YAC selecn [selection]. Windows

Publication/Creation

1995-2001

Persistent URL

https://wellcomecollection.org/works/u5twyy7z

wellcome collection

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Good libs: (7/11)

Y4BE1 II Tm11
Y698 V
Y51H1 III Tm12
Y57911 IV Tm13

41707 I Tm14 enzymatic

(High yeast:

Y3986 V
Y51H5 IV
Y37A1 VI)

In progress/current regime:

Y67A10 IV
Y8193 II
Y53C12 II
Y615A5 IV

Y3998 W1A II
Y6491D IV
Y59A8 V

Y17B7 II
Y53F4 III
Y52E2 III } for Tm12

25 H2O
3 buff
5 dNTPs
1.25 oligo
0.3 Taq

19 H2O
2.5 buff
4 dNTPs
1 each oligo
0.5 Taq

Dijay

YAC GROWTH

1 Streak YAC on -URA plate. Grow for 2 days at 30 C

2 In 500ml sterile flask add;

200ml -URA Broth
20ml Glucose (20%)
2ml Tryp (5mg/ml)
4ml Leu (5mg/ml)

Large blob of YAC. Shake at 200rpm, 30 C, 24hrs.

YAC HARVEST

1 Melt (1% LGT agarose, 0.1M EDTA) at 100 C, hold at 42 C.

2 Distribute medium equally into 4 blue-cap 50ml tubes. Spin at 2000rpm, 5mins. Discard supernatent and allow to drain.

3 Add 10ml EDTA (50mM, pH8) to one tube, dissolve pellet and collate into next tube until you are left with one tube. Spin at 2000rpm, 5mins and discard supernatent. Pellet should be approx. 2-3ml.

4 Resuspend pellet with EDTA (50mM, pH8) to a final volume of 20ml.

5 In 2 NUNCLON 24-well plates dispense 0.4ml YAC solution into each well. There is enough to make 48 plugs (long-type variety).

6 Spin plates at 3000rpm, 2mins. Aspirate the supernatent with a drawn pateur. Vortex pellet hard.

7 Add 20ul EDTA (50mM), vortex gently.

8 Add 16ul solution I to first row, vortex gently. Add 16ul solution I to second row, vortex gently.

9 Prepare mould by sealing one side with magic tape.

10 To each well of the first row in turn add 120ul (1% LGT agarose, 0.1M EDTA), pump 2-3 times without sucking air, transfer to mould sealing the edges.

11 Add 16ul solution I to third row, vortex gently then perform step 10 on second row. Repeat for all the rows until both plates complete.

12 Once mould full, leave in fridge for 3-4mins until agarose has set. Meanwhile, prepare 2 more NUNCLON 24-well plates with 0.4ml solution II in each well.

13 Remove tape from the mould and push out plugs, one for each well with plunger. Seal with parafilm and incubate at 37 C overnight.

14 Aspirate with drawn pasteur, protecting the plug with a spatula Add 0.4ml solution III to each well, seal with parafilm. Incubate at 50 C, 1.5 days.
15 Aspirate with drawn pasteur, protecting the plug with a spatula. Add 0.4ml EDTA (0.5M, pH9) and store in fridge.

SOLUTIONS

Solution I
1mg zymolase 100T
1ml SCE (Sorbitol (1M),Sodium Citrate (0.1M),EDTA (0.06M,pH7))
50ul beta-mercaptoethanol

Solution II
20ml EDTA (0.5M,pH9)
1.5ml beta-mercaptoethanol

Solution III
20ml EDTA (0.5M,pH9)
20mg proteinase K
0.67ml Sarcosyl NL30

PULSED-FIELD GEL ELECTROPHORESIS

1 Prepare gel (350ml 0.5 X TBE + 2.45g LGT agarose). Boil, cool. Tape a turntable with tape to form a wall. Pour gel mix, support a comb by a horizontal rod. Set in cold room for at least 2hrs.

2 Soak plugs at least 1hr in 2 changes of 0.05 X TBE.

3 Remove comb, fill wells with 0.5 X TBE and insert plugs with 2 bent spatulas.

4 Drain old buffer from gel chamber by disconnecting back hose and tilting slightly. Reconnect hose and refill chamber with approx. 2L 0.5 X TBE.

5 Mount turntable, check field direction and rotation. RUN at 150v, 35s, 27hrs. These conditions are suitable for YACS of 250-450Kb.

6 Wells are cut after approx. 5hrs to reduce background smears.

7 After the run, the gel is cut away from the velcro and placed into a tub containing 500ml distilled water. Ethidium Bromide is added (50ul, 10mg/ml) and the gel stained for 1.5-2hrs.

8 The ethidium bromide is removed by syringe to a waste can and the gel washed in 1L of 0.5 X TAE.

9 The gel is viewed on a glass plate on a long wave box and the YAC strip excised with a double scalpel. It is stored in the fridge until extraction.

YAC EXTRACTION

1 Add 30ul NaCl (1M) and 1/100th (v/v) 50 X TAE to YAC strip and melt at 64 C (15-20mins).

2 Equally distribute 1ml to eppendorf tubes (2ml) and add 1ml phenol. Shake, keep on ice for 5mins, spin at 13000rpm for 5mins. Take upper layer.

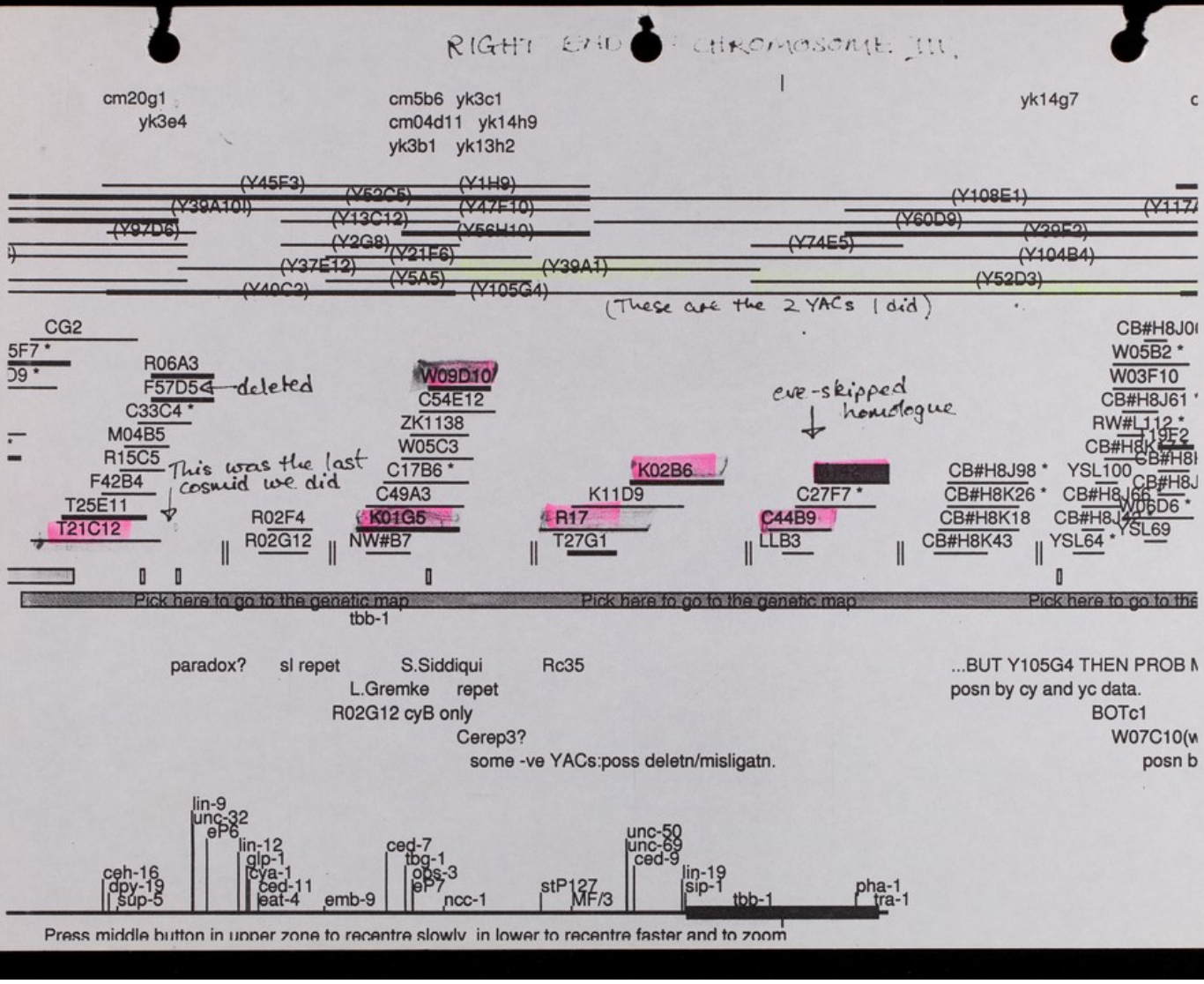
3 With remaining white emulsion layer in each tube, collate into one tube and spin as step 2. Add upper layer to other layers taken.

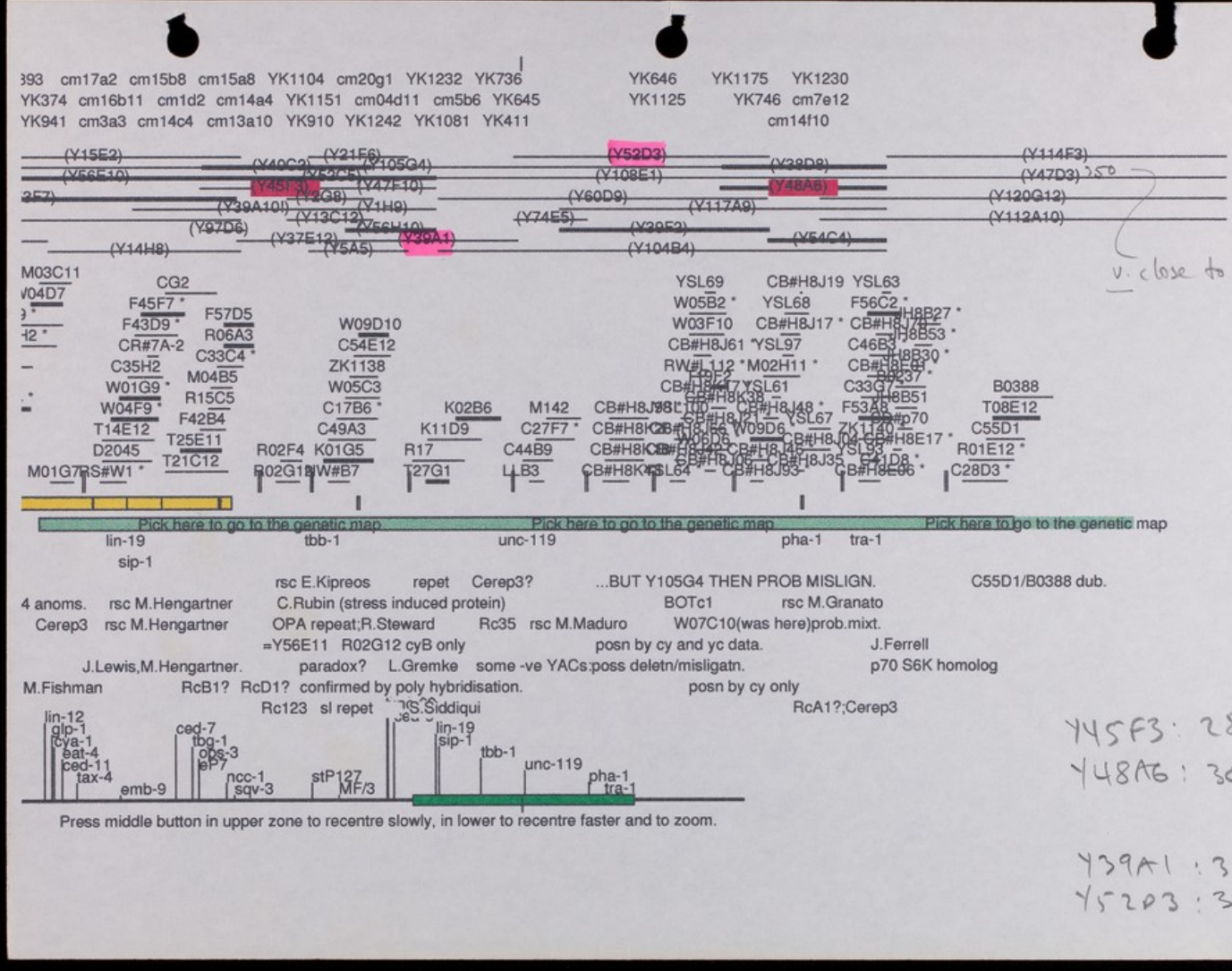
4 Add 1ml phenol and repeat step 2.

5 Add phenol/chloroform (500ul each) to each tube. Ice 2mins, spin 13000rpm, 2mins. Take upper layer.

6 Add 1ml iso-butanol to each tube. Spin 13000rpm, 2mins. Discard upper layer. Repeat until you end up with approx. 300ul.

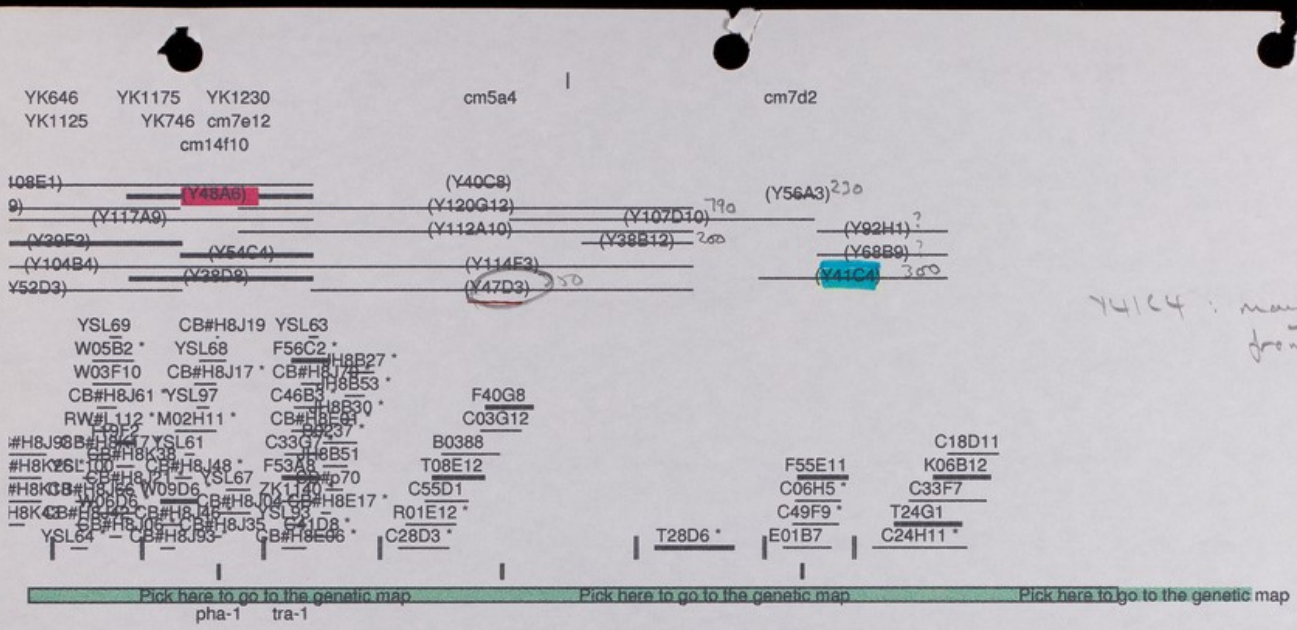
7 Transfer to an eppendorf tube (1.5ml), add 700ul chilled ethanol (96%), store at -20 C overnight.


RIGHT END OF CHROMOSOME III.
cm20g1
yk3e4
cm5b6 yk3c1
cm04d11 yk14h9
yk3b1 yk13h2
yk14g7
(Y45F3)
(Y52C5)
(Y1H9)
(Y39A10l)
(Y13C12)
(Y47F10)
(Y97D6)
(Y2G8)
(Y56H10)
(Y21F6)
(Y37E12)
(Y39A1)
(Y5A5)
(Y40C2)
(Y105G4)
(Y108E1)
(Y60D9)
(Y74E5)
(Y39F2)
(Y104B4)
(Y52D3)
(These are the 2 YACs I did)
CG2
R06A3
F57D54
deleted
C33C4 *
M04B5
R15C5
This was the last cosmid we did
F42B4
T25E11
T21C12
W09D10
C54E12
ZK1138
W05C3
C17B6 *
C49A3
K01G5
NW#B7
R02F4
R02G12
K11D9
R17
T27G1
K02B6
eve-skipped homologue
C27F7 *
C44B9
LLB3
CB#H8J98 *
CB#H8K26 *
CB#H8K18
CB#H8K43
YSL100
YSL64 *
W05B2 *
W03F10
CB#H8J61
RW#L112 *
YSL69
Pick here to go to the genetic map
tbb-1
Pick here to go to the genetic map
Pick here to go to the genetic map
paradox?
sl repet
S.Siddiqui
Rc35
L.Gremke
repet
R02G12 cyB only
Cerep3?
some -ve YACs:poss deletn/misligatn.
...BUT Y105G4 THEN PROB
posn by cy and yc data.
BOTc1
W07C10(w
posn b
lin-9
unc-32
eP6
lin-12
glp-1
cya-1
ced-11
eat-4
emb-9
ceh-16
dpy-19
sup-5
ced-7
tbg-1
ops-3
eP7
ncc-1
stP127
MF/3
unc-50
unc-69
ced-9
lin-19
sip-1
tbb-1
pha-1
tra-1
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom



Y45F3: 280
Y48A6: 300

Y39A1: 370
Y52D3: 300.



Y41C4 : may be seperable from 2nd chrom.

...BUT Y105G4 THEN PROB MISLIGN. p70 S6K homolog ...Y66A7,Y47D3,103F12,Y114F3,Y112A10...

S.Siddiqui Rc35 posn by cy only EXTENT OF YACs NOT KNOWN; need yc's.

L.Gremke repet posn by cy and yc data. C55D1/B0388 dub. posn by cy only

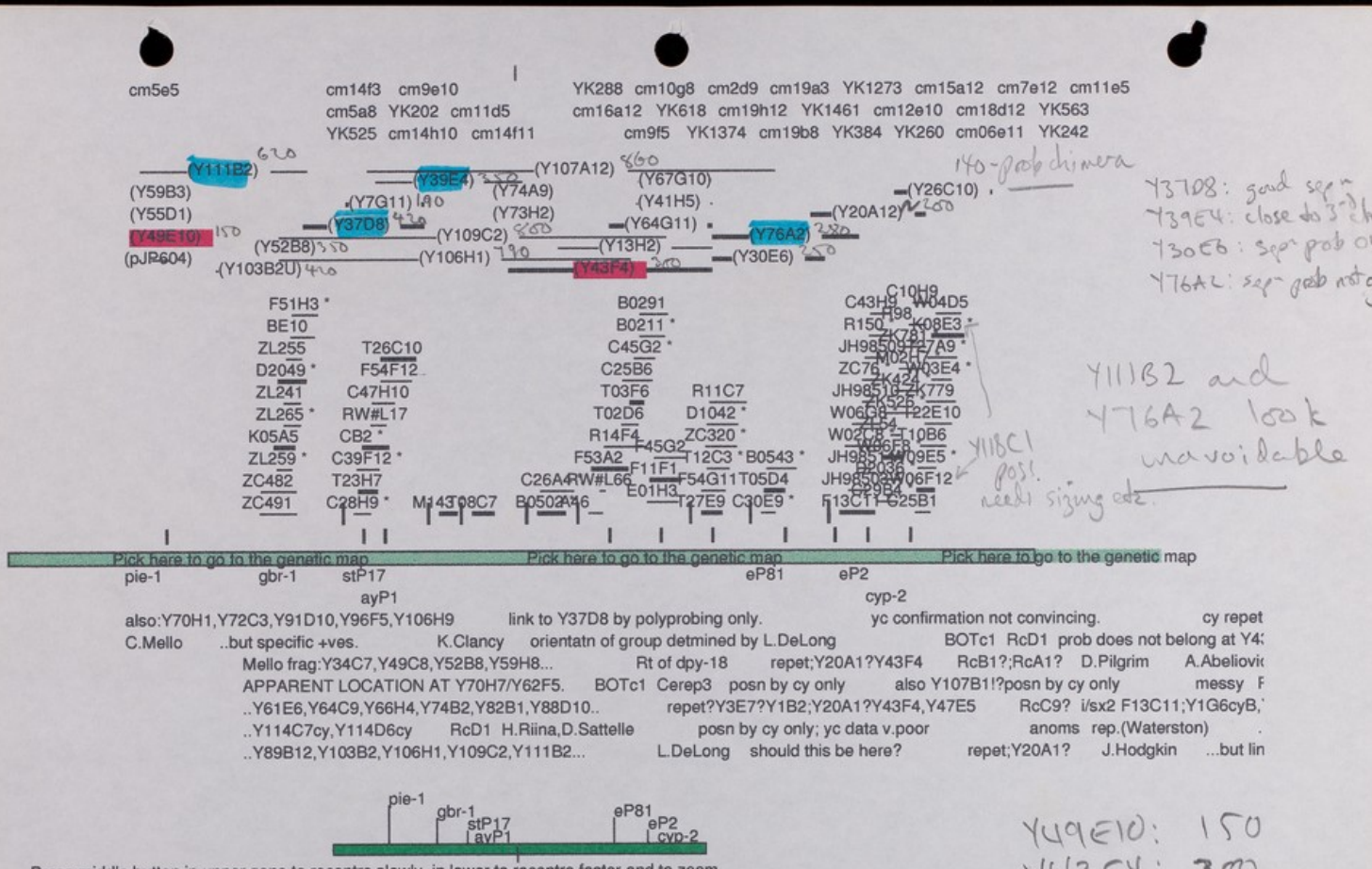
some -ve YACs:poss deletn/misligatn. RcA1?;Cerep3 ...Y75E12,Y120G12,Y107D10,Y67D1...

2G12 cyB only Cerep3? J.Ferrell ORDERING OF GROUPS NOT CLEAR; PRECISE...

rsc M.Maduro BOTc1 W07C10(was here)prob.mixt. Van Auken F450/dpy-18 probe:Y61F5...

ridisation. rsc M.Granato ...Y1134D3,Y119A9.

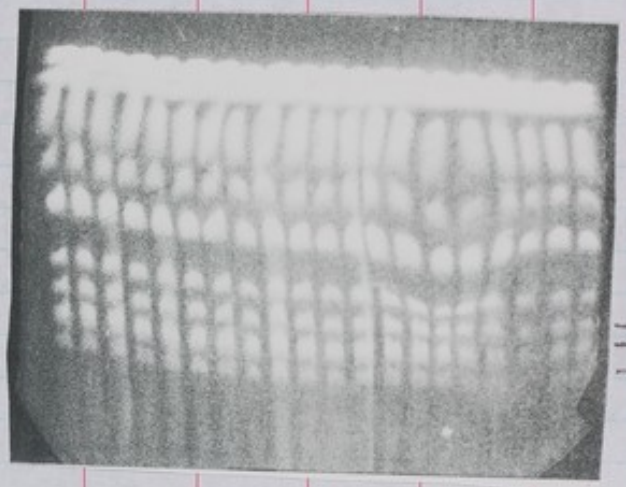
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.


cm5e5
cm14f3 cm9e10
cm5a8 YK202 cm11d5
YK525 cm14h10 cm14f11
YK288 cm10g8 cm2d9 cm19a3 YK1273 cm15a12 cm7e12 cm11e5
cm16a12 YK618 cm19h12 YK1461 cm12e10 cm18d12 YK563
cm9f5 YK1374 cm19b8 YK384 YK260 cm06e11 YK242
(Y111B2)
(Y59B3)
(Y55D1)
(Y49E10)
(pJR604)
(Y52B8)
(Y103B2U)
(Y39E4)
(Y7G11)
(Y37D8)
(Y109C2)
(Y106H1)
(Y107A12)
(Y74A9)
(Y73H2)
(Y43F4)
(Y67G10)
(Y41H5)
(Y64G11)
(Y13H2)
(Y26C10)
(Y20A12)
(Y76A2)
(Y30E6)
F51H3
BE10
ZL255
D2049
ZL241
ZL265
K05A5
ZL259
ZC482
ZC491
T26C10
F54F12
C47H10
RW#L17
CB2
C39F12
T23H7
C28H9
M143.08C7
B0502A6
B0291
B0211
C45G2
C25B6
T03F6
T02D6
R14F4
F53A2
F45G2
F11F1
C26A4
RW#L66
E01H3
R11C7
D1042
ZC320
T12C3
B0543
F54G11
T05D4
T27E9
C30E9
C10H9
C43H9
W04D5
R150
K08E3
ZC76
W03E4
JH98510
W06G6
T22E10
W02C8
T10B6
JH985
F13C11
G25B1
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
pie-1
gbr-1
stP17
ayP1
eP81
eP2
cyp-2
also:Y70H1,Y72C3,Y91D10,Y96F5,Y106H9
link to Y37D8 by polyprobing only.
yc confirmation not convincing.
cy repet
C.Mello
..but specific +ves.
K.Clancy
orientatn of group detmined by L.DeLong
BOTc1 RcD1 prob does not belong at Y4
Mello frag:Y34C7,Y49C8,Y52B8,Y59H8...
Rt of dpy-18
repet;Y20A1?Y43F4
RcB1?;RcA1?
D.Pilgrim
A.Abeliovi
APPARENT LOCATION AT Y70H7/Y62F5.
BOTc1 Cerep3
posn by cy only
also Y107B1!?posn by cy only
messy
..Y61E6,Y64C9,Y66H4,Y74B2,Y82B1,Y88D10..
repet?Y3E7?Y1B2;Y20A1?Y43F4,Y47E5
RcC9?
i/sx2 F13C11;Y1G6cyB,
..Y114C7cy,Y114D6cy
RcD1
H.Riina,D.Sattelle
posn by cy only; yc data v.poor
anoms
rep.(Waterston)
..Y89B12,Y103B2,Y106H1,Y109C2,Y111B2...
L.DeLong
should this be here?
repet;Y20A1?
J.Hodgkin
...but lin
pie-1
gbr-1
stP17
ayP1
eP81
eP2
cyp-2
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.
140-prob chimera
Y37D8: good sep
Y39E4: close to 3' chr
Y30E6: sep prob OK
Y76A2: sep prob not good
Y111B2 and Y76A2 look unavoidable
Y11BC1 pos!
needs sizing adz.
Y49E10: 150
Y43F4: 300

19/3/96

Y45F3 Bijay/JES plugs etc

23hr 150V 50sec switch.
Top removed after 5 hr



AC excision

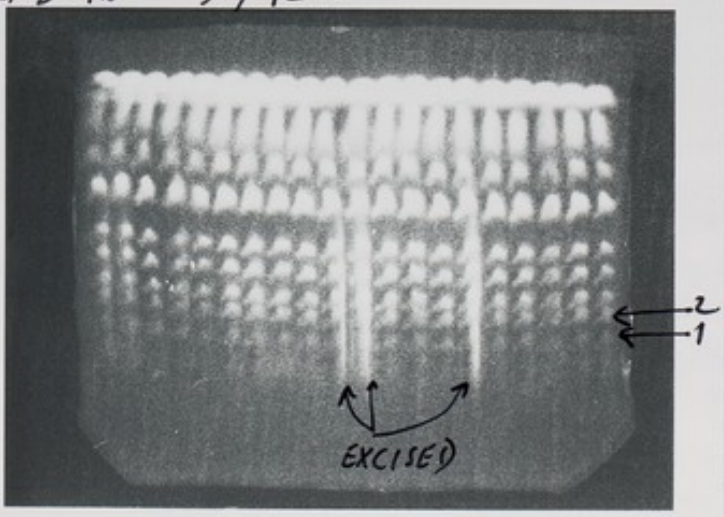
4-6 ml/gel.
Frozen storage

This end discarded - end view showed DNA run out.

Staining : 100 ml H_2O + 10 ml 10 mg/ml EtBr
2 hr + gentle shake

Wash 45' 0.5x TAE

21-3-96 Y49E10



1 = YAC, 2 = YEAST.

PRELIMINARY RESULTS (no floating points!)

Statistics of Y111B2 620kb, sep^n ok III (pic-1)
YEAST = 40%
SEQVEC = 5%
CLONVEC = 0%
WORM = 55%

Statistics of Y48A6 300kb, good sep^n III (pha-1)
YEAST = 42%
SEQVEC = 7%
CLONVEC = 14%
WORM = 36%
W05B2
W09D6

Statistics of Y37D8 420kb, good sep^n III (pic-1)
YEAST = 36%
SEQVEC = 45%
CLONVEC = 0%
WORM = 18%

Statistics of Y76A2 280kb, good sep^n III (..)
YEAST = 24%
SEQVEC = 0%
CLONVEC = 14%
WORM = 61%

Statistics of Y43F4 300kb, good sep^n III (..)
YEAST = 45%
SEQVEC = 1%
CLONVEC = 1%
WORM = 50%

Genuine c.e reads? cf. known cosmid data.

What status of other slices.?

YAC	COSMID	HITS
Y48A6	W09D6	Y48A6c7.s1t
Y37D8	ZK1010	Y48A6c11.s1t Y48A6c7.s1t
	F14F7	Y48A6c7.s1t
	F54F12	Y48A6c7.s1t
Y43F4	F56A8	Y43F4a1.s1t Y43F4d9.s1t Y76A2c1.s1t
	F53A2	Y48A6c7.s1t
	T03F6	NO MATCHES
	F45G2	Y43F4a8.s1t Y43F4c10.s1t Y43F4d3.s1t
	T27E9	Y111B2b6.s1t Y43F4e5.s1t Y43F4e8.s1t Y48A6c7.s1t
Y76A2	T27E9	Y111B2b6.s1t Y43F4e5.s1t Y43F4e8.s1t Y48A6c7.s1t
	T25C8	NO MATCHES

NAME Y111B2

	1	2	3	4	5	6	7	8	9
ROW a	27	274	dirty	-	pUC18	78	313	136.S	-
ROW b	dirty	-	160.S	215.S	-	90.S	390	dirty	-
ROW c	.	.	.	.	.	.	.	.	.
ROW d	.	.	.	.	.	.	.	.	.
ROW e	.	.	.	.	.	.	.	.	.
ROW f	.	.	.	.	.	.	.	.	.
ROW g	.	.	.	.	.	.	.	.	.
ROW h	.	.	.	.	.	.	.	.	.

NAME Y37D8

	1	2	3	4	5	6	7	8	9
ROW a	387	-	0.S	pUC18	-	-	dirty	pUC18	pUC18
ROW b	.	.	.	.	.	.	.	.	.
ROW c	.	.	.	.	.	.	.	.	.
ROW d	.	.	.	.	.	.	.	.	.
ROW e	.	.	.	.	.	.	.	.	.
ROW f	.	.	.	.	.	.	.	.	.
ROW g	.	.	.	.	.	.	.	.	.
ROW h	.	.	.	.	.	.	.	.	.

NAME Y48A6

	1	2	3	4	5	6	7	8	9
ROW a	55.S	30.C	152.S	-	-	dirty	dirty	dirty	dirty
ROW b	69.S	-	dirty	-	119.S	dirty	dirty	pYAC4	-
ROW c	-	-1.S	-	-	pUC18	-	439	125.S	-
ROW d	dirty	-	pUC18	pYAC4	-	-	404	162.S	pUC18
ROW e	dirty	117	dirty	dirty	dirty	dirty	dirty	dirty	dirty
ROW f	dirty	pYAC4	dirty	dirty	dirty	dirty	dirty	dirty	dirty
ROW g	dirty	pYAC4	dirty	pUC18	-	-	-	pYAC4	pYAC4
ROW h	dirty	dirty	161	-	-	69	dirty	56.S	50.S

NAME Y43F4

	1	2	3	4	5	6	7	8	9
ROW a	87.S	264.S	-	-	533	-	-	264	43
ROW b	-	50.S	-	172.S	89.S	-	-	-	486
ROW c	-	-	96.S	149.S	-	-	78.S	-	-
ROW d	-	-	520	161.S	24.S	pUC18	dirty	204.S	495
ROW e	309.S	-	dirty	9.C	552	485	-	258	-
ROW f	.	.	.	.	.	.	.	.	.
ROW g	.	.	.	.	.	.	.	.	.
ROW h	.	.	.	.	.	.	.	.	.

NAME Y76A2

	1	2	3	4	5	6	7	8	9
ROW a	258	-	97.S	-	-	-	398	477	305
ROW b	pYAC4	218	83.S	-	246	pYAC4	255	42.S	-
ROW c	43.S	-	162.S	111	pYAC4	pYAC4	250	213	252
ROW d	27.S	-	165.S	89.S	251	158.S	-1.S	pYAC4	63.S
ROW e	100.S	-	pYAC4	-	214	-	249	-1.S	pYAC4
ROW f	.	.	.	.	.	.	.	.	.
ROW g	.	.	.	.	.	.	.	.	.
ROW h	.	.	.	.	.	.	.	.	.

YAC	cosmid	6/18/96
Y48A6	W09D6	(40 kb sg)
Y37D8	ZK1010	(37 kb sg)
	F14F7	(41 kb sg)
	F54F12	(36 kb sg)
Y43F4	F56A8	(37 kb sg)
	F13A2	(41 kb sg)
	T03F6	(finished)
	F45G2	(36 kb sg)
	T27E9	(35 kb sg)
Y116A2	T27E9	(35 kb sg)
	T25C8	(29 kb sg)

111

Sel

Poss's

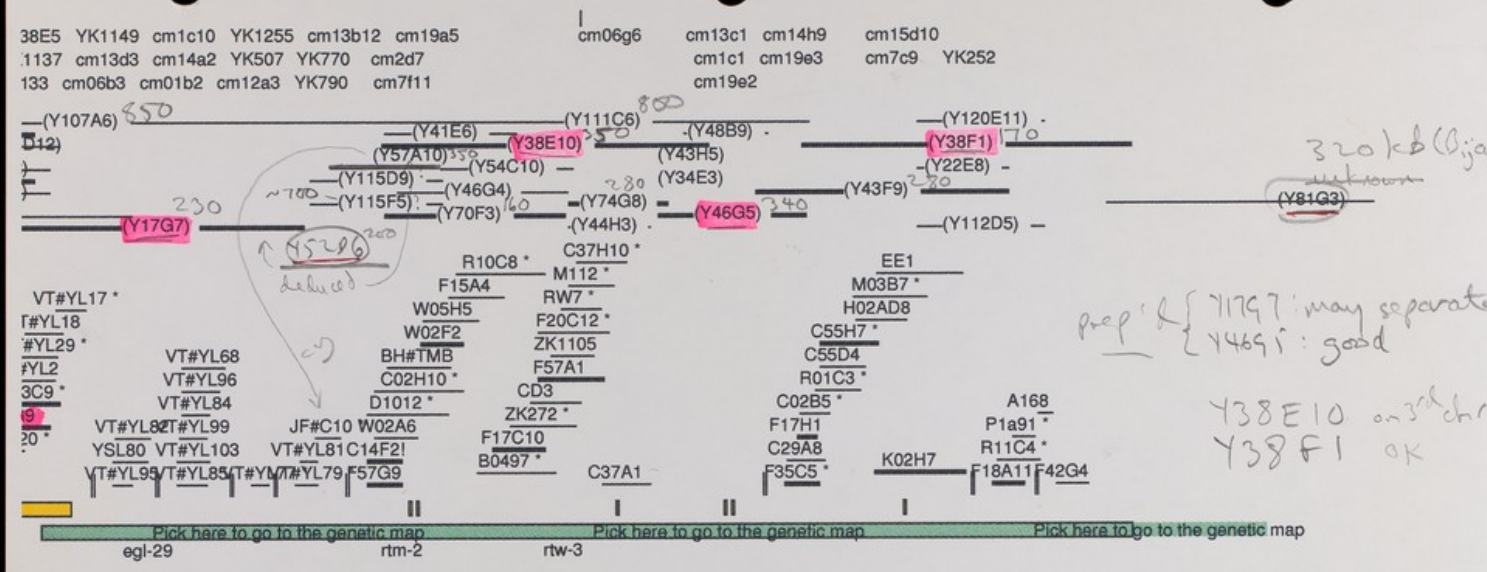
✓ Y45F3 280
✓ Y39A1 } early mayg efforts. What is status?
✓ Y52D3 }
✓ Y48A6 300

Y4703 or Y107D10?

✓ Y41C4 300 - for window draister (Bijay)

//

✓ Y49E10 150
✓ Y111B2 620
✓ Y37D8 420
✓ Y39E4 350 — for window draister (bijay)
✓ Y43F4 300
✓ Y76A2 280



...by single large YAC. posn by cy only;could be more extensive. C37A1 mix? Cerep3 LESS THAN 225kB TO F29C12....

@Y17G7 A.Papp posn by cy only;could be more extensive. sl.repet.,thus dub.

VT#YL77 & 79 cy+ve WAS JOINED TO Y67C11 BY WEAK CY.DISMNTLD posn by cy only. JOIN BY cm19c10. HIGHLY UNCONFIRMED.

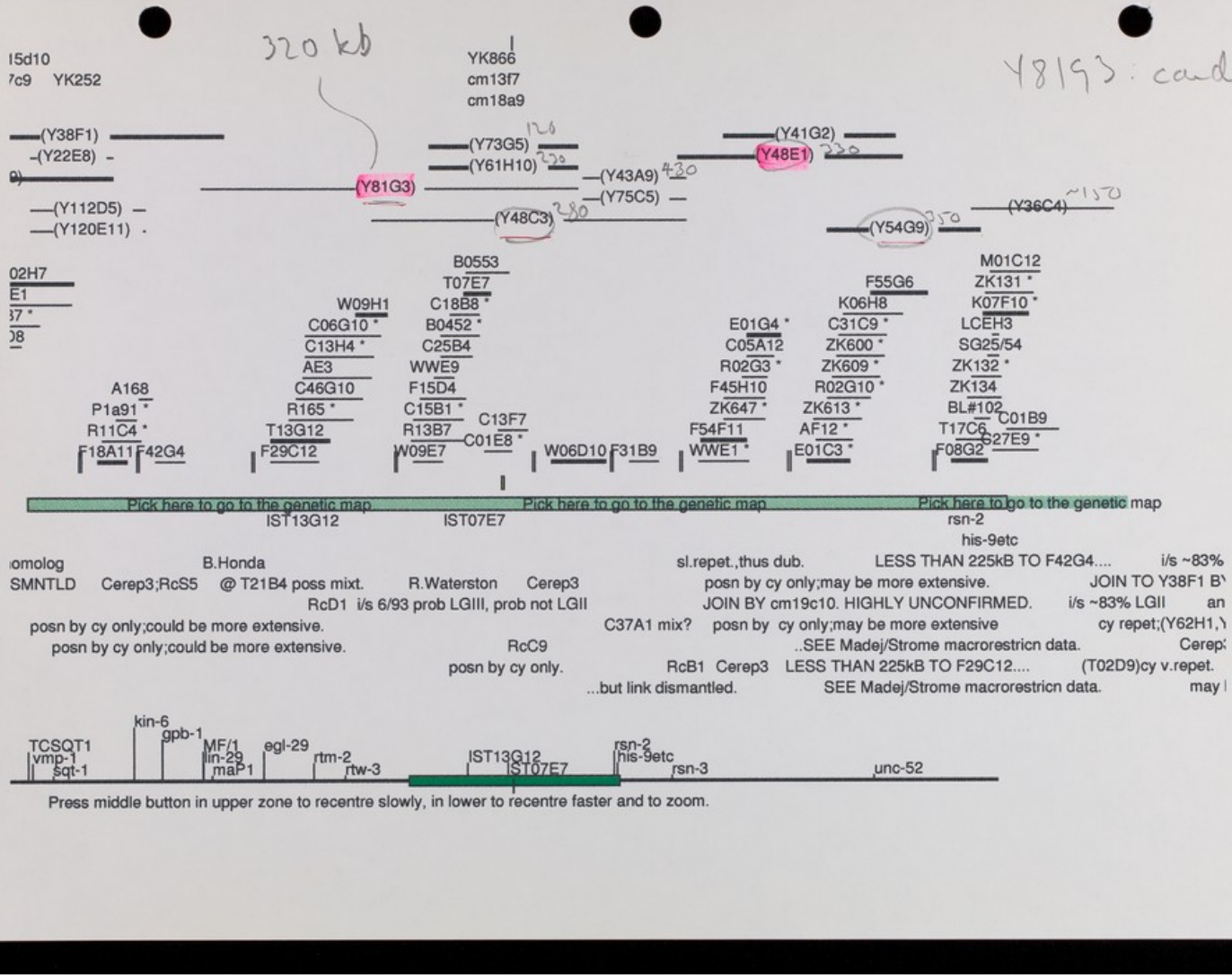
@Y17G7 A.Papp was at C38E12 (poss) by f/p. R.Waterston ...but link dismantled.

A.Papp;BOTc1 rsc A.Newman o/l VT#'s dubious Cerep3;RcS5 T21B4 poss mixt. posn by cy only;may be more extensive.

_IN29 RcS5 VERY FLIMSY JOIN: poor yc data and... B.Honda i/s 6/93 prob LGIII, prob not LGII ..SEE Madej/Strome macrorestricn d

band o/l VT#YL34 @Y17G7 A.Papp J.Fleming; rRas homolog @ RcD1 RcC9 Cerep3 RcB1 posn by cy only;may be more extensive

Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.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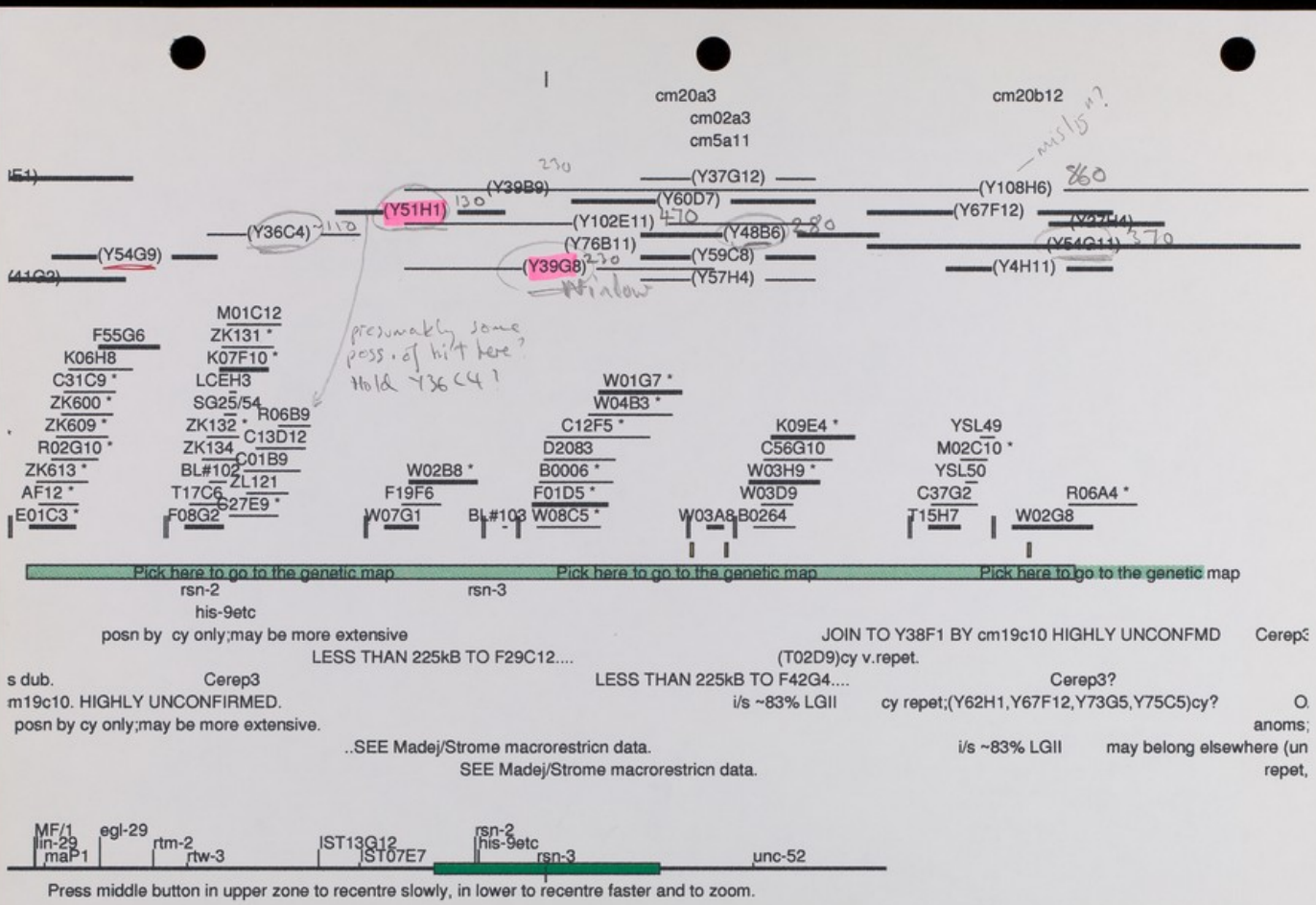
I5d10
?c9 YK252
YK866
cm13f7
cm18a9
(Y38F1)
(Y22E8)
(Y112D5)
(Y120E11)
(Y81G3)
(Y73G5)
(Y61H10)
(Y48C3)
(Y43A9)
(Y75C5)
(Y41G2)
Y48E1
(Y54G9)
(Y36C4)
02H7
E1
B0553
T07E7
C18B8 *
B0452 *
C25B4
WWE9
F15D4
C15B1 *
R13B7
C01E8 *
C13F7
W09E7
W09H1
C06G10 *
C13H4 *
AE3
C46G10
R165 *
T13G12
F29C12
A168
P1a91 *
R11C4 *
F18A11
F42G4
W06D10
F31B9
E01G4 *
C05A12
R02G3 *
F45H10
ZK647 *
F54F11
WWE1 *
F55G6
K06H8
C31C9 *
ZK600 *
ZK609 *
R02G10 *
ZK613 *
AF12 *
E01C3 *
M01C12
ZK131 *
K07F10 *
LCEH3
SG25/54
ZK132 *
ZK134
BL#102
C01B9
T17C6
G27E9 *
F08G2
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
IST13G12
IST07E7
rsn-2
his-9etc
omolog
B.Honda
SMNTLD Cerep3;RcS5 @ T21B4 poss mixt.
R.Waterston Cerep3
RcD1 i/s 6/93 prob LGIII, prob not LGII
posn by cy only;could be more extensive.
posn by cy only;could be more extensive.
RcC9
posn by cy only.
sl.repet.,thus dub.
LESS THAN 225kB TO F42G4.... i/s ~83%
posn by cy only;may be more extensive.
JOIN TO Y38F1 B
JOIN BY cm19c10. HIGHLY UNCONFIRMED.
i/s ~83% LGII an
C37A1 mix? posn by cy only;may be more extensive
cy repet;(Y62H1,
..SEE Madej/Strome macrorestricn data.
Cerep
RcB1 Cerep3 LESS THAN 225kB TO F29C12....
(T02D9)cy v.repet.
...but link dismantled.
SEE Madej/Strome macrorestricn data.
may
kin-6
gpb-1
TCSQT1
vmp-1
sqt-1
MF/1
lin-29
maP1
egl-29
rtm-2
rtw-3
IST13G12
IST07E7
rsn-2
his-9etc
rsn-3
unc-52
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.



posn by cy only;may be more extensive

LESS THAN 225kB TO F29C12....

JOIN TO Y38F1 BY cm19c10 HIGHLY UNCONFMD

(T02D9)cy v.repet.

Cerep3

s dub.

Cerep3

LESS THAN 225kB TO F42G4....

Cerep3?

m19c10. HIGHLY UNCONFIRMED.

i/s ~83% LGII

cy repet;(Y62H1,Y67F12,Y73G5,Y75C5)cy?

posn by cy only;may be more extensive.

anoms;

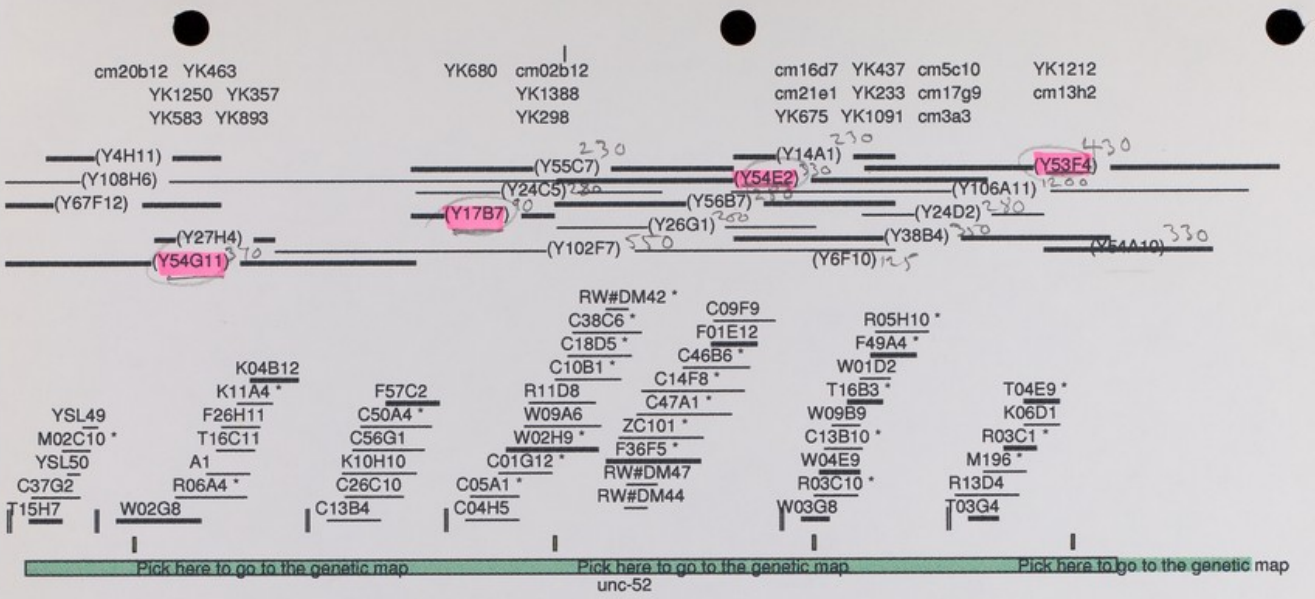
..SEE Madej/Strome macrorestricn data.

i/s ~83% LGII

may belong elsewhere (un

SEE Madej/Strome macrorestricn data.

repet,



38F1 BY cm19c10 HIGHLY UNCONFMD repet,so dub W02B8etc,W07G1 somewhere here?

