

OLIVER SMITHIES

Dept. of Pathology & Laboratory Medicine
University of North Carolina
Chapel Hill NC 27599-7525

Dec. 13th 1997.

Oliver Smithies.

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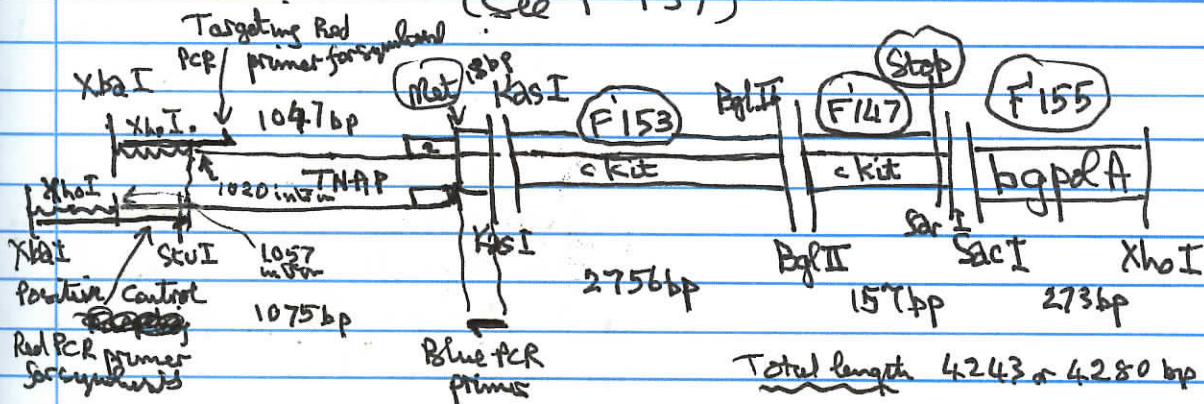
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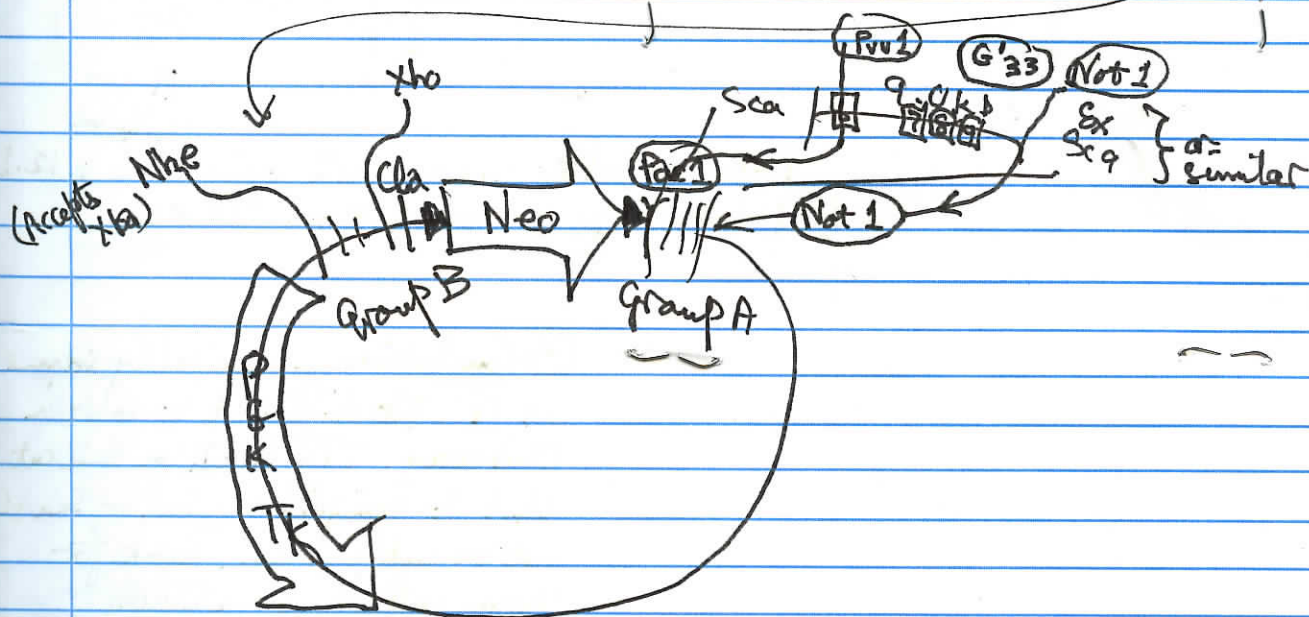
Sat. Dec. 13th

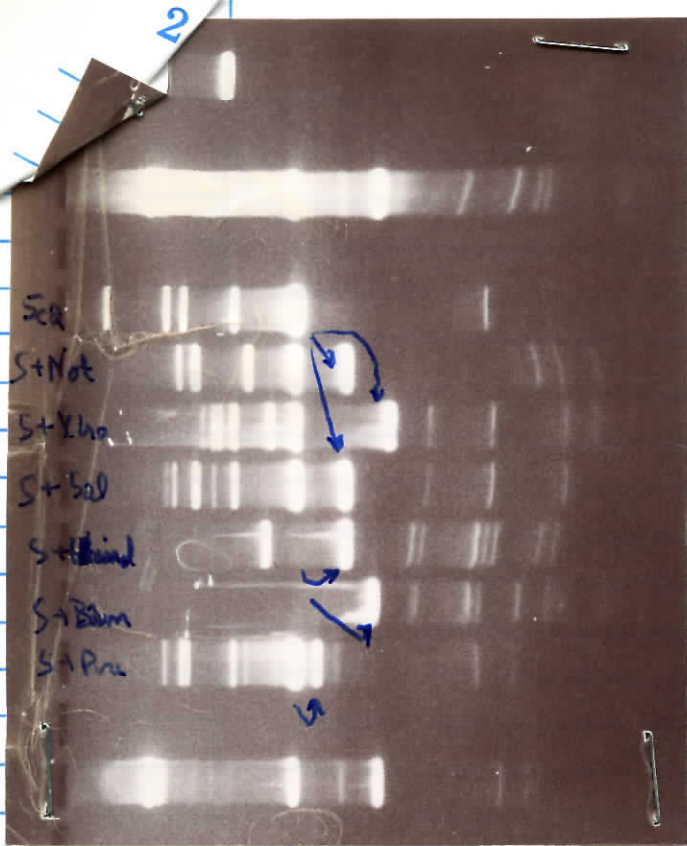
Review of assembly of ckit/TNAP

(See F'139)



TNAP fragment still to make. Whole can be assembled in XbaI/Xho cut BS & cut out later for insertion into pMDel

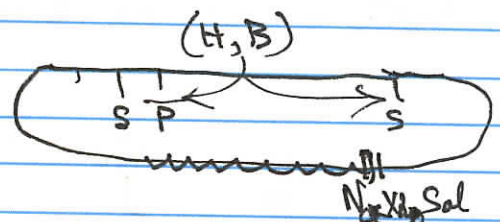




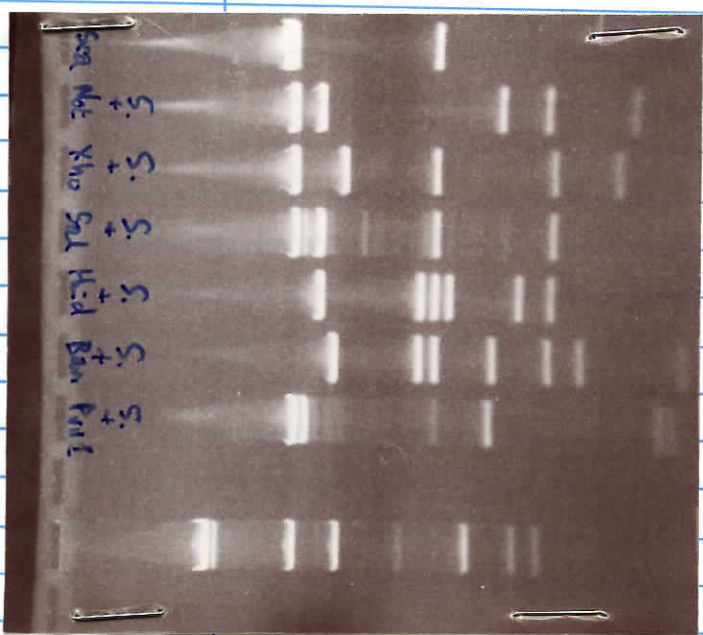
Gel had a buffer problem.

① Conclusion? Second enzyme incomplete on all samples, but by intensity, positions all ^{do} cut. Pvu 1 may give one good end. Not, Sal & Hind are together (presumably in a linker) that doesn't have a Pvu 1 site. Xho & Bam probably cut in the fragment. ~~Dependent~~ Possibly the p14.1 plasmid gives two overlapping Sca fragments, so the 9.0 kb one is not cut by Not, Xho & Sal & is shortened by Pvu.

Map would be:

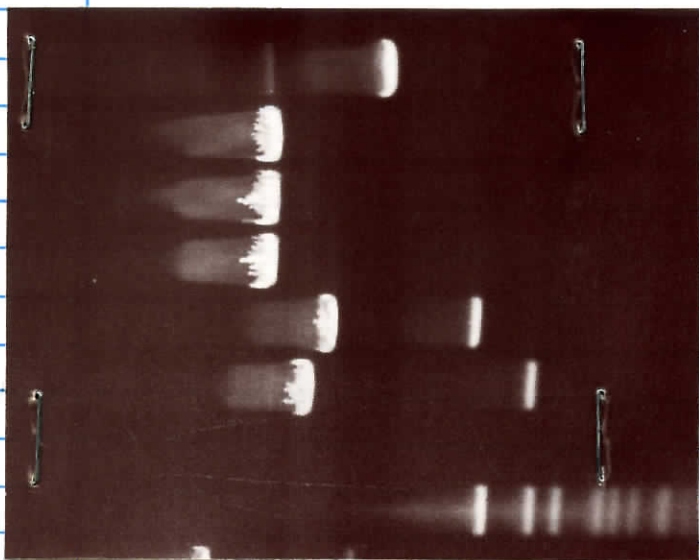
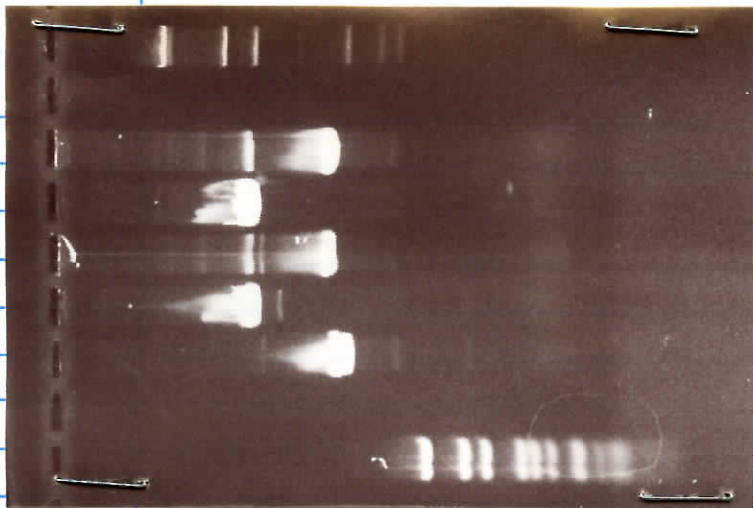


Probably better to use Pvu I to Not I on p12.1
- but total size w'd be 4.3 + 6.1 + ~10



② Conclusion - basically same as ①. Too difficult to solve in this way. Try Afl II on Sca cut 14.1 to simplify (Sca - Sca sh'd not be cut) & then look for non-cutters or slightly shorteners. Not & Xho look best but (or no good at all!).

Agar M
Sca
S. + Not
S. + Xho 1
S. + Sal 1
S. + Hind 3
S. + ~~Sam~~ H1
S. + Pvu 1
W
Ag M
W
Bye



A+Xho

A+Xba

① Conclusion:
 Site I not cutting.
 Inspection of
 Sequence data
 shows overlapping
 Dam methylase
 site! Dam site
 AGGCCTGG
Site site.

② Afl II is there but
 comparatively far
 (Xba) ← 1.3 —*— 1.7 → (Xho)
 (ExSca) (ExSca)

- not important yet.

2 μ l RNase
per 10 ml gel
M 2:45 pm
4:10 PM
0.8% Hx4 Max

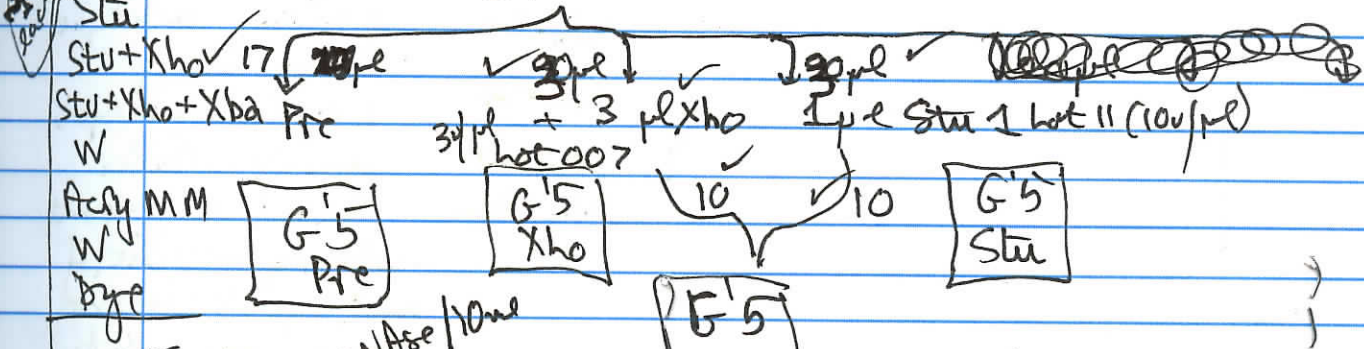
Mon. Dec. 15th

Stu 1?
(Sca) $\frac{1}{3.1}$ (Sca)

Recheck of Stu 1 on Sca - Sca

① Dye
Water
Ag MM
W
Pre
Xho
Stu

Take $\checkmark$ 4 μ l F'145 #1 mini
 $\checkmark$ + 8 μ l 10 $\times$ RS + 0.5M NaCl
 $\checkmark$ + 50 μ l ddw



9:30 am
0.8% 2 μ l RNase / 10 ml
Max

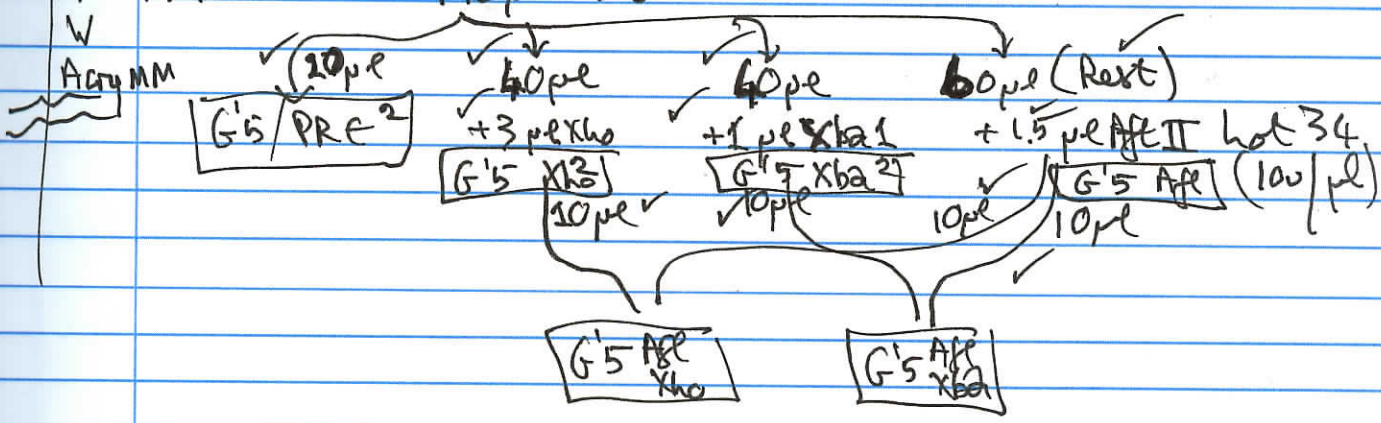
37° 9:45 am

② Dye
Pre
Xho
Xba
Afl
A+Xho
A+Xba
W
Ag MM

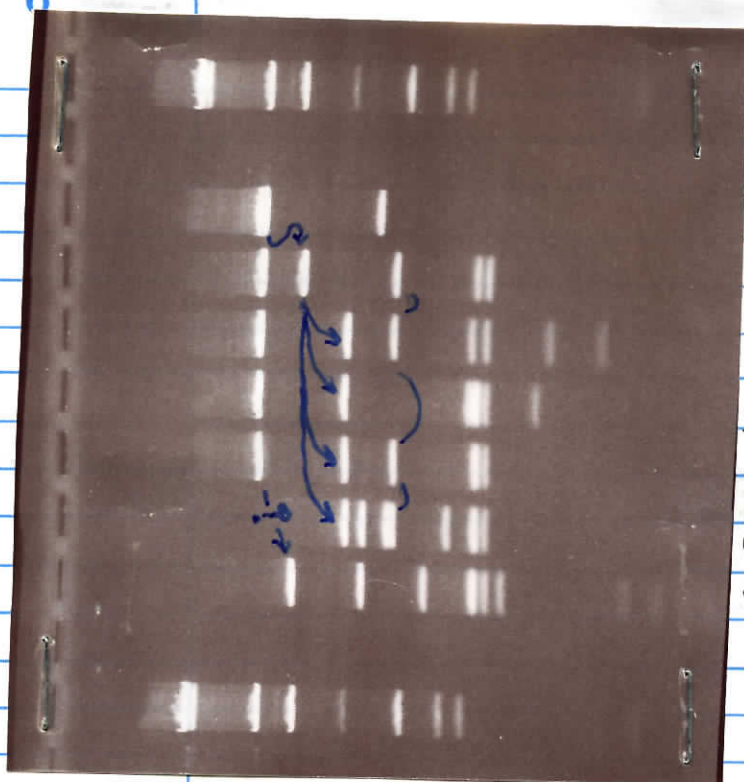
11:30 am
10 μ l + $\frac{1}{2}$ μ l Xba I lot 30 (200 μ l)
G'5
SXX

2:20 pm

Take $\checkmark$ 4 μ l F'145 #1 mini
 $\checkmark$ + 16 μ l 10 $\times$ RS + 0.5M NaCl
 $\checkmark$ + 140 μ l ddw



37° 5:45 pm



①

Conclusion: Afl II removed the
marking band. Not I, Xho I &
Sal I do not cut the Sca^{9kb} Sca

Sca

Can $\therefore$ change one (3')

Sca + Afl

of the 2 Sca sites

S.A. + Not

to Not and use

Pvu2 - Not I insert

S.A. + Xho

of 6.5 kb. Can distinguish

S.A. + Ssp

the two sites by

a Pvu/Not digesting

S.A. + Hind

same size as Pvu/Sca.

S.A. + Bam

Can put this fragment

into Msp Sal first, if

nec^y. Since it does not

cut with Xho, but

don't know Xho status.

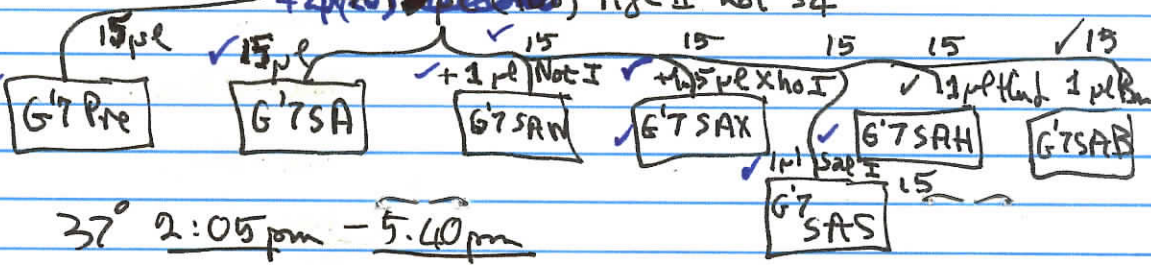
Can't
Not to

Tues. Dec. 16th ~~Alc~~ ^{Seq} | ~~9~~ ⁶ ~~10~~ ¹¹ | Seq |

looking for Nan-cutters in 9 kb Seq

(Pre) Sca
Sca Aft
SA + Not
SA + No
SA + Sal ✓
SA + H-land ✓
SA + Barn
W
M M
W
Dyl

Take whole of G_3 s.a (110 yr)

$$+2p(10) \cdot p(10) \text{ Afl II lot 34}$$


37° 2:05 pm - 5:40 pm

Wed. Dec. 17th

Assembly confederation

Almost certainly the 3' homology can be either the 9.0 kb ScaI - ScaI or the 6.5 kb PvuI - ScaI fragments modified to insert bi-directionally into Not I or oriented into PvuI & Not I. Technically it may be easiest to ligate phosphorylated Not I linkers onto the blunt ScaI piece, cut with Not I & reclaie for purification. Need to know if any of 5' sequences are cut with Not I.

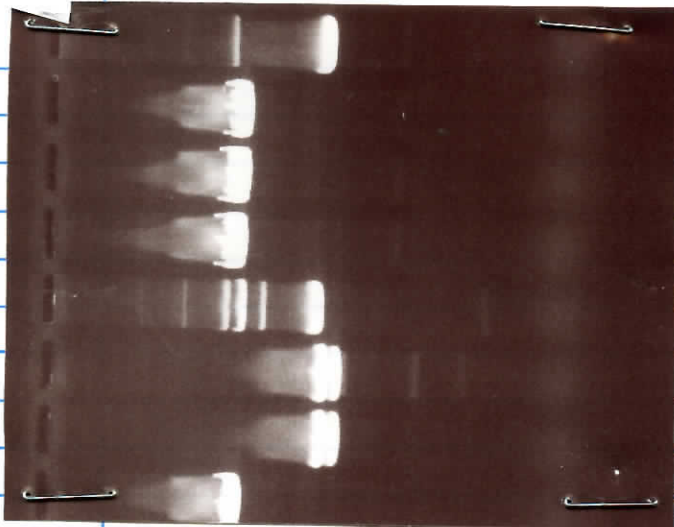
Can't will
Not twice!

Note 2 None in bptt, ckit, TNAP coding. Need to check Scs 3.1 [2] Scs
Also good to check Scs 9.0 Scs for XLA

Xho I None in bp A by Sac I to Xho I, ckit, TNAP 3.1. Need to check Sca I $\xrightarrow{9.0}$ Sca

Para 1 None in bp A, skit, Need to check Sca 1 3.1 ~~1~~ Sca

9.0 can use: Xho, Sal, Xba, Not, Spe, PvuI, NheI
Cannot use: Hind, Bam



(1) Pre

Xba

Xba Not

Not

Pvu 1

Xba+Xho+Pvu 1

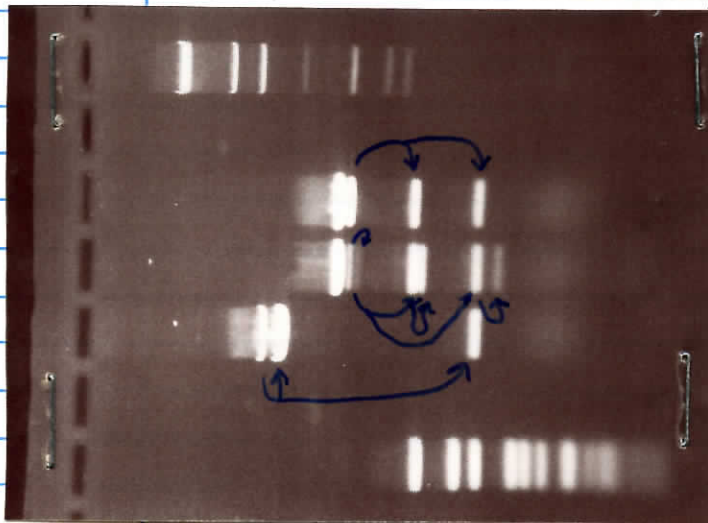
Xba+Xho

Xho

Conclusion: Demonstrates
absence of Not 1 in
3.1; Pvu 1 too unspecific
to tell. #

INSERT

G'11
Sca Aff
SA+Xba



↑
3.1 XK

(2) Conclusion: ~~3.1 does not cut with~~

Xho, Xba, Sca

Pvu Xho, Xba, Sca

Xba, Not 1, Sca

3.1 does not cut with
either Xba or Pvu 1
(or Not 1)
Pvu cuts BS 2x; Sca cuts 1x

ON
Bye
w
Pre
Xb
Xba
Na
Pvu
PX
XbX
Xho
W
M
W
Bye
32°
E
B
7090
ON
Pvu
Bye
W
A
W
X
P
X
W
A
W
Bye

0.8% HX4 2p RNAse / 10ml ^{winter sites}

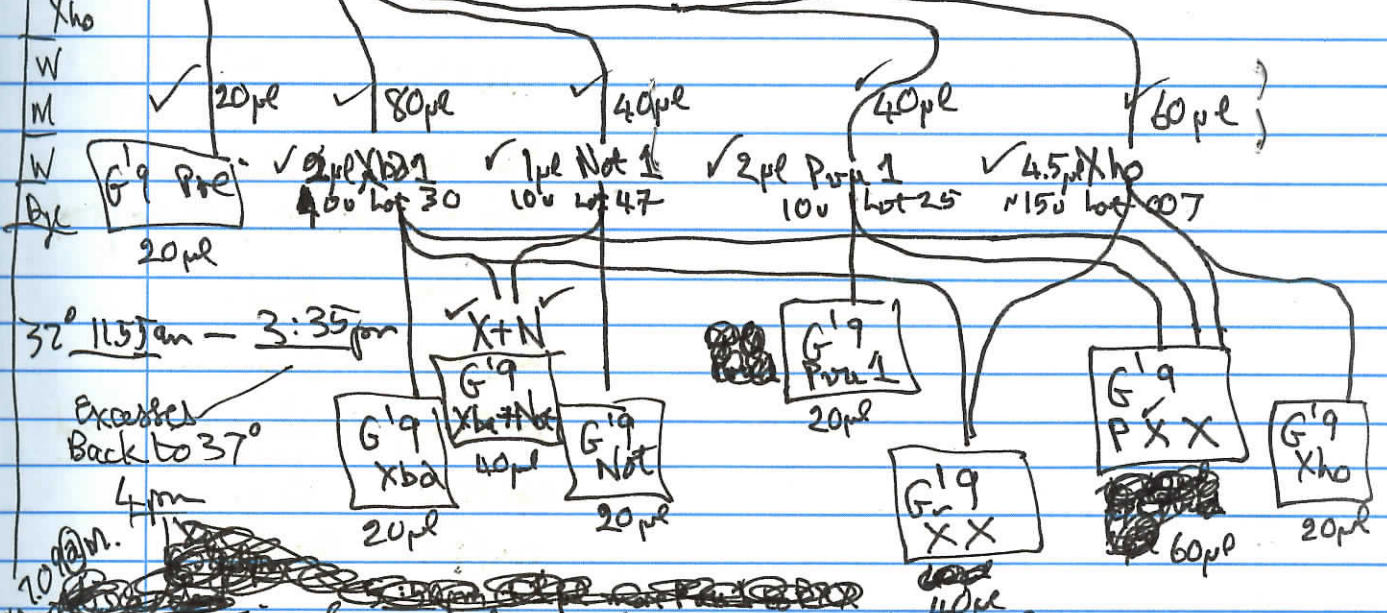
Bye w ①

Wed. Dec. 17th Not (Sca) 3.1 2 (Sca) linker site Xho

Pre
Xba
Xba+Not
Not
Pvu1
PXXho
XbaXho
Xho

3.1 Sca-Sca check for Not Xba & Pvu1

Take 6 pL F145 #1 mini
✓ 24 pL 10XRS + 1.0 M NaCl
✓ 210 pL d.i.d.w



This should check for a second Not within the 3.1, & look for a Pvu1 other than the one in BS.

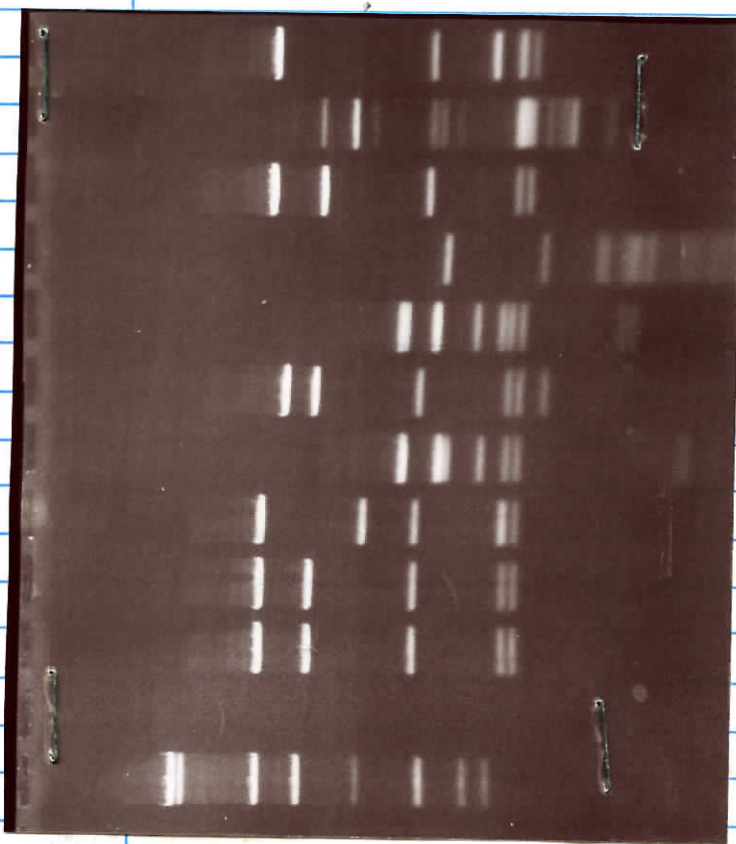
6:05 pm + 1 pL more Pvu1 to PXX → 1 pL Sca1 to PXX & XX
7:00 am! + 1/2 pL to Xba+Not cuts BS near Pvu1 (100 bp)

Xho, Xba, Sca
Pvu1, XXS
Xba, Not 1, Sca
W
Agar M.N.
W
Dye

$$\text{Sca} \rightarrow \text{Agl}$$


① Conclusion: ✓ ✓ ✓
XbaI does not cut 9 kb Σ - Σ

①



SA. + X_{ba} ② Conclusion

K_{pin} Neg.

Pac looks ok, but no proof

Sty Neg.

• Pos Neg.

• **Nhe** - 2 site near one end
- check which

2. Aut Neg.

~~Spe~~ Good

Time looks ok, but no proof

Sca + Afl

~~12:18 PM~~
~~12:15 PM~~
OST
Bx
N
Fill Kpn
Pac [] S
Sax + Sax
Stg
Bx
Nhe
AOT
Spe
90
W
MM
W
bu
92

K. S.

20

30
125

8.3

12

↓
to Sea-Ser

Seq G'9
① Sea + Aff
SA + Xba.

Wed. Dec 17th



Looking for more non-cultures in 9 kb Sea

MM
OST 4x4 Max
G'11 SA + Xba
G'11 Sea Aff
p14.
St
Br
Nhe
Avt
Spe
Pme
9(SA + Xba)
W
MM
W
Dye
②

Take 2 µl (~5 µg) p14.1 G'3
✓ 35 µl 10xRS + 1M NaCl
✓ 313 µl H₂O
✓ 4 µl Sea I lot 17 (100 µl)
✓ 4 µl Aff II lot 34 (100 µl)
40 µl
200 µl Lot 30
✓ + 1 µl Xba I
G'11 SA
G'11 SA + Xba
G'11 SA

} For future use.

37° 1:25 pm to 3:35 pm & 4 pm — 5:40 pm Freeze

Thurs. Dec 18th

Testing for ~~other~~ other enzymes

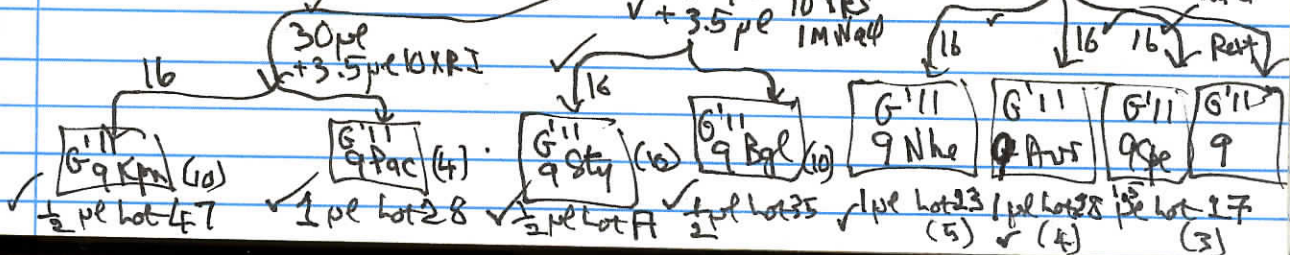
Take whole of G'11 SA + Xba (303 µl)
+ 30 µl 8M NaCl
+ 800 µl EtOH

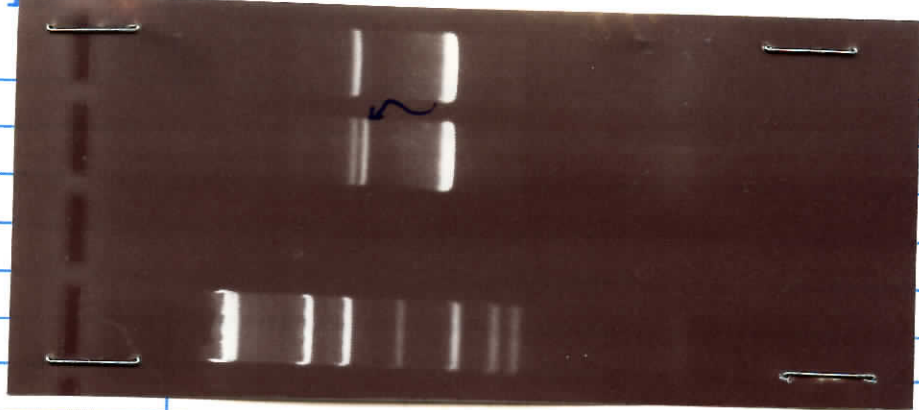
8 µl + 2 µl NEB #4
+ 1.5 µl
9 Pme
lot 10 (4)

Kaelt's Pme I
37° 50 mM NaCl Nhe I, Avt II, Spe I
3:35 pm

100 mM Sty I (nucleicase), Bgl II

12 pm R1 Kpn I, Pac I





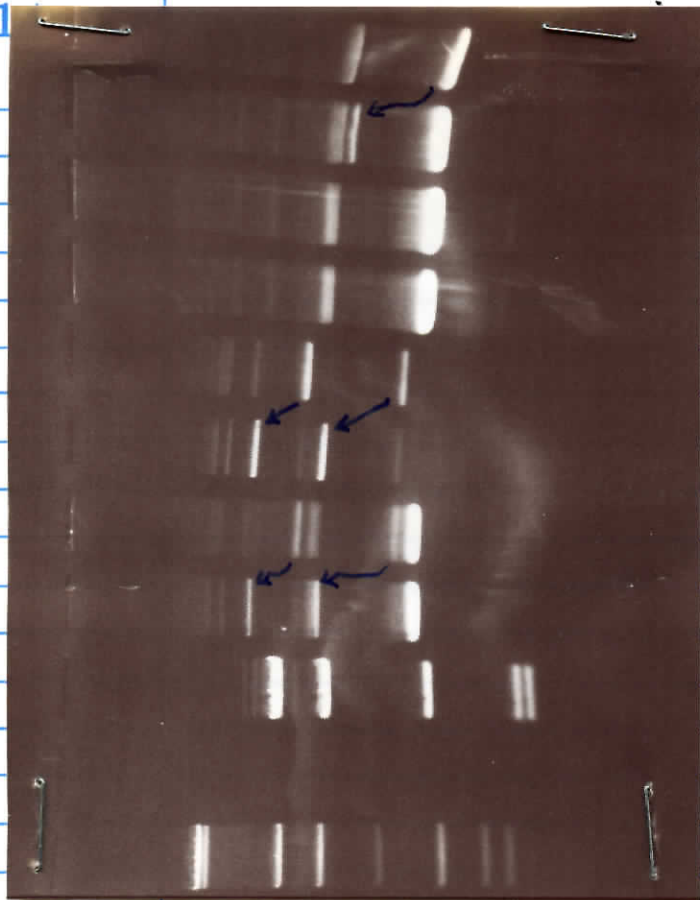
① Conclusion :

Dup Del was poor
choice, almost exactly
same size as Sea-Sea
BS. Repeat G'15 with
Duplod & more controls.

See G'14.

7/10/12
G'15
G'15

32° 4:10pm 5:10pm



6'13

① Conclusion: Pac worked fine, and 3.1 has no sites - but

Sca-Sca Pre

Sca-Sca Pac

Deep Red Pre

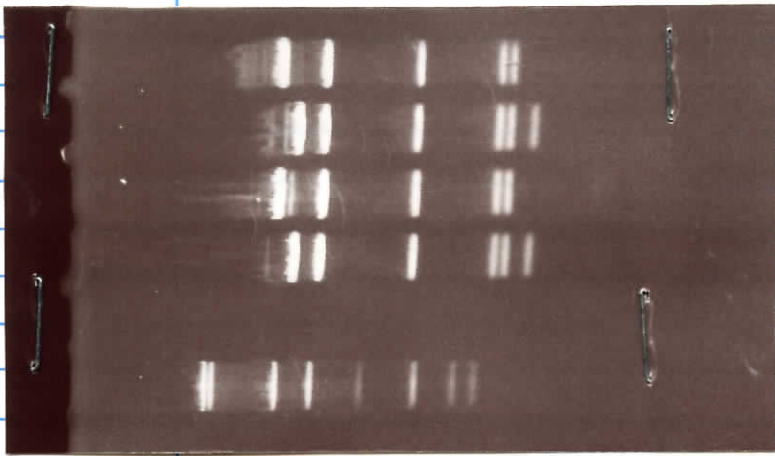
Deep Red Pac

Book Pre

Book Pac

9 kb Sca+Afl

But Cod has some ancient dimers. Sca, Afl p14.2 is fine



②

SA

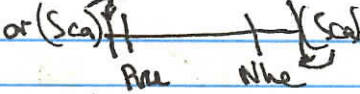
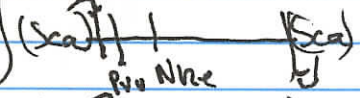
SANhe

SAPw

NP

SA Bst

Digestion with conclusions: Fvu is poor (as usual - add 1 ul more for overnight) but order is either



and neither is good.

~~Not a better choice~~

Q1 9:10am
 10:10am
 0.8
 2pe RNAse

Thurs. Dec. 18th

Pac I in Sca^{3.1} Sca (contd.)

Dup W ①

G'13 Pre3.1

Pac3.1

Pre F'

Pac F'

Pre F'

Pac E'

Pre E'+F'

Pac E'+F'

G'13/9 Sca-Aff

W

MM 37° 7.05 pm

W

Dye

Dup Ad was a poor choice of control - it's 6.1 kb,

almost exactly same as Sca-Sca BS

∴ take

2pe F'145 #1 mini + 60pe 1xRI salts

1pe E'137 Dup Cod (1kb)

30pe

+1pe Pac I

+1pe Pac I

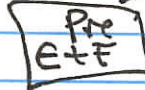
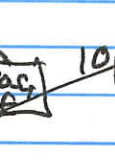
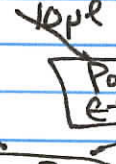
30pe

Pre F'145

Pac F'145

Pac E'137

Pre E'137



General Conclusion: can use Pvu I TNAp Not I 8 Kb as 3' homology into Pac I & Not I Group A sites.

②

∴ can use Xba Xho TNAp ckit Xho

as 5' homology into Nhe I & Xho I Group B sites.

Because of the Nhe I site in 8 kb, the order w'd have to be probable 5' first. Worth finding out where the Nhe site is located, altho' lengths suggest it is in 8 kb

Take G'13 Sca Aff

Q1 9:10am
 10:10am
 0.8
 2pe RNAse

G'15 SA

SANhe

SAPvu

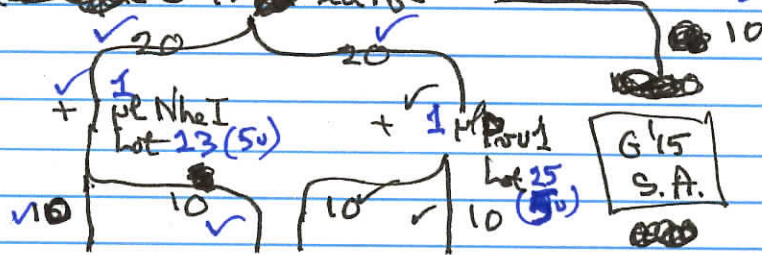
SANP

W

MM

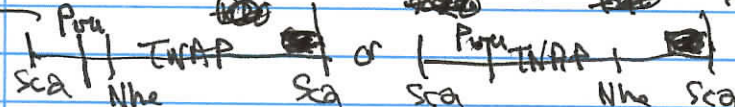
W

Dye



37° 2:40 pm

6.25 pm



+1pe more Pvu I + 10pe + KCl to 50 mM (correct AS) 1xRS 50 mM NaCl

12 noon
 12:30 9.4X4
 2.5 10 and Max
 2.5 RNase

Thu Dec. 19th

Sca 9.0 Sca

BS Pre G'17
 BS Post Not G'17

Not ends on 9.0

Not Not

Take 100 μ l ($\sim 3 \mu$ g) G'13 9 Sca Afe
 + 10 μ l 8M NH₄Ac
 + 275 μ l EtOH

Freeze. Cent - Dry & back into 50 μ l

G'17 Sca Afe 14.1
 + 3 μ l Phosphorylated Not I linkers p(A G C B G C G C T) G'17
 + 6 μ l 10x RS
 + 3 μ l T4 ligase (white) lot 970
 37° 3:45 to 5:45 pm

+ 6 μ l 1.0 M NaCl for 5 min
 + 6 μ l 10x RS
 + 1 μ l Not I lot 47 (100 μ l)
 37° 6 pm to 11:30 am

When using Pout I, add KCl in future

Possible mixup with this sample
 Run prep gel & ligate to BS Not I CIAP & then isolate
 Pout I - Not I for 3' homolog

Bluescript Not / CIAP
 Take 16 μ l (5 μ g) Bluescript
 + 10 μ l 10x RS + 1M NaCl
 + 74 μ l ddw
 + 2 μ l Not I lot 47 (100 μ l)
 37° 5:10 pm to 11:30 am
 + 20 μ l 5x CIAP buffer (Invitrogen)
 + 2 μ l hot stock CIAP
 30 min 55° from 12:13 pm to 12:43 pm
 1X phosphate 2x CIAP. Rest to N.M. 0.01 to 0.02 μ g / μ l
 EtOH ppt & back to 200 μ l



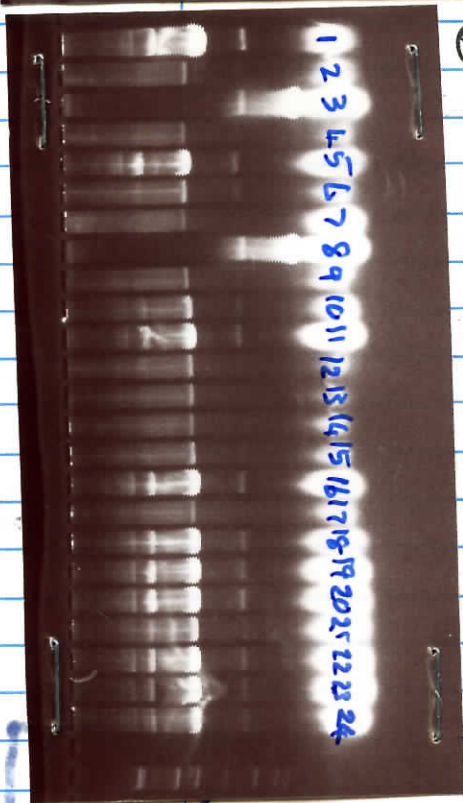
①

Conclusion:

Watch for excess
of BS Not CIAP, prob.
concⁿ is 0.02 pg/ul.
9.0 is better than expected



② Conclusion: If nec.
to repeat, decrease
concⁿ of BS Not
by factor of 4.



③ Conclusion: looks like 100% of whites (22/24)
Process 1 (may be different), 18, 19 & 20.

G'19
18

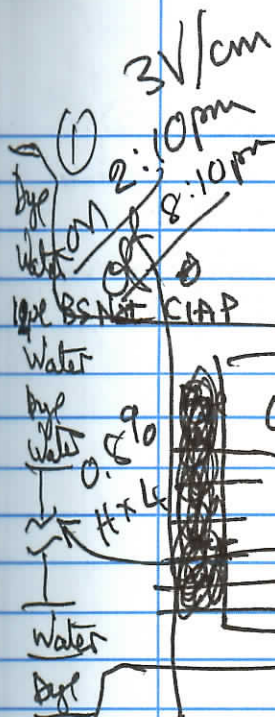
was used later
on H'27

①
Dye
Water
10x BSN
Water
Dye
Water
0.9
H
Water
Dye
11.0
11.0
0
Dye
W
G'19
W
MN
W
1.3
Dye
W
1
24
W
MN
W
Dye
H

Not ~~9:00~~ ~~11:00~~ ~~19~~
TNAP

Sat. Dec. 20th

See 9.0 See cloning
Not Not



$\pm \sim \frac{1}{2}$
 $\pm$ } 2 or 3 slots
only!

② 11:05am
11:55am
0.8% H x L
max

Quick ligation

Take 100 pl G'18 9kb Not CIAA
+ 2 pl G'17 BS Not CIAP
+ 255 pl EtOH
Freeze. Cent. Dry. Back up into
15 pl 1x p95 as G'19
+ 1 pl T4 ligase
Cold room from 9:55 pm to 5:05 pm

Repeat ligation

100 pl ✓
 $\frac{1}{2}$ pl ✓
253 pl ✓
✓

G'19
LIGN

G'192
LIGN

4+13
G'19
Pre lig

Transformation

Blue Top G'19 80 pl
G'19 hq 3 pl
OC 5:11pm to 5:50 pm
~90 sec 42°

4+13
G'19
Post lig

Platch + 40/40 pl

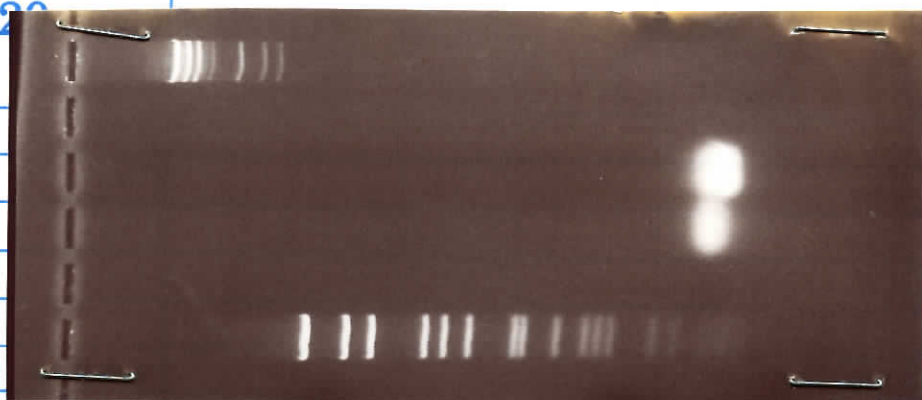
(Many, many V. wet)
Rest 20, 5, 20, 14, 5
14, 18, 16, 1
6pm - 10:00am

G'19 minus 1-24 32° (2 blue) 1x AMP NZY 4:30 pm

③ 12:30pm
0.8% H x L
max

1) G'19 minus
24 W
MN
W

front - look
white out number
white by factor
of 10.



