
Illinois 60005

Amersham Denmark ApS
Abildgårdsparken 2
DK-3460 Birkerød

STABILITY, AND STORAGE RECOMMENDATIONS

To minimize decomposition, stocks of deoxyadenosine 5'-[α-^{32}P]triphosphate, triethylammonium salt solution at the above concentration should be stored at -20°C. Under these conditions decomposition does not usually exceed 2% per week.

PACKAGING

Deoxyadenosine 5'-[α-^{32}P]triphosphate, triethylammonium salt is supplied in solution* at 370 MBq/ml, 10 mCi/ml on the reference date in polypropylene V vials vials supported in "Duoseal" vials ("Duoseal V-vial").

For most applications the solution may be used directly. If it is necessary to remove the water, this is best achieved in vacuo.

*made 5 mmolar with 2-mercaptoethanol as a stabilizer.

PREPARATION

Deoxyadenosine 5'-triphosphate labelled with phosphorus-32 at high specific activity in the alpha phosphate group is prepared from inorganic [^{32}P]phosphate via deoxyadenosine 5'-monophosphate which after purification, is then enzymatically converted to the triphosphate. dATP is purified using high performance liquid chromatography (HPLC) employing a system which ensures separation of both radioactive and ultraviolet absorbing impurities

---

BIOLOGICAL TESTING

Each batch of the deoxyribonucleoside 5'-[α-^{32}P]triphosphates from Amersham International is tested in a 'nick translation' procedure based on published methods [1,2,3].

The percentage of the [α-^{32}P]dNTP incorporated and the specific activity of the labelled DNA product depend primarily on the relative concentrations of the DNA and labelled nucleotide [4].

Typical results obtained after a 5 hour incubation using 750 picomoles of an [α-^{32}P]dNTP per µg of DNA, together with an excess of the three appropriate unlabelled dNTPs, are summarised in the table below:

dNTP	Code	% dNTP Incorporated in λ DNA	Specific Activity* of DNA (dpm/µg)
dATP	PB.164/10164/10384)		
dCTP	PB.165/10165/10385)	40-60	~ $2\text{-}4 \times 10^8$
dGTP	PB.166/10166/10386)		
dTTP	PB.167/10167/10387)		
dATP	PB.204/10204/10474)		
dCTP	PB.205/10205/10475)	40-60	~ 1.5×10^9
dGTP	PB.206/10206)		
dTTP	PB.207/10207)		

* as [^{32}P]d-NTP ref. date)

Results obtained by the customer will vary with the conditions used and the DNA being labelled.

REFERENCES

1. RIGBY, P.W.J., DIECKMANN, M., RHODES, D., and BERG, P. Labelling deoxyribonucleic acid to high specific activity *in vitro* by 'nick translation' with DNA polymerase I. *Journal of Molecular Biology,* Vol. 113, pp. 237-251, 1977.

2. MACKEY, J.K., BRACKMANN, K.H., GREEN, M.R., and GREEN, M., Preparation and characterization of highly radioactive *in vitro* labelled adenovirus DNA and DNA restriction fragments. *Biochemistry,* Vol. 16, pp. 4478-4483, 1977.

3. MANIATIS, T., JEFFREY, A., and KLEID, D.G. Nucleotide sequence of the rightward operator of phage λ. *Proceedings of the National Academy of Sciences,* U.S.A., Vol. 72 pp. 1184-1188, 1975.

4. "Labelling of DNA with ^{32}P by nick translation," Technical Bulletin TB.80/3, Amersham International. Available from Amersham.

U.V. recA:
streaks from dil^n prior to O/N. Also reassayed after O/N.

104 6
CES201
DB1317
TG1 no growth (old O/N)

2/6

Various library platings.

a) Lorist/Mbo (28/9/85) / 1046

0.1 ml LoristMbo (should be ~1000 col^ns)
0.1 ml λ dil
0.2 ml O/N 1046
20'
1.4 ml CY
90'
Plate 12 x 150 μl

O.K. ~130 / plate

b) Test platings on RecBC strains

TGLorist (7/3/86) and pSB8/R1 (21/8/85)
on CES201, DB1317 and 1046

40 μl λ dil
2 μl library
80 μl O/N bugs
20'
200 μl CY
30' (pSB8) or 90' (TGL) 37°
150 μl plated.

16 hr 37°

pSB8/R1	1046	550
" "	CES201	75
" "	DB1317	210
TGL	1046	350
TGL	CES201	only v. small col^ns at 16 hr
TGL	DB1317	- - -

Further incub^n (9.00 am → 9.00 am) 40 hr ~~total~~

TGL CES201 12 v. small colonies
TGL DB1317 80 colonies, v. variable sizes.

23/6

EcoK⁻ (Gigapack) packaging of N2Mbo/pJB8 recombinants.

Ligation:
2 H_2O
0.5 each arm pJB8 (JCS)
2 N2Mbo frags (~0.3 μg) (JCS)
1 10×C
1 10mM rATP
1 DTT
1 Bentley ligase.

o/n 15°C

① 5 μl ligⁿ into Gigapack red tube
+15 μl " yellow "

2 hrs r.t.
+ 0.5 ml λ dil / 20 μl $CHCl_3$

② 2 μl packaged with A.C. 19/2 pack. mix:
20 CH, rATP
2 100mM rATP
2 ligⁿ
into 50 μl 19/2 pack. mix
60' 37°
+ DNase mix as per Mullin protocol
30' 37°
+ 0.5 ml λ dil / 20 μl $CHCl_3$.

10 μl each
0.1 ml λ dil
0.2 ml 1046 o/N (not recA tested)
20' 37°
0.5 ml CY
30' 37°
plate 150 μl :

EcoK⁻ 40 : ~12,000 ~~total~~
A.C. 18 : ~6,000 "

(but 2/5 packaged AC mix, so efficiency about =)

30/6/86

12 colonies picked from C.K. mab.5 probing of 23/1 library filters.

1/7/86

Plating of EcoK⁻ N2r1b0/pS38 for f/p

25 μl 23/6 packaging
0.1 ml λ dil
0.3 ml 1046 O/N (RecA U.V. assayed O.K.)
20' 37°
0.5 ml CY
30' 37°

6x150 μl plated 75 μg/ml Amp plates.
(Should give ~ 100 col/plate)

Actually ~ 50/plate.
(Keep at this density for future platings)

5/7
2x above plating; same bugs (UV OK)
Only ~ 18/plate.

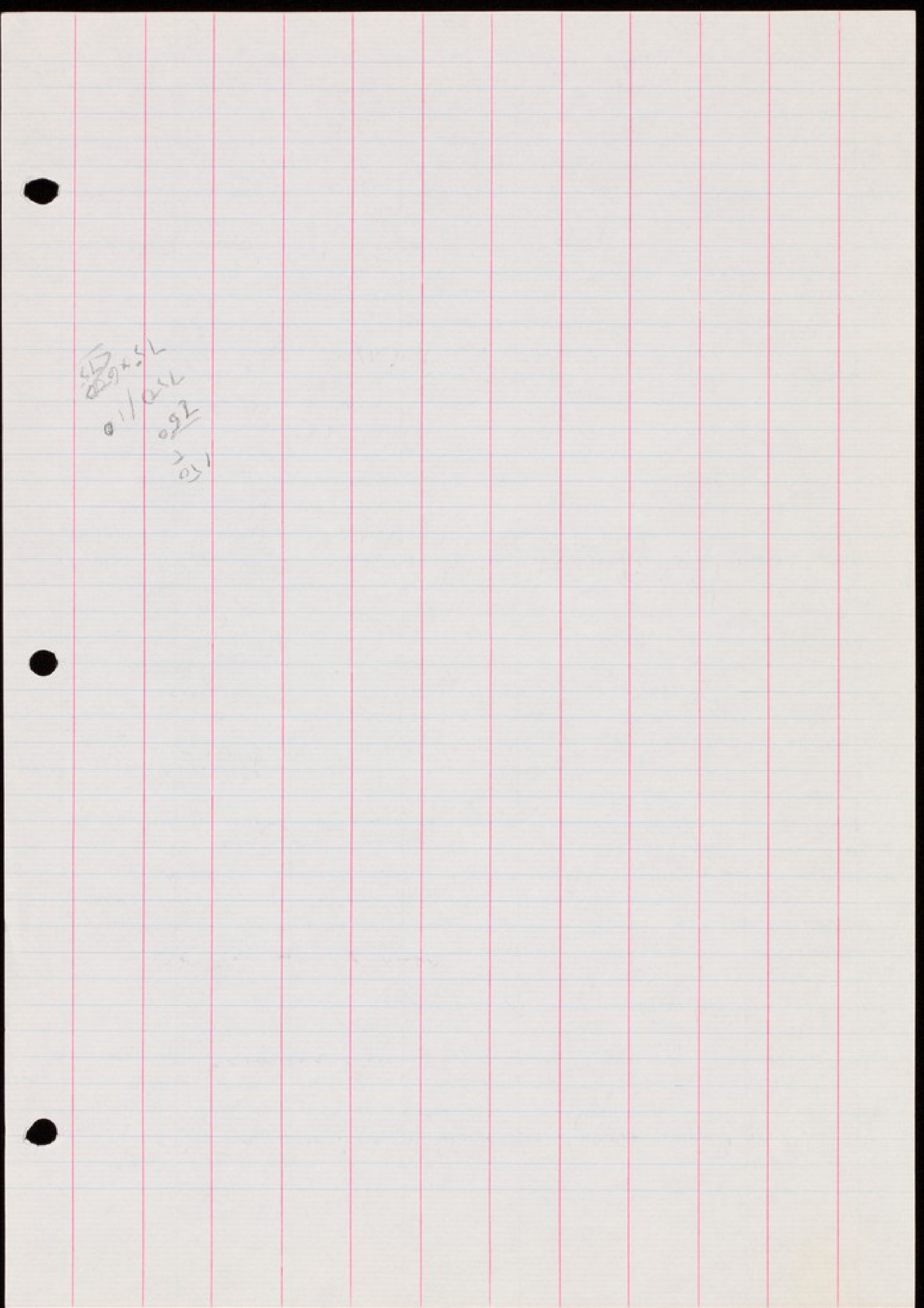
11/7
2x 1/7 plating
Fresh O/N bugs

23/7
100 μl library
0.4 ml λ dil
1.2 ml 1046
20'
2 ml CY
35'

(UV OK)

23 x 150 μl

~1500 colonies total



25/7/86.

EcoK⁻ packaging of Lorist B/Mbo ligation

Ligation was frozen unpackaged half of 28/7 reaction.
This should be enough for ~7500 cosmids under packaging conditions used at that time.

Stratagene mixes.
+1500X dil final.

Assay.

0.1 ml λdil
10 μl package
0.2 ml 1046 (3 days old).
20' 37°
0.5 ml CY
45' 37°

Plate		
150 μl	140	colonies
50	32	"
10	6	"

FOI→

25/4/86

Ligation LORIST2 (terminator)/N2Mbo
for EcoK⁻ packaging.

As 7/3/86. (Same vector and fragments etc)

O/N 14°

10µl taken for Stratagene EcoK⁻ packaging

Remaining ½ of ligation -20°C.

7/8
Assay.

10µl library
0.1ml λ dil
0.2ml 1046 (fresh O/N U.V. tested)
20' 37°
0.5ml CY
50' 37°

Plating 150µl 40. 20 cosmid/µl ≤ ≃10000 total
50µl 6
10µl 0

Plating for growth

40 library
0.4 λ dil
0.8 1046
20' 37°
2, CY
50' 37°; 200µl platings

Only ~30/plate.

Streaked
To be designated F (i.e. F01A1 etc)

15/8

Further plating LORIST2/Mbo E coK⁻

60 library
0.4 λ dil
0.8 1046
20' 37°
2ml CY
50' 37°

200 μl platings.

~ 120/plate 15 plates.

Probably ~~at~~ a bit too dense, but useable.

11/8

Further plating LORIST2/Mbo E coK⁻
1046 and ED8767

25 μl library
0.2 ml λ dil
0.4 ml O/N 1046 or ED8767
20' 37°
1ml CY
50' 37°

200 μl/plate

17/9/86

Prep. N2 Sau3A partial fragments.

(12/9 attempt all overdigested).

	a	b	c	d		
N2 DNA	0.3	0.3	0.3	0.3	ml	(JES 15/9/86)(200µg/ml?)
H_2O	0.15	0.15	0.15	0.15	ml	} 650 } 200
10×H	50µl	50	50	50	µl	} 200
Sau3A 6ku/ml /10	3.5	2	1	0.5	µl	

37° 2hr
Ice

0.4% HGT 200ml TBE assay

1) 1µl N2
2,3) 2µl 12/9 d (minimal digest), /10
4-11) 2µl 17/9 a-d and a-d/10 alternating
12) 2µl 30µg/ml λH3 + 3µl 10µg/ml λ
13) N2/10.

19mA 15 hr.

N2 17/9 d 17/9 a b c d λH3 N2/10

b-d phol extracted 1X
with 500µl phol-TE
1× ether;
Divided/2; EtOH pptd; 70% EtOH wash

v. brief dry
+ 20µl TE
4° resuspension

a Further 2hr 37°C

e Additional digest
as above;
+0.5µl 6ku/ml Sau3A
2hr 37°.

0.2 N2
2µl 18/9 a
" " a
1µl 17/9 b/(after EtOH ct) (0.25→2.5')
" " c
" " d
λ/H3
Cetacea Sma frag.

a, e phenol, EtOH etc. as b - d
+ 20µl TE
4° resuspension.

a - e combined for prep. 0.4% LGT TBE 300ml gel
(220µl).
+ 100 dyes (Max. buffer vol. to prevent egress of sample from slot!)
80µl / 13mm slot.
20V 24mA (5.30 → 11a.m.) Could have run further.
Post stained 35' + 50µl 10mg/ml EtBr
Accidently exposed to some short wave, U.V.
6 bands cut (1 - 6 bottom → top)
2 - 5 worked up
2 x phenol, iso-butanol etc.
→ 300µl
+ 30µl 3M NaAc
800 EtOH.
-20° 2 hrs.
Visible ppt in all (Lots in 5)
70% EtOH wash
Brief dry
40µl TE
1µl on 0.4% HGT minigel
0.5µl and 0.05µl on 0.4% HGT 200ml O/N gel

0.5μl λH2 0.5μl 2 0.5μl 3 0.5μl 4 0.5μl 5 λ, λH3

19mA 25V
16hr.

5 (40μl) + 60μl TE
+ 60μl dyes

Loaded on 6 cm width of 0.4% 300ml LGT TBE gel
with λ, λH3 markers

25V 22mA (3.00pm → 11am) 20 hrs.
Post-stained (50μl 10mg/ml Eth Br)
60'

3 slices to top of band eluted
Largest 2 fragments (2, 3) worked-up.
+ 20μl TE final.

200ml 0.4% HGT TBE gel   0.5μl 2, 3 → 15 dyes
26V (8.30 →   → 3 → 12 dyes
(Gel slots seemed a bit 'lumpy')

each marker lane { 2 30μg/ml λH3
{ 2 10μg/ml λ

2 3

Could be bigger, but worth proceeding.
(2 too small)
Prob. about 1μg ~~total~~

22/9/86

Prepⁿ LORIST6 (C.R. 0.5μg/μl) for cloning

20μl (10μg) LORIST6
3   10 × H⁻
5   H₂O
2   25 u/μl Bam H1.
  2 h 37°

a b

0.8% gel (post-stained)
a 0.2μl uncut Lor6
b 0.3μl Bam ..
Either not cut or linears & circles not resolved.
Run 2nd sample in same gel, longer run

a b c

a, b as above
c 0.5μl Lor2 Bam i.e. ~0.1g
NB loaded late as an afterthought
– blue dye had gone ~1cm.

Still not much resolution – assay by ligation.

Prepⁿ λ Sau3A frags. for vector test.

4μl 0.5μg/μl Biolab λ DNA
2 μl 10 × H⁻
13 μl H₂O
1 μl 6 u/μl Sau3A1.
  2 h 37°
1μl 2 × phenol
  2 × ether.

O.K.

23/9

LOR6 Bam 2 x phenol
Etoh pptd. (dry ice)
+ 10 TE.

(Remove 1µl
+ 9µl TE)

+ 40µl 50mM Tris pH 8.0
10mM EDTA

1µl CAP phase (22 units, Boehringer)

15' r.t.

2 x phenol
EtOH ppt, wash

+ 25 TE

LOR6 Bam, ph'ase ~~test~~. (....)

3X Buffer ~~10~~ 5 10 x C
~~10~~ 5 10mM r ATP
~~10~~ 5 0.1M DTT

a) 0.2 µl uncut LOR6 (~0.1µg) (0.5µg/µl)
7 H_2O
3 buffer
0.2 ligase (DB blunt)

b) 2 µl LOR6 Bam (0.05µg/µl)
5 H_2O
3 buffer
0.2 ligase

c) 0.5 µl LOR6 Bam phase (0.2µg/µl)
7 H_2O
3 buffer
0.2 ligase

d) 0.5 LOR6 Bam phase (0.1µg/µl)
1 µl λ S3A
6 H_2O
3 buffer
0.2 ligase

12.30 → 3.30

4 µl for gel

Also e) 0.1 µl uncut LOR6 (0.05µg)
f) 0.25 µl TG LOR2 Bam (0.1µg)

a b c d e f

24/9
Conc^n assay of LORIST6 Bam phage relative to
TG LORIST2 Bam phage
(see 7/7/86 ligation).

Looks to be only ~1/5 conc^n.

24/9
Trial ligation and packaging of N2pSau3A (17/9) and LOR6

2.5 µl LORIST6 Bam phage
2 µl N2pSau3A frags (cut 3, largest frags)
1 10xC
1 rATP 10mM
1 0.1M DTT
1.5 H_2O.
1 ligase (DB blunt)

o/n 14°.

All packaged (EcoK+ AC mix)

+ dil → 600 µl

25/9 Assay

10 µl library
0.1 ml λ dil
0.2 ml 1046
20' 37°
0.5 CY
50' 37°

Plate 150 µl x2 . 183, 204
50 µl x2 40, 46
10 µl x2 2, 6

Numbers a bit inconsistent
Taking 50 colonies / 50 µl, then 800 / 10 µl
= 48,000 total

29/9
Ligation of N2 pSau3A (17/9.3) and LoR6 for EcoK⁻ packaging

Ligation of 24/9/86

EcoK⁻ packaged (Stratagene)

+ 300 λ dil.

Assay.

5 μl package
0.1 ml λ dil
0.2 ml 1046 o/N (not U.V. recA assayed)
20' 37°
+0.5 ml CY
50' 37°

Plating 2x 0.15 ml 220, 280 250
0.05 ml 65, 90 75
0.01 ml 4, 9 7

Say 230/0.15 ml = 1200/5 μl ≃ 70,000 total.

(f/p's of 200 of these showed ~ 1:4.8 ribosomal)

15/10/86
14cm plating of 29/9 LoR6/pS3A EcoK⁻
0.4 ml λ dil
0.15 ml LoR6 EcoK⁻ library (½)
0.8 ml ED8767
20' 37°
1 ml CY.
50' 37°
0.4 ml x 6 14cm 30 μg/ml Kan/TYE plates
37° (9.30 → 11 pm)

Subsequent count from probed film showed ~2400/plate

23/9/86

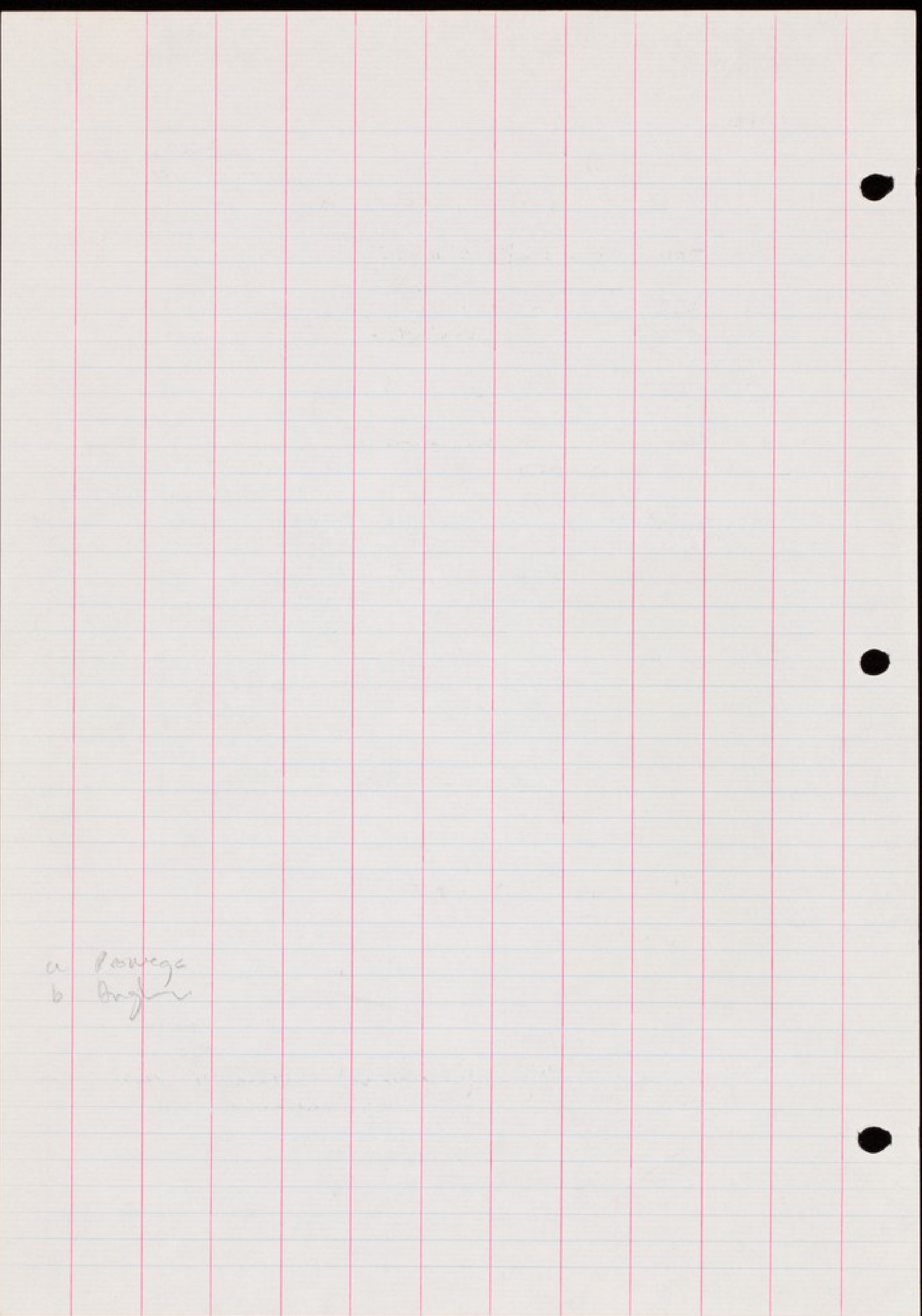
Attempted recovery of colonies from -70°
glycerol cultures without thawing
i.e. scraping of surface by toothpick

8 clones C08D7
C14E7
C17E1
C18G6
C26F5
C31G1
C37E1
C47B7

C08D7, ~~C14E7~~ and C26F5 failed to grow.
C14E7 gave v. poor blob in streak
C17E1 and C18G6 gave ½ doz small colonies
Thaw these, dibble (to bottom of tube, into pellet) and streak.
Mix, Refreeze and try subsequent surface scrape

All gave adequate streaks from thawed tube

C08D7 and C26F5 gave a few colonies from
scrape of refrozen mixed tubes;
C14E7, C17E1 and C18G6 gave v. heavy streaks
from ~~the~~ scraping



a Pouvega
b Anglia

9/10/86

Preliminary expts. for SP6/T7 probing.

2 x 4 ml 'T' series clones grown

1 T01C6 (unc 86-3 left)
2 T23C10 (" right)
3 T05B2 (Lin-12 left)
4 T05G5 (" right)

Giving 4 x 25 μl each final.

Purification for transcription.
(RNase / phol / ether / EtOH).

1 x 25 μl each
2.5 μl 10xH
1 μl 10mg/ml RNase
30' 37°.
(Ice 30')
2 x 15 μl phenol
1 x ether (aqueous v. 'milky')
NaAc, EtOH -20° 60'
Looks v. 'salty'
Dry
+ 100 μl 0.3M NaAc
200 μl EtOH
-20° 30'
70% EtOH wash
Looks much cleaner
1, 2 + 10 μl H_2O
3, 4 + 10 μl TE

1 μl for 0.4% ag. minigel.

Note: 1) material apparently stuck in wells
2) RNA still present

But since appears much cleaner from ppt., try transcribing anyway.

OVER

Control DNA's:

14/10/86

T01C6, T23C10 minipreps repptd + 10µl TE.

[20µl reactions; 5µCi 400 Ci/mol α32P UTP)

4 H_2O
4 5x transcription buffer
0.2 10mg/ml BSA
2 0.1M DTT
0.5 (12.5 u) RNasin
4 2.5 mM rNTP's (A, C, G)
5 DNA
0.5 (5µCi) α32P-UTP 400 Ci/mmol)
5 u SP6 or T7 pol (0.2µl or 0.1µl respec.)

37° ~~40°~~ 60'

a) T01C6 purifd.
b) T05B2 "
c) T01C6 straight miniprep
d) T05B2 "

1 x $CHCl_3$ extn.
Ice.

1µl TCA pptn.

T01C6 'purified' 18.5% incorpn
T01C6 'crude' minip. 20% "
T05B2 'purified' 31% "
T05B2 'crude' 42%.

PROG 10

CNT CH 1 1 TIMES
SCR =NO
AQC =NO
CALC= 1
PST = 1.00 MIN
CH 1 1.00 2 SIGMA %
0 LL
1000 UL

POS	CH 1	2S%	TIME	
231	881620.0	.9	.05 a°	18.5%
232	163788.0	.9	.25 a	
233	306435.7	.9	.14 b	31%
234	868840.0	.9	.05 c°	
235	171225.0	.9	.24 c	20%
236	939660.0	.9	.05 d°	42%
237	393000.0	.9	.11 d	
238	990380.0	.8	.05 b°	

(Should have done obvious control - transcripn of non-promoter cosmid. Try this; also run gel of transcripts).

15/10/86

λ
33d e e

4hr expos.

15/10

Control for 14/10 T7 transcriptions.

C49A2 (pJ38 clone)
minIprep repptd.
+ 10 µl TE

e) 5 µl taken for T7 transcripⁿ as 14/10.
37° 60'
1 µl for TCA pptⁿ (after CHCl₃ extⁿ 1X)

C49A2 (no promoter, crude) 0.8% incpⁿ

```
PROG 10

CNT CH  1      1 TIMES
SCR =NO
AQC =NO
CALC=          1
PST =       1.00 MIN
CH 1        1.00 2 SIGMA %
               0 LL
            1000 UL
POS       CH 1    2S%   TIME

 47   782616.6    .9    .06
 48     6647.0   2.4   1.00
```

1 µl a - e 4% TBV gel

(a - e [a ballsed-up loading] 1 µl + 2 for [illegible] 3'80°
marker e " " "]
a - e 1 µl + 1 µl dyes).

RNaseD / pol samples look degraded.
'crude' miniprep transcripts look pretty good
- products up to ~ 1500 b.p.
Try transcribing LiCl pptd. minipreps.
25 µl minipreps (TOKG, TOTB2 9/10/86)
+ 175 µl H₂O
+ 200 µl 5M LiCl.
Ice 60'.
4' spin
+ 400 isopropanol
-20° 20'
4' spin ; EtOH wash ; Dry

+ 10 TE

over

T01C6 LiCl
T05V32 LiCl
T01C6 crude
T05V32 crude
10ng λ

20/10

T7 transcripⁿ of T01C6 (a), T05V32 (b) LiCl
as 14/10/86.

a) Promega RNasin
b) Anglian ".

1µl for TCA pptn
1µl for 4% acryl. gel
Also 1µl EtOH pptd 14/10 c, d (+15' TC final)

T01C6 LiCl 38% incorpn
T05V32 LiCl 43% –

why total counts reduced by 1/2?

Looks pretty good.

PROG 10

CNT CH 1 1 TIMES
SCR =NO
AQC =NO
CALC= 1
PST = 1.00 MIN
CH 1 1.00 2 SIGMA %
0 LL
1000 UL

POS	CH 1	2S%	TIME
275	535225.0	.9	.08
276	202790.0	.9	.20
277	531375.0	.9	.08
278	230483.3	.9	.18

22/10

Probing of 16/10 A1 filter set with T01C6 LiCl/
T7 probe (20/10/86)

(T01C6 looks to give more 'specific' product,
than T03B2, in both 20/10 and 14/10 expts.)

(16/10 A1 filter set is low density pS3A/LORIST6
EcoK+ library (24/9/86)),
Filters U.V. exposed 3').

Probing as 4/10/85 protocol
Entire transcript used (unincorporated label not
removed).
As protocol except 1 tube, Anglian vanadyl
nucleotides added to 150 ml hyb. mix.

23/10/86

Probing of 16/10 A2 filter set with

T01C6
T05B2 } (20/10 transcript – kept at -20°C)
T0595
T23C10 } T7 transcripts

T0595, T23C10 LiCl purified as 15/10

5 μl of these (1/2) and remaining half of T01C6 from 15/10 T7 transcr. Ded as 14/10/86. (Promega RNasin)
(1 μl retained for TBU gel.)