ɜt.

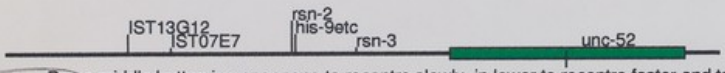
Cerep3?

repet;(Y62H1,Y67F12,Y73G5,Y75C5)cy? U2-3,U2-4;T.Blumenthal,J.Thomas Rc35 Cerep

anoms; sl poss eP1 RcC9 i/s ZK134 X 48-72 O/L Y51H1 good by lambda grid (28/9) may be mislign

i/s ~83% LGII may belong elsewhere (under Y51H7 etc). S.Emmons rsn-3 here somewhe

Cerep3 O/L Y36C4 good by lambda grid(28/9) U2-5,U2-6;T.Blumenthal,J.Thomas B0563etc<removed



Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.

Selection:

10/5/96

Y1797 230 kb
Y469 340 kb

27/6/96

Y38E10 310 kb
Y38F1 170 kb

31/8/96

Y48E1 370 kb (JES)

19/9/96

Y549 11 (370)
Y17B7 (90)
Y54E2 (330)
Y53F4 (430)

22/10/96

Y8193 (320)
Y53C12 (280)

28/10/96

Y398 WIA (230)

1,,
Summary-line: 18-Oct zarkower@lenti.med.umn.ed #Re: mab-3 etc.
Mail-from: From zarkower@lenti.med.umn.edu Fri Oct 18 17:24:51 1996
Received: from mhub2.tc.umn.edu by sanger.ac.uk (4.1/SMI-4.1)
        id AA08895; Fri, 18 Oct 96 17:24:38 BST
Return-Path: <zarkower@lenti.med.umn.edu>
Received: from lenti.med.umn.edu by mhub2.tc.umn.edu; Fri, 18 Oct 96 11:22:19
[illegible]te: Fri, 18 Oct 96 11:22:12 CDT
Received: from [134.84.112.176] (x112-176.med.umn.edu) by lenti.med.umn.edu; F
X-Sender: zarkower@lenti.med.umn.edu
Message-Id: <v01530503ae8d52b24b6b@[134.84.112.176]>
Mime-Version: 1.0
Content-Type: text/plain; charset="us-ascii"
To: alan@sanger.ac.uk
From: zarkower@lenti.med.umn.edu (David Zarkower)
Subject: Re: mab-3 etc.

*** EOOH ***
Return-Path: <zarkower@lenti.med.umn.edu>
Date: Fri, 18 Oct 96 11:22:12 CDT
X-Sender: zarkower@lenti.med.umn.edu
Mime-Version: 1.0
Content-Type: text/plain; charset="us-ascii"
To: alan@sanger.ac.uk
From: zarkower@lenti.med.umn.edu (David Zarkower)
Subject: Re: mab-3 etc.

Dear Alan,

You've made my day (and trust me, that took some doing today)! I do think it would be a great plan to put Y53C12 into production and for us to proceed with our current plans in the meantime. If we had even some shotgun sequence from inside the bridge we could start pulling out clones [redacted] fingerprint and test for rescue, and we might be able to find an orphan cosmid contig or get more fosmids. Also, from our standpoint, even if our Tc1 screen works, having some sequence or physical map in the region would be a huge help. One could make a case that as a biggish bridge in a gene-dense and otherwise sequenced region, this one makes sense to tackle now.

I pulled out my notebook and had a look at the PFG picture, and the YAC runs just below the second chromosome, if I have my names right - the chromosome that is about 300 kb. There is reasonable separation - about the same width as the YAC band. We did two runs on your PFG apparatus at the LMB; in one the YAC and chromosome were a poorly resolved doublet, and in the second longer run under slightly different conditions there was better resolution and I would guess there was not much yeast DNA around (the YAC was about 1/4 of the distance from chromo 2 down to chromo 1). I could certainly send you what is left of my prep if you are interested, but I suspect there isn't enough DNA to get a decent quality library. I also have the PFG conditions, as recorded by Rachel Aronoff, with whom I shared the gels, in case those are of interest: run length was 54.5 hr, 1% LMP gel, 150 V, about 150 mA; times: 18s, 16h; 21s, 5h; [23s, 3h; 28s, 18h - maybe this part could be longer]; 33s, 6h; 38 s, 6h.

If you think lambda walking would be sufficiently fast, I'll look into vectors - let me know if it makes any difference to you in terms of fingerprinting etc. I was just going to see which commercial kits seemed to have the best system for making end probes and getting rapid turnaround between steps. I also intended to use CES200 unless a better host strain [redacted]rns up.

Thanks again for the good news. You guys are giving the field a huge boost, and our pleading and wheedling is just evidence of it!

Best wishes,

Dave

11

✓ 41797 230 200 Y52D6? Y57A10? 320

✓ Y38E10 310 - for window transfer (Vijay)

✓ Y46955 340

✓ Y38F1 170

22/10 ✓ Y48193 320

Y48C3 280 attempted window transfer 1/10

✓ Y48E1 330

Y5459 310

Y36C4? 150 Hold for Y51H1 data?

✓ Y51H1 130

✓ Y3958 230 attempted window transfer 1/10

Y4886 280 " "

✓ Y5491 270

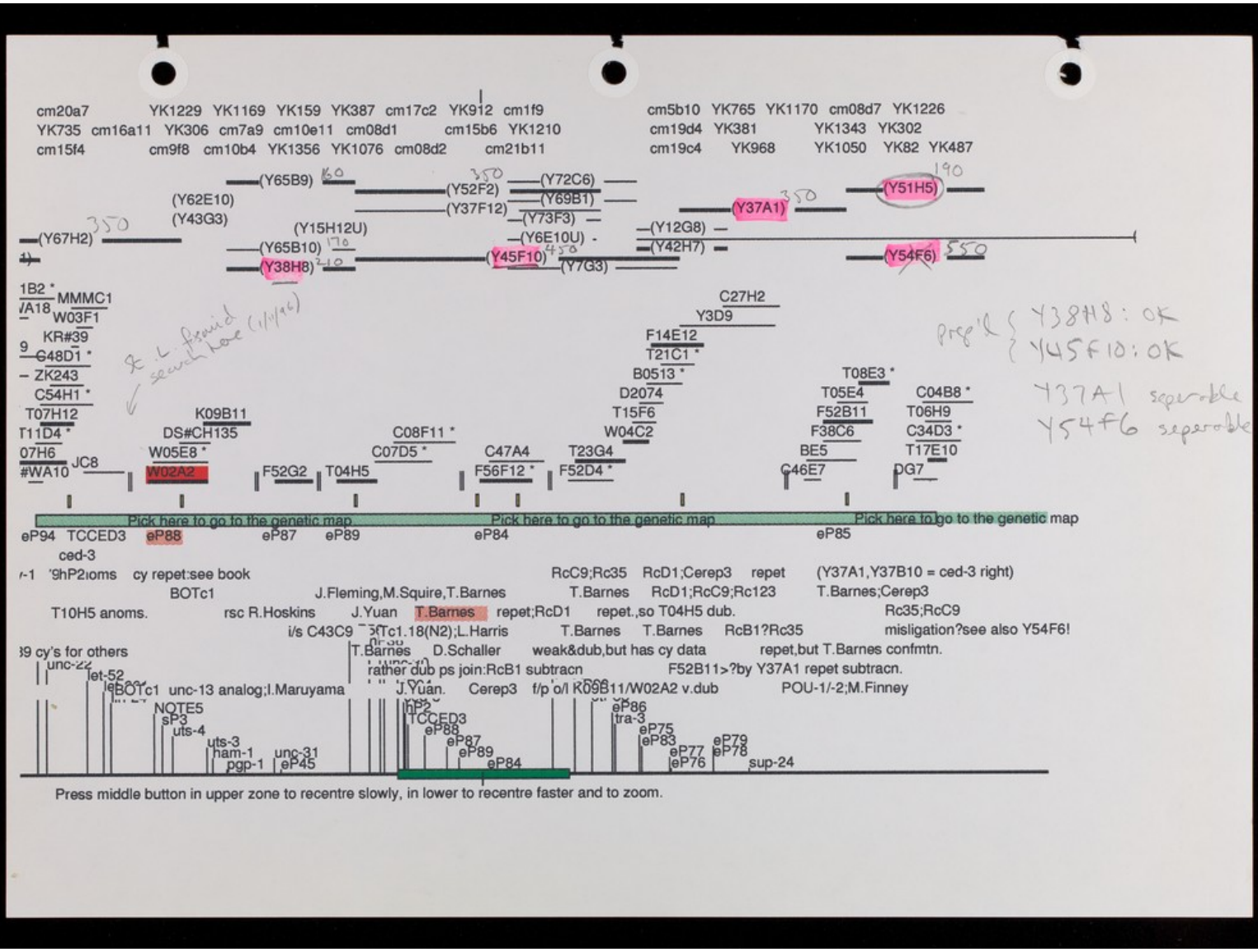
✓ Y17B7 90

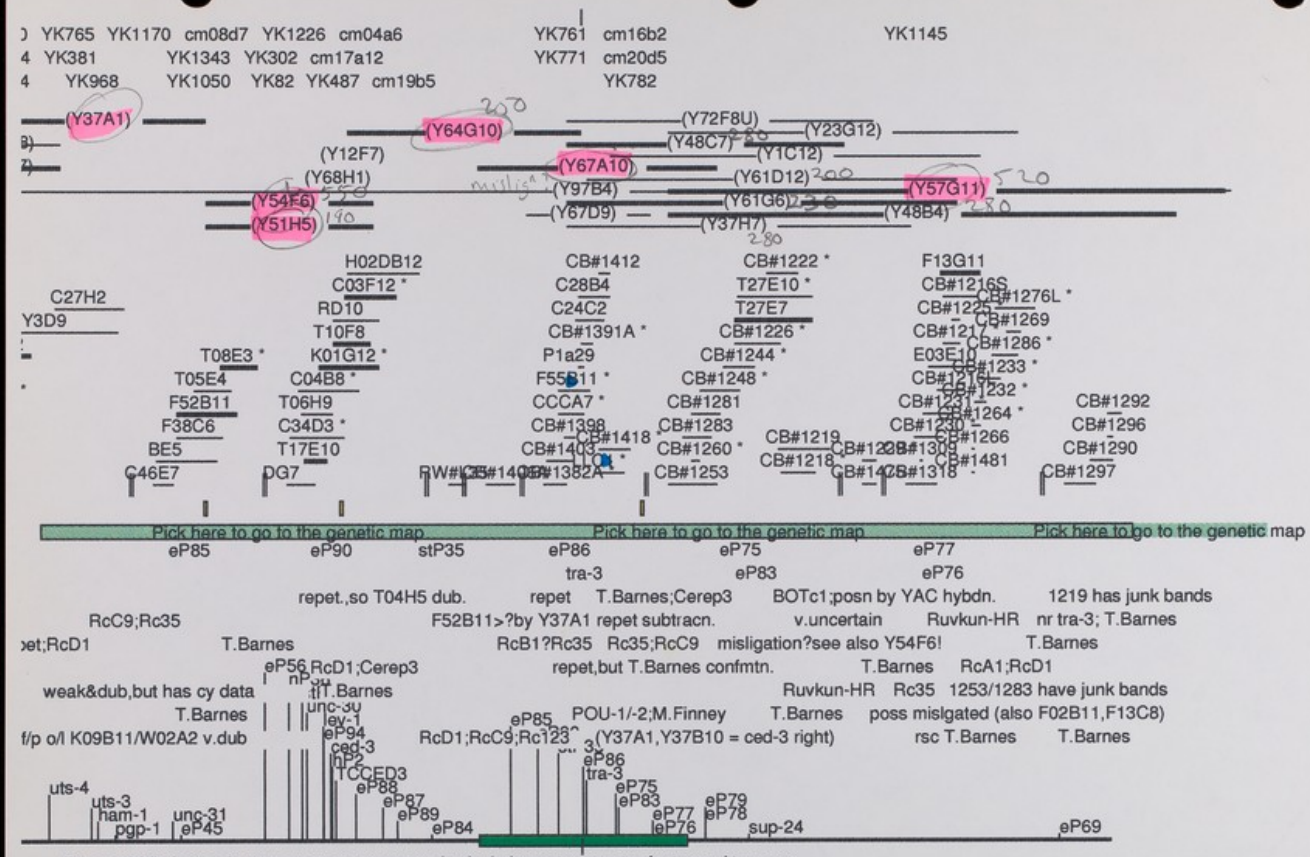
✓ Y54E2 330

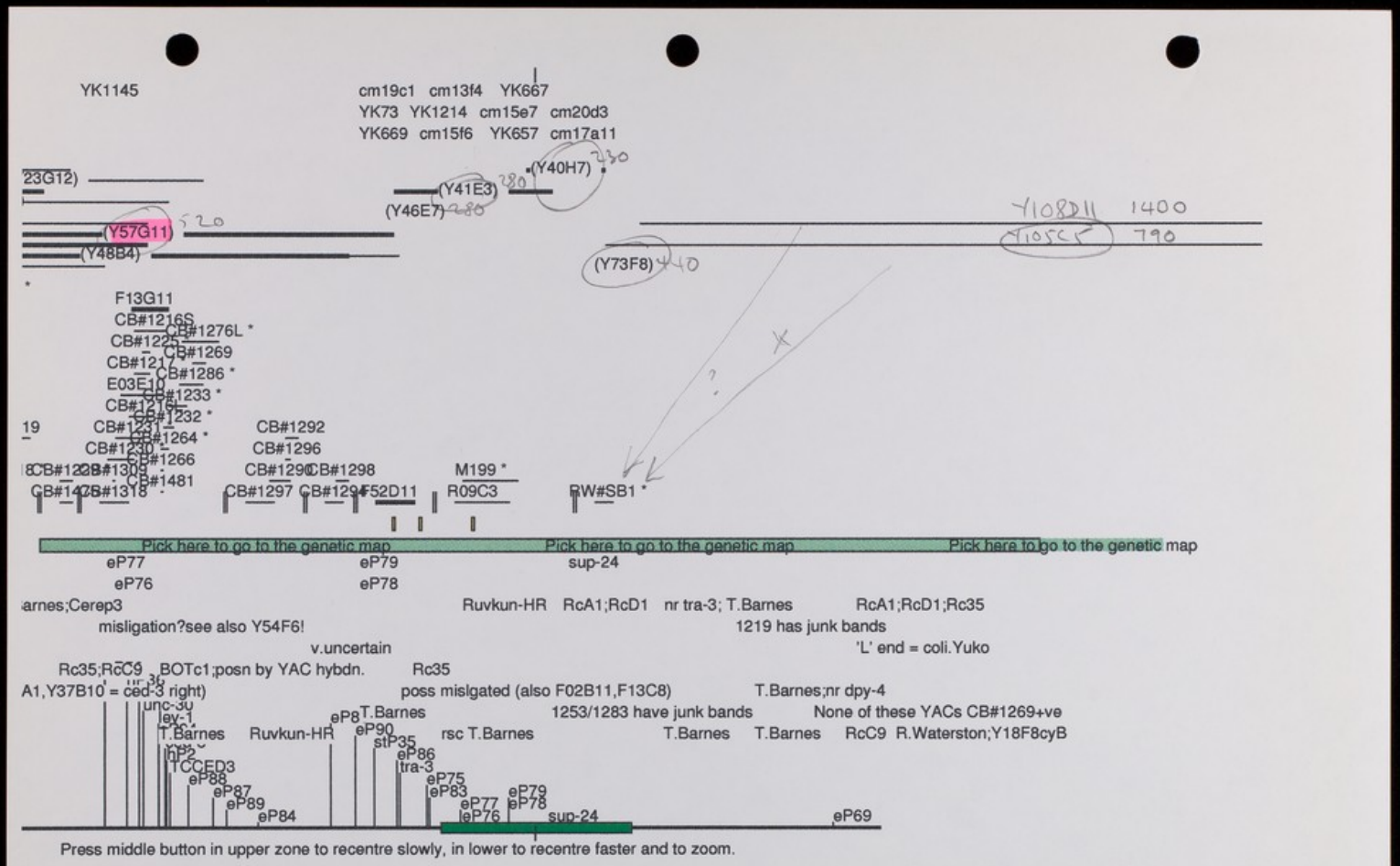
✓ Y53F4 430

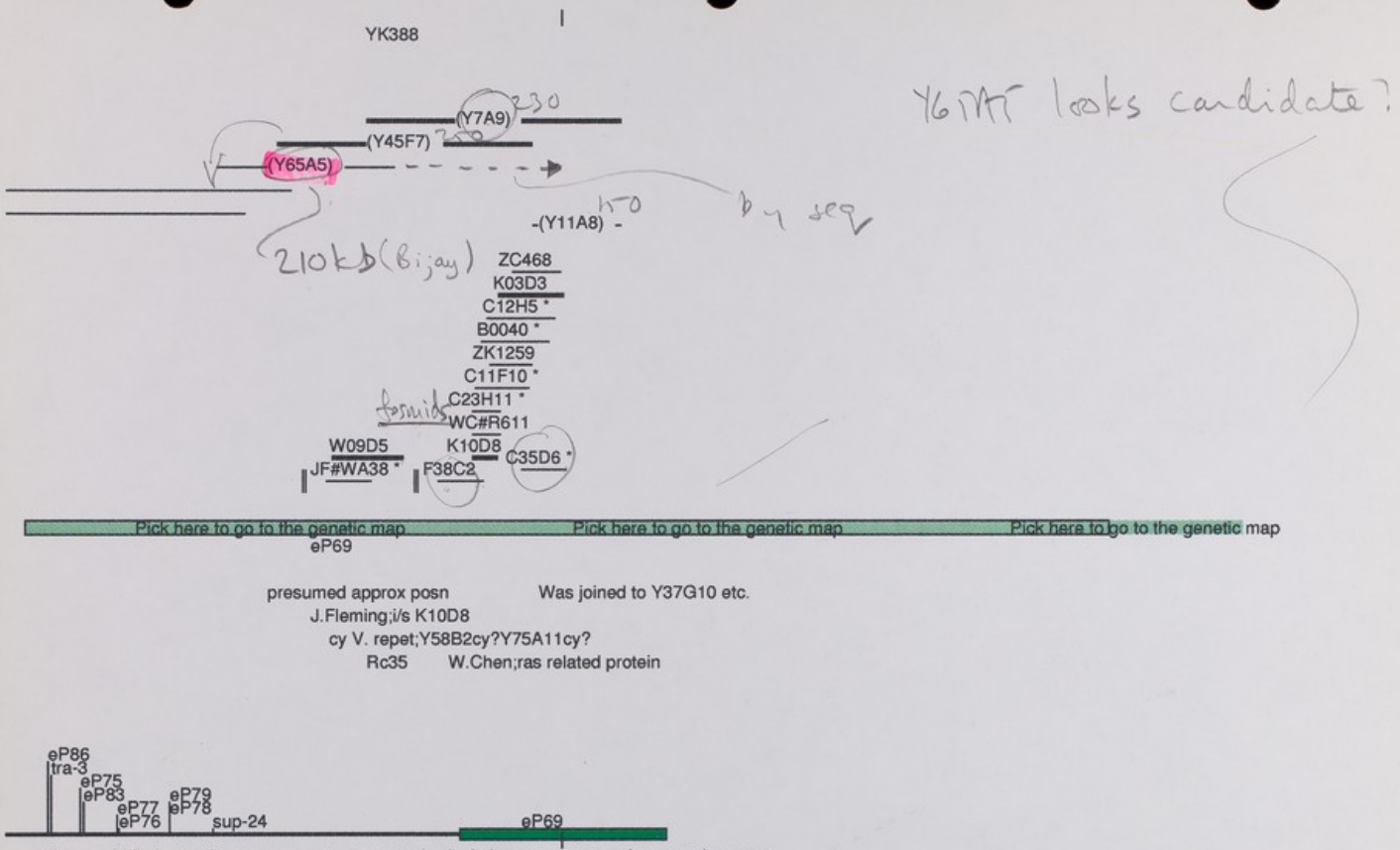
Cluster

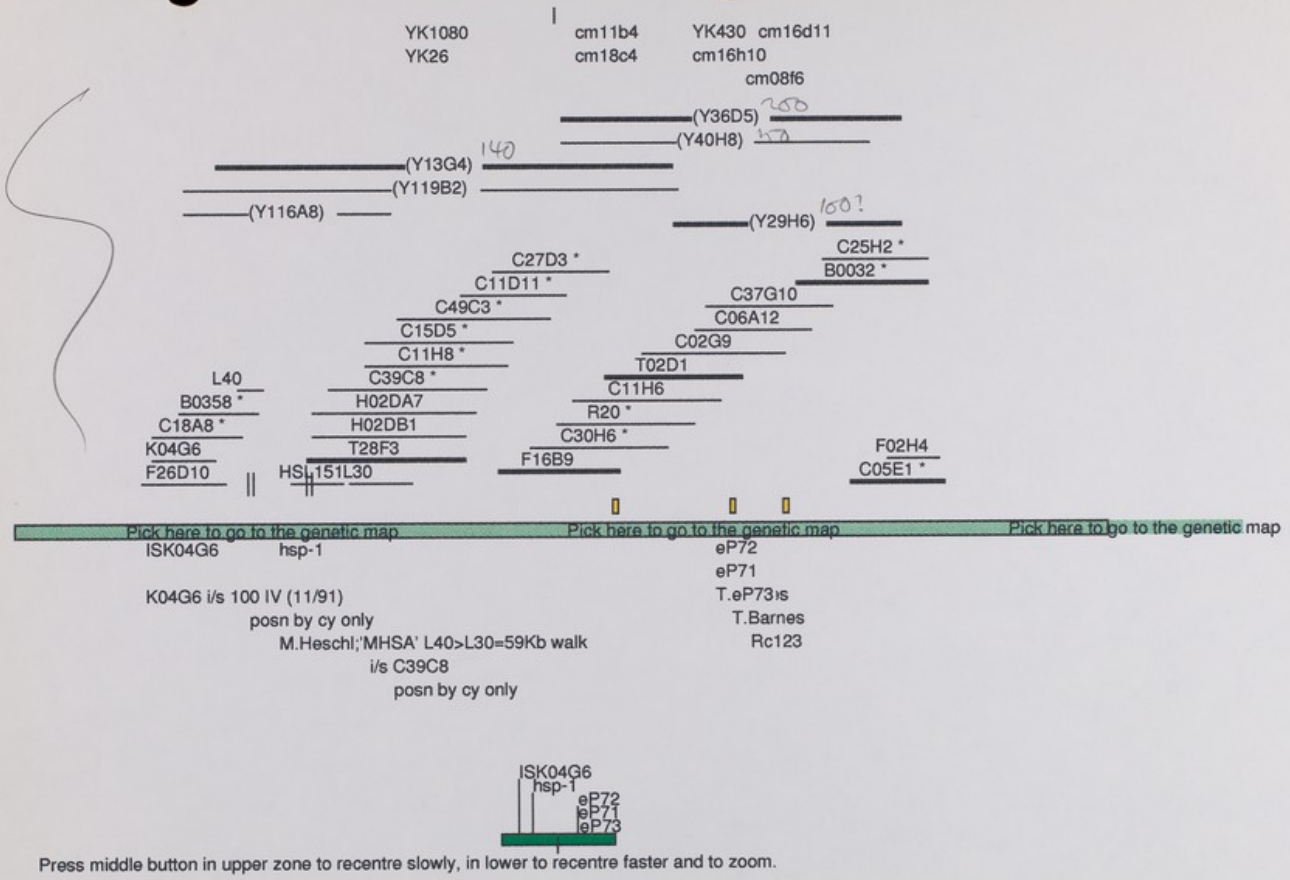
22/10 ✓ Y53C12 280 (Zark says seperate - see in 'Notes') mab-3.


cm20a7 YK1229 YK1169 YK159 YK387 cm17c2 YK912 cm1f9
YK735 cm16a11 YK306 cm7a9 cm10e11 cm08d1 cm15b6 YK1210
cm15f4 cm9f8 cm10b4 YK1356 YK1076 cm08d2 cm21b11
cm5b10 YK765 YK1170 cm08d7 YK1226
cm19d4 YK381 YK1343 YK302
cm19c4 YK968 YK1050 YK82 YK487
(Y65B9)
(Y62E10)
(Y43G3)
(Y67H2)
(Y15H12U)
(Y65B10)
(Y38H8)
(Y52F2)
(Y37F12)
(Y72C6)
(Y69B1)
(Y73F3)
(Y6E10U)
(Y45F10)
(Y7G3)
(Y37A1)
(Y51H5)
(Y12G8)
(Y42H7)
(Y54F6)
C27H2
Y3D9
F14E12
T21C1 *
B0513 *
D2074
T15F6
W04C2
T23G4
F52D4 *
C47A4
F56F12 *
C08F11 *
C07D5 *
T04H5
F52G2
W02A2
W05E8 *
DS#CH135
K09B11
T08E3 *
T05E4
F52B11
F38C6
BE5
C46E7
C04B8 *
T06H9
C34D3 *
T17E10
PG7
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
eP94 TCCED3 eP88 eP87 eP89 eP84 eP85
ced-3
BOTc1 J.Fleming,M.Squire,T.Barnes T.Barnes RcD1;RcC9;Rc123 T.Barnes;Cerep3
T10H5 anoms. rsc R.Hoskins J.Yuan T.Barnes repet;RcD1 repet.,so T04H5 dub. Rc35;RcC9
T.Barnes D.Schaller weak&dub,but has cy data repet,but T.Barnes confmtn.
rather dub ps join:RcB1 subtracn F52B11>?by Y37A1 repet subtracn.
unc-13 analog;I.Maruyama J.Yuan. Cerep3 f/p o/l K09B11/W02A2 v.dub POU-1/-2;M.Finney
let-52
NOTE5
sP3
uts-4
uts-3
ham-1
unc-31
pgp-1
eP45
eP84
eP89
eP87
TCCED3
eP88
eP86
tra-3
eP75
eP83
eP77
eP76
eP79
eP78
sup-24
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.


YK765 YK1170 cm08d7 YK1226 cm04a6
YK381 YK1343 YK302 cm17a12
YK968 YK1050 YK82 YK487 cm19b5
YK761 cm16b2
YK771 cm20d5
YK782
YK1145
(Y37A1)
(Y64G10)
(Y72F8U)
(Y48C7)
(Y23G12)
(Y12F7)
(Y1C12)
(Y67A10)
(Y68H1)
(Y97B4)
(Y61D12)
(Y57G11)
(Y54F6)
(Y51H5)
(Y67D9)
(Y61G6)
(Y48B4)
(Y37H7)
H02DB12
CB#1412
CB#1222 *
F13G11
C03F12 *
C28B4
T27E10 *
CB#1216S
C27H2
RD10
C24C2
T27E7
CB#1276L *
Y3D9
T10F8
CB#1391A *
CB#1226 *
CB#1225
CB#1269
T08E3 *
K01G12 *
P1a29
CB#1244 *
CB#1217
CB#1286 *
T05E4
C04B8 *
F55B11 *
CB#1248 *
E03E10
CB#1233 *
F52B11
T06H9
CCCA7 *
CB#1281
CB#1232 *
CB#1292
F38C6
C34D3 *
CB#1398
CB#1283
CB#1264 *
CB#1296
BE5
T17E10
CB#1403
CB#1260 *
CB#1219
CB#1266
CB#1290
C46E7
DG7
CB#1382A
CB#1253
CB#1218
CB#1481
CB#1297
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
eP85
eP90
stP35
eP86
eP75
eP77
tra-3
eP83
eP76
repet.,so T04H5 dub.
repet T.Barnes;Cerep3
BOTc1;posn by YAC hybdn.
1219 has junk bands
RcC9;Rc35
F52B11>?by Y37A1 repet subtracn.
v.uncertain
Ruvkun-HR
nr tra-3; T.Barnes
RcD1
T.Barnes
RcB1?Rc35 Rc35;RcC9
misligation?see also Y54F6!
T.Barnes
eP56 RcD1;Cerep3
repet,but T.Barnes confmtn.
T.Barnes
RcA1;RcD1
weak&dub,but has cy data
T.Barnes
Ruvkun-HR
Rc35
1253/1283 have junk bands
T.Barnes
ley-1
eP85
POU-1/-2;M.Finney
T.Barnes
poss mislgated (also F02B11,F13C8)
f/p o/l K09B11/W02A2 v.dub
eP94
RcD1;RcC9;Rc123
(Y37A1,Y37B10 = ced-3 right)
rsc T.Barnes
T.Barnes
ced-3
eP86
TCCED3
tra-3
uts-4
eP88
eP75
uts-3
eP87
eP83
eP79
ham-1
eP89
eP77
eP78
unc-31
eP84
eP76
sup-24
eP69
pgp-1
eP45
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.


YK1145
cm19c1 cm13f4 YK667
YK73 YK1214 cm15e7 cm20d3
YK669 cm15f6 YK657 cm17a11
(Y40H7)
(Y41E3)
(Y46E7)
(Y57G11)
(Y48B4)
(Y73F8)
Y108D11 1400
Y105C5 790
F13G11
CB#1216S
CB#1276L *
CB#1225
CB#1269
CB#1217
CB#1286 *
E03E10
CB#1233 *
CB#1216L
CB#1232 *
CB#1231
CB#1264 *
CB#1230
CB#1266
CB#1481
CB#1292
CB#1296
CB#1290
CB#1298
M199 *
CB#1297
CB#1294
F52D11
R09C3
RW#SB1 *
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
eP77
eP76
eP79
eP78
sup-24
Ruvkun-HR RcA1;RcD1 nr tra-3; T.Barnes RcA1;RcD1;Rc35
misligation?see also Y54F6!
1219 has junk bands
v.uncertain
'L' end = coli.Yuko
Rc35;RcC9 BOTc1;posn by YAC hybdn. Rc35
poss mislgated (also F02B11,F13C8) T.Barnes;nr dpy-4
1253/1283 have junk bands
None of these YACs CB#1269+ve
T.Barnes
Ruvkun-HR
rsc T.Barnes
T.Barnes
T.Barnes
RcC9 R.Waterston;Y18F8cyB
unc-30
lev-1
TCCED3
eP88
eP87
eP89
eP84
eP8
eP90
stP35
eP86
tra-3
eP75
eP83
eP77
eP76
eP79
eP78
sup-24
eP69
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.


YK388
Y61A? looks candidate?
(Y7A9)
(Y45F7)
(Y65A5)
-(Y11A8) -
by seq
210kb (Bijay)
ZC468
K03D3
C12H5 *
B0040 *
ZK1259
C11F10 *
C23H11 *
fosmids
WC#R611
W09D5
K10D8
C35D6 *
JF#WA38 *
F38C2
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
eP69
presumed approx posn
Was joined to Y37G10 etc.
J.Fleming;i/s K10D8
cy V. repet;Y58B2cy?Y75A11cy?
Rc35
W.Chen;ras related protein
eP86
tra-3
eP75
eP83
eP77
eP76
eP79
eP78
sup-24
eP69
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.



Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.

Selection → production

10/5/96

Y38 H8 210 kb
Y45 F10 450 kb

27/6/96

Y37 A1 350 kb
Y54 F6 ~~510~~ kb (error).

25/9/96

Y51 H5 190 kb

2/10/96

Y67 A10 200 kb
Y57 G11 ~~520~~ kb

22/10 Y65 A5 210 kb

28/10 Y64 G10 210 kb

IV

Rod's

✓439H8 210
✓445F10 450
✓437A1 350
✓451H5 190
✓464G10 210
✓467A10 200
✓457G11 520

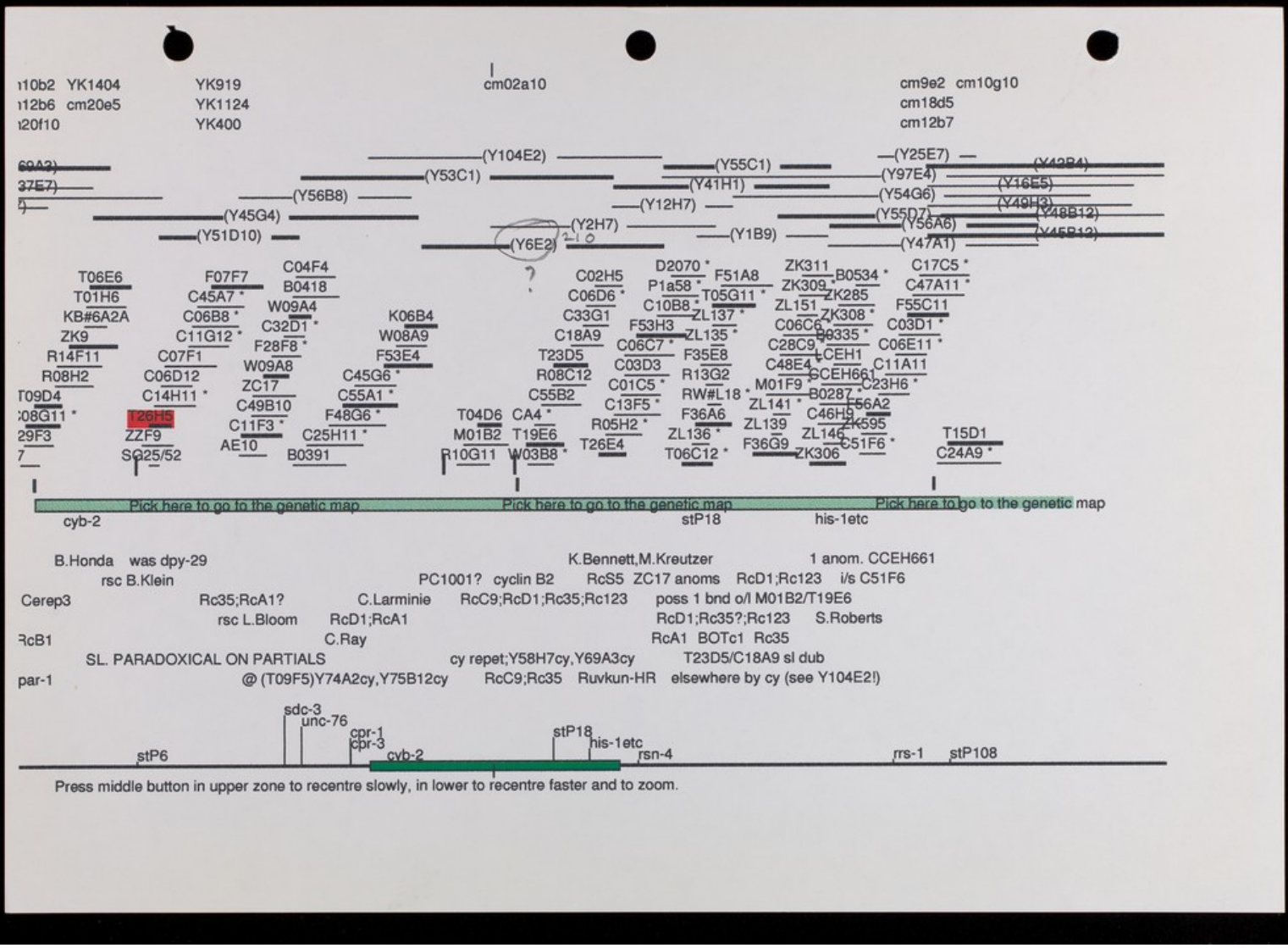
441E3 250 → window?
440H7 270 → window??
473F8 440
4105C1 790
465A1 210
47A9 230 → window?

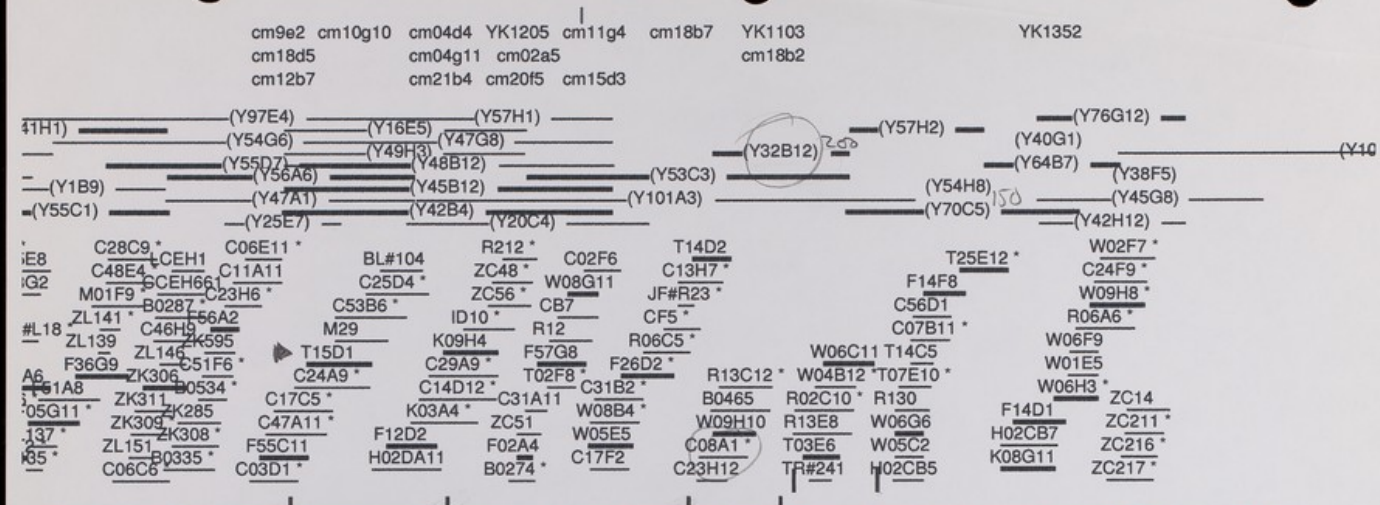
2240 ✓

//

hsp-1 contig. Look carefully at ends.

for window drawer (Bijay)


cm02a10
cm9e2 cm10g10
cm18d5
cm12b7
YK1404
YK919
YK1124
YK400
cm20e5
(Y104E2)
(Y53C1)
(Y56B8)
(Y45G4)
(Y51D10)
(Y6E2)
(Y2H7)
(Y55C1)
(Y41H1)
(Y12H7)
(Y1B9)
(Y25E7)
(Y97E4)
(Y54G6)
(Y55D7)
(Y56A6)
(Y47A1)
(Y16E5)
(Y49H3)
(Y48B12)
(Y45B12)
T06E6
T01H6
KB#6A2A
ZK9
R14F11
R08H2
C07F1
C06D12
C14H11
T26H5
ZZF9
SC25/52
F07F7
C45A7
C06B8
C11G12
C04F4
B0418
W09A4
C32D1
F28F8
W09A8
ZC17
C49B10
C11F3
AE10
C25H11
B0391
K06B4
W08A9
F53E4
C45G6
C55A1
F48G6
C02H5
C06D6
C33G1
C18A9
T23D5
R08C12
C55B2
T04D6
M01B2
R10G11
CA4
T19E6
W03B8
T26E4
C06C7
C03D3
C01C5
C13F5
R05H2
D2070
P1a58
C10B8
F53H3
F35E8
R13G2
RW#L18
F36A6
ZL136
T06C12
F51A8
T05G11
ZL137
ZL135
ZK311
ZK309
ZL151
C06C6
C28C9
C48E4
M01F9
ZL141
ZL139
F36G9
B0534
ZK285
ZK308
B0335
CEH1
CCEH661
B0287
C46H9
ZK595
ZL146
ZK306
C17C5
C47A11
F55C11
C03D1
C06E11
C11A11
C23H6
F56A2
C51F6
T15D1
C24A9
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
cyb-2
stP18
his-1etc
B.Honda was dpy-29
rsc B.Klein
K.Bennett,M.Kreutzer
1 anom. CCEH661
PC1001? cyclin B2
RcS5 ZC17 anoms
RcD1;Rc123
i/s C51F6
Cerep3
Rc35;RcA1?
C.Larminie
RcC9;RcD1;Rc35;Rc123
poss 1 bnd o/l M01B2/T19E6
rsc L.Bloom
RcD1;RcA1
RcD1;Rc35?;Rc123
S.Roberts
RcB1
C.Ray
RcA1 BOTc1 Rc35
SL. PARADOXICAL ON PARTIALS
cy repet;Y58H7cy,Y69A3cy
T23D5/C18A9 sl dub
par-1
@ (T09F5)Y74A2cy,Y75B12cy
RcC9;Rc35 Ruvkun-HR
elsewhere by cy (see Y104E2!)
sdc-3
unc-76
cpr-1
cpr-3
stP18
his-1etc
stP6
cyb-2
rsn-4
rrs-1
stP108
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.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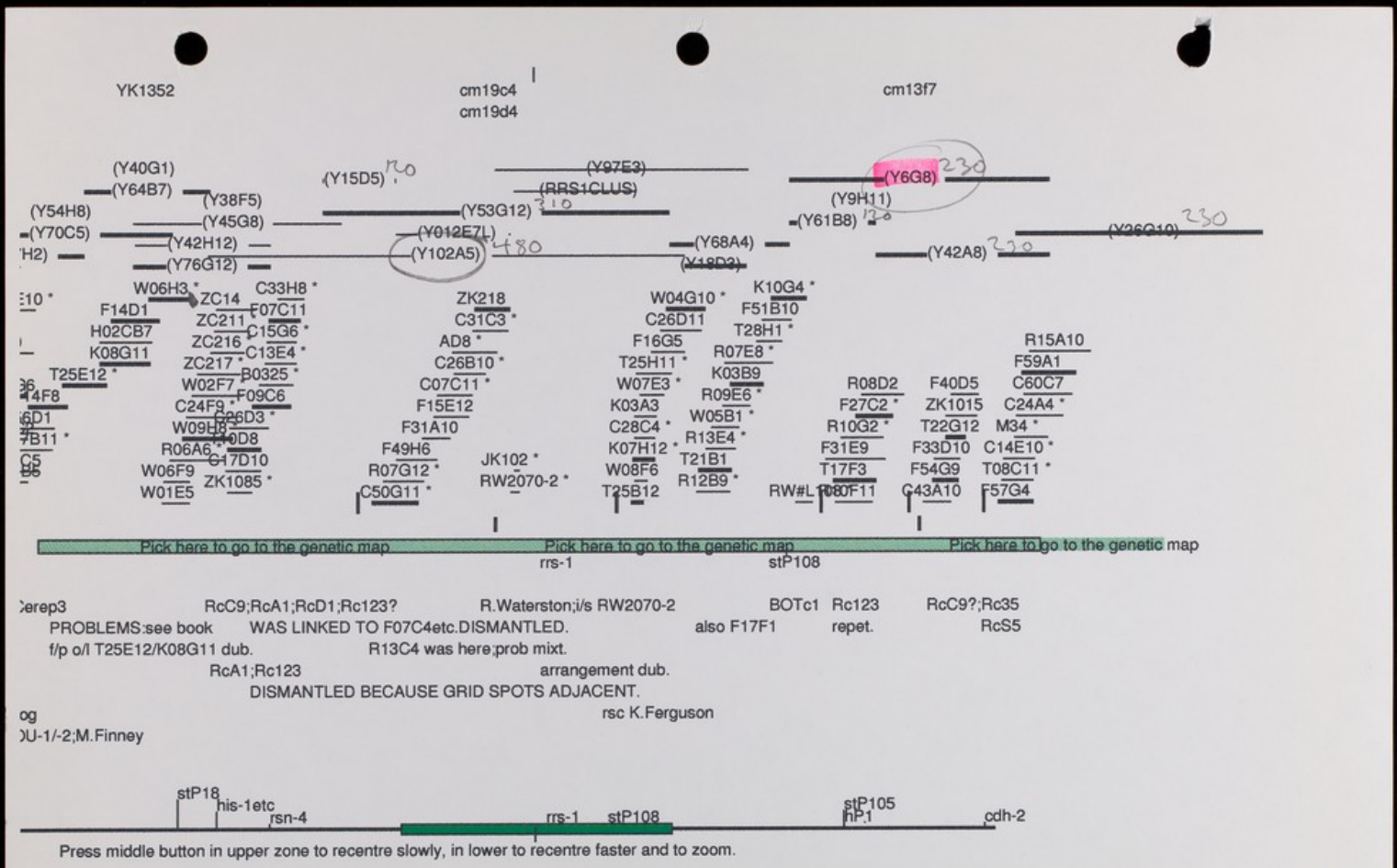
cm18d5 cm04g11 cm02a5 cm18b2
cm12b7 cm21b4 cm20f5 cm15d3
(Y97E4) (Y57H1) (Y57H2) (Y76G12)
(Y54G6) (Y16E5) (Y47G8) (Y32B12) (Y40G1)
(Y49H3) (Y64B7)
(Y55D7) (Y48B12) (Y38F5)
(Y1B9) (Y56A6) (Y45B12) (Y53C3) (Y54H8)
(Y55C1) (Y47A1) (Y101A3) (Y45G8)
(Y25E7) (Y42B4) (Y20C4) (Y70C5) (Y42H12)
C28C9 * C06E11 * R212 * T14D2 T25E12 * W02F7 *
C48E4 CEH1 C11A11 BL#104 ZC48 * C02F6 C13H7 * C24F9 *
SCEH661 C25D4 * W08G11 JF#R23 * F14F8 W09H8 *
M01F9 * C23H6 * ZC56 * C56D1
B0287 * F56A2 C53B6 * CB7 C07B11 * R06A6 *
ZL141 * C46H5 M29 ID10 * R12 CF5 * W06F9
ZL139 ZK595 K09H4 R06C5 * W06C11 T14C5 W01E5
ZL146 T15D1 F57G8 R13C12 * W04B12 * T07E10 * W06H3 *
F36G9 C51F6 * C29A9 * F26D2 * B0465 R02C10 * R130 ZC14
ZK306 C24A9 * T02F8 * W09H10 R13E8 W06G6 F14D1 ZC211 *
F61A8 B0534 * C14D12 * C31B2 * C08A1 * T03E6 W05C2 H02CB7 ZC216 *
ZK311 C17C5 * C31A11 C23H12 TR#241 H02CB5 K08G11 ZC217 *
ZK285 K03A4 * W08B4 *
ZK309 C47A11 * ZC51 W05E5
ZK308 F12D2 F02A4 C17F2
ZL151 F55C11 H02DA11 B0274 *
B0335 *
C06C6 C03D1 *
Pick here to go to the genetic map
his-1etc
rsn-4
Pick here to go to the genetic map
Pick here to go to the genetic map
RcD1;Rc35?;Rc123 BOTc1 Rc35 RcD1 RcD1;RcA1;Cerep3 <C14G5? POU-1/-2;M.Finney
poss 1 bnd o/l M01B2/T19E6 S.Roberts Ruvkun J.Fleming;Rb homolog Cerep3
RcD1;Rc123 Rc35 B.Cali PROBLEMS:see book
T23D5/C18A9 sl dub RcC9 f/p o/l T25E12/K08G11 dub.
RcA1 elsewhere by cy (see Y104E2!) U2-7;T.Blumenthal,J.Thomas
i/s C51F6
1 anom. CCEH661
sdc-3
unc-76
cpr-1
cpr-3
cyb-2
stP18
his-1etc
rsn-4
rrs-1
stP108
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.

32 %


YK1352
cm19c4
cm19d4
cm13f7
(Y40G1)
(Y64B7)
(Y38F5)
(Y54H8)
(Y45G8)
(Y70C5)
(Y42H12)
(Y76G12)
(Y15D5)
(Y97E3)
(RRS1CLUS)
(Y53G12)
(Y012E7L)
(Y102A5)
(Y6G8)
(Y9H11)
(Y61B8)
(Y68A4)
(Y18D2)
(Y42A8)
(Y26G10)
W06H3
ZC14
C33H8
F14D1
F07C11
ZC211
H02CB7
C15G6
ZC216
K08G11
C13E4
ZC217
T25E12
B0325
W02F7
F09C6
C24F9
W09H8
R06A6
C17D10
W06F9
ZK1085
W01E5
ZK218
C31C3
AD8
C26B10
C07C11
F15E12
F31A10
F49H6
R07G12
C50G11
JK102
RW2070-2
W04G10
C26D11
F16G5
T25H11
W07E3
K03A3
C28C4
K07H12
W08F6
T25B12
K10G4
F51B10
T28H1
R07E8
K03B9
R09E6
W05B1
R13E4
T21B1
R12B9
R08D2
F27C2
R10G2
F31E9
T17F3
F40D5
ZK1015
T22G12
F33D10
F54G9
C43A10
R15A10
F59A1
C60C7
C24A4
M34
C14E10
T08C11
F57G4
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
rrs-1
stP108
Cerep3
RcC9;RcA1;RcD1;Rc123?
R.Waterston;i/s RW2070-2
BOTc1
Rc123
RcC9?;Rc35
PROBLEMS:see book
WAS LINKED TO F07C4etc.DISMANTLED.
also F17F1
repet.
RcS5
f/p o/l T25E12/K08G11 dub.
R13C4 was here;prob mixt.
RcA1;Rc123
arrangement dub.
DISMANTLED BECAUSE GRID SPOTS ADJACENT.
rsc K.Ferguson
stP18
his-1etc
rsn-4
rrs-1
stP108
stP105
cdh-2
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.