①
 Conclusion: No sign
 of working. Increase
 template & try
 again with co-primer
 (see F162 & below).

an 11.15

①

Oligo # 17667	5'	Oligo Name: G'20 Blue Target Co-primer	3'
Date made: 12-22-97		ACT GTC TAG ACT CCA	
LENGTH: 19		GGG C dC	
% G+C: 58%			
Oligo # 17668	5'	Oligo Name: G'20 Blue Control Co-primer	3'
Date made: 12-22-97		ACT GTC TAG ACT CCA	
LENGTH: 20		GTT CC dC	
% G+C: 50%			
Oligo # 17669	5'	Oligo Name: G'20 Red Co-primer	3'
Date made: 12-24-97		ATC CCA GGC GCG GCG	
LENGTH: 17		AG dG	
% G+C: 76%			

an 11.15 am
 290 10V/cm

Mon. Dec. 22nd

Target
 TNAP

21

① Δ_{AgMM} 5' homolog. TNAP/c kit PCR Control

Black Control
 Blue Target

$n = 90$ (500 μe)

AgMM

20x PCR 25 ~~100~~ μe ✓

W

ddw 398 μe ✓

Agc

2 NTP's 2 μe ✓

DMSO 50 μe ✓

F158 Red c kit TNAP primer 10 μe ✓

~~2.5 μe p13/2 from G.M.~~

G'21 1 μe Template ~~243~~ 243 ✓ diluted 1:10 (w 0.3 μg / μl) in 0.001M EDTA

✓ 5 μe F'162 Target TNAP Blue Synthesis primer

✓ 2.5 μe Lot - 837

Stratagene
 pfu DNA
 polymerase
 cloned (2.50 μe)

✓ 2.5 μe

✓ 5 Blue label

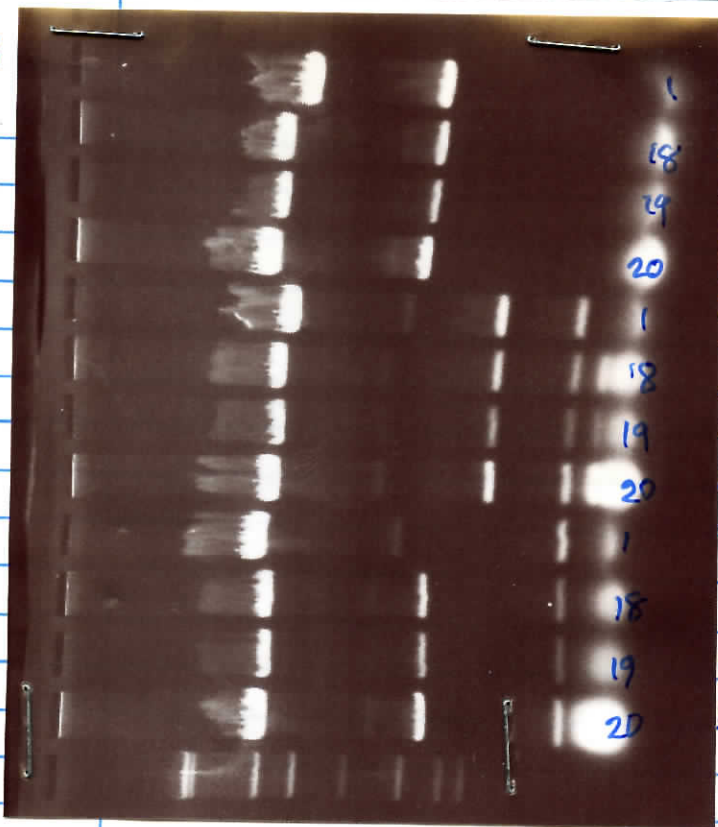
✓ 5 Black label

Start 6.10 pm ✓

If unsuccessful, make co-primer.

If successful, ppt. & digest prior to prep. gel.

← This!

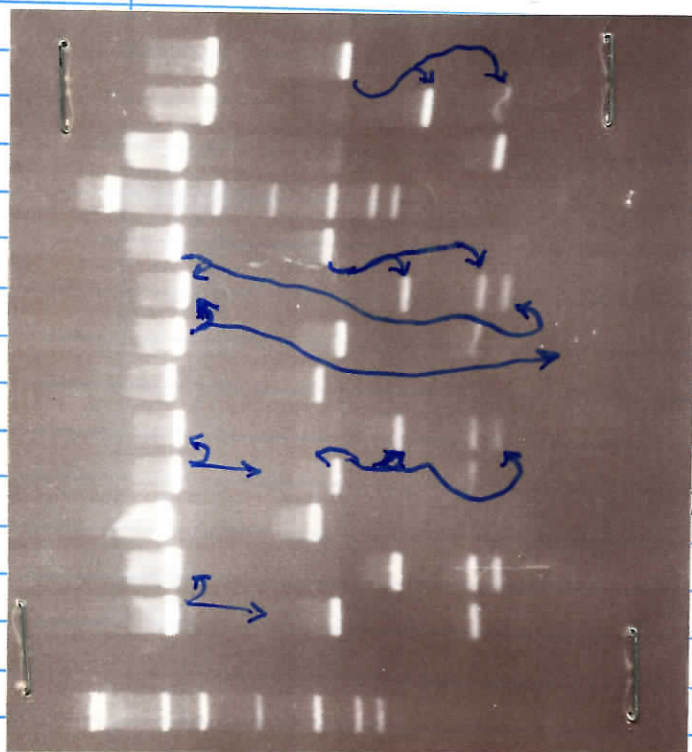


① Conclusion from ① & ②

~~All~~ All cut out Not BS at 3.1 kb
#1 this smaller insert < 9 kb

Note 18, 19, 20 are identical; they contain a 9 kb Not frag.^t which is shortened by Pvu1. They are all expected

Note May be desirable to make the Pvu1-Not1 frag.^t from plasmid grown in Dam⁻; the methyl group of inhibit ligation



②
1

18

19

20

OM

Apr 1

m/1:15pm

P

w

1

1

1

18

18

19

1

2

2

2

2

2

2

12/2

Sat 3.1 kb
st < 9 kb
l; then
frag.
by

valle
- Nat/1
the
in bit

12/20

0.8% H₂O max

W

①

Tues Dec. 22nd

23

Not 9 kb Not
Pvu1 TNAp

Boer. Mann.

lot 38 1995

G' 21 minis

Take 96 pl ddw
12 pl 10xRS + 1M NaCl

12 pl 0.5% MKCl + 10xRS

+ 8 pl Pvu1 (different lot) 4u/pl
1995 vs. 1994!

TO 8 pl ddw

12 pl 10xRS + 1M NaCl

5 pl Not 1 lot 47 (100/pl)

1 Not
18 Not
19 Not
20 Not
1 PN
18 PN
19 PN
20 PN
1 Pvu
18 Pvu
19 Pvu
20 Pvu

30 pl 30 pl 30 pl 30 pl 30 pl 30 pl 30 pl 20 pl
1 Pvu 18 Pvu 19 Pvu 20 Pvu 1 Not 18 Not 19 Not 20 Not

1 pl each mini

11/15pm

0.8% H₂O x 4R 2/3 max

3:50pm

10 pl each 10 pl

W

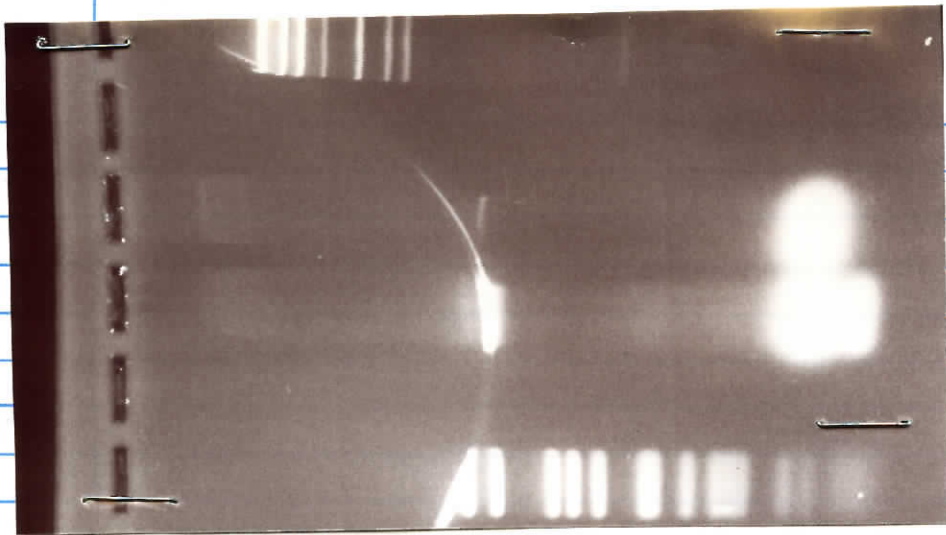
1 PN 18 PN 19 PN 20 PN

Thurs. Dec. 25th Christmas Day

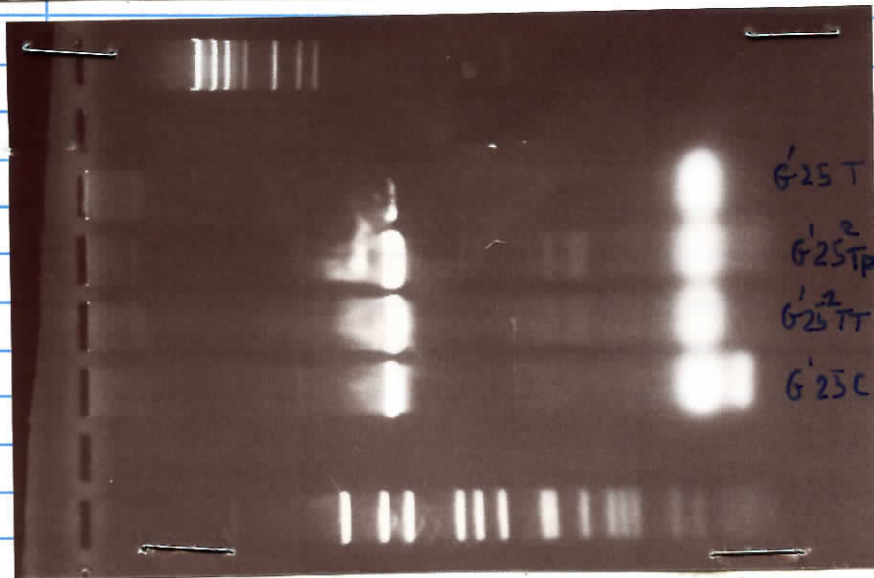
Added 17 pl ddw to all (dried from naturally)

Agm samples & re-run in better order + PNAse

1 Not
1 PN
1 Pvu
18 Not
18 PN
18 Pvu
19 Not
19 PN
19 Pvu
20 Not
20 PN
20 Pvu
W
Agm
W
Agm



① Tag worked well. Pfu beginning; 1 enzyme target



② Target, Pfu worked fine. Proceed with G'25 TP & G'25 C with Xba & Ksa

On 12-20
~~Ag~~
~~W~~
~~Bl~~
~~Bl~~
~~W~~
~~Ac~~
~~W~~
~~By~~
 11-20
~~Ag~~
~~W~~
~~AgM~~
~~W~~
~~Blue~~
~~G'25~~
~~G'2~~
~~Bl~~
~~W~~
~~Acc~~
~~W~~
~~By~~
 12-20

1.5g Hx1
10V/100

TNAP kit
- 81Kb

Wed. Dec. 24th (Christmas Eve)

Agw
W
Blue G'25
Black G'25
W
AgMM
W
byr

Multiple primer by oh fusion
n=20

- ✓ 20x PCR 25 µl
- ✓ ddw 360 µl
- ✓ dNTP's 2 µl
- ✓ DMSO 50 µl
- ✓ F'158 Red ckit TNAP primer 10 µl
- ✓ G'20 Red Co-primer 10 µl
- ✓ 4 µl template diluted 1:10 in QOOLM-ETA (20.3 µg/µl)

- 230 µl → ✓ 5 µl F'162 Target TNAP Blue Synthesis Primer
- 230 µl → ✓ 5 µl F'162 Control TNAP Blue Synthesis Primer
- ✓ 5 µl G'20 Target Co-primer
- ✓ 5 µl G'20 Control Co-primer
- ✓ 2.5 µl G'21 Pfu µl
- ✓ 10 µl G'21 Regular lab Tag
- ✓ 5 Blue tubes
- ✓ 5 Black tubes

1.5g Hx1
10V/100

AgMM
W
Blue G'25
G'25² TP
G'25² TT
Black G'25² C
W
AgMM
W
byr

Thurs. Christmas Day

Start 1:35 pm 21 min 90°
35 cycles 7 sec. 40°
10 min 60°

- ✓ 20x PCR 25 µl
- ✓ ddw 360 µl
- ✓ dNTP's 2 µl
- ✓ DMSO 50 µl
- ✓ F'158 Red ckit TNAP primer 10 µl
- ✓ G'20 Red co-primer 10 µl
- ✓ 4 µl p13.2 template G'21 1/10
- ✓ 10 µl F'162 Target TNAP Blue primer
- ✓ 10 µl G'20 Target TNAP Blue co-primer
- 240 µl → ✓ (4x) 10 µl G'21 Pfu µl
- 240 µl → ✓ 10 µl G'21 Regular lab tag
- ✓ 5 Blue tubes
- ✓ 5 Black tubes

Start 4:50 pm



Reverse photo.
 ① Conclusion: All four
 K were correct. Use A, D & I
 I for bulk. Start with D.

D

A

KN

IN

DN

AN

G'19 Min "9kb"

G'27
Pooled minis
100 I & K

G'27
18D

Used

On 2:10 PM
 Dye
 W
 A P
 D
 I
 K
 AN
 DN
 IN
 KN
 W
 MM
 W
 Dye

D&I
A.

on 2:10pm
0.8% RNAse
2/3 Max

Fri Dec. 26th (Boxing Day)

Dam for G'19 #1
(See G'22 comment)

Dam for 9 kb

Transformative

A Proo. comp

D G'27 → Dam

I

3/6/97 re-freeze after use

FB 446

80 µl

1/10 G'19 #18 mini

1 µl

0° 12:30 to 12:53 pm

43-42°

90 sec

Plated on 1XNZY Amp

37° 1 pm - 11 am

Rest, 20, 5, 20, 15, 16

Many! ①

W 37°

1:30 pm

G'27 A, B, C, D

4 colonies to 1XNZY Amp

11 pm Funny - only A is growing properly.

Picked E-N (10) more 11 pm 37°
varying sizes.

~ 1030 am Following gave workable growths - including plate -
A++, D++, I++, K+. Proceeded.

less time

Take 20 µl 10X RS + 1M NaCl

+ 180 µl ddw

18A 2 µl

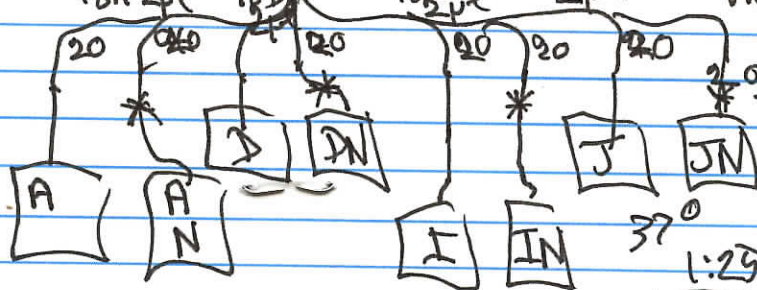
18D 2 µl

18I 2 µl

18K 2 µl

G'27 2 µl

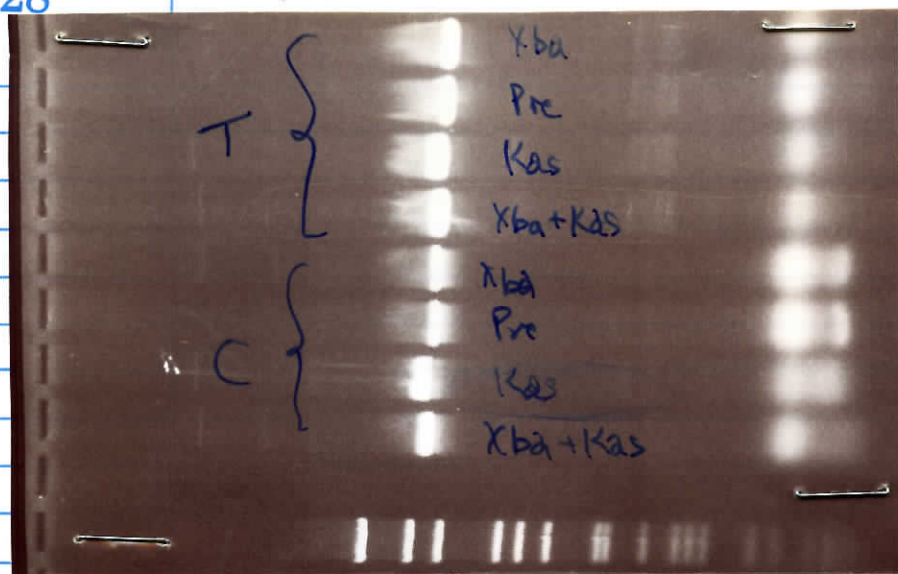
18A, D, I, K



+ 1 µl NaCl
Lot 47
100 µl

1:29 pm - 1:55 pm!

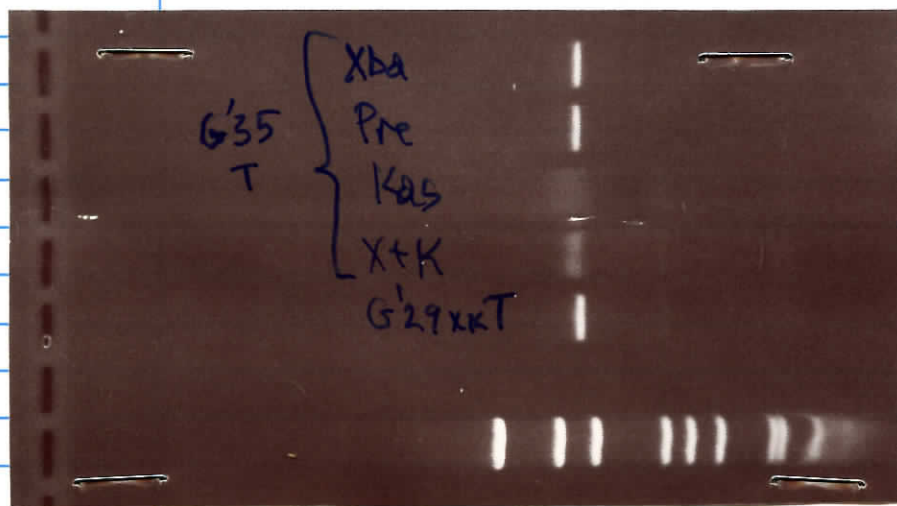
back to 37° at 2:10 pm
Frozen 4:40 pm



Conclusion ① ^{C > T in length}
 but they are not working. Ep
 is odd. May have
 to pre purify to get
 rid of linker prior
 to ligation. Try
 more enzyme
 over time.



Conclusion ② Cant
 tell - sh'd have
 included an ligation
 salt again on
 return from U.K.



← See G'34 & 35
 for details
 This is a approx.
 equivalent to T conc/10

11:37 am

XT	X
PreT	Pre
KT	K
XKT	X
XC	X
PreC	Pre
KC	K
XKC	X
	W
	Mark
	W
	Dye
②	
17+17	XK
10+10	G'2
17+17	X
Black 10+10	G'
	W
	A
	W
	A

~~11.37 am~~
~~9.45 AM~~
11.50 AM
10/11/2018

Sat Dec. 27th Xbox v.1.13 Target 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 4

	XT
XT	XT
PreT	PreT
KT	KT
XKT	XKT
XC	XC
PreC	PreC
KC	KC
XKC	XKC
	W
	Marker
	W
	Byte

Target & Control 3' kb 5' fusion
G²⁹ Take whole (24bp) G²⁵ TP ✓

and (168 μ l) $G'_{25}G$ ✓ ✓ ✓
 $+ \frac{1}{10}$ vol - 8M NH_4Ac 678 μ l 463 μ l
 ≈ 2.5 vol EtOH.

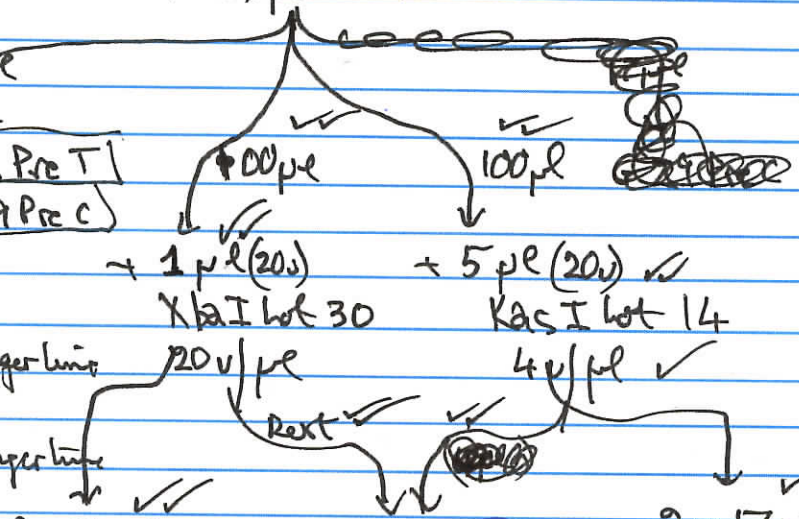
Freeze Cent. Dry & back up into

Air cool
-70°C
Nia Hpts.

500 µl 0.001M EDTA → G'29 T conc (Blue)
250 µl → G'29 C conc (Black)

Take 200 µl G'29 T conc (Blue)
+ 23 µl 10% RS + 0.5M NaCl

②
1000 ft
6' x 17 ft
↓
G' 29
G' 29



17+17 8+10	XKT ² "Pre"	
	G'29 XKT longer time	
17+17 8+10	XKc ² "Pre"	
	G'29 XKc longer time	
	W	
	Acc. M	2 x 17
	W	$\frac{1}{G'29}$
	Age	$\frac{1}{G'29}$

$2 \times 17 \mu\text{p}$
 $G'_{29} \times T$
 $G'_{29} \times C$

G'29 xkT

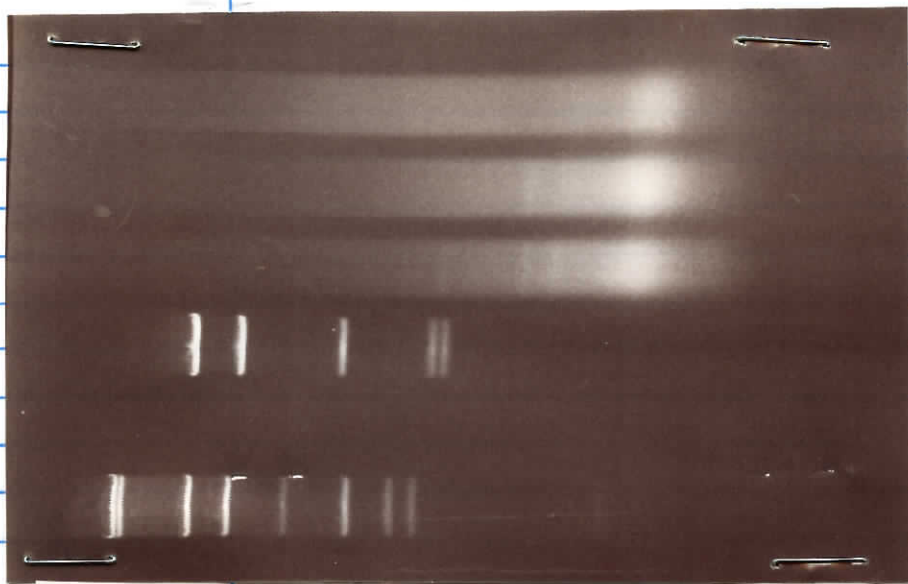
G'29 xkC

$\downarrow$ ✓✓
 $2 \times 17 \text{ Pf}$
 $\boxed{G' 29 \text{ KT}}$
 $\boxed{G' 29 \text{ KC}}$

37° 3pm
↑
11pm
Fridge

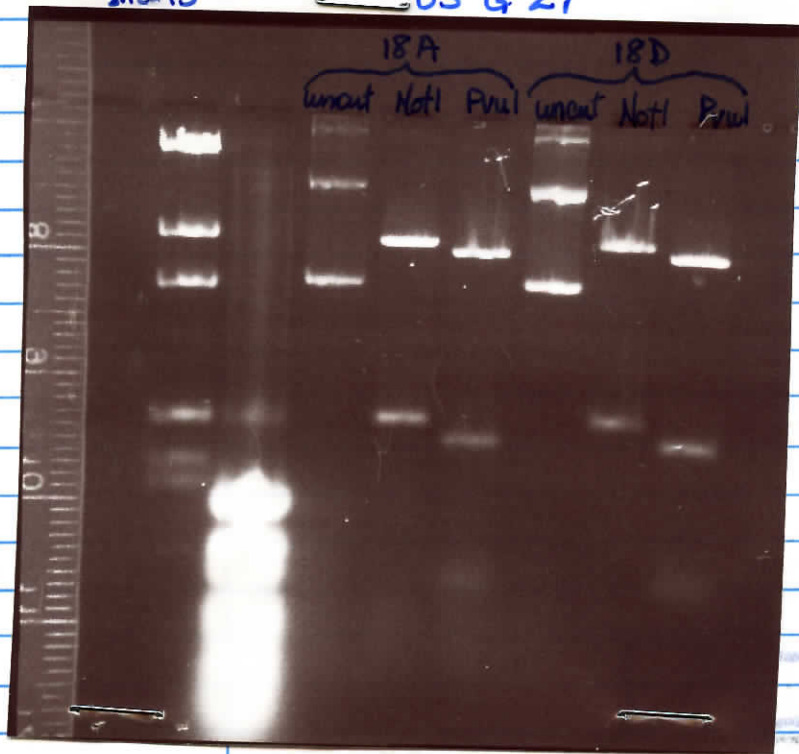
1pm Sunday - each + 1pl more XbI
 1
 8 1pl more KASI
9:30am Mon

XKT 2" Pro
XKC 2" Pro saved & detailed to 50%



SH2143

DS Q'27



⑤ Conclusion
 What a mess! It
 won't stand this
 incubation or digestion. Grow
 up a proper
 min.
 Degrades without any!

① 9/10/05
 9/11/05
 Dp
 W
 G'31
 G'31
 G'31
 G'11
 W
 Mark
 W
 Jy

10

Use 4.
 +14 for

① 11:05 am
 11:15 am
 0.80g HX4
 0.80g R Max

Sun. Dec. 28th

Prv1
 11:17 6
 TNAP
 11:17 6

De
 G'31 Pre
 G'31 Prv
 G'31 18D/PN
 G'11 "9E1"
 W
 Marker
 W
 dye

Bulk prep.
 ✓ Take (30 μ l G'27 (dam) 18D
 ✓ 20 μ l 10xRS + 1M NaCl
 ✓ 130 μ l ddw
 ✓ 20 μ l 10xRS + 0.5M KCl
 ✓ + 5 μ l Boer. Mann. Prv1 lot 38 40 μ l
 ✓ + 2 μ l Not 1 lot 47 (100 μ l)

17 μ l ✓
 G'31
 Pre

17 μ l ✓
 G'31
 Prv

G'31
 18D/PN

37° 4:50 pm 9:30 am

Monday Dec. 29th

To U.K. for New Year with N.M.

Thurs. Jan 6th 1998 (Dam-)

Sylvia grew up G'27 18A² & 18² (both in FB 446)
 - both behaved v the same. Now available for
 Prv1 / Not 1 fragment preparation with absence of ligation
 difficulties. Expect

Not 1 2 Not Prv1 in lac Z

✓ Take 52 μ l (10 μ g) G'27 18A SH 2143 0.1925 μ g/ μ l
 ✓ 20 μ l 10xRS + 1M NaCl
 ✓ 108 μ l ddw
 ✓ 20 μ l 10xRS + 0.5M KCl

G'31²
 18A²
 ~ 0.05 μ g/ μ l

10 ~~0.05~~ μ l
 G'31²
 Pre

+ 10 μ l (400)
 Boer. Mann. Prv1
 lot 38 40 μ l

+ 4 μ l (400)
 Not I lot 47
 100 μ l

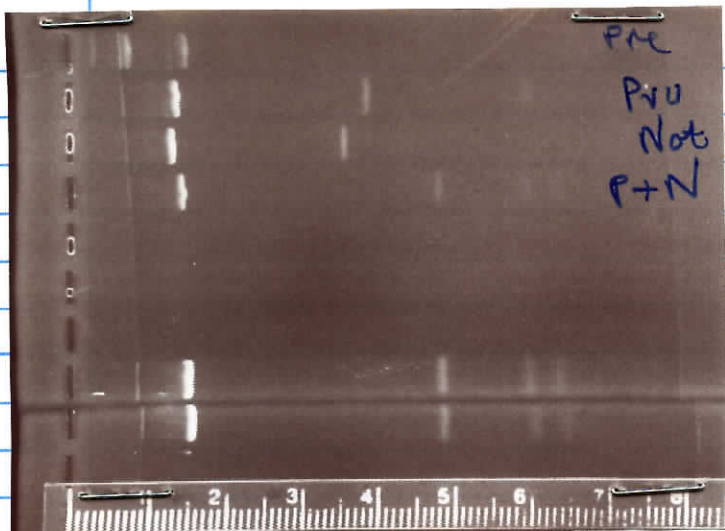
Use 4
 + 14 for exp

10 μ l
 G'31²
 Prv

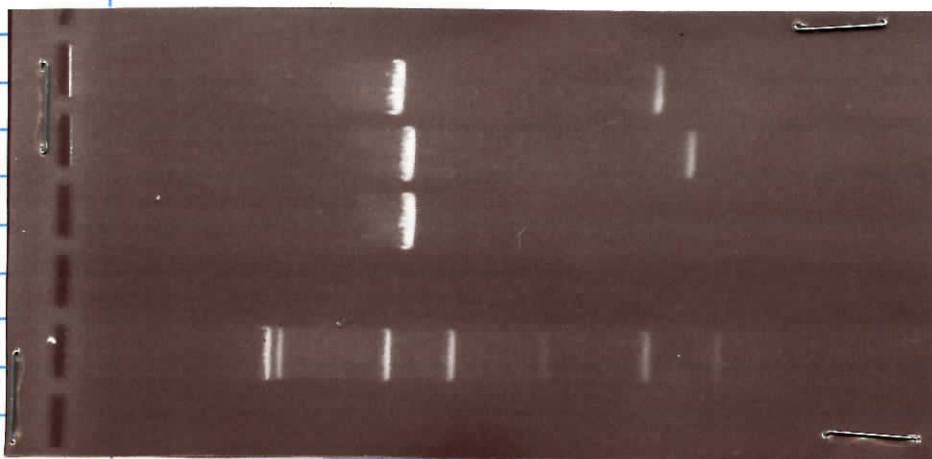
18A² G'31²
 PN

37° 2:25 pm
 4:25 pm to 6:00

10 μ l
 G'31²
 Not



① Conclusion: Both experiments worked to completion. Spots are as expected. Cut made successfully, at 1.2 cm & 1.8 cm.



② Conclusion: Product is likely ~~Pru~~ Not & is in fine yield & purity

6pm
9.5

①

A9

②
1:1
2

~ 8+8
~ 8+8
Unkilled

7

miss
Sides

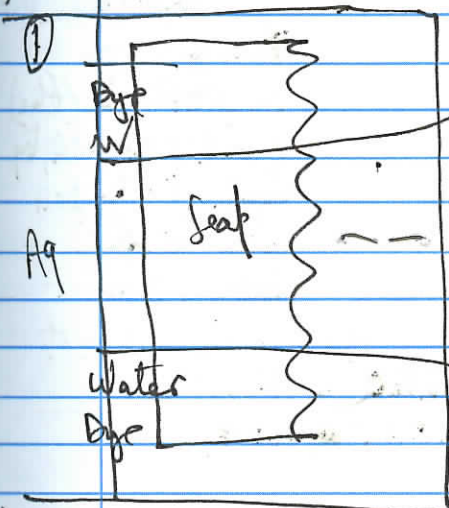
1.2 cm

Not
city

6pm → 0.8
9.50m
0.8% Hx4

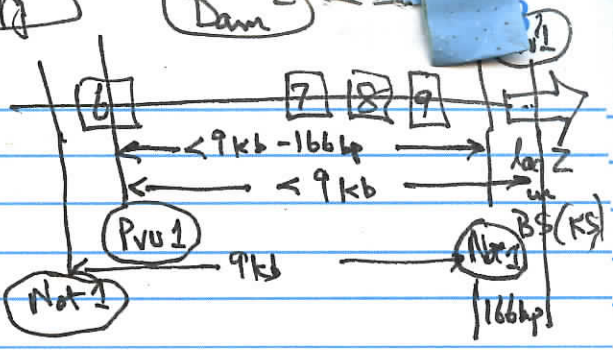
3' homolog
Tues. Jan 6th

Dam



4+14 G'31² Prc
4+14 Pvu
4+14 Not
4+14 PN
W

Dye
Water
~158lbs
1/2X diluted G'31² PN
(~150pl total volume)
(~1/2 is left)



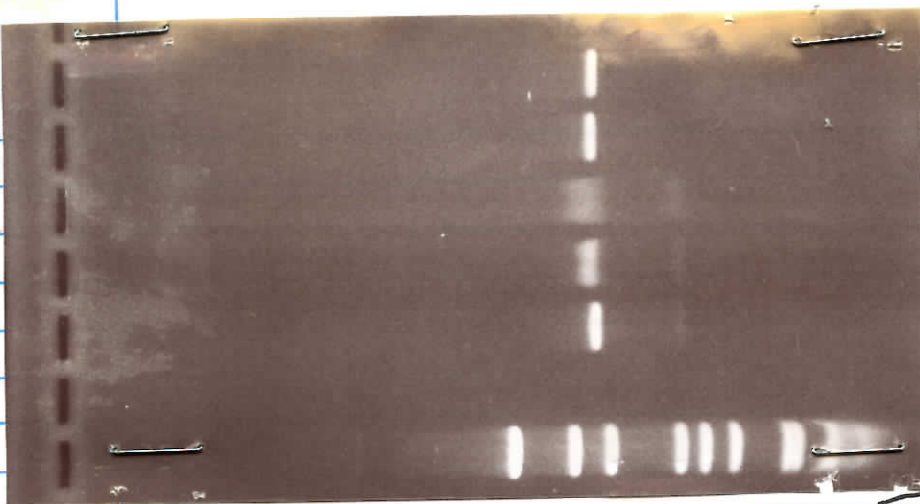
② 1:55pm
4:30F
0.8% Hx4
0.8% max

Recovered into 75 pl 0.001M EDTA as

G'33
P6-9N

Dam

Dye
Water
~8+8 G'31² Not
~8+8 G'31² Pvu
Initial G'33 P6-9N
W
Marker
W
Dye



① Conclusion:

~~Got~~ Trouble with
 Kas digestion - or
 e.p. ~~again~~ Xba
 gets ~~big~~ smaller!
 On this test
 G'29 x K T looks
 good - it is
 smaller than
 G'35 Xba.

Problem is most
 likely in e.p. not
 in digestion.

Run gel on 40H/40Q extracts of G'29 x K T & C
 Run prep-gel on vs. G'35 Pre & Xba & X K



9.150 am
 345 G'29
 Water
 Acet. M
 W
 Dye

5 homologs

Tues. Jan 6th

Xba^{1.1} [EK]

Kas
[EK]

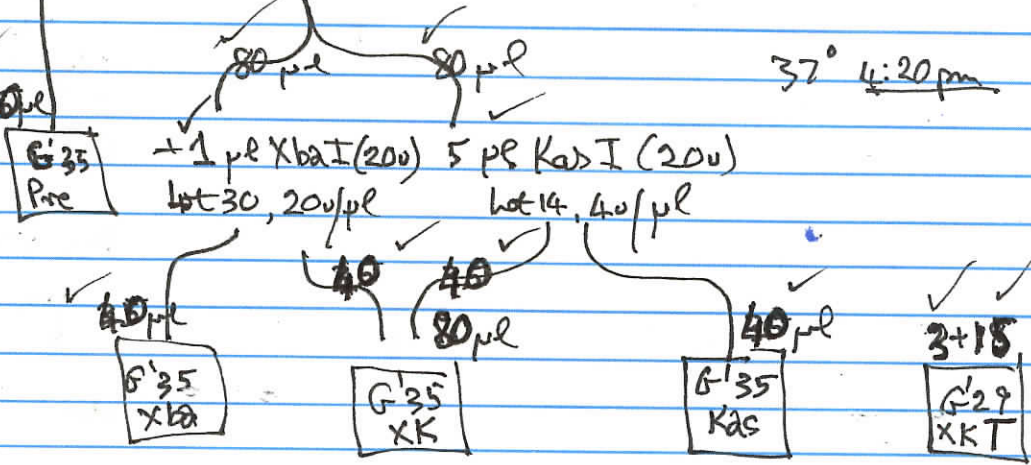
Conditions for Xba & Kas digestion
of G'29 T conc (blue) & later, for G'29 C conc.

Possible that amount of primer etc. is inhibitory; ∴ test lower conc. digests:

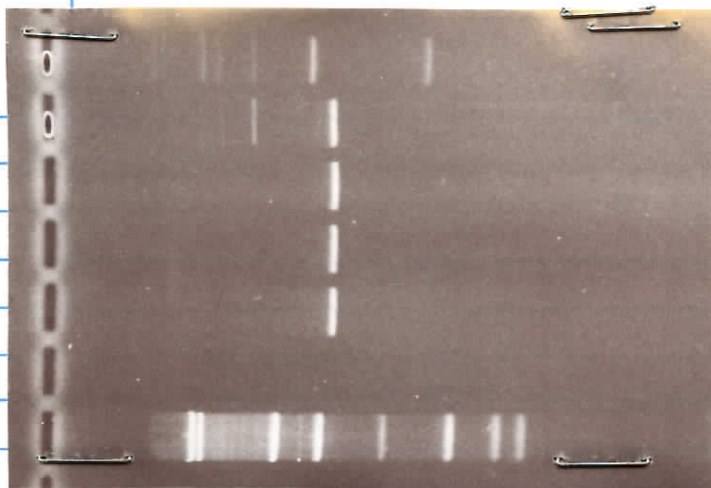
4.50 am
HX 1 10V/cm
1.56
Dye
Water
G'35 Xba
G'35 Pre
G'35 Kas
G'35 XK
345 G'29 XKT
Water
Acr. M
W
Dye

Take
✓ 20 µl G'29 T conc (blue)
✓ + 20 µl 10x RS + 0.5 M NaCl
✓ 160 µl H₂O

37° 4:20 pm



CARE
Use Dam - dup del
when cutting with
Pac 1



Conclusion

A little better Pac I need
 & can ^{then} proceed. Xba &
 Not as secure & protective
 (jointly) from spurious
 colonies. Added more
 Pac I & gave $1\frac{1}{2}$ more
 at 37°



② Conclusion: Beautiful
 proportions & ligation.
 Proceed.

Vector

1:30 m
 1:30 p
 0-0

① Dye
 W

4+12 Pre

4+12 Pac

4+12 D4

4+12 Not

4+12 Xba

W

Ag. M

W

Dye

② on

Ag. M

Water

4+12 Pre ligation

4+12 Port

W

M.M

W

Dye

Ligation

to
ACCEPTA

Insertion of $\frac{1}{2}$ homology

[illegible]

Vector

✓ Since Sca^I has no Xho or Xba sites, can put 3' homol. into Suppel as 1st assembly. (5' homol. ckit, hpaI have no Not sites).

①	Dys
4+12	Pre
4+12	Pac
4+12	DD+
4+12	Not
4+12	Xbs

A.M.

~~(2) $\frac{m}{\rho} = \frac{L}{\rho} = \frac{1}{\rho} \int_0^L \rho dx$~~

6th Prelim.

4/12 Part 1

MM

W.

Dye



(Lig)

20



Diagram showing restriction enzyme sites: **PacI**, **Xba**, and **Not**.

✓ To reduce background on this attempt without purification

} G37 Taken 5.6 μ l (5 μ g) D59 Dam DD E51 0.9 μ g/ μ l
 ✓ 20 μ l 10X R1 salts
 ✓ 2174 μ l ddw
 0.025% $\sim$ G37 mix

✓ 4 pL	✓ 65 pL	✓ 65 pL	✓ 65 pL
12) G'37 Pre	5 pL ✓ Pac I (20 u) hot 28, 40 u/pL	1 pL ✓ Xba I (20 u) hot 30, 30 u/pL	2 pL ✓ Not I (20 u) hot 47, 100 u/pL

Diagram illustrating the structure of a DNA molecule with various restriction sites and their corresponding fragments:

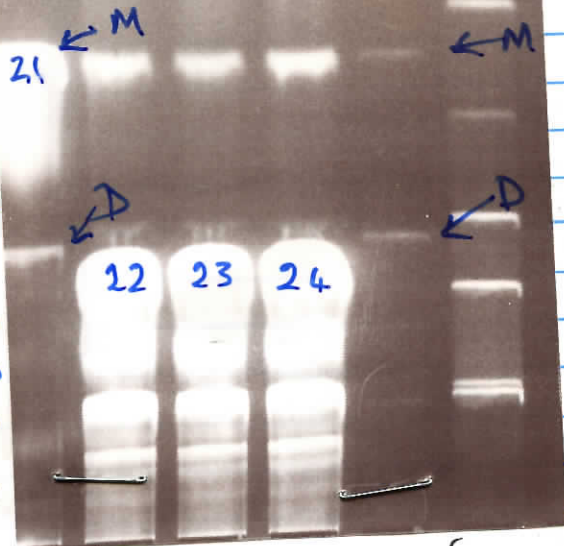
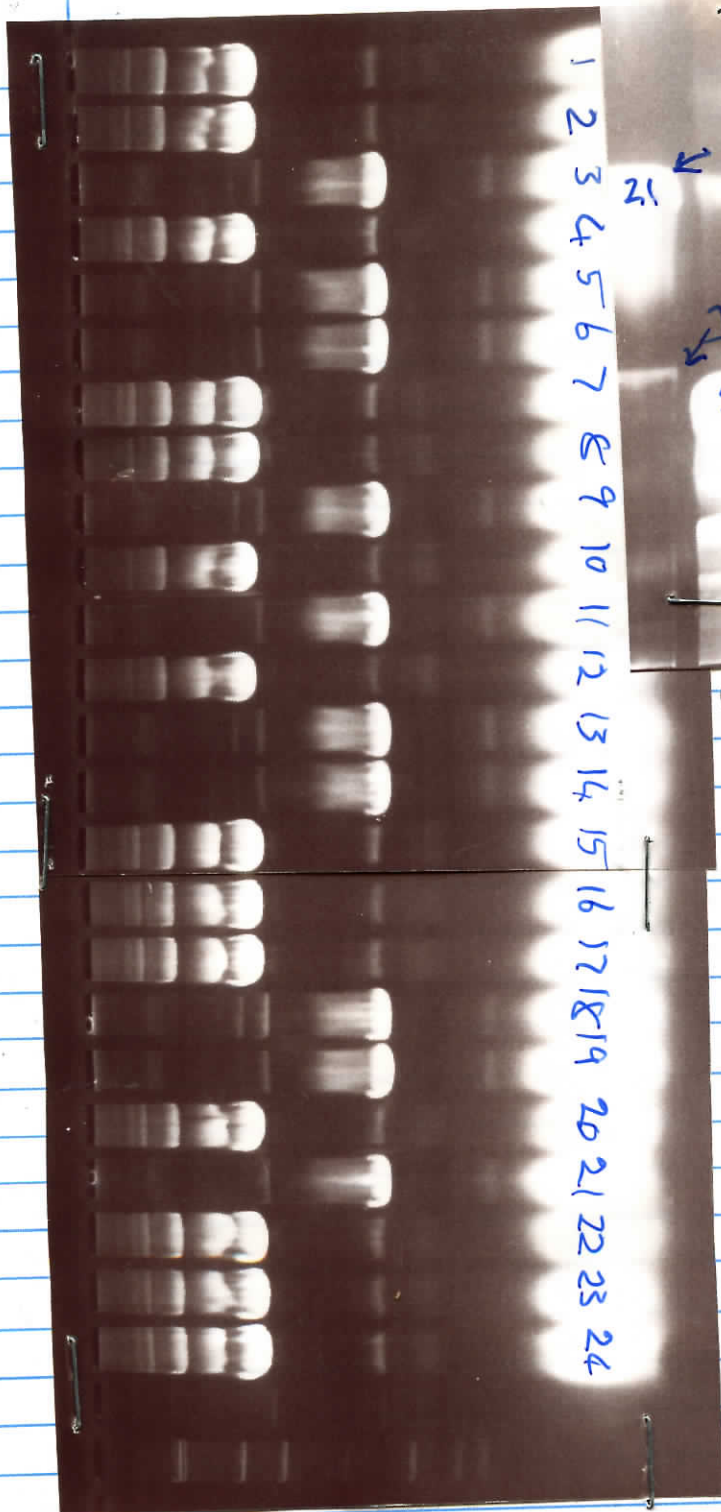
- Fragment 1: $G^I 37$ (412 bp)
- Fragment 2: $G^I 37$ Pac (412 bp)
- Fragment 3: $G^I 37$ Xba (412 bp)
- Fragment 4: $G^I 37$ Not (412 bp)

The diagram shows the DNA molecule with restriction sites marked by arrows and the resulting fragments labeled with their sizes in base pairs (bp).

4 μ e $\leftarrow$ DDPXN 37° 10 am to 12 pm to 350 pm
+12 fore P.
68°-65° for 20 min $\leftarrow$ 5.20 pm to +5 μ e
more Pac 1

Ligature

✓ Take 20 µl G'37 D₅PXN Undiluted but heated for 20 min at 65°
 ✓ 30 µl G'33 Pb-9N Undiluted
 6 µl ~~100x NH₄Ac~~
 165 µl EtOH. Freeze. Cent. Dry. Back up to 20 µl
 1.6 µl T4 ligase (low conc.) lot 970 Cold room 1 x p15
 15° 9:30 to 10:48 A.T. 10:48 to 11:27, 37° 11:27 to 12:15 pm
 5pm Freeze ← To cold



Conclusion: 14/24
 with insert - excellent
 yield with live shake
 (still suspended at harvest)

Feb 5th 02/05

streaked out NZY 1X Arg

37° 3pm

G'39DD3'(22)

Sam
 12:30pm
 12:50pm
 0.8
 Dye
 W
 1
 2
 1
 1
 1
 1
 23
 24
 1/40 DupD
 A
 w
 Dye

Lox
 ↗

Sun 12:30pm.
 12:45pm
 0.86 Hx1 Max 1/2

Fire. Sam 9th

(3' hamol. (contd))

Transformation



6:39 Red top DH5α 8 pl ✓
 G'39 wign 2 pl ✓
 0° 5:06pm to 6:18pm

23 90sec 42° - 43° NZY
 24 Dup Del D'59 Mini Plate Rest 20, 5, 20, 5, 16, 5, 16 1x Amp 37° 6:30pm
 1/20 Agm W 6:45pm Minis G'39 1-24 (17) 1x NZY → 11:30am
 Dye 11:30am Sun

Man. Sam 12th

See also

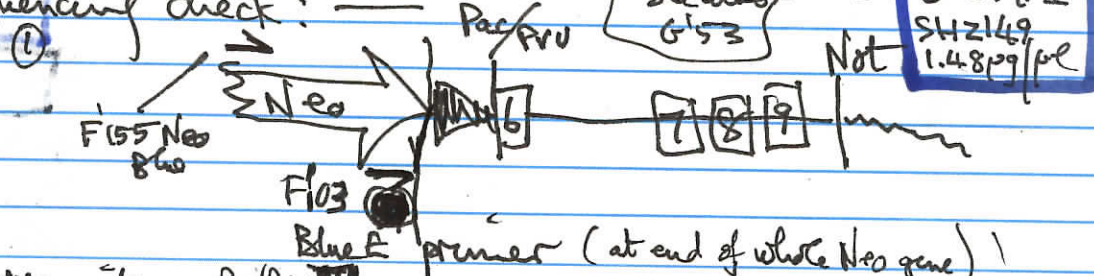
G'78 → G'39-22

2.11.98
 2.4199/pl

(G'39) Processed #'s 1, 2, 22 & 23 individually.
 Pooled rest for processing (4, 7, 8, 10, 12, 15, 16, 17, 20, 24)
 later if nec.