Hybrdn as 14/10/85 (vanadyl nucleotides had turned hyb mix blue rather than original green after 48 hr at 4°C) (prob better to add van. nucs. only to solution to be used)

Washing 1x SCP/1%SDS 4x 10' 50° → r.t.
except 2A2, 3 washes 1xSCP/1% 10' 50° r.t.
plus 10' 65° 0.5x SCP/1%SDS

Quick rinse 3mM Tris Cl pH8.0

(JES picked +'s from 22/10 and 23/10 probings.
('+'s from 22/10 not visible in 23/10 – too much cumulative background?)
= ZK15 – 46.

→ ZK17, 27 UNC86L (T01C6) internal
ZK29, 30, 34, 35, 37, 38, 42 UNC86R (T23C10) external

both from T01C6 individual probing (22/10)

27/10/86.

Probing of 16/10 B3 filter set with
TO5B2, TO5g5, T2SC10 LiCl T7 transcripts

Filters 3' U.V.

5 & 6. soaked in 1x SCP 1% SDS
Colonies rubbed off with gloved finger
Washed 2x in 1x SCP 1% SDS
Quick wash in 3mM Tris pH 8.0
Dried. (4 hr r.t)

Probes prep'd as 14/10/86.
Combined.

Hybdn.
2.5 ml 100x Denhardts (Filtered 0.45μm)
12.5 20x SSC
0.5 10% SDS
0.5 10mg/ml SSDNA denat.
25 formamide
1ml vanadyl nucleotides
8 ml H_2O

18 ml added to filters, sealed in bag (30' 50° prehybdn.
('Soln not heated before adding).

Probe water bath 100° 5'.
Added to 18 ml hybdn mix
Added to filter bag.
50°C 16 hr.

Washing
1,3 10' in 2x SSC + 2μg/ml RNase A
Then
1,2,5 3 x 10' 2x SSC 0.1% SDS r.t
1 x 20' 0.2x SSC 0.1% SDS 65°
Quick rinse 3mM Tris pH 8.0

3,4,6 4x 1x SCP 1% SDS 50°C → r.t
Quick rinse 3mM Tris pH 8.0

(RNased filters washed separately from others). OVER

~~5ES picked ti from 23/10 and 27/10 probings~~

3/11/86

SP6 probes

TO1C6
TO5B2
TO595

LiCl's

<u>Mix</u>

16 H_2O
16 5xTB
0.8 BSA
8 DTT 0.1M
2 RNasin
16 rNTP's 2.5mM
2 α UTP32

15 mix
5 DNA
0.2 30u/μl SP6 pol.

60' 37°

1μl for ~~[illegible]~~ 4% TBU gel

– Reaction appear o.k., but v. weak (5 – 10x down re. similar T7 reacn).
Try i) increase nucleotide concn
ii) longer time of incubation.

(see 11/11)

11/11/86

Further attempt SP6 transcription of LiCl purified cosmids.

1 T01C6
2 T05B2
3 T0595

a) Increase (→10mM) rNTP's and 2x label and 2x enzyme
b) As a, + 4 hr incubn

Mix
14 H_2O
16 5x TB
0.8 BSA
8 DTT 0.1M (fresh)
2 RNasin
16 10mM rNTP's
4 αUTP32

2x 7.5 mix
2.5 DNA
~0.2 30u/μl SP6 (x2)

a) 60' 37°
b) 4.5 hr 37°.
O/N -20°

4% TBU gel (1a, 1b, 2a, 2b, 3a, 3b)

– Washout
– only faint streaks on gel.

13/10/86

Prep. of LORIST2/N2 Sau3A library for probing

(27/9 LORIST6/Sau3A library was 20% ribosomals)

1.5 µl LORIST2 Bam phase (Toby) (~0.3µg/µl)
4 µl 17/9 #3 frags.
2 10×C
2 rATP 10mM
2 0.1M DTT
6.5 H_2O
2 DB blunt ligase (J&S)

O/N 14°C.

10µl for Stratagene EcoK⁻ packaging
+ 300µl λ dil

5µl package
0.1ml λ dil
0.2ml GD8767 (u.v. o.k.)
20' 37°
0.5ml L/
50' 37°
Plate 150µl ×2
50µl : 18, 33 say 25
10µl : 2, 8
= ~~20,000~~ 24000 total.

14/10
Packaging repeated on remaining half of ligation.
+ 300 λ dil.
Assay as above.

150µl ×2	60, 70		
50	16, 23	say 20	
10	4, 5		

≃ 20,000.

t/p's gave ~50% ribosomals!

17/10

Ligation 5C3 Mbo frags / TG LORIST (LOR2)
- more cosmids for filter library
- as 7/7/86 i.e.:

1 TG LORIST -2
2 N2 Mbo (5C3)
2 rATP 10mM
2 0.1M DTT
9 H_2O
2 DB ligase
o/N 14°.

½ for stratagene EcoK⁻ packaging.
+ 600 λ dil final

Assay on 1046 and ED8767 (1046 fresh o/N sub
ED8767 2 days old)

10 μl library
0.1 λ dil
0.2 bugs.
20' 37°
0.5 CY
50' 37°

Plating 2x 100 μl

1046 120, 132 126 = 600500 ~~total~~

ED8767 60, 72 66

Librarys as at 1/11/86

EcoK+

pJB8	R1	21/8/85	200 μl	
pJB8	Rc2	6/11/85	1000 μl	
pJB8	R1	28/2/86	400 μl	
LORIST B	Mbo	28/4/86	200 μl.	K's
TG LORIST B	Mbo	7/3/86	500 μl.	← T's
TGL/Mbo (7/3)		3/5 pack.	1000 μl.	(→ Marty)
pJB8	Mbo	24/6/86	500 μl	
LORIST 2	S3A	4/11/86	600 μl	
LORIST 6	S3A	24/9/86	600 μl.	

EcoK−

pJB8	Mbo	24/6	300 μl	E's
LORIST B	Mbo	25/7	500 μl	(see 25/7/86)
LORIST 2	Mbo	26/7.	400 μl	← F's
LORIST 6	S3A	30/9/86	200 μl	F29 → (see 4/2/87)
LORIST 2	S3A	14/10/86	300 μl	
LORIST 2	S3A	15/10	400 μl	
LORIST 2	Mbo	17/10/86	600 μl.	F25, 26

Ligations −20°

pJB8	Mbo	20/2/86	
LOR2	Mbo	25/7/86	← F's
LOR2	Mbo	17/10/86	
LOR2	S3A	4/11/86	

22/10/86

Assay of 7/3/86 LORIST2/Rho Eco K+ library

50 µl λ dil
1 µl library
100 µl 1046 or ED8767
20' absorptn
0.25 ml CY
50' expression

Plate 2 x 100 µl each (30 µg/ml Kan plates (fresh))

1046	38, 44	41	160/µl	≏ 30,000 total
ED8767	30, 32	31		≏ 60,000 "

28/10/86

Platings

30/9 LOR6/Sau3A Eco K-
14/10 LOR2/Sau3A Eco K-

5 µl library
0.1 λ dil
0.2 1046 (fresh o/N U.V. O.K)
20' 37°
0.5 CY
50' 37°

LOR6	Plate	4 x 50	8	total!
LOR2	"	4 x 150	26	" !

(Also no phage control → 0
Something badly wrong!
These were new batch of plates.
Repeat plating (see over leaf)

29/10/86

LoR2 & 6 Sau3A libraries
Also 14/11 pS38 / Sau3A library (bug control)

5 µl library
0.1 ml λ dil
0.2 ml bugs
20' 37°
0.5 ml CY

30' 37° Amp
50' 37° Kan.

Amp - plate 1 x 150 µl

Kans - plate 1 x 150 µl a) Ac plates (as previous)
b) N.Dibb "

~~pS~~ pS38 / Amp O.K.

LoR2 & 6 — virtually zero! (Threw plates before counting)

3/11/86.

Repeat attempts at LOR2, LOR6/N2pS3A (AC 22/9) ligations & packaging (EcoK+) and f/p'. (Previous efforts gave v. high ribosomal count and then apparently died).

- LOR6/pS3A EcoK+ already exists: 24/9/86 (not f/p'd).

LOR2/pS3A lig^n.

1.5 TG LORIST2 Bam phase
4 N2 pS3A frags (22/9/86)
2 10XC
2 rATP 10mM
2 0.1M DTT
9 H2O (should have been 6.5)
2 DB ligase.

o/n 14° (Package 1/2 EcoK+).

Assay
0.1 λ dil
2 μl library
0.2 1046
20' 37°
0.5 CY
50' 37°

uv bugs good

Plate 150 μl

LOR2 : 2 3000?
LOR6 : 10 15000?

10/11/86

Plating LORIST6/Mbo EcoK⁻ 25/4/86

0.1ml } dil
10μl library
0.2 1046 } (3/11 growth; U.V. ok)
20' 37°
+ 0.5ml CY

Plating 150μl (Gave 140 colonies when fresh)

200+ colonies/plate (v. variable in size)
(60,000+ ~~total~~)

5/11/86

Preliminary assay of 'T' bank for probing plating

a) 50 μl λ dil
1 μl library 7/3 LORZ/Mbo EcoK+
100 μl 1046
20'
0.25 CY
50'

Plate 2x100 μl = 30, 36 = 60,000 total

Also little method for large-volume phage stock plating

1 ml bugs
1 μl library
20' 37°
2.5 ml CY (37°)
50' 37°
Spin 5' 3K IEC
+ 400 μl CY

Plate 2x100 μl = 0.

11/11 Trial large-scale plating (1 x 14cm plate)

80 μl library (7/3 LORZ/MBO) U.V. bugs O.K.
200 μl 1046 O/N
20' 37°
200 CY
50' 37°

All spread – only 600 colonies ~~total~~!

12/11/86

(ε 10K⁻)

Assay of 17/10 Lac Z/Mbo library - various conditions (with view to large scale plating for filters.

In Eppendorf tubes, 37° oven.

1. Normal small scale

25 µl λ dil
2.5 µl library
50 µl 1046 — 22
20'
125 µl CY.
50'

2. As 1. but 40' absorption — 60

3. No λ dil

75 µl bugs
2.5 µl library — 0
20'
125 µl CY — Error?
50'

4. Proportional large scale

5 library — 60
10 bugs
20'
13 CY
50'

5. As 4 but TYE not CY. — 46

13/11/86

Packaging (EcoK+) of remaining ½'s of previous ligations

~10 μl 25/7/86 or 17/10/86 LOR2/Mbo lig^n.
1 μl 0.1M r ATP
20 μl CH buffer

to 50 μl 9/7 pack mix.

Assays

	Various LOR2/Mbo librarys			/μl
1.	7/3/86 EcoK+	11, 16	13	21
2.	17/10/86 EcoK−	5, 5	5	8
3	26/7/86 EcoK−	3, 6	4	7
4	13/11/86 (17/10 lig^n) EcoK+	15, 16	15	24
5	" (25/7 lig^n) EcoK+	15, 24	19	30

100 μl λ dil
5 μl library
200 μl 1046
20' 37°
500 μl CY
50' 37°

Plate 2 x 100 μl

12/11/86

RecD plating

100 μl λ dil
5 μl 21/8 pS38/N2R1 library
200 μl O/N DB1317
20' 37°
400 μl CY
30' 37°

Plating 100 μl x 7.

(These will be R16 →)

~350 total

14/11/86

Probing of old filter sets (to test for viability) with previous used probes from Caterina. (used successfully on low density filterset (14/10)

Set	Probe
R23/1 (1)	PC#1
11/12 (3)	C20.11A

16/11/86

Assay various librarys using new 1046 bug growths.

Bugs. a) AC 12/10 plate colony
b) SES 15/11 " " (small) (fresh from -20 pot)
c) " " " " (") " "
d) " " " " (large) " "

u.v's all OK.

		colonies
a)	7/3/86 LORZ/Mbo Eco K+	125
b)	17/10/86 " " Eco K-	90
c)	13/11/86 (25/4 lig-) " " Eco K+	155
d)	25/7 LORB/Mbo	120

0.05 ml λ dil
2 μl library
0.1 ml 1046 growth a.
20' 37°
0.25 ml C Y
50' 37°

100μl plated

e)	as c, 1046 growth b.	150
f)	" " " " c.	175
g)	" " " " d.	74
h)	" " " " AC week old subculture	10
i)	" " , CY + 10mM $MgCl_2$	125

Looks like AC. subculture 'off'.

17/11/86

Trial 14 cm platings LORZ/Mbo libraries
a) 17/10/86 EcoK⁻ (F25, 26)
b) 13/11 (25/7 lign) EcoK+

75 μl λ dil
35 μl a) or 20 μl b)
150 μl 1046 SCS/c (see 16/11)
20' 37°
0.2 ml CY
50' 37°

All plated
5.30 → 9.00 am

(+20%)
Look about right density possibly slightly low.
Slightly overgrown at 15.5 hrs.

18/11/86

6 x 14 cm plating of 17/10/86 EcoK⁻ LORZ/Mbo.

0.45 ml λ dil
0.25 ml library
0.9 ml 1046 SCS c (see 16/11)
20' 37°
0.9 ml CY
50' 37°

Plating 6 x 0.4 ml

2.4
1.6
0.9

21/11/86

o/n tube growths for probing clones

5ml 30μg/ml Kan.

			TBUg/gel	probing	Picking
1	K06H3	.	ok	18/11 A1	
2	K07F5	.	ok	18/11 A1	2K 112-126
3	T13G10	.	ok	18/11 A1	
4	T20G3	.	ok	18/11 A2	
5	T22C9	.	ok (low bkgd)	18/11 A2	2K103-111
6	K10B6	.	ok	18/11 A2	
7	K11E5	.	ok	18/11 A3	
8	T05A7	.	ok	18/11 A3	2K92-102
9	T07B11	.	ok (long transcript)	18/11 A3	
10	T15F9	.	ok	18/11 A4	
11	T20A7	.	ok	18/11 A4	2K57-91
12	F13C11	.	ok (low bkrd)	18/11 A4	
13	W06F8	.	ok	18/11 B3	
14	T01C6	.	weak	18/11 B1	
15	T05G5	.	weak	18/11 B1	
16	T05H7	.	blank	—	
17	T04D10	.	ok	18/11 B2	
18	T22H8	.	long transcript	—	
19	F01G10	.	ok	18/11 B2	
20	F11G11	.	sl weak	18/11 B4	
21	F12A10	.	ok	18/11 B2	
22	F13E12	.	not loaded	18/11 B3	
23	F18E7	.	sl weak	18/11 B4	
24	W02D1	.	weak	18/11 B4	
25	W07H7	.	ok	18/11 B3	
26	T05B2	.	blank	—	

2x 2ml alkali lysis
1x EtOH ppt
+ 0.2ml H_2O
0.2ml 5M LiCl
60' 0°
4' spin
+ 400 iso-propanol to super.
20' -20°
2' spin
EtOH wash ; dry ; + 10μl TE

24/11/86

Prep^n of T7 probes from 21/11 DNA preps.

Mix: 110 H2O
105 5x transcript buffer
5 10 mg/ml BSA
50 0.1M DTT
10 RNasin
105 2.5mM rNTP's
13 (130 µCi) α32P UTP 400 ci/mol
3 µl T7 pol.

15 min
5 DNA

60' 37°
→ ice

1 µl each + 1 µl dyes for 4% TBU gel
(Gap over 10; #22 lost).

– Mostly fairly similar, except 14-15 (T01C6, T05G5) rather weak
(UNC86L, LIN12R)
16, 26 (T05H7 and T05B2) didn't work.
(SUP5L, LIN12L)

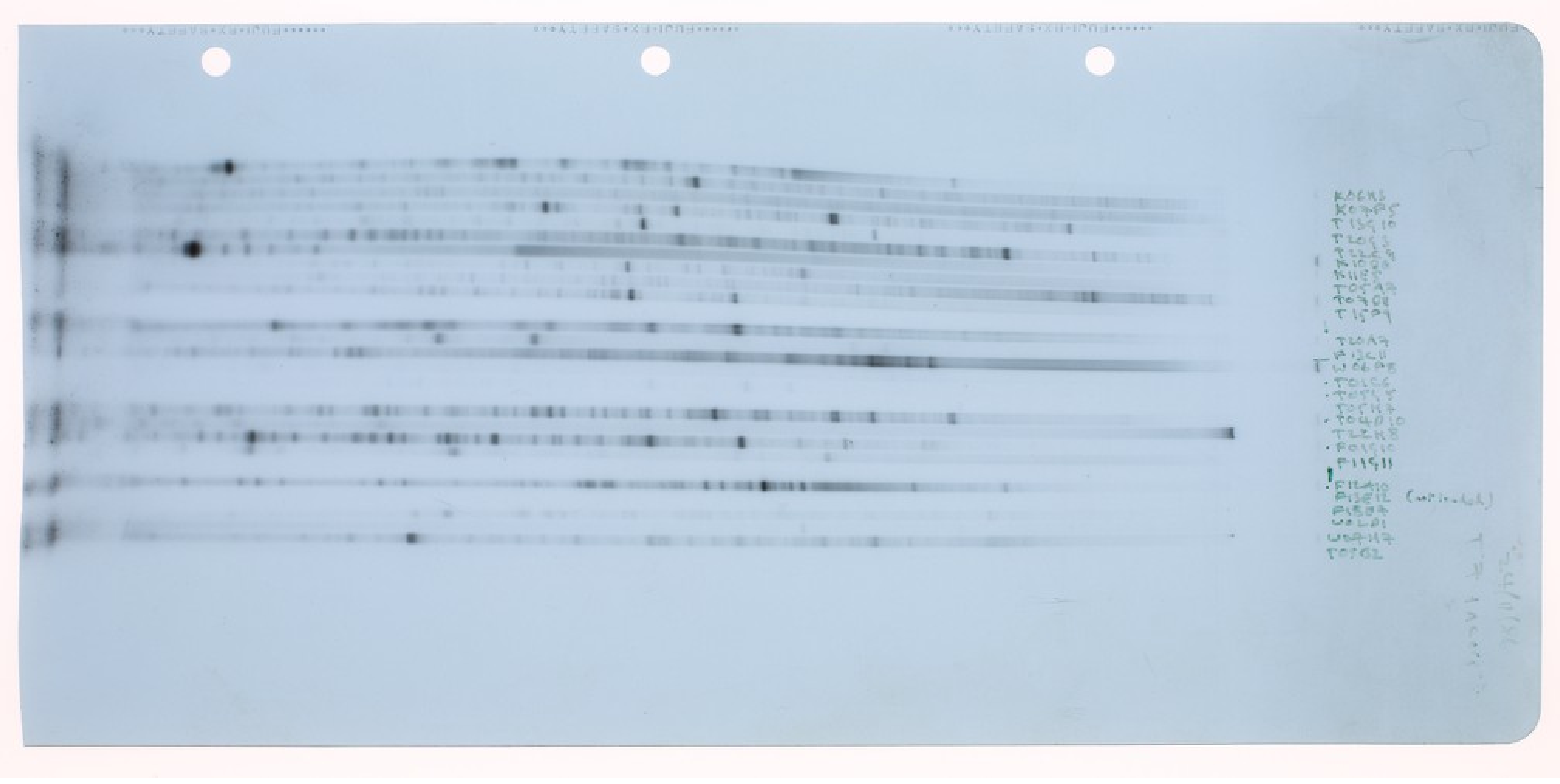
Probing of 18/11 A1-4 filter sets with mixtures of 3 above probes.

a, b) Sets 1, 2 Probes K06H3, K07F5, T13G10 (set 1)
" T20G3, T22C9, K10B6 (set 2)

Usual 4/10 probing conditions. (+ vanadyl nucls)
(Hyb. mix spun 3K 10' I.E.C.)

c) Set 3 Probes K11E5, T05A7, T07B11
As a and b except + poly A, poly C 10mg/ml
(+ 0.02ml 20mg/ml)

d) Set 4 Probes T15F9, T20A7, F13C11
As a and b except + Yeast RNA (Na) (#1590360)
(+ 0.2ml 10mg/ml = 50mg/ml.



24/11 (contd.)

(Extra goodies i.e. poly A, C and yeast RNA added to H_2O/probe/ssDNA prior to 10' boil).

O/N 68°.

4x 1XSCP 1% SDS washes 50° → r.t.
1x ~~5~~ 5mM Tris pH 8.0

(Repeat ~~T01C6, T05G5~~, T05H7, T05B2)
T12H8 (v. long) (blank) (blank).

— Background on all filters v. high
(Also some apparently not washed properly).
but yeast RNA looks best.

26/11/86

More trial probing conditions.

1) T01C6 / T05G5 (both weak transcripts)
4/10/85 hybdn condition + 500μg/ml yeast RNA
~~Filter set~~ 18/11 B1

(A) Prehyb. soln: 18 H_2O
150μl 10mg/ml ss DNA
36 mg yeast RNA (Na)
10' boil
↓
9ml prehyb
+ 9ml hyb. mix (dextran sulphate etc)
→ filters

(B) 9 ml H_2O
75 μl 10mg/ml ssDNA
probe
10' 100°
+ 9 ml hyb mix
→ filters

26/11 (cont.)

F12A10 / T04D10 / F01G10 filter set 18/11 B2

As B1, except prehyb. containing 2% SDS.
(1% SDS final?)

O/N 68°

Washing

4 x 68°C 20' 0.5 x SCP 1% SDS

27/11

Probing of 18/11 B3 with W06F8, W07H7, F15E12
" " 18/11 B4 with F11G11, F18E7, W02D1

As 18/11 B2 (26/11) i.e. + SDS and 500 μg/ml yeast RNA
but prehybridising at 68° for 1 hour.

– awful – nothing to pick.

(From this set, the following definitely need repeating
i.e. gave no pickable +'s or no T7 incorp^n
T05H7
T05B2
W06F8
W07H7
F15E12
F11G11
F18E7
W02D1
)

25/11/86

Assay bug culture prior to repeat 14cm plating of 17/10/86 EcoK⁻ Bank.

1046 colony from JES 14/11/86 plate
O/N.

~~0.05 ml~~ 0.05 ml d dil
2 μl 17/10/86 library
0.1 ~~ml~~ ml 1046 24/11
20' 37°
0.25 ml CY
50' 37°.

Plate 100 μl
(Should give ~100 colonies) (see 16/11/86)
Actually 60 prob ok.

27/11/86

6 x 14 cm platings of 17/10/86 EcoK⁻ corr/lib

0.45 ml λ dil
0.30 ml library
0.9 ml 1046
30' 37° (H_2O bath).
1.0 ml CY
60' 37° (H_2O bath)

(v.v. OK)
(assay OK)

Plate 6 x 0.4 ml (1 soaked in v. slowly - 10'
others pretty fast 2'-3').

(6.30 pm → 10 am)

28/11/86

Growths for riboprobes.

1 K07H8
2 K08E7
3 K09F8
4 K11G4
5 E02G7
(E04E1 didn't grow)
(F02B7 " ")
6 F07A5
7 F07B11
8 F08B5
9 F10E5
10 F15D7
11 F17E9
12 F21D5
13 T09A12
14 T10H5
15 T13C12
16 T16B2
17 T16H8
18 T19G9

2/12
T7 transcripts (as 24/11/86)

		Results of gel
Repeats of 21/11 growths	T05H7	too weak
	T05B2	too weak
	W06F8	poss (high MW Bkgd)
	W07H7	OK
	F13E12	OK
	F11G11	weak
	F18E7	too weak
	W02D1	poss.
28/11 growths	K07H8	good
	K08E7	good
	K09F8	poss
	K11G4	high MW Bkgd

For probing, combine a) W07H7 (4), F13E12 (5), K07H8 (9) (take 1/2)
b) F11G11, K08E7, K09F8

Probably forget rest.

3/12/86

Probing of 23/1 4 filter set with T7 riboprobes

(Probing of pJB8/R1 cosmid, to find out if high background on 10/11 filters is due to vector binding of some sort).

(These filters last used for JH98503 17/4 probing - dont think they have been boiled and should be O.K.).

Basic 4/10 hybridis^n with mixture of 1/2 of W07H7, F13E12 and K07H8 T7 28/11 transcripts.

+ vanadyl nucleotides
hyb mix spun 3K 10' prior to use

4/12/86. — forgot to U.V.!

Probing of 27/11 B4 (cos2) filter set with T7 riboprobes
- higher temp hyb^d - 75°C
- higher temp wash - 65°C?

Sol^n A) 9 ml H_2O
20 mg ~~ribosomal~~ yeast RNA

Sol^n B) 9 ml H_2O
1 ml vanadyl nucleotides
5 ml W07H7, F13E12, K07H8

A & B 10' 100°; ice 10'
+ 9 ml dext. sulph. hyb. mix

75° o/N
Bag not allowed to cool; opened under 0.2xSCP 1% SDS at ~70°C
3x wash in 0.2xSCP 1% SDS 70° → r.t.
1x brief wash 3mM Tris pH 8.0

11/12/86

Probing of 27/11 B1 & B2 filter sets.

B1 W07H7 F13E12 K07H8 (same as 4/12/86 which apparently gave 1 strong +ve)
(gel - remains - of these clones, also used to probe #1 colonies 7/12/86 - no +'ves)

B2 F11G11 K08E7 K07F8

+ 50 μg/ml yeast RNA Na
+ variously nucleotides.

70° o/N (cf 4/12 75°)
4 x wash 0.5 x SCP (cf 4/12 0.2 x SCP)
65° → r.t. (cf 4/12 70° → r.t.)

(Bags opened under SCP 0.5x at ~65°).

(Flashed film ; screen ; -70°)

10/12/86

f/p ZK 57 etc → ZK164 (64, 83 missing)
123, 125
136, 145
155, 161

1-30 no RNase
31-60 + 1/10th std RNase (0.3 μl 10mg/ml RNase → 160 μl mix)

61-end + normal RNase (2.5 μl 10mg/ml RNase → 160 μl mix)

13/12/86

f/p ZK 106 - 158 (123, 125, 136, 145, 155 missing)

Also ZK 83
LH8 RG4 LE4 5D11 22E10

No RNase.

16/12

Linearisation of lorist cosmids prior to T7 transcript

Previously strong transcript W07H7 F13E12
" poor " K08E7 K09F8

Xba linearisation
5 DNA
1.2 high salt buffer 5X (Maniatis)
1 Xba 20u/μl 8/86
2h 37°
0.5 for agarose
– didn't cut at all.

Dilute + 10μl (5E3 mix)
H_2O 115
10X Hi 35
RNase 0.5
1M Spermidine 1

3 H_2O

1μl 20u/μl Xba 12/86

Also 2nd K08E7 digest
14 mix
5 DNA
1 12/86 10u/μl Xba

2h 37°.

Look O.K.

EtOH ppt x wash
+10μl H_2O

uncut (longer run)

W07H7
F13E12
K08E7
K09F9
K08E7(2)

50ng λ HIII

17/12

Transcrip of Xba-cut cosmids.

Mix
55 H_2O
52 transcrip buffer 5X
2.5 10mg/ml BSA
25 0.1M DTT
5 RNasin
52 2.5 mM rNTPs
7 UTP^{32}
1.5 T7 pol

15 mix
5 (½) Xba-cut DNA

Also 15 mix - T7 pol
5 K08E7(2)
0.2 SP6 pol

60' 37°

10µl removed → -20°

120' 37°

1µl each 4% gel.

Awful - couple of heavy bands right near bottom & v. faint streaks up after o/N exposure.