1,,
Summary-line: 4-Sep cooks@mendel.Berkeley.EDU #Re: Re:YAC Y58A8
Mail-from: From cooks@mendel.Berkeley.EDU Thu Sep 5 04:43:17 1996
Return-Path: <cooks@mendel.Berkeley.EDU>
Received: from mendel.Berkeley.EDU by sanger.ac.uk (4.1/SMI-4.1)
        id AA20841; Thu, 5 Sep 96 04:43:15 BST
Received: (from cooks@localhost) by mendel.Berkeley.EDU (8.7.5/8.7.5) id UA
Date: Wed, 4 Sep 1996 20:41:36 -0700 (PDT)
From: Edith Cookson <cooks@mendel.Berkeley.EDU>
To: alan@sanger.ac.uk
Subject: Re: Re:YAC Y58A8
In-Reply-To: <9609041133.AA14450@sanger.ac.uk>
Message-Id: <Pine.SUN.3.91.960904203441.9649A-100000@mendel.Berkeley.EDU>
Mime-Version: 1.0
Content-Type: TEXT/PLAIN; charset=US-ASCII

*** EOOH ***
Return-Path: <cooks@mendel.Berkeley.EDU>
Date: Wed, 4 Sep 1996 20:41:36 -0700 (PDT)
From: Edith Cookson <cooks@mendel.Berkeley.EDU>
To: alan@sanger.ac.uk
Subject: Re: Re:YAC Y58A8
In-Reply-To: <9609041133.AA14450@sanger.ac.uk>
Mime-Version: 1.0
Content-Type: TEXT/PLAIN; charset=US-ASCII

On Wed, 4 Sep 1996 alan@sanger.ac.uk wrote:

> Dear Edith
> Many thanks for the offer of the Y95G10 fosmids. We certainly would like
> to have these for sequencing. Can you remind me of the size of Y95G10? (I'm
> not sure I ever knew). Would it be useful for us to fingerprint map some
> number of your fosmid subclones?
> Good luck with the rescue!
> Alan
>
Hi Alan
        Great to hear that you are interested in the clones. I'm sure it would be useful to fingerprint map them as independent confirmation that they overlap. Shall I send you DNA (Qiagen pure) or stabs, or both ? Let me know and I'll fedex them immediately !
        YAC Y95G10 is 450Kb on PFG. Y59A8 is around 350Kb-The two span the cosmid gap between K03D8 and C48B12.

        Thanks again

        Edith

1,,
Summary-line: 15-Jan cooks@mendel.Berkeley.EDU #Re: fosmids
Mail-from: From cooks@mendel.Berkeley.EDU Thu Jan 16 00:42:25 1997
Return-Path: <cooks@mendel.Berkeley.EDU>
Received: from mendel.Berkeley.EDU by sanger.ac.uk (4.1/SMI-4.1)
        id AA22917; Thu, 16 Jan 97 00:42:23 GMT
Received: (from cooks@localhost) by mendel.Berkeley.EDU (8.7.5/8.7.5) id QAA23
Date: Wed, 15 Jan 1997 16:38:58 -0800 (PST)
From: Edith Cookson <cooks@mendel.Berkeley.EDU>
To: alan@sanger.ac.uk
Subject: Re: fosmids
In-Reply-To: <9701140950.AA00635@sanger.ac.uk>
Message-Id: <Pine.SUN.3.91.970115161533.21400A-100000@mendel.Berkeley.EDU>
Mime-Version: 1.0
Content-Type: TEXT/PLAIN; charset=US-ASCII

*** EOOH ***
Return-Path: <cooks@mendel.Berkeley.EDU>
Date: Wed, 15 Jan 1997 16:38:58 -0800 (PST)
From: Edith Cookson <cooks@mendel.Berkeley.EDU>
To: alan@sanger.ac.uk
Subject: Re: fosmids
In-Reply-To: <9701140950.AA00635@sanger.ac.uk>
Mime-Version: 1.0
Content-Type: TEXT/PLAIN; charset=US-ASCII

Hi Alan

It occurred to me last night that since Y59A8 is in the offing for sequencing, you are probabely planning to also do Y94A7. Is this right ? It is the smallest (170Kb) of the YACs spanning K03D8 to Y59A8 and runs faster than the smallest yeast chromosome. I am very excited, of course, that you guys are working in this region ! Do you have any rough timescale of when you will start to get sequence data ?

Best wishes

Edith

Think I misinterpreted 4/9 mail
i.e. Y59A8 + another YAC (Y95G10, Y94A7sg)
are required to span K03D8/C48B12

1,,
Summary-line: 16-Sep cooks@mendel.Berkeley.EDU #Re: Re:YAC Y58A8
Mail-from: From cooks@mendel.Berkeley.EDU Mon Sep 16 18:14:35 1996
Return-Path: <cooks@mendel.Berkeley.EDU>
Received: from mendel.Berkeley.EDU by sanger.ac.uk (4.1/SMI-4.1)
        id AA21756; Mon, 16 Sep 96 18:14:31 BST
Received: (from cooks@localhost) by mendel.Berkeley.EDU (8.7.5/8.7.5) id KAA01
Date: Mon, 16 Sep 1996 10:12:14 -0700 (PDT)
From: Edith Cookson <cooks@mendel.Berkeley.EDU>
To: alan@sanger.ac.uk
Subject: Re: Re:YAC Y58A8
In-Reply-To: <9609060849.AA01739@sanger.ac.uk>
Message-Id: <Pine.SUN.3.91.960916093928.28585A-100000@mendel.Berkeley.EDU>
Mime-Version: 1.0
Content-Type: TEXT/PLAIN; charset=US-ASCII

*** EOOH ***
Return-Path: <cooks@mendel.Berkeley.EDU>
Date: Mon, 16 Sep 1996 10:12:14 -0700 (PDT)
From: Edith Cookson <cooks@mendel.Berkeley.EDU>
To: alan@sanger.ac.uk
Subject: Re: Re:YAC Y58A8
In-Reply-To: <9609060849.AA01739@sanger.ac.uk>
Mime-Version: 1.0
Content-Type: TEXT/PLAIN; charset=US-ASCII

On Fri, 6 Sep 1996 alan@sanger.ac.uk wrote:

> Hi Edith
> Thanks for the YAC size info.
> In your earlier email you said that you had subcloned Y95G10 into fosmids.
> Was this made from purified YAC? Should we fingerprint a set of fosmids to
> try and fill the entire bridge? In the mean time, could you send DNA and sta
> of the set you are interested in?
> We will need bugs when we come to prep material for sequencing.
>
> Alan
>
Hi Alan

I sent the stabs and DNA last week, so you have probabely received them by now. I pulled them out of a library made from total DNA from strain Y95G10 (if you have a protocol for making libraries from purified YAC, I'd love a copy !). A while ago, I isolated a phage clone from another sub-library using a PCR fragment from the left end of Y59A8(I got the primer sequence from you). I used the phage insert to screen my fosmid library and got 3 duplicate positives. These are clones 1, 2 and 4. Clone 4 clearly contains approx. 35 Kb worm DNA, and looks the same as genomic DNA on blots. Clones 1 and 2 pick up only 9Kb of genomic DNA and this does not look the same as the YACs. I know that 1 and 2 hybridise with clone 4, so it is definately worth fingerprinting rthem. This region is so rich in repeat DNA that I have to compete all my probes to see any clear bands on blots. I guess this will complicate the analysis.

Clones 5, 7 and 8 were pulled out using a 1 Kb Hin D111 fragement om the left end of clone 4. Clone 8 looks kosher with respect to genomic DNA, but 5 and 7 both only pick up a 9Kb fragment on Eco R1 digests of genomic DNA. Again this might be because of repeat DNA(ie that the band actually represents several tandem repeats). I know that all the clones have sequences in common from hybridisation and double digests (and that they all map to my region. So I think it's definately worth looking at all of them. I'm making DNA from the phage clone today, and

will send it, since it would be good to see the extent of overlap of all the clones I have

Thanks again, and let me know if you have any questions

Edith Cookson

DEAR ALAN.

HERE ARE THE FOSMID CLONES (#1, 2, 4, 5, 7 + 8) ENCLOSED ARE STABS + DNA SAMPLES (~10 μg OF EACH. A COUPLE OF THE CLONES LOOK ~~&~~ STRANGE ON GENOMIC BLOTS IN THAT THEY ~~@~~ PICK UP ONLY 1 9Kb EcoRI BAND. THIS COULD BE SEVERAL COPIES OF A REPEAT, OR IT COULD MEAN THAT THE CLONE IS A CO-LIGATION OF WORM + YEAST DNA (SINCE I MADE LIBRARY FROM ~~to~~ WHOLE YEAST STRAIN). I'M STILL WORKING ON WHAT'S GOING ON, AND WILL E-MAIL YOU.

THANKS.

EDITH COOKSON.

For flp.

In fridge

```
project name = cen2hs
number of clones = 24253, number of bands = 422374
first clone in set = pFos1
last clone in set =


first clone in set =
last clone in set =


tolerance in tenths of mm =      7 (main)        10 (difmap)
match probability cutoff =  1.0e-04
analytical probability cutoff =  1.0e-11

-----start-of-mapsub-output-----@
1207-pFos1   ( 10b,   0)
                          4 matches  1044-CB#1237 (  4b,   0)   3.0e-06     0     6d
                          4 matches  1058-YSL487  (  4b,   0)   3.0e-06     0     0
                          4 matches  1058-YSL493  (  4b,   0)   3.0e-06     0     0
                          4 matches  1043-CB#1220 (  5b,   0)   1.5e-05     0     0
                          4 matches  1044-CB#1255 (  5b,   0)   1.5e-05     0     0
                          4 matches  1058-YSL490  (  5b,   0)   1.5e-05     0     0
                          4 matches  1022-YSL117  (  6b,   0)   4.2e-05     0     0
                          4 matches  1043-CB#1228 (  6b,   0)   4.2e-05     0     0
                          6 matches   207-C47A2   ( 21b, 313)   6.0e-05  5003     4   C10B3
                          4 matches  1059-CB#1422 (  7b,   0)   9.5e-05     0     0
                  difmap:  1 matches   224-M7     ( 21b, 423)   5.9e-01  2653     5
                  difmap:  5 matches   621-F14A2  ( 21b, 313)   7.9e-04  5065     5   M03G9
                  difmap:  2 matches   854-CT#11  ( 12b, 313)   8.5e-02  5121     4
                  difmap:  3 matches   890-EK287  ( 12b, 313)   1.1e-02  5120     4   D10B2
                  difmap:  2 matches   220-CF3    ( 13b, 674)   9.8e-02  3084     4   C30E11
1207-pFos2   ( 16b,   0)
                          9 matches   927-NY18   ( 38b, 369)   1.5e-04  1678    7d   canon
                          6 matches   211-AC4    ( 15b, 313)   2.4e-04  4216     3   R109
                          7 matches   302-C50F2  ( 22b, 465)   2.5e-04  1160     4
                          6 matches    87-C13B5  ( 16b, 377)   3.6e-04  1454     2   canon
                          7 matches   375-K06B12 ( 24b, 377)   4.2e-04     3     2
1207-pFos4   ( 27b,   0)
                          7 matches   271-R03B8  ( 15b, 423)   5.1e-04  2956    20d
                          8 matches  2028-H14I22 ( 21b,   0)   1.1e-03     0     8
                          9 matches   353-K03D7  ( 26b, 313)   1.2e-03    29     6   canon
                          9 matches   107-C18D4  ( 27b, 313)   1.6e-03     5     8   canon
                          9 matches    21-C01F1  ( 27b, 369)   1.6e-03  2755     6   canon
1207-pFos5   ( 28b,   0)
                          7 matches  2006-H03K20 ( 10b, 674)   2.0e-05  3625    21d  H04B20
                          7 matches   404-T06D1  ( 13b, 674)   2.1e-04  1727     6   M01F8
                          7 matches   638-F18D7  ( 13b, 674)   2.1e-04  1727     5   M01F8
                          6 matches   829-C20.14C ( 11b, 674)  5.6e-04   186     5   C20.11A
                          7 matches   577-W09A5  ( 14b, 423)   3.7e-04  2272     7   W03A12
1207-pFos7   ( 24b,   0)
                          9 matches   297-C49A6  ( 22b, 465)   1.2e-04  1323    15d  C14H7
                         11 matches     0-B0416  ( 34b, 674)   1.2e-04  4815     7
                          7 matches   675-P28F7  ( 15b, 674)   2.6e-04  2631     6   C09D10
                          8 matches   599-F08F1  ( 20b, 674)   3.4e-04  4426     5
                          5 matches   488-T27C1  (  8b, 674)   3.7e-04  2082     3   T28E4
1207-pFos8   ( 23b,   0)
                         10 matches   289-CD10   ( 26b,   0)   5.0e-05     0    13d
                          9 matches   791-F57B2  ( 24b, 423)   1.7e-04  4126     4
                          6 matches   728-F42B4  ( 12b, 377)   3.7e-04   588     3
                          8 matches   392-T03C1  ( 21b, 313)   3.8e-04  3522     5   C15C1
                          7 matches     0-B0494  ( 17b, 674)   5.1e-04  2760     5   C37H3
                  difmap:  7 matches   745-F45C7  ( 30b, 369)   1.5e-02  2169     8   F14D2    limbo
                  difmap:  4 matches   779-F54B4  ( 11b, 313)   1.5e-02  1555     5   C10B12
                  difmap:  5 matches   514-K10B10 ( 17b, 313)   1.7e-02  1555     6   C10B12
                  difmap:  8 matches  2014-H17J18 ( 36b,   0)   1.1e-02     0     8
                  difmap:  7 matches   708-F36G7  ( 28b,   1)   1.1e-02   259     7   F09C3
1207-Fosx1   ( 23b,   0)
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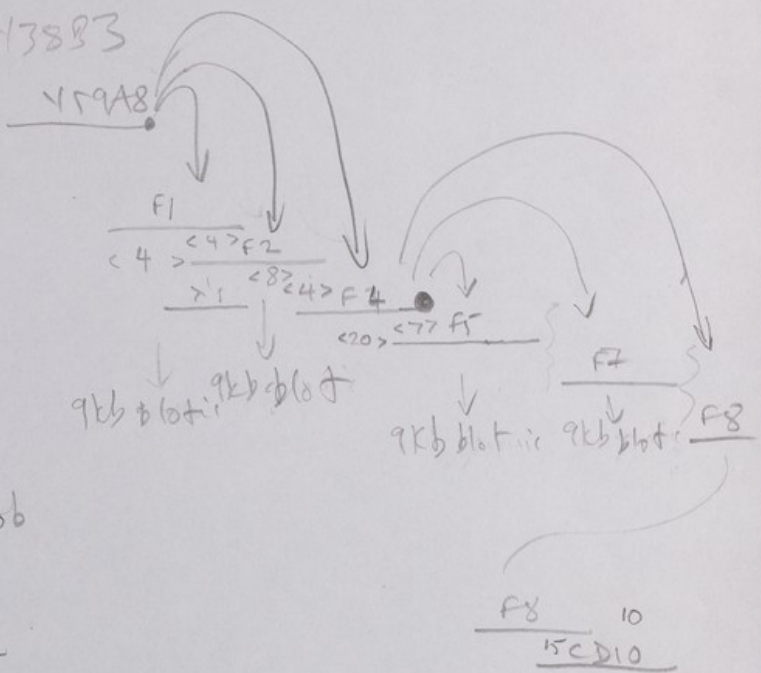

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                 18 matches   667-P26E5   ( 25b, 369)   2.4e-14  3395     5d
                 15 matches   475-T24B8   ( 18b, 369)   2.1e-13  3383     4
                 17 matches   694-P33D3   ( 24b, 369)   3.4e-13  3395     2    C12B6
                 16 matches   266-R02F6   ( 22b, 369)   1.4e-12  3383     3    C42H2
                 15 matches   188-C42H2   ( 23b, 369)   8.2e-11  3383     2    canon
1207-Fosx2   ( 35b,   0)
                 25 matches   708-P36N12  ( 31b, 423)   1.0e-16  2652    10d
                 25 matches   611-F11E9   ( 33b, 423)   1.5e-15  2652     9    C14D5
                 25 matches    33-C14D5   ( 36b, 423)   2.7e-14  2652     3    canon
                 13 matches  1022-Y5L109  ( 17b, 423)   9.3e-09  2646     2
                 15 matches   106-C17E9   ( 23b, 423)   2.1e-08  2652     4
                 14 matches  1074- ZL183  ( 15b,   0)   1.2e-11     0     2
                 13 matches  1073-ZL170   ( 14b,   0)   8.2e-11     0     1
                 14 matches  1073-ZL168   ( 20b,   0)   1.6e-08     0     2
                  9 matches  1074-ZL177   ( 13b,   0)   7.8e-06     0     2
```

H02E13

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DEPARTMENT OF MOLECULAR & CELL BIOLOGY
BARKER HALL / KOSHLAND HALL

401 BARKER HALL
BERKELEY, CALIFORNIA 94720-3204
FAX: (510) 642-7000

FAX COVER SHEET
(510-642-7000)

DATE: 30.1.97

TO ALAN COULSON.
44-1223-494919.

CONTACT PHONE: 44-1223-494940.

FROM: EDITH COOKSON.

LAB: MEYER, UC, BERKELEY.

Total number of pages transmitted including this: 2
If you have a problem, phone (510) 643-5582

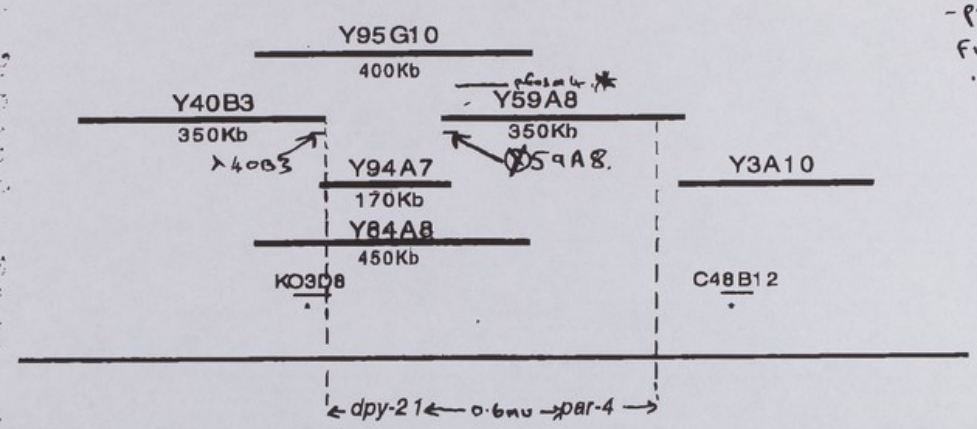
Hi ALAN.

- HERE IS THE MAP OF DY4-21 YACS ETC, ACCORDING TO MY INVESTIGATIONS
- SO TO GET THE WHOLE REGION BETWEEN K0308 + C48B12 WILL REQUIRE ~~SOL~~ SEQUENCING SUB-LIBRARIES OF Y94A7 + Y59A8.

- HAVE YOU FOUND ANY ORFs YET!

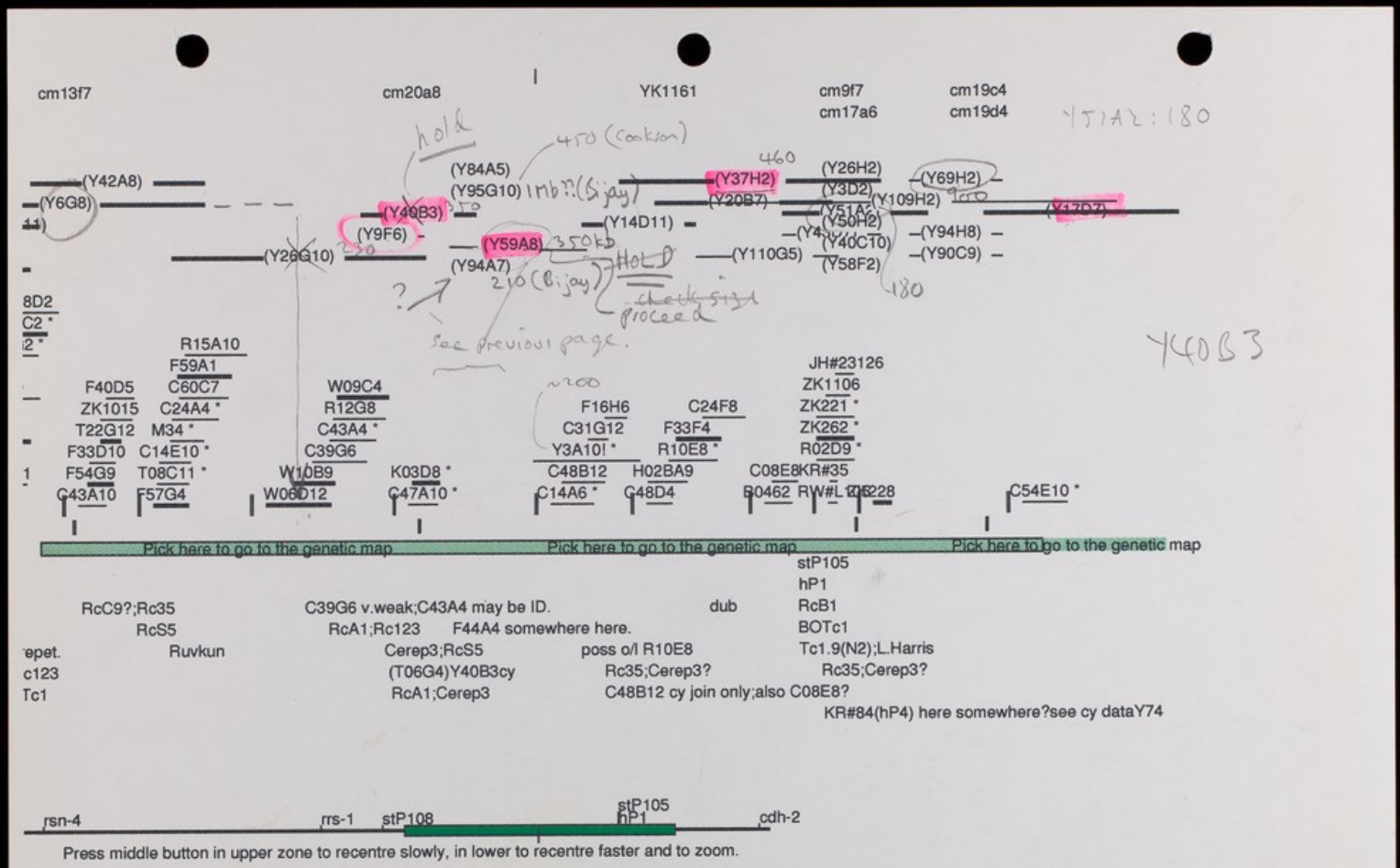
EDITH.

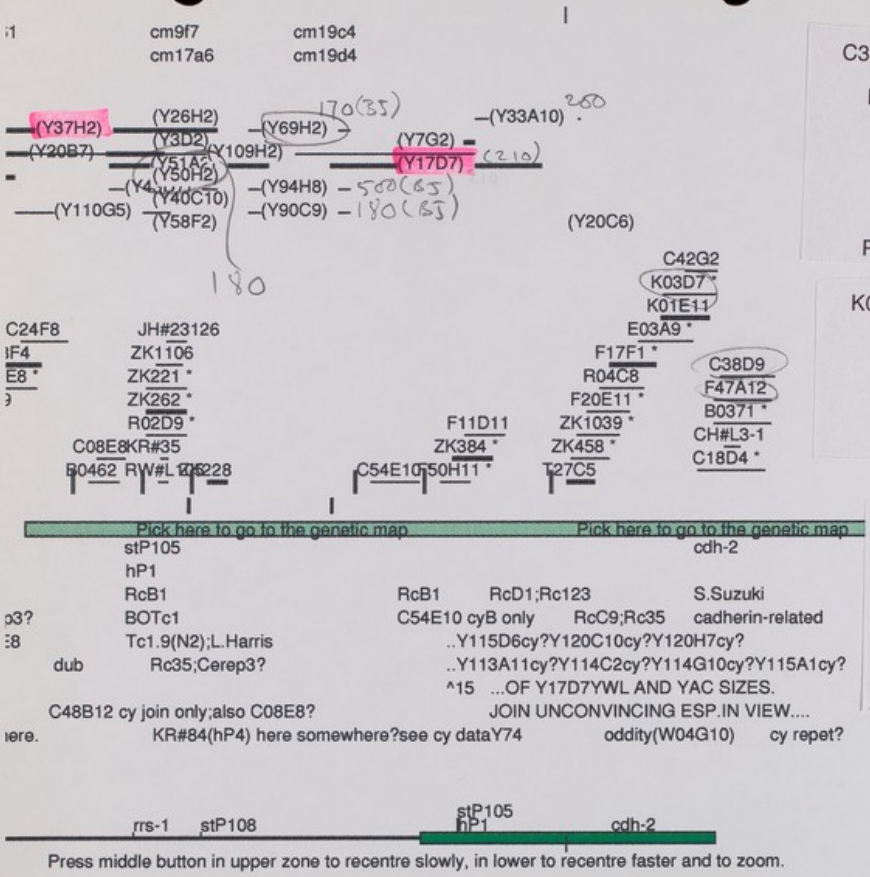
DPY-21 GENOMIC REGION.



genetic map

→ pfos 4 + pfos 8 HERE.
- pfos 8 EXTENDS FURTHER INTO Y59A i.e. TO THE RT.


cm13f7
cm20a8
YK1161
cm9f7
cm17a6
cm19c4
cm19d4
(Y42A8)
(Y6G8)
(Y84A5)
(Y95G10)
(Y40B3)
(Y9F6)
(Y26G10)
(Y59A8)
(Y94A7)
(Y14D11)
(Y37H2)
(Y20B7)
(Y26H2)
(Y3D2)
(Y51A5)
(Y50H2)
(Y40C10)
(Y58F2)
(Y110G5)
(Y109H2)
(Y69H2)
(Y94H8)
(Y90C9)
(Y17D7)
hold
450 (Coulson)
460
Imb?! (Bijay)
350kb
Hold
check sizes
proceed
210 (Bijay)
180
see previous page.
Y40B3
8D2
C2 *
2 *
R15A10
F59A1
F40D5
C60C7
ZK1015
C24A4 *
T22G12
M34 *
F33D10
C14E10 *
F54G9
T08C11 *
C43A10
F57G4
W09C4
R12G8
C43A4 *
C39G6
W10B9
W06D12
K03D8 *
C47A10 *
F16H6
C24F8
C31G12
F33F4
Y3A10 *
R10E8 *
C48B12
H02BA9
C14A6 *
C48D4
C08E8
B0462
JH#23126
ZK1106
ZK221 *
ZK262 *
R02D9 *
KR#35
C54E10 *
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
stP105
hP1
RcC9?;Rc35
RcS5
Ruvkun
C39G6 v.weak;C43A4 may be ID.
RcA1;Rc123
F44A4 somewhere here.
Cerep3;RcS5
(T06G4)Y40B3cy
RcA1;Cerep3
dub
poss o/l R10E8
Rc35;Cerep3?
C48B12 cy join only;also C08E8?
RcB1
BOTc1
Tc1.9(N2);L.Harris
Rc35;Cerep3?
KR#84(hP4) here somewhere?see cy dataY74
epet.
c123
Tc1
rsn-4
rrs-1
stP108
stP105
hP1
cdh-2
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.



C38D9

Remark
PCR_remark
PCRB:Y39A2,Y42A6,Y42A8,Y42H2,Y43H12
PCRB:Y45H5,Y61B12,Y64E8,Y71F7,Y71G4
PCRB:Y6G8
PPCRY:Y101H7,Y114E2,Y117B1,Y117G6,Y119E4
PPCRY:Y119H2,Y120H2,Y112C10,Y119A8
Position

K03D7

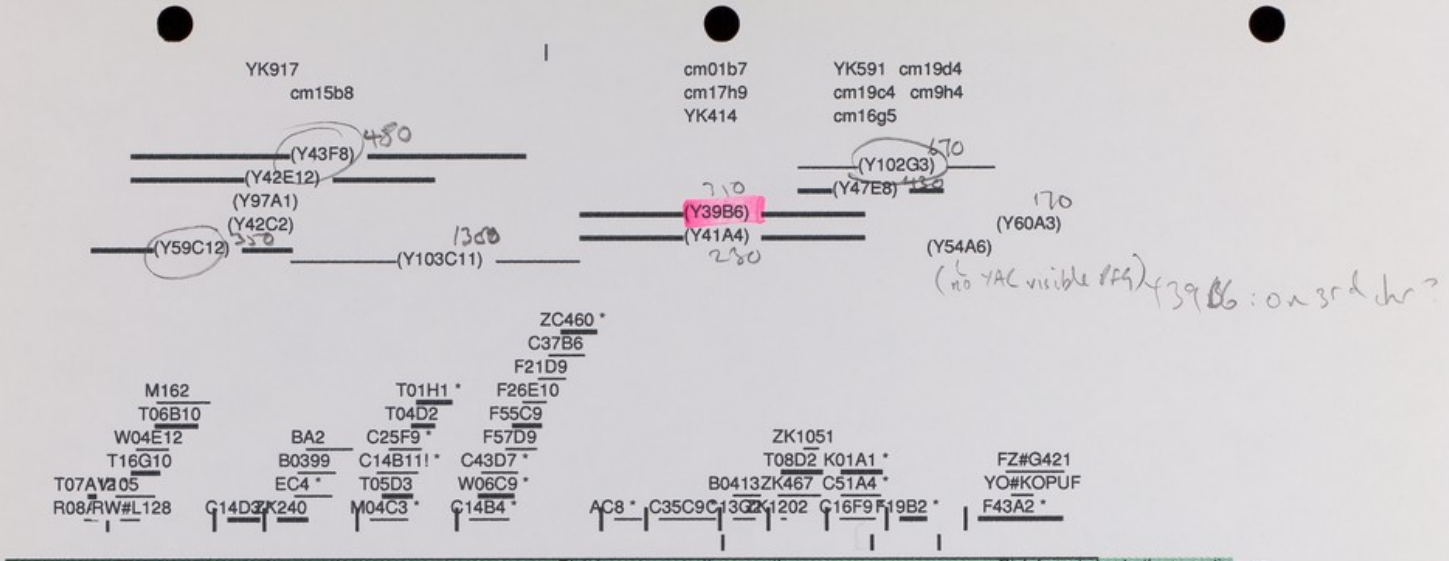
Remark
Y_remark Y112C10cy,Y118F8cy
Position
Pmap **ctg313** 6192 6218
FingerPrint
Gel_Number 353

F47A12

Remark
General_remark cy repet?
Y_remark
Y112C10cy,Y113A1cy,Y115H1cy,Y117E9cy
Y118D11cy,Y119E4cy?Y119H2cy?Y120H11cy?
Position
Pmap **ctg313** 6217 6245
FingerPrint

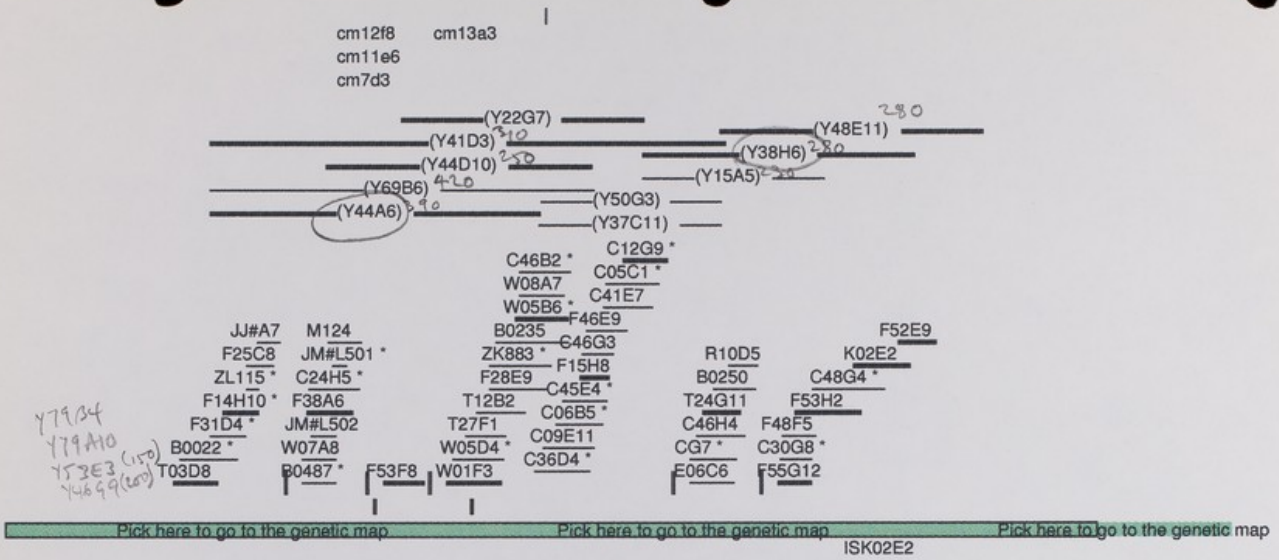
F17F1

Remark
Y_remark
Y68A4
Y105H8cy
Position



'L' end not C.e.? Yuko % Rc123 ORDERING OF GROUPS V DUBIOUS No apparent YAC on PFG.
BOTc1 repet,so dub;RcD1? f/p o/l in error? these cy+s need yc! RcA1
genetic % approx. rather repet;Rc35 RcC9 repet,but strong +'s
cy repet;(Y59C12,Y63F10,Y65A1,Y75A4)cy? RcC9;RcD1;Rc35;Rc123 C.Wicky;(telomeric repeat)
RcA1;Cerep3 ZK240 messy poor probing; unreliable confirmation.
rather dub psjoin f/p location probable
was linked to Y47A7 by cyB early embryonic.

Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.



BF10 1anom.band F33E7:oddity Cerep3 i/s VR?

M.Azzaria,J.McGhee

HNF-3 TF/forkhead homolog.

S.MANGO 'rol-9' probe:Y44A6,Y47E12....

...Y47F12,Y79B4,Y79A10,Y79G10,Y82E1

Cerep3

K04B7 here but cant make sense

Y79B4 :
S. Mango λ library

ISK02E2

Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.

Selections

10/5/96

41707 210 kb
43712 460 kb

27/6/96

43986 310 kb
44053 310 kb

8/9/96
~~45948 350kb (yes)~~ hold - check size
4698 (230 kb) (yes)

28/10
45948 (350)

V

Poss?

Y6E2 210
Y32612 200
Y70C1

← prb should adapt other methods to here.

Y102AJ 480

✓ Y698 220

Y26910? 230

✓ Y40B3 250 withdraw?
✓ Y59A8 250? field pending size check: 350
✓ Y37H2 460
Y51A2 180
Y69H2 170 ~~or Y94H8 or Y90C9 (need sizing)~~.
✓ Y17D7 210

Y33A10 200
Y112C10 or Y119E4 or Y119H2 (need sizing)

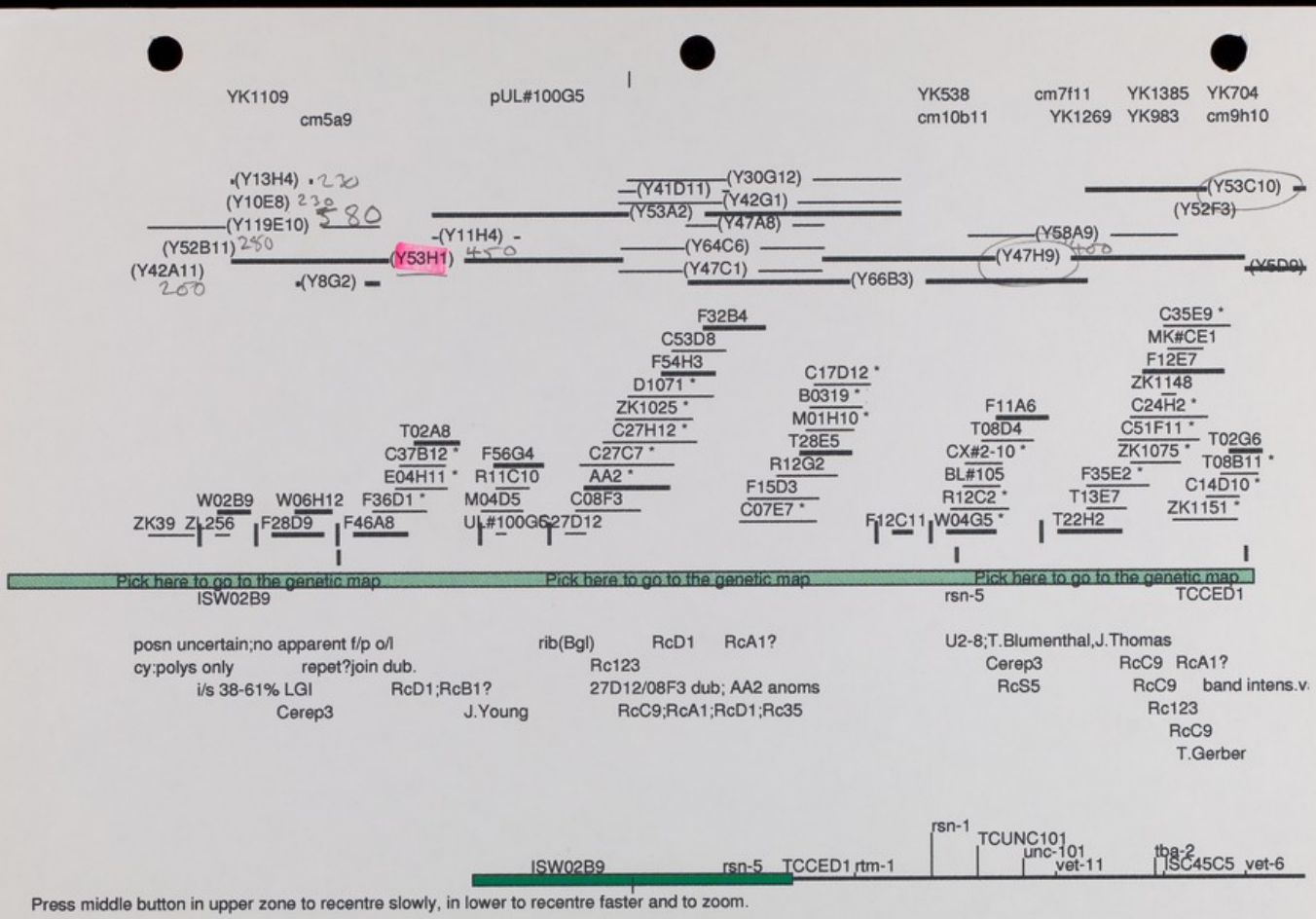
//

Y59C12 350 → window? (3rd dr)
Y43F8 480
✓ Y39B6 310
Y10293 670 → attempted window transfer 14/10

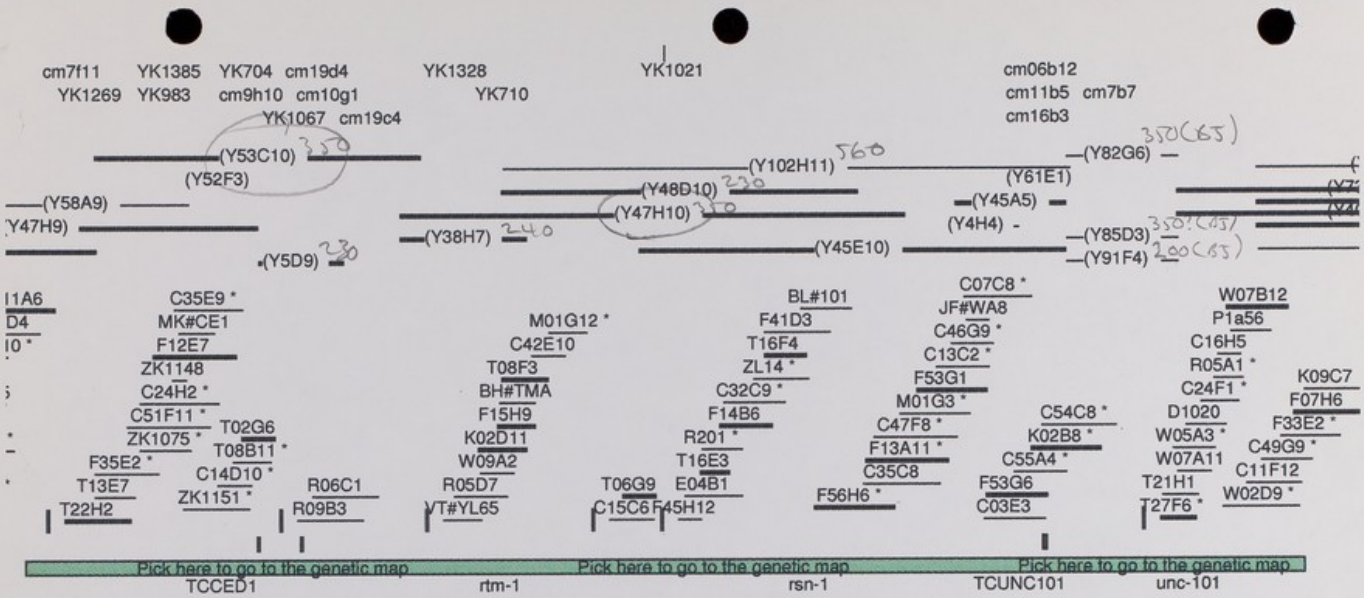
//

Y44A6 390
Y38H6 280

H



Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.



pYACL & pYACR :double sequences F45H12 del? U2-1a,U2-1b,U2-2 J.Lee convincing).BUT SEE SOUTHERN 11/1.

Blumenthal,J.Thomas RcC9;Rc123;RcS5 @ was i/s LGIV 42-52% T.Blumenthal,J.Thomas (cont)split to allow Y48F4/Y5F2 (more

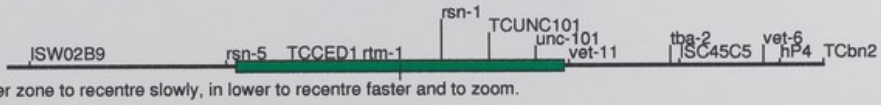
arep3 RcC9 RcA1? Cerep3 C42E10/M01G12 sl dub F41D3/F56H6 dub Rc35 extent of these not known;cy only

RcS5 RcC9 band intens.vars. B.Honda;i/s F15H9 was i/s LGIV 39-53%...

Rc123 M01G12? RcS5 T16E3=yc -ve;RcA1 ...but i/s 6/93 = LGI? not IV

RcC9 i/s F53G1 rsc J.Lee

T.Gerber J.Fleming



Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.

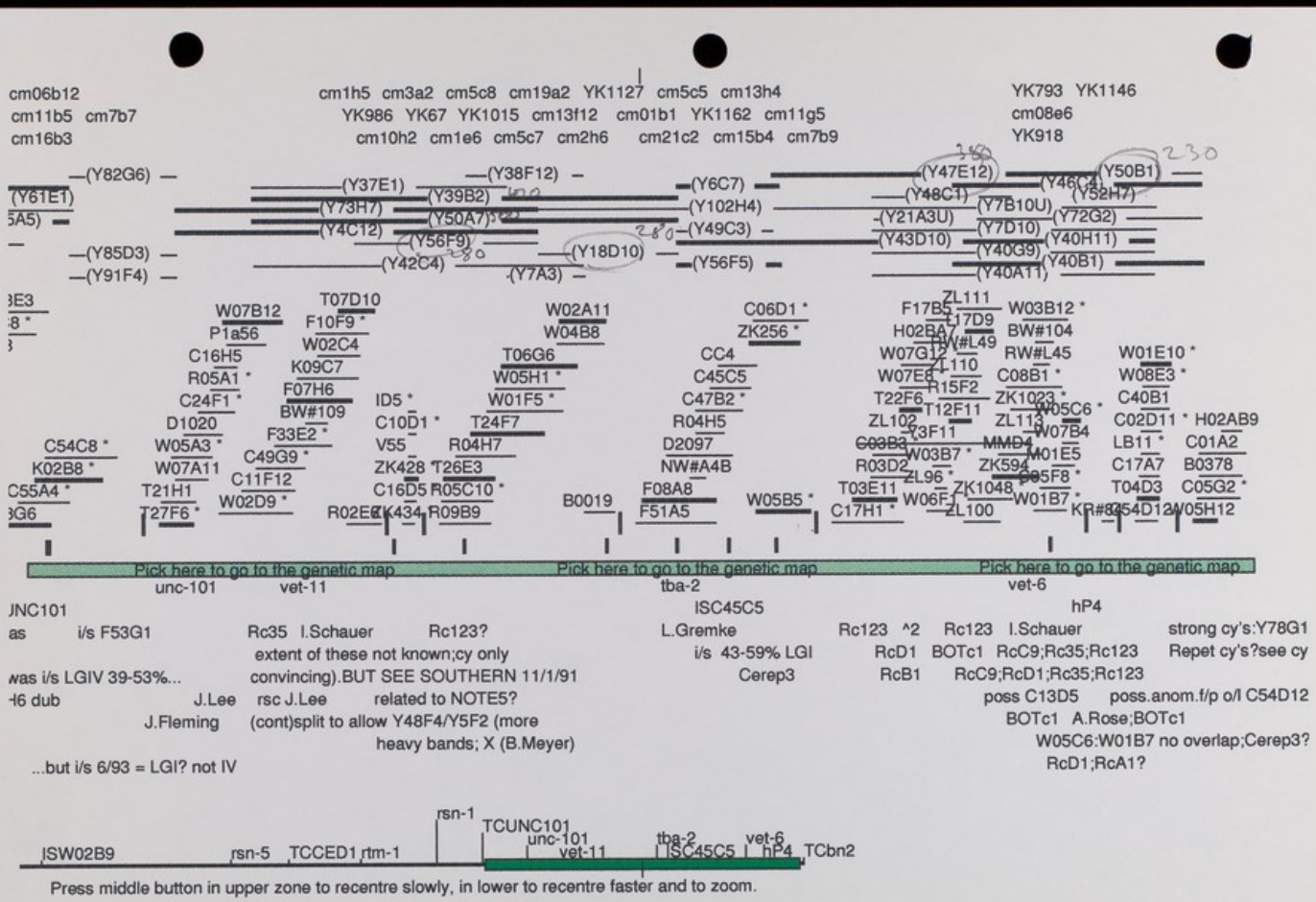
1, filed,,
Summary-line: 18-Feb th29@cornell.edu #doubts on the physical map a
Mail-from: From th29@cornell.edu Wed Feb 19 02:33:19 1997
Return-Path: <th29@cornell.edu>
Received: from postoffice2.mail.cornell.edu by sanger.ac.uk (4.1/SMI-4.1)
id AA12819; Wed, 19 Feb 97 02:33:17 GMT
Received: from [128.84.203.101] (FASTPATH2B.TN.CORNELL.EDU [128.84.203.101])
X-Sender: th29@postoffice4.mail.cornell.edu
Message-Id: <v02130500af2e37c49488@[128.84.203.101]>
Mime-Version: 1.0
Content-Type: text/plain; charset="us-ascii"
Date: Tue, 18 Feb 1997 21:30:38 -0500
To: alan@sanger.ac.uk
From: th29@cornell.edu (Tak)
Subject: doubts on the physical map and cosmid T26E3 and R09B9

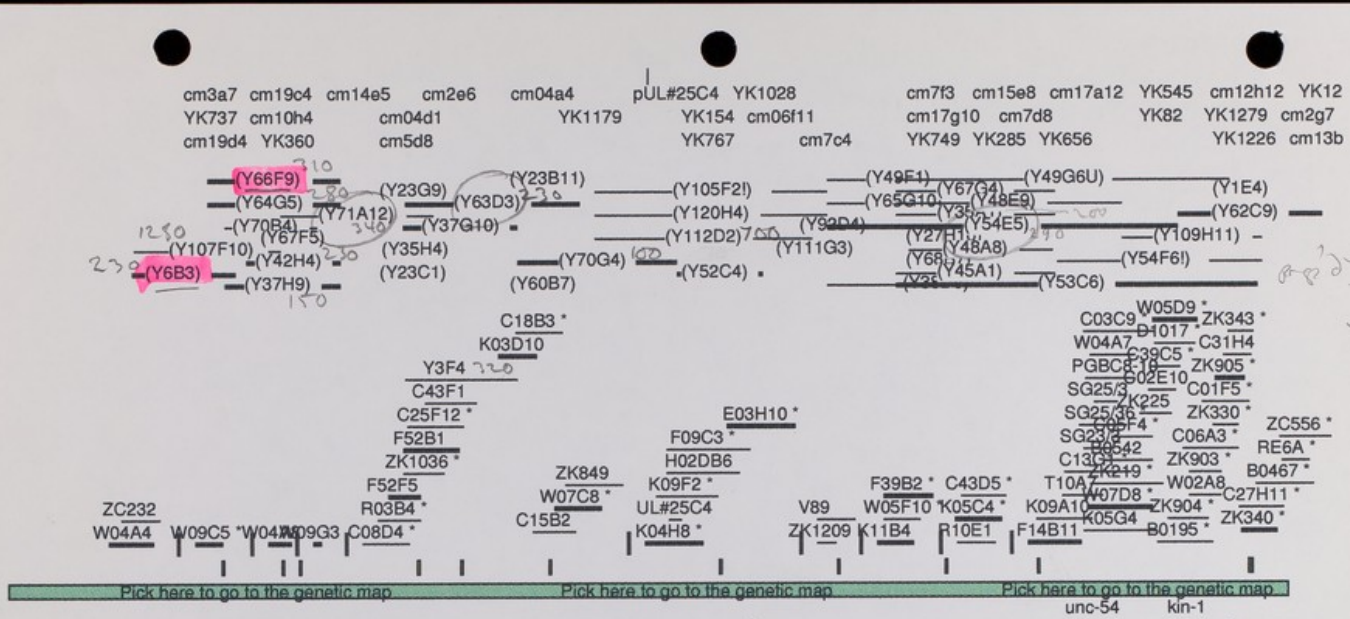
*** EOOH ***
Return-Path: <th29@cornell.edu>
X-Sender: th29@postoffice4.mail.cornell.edu
Mime-Version: 1.0
Content-Type: text/plain; charset="us-ascii"
Date: Tue, 18 Feb 1997 21:30:38 -0500
To: alan@sanger.ac.uk
From: th29@cornell.edu (Tak)
Subject: doubts on the physical map and cosmid T26E3 and R09B9

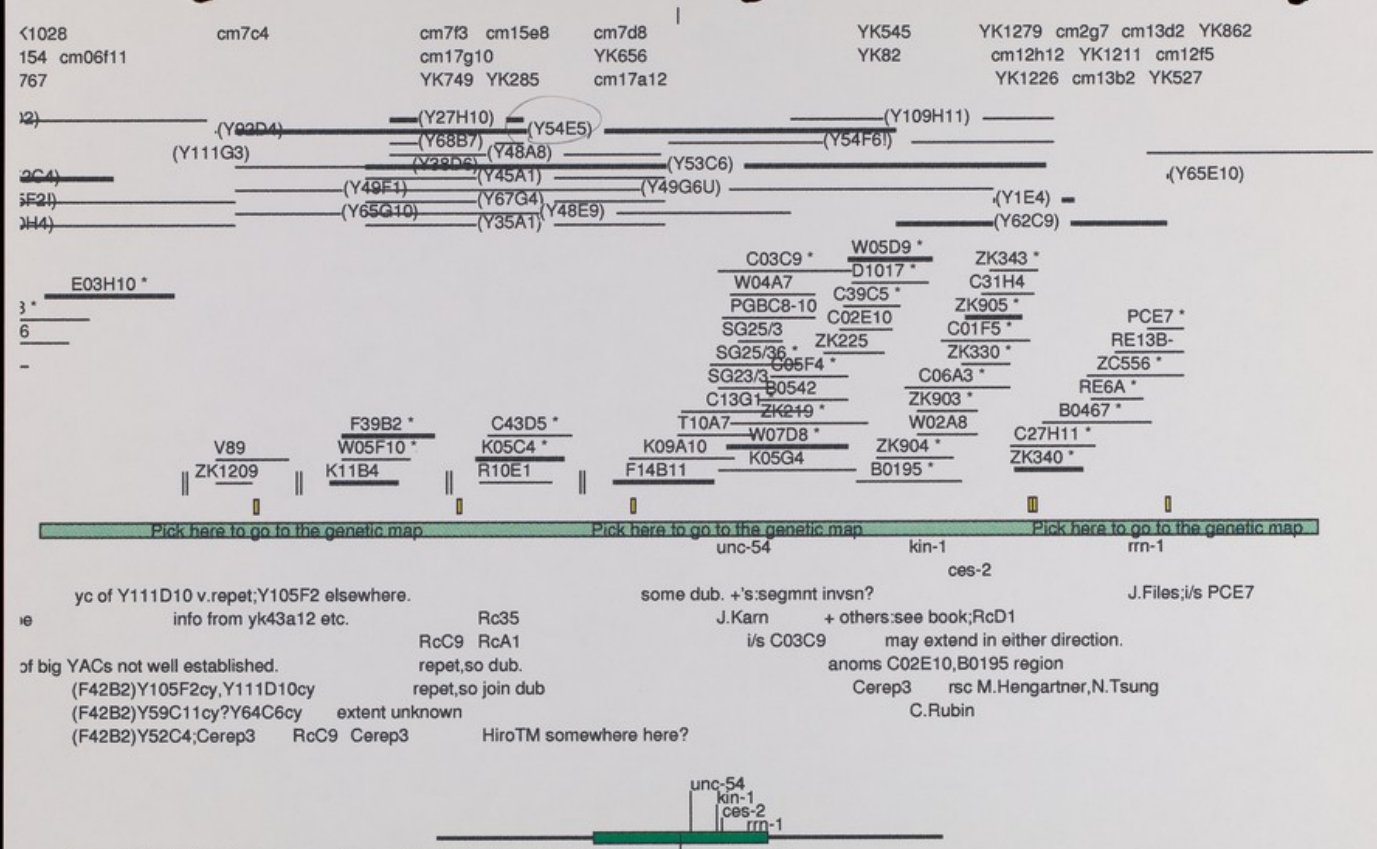
Dear Dr. Coulson:
I had one puzzling result when I was trying to align the cosmids between W05A3 and W02A11. To further define the overlapping relationship of these cosmids, I did a southern hybridization with a 1.5 Kb EcoRI fragment of R02E6 to probe cosmids in the region. A 1.5 Kb E.coRI band of R09B9 was lit up as the same intensity as the one of R02E6. This leads me to think that R02E6 overlaps with R09B9. That is to say, there is no gap at all between R02E6 and R09B9 as shown on the physical map.
I have one more line of evidence to support the argument that the tiny cosmids bunch between R02E6 and R09B9 do not belong there. Using PCR-based deletion mapping, I found that hDf15 deletes W02D9, R02E6, W01F5 but not C10D1. The result could be simply explained by the possibility that C10D1 is not between R02E6 and W01F5.
To further map this region, I want to ask for two cosmids: R09B9 and T26E3. I am asking for R09B9 again because I want to confirm my result mentioned above. Thank you for your assistance. I look forward to your feedback on my thoughts.