Streaked at G'39 #1 for Sylvie for purified mini

Sequencing check:



Slide into Pac/Pvu
 Asc I inverted Ex. Pac
 LoxP G G C G C C T T A A T C G G A C T G G T A C T C
 Ex. Pvu I

Dup/Del

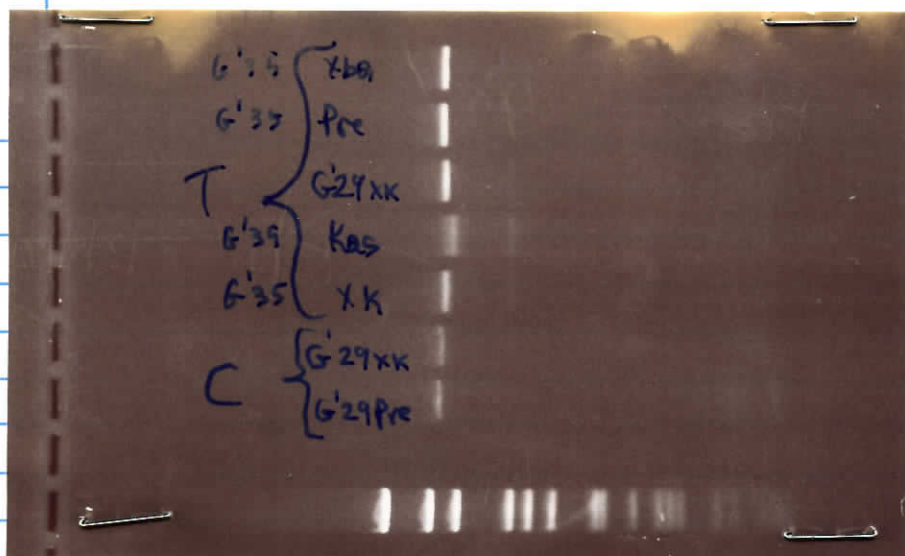
50bp from 3' end of primer

Exon 6

① Didn't work on crude mini. gr.

② On purified

③ Try F'155 Neo Blue (brown track)



① Conclusion

Ep is now believed
looks as if Xba
is working, but
only dilute DNA
with KAS is
digesting, & then
not complete.

Try running
prep. gel

on G'29 Xba T & C

& redigesting product.

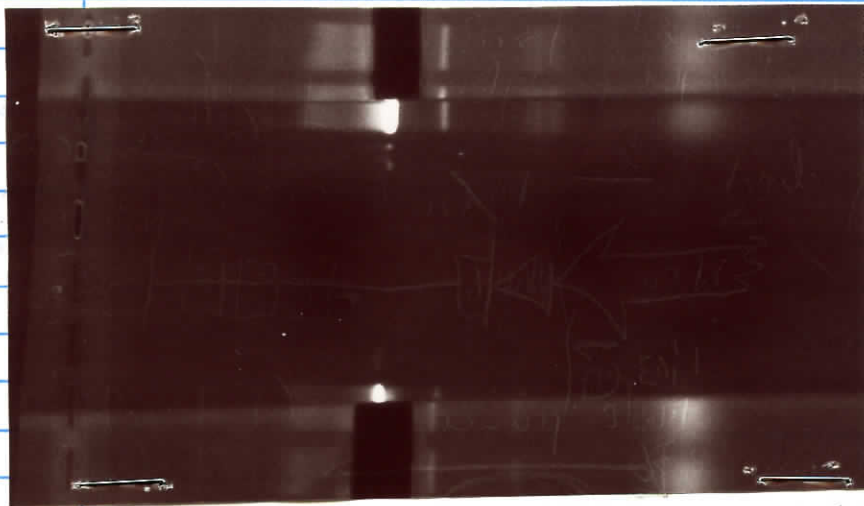
Compare with G'29 conc. (for Xba)

& with self for KAS. KAS is
the problem. Note, however,

that on long digests, the
primers have degraded.

Compare G'29 vs G'28 (0.05%)
& G'40.

Can : try simple ϕ OH/HOQ $\rightarrow$ EtOH on G'29 & redigest.
CIAA



② conclusion Good ap.

& cuts. Recovered into

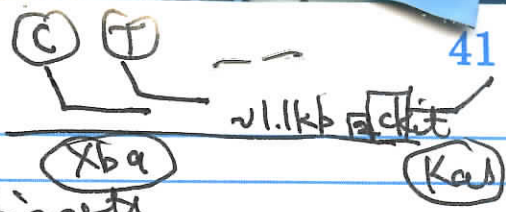
0.001M EDTA

a) G'29/41 T 100 pl

b) G'29/41 C 50 pl

expected T is smaller
than C. Proceed
with digestion.

Thurs Jan 9th



Better e.p. on PCR digests

12:17pm
1:00pm
1:50pm
8V/100

Checking on previous table, which suggest e.p. not highest. is problem:

G'29 XK T (extended time) } are both available
XK C (extended time) } in sufficient amount to do H or whatever

G'35 Xba (T) G'35 Xba available & runs OK, smaller than
G'35 Pre (T) G'35 Pre which is also available.
2+15 G'29 XK T (extended time) ∴ propose to run a
G'35 Kas (T) gel on (2+15) G'29 XK T - heated?
G'35 XK (T) (2+15) G'29 XK C - heated?
2+15 G'29 XK C (extended time) versus (undiluted) G'35 Xba (T) should tell if OK
1.5+15 G'29 C conc ∴ G'35 Pre (T) to proceed.

Then to prep gel on last two, if OK any.
Decided to heat all:

G'31² Pre, G'35 Xba, G'35 Pre, G'35 Kas, G'35 XK,
G'29 XK T, G'29 XK C all heated 65° 11:15am to 11:37am

All e.p.s on the heated samples (except G'29 was not heated).
68°

Purifying G'29 XK T & C and T & C concs

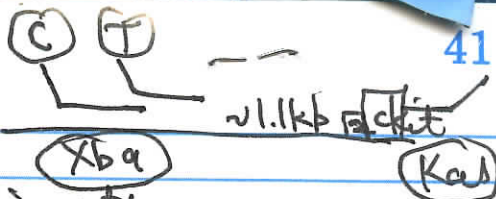
Slot
Agarose
1.5%
10:15am
10:35am
11:35am

10-120 µl G'29 XK T and XK C 1X φOH/HOQ ✓
2X CIAA. ✓

Recovered ~95 µl
Fri. Jan 9th

G'29
XK T φOH etc.
W
Dye
W
G'29
XK C φOH etc.
W
Dye

Thurs Jan 9th



Better e.p. on PCR digests

Checking on previous test, which suggest e.p. not highest. is problem:

G'29 XK T (extended time) } are both available
 & XK C (extended time) } in sufficient amount to do or whatever

G'35 Xba (T) } G'35 Xba available & runs ok, smaller than
 G'35 Pre (T) } G'35 Pre which is also available.

2+15 G'29 XK T (extended time) } propose to run a
 G'35 KAS (T) } gel on (2+15) G'29 XK T - heated
 G'35 XK (T) } (2+15) G'29 XK C - heated
 2+15 G'29 XK C (extended time) } versus (undiluted) G'35 Xba (T) should tell if ok
 1.5+15 G'29 C conc } & G'35 Pre (T) to proceed.

Then to prep gel on last two, if okay.
 Decided to heat all.

G'31² Pre, G'35 Xba, G'35 Pre, G'35 KAS, G'35 XK,
 G'29 XK T, G'29 XK C all heated 65° 11:15 to 11:37am

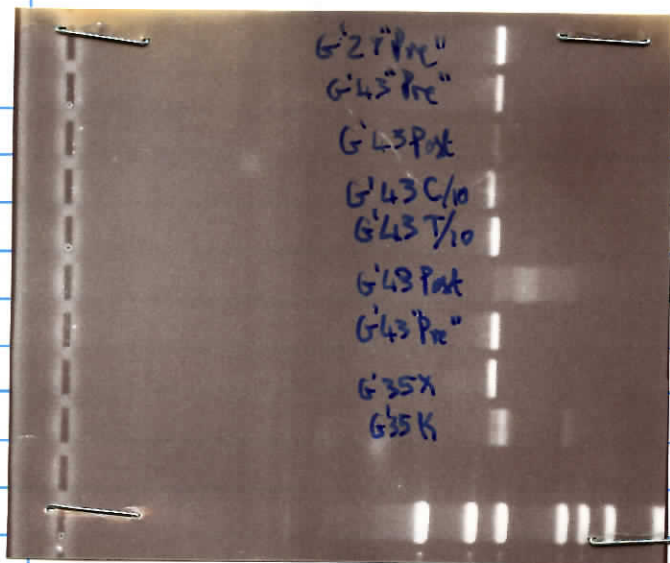
All e.p.s on the heated samples (except G'29 was not heated).

Purifying G'29 XK T & C and T & C concs

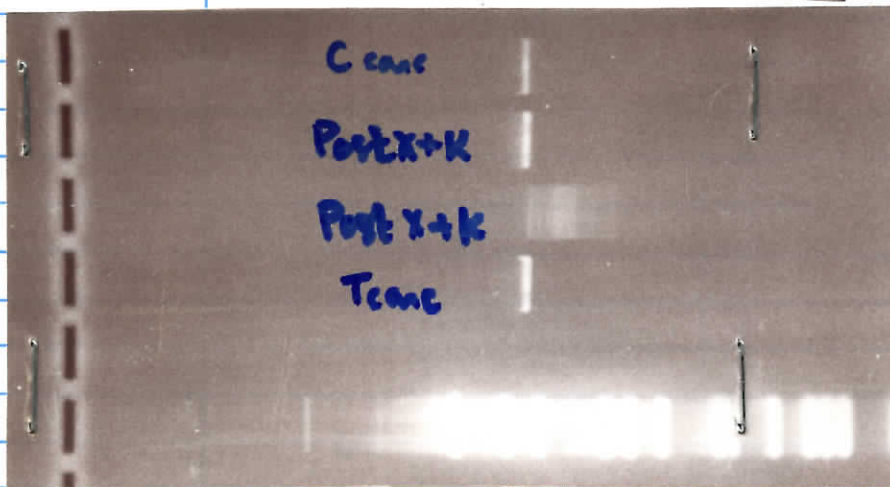
10-120 pl G'29 XK T and XK C 1X PH/NOQ ✓
 2X CIAA. ✓

Recovered ~95 pl
 Fri. Jan 9th

G'29
 XK C



① Conclusion: No help for getting Kas better. Remake more starting material & work on Kas first - can re-try Var I (see G'35K) Meanwhile try pOH/HOQ on G'43C & T ~~post~~ heated



② Conclusion: Not significantly different from heating. Look like excess enzyme with secondary activities

1.59 H₂O
on 11.0
0.11 H₂O

①
5+10 G
5+10 G
5+10
(2+18) G
(2+18)
5+10
5+10
Whatever

Any

1.59 H₂O
on 11.0
0.11 H₂O

10.

10

Fr. Jan 9th

1.1 Kb
Xba
Kas

More Kae digests

Take 40 μ l G'29/41 T & G'29/41 C

+ 5 μ l 10x NEB buffer 2

+ 25 μ l 10x NEB buffer 2

+ 1 μ l (20u) Xba I lot 30 (20u/ μ l)

+ 5 μ l (20u) Kas I lot 14 (4u/ μ l)

G'43 Pre T

G'43 T

G'43 C

G'43 Pre C

1.5 μ l
100/10

5+10
G'43
G'29 Pre C (x+K) Post T

37^o 6:15 to 10am

65^o min

5+10
G'43
Post C

5+10 G'43 Pre C (chiefly x)
5+10 G'43 Post C (x+K)
(2+18) G'43 Conc (ex G'29) /10
(2+18) G'43 T conc (ex G'29) /10
5+10 G'43 Post T (x+K)
5+10 G'43 Pre C (chiefly x)

What's G'35 Kae
G'35 Kas

W
MM
W
Dye

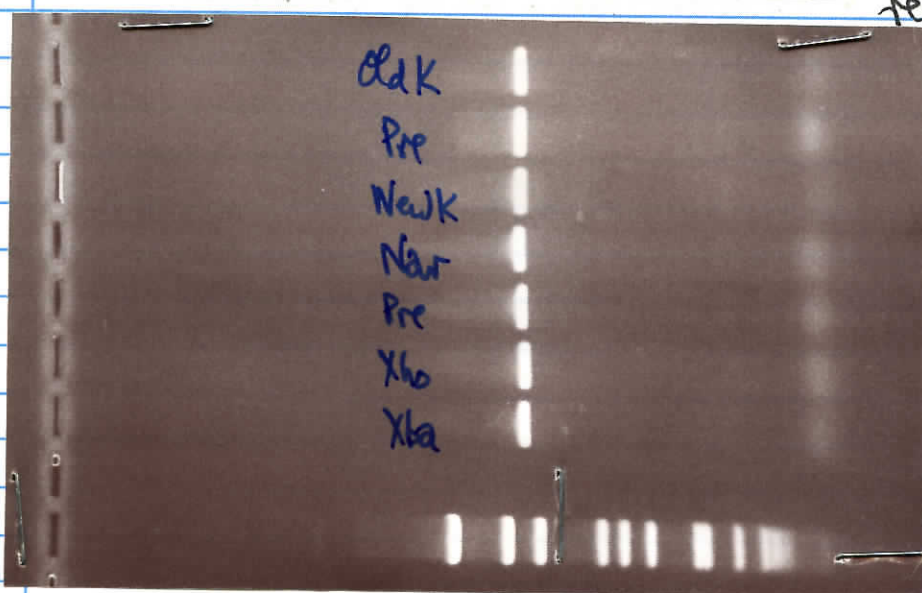
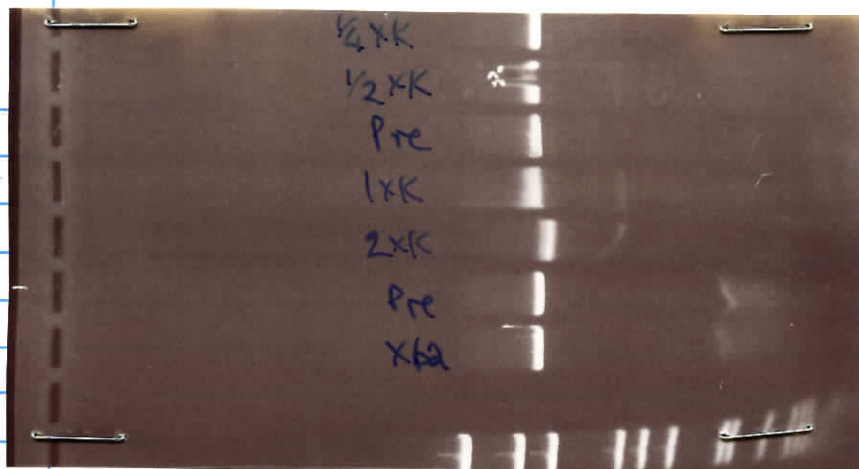
1.5 μ l
100/10
Dye
Water

G'43 Conc /10
10+5 G'43 Post C (x+K) Heat at 60^o C
10+5 G'43 Post T (x+K) " " "
G'43 T Conc /10

W
MM
W
Dye

1x 60H / CIAA 2x

5.8.2001



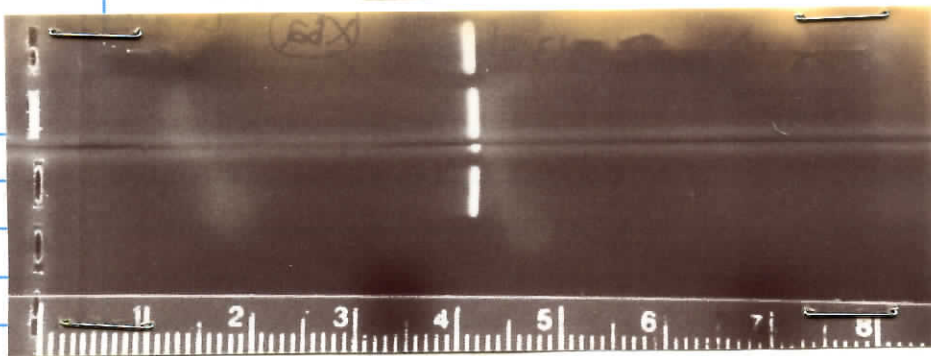
① Conclusion: Heating is not working. Kas - but still incomplete & unsatisfactory. Also Xba is not working. Try Xho & a different batch of Kas, and redesign.

② Conclusion: I cannot believe that none are working. More likely all are working in this buffer.

PROCEED with T

② Conclusion: I cannot believe that none are working. More likely all are working in this buffer. PROCEED with T

37
Page
Old K
Pre R
New
Na
Pre
Xho
Xba
W
NM
W /
Pre /



Conclusion:

Obs ↓ in size.

Good cut.

Proceed.

Recovered into

100 μ l as $G'47$

after por, CIAA ~~fragt~~
 & type ~

water
 called I (extra) 1
 probe

XKT

Tues 13 ch
Wed. Jan 14

Xba Targeting 17
X 1.1 kb
ckit
Kad

Bulk prep. Xba/Kas 1.1 kb
with G'29 conc T

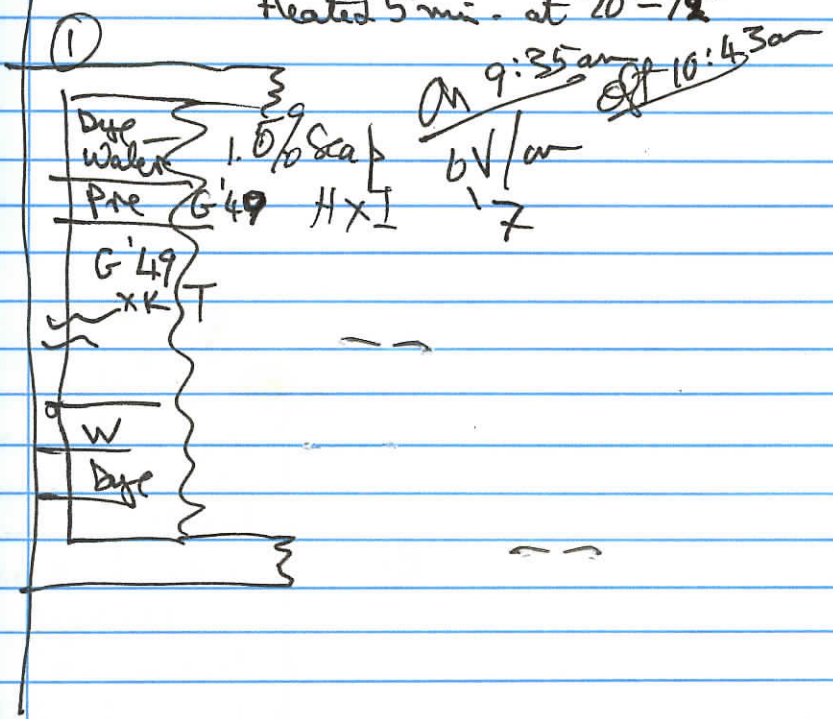
Take 40 μ l G'29 T conc
✓ 20 μ l 10x R1 salts
✓ 140 μ l ddw

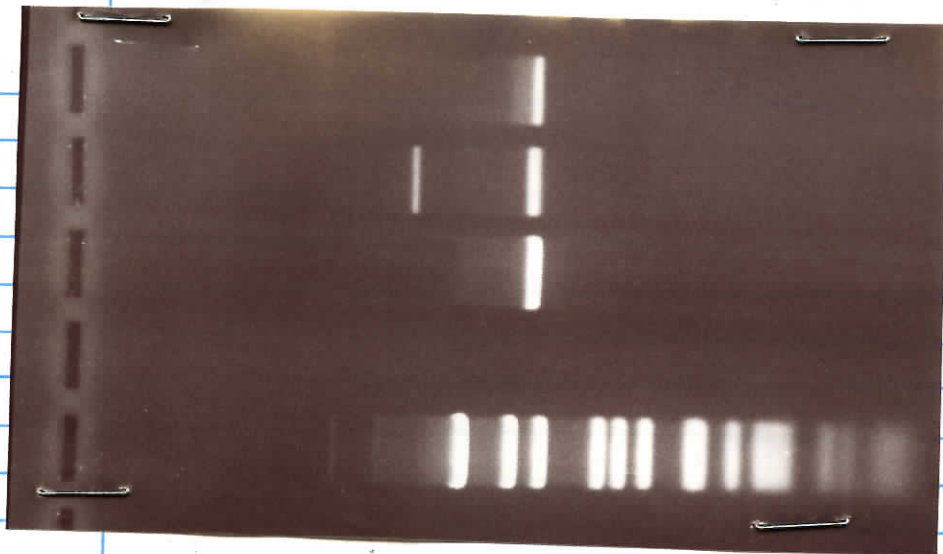
G'49
XKT

✓ 10 μ l Kas I (current) lot 14 4u/ μ l
✓ 3 μ l Xba I lot 30 20u/ μ l
30 μ l
Pre G'49

32° 6 pm to 9:30 pm (cold)

Heated 5 min. at 70-72°





Conclusion:

Dimension is OK,
but former is
very faint.

Repeat test
with G'47 XKT
Frag²

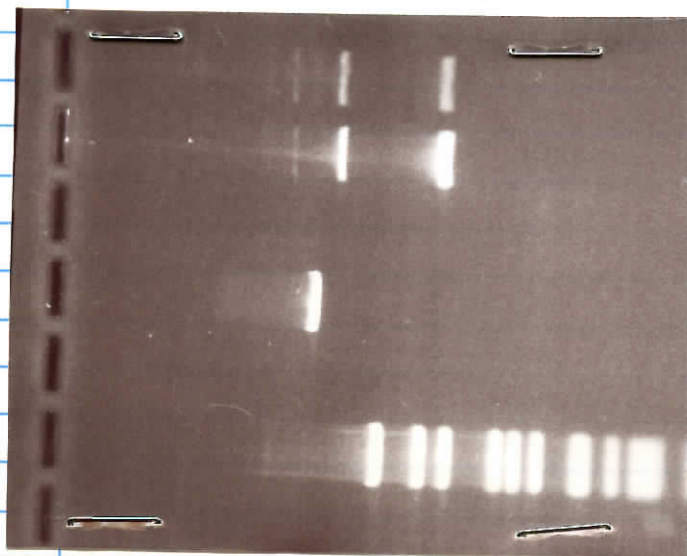
if nec^y, do
two stage: Kos
then Xba.



②

Conclusion: On
this test the new
product is no
better - but
result is odd
altogether.

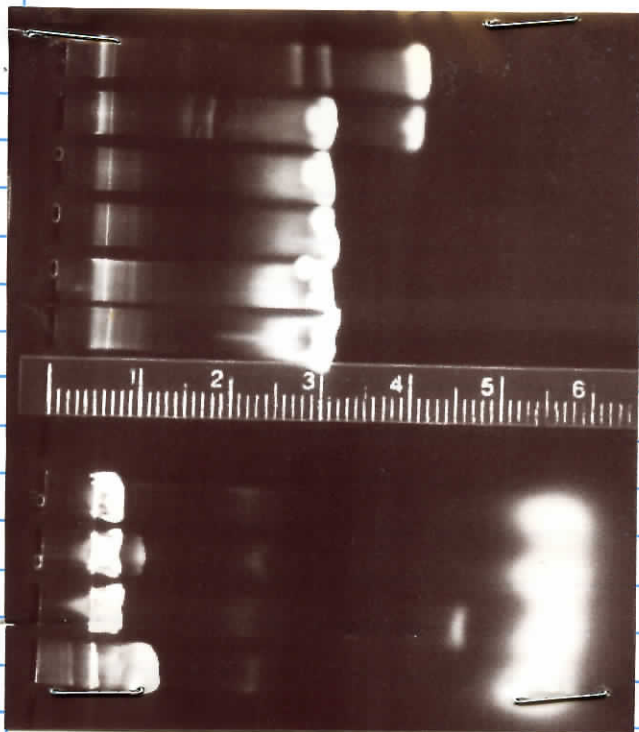
Repeat with full
strength G'47 XKT Frag²
1 or repeat G'9/41



③

③ Conclusion: Look as if
G'47 XKT is less ^{in amount or less} ~~than~~
than G'29/41, altho' it ligates
slightly better, however, may

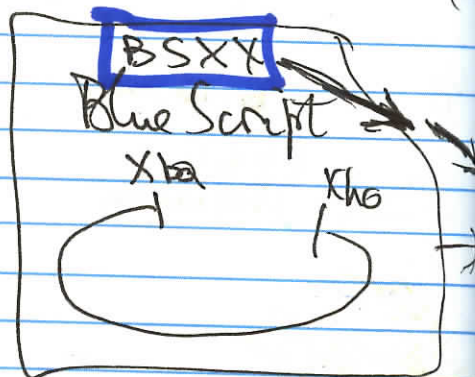
need to go to Xba anyway, altho'
this can be done after assembly in BS.



G'51BSXX recovered as
into 300 μ l 0.001M EDTA

① Conclusion: Xho
looks good; Xba may
be incomplete.
Prepare fragment
→ recut with
Xba I and EcoRI
some other for safety.
Dilute the rest of
the G'39#1 series with
1X R1 salts & re-run, after
more Xba - or
better repeat the
Xba expt. with
new DNA (now
available)

G'50 BSXX Xho



① 0.16.40

Dye
Water

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

G'51

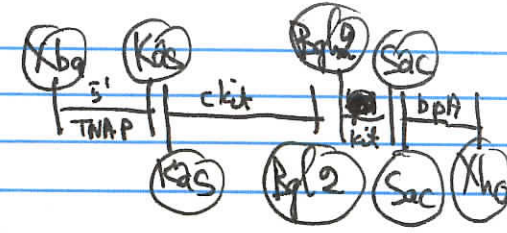
G'51

Xho
Xba may
to
sent
oh
Eco RI
rest of
series w
after
- or
the
work
now

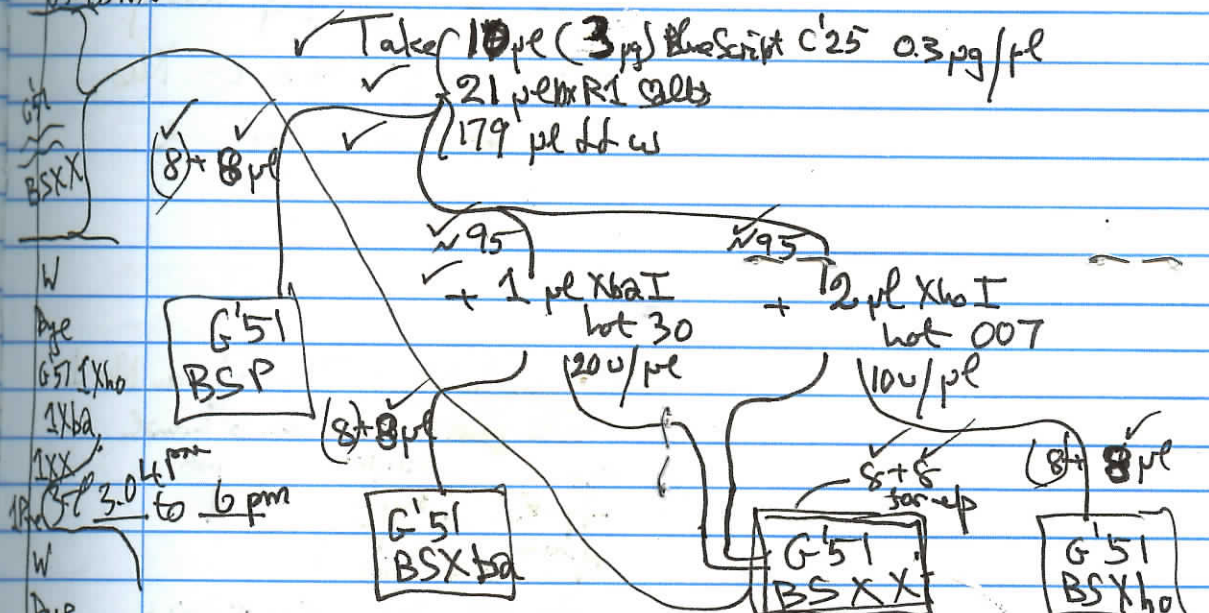
0.6:40pm
off 10:10am
0.8b
Hx4
Ag+Scap.
0.5V/cm

← Too low (same error) 1 to 3V/cm 9am
Wed. Jan. 14th

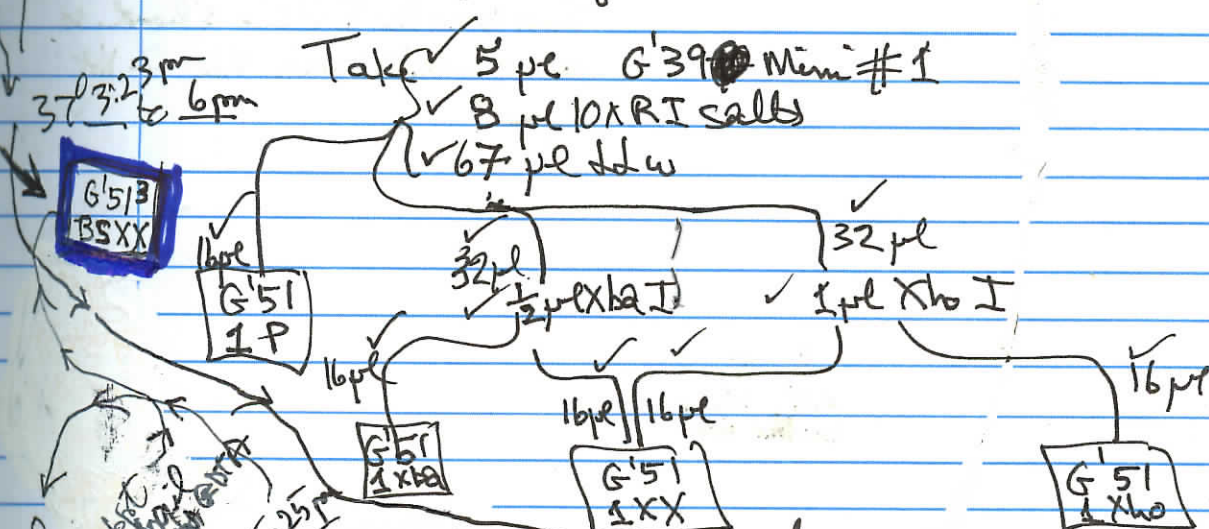
Preparation for 5' homology assembly



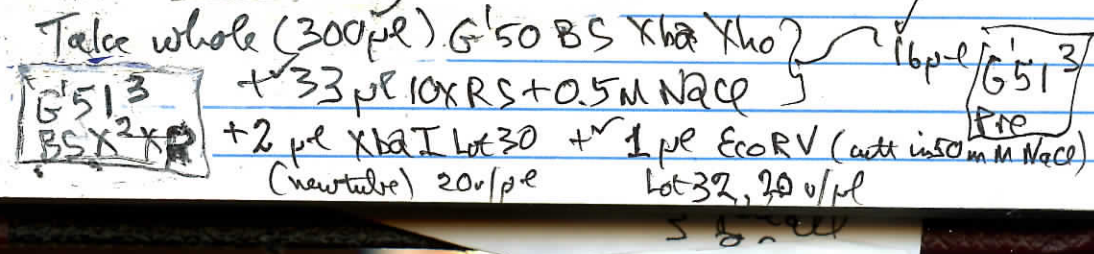
BlueScript Xba/Xho



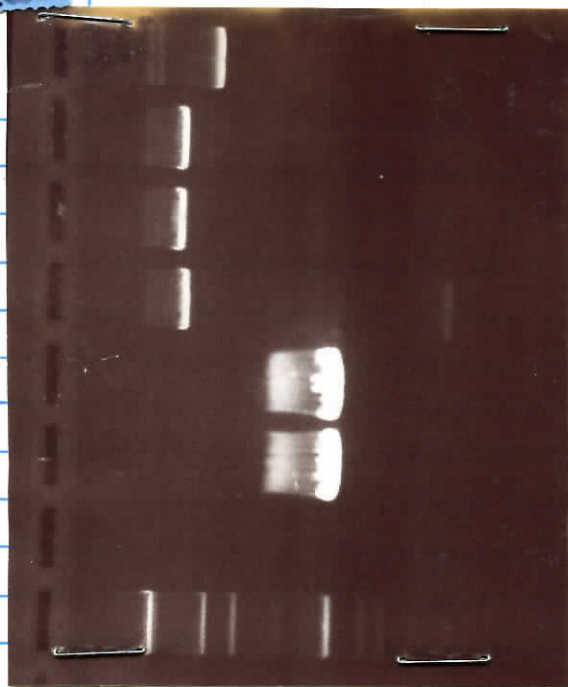
Check on G'39 #1 for (single) Xho & Xba with Web cut out by back



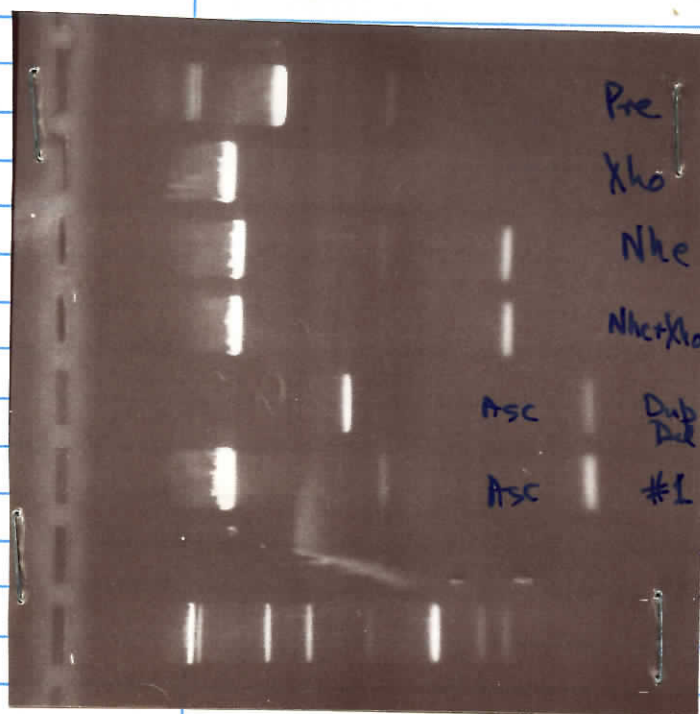
Thurs. Jan 15th



14:00H/100
2XClA9
37°C 1pm
0.00m
5:25pm

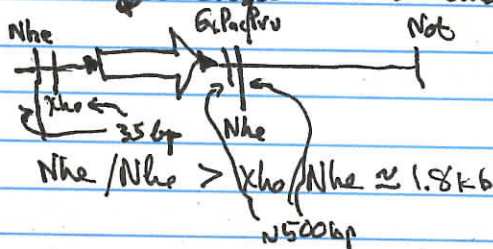


① All OK, but she'd have better
~~Nhe~~ for Xba — this
 is known to be bad! Forget ~~the~~
~~the~~ logic — need
 to do 3' second if using
 Xba. ∴ go back to Xho,
 or do some tricks.



↑ 1.8 kb
 ↑ 1.2 kb
 Xho Nhe

② Conclusion: There is an Nhe 1
 site ~~site~~ inside the 3' band.



There is no Asc I site in 3' band. so
 can reconstitute with Asc — Not
 if nec^y

① G-10
 W
 G
 Ag
 2 1P
 3 1K
 4 1N
 5 1Y
 6 G-53
 7 55
 8 W
 9 M
 10 W
 11 10p
 12 16
 13 G-10

Thurs. Jan 15th

Dye
Water
G'53 1P

Repeat Check on Xho & Xba in G'39#1

1Xba
1Xho
1XX

Take $\frac{1}{2}$ pl ~~G'39#1~~ SH Z149 1.48 μ g/pl
+ 8 μ l 10X RS + 0.5 M NaCl
+ 72 μ l LbW

G'53#1

Rename

To Marker
W
Dye

16' 32' 32'
+ 1 μ l Xba I ✓ + 1 μ l Xho ✓
Lot 30 (200) Lot 007 (100)

G'53 3Pre
G'51 3 BSX²XR
W

G'53
1Xba

G'53
1XX

G'53
1Xho

37° 12:10 pm

5:25 pm

Dye
W
IP
1Xho
1Nhe
1XN

Fri. Jan 16th

Logic Error or Forgotten Conclusion

If Nhe is inside Pvu 1 of 3' — as previous data without same tracks
show (G'15) — cannot put in 5' second / . Need to

G'53#1 Asc

Also check Asc I for pass. excision. Asc I $\rightarrow$ Asc Nhe
~~some time needed~~

Take 1 μ l G'53#1

+ 8 μ l 10X RS + 0.5 M NaCl
+ 72 μ l LbW

16 μ l
G'53²
1P

32 μ l
1 μ l Xho Lot 007 (100)

32 μ l
2 μ l Nhe I Lot 23 (50 μ l)

Take ~~G'53#1~~

4 μ l 10X NEB #4
+ 36 μ l LbW

+ 2 μ l Asc I Lot (100 μ l)

G'53²
1Xho

G'53²
1XN

G'53²
1Nhe

G'53#1
Asc

B'59DD
Asc



3.0
2.8

1.1

0.27 0.16

① Conclusion

V. nice proportion
- only fastest difficult
because of e.p. fault. overh
looked v. promising.
BS is a trace high

①

A

37

BS 3.0
 Xba
 TNAP
 K_{as} K_{at} A_{ap} B_{gl} S_{ac} X_{ho}
 1.1 2.8 0.16 0.27
 First assembly into BS XX

First assembly of 5' hand with 5 different
 Xba K_{as} B_{gl} S_{ac} X_{ho}
 CTAG GCGC GATC AATT TGA
 Take 10 µl G'47 XKT fragment
 + 10 µl F'153 ckit conc. KB
 + 5 µl F'147 BSKT conc
 + 20 µl F'155 bpA/SX conc
 + 2 µl G'51³ BSXX
 + 5 µl 8M NH₄Ac
 + 130 µl EtOH

Can cut out
 Xho — Xho
 later to put
 into 3' hand.
 if successful -
 Most uncertain
 frag. is the XKT.

① 2:45pm
 50pm
 1.59 to 4X1
 Rashes changed
 10/10/10
 Dye
 W
 G'55 Pre
 W
 Acc MM
 W
 Dye

Freeze. Cent. dry & back up into 20 µl 1x p95 hiq. buffer
 as G'55 hiq.

+ 1.5 µl T4 ligase Lot 970 (dilute)
 6 µl to 10 µl

G'55 Pre

To 150 9:40pm to 6pm
 Cold 6pm to 12:40pm sat RT 12:40 to 1:15
 37° 1:15 to 1:50pm

Cold until 9:30am
 Jan 23rd (Friday)

Sat Jan 17th
 Transformation

G'39 JH5d 80 µl
 G'55 hiq 30 µl
 00 1:52pm to 2:37 (45 min.)
 42° 90 sec ✓

Mon
 10:30am
 3, 3, 3 daily

Plate on 1x Amp NZY with 40 µl IPTG per plate
 40 µl XGal

37° Sun 3pm
 Picked 24
 to 1x Amp NZY - good
 and 50/50
 IPTG/XGal
 NZY

Rest 20/5 20/16 5/16 37° 3pm
 Rest 5/5 20/16 20/16
 (10) ①

NM's

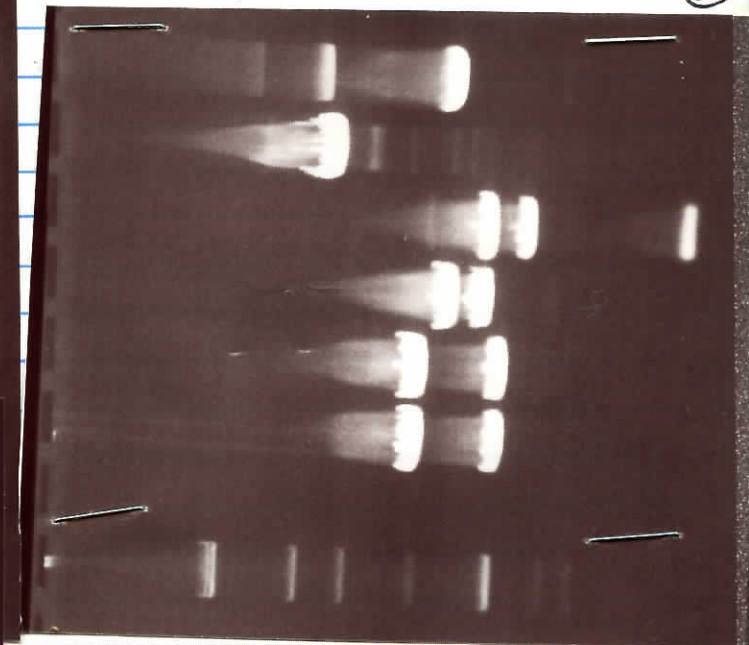
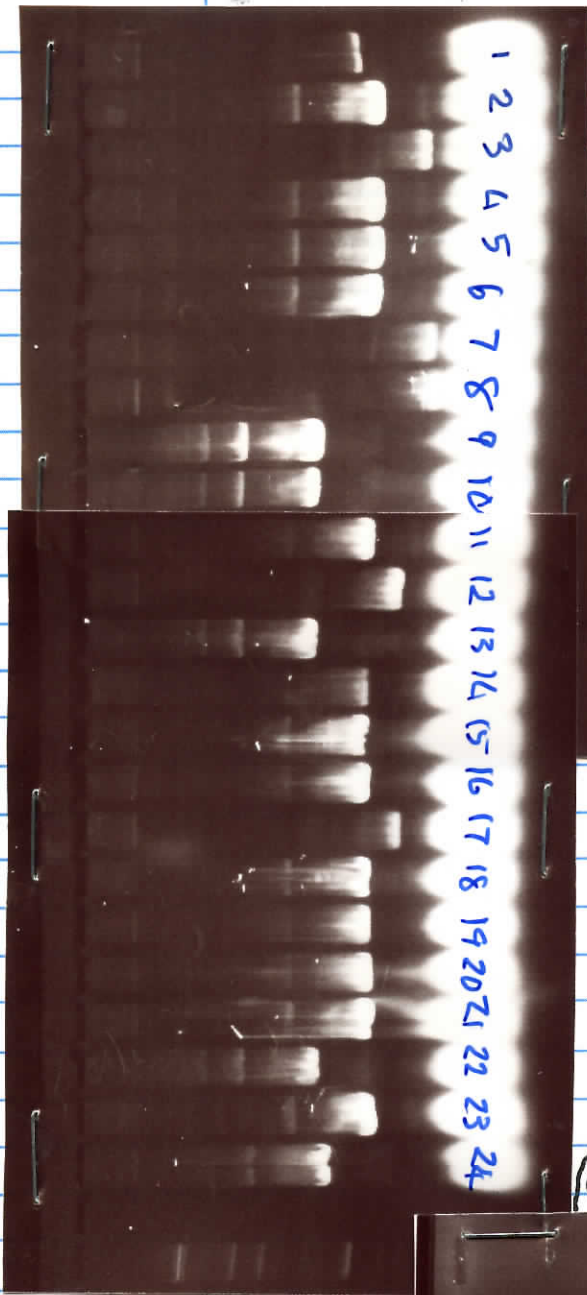
10am cold - 2pm

~ 75% White

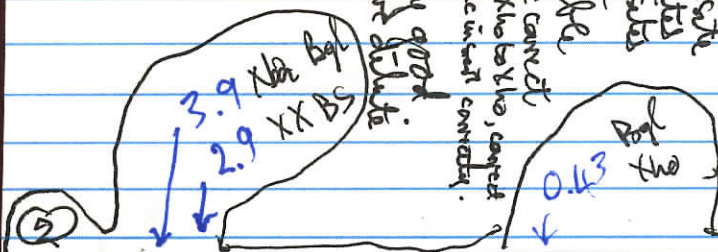
G'55
 5' hand

① Several reasonable candidates.
Processes 9 10 13 22 & 24

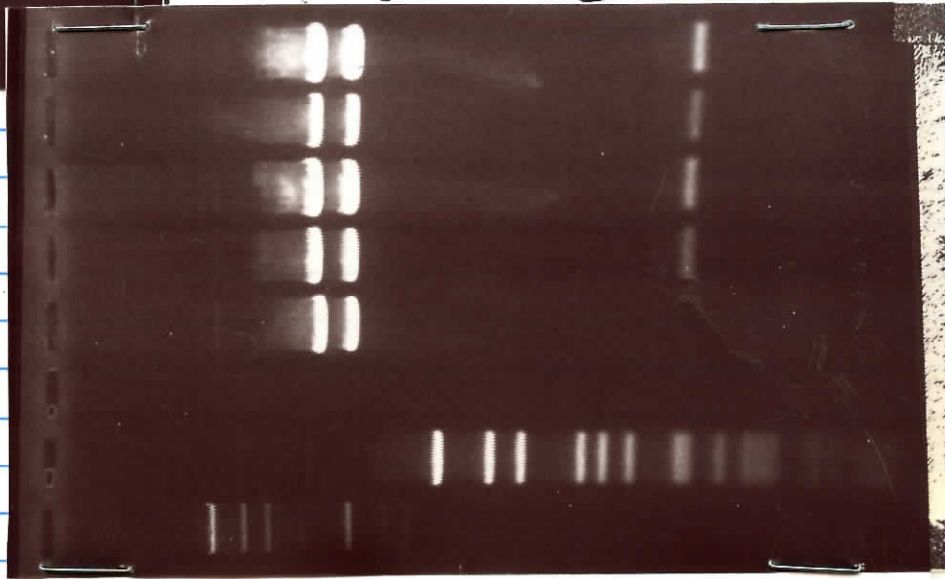
(w)



③ Single Kas I site
Two Sac I sites
Two Xho I sites
Xho Xba not
detectable
Xho sites are correct
Sac to Sac < Xho to Xho, correct
Kas and Sac - Sac incorrect, correct.
looks very good.
Be sure most dilute
for size
premixer.
Bgl is also
OK



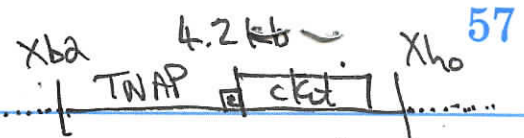
② Boy oh boy!
Correct from 24
with 5 fragments!!
The other is shorter
on both ends (#24)



①
②
③
Pre
Kas
Kas
Sac
Xho
Xho
W
Agarose
W
Bgl
3.9
0.572
Kas

0.810 H₂O Max

Mon Jan 19th



5' hand - (contd.)