16/12/86

library assays

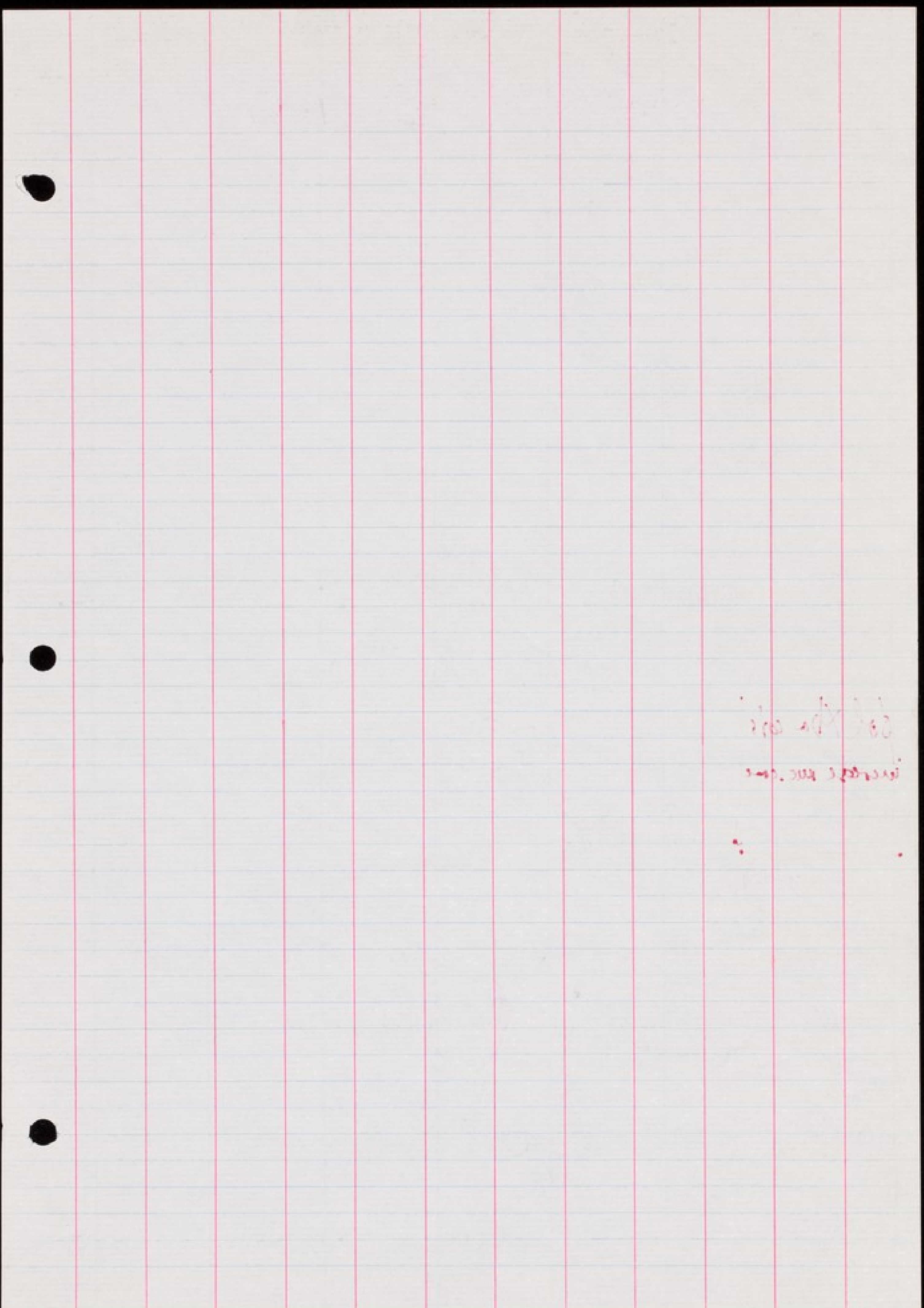
a) 13/11/86 (17/10) lig⁻) LOR2/Mbo EcoK+
b) " (25/7 lig⁺) " " "
c) 30/9 LOR6/pS3A EcoK⁻
d) 14/10 LOR2/pS3A EcoK⁻

0.1 λ dil
5 µl library
0.2 1046 ——— (small colony selected from plate of glycerol stock of small colony growth from 5 ES stock plate (see 16/11) U.V. OK Maintained small colony morphology on plating)
20' 37°
0.8 L
50' 37°

a, b 2 x 0.1ml plated

c, d 3 x 0.1ml plated

		TOTAL
a) 13/11 (17/10 lig⁻) LOR2/Mbo EcoK+	9	~8000
b) " (25/7 ") " " "	17	~16000
c) 30/9 LOR6/pS3A EcoK⁻	80	
d) 14/10 LOR2 " "	10	



2ml LiDs 13/11 (17/10 and 25/7 lig^ns)
0.1 λ dil
0.2 bugs
20'
0.5 CY
50'
Plating 150 μl

VS very small colony
S small colony

25/7 lig^n

1046 VS	64
1046 S	45
ED8767 VS	71
ED8767 S	50

17/10 lig^n

1046 VS	0
1046 S	2
ED8767 VS	70
ED8767 S	56

} 5 in Pries old plates (drier than others)
↑ ~3 weeks

60
5
300 /
150 / μl
15000
6
[90000]
15

150 | 5000
33 μl

pol Xba cos's
increase mic. conc

a) Spin down 1 ml bugs ED8767 VS
Resuspend in 0.1 ml λ dil
+ 15 μl library (= 2250 phage) (25/7 lig^n)
20'
+ 0.3 ml TYE
60'

400 tot

b) 0.2 ml bugs ED8767 VS
0.01 ml of mg SO4
+ 15 μl library
20'
+ 0.2 TYE
60'.

24/12 extra
C467
HH ϕ7 1, ϕ72
JH 6, 8, 9, 10

Spence
Hecht.

- All plated 14cm plats

HH M13

H_2O	60
10xH	17
RNase	.25
Spermid.	0.5

1 DNA
3 mix
0.5 R1
0.5 Bam.

19/1

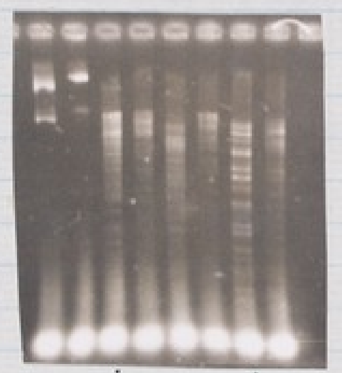
Hae III linearisation for SP6/T7 transcripⁿ

10µl Lill miniprep DNA
2 10×H
7.5 H_2O
0.5 Hae III 8u/µl.

F15D7
F17E9
F21D5
F07A5
F02B7
T09F12

2hr. 37°.

0.8% agarose gel
1µl F15D7°
1µl F17E9°
1µl digests.



Digestⁿ looks O.K.

1× phenol
EtOH pptⁿ ; EtOH wash ; dry
+10µl H_2O

OVER

	LOR2 posⁿ		Nearest Hae III site
(T7	5530	→	5509
SP6	40	←	319

(18bp → promoter)

SP6 & T7 transcript of HaeIII digested cosmids (previous page)

Mixes

H_2O	20	
5x transc. buff.	20	
10mg/ml BSA	1	
0.1M DTT	10	
RNasin	2.5	
10mM rNTP's	20	(A, C, G)
α^{32}P UTP	2.5	(400 ci/mol 10 μCi/μl)
SP6 pol	1	(30 u)
or T7	1	(70 u)

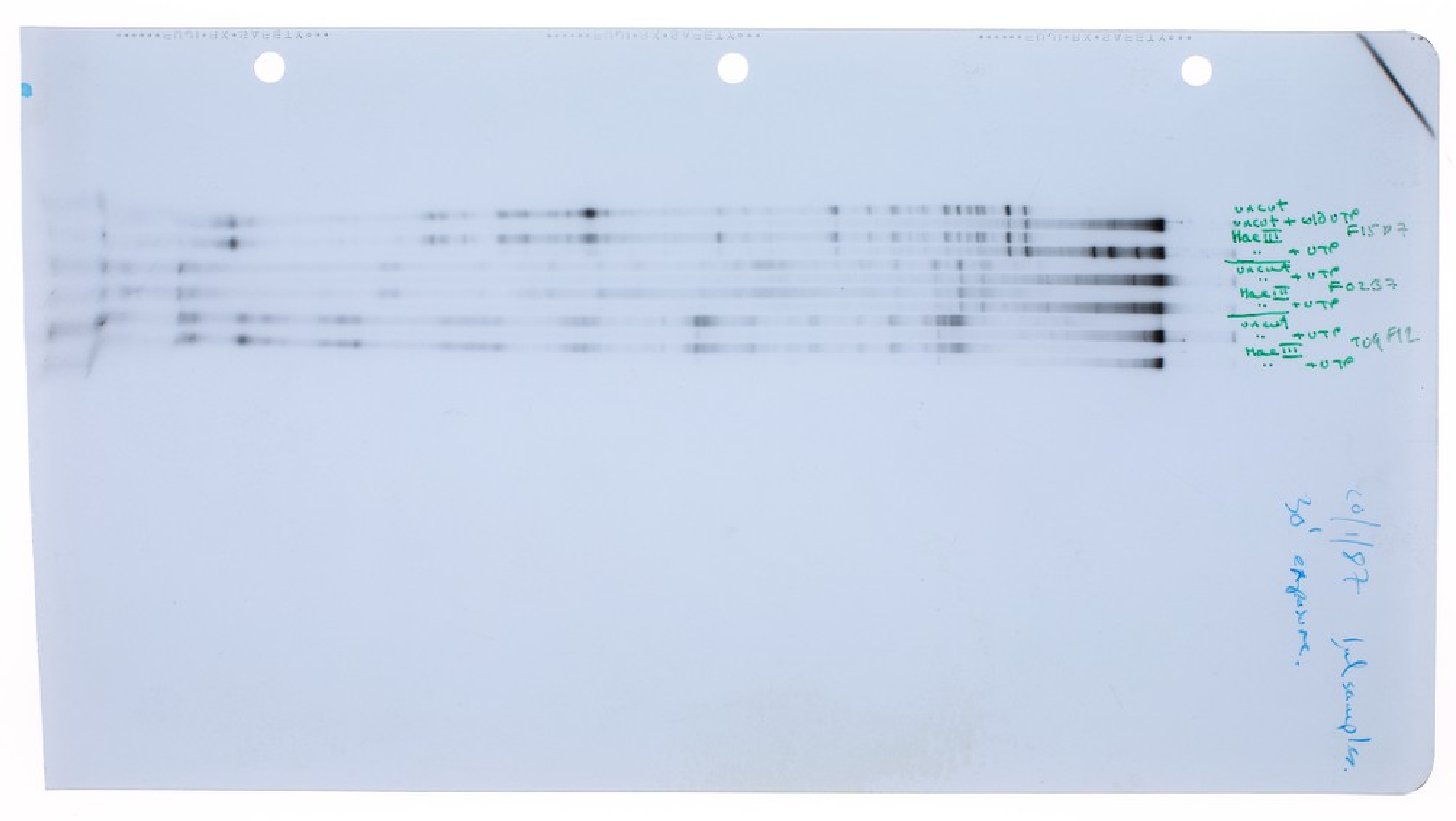
2.5 DNA (unused ½ of HaeIII digest in 'PROBE MINIPREP' BOX.)
7.5 mix

37° 60'

~~20° O/N~~

1μl for 4% TBU gel

– no incorpn.


uncut
uncut + cold UTP
F15D7
HaeIII
" + UTP
uncut
" + UTP
F02B7
HaeIII
" + UTP
uncut
" + UTP
T09F12
HaeIII
" + UTP
10/1/87 1ul samples.
30' exposure.

20/1/87.

Further effort at T7 transcripⁿ.

Changes

1) rNTP's pH'd
2) + cold rUTP to some reacⁿ.
3) uncleaved v. Hae III cleaved.

Mix		
	H_2O	20
	5×TB	20
	BSA	1
	0.1M DTT	10.
	RNasin	2.5
	2.5mM rNTPs	20
	$\alpha^{32}P$ UTP	2.5
	T7 pol	2

a) 6.5 mix
   2.5 uncleaved Li-miniprep DNA

b) 6.5 mix
   2.5 uncleaved Li-miniprep DNA
   1 cold 0.1mM UTP

c) 6.5 mix
   2.5 cleaved DNA Hae III 19/1

d) 6.5 mix
   2.5 cleaved DNA
   1 0.1mM UTP cold

× 3 cosmids
① F15D7
② F02B7
③ T09F12

(1a-d, 2a-d, 3a-d)

Promising! — reacⁿs + cold UTP v. long hot transcripts (few 'stops') but little apparent difference between intact cosmids × Hae III cut cosmids (except F15D7 which has some extra bands of 2Kb +) — Hae III digests could be partial or just long fragments including promoter.

Try better regulated different digests or JES idea to sonicate cosmids.

Better incorpⁿ in general — could be pHing of rNTP's or 2X T7 pol.

21/1
Cosmid miniprep sonication (for riboprobe prep.)

K07H8 15 μl total
T1999 20 μl total

5 DNA
+ 15 TE

Sonication in 1.5 ml Eppendorf
3 x 40" full power, ~1mm from probe (centre of radius of probe)

1% ag. gel

a) λH3 30 μg/ml 5
½ TE 2
½ dye mix 2

b) 1
½ 4 TE
½ 2 dye mix

c) 0.5 μl unsonicated K07H8
2.5 TE
1 dye

d) 2 μl sonicated K07H8
1 TE
1 dye

e) 0.5 μl unsonicated T1999
2.5 TE
1 dye

f) 2 μl sonicated T1999
1 TE
1 dye

T1999 looks deleted

g, h) as a, b

a h

K07H8, T1999 EtOH pptd
-70°C o/N
EtOH wash, dry
+ 5 H2O

22/1/86

Restriction digestion of cosmids for riboprobe transcrip^n
& parallel sonication expt.

a	K07H8	Rsa I
b	T1999	Rsa I
c	T16H8	Rsa I
d	"	Hae III
e	T16B2	Rsa I
f	"	Hae III

5 µl DNA
7 µl Hin / RNase / Spermidine
7 H_2O
0.5 Rsa I (10u/µl)
or 0.5 Hae III (8u/µl)

(H_2O 115
10x H 35
10mg/ml RNase 0.5
1M Spmd^n 1)

90' 37°
1 µl for 1% ag. gel

May have been better to omit RNase and keep RNA around during transcrip^n

λ H3 a f g h

g = 0.25 µl K07H8 sonicated after EtOH ppt^n
h = 0.25 µl T1999 sonicated after EtOH ppt^n

Looks O.K.

1x phenol
EtOH ppt & wash
+ 7.5 H_2O (recovery looks OK on gel of this).

(Sonication of C49A2 for control transcrip^n
C49A2 miniprep used as control 15/10/86
LiCl pptd; EtOH pptd & washed; dry; + 10 µl TE
5 DNA
15 TE
Sonicated as K07H8, T1999 21/1
Looks OK on 1% minigel; EtOH ppt, wash, dry + 5 H_2O)

26/1

SP6/T7 transcripts of sonicated and restricted cosmid DNA's (21/1 etc)

Mixes

H_2O	50 µl
5xTB	50
BSA	2.5
0.1M DTT	25
30u/µl RNasin	6
2.5mM rNTP's	50
α32P UTP	6.25
cold 1mM UTP	3

(pH~7.0)

95 µl + 5 µl SP6 pol

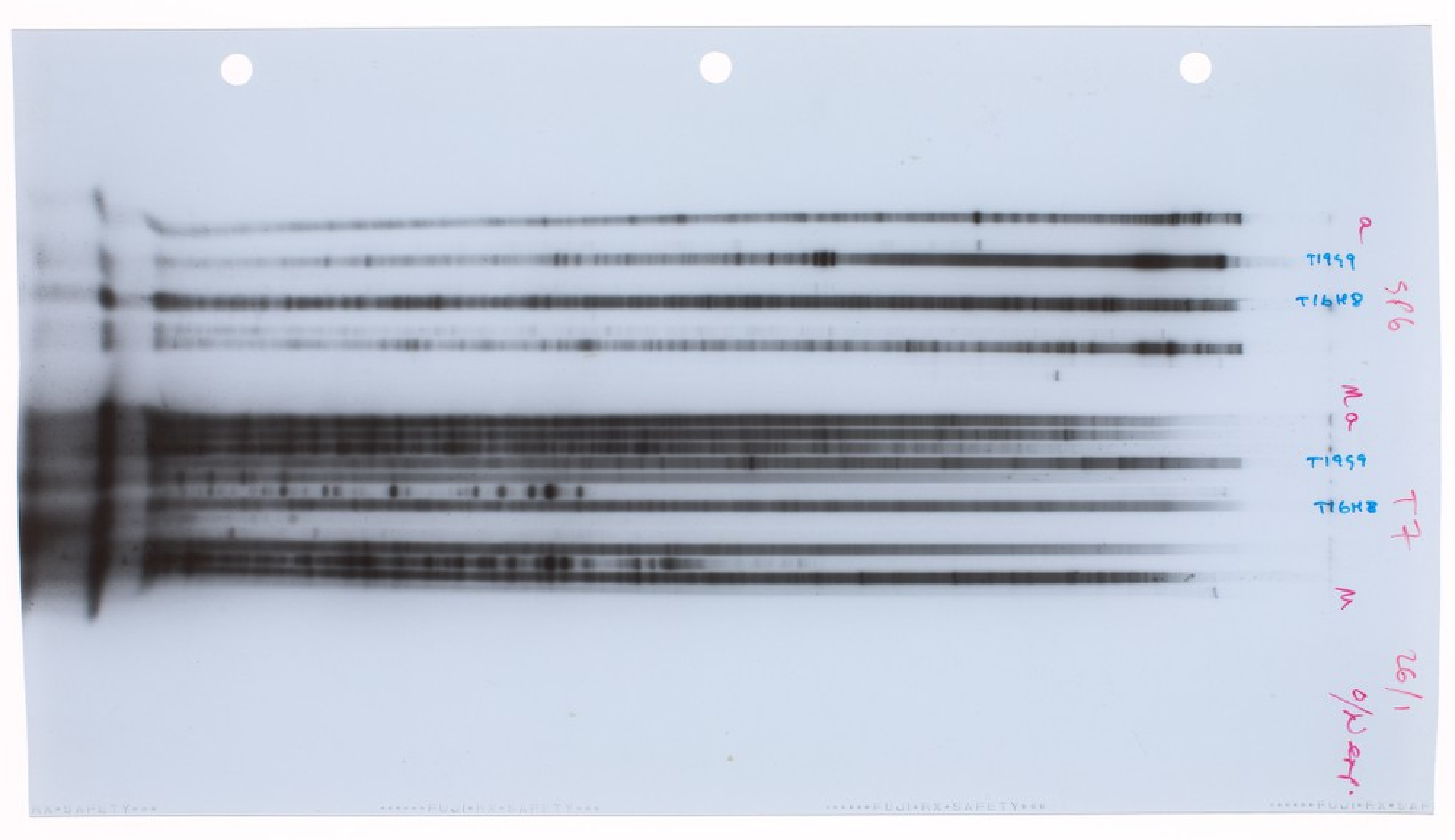
95 µl + 5 µl T7 pol

6.5 mix
2.5 DNA viz:

Duplicated for T7

a K07H8 uncut
b " sonicated
c " Rsa
d T1999 uncut
e " sonicated
f " Rsa
g T16H8 uncut
h " Rsa
i " Hae III
j T1682 uncut
k " Rsa
l " Hae III
m C49A2 sonicated (no promoter control)

60' 37°C
1 µl + 2 µl form./dye for 4% TBU gel



a
T1959
T1648
SP6
Ma
T1959
T1648
T7
M
26/1
o/n exp.
FUJI RX SAFETY

28/1

Probing of 18/11 A1-4 filter sets with T16H8 and T1959 SP6 & T7 riboprobes from 26/1

<u>NaOH 'unblocking' of filters:</u>

Each set submerged individually in 0.4N NaOH (250ml in sandwich box) 15'
Individually into 1.5M NaCl
0.5M Tris-Cl pH7.5 (500ml)
Then through 2 x SSPE
Air Dried. 3hr.

<u>Prehybdn</u> (as Amersham booklet p36)

i.e. 5 x SSPE
50% deionised formamide
5 x Denhardts
0.5% SDS
0.02mg/ml denatured SS DNA

(16 filters in 250ml prehyb. soln) in xtallising dish

50°C (occasional slight ~~agitation~~) ~~3 hr~~ 2hr.

<u>Hybdn.</u>

A1	SP6 T16H8	(26/1 SP6g)
A2	T7 T16H8	(" T7g)
A3	SP6 T1959	(" SP6d)
A4	T7 T1959	(" T7d)

Hybdn as Amersham booklet (p36)
i.e. in 5 x SSPE
50% formamide
5 x Denhardts
0.5% SDS
0.02mg/ml denatd. SS DNA
Also + 1ml/100 mix of vanadyl nucleotides (AST)

Probe not denatured.

o/N 50°C

OVER

Washing

2x SSPE 1% SDS 50°→r.t. 30'
" " " 15' x 2

Quick rinse 3mM Tris pH8.0

4 day -70° flashed film screen exposure.

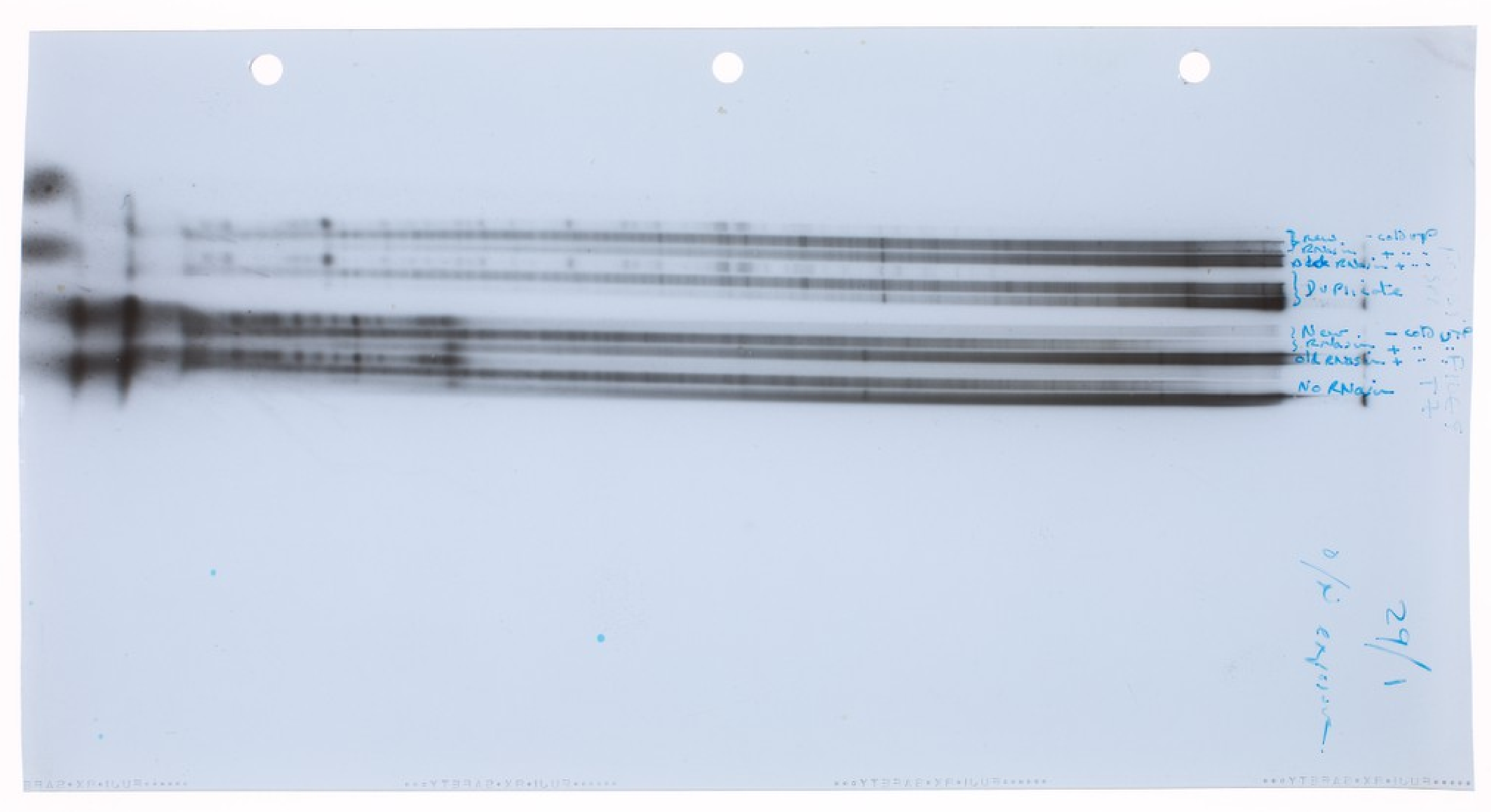
A1 — SP6 T16H8 — good looking clean +'s
No background
(This probably 'best looking' probe)

A2 — T7 T16H8 — blank - poss. one weak spot

A3 — SP6 T1999 — even background only

A4 — T7 T1999 — even background only.

John did probing of this (to ~~attempt~~ to provide background to +'s) but added too much probe. +'s probably swamped.


Duplicate
No RNasin
29/1
o/n exposure

29/1
Reassessment of effect of cold UTP on riboprobe transcription.
Also assay of new batch of Promega RNasin

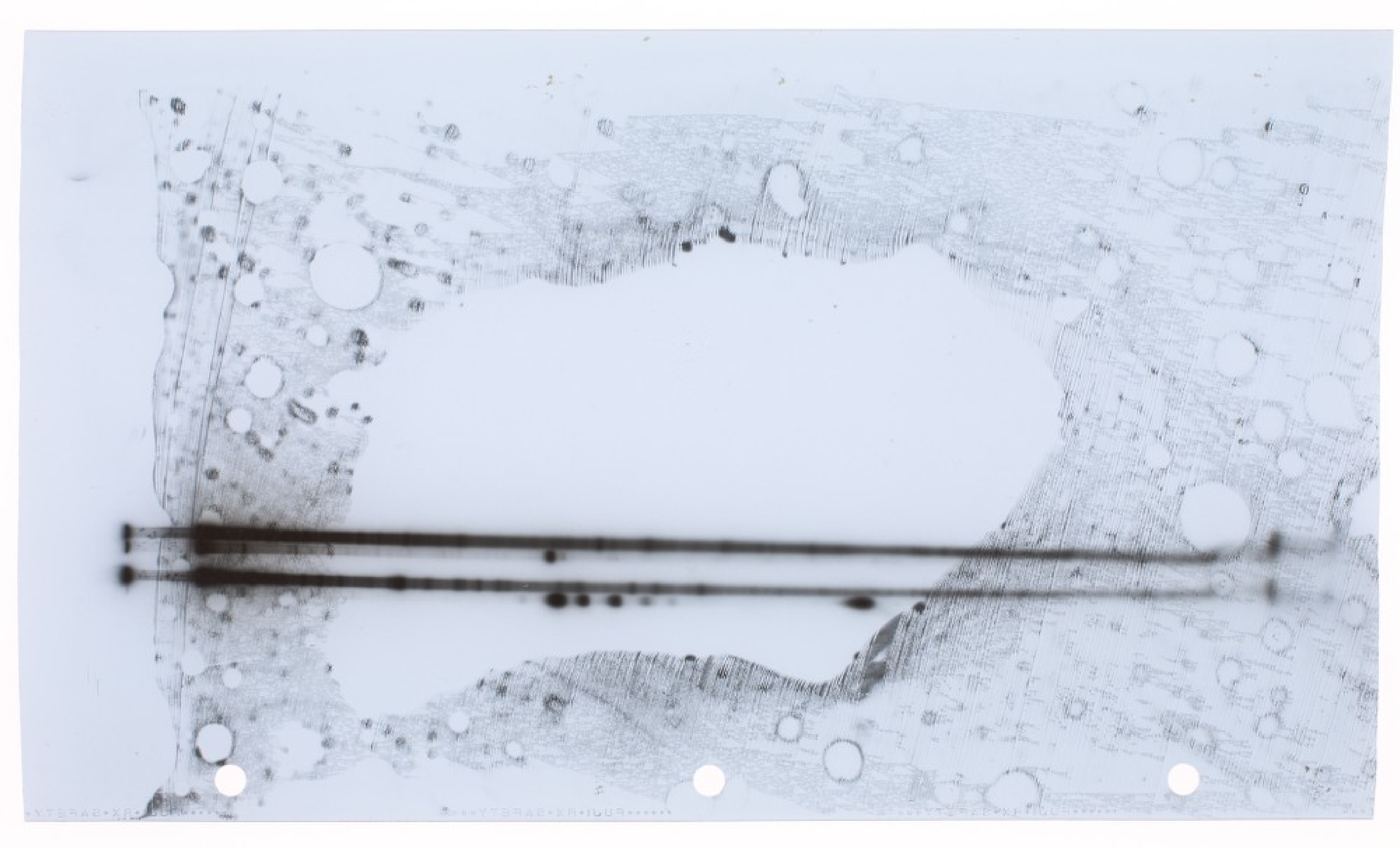
F08B5 and F10E5 LiCl pptd
EtOH ppted & washed; dried
+16 µl H_2O

Mix		
	H_2O	2.5
	5xTB	20
	BSA	1
	0.1M DTT	10
	2.5mM rNTP's	20
	$\alpha^{32}P$ UTP	2.5

37.5 + 2 T7 pol
37.5 + 2 SP6 pol

2.5 DNA
a) No cold UTP) 0.2 µl new RNasin (batch P136)
b) + 1 0.1mM UTP)
c) + 1 0.1mM UTP +old RNasin 0.2 µl

Meant a-c to be SP6, d-f to be T7 but balled up – F08B5 a-f all SP6
F10E5 a-f all T7
i.e. a-c and d-f in each case duplicate sets, except F10E5 e – no RNasin.
60' 37°; 1 µl for 4% gel
–
cold UTP obviously enhances ~~extent~~ of transcription.
New RNasin looks pretty useless – no better than none, unless adding too much is inhibitory.
Backgrounds generally v. high, looks like generally less specific banding than e.g. 26/1



K10B6 T20A7

SP6 T7 SP6 T7

50' exposure.

4/2/87

Gold LiCl of minipreps DNA's prior to SP6 and T7 transcrip^n

K10B6 and T20A7
10 μl
+ 10 μl TE

+ 15 phenol

Aqueous + 50 H_2O
70 5M LiCl
60' 0°C

-N ppt
Subsequently realised that these had already been LiCl pptd once.

EtOH ppt super
-20°C o/N
EtOH wash ; dry
+ 10 μl H_2O

Mix
10 H_2O
10 5×TB
0.5 BSA
5 0.1M DTT
.1 RNasin (p136)
10 2.5mM rNTP's
2 α32P NTP (2 weeks old)

19 / 19
+1 SP6 pol +1 T7 pol

a) 2.5 K10B6	1 0.1mM UTP	6 SP6 mix
b) " "	"	6 T7 "
c) " T20A7	"	6 SP6 mix
d) " "	"	6 T7 mix

60' 37°
1 μl for 4% gel
Probe with K10B6 SP6 & T7

OVER

4/2 Probings

Filter set 18/11 A2 26/1 f and k T7 transcripts
i.e. T1999 Rsa and T16B2 Rsa T7's

18/11 A3) 4/2 a & A4) 4/2 b
i.e. K10B6 (pol) SP6 & T7.

2 hr prehyb in same prehyb mix as used for 28/1 expt. (mix stored at -20°)

Hybdn as 28/1.

(Since signals from previous expt so low, filters not washed prior to re-use),

- K10B6 T7 gave pickable +'s (adequate background after 4 day exposure) - picked.