Tak Hung

Tak Hung
433 Biotechnology Building
Field of Genetics and Development
Cornell University
Ithaca, NY 14853
U. S. A.
Tel: 607-254-4804
Fax: 607-255-6249


cm06b12
cm11b5 cm7b7
cm16b3
cm1h5 cm3a2 cm5c8 cm19a2 YK1127 cm5c5 cm13h4
YK986 YK67 YK1015 cm13f12 cm01b1 YK1162 cm11g5
cm10h2 cm1e6 cm5c7 cm2h6 cm21c2 cm15b4 cm7b9
YK793 YK1146
cm08e6
YK918
(Y82G6)
(Y61E1)
(Y85D3)
(Y91F4)
(Y37E1)
(Y38F12)
(Y39B2)
(Y73H7)
(Y50A7)
(Y4C12)
(Y56F9)
(Y42C4)
(Y18D10)
(Y7A3)
(Y6C7)
(Y102H4)
(Y49C3)
(Y56F5)
(Y47E12)
(Y48C1)
(Y21A3U)
(Y43D10)
(Y50B1)
(Y46C4)
(Y52H7)
(Y7B10U)
(Y7D10)
(Y72G2)
(Y40G9)
(Y40H11)
(Y40B1)
(Y40A11)
W07B12
P1a56
C16H5
R05A1 *
C24F1 *
D1020
C54C8 *
K02B8 *
C55A4 *
T07D10
F10F9 *
W02C4
K09C7
F07H6
BW#109
F33E2 *
C49G9 *
C11F12
W02D9 *
W05A3 *
W07A11
T21H1
T27F6 *
ID5 *
C10D1 *
V55
ZK428
C16D5
R02E4
ZK434
T24F7
R04H7
T26E3
R05C10 *
R09B9
W02A11
W04B8
T06G6
W05H1 *
W01F5 *
B0019
C06D1 *
ZK256 *
CC4
C45C5
C47B2 *
R04H5
D2097
NW#A4B
F08A8
F51A5
W05B5 *
ZL111
F17B5
H02BA7
W07G12
W07E8
T22F6
ZL102
C03B3
R03D2
T03E11
C17H1 *
L17D9
BW#L49
T110
R15F2
T12F11
Y73F11
ZL96 *
W06F1
ZL100
W03B12 *
BW#104
RW#L45
C08B1 *
ZK1023 *
ZL113
W03B7 *
MMD4
ZK594
ZK1048
W01E10 *
W08E3 *
C40B1
W05C6 *
W07B4
M01E5
P05F8 *
W01B7 *
KR#8
C02D11 *
H02AB9
LB11 *
C01A2
C17A7
B0378
T04D3
C05G2 *
C54D12
W05H12
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
unc-101
vet-11
tba-2
vet-6
ISC45C5
hP4
JNC101
as i/s F53G1
Rc35 I.Schauer Rc123?
extent of these not known;cy only
was i/s LGIV 39-53%...
convincing).BUT SEE SOUTHERN 11/1/91
-16 dub J.Lee
rsc J.Lee related to NOTE5?
J.Fleming
(cont)split to allow Y48F4/Y5F2 (more
heavy bands; X (B.Meyer)
...but i/s 6/93 = LGI? not IV
L.Gremke
i/s 43-59% LGI
Cerep3
Rc123 ^2 Rc123 I.Schauer
RcD1 BOTc1 RcC9;Rc35;Rc123
RcB1 RcC9;RcD1;Rc35;Rc123
poss C13D5 poss.anom.f/p o/l C54D12
BOTc1 A.Rose;BOTc1
W05C6:W01B7 no overlap;Cerep3?
RcD1;RcA1?
strong cy's:Y78G1
Repet cy's?see cy
rsn-1
TCUNC101
unc-101
vet-11
tba-2
ISC45C5
vet-6
hP4
TCbn2
ISW02B9
rsn-5
TCCED1
rtm-1
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.


cm3a7 cm19c4 cm14e5 cm2e6 cm04a4 pUL#25C4 YK1028 cm7f3 cm15e8 cm17a12 YK545 cm12h12 YK12
YK737 cm10h4 cm04d1 YK1179 YK154 cm06f11 cm17g10 cm7d8 YK82 YK1279 cm2g7
cm19d4 YK360 cm5d8 YK767 cm7c4 YK749 YK285 YK656 YK1226 cm13b
(Y66F9)
(Y64G5)
(Y71A12)
(Y23G9)
(Y63D3)
(Y23B11)
(Y105F2!)
(Y120H4)
(Y112D2)
(Y111G3)
(Y49F1)
(Y67G4)
(Y49G6U)
(Y1E4)
(Y62C9)
(Y109H11)
(Y107F10)
(Y67F5)
(Y42H4)
(Y6B3)
(Y37H9)
(Y35H4)
(Y23C1)
(Y37G10)
(Y70G4)
(Y60B7)
(Y52C4)
(Y92D4)
(Y65G10)
(Y48E9)
(Y54E5)
(Y27H1)
(Y48A8)
(Y45A1)
(Y54F6!)
(Y53C6)
C18B3 *
K03D10
Y3F4
C43F1
C25F12 *
F52B1
ZK1036 *
F52F5
R03B4 *
ZC232
W04A4
W09C5 *
C08D4 *
ZK849
W07C8 *
C15B2
E03H10 *
F09C3 *
H02DB6
K09F2 *
UL#25C4
K04H8 *
V89
ZK1209
F39B2 *
C43D5 *
W05F10
K05C4 *
K11B4
R10E1
F14B11
W05D9 *
C03C9
ZK343 *
D1017 *
W04A7
C31H4
C39C5 *
PGBC8.10
ZK905 *
S02E10
SG25/3
C01F5 *
ZK225
SG25/36
ZK330 *
C06A3 *
ZC556 *
RE6A *
ZK903 *
B0467 *
T10A
W02A8
W07D8 *
C27H11 *
K09A10
ZK904 *
K05G4
ZK340 *
B0195 *
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
unc-54
kin-1
ces-2
was joined to K09G11etc by Y111F12
RcD1;RcC9
1280kb YAC!
sl.repet.
RcC9 Cerep3;RcS5
RcB1
Was inadvertantly joined to K03D3
J.Young,I.Hope RcC9 extent unknown
extent of big YACs not well established.
(F42B2)Y105F2cy,Y111D10cy
(F42B2)Y59C11cy?Y64C6cy
info from yk43a12 etc.
(F42B2)Y52C4;Cerep3 Cerep3 repet,so dub.
yc of Y111D10 v.repet;Y105F2 elsewhere.
Rc35 some dub. +'s:segmnt invsn?
i/s C03C9 C.Rubin
RcC9 HiroTM somewhere here?
repet,so join dub
may extend in eithe
RcA1 J.Karn Cerep3 rsc M.Hen
anoms C02E10,B019
+ others:see book;RcD
unc-54
kin-1
ces-2
rrn-1
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.



yc of Y111D10 v.repet;Y105F2 elsewhere. some dub. +'s:segmnt invsn? J.Files;i/s PCE7

ıe info from yk43a12 etc. Rc35 J.Karn + others:see book;RcD1

RcC9 RcA1 i/s C03C9 may extend in either direction.

of big YACs not well established. repet,so dub. anoms C02E10,B0195 region

(F42B2)Y105F2cy,Y111D10cy repet,so join dub Cerep3 rsc M.Hengartner,N.Tsung

(F42B2)Y59C11cy?Y64C6cy extent unknown C.Rubin

(F42B2)Y52C4;Cerep3 RcC9 Cerep3 HiroTM somewhere here?

Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.

Selections

10/5/96

453H1	450 kb
466F9	310 kb.

27/6/96

46B3	230 kb

I

pos.

Y119E10 (needs sizing).

✓ Y53H1 450
Y47H9 400
Y53C10 350 → window!
Y47H10 350 separable?
Y82G6 or Y85D3 or Y91F4 200 (~~need sizing~~)
Y56F9 280
Y18D10 280
Y47E12? 380 seems like a big YAC for a possibly small gap.
Y50B1 230

//

✓ Y6B3 270
✓ Y66F9 310
Y71A12 340 → attempted window 14/10
Y63D3 270 → window?
Y112D2 or Y120H4 (need sizing)
Y54E5 290 separable??

Places on X that look as though they might benefit from a YAC

T2007 / T02F6 / F46F6	Y49A10	100 kb
T04F8 / R14H4 / C34E11	Y102F5	400 kb
CC9 / F55G7	Y62H9	70 kb
R09A8 / C02B4	Y45C8	200 kb
F54B11 / C40C9	Y12A6	170 kb
K11E4 / (C49A1) / F19D8	Y6D6	180 kb
C02F11 / W01A6 / K02B9	Y26E6	180 kb
K04G11 / T24C2 / T14C1 / C44H4	Y52F7	320 kb
K01B4 / F42D1	Y3B8	200 kb
F31F6 / T2898	Y70D2	130 kb
K11C2 / F09B12	Y37H4	350 kb (big, but smallest)
T10B10 / T03G12	Y69B3	100 kb
C02D4 / C33A11	Y57C10	230 kb
R03A10 / F59D12 / K09E9	Y53G9	280 kb
F02B5 / ZK662 / F59F4	Y69A9 / Y7A5	140 / 210 kb
F53H4 / F56B4	Y67D11	140 kb
ZK1073 / T01F10	Y33B12	100 kb

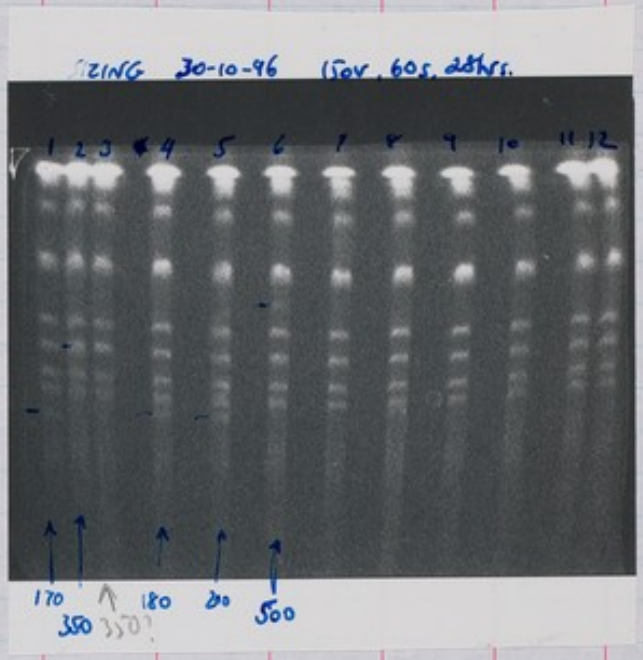
sizing etc

8-12

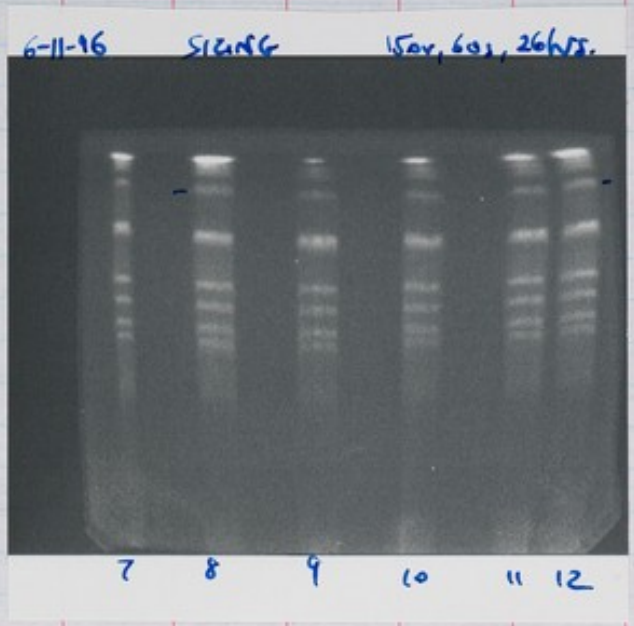
31/10

Vijay sizing

1	Y6 9H2	170
2	Y8 2G6	350
3	Y8 5D3	350?
4	Y9 0C9	180
5	Y9 1F4	200
6	Y9 4H8	500
7	Y11 2C10	
8	Y11 2D2	
9	Y11 9E4	
10	Y11 9F10	
11	Y11 9H2	
12	Y12 0H4	



7-12 to be rerun (expecting large)

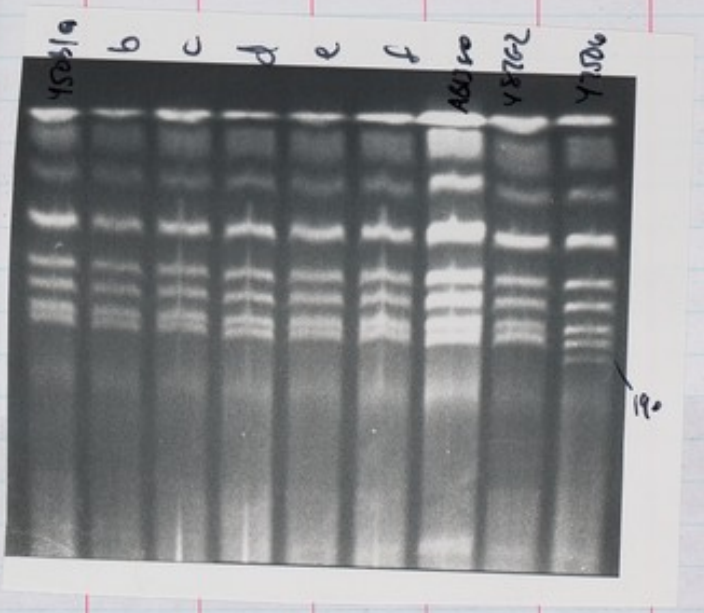


To be rerun again (much longer time run)

Plated 8/1/97

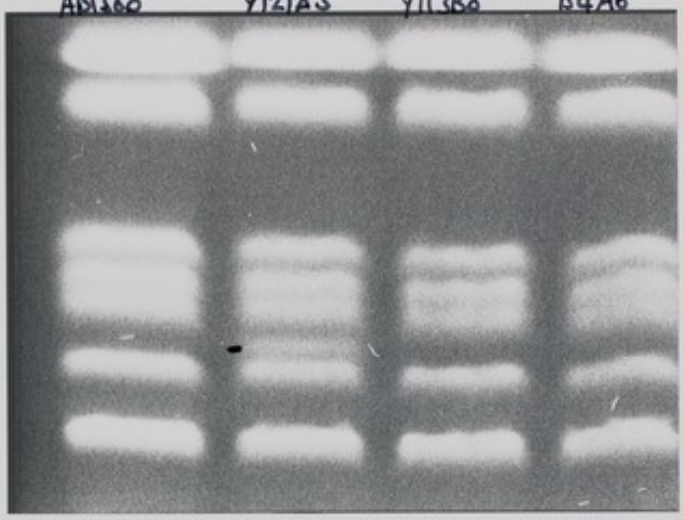
Y38H6
Y5081
} for preliminary gel to assess need for sizing gel for window

Y112C10
Y112D2
Y119E4
Y119E10
Y119H2
Y120H4
} for sizing; tried 7/10; need longer gel

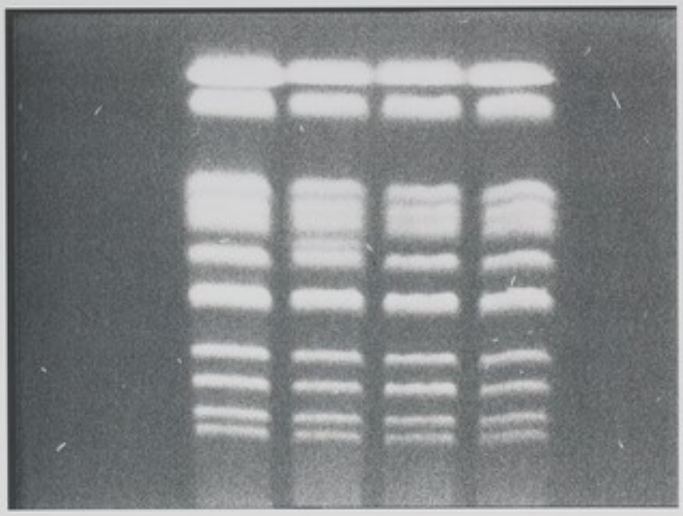


SIZING 8-5-97

AB1380 Y121A3 Y113B8 Y24A6



700kb



260

330

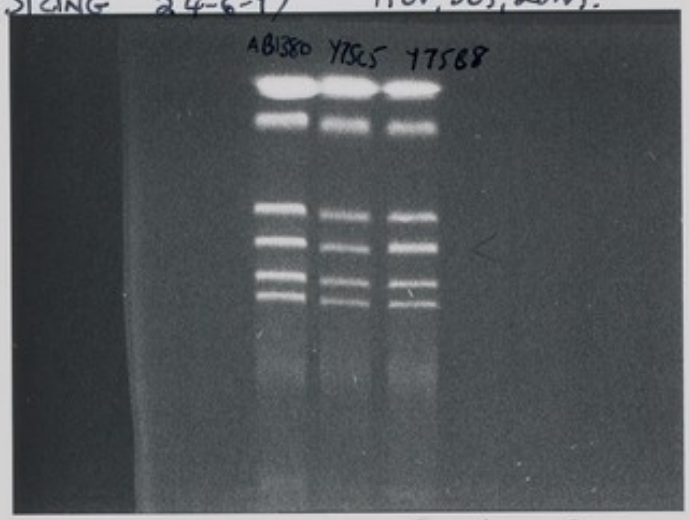
Y48E1

20/1/97 Comparison of original PFG / Bijay PFG.

580
450
370
280
230

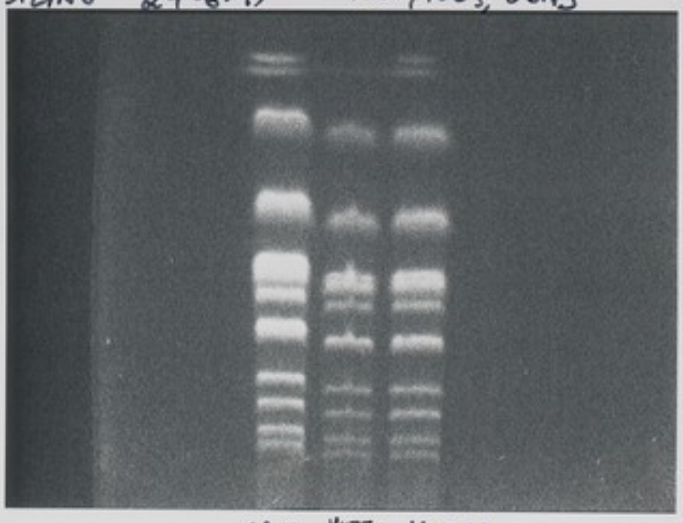
		orig.	bijay.
5/96	Y43F4	300 =	
	Y76A2	280	250
	Y48A6	300 =	
	Y39E4	350 =	
	Y41C4	280 =	
	Y111B2	620 =	
6/96	Y17G7	230 =	
	Y38H8	210 =	
	Y45F10	450 =	
	Y46G5	340 =	
7/96	Y37H2	460	450
	Y46G5	340	~320?
	Y53H1	450	sl fast?
	Y66F9	310	~300? (mid 280-350 → just above 280).
	Y38H8	210 =	
	Y46G5	340	~310? (orig. just below 350 → midway 280/350) (looks faster than 27/6)
	Y53H1	slow or better sep?	
	Y38F1	170 =	
	Y39B6	350	sl fast
	Y38E10	350 =	
	Y6B3	?	
10/96	Y37A1	350 =	
	Y40B3	350	320?
	Y54F6	550	~500?
	Y67A10	200 =	
	Y57G11	520	480? nearer 580 → nearer 450
	Y53C12	280	~260
	Y64G10	orig poor.	
	Y59A8	?	
	Y54E2	330	300? nearer 350 → midway 280/350
	Y53F4	430 =	
	Y47H9	460	350 midway 310→450 → on top of 350
	Y53H1	450 =	
	Y76A2	280	250
12/96	Y49A6	300 =	
	Y37D8	420	sl fast
	Y39B6	350	double, both fast
	Y39A1	350 = ?	
	Y46G5	340	~310. as 7/96
	Y43F4	300 =	
	Y52D3	300	~280

SIZING 24-6-97 150v, 50s, 28Hrs.



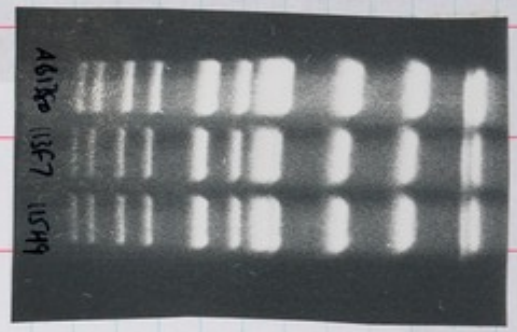
window?

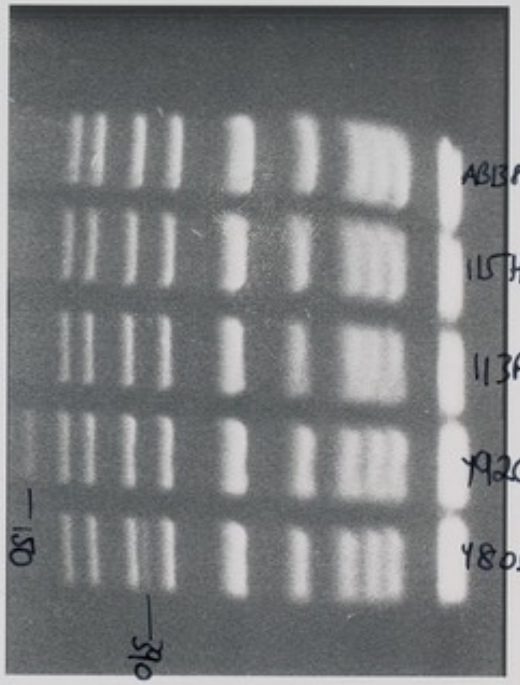
SIZING 24-6-97 150v, 100s, 36Hrs



ABI380 YTTC5 Y113C8

30/6/97





Alan,

A couple of YACs couldn't be separated and require windows.

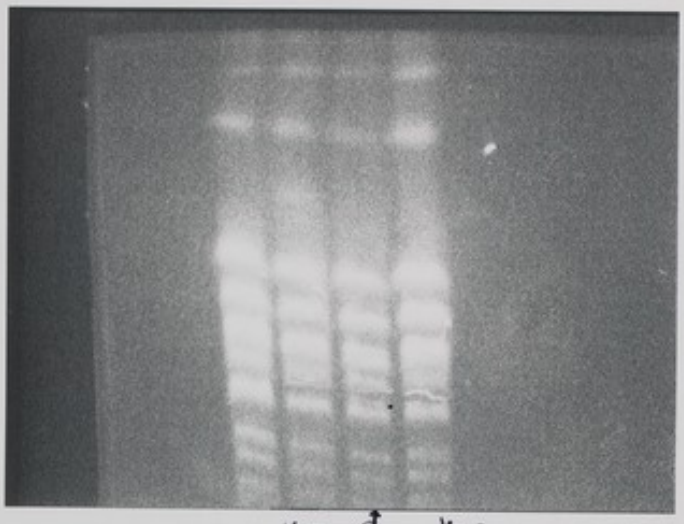
Y80D3 (390kb)

Y73F8 (310kb) - Looks OK in your book (as usual!) but not on my runs!

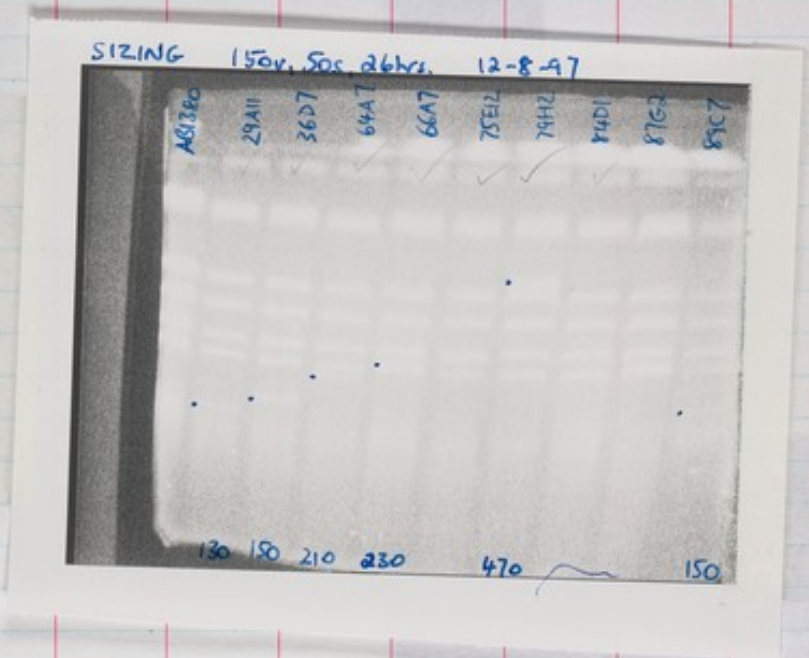
Cheers

Beej.

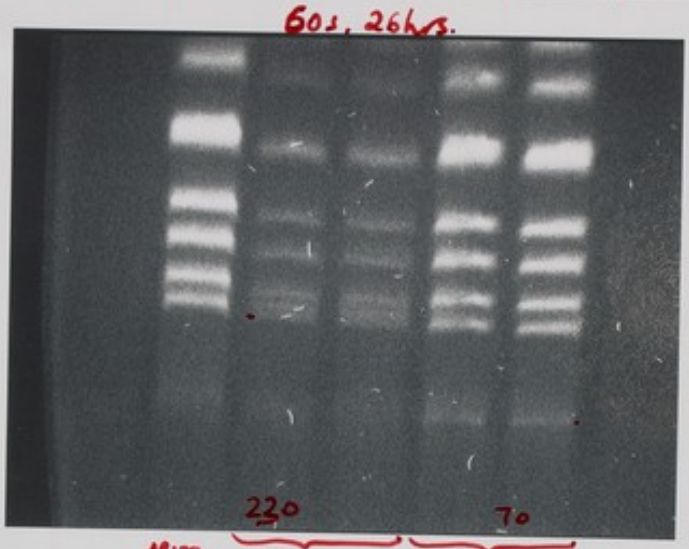
16/7/97



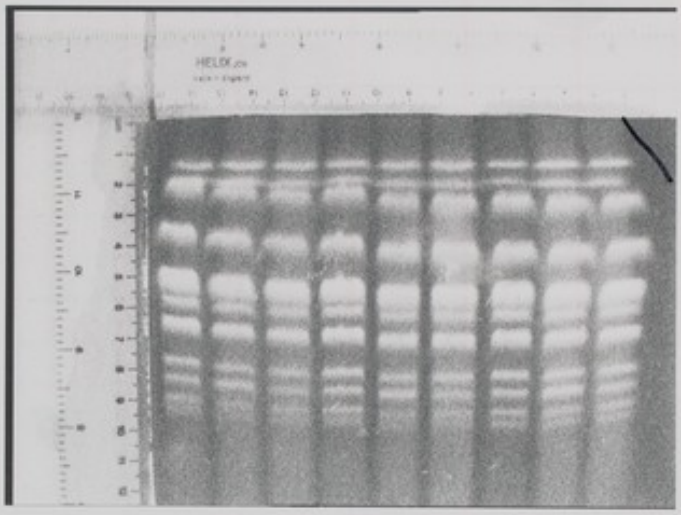
AB Y117 G00 Y113
1380 FS Y7525 B8



60s, 26kV.



17/9
Gel for Southern blot sizing



AB1380
Y11368
Y5446

Bijay prepped Y11388, Y1446, AB1380 blocks.
(run conditions?).

Gel ~~treat~~ment:

1. HCl 2 x 500ml 0.25M 15'
   (0.25M HCl = 28.5 ml/l of 32%).
2. 1 x 500ml 1.5M NaCl 0.5M NaOH 15'
   1 x 1000 ml " " 45'
3. 1 x 1000ml 1M Tris pH 8.0 1.5M NaCl,
   or 2 x 500 ml 0.5M Tris 7.4, 1.5M NaCl (15')

Blot into Hybond-N+ in 0.5 x SSPE o/n

Quick rinse in 0.5 x SSPE
Air dried
UV. fixed (2' transilluminator).

24/9 Probing with mp19.

50ng DNA
16 µl H_2O
100° 5'
ice

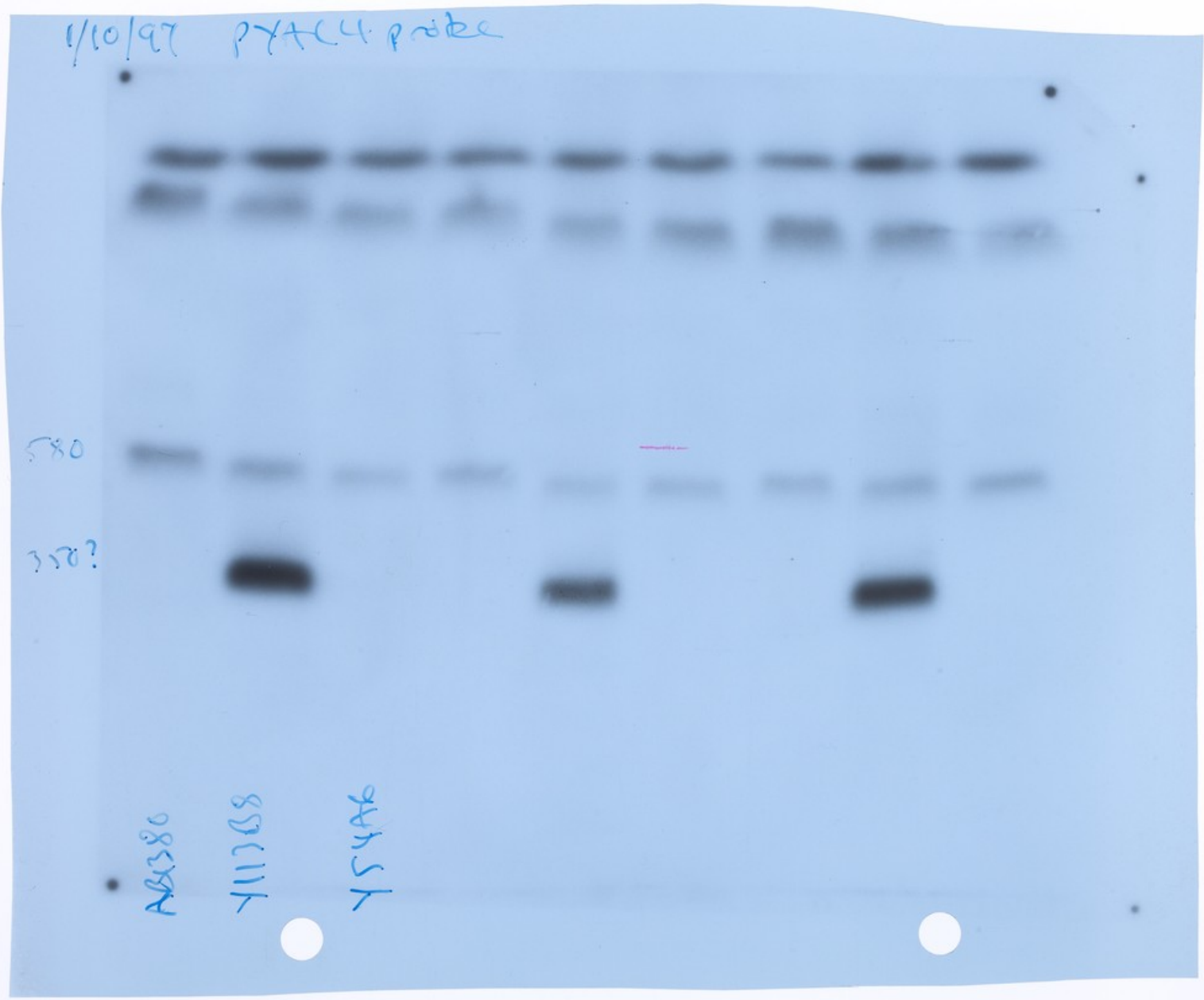
+ SOLB
- BSA 10 mg/ml
- 0.5 mM CTGTP
- 800 Ci/mmol αdATP^{32}
- 1 Klenow 5 u/µl
o/N

→ 5 µl H_2O
50 µl SS DNA
10' 100°
20' ice
+ 5 µl SCP/Boehringer sulphate

filter pre-welted in 1/2 x hyb mix + SS DNA
Seal bag hyb
o/N 68°
3 x washes 0.5 x SCP 0.1% SDS 50° → r.t. over

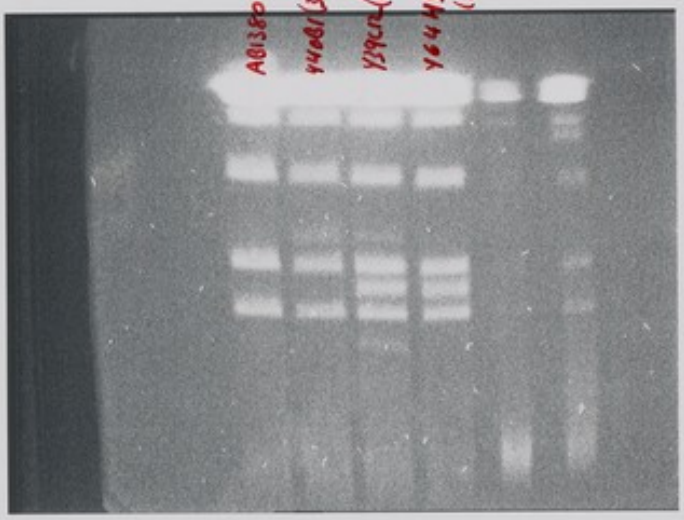
Probing failed.
Suspect used γ ATP!

1/10 Hybdn of same filter with pYAC4 (Rat fra)
See A/R.
Y113B8 = 350 kb. (window → W2).
Y54A6 - no sign!


1/10/97 PYAC4 probe
580
330?
AB380
YAC 1158
YAC 4454

7/10/97

Separate ~~test~~s:



15/10

0/2

~800.

AB1380

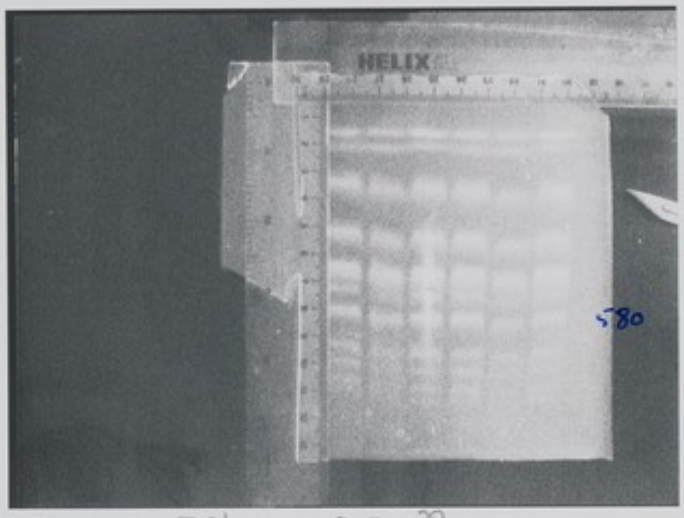
Y11357

AF273

9m sperm DNA

15/10.

Southern sizing of Y11367.
Blocks prep'd by Rick Dobson / Bijay.
Gel 100 secs 36 hr (150 v).
Blotting on 17/9 gel.



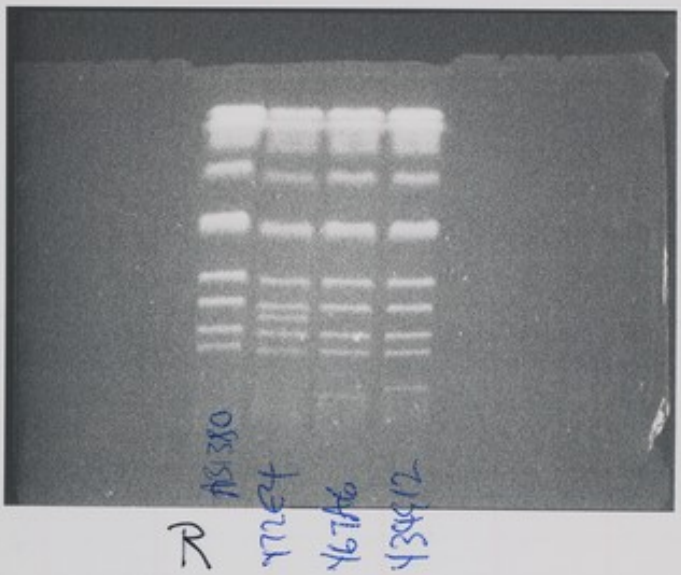
100 secs 36 hr (150 v)
Y11367
Y7368

Y7368 = 480

Radha to probe ∂ pYAC4

So Y11367 ≅ 800 kb.
Window transfer → WB

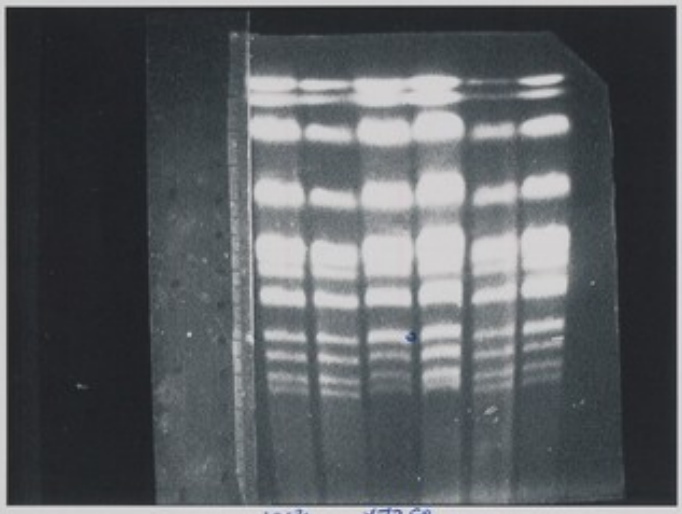
19/10



Sizing of YAC detected in 'small YAC search' for F35C12/F10C3.
Y67A6 and Y34G12 both good.

5/11

Southern blot



1380 YT3F8
Y11595

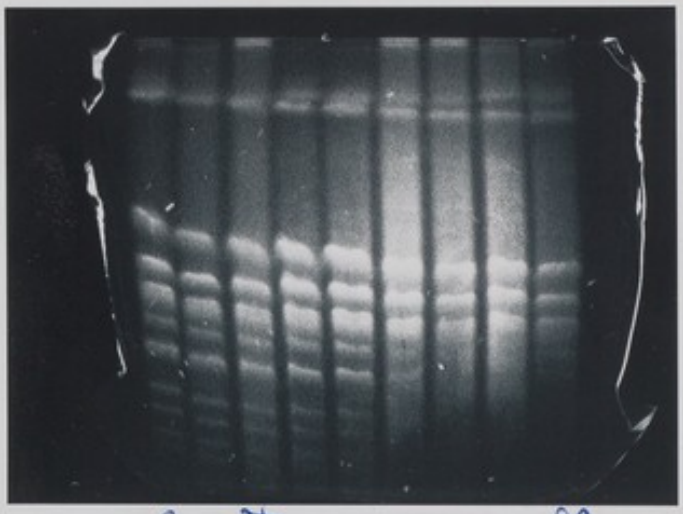
poke control

No sign of Y11595.

Try dropping plug from plate scrape.