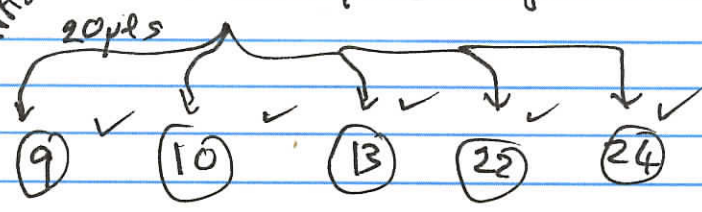
Initial check on correct product by: -
EcoRI XbaI XhoI & BglII with 1.5% gel

Ag MM
W
Dye

Take 12 µl 10x RI salts
+ 93 µl ddw

+ 3 µl (60v) XbaI lot 30 (20v/µl)
6 µl (60v) XhoI lot 007 (10v/µl)
6 µl (60v) BglII lot 35 (10v/µl)

Dye
On 1:25 for
5.05
1.5
+ RNase



37° 2:38 pm

1 µl each
of 5' 57
mm

All 5 streaked out
to incubator ~ 2 pm.

4:08 pm
back to 37°

Acryl MM
Ag MM
Dye

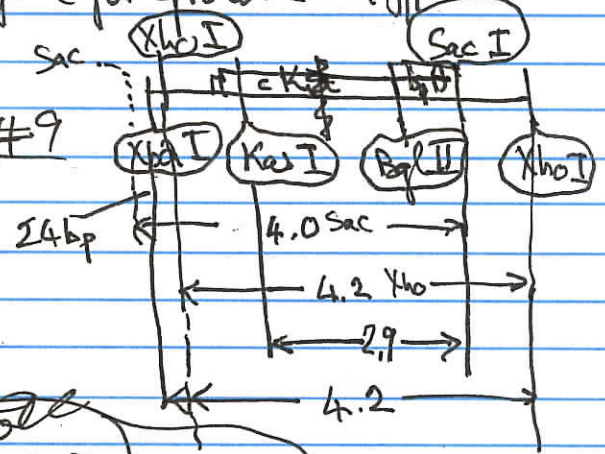
G'57 #9

to Sylvie for growth 1.2 µg/µl
SHZ155 ← Serials G'93

Thurs. Jan 20th

More check on G'57 #9

Take 15 µl 10x RI salts
130 µl ddw
4 µl G'57 Mini #9



G'57²
Pre

30
1/2 µl SacI
Lot 48
20v/µl

40
1 µl XhoI
Lot 007
10v/µl

1 µl XbaI
Lot 30
20v/µl

3 µl KasI
Lot 14
4v/µl

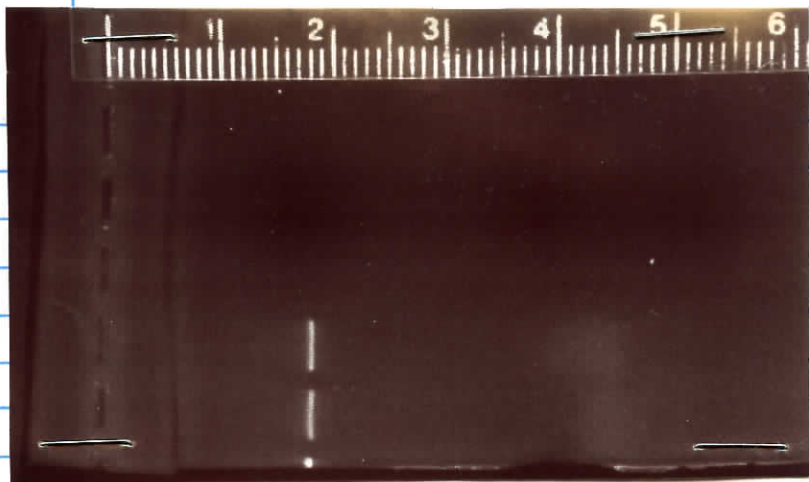
G'57²
Sac

G'57²
Xho

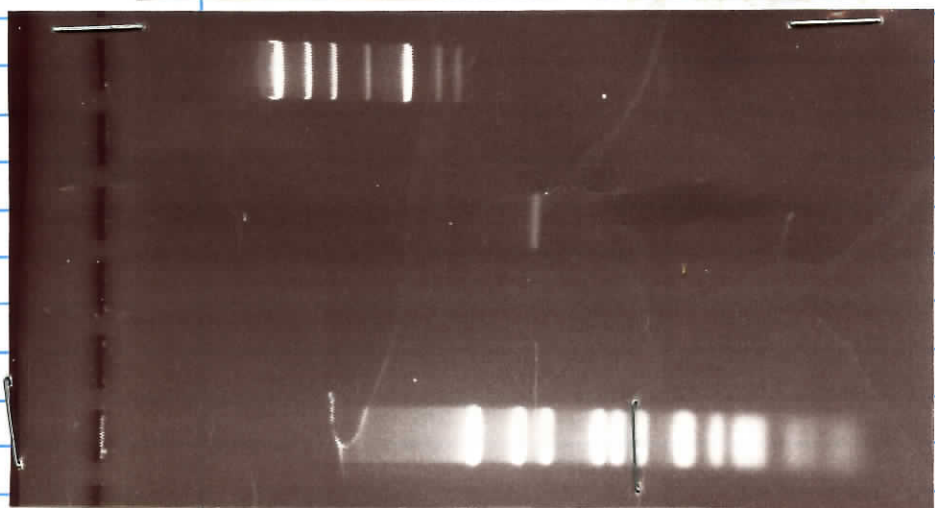
G'57²
XX

37° 12:15 pm
3:45 pm
Not thinking!

5.8.2004

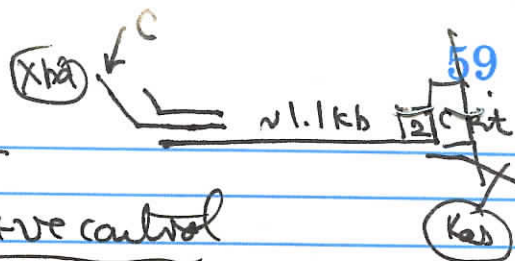


② Conclusion
 Proceed, altho' size
 difference is v. detectable.



4-22-74
 On 4-22-74
 Rye
 wife
 Rye
 Rye
 Rye
 W.
 ACR
 W
 Dye
 1.59

Thurs. Jan 20th



Catching up on PCR +ve control

Since G'47 XKT was apparently successful, can repeat procedure for XKC. 46 μ l of G'29 C conc is available. Proceed using 20 μ l (i.e. $\frac{1}{2}$ amount used for XKT).

- ✓ Take 20 μ l G'29 C conc
- ✓ 10 μ l 10 $\times$ RT salts
- ✓ 70 μ l ddw
- ✓ 5 μ l KcsI
- ✓ 1.5 μ l Xba I

Pre G'59

G'59
XKC

Lot 4 4 μ l/ μ l

Lot 30 20 μ l/ μ l

8+8

37° 10:25 am to 2:10 pm Cold

Heat 5 min. at 70° $\pm$ 2°

Post G'59

1
Agarose
Gel
Electrophoresis
Buffer
Dye
Water
PCR
Mixer
Agarose
Gel
Electrophoresis
Buffer
Dye
Water
PCR
Mixer

1 hr @ 6V/cm

1.5% Seap

H+I 6V/cm

Cut & processed to 50 μ l 0.001M as EDTA

G'59
XKC
Fragt

Wed. Jan 21st

Ligation test on G'59 XKC Fragt

- ✓ Take 10 μ l G'59 XKC Fragt
- ✓ + 20 μ l 10 $\times$ p95
- ✓ + 8 μ l ddw
- ✓ + 1 μ l T4 ligase

10 μ l G'59 Pre lig

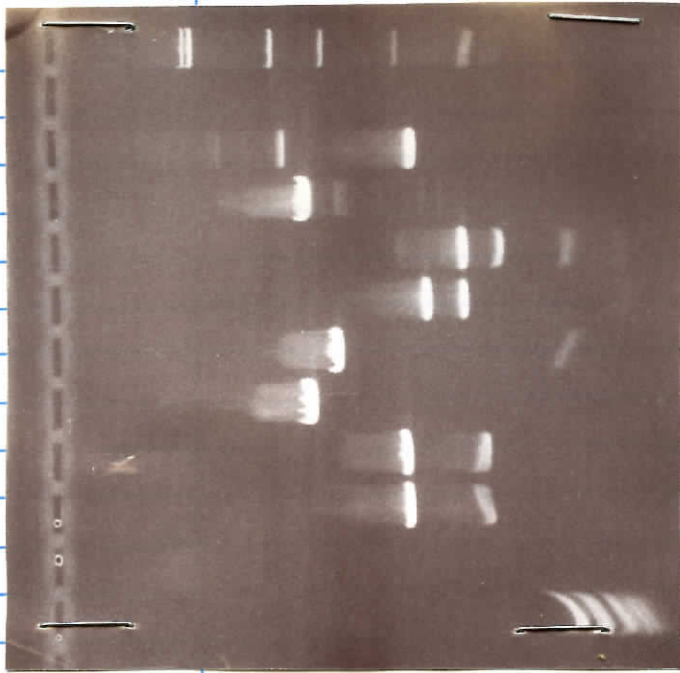
+5 μ l trap
G'59
LIG N

150 11 am to 12:45 pm
RT 12:45 pm to 2 pm

37° 2 pm to 4 pm

PCR
Mixer
W
Dye
1.5% Agarose
10V/cm

5 8:24



~~Handwritten scribbles and illegible text.~~

0.81
on 5:4
off

Handwritten notes and symbols, including a vertical line and some illegible text.

This
mean
after
the
sit
is

~~on 5:40 pm~~
~~off 9:40 pm~~

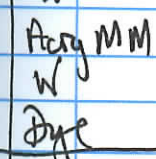
20 μ l of $G' 57^2$ Pre + $\frac{1}{2}$ μ l Xba I hot 30 (20 μ l)
10 μ l



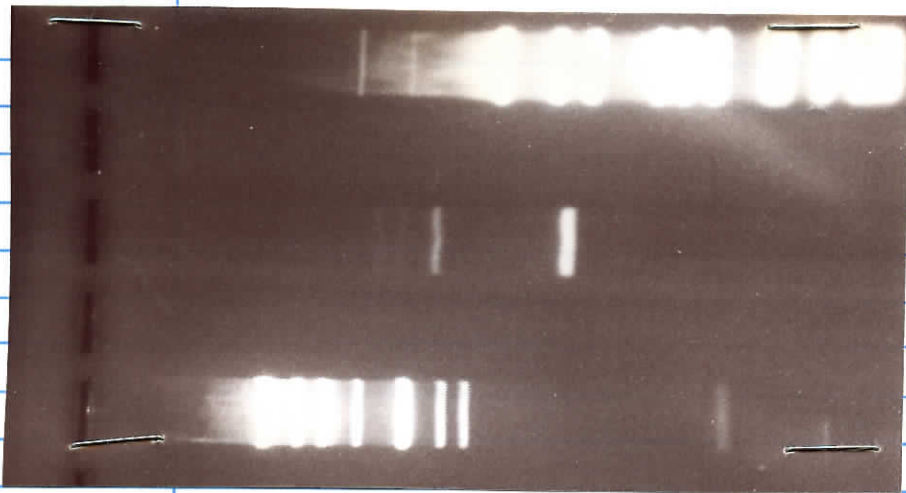
Back to 37°

Get ~~Buffer~~ problems!! Again!!

Notes:
Xho is single, & Kae single is confirmed.



This means
attending
into the
site only
is best.



① Conclusion:
Acceptable ligation
under these conditions

On 11/1

①

On 11:20 am 11:56 159 HX1 10/1/01

①
Agg
W
AGMM
W
G'63 ligⁿ
W
AGMM
W
Dye

Agg
Conditions

Wed. Jan 21st

~~Agg~~
~~W~~
~~AGMM~~
~~W~~
~~G'63 ligⁿ~~
~~W~~
~~AGMM~~
~~W~~
~~Dye~~

63

Repeat of ligata test for G'59 XKC frag^t

✓ Take 15 μ l G'59 XKC frag^t
✓ +1.5 μ l p95 10X ligⁿ buffer
✓ +1 μ l T4 ligase hot
Cold room ~6pm to 11am

G'63
LIGⁿ

5 8-944

Sac/Sac
digest
of
A.
~~10~~
Sac

Marktmappe 2024

✓ 44 pe 10 x RI salts

✓ 290 ✓ 390' pe ddw

220 ✓

✓ 30 ml Xba I Lot 30 (200/μl)

6 μ l BqCII lot 35 (100/ μ l)

✓✓✓✓ 5x 40pl

+2 p.e KasI wt 14

+ 1 pe B/LT

+1/2 pe bac I 12-48
200/41

Lot 007

29X

$$Xb_2 + Kac$$
$$Y_{ba} + B_{gl}$$

$Xba + Sac$

Xba + Xho

3:55 pm
9 HkK + KKA
2/3
Bgl

$B_{gl} + K_{ds}$

Bgl + Xba

Bgl + Sac

Bgl + Xho

+ 2nd Kar = 1

$$+ \frac{1}{2} \text{I}^- \text{XbaI}$$

+ $\frac{1}{2}$ μ l Sac I

+ 1 pl Xhc

Bgl
 Ag N M
 XBA

$$5 \times 40$$
$$N_2 + 3H_2 \rightarrow 2NH_3$$
$$Xba + Sac$$
$$X_{A2} > R_{A2}$$
$$XBa + K_2S$$

W	
---	--

ACR M M

✓	
---	--

$$R_L + G_c$$

2019.10.10

Bgl + Nho

BqL + ~~QAS~~ KAS

$$\text{Bgl} + \text{X h2}$$

W

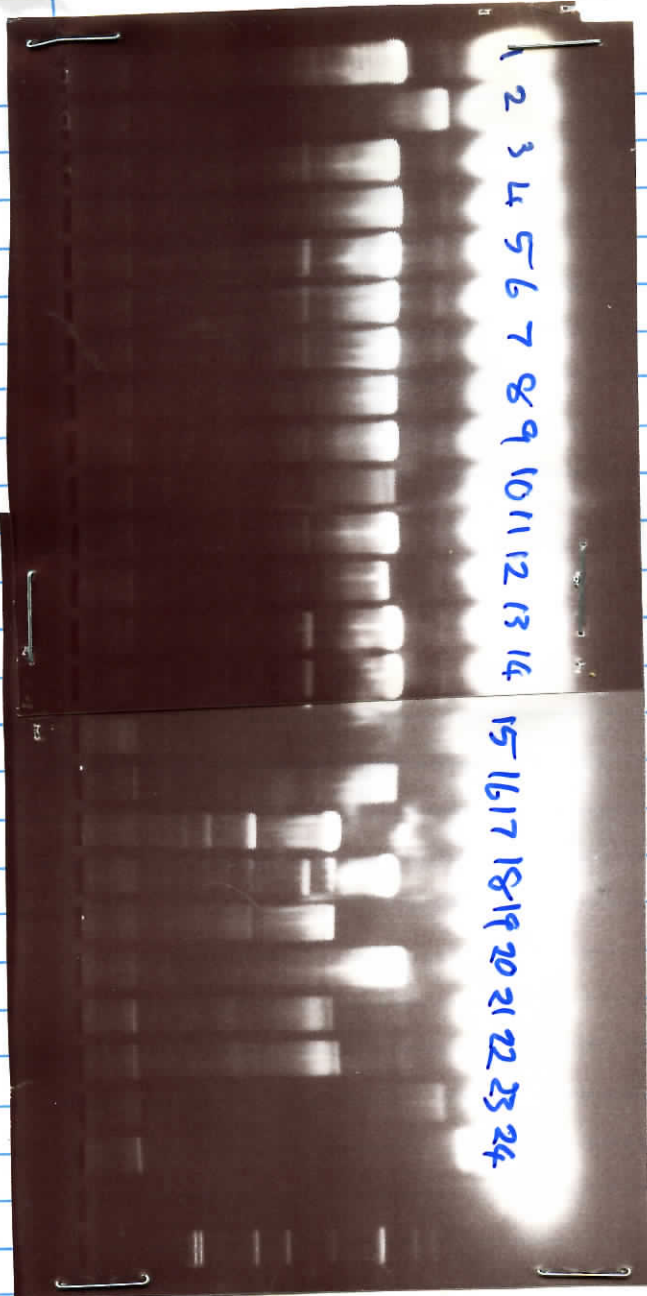
AgMM

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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And

37° 10:50 am to 1 pm ² 1:40 pm to 4.15 pm Fragen
RT

3 15 24



Conclusion: #17
 looks good, and
 19, 21 & 22 are
 probably OK.
 go ahead with
 all 4 & streak
 out also.

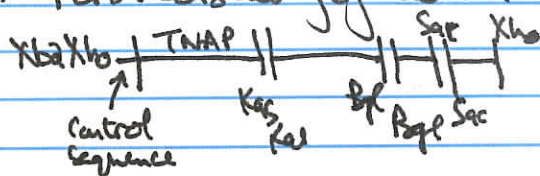
Sum streaked out
 into 1x Amp MZY 4:30 pm

Re-streaked out Feb. 5th
 as G'67 CONTROL (17) 02/05

012:00
 01
 02
 03
 04
 05
 06
 07
 08
 09
 10
 11
 12
 13
 14
 15
 16
 17
 18
 19
 20
 21
 22
 23
 24
 W
 Agm
 W
 Dye

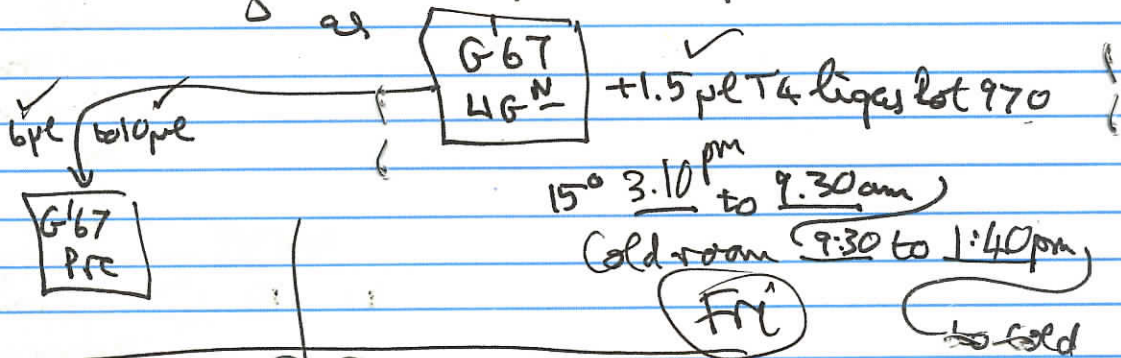
2.10.75
3:30 pm
0.8/0 Hx4
Sum

CONTROL 67
Thurs Jan 22nd First assembly of control



- Take 20 µl G'59 XKC frag t
- ✓ 10 µl F'153 kit conc KB
- ✓ 5 µl F'147 BS kit conc
- ~~10 µl F'153 kit conc~~
- ✓ 20 µl F'155 bpA/SX conc
- ✓ 1.5 µl G'51³ BSXX
- ✓ 6 µl 8M NH₄ Ac
- ✓ 56 µl EtOH

Freeze. cent. D₂O, & back up into 20 µl p951X lig. buffer



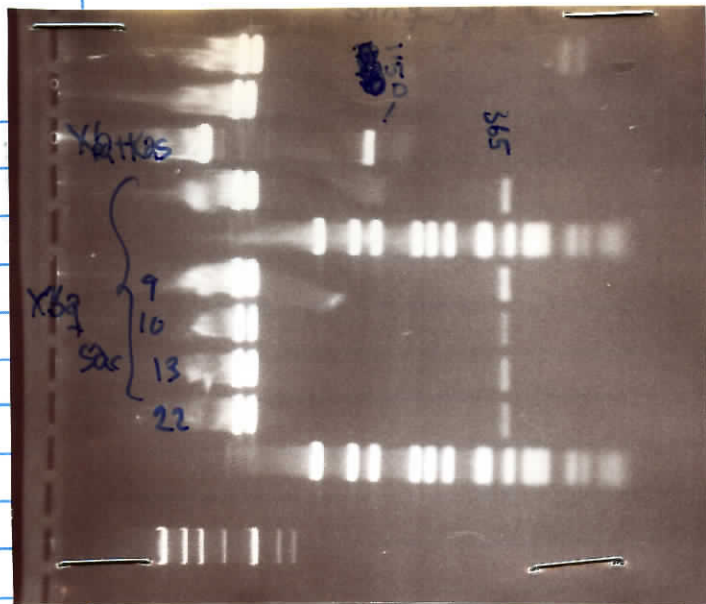
G'55+similar	DH5α (G'67)	80 µl
	G'67 lig-	3 µl
	0° 1:43 pm to 2:26	
	90 sec 40°-42.5°	

Plated on 40+40 1XNZ4 Rext 20, 3X5, 3X24 5
16'16

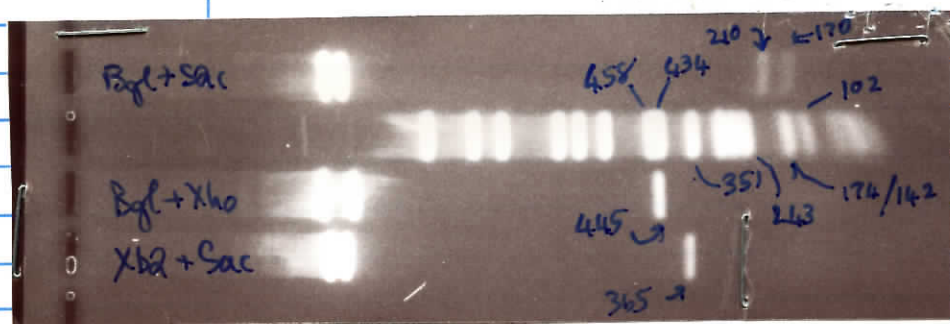
looks like >50% are white
37° 2:45 pm - 11 am

Sat Minis G'67 1-24 37° 12:45 pm ~ 10:30

#2 & #23 are blue.

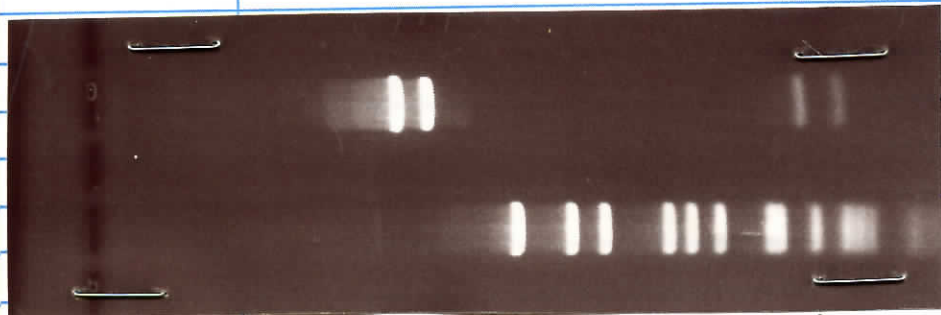


① Conclusion: All four clones are identical. The 365 bp Sac piece requires explanation.



② Conclusion: N.M. Suggests Sac I in clat. Sequence confirms 199 bp 5' to Bgl2. ... expectⁿ is Sac Bgl Xho

correct
 199 → 152 → 273 →
 (358) (273)
 From Xho+Sac
 ③ Conclusion: within tolerance



11.30am
12.10pm
1.5% Hx1 + RNase
100/cu

Error - intended Bgl + Xho

Thurs Jan 22nd

Xba + Sac on all candidates for 5' homologs

Dye
W

Ac M M

G'65 Bgl + Sac

G'65 Bgl + Xba

G'65 ~~Xba~~ + Sac

G'65 Xba + Sac

Take ✓ 9 µl RI salts 10x

✓ 79 µl ddw

✓ 1 µl Xba I lot 30 (200/µl)

✓ 1 µl Sac I lot 48 (200/µl)

Ac M M

G'69 (9)

G'69 (10)

G'69 (13)

G'69 (22)

W

Ac M M

Ac M M

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

1/2 µl each mini

(9)

(10)

(13)

(22)

37° 5:20pm

100µm

5:20pm

5:20pm

5:20pm

5:20pm

5:20pm

5:20pm

5:20pm

5:20pm

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5:20pm

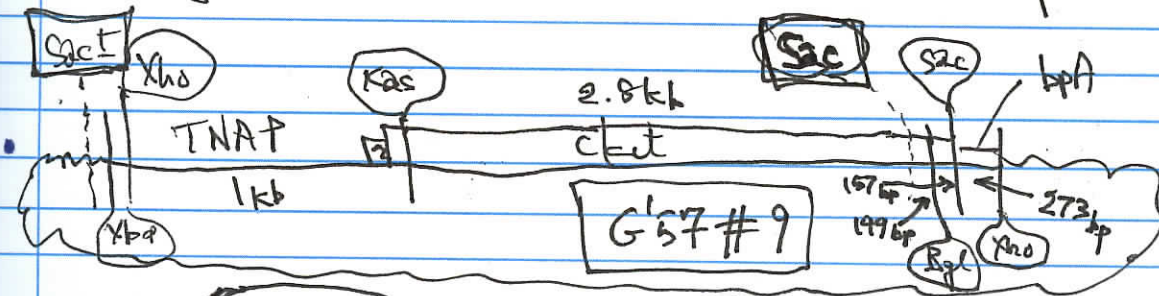
5:20pm

5:20pm

Need XhoI + SacI

Take G'65 Xba + Xho (25 µl) + 1/2 µl Sac I (lot 48; 200/µl)
32° 3:55pm — 5:10pm

General conclusion — all is well; proceed!



Use Xho / Xho to go into G'39#1 for targeting construct

5:20pm



Model 377
Version 3.0
ABI100

Version 3.0
ABI100

980123C1_20_Shiller
0120.01
20-Shiller
and 20

Signal G:43 A:58 C:63 T:60
DT (dR Set Any-Primer)
96051547 DR LR9709 Instr File
Points 1121 to 10624 Base 1: 1121

Mon, Jan 26, 1998 11:05 AM
Fri, Jan 23, 1998 04:15 PM
Spacing: 9.00(-9.00)

1. seq

G39#1

Ex Bama
Ex Bama
Ex Bama

Lox OK

Pac I

Page 1 of 2



F155 Neo Bkg
Neo OK
Mishy
Datta
Lox OK
But linker still here

F103 Blue is inside the Selex - leave future.

Remap some more candidates

NCCTANNA NNGG TTTTNGCCCTNNG C TNNNN GCTGAGGAGN CCA TGNNG C NCC C NTTNGC NGTTT A TT NC GC NCCCTT C TAN N GA GGC CTNG G CT CTT GANTNTT NG GAN CNGANC N NNG TANG CNGG N N N N N C
580 590 600 610 620 630 640 650 660 670 680 690 700 710 720

NNNN N NNC G T NG GNN CN ACT NC GNNG TG N NCT TT G GNGNN C GNC C TTTCNN GNN CTNN C AAG GAT TC NTT TANTAN TIN NT NNTTNNNG GCTNT N NNTN ANG GC TN CNG NNG NC CN N N G TANA CNG TCG CC G N
730 740 750 760 770 780 790 800 810 820 830 840 850 860 870

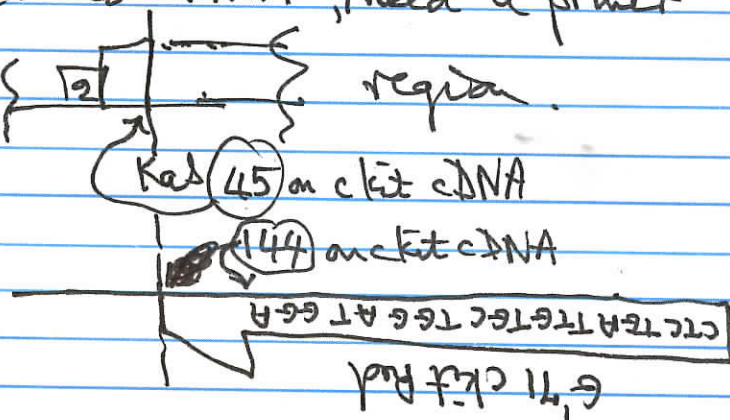
Sat Jan 24th

Sequencing tests on targeting construct

Standard forward & reverse sequencing primer for BlueScript can determine ends of 5' homology.

For the ckit junction to TNAP, I need a primer (red) from ckit into region.

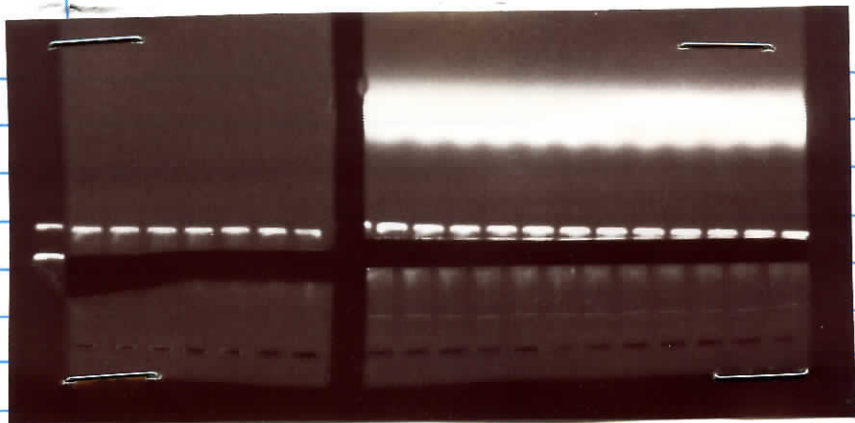
G'41 ckit Red



For the 3' homology first try with F'103 Blue E (just 5' to 10X in Neo) did not work. repeated with F'155 Neo Blue (just 5' to Stop codon of Neo).

Result shows (i) that PMCI Neo was used in making DupDel (not PMCI Neo folA). Error may have been when N.M. had S.H. grow up both together. My entry is W87, B'129, A'11, & B'51 - but stop in making DupDel. Fortunately, D'46 Red (22) min is still available.

(ii) that 3' hand. did not go in correctly since Pac is still there. Check out other G'39 candidates.



Conclusion:
Good cut target;
adequate cut control

G-732
cut target
adequate

Sat. - Jan. 26th
Xho fragments for assembly

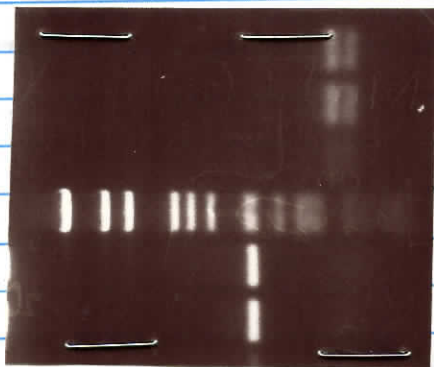
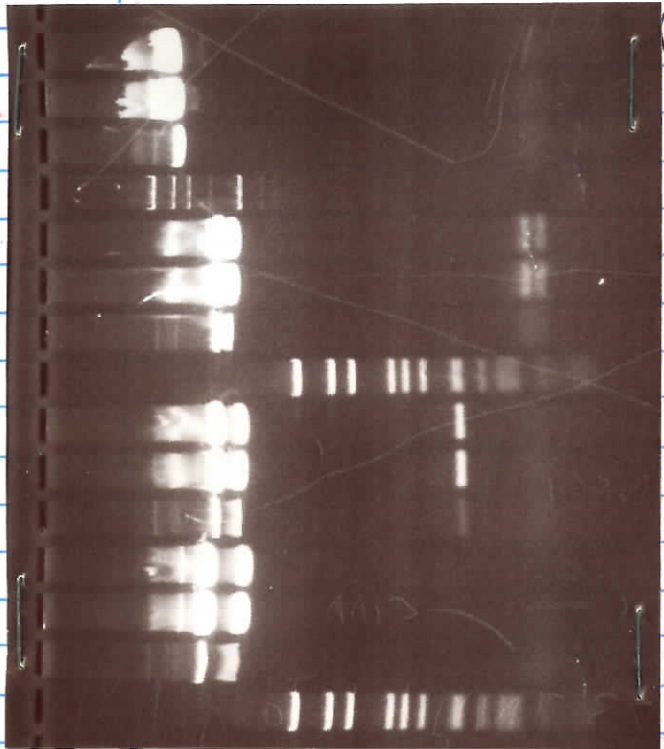
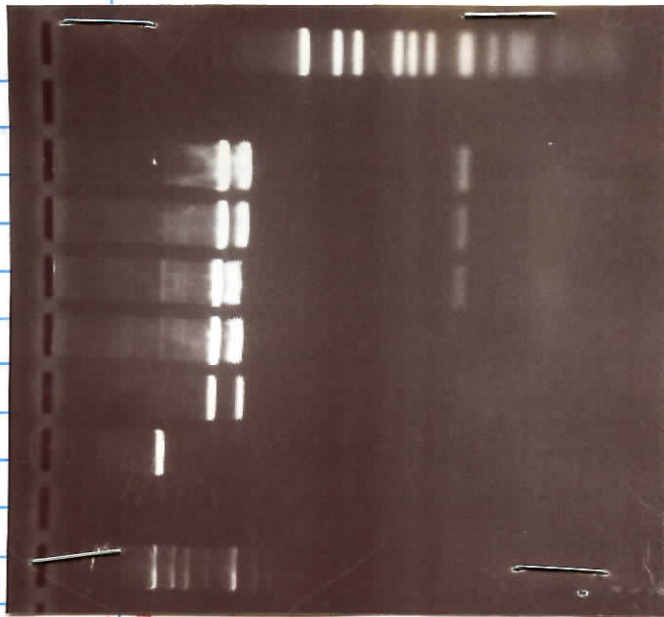
~~CANCEL~~
~~G'53 #1~~
CIAP treated
Xho Xho
Not 3' hand
Xho TNAP kit Xho
G'57 #9

Take 32 μ l 10x R1 salts
288 μ l ddw
4 μ l Xho I hot 007 (100 μ l)
120 μ l
200 μ l
+ 2.5 μ l (3 μ g) G'57 #9
+ 3.4 μ l (5 μ g) G'53 #1
G'73 1.20 μ g/ μ l St 2.155
G'73 TNAP Kit Target Xho
G'73 DD3 Xho
G'73 DD3 Xho

37 $^{\circ}$ 4:30pm to 10:30am 70 $^{\circ}$ - 75 $^{\circ}$ for 10 min
G'73 DD3 Xho + 45 μ l IMT res pH 8 + 2 μ l CIAP lab stock
55 $^{\circ}$ C for 30 min 11:52 to
1x 004 / HQ; 2x CIAP
CANCEL

04:5:30pm
09:30am
0.8 μ l
0.5 μ l/cm

8 slots
14 slots
G'73 TNAP Kit Target Xho (Blue)
G'81 TNAP Kit Control Xho (Black)
G'73 TNAP Kit Target Xho for
G'81 TNAP Kit Control Xho for
Both cut & extracted
at usual to CIAP
Rest (150 μ l) into 20 μ l



① Conclusion: (17) (19) & (21) are possible with a fairly clear indication that 17 & 19 differ from 21 in length of the TAAAT pattern.

~~Target #9~~
~~Control #17~~
~~agree~~

② Conclusion:
Target #9 } agree.
Control #17 }

Proceed

#21 is not recognizably different but at lower yield

Bgl ✓

Sac ✓

Xho ✓

Kas ✓

Re photo.

②

9 Bgl

17 Bgl

21 Bgl

9 B+Sac

17 B+Sac

21 B+Sac

AC M

9 B+Kas

17 B+Kas

21 B+Kas

AC M

9 B+Xho

17 B+Xho

21 B+Xho

AC M

9 B+Xho

17 B+Xho

21 B+Xho

AC M

9 B+Xho

17 B+Xho

21 B+Xho

AC M

9 B+Xho

17 B+Xho

21 B+Xho

AC M

9 B+Xho

17 B+Xho

21 B+Xho

AC M

9 B+Xho

17 B+Xho

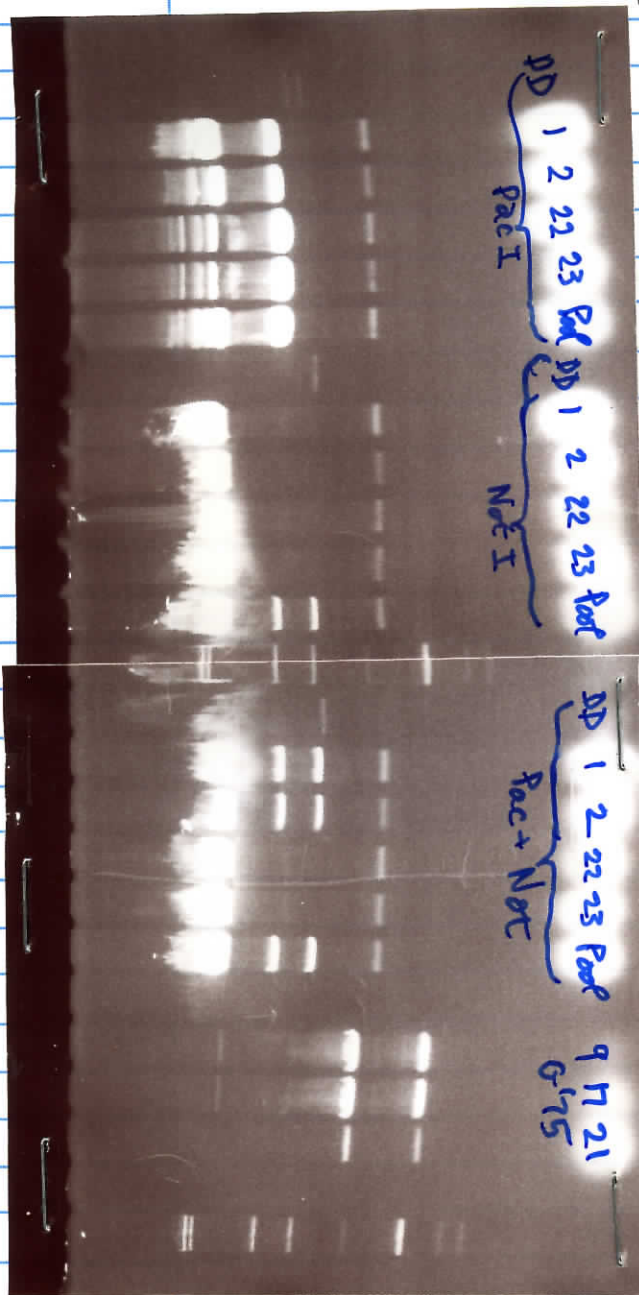
21 B+Xho

AC M

on 10. Co
off

By
w
AD
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81
82
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86
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88
89
90
91
92
93
94
95
96
97
98
99
100

✓ 481
✓ 55
✓



① Conclusion:

①, ② & Pool are cut (incompletely) with Pac.

②② & ②③ are not cut with Pac

All are cut with Not I & pool has some Not-Not fragment in it.

②② & ②③ look more likely than rest. Sequence both.

1 PCR control
G'75 candidates for 5' hand, are all 3' identical after Bgl & KAS & same as target 5' hand / Proceed on G'64

on 3.25p

0.8% HxL max

①

primers

D
w
DD
1
2
22
23
Pool
DD
1
2
22
23
Pool
AGM
DD
1
2
22
23
Pool
w
G'75 B
G'75 B
G'75 B
w
AGM
w
w
Day

G'77

9/5

17/5

21/5

Thurs. Jan 27th
Check on G'39 candidates

Sequence on G'39 mini #1 shows incorrectness (G'70)
 Pac I, Bam I & Sal I are still present. ∴ test other G'39 candidates

Take 20 µl 10x R1 salts
 200 µl ddw
 200 µl

20 µl Pac I lot 28 (40 µl)
 10 µl Not I lot 47 (100 µl)

~~20~~ Pac

~~20~~ PN

~~20~~ Not

lotto
PN

lotto
N

1 µl of
G'39
Minis
added

①
P

②
P

②②
P

②③
P

Pool
P

37° 12:15 pm
 ~ 3 p.m.

1 µg
D59 DD
2.7 µg/µl

G'77
D59 del
0.1 µg/µl
2 µl 0.001 M EDTA

something wrong here

Wed Jan 28th
 streaked out G'39 # 22 & #23 on NZY Amp
 37° 10 am

3:25 pm
 08/4/4
 MAX

Dye
W

DD

1

2

22

23

Pool

DD

1

2

22

23

Pool

ABM

DD

1

2

22

23

Pool

W

W

W

W

W

W

W

W

W

W

W

W

W

W

W

W

W

W

W

W

W

W

Tues. Jan 27th

More sequence data — on 5' hand $\approx 20\text{ng}$
 Laura Livingston 6-3763 (or help) says 10 pMoles of
 primer & 700 ng of template in 20 μl for a sequence.

G'57(9) F1 (Blue) M13 F1 primer 2 pm/ μl G'79 5 μl ✓
 G'57 #9 (G'73) 1.2 $\mu\text{g}/\mu\text{l}$ → G'79/G'57#9 (2.3 μl + 37 μl) 10 μl ✓
 G'57(9) R3 (Red) M13 R3 primer 2 pm/ μl G'79 5 μl ✓
 → G'79/G'57#9 10 μl ✓
 ddw 5 μl ddw

Results: The 5' end is good with Xba, Xho & other 2 sequence. ✓
 The 3' end is good with Rho bpa / SacI / sup kit / Bgl II / ckit... (Data on G'86)

← ^{somewhat} probably too much DNA
 G'39 #22 mini 2 μl
 ✓ F'155 Neo Blue primer 100 5 μl
 ✓ ddw ~~1000 μl~~ 13 μl } OS
 G'79
 22

0.4 μg of G'39 #22 would be in about 2 μl looking at G'76 gel.
 DNA

10 pMoles of primer $\equiv 5000 \times 10 \text{ pg} = 50 \text{ ng} = 0.05 \text{ } \mu\text{g}$

Assuming primer is 1 $\mu\text{g}/\mu\text{l}$ this is 5 μl of primer / 100 ← in 0.01M EDTA

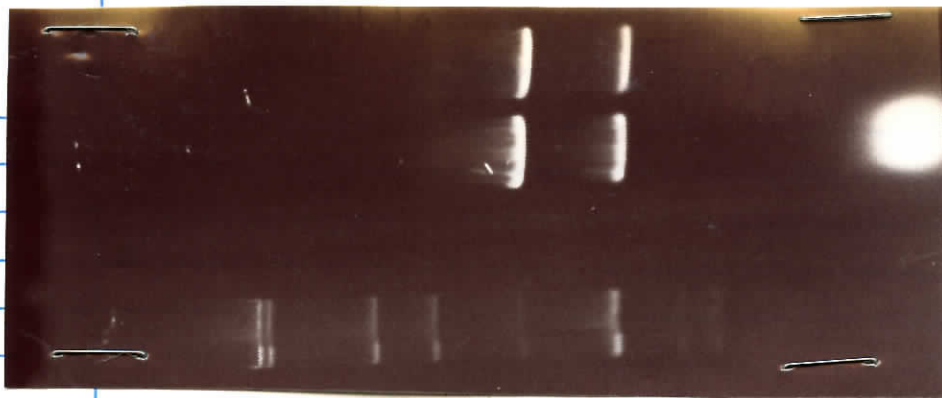
Results: Possibly w/ pl mini in future. #22 is correct on 5' end

#9
 → G'57 (G'73) 1.2 $\mu\text{g}/\mu\text{l}$ / 10 10 μl ✓
 G'71 ckit Red primer / 100 (stock 0.5 = 1 $\mu\text{g}/\mu\text{l}$) 5 μl ✓
 ddw 5 μl ✓
 G'79
 ckit

Results: Fine sequence. Function is exactly as planned.

THAS
Emb

80



—

Wed. Jan 28th Black (control) Xho
 TNAP kit ckit Xho

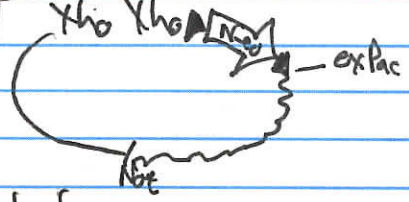
5' homology (contd.) Blue (Targeting)

- Take ✓ 20 µl 10x RI salt
 ✓ 170 µl ddw
 ?shd have used 10 µl ✓ 5 µl G'67 mini #17
 ✓ 5 µl Xho I lot 007 (100/µl)
 37° 11:45 to 2:30 pm → 70°C for 10 min
 75°C

G'81
 Control
 TNAP KIT
 Xho

Vector + 3' homology (repeat) CIAP

- Take ✓ 32 µl 10x RI salt
 ✓ 280 µl ddw
 ✓ 8 µl Xho I lot 007 (100/µl)



G'81
 Xho
 22
 16 µl Part

160 µl
 5 µl G'39 }
 Mini 22 }
 37° 2:35 to 11 am

160 µl
 5 µl G'39 }
 Mini 23 }

G'81
 Xho
 23
 16 µl Part

- + 40 µl 1M Tris pH 8 (5x CIAP buffer) + 40 µl
 + 2 µl lab stock CIAP
 55° for 30 min 11:50 am to 12:28 pm
 1x 0.04M HEPES 2x 0.1M

3 pm
 0.80 M NaCl
 0.80 M NaCl

G'81 Xho
 22 CIAP

G'81 Xho
 23 CIAP

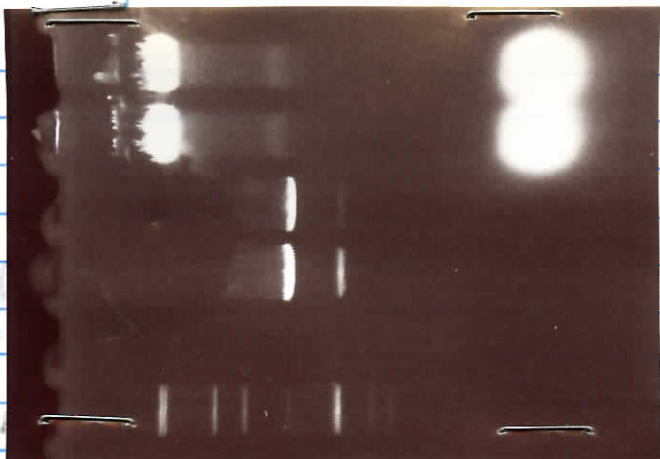
- 10 to 6 µl G'73 TNAP kit (Target) Xho
 10 to 6 µl G'81 (Control) TNAP kit Xho

Blue (from purified DNA)
 Black (from mini)

G'81 Xho
 22
 G'81 Xho
 23

W
 10 markers
 W
 DTC

Prep. gel on G'73



① Conclusion Target TNAP kit
is borderline quality. Control
TNAP kit is definitely not
acceptable. Upgrade format.
Repurify better.

AP list
Control
at

TNAP KIT

Ex Pac

Thurs. Jan 29th

Final assembly

Blue target

Black control } Hold

3' home
2.45pm
0.8% off mark
decreased to 25%

First check of conc.