T1999 & T16B2 Rsa T7's gave weak +'s with inadequate bkgrnd after 4 days - reexposed for 2 weeks.

K10B6 SP6 gave pretty even background only - probably no +'s.

T20A7 SP6 & T7 reacn abandoned.

4/2/87 (30/9 library)

Plating of LOR6/pS3A E.coK⁻ clones - should become F31 →
(F29 + 30 from this batch having given a high % of joins).

0.1 λ dil
5 μl library (from ~100 μl remaining)
0.2 ml o/n 1046 a

20' 37°
0.5 CY
50' 37°

Plating 5 × 150 μl.

(U.V. assay of 1046 a - c grown - from small colonies on 17/12/86 plate of 5C5 stock)
~ U.V. ok)

~100 / plate.

24/2
Further plating as above (bugs subculture of those used above).

U.V. assay

K06H3
T13G10
T22C9
T15F9
SP6
T13G10
T22C9
T15F9
T7
16/2

16/2
SP6 & T7 transcripts of 21/11/86 prep'd DNA's.
(LiCl ppte)

KO6H3 (MSP45R) (T7 int) SP6 only
T13G10 (MAB5R) SP6 & T7
T22C9 (MSP71L) SP6 & T7
T15F9 (MSP77L) SP6 & T7

Mix
17 H_2O
17 5xTB
1 BSA
9 DTT
2 RNasin
17 2.5mM rNTP's
3 $\alpha^{32}P$ UTP (fresh)

36 / 30

5μl NEN 9ku/μl SP6 pol
(previously 2 NEN 30ku/μl)

2 T7 pol

9 mix
4 DNA
1.5 0.1mM cold UTP

60' 37°

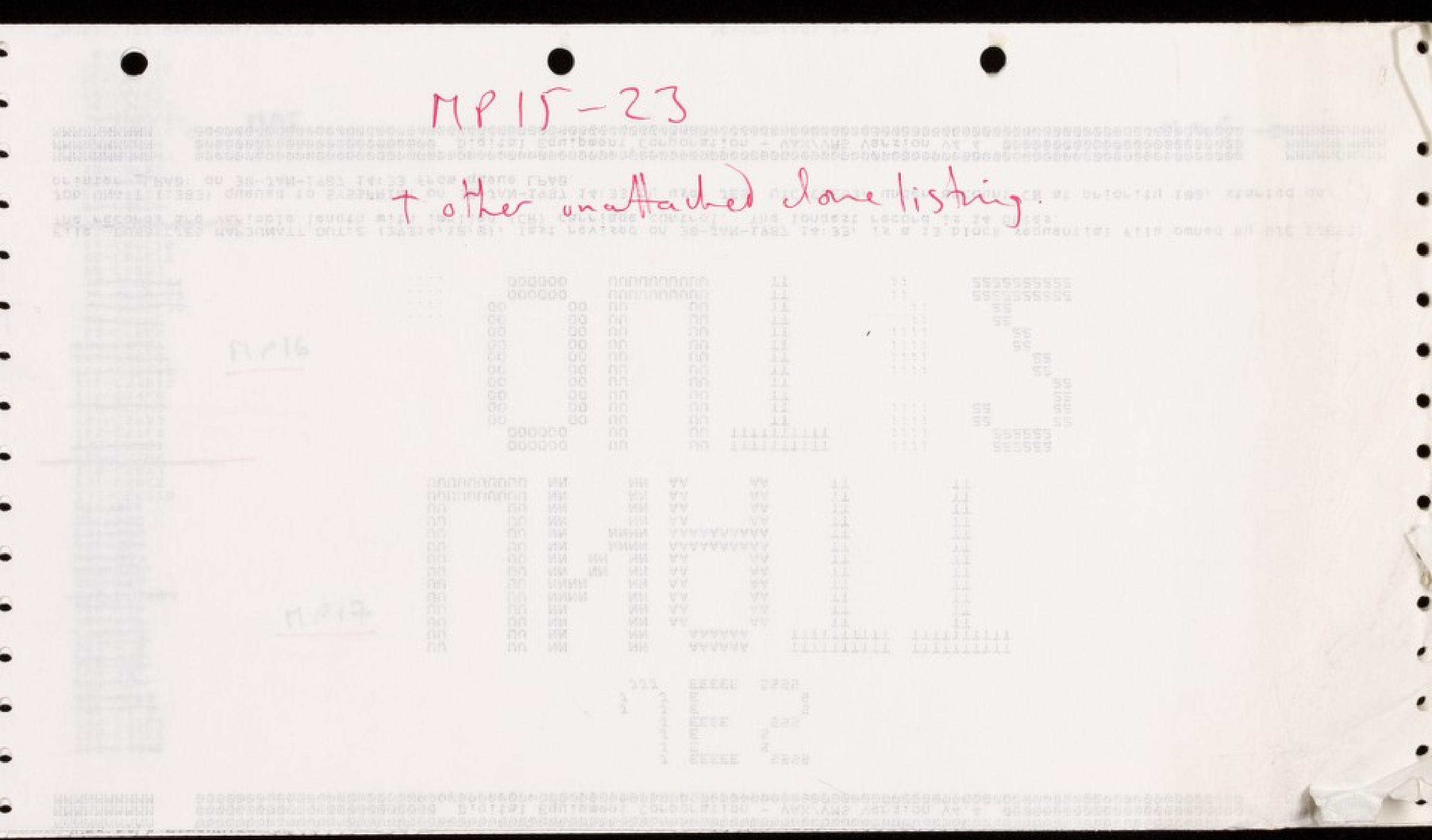
← 1μl for 4% TBU gel.

Probing:
18/11 A1, A3, A4 NaOH unblocked

Prehyb & hybdn as 28/1/86

SP6 KO6H3 - 18/11 A1
other SP6's combined - A3
T7's " - A4

Washing as 28/1


MP15-23
+ other unattached clone listing.
MP16
MP17

MMMMMMMMMM 0000000000000000000000000 Digital Equipment Corporation - VAX/VMS Version V4.4 0000000000000000000000000 MMMMMMMMMM

JES

UNATT

.OUT;2

File _DUB3:[JES.MAP]UNATT.OUT;2 (39314,12,0), last revised on 30-JAN-1987 14:33, is a 13 block sequential file owned by UIC [JES].
The records are variable length with implied (CR) carriage control. The longest record is 14 bytes.

Job UNATT (1383) queued to SYS$PRINT on 30-JAN-1987 14:33 by user JES, UIC [JES], under account CB at priority 100, started on printer _LPA0: on 30-JAN-1987 14:33 from queue LPA0.

MMMMMMMMMM 0000000000000000000000000 Digital Equipment Corporation - VAX/VMS Version V4.4 0000000000000000000000000 MMMMMMMMMM

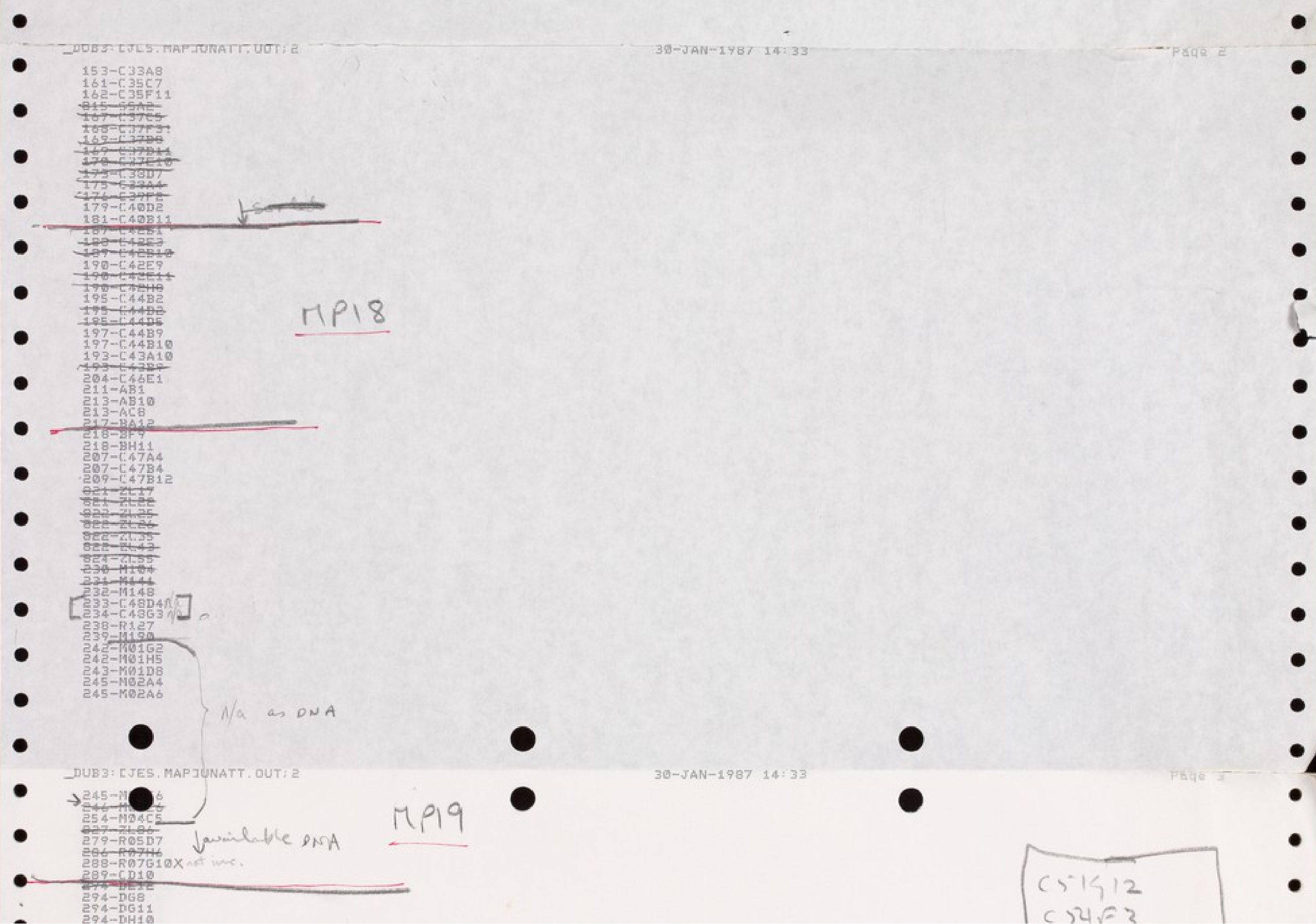
0-B[illegible]53
23-C[illegible]B10
25-C02A1
26-C02H3
28-C02G10
~~30-[illegible]~~
32-C03H12 ← MP15
40-D1030
~~60-[illegible]~~ x
48-C04B3
~~49-C04F3~~
53-C05D3

MP15 →

0-B[illegible]3
23-C[illegible]10
25-C02A1
26-C02H3
28-C02G10
~~30-SG25/24~~
32-C03H12 ← MP15
40-D1030
~~60-D2370~~ X
48-C04B3
~~49-C04F3~~
53-C05D3
~~53-C05D6~~
~~54-C05G4~~
57-C06D2
59-C06C10
60-C06E12
65-C08B3
~~79-SG25/44~~
~~79-SG25/33~~
~~79-SG25/34~~
~~78-C11H5~~
83-C12E2
~~104-C17E6~~ MP16
~~109-C18D8~~
~~111-C22F11~~
115-C24B12
116-C24F8
~~118-C25H5~~
121-C26B3
122-C26F6
~~123-C26G1~~
~~126-C27H2~~
130-C28A3
131-C28E2
133-C28G10
~~501-A15~~
~~501-A20~~
~~501-A22~~
~~501-A23~~
~~502-A43~~
~~502-A46~~
~~502-A47~~
~~807-D1360~~ X
~~504-A70~~ MP17
~~504-A75~~
~~504-A76~~
504-A78
~~504-A81~~
504-A87
~~504-A89~~
~~505-A168~~
~~505-A172~~
144-C31F5
152-C33E1
~~152-C33G5~~

MP15 →

153-C33A8
161-C35C7
162-C35F11
~~815-SSA2~~
~~167-C37C5~~
~~168-C37F3~~
~~169-C37B8~~
~~169-C37D11~~
~~170-C37E10~~



153-C33A8
161-C35C7
162-C35F11
~~815-S5A2~~
~~167-C37C5~~
~~168-C37F3~~
~~169-C37B8~~
~~169-C37B11~~
~~170-C37E10~~
~~173-C38D7~~
~~175-C39A4~~
~~176-C39F2~~
179-C40D2
181-C40B11
~~187-C42B1~~
~~188-C42E3~~
~~189-C42B10~~
190-C42E9
~~190-C42E11~~
~~190-C42H8~~
195-C44B2
~~195-C44D2~~
~~195-C44D6~~
197-C44B9
197-C44B10
193-C43A10
204-C46E1
211-AB1
213-AB10
213-AC8
~~217-BA12~~
218-BF9
218-BH11
207-C47A4
207-C47B4
209-C47B12
~~821-ZL17~~
~~821-ZL22~~
~~822-ZL25~~
~~822-ZL26~~
~~822-ZL35~~
~~822-ZL43~~
~~824-ZL55~~
~~230-M104~~
~~231-M141~~
232-M148
[233-C48D4 N/A]
[234-C48G3 N/A]
238-R127
239-M190
242-M01G2
242-M01H5
243-M01D8
245-M02A4
245-M02A6

MP18

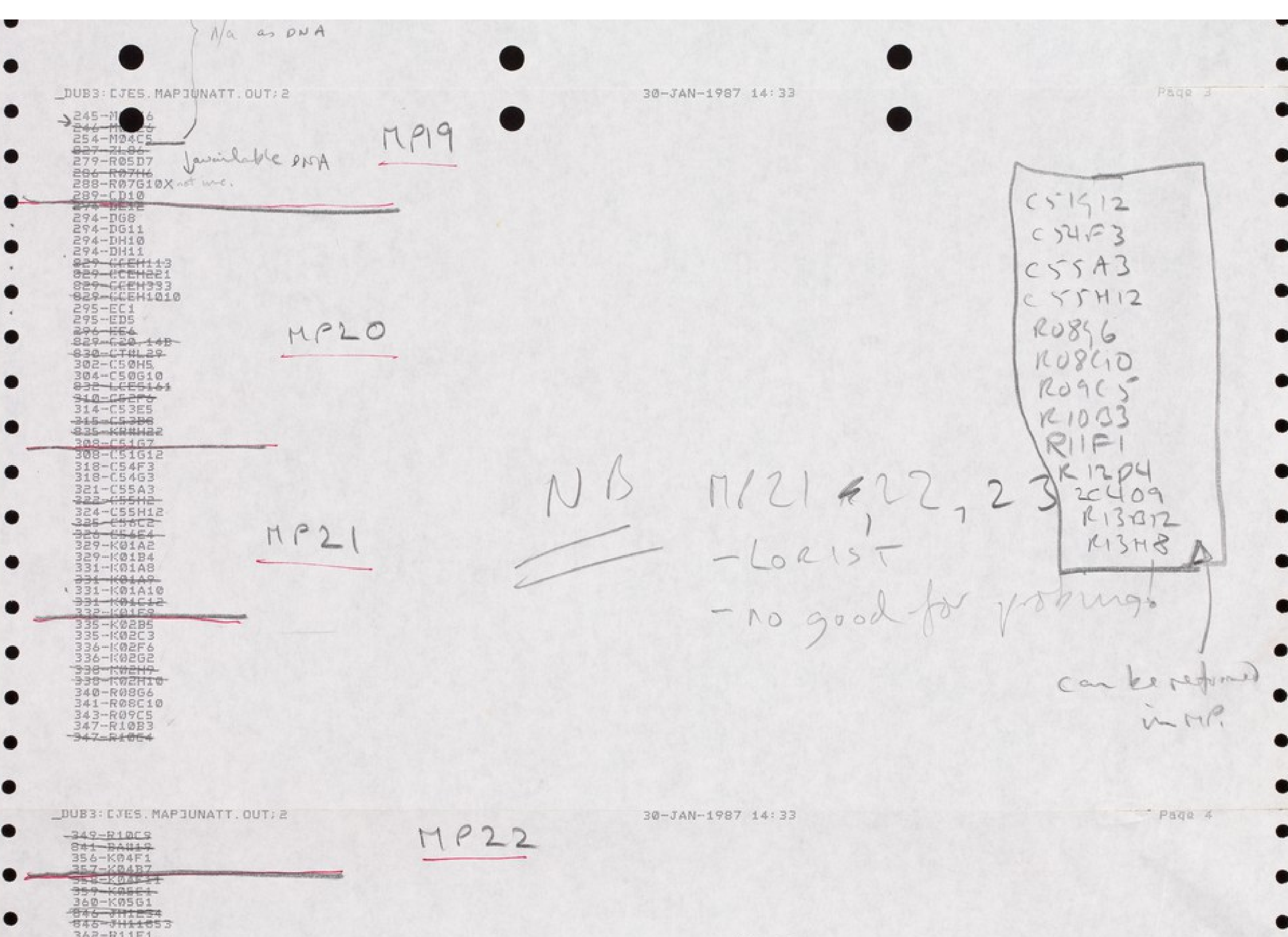
N/a as DNA

→ 245-M 6
254-M04C5
~~827-ZL86~~
279-R05D7
~~286-R07H6~~
288-R07G10X not avail.
289-CD10
294-DG8
294-DG11
294-DH10

MP19

available DNA

C51G12
C54F3



N/a as DNA

→ 245-M[illegible]6
~~246-M[illegible]~~
254-M04C5
~~827-ZL86~~
279-R05D7
~~286-R07H6~~
288-R07G10X not use.
289-CD10
~~294-DE12~~
294-DG8
294-DG11
294-DH10
294-DH11
~~829-CCEH113~~
~~829-CCEH221~~
~~829-CCEH333~~
~~829-CCEH1010~~
295-EC1
295-ED5
~~296-EE6~~
~~829-C20.14B~~
~~830-CT#L29~~
302-C50H5
304-C50G10
~~832-LCES161~~
~~310-C52F6~~
314-C53E5
~~315-C53D8~~
~~835-K##H22~~
~~308-C51G7~~
308-C51G12
318-C54F3
318-C54G3
321-C55A3
~~322-C55H2~~
324-C55H12
~~325-C56C2~~
~~326-C56E4~~
329-K01A2
329-K01B4
331-K01A8
~~331-K01A9~~
331-K01A10
~~331-K01C12~~
~~332-K01F8~~
335-K02B5
335-K02C3
336-K02F6
336-K02G2
~~338-K02H9~~
~~338-K02H10~~
340-R08G6
341-R08C10
343-R09C5
347-R10B3
~~347-R10C4~~

unavailable DNA

MP19

MP20

MP21

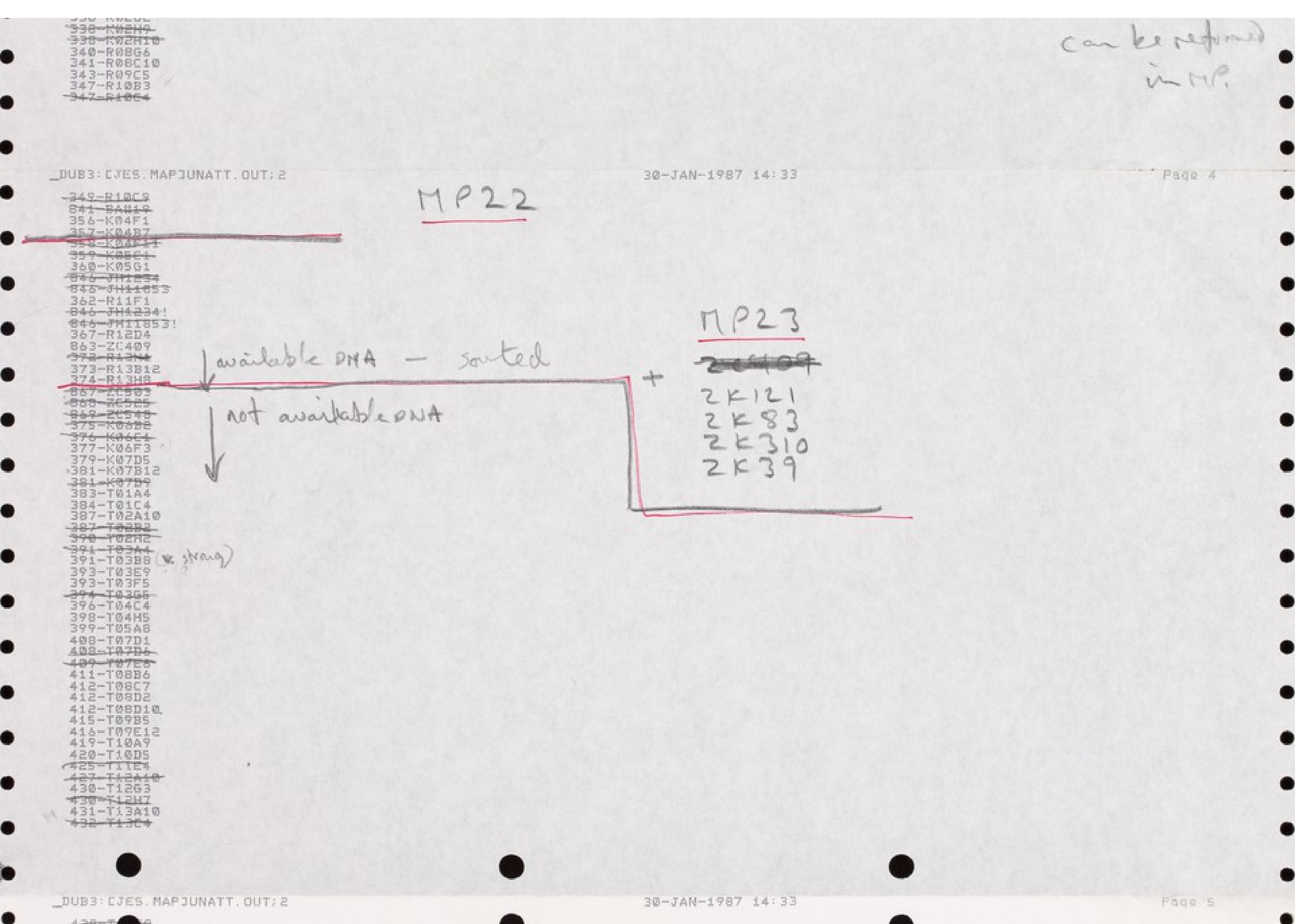
C51G12
C54F3
C55A3
C55H12
R08G6
R08C10
R09C5
R10B3
R11F1
R12D4
2C409
R13B12
R13H8

NB M/21, 22, 23
- LORIST
- no good for probing!

can be retrieved in MP.

~~349-R10C9~~
~~841-BA#19~~
356-K04F1
~~357-K04B7~~
~~358-K04F11~~
~~359-K05E1~~
360-K05G1
~~846-JH1234~~
~~846-JH11653~~
362-R11F1
~~846-JH1234!~~

MP22



~~338-K02H9~~
~~338-K02H10~~
340-R08G6
341-R08C10
343-R09C5
347-R10B3
~~347-R10C4~~

can be performed in MP.

_DUB3:[JES.MAP]UNATT.OUT;2 30-JAN-1987 14:33 Page 4

MP22

~~349-R10C9~~
~~841-BA019~~
356-K04F1
~~357-K04B7~~
~~358-K04F11~~
~~359-K05E1~~
360-K05G1
~~846-JH1234~~
~~846-JH11853~~
362-R11F1
~~846-JH1234!~~
~~846-JH11853!~~
367-R12D4
863-ZC409
~~372-R13H4~~
373-R13B12
374-R13H8

available DNA — sorted

not available DNA

~~867-ZC503~~
~~868-ZC525~~
~~869-ZC540~~
~~375-K06B2~~
~~376-K06C1~~
377-K06F3
379-K07D5
381-K07B12
~~381-K07D9~~
383-T01A4
384-T01C4
387-T02A10
~~387-T02B2~~
~~390-T02H2~~
~~391-T03A4~~
391-T03B8 (v. strong)
393-T03E9
393-T03F5
~~394-T03G5~~
396-T04C4
398-T04H5
399-T05A8
408-T07D1
~~408-T07D6~~
~~409-T07E8~~
411-T08B6
412-T08C7
412-T08D2
412-T08D10
415-T09B5
416-T09E12
419-T10A9
420-T10D5
~~425-T11E4~~
~~427-T12A10~~
430-T12G3
~~430-T12H7~~
431-T13A10
~~432-T13C4~~

MP23

+ ~~ZC409~~
ZK121
ZK83
ZK310
ZK39

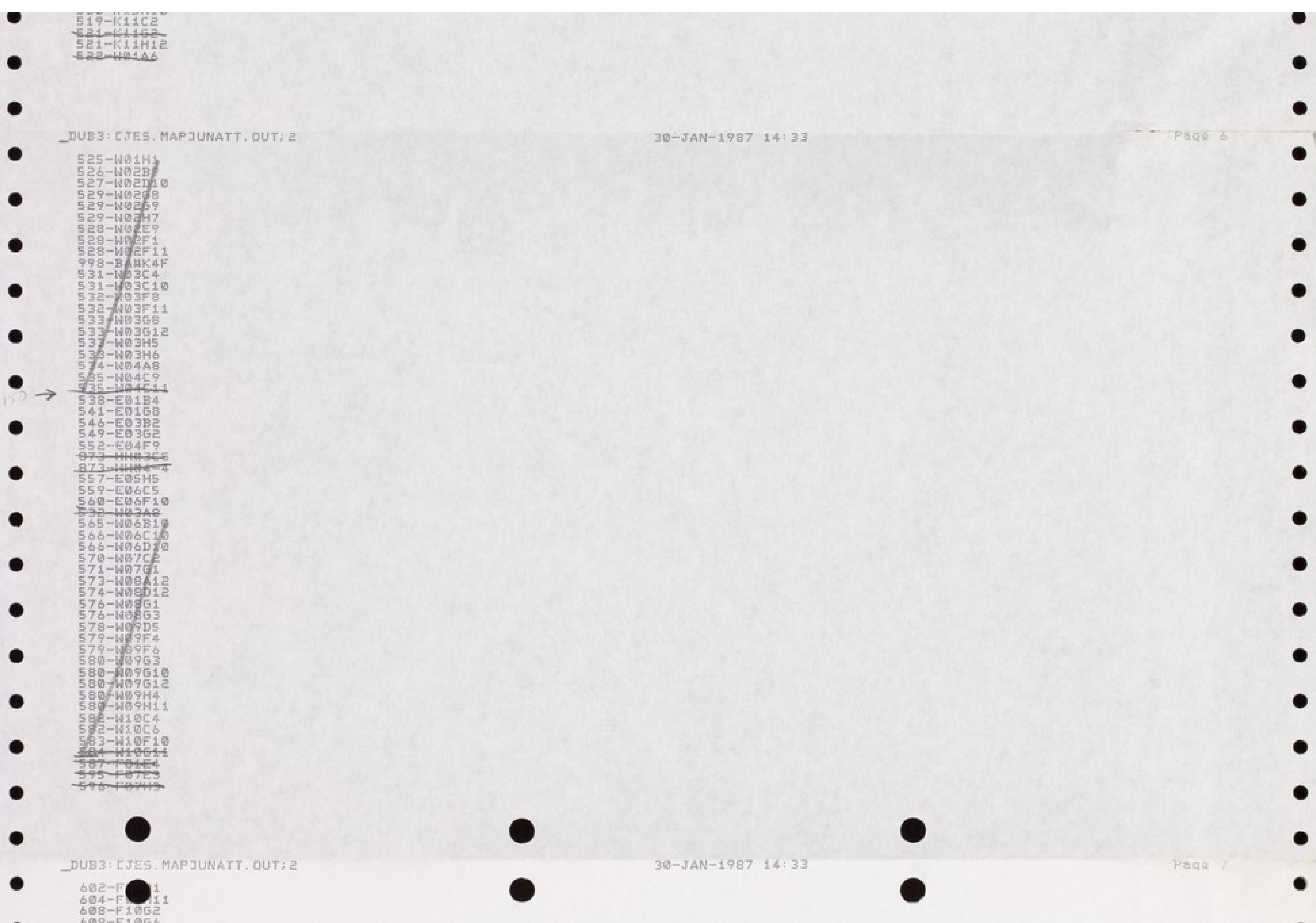
_DUB3:[JES.MAP]UNATT.OUT;2 30-JAN-1987 14:33 Page 5

~~438-T...9~~
~~438-T...10~~
~~438-T14H11~~

~~432-T13E4~~

~~438-T[illegible]9~~
~~438-T[illegible]H10~~
~~438-T14H11~~
439-T15B10
~~442-T15G4~~
~~443-T16B4~~
456-T19C12
~~461-T20E8~~
465-T21E3
~~465-T21E10~~
467-T22A7
469-T22E2
469-T22E11
470-T22G12
470-T22H9
473-T23E1
473-T23F12
474-T23G4
474-T23H2
~~476-T24B[illegible]~~
~~476-T24[illegible]~~
~~477-T24E6~~
~~477-T24F12~~
478-T24G2
~~478-T24H9~~
~~479-T25B11~~
~~482-T25G11~~
482-T25H8
483-T26B5
483-T26B7
484-T26C10
485-T26F2
488-T27C4
488-T27C5
~~487-T27E1~~
~~495-R14E7~~
~~495-R14F2~~
~~496-R14G8~~
496-R14H11
506-K08A3
506-K08B11
507-K08D11
~~837-PC#4-15~~
~~837-PC#4-10~~
~~510-K09A4~~
510-K09B11
~~513-K09H5~~
~~513-K09H7~~
~~497-R15D10~~
~~498-R15E6~~
~~500-R15G7~~
~~500-R15H10~~
519-K11C2
~~521-K11G2~~
521-K11H12
~~522-W01A6~~