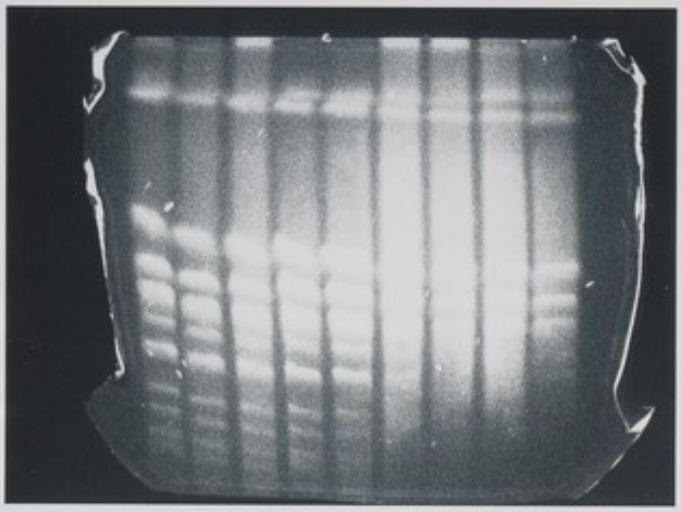
ΔPS1080
ΔPS1
PS1

24/11
Sizing



AS1380
V82A10
V8257
V8752
V8093W3B

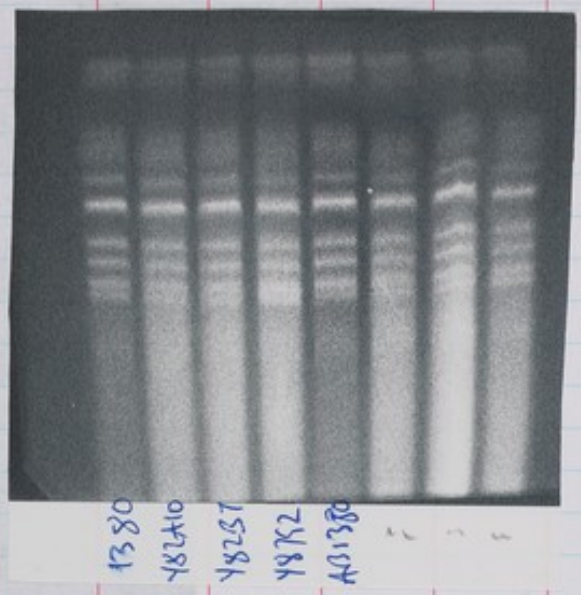
AS1380
V82A10
V8257
V8752
V8093W3B



Will require Southern. Gel to be repeated

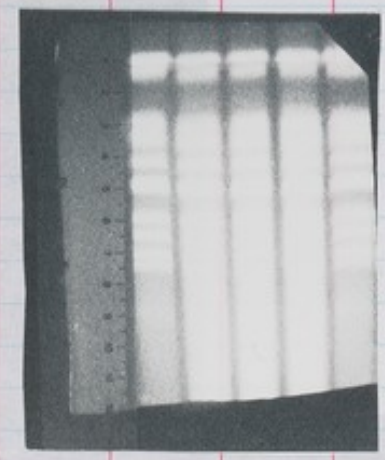
(over)

26/11



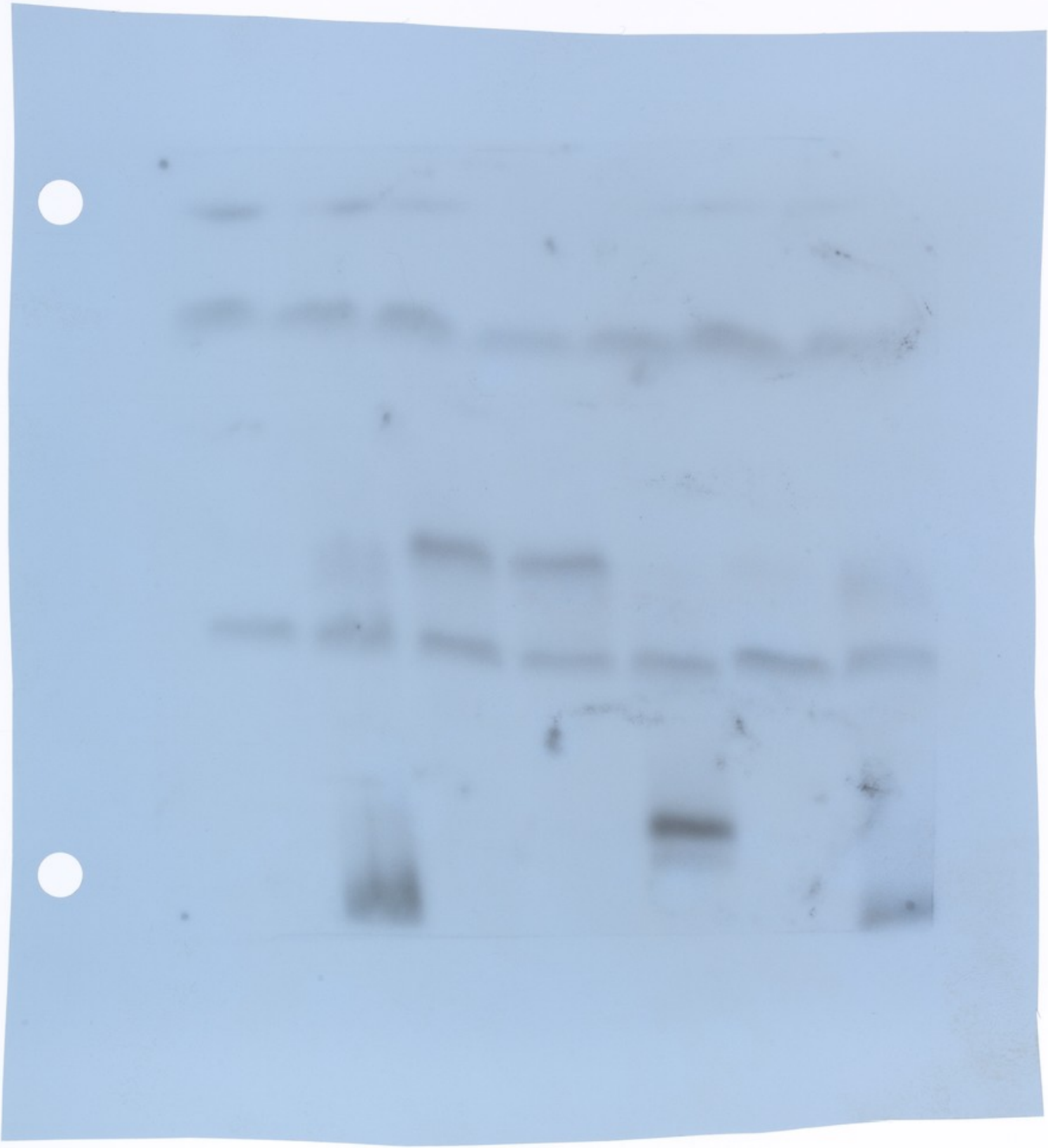
Some indication of YAC in Y8252 at 280??
But not obvious in duplicate

Blot:



Southern Y8252 at 280. - for window
Y8240: Poss v. weak band at ~ 230
Y8207: can't see

A9138c
A9124c
A9127
A9125
A9123c



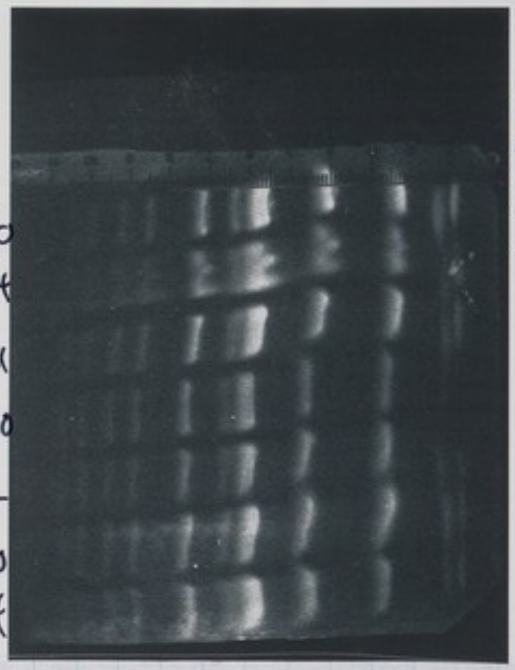
~850

280

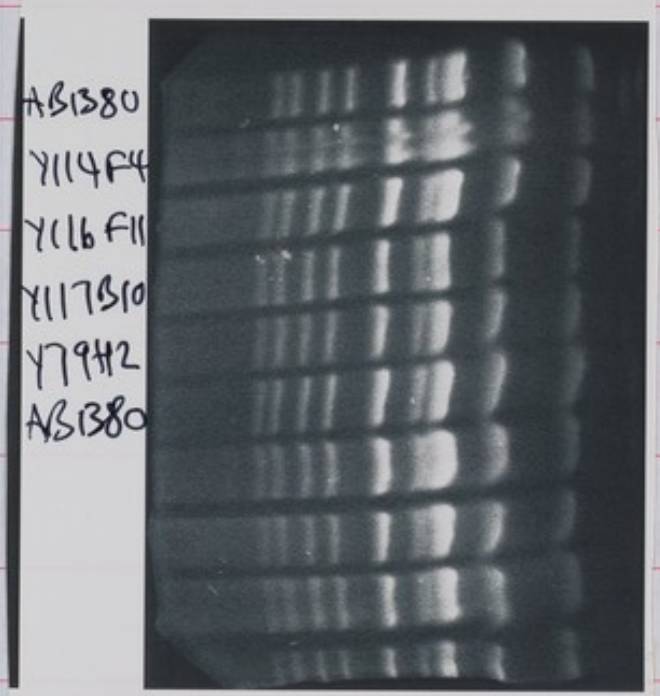
200

Blotted 1/12/97

For Southern

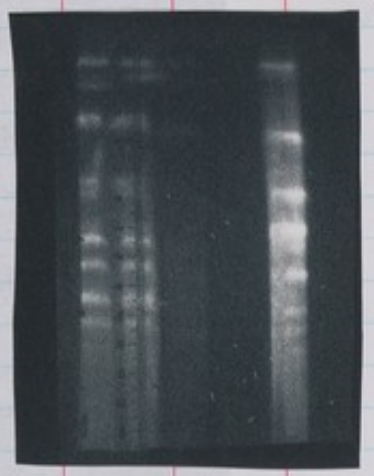


30/11/97
Siginis



11/12/97

Gel for Southern:

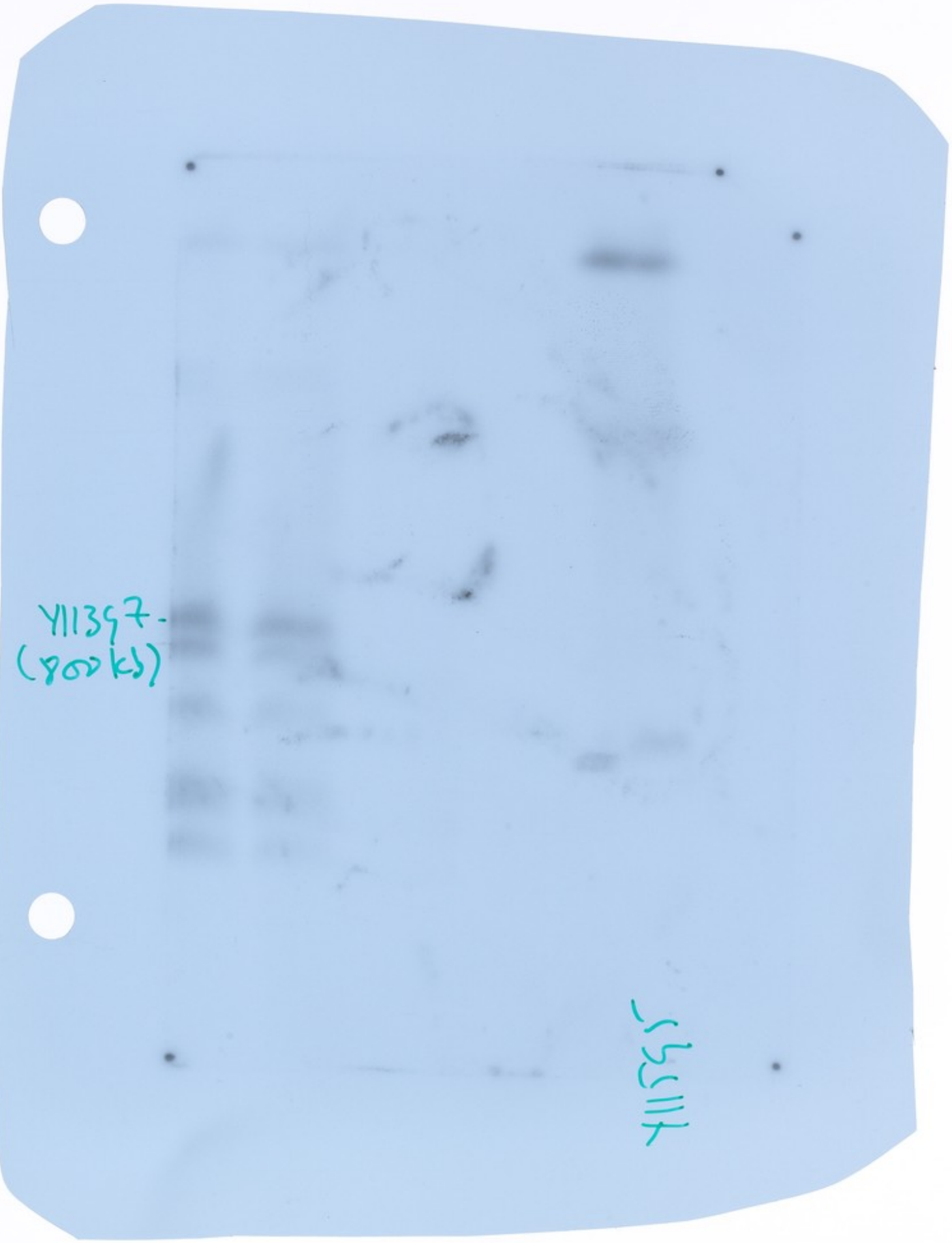


W6

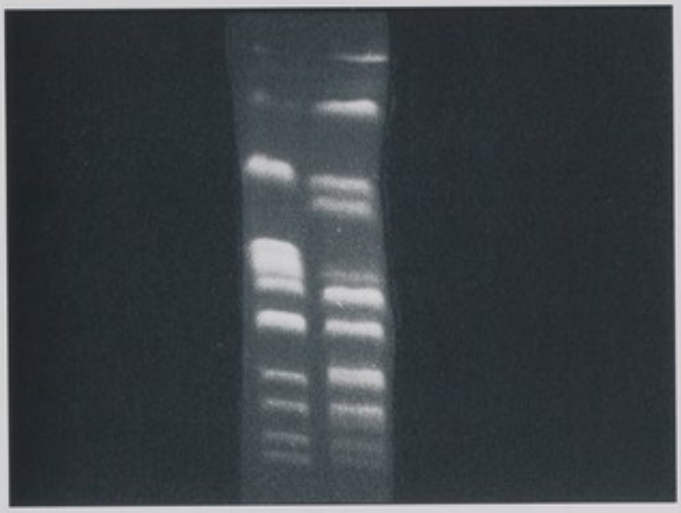
↑ Y11595, plug made from plate scrape (no signal on southern of plug made from liquid culture).

Y11595W6 Band C.

No obvious tre YAC band but need better gel with more suitable control.


Y11397-
(800 kb)
ハンドリ

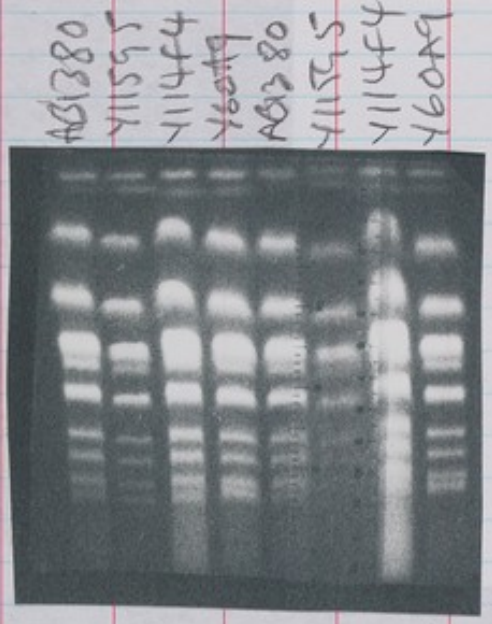
19/11/97



Not blotted - must be run with ABI 3730

15/1/98

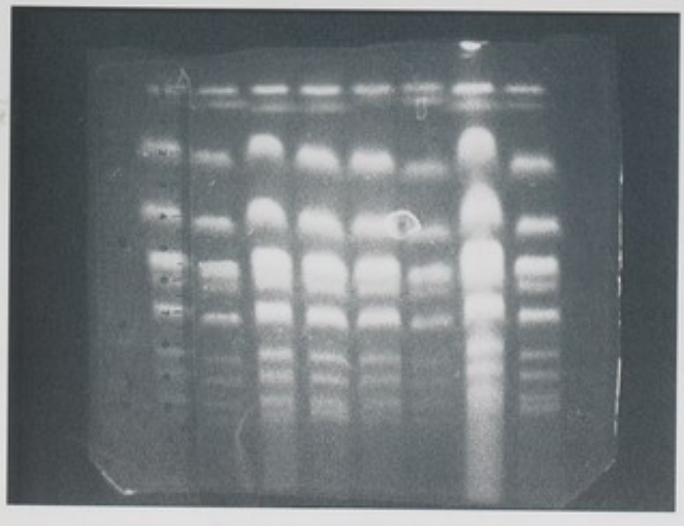
Southern



Y115G1 = plate-scrape plugs

Y114F4 = probable disintegrating YAC

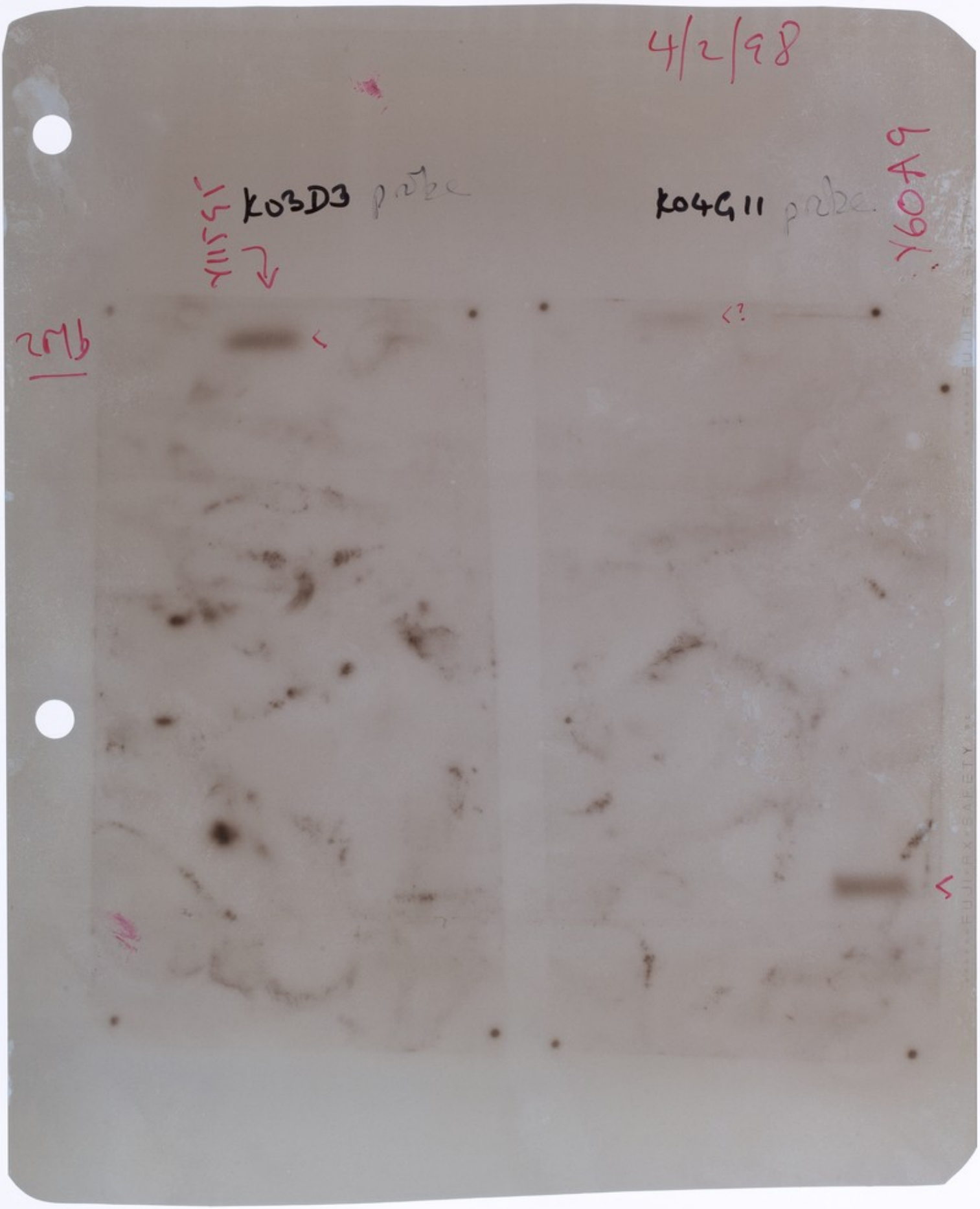
Y60A9 = 270 kb

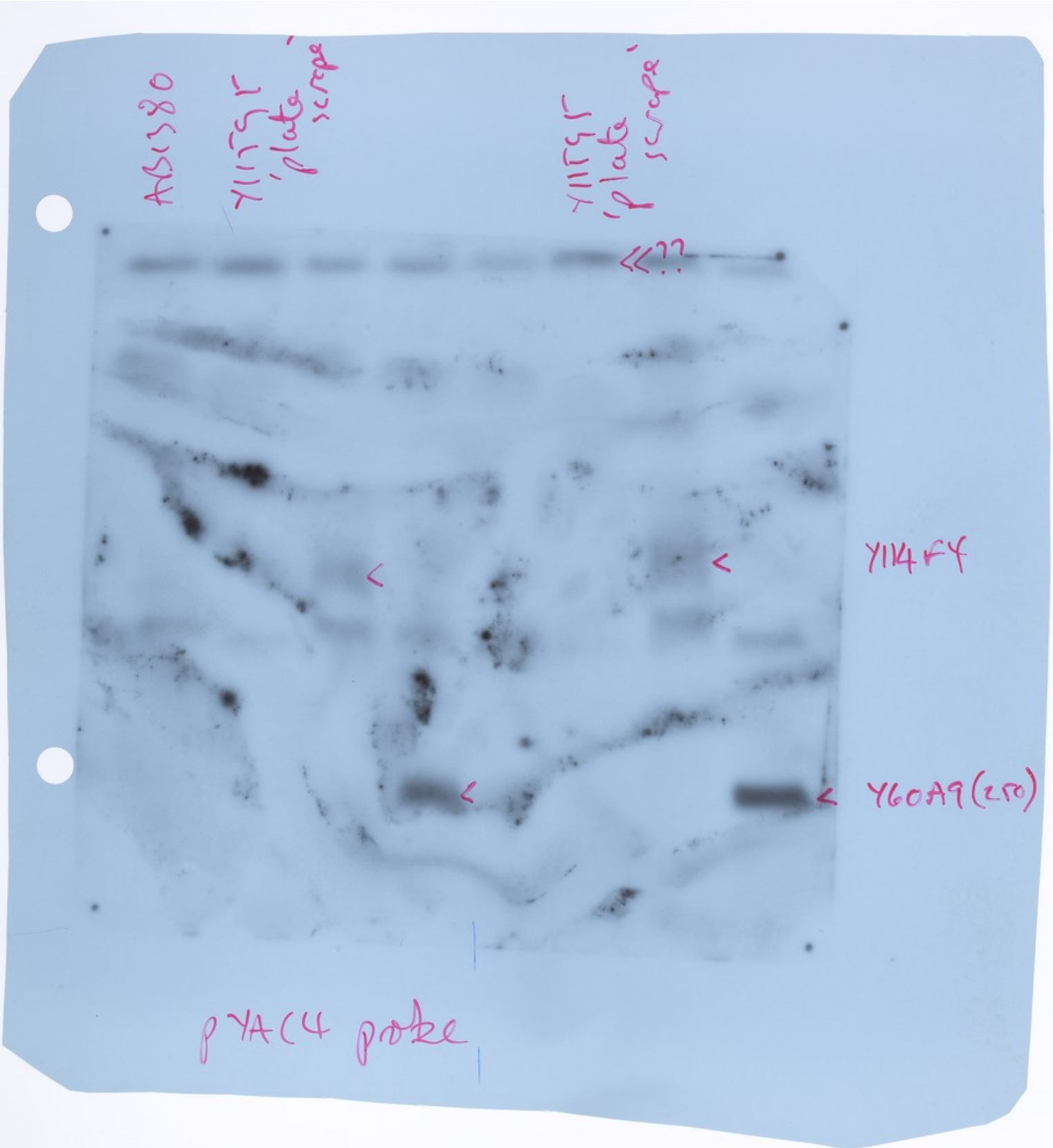


4/2/98

K03D3 probe

K04G11 probe




ABC1580
Y114F4 plate scrape
Y114F4 plate scrape
??
Y114F4
Y60A9(210)
pYAC4 probe

1155+5 poss running with top band ??!
Note: This last weaker by ETLRR.