Mini (22) G'81 Xho ~~Part~~ Post
Mini (23) G'81 Xho ~~Part~~ Post
G'73 TNAP kit Target Xho Conc (Blue)
G'81 TNAP kit Control Xho Conc (Black)
AGMM
W
Dry

Ligation

- ✓ Take 8 ~~pl~~ (22) G'81 Xho ~~CIAP~~
- ✓ +25 ~~pl~~ G'73 TNAP kit Target Xho Conc (Blue)
- ✓ 3.7 ~~pl~~ 8M NH₄ Ac
- ✓ 92 ~~pl~~ 600H

Freeze - Cent. Dry. Back into 15 pl x p95 as

Cold room 5:30pm

+ 1 pl T4 ligase (Histo) lot 067

G'83
WGN

5 to 10 as

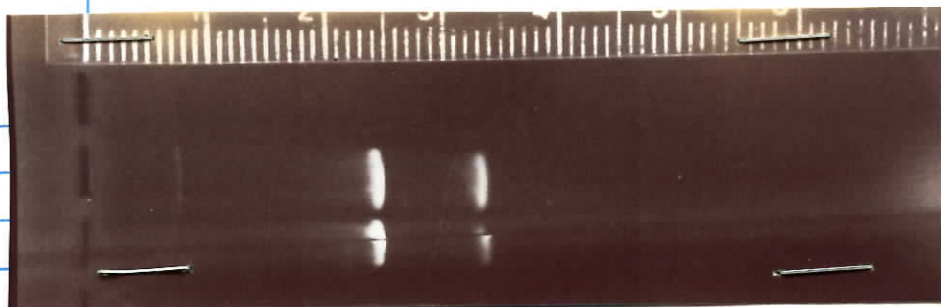
G'83
Pre high

11.10am

5 to 10

as G'83
Post high

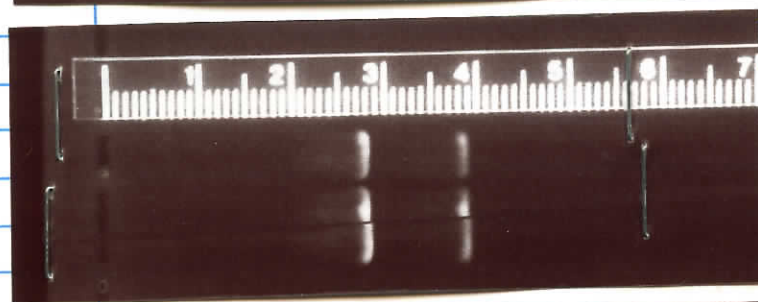
See G'89 Fri Jan 30th
Cold



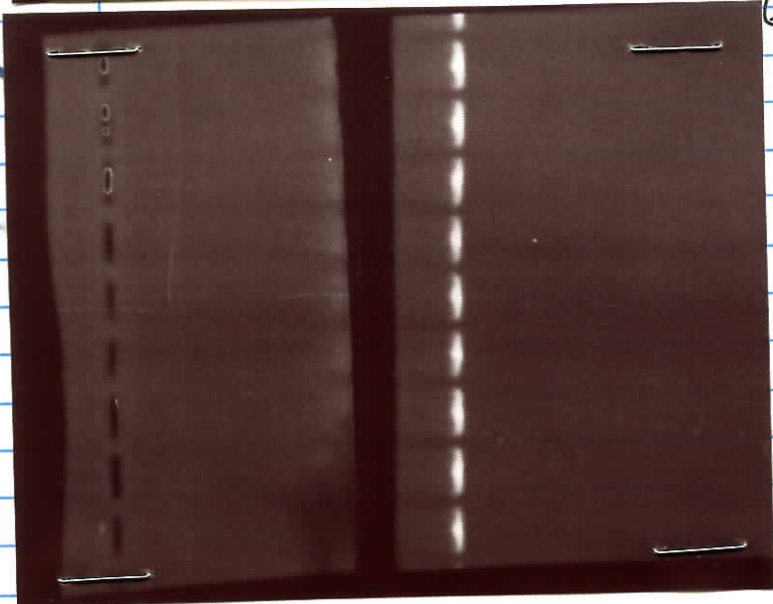
Conclusion
① Good separation



② conclusion:
G' 83 ligⁿ is good enough to proceed.
Purity of INAP kit Carboxyl is fully adequate.
Amount of vector in ligⁿ was good.



③ Conclusion: Good cut



①

Dye

W

G' 81

W

Dye

on

②

5-

5

Vindil

operation

Thurs Jan 29th

Clean up of TNAP kit Xho Control

✓ Whole of G'81 TNAP kit Xho Control Conc
 + 400 µl of G'81 TNAP kit Xho Control CIAA
 + 1.1 ml G'81
 Freeze. Cent. Dry. Back into 60 µl 0.001M EDTA
 as G'85 TNAP kit Control Grade

✓ Load ✓
 6:10 pm
 9:00 am
 1.1 ml/cm

W
 Dye

G'81 TNAP kit Xho Control (same load as G'73)
 G'85 TNAP kit Control Grade

Cut, good. recovered as
TNAP kit Xho G'85 CONTROL CONC

Fri Jan 30th

into 45 µl 7 mm have
 low 50 µl pellet

on 11:45 am
 12:30 pm
 0.8 to max

Back up repeat of TNAP kit Xho Target

Set up as G'73²

37° 9:15 am to 1:45 pm

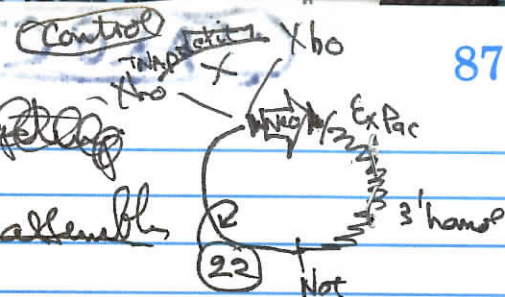
(2) App
 Water
 5+10 Pre G'83 high
 5+10 Post
 TNAP kit Xho CONTROL conc G'85
 Marker
 W
 Dye

(3) App
 W
 0.8 Hx4
 10 slots
 W
 Dye

70°-75° ~ 10 min
 2:30 pm
 0.8 Hx4
 0.8
 6:30 pm

Good 970 mg
 Cut & recovered
 into 80 µl
 as G'85 conc
TNAP kit Xho TARGET

5 8 2 2 2 2

Fri. Jan 30th~~Calad~~ construct final assembly

G'81

Take 8 μ l (22) Xho CIAP✓ + 18 μ l TNAP kit Xho CONTROL conc. G'85✓ 3 μ l 8M NH_4Ac ✓ 73 μ l EtOHFreeze, Cent, Dry $\rightarrow$ back up into 15 μ l p95

as

G'87
Control
lig.+ 1 μ l T4 ligase hot 067

✓ 5+10

G'87

Pre lig.

Cold 2:10 pm

Transformer

G'62 DH5 α	$\sim 75 \mu$ l
G'87 Control lig.	3 μ l

0° 3:15 pm 3:59 pm

90secs 40°

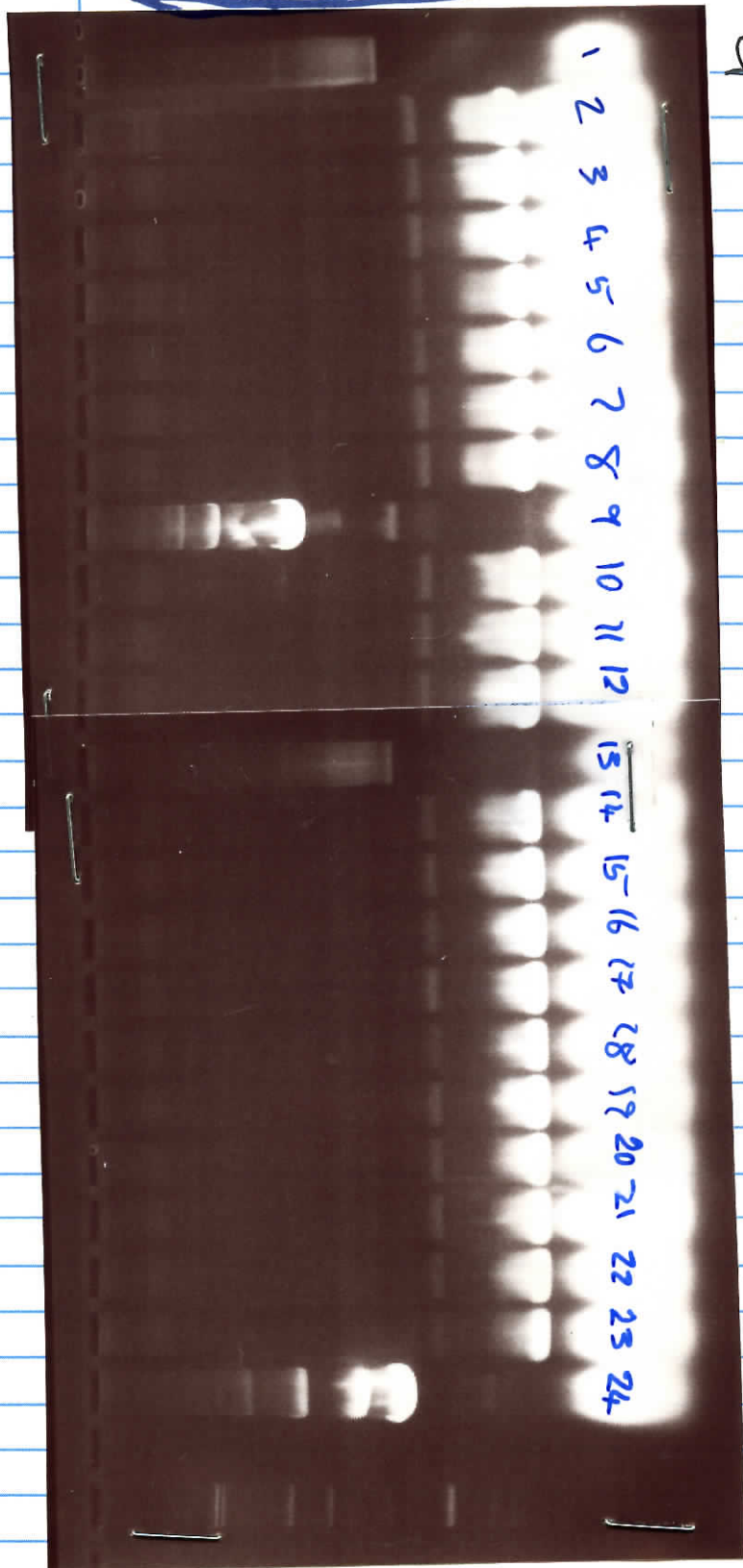
G'87 Control	Plate	Rest	20, 5, 20, 5	1XNZY	37°	408
			10, 16	Amp	10 pm	2m

CONTROL
G'87 Minis 1-24
picked to 1XNZY Amp 37° 2m pm to Mon. am

Gel on G'93

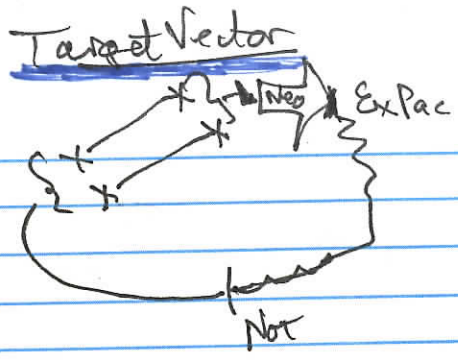
TARGET

G' 89 T



Conclusion: It was wise
to make target backup.
Only #9 is pers. & it
looks like 103!

11:15am
12:30pm
0.86 HX4 Max
G'89 TARGET BLUE mini
Fri. Jan 30th



Transformation

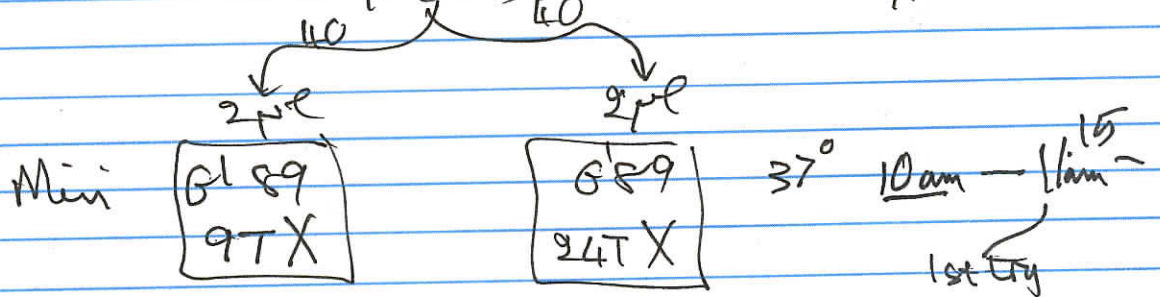
22	G'67	DH5 α	80 μ l	✓
23		G'83 h ⁺	3 μ l	✓
24				✓
W			0° 2pm to 2:40pm	
Marker			42° 90sec	✓
W				
G'89			Plate 1K Amp NZY Rest, 20, 5, 20, 5	37° 2:55pm

24 Min G'89 (Blue) T 1 ... 24 NZY ← 305pm
37° Amp
4:30pm
n 10am Sun

Processed G'89 (9T) & (24T) to 50 μ l

Mon. Feb 2nd

Take 8 μ l 10x R1 salts
✓ 70 μ l H₂O
✓ 2 μ l (20v) Xho I lot 007 (100 μ l)



Sun. Feb 1

Repeat of target construct assembly

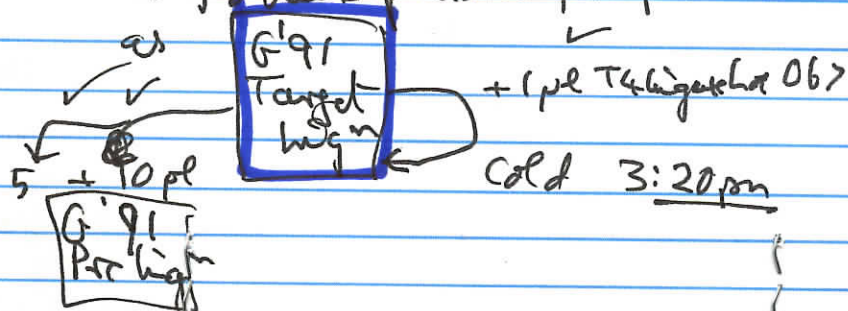
Take 8 μ l G¹81 (22) Xho CIA P

+ 20 μ l TNAP kit Xho TARGET conc G¹85

+ 3 μ l 8M NH₄Ac

+ 18 μ l EtOH

Freeze, Cent. - dry & backup into 15 μ l p95



Mon Feb 2nd

Transformation

DH5 α G¹81 80 μ l ✓

G¹81 high 3 μ l ✓

0° 3:15 pm to 3:50 pm ✓
mon mon

90 sec. 42° ✓

Plated 20, 5, 20/16, 5/16 1X Amp NZY 37° 4:05 pm

(16) (8)

Tues 3:20 pm

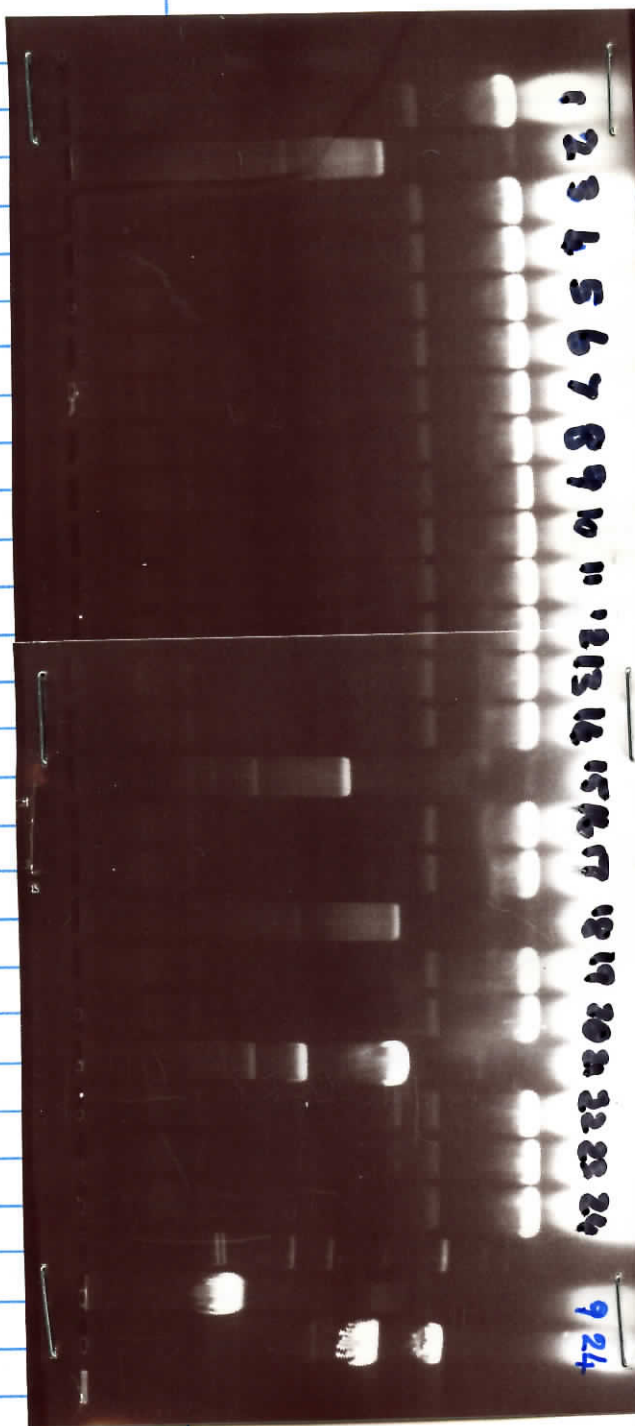
Picked to mini G¹91T

1X Amp NZY

#1-25

37° 5:10 pm

✓ 10 am

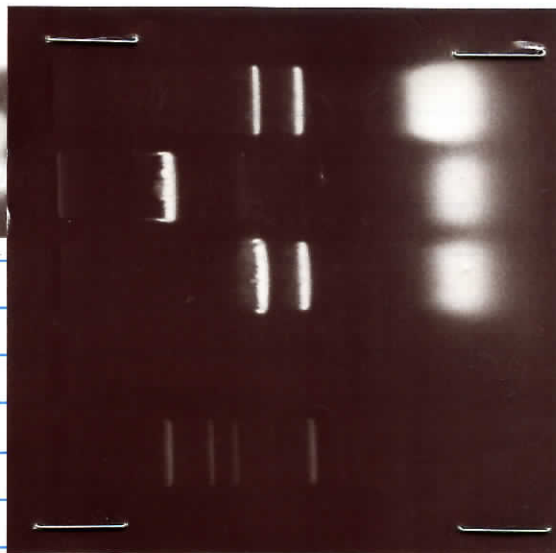


(2) Conclusion: Probably
(15) was BS TNAP kit BS.
No good.

Conclusion (2) & (21) are probably
the same and equivalent to
(24) kit, which is TNAP^{kit BS} again.
(9) kit does not cut ~~well~~ twice
with Xho, & so is rejected, altho'
its size is nearly correct
Dup Del is 6 kb
TNAP kit is 4.3 kb
3' handle is ~ 8 kb
18-19 kb

So (15) is possible. Check it out
Meanwhile ~~good~~ repeat of
target ligation. ^{transform} Pick another
24 from G^{87C}.

Note - orientation is ~~important~~
important for PCR control
can be shown by PCR or enzyme



11:25
~~0.9 #x4~~
~~3/4 Max~~
 at 1:15 PM

Ma. Feb 2nd

Final Control Control checks

Dye
~~1~~
 2

G'87C

24

G'89 9TX(ho) w/ 1/2 digit

G'89 24TX(ho)

Ag MM

W

Dye

5:10 PM
 6:15 PM

Straked out (15) G'87 32° ~ 3 PM Mar.

Proceeded min to 50 pl

✓ 4 pl 10X RI buffer

✓ 35 pl ddw

✓ 1 pl Xho lot 007 (100 pl)

✓ 2 pl G'87 (15)

Xho
 G'93

32° 3:48 PM to 5:26 (first)

G'93Xho

G'89 9TX(ho)

G'89 24TX(ho)

W

W

W

Dye

(3) Max
 9 #x4
 0.8
 4:30 PM

4:30 PM

Minis
 G'93

Dye

W

25

26

47

48

W

MM

W

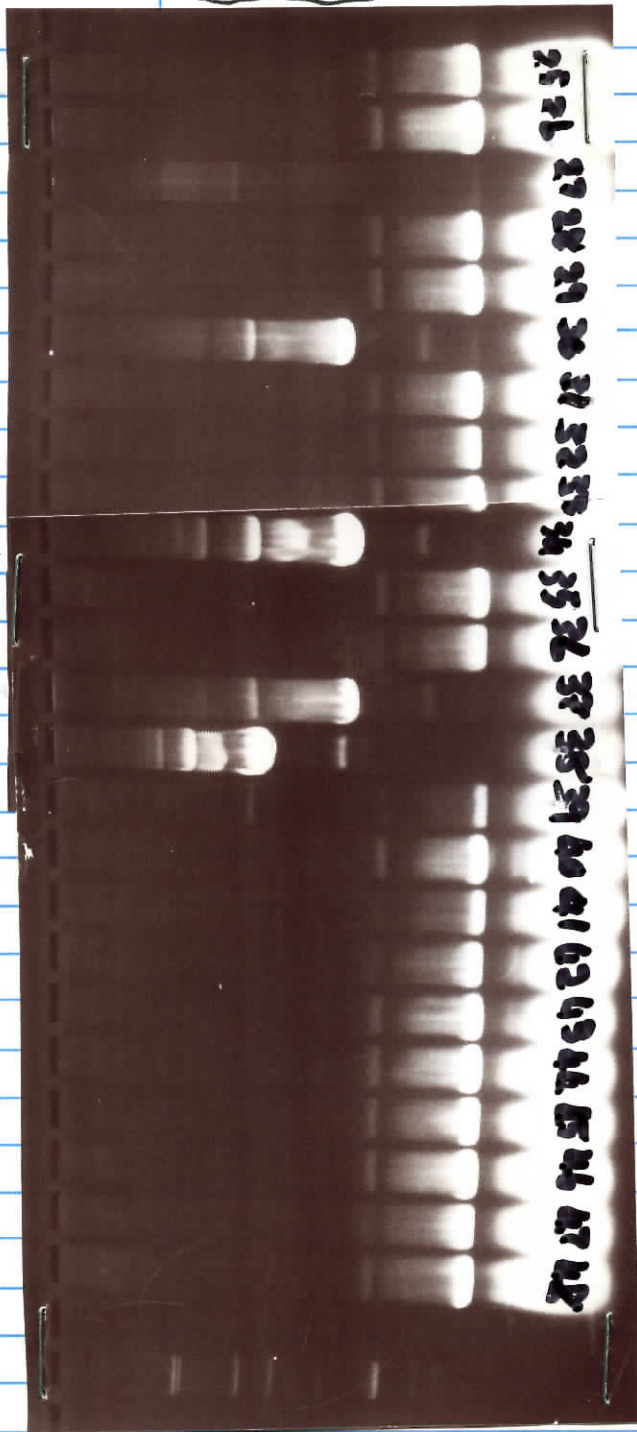
Dye

Ma. Feb 2nd
 Minis G'87 #25 - #48 1X Amp NZY 32° 4:45 PM

Gell G'94
 Photo

over
 page →

G'87 Control
minis



← ③ G'93 gel G'87 minis

Conclusion:

G'87 Mini (38) C is possible

Processed to 50 μ l

4 μ l 10x A1 salts

✓ 35 μ l d.d.w

1 μ l (38) C G'87 min

1 μ l XhoI hot 007 (100 μ l)

37° 7:10^{pm} to 2:10^{am}

Streaked out on 1X Amp N2Y

#38
37° 6:45^{pm}

G'95 C
17

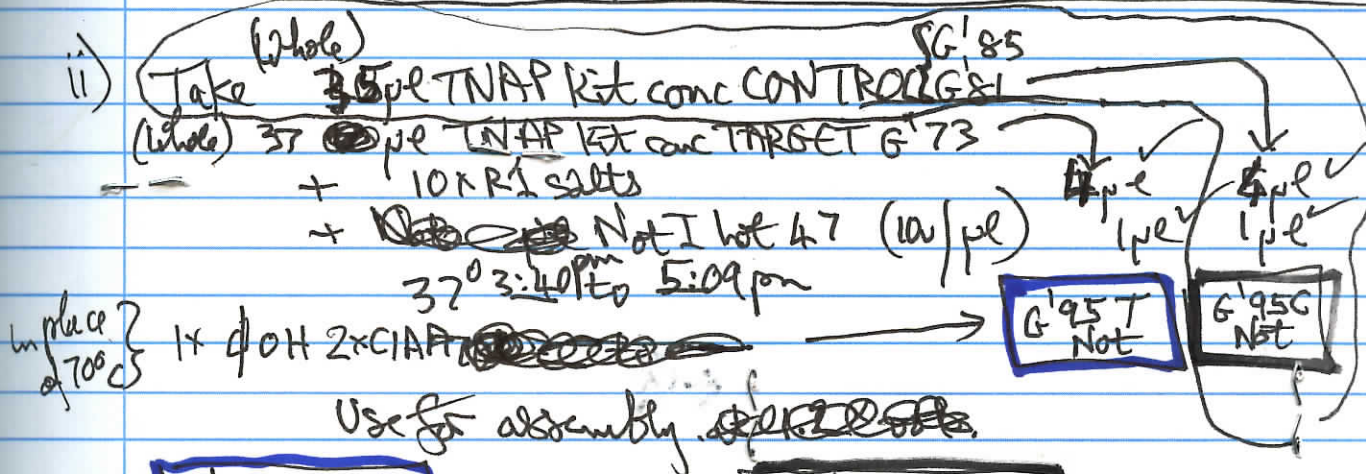
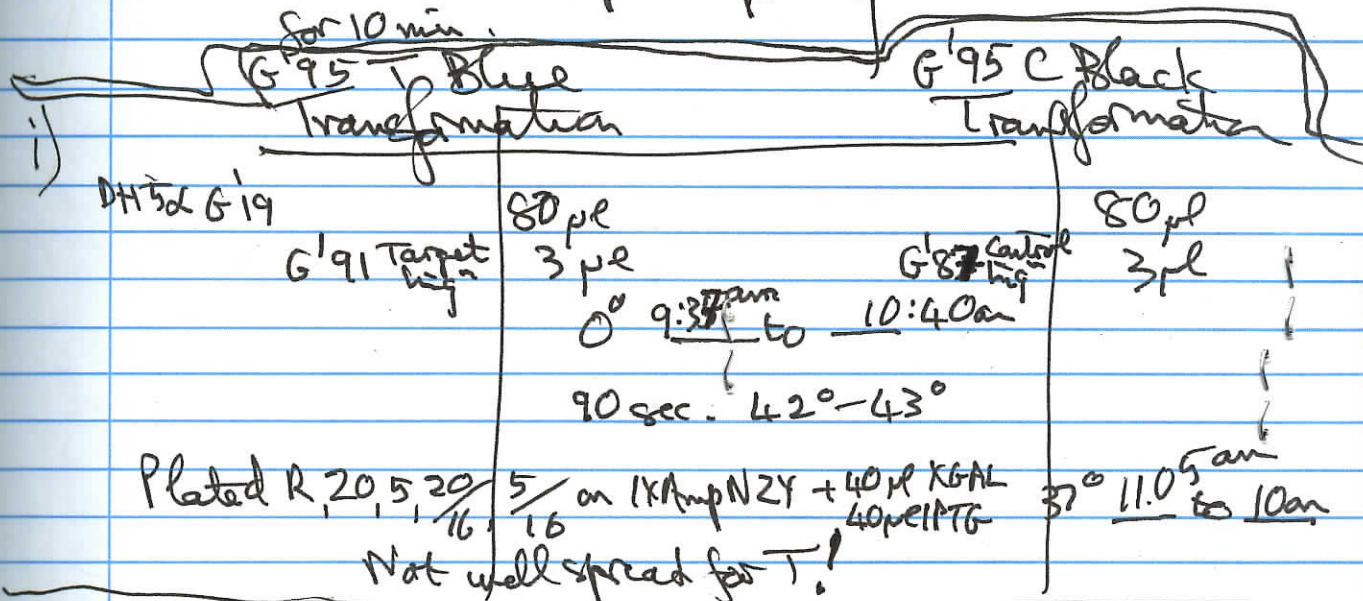
SHA 4 1.62 μ g/ μ l

See G'98

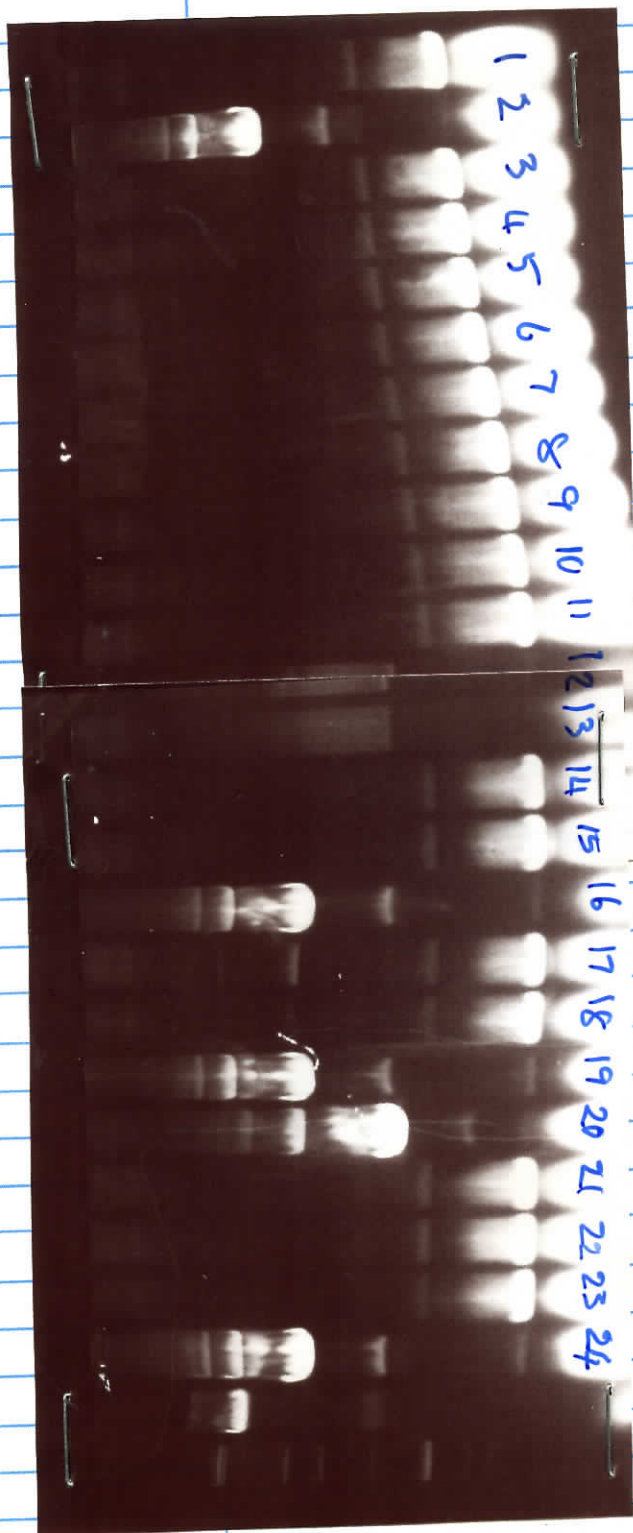
Tues. Feb 3rd

Improvements in final step

- i) Replate both of best ligations on IPTG/XGal to ~~eliminate~~ ^{reduce} BS problem
- ii) ~~Put~~ the purified X-TNAP kit Xho fragments with Not I before ligation. Inactivate with 70°C for 10 min.



- | | |
|------------------------------|--------------------|
| ✓ 8 μl G'81 (22) Xho CIAP | ✓ 8 μl |
| ✓ 20 μl G'95 T Not | ✓ 20 μl G'95 C Not |
| ✓ 3 μl 8M NH ₄ Ac | ✓ 3 μl |
| ✓ 78 μl EtOH | ✓ 78 μl |
- Freeze. Test. Dry. Back into 10 μl 1X p95 + 1 μl T4 lig. lot 067 Cold 7 pm

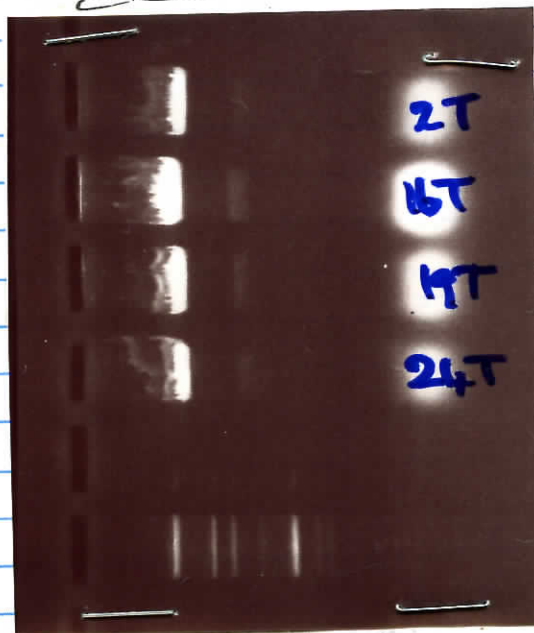


38C
↑

① Conclusion: 691 is a better
ligation - now 4 candidates.
With Xgal & IPTG it sh'd be OK.
38C is not correct.

Process ② ⑬ ⑱ ~~24~~ to 50 μ l
+ test.

② Conclusion



① 1:20
1:10
1:5
1:2

Dye
W
1
2
23
24
AG
AG
T

② W
S
I

1:20pm
 2mm with stop repair.
 0.8% MAX

Tues. Feb 3rd

G'87 Control Minis #25-48
 G'93 gel as G'94 #38C is possible.

Dye
 W

1

2

23

24

AGMM

W

Dye

W

Dye

W

2TX

16TX

19TX

24TX

W

MM

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

G'91T

Minis

Target

G'94/38CXho

Control

AGMM

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

G'91T Target Minis #1-24

G'94/38CXho Control

AGMM

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

W

Dye

17pe 10XR1

140pe 12W

4.2 2pe (420) Xho I Lot 007 (100/pe)

40 40 40 40

1pe 10XR1

140pe 12W

4.2 2pe (420) Xho I Lot 007 (100/pe)

40 40 40 40

1pe 10XR1

140pe 12W

4.2 2pe (420) Xho I Lot 007 (100/pe)

40 40 40 40

1pe 10XR1

140pe 12W

4.2 2pe (420) Xho I Lot 007 (100/pe)

40 40 40 40

1pe 10XR1

140pe 12W

4.2 2pe (420) Xho I Lot 007 (100/pe)

40 40 40 40

1pe 10XR1

140pe 12W

4.2 2pe (420) Xho I Lot 007 (100/pe)

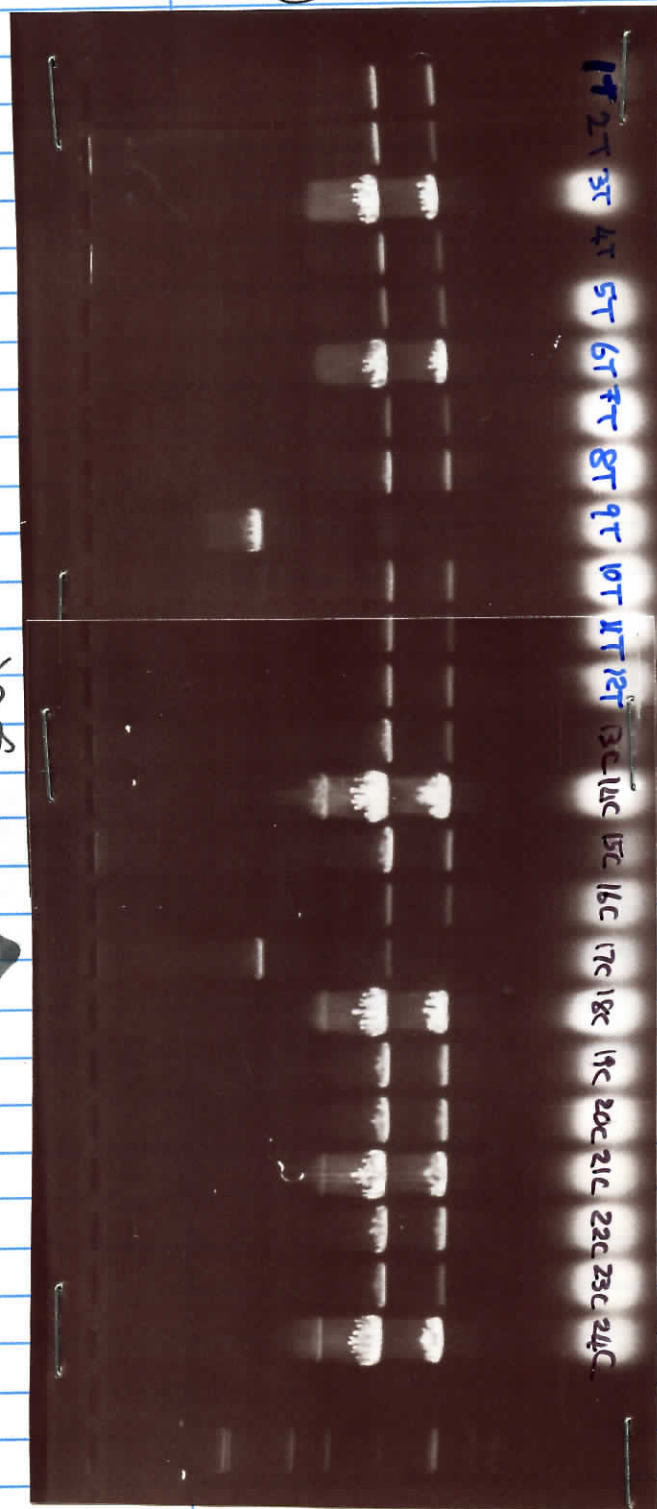
37° 4:40pm to 5:30pm
 1st try back to 37°

If none are +ve, reconsider what has gone wrong.

None are positive. G'81 may be wrong.
 Repeat. CIAP may have failed or whatever -
 probably single stranded w/alt probat.
 Better - make the Xho TNA kit Xho frag.
 - remake the CIAP

Xho Xho

G'95 minus



G'95
minus



① Conclusion: The Xgal really worked! But clearly the ~~most~~ colonies are "TNAP kit BS" and "DD3' homology".

The insert is not being ligated into vector? try very low amount of ~~DD3'~~ DD3' without CIAP plus large excess of Not-cut Xho TNAP kit Xho. But first look at the results of G'95 (Not) lig⁺ is not good.

!! Gave up too soon!!
Ret-inspection shows that G'95/17C looks good, but faint streaked out. Now check orientation, & ~~re-pick~~ pick more.
3:50pm Fri Feb 6th

← See G'94 for growth

G'95 minus after Xho.
1T
2T
3T
4T
5T
6T
7T
8T
9T
10T
11T
12T
13C
14C
15C
16C
17C
18C
19C
20C
21C
22C
23C
24C

G'94
G'95

Wed. Feb 4 ch

3:20p
4:50p
0.8% Hx4
Wax

(am)

G'95 Minis + Xho T and C on XGal / IPTG

Best success rate sh'd be the G'95 T & C Not lig⁴

Meanwhile pick 12 T and 12 G from XGal / IPTG G'95

G'95 T (17) - (12) G'95 C (13C) - (24C)

1x Amp NZY 37° 3pm

Pretty well all the secure or near so whites available

G'99T Transformation

(Not)

Green F'129 DH5d

80 μ l

(Not)

G'95 T lig²

3 μ l

0° 4:10pm

5.0pm

42°-42.5° 90sec ✓

Plated on XGal / IPTG 1x Amp NZY 37° 5:15pm

Rest 20, 9 20, 5

40 40

(23) (11) (6) (6)

→ Processed to 100 μ l (for pipetting ease) G'95/T 1-12; G'95/C 13-24

G'95 T Xho digests

Take + 50 μ l 10x R1 buffer

435 μ l ddw

G'99 T Xho digests

+ 10 μ l (100v) Xho I Lot 007 (4v/sample)

+ 20 μ l for 1 μ l of each mini 1T-12T; 13C-24C

37° 12:25pm - 3:00pm

(Not)

Suspicious color; no blues in any of plates - too good to be true. Process 24 if no signal later

One last try before starting final step again. Pick 24

to NZY 1x Amp 37° 4:40pm There

G'99 T Minis #1 - 24

(Not)

37° 2:55pm G'99 T 1-24 Xho digestion

4:05pm

Gel G'105

(Not)



Model 377
Version 3.0
ABI100
Version 3.0

020Sm10205079
Lane 2

Signal G:225 A:265 C:249 T:241
DT (dR Set Any-Primer)
97022467 DR LR9710a Instr File
Points 1088 to 10624 Base 1: 1088

Sat, Feb 07, 1998
Fri, Feb 06, 1998

G101

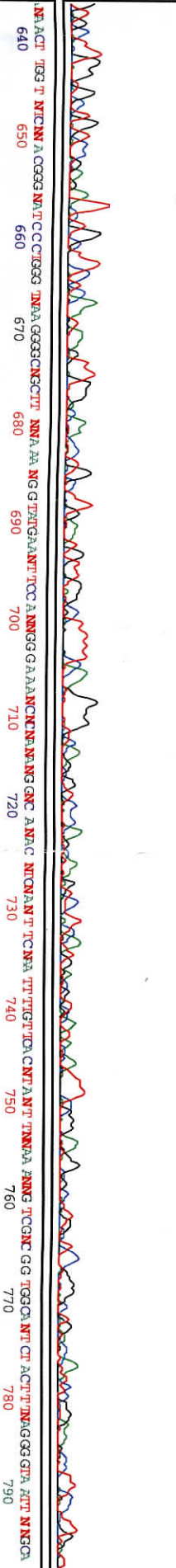
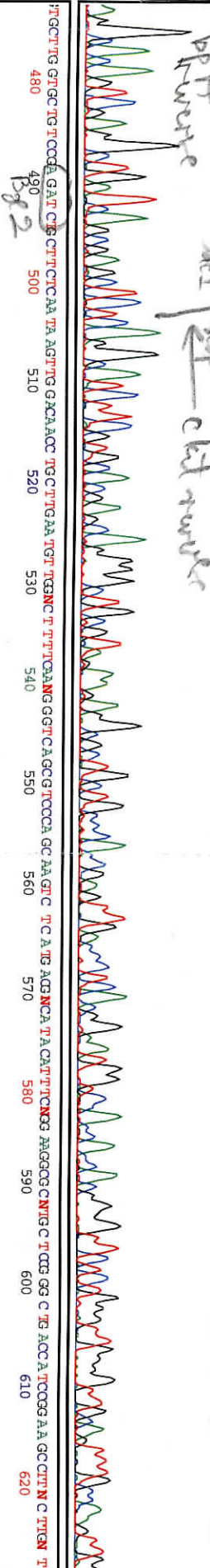
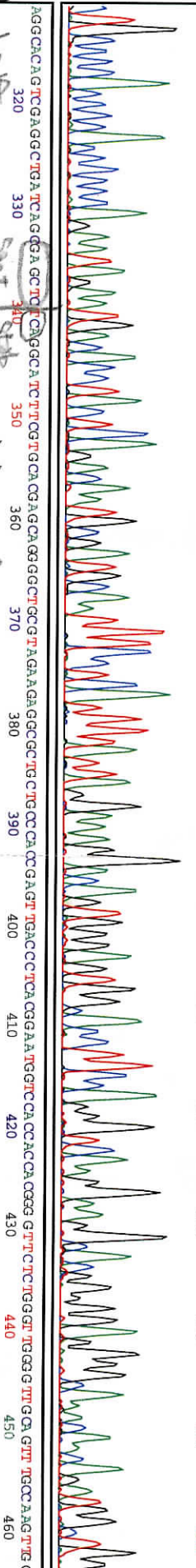
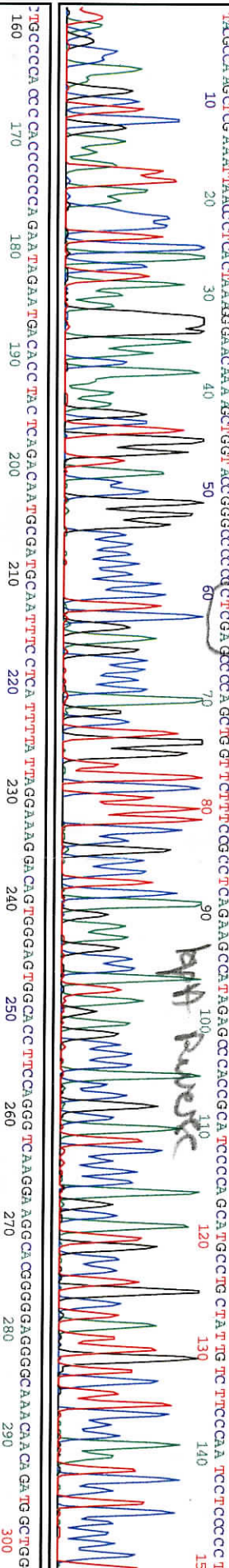


Model 377
Version 3.0
ABI100
Version 3.0

980206Da030Sm10205080
030Sm10205080
Lane 3

Signal G:102 A:95 C:131 T:88
DT (dR Set Any-Primer)
97022467 DR LR9710a Instr File
Points 1067 to 10624 Base 1: 1067

Sat, Feb 07, 1998
Fri, Feb 06, 1998
Spacing: 10



Thurs. Feb 5th

Review of final step

TARGET
TNAP kit G'57 (9) is fully checked with Xho's on both ends
→ it digests properly with Xho. Available as purified DNA.

Mini — streaked out for prep.
CONTROL
TNAP kit G'67 (17) checks out on digests — needs to be
sequenced with standard forward & reverse BS primers
kit Red — c.f. G'86 data on G'57 (9) since orientation not known
later G'78 (Sequences (G'100) check out correctly c.f. G'86)

G'101 17FI	RX #79	M13FI primer 2pm/μl G'79	5μl
		G'67 (17) mini	2μl ✓
		Water	13μl ✓
G'101 17R3	RX #80	M13R3 primer 2pm/μl G'79	✓ 5μl
		G'67 (17) mini	✓ 2μl
		Water	✓ 13μl

known
orientation
02/05/98

Hold
pending
CR1
17FI, all
that is
needed.