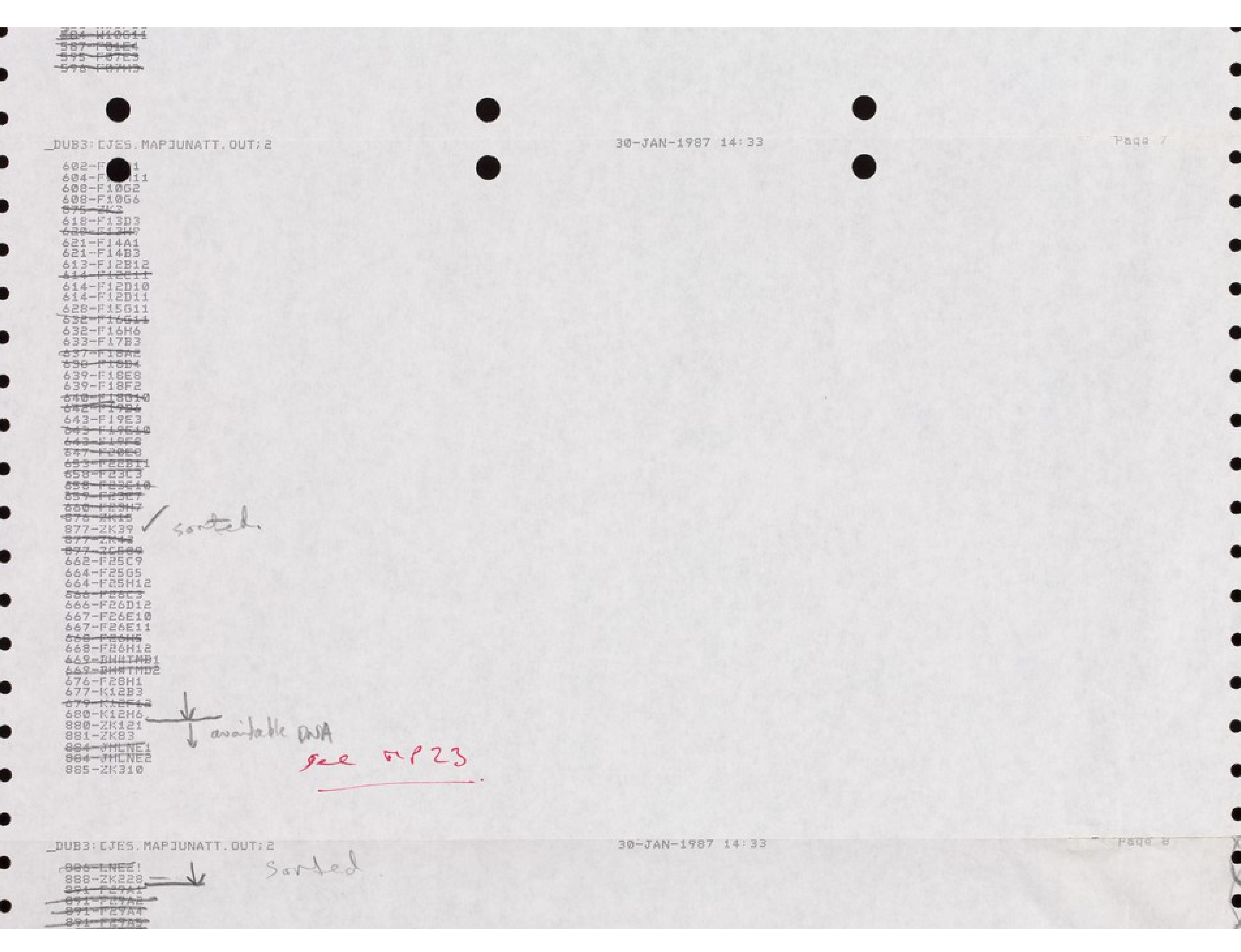
525-W01H1
526-W02B[illegible]
527-W02D10
529-W02G8
529-W02G9
529-W02H7
528-W02E9
528-W02F1
528-W02F11



519-K11C2
521-K11G2
521-K11H12
522-W01A6

525-W01H1
526-W02B7
527-W02D10
529-W02G8
529-W02G9
529-W02H7
528-W02E9
528-W02F1
528-W02F11
998-BANK4F
531-W03C4
531-W03C10
532-W03F8
532-W03F11
533-W03G8
533-W03G12
537-W03H5
538-W03H6
534-W04A8
535-W04C9
535-W04C11
538-E01B4
541-E01G8
546-E03B2
549-E03G2
552-E04F9
873-WHW3C6
873-WHW4 4
557-E05H5
559-E06C5
560-E06F10
532-W03A8
565-W06B10
566-W06C10
566-W06D10
570-W07C2
571-W07G1
573-W08A12
574-W08D12
576-W08G1
576-W08G3
578-W09D5
579-W09F4
579-W09F6
580-W09G3
580-W09G10
580-W09G12
580-W09H4
580-W09H11
582-W10C4
582-W10C6
583-W10F10
584-W10G11
587-F01E4
595-F07E3
598-F07H3

602-F[illegible]1
604-F[illegible]11
608-F10G2
608-F10G6
875-JK3
618-F13D3

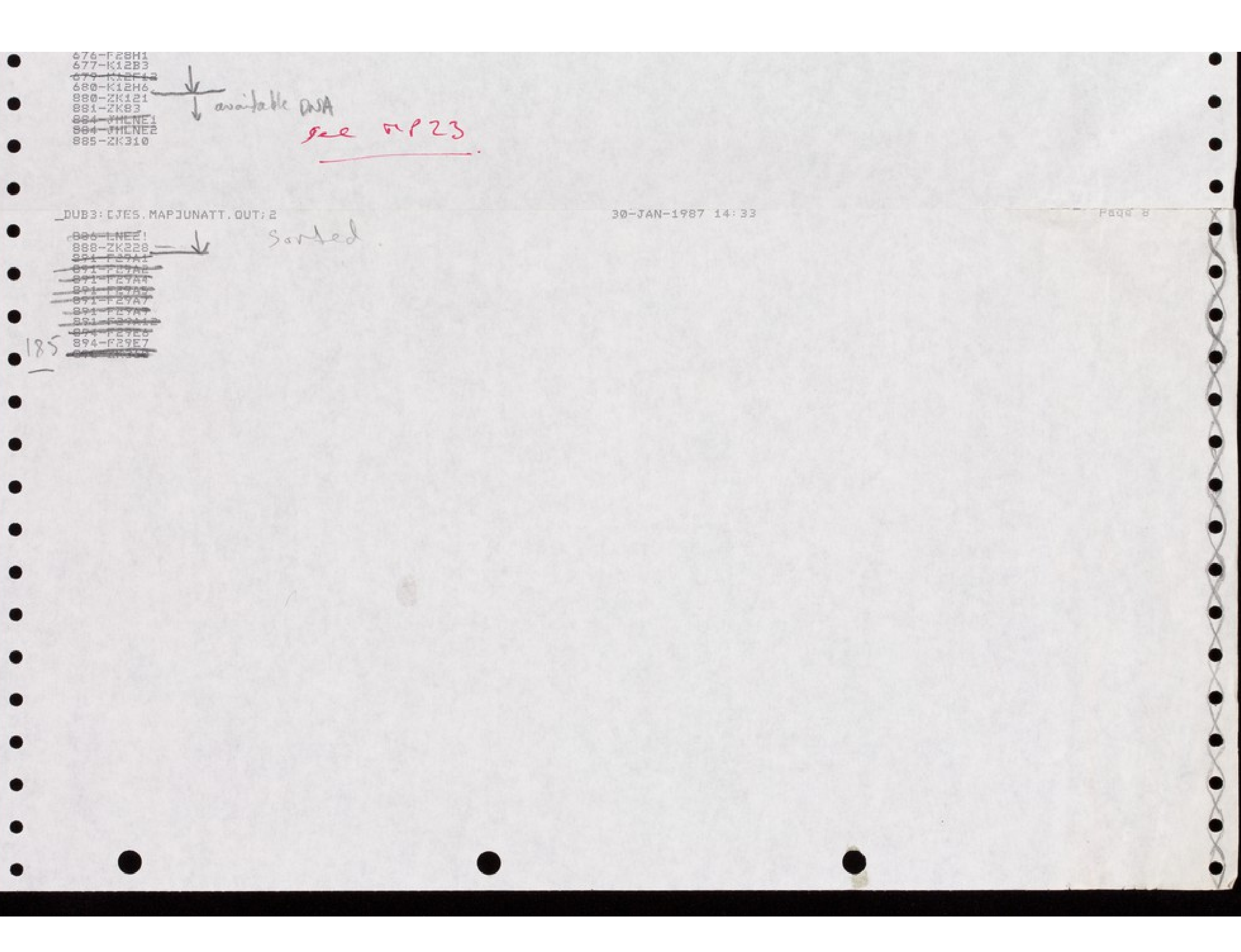


~~584-H10G11~~
~~587-F01E4~~
~~595-F07E3~~
~~596-F07H3~~

602-F[illegible]1
604-F[illegible]11
608-F10G2
608-F10G6
~~875-ZK2~~
618-F13D3
~~620-F13H9~~
621-F14A1
621-F14B3
613-F12B12
~~614-F12C11~~
614-F12D10
614-F12D11
628-F15G11
~~632-F16G11~~
632-F16H6
633-F17B3
~~637-F18A2~~
~~638-F18B4~~
639-F18E8
639-F18F2
~~640-F18G10~~
~~642-F19B6~~
643-F19E3
~~643-F19E10~~
~~643-F19F8~~
~~647-F20E8~~
~~653-F22B11~~
~~658-F23C3~~
~~658-F23C10~~
~~659-F23E7~~
~~660-F23H7~~
~~876-ZK15~~
877-ZK39 ✓ sorted.
~~877-ZK42~~
~~877-ZC600~~
662-F25C9
664-F25G5
664-F25H12
~~666-F26C3~~
666-F26D12
667-F26E10
667-F26E11
~~668-F26H5~~
668-F26H12
~~669-DH#TMD1~~
~~669-DH#TMD2~~
676-F28H1
677-K12B3
~~679-K12F12~~
680-K12H6 ↓
880-ZK121 ↓ available DNA
881-ZK83
~~884-JHLNE1~~
~~884-JHLNE2~~
885-ZK310

see p23

~~886-LNE2!~~ Sorted
888-ZK228 ↓
~~891-F27A1~~
~~891-F27A2~~
~~891-F27A4~~
~~891-F27A5~~



676-F28H1
677-K12B3
~~679-K12F12~~
680-K12H6
880-ZK121
881-ZK83
~~884-JMLNE1~~
~~884-JMLNE2~~
885-ZK310

available DNA

see MP23

~~886-LNE2!~~
888-ZK228
~~891-F29A1~~
~~891-F29A2~~
~~891-F29A4~~
~~891-F29A5~~
~~891-F29A7~~
~~891-F29A9~~
~~891-F29A12~~
~~894-F29E6~~
894-F29E7
~~[illegible]~~

Sorted

185

16/12/86

Mixed probe preps of unattached clones.

See attached printout for MP15-23 constituents

5 μl each DNA
(50 μl total)
20 5×R1 R1 buffer
0.5 M spermidine
0.5 10 mg/ml RNase
30 H_2O
2 25 u/μl EcoR1

5×R1 buffer 500 mM NaCl
250 M Tris pH
50 $MgCl_2$ M
50 .1M DTT
150 H_2O

2 hr 37°
1 μl on mini gel looked ok.

EtOH ppt.
+ 25 TE
10 dyes

Also 2 pTB8 600 μg/ml
3 H_2O
5 mix
0.5 R1

0.6% 250 ml LGT TBE gel.
1 cm slots.

(MP 21-23 abandoned
– contain Lor11T)
– R1 digest will contain vector)

pTB8 MP15 16 17 18 19 20 21

over

Bands cut out as shown.
1x phenol extract.

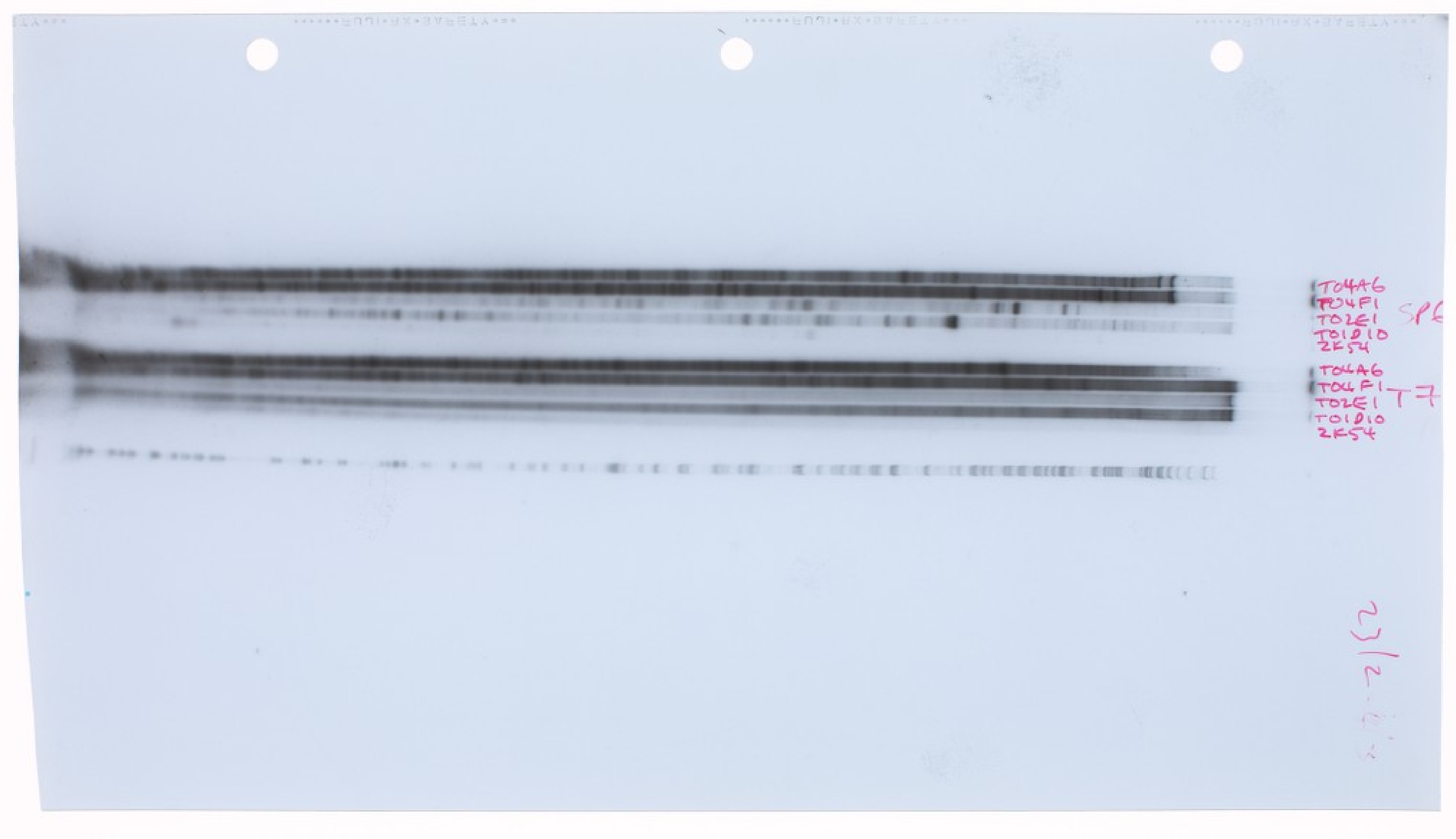
Balls-up - added isopropanol to concentrate rather than butanol!

+ 10μg glycogen (sorvall tubes vol ≈ 10ml)
o/N -20°C.

Numbering may have got ballsed-up.

Nick trans. of MP15 & 16
As 13/12/85
½ (10μl) labelled.
All nick trans. used to probe 18/11 A1 & A2
as 4/10/85 protocol

1/20 MP
15 20


T04A6
T04F1
T02E1
T01D10
2K54
SP6
T04A6
T04F1
T02E1
T01D10
2K54
T7

25/2

Clones from JES growths for riboprobe prepn.

TO4A6 (MABFL)
TO4F1 (MLCIIR)
TO2E1 (LINIOR)
TO1D10 (UBQ1R)
2K54 (MLCIIL).

+ 75 H_2O
100 5M LiCl
60' 0°
3' spin
Super + 0.5 ml EtOH
-70° 30'
Spin; wash; dry; + 10 μl H_2O

~~3~~ 4/3
2x5 μl for SP6 + T7 reactions as 16/2

← 1 μl gel

		Filter set
Combine	TO4A6 + TO4F1 SP6	18/11 A1
	TO2E1 + TO1D10 SP6	18/11 A2
	TO4A6 + TO4F1 T7	18/11 A3
	Rest SP6 + T7.	18/11 A4

50% formamide hybridisation.

(5A2 in with A3 by mistake)

16/3/87

Growths for riboprobing.

F25B5	UBQ1L
ZK180	MCCI2R
F13C11	EP2L
ZK637	LIN12L
T13910	MAB5R
K01A9	matl. (prob LIN12L)

2x2 ml workups.
LiCl etc.
+ 20 μl H_2O final.

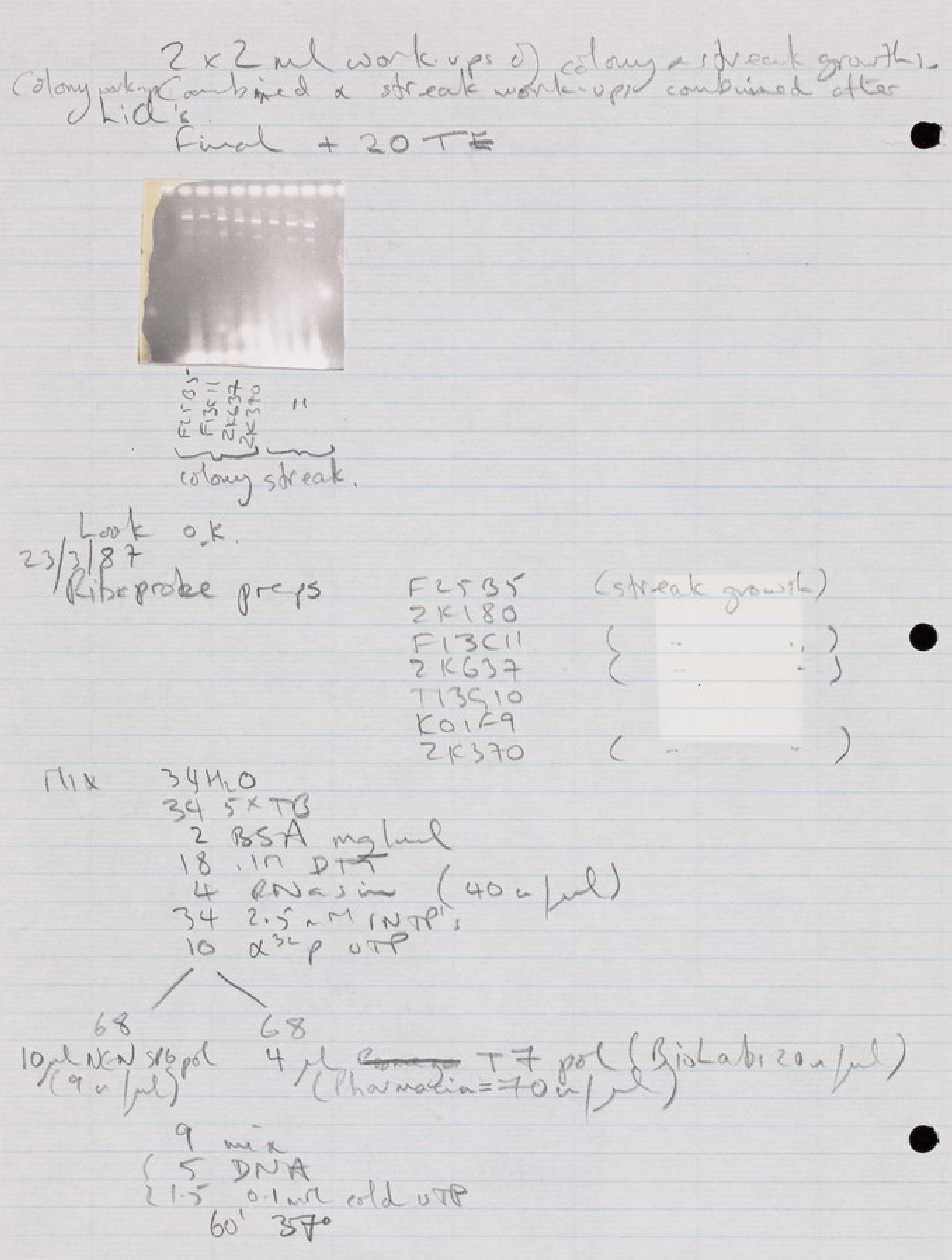
1 μl for 0.5% ag. gel

F25B5, F13C11, ZK637 badly deleted.
colony purify, restreak + grow.

Regrowths.

F25B5, F13C11, ZK637, (also ZK370)
colony purified and streaked.
5 ml growths of single colonies and
1/2 streaks (6.00 → 10 am)
30 μg/ml Kan.

F25B5 & F13C11 colony growths grew v. slowly
- left for further 8 hours.
O/N 4° prior to work-up.



2 x 2 ml work-ups of colony & streak growths.
Colony work-ups combined & streak work-ups combined after LiCl's.
final + 20 TE

Look o.k.

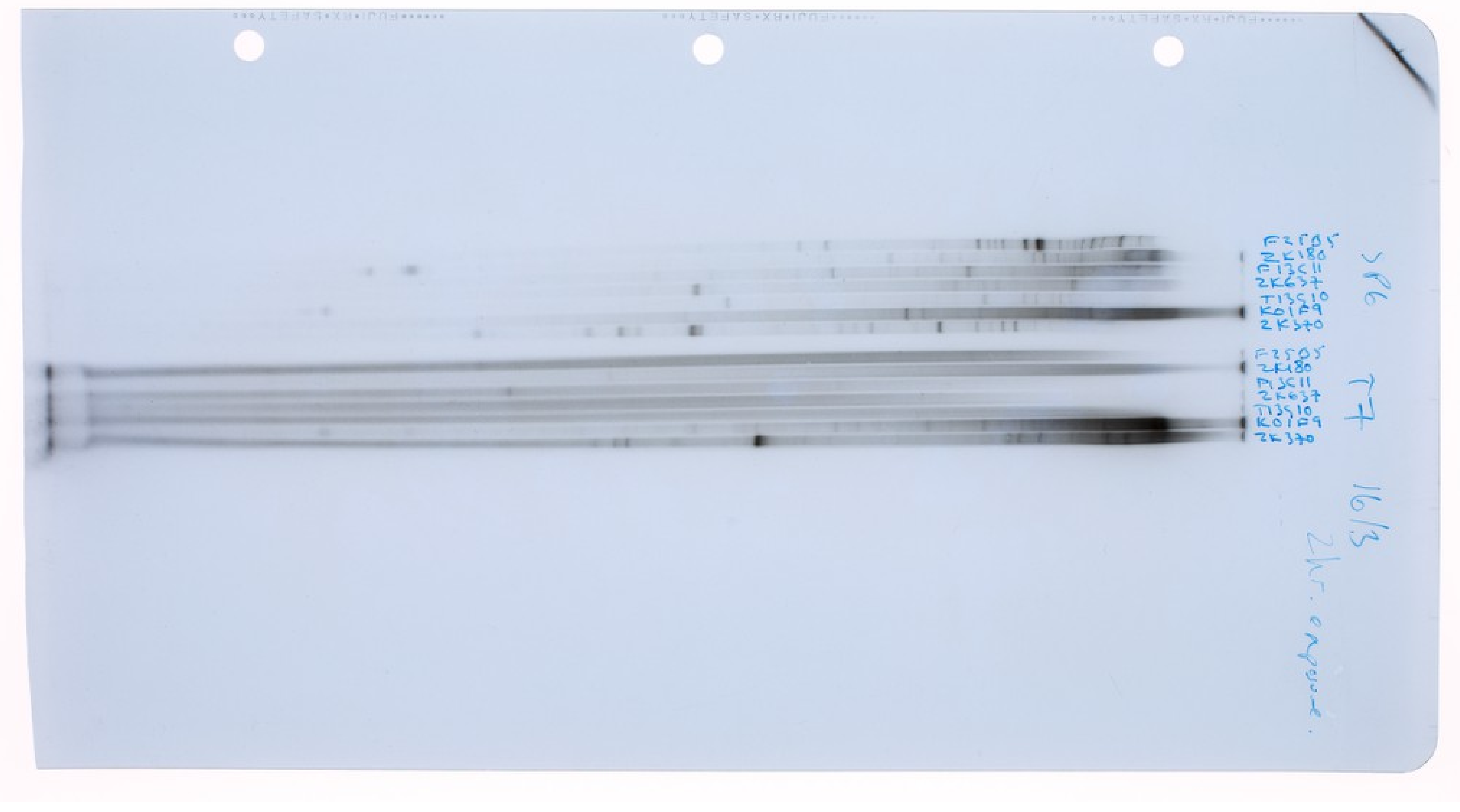
23/3/87
Riboprobe preps

F25B5 (streak growth)
ZK180
F13C11 (" ")
ZK637 (" ")
T13G10
K01F9
ZK370 (" ")

Mix
34 H_2O
34 5 x TB
2 BSA mg/ml
18 .1M DTT
4 RNasin (40 u/μl)
34 2.5 mM rNTP's
10 $\alpha^{32}P$ UTP

68 — 10 μl NEN SP6 pol (9 u/μl)
68 — 4 μl T7 pol (BioLabs 20 u/μl) (Pharmacia = 70 u/μl)

9 mix
5 DNA
1.5 0.1 mM cold UTP
60' 37°



F25D5
ZK180
F13C11
ZK637
T13G10
K01F9
ZK370
F25D5
ZK180
F13C11
ZK637
T13G10
K01F9
ZK370
SP6
T7
16/3
2hr. exposure

O/N expos.

Probing 25/3

SP6's only

18/11 A1 F13C11
2 K01F9
3 ZK637
4 ZK370.

(Probe with F25B5, ZK180 and F13G10 SP6 if these look promising).

Unblocking. prehyb. hyb. and wash as 28/1

15/3

Prep pOU61cos vector DNA

Colony from transformation of JM101 by
DNA provided by Vroni Knott inocculated into
10ml 2xTY / 100 μg/ml Amp O/N growth @ 37°.

Into 1l 2xTY / 100 μg/ml amp.
O/N 37°.
Alkali lysis work-up
LiCl pptd after 1st EtOH
EtOH pptd.
φol extracted
$CHCl_3$ ..
Iso propanol pptd
EtOH washed
+ 300 μl TE
1 μl and 1 μl DNA/10 for 0.5% ag. gel
with 1 μl 0.5 μg/μl λ Hind III
.. 0.1 μg/μl ..
.. 0.05 .. ..
.. 0.01 .. ..

Looks like 1 μl pOU61cos/10 ≃ 0.02 μg
1 μl .. ≃ 0.2 μg
≃ 60 μg total.

OVGR.

Bam digest of pOU61 cos

50 pOU61 ($\simeq 10 \mu g$)
6 10×H
0.25 M Spermidine
0.25 10 mg/ml RNase
4 µl 25u/µl Bam H1.

90' 37°.
Ag gel
Digest incomplete
+ 2µl Bam H1
2hr 37°
Ag gel
Digest o.k.

Gel purified on 0.5% LGT minigel (100 ml)
Band 3× phenol (Band vol. ≃ 1 ml)
CHCl3
iso prop ppt
EtOH wash
+ 40µl 50mM Tris pH 8.0
10 mM EDTA
1µl 22u/µl CIP
20' r.t.
2× phenol
EtOH ppt
+ 20 TE
Ag gel with stds.
Looks like 0.2 – 0.5 µg/µl

a 0.5µg TGL l...
b 1µl pOU61 cos
c 1µl 0.5µg/λ λH3
d 1µl 0.1µg/λ "

20/3

Ligation pOU61cos Bam phase/N2 pS3A 3 frags

a) 2 µl pOU61cos Bam phase (15/3)
2 µl N2 pS3A 3 (17/9/96)
1 10×C
1 10mM rATP
1 0.1M DTT
2 H_2O
1 DB blunt ligase
o/n 14°

b) 0.5 pOU61 cos
1 10×C
1 rATP 10mM
1 DTT
5.5 H_2O
1 ligase
o/N 14°

c) 1/2 b
1 µl pS3A 2 (17/9)

(b & c for ag. gel)

b c a

Packaging

All a) Gigapack + (E.coK-)
+ 500 λ dil

Assay
0.1 λ dil
5 µl package
0.2 1046 o/N
20' 37°
0.5 CY
30' 37°

250 µl plated
(Plates seemed v. dry)
- 1 colony!

28/3/87

Further Bam/ph'ase of pOU61cos (15/3)

50μl (~10μg) pOU61cos purified on 0.5% LGT gel.
Phenol extracted 3X
$CHCl_3$ 1X
Iso-prop. pptn, EtOH wash
+ 20μl H_2O

30μl Bam digest.
1μl HGT gel. digest looks o.k.

EtOH pptd. + washed.

40μl 50mM Tris pH8.0
10mM EDTA
1μl 22u/μl CIP
30' r.t.