– 1155+5 is at 2216 (see 4/2 posting)

```
BABYL OPTIONS: -*- rmail -*-
Version: 5
Labels:
Note:   This is the header of an rmail file.
Note:   If you are seeing it in rmail,
Note:    it means the file has no messages in it.
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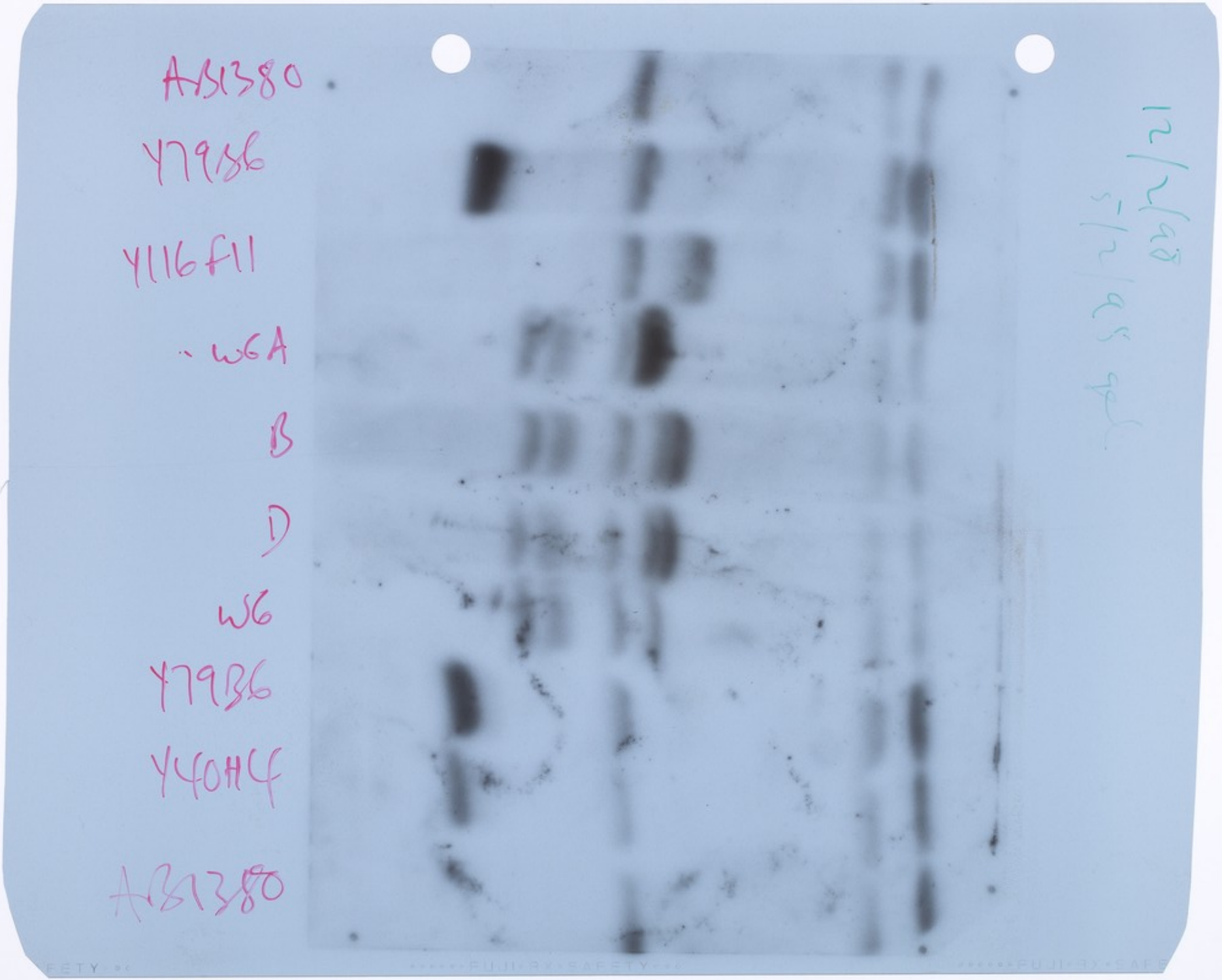
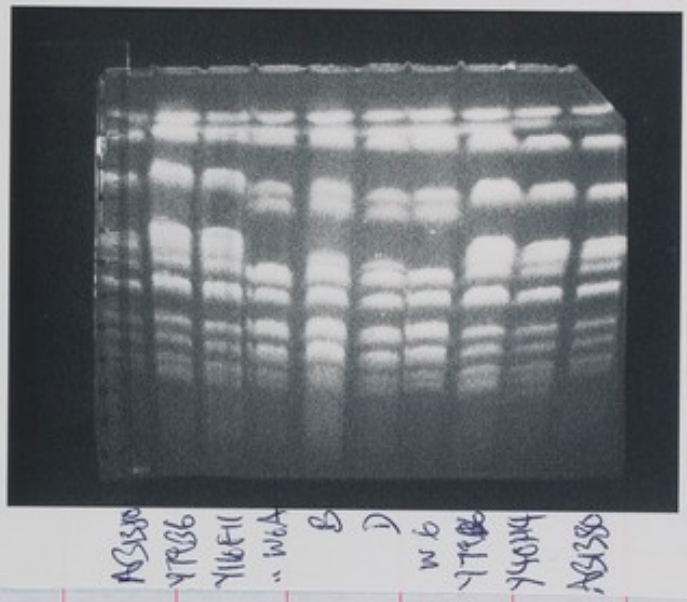

AB1380
Y7986
Y116F11
wGA
B
D
W6
Y7986
Y40HC
AB1380
12/7/98
5-12/98 gel

5/2/98

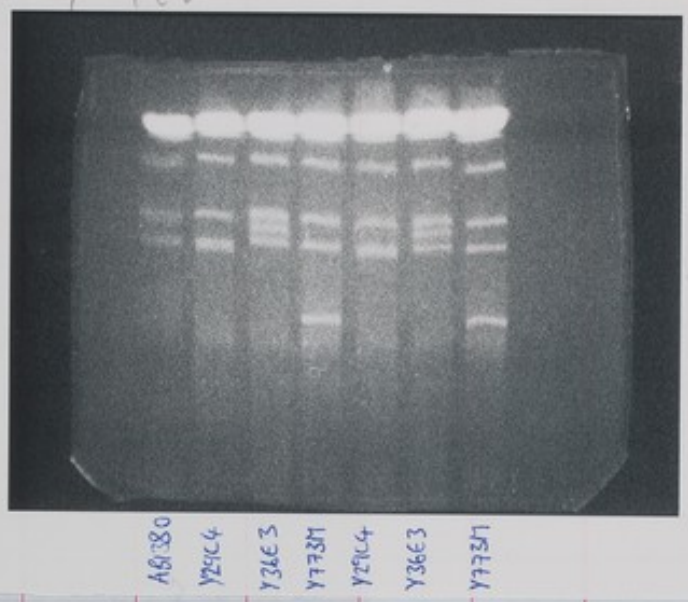
47986 sizing



for Southern

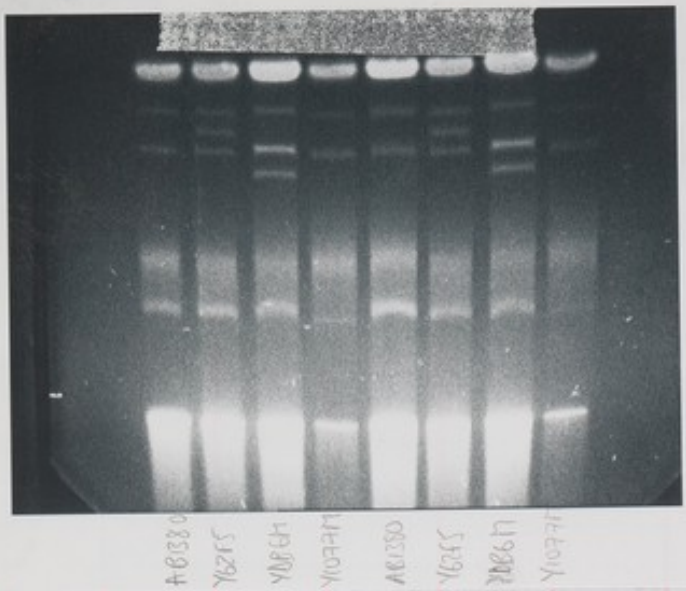
12/2/98 Sizing

12/2/98

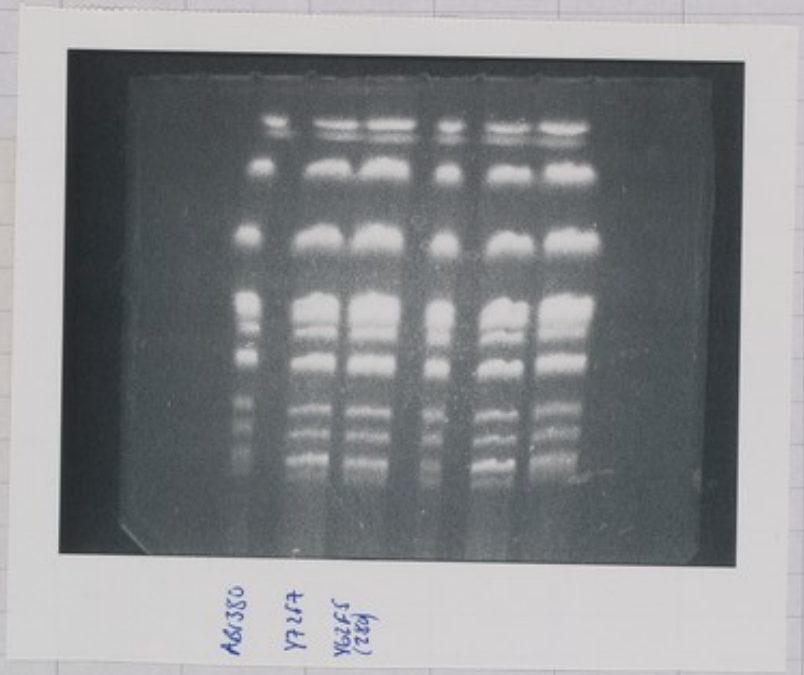


Y29C4 = 230 kb
Y36E3 = 260 kb ← Prep.

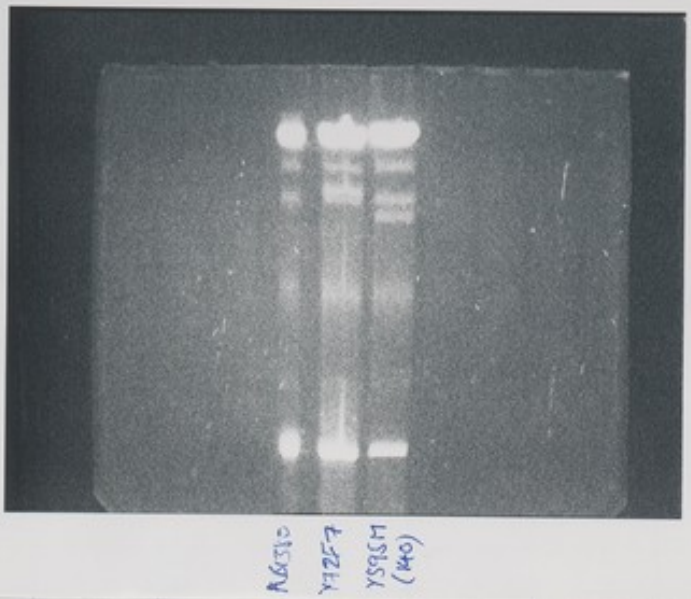
18/3/98



9/4



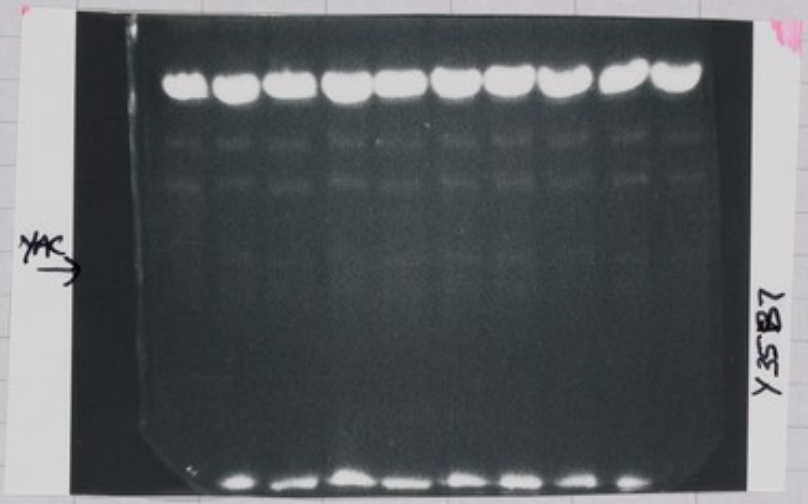
YZ2F7 sizing 280 kb
Not necessarily required for library.



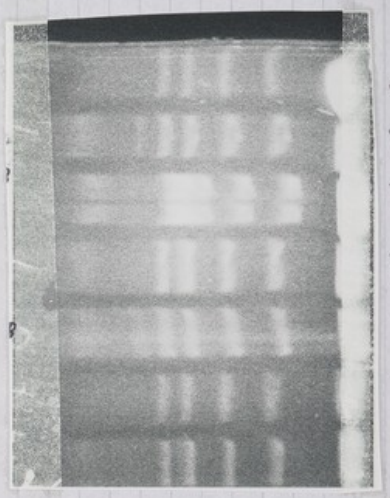
16/4
Seq~. test
YAC band a bit
suspiciously strong.

12/1/99

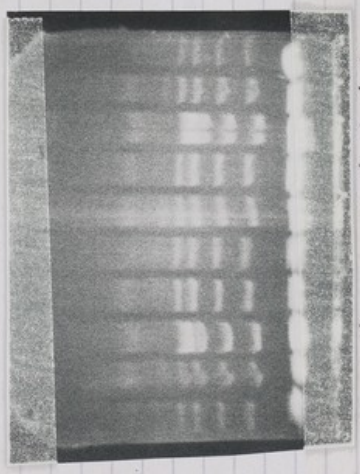
Y35B7 (II R ? for exploratory shotgun ?)



V. small < ~100 kb ?

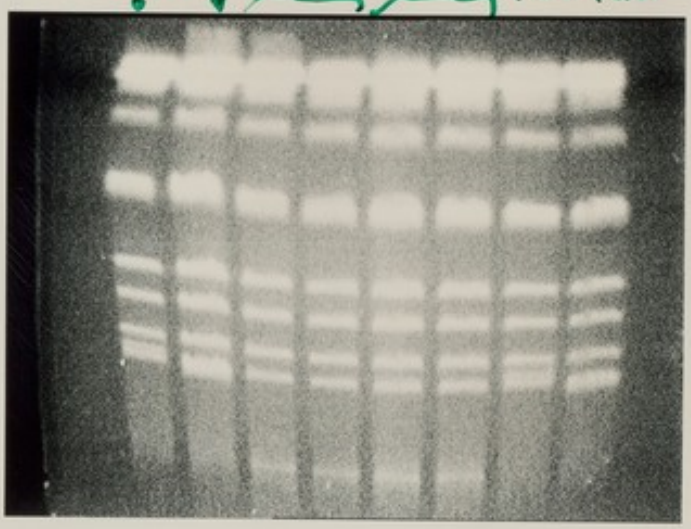


AB 1380
Y66D12
Y75E12
Y8762W2B
Y66D12
Y8762W2B



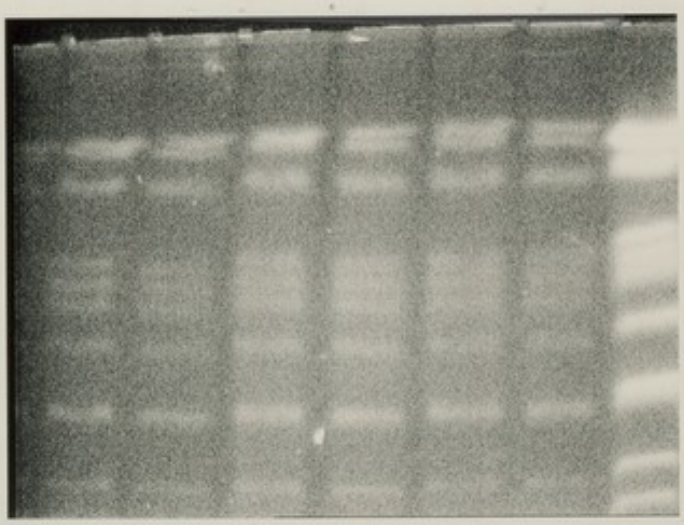
AB-1380
Y66D12
Y75E12
Y8762W1B
Y66D12
AB-1380
Y75E12
Y8762W1B
Y66D12
AB-1380

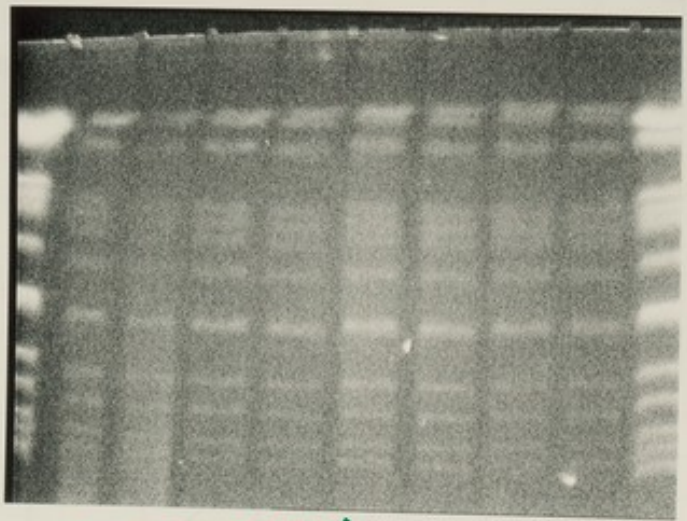
8/5/00



25/9/00

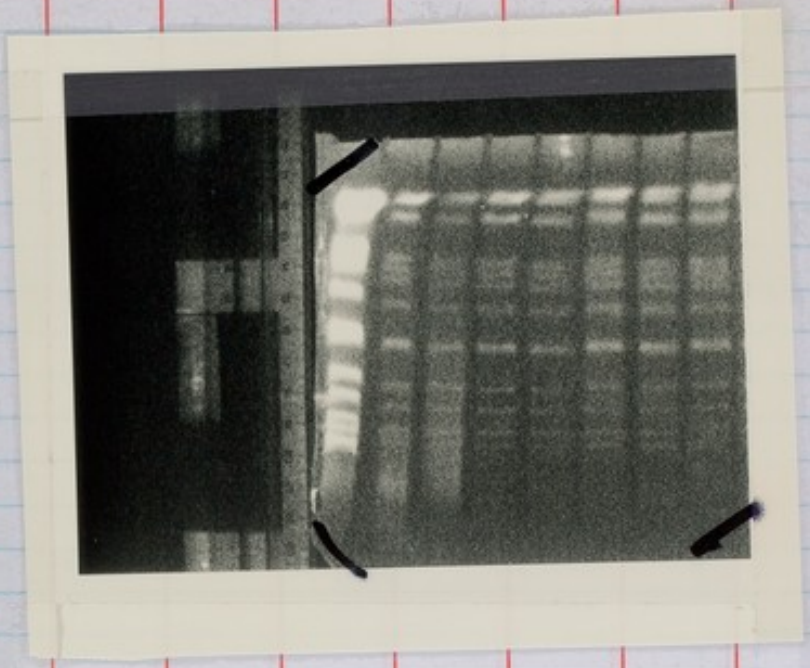
Sizing of Y113D12.
YAC not apparent on earlier gel.
Try Southern
David Johnson set:





Y113D12 on box
90s, 45hrs marker 4A1

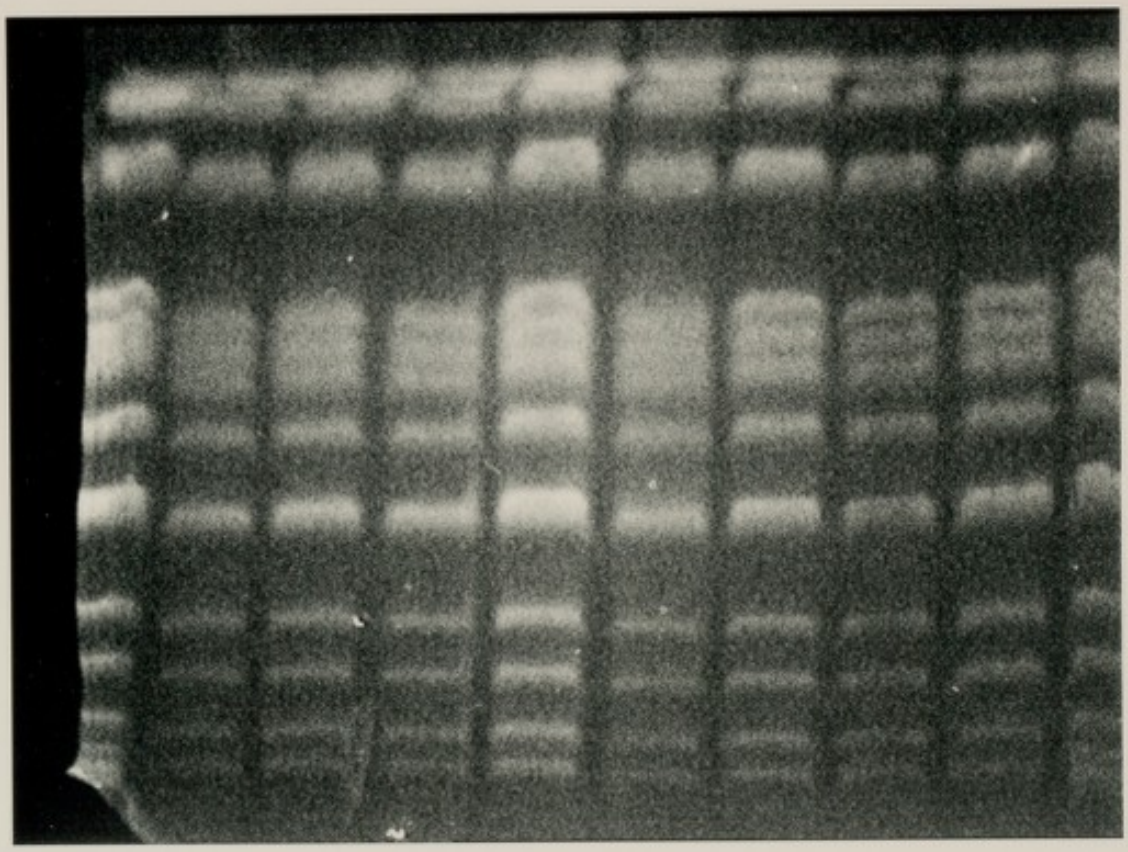
over

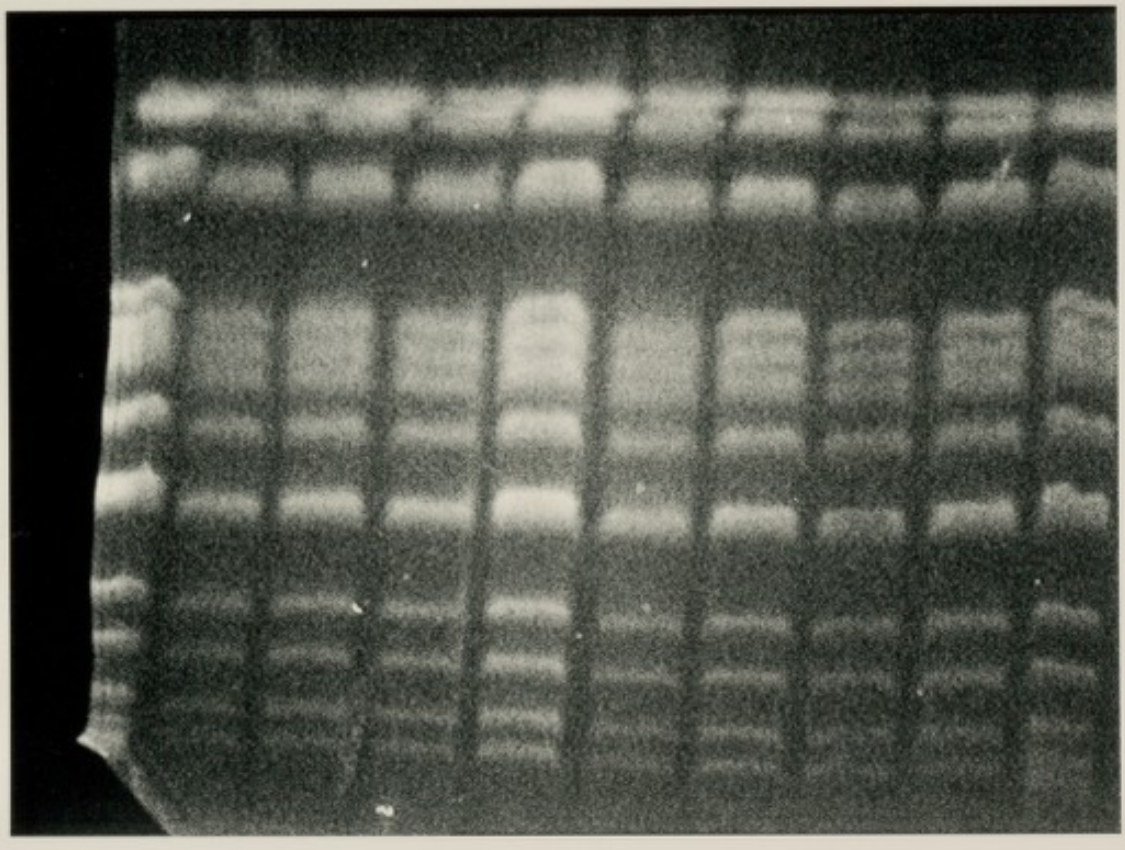


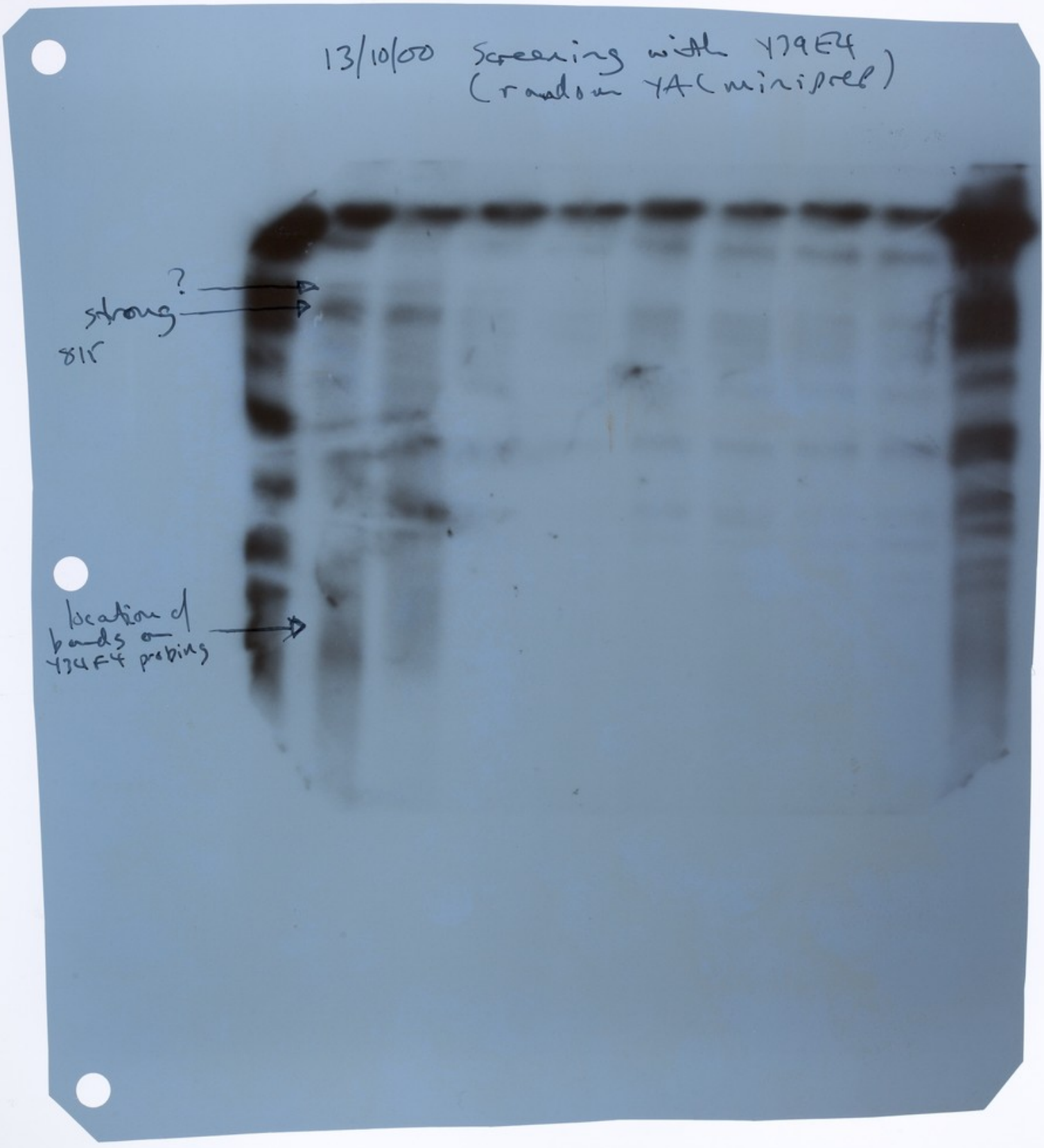
10' acid prior to denat.ⁿ/neutralisⁿ
Hybond-N blot. 0.1x SSC

To probe with
a) Y34F4
[b) YAC miniprep.]

26/9. Radha Y34F4 probe.
O/N exposure.
Only 1st 2 lanes have signal.
NB Not at par of smear by measurement
- signal seems actually to be
closer to 230 kb chromosome.
Probe with 710195 (other side of gap)
(Then probe with yeast miniprep.)






13/10/00 Screening with Y79E4
(random YAC minipool)
?
strong
815
location of
bands on
Y79E4 probing

Y113D12 Southern probed with Y34F4 fragment
26/9/21

Y113D12 Smutter blot- screening
with MO165 fragment-
2/10/21C - Weekend Exp.

(Hypothesis is that YAC is throwing out central portion).

11/10/00

Rough probing

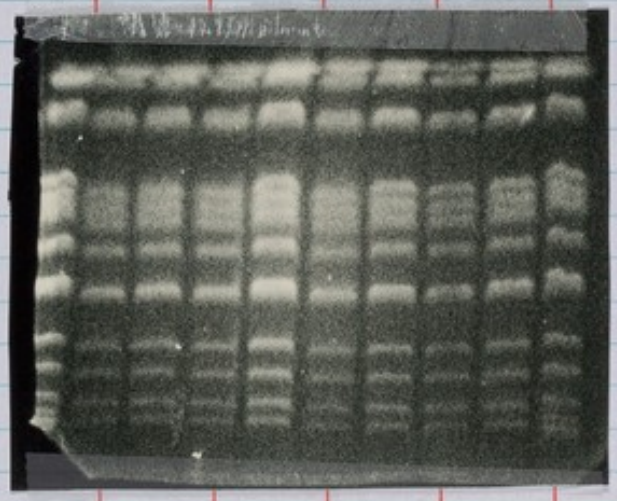
Same banding pattern as 43454 probe, but v. weak.

Can't be certain they are not residue of 43454 probing (filter was stripped).

(Gotta do whole YAC probing then repeat rough).

10/10/08

Clone individual colony pool 1
Y113D12 (Dave Johnson)
Direct inoculation of small single colonies into 200 ml.



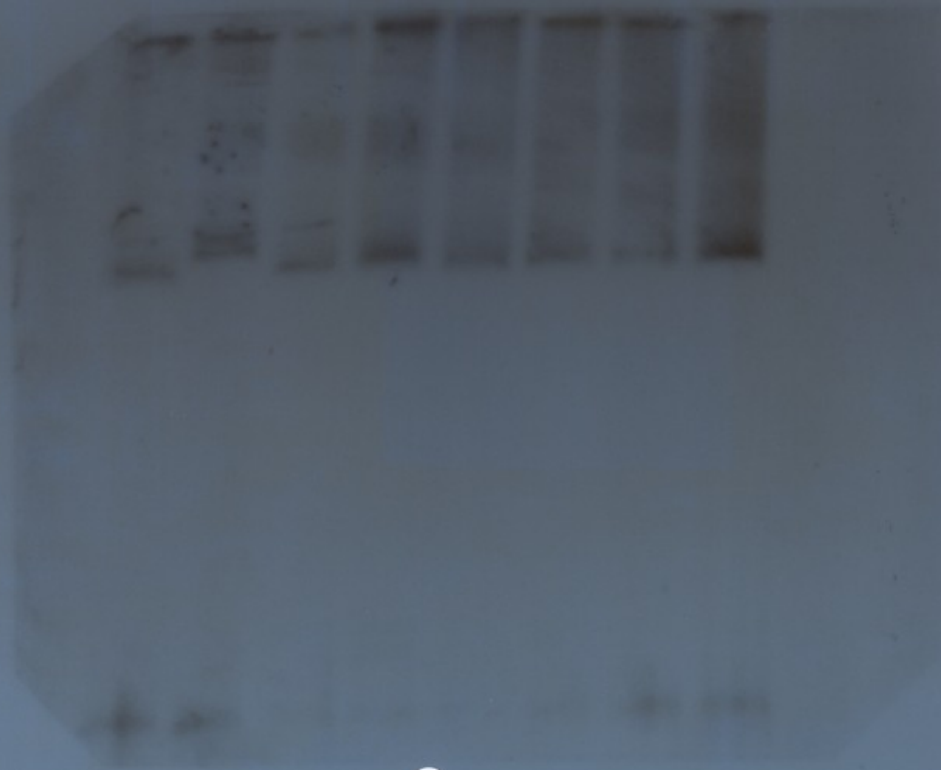
No obvious sign of YAC, either possibly intact at ~200 kb or breakdown product at ~200 kb

Blotted for Rad1 probe.

18/10/2010

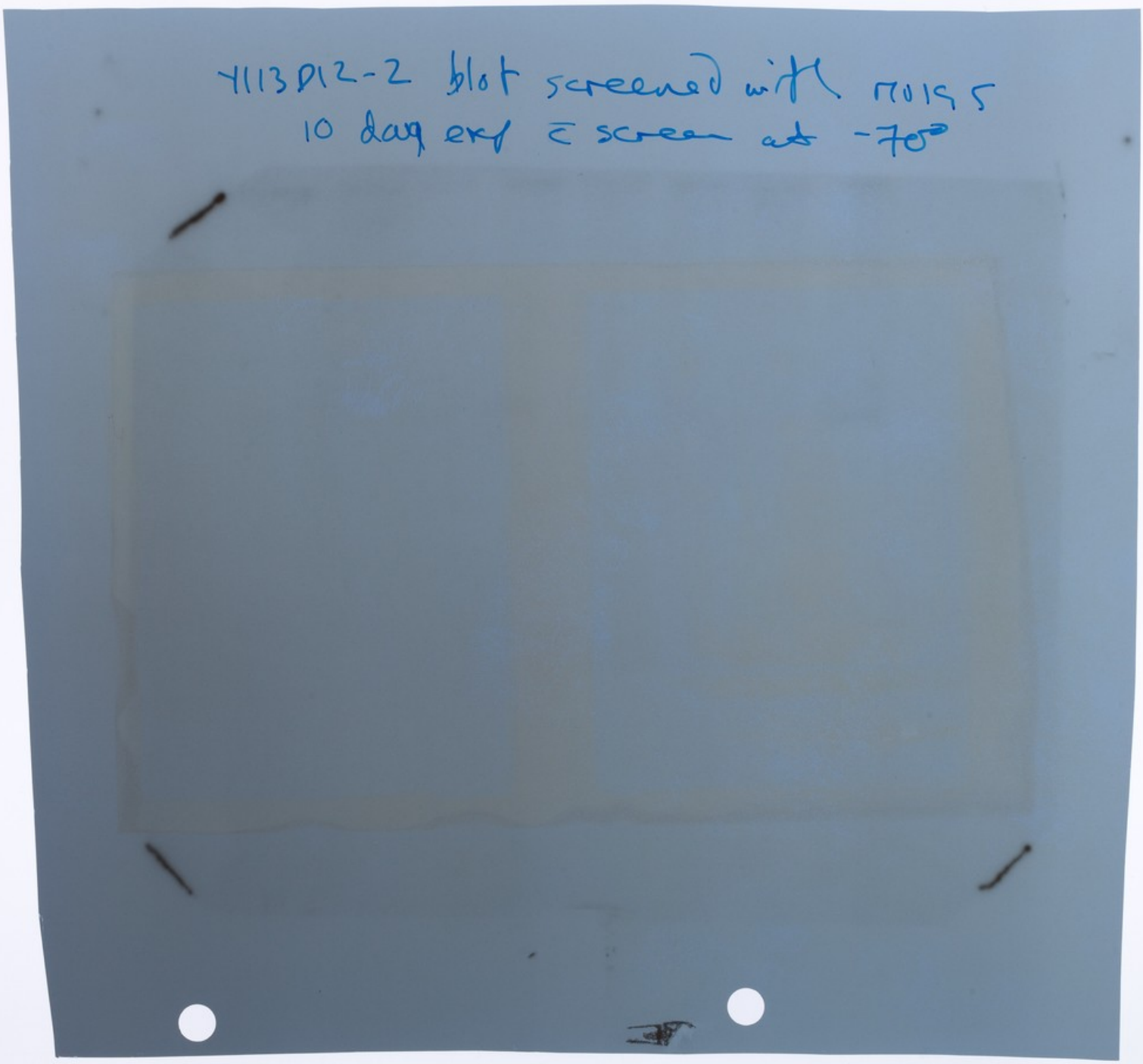
X113D12 Southern Blot - #2 Screened with y34F4 fragmen-

Exp.- Friday → Tuesday @ -70°C E screen

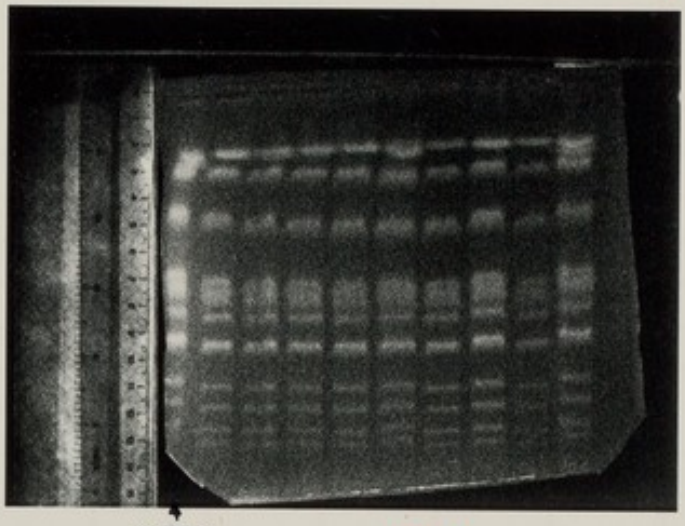


FUJI SAFETY

34/10/00
X113012-2 Π0195.2 48 hr exp.

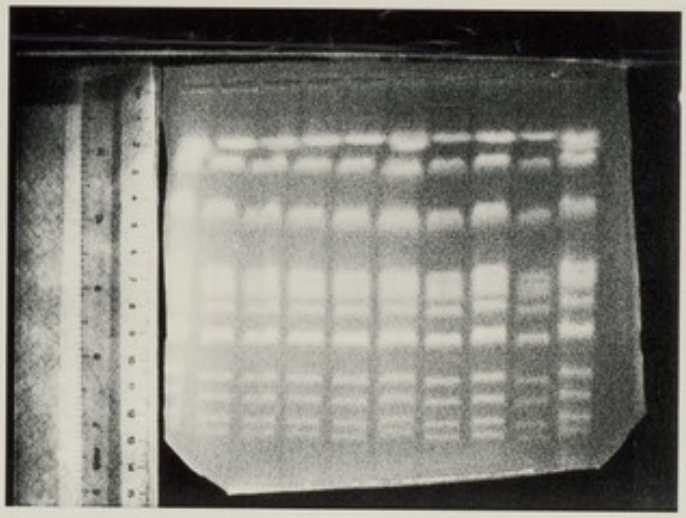

Y113D12-2 blot screened with mouse
10 day exp @ screen at -70°

31/10



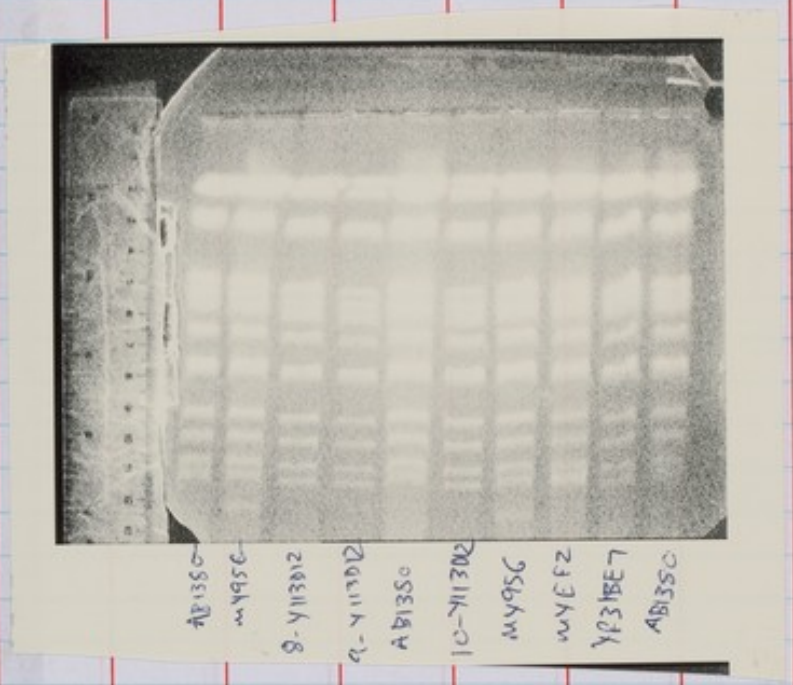
my456

YP3612.



31/10/00

3/10/00



90 sec 40 hr 150V

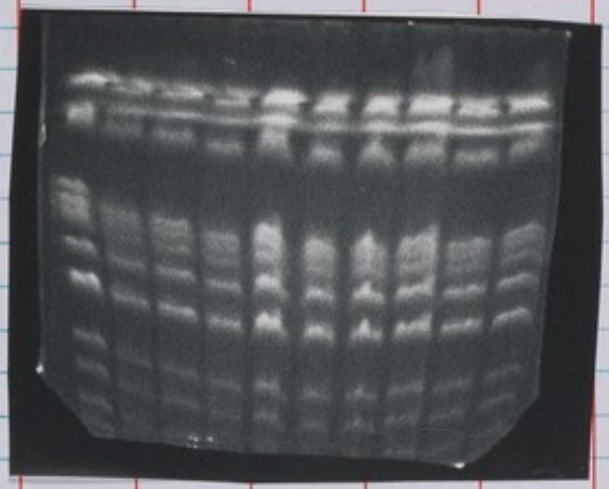
9/2/01

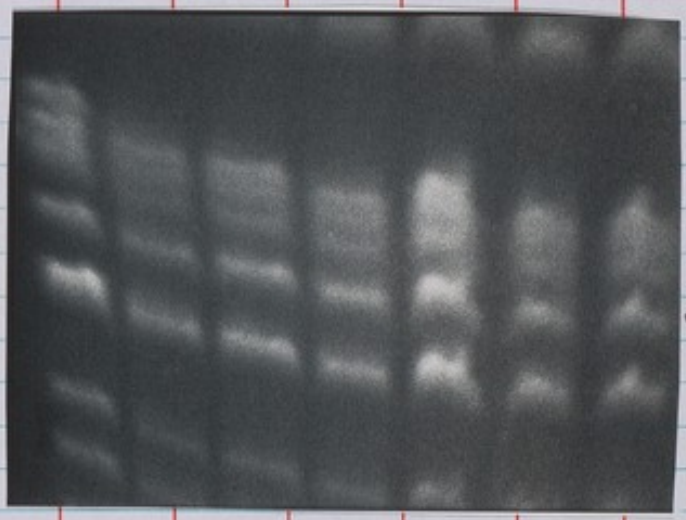
yX60D10

chr 20 YAC

Prep as a result of earlier muddle.

Size unknown (400 kb +)





680 kb band looks relatively strong so speculate that YAC co-migrating

David to initiate window transfer to WS on that basis.

Blot for Southern.

70 sec 45 hr.

Jane Holden / Paros to pick

29/2/01

Peculiar — +ve as predicted (680) if measured from bottom, but at ~815 if measured from top. Don't understand this — gel broke during pretreatment but I don't think I lost 1.5 cm from either end! Shape of band looks more like 680 kb

Analytical sizing needed of:

Y115D9
Y115F5
Y81G3
Y65A5
Y59A8

Y94A7
Y84A5
Y95G10

Streak:
70secs

Predive:

II Y17G7, Y46
IV Y38H8, Y
V Y17D7, Y37H2 210, 460
I Y53H1, Y66F9 450, 310

Selected 27/6/96

II Y38E10, Y38F1 310, 170
IV Y37A1, Y54F6 320, 180
V Y39B6, Y40B3 310, 310
I Y6B3 230

Bijay sizing

Y6 SAT. 210 kb
Y8193 320 kb

YAC's to Date 27/6/96

Chromosome	
3	Y48A6(300), Y45F3(280), Y49E10(150) Y43F4(300)
3	Y111B2(620), Y76A2(280), Y39E4(350)● Y37D8(420), Y41C4(300)⊛
1	Y53H1(450), Y66F9(310)
2	Y17G7(230), Y46G5(340)
4	Y38H8(210), Y45F10(450)
5	Y17D7(210), Y37H2(460)

Analytical sizing needed of:

Y115D9
Y115F5
Y8193
Y65A5
Y59A8
Y94A7
Y84A5
Y95910

Streak:
70secs

Predive:

II	Y1797, Y4695	230, 340
IV	Y38H8, Y45F10	210, 450
V	Y17D7, Y37H2	210, 460
I	Y53H11, Y66F9	450, 310

Selected 27/6/96

II	Y38E10, Y38F1	310, 170
IV	Y37A1, Y54F6	310, 170
V	Y3986, Y40B3	310, 310
I	Y6B3	230

Bijay sizing

Y65A5 210 kb
Y8193 320 kb

Sijay

Bijay

Y59A8
Y65A5 210
Y81C3 320
Y84A5
Y94A7 210
Y95C10 ~1Mb?
Y115D9
Y115F5 ~700

31/08/96

Y148E1 (330kb) JES.

8/08/96

~~Y1-9A8 (~~~~kb (~~kbps) JES~~

Y6G8 (230kb) JES.

19/09/96

Y51H1 (II) (130kb) JES

23/9/96

Y549I1 (II) (370)
Y17B7 (II) (90)
Y54E2 (II) (330)
Y53F4 (II) (430)

21/9/96

Y51H5 (IV) (190kb) JES

2/10/96

Y579I1 (IV) (520) JES
Y67A10 (IV) (200) ~~JES~~

22/10

Y81G3 (II) 320
Y13C12 (II) 280 - cluster, but Zarkower says >50 kb gap. Zarkower says separates.

Y65A5 (IV) 210

28/10

(Y39G8 W1A (II) 230 - ~~start~~ again)
Y64G10 (IV) 270
Y59A8 (V) 310

((4 from Bijay for repeping: Y1~~7~~57 (90 kb)
Y54G11 (370 kb)
Y~~5~~42 (330 kb)
Y5~~3~~F4 (430 kb)

Better recycle Y39G8W1A plate (assuming BJ really did take the wrong band) My gel wasn't so great, let's start again

(JH)

(280).
~ transfer))

Y5 3F4 (II) 430
Y5 4E2 (II) 330

13/11

Y53H1 (I) 450
Y47H9 (I) 400
Y91F4 (I) 200
} for Tm 14

18/11

Y39G8W1A (II) 230
Y48A6 (III) 300
Y76A2 (II) 280 ← PFC still fuzzy

Colony purified
Pin to streak for
inoculation

OVER

22/10

Y81 93 (II) 320
Y13 C12 (II) 280 - cluster, but Zarkower says >150 kb gap. Zarkower says separates.

Y65 A5 (IV) 210

28/10

(Y39 98 W1A (II) 230 - start again)
Y64 G10 (IV) 270
Y5 9A8 (V) 310

((4 from Bijay for repeping: Y1 ~~A~~57 (90 kb)
Y5 4G11 (370 kb)
Y ~~A~~E2 (330 kb)
Y5 ~~3~~F4 (430 kb)

A1 5 Y51H5 (190kb) repeat (5th)
A1 10 Y48C3 W1A ~~for~~ prep (280).
- good window transfer))

5/11

Y1 7B7 (II) 90 re-prep
Y5 3F4 (II) 430 "
Y5 4E2 (II) 330 "

13/11

Y5 3H1 (I) 450
Y4 7H9 (I) 400 } for Tm 14
Y9 1F4 (I) 200

18/11

Y39 98W1A (II) 230 Colony purified
Y48 A6 (III) 300 pin to streak for
Y76 A2 (II) 280 ← PFC still fuzzy inoculation

OVER

25/11

Y59A8 ? repeat (orig. too fuzzy)
or leave till later if others more
urgent

Y53H1 repeat
(Y47H9 for window).

25/11/96

Y51H5	IV	190 kb	colony purified
Y39B6	V	310 kb	
Y37D8	III	420 kb	
Y52D6	II	200 kb	

Note:

repeat Y59A8? (orig. too fuzzy)

Y102A5 for Tm II? (hmA ms-1)

2/12

Y6B3	(I)	230 kb	colony purified
Y39A1	(III)	310 kb	"
Y46G5	(II)	340 kb	"
Y59A8	(V)	350 kb	"

9/12

Y53C10W2B	(I)	350
Y54E5W2A	(I)	290
Y37H2W3A	(V)	460
Y51A2	(V)	~~X 180 X~~

(Y70S10
Y51A2 repeat (not 180kb)) → window?

23/12/96

Y70S10	(III)	100
Y43F4	(III)	360
Y71A12NRG	(II)	340
Y9F6	(V)	190

30/12/96

Y52D3	(III)	300
Y38F1	(III)	170
Y32H8	(IV)	210
Y47H9W3A	(II)	405

7/1/97

Y39E4W2A	(III)	350
Y45F10W3A	(IV)	410
Y49A10	(III)	100
Y48C3W2A	(II)	280

13/1/97

Y54S9	(II)	350
Y41E3W2C	(IV)	280
Y69H2	(III)	170
Y91F4	(II)	200

Y102A5 480 (rrs-1)
Y6G8 230 good library available, but odd preliminary analysis. Tm
Y26G10 230 window transfer underway >W1
Y40B3 350 hold
Y59A8 350 in library prepn (2/12)(28/10 v.fuzzy)
Y37H2 460 good window transfer (W3A)
Y51A2 180
Y69H2 170
Y17D7 210 in sequence, Tm14 (enzymatic library)
Y33A10 200 may be displaced due to Y6G8 paradoxical preliminary ana
Y112C10 or Y119E4 or Y119H2? being sized

Y59C12 350 sepn OK
Y43F8 480
Y39B6 310 in library prepn (25/11); earlier library high yeast
Y102G3 670 window transfer may be OK, but size discrepancy; Y102G3W

Y44A6 390
Y38H6 280 may need window transfer (preliminary PFG)

NCI
1 Y119E10 being sized
2 Y53H1 450 in library prepn (13/11); for Tm14
-> 3 Y47H9 400 window transfer underway (>W2)
4 Y53C10 350 * good window transfer (W2B)
5 Y47H10 350 sepn prob OK (but preliminary PFG)
6 Y91F4 200 13/11 library 30% yeast; reprep?; for Tm14
-> 7 Y56F9 280 window transfer underway >W2
-> 8 Y18D10 280 window transfer underway >W2
9 Y47E12 380 ? big YAC for poss small gap; try other methods first?
-> 10 Y50B1 230 sepn may be OK (but preliminary PFG)

11 Y6B3 230 in library prepn (2/12);sepn should be OK
12 Y66F9 310
13 Y71A12 340 window transfer good (but unnecessary): use Y71A12W2B
-> 14 Y63D3 230 window transfer awaiting PFG assay (22/11);failed;needs
15 Y112D2 or Y120H4 being sized
-> 16 Y54E5 290 * good window transfer (W2A)

from SO, 11/12/96

9/12

Non-cluster YAC selections

No comment = nothing happening yet.

NCIII

	Y45F3	280kb	
*	Y39A1	350	in library prepn (2/12);sepn should be OK
	Y52D3	300	sepn should be OK at 20sec.
*	Y48A6	300	in library prepn (18/11); for Tm11
	Y47D3	350	window transfer >W2 underway
	Y41C4	300	window transfer failed at 1st stage 2x.
	Y49E10	150	
	Y111B2	620	for window transfer >W4 for Tm11
*	Y37D8	420	in library prepn (25/11) for Tm11
	Y39E4	350	good window transfer (W2A)
	Y43F4	300	
*	Y76A2	280	sepn OK; in library prepn (18/11); for Tm11

NCII

	Y17G7	230	window transfer underway >W1
*	Y52D6	200	in library prepn (25/11)
	Y38E10	350	good window transfer (W2A)
*	Y46G5	340	in library prepn (2/12)
	Y38F1	170	
	Y81G3	320	in library prepn (22/10):v.high yeast (excision error?);
	Y48C3	280	good window transfer
	Y48E1	330	in sequence, Tm11
	Y54G9	350	
	Y36C4	150	? hold for Y51H1 data, which may o/l R06B9
	Y51H1	130	in sequence, Tm12
	Y39G8	230	good window transfer; W1A in library prepn.(28/10);wrong
	Y48B6	280	possible good window transfer, but prob OK without.
	Y54G11	370	
	Y17B7	90	in sequence Tm12
	Y54E2	330	in sequence Tm12
	Y53F4	430	in sequence Tm12

ClusterII

*	Y53C12	280	library available(21/11)(mab-3 bridge; Zarkower says est

NCIV

	Y38H8	210	
	Y45F10	450	good window transfer (W3A)
	Y37A1	350	
*	Y51H5	190	in library prepn (25/11); earlier library high yeast
*	Y64G10	250	library available (3/12)
	Y67A10	200	in sequence, Tm13
	Y57G11	520	in sequence, Tm13
	Y41E3	280	good window transfer (W2C)
	Y40H7	230	window transfer awaiting PFG assay (22/11)
	Y73F8	440	
	Y105C5	790	for window transfer >W6
*	Y65A5	210	library available (26/11)
	Y7A9	230	window transfer awaiting PFG assay (22/11)

(hsp-1 contig requires further analysis)

NCV

	Y6E2	210	clusterish; try other approaches first?
	Y32B12	200	
	Y70C5	150	

Y102A5 480 (rrs-1)
Y6G8 230 good library available, but odd preliminary analysis. Tm
Y26G10 230 window transfer underway >W1
Y40B3 350 hold
• Y59A8 350 in library prepn (2/12)(28/10 v.fuzzy)
> Y37H2 460 good window transfer (W3A)
Y51A2 180
Y69H2 170
Y17D7 210 in sequence, Tm14 (enzymatic library)
Y33A10 200 may be displaced due to Y6G8 paradoxical preliminary ana
Y112C10 or Y119E4 or Y119H2? being sized

Y59C12 350 sepn OK
Y43F8 480
• Y39B6 310 in library prepn (25/11); earlier library high yeast
Y102G3 670 window transfer may be OK, but size discrepancy; Y102G3W

Y44A6 390
Y38H6 280 may need window transfer (preliminary PFG)

NCI
Y119E10 being sized
• Y53H1 450 in library prepn (13/11); for Tm14
Y47H9 400 window transfer underway (>W2)
> Y53C10 350 good window transfer (W2B)
Y47H10 350 sepn prob OK (but preliminary PFG)
? Y91F4 200 in library prepn (13/11); for Tm14
Y56F9 280 window transfer underway >W2
Y18D10 280 window transfer underway >W2
Y47E12 380 ? big YAC for poss small gap; try other methods first?
Y50B1 230 sepn may be OK (but preliminary PFG)

• Y6B3 230 in library prepn (2/12);sepn should be OK
Y66F9 310
Y71A12 340 window transfer good (but unnecessary): use Y71A12W2B
Y63D3 230 window transfer awaiting PFG assay (22/11)
Y112D2 or Y120H4 being sized
> Y54E5 290 good window transfer (W2A)

In lib prep or lib available:

III 4/12
II 3/18
IV 3/17
V 2/20 9/12 +2
I 2/16 9/12 +2

16/12

Non-cluster YAC selections

lib

No comment = nothing happening yet.

NCIII

	Y45F3	280kb	
	Y39A1	350	needs window transfer
	Y52D3	300	sepn should be OK at 20sec.
>	Y48A6	300	library available (16/12); for Tm11
	Y47D3	350	window transfer >W2 underway
	Y41C4	300	window transfer failed at 1st stage 2x.
	Y49E10	150	
	Y111B2	620	for window transfer >W4 for Tm11
~~lesbos~~lig	Y37D8	420	in library prepn (25/11) for Tm11
	Y39E4	350	good window transfer (W2A)
	Y43F4	300	
>	Y76A2	280	library available (16/12); for Tm11

NCII

	Y17G7	230	window transfer underway >W1
DNA	Y52D6	200	in library prepn (25/11)
	Y38E10	350	good window transfer (W2A)
DNA	Y46G5	340	in library prepn (2/12)
	Y38F1	170	
	Y81G3	320	in library prepn (22/10):v.high yeast (excision error?);
	Y48C3	280	good window transfer
o	Y48E1	330	in sequence, Tm11
	Y54G9	350	
	Y36C4	150	? hold for Y51H1 data, which may o/l R06B9
	Y51H1	130	in sequence, Tm12
>	Y39G8	230	library available (W1A)(16/12)
	Y48B6	280	possible good window transfer, but prob OK without.
	Y54G11	370	
	Y17B7	90	in sequence Tm12
	Y54E2	330	in sequence Tm12
	Y53F4	430	in sequence Tm12

ClusterII

>	Y53C12	280	~~library available(21/11)~~(mab-3 bridge; Zarkower says est

in sq Tm14

NCIV

	Y38H8	210	
	Y45F10	450	good window transfer (W3A)
	Y37A1	350	
lig	Y51H5	190	in library prepn (25/11); earlier library high yeast
>	Y64G10	250 •	library available (3/12)
o	Y67A10	200 •	in sequence, Tm13
o	Y57G11	520 •	in sequence, Tm13
	Y41E3	280	good window transfer (W2C)
	Y40H7	230	window transfer awaiting PFG assay (22/11):no good; star
	Y73F8	440	
	Y105C5	790	for window transfer >W6
>	Y65A5	210•	library available (26/11)
	Y7A9	230	prob good window transfer (see 10/12); W1A (bottom band)

(hsp-1 contig requires further analysis)

NCV

	Y6E2	210 |	
	Y32B12	200 }	clusterish; try other approaches first?
	Y70C5	150 |	

Tm 11

Y102A5	480	(rrs-1)
Y6G8	230	~~good library available, but odd preliminary analysis. Tm~~ (in seq Tm 11
~~Y26G10~~	~~230~~	~~window transfer underway >W1~~
~~Y40B3~~	~~350~~	~~hold~~ 49f6
Y59A8	350	in library prepn (2/12)(28/10 v.fuzzy)
Y37H2	460	W3A in library prepn (9/12)
Y51A2	180	in library prepn (9/12)
Y69H2	170	
Y17D7	210	in sequence, Tm14 (enzymatic library)
Y33A10	200	may be displaced due to Y6G8 paradoxical preliminary ana
Y112C10 or Y119E4 or Y119H2? being sized		
Y59C12	350	sepn OK
Y43F8	480	
Y39B6	310	in library prepn (25/11);(earlier library high yeast); f
		Y41A4 poss alt (but would require window?)
Y102G3	670	window transfer may be OK, but size discrepancy; Y102G3W
Y44A6	390	
Y38H6	280	may need window transfer (preliminary PFG)

NCI

Y119E10		being sized
Y53H1	450	in library prepn (13/11); for Tm14
Y47H9	400	window transfer underway (>W2)
Y53C10	350	W2B in library prepn (9/12)
Y47H10	350	sepn prob OK (but preliminary PFG)
Y91F4	200	13/11 library 30% yeast; reprep?; for Tm14
Y56F9	280	window transfer underway >W2
Y18D10	280	window transfer underway >W2
Y47E12	380	? big YAC for poss small gap; try other methods first?
Y50B1	230	sepn may be OK (but preliminary PFG)
Y6B3	230	in library prepn (2/12);sepn should be OK
Y66F9	310	
Y71A12	340	window transfer good (but unnecessary): use Y71A12W2B
Y63D3	230	window transfer awaiting PFG assay (22/11);failed;needs
Y112D2 or Y120H4		being sized
Y54E5	290	W2A in library prepn (9/12)

7/1/97

\ Non-cluster YAC selections

No comment = nothing happening yet.

NCIII

Y45F3	280kb	
Y39A1	350	needs window transfer
Y52D3	300	in library prepn (30/12); sepn should be OK at 20sec.
Y48A6	300	library available (16/12); for Tm11
Y47D3	350	W2 transfer under PFG assay
Y41C4	300	W2 transfer under assay (W2A only):no good; needs repeat
Y49E10	150	
Y111B2	620	for window transfer >W4 for Tm11
Y37D8	420	in library prepn (25/11) for Tm11
> Y39E4	350	good window transfer (W2A)
Y43F4	300	in library prepn (23/12)
Y76A2	280	library available (16/12); for Tm11

NCII

Y17G7	230	W1 transfer under PFG assay; poss OK - needs 230-280 exp
Y52D6	200	in library prepn (25/11)
Y38E10	350	good window transfer (W2A)
Y46G5	340	in library prepn (2/12)
Y38F1	170	in library prepn (30/12)
Y81G3	320	in library prepn (22/10):v.high yeast (excision error?);
> Y48C3	280	good window transfer (W2A)
Y48E1	330	in sequence, Tm11
Y54G9	350	
(Y36C4	150	? hold for Y51H1 data, which may o/l R06B9: apparently n (kt,19/12) but prob does overlap F08G2, so F08G2>R06B9 i Y36C4 redundant).
Y51H1	130	in sequence, Tm12
Y39G8	230	library available (W1A)(16/12)
Y48B6	280	possible good window transfer, but prob OK without.
Y54G11	370	
Y17B7	90	in sequence Tm12
Y54E2	330	in sequence Tm12
Y53F4	430	in sequence Tm12

ClusterII

Y53C12	280	(mab-3 bridge; Zarkower says estimated >100kb gap). In sequence Tm14 (16/12)

NCIV

Y38H8	210	in library prepn (30/12)
> Y45F10	450	good window transfer (W3A)
Y37A1	350	
Y51H5	190	in library prepn (25/11); earlier library high yeast
Y64G10	250	library available (3/12); in sequence, Tm13.
Y67A10	200	in sequence, Tm13
Y57G11	520	in sequence, Tm13
Y41E3	280	good window transfer (W2C)
Y40H7	230	window transfer awaiting PFG assay (22/11):no good; star
Y73F8	440	
Y105C5	790	for window transfer >W6
Y65A5	210	in sequence Tm13(7/1)
Y7A9	230	prob good window transfer (see 10/12); W1A (bottom band)

(hsp-1 contig requires further analysis)

Y54E6	330	Cluster; good library made inadvertantly (Tm13?)

NCV

Y6E2 210 |
Y32B12 200 } clusterish; try other approaches first?
Y70C5 150 |

Y102A5 480 (rrs-1)
Y6G8 230 Odd preliminary analysis. In sequence Tm11 (14/11)
(Y26G10 230 good window transfers (W1B,C,D) but cancelled)
(Y40B3 350 prob.cancel)
Y9F6 190 in library prepn (23/12)
Y59A8 350 in library prepn (2/12)(28/10 v.fuzzy)
Y37H2 460 W3A in library prepn (9/12)
Y51A2 450 in library prepn (9/12); not 180kb as in acedb!
Y69H2 170
Y17D7 210 in sequence, Tm14 (enzymatic library)
Y33A10 200 may be displaced due to Y6G8 paradoxical preliminary ana
Y112C10 or Y119E4 or Y119H2? being sized

Y59C12 350 sepn OK
Y43F8 480
Y39B6 310 in library prepn (25/11);(earlier library high yeast); f
Y41A4 poss alt (but would require window?)
Y102G3 670 window transfer may be OK, but size discrepancy; Y102G3W

Y44A6 390
Y38H6 280 may need window transfer (preliminary PFG)

NCI

Y119E10 being sized
Y53H1 450 in library prepn (13/11); for Tm14
Y47H9 400 W3A in library prepn (30/12)
Y53C10 350 W2B in library prepn (9/12)
Y47H10 350 sepn prob OK (but preliminary PFG)
Y91F4 200 13/11 library 30% yeast; reprep?; for Tm14
Y56F9 280 W2 transfer under PFG assay; poss OK - need 230-280 expa
Y18D10 280 window transfers good; prep Y18D10W2D
Y47E12 380 ? big YAC for poss small gap; try other methods first?
Y50B1 230 sepn may be OK (but preliminary PFG)

Y6B3 230 in library prepn (2/12);sepn should be OK
Y66F9 310 hold for data from flanking YACs?
Y71A12 340 Y71A12W2B in library prepn (23/12)
Y63D3 230 window transfer awaiting PFG assay (22/11);failed;needs
Y112D2 or Y120H4 being sized
Y54E5 290 W2A in library prepn (9/12)

X
> Y49A10 100 for Tm 14

5/11/96

~jes/report/yac.dat

	yeast%	cvec%	svec%	pass%	titre/ul
Y54E6					
Y48E1 -11	9	7	1	78	8E4
Y39B6	16	6	51	22	5E3
Y6G8	6	5	5	77	4E4
Y51H1 -12	8	6	4	71	
Y51H5	19	4	4	65	
Y37A1	21	4	0	71	9E4
Y57G11 -13	0	8	0	85	1E5

Y17D7 (ans)-14

1.5 - 2 kb pUCs.

(~rnd/Yname).

10 plates

III
II
IV
V
I
X

11	=	3000	, + 1
15	=	4230	, + 2 (~ 400).
13	=	4350	
14	=	4640	, + 2 (~ 500?)
12	=	3740	, + 3 (~ 800?)
65		19960	8

(34 in process at 4/10/96).

17	=	3500	
82		23460	

1,,
Summary-line: 16-Oct zarkower@lenti.med.umn.ed #mab-3
Mail-from: From zarkower@lenti.med.umn.edu Thu Oct 17 00:43:43 1996
Received: from mhub0.tc.umn.edu by sanger.ac.uk (4.1/SMI-4.1)
        id AA19225; Thu, 17 Oct 96 00:43:41 BST
Return-Path: <zarkower@lenti.med.umn.edu>
Received: from lenti.med.umn.edu by mhub0.tc.umn.edu; Wed, 16 Oct 96 18:41:29
Date: Wed, 16 Oct 96 18:41:25 CDT
Received: from [134.84.112.176] (x112-176.med.umn.edu) by lenti.med.umn.edu; W
X-Sender: zarkower@lenti.med.umn.edu
Message-Id: <v01530501ae8b1f7df31c@[134.84.112.176]>
Mime-Version: 1.0
Content-Type: text/plain; charset="us-ascii"
To: alan@sanger.ac.uk
From: zarkower@lenti.med.umn.edu (David Zarkower)
Subject: mab-3

*** EOOH ***
Return-Path: <zarkower@lenti.med.umn.edu>
Date: Wed, 16 Oct 96 18:41:25 CDT
X-Sender: zarkower@lenti.med.umn.edu
Mime-Version: 1.0
Content-Type: text/plain; charset="us-ascii"
To: alan@sanger.ac.uk
From: zarkower@lenti.med.umn.edu (David Zarkower)
Subject: mab-3

Dear Alan,
I wanted to get some info on the mab-3 region from you so we can plan our next move. My student is finally settling in and getting geared up. My main question for you is do you have any near-term plans for the Y53C12 bridge that appears to contain mab-3? You had mentioned the possibility of long PCR at some point, but I'm pretty sure the bridge is in the 100-150 kb range (we got CB#1162 from Jonathan to test the remote possibility that it and the nearest fosmid on the other side overlap, but we're not holding our breath). It seems to me that the current options are for you guys to try sequencing one of the spanning YACs like Y53C12, or for us to try everything we can think of.

If there are no plans for the genome project to go after that region (and I'm writing to you because I'm assuming that region is a Cambridge region), we have several things in mind. First, we are continuing to make fosmid end probes to send Stephanie to see if we can extend the existing contigs with fosmids. This is not very likely because probing with purified Y53C12 DNA did not get us anything new, but there was a lot of background from repetitive clones, so it is worth a try. Second, we are setting up a Tc1 screen based on the fact that mab-3 mutations activate a gfp reporter in the male gut. This may solve our problem if mab-3 is a Tc1 target, but won't help you much. Third, we will probably make smaller YAC derivatives by recombination, using our fosmid end-sequences. These could then be subcloned into phage to be tested for mab-3 rescue, and might also help you with your sequencing efforts. Fourth, and I hope finally, we could try a phage walk from the left side of the bridge, using libraries made from yeast containing the three YAC clones that cover the bridge. Caroline Shamu tried walking from the right side using libraries that John Sulston made from purified YAC DNA, but got stuck, and CB#1162 was the best extension she got. Nobody has tried to go in from the left because it probably is the long way, but at this point it seems worth a try.

Ok, so that's where we stand. Obviously what I would most like to hear is that you are currently sequencing purified Y53C12 and should have finished sequence in several weeks! If there is no near-term plan for that region, I would very much appreciate any advice you could give me on choice of vector and host for the walk. I had thought lambda might makes sense because we are looking at a region of about 100-150 kb, and with lambda

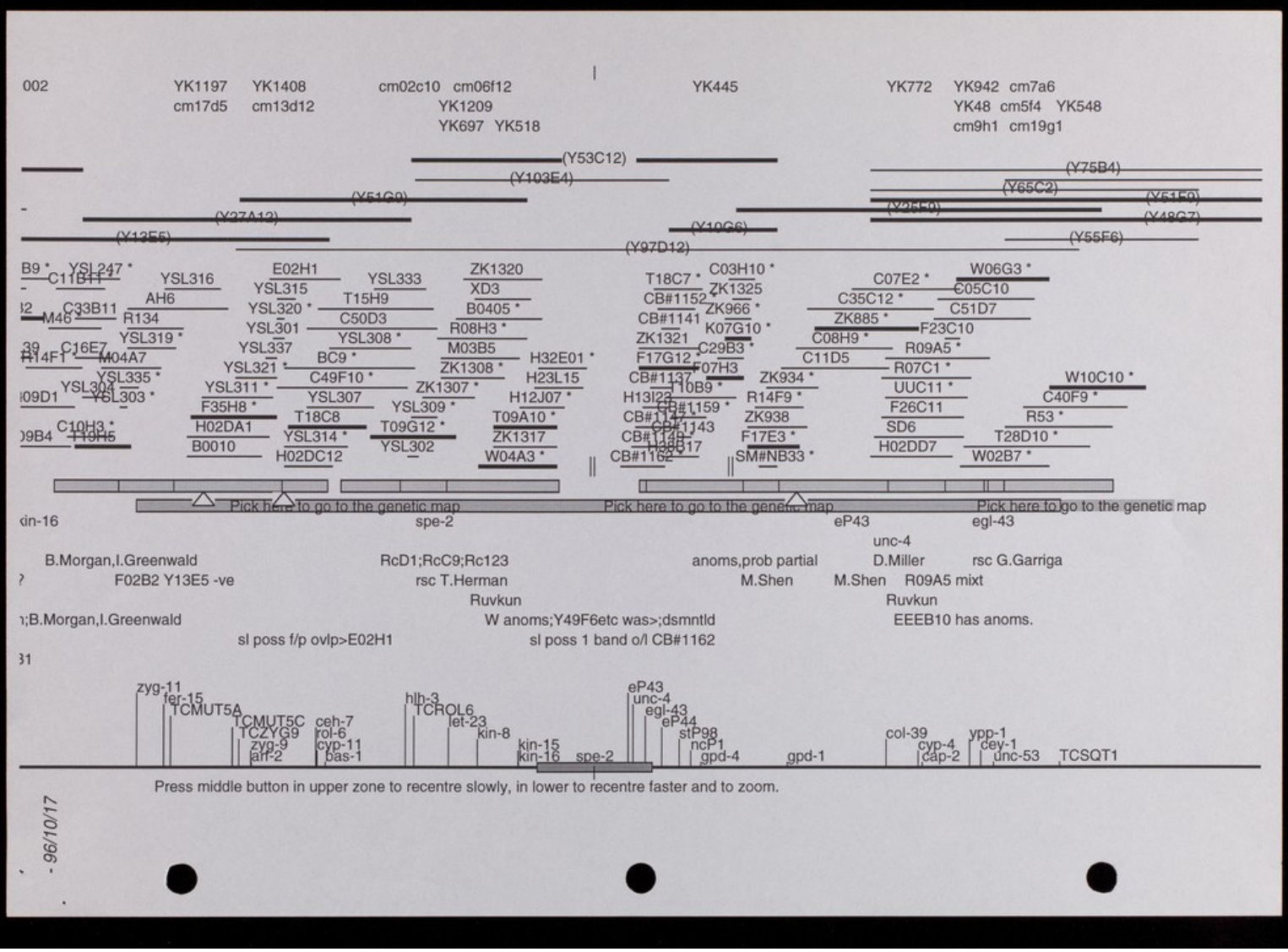
clones the number required to span it would be reasonable, and the logistics of isolating the gene after rescue also would be reasonable. We could try cosmids or P1 vectors, but in that case while very few clones would be needed to span the region, getting our gene out might be a lot of work and require lambda subcloning anyway. You have a lot more experience with this, so suggestions please! The final issue is whether the Cambridge group would be interested/willing to fingerprint clones if I could assemble a contig by walking. Seems like something that would be mutually beneficial, but I don't want to impose. Sorry to go on at such length, but we need, in the local vernacular, to fish or cut bait, and I want to make sure we don't waste a lot of effort if the professionals are already working on the problem. Regards to all. I'm about to start teaching for the first time, and appreciate the postdoc lifestyle all the more! Our first snow is forecast for tomorrow; summer is short in these parts.

Best wishes,
Dave

1,,
Summary-line: 17-Oct zarkower@lenti.med.umn.ed #mab-3 etc.
Mail-from: From zarkower@lenti.med.umn.edu Thu Oct 17 23:23:11 1996
Received: from mhub2.tc.umn.edu by sanger.ac.uk (4.1/SMI-4.1)
        id AA00402; Thu, 17 Oct 96 23:22:41 BST
Return-Path: <zarkower@lenti.med.umn.edu>
Received: from lenti.med.umn.edu by mhub2.tc.umn.edu; Thu, 17 Oct 96 17:20:29
Date: Thu, 17 Oct 96 17:20:24 CDT
Received: from [134.84.112.176] (x112-176.med.umn.edu) by lenti.med.umn.edu; T
X-Sender: zarkower@lenti.med.umn.edu
Message-Id: <v01530502ae8c61edb794@[134.84.112.176]>
Mime-Version: 1.0
Content-Type: text/plain; charset="us-ascii"
To: alan@sanger.ac.uk
From: zarkower@lenti.med.umn.edu (David Zarkower)
Subject: mab-3 etc.

*** EOOH ***
Return-Path: <zarkower@lenti.med.umn.edu>
Date: Thu, 17 Oct 96 17:20:24 CDT
X-Sender: zarkower@lenti.med.umn.edu
Mime-Version: 1.0
Content-Type: text/plain; charset="us-ascii"
To: alan@sanger.ac.uk
From: zarkower@lenti.med.umn.edu (David Zarkower)
Subject: mab-3 etc.

Dear Alan,
I quick addendum to my note of yesterday: To explore further the idea of convincing you (the genome project that is) to sequence Y53C12 YAC DNA, I wondered if it would be a more tempting prospect if we were to make a smaller derivative of Y53C12. We don't know the orientation of it, but could find out, and then recombine one end off, which should get the size down by several cosmid lengths. The only drawback other than the time it would take to make and characterize the derivative is that such a DNA might be harder to purify cleanly than Y53C12, which separated quite well for us from the yeast chromosomes on a pulsed field gel. I don't even know if you are the one to bother about this, so do let me know if I am barking up the wrong tree!
Best wishes,
Dave


002
YK1197 YK1408 cm02c10 cm06f12
cm17d5 cm13d12 YK1209
YK697 YK518
YK445
YK772 YK942 cm7a6
YK48 cm5f4 YK548
cm9h1 cm19g1
(Y53C12)
(Y103E4)
(Y51G9)
(Y37A12)
(Y13E5)
(Y97D12)
(Y10G6)
(Y75B4)
(Y65C2)
(Y51F9)
(Y48G7)
(Y55F6)
YSL247 *
C11B11 *
YSL316
AH6
C33B11
R134
YSL319 *
C16E7
M04A7
YSL335 *
YSL303 *
C10H3 *
T19H5
E02H1
YSL315
YSL320 *
YSL301
YSL337
YSL321 *
YSL311 *
F35H8 *
H02DA1
B0010
YSL333
T15H9
C50D3
YSL308 *
BC9 *
C49F10 *
YSL307
T18C8
YSL314 *
H02DC12
ZK1320
XD3
B0405 *
R08H3 *
M03B5
ZK1308 *
ZK1307 *
YSL309 *
T09G12 *
YSL302
T09A10 *
ZK1317
W04A3 *
H32E01 *
H23L15
H12J07 *
T18C7 *
CB#1152
CB#1141
ZK1321
F17G12
CB#1137
H13I23
CB#1147
CB#1148
CB#1162
C03H10 *
ZK1325
ZK966 *
K07G10 *
C29B3 *
F07H3
T10B9 *
CB#1159 *
CB#1143
H08B17
SM#NB33 *
C07E2 *
C35C12 *
ZK885 *
C08H9 *
C11D5
ZK934 *
R14F9 *
ZK938
F17E3 *
C05C10
C51D7
F23C10
R09A5 *
R07C1 *
UUC11 *
F26C11
SD6
H02DD7
W06G3 *
W10C10 *
C40F9 *
R53 *
T28D10 *
W02B7 *
Pick here to go to the genetic map
Pick here to go to the genetic map
Pick here to go to the genetic map
kin-16
spe-2
eP43
unc-4
egl-43
B.Morgan,I.Greenwald
RcD1;RcC9;Rc123
anoms,prob partial
D.Miller
rsc G.Garriga
F02B2 Y13E5 -ve
rsc T.Herman
M.Shen
M.Shen R09A5 mixt
Ruvkun
Ruvkun
;B.Morgan,I.Greenwald
W anoms;Y49F6etc was>;dsmntld
EEEB10 has anoms.
sl poss f/p ovlp>E02H1
sl poss 1 band o/l CB#1162
zyg-11
fer-15
TCMUT5A
TCMUT5C
TCZYG9
zyg-9
arf-2
ceh-7
rol-6
cyp-11
bas-1
hlh-3
TCROL6
let-23
kin-8
kin-15
kin-16
spe-2
eP43
unc-4
egl-43
eP44
stP98
ncP1
gpd-4
gpd-1
col-39
cyp-4
cap-2
ypp-1
cey-1
unc-53
TCSQT1
Press middle button in upper zone to recentre slowly, in lower to recentre faster and to zoom.
- 96/10/17

1,,
Summary-line: 18-Oct to: alan #zark
Mail-from: From alan Fri Oct 18 10:50:55 1996
Return-Path: <alan>
Received: from rona.sanger.ac.uk by sanger.ac.uk (4.1/SMI-4.1)
id AA03509; Fri, 18 Oct 96 10:50:54 BST
Date: Fri, 18 Oct 96 10:50:54 BST
From: alan
Message-Id: <9610180950.AA03509@sanger.ac.uk>
Received: by rona.sanger.ac.uk (4.1/SMI-4.1)
id AA21694; Fri, 18 Oct 96 10:48:49 GMT
To: alan
Subject: zark

*** EOOH ***
Return-Path: <alan>
Date: Fri, 18 Oct 96 10:50:54 BST
From: alan
To: alan
Subject: zark

>From alan Fri Oct 18 10:47:25 +0000 1996
To: zarkower@lenti.med.umn.edu
In-reply-to: <v01530502ae8c61edb794@[134.84.112.176]> (zarkower@lenti.med.umn.
Subject: Re: mab-3 etc.

Yo Dave
I was about to reply and say that, since you reckon the gap to be over 100kb, we should perhaps put Y53C12 into production. I was also going to say that sin our only PFG of the YAC, it runs on top of the second chromosome the whole process would be rather lengthy since I am trying to move these co-migrating YACs into Eric Green's 'window' strains in order to avoid sequencing more than 50% yeast. However, since you say that the YAC is, in fact, seperable (does it run slightly slower than the second yeast chromosome?) this should simplify things. But you have to be aware that we are only just getting going on the YAC sequencing and the whole process is inevitably lengthy anyway. In addition there is already a queue of YACs in the system at various stages (we have most been concentrating on YACs from the arms in the expectation that some proporti of bridges in the clusters will be filled by long-range PCR, fosmids etc, effo for which are ongoing). It is very hard for me to put a time on when useful in for Y53C12 might start to appear. But I don't think it can be consisered 'near Consequently, I wonder if you shouldn't try the lambda walk? We obviously have unknowns either way. Should we just embark on both?
We certainly would fingerprint any lambda clones you generate (I would think l are the things to go for).
Best wishes
Alan

WINPAWT

Construction of YLBW1A (Replacement to YLBW1)

March, 1999

PCR product generated with OM1625 (upstream primer) and OM1626 (downstream primer) using pBM2815 as template to yield HIS3 with flanking homology provided by primers.

OM1625: 5'gttactttattctgctttaacgccattatgattatacaattgtatGGCCTCCTCTAGTACACTC3'
OM1626: 5'gtgaaataaaataaaggttttaatatacaggttaaaaaataagtaGCGCGCCTCGTTCAGAATG3'

Notes:

1. HIS3 SEQUENCE IN CAPS.
2. Flanking Chromosome 1 sequence between YAL011 and MDM10 in lower case

PCR product inserted by recombination into the intergenic region between YAL011 and MDM10 in YPH925. Subsequent yeast strain was then transformed with BamH1+Not1-cut pLB553, and the resulting Ura+His- colonies were selected and analyzed by PFGE. 4/4 had a fragmented chromosome 1. Each was subjected to 5FOA treatment to pop-out the URA3 within pLB553, with 4/4 again containing the fragmented chromosome 1. This new strain was named YLBW1A, and its replaces YLBW1 in the window strain set.

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11706–11710, December 1995
Genetics

Isolation of yeast artificial chromosomes free of endogenous yeast chromosomes: Construction of alternate hosts with defined karyotypic alterations

(chromosome fragmentation/*kar1*-mediated transfer/genome mapping)

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*Diagnostic Development Branch, National Center for Human Genome Research, National Institutes of Health, Bethesda, MD 20892; and ‡Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110

Communicated by Francis S. Collins, National Institutes of Health, Bethesda, MD, August 16, 1995 (received for review May 30, 1995)

ABSTRACT **An intrinsic feature of yeast artificial chromosomes (YACs) is that the cloned DNA is generally in the same size range (i.e., ≈200–2000 kb) as the endogenous yeast chromosomes. As a result, the isolation of YAC DNA, which typically involves separation by pulsed-field gel electrophoresis, is frequently confounded by the presence of a comigrating or closely migrating endogenous yeast chromosome(s). We have developed a strategy that reliably allows the isolation of any YAC free of endogenous yeast chromosomes. Using recombination-mediated chromosome fragmentation, a set of *Saccharomyces cerevisiae* host strains was systematically constructed. Each strain contains defined alterations in its electrophoretic karyotype, which provide a large-size interval devoid of endogenous chromosomes (i.e., a karyotypic "window"). All of the constructed strains contain the *kar1-Δ15* mutation, thereby allowing the efficient transfer of a YAC from its original host into an appropriately selected window strain using the *kar1*-transfer procedure. This approach provides a robust and efficient means to obtain relatively pure YAC DNA regardless of YAC size.**