G'101 17Kit	G'71 kit Red primer/100	5μl
	G'67 (17) mini	2μl
	Water	13μl

Mini — re-streaked out for prep.
3' HOMOLOG-Y G'39 (22) has the correct ex. fac/ex. inv. junction
DVP. DEL → runs into TNAP ex. fac. It cut with Xho (G'82)
→ was treated with CIAP. Preparation on G'39-22
Tokano

Standard ABI mix

Usual primer prep (in 500μl 0.001M-ESTA)
shd be diluted 1/100; then take 5μl
Typical mini 1μl or 2μl
Water to 20μl

or 0.7μg
of pure DNA

Fri. Feb 6th

Recovery steps for final assembly

[Compare G'81]

✓ Take 16 pe 10 x R1 salts

+ 142 pe ddw

+ ✓ 2 pe G'39 (22) min

+ ✓ 2 pe Xho I hot 007 (100/pe)

37° 9:50^{am} to 9:50^{pm}

G'103
22Xho

15
G'103
PTE
22X

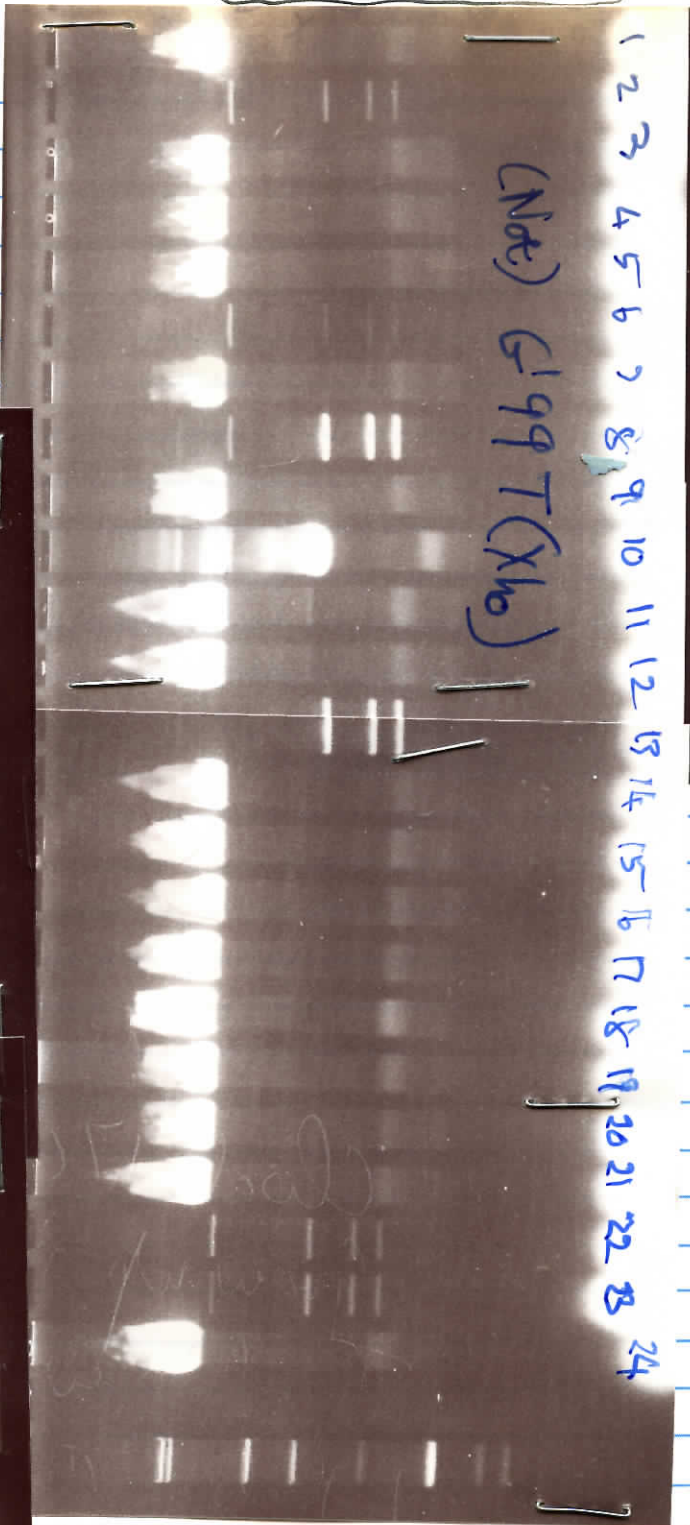
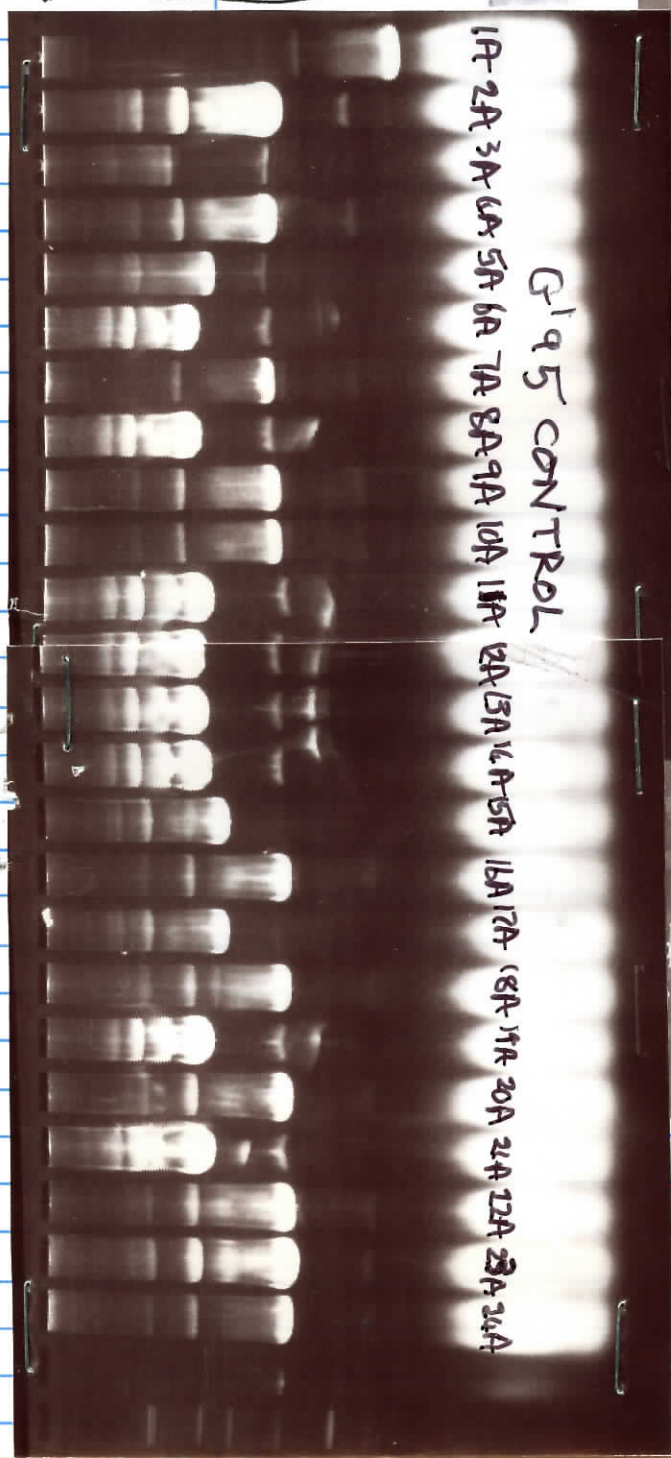
G'103
PortX

70° ≥ 15 min
Frozen pending G'99 data

Conclusion:
 None are correct. Weird
 3 out of 40 trans somewhere

(G'99T(Not) Xho)

2 (G'95 Control minus)



1A prob. BS
2A 4A 7A 9A 10A 16A 18A 20A 22A 23A 24A
prob. BS
5A 11A 15A 17A prob. BS
3A ?

April 30 pm
~~0.8% Hx4 Max~~ → to 7/3 at 4:40 pm
 Fri Feb 6 d

Dye
 w
 9T
 G'99T (1) Xho
 G'99T (2) Xho
 9T

G'99T (23) Xho
 G'99T (24) Xho
 w
 Water
 w
 Dye

G'99T
 minus Xho digests
 (Not)

G'99T
 Target

more minus Xho

Control
 G'95C

more minus

11 am
~~0.8% Hx4 Max~~

Picked 24 more from G'95C as 1A - 24A
 minus ~ 6 pm - ~ 10 am Sat.

All are Control

5C 1A
 2A
 Processed to K0Ac

G'95 Control
 minus 1A - 24A

Conclusion:

(6A) (8A) (12A) (13A) (14A) (19A) (21A)

- all look promising
 Processed to 100 pp

23A
 24A

w

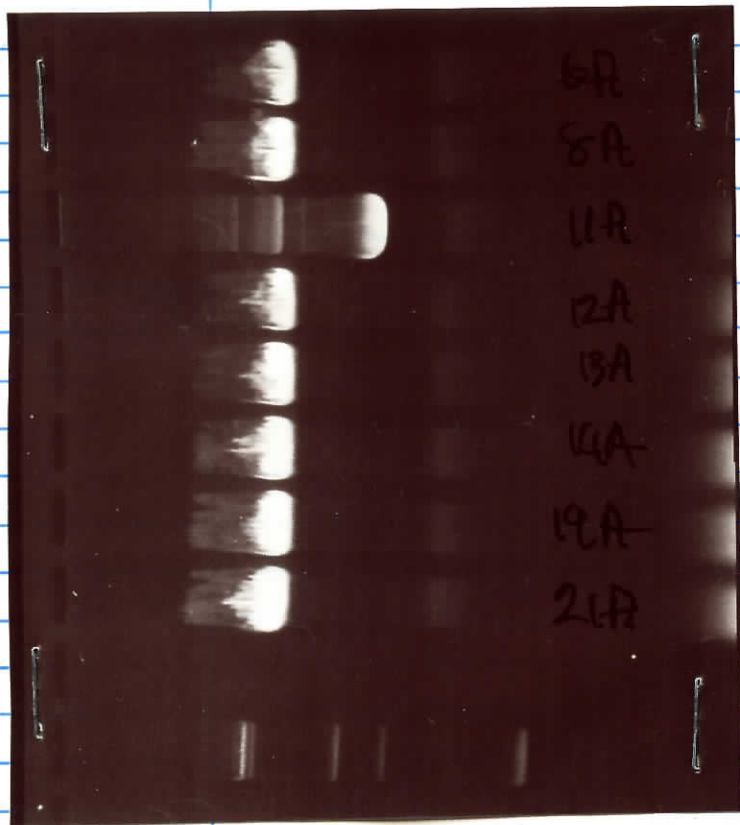
MM

w

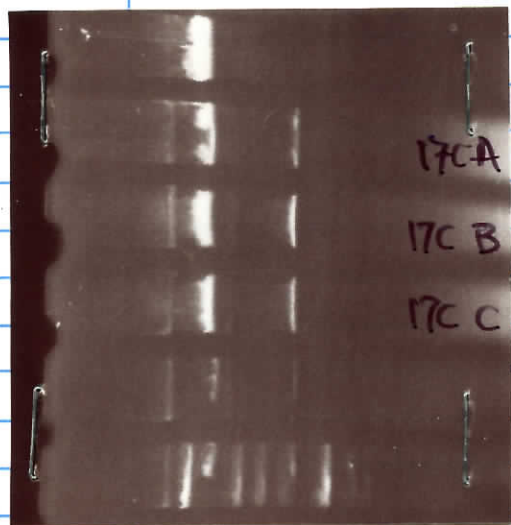
Dye

3A 24A

G95C minus (1)



Conclusion: No good. Abundant
this lot 2 pursue 17C →
fresh start



(2) Conclusion: Confirms 17C, despite
poor gel, but it's a poor grower!
Proceed to check orientation.

ambly...
Dye
GA
8A
11A
12A
13A
14A
19A
21A
W
MM
W
Dye

1:1
Dye
W
G
G
W
MM
W

11:40 AM
0.86 MAY

1

Sat. Feb 7th

Control Xho G'95C

Target Minis? G'95T
more

6A
8A
11A
12A
13A
14A
19A
21A

G'95 Control Xho

Take ✓ 18 µl 10xRI Salts

✓ 157 µl ddw

✓ 4.5 µl (45u) Xho lot 007 (10u/µl)

✓ 20 µl's + 1 µl of G'95 Minis (100 µl)

G'95C 6A 8A 11A 12A 13A 14A 19A 21A

37° 2:41 PM to 4:31 PM

Only 16 G'95T (Target) minis with XGal / IPTG were ~~all~~
tested. Picked another 12 (assuming 95C were as they appear
call that I could) 13 - 24 37° ABANDON
and another 12 from G'99T 25 - 36 3:35 PM Sat

Also 3 isolated colonies from G'95C (17C) for minis. ~7 PM

G'95C 6A / 4 Xho

Sun. Feb 8th

G'95C² 17C A

" 17C B Xho ✓ 6 µl 10xRI Salts

" 17C C ✓ 525 µl ddw

W ✓ ✓ 1.5 µl (15u) Xho I lot 004

MM ✓ 20 µl

W ✓ 1 µl

Dye

G'95C² 17CA
X

G'95C² 17CB
X

G'95C² 17CC
X

37° 11:37 AM

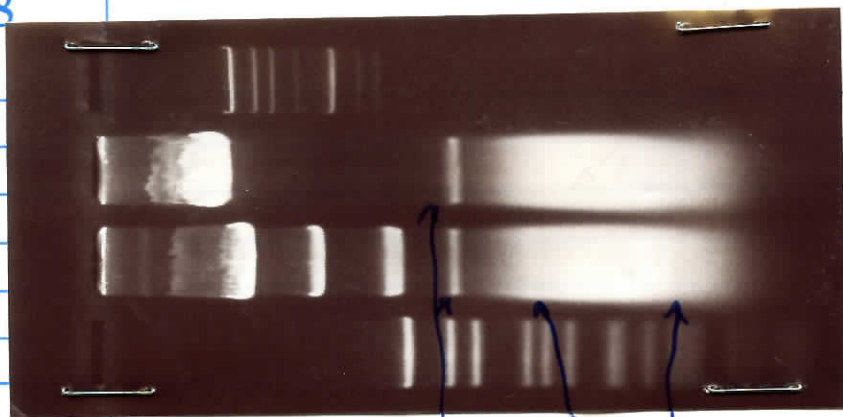
~1:30 PM

Orientation can be checked, I think, with Asc I
+ KasI (or RseI etc.)

G'107 test

of G'95C #17

5.5.2004



Not exactly sure
See G'110

①

Conclusion:

framing, but
ch' I have KAS also

1.56
(1)

0

17

17

17

17

17

17

17

1.5g Hx/
Crownase Max Error

Sun. Feb. 8th

Orientation check on 17C

①
Dye
W
17Asc
17Asc Kas
Ac MM
W
Dye

Quick & dirty:

Take
✓ 28 μ l 10xR1 salt
✓ 28 μ l H₂O
✓ 8 μ l 17C G'95C² 17C B mini
✓ 12 μ l Asc I lot 5 (100/ μ l)

37°

3:20 pm
5:20 pm

G'109
17Asc

G'109
17Asc/Kas

Asc — Kas correct orientation will give ~ 3 kb band
- - - incorrect " " ~ 1.1 kb band
Better to have included G'109 Kas!

Practically it looks good — no band at 1.1
— band at ~ 3.5

DNA is plenty. Repeat with 1% gel & RNA & Kas alone.
Can use same diagnostic with Asc + others vs. Asc & others alone.

Thurs. Feb 12th
17C (contd.)

Compare G'95 (17C) Asc, Kas, Bgl 2, Asc+Kas, Asc+Bgl 2
with G'39(22) DD3' & same enzymes.

Correct orientation will give ~~2~~ Kas-Asc of 3.2 kb
& a Bgl 2-Asc of ~ 0.45 kb
Incorrect will give Kas-Asc of ~ 1.1 kb
& a Bgl 2-Asc of ~ 4 kb

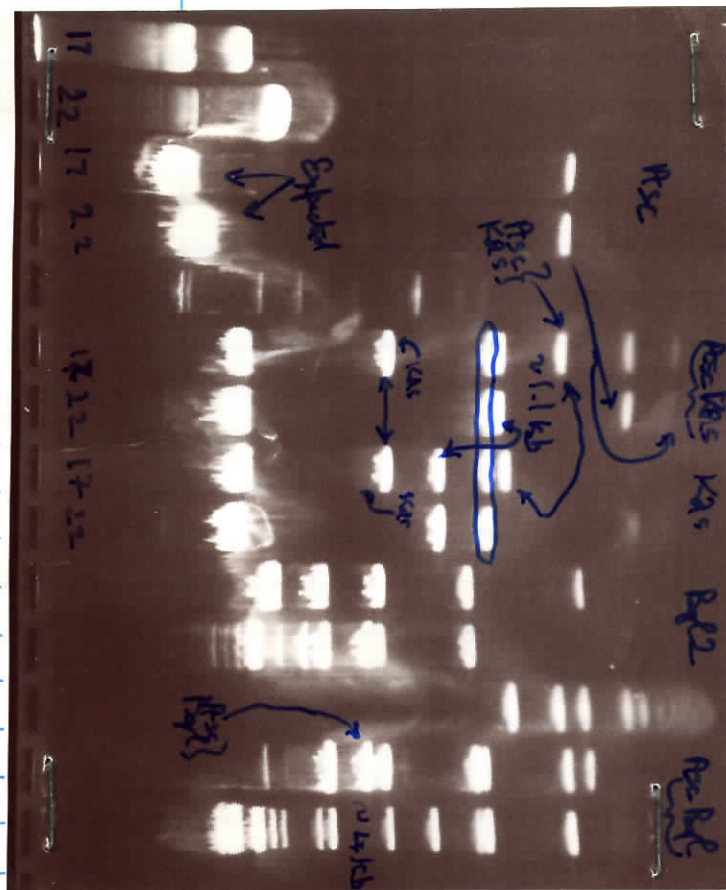
These bands will be in addition to any in DD3' which will
lose ~~or shorten~~ a band in each case.

Once confirmed, can check with PCR, or sequencing for all.

5.5.94

Oligo #
Date made:
LENGTH: 20
% G+C: 50%

Oligo Name: G111 TK 4731 (Blue)
5' T A A A G C G C A T G C T C C 3'
A G A C T
[] [] [] [] [] [] [] []



Conclusion: lot, there is a Kas site in Neo ~450bp from Asc 5' + ~770bp from Asc 3' in PD.
2nd, Asc+Kas → 1.1 kb band in 17 and no 3 kb band that wasn't there with Kas alone.

3rd Asc+Bgl → ~4kb band and no (visible) band < 0.5kb but this end of gel is poor. Looked like incorrect orientation.

Proceed with sequence check & re-make the targeting construct.

CANCELLED
Could take #17 & cut it with Xho & then cut 90% with Not, mix & ligate very conc. to get inversion candidates.

take 5 μ l 10X R1 buffer
+ 42 μ l ~~water~~ μ l μ l w
+ 1 μ l G111 (1.6 μ g)
+ 2 μ l XhoI lot 007 (60 μ l)

5 μ l
37°

45 μ l
+ 1 μ l NotI lot 47 (1.6 μ g)

Newtubet

G110
17X

G110
17XN

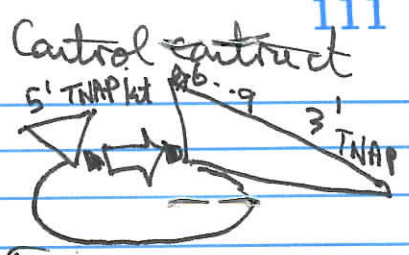
Add 40% HOQ. Mix. 2X CIAA

+ 1 μ l 8M NH₄Ac + 1 μ l EtOH

Each plus 90 μ l H₂O
+ 13 μ l NH₄Ac
+ 40% HOQ, mixed CIAA, EtOH & back into

0.8% H₂O max

Thurs. Feb 12th



Check on 17C orientation

- Pre 17
- Pre 22
- Asc 17
- Asc 22
- ACRMM
- Asc Kas 17
- Asc Kas 22
- 17 Kas
- 22 Kas
- Bgl 17
- Bgl 22
- ACRMM
- Asc Bgl 17
- Asc Bgl 22
- W

Will need to confirm 5' orientation on any candidates in the group B site(s) of Dup Del.
A single sequence will do it for less labor than the mapping. ∴ design a primer from PGK promoter region

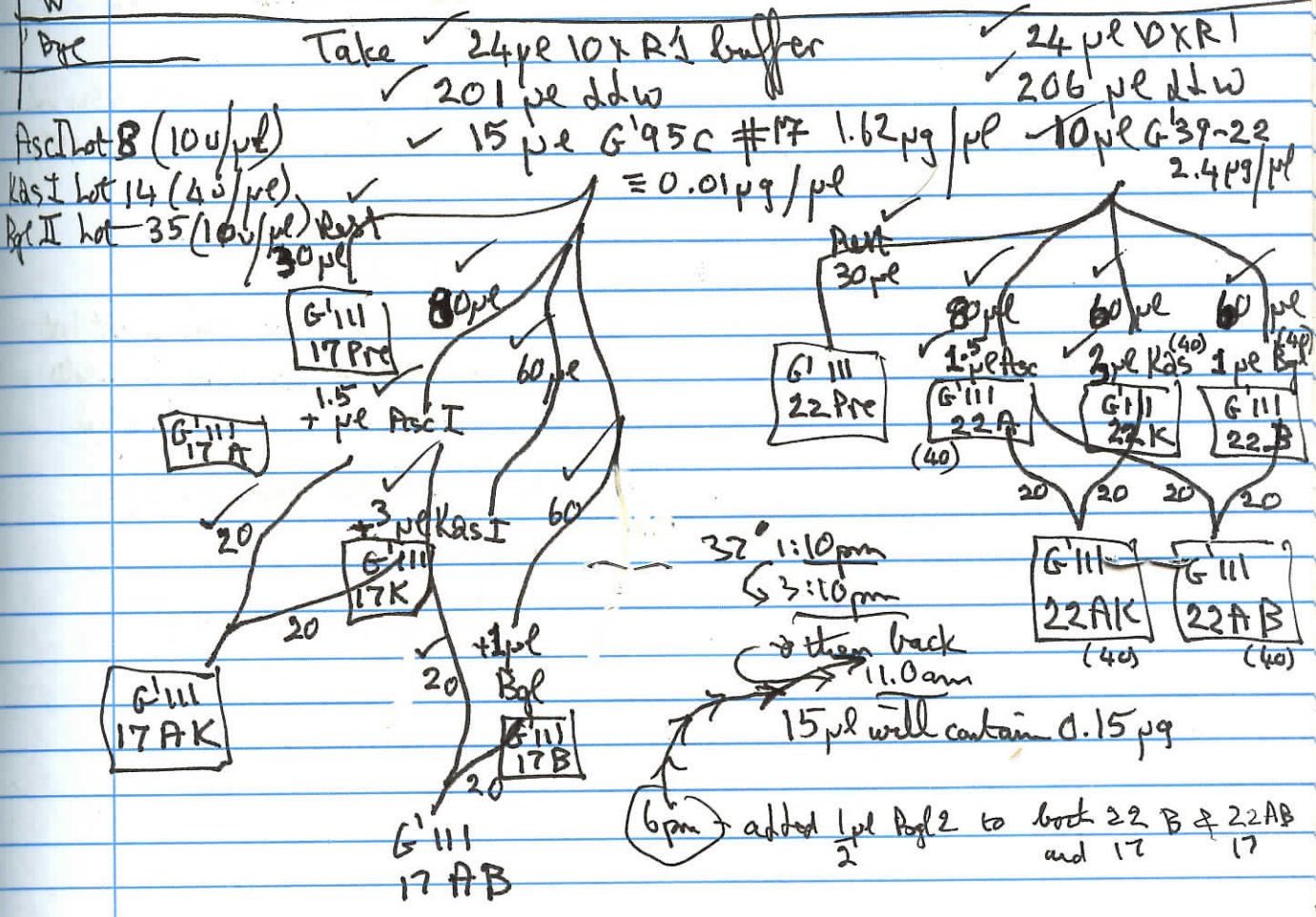
red re. TK
blue re. DB

4731 4750

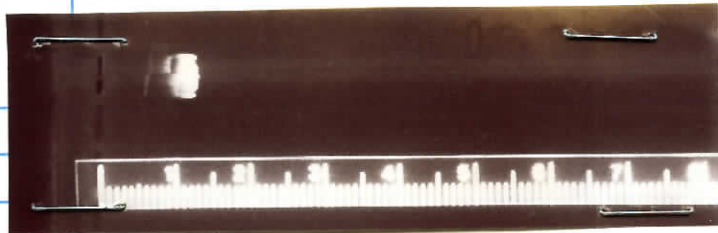
ITRAAGCGCATGCTCCAGACT

20, 50% GC, T_m 60°

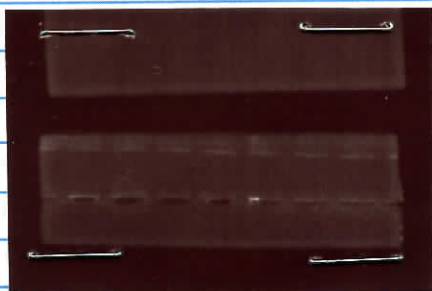
G'III TK 4731



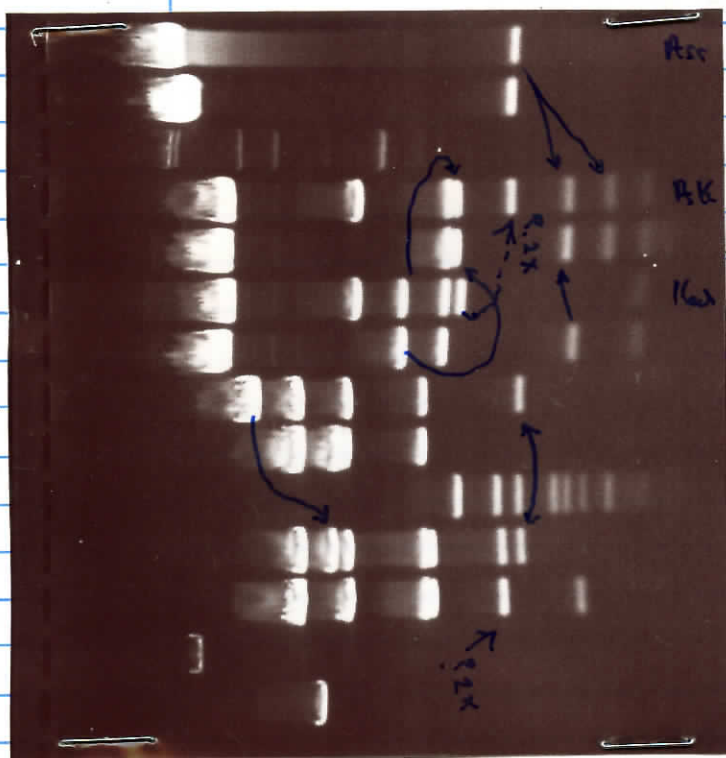
3 B cells



15mm
5mm



① Conclusion: looks good (only slow blue left angel / + warm).
Cut is good.



② Conclusion:

Asc Kas Extra at 1.1 kb ~~and~~
with loss of 1 at ~800. Nothing at
~~1.1 kb~~ in Kas ~~at~~ 3.2 kb.

(17)

Asc Bgl I extra at 1 kb & at ~4.5 kb with
one loss from 650 bp. Nothing at 450 bp.

Still looks to be wrong
orientation.

Best check for mini first test
is probably Asc + Kas in R1 salts
with 0.8% Ag H₂O + RNase gel.

← DD3' Xho purified (5 pL)

← TNAPK2 Xho Conc (15 pL)

overnight
Samples from G111
5 pL
15 pL
A
K
R
A
T

113

Xho Xho Xho $TNAp$ Ret

Neo

3' homolog

~~M 6:40 pm 1.5 V/cm
 8:19.40 am~~

①

Purification & ligation
for Target construct

Bye
Walet

0.8%

Leaf

$$-H \times L$$
w

Dy

$\sim 9 \text{ slots } G'_{103}$

22Xho (after heating)

Recovered into 150 μ l as

$G^1 113 \text{ DD} 3' X_{ho}$ (see $G^1 123$)

Review of available X_{ho} TNAP kit X_{ho} fragments

G-85	TNAP kit	Xho	TARGET conc
------	----------	-----	-------------

G'95 T Not } used but never proven successful.
F'95 C Not }

G'95 C Not

B'73² TNAP kit Xho TARGET (Rest of sample used for -)

This is good but not checked by u.p.

Asc 17

Acc 22

Aug 11, 1911

Acc 148 17

Ver. 16229

KAS 17

Kas 22

Page 17

Bgl 22

ACRMM

Asc BqC 17

Asc Bgl 22

G'113 DD3' Xha

G'85 TNAP Kit Xho TARGET Conc

h

Dy

Some pDR

G'113 hign T

Take 15 μ l G'113 DD3' Xho

✓ 15 µl GIBCO TNAP kit Xho TARGET

✓ 3 pl 8M NH_4Ac

✓ 82.5% EtOH

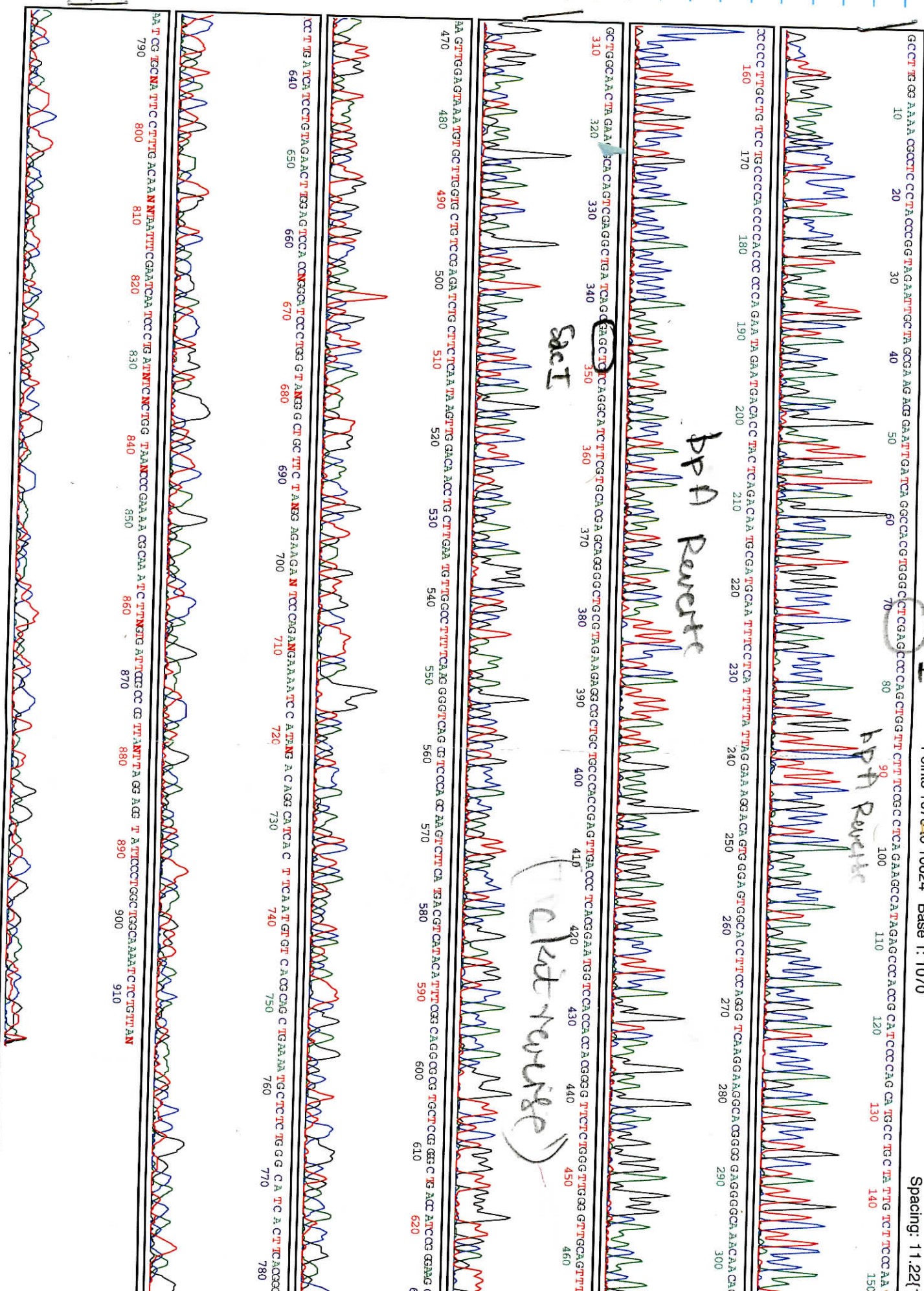
Freeze. Kant. Dry. Back into

TNARIC: 6pe 1x p95

3' $\rightarrow$ 5' T4 ligase

Cell room 16pm Fri 14 Nov RT
at 3pm 374

3 5000



115

Sequencing G'95C #17 1.62 pg/pl

Take $\rightarrow 5 \mu\text{l} \checkmark \equiv 25 \text{ ng}$

for 200 ng of G195C #17, dilute 1.62 pg/μl stock
in 15 μl ✓ 9.9 μl + 97 μl 0.001M

as G'95C#17/700/15pe

$\hookrightarrow 15 \mu\text{e} \checkmark \approx 700 \text{mg}$

Shows that orientation is, as expected, incorrect.

$$\frac{02}{13} / 98$$

Repeating more of 6'87C high.

G'19	DH5 α	80 μ l
G'19	87C lig ⁺	3 μ l
0 $^{\circ}$ C	3:32	4:42
42 $^{\circ}$ C		90 sec

42°C 9088e ✓
Plated as G' 87C / 115 1xN2Y XGal / IPTG Fri
Rest 20², 20, (5, 20/16, 5/16) 37° 4:50 pm

24 mins as G' 115 / 5# 1XAMP NZY whites 37° 4:30 pm — 110 am
to cold for hold

3500

2/16/98

Oligo #

8163

Oligo Name: G'117 TK Blue
5' T T C C A G G C A T C A A A C 3'
T T C A G G
T C A G G
50%

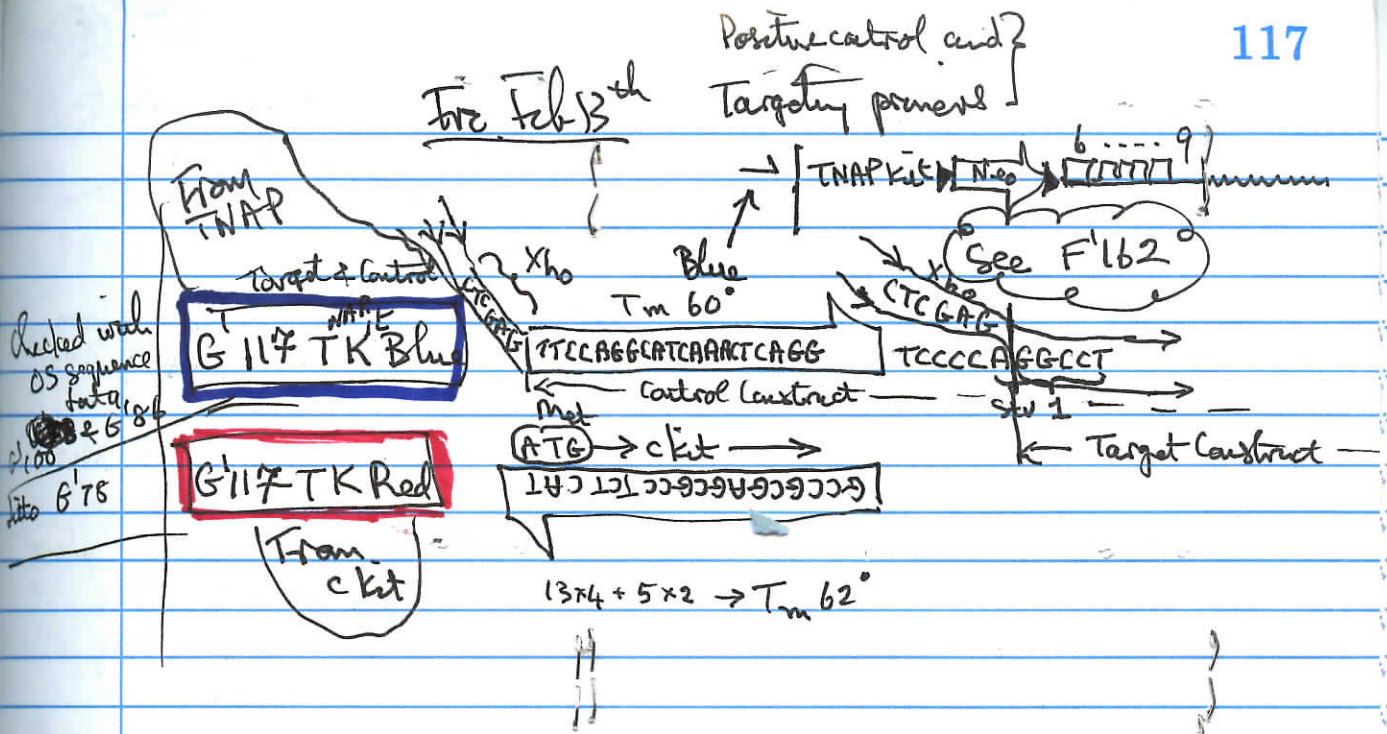
WTO 500pe 0.001M EDTA

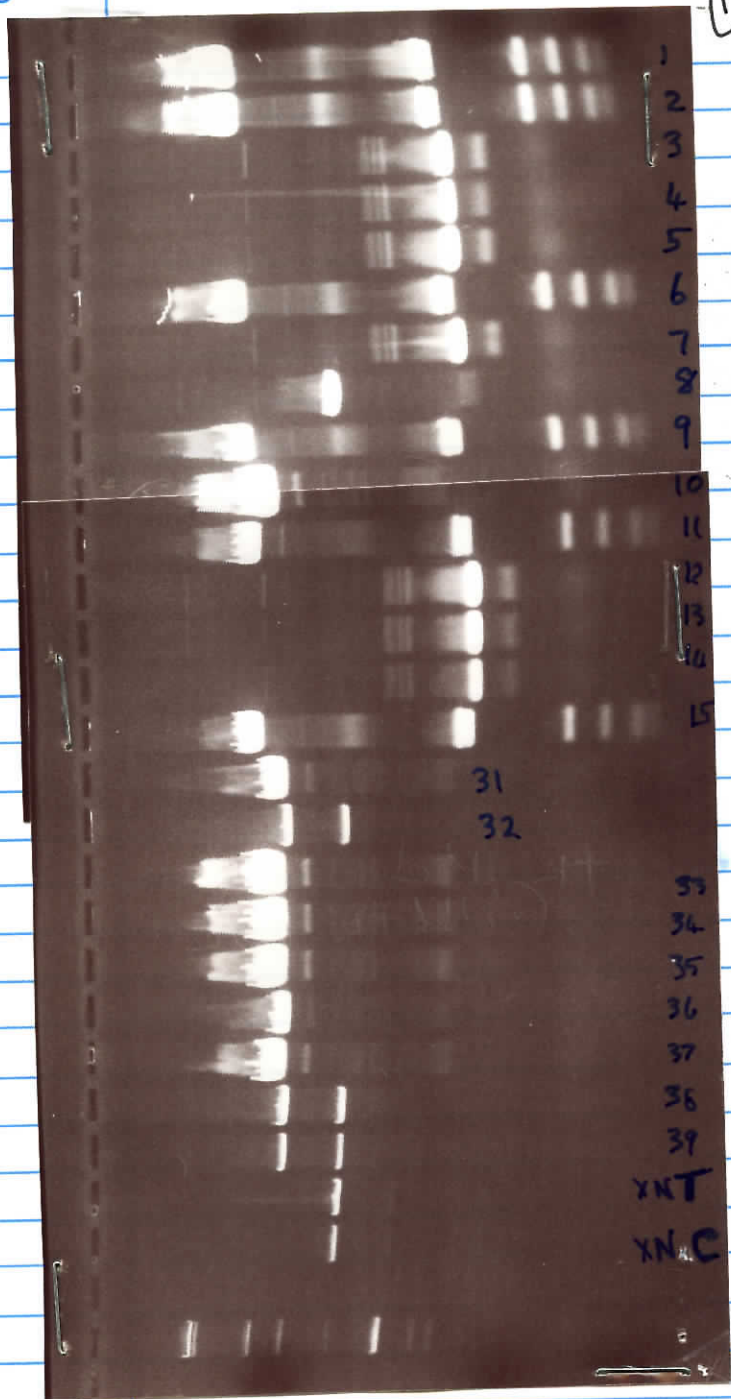
1XCIAA

8164

Oligo Name: G'117 TK Red
5' G C C G C G A G C G C C T C T 3'
G C C G C G A G C G C C T C T
C A T
18%
72%

later comment NM think --GG is poor
but --CAT will be okay.





① conclusion: No correct;

recovered DD3' 6x
Blue Script(?) 7x ← from
G119

None include DD3'

TNAPc kit BS 6x ← from
which cuts 1x with base G195C
with Pse 3x

Remake Xho DD3' Xho
with CIAAP & gel
purification. Combine
with today's XNT &
XNC conc & try,
try again

later confirmed

① 3:50
on 3:50

Page

1

2

14

15

31

32

38

39

13 G112

13 G112

AG

stap-
forms-
t;
on
119
from
G'95C
cho
el
gubine

3:50 pm
5:30 pm
G'119 Hx4 2pl
RNase max

Sat. Feb 14 d N.M. day!

TARGET
TNARKE



Age
1 }
2 } G'119
14 }
15 }

G'113 lig

Transformation

G'119 Yell H5 d 80 μ l ✓
G'113 lig 2 μ l ✓

31 }
32 } G'95C
38 }
39 }

4:40 pm 5:15 pm
90 sec 42°
plated XGal/1PTG

37° 5:25 pm

G'121 XNT conc
G'121 XNC conc
W
AGMM

~ 20 colonies
total (all look white)

~ 11 am Cold 1 am

Final Total 2

G'119 Minis 1-15
To 100 μ l

1xAMP NZY ~ 3 or 4 maybe blue.
37° 5:50 pm Sun

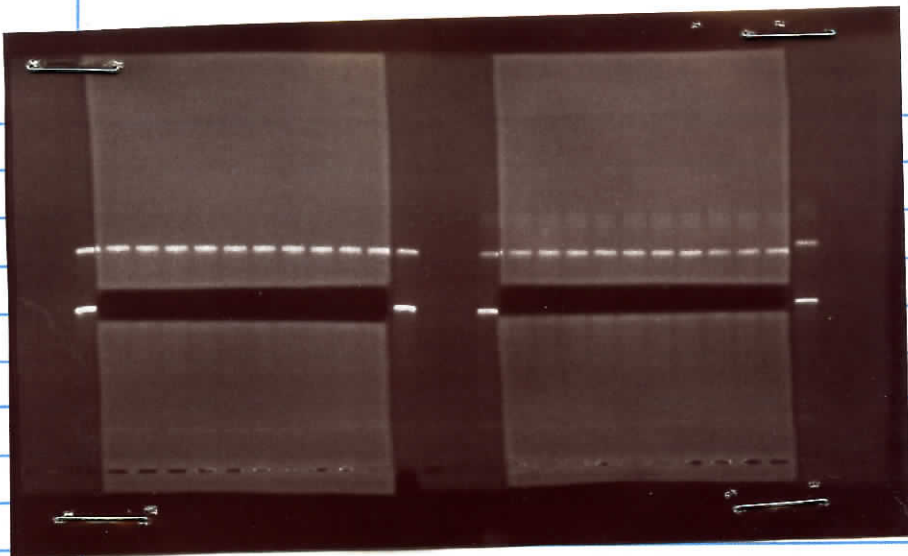
Process of G'115 C at same time using
label G'95C (30) - (39), check with Asc + Kas.

Take 50 μ l 10x R1 buffer

This was
an error
- but didn't
matter
(total 550
not 500)

✓ 462 μ l ddw
12.5 μ l Asc I lot 8 (100/ μ l) (50/Rx)
25 μ l Kas I lot 14 (40/ μ l) (40/Rx)

20 μ l
1 μ l G'119 minis 1-15 → G'95C minis 31-39
37° 11:35 am to 3:25 pm



① Conclusion:
Good cuts, into
100 μ l as
G'121 XNT conc
G'121 XNC conc

Sun Feb 15th

Remake of the
fragments

¹²¹
K_{ho} TNAP_{kit} K_{ho}

Target & Control TNAP_{kit} again

Take	Blue Target	Black Control
✓	40 μ l 10x R1 salts	
✓	354 μ l ddw	
✓	3 μ l K _{ho} I hot 007 (100/ μ l)	
✓	3 μ l Not I hot 47 (100/ μ l)	New tube

200 μ l
G'57 #9 [T] 1.2 μ g/ μ l
G'73

200 μ l ✓
G'67 (17) 1.86 μ g/ μ l
[C]

(2 μ g total)
+1.7 μ l ✓
G'121
~~XXXXXXXXXX~~
XNT Conc

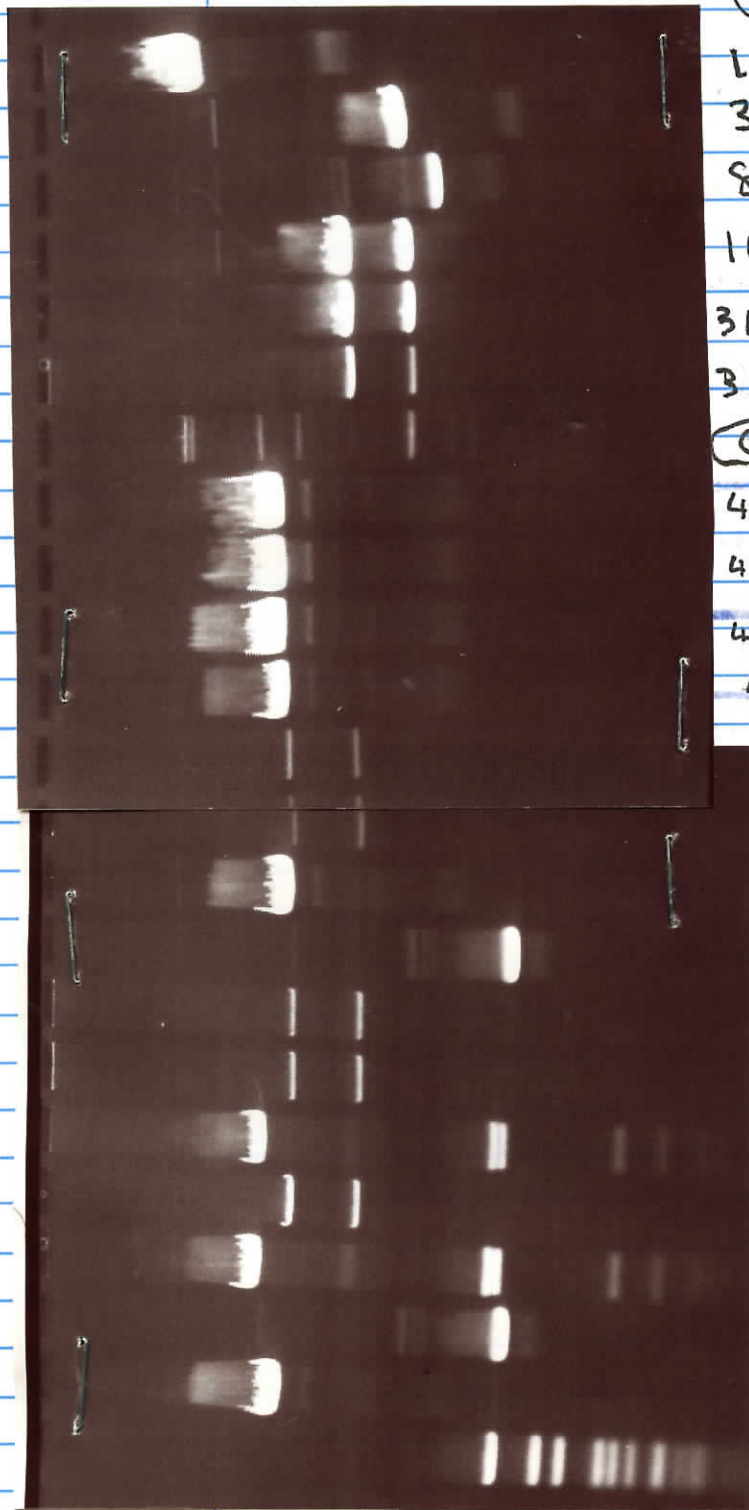
+1.1 μ l
G'121
XNC Conc
(2 μ g total)

37° 1.33 pm to 5.05 pm
70° 5:10 pm to 5:20 pm
Proceeded to 100 μ l as

① 6:10 pm
On 10 am
0.8% H₂O₂
IV

Dye	
Water	
XNT	Whole sample ~ 12 slots
Dye	Seaf.
Dye	
XNC	~ Whole sample ~ 12 slots
W	
Dye	

3 5:44



① Conclusions

- 1 DD3 with no insert
3 Blue Script
8 Does not appear to cut with Xho

10 } These are
31 } all TNAPket
in BlueScript

32 $\frac{1}{2}$ looks the same but ~~different~~
~~different~~ ~~different~~ ~~different~~
G 95C Asc + Kac of opposite orientation

40

41

42

4

10

10

10

1

1



10

100

1

• TNAP ckt
BS
2 orientahan!