3x phenol
1x $CHCl_3$
EtOH + wash; dry.

+ 20 H_2O.

0.2μl 19/3 Bamphase
0.2μl 28/3 "
1μl uncut 15/3
0.5μl Xba
0.5μl Xba

Xba cut hasn't worked (forgot RNase)

Try trial ligations of 19/3 + 28/3 Bamphase material with λ Sau3A digest (tested on gel - looks OK)

7/4

Xba digest pOU61cos

50µl (~10µg) pOU61cos
5 10x Hin
2.5 M NaCl
1 M Tris
5 Xba I 20u/µl.
2h 37°
0.5 & 0.1 µl for gel - see 28/3.
- didn't cut - try adding RNase.

Ligation assays. 8/4

a) 0.2µl 19/3 Bamphase pOU61cos
0.2µl λ Sau3A
9 ligⁿ mix
1 ligase OB blunt

ligⁿ mix x 10 cmix
10 DTT
10 5 ATP
60 H_2O

b) 0.2 28/3 Bamphase pOU61cos
0.2 λS3A
9 ligⁿ mix
1 ligase

c) as b, no λ Sau3A
O/N 14°C
5µl + 1µl dyes
A(3od) ~ 0.1 unligated 28/3 Bam phased pOU61cos

abcd

Both 19/3 & 28/3 look O.K. & comparable

8/4

Ligⁿ of N2pS3A / pOU61 cos 28/3 Bam ph'ased

4μl pOU61cos 28/3 Bam ph'ase
2μl N2pS3A 3 (17/9).
1 10×C
1 DTT 0.1M
1 10mM rATP
1 ligase (OS blunt)

O/N 14°.

Packaging.

Gigapack +
2hr r.t.
+ 300μl λ dil.

Assay:

a) 10μl library
0.1ml λ dil
0.2ml 1046 O/N
20' 37°
0.5ml CY
50' 37°

b) as a
but 30°
30' absorpⁿ
80' expression

Plate 200μl.

a) 3 colonies

b) 0.

21/4
Religation of failed Xba digest of
pOU61cos (7/4).

+ 0.5 10mg/ml RNase
0.5 1M spermidine
5 µl 20-/µl Xba I.
2 h 37°.

Xba 0

~0.2 µl for 0.5% gel
also 1 µl pOU61cos/10

looks o.k.

0.5% LGT gel
Band melt extracted; 3x phenol
2x butanol
150 prop. pptn. +glycogen

No recovery! forgot salt!
+ 60 µl 3M NaAc
2 h -20°
EtOH wash, dry; + 40 µl 50mM Tris pH 8.0
10 mM EDTA

1 µl on gel - looks o.k.

Phase 30 µl. + 1 µl 22 u/µl CIP
30' r.t.
+ 40 H_2O
8 10XSTE (CSH)
2.5 10% SDS
68° 15'
2x phenol
EtOH ppt.
+ 20 H_2O

Bam dig^n of pOU61cos Xba ph'ase

12.5 μl pOU61cos Xba ph'ase (½)
2.5 μl H_2O
10 restric^n mix (– RNase) H_2O 115
1 23 u/μl BamH1   10×H 35
2 hr 37°   1M Spd^n 1

Gel a) 0.1 μl pOU61cos Xba ph'ase
b) 0.2 μl " " " Bam

a b

Not sure about digest

+ 1 μl Bam
60' 37°

2× phenol
EtOH ppt^n, wash, dry ; + 20 μl H_2O.

Gel

1 μl pOU61cos/10
0.1 μl Xba phos 2x
0.5 μl Xba phos Bam
0.1 μg λ H3
0.2 μg λ H3

why large arm so weak re. small?
Rerun another gel
– Same.
Try R1 & Cla1 digests of Xba cut stuff
Also small scale BamH1

0 Bam R1 Cla λH3

Look O.K
so large frag. are lost
during phenol / EtOH ?

Repeat Bam H1 digest on remaining
half of Xba ph'ased material.

10μl Xba phased pOU61cos
5μl H_2O
10μl restrictⁿ mix (- RNase)
1μl 23u/μl Bam H1
2 hr 37°.
+ 1μl 0.1M EDTA
5' 68°
2x phenol (+TE → 40μl)
1x chloroform
Etoh pptⁿ + wash; dry; + 15μl H_2O
1μl for gel (with 0.1μg λH3)

Top band still reduced!
Try ligation anyway.

(1st pol from extraction extracted
with 80μl TE and 40μl $CHCl_3$.
(Looking for 'missing' fragment. ✱ over.

30/4/87

Lig^n pOU61cos Xba phase Bam and N2 pS3A/3 (17/9)

6 μl vector (previous page)
3 μl pS3A frags.
1.5 μl 10×C
1.5 μl 0.1M DTT
1.5 μl 10mM rATP
1.5 ligase

O/N 14°

5 μl for Gigapack + packaging (E.coK⁻)

+ 0.5 μl λ dil. (+$CHCl_3$)

Assay

a) 5 μl library
0.1 λ dil
0.2 1046 O/N
20' 37°
0.5 CY
30' 37°
Plate 150 μl
3 colonies

b) 5 μl library
0.1 λ dil
0.2 1046 O/N
30' 30°
0.5 CY
60' 30°.
Plate 150 μl
0 colonies.

* Large fragment was extracted from phenol by $CHCl_3$
EtOH pptd; + 10 μl H_2O
1 μl for gel with 0.1 μg λ H3

This 10 μl added to remaining 10 μl of 'small arm'

λH3

31/4/87
Isolation of DNA for f/p from Waterston NY yeast clone DNA in agarose. (See letter in file)

1/2 slices ~~attempted~~ elution by electrophoresis onto GF/C filters, essentially as in CSH manual.

1/5 of final sample run on gel - no obvious product. (possible very low yield in NY4)

+ 2.5 μl final vol (optimistically f/p'd - see clone log).

1/5/87
Attempted isolation of part of DNA from NY55 HGT band by electrophoresis into LGT + subsequent phenol extraction.

Slice soaked in 1/10x TBE 20'

1μl EtBr Sealed in 0.5% LGT 50 ml gel. (TBE)

50 ml TBE

3hr 20mA

Band just in LGT

Cut out; 1/2 worked up by JZS f/p'd (see clone log).

		16	16	23	18	22	17	18	19	21	17	
		11	16	17	18	21	25	38 (~~28~~)	29	30	41	~~45~~
16	211											
16	216	13										
23	217	16	13									
18	218	14	15	14								
22	221	15	13	22	14							
17	225	12	12	12	12	12	—					
18	238	14	15	14	17	14	12	—	15	~~12~~		
19	229	14	14	15	15	15	11					
21	230	12	11	19	12	19	(10)	12	14	—		
17	241	14	14	14	15	14	12	16	15	12	~~15~~	
15	245	13	14	13	14	13	12	14	13	11	13	—

~~245~~

1 7 11 (3)

16

L 217

18 R

L 221

225 MAY BE BADLY DIGITIZED

238 R

229

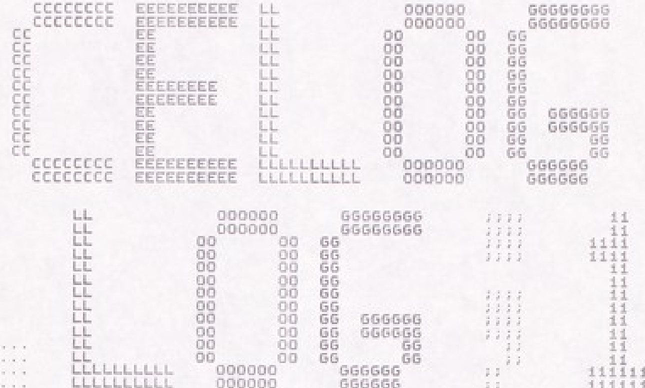
L 230

241 R

245

C10F9
C12C10
C16F2
C16C3

C13F10



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Job CELOG (164) queued to SYS$PRINT on 13-JAN-1986 13:30 by user JES, UIC [JES], under account CB at priority 4, started on printer _LPA0: on 13-JAN-1986 13:30 from queue LPA0.

0000000000 555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555 0000000000
0000000000 555555555555555555555555 Digital Equipment Corporation - VAX/VMS Version V4.1 555555555555555555555555 0000000000
0000000000 555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555 0000000000


```
$SET NOVERIFY
All users of crystallographic programs please see info on new program versions.
Type $PRD NEWLCF to print file [public.xtal.doc]newlcf.doc
MONDAY 13-JAN-1986 13:29:40
%SET-W-NOTSET, error modifying DUA0:
-CLI-E-IVDEVTYPE, invalid device type - specify a mailbox device
NEW VERSION OF DBAUTO IN OPERATION. CHECK JOINS CAREFULLY
  project name =
CEN2HS
number of clones=  7955 number of bands=166626
80000
30
2
   minimum contig size to print (kb) =
150
   last clone analysed =
ZC156
     23          111 bands          157 kb
     26          217 bands          235 kb
     37          208 bands          278 kb      VIT3,4    (X)
     91          122 bands          165 kb
    161          153 bands          209 kb      SUP5      (III)
    254          198 bands          233 kb
    261          166 bands          227 kb
    307          145 bands          198 kb
    350          189 bands          251 kb
    377          194 bands          249 kb      LIN12     (III)
    549          130 bands          208 kb      VIT1       (X)
    619          111 bands          162 kb
    637          268 bands          352 kb      EP7       (III)
    655          132 bands          174 kb
    674          137 bands          155 kb      COL3       (IV)
    680          111 bands          152 kb      LIN10      (I)
    828          266 bands          366 kb      MSP1424   (II)
    875          138 bands          155 kb
 clones analysed=  7664
 clones in contigs=  7207
 contigs=   873
 total bands in contigs= 36585
 % genome in contigs = 62.3
 mean contig size (kb) =     57
 contig occupancy =  8.3
 unattached clones =   457 415
 max % genome in unattached clones = 17.1
FORTRAN STOP
  JES          job terminated at 13-JAN-1986 13:30:48.92

  Accounting information:
  Buffered I/O count:              99     Peak working set size:    500
  Direct I/O count:               628     Peak page file size:     1395
  Page faults:                   3747     Mounted volumes:            0
  Charged CPU time:     0 00:00:30.82     Elapsed time:     0 00:01:12.33
```

160 / 5 ml
600
32
18000

161-C35B12 (5b, 604)
0
next clone for current contig/contig control=

C35B12
C16H12 *
C38C2
C06H4 *
C04G10 *

B0463 *
B0507
C48E1
ZC157
ZC169 *
C02G4
R09H
ZC170
C31A1
ZC178

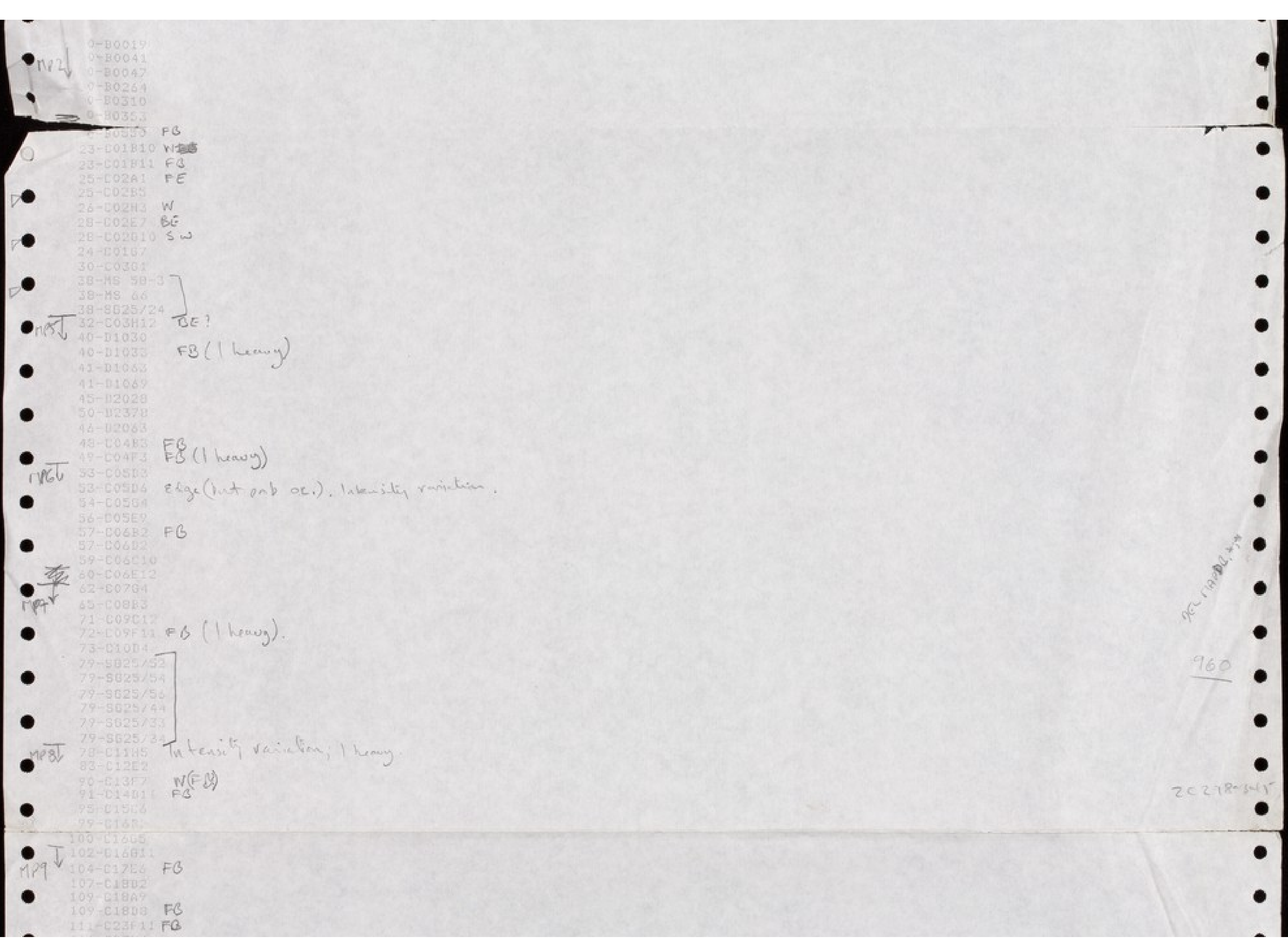
mattacked 10/2/86

unattached 10/2/86

```
Username: JES
Password:
        Welcome to VAX/VMS version V4.2 on node CV
    Last interactive login on Monday, 10-FEB-1986 09:14
    Last non-interactive login on Sunday,  9-FEB-1986 17:43
---New versions of many crystallographic programs now default: all problems
to Phil Evans. Old versions may be accessed following the command XCOMOLD
---Old FFT reinstated pending investigation, new GENSFC and SFC now default
MONDAY 10-FEB-1986 09:39:30
%DCL-I-SUPERSEDE, previous value of PIRSYSTEMP has been superseded
VERY NEW VERSIONS OF ANALYSEQ, ANALYSEP AND DIAGON IN OPERATION.
PLEASE REPORT PROBLEMS HOWEVER SLIGHT
$ MAP
$ CONTIG
     project name =CEN2HS
number of clones=  8364 number of bands=176027
   clone name/global control=CHECK

 menu:-
   1   read clone data
   2   read contig lists, search for low occupancy
   4   overlap analysis
   5   list files
   6   overlap histogram
   7   type contigs
   8   list set of overlapping clones

 option number=7

   contig number (-1 for contig 0) = -1
   file for output (defaults to terminal)

   0-B0019
   0-B0041
   0-B0047
   0-B0264
   0-B0310
   0-B0353
   0-B0559  FG
  23-C01B10 W
  23-C01B11 FG
  25-C02A1  FE
  25-C02B5
  26-C02H3  W
```

FE fuzzy edge
BE bent edge
SW weak slightly
FD few bands.
VW very weak.

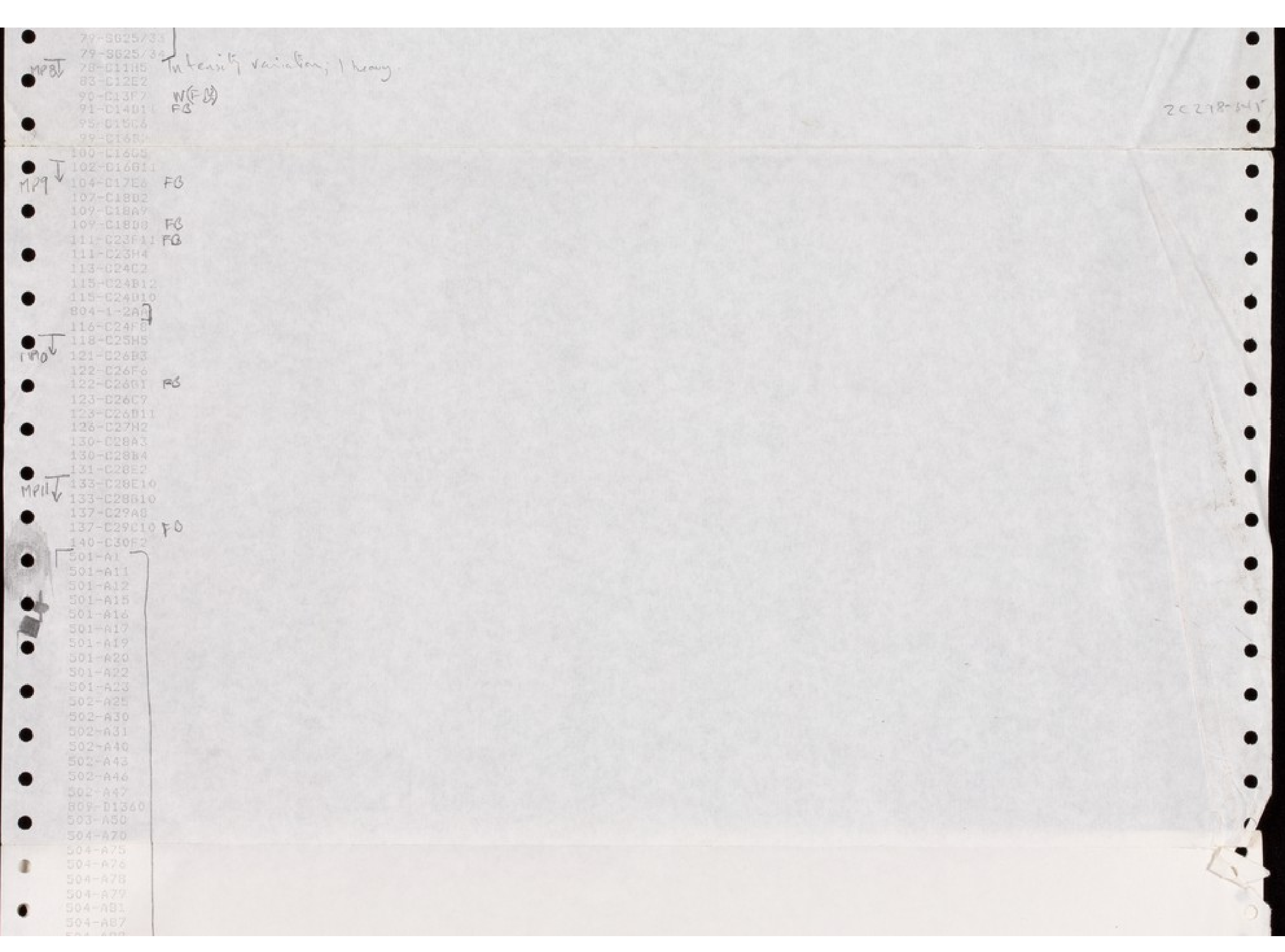
M/2



MP2
0-B0019
0-B0041
0-B0047
0-B0264
0-B0310
0-B0353
0-B0553 FB
23-C01B10 W
23-C01B11 FB
25-C02A1 FE
25-C02B5
26-C02H3 W
28-C02E7 BE
28-C02G10 SW
24-C01G7
30-C03G1
38-MS 58-3
38-MS 66
38-SG25/24] BE?
MP5
32-C03H12
40-D1030
40-D1033 FB (1 heavy)
41-D1063
41-D1069
45-D2028
50-D2378
46-D2063
48-C04B3 FB
49-C04F3 FB (1 heavy)
MP6
53-C05D3
53-C05D6 Edge (but prob ok.). Intensity variation.
54-C05G4
56-C05E9
57-C06B2 FB
57-C06D2
59-C06C10
60-C06E12
MP7
62-C07G4
65-C08B3
71-C09C12
72-C09F11 FB (1 heavy).
73-C10D4
79-SG25/52
79-SG25/54
79-SG25/56
79-SG25/44
79-SG25/33
79-SG25/34] Intensity variation; 1 heavy.
MP8
78-C11H5
83-C12E2
90-C13F7 W(FB)
91-C14D11 FB
95-C15C6
99-C16B
100-C16G5
MP9
102-C16B11
104-C17E6 FB
107-C18D2
109-C18A9
109-C18D8 FB
111-C23F11 FB

960

ZC278-3-45


79-SG25/33
79-SG25/34 Intensity variation; 1 heavy.
MP8 78-C11H5
83-C12E2
90-C13F7 W(FB)
91-C14D11 FB
95-C15C6
99-C16B?
100-C16G5
102-C16G11
MP9 104-C17E6 FB
107-C18D2
109-C18A9
109-C18D8 FB
111-C23F11 FB
111-C23H4
113-C24C2
115-C24B12
115-C24D10
804-1-2AA
116-C24F8
118-C25H5
MP10 121-C26B3
122-C26F6
122-C26G1 FB
123-C26C7
123-C26B11
126-C27H2
130-C28A3
130-C28B4
131-C28E2
MP11 133-C28E10
133-C28G10
137-C29A8
137-C29C10 FO
140-C30F2
501-A1
501-A11
501-A12
501-A15
501-A16
501-A17
501-A19
501-A20
501-A22
501-A23
502-A25
502-A30
502-A31
502-A40
502-A43
502-A46
502-A47
809-D1360
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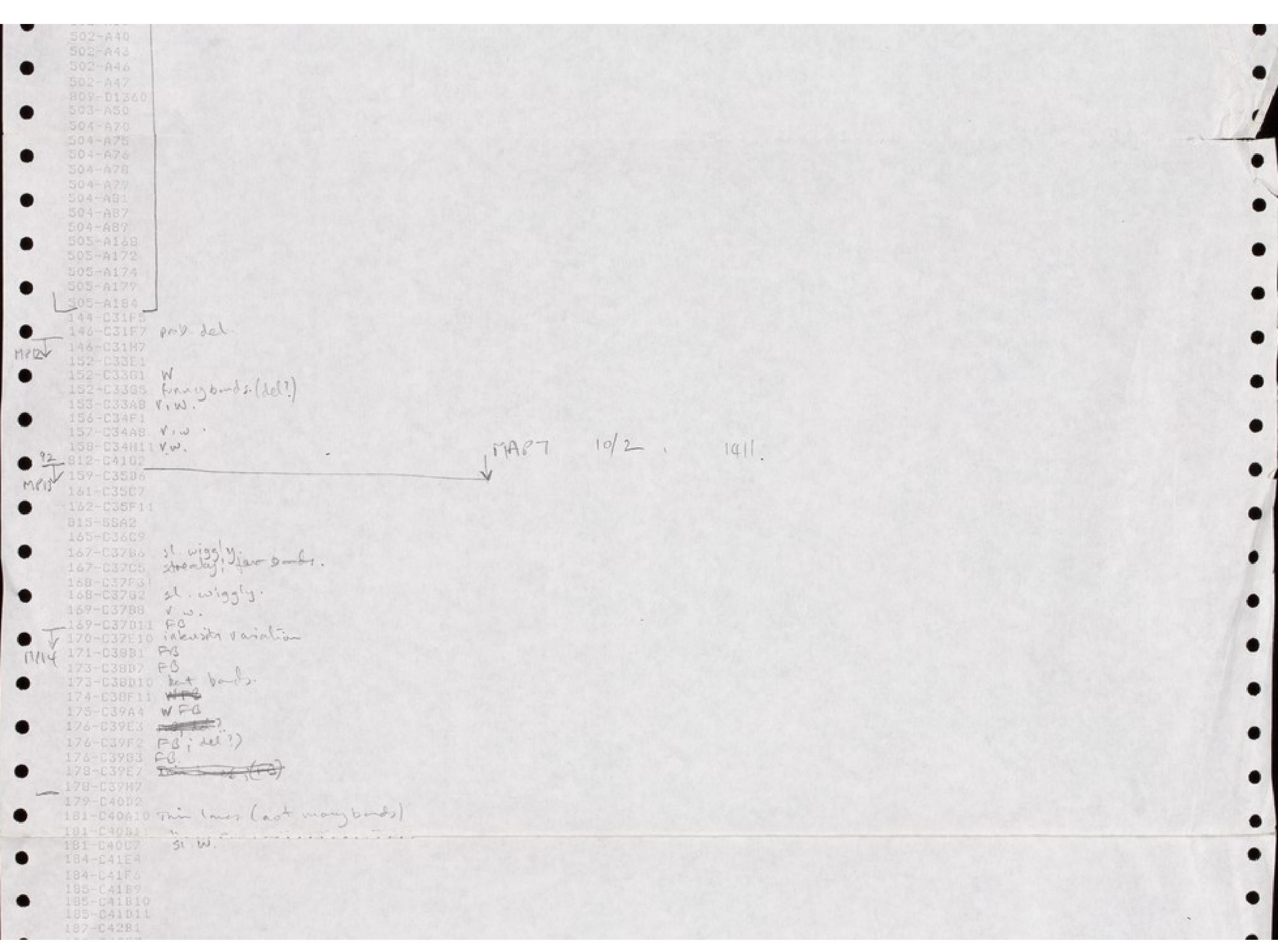
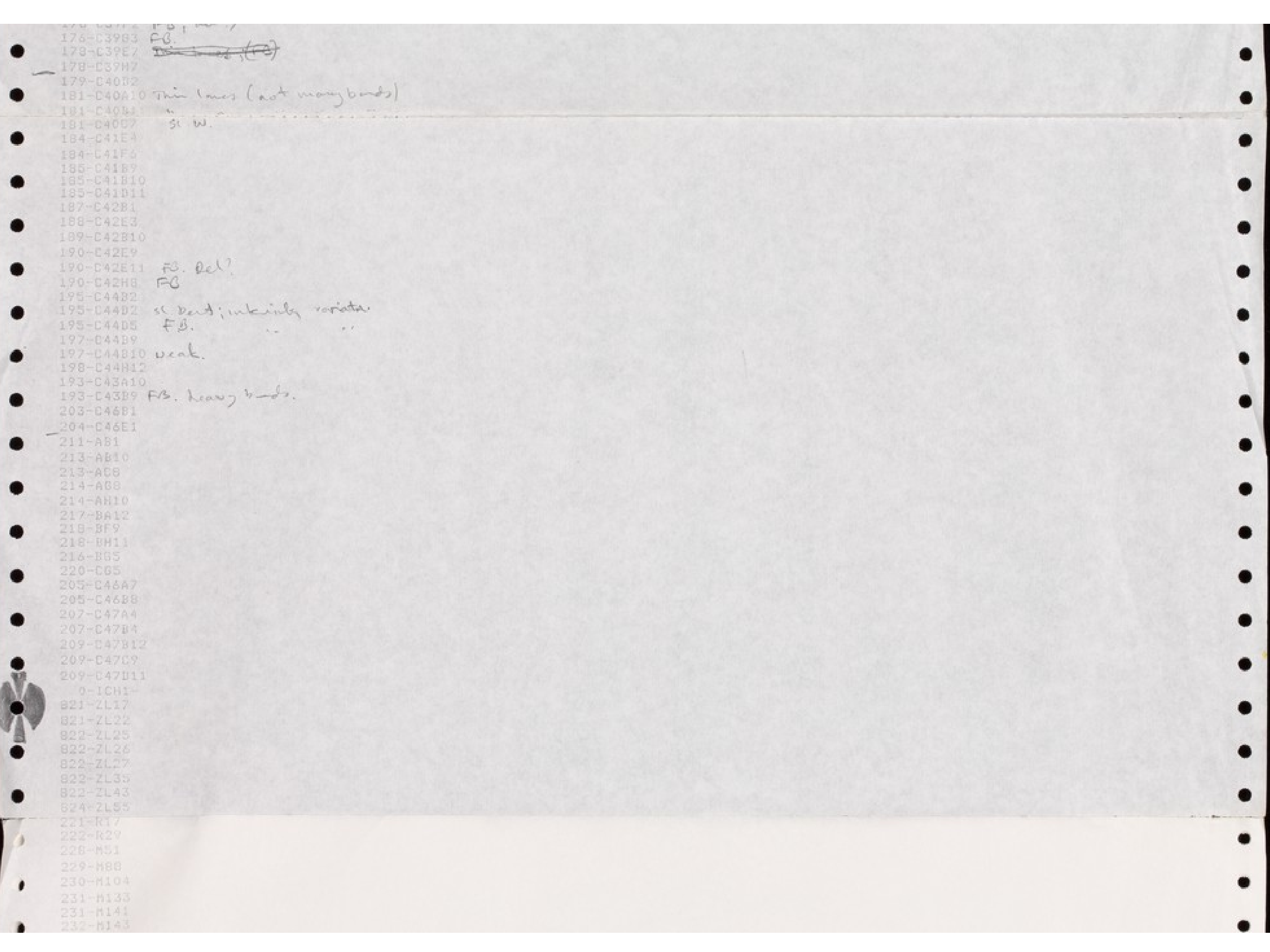
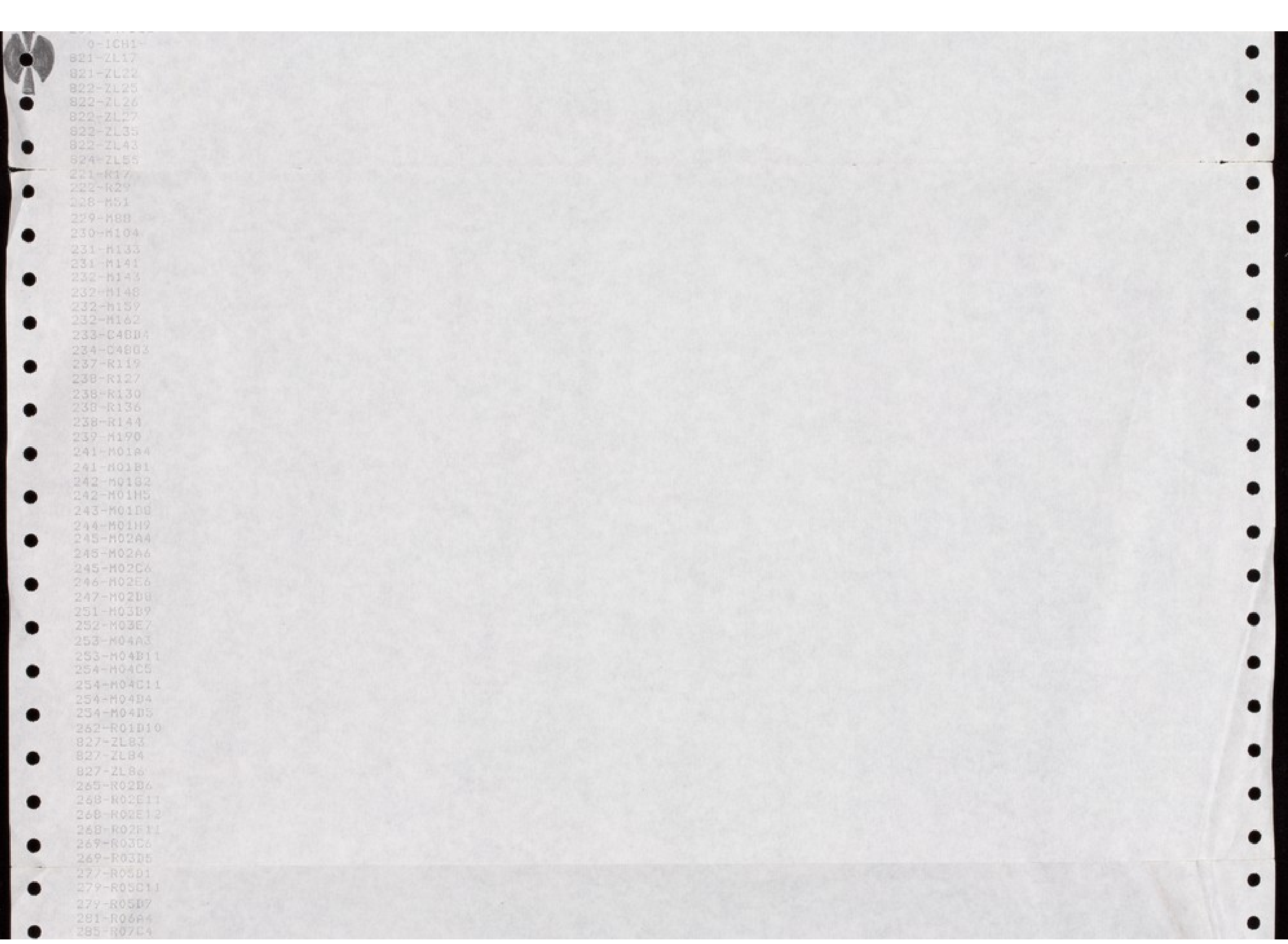
504-A70
504-A75
504-A76
504-A78
504-A79
504-A81
504-A87
2C278-345