The development of yeast artificial chromosome (YAC) cloning (1, 2) has greatly advanced the ability to analyze complex genomes. Specifically, segments of DNA spanning upwards of 1000 kb (or more) can be isolated in YACs, thereby allowing studies that were previously unapproachable using bacterial-based cloning systems. However, YACs have several inherent properties that reduce accessibility to the cloned DNA; in particular, YACs are (*i*) maintained in roughly single-copy in yeast cells, (*ii*) generally in the same size range (i.e., ≈200–2000 kb) as the 16 endogenous yeast chromosomes, and (*iii*) linear DNA molecules with the same general structure as other yeast chromosomes. As a consequence, the isolation of purified YAC DNA, most often performed by preparative pulsed-field gel electrophoresis (PFGE) (3–7), yields relatively small amounts of material for subsequent manipulation. Frequently, the purification of YAC DNA is further hindered by the presence of comigrating or closely migrating endogenous yeast chromosomes, which inevitably results in the presence of large amounts of unwanted yeast DNA. Contaminating yeast chromosomes can hamper subsequent applications that demand highly purified preparations of YAC DNA, such as exon trapping/amplification (8–10), direct cDNA selection (11, 12), construction of random libraries for DNA sequencing (13), and other methods where smaller-insert subclones must be obtained from the purified YAC DNA (14).

To ameliorate this problem, we have developed a strategy that allows the reliable isolation of YAC DNA free of endogenous yeast chromosomes. Specifically, we constructed a series of *Saccharomyces cerevisiae* host strains whose endogenous chromosomes have been systematically altered by recombination-mediated chromosome fragmentation (15, 16). Each strain represents the product of a different set of fragmentation events and contains a unique chromosome-free region (i.e., a "window") in its electrophoretic karyotype. Using the recently described *kar1*-transfer procedure (17, 18), a YAC of interest can be readily moved from its original host into one of these newly constructed strains, such that the YAC will migrate within the host's window during PFGE and thus can be isolated free of endogenous yeast chromosomes. Here we describe the construction of the yeast window strains[¶] and demonstrate that YACs of various sizes can be transferred into the new hosts for subsequent isolation. This approach eliminates a major limitation of YAC cloning and should facilitate the direct use of YACs for a larger array of molecular genetic techniques.

MATERIALS AND METHODS

Construction of a Universal Fragmentation Vector. pLB503 (Fig. 1) was constructed from the following components using standard molecular biology methods (6, 21): (*i*) a backbone of pBluescript (Stratagene), (*ii*) an *Xho* I fragment of pYAC4 containing the *Tetrahymena* telomeres and intervening stuffer (1), (*iii*) the yeast *URA3* gene flanked by direct repeats [*Salmonella hisG* sequences (20)], and (*iv*) a PCR-generated fragment containing the yeast CEN4 sequence (GenBank no. X70276). Additional details about plasmid construction are available on request.

Yeast Chromosome Fragmentation. *Strategy 1.* Two pairs of oligonucleotide primers were used for PCR amplification of adjacent segments of a target gene sequence. For each target, the two segments were designed such that they were slightly different in size, were completely within the open reading frame of the gene, contained engineered *Not* I sites at their internal ends (via the artificial placement of a *Not* I recognition sequence within the internal-most PCR primers), and were devoid of a *Bam*HI, *Sac* I, *Xba* I, or an additional *Not* I site. The resulting two PCR products were digested with *Not* I, ligated, and used as template for a second round of PCR that employed the two external-most primers (i.e., the ones devoid of *Not* I sites). The final PCR product, which represented a disrupted target gene sequence (lacking 5′ and 3′ portions of coding sequence) with an asymmetrically placed, internal *Not*

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Abbreviations: YAC, yeast artificial chromosome; PFGE, pulsed-field gel electrophoresis.

†Present address: Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.
§To whom reprint requests should be addressed.

¶The window strains reported here are freely available to other investigators. Please contact the corresponding author by FAX (301-402-4735) or e-mail (egreen@nchgr.nih.gov).

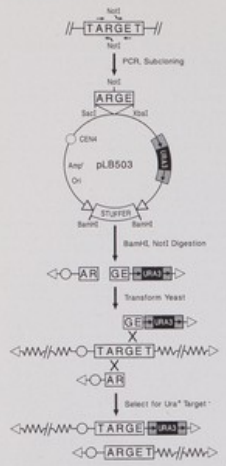

FIG. 1. Fragmentation of a yeast chromosome by recombination at a natural endogenous target (strategy 1). The plasmid pLB503 contains a yeast centromere sequence (CEN4), two *Tetrahymena* telomeres (triangles), a stuffer fragment (1, 19), and the *URA3* gene flanked by direct repeats (20). A target corresponding to a sequence fully contained within the open reading frame of a yeast gene required for prototrophy was engineered to contain an internal *Not* I site (and a small deletion) and subcloned into pLB503 (via unique *Sac* I and *Xba* I sites) in both orientations (only one orientation is depicted). Following digestion with *Not* I and *Bam*HI, the resulting vector fragments are transformed into yeast, where homologous recombination of the target sequences results in fragmentation of the endogenous chromosome (depicted with wavy lines) to yield two smaller derivative chromosomes. The resulting yeast cell is Ura$^+$ and auxotrophic due to disruption of the target gene. Of note, an earlier generation vector (pLB301) is similar to pLB503 but contains the *HIS3* gene on the CEN4-containing side of the *Sac* I/*Xba* I cloning site. pLB301 was only used to fragment chromosomes I and XI at the *CYS3* and *MET14* genes, respectively (see Table 1).

I site, was directly subcloned into pT7Blue (Novagen). The orientation of the cloned insert in multiple isolates was established by digestion with *Xba* I and *Not* I and/or by PCR analysis using T7 and T3 primers in combination with the two internal-most target-specific primers. An isolate containing the target in each orientation was selected, the *Bam*HI site was destroyed by *Bam*HI digestion, Klenow treatment, and self-ligation (21), and the target was excised by digestion with *Xba* I and *Sac* I and cloned into *Xba* I/*Sac* I-digested pLB503 (Fig. 1). Details about the primers used for PCR amplification of target sequences (in strategy 1 and strategy 2) are available on request.

Yeast were transformed with *Bam*HI/*Not* I-digested fragmentation vector using the lithium acetate method (22) and plated on SC-Ura plates (23). Ura$^+$ transformants were tested for disruption of the target gene (i.e., auxotrophy) by replica plating onto the appropriate dropout medium (e.g., SC-Ura-Met for the *MET14* target). The frequency of auxotrophs among the Ura$^+$ transformants was generally quite low, typically ranging from 1% to 10%, and auxotrophs containing an appropriately fragmented chromosome were only recovered with the target sequence in one (of the two) orientations. Auxotrophs for the target gene were analyzed by PFGE to verify the presence of an appropriately fragmented chromosome. Following each fragmentation event, strains were propagated on SC medium containing 1 g of 5′-fluoroorotic acid per liter (23) to select for isolates that had excised the *URA3* gene via the flanking direct repeats (24).

Strategy 2. A DNA fragment containing the yeast *HIS3* gene flanked by target sequences was generated by PCR (Fig. 2). Primers containing a 45-bp target sequence (on the 5′ end) and a 19-bp *HIS3*-specific sequence (on the 3′ end) were used for

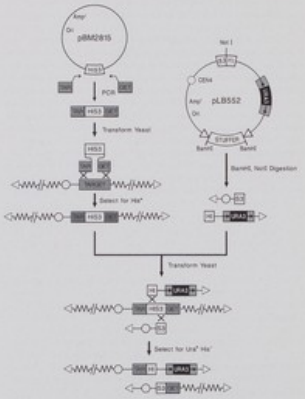

FIG. 2. Fragmentation of a yeast chromosome by recombination at an artificially integrated target (strategy 2). A target-specific integrative fragment containing the *HIS3* gene is generated by PCR using the plasmid pBM2815 (which contains the *HIS3* gene subcloned into pBluescript) as template and primers containing 45 bases of appropriate target sequence at their 5′ ends. Following yeast transformation, integration into an endogenous chromosome occurs by homologous recombination between the target sequences flanking the *HIS3* gene. Authentic integrants, containing the *HIS3* gene at the correct target site, are then transformed in parallel with *Bam*HI/*Not* I-digested fragments derived from pLB552 (depicted here) and pLB553 (identical to pLB552 except with the *HIS3* target sequences in the opposite orientation). Virtually all Ura$^+$His$^-$ transformants contain two smaller derivative chromosomes resulting from fragmentation at the integrated *HIS3* gene. Note that the relative positioning of the two parts of the *HIS3* target sequence in pLB552 and pLB553 is distinct from the approach used for subcloning target sequences in strategy 1 (see Fig. 1). Strategy 2 was designed to better ensure that no homologous sequences remained in the two derivative chromosomes following fragmentation.

PCR amplification of the *HIS3* gene using DNA from the plasmid pBM2815 as template (Fig. 2). The resulting PCR product was used to transform yeast directly (25) as described above and cells were plated on SC-His medium. The fraction of His$^+$ transformants containing authentic integration of the ≈1-kb *HIS3* gene at the appropriate site varied widely among the different targets. As a result, we found it essential to test the resulting transformants by PCR using primers that flank the expected integration site to identify authentic integrants. The resulting strains were then transformed (as above) in parallel with the *HIS3*-specific fragmentation vectors pLB552 and pLB553. The latter vectors, which contain engineered *HIS3* target sequences in opposite orientations (see Fig. 2), were constructed by subcloning PCR-generated fragments (≈500 bp each) of the *HIS3* gene into pLB503. Disruption of the *HIS3* gene in the resulting Ura$^+$ transformants was tested by replica plating to SC-Ura-His. Generally, 10–15% of the Ura$^+$ transformants were His$^-$, and virtually all Ura$^+$His$^-$ strains contained the expected fragmented chromosome, as assessed by PFGE. Auxotrophs were processed as described above for strategy 1.

Transfer of YACs into Window Strains. YACs were transferred from their original host into an appropriate window strain (Table 1) using the *kar1*-transfer procedure (17). Briefly, 5×10^8 cells each of a YAC strain (*MAT*a) and a window strain (*MAT*α) were combined, incubated at 30°C for 6 h, plated on AHC medium (26) containing 3 mg of cycloheximide per liter, and incubated at 30°C for 3–4 days. Two major types of Cyh^R colonies were typically encountered: YACs appropriately transferred into the recipient window strain (Ura$^+$Trp$^+$*MAT*α) and YAC-containing diploid cells (Ura$^+$Trp$^+$*MAT*a/α). An efficient approach for distinguishing between these two types of colonies is by direct PCR analysis of yeast cells using *MAT*-specific primers (27). In our experience, 85–95% of the resulting colonies are *MAT*α, with virtually all of these containing the window strain nucleus along with a transferred YAC. Successful YAC transfer was verified by PFGE analysis of the resulting *MAT*α clones in parallel with the original YAC strain and the recipient window strain (e.g., see Fig. 4).

RESULTS

Construction of Window Strains. A set of nine yeast strains (Table 1), each containing defined areas in the electrophoretic karyotype devoid of endogenous chromosomes, was systematically constructed by recombination-mediated chromosome fragmentation (15, 16). Our general approach involved the transformation of yeast cells with two small linear DNA fragments, each terminated on one side with a functional telomere sequence and on the other side by a target sequence also present in an endogenous chromosome (Figs. 1 and 2). One fragment contained a yeast CEN sequence, while the other harbored a marker (the *URA3* gene) used for selection of transformants. Recombination across the common (i.e., homologous) target sequence present in each fragment resulted in the introduction of the *URA3* gene, the disruption of the endogenous target gene, and the creation of two smaller derivative chromosomes from the starting endogenous chromosome. Since only one orientation of the target sequence (relative to the CEN- and *URA3*-containing fragments) resulted in the formation of two stable (i.e., monocentric) derivative chromosomes, parallel experiments using the target sequence subcloned in both orientations within the fragmenting vector were always performed.

Two strategies for chromosome fragmentation were employed. Initially, genes required for prototrophy (e.g., *CYS3, MET10, THR4, THR1, HIS1,* and *MET14*; see Table 1) were used as the targets for fragmentation (strategy 1). In these cases, target sequences within the coding region of the gene were subcloned in both orientations into pLB503 (Fig. 1). Subsequent restriction enzyme digestion released the two fragmenting DNA molecules, which were transformed into yeast. This strategy was effective, accounting for 6 of the 15 chromosome fragmentation events required for constructing the strains listed in Table 1. However, this approach was found to be limiting, because for some chromosomes, appropriate genes with known DNA sequence (for convenient generation of PCR primers) either were not available or were located near a chromosome end, which would have yielded a derivative chromosome that remained within the desired window. Fur-

Table 1. Yeast strains containing windows in their electrophoretic karyotype

Strain	Genotype	Target(s)	Window, kb
YLBW1	*MATa leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15* ***cys3***	*CYS3* (I)	≈140–280
YLBW2	*MATa leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15* ***met10 thr4***	*MET10* (VI), *THR4* (III)	≈250–450
YLBW3	*MATa leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15* ***met10 thr4***	*MET10* (VI), *THR4* (III), *HIS5* (IX)	≈310–590
YLBW4	*MATa leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15* ***thr1***	*HIS1* (V), *THR1* (VIII)	≈450–680
YLBW5	*MATa leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15* ***met14***	*MET14* (XI)	≈590–755
YLBW6	*MATa leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15*	*ARG3* (X), *LEU4* (XIV), *RAD16* (II)	≈680–950
YLBW7	*MATa leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15*	*RAD16* (II), *ILV2* (XIII), *AB97* (XVI)	≈810–1120
YLBW8	*MATa leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15*	*LEU1* (VII), *ADE2* (XV)	≈985–1640
YLBW9	*MATa leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15*	*GAL3* (IV)	≈1140–2000*

YLBW1–YLBW9 are YPH925-derived yeast strains containing windows in their electrophoretic karyotype. Indicated for each strain are the fundamental YPH925 genotype (italics) with any additional features (due to disruption of a gene required for prototrophy) indicated in boldface italics, the target sequence(s) used for fragmentation (with each corresponding chromosome number given in parentheses), and the approximate size range of the resulting window. In many instances, the site of fragmentation resided upstream or downstream of the indicated target gene, and thus the gene itself was not actually disrupted by the fragmentation event. The approximate sizes (in kb) of the resulting fragments for each chromosome are as follows: I (110, 140); VI (50, 230); III (110, 220); IX (140, 310); V (270, 320); VIII (150, 440); XI (240, 440); X (280, 475), (350, 460); XIV (330, 480); II (340, 500); XIII (460, 490); XVI (300, 685); VII (540, 580); XV (530, 600); and IV (500, 1140).
*The precise size of the window varies, since the length of the ribosomal repeats contained in chromosome XII is polymorphic in different isolates.

thermore, the construction of multiple chromosome-specific fragmentation vectors became a slow and tedious process.

As an alternative, a second strategy for chromosome fragmentation was utilized. In this case, a universal sequence (i.e., the *HIS3* gene) was "artificially" positioned at a desired site in an endogenous chromosome by integrative recombination (28) (Fig. 2) and the chromosome was then fragmented at this site by an approach analogous to strategy 1 [using common fragmentation vectors (pLB552 and pLB553) containing cloned *HIS3* gene sequences]. The initial integration of the *HIS3* gene at a site of interest was accomplished by transforming yeast with a PCR-generated *HIS3* gene fragment flanked on each side by a 45-bp chromosomal target sequence (25) (Fig. 2). The target sequences used for directing integration of the *HIS3* gene into an endogenous chromosome typically corresponded to a noncoding region near a known sequenced gene (e.g., *HIS5, ARG3, LEU4, RAD16, ILV2, ARO7, LEU1, ADE2,* and *GAL3*; see Table 1). Chromosomes containing the *HIS3* gene at the desired site were then fragmented using either pLB552 or pLB553 (Fig. 2), depending upon the orientation of the target sequence on the chromosome.

Using the strategies described above and in the legends to Figs. 1 and 2, we constructed a set of yeast strains that together provide a series of overlapping windows covering the typical size range of YACs. In all instances, YPH925 (*MATα leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2*^{R} *kar1-Δ15*) (29) was used as the starting strain, since it (*i*) is auxotrophic for a number of yeast genetic markers that are routinely used for YAC selection and subsequent manipulation (e.g., *URA3, TRP1, HIS3, LEU2, ADE2,* and *LYS2*) and (*ii*) contains the *kar1-Δ15* mutation, allowing the strain to be a YAC recipient during the *kar1*-transfer procedure (17, 18). In some instances, fragmentation of a single chromosome (e.g., chromosome I) was sufficient to create a desirable window for subsequent YAC isolation. However, in four cases (e.g., the doublet of chromosomes V and VIII at 590 kb; the doublet of chromosomes XIII and XVI at 950 and 985 kb, respectively: the doublet of chromosomes VII and XV at 1120 and 1130 kb, respectively; and the triplet of chromosomes X, XIV, and II at 755, 810, and 840 kb, respectively), a window of desired size could only be created by the successive fragmentation of multiple chromosomes. For this purpose, the fragmentation vectors used in strategy 1 and strategy 2 (Figs. 1 and 2) were engineered to contain the *URA3* gene (for positive selection of yeast transformants) flanked by direct repeats. Thus, after each round of fragmentation, the *URA3* gene was eliminated from the yeast strain by growth on 5′-fluoroorotic acid [which allows selection of clones that have excised the *URA3* gene via recombination of the flanking direct repeats (20)], thereby enabling another round of fragmentation with *URA3* selection. In the end, we constructed the nine strains listed in Table 1 by employing strategy 1 and/or strategy 2 to fragment up to three endogenous chromosomes. Importantly, these nine strains together provide an overlapping set of electrophoretic windows extending from ≈140 kb to ≈2000 kb (Fig. 3).

Transfer of YACs into Window Strains. Key to the utilization of the window strains for YAC isolation is the ability to transfer a YAC of interest into an appropriately selected strain in a convenient and efficient fashion. The presence of the *kar1-Δ15* mutation in all the newly constructed strains allows the transfer of a YAC from its starting host (e.g., AB1380) into a window strain. YACs have been successfully transferred into each of the window strains listed in Table 1. More than 30 different YACs, ranging in size from 240 kb to >1 Mb, have been transferred, with three examples shown in Fig. 4. In general, 80–90% of the Ura$^+$Trp$^+$Cyh^R colonies are *MATα*, and virtually all of these *MATα* clones represent authentic YAC-transfer events. Significant differences in the growth characteristics or the frequency of authentic YAC transfers were not observed among the different strains. Furthermore,

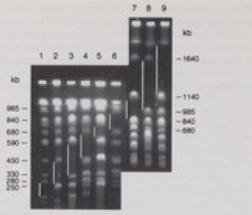

FIG. 3. PFGE analysis of window strains. The chromosomes of the various yeast window strains (Table 1) were separated by PFGE (lanes 1–6, 30–90 sec switching time, 24 h; lanes 7–9, 80–150 sec switching time, 36 h) and detected by ethidium bromide staining. YLBW1–YLBW9 were analyzed in lanes 1–9, respectively. The windows in the electrophoretic karyotypes are indicated by white lines. For PFGE, 1% agarose gels were electrophoresed in 0.5× TBE (1× TBE: 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3) with the following parameters: 6 V/cm, 120° angle, 12°C.

the fragmented chromosomes and the windows themselves have generally remained unchanged during YAC transfer. Similarly, there has been no evidence of additional YAC instability following transfer, beyond that encountered with the YAC in its original host strain. Thus, similar to previous experience (17, 18), we find that transfer of YACs by the *kar1*-transfer procedure is highly robust and efficient, thereby providing a reliable method for moving YACs into the new window strains.

DISCUSSION

The YAC cloning system has rapidly become a critical tool for the dissection of complex genomes and for the study of large genes and their associated regulatory elements. One limitation

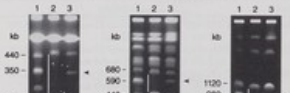

FIG. 4. Transfer of YACs that comigrate with endogenous chromosomes into window strains. Three YACs [yWSS2319, yWSS2764, and yWSS647 (lane 1 in *A–C*, respectively), previously described (30)] each comigrated with an endogenous yeast chromosome(s). YLBW2, YLBW4, and YLBW7 (lane 2 in *A–C*, respectively) contain windows of the appropriate size for these three YACs. Using the *kar1*-transfer procedure (17, 18), each YAC (arrowhead) was transferred into the corresponding window strain, thereby allowing its free migration within the available window (lane 3 in *A–C*, respectively). The chromosomes of the various yeast strains were separated by PFGE (*A*, 15–35 sec switching time, 24 h; *B*, 60–120 sec switching time, 24 h; *C*, 80–150 sec switching time, 32 h) and detected by ethidium bromide staining. Additional details about the PFGE conditions are the same as in Fig. 3. Note that there are some natural size polymorphisms between certain chromosomes in YPH925 and AB1380.

of YACs is that the cloned DNA cannot be readily purified in large quantities, even by preparative PFGE. This problem is exacerbated when a YAC is the same size as one or more of the endogenous yeast chromosomes. Even when a YAC is slightly different in size than an endogenous chromosome(s), PFGE-based separation can often be tedious and challenging on a preparative scale, especially when high concentrations of yeast DNA are loaded onto the gel and low-melting-point agarose is employed, which requires different electrophoretic conditions than regular agarose. Thus, the subsequent utilization of a YAC that is similar in size to an endogenous yeast chromosome(s) may be limited, especially for applications that demand relatively pure preparations of the cloned DNA.

To minimize this limitation, we have devised an approach that greatly facilitates PFGE-based isolation of YAC DNA away from endogenous yeast chromosomes. Our approach capitalized on two important advances in yeast genetics: homologous recombination-based chromosome fragmentation (15, 16) and movement of YACs from one yeast cell to another by the *kar1*-transfer method (17, 18). Here we report the construction of a set of yeast host strains whose chromosomes have been systematically altered to produce large windows in their electrophoretic karyotypes. These strains were produced by up to three sequential rounds of recombination-mediated chromosome fragmentation using the strategies depicted in Figs. 1 and 2. By careful selection of the yeast targets that served as fragmentation sites (based on their relative positions on the yeast chromosomes), each resulting strain contains a window covering a different size range within the electrophoretic karyotype. Importantly, the presence of the *kar1-Δ15* mutation allows each newly constructed strain to act as a YAC recipient following mating with a YAC-containing strain. Thus, a YAC of any size can now be efficiently transferred into an appropriately selected window strain and purified by PFGE without concern about the presence of closely migrating endogenous yeast chromosomes.

Other approaches to deal with the problem of YAC DNA isolation were also considered. For example, one possibility was to create a set of fragmentation vectors that could be used to fragment a closely migrating yeast chromosome in the starting YAC strain. Inherent problems with this approach include (*i*) the high frequency of resulting transformants that do not contain a fragmented chromosome, which would have to be screened through and analyzed in every experiment, and (*ii*) the need to successively perform two (or more) rounds of chromosome fragmentation in the case of YACs that migrate close to two (or more) endogenous yeast chromosomes (such as chromosomes V and VIII at 590 kb or chromosomes X, XIV, and II at 755, 810, and 840 kb, respectively). Thus, constructing a universal set of strains with preexisting windows was viewed as more desirable than performing chromosome fragmentation in every instance where a YAC was similar in size to an endogenous chromosome. A second possibility for creating karyotypic windows was by the recombination of endogenous chromosomes to yield larger DNA molecules. For example, established recombinase systems from bacteriophage P1 (31) or *Zygosaccharomyces rouxii* (32) could, in principle, be utilized for systematically recombining endogenous yeast chromosomes. However, this approach seemed less straightforward and potentially more problematic than the strategies we utilized for chromosome fragmentation.

We have found that *kar1*-mediated transfer of a YAC from its original host into a window (or other *kar1-Δ15*-containing) strain is a highly robust procedure. Other endogenous chromosomes can move into the recipient strain along with the YAC at a frequency as high as 20% (17, 18). However, for the window strains, the cotransfer of a YAC and an endogenous chromosome that migrates within the window should occur at a frequency of at most 2–3% and will likely not represent a significant problem. Armed with the newly constructed window strains and a reliable and routine means for moving YACs from one host to another, it is now possible to isolate YAC DNA away from other yeast chromosomes in an efficient fashion.

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TRANSFER OF YACs INTO YEAST "WINDOW" STRAINS

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Version 10/95

BACKGROUND

The isolation of YAC DNA by preparative pulsed-field gel electrophoresis (PFGE) is often hampered by the presence of closely migrating or co-migrating endogenous yeast chromosomes. We have developed an approach for the reliable isolation of YAC DNA free of endogenous yeast chromosomes regardless of YAC size [described in detail in Hamer *et al.* (submitted) and illustrated in Figure 1]. Our general strategy involves: (1) The use of an established set of yeast strains with chromosome-free areas (i.e., "windows") in their electrophoretic karyotypes (Figure 2 and Table 1); (2) The transfer of a YAC of interest into an appropriate "window" strain (Figures 3 and 4); and (3) The isolation of YAC DNA by preparative (PFGE).

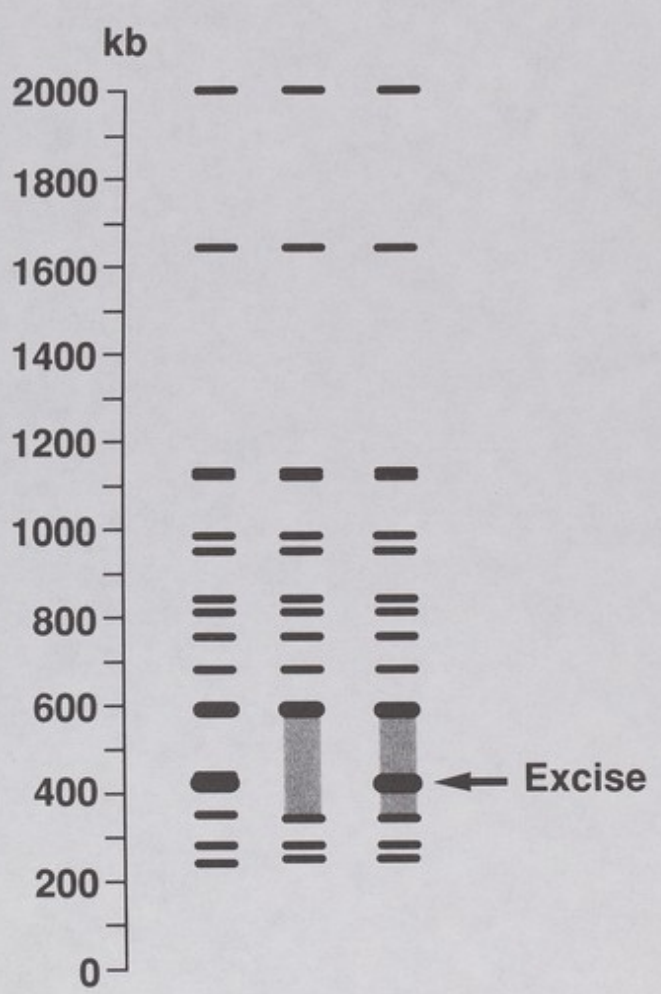

Figure 1: Isolation of YAC DNA Following Transfer into a Yeast "Window" Strain. YAC DNA is typically isolated by preparative PFGE, with the YAC DNA band simply being excised from the gel. However, as shown in the left lane, this approach can be confounded by the presence of closely migrating (or co-migrating) endogenous yeast chromosomes. A solution to this problem is depicted. The center lane shows the results of PFGE analysis of a yeast strain whose chromosomes have been systematically altered to create a chromosome-free "window" in the electrophoretic karyotype (shaded area). Following transfer into the "window" strain, the YAC can be readily excised free of endogenous yeast chromosomes, as shown in the right lane.

To construct the "window" strains, we used recombination-mediated chromosome fragmentation as described by Vollrath *et al.* (1988). Yeast cells were transformed with two small linear DNA molecules: one harbored a centromere, while both contained a telomere and a sequence homologous to a target region on an endogenous yeast chromosome. Recombination across the homologous regions resulted in the fragmentation of the endogenous chromosome and the formation of two smaller derivative chromosomes (which then typically migrate faster than the intact target chromosome). We have created a set of "window" strains (each with a unique "window") by the strategic fragmentation of different combinations of yeast chromosomes (Figure 2).

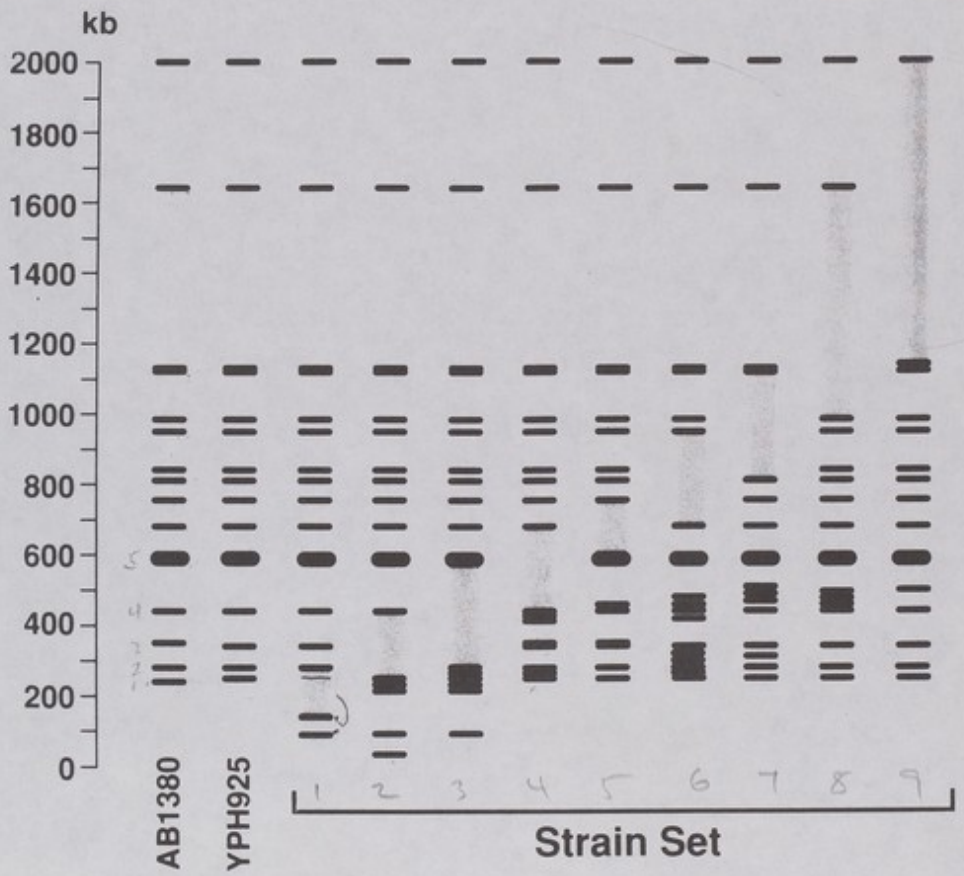

Figure 2: PFGE Analysis of the Yeast "Window" Strains. The results of analyzing the set of 9 "window" strains by PFGE are schematically illustrated. The first 2 lanes from the left show the electrophoretic karyotypes of AB1380 (the most common YAC host strain) and YPH925 (the strain used to construct the "window" strains), respectively. The next 9 lanes show the set of "window" strains. Note the overlapping electrophoretic "windows" (depicted as shaded boxes). Some "windows" resulted from fragmentation of a single chromosome, while others resulted from the successive fragmentation of 2 or 3 chromosomes. There is a complete overlap from one "window" to the next, starting with the "window" at 150-280 kb and ending with the "window" at ~1140-2000 kb (see Table 1). Importantly, these "windows" cover the size spectrum of most YACs, and include the regions where YACs are frequently co-migrating with closely-spaced yeast chromosomes (e.g., the doublet of yeast chromosomes at 590 kb, the triplet of yeast chromosomes from 755 kb to 840 kb).

Table 1: Yeast Strains Containing "Windows" in Their Electrophoretic Karyotypes. The "window" strains YLBW1 through YLBW9 are listed along with each respective genotype, target(s) for fragmentation [with the corresponding chromosome(s) indicated in parenthesis], and size range of the "window". The upper size of the "window" in YLBW9 varies due to the presence of ribosomal repeats in chromosome XII (indicated by a "*").

Strain	Genotype	Target(s)	"Window" (kb)
YLBW1	*MATα leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15* ***cys3***	CYS3 (I)	~150-280
YLBW2	*MATα leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15* ***met10 thr4***	MET10 (VI) THR4 (III)	~250-450
YLBW3	*MATα leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15* ***met10 thr4***	MET10 (VI) THR4 (III) HIS5 (IX)	~310-590