TNAcket
BS
2 orientati a 2

DD3'

No cut

But again, no ~~all~~ complete products

①

54

2

10



③

14

(10)

(31)

32

1

159

40

41

57

27

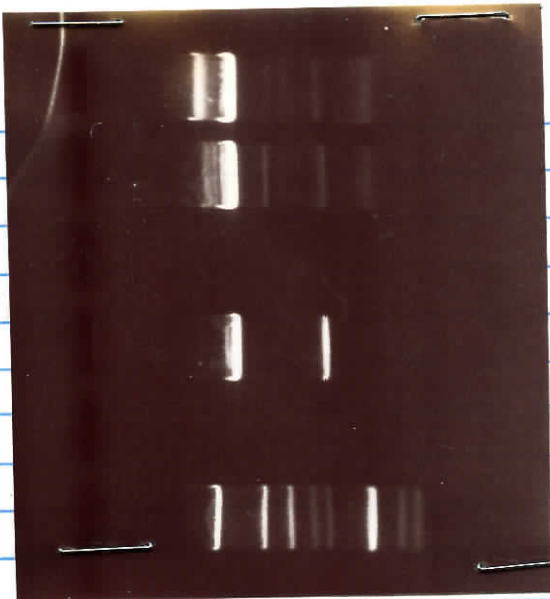
54

Hto

6

A

124



Conclusion:
Plenty of DD3' CIAP
still left, signs of p₁ appear
no no X₁ TNAP but X₁ left
Proceed w/ transfor

Xho on G'127² is
complete.

See G'128

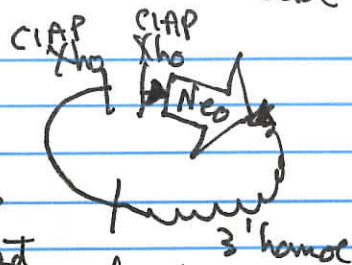
G'125
T1 2.16g/pe
SH 213

CIAP
Flyover
left
uniform

Tues. Feb. 17th

X TNAP kit X Target Control (with Not treatment) 125

Fresh start with fully purified fragments



The new TNAP kit Xho frags are about as conc! as last time (compare G'112 gel with G'118 gel)

11:40 AM
0.5 HX
W
G'125 T Hig
G'125 C Hig
W

Take V45 μ l G'121 XNT conc
✓ + 45 μ l G'113 DD3' Xho CIAP
✓ + 9 μ l 8M NH₄ Ac
✓ + 248 μ l EtOH

G'125
THIG^N

Blue

Freeze. Cent. Dry. Back up into 10 μ l 1x p95 buffer.
Briefly heated to 50°C. Cool. 1 μ l T4 ligase lot 067
Cold room 50-53°C to

AG MM
W
W
W

Take 45 μ l G'121 XNC conc
✓ + 45 μ l G'113 DD3' Xho CIAP
✓ + 9 μ l 8M NH₄ Ac
✓ + 248 μ l EtOH

G'125
C HG^N

Black

Freeze. Cent. Dry. Back up into 10 μ l 1x p95 buffer.
Briefly heated to 50°C. Cool. 1 μ l T4 ligase lot 067
Cold room 3:15 PM to 10:15 15°C to 1345 37°C 1:15 PM to cold

x3 μ l + 12
G'127²
at 30 min.

Transformation

OS mix
(HK cells)

DH5 α

G'125 Hig^N

00 3:25 PM to 5:35 PM

42 $\pm$ 10 290 sec

Plated Rest, 20/5, 20/16, 5/16 on 1x Amp NZY (PTG/XG2)
37 $\pm$ 10 min to 1:30 PM
(5 T was 20/16) 2x T 20/16 to cold

G'125 T
Blue

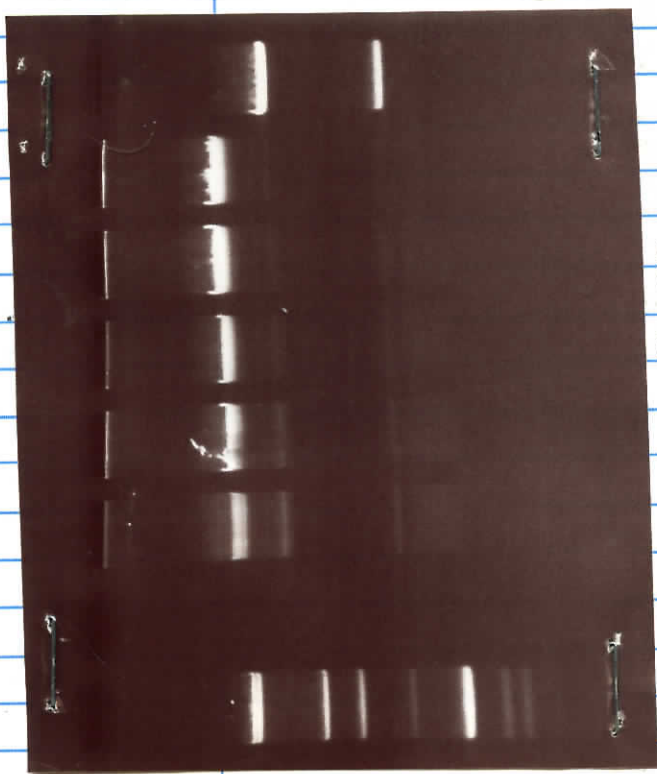
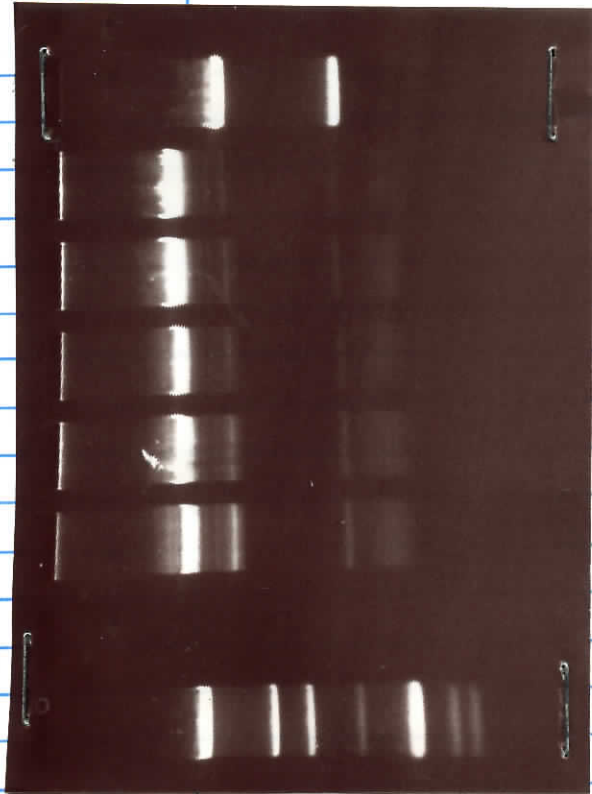
G'125 C
Black

80 μ l
3 μ l T

80 μ l
3 μ l C

2:30 PM Th. picked 24 min G'125 T - no blue visible
to 1x Amp NZY 37 $\pm$ 3 PM
small. G'125 C ditto 4:20 PM First bar 50 off 9 AM to cold

3:50 PM



①

Conclusion: Very successful
expt. - clear effects
of conc. Transform
with 1X and 1/8X

1 μ l 8 μ l

Possible that staining
intensity indicates
that 1/8 is circles, while
1 is linear

See photo of result
on G'129

All products were
D3, I can't beat
self ligation always!

lighter
photograph

①

11:16a
0.80 4x4
WSS

Dx
W
C/8 (1/2)
2X
1X
1/2X
1/4X
1/8X
W
AGMM
W
Dye

G'127
20
G'1
17

12:
3
to c
pend
1K00H/
2X dff

6/1x
T
Wed
Thurs

86 HX4
W2X

1/8 (1x)

2X
X
1/2 X
1/4 X
1/8 X
GMM

eye

G'127 ligational

20pg
G'127 2
17Xho 40
340
12.4
8
12:45 pm
3:10 pm
to cold,
pending
100% H2O
2X DNA

Tues Feb 17th
Investing G'95C #17

Control

127

Since competition is
solely conc. dependent
not ratio

Crazy thought: Cut with Xho → re-ligate at ~~high~~ conc.
→ just test the ligation. Check ligation first.

Take 10 μl 10X RI 200
85 μl H₂O

G'127 0.05 pg/μl
17Xho

3.1 μl G'95C #17 1.62 pg/μl (5 pg total)
2 μl (200) Xho hot 007 (10 μl/μl)
37° 6:50 pm 10:15 am

+ 10 μl 5M NH₄Ac 80° ~ 10.2 to ~ 10.35 (10 min)
+ 275 μl EtOH, Freeze. Cent. Dry. Back into 25 μl 1x p95
Heat 50°C briefly.

No ves. DNA.

4.5 μl aliquots

so set up repeat

Required 200 μl 1x p95

61x p95 + 0 + 4.5
T4 pol. 2x 1
ligate cold room accurate
1 μl + 1/4
G'127 2X
G'127 1
G'127 1/2
G'127 1/4
G'127 1/8
G'127 1/16

(~ 0.9 pg / 1x)

Requires 12 μl T4
Cold, Frigor
Freezer

→ then decide
which to use
for transformation

Transformation

	G'127 1x	G'127 1/8x
(G'125) DH5d	80 μl	80 μl
high	1 μl	8 μl
0° 1:20 pm to 2:05 pm		

Plated 1x Amp NZY Ret 20, 5, 20/16, 5/16 37° 2:30 pm

Two to three times
more colonies with 1/8 than 1

G'127 Minis #1-12 (1x) #13-24 (1/8x) 37° 3 pm

3 small

TARGET

①

Conclusion: Presumed correct: (1)(3)(15)

TARGET

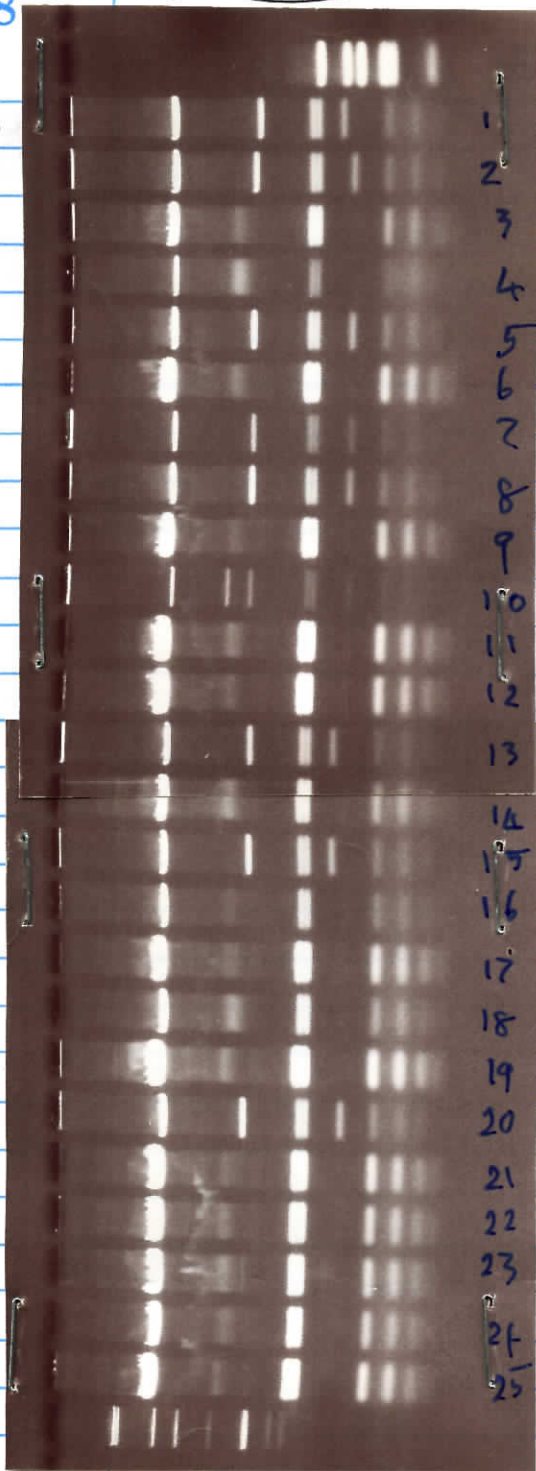
Presumed reverse: (2)(5)(7)(8)(20)

All others are DP3', except 10 which may have two inserts.

② All are DP3' - so concn. does not help with DH5 α . Try a RecA⁺ strain.

③ Conclusion: Gel on G'130: (13C) & (23C) are correct

(9) may have two inserts.



ON 6:15
 1
 2
 27
 2
 2
 2
 F
 5
 N5
 N5
 N5
 RNA
 N5 13G
 1C
 2C
 23C
 24C
 N5 5G
 16
 2

6:15 pm
 0.8% HX4
 + RNase Max
 1/20
 ~3V/cm
 Fri. Feb. 20th

Sun Feb 22nd 129
 NM ← day

- 1 PCR M
- 2
- 23 G'125T
- 24
- 25
- AG MM
- W
- Dye
- 5:12 pm
- 5:16 pm
- 0.8% HX4 RNase Max
- PCR M
- 1 G'125T
- 2
- 23 G'127C
- 24
- 25 G'125T
- AG MM
- 8 HX4
- 8 RNase Max
- 13 G'125T
- 1C
- 2C
- 23C
- 24C
- 5 G'125T
- AG MM
- Dye

Tests of G'125T ligation and G'125C

Processed (G'125) 1T to 25T to 100 μ l 0.001M EDTA

- ✓ Take 55 μ l 10x R1 buffer
- ✓ 454 μ l ddw
- ✓ 13.8 μ l Asc I Lot 8 (100/ μ l) 50/Rx
- ✓ + 27.5 μ l K25 I Lot 14 (40/ μ l) 40/Rx

20 μ l - - -
 + 1 μ l minis G'125 1T to 25T 37° 2:15 pm to 5:35 pm

Test of G'127C ligation

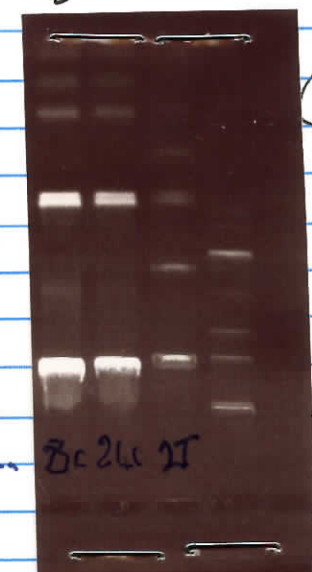
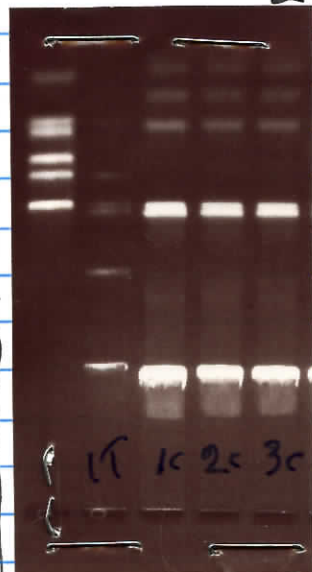
Processed G'127C 1...12, 13/8 ... 24/8 to 100 μ l

- ✓ Take 50 μ l 10x R1 buffer
- ✓ 413 μ l ddw
- ✓ + 12.5 μ l Asc I Lot 8 (100/ μ l) 50/Rx
- ✓ + 25 μ l K25 I Lot 14 (40/ μ l) 40/Rx

20 μ l - - -
 + 1 μ l minis G'127C 1...12, 13/8 ... 24/8 37° 12:30 pm

Test of G'125C ligation
 10-24C
 37° 13:22
 Sun 1 pm
 3:55 pm

② Conclusion:
 Didn't help! All
 are DP3! Try RecA⁺
 Process G'125C minis



G'127
 Inversion
 See G'126-7

3 bands

Control

← Marked clipped
in photo

BT

(13T)

1C

2C

3C

4C

5C

6C

7C

8C

9C ←

10

11C

12C

13C ←

14C

15C

16C

17C

18C

19C

20C

21C

22C

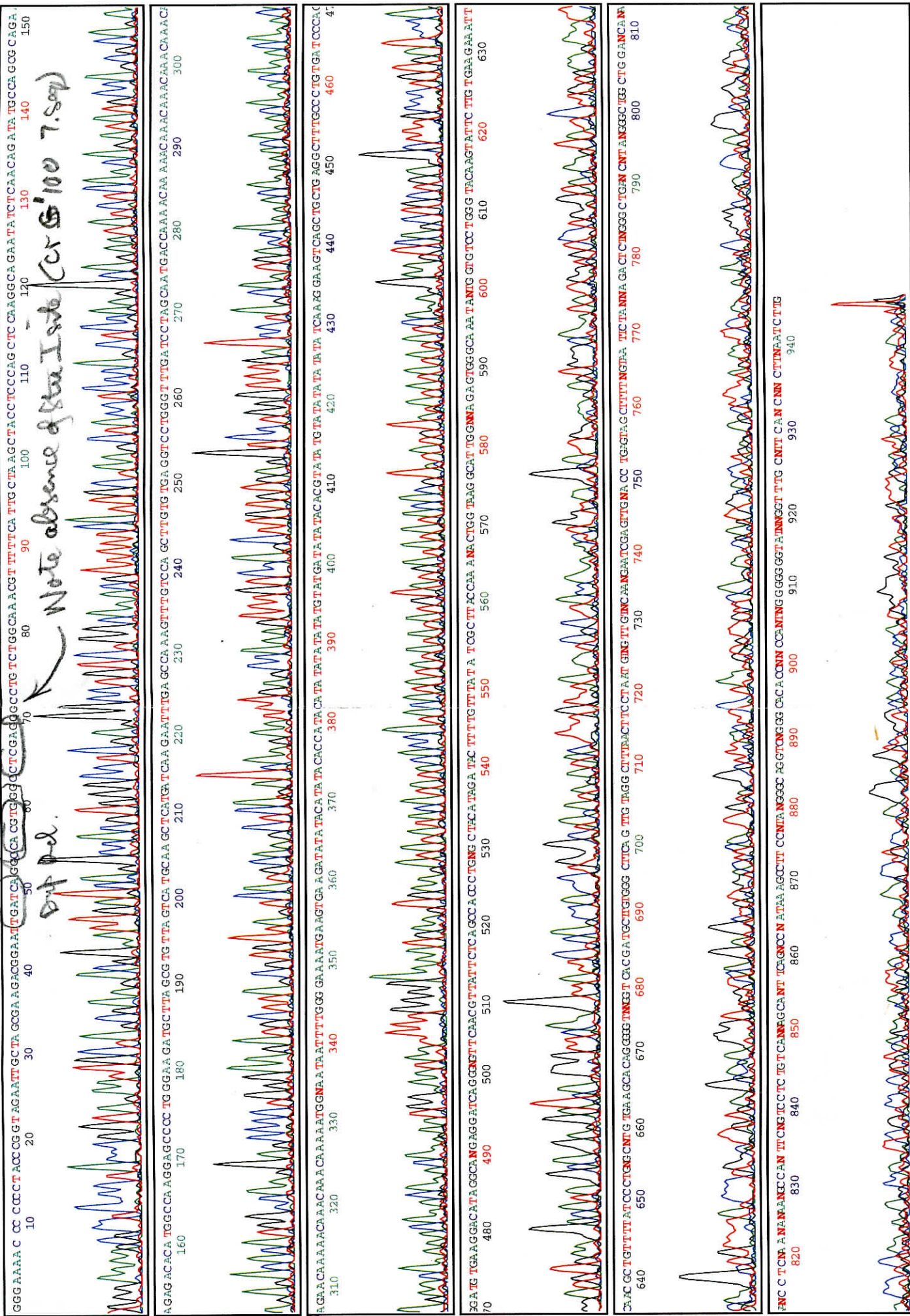
23C

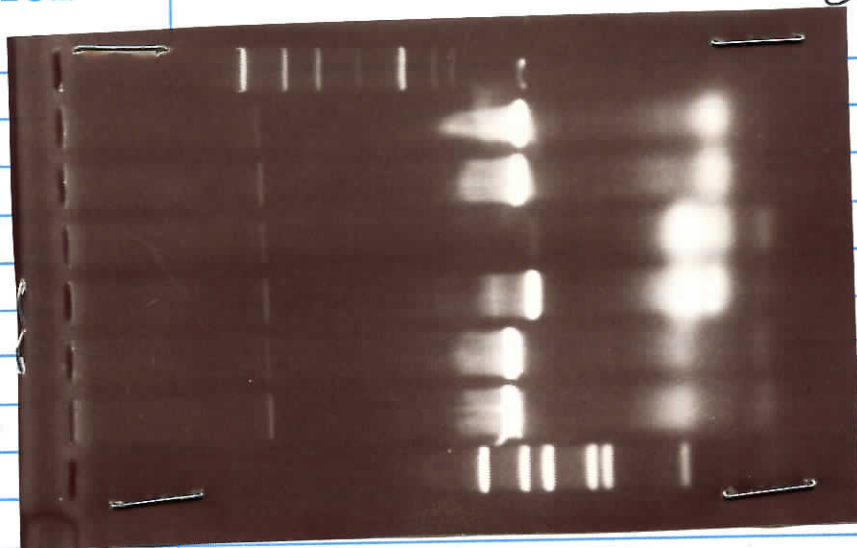
24C

5T

(5T)

← CONTROL





①

Conclusion:

All 4 universal
work with 1 slightly
shorter than
control 23. And
Target 1 does
not work
with diagnostic
pair of
primers:

G'117 Blue + Red

Case all agree

Good news!

Sequence in this region
is also OK. (see G'134)

TWAP KIT primer mix

G-132

Use Ipe / Rx

150 μ l G'117 TK Blue
+ 150 μ l G'117 TK Red

①	822 pm
light	1.59 Hz
1	1V
Universal	23V
Target	1C
Longest	23C
Universal	1LV
Red	23LV
Free	ACB
	W
	24

1.5% H₂O₂
10V

4 months from conception
3 months from start
Mar. Feb 23rd

Re-name TK 4731 133
tk 4731

Agarose
W
PCR MM
1U (T)
23U (C)
1C (T)
23C (C)
1LU (T)
23LU (C)
PCR MM
W
Agarose

PCR tests on G'125 (T1) and G'125 (23C)

Universal
Target
Control

T1 sh'd both be +ve with G'111 ^{primase kinase} tk 4731 ^{Blue} primer
and G'117 TK Red

T1 sh'd be -ve with G'117 TK Blue
and G'117 TK Red

23C sh'd be +ve with G'117 TK Blue
and G'117 TK Red

At a later time can set up a tail DNA short PCR
for genotyping and for ~~control~~ control +ves after
Cre treatment etc. by using a Tissue Non-specific
Alkaline Phosphatase ^{Blue} primer from within ~~entron~~ 2.

longer
universal

Could ~~use~~ use G'71 c kit Red in place of G'117 TK Red
- best with tk 4731

Since there are all minis, do some sample tests first.

Mini DNA
all
diluted
1+20 in 0.001M EDTA

		n=16		n=1		On 12:30pm	
G'		10xPCR	20 µl ✓	1.25		35 cycles	
G'133		dNTPs	290 µl ✓	18 µl		90° 1 min.	
G'133		DMSO	1.6 µl ✓	0.1		40 7 sec.	
Tag		40 µl ✓	2.5			60 10 min.	
		16 µl (365 n=16)	1			23 (n=1)	
(n=15)		5/16					
Blue G'111 primer		115 µl	2.5 µl ✓	115 µl	2.5 µl ✓	(345 n=15)	
Red G'117 primer		2.5 µl ✓	2.5 µl ✓	2.5 µl ✓	(n=5)	0.5	
Blue G'117 primer		2.5 µl ✓	2.5 µl ✓	2.5 µl ✓	(n=5)	0.5	
(n=12)		48		48			
Mini DNA		G'125 (T1)	2 µl ✓	G'125 (23C)	2 µl ✓	G'71 c kit Red	
		50 µl	50 µl	50 µl	50 µl	(n=12)	
(n=12)		1U	23U	1C	23C	1LU	23LU

5 5 5 5 5

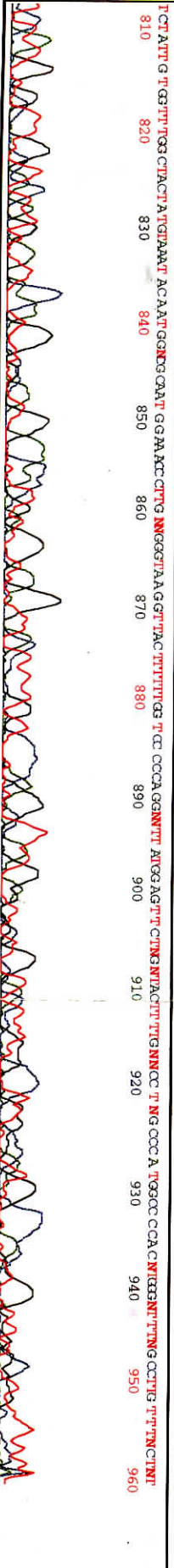
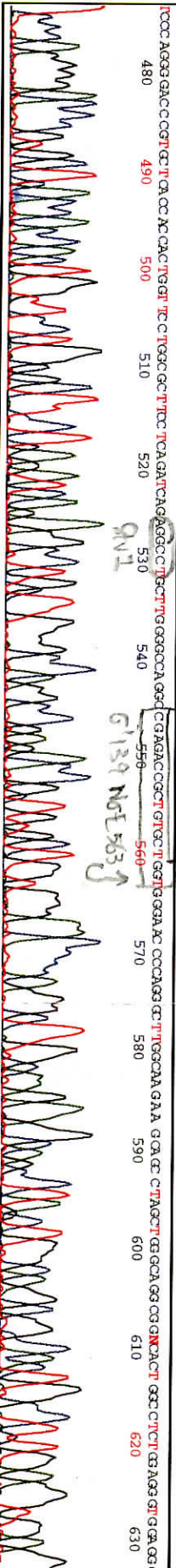
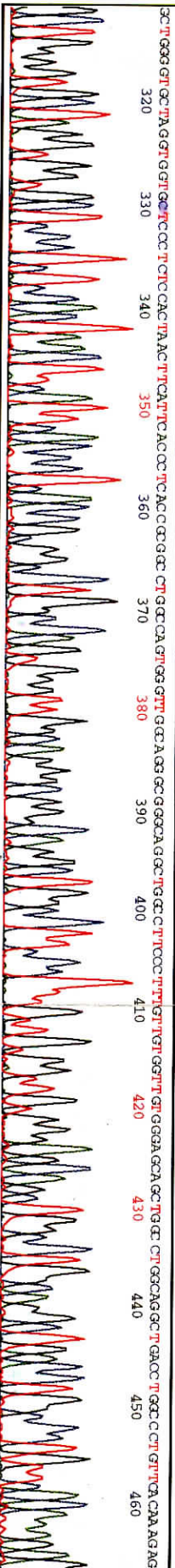
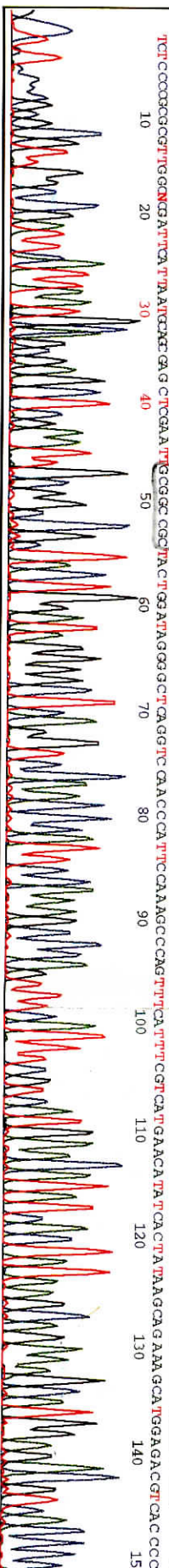
2305mi0225581
Lane 23 Not

Points 1081 to 10624 Base 1: 1081

Pa

Sat, Feb 28, 1998 0
Fri, Feb 27, 1998 0

Spacing: 11.0



Tues. Feb 24th

59
1 pl ✓
14 pl ✓
5 pr

Construct check

Primer sequence check

Works well

Don't work

OK

OK

G'39 #22 for DD3'

OK

G'125 T(1) for TNAP intron

One more sequencing check

→ universal primers for both ends of both inserts

Equivalent of tk 4731 ^{Blue} for group A inserts ~~Blue~~ would be:-

This is (like tk 4731) a universal primer ^{for group B}

HHHGGGHHHGGGHHHGGGHHHGGGHHH

Vector 39 10.8eq 58

G'135
V58 Red

Km ≈ 60°

for group A 3' end

Other ~~two~~ primers would be

~~HHHGGGHHHGGGHHHGGGHHHGGGHHH~~

This is a universal primer

HHHGGGHHHGGGHHHGGGHHHGGGHHH

XOY: 0333 04X 031

Error here! 4895

4874

This is a universal primer

GCT TTA CGG TAT CGC CGC T

(with corrected AUPDEL can use F'103 Blue E)

5895

5913

This is a universal primer

TAA AGC GCA TGC TCC AGA CT

4731

4750

G'135
5' Neo Red

Km ≈ 62

for group B 3' end

F'155
Neo Blue

for group A 5' end

G'111
tk 4731 Blue

9.8eq

for group B 5' end

Oligo Name: V58 Red 3'

AA	G	AGC	GCC	CAA	TAC
GC	AA				

5' Oligo Name: 5' Neo Red 3'

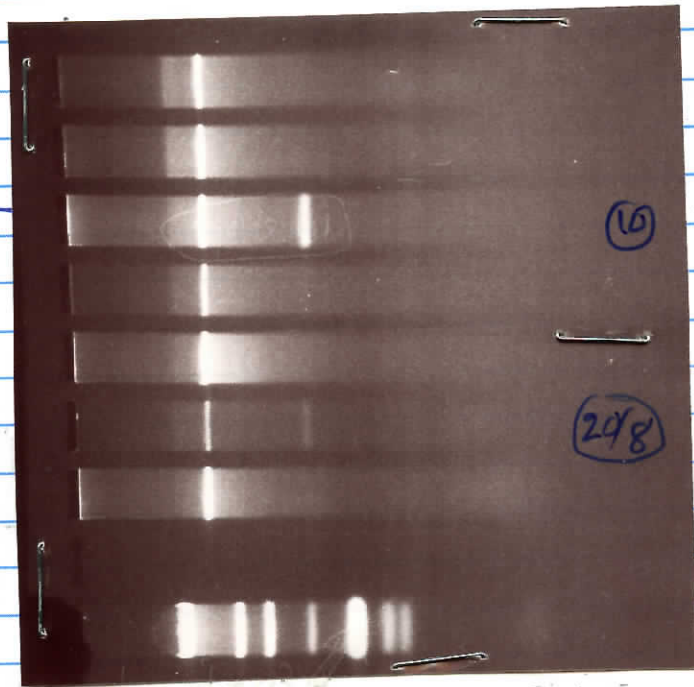
AA	C	CAC	ACT	GCT	CGA
AA	T	TCA	T		

3 5' end

conclusion ①: looks to have made no difference
 — probable insert from each ligation. Check
 with more DNA

BUT IT W'D HAVE GIVEN ME A PRODUCT
 at same frequency as the CIAA+ lane

conclusion ②
 confirm



⑩

← ⑩

248

← 20/8

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Thurs. Feb 26th

Rec A⁺ test of G'127C ligation

1 } G'127 (Black)
 2 } 1X highⁿ
 11 } Xho.
 12 } G'127
 13 } 1X highⁿ
 14 } Rec A⁺
 23 } (Blue) Xho
 24 }
 W
 MM
 W
 Dye

G'127 1X	G'127 1/8X
80 ✓	80 ✓
1	8 ✓
10:50 am	11:58 am

Plate each 42° 1X
 15 sec 7 6 1 0 1X
 Rest 20.5 20/16.5 16 37° 12:25 pm
 1X Amp N 2Y 20.5 20/16.5 16 1/8X

FB440 was from Sanger (GM48) and has genotype:
 (FB440) F⁻ dcm⁻ dam⁻ gal⁺ ara-14 lacY thr1

levb Thi-1 la(ao)n R31 TSR-78 (SupE44?)

Thurs 1/8X again ~ 3X 1X

Picked 1-12 from 1X & 13/8 - 24/8 from 1/8X
 by 1X Amp N 2Y 32° 10:30 am - 9:15 pm Fri → Cold
 Processed to 100 µl Sat.

Take 50 µl 10X RT salts

438 µl H₂O

12.5 µl Xho hot 00Y (100 µl)

20 µl + 1 µl each mini

37° 5:28 pm to 6:30 (to cold)

16 µl + 4 µl ... (3) (4) (10) (11) (20/8) (22/8)

12:02 pm Sun 2:45 pm

G'137/2

3 small

G138 G125 (T1) Pure Nat 718 primer



Model 377
Version 3.0
ABI100
Version 3.0
980317Daz3OSmi0316064
23OSmi0316064
Lane 23

Signal G:397 A:232 T:160 C:379
DT (BD Set Any-Primer)
97022467 DR LR9710a Instr File
Points 1182 to 1180 Base 1: 1182

11.29

Page 1 of 2
Wed Mar 18 1998 10:28
Tue Mar 17 1998 16:54
Spacing: 11.63(11.63)

GAACCATGTCTCTCTGAGC GATGAAGCC CAGT GATGTGGAACTTCTTTTTCATGGGTGACGCTTTCAGGCTGGATGCTCTTCA CAGCCAGGACTGAGAGAAAGAC GTCTCAAGGAAGATGGCTTCTCTCTTGC CAGGAA GCCCC

(BestFit) SEGMENTS from: t.dat March 18, 1998 14:59

(Nucleotide) WORDSEARCH of: /export/home/nobuyo/oliver/tnap.seq check: 6823
from: 1 to: 2459
LOCUS MUSPHOPA 2459 bp mRNA
DEFINITION Mouse placental alkaline phosphatase mRNA, complete cds.
ACCESSION J02980
NID G200345
KEYWORDS alkaline phosphatase; orthophosphoric-monoester phosphohydrolase.

AvMatch: 3.84 AvMismatch: -6.00 GapWeight: 50 LengthWeight: 3

Match display thresholds for the alignment(s):
: = IDENTITY
: = 3
: = 1

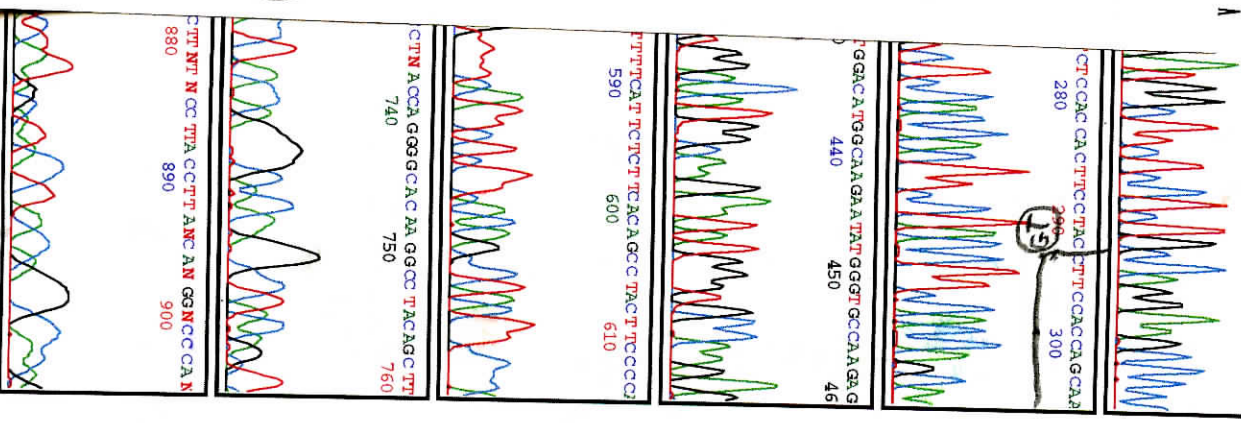
tnap.seq

check: 6823 from: 1 to: 2459 /Reverse
/export/home/nobuyo/oliver/tnapitnot.seq check: 6870 from: 844 to: 2736
Gaps: 2 Quality: 1302 Ratio: 7.356 Score: 139 Width: 7 Limits: +/-8

1187 CCTGCCTCCTTCCACCAGC.AAGAGAAGCCCTTTGAGGTTTGGTCAGG 1139
2117 cttcctaccctccaccaggaagaagaagcctttaggttttggtcaggt 2166
1138 ATCCGGAGGGCCACCTCCACCATCTCGGAGAGCGAAGGTCAGTCAGGT 1089
2167 atccggagggccaccctccaccatctcgagagcgaaggttcagtcaggtt 2216
1088 GTTCCGATTCAATTCTATCTGTCATGTCCCGGGGCTCAAGAGACCTAAGA 1039
2217 gttccgalttcaattcatactgcatgtccccgggctcaagagacgtgtg 2265
1038 GGTAAGTCCACCTCGAGGGGTCAAGGGC 1011
2266 acatggcaagaatatggtgccaagagc 2293

Exon 9 (rev.)

mis type



Ma. March 2nd

More TNAP Kit Sequences

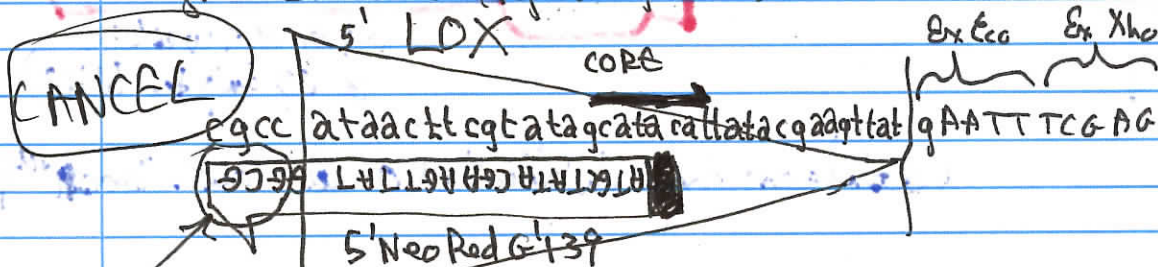
Starting outside NOT w/ 58 Red (universal primer) did not yet reach exon 9. Nice sequence around 560 gives good primer for extension: —

CGAGACCGCTGTGCTGGT

will be G'139 Not 563

$$T_m = 2 \times 6 + 4 \times 12 = 60^\circ \quad 563 \text{ in G'134 seq}$$

The universal primer, ~~also~~ 5 Neo Red next to lox, did not work although it checks with ~~some~~ sequence from DUPDEL. Try using LOX into Asc I inverter



Specific for this 5' LOX. Not very secure w/ 3 competitive sequences — one is too close. ? CANCEL. Anyway already made as F86 Red

F'101 Red D

~~But the go back into Neo and use the repeated sequence~~
~~the promoter/enhancer~~ ~~it worked~~ yet it overlaps completely with the last 17 bp of 3' LOX Blue i.e. only 4 bp left at the 5' end! Let's go back into Neo and use F'101 Red D — it works uniquely. Can be the new universal Red primer for Group B

Sequencing as #60 March 2, 1998 TINEOR

Take	G'125 (T1) mini	1 µl ✓
	Water	14 µl ✓
	F'101 Red D/100	5 µl ✓

Sequencing as #61 TINEOR

Take	G'125 (T1) mini	1 µl ✓
	Water	14 µl ✓
	G'139 Not 563/100	5 µl ✓

See G'143

Redesign G'135 5' Neo Red

See G'143

Worked fine but too far away

G141 Thores OS Dupl del poly 11
Doup Del B(lue) (Damm)
0.47 ~~1.84~~ / 1e

Dup Del R(ed) (Damm-)
 SHA26 0.24pg/pl
 G'140

```
887.seq check: 5847 from: 1 to: 845
/afs/isis.unc.edu/home/n/o/nobuyo/oliver/osdupdel.seq check: 4439
ASSEMBLE August 13, 1995 16:20
Gaps: 3 Quality: 1935 Ratio: 9.085 Score: 170 Width: 6 Limit:
```

Dup Del B (luc) (Darm +)
Q: 75pg/yr KC AM 1-158
G: 140

Dup Del R(ed) (Damm +)
 0.5pg/1st KC AMI-158
 G'140

(Neo is reverse of OS dup tel 6142)

en has duplicates of all four. Dec 10th, 2001

Del Blue may
have o base (4898G)
missing - it is

present in F101 at 60

Port K.C. 04/27/07 has
used it OK several times

Submitted
04/23/0-

887
889

1 ml
F155²
NeoBlue

1 ml 2
G140
Dupl. 1.3/29/1 ml

18 Feb 1962

Thurs March 5th

DUPDEL with ¹/₂ in Dam

Correct & Dup Dels in Dam

Kathleen Karon, using D53 ASP Conc 7.
the correct (peft) version of pM1 Neo, obtained
DUPDEL B (blue) & DUPDEL R (red) for reverse.

Today transform to FB

Transformation	Correct DDB (Dam)	Reverse DDR (m)
G'27 FB 446	80 pl	80 pl
KC Black 32 mini	1 pl	1 pl
KC Blue 2 mini	—	1 pl
0° C 11 am to 12:45 pm		
90 x c. G2° 43°		
Back plate R, 20, 5, 20/16, 5/16		
1 x Amp NZY 37° 1 pm — 10 am		

Gave to S.H. as DUPDEL B (blue) = Correct
Dam
→ DUPDEL R (red) = Reverse
Dam

for growth

The normal DH5α growths are
Kathleen 7 AM1 159 (32) Correct } See page 6140
Karon AM1 159 (2) Reverse }

osdupdel B
(was #32)
G'141 + pefA

osdupdel R
(was #2)

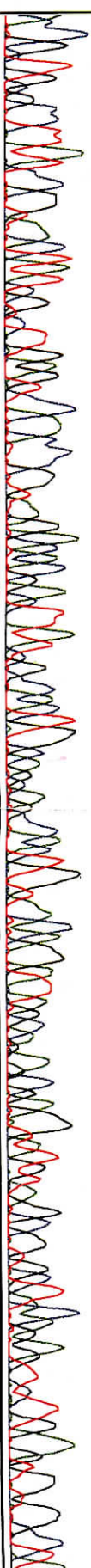
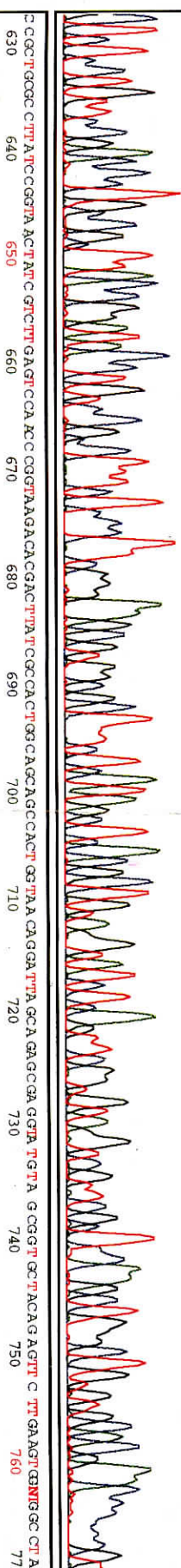
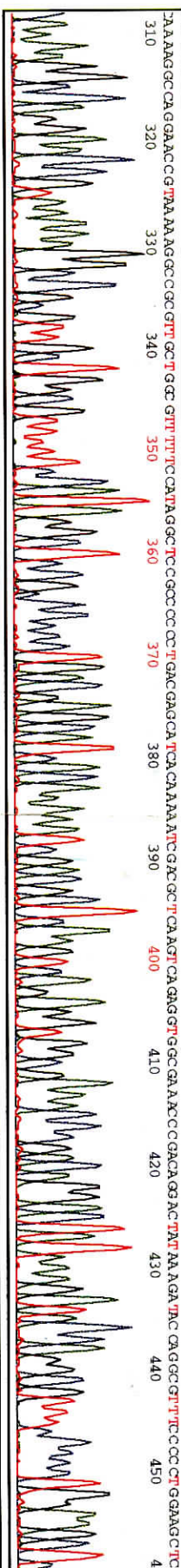
to N7

part of diagram in Diagram F103 Blue is primer

Model 377	9803324Ca46OSmI0323007
Version 3.0	
ABI1100	46OSmI0323007
Version 3.0	
	PhsI Lane 46

Signal G:195 A:113 T:90 C:153
DT {BD Set Any-Primer}
96051547 DR LR9709 Instr File
Points 1294 to 10624 Base 1: 1294

Wed, Mar 25, 1999
Tue, Mar 24, 1999
Spacing: 11



TTCTACTANAGACAGTATTGGTATCTGGCCTCTGCTGAAACAGTINT

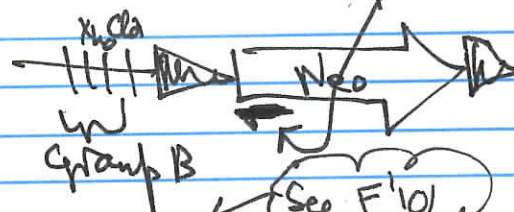
Standard
ABI sequencing
protocol

1 µg of DNA
or 1 µl of
+ 5 µl of standard 100x
diluted water to 20 µl

Fri. March 6th

DUPDEL Sequencing
& (T1)

DUPDEL
universal primers



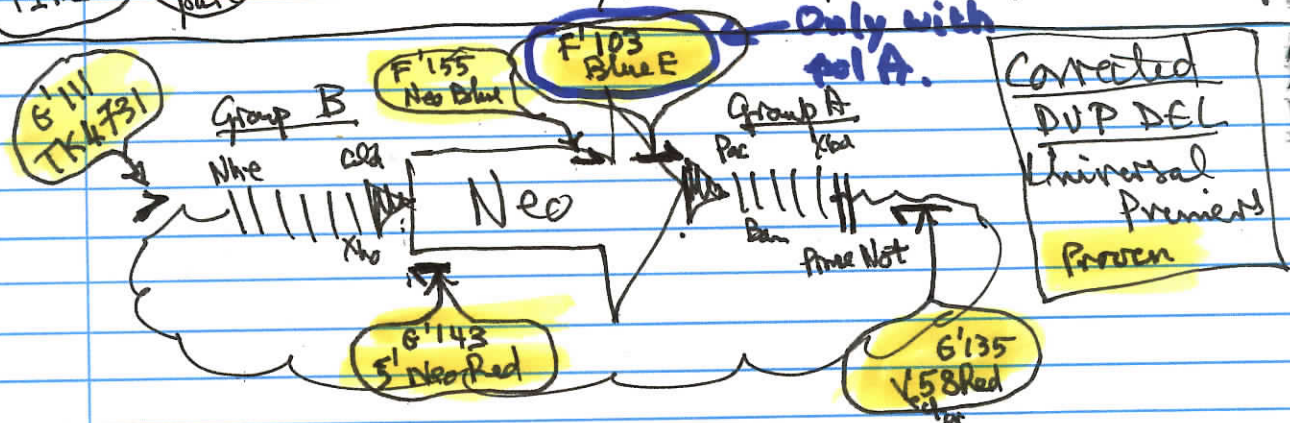
G'139 Result shows that F'101 Red adds well - but is a long way from insert. The extra 7 bp explains failure of 5' Neo Red G'135. Redesigned the 5' Neo Red primer to correct this problem.