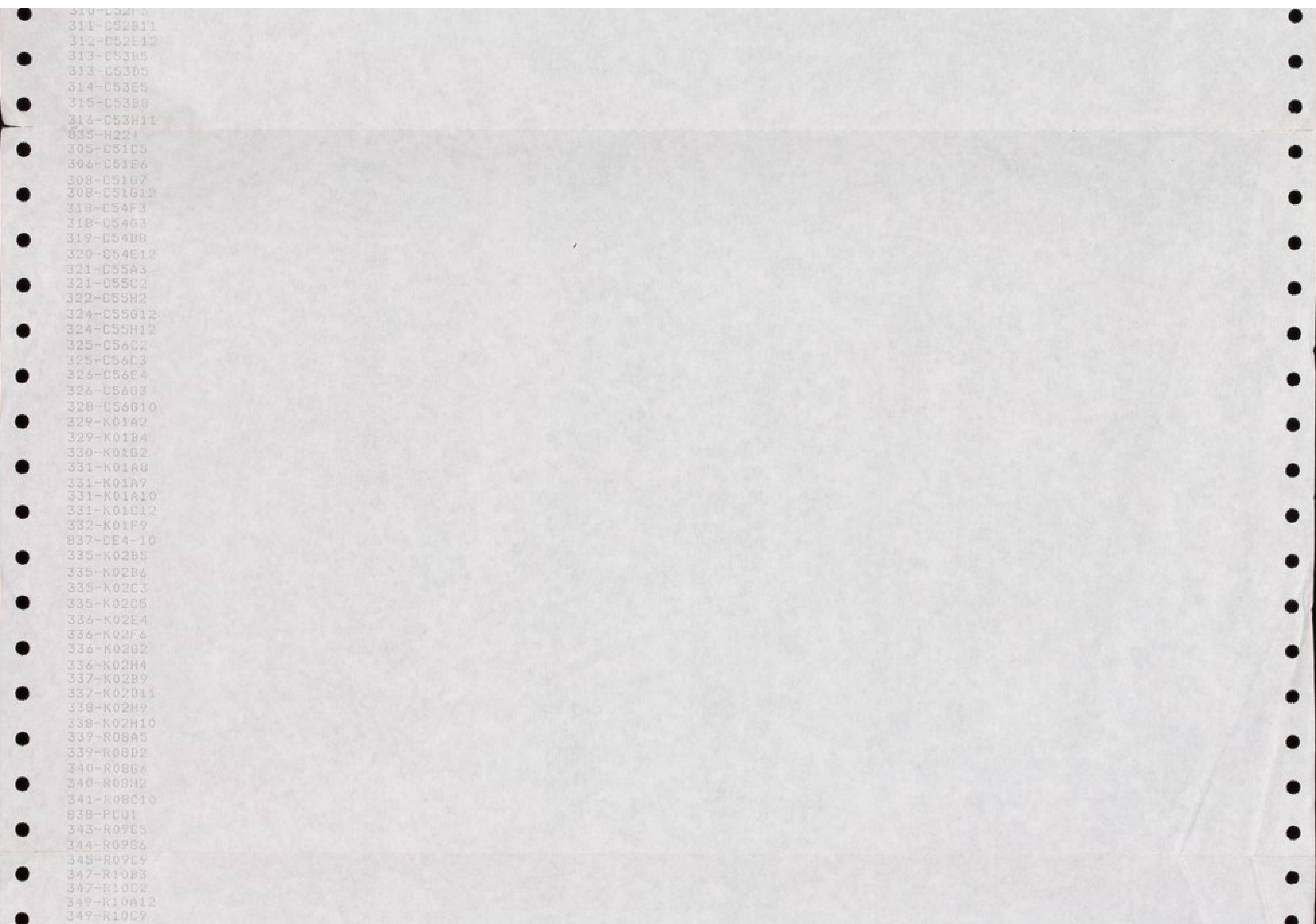


502-A40
502-A43
502-A46
502-A47
809-D1360
503-A50
504-A70
504-A75
504-A76
504-A78
504-A79
504-A81
504-A87
504-A89
505-A168
505-A172
505-A174
505-A179
505-A184
144-C31F5
146-C31F7 prob. del.
MP12
146-C31H7
152-C33E1
152-C33G1 W
152-C33G5 funny bands. (del?)
153-C33A8 v.w.
156-C34F1
157-C34A8 v.w.
158-C34H11 v.w.
92
812-C41G2
MAP7 10/2. 1411.
MP13
159-C35D6
161-C35C7
162-C35F11
815-SSA2
165-C36C9
167-C37B6 sl. wiggly
167-C37C5 streaky; few bands.
168-C37F3
168-C37G2 sl. wiggly.
169-C37B8 v w.
169-C37D11 FB
170-C37E10 intensity variation
M14
171-C38B1 FB
173-C38D7 FB
173-C38D10 bent bands.
174-C38F11
175-C39A4 W FB
176-C39E3
176-C39F2 FB; del?)
176-C39G3 FB.
178-C39E7
178-C39H7
179-C40D2
181-C40A10 Thin lines (not many bands)
181-C40B1
181-C40C7 sl. w.
184-C41E4
184-C41F6
185-C41B9
185-C41B10
185-C41D11
187-C42B1


176-C39B3 F.B.
178-C39E7 ~~[illegible]~~
178-C39H7
179-C40D2
181-C40A10 Thin lines (not many bands)
181-C40B1 "
181-C40C7 sl. W.
184-C41E4
184-C41F6
185-C41B9
185-C41B10
185-C41D11
187-C42B1
188-C42E3
189-C42B10
190-C42E9
190-C42E11 FB. Del?
190-C42H8 FB
195-C44B2
195-C44D2 sl. Del; intensity variation.
195-C44D5 F.B. " "
197-C44D9
197-C44B10 weak.
198-C44H12
193-C43A10
193-C43B9 FB. heavy bands.
203-C46B1
204-C46E1
211-AB1
213-AB10
213-AC8
214-AG8
214-AH10
217-BA12
218-BF9
218-BH11
216-BG5
220-CG5
205-C46A7
205-C46B8
207-C47A4
207-C47B4
209-C47B12
209-C47C9
209-C47D11
0-ICH1-
821-ZL17
821-ZL22
822-ZL25
822-ZL26
822-ZL27
822-ZL35
822-ZL43
824-ZL55
221-R17
222-R29
228-M51
229-M88
230-M104
231-M133
231-M141
232-M143


0-ICH1-
821-ZL17
821-ZL22
822-ZL25
822-ZL26
822-ZL27
822-ZL35
822-ZL43
824-ZL55
221-R17
222-R29
228-M51
229-M88
230-M104
231-M133
231-M141
232-M143
232-M148
232-M159
232-M162
233-C4BD4
234-C4BG3
237-R119
238-R127
238-R130
238-R136
238-R144
239-M190
241-M01A4
241-M01B1
242-M01G2
242-M01H5
243-M01D8
244-M01H9
245-M02A4
245-M02A6
245-M02C6
246-M02E6
247-M02D8
251-M03B9
252-M03E7
253-M04A3
253-M04B11
254-M04C5
254-M04C11
254-M04D4
254-M04D5
262-R01D10
827-ZL83
827-ZL84
827-ZL86
265-R02D6
268-R02E11
268-R02E12
268-R02F11
269-R03C6
269-R03D5
277-R05D1
279-R05C11
279-R05D7
281-R06A4
285-R07C4

827-ZL86
265-R02D6
268-R02E11
268-R02E12
268-R02F11
269-R03C6
269-R03D5
277-R05B1
279-R05C11
279-R05B7
281-R06A4
285-R07C4
286-R07H6
287-R07C12
288-R07G10
289-CD10
273-R04B3
273-R04B5
274-R04G4
275-R04C8
294-DE12
294-DG8
294-DG11
294-DH10
294-DH11
829-LCEH3
829-CCEH113
829-CCEH221
829-CCEH333
829-CCEH1010
830-DBL2D
295-EB2
295-EC1
295-ED5
296-EE6
829-C2D.14B
831-LGA3
831-LGB8
297-C49A1
830-CTL29
301-C50D5
302-C50H5
304-C50F7
304-C50G10
304-C50H11
832-LCES161
309-C52D5
310-C52F6
311-C52B11
312-C52E12
313-C53B5
313-C53D5
314-C53E5
315-C53B8
316-C53H11
835-H221
305-C51C5
306-C51E6
308-C51G7
308-C51G12
318-C54F3
318-C54G3
319-C54D8


311-C52B11
312-C52E12
313-C53B5
313-C53D5
314-C53E5
315-C53B8
316-C53H11
835-H221
305-C51C5
306-C51E6
308-C51G7
308-C51G12
318-C54F3
318-C54G3
319-C54D8
320-C54E12
321-C55A3
321-C55C2
322-C55H2
324-C55G12
324-C55H12
325-C56C2
325-C56C3
326-C56E4
326-C56G3
328-C56G10
329-K01A2
329-K01B4
330-K01G2
331-K01A8
331-K01A9
331-K01A10
331-K01C12
332-K01F9
837-CE4-10
335-K02B5
335-K02B6
335-K02C3
335-K02C5
336-K02E4
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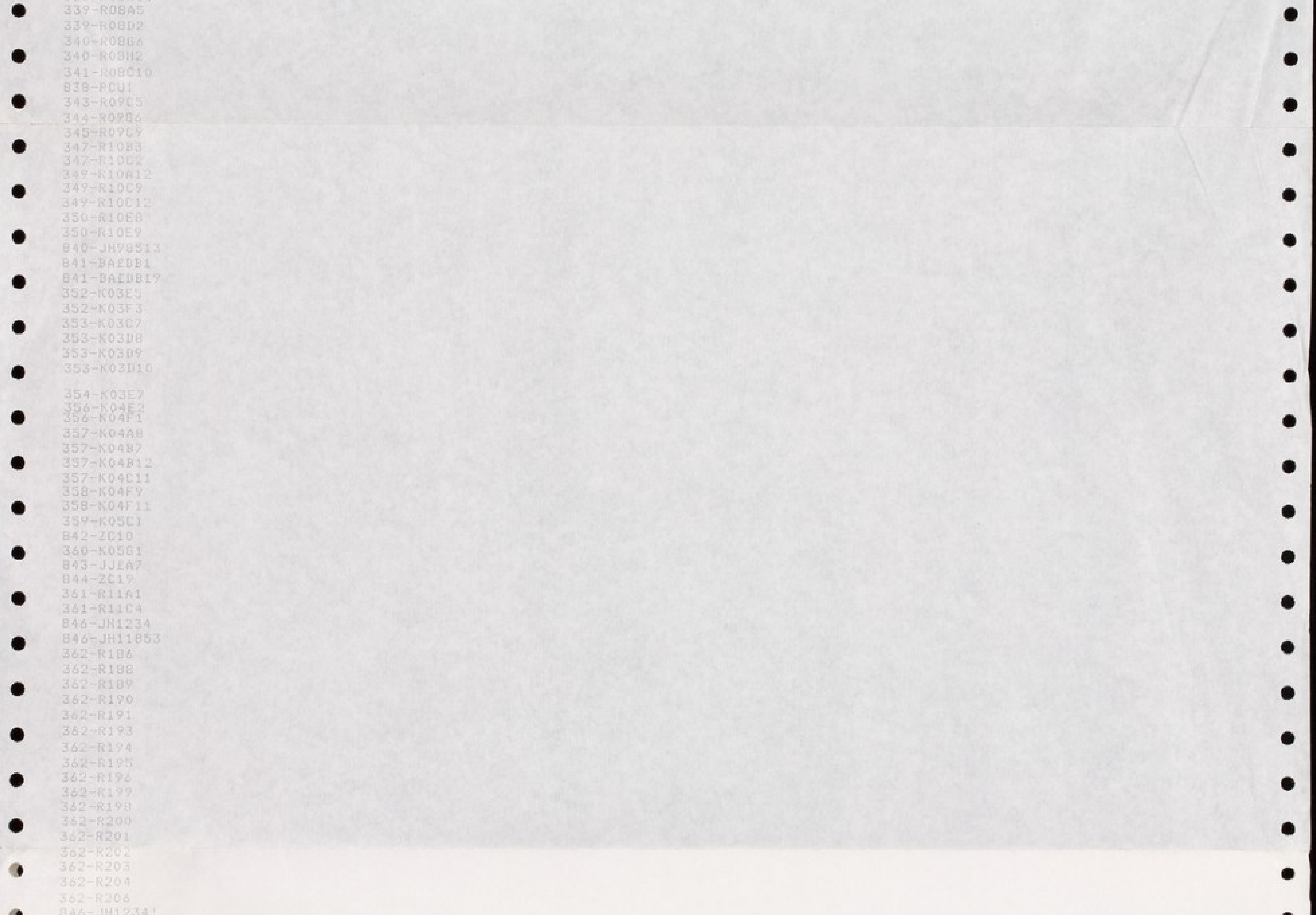
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844-ZC19
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846-JH11853
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362-R189
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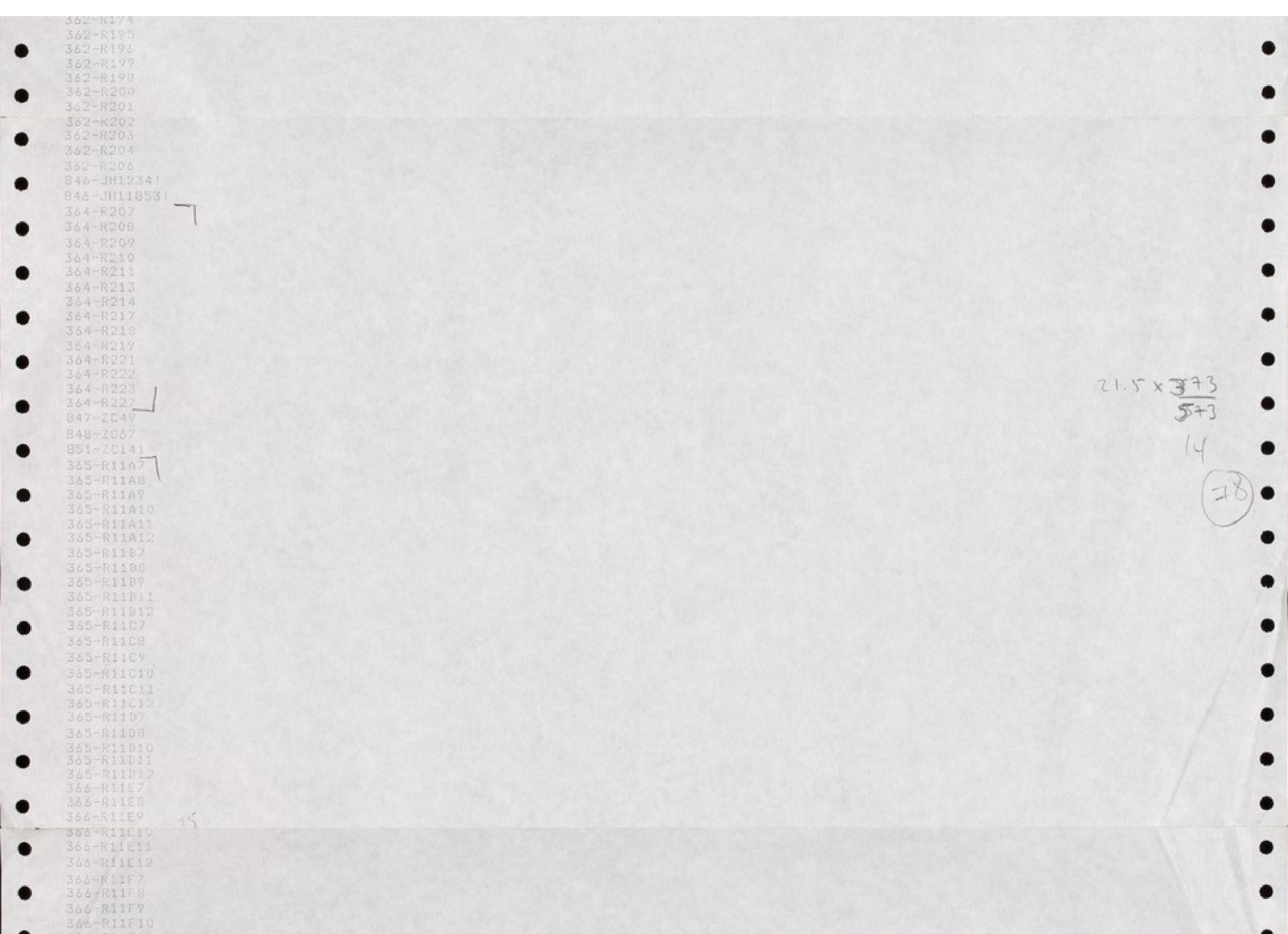

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364-R208
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364-R227
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366-R11E12
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366-R11F8
366-R11F9
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366-R11H11
366-R11H12
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367-R12A2
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367-R12A5
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367-R12B2
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368-R12E2
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368-R12E6
368-R12F2
368-R12F3
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368-R12G3
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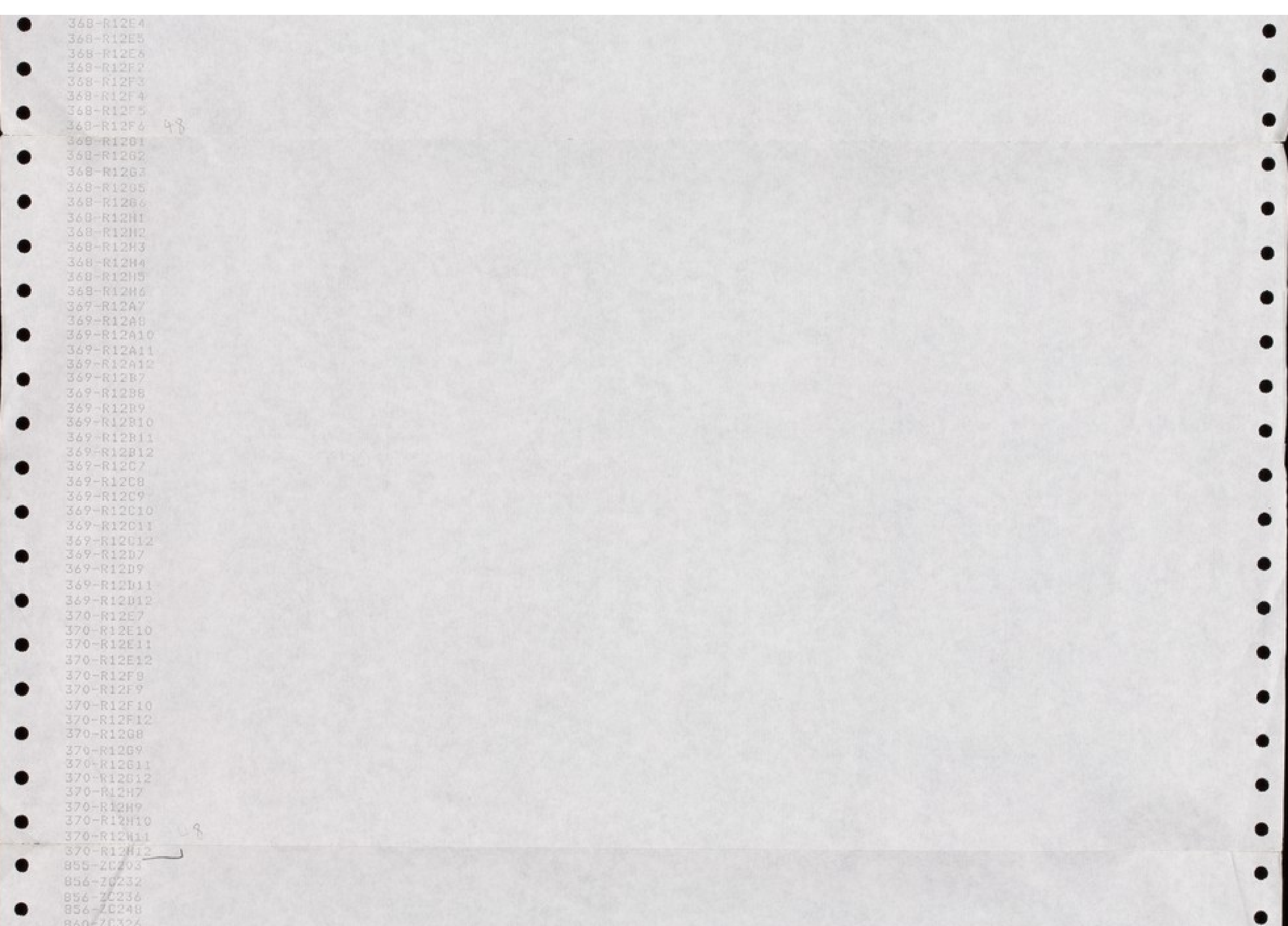
368-R12H1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368-R12E6
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368-R12F6 48
368-R12G1
368-R12G2
368-R12G3
368-R12G5
368-R12G6
368-R12H1
368-R12H2
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368-R12H4
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370-R12G11
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370-R12H7
370-R12H9
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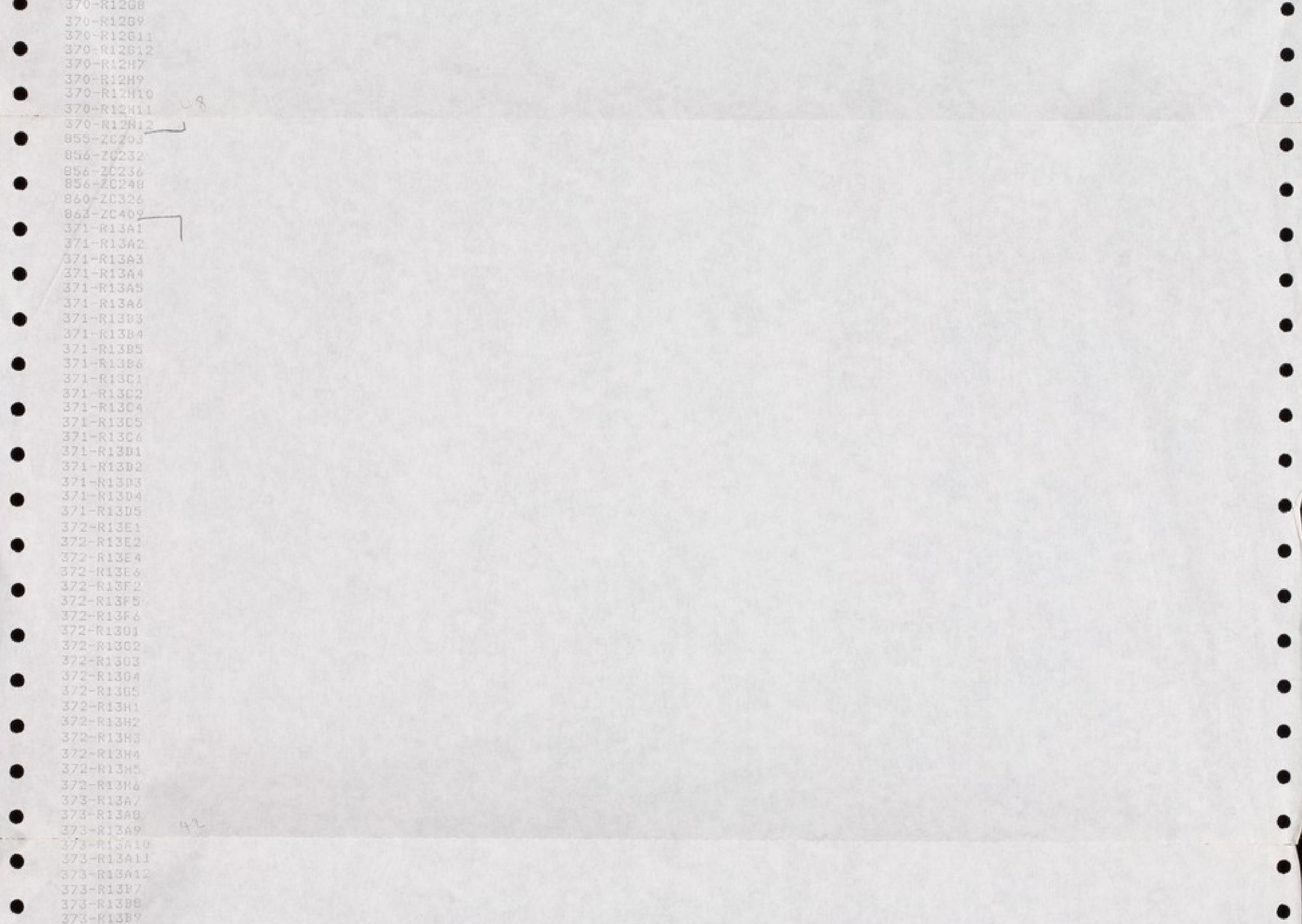

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370-R12G12
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370-R12H9
370-R12H10
370-R12H11
370-R12H12
855-ZC203
856-ZC232
856-ZC236
856-ZC240
860-ZC326
863-ZC409
371-R13A1
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371-R13A4
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371-R13A6
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371-R13B6
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373-R13A7
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373-R13A9
373-R13A10
373-R13A11
373-R13A12
373-R13B7
373-R13B8
373-R13B9
373-R13B10

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372-R13H4
372-R13H5
372-R13H6
373-R13A7
373-R13A8
373-R13A9      42
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373-R13A11
373-R13A12
373-R13B7
373-R13B8
373-R13B9
373-R13B10
373-R13B11
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373-R13C11
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374-R13G9
374-R13G10
374-R13G11
374-R13G12
374-R13H7
374-R13H8
374-R13H9
374-R13H12
               34

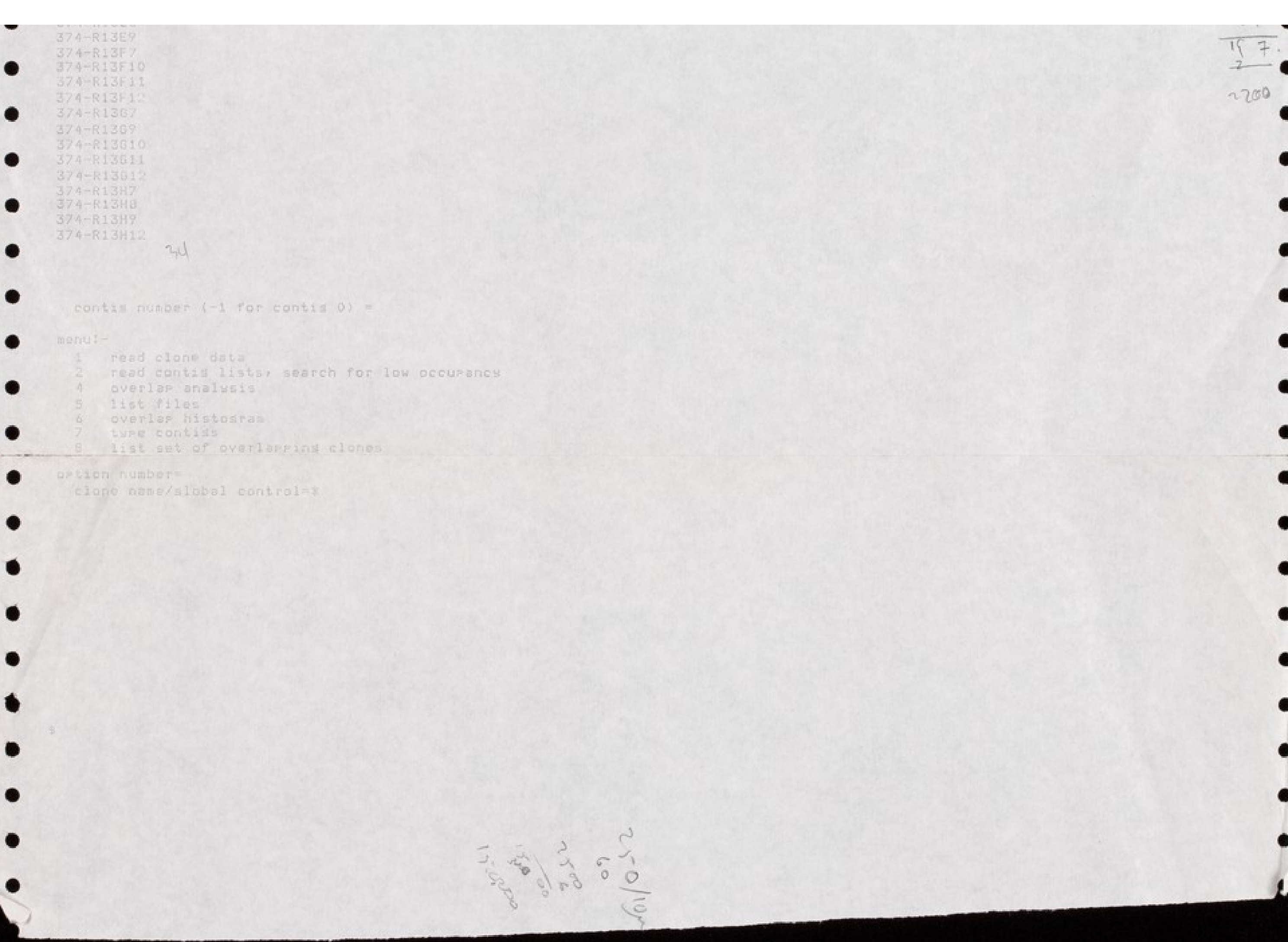

  contig number (-1 for contig 0) =

menu:-
  1   read clone data
  2   read contig lists, search for low occupancy
  4   overlap analysis
  5   list files
  6   overlap histogram
  7   type contigs
  8   list set of overlapping clones

option number=
  clone name/global control=$
```

34
42
48
48
21

1?7.
2
~200



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374-R13F11
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374-R13G9
374-R13G10
374-R13G11
374-R13G12
374-R13H7
374-R13H8
374-R13H9
374-R13H12


  contig number (-1 for contig 0) =

menu:-
   1   read clone data
   2   read contig lists, search for low occurancy
   4   overlap analysis
   5   list files
   6   overlap histogram
   7   type contigs
   8   list set of overlapping clones
option number=
  clone name/global control=*
```
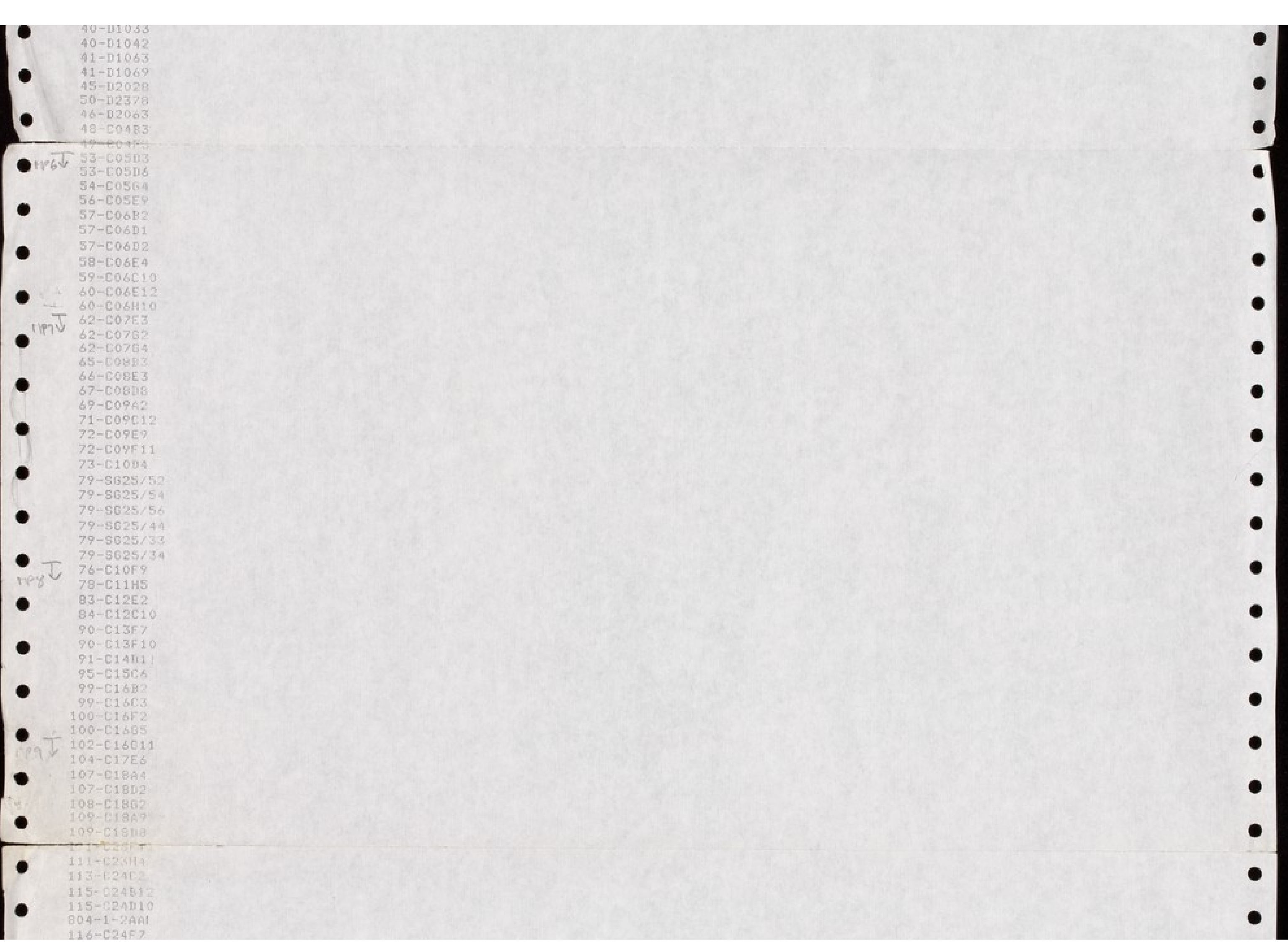

```
   1   read clone data
   2   read contig lists, search for low occurancy
   3   contig statistics
   4   overlap analysis
   5   list files
   6   overlap histogram
   7   type contigs
   8   list set of overlapping clones

option number=7

    contig number (-1 for contig 0) = -1
    file for output (defaults to terminal) =

     0-B0001
     0-B0019
     0-B0041
     0-B0047
     0-B0264
     0-B0310
     0-B0353
     0-B0550
    23-C01B10
    23-C01B11
    25-C02A1
    25-C02B5
    26-C02E2
    26-C02G4
    26-C02G6
    26-C02H3
    28-C02E7
    28-C02G10
    24-C01G7
    30-C03G1
    38-MS 58-3
    38-MS 66
    38-SG25/24
    32-C03F7
    32-C03H12
    40-D1030
    40-D1033
    40-D1042
    41-D1063
    41-D1069
    45-D2028
    50-D2378
    46-D2063
    48-C04B3
    49-C04F3
    53-C05D3
    53-C05D6
    54-C05G4
    56-C05E9
    57-C06B2
    57-C06D1
    57-C06D2
    58-C06E4
    59-C06C10
    60-C06E12
    60-C06H10
```

Unattached 13/1/86

145↓

146↓



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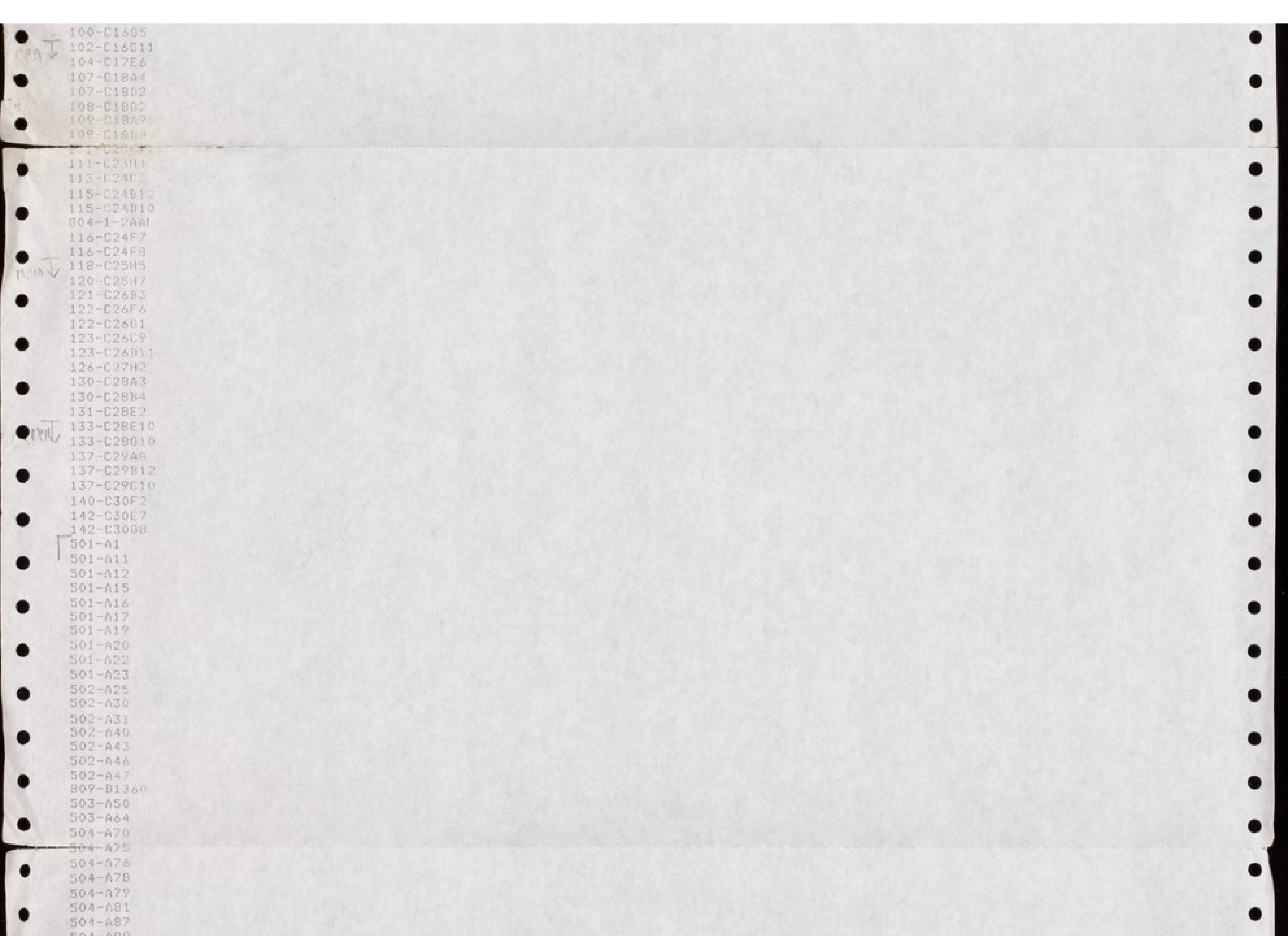
40-D1042
41-D1063
41-D1069
45-D2028
50-D2378
46-D2063
48-C04B3
53-C05D3
53-C05D6
54-C05G4
56-C05E9
57-C06B2
57-C06D1
57-C06D2
58-C06E4
59-C06C10
60-C06E12
60-C06H10
62-C07E3
62-C07G2
62-C07G4
65-C08B3
66-C08E3
67-C08D8
69-C09A2
71-C09C12
72-C09E9
72-C09F11
73-C10D4
79-SG25/52
79-SG25/54
79-SG25/56
79-SG25/44
79-SG25/33
79-SG25/34
76-C10F9
78-C11H5
83-C12E2
84-C12C10
90-C13F7
90-C13F10
91-C14B11
95-C15C6
99-C16B2
99-C16C3
100-C16F2
100-C16G5
102-C16D11
104-C17E6
107-C18A4
107-C18D2
108-C18G2
109-C18A9
109-C18B8
111-C23H4
113-C24C2
115-C24B12
115-C24D10
804-1-2AAI
116-C24F7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[illegible]
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113-C24C2
115-C24B12
115-C24D10
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116-C24F7
116-C24F8
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501-A11
501-A12
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501-A16
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501-A19
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501-A23
502-A25
502-A30
502-A31
502-A40
502-A43
502-A46
502-A47
809-D1360
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504-A70
504-A75
504-A76
504-A78
504-A79
504-A81
504-A87
[illegible]

502-A43
502-A46
502-A47
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505-A168
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505-A179
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144-C31F5
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152-C33G1
152-C33G5
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158-C34H11
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161-C35C7
162-C35F11
815-SSA2
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167-C37B6
167-C37C5
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168-C37G2
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169-C37D11
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173-C38D7
173-C38D10
174-C38F11
175-C39A4
176-C39E3
176-C39F2
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178-C39[illegible]
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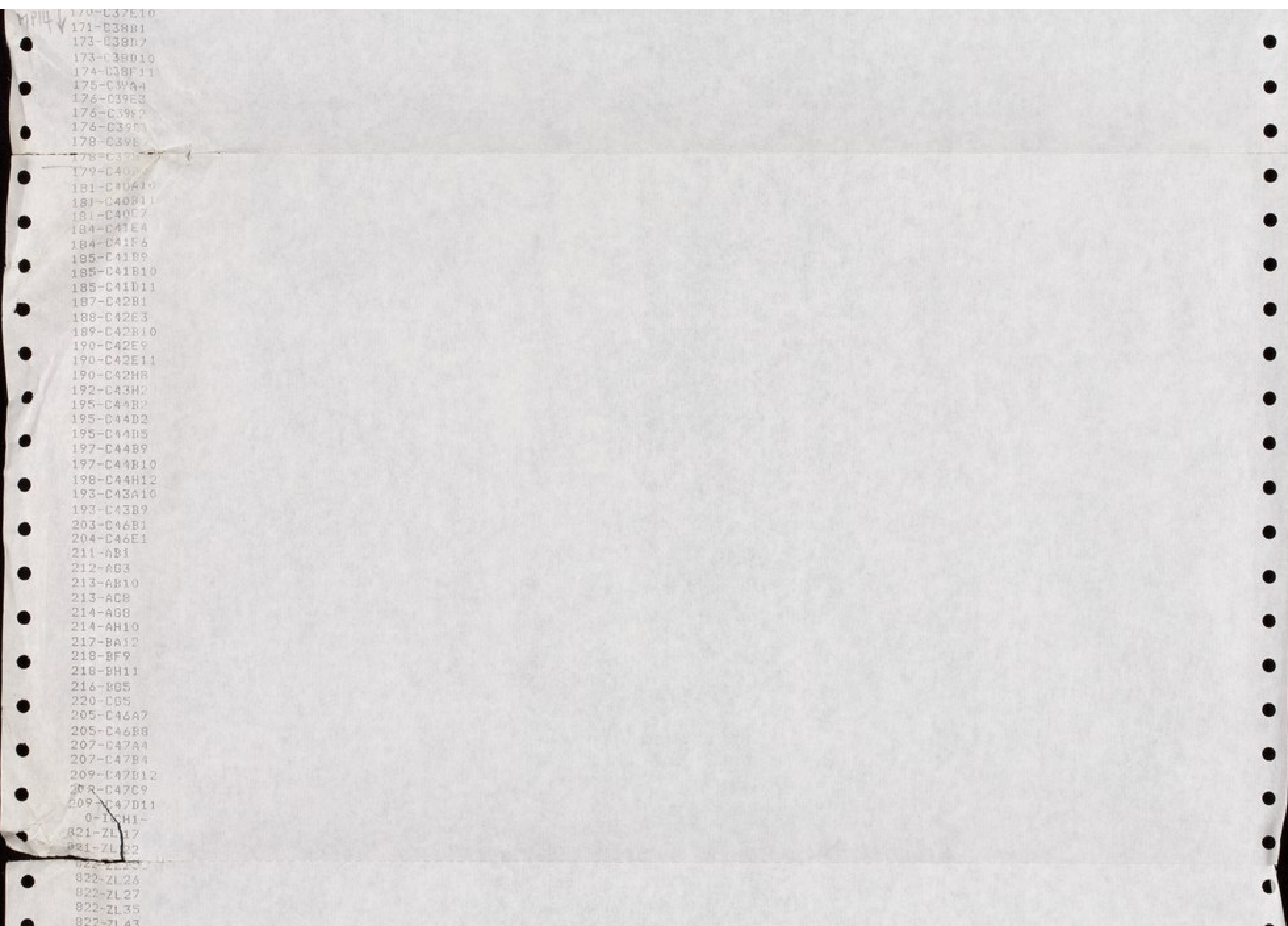
MP12
M1
MP13
MP14



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185-C41D11
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195-C44D2
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197-C44B9
197-C44B10
198-C44H12
193-C43A10
193-C43B9
203-C46B1
204-C46E1
211-AB1
212-AG3
213-AB10
213-AC8
214-AG8
214-AH10
217-BA12
218-BF9
218-BH11
216-BG5
220-CG5
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205-C46B8
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207-C47B4
209-C47B12
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209-C47D11
0-I[illegible]H1-
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821-ZL[illegible]22
822-ZL[illegible]
822-ZL26
822-ZL27
822-ZL35
822-ZL43

209-C47B12
20R-C47C9
209-C47D11
0-ICH1-
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822-ZL27
822-ZL35
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824-ZL55
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222-R29
228-M51
229-M88
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231-M141
232-M143
232-M148
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232-M162
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234-C48B3
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268-R02F11
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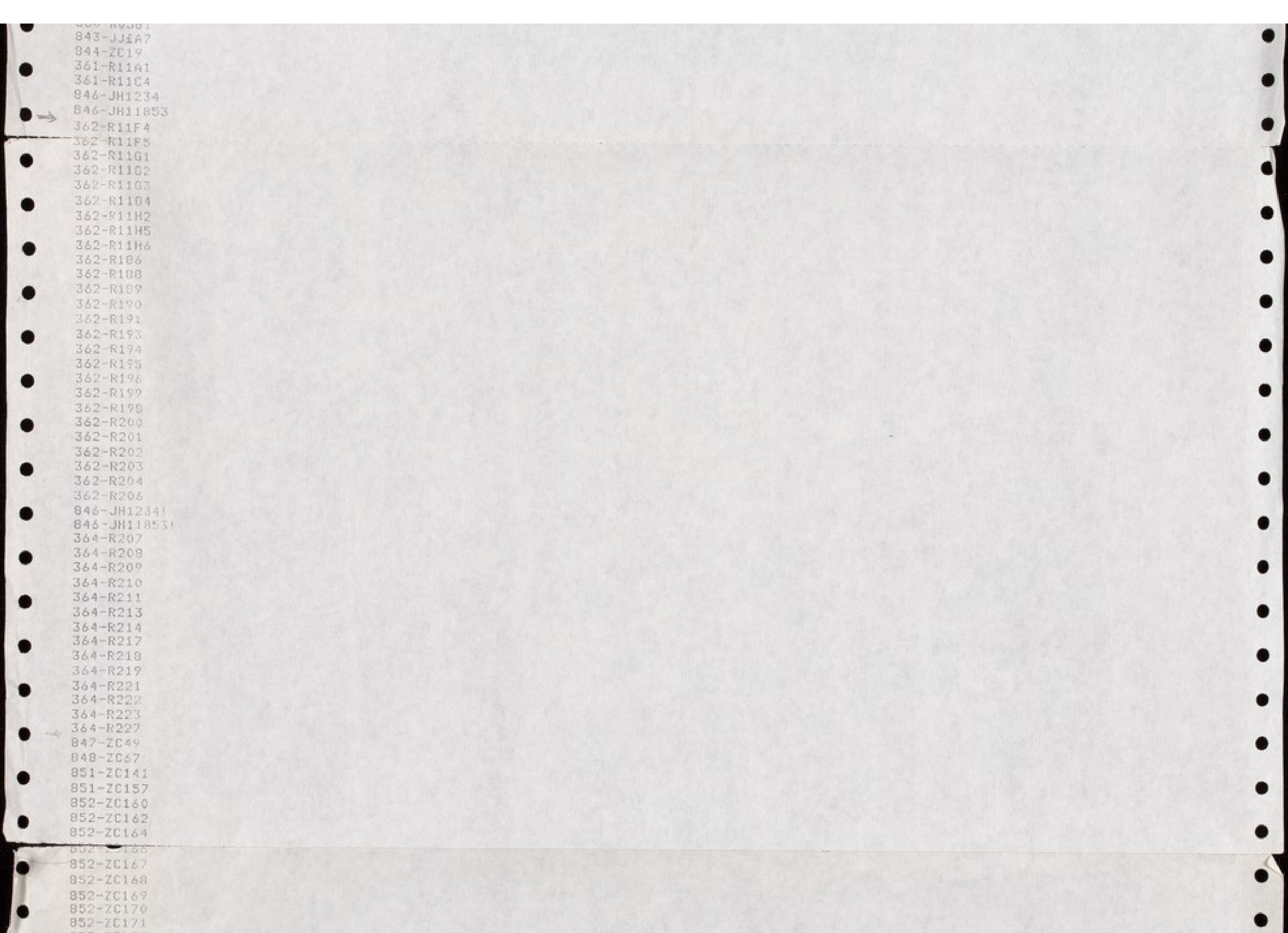
346-R09H7
347-R10B3
347-R10C2
349-R10A12
349-R10C9
349-R10C12
350-R10E8
350-R10E9
840-JH98513
841-BA£DB1
841-BA£DB19
352-K03E5
352-K03F3
353-K03C7
353-K03D8
353-K03D9
353-K03D10
354-K03E7
356-K04E2
356-K04F1
357-K04A8
357-K04B7
357-K04B12
357-K04C11
358-K04F7
358-K04F11
359-K05C1
842-ZC10
360-K05G1
843-JJ£A7
844-ZC19
361-R11A1
361-R11C4
846-JH1234
846-JH11853
362-R11F4
362-R11F5
362-R11G1
362-R11G2
362-R11G3
362-R11G4
362-R11H2
362-R11H5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844-ZC19
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362-R11G1
362-R11G2
362-R11G3
362-R11G4
362-R11H2
362-R11H5
362-R11H6
362-R186
362-R188
362-R189
362-R190
362-R191
362-R193
362-R194
362-R195
362-R196
362-R199
362-R198
362-R200
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362-R202
362-R203
362-R204
362-R206
846-JH12341
846-JH118531
364-R207
364-R208
364-R209
364-R210
364-R211
364-R213
364-R214
364-R217
364-R218
364-R219
364-R221
364-R222
364-R223
364-R227
847-ZC49
848-ZC67
851-ZC141
851-ZC157
852-ZC160
852-ZC162
852-ZC164
852-ZC167
852-ZC168
852-ZC169
852-ZC170
852-ZC171
852-ZC172

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[illegible]
364-R223
364-R227
847-ZC49
848-ZC67
851-ZC141
851-ZC157
852-ZC160
852-ZC162
852-ZC164
852-[illegible]
852-ZC167
852-ZC168
852-ZC169
852-ZC170
852-ZC171
852-ZC172
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852-ZC177
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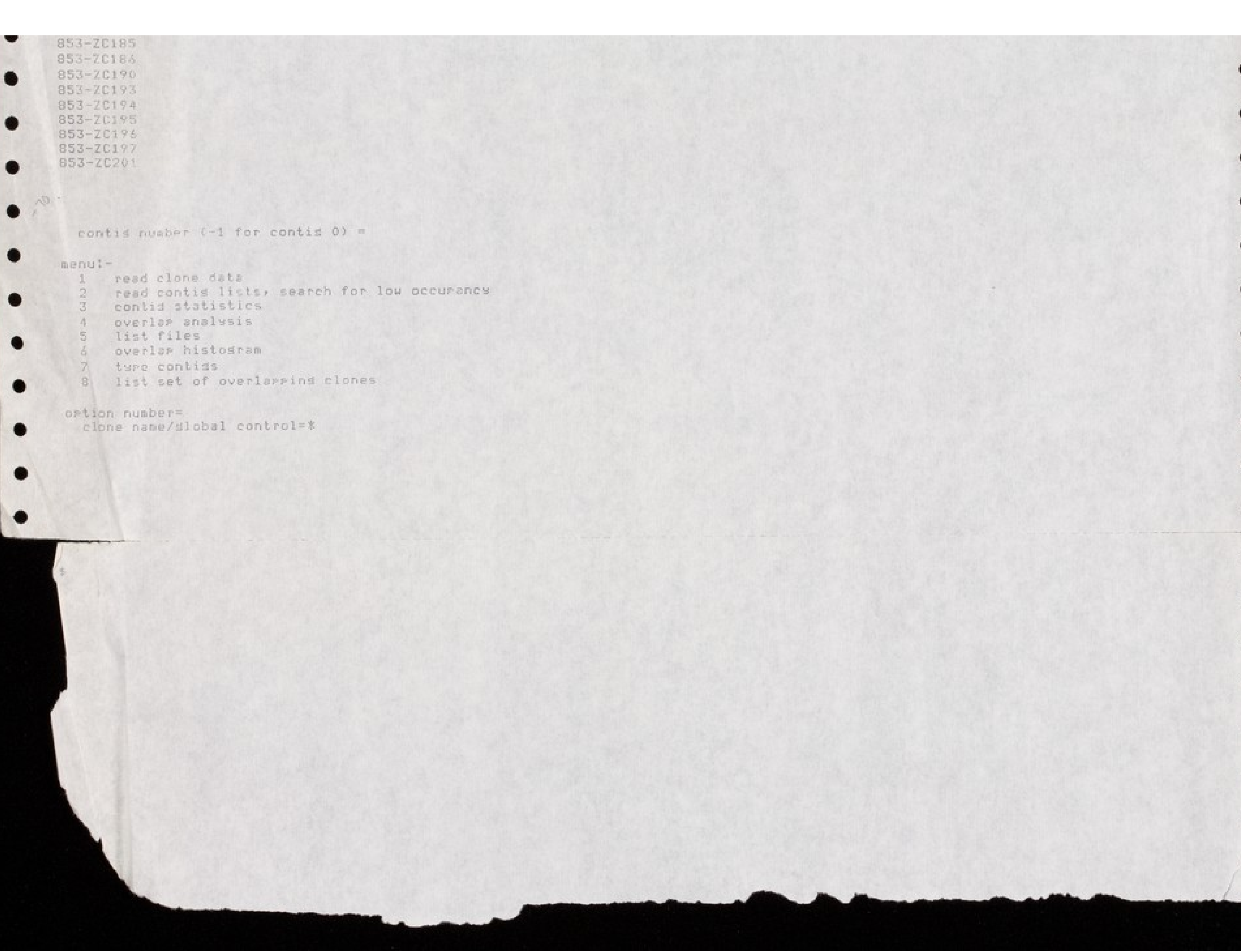
852-ZC180
852-ZC181
853-ZC185
853-ZC186
853-ZC190
853-ZC193
853-ZC194
853-ZC195
853-ZC196
853-ZC197
853-ZC201


  contig number (-1 for contig 0) =

menu:-
  1   read clone data
  2   read contig lists, search for low occupancy
  3   contig statistics
  4   overlap analysis
  5   list files
  6   overlap histogram
  7   type contigs
  8   list set of overlapping clones

option number=
  clone name/global control=*
```



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853-ZC185
853-ZC186
853-ZC190
853-ZC193
853-ZC194
853-ZC195
853-ZC196
853-ZC197
853-ZC201


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 option number=
  clone name/global control=*
```