G'143 5' Neo Red

Universal
DUPDEL
Primer

To sequence as #62
NOT 541
T1 pure
Take G'143/4.7 µl + 95.3 µl Diluted
G'125 T1
Water
G'139 Not 541/100 5 µl
This will have 1 µg

To sequence as #63
T1 Neo R
T1 pure
Take G'143/G'125 T1 Diluted
Water
G'143 5' Neo Red/100
10 µl
5 µl ✓
5 µl ✓
O.S. questionable that this will better be 5 µg for such a large plasmid



To sequence
March 16th
as #64
NOT 718
T1 pure

Take G'143/G'125 (T1) Diluted
Water
G'139 Not 718/100

10 µl ✓
5 µl ✓
5 µl ✓

Good result
Reached exon 9
reverse at last

To sequence
March 23rd

as #65
Blue Dam

Good result

Take Pure DupDel B (blue) Dam
F'103 Blue E/100
Water
G'141 5' Neo Red

10 µl ✓
5 µl ✓
5 µl ✓

5 µl

Inactivation of the Mouse *HPRT* Locus by a 203-bp Retroposon Insertion and a 55-kb Gene-Targeted Deletion: Establishment of New *HPRT*-Deficient Mouse Embryonic Stem Cell Lines

Hirohisa Tsuda,^{*,1} Catherine E. Maynard-Currie,^{*} Laura H. Reid,[†] Takayuki Yoshida,[‡] Koji Edamura,[‡] Nobuyo Maeda,[†] Oliver Smithies,[†] and Aya Jakobovits^{*}

^{*}Cell Genesys, Inc., 322 Lakeside Drive, Foster City, California 94404; [‡]Life Science Research Laboratory, Japan Tobacco, Inc., 6-2 Umegaoka, Aoba-ku, Yokohama, Kanagawa 227, Japan; and [†]Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, North Carolina 27599-7525

Received January 3, 1997; accepted April 15, 1997

To obtain useful hypoxanthine phosphoribosyltransferase (*HPRT*)-deficient mouse ES cell lines, two different methods were employed: (i) selection of spontaneous 6-TG-resistant mutants and (ii) gene targeting of the *HPRT* locus. The first approach resulted in the establishment of E14.1TG3B1, a spontaneous *HPRT*-deficient cell line with an insertional mutation of 203 bp in the third exon of the *HPRT* gene. The insert is highly homologous to the B2 mouse repetitive element and has all the expected retroposon characteristics, thus providing an example of gene inactivation by retroposon insertion. This clone exhibited stable 6-TG resistance and high germ-line transmission frequency. Thus E14.1TG3B1 is a useful ES cell line for modifying the mouse genome using the *HPRT* gene as a selection marker and for transmissibility at a high frequency into the mouse germ line. The second approach resulted in a 55-kb deletion of the mouse *HPRT* locus, demonstrating the feasibility of replacement-targeting vectors to generate large genomic DNA deletions. © 1997 Academic Press

INTRODUCTION

Mouse embryonic stem (ES) cells have proven powerful for *in vitro* selection of genetic modifications in the mouse genome and for their subsequent transmission to the mouse germline (Capecchi, 1989; Koller and Smithies, 1992). Such genetic alterations include gene-targeted mutations and introduced transgenes. This approach relies on the ability to efficiently select for clones with the acquired genetic alterations, as well as on the capability of the altered ES cell line to trans-

mit the modification to the mouse germ line at high frequency.

One of the most powerful selection systems for ES cells is the combination of a hypoxanthine phosphoribosyltransferase (*HPRT*) expression cassette in conjunction with *HPRT*-deficient ES cell lines. The X chromosome-linked *HPRT* gene is an attractive selectable marker, being well characterized and available for either positive selection using HAT (hypoxanthine, aminopterin, thymidine) or negative selection using 6-thioguanine (6-TG) (Melton *et al.*, 1984). Derivation of *HPRT*-deficient variants is facilitated by the need to inactivate only a single *HPRT* locus in male ES cells via spontaneous or gene-targeted-derived mutations and by the ability to select directly for loss of *HPRT* function (Hooper *et al.*, 1987; Kuehn *et al.*, 1987). For these reasons, the *HPRT* gene has served as a powerful tool to assess new mechanisms for gene inactivation in ES cells (Reid *et al.*, 1990; Valancius and Smithies, 1991; Zheng *et al.*, 1991; Ramirez-Solis *et al.*, 1995). However, only a few of the generated *HPRT*-deficient ES cell lines were established as cell lines capable of introducing genetic modifications into the mouse germ line. Experiments with these cell lines (Hooper *et al.*, 1987; Kuehn *et al.*, 1987; Selfridge *et al.*, 1992) indicated the benefit of generating additional *HPRT*-deficient cell lines with improved germ-line transmission capability.

In this paper we describe new *HPRT*-deficient ES cell lines generated by inactivating the *HPRT* gene by two mechanisms: spontaneous retroposon insertion and gene-targeted large-DNA deletion.

Selection for spontaneous *HPRT* variants led to the identification of a new *HPRT*-deficient cell line, E14.1TG3B1, having a 203-bp insertion at the *HPRT* exon 3. This insert is highly homologous to the mouse B2 repetitive element and shows the characteristics of a retroposon insertion. The high level of germ-line transmission exhibited by E14.1TG3B1 validates its

Sequence data from this article have been deposited with the BL/GenBank Data Libraries under Accession No. D85770.

To whom correspondence should be addressed at present address: Life Science Research Laboratory, Japan Tobacco, Inc., 6-2 Umegaoka, Aoba-ku, Yokohama, Kanagawa 227, Japan.

Fri. April 3rd Ex. Feb 6th, 1996

Aya Jacobovits / Hirohisa Tsuda

and Nobuyo Maeda

Rebind to make E14 → E14TG2a

SH has

↑ MP 5X G'145
SH X 52 0.6675 pg/μl

← Test - shd be X

N.M.

MP5 G'145
Z 24
0.35 pg/μl

Definitely MP5

↑ MP 5X

SH has

↑ E2 G'145
SH X 52 1.717 pg/μl

Camolabeller PM4

↑ MP 4
0.825 pg/μl
SH X 52

SH has

Original

H.T. label still available

Hargrave et al. 2167-2175
 Blood 81 (1993)

AAV Constructs

April 12th, 1998

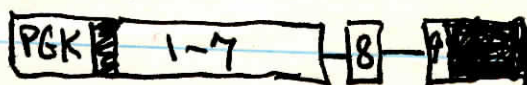
Subunits:

CD34+ bm cells @ 1 in 20 with MOI of 10⁵
 less purified AAV may require MOI of 10⁴ or more.
 work better.

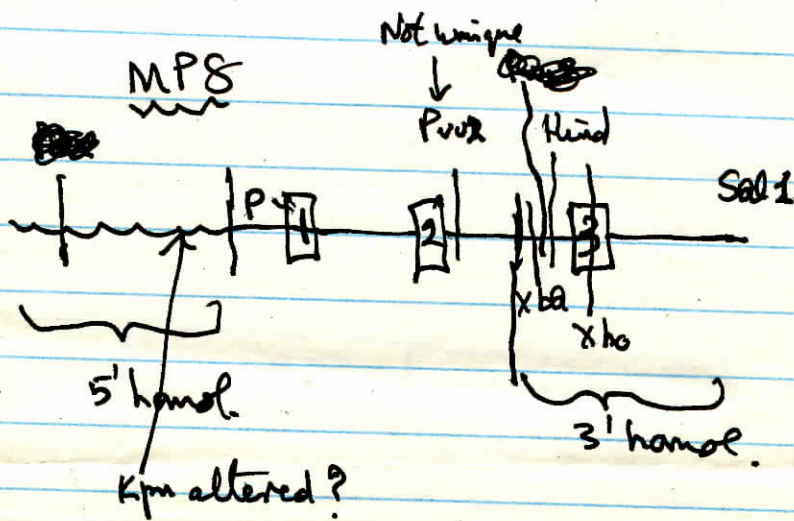


1.6 kb

HPRT minigene

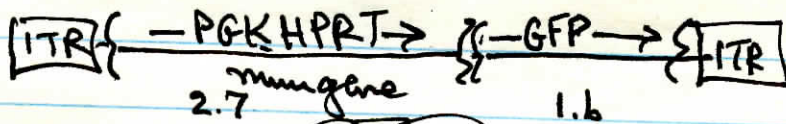


2.7 kb



Possible constructs:

Double
 Minigene
 GFP/HPRT



2.7 minigene 1.6

4.3 kb

Yes

- but better
 over page

Fade has it

Friday April 17th

New direction for HSC gene targeting

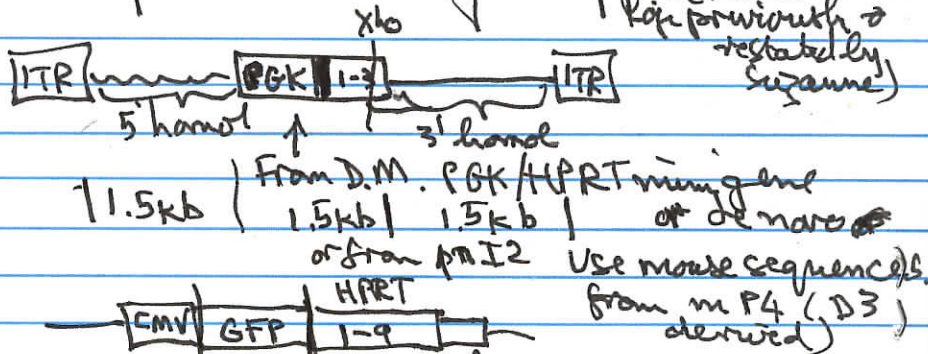
Summary of expts. carried out by Koji Nikkuni shows no indication of gene targeting at HPRT locus, and no transformation from HAT^S to HAT^R with HPRT minigene or to 6418^R with Neo. Assume that DNA delivery by electroporation is the problem $\rightarrow$ look for a new possible route.

Recent report by ~~Ben~~ Russell et al. in Nature Medicine suggests that AAV can be used for homologous recombination with HeLa cells and normal human fibroblasts at the HPRT locus. Review of literature (including Ludo Samulski, Art Nienhuis $\rightarrow$ Elip Vanman) indicates that clonogenic hematopoietic cells can be transduced - at very high MOI (10^8 for 90%) with highly purified AAV, and at much lower MOI's with code AAV ($\sim 10^3$). Can test h.r. with less pure preps., assuming that others will solve the problem of $\downarrow$ MOI needed. In any case, it is the principle of (h.r.) in HSC that we are testing.

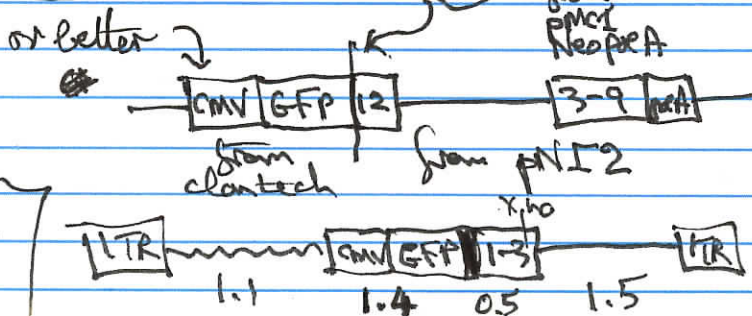
Following constructs look workable, and include the additional idea that finding cells earlier than needed for colonies to develop would be very helpful. (discussed with Koji previously $\rightarrow$ restated by Suzanne)

Order

A) Sample h.r. vector
S. will make this



B) Plasmid to test GFP/HPRT fusion



1

2

C) GFP/HPRA Neo pA fusion

hara's constructs have human DNA.

3 cells

Fri May 1st

First GFP HPRT Mini Design

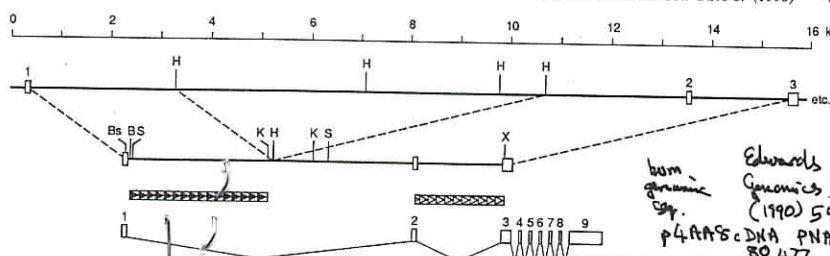
Using Laura Reid's constructs (PNAS) 87 4299 (1990)
 & CLONTECH's (enhanced) G (green) F (fluorescent) P (protein) - C
 vector, the following construct was designed. It
 corresponds to G'142 (1)

Now in MISCELLANEOUS

G'149 pnNI
 0.70 µg/µl
 Seigo Hatada F13 0.7 µg/µl

Genetics: Reid et al.

Proc. Natl. Acad. Sci. USA 87 (1990) 4301



Edwards
 Genomics 6
 (1990) 543-108
 p4AAS.cDNA PNAS (1983)
 80 477

Construct	size (kb)	percent control plasmid	test plasmid colonies	control plasmid colonies
pnI	1.6	1	2	84
pnNI	1.6	1	1	84
pI1,2	9.1	107	690	268
pnI1,2	9.1	100	548	275
pnI2	3.3	23	47	21
pnI1	7.4	1	231	548
pnI1Δa,2	5.2	30	183	110
pnI1Δb,2	5.4	42	130	124
pnI1Δc,2	8.1	110	335	252
pnI2(I1S)	6.0	224	587	747
pnI2(I1R)	6.0	172	556	470
pnNI(I2S)	3.4	<1	0	0
pnNI(I2R)	3.4	<1	0	0

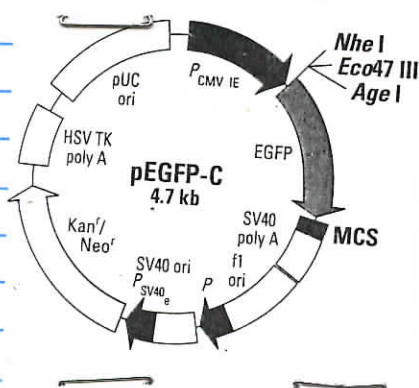
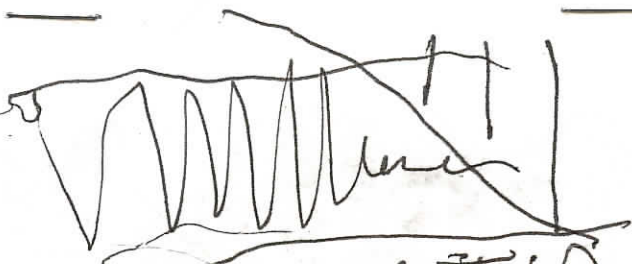


Fig. 1. Influence of DNA from introns 1 and 2 on the expression of human HPRT minigenes in mouse ES cells. (Upper) The line immediately below the scale depicts genomic DNA including the first three exons of the human HPRT gene. The origin of fragments from this genomic region and restriction enzyme sites used to construct the HPRT minigenes are illustrated below. (Lower) The structure of a particular HPRT minigene is shown on the left; its size and relative expression in ES cells as a percentage of the control plasmid are shown in the table on the right. For each plasmid, the data from which this expression value was calculated are shown as the number of HAT-resistant ES cell colonies obtained after transfection with that plasmid over the number of HAT-resistant colonies obtained in a parallel transfection with the control plasmid pnI1,2. In some cases (denoted by asterisks) pHP5 was used as the control plasmid. Introns are represented as lines; exons are depicted as open boxes. Shaded exon boxes contain the introduced Nco I site. Dotted lines indicate regions of the intron DNA that have been deleted. Intron fragments located outside of their normal position in the gene are shown as boxes with internal arrowheads. These arrowheads indicate the orientation of the fragment in the HPRT minigene. The 5' TK promoter with mutant polyoma enhancer and the 3' simian virus 40 polyadenylation signal present in all minigenes are represented by solid boxes and boxes with crosses, respectively. B, BamHI; Bs, BsaIII; C, Cla I; H, HindIII; K, Kpn I; N, Nco I; S, Sma I; X, Xho I. Not all sites for each restriction enzyme are illustrated.



Establish TOP

1) SLOPE

2) Intercepts

Extrapol. Sigs /

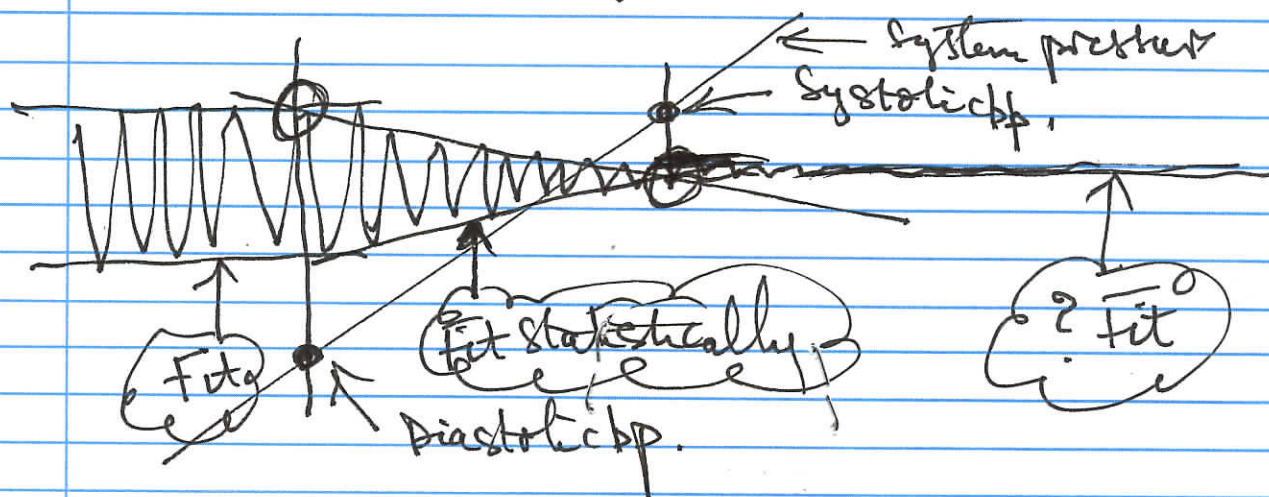
Calcs, D o/m

Fri May 1st

B.P. measurements

Recent data suggests a systematic decrease in bps. measured by the Rogers machine vs. older data of $> 110 \text{ mm Hg}$. Changes in the algorithm may be responsible.

New idea - Jay R. will test - :-



$mu/+ \times Atr1 -/-$ $\rightarrow$ $Ren 8x$
 $\downarrow$
 $mu+/-$ $+/+$
 $Atr+/-$ $Atr+/-$
 Ren Ren
 $?x$ $2x$

What Ren genotype

Check with
 $mu =$ AGT
 Ren
 eNOS
 ANP
 etc.

≥ 5
 $W_{t.1}$
 animals
 for each exp.

Fri. May 1st

Testing for interacting loci vis à vis hypothesis

Discussions with N.M., Bill & Alexandra Dore in Madras, Cal.
April 25th led me to suggest that the earliest phenotype
for detecting hypert. or bp.-affecting mutations is
the level of Renin plasma, since Agt 1/0 and
Atr 12 1/0 animals have at least 2 x renin but fairly a
few months. Dore has been breeding ENV mutant to an indicator
strain to see modifiers :-

$\text{ENU} \left. \begin{matrix} \text{mut} / + \end{matrix} \right\} \times \text{apc} \begin{matrix} + / - \\ (?) \\ (-) \end{matrix} \rightarrow \begin{matrix} \uparrow \text{ or } \downarrow \text{ in } \# \text{ of} \\ \text{polyps etc. when} \\ \text{modifier is present} \end{matrix}$

Thinking of Agt 0/0 x mut +/- $\rightarrow$ Agt 1/0 { mut +/-
as indicator { mut +/-

which ~~would~~ detect effects of mutation

Talking to H. S. Kim suggests Δ tr1a are ^{mutant} better since 0/0 survives.

Can test ~~is~~ first with known mutants: -

Test locu + / - x Atr 1a 0/0 $\rightarrow$ 1/0 Atr 1a 1/0 Atr 1a 0/0
T.L. + / - T.L. + / +

Thus can establish the positive, negative or zero interaction between Atr12 locus (RAS) and :-

Renin can be measured on retro orbital venous sample without anesthesia.

Agt
Amp
Npra
Nprc
Atr 1a (itself)
Atr 1b
e NOS
COX-1
COX-2
etc.

these test
If results warrant
it could consider
setting up an FNU
screen.

Oligo # _____
 Date made: _____
 LENGTH: 18
 % G+C: 33%

5' Oligo Name: Xba Spe Xba Blunt G'154 3'

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

Alternative Plan

Sat. May 9th

pSSV9

Cut with Xba I (→ Bam)
 Blunt end
 CIAP treat.
 Purify

pT2

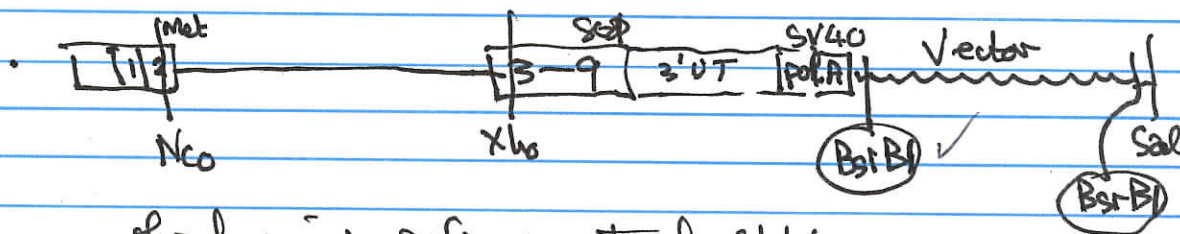
Cut with Bst B1
 Purify

Blunt end ligate & transform

Tues. May 5 ch

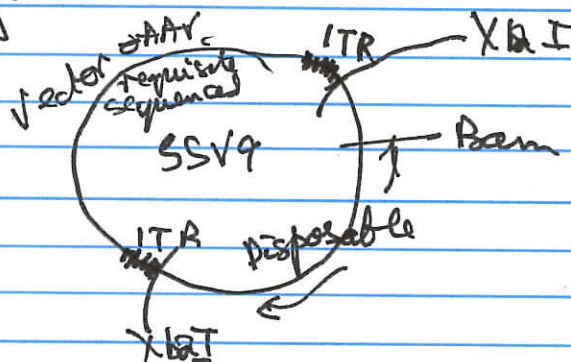
Construction of ITR HPRT^{mini} ITR

Can take p_{nI2} & cut with Bst B1 (blunt):

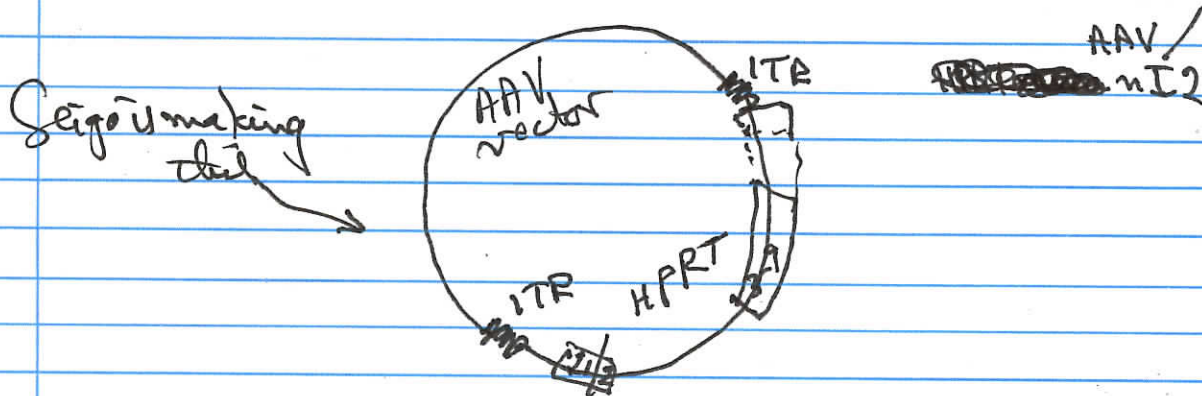


which gives 2 fragments of ~4kb.
ligate on ~~XbaI SpeI XbaI~~ linker (G'154)
Cut with SpeI and purify
↑ does not cut p_{nI2}.

Take SSV9 (pSub 201), cut with XbaI, treat with CIAP and purify.



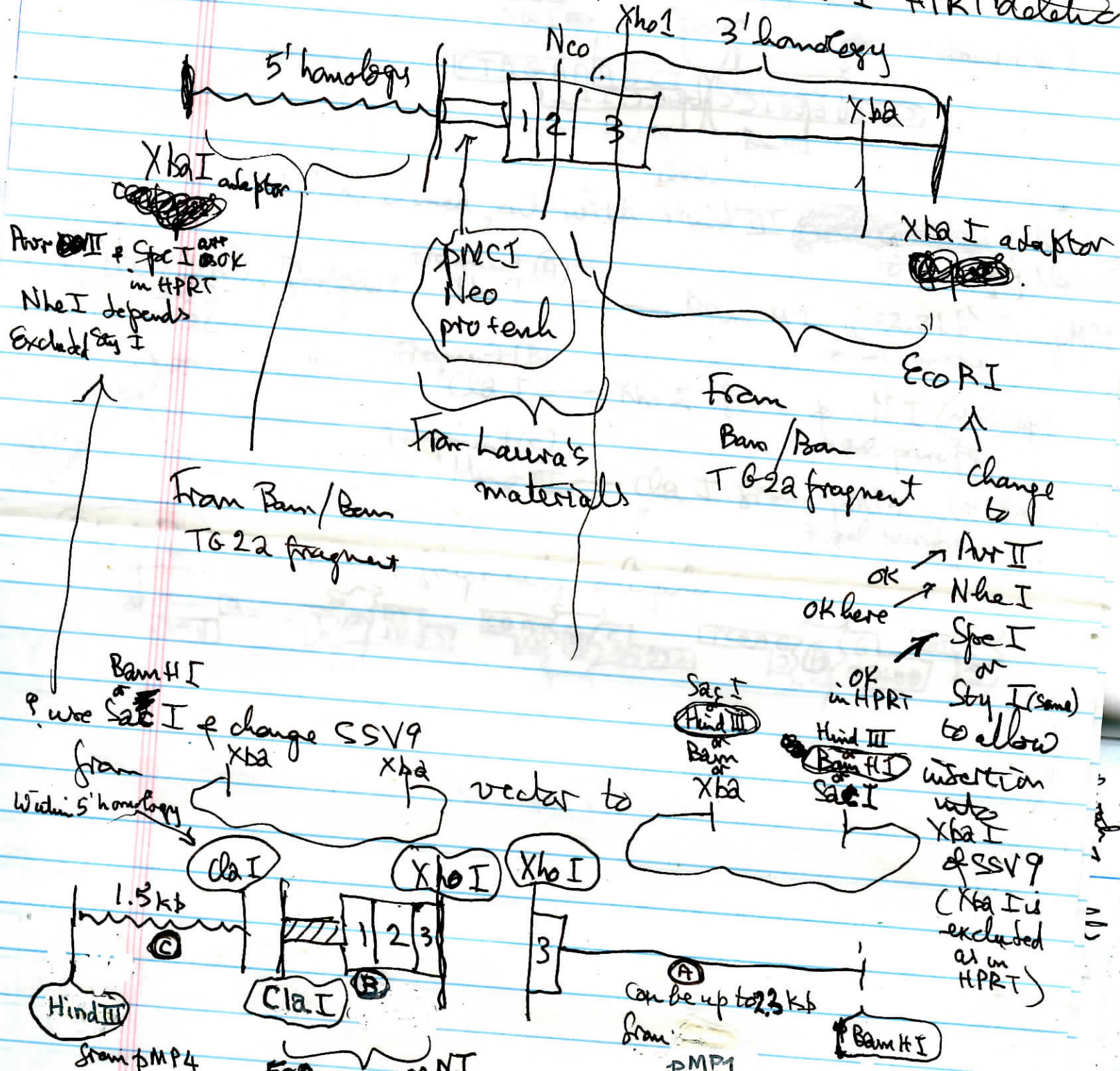
ligate two fragments to give: -



Mar. May 25th, 1998

(1)

First construct for h.r. that is length compatible with AAV and will correct HM-1 HPRT deletion



All with
1-1.5 H₂O ES cells
HAT R
with no feeder

Sun May 24th

Back from IFR
trip in C182 to
Stagnary Alaska
with F.M. & G.B.

157

Initial test with smaller HPRT construct
(Seigo's data)

HAT R colonies
Total as feeders

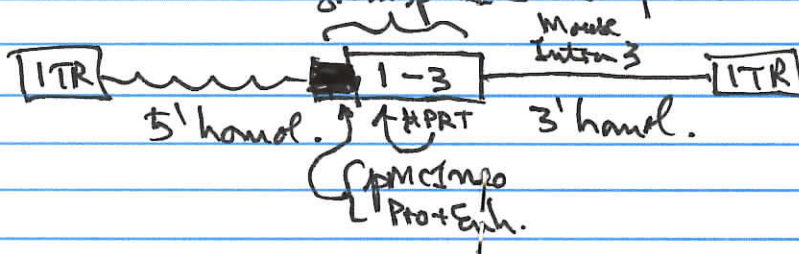
08	(1)	pn I2 cut with Nde I & Sal I	563 (Random)
16	(2)	mp 8 Neo cut with Bam HI	173 H.R.
77	(3)	pn I2 cut with Pvu I	194 (Random)
89	(4)	pn I2 cut with Bst BI	358 (Random)

All with 2.5×10^5 ES cells
Conclusion:

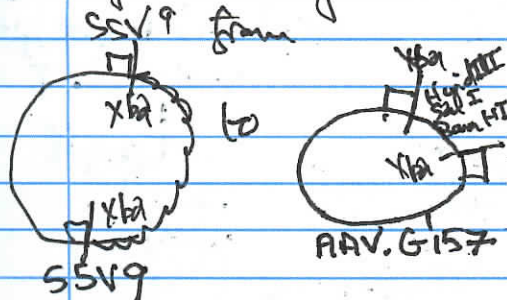
pn I2 is a good starting construct
that is ~~4~~ 4 kb.

Mon. May 25th

Next construct is simple h.v. 4 kb construct
from pn I2 and pNI

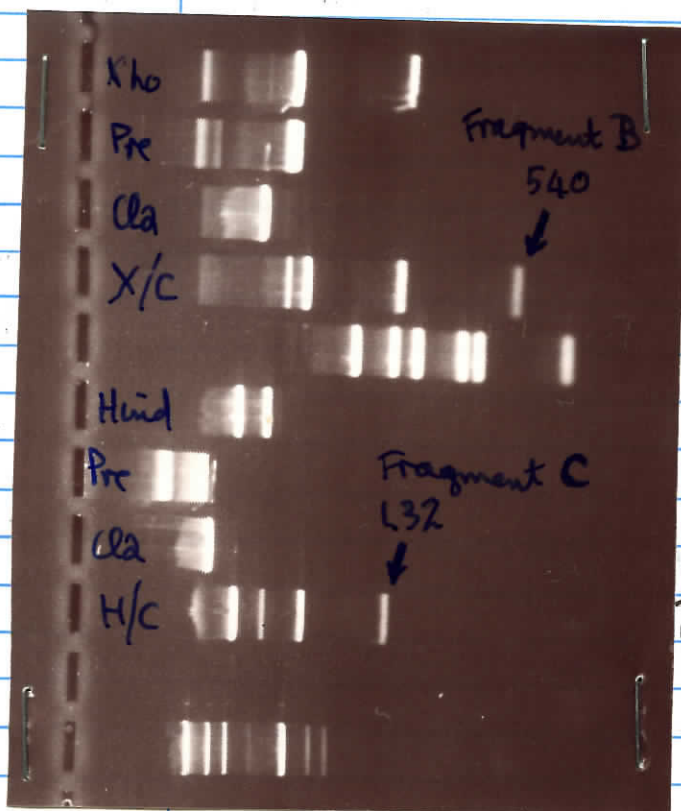


Oligos to change



Oligo #	5'	Oligo Name: G'157 (Xba Hind Sal Bam Xba Blue)	3'
Date made:		CTA GAA AGC TTG TCG	
LENGTH: 24		ACG GAT CCT	
% G+C: 50%			
Oligo #	5'	Oligo Name: G'157 XASBX Red	3'
Date made:		CTA GAG GAT CCG TCG	
LENGTH: 24		ACA AGC TTT	
% G+C: 50%			

Into 0.5 ml TE & IXCIAA



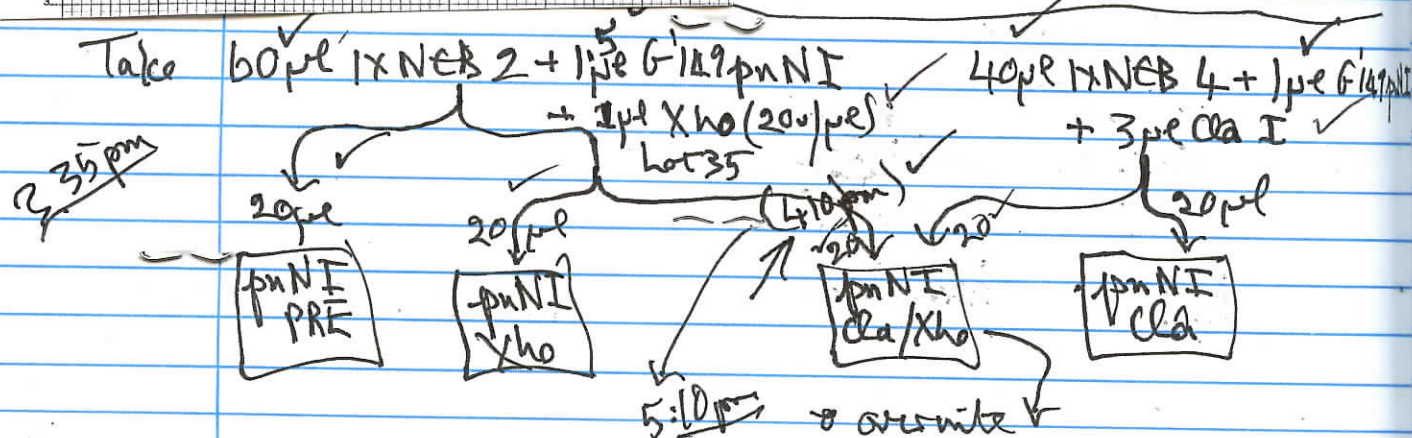
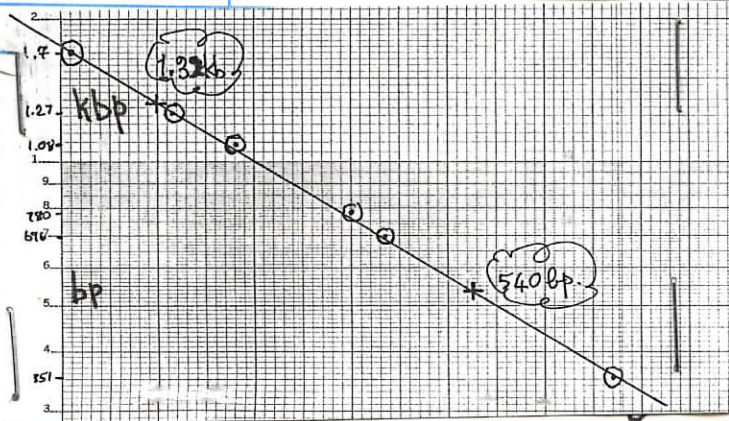
This PCR marker is odd - with the *Hae* III digest

Conclusion: *Cla* has a single site in (pN1) which cuts fine; 2 *Xho* I sites; double digest gives the (presumably) 0.5 kb fragment (540bp) *min*

Confirmed PCR marker identification

Cla has 1 site in (pMPL4) which cuts fine; *Hind* III has 2 sites; double is working *well*.
Fragment is 1.3 kb *min*

This PCR MM has 1696, 1268, 1075, 780, 626 & 351
Usual *Hae* III are missing 605, 450



missing
a
which
double
20ly)
after
further
which
sites:
075
'51'
5.456p43

On 5:25 pm
Off 5:55 pm
1.5% Ag
Hx1
Nmax

Tues. May 26th

Clal pN1 Xho ~500bp 159
Hind pMP4 Clal ~1.5 kb

looking at clal cleavage sites

Dye
W
pN1 Xho
I Pre
I Clal
I Clal/Xho

Dam methylation of GATCGAT will block
AarMm

pMP4 Hind
4 Pre
4 Clal
4 Clal
W

~43% of Clal cleavages.

Fragment B from pN1 is Clal ~500bp Xho I
Sequence at Clal is --ttatcATCGATgtc--

should be okay.

is Hind ~1.5 kb Clal; the Clal site
Not G Not C

Fragment C from pMP4

Sequence data: ??ATCGATtgggt....

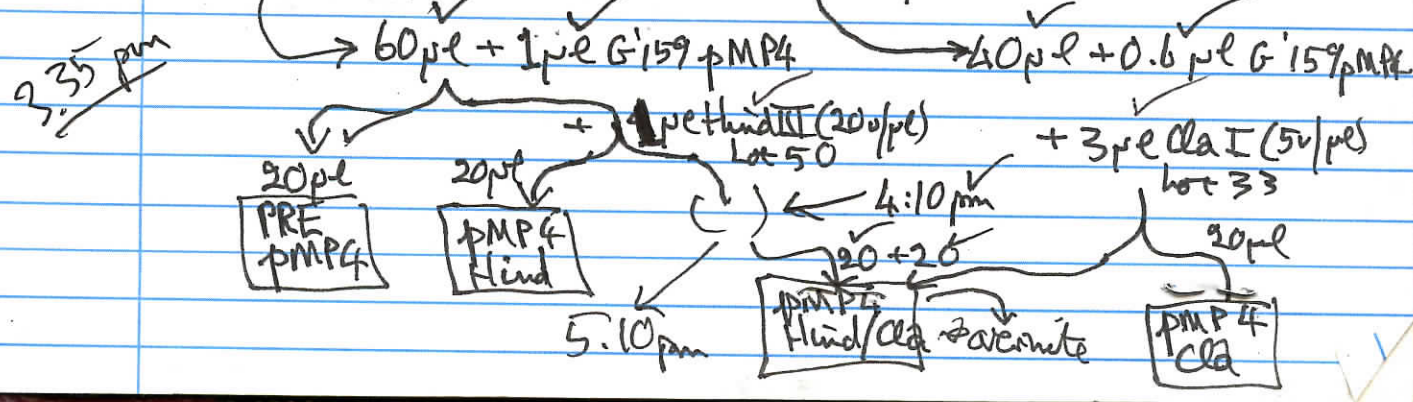
Uncertain OK

Thus B is okay & C may be okay without recloning.

Tests:-

SH X52 G'159 pMP4 0.825 pg/ul
Seigo F13 G'149 pN1 0.70 pg/ul

Take (20 ul 10x NEB 2) Xho (20 ul 10x NEB 4 (Kcl)) Clal
(2180 ul ddw) (180 ul ddw)



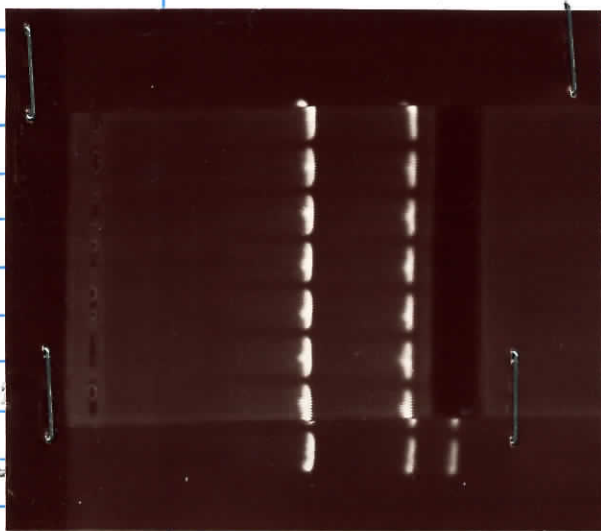
10



① Conclusion: Fragment A
looks secure at 2.2 kb, run prep gel.
The Cla/Hind & Cla/Xho digests did not
improve. Use constant digestion.

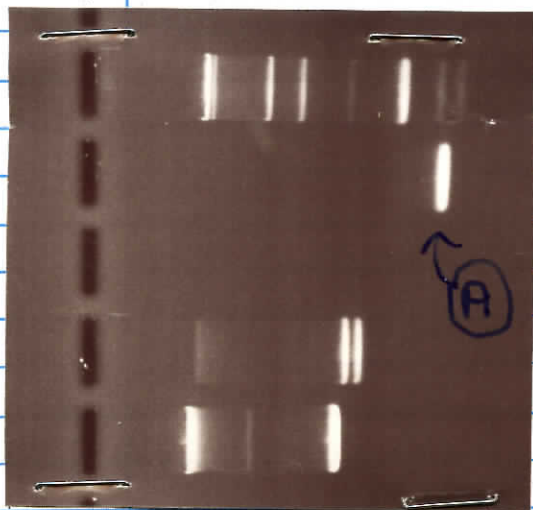
Fragments (A + B + C) = $(1.32 + 0.52 + 2.2) \geq 4.3$ kb, so length is OK

97



② Good cuts.

③



③ Conclusion Fragment A
is fine. Xho 3 ————— Bam
pSV9 cuts well - some losses.

③

Day	
W	
RG	
C	G
	W
	G
	G
	W
	R

(161)

Wed. May 27th

Xho¹ PMP1 Bam ~2.3kb
(A)

Making (hooking at) fragment A

The only pMP1 material that I can find currently is T73² MP1 Bam from Jan 1991 - it sh'd be 0.25µg/µl & is in 1X RI salts.

Take 10µl (2.5µg) T73² MP1 Bam

+ 14µl 10X RI salts

+ 10µl ddw

+ 126 + 1/2µl XhoI (10 u) lot 35 (20u/µl)

37° at 10:25am → 12:35pm

G'161
MP1 B/X

~1.50 Hx2.5
~1.25µm
~2.30µm
G'161
Pre

Agg (1)

Ag MM

MP1/BX G'161

MP1 Pre G'161

ACR MM

pMP4 Hind/Clb average G'159

pN1 Xho/Clb average G'159

W

~5.05µm

0.8% Ag/Seal

off 10am

Agg water

(2)

2.2kb

Fragment A
recovered into 60µl 0.001M EDTA

as

G'161
FRAG. A

(3)

Dye

Xba Vector

Wed. May 27th

Take 15µl 10X ~~RI~~ salts

+ 31µl ddw (2µg)

+ 2.6µl G'161 pSSV-7 (Seig H F17 0.76µg/µl)

+ 1/2µl XbaI lot 30 (20u/µl)

37° 5:40pm to ~7:45pm to cold

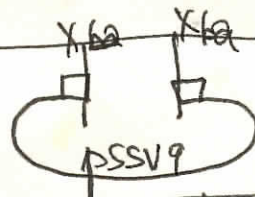
+ 15µl 8M NH₄Ac + 385µl EtOH

Recovered into 100µl 0.001M EDTA as

G'161
pSSV9/Xba

~0.2µg/15µl

G'161
pSSV/X stock



A prep gel

this is OK

Bam
losses.

162

Thurs May 28th
linker ligation to ΔSSV9

Take 30 μl (no. 4 μl) G'161 SSV9/X(ha) stock

✓ 1 μl G'157 XHSBX Blue
✓ 1 μl G'157 XHSBX Red
✓ 3.5 μl 10X p99 ligation buffer
150 μl 2 μl T4 ligase (dilute) lot 67
150 μl to 3:40, 37° 3:40 to 5:55 — to cold room.

G'162
LIG^N

8 to 8
G'162
PRE

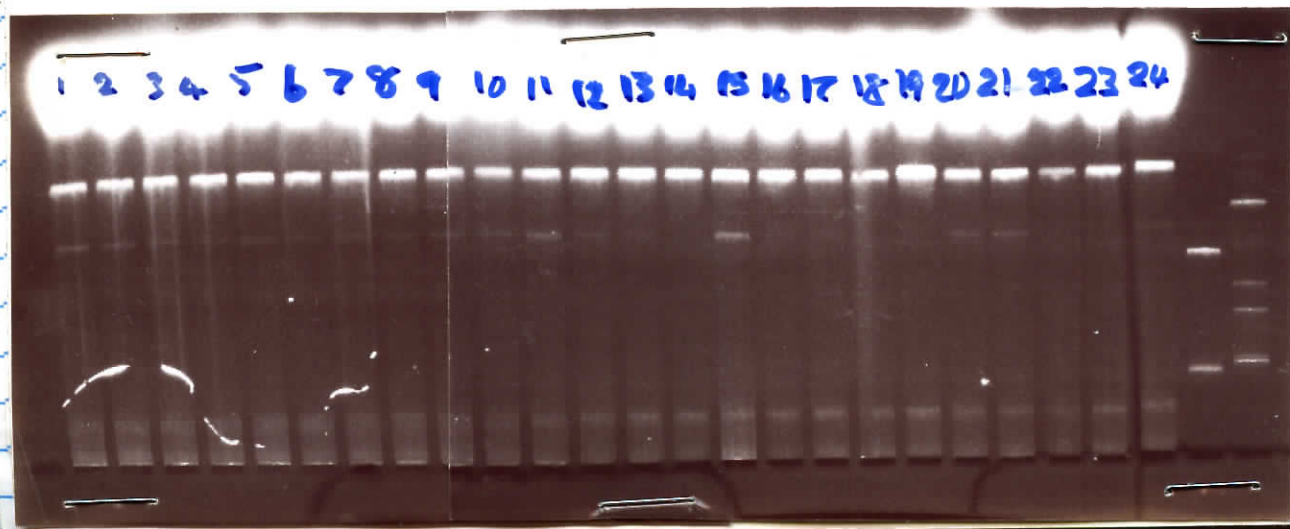
✓ 5 μl ~~10X PBS~~ 80°C for 2 min. ~~10X PBS~~
115 μl ddw + 2.5 μl 10X PBS
G'162
DIL^N LIG^N

Transformation

on 2/10/01
G'162 2/12/01
0.89 Hx4
Max
23
24
G'161
2 1/2 μl 1/2 μl
PSV9
PBM
W
D

G'162 5/28 DH5d 80 μl ✓
G'162 DIL^N LIG^N 8 μl ✓
0°C 6:15 pm to 6:50 pm
42° 90 sec ✓ (730) (2)

Plated on 1X Amp NZY at Rest, 20, 5, 20/16, 5/16 37° 7 pm
G'162 (1) — (24) to NZY 1X Amp 37° 2:30 pm Fri
Fast shake. 9:00 am Sat



Boorum & PeaseTM

Columnar Books • 10 3/8 in. x 8 3/8 in.

150 Pages	300 Pages	Ruling	Width of Desc. Space	Units Per Column
Record Ruled				
21-150-R	21-300-R	Record	—	—
Columnar / Ledger / Quad Ruled				
Single Page Forms				
21-150-2	21-300-2	2 Columns	3 3/8"	10,000,000
21-150-3	21-300-3	3 Columns	3 3/8"	1,000,000
21-150-4	21-300-4	4 Columns	2 3/8"	1,000,000
21-150-5	21-300-5	5 Columns	1 5/8"	100,000
21-150-6	21-300-6	6 Columns	1 3/8"	10,000
21-150-7	—	7 Columns	1 3/8"	10,000
21-150-DEL	—	D.E. Ledger	1 3/8"	10,000,000
21-150-Q	21-300-Q	Quad-5 Sq.	—	—
Double Page Forms				
21-150-8	21-300-8	8 Columns	3 5/8"	10,000,000
21-150-10	21-300-10	10 Columns	4 1/4"	1,000,000
21-150-12	21-300-12	12 Columns	2 3/8"	1,000,000



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RMN 012997

Conclusion. All are
shorter. Better text
with Sal I & then
Hind, Ben & Kba.
Continued on H'3