YLBW4	*MATα leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15* ***thr1***	HIS1 (V) THR1 (VIII)	~450-680
YLBW5	*MATα leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15* ***met14***	MET14 (XI)	~590-755
YLBW6	*MATα leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15*	ARG3 (X) LEU4 (XIV) RAD16 (II)	~680-950
YLBW7	*MATα leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15*	RAD16 (II) ILV2 (XIII) ARO7 (XVI)	~810-1120
YLBW8	*MATα leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15*	LEU1 (VII) ADE2 (XV)	~985-1640
YLBW9	*MATα leu2-Δ1 trp1-Δ63 ura3-52 ade2-101 his3-Δ200 lys2-801 cyh2^R kar1-Δ15*	GAL3 (IV)	~1140-2000*

KAR-CROSS TRANSFER OF YACs INTO YEAST "WINDOW" STRAINS

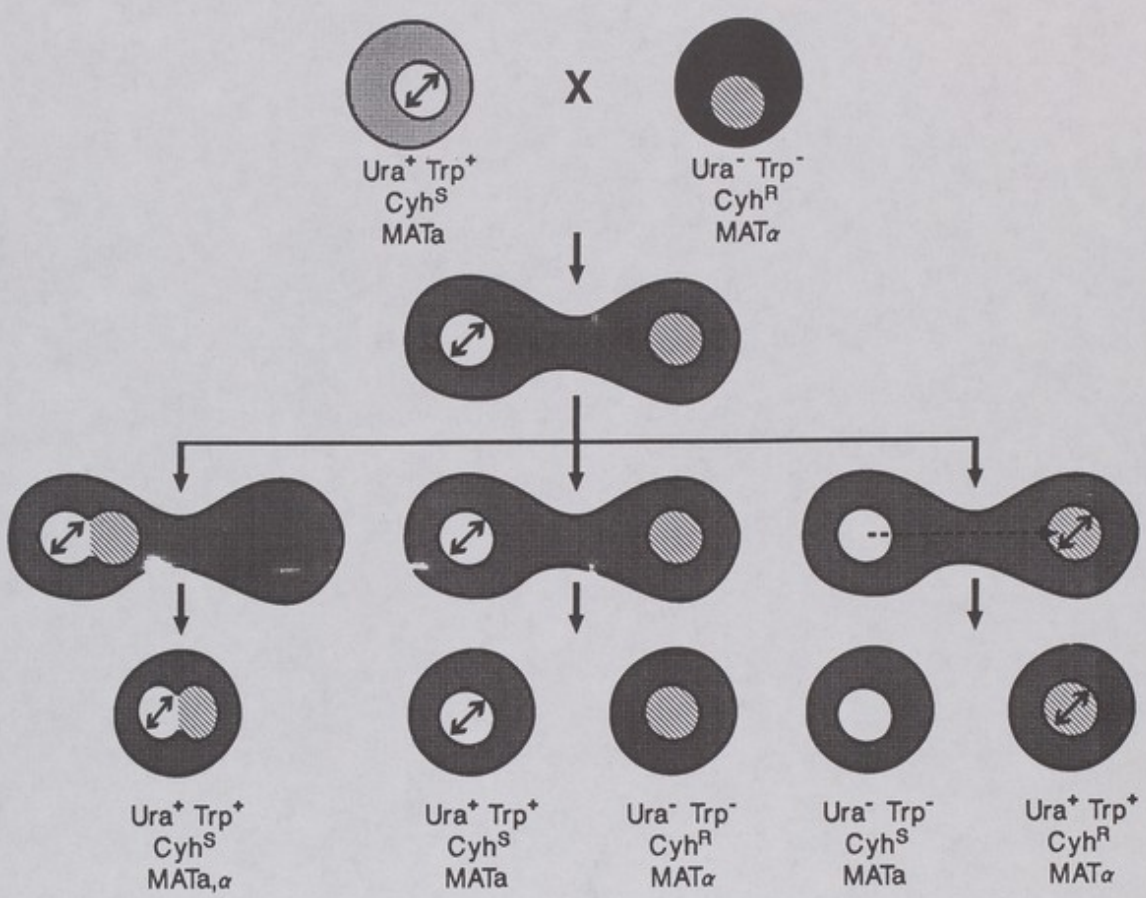

Figure 3: Transfer of YACs into New Hosts by Kar Crossing. YACs can be transferred into new host strains by the *kar*-cross method developed by Spencer *et al.* (1994) and Hugerat *et al.* (1994). Shown in the upper left is a typical YAC in its cycloheximide-sensitive (Cyh^S) host of mating type **a** (e.g., AB1380). The YAC renders the cell Ura^+Trp^+. Shown in the upper right is a "window" strain (i.e., the recipient cell). This strain is cycloheximide resistant (Cyh^R), mating type α, and Ura^-Trp^-. Normally, when yeast cells of opposite mating type mate, nuclear fusion (karyogamy) follows cell wall fusion to form a diploid cell. However, strains with a mutation in the *kar1* gene are deficient in karyogamy. All the "window" strains are *kar1-Δ15* mutants. In crosses involving a *kar1-Δ15* mutant, haploid progeny budding off from the heterokaryon share parental cytoplasm but have one or the other of the parental nuclei (because the nuclei remain unfused). At a frequency of ~10^{-3} to 10^{-4}, a chromosome from one nucleus is transferred to the other. When the transferred chromosome is a YAC, the resulting recipient strain is $Ura^+Trp^+Cyh^R$ (lower right). The *kar1-Δ15* mutation is slightly leaky, so that some diploid cells are formed (lower left), and-- since the Cyh^S allele is dominant-- these diploids are sensitive to Cyh. However, at relatively low frequencies, these diploids can become Cyh^R (presumably from the loss of the Cyh^S allele through mitotic recombination or chromosome loss). Since these diploids are also Ura^+Trp^+, they must be distinguished from the products of authentic YAC-transfer events, and this can be conveniently performed by assessing the mating type of the cells. Diploids are heterozygous at the mating type (*MAT*) locus (**a**/α), while the desired $Ura^+Trp^+Cyh^R$ haploid cells are mating type α.

Storage and Propagation of Yeast "Window" Strains

The *kar1-Δ15*-containing yeast "window" strains should be stored at -80°C in sterile 20% glycerol. For YLBW2-9, routine propagation should be performed in YPD medium. For YLBW1, routine propagation should be performed in YPD supplemented with 40 mg/liter of cysteine (Sigma C-7880). YLBW1 was created by fragmentation within the *CYS3* gene of yeast chromosome I, making it an auxotroph for cysteine. At low frequencies, the derivative smaller chromosomes in this strain tend to recombine, "reforming" the starting chromosome I (thereby eliminating the "window") and restoring cysteine prototrophy. This effect can be minimized by the inclusion of extra cysteine in the medium used to culture YLBW1.

Kar-Cross Transfer of YACs

The basic procedure for transferring YACs into the "window" strains (or other YPH925-derived strains) by *kar*-crossing is depicted in Figure 4.

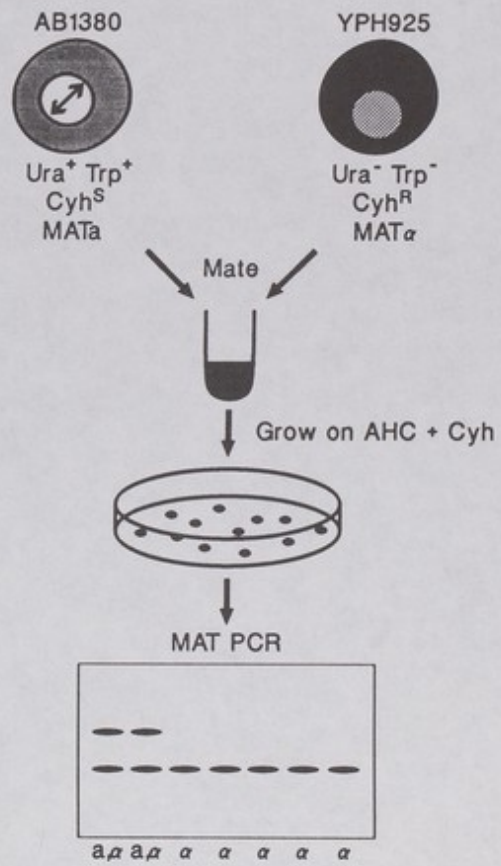

Figure 4: Experimental Procedure for Performing a Kar-Cross. A YAC clone (e.g., in host AB1380) is mated with a "window" strain derived from the *kar1-Δ15*-containing YPH925. The cells are plated on AHC medium (to select for the YAC) containing cycloheximide (to select for the "window" strain nucleus and against the starting YAC host nucleus). Most of the resulting clones represent authentic YAC-transfer events (haploids), while a minor fraction are diploids that have become Cyh^R. These can be distinguished by assessing the mating type, performed either by *MAT*-specific PCR (see below) or a replica plate test. Virtually all *MATα* cells will contain the YAC in the recipient "window" strain.

PROTOCOL

1. Inoculate 25 ml of YPD broth in a 50-ml tube with an appropriately-selected "window" strain (see Figure 2 and Table 1) and inoculate 25 ml of AHC broth in a 50-ml tube with the YAC clone.

   Note: For YLBW1, use YPD broth containing 40 mg/liter of cysteine (Sigma C-7880).

2. Incubate at 30°C shaking at 250-300 rpm for 1 overnight.

3. Determine the cell concentration in each culture using a hemocytometer.

4. Mix $5x10^6$ cells from each culture in a 50-ml tube.

   Note: The rest of the cells from each culture can be used to prepare agarose-embedded high-molecular-weight yeast DNA (see attached protocol). These DNA samples can be used as controls during PFGE analysis to verify authentic YAC transfer into the "window" strain.

5. Centrifuge the cell mixture for 5 minutes at 4000 rpm, discard the supernatant, and add 1 ml of fresh YPD broth.

6. Resuspend the cells and incubate stationary at 30°C for 6 hours.

7. Plate 300 µl and 500 µl aliquots of the cell mixture on separate AHC+Cyh plates.

   Notes:

   It is important that the AHC+Cyh plates are poured "thick" (i.e., contain 35-40 ml of medium in an 85-mm diameter petri plate).

   For YLBW1, use AHC+Cyh containing 40 mg/liter of cysteine (Sigma C-7880).

8. Incubate plates at 30°C for 4-5 days.

   Note: Typically, the faster-growing diploid cells will be seen after 3 days. Wait and analyze the colonies that become visible on the 4th and 5th day.

1 ml

100 µl M Ammonium sulphate

400 µl Tris

25 µl $MgCl_2$

475 µl H_2O

9. Assess the mating type of 10 independent, medium-size colonies by *MAT*-specific PCR analysis (Huxley *et al.*, 1990), as follows:

A. Prepare an appropriately-sized master PCR cocktail based on the number of samples to be analyzed. Be certain to include 3 extra reactions for testing: (1) a known *MAT***a** strain (e.g., a YAC in an AB1380 background), (2) a known *MAT*α strain (e.g., a "window" strain), and (3) a blank sample (no added yeast cells).

	Per Reaction (5 µl)	
Sterile Water	3.35 µl	3.65
5X PCR Buffer [50 mM Tris-HCl (pH 8.3), 250 mM KCl, 12.5 mM $MgCl_2$]	1.0 µl	0.5 10X
dNTP Mixture (@2.5 mM)	0.4 µl	0.8 1.2 mM
Primer Mixture (3 primers, each at 25 µM)	0.2 µl	Dry Supplied at ~30 pmol/µl ≈ 25µM
WIN1 5'AGTCACATCAAGATCGTTTATGG3'		
WIN2 5'GCACGGAATATGGGACTACTTCG3'		
WIN3 5'ACTCCACTTCAAGTAAGAGTTTG3'		
AmpliTaq Polymerase (5 units/µl)	0.05 µl	
		5

B. Dispense 5 µl of the cocktail to the bottom of each PCR tube.

C. Lightly touch the pointed end of a sterile toothpick to each yeast colony, place in a tube containing the PCR cocktail, and twirl the toothpick briefly to suspend the cells.

Notes: A "large" amount of yeast cells will inhibit the PCR. The optimal amount to add is a "barely visible" collection of cells at the tip of a toothpick. Be absolutely certain <u>not</u> to touch the agar with the toothpick, as components in the medium can inhibit PCR.

D. Overlay samples with mineral oil (if necessary), and then subject samples to 35 cycles of 92°C for 1 minute, 58°C for 2 minutes, and 72°C for 2 minutes.

Note: These conditions have been established using a Perkin-Elmer TC1, 480, and 9600 thermal cyclers. The use of another brand of thermal cycler may require prior optimization to establish the appropriate conditions.

10. Analyze PCR products by agarose or acrylamide gel electrophoresis.

PCR Product(s)	*MAT* Type	Frequency	Interpretation
404 bp	*MAT*α	Most	Desired YAC Transfer
404 bp and 544 bp	*MAT*α/**a**	Intermediate	Diploid
544 bp	*MAT***a**	Rare	Starting YAC Strain

11. Select at least 2 *MAT*α colonies, and inoculate each into a separate 25-ml AHC culture (in a 50-ml tube).

12. Incubate shaking at 30°C for 2 days.

13. Prepare agarose-embedded high-molecular-weight yeast DNA (see attached protocol).

14. Analyze purified DNA by PFGE (see Table 2 for suggested conditions). Include the following samples: (1) a lambda concatamer; (2) the initial YAC clone; (3) the "window" strain; (4) each of the YAC-transfer candidates (*MAT*α colonies from step 11).

    Note: Because of the presence of "remnant" homologous sequences among the various fragmented chromosomes (deriving from the fragmentation vectors), there is the possibility for recombination (and therefore the formation of new derivative chromosomes). Therefore, the starting "window" strain should always be tested by PFGE in parallel with the initial YAC clone and all YAC-transfer candidates. Such analysis allows confirmation that the "window" is still devoid of endogenous yeast chromosomes.

Table 2: Suggested Parameters for Analysis of "Window" Strains by PFGE. The indicated conditions are based on the use of a Bio-Rad CHEF System, 1% SeaPlaque GTG agarose (FMC), 0.5X TBE maintained at 12°C, and a 120° angle used during electrophoresis.

Strain	"Window" (kb)	Switching Time (Linear Gradient)	Total Time of Electrophoresis
YLBW1	~150-280	15-25 seconds	24 hours
YLBW2	~250-450	20-35 seconds	24 hours
YLBW3	~310-590	35-60 seconds	24 hours
YLBW4	~450-680	50-70 seconds	24 hours
YLBW5	~590-755	50-80 seconds	36 hours
YLBW6	~680-950	60-100 seconds	36 hours
YLBW7	~810-1120	70-120 seconds	48 hours
YLBW8	~985-1640	80-130 seconds	48 hours
YLBW9	~1140-2000	100-160 seconds	48 hours

Preparation of "High Quality" Yeast DNA for Gel-Purifying YACs

[Adapted from Southern et al. (1987) and Anand et al. (1989)]

1. Inoculate each YAC clone in 25 ml of AHC medium (in a 50-ml tube), close caps to the point where they will remain in place during vigorous shaking (or spinning), and incubate at 30°C shaking at 250-300 rpm (or spinning) for 2 overnights.

   Note: Alternatively, a 25-ml YPD culture can be inoculated and incubated for a single overnight at 30°C. YPD medium must be used for culturing yeast strains devoid of a YAC.

2. Centrifuge the culture at 2000 rpm for 10 minutes and discard supernatant.

3. Add 20 ml of 50 mM EDTA (pH 8.0), resuspend cells by gentle shaking, repeat step 2, and briefly invert tube to drain.

4. Add 100 µl of **Solution I** per 200 µl of cell pellet and resuspend cells by gentle vortexing at the lowest speed.

Solution I [1 M sorbitol/20 mM EDTA/14 mM 2-mercaptoethanol]
91 g of Sorbitol 20 ml of 0.5 M EDTA (pH 8.0) 500 µl of 2-Mercaptoethanol dH_2O to 500 ml

5. Add 25 µl of 10 mg/ml Zymolyase-20T (ICN, prepare fresh in sterile dH_2O) per 100 µl of **Solution I** added in step 4.

6. Immediately add 1 to 1.3 volumes of 2% SeaPlaque GTG agarose (FMC) prepared in **Solution I**, melted, and equilibrated at 50-60°C. Using a blue tip, mix thoroughly but carefully (bring up and down 3-4 times with the pipetman, avoiding formation of air bubbles), and place mixture in an individual plug mold that is sealed on the bottom with tape.

7. Chill the mold at 4°C until the agar is solidified.

8. Dislodge the solidified plug using a bent 21-gauge needle or a pasteur pipette and place in an individual well of a 6-well culture plate containing 8-10 ml of **Solution II**.

Solution II [1 M sorbitol/20 mM EDTA/14 mM 2-mercaptoethanol/ 10 mM Tris (pH 8.0)/0.5 mg per ml Zymolyase-20T]
99 ml of **Solution I** 1 ml of 1 M Tris (pH 8.0) 50 mg of Zymolyase-20T (ICN) [Note: Add immediately before use]

9. Incubate at 37°C for 2-4 hours.

10. Carefully remove solution (e.g., using an aspirator) and add 8-10 ml of **Solution III**.

Solution III [1% lithium dodecyl sulfate/100 mM EDTA/ 10 mM Tris (pH 8.0)]
5 g of Lauryl Sulfate, Lithium Salt (Sigma L-4632) 100 ml of 0.5 M EDTA (pH 8.0) 5 ml of 1 M Tris (pH 8.0) dH_2O to 500 ml FILTER STERILIZE AND STORE AT ROOM TEMPERATURE

11. Incubate at 37°C overnight.

12. Repeat steps 10 and 11.

13. Carefully remove solution, add 8-10 ml of 0.5 M EDTA (pH 8.0), incubate at room temperature overnight, and repeat twice.

    Note: Alternatively, plugs can be taken immediately from **Solution III** and loaded in a gel (as long as the gel is then equilibrated overnight in the circulating buffer prior to electrophoresis).

14. Store plugs in 0.5 M EDTA (pH 8.0) or TE (pH 8.0) at room temperature or at 4°C.

TIPS FOR IMPROVED VISUALIZATION OF YEAST CHROMOSOMES AFTER PFGE

1. Gel Volumes:	Large Bio-Rad Casting System	180 ml
	Small Bio-Rad Casting System	85 ml

2. Sample Loading:
- Create loading "trough" by excising entire top 1-2 cm of gel
- Place plug directly on side of remaining gel (in trough)
- Be certain that there is no air trapped between plug and gel
- Seal plug in place with melted agarose equilibrated to 50°C
- Pour in rest of melted agarose to fill "trough"

3. Equilibration:
- Equilibrate gel in 0.5X TBE at 12°C overnight in gel chamber
- Circulate TBE with pump set at "90"
- Remove as much air from the tubing as possible
- Be certain that the TBE is well circulating

4. Electrophoresis:
- Lower amperage to ~100-115 mA by removing TBE from chamber
- Level of TBE should be only 2-3 mm above the gel
- Be certain that the gel chamber is level

MEDIA

AHC Medium

A rich -Ura-Trp ("double drop-out") complete medium for the routine culturing of YACs. The acid-hydrolyzed casein contains no vitamins or nucleosides and is low in salt, but provides virtually all of the necessary nutrients for yeast growth except for uracil, tryptophan, and adenine. Adenine is added such that growth is limited, and the red pigment forms as a consequence of the nonsuppressed *ade2* mutation.

1. Dissolve the following components in water:

	Per 1 liter:
Yeast Nitrogen Base Without Amino Acids (Difco 0919-15)	6.7 g
Casein Hydrolysate Acid, Low Salt (U.S. Biochemicals 12852)	10.0 g
Adenine Hemisulfate•H_2O (Sigma A-9126)	0.02 g

Note: Instead of the above yeast nitrogen base without amino acids, 1.7 g/liter of yeast nitrogen base without amino acids and without $(NH_4)_2SO_4$ (Difco 0335-15) plus 5 g/liter of $(NH_4)_2SO_4$ can be substituted.

2. Bring volume to 950 ml and adjust pH to 5.8 (with HCl).

3. For plates, add 20 g/liter of Bacto agar

Note: Difco brand is highly recommended, otherwise a precipitate may form upon autoclaving.

4. Autoclave 20 minutes (for 1 liter) and allow to cool (to 65-80°C).

5. Add 50 ml of sterile 40% (w/v) glucose per liter and mix well.

Note: Alternatively, 20 g of solid glucose per liter can be added before autoclaving; however, the resulting medium will be markedly darker in color (although still usable for YAC culturing).

6. For AHC+Cyh, add sterile cycloheximide (Sigma C-7698) to a final concentration of 3 µg/ml.

YPD Medium

A general purpose non-selective, complex medium for the routine culturing of yeast. Yeast cells grow vigorously, regardless of the presence of a YAC. Promotes growth to a high density, but is not selective for YACs.

1. Dissolve the following components in water:

	Per 1 liter:
Yeast Extract (Difco 0127-01)	10 g
Peptone (Difco 0118-01-8)	20 g

2. Bring volume to 950 ml and adjust pH to 5.8 (with HCl).

3. For plates, add 15 g/liter of Bacto agar.

4. Autoclave 20 minutes (for 1 liter) and allow to cool (to 65-80°C).

5. Add 50 ml of sterile 40% (w/v) glucose per liter and mix well.

Note: Alternatively, 20 g of solid glucose per liter can be added before autoclaving; however, the resulting medium will be markedly darker in color (although still usable for yeast culturing).

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(p138)

-trp-ura medium # plates (1ℓ)

(1ℓ) YPD broth and plates ~~+ 40 mg/l cysteine~~

(1ℓ) " " " " + 40 mg/l cysteine

(1ℓ) -trp-ura plates + 3 µg/ml cycloheximide

(1ℓ) -trp-ura plates + 40 mg/l cysteine
+ 3 µg/ml cycloheximide

Plates should be poured thick
(35-40 ml / plate)

round plates

20/9/96

Winlow strain storage.

Shipment buds puddled in 20% glycerol in -ura medium (3 -ura : 2 50% glycerol).
-70 storage.

Buds streaked on YPD plates
2 day growth; variable growth but all have colonies.

21/9
YLSW1 replated on YPD + cysteine
a) bud streak (stored 4°)
b) from glycerol
c) from YPD plate.

~~30/9~~
~~o/n growths~~

YPD
YPD + cysteine

-trp -ura
-trp -ura + cycloheximide
-trp -ura + cycloheximide + cysteine

Pink slipgoes.

Haemocytometer (5 x 10^6 cells)
(8 µl)

Seal coverslip (Newton rings)
8 µl culture

Empty plate
Haemocytometer on top
Green objective
Turn device on at back ; light
Press → once
Press → again
Use joy stick to move stage
Count 5 big squares (each = 16 small)
(Total).
Total x 5×10^4 = cells/ml

(5×10^6/ml ≃ 20/ big square).
Switch off device, monitor (2 switches).

24/9/96

YACs for window transfer:

		YLBW
Y3998	(230kb)	1
Y4886	(280kb)	2
Y48C3	(280kb)	2

30/9

O/N growth:

YLBW1 YPD + cysteine
YLBW2 YPD
Above YACs -trp -ura
25 ml 2.30 → 9.00am
YLBW1 very thin.

1/10

Haemocytometer assay.
10xD readings:

	YLBW1	YLBW2	Y3998	Y4886	Y48C3
5 square	26	271	242	198	188
x10	260	2710	2420	1980	1880
x5x10^4 (=cells/ml)	1.3×10^7	1.3×10^8	1.2×10^8	9.9×10^7	9.4×10^7
5×10^6 cells	0.38ml	0.038	0.04	0.05ml	0.05

OVER

1/10

YAC transferance

a	0.4 ml	YLBW1	50 ml Falcon
	40 µl	Y3998	
b	40 µl	YLBW2	
	50 µl	Y4886	
c	40 µl	YLBW2	
	50 µl	Y48C3	
d	0.4 ml	YLBW1	
e	40 µl	YLBW2	
f	40 µl	Y3998	
g	50 µl	Y4886	
h	50 µl	Y48C3	

a, d: spin 4K 5'
decant
+ 1 ml YPD

others: no spin
+ 1 ml YPD

30° (~~stationary~~) 6 hr 2.15 pm 8.15 pm

300 µl and 500 µl aliquots plated
a, d -trp/-ura + cycloheximide + cysteine

others -trp -ura + cycloheximide
and fish -trp -ura
in cubs 30° 1/10 →

(300 and 500 µl reluctant to absorb)

3/10
am
< 2 days

f, g, h – trp –ura : v. dense growth.

others difficult to distinguish early growth from surface effects caused by spreading ~~too~~ large volumes of culture.

Also some bacterial contamination (c 300) prob caused by extensive spreading time.

4/10
am
< 3 days

a	300µl	13	(3 'large').	
	500	16	(7 ")	
b	300	29	{ 4 " }	+ many tiny 'colonies' *
	500	23	{ 3 " }	
c	300	25	{ 4 " }	(+ contam?)
	500	41	{ 6 " }	
d	500	8	(1 "?)	
e	500	2	(1 ")	+ similar number of tiny colonies as b 500.
f	500	0		
g	500	0		
h	500	0		

* 'Leaky' –ura –trp? (f–h indicate that cycloheximide OK).

Should plate YKBW's on –ura–trp.

over

6/10/96

~ 5 days incub^n

a	200 µl	~32	colonies various sizes, not pink
	500	60	" " " , not pink
b	300	~75	, mostly pink
	500	65	" "
c	300	75	mostly pink
	500	90	" "
d	500	~20	v small (poss no growth days 2-3?)
e	500		v small 'bkgnd' colonies
f	500	0	
g	"	0	
h	"	0.	

Re-streaks:
3 'large' and 3 'small' colonies from a, b, c were restreaked on -trp-ura + cyclohex (+cys, a)
2 day 30°:
a Large colonies more advanced
b as a (one selected (ura is bacterial)
c all growths generally slower
(Reincubate).

YLBW1 / -trp-ura + cyclohex + cys : No growth

YLBW2 / -trp-ura + cyclohex : No growth
(Reincubate).

Background colonies from 1/10 b : No growth
(-trp-ura + cyclohex)
(Reincubate)

7/10/96

PCR assays of various a, b, c colonies.

Mix:
(24 reacn)

150	95 H_2O
20	13 10× PCR buffer (MAS)
30	21 1.25 mM dNTPs (MAS)

7.5 Added to 5 µl each oligo (dried) assumed ~30 µM as delivered.

2 + 1.3 µl 5 u/µl Taq (MAS)

5 µl / reaction 0.5 ml tube.

a - c Toothpicked colony from each YAC (Y3998, Y4886, Y4805)

d, e YLBW1, 2

f Blank

g - l 3 large, 3 small colonies from 1/10 a.

m - r " " 1/10 b

s - x " " 1/10 c

Oil overlay.

92° 1'
58° 2'
72° 2'
× 35
35° hold.

+ 15 µl TE 10:0.1, mix and quick spin. (Careful remove aqueous from under oil!).

5 µl + 1 µl dye

5 µl onto 1.4% gel (λ HindIII + ΦX HaeIII markers)
35 mA

Blue

Y3998/YLBW1 1/10/96

Y48B6/YLBW2 1/10/96

Y48C3/YLBW2 1/10/96

Small colonies appear to be the diploid. This is opposite of what the protocol says i.e. that the diploids grow faster.

6/10

Platings for window transfer

Y711A12 II 340 (for YLBW2)

Y53C10 I 350 (for YLBW2) Failed plate growth

Y10293 V 670 (for YLBW4 and 5).

Y53C10 restreaked 8/10.

8/10.

Continuation of 1/10 expts.

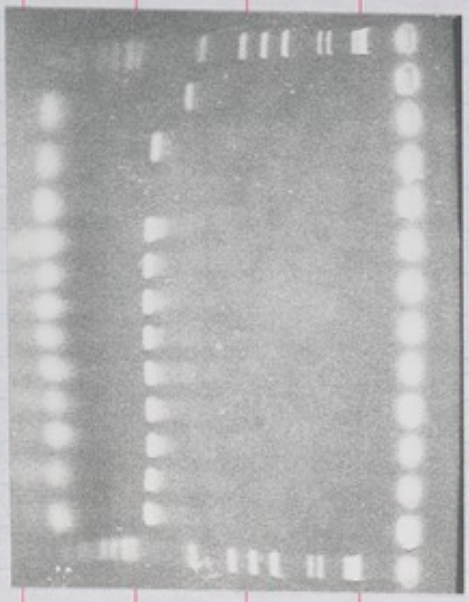
3 large colonies from a, b, c selected for PCR and liquid culture in -trp-ura.

Y3998W1a-c

Y48B6 W2a-c

Y48C3 W2a-c

PCR on 7/10.



Y3998

Y48B6

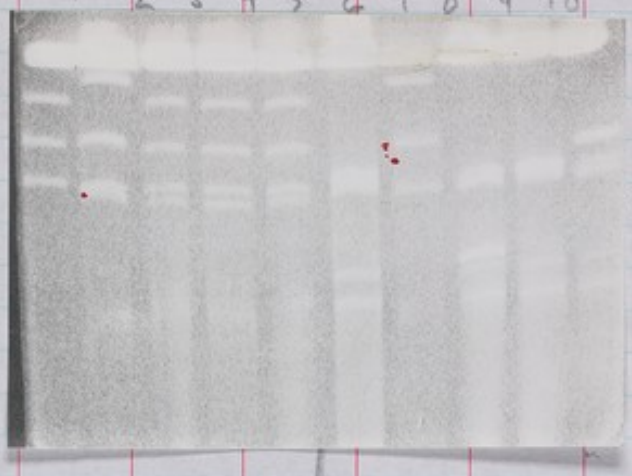
Y48C3

All look good.

i.e. Mat α

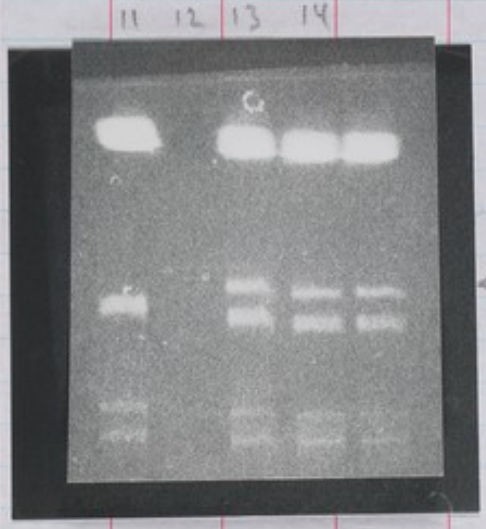
15/10

1/10 expt., Bijay gel (Reruns on top)



1	YLBW1
2	Y3998 (230 kb)
3	Y3998W1A
4	" B
5	" C
6	YLBW2
7	Y4886 (280 kb)
8	Y4886W2A
9	" B
10	" C

150 V 20sec 24hr



11	YLBW2
12	Y4886 (floated out) (280kb
13	Y4886W2A
14	" B
15	" C

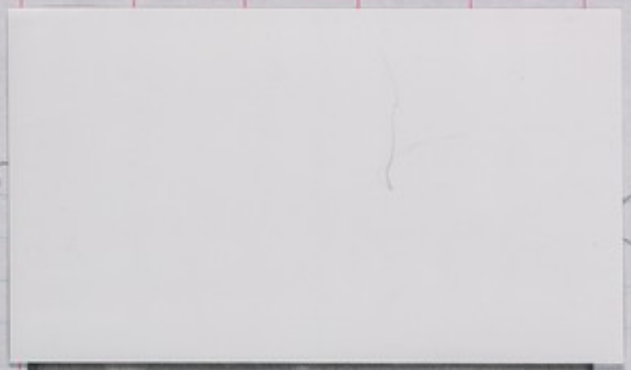
Later gel overleaf

Looks like 'window chromosome' has reformed.

Note:

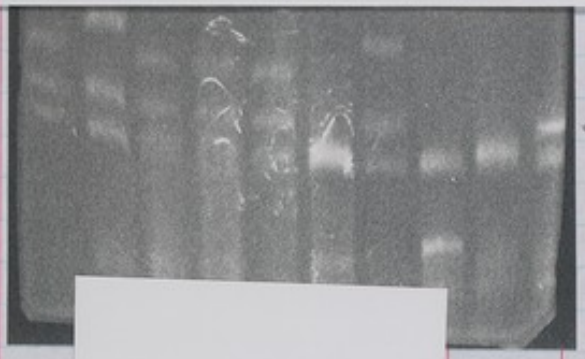
1. Should do preliminary gel at optimal separation prior to transfer – original analysis not adequate to determine whether or not separable.
2. Should run AB1380 (in hand) – can't rely on multiple YACs on same gel to indicate host chromosomes.
3. 1A YAC v. weak in Y4886. Why overly strong

over



(Reruns on top)

1 YLBW1
2 Y3998 (230 kb)
3 Y3998W1A
4 " B
5 " C
6 YLBW2
7 Y4886 (280 kb)
8 Y4886W2A
9 " B
10 " C



150 V

11 YLBW2
12 Y4886 (floated out) (280kb)
13 Y4886W2A
14 " B
15 " C

Later gel overleaf

Looks like 'window chromosome' has reformed.

Note:
1. Should do preliminary gel at optimal separation prior to transfer — original only is not adequate to determine whether or not separable.
2. Should run ABI380 (in hand) — can't rely on multiple YACs on same gel to indicate host chromosomes.
3. If YAC v. weak in Y4886, why overly strong

over

in Y4886W2C ?

31/10/96

Pic borrowed for poster

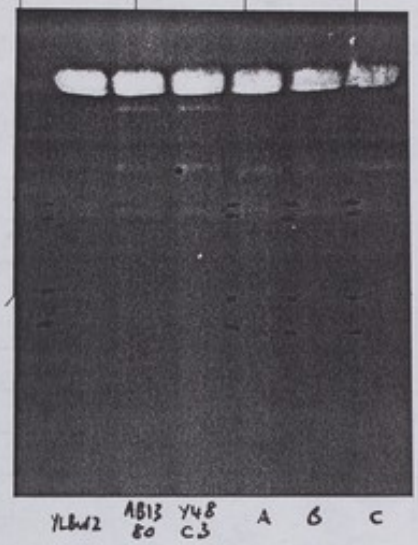
New block Y48C3.
inc. AB380.

Looks v. good.

Forward Y48C3W2A for stripping.

in Y48B6W2C ?

31/10/96



New block Y48C3.
inc. ABI380.

Looks v. good.

Forward Y48C3W2A for pooling.

14/10

a 300µl 1 large, 4 'medium', 3 small
300µl 3 'medium', 2 small

b 300 1 medium
300 1 medium 2 small

c 300 3 large, 4 medium 1 small
300 3 large, 3 medium 1 small

d (5 contaminating, non-yeast.)

e 0

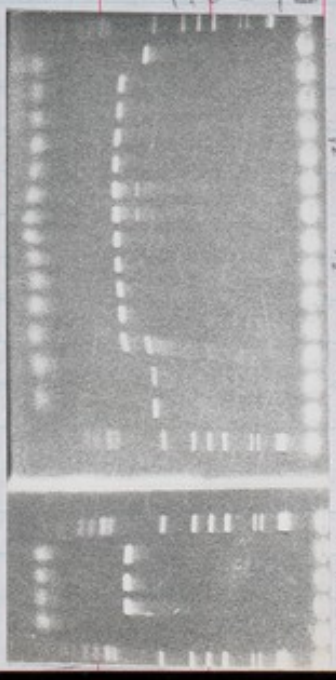
f 1 medium, 2 small

g 0

h 0

i 0

Pick for PCR and O/N culture 4 colonies from each d a - e, i
Also Y71BW 2, 4, 5
Y71A12
Y10293

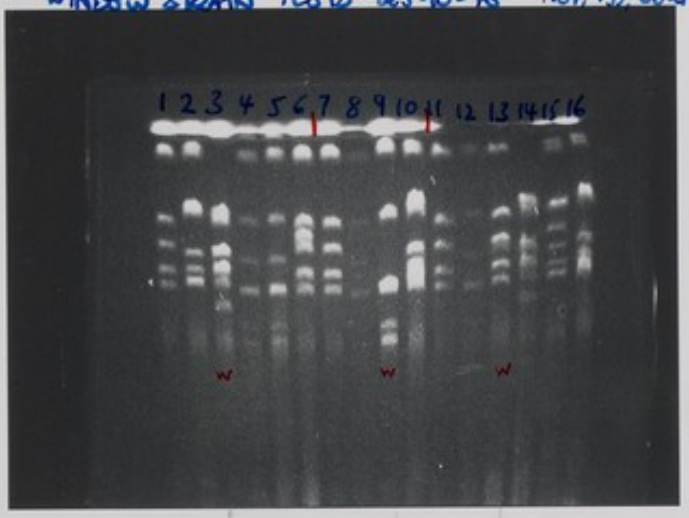


Bijay to proceed with
Y71A12W2 B, C, D
Y10293W4 A, (D - to be grafted)
Y10293W5 A, B, C

OVER

72 H2O
10 PCR
16 dNTPs
1 pol

WINDOW STRAIN TESTS 25-10-96 15hrs, 45s, 26hrs



1 AB1380
2 YTIA12 (340)
3 YLBW2
4 YTIA12W2B
5 " C
6 " D.

7 AB1380
8 Y10293 (670)
9 YLBW4
10 Y10293W4A -

11 AB1380
12 Y10293
13 YLBW5
14 Y10293W5A
15 " B
16 " C

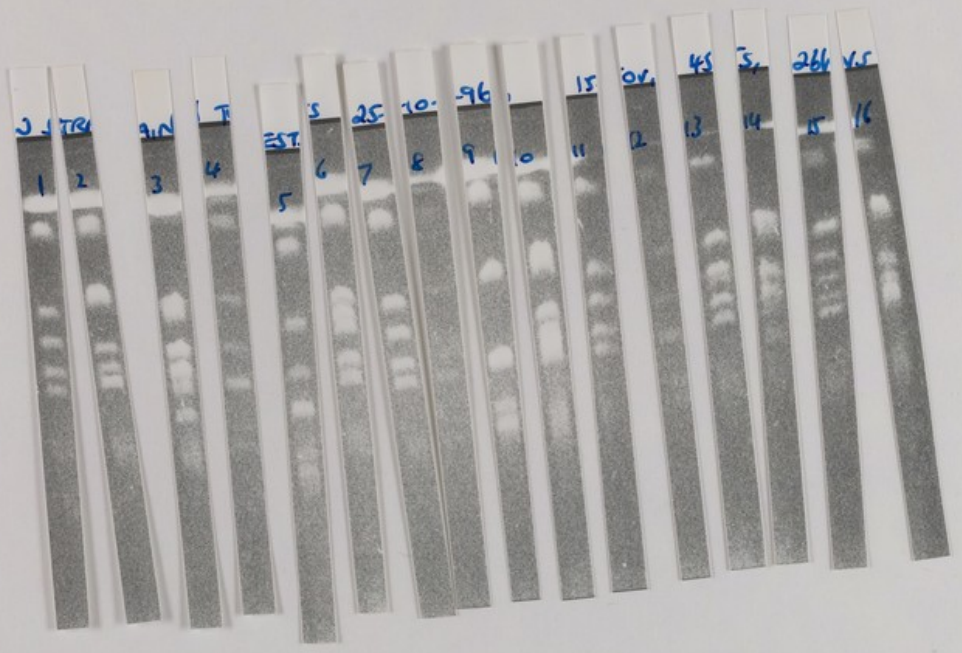
Obviously bad muddle here. Can't resolve properly. See next page for best guess.
At which stage did muddle occur?
Assay glycerol stocks.

28/10 Streak all relevant glycerols on YPD
-ura -trp
-ura -trp +cyclo

	YPD	+-U	+-U+cyc.
YTIA12	✓	✓	×
YLBW2	✓	×	×
YTIA12W2B	✓	✓	✓
" C	✓	✓	✓
" D	✓	✓	✓
Y10293	✓	✓	×
YLBW4	✓	×	×
Y10293W4A	✓	✓	✓
~~Y10293~~			
YLBW5	✓	×	×
Y10293W5A	✓	✓	✓
B	✓	✓	✓
C	✓	✓	✓

All behave as expected
Bijay to make blocks

3/11
These platings used to initiate growth for re-preparing of blocks by Bijay
(see +2 pages)



DO NOT REMOVE FROM SLEEVE

Possible

1 AB1380 ~~(+?)~~ (or big YAC?)

~~15~~ ~310 kb YAC

9 YLBW2

4 " +~310 kb YAC

5 " "

8 " "

12 " "

---

3 YLBW4 (+?) ??

14 " (+?)

(should be only 2 YLBW4's.)

13 ~340 kb YAC (Y71A12)?

7 AB1380 (or big YAC?)

11 AB1380 (" " ")

2 } YLBW5. (+?)

10

16

~~2~~

~~6~~

~~10~~

~~16~~ }

where YLBW5?

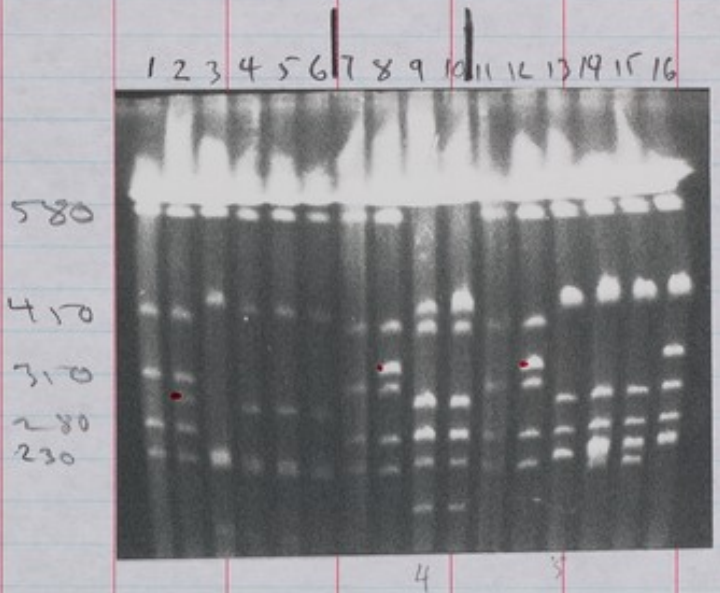
#2 #5 #6 #9 #10 #16

probes provided by the community. In addition, repeat sequences can cause severe problems. Figs N - N show a variety of probings having different degrees of interpretability. (Efforts were made to overcome these problems by prehybridistion to eliminate repetitive sequences from probes (ref Southern), but without success).

Directed efforts

A few recalcitrant regions have been mapped in some detail as a result of intensive efforts to locate a particular gene. A good example of the complexities that can arise is seen in the cloning of tra-1 (LGIIIR) (Hodgkin 1987, 1993). Attempts to clone this gene by transposon tagging had been unsuccessful. A number of Bergerac-derived Tc1 polymorphisms were identified and mapped relative to genes surrounding the tra-1 locus. None appeared likely to be within 200 kb of tra-1 (interpolating between genetic and physical distances using wabt was thought to be a typical metric for the chromosome arms). Unique fragments adjacent to three Tc1 insertions (eP1, eP2 and eP10) were selected as start points for walking. No positive phage (lambda 2001, plated on the standard rec+ host Q358) were found by hybridisation with the eP1 fragment (believed to be the closest to tra-1) but two YACs were detected. These in turn led to the identification of a small previously unmapped cosmid contig. RFLP analysis of rearrangement alleles showed that this contig was in the region of tra-1. The cosmid contig was oriented by differential screening of lambda clones with the two cosmids that wholly represented the cosmid contig. Walking in the required direction from this contig was only achieved by the isolation of phage clones from an unamplified library of lambda 2001 clones plated on the rec- host CES 200 (recBC sbcB) (Wyman et al). (Other hosts were assayed but were not as satisfactory). Extension of the cosmid contig was determined by fingerprinting of the isolated lambda phage. These extending phage then led to the isloation of a further YAC which was used to indentify two more small cosmid contigs. Overall, the region around tra-1 was found to contain at least four sites that cannot

11/11



1	ABJ380
2	Y71A12 (340)
3	YCBW2
4	Y71A12W2B
5	" C
6	" D
7	ABJ380
8	Y1029J3 (670).
9	YCBW4
10	Y1029J3W4A
11	ABJ380
12	Y1029J3
13	YCBW5
14	Y1029J3W5A
15	" B
16	" C

So:

Y71A12 transfer (B, C, D) all good but unnecessary.

Y1029J3 transfer to YCBW4 probably failed but 4A appears to be not of expected size i.e. apparently 400 rather than 670. Y1029J3W4A does look to have an additional band at ~ 470.

If Y1029J3 really is ~400, transfer to Y1029J3W5C may be good. But A and B appear to have gained products at ~ 200 kb. Perhaps Y1029J3 is unstable?

(Probing 1 cosmid grid with ~670 kb band gave poor probing but generally in accordance with cy and PCR data).

18/10.

Bijay info on co-migrating YACs that require window transfer:

			Window
Y39E4	(350 kb)	III	2
Y41C4	(300 kb)	III	2
Y45F10	(450 kb)	IV	3
Y37H2	(460 kb)	V	3
Y38E10	(350 kb)	II	2

Streaked 21/10/96.

To check for necessity for window transfer on preliminary gel:

IV	Y41E3	280 kb
	Y40H7	230 kb
	Y7A9	230 kb
V	Y59C12	710
I	Y53C10	350
	Y67D3	230
	Y54E7	290

YACs that haven't separated from Yeast chromosomes

3	Y39E4	(350 kb)	on 3rd
4	Y41C4	(300 kb)	on 2nd
5	Y45F10	(450 kb)	on 4th
1	Y37H2	(460 kb)	on 4th
2	Y38E10	(350 kb)	on 3rd

23/10.

Inoculations for transfer expts

10 ml inocs of

		Window
Y39E4	} -trp -ura	2
Y41C4		2
Y41F10		3
Y37H2		3
Y38E10		2
YLBW2	} YPD	
YLBW3		

All 24 hr 30° except YLBW3 - 36hr

	Y37H2	Y38E10	Y39E4	Y41C4	Y41F10	W2	W3
10 x 10 Sq	117	209	185	107	287	226	180
x 10	1170	2090	1850	1070	2870	2260	1800
cell/ml x 5×10^4	5.6×10^7	1×10^8	9×10^7	5×10^7	1.4×10^8	1.1×10^8	9×10^7
5×10^6 cells	90 µl	50 µl	55 µl	100 µl	35 µl	45 µl	55 µl.

Glycerols of all. (25/10)

25/10

	µl		200 µl plating	
1.	90	Y37H2	1	1 small
	55	YLBW3	2	0
			3	2 small
2	50	Y39E10	1	7m ~10 small
	45	YLBW2	2	1L 7m ~10 s
			3	7m ~10s
3	51	Y39E4	1	3L 11m ~10s
	45	YLBW2	2	3L 1m 4s
			3	4L 6m ~10s
4	100	Y41C4	1	1s 3 v.s.
	45	YLBW2	2	1s 2 v.s.
			3	2 v.s.
5	35	Y45F10	1	24 various
	55	YLBW3	2	2m 12s
			3	3L 15m 10s
6	45	YLBW2	1	0
7	55	YLBW3	1	0
8	90	Y37H2	1	0
9	50	Y38E10	1	0
10	51	Y39E4	1	0
11	100	Y41C4	1	5s
12	35	Y45F10	1	0
13	0		1	0

Spin, super not discarded.
+ 1 ml YPD

6hr (8:30 → 2:30).

3 x 200 µl 1-5 tyr-ura + cycloh ex
1 x 200 µl 6-13 " "

OVER

29/10

Restreaking of 25/10 expt colonies -trp -ura + cyclo.

1. Y37H2 YLBW3 2 small
2. Y38E10 YLBW2 1 L 3M 2S
3. Y39E4 YLBW2 3L 2M 1S
4. Y41C4 YLBW2 ~~3S~~ ~~4HS~~ only 2 grow.
5. Y45F10 YLBW3 3L 2M 1S

(These to test by PCR and make glycerol stocks)

(Note Appears to be some correlation of colony numbers with initial cell count, although mating volumes adjusted accordingly).

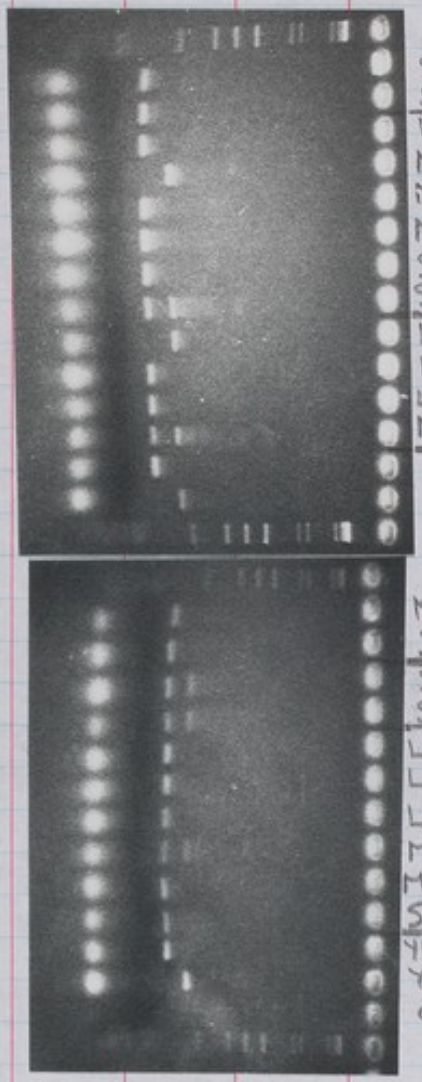
1 and 4 most likely to need repeat mating.

22 for PCR + YWLB2, Y37H2, 0.

As 7/10.

1. 1/1 → 3/4 + YWLB2, Y37H2
2. 3/5 → 5/6 + " , + " , + 0

- Total blank! Try again



· = miniglycerols : Y37H2W3 A, B
Y38E10W2A-D
Y39E4W2 A-D
Y45F10 W3A-E.

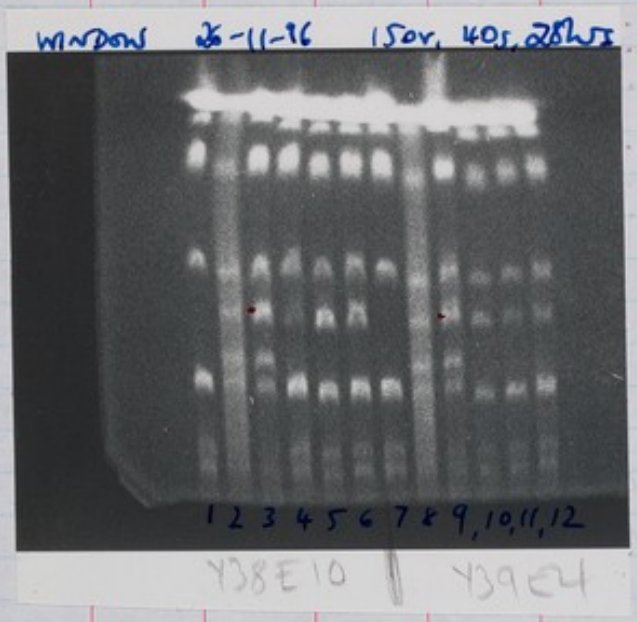
(Y41C4 needs repeating)

13/11 Plated for PFG analysis:
Y37H2W3A, B
Y38E10 W2 A, B, C
Y39E4 W2 A, B, C
Y45F10 W3 A, B, C
W2, W3
Y37H2, Y38E10, Y39E4, Y45F10

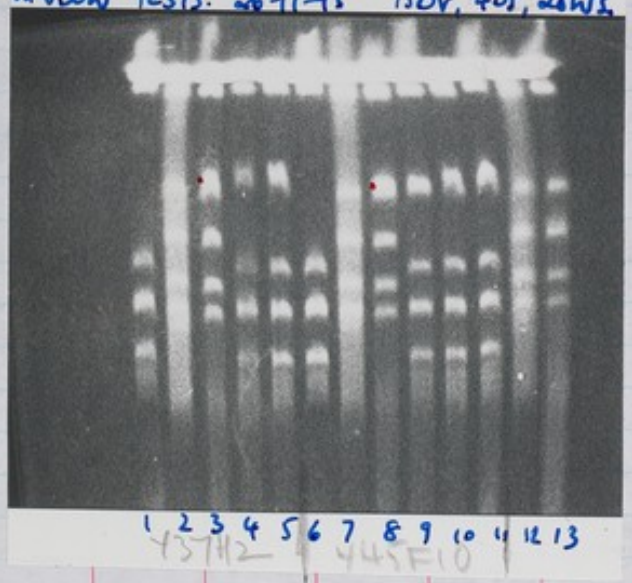
For PFG analysis:

OVER

26/11



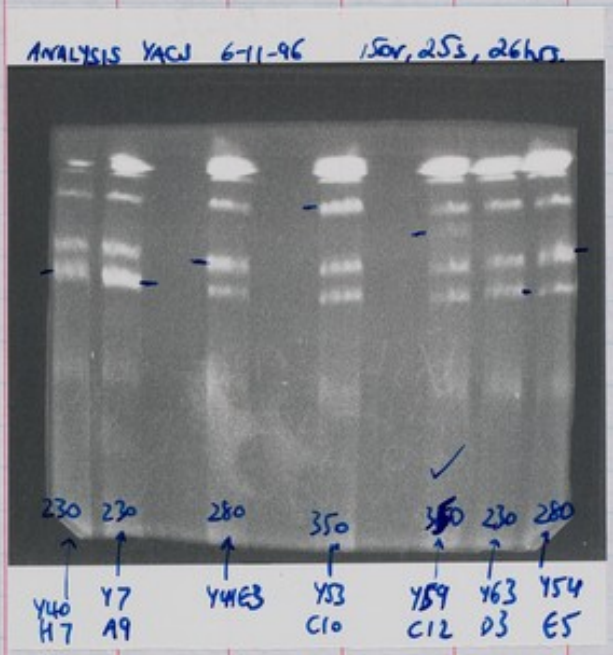
1	YLBW2	
2	AB1380	
3	Y38E10	(350)
4	"	W2A
5	"	W2B
6	"	W2C
7	YLBW2	
8	AB1380	
9	Y39E4	(350)
10	"	W2A
11	"	W2B
12	"	W2C



1	YLBW3	
2	AB1380	
3	Y5TH2	(460)
4	"	W3A
5	"	W3B
6	YWBW3	
7	AB1380	
8	Y45F10	(450)
9	"	W3A
10	"	W3B
11	"	W3C
12	AB1380	
13	Y47H9	

All analysed colonies look good transfers.
Y47H9 needs window transfer (→ W2).

Confirmation of necessity for window transfer



So Y46H7
Y7A9
Y41E3
Y53C10
Y63D3
Y54E5
all need transfers.

Also outstanding:
Y41C4 (repeat) → YLBW2.

10/11/96
All above, + YWLB1 (+yr), YLBW2
25 ml liquid cultures.

12/18

	Y7A9	Y40H7	Y41C4	Y41E3	Y53C10	Y54E5	Y6303	YW1	YW2
10xD 559	224	235	159	226	246	257	246	225	224
x10	2240	2350	1590	2260	2460	2570	2460	2250	2240
cells/ml x5x10⁴	1.1x10⁸	1.2x10⁸	8x10⁷	1.1x10⁸	1.2x10⁸	1.3x10⁸	1.2x10⁸	1.1x10⁸	1.1x10⁸
5x10⁶cell	45µl	45	80	45	40	40	45	45	45

1	Y7A9	W1	~16 large, 3? pinkish, ~12 small	per plate
2	Y40H7	W1	~12 large, 3? pinkish, ~12 small	per plate
3	Y41C4	W2	1 large pink + 3-15 small	per plate
4	Y41E3	W2	~7 large pink, ~20 small	per plate
5	Y53C10	W2	~12 large pink, ~12 small	per plate
6	Y54E5	W2	~4 large pink, ~12 small	per plate
7	Y6303	W1	~20 large ~1/3 pink, ~12 small	per plate
8	Y7A9		0	
9	Y40H7		0	
10	Y41C4		12 v. small	*1
11	Y41E3		0	
12	Y53C10		9 smallish	*2
13	Y54E5		0	
14	Y6303		0	
15	W1		13 small/medium whites.	*3
16	W2		0	
17	0		0	

No 'fog after mixing of YAC/window vols.
+ 1ml YPD + cycloheximide (W1) or YPD (W2's)
6 W 30° (10.45 - 4.45)
3 x 200µl 1-7 plated (-t, -U, +cycl, or +gcd +cyc)
1 x 200µl 8-17

#1 control gave colonies 21/10 attempt
#2 fresh cycloteximide plates req'd?
#3 bothering: Pink colonies from transfers probably worth proceeding.

Restreaking:

4 large colonies (3 pink, 1 w) from each transfer restreaked on trp-ura cyclo. 1-3 4

19/11

PCR ~~testing~~ of restreaks.

7.5	each oligo, dried
140	H_2O
20	buffer
30	dNTPs
2	Taq
192	

over

Y7A9

Y40H7

Y41C4

Y41C4

Y41E3

Y53C10

Y54E5

Y54E5

Y63D3

minigly cerols: 22/11/96

Y17A9W1A-C
Y140H7W1A-C
Y141E3W2A-D
Y153C10W2A, B
Y154E5W2A, B
Y163P3W1A-D
(19)

-70: 1st batch in YKBW-2

Y41C4 failed again

⇒ wrong! Y41C4-4 needs gel assay!
Minigly cerol = Y41C4W2A. (23/11/96).

24/11/96

25 ml preps of A-C (or A, B) started.
(-Ura-Trp)

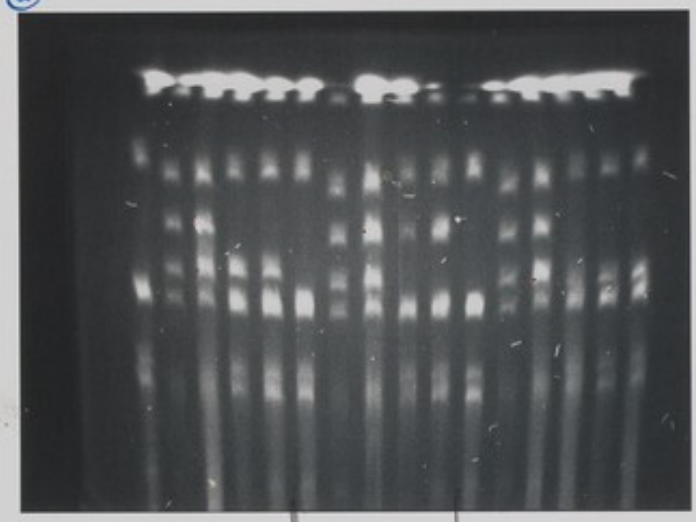
Also W1 (YPD+ags), W2 (YPD), AS1380 (YPD)

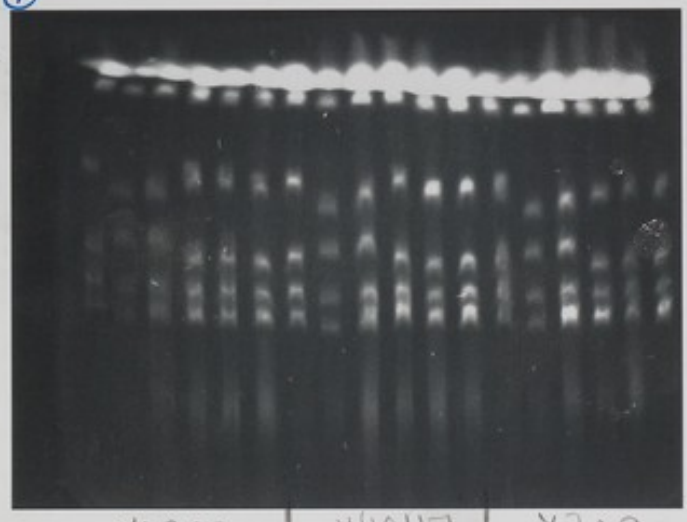
2 ml of 50% inoculum

3+2
4+3+3

30

3/12
35 sec, 28 hr (B. Jan)





② YLBW2
AB1380
Y54E7
Y54E7 W2A
" W2B

YLBW2
AB1380
YT3C10
YT3C10 W2A
" W2B

YLBW2
AB1380
Y41E3
Y41E3 W2A
" W2B
" W2C

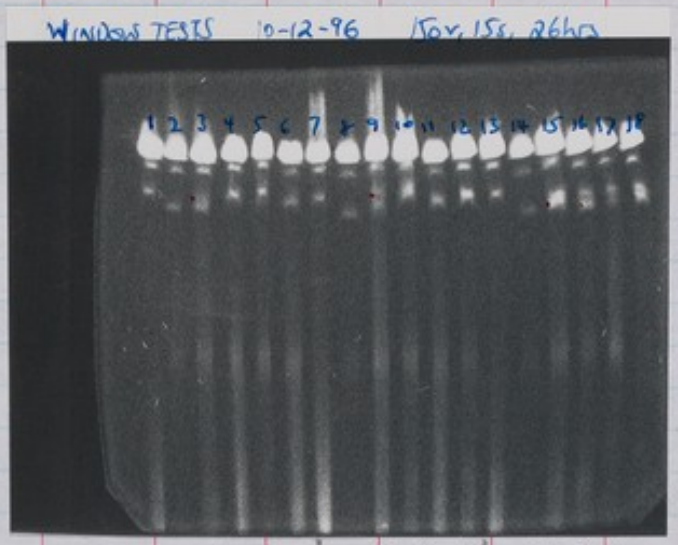
① YLBW1
AB1380
Y6303
Y6303 W1A
" W1B
" W1C

YLBW1
AB1380
Y40H7
Y40H7 W1A
" W1B
" W1C

YLBW1
AB1380
Y7A9
Y7A9 W1A
" W1B
" W1C

YLBW2's all look good.
But YLBW1's not good; window drawn.
has recombined.
Bijan will run better gel of these.
Either original YLBW1 window frazier
should be separable

10/12
Repeat of 3/12 (1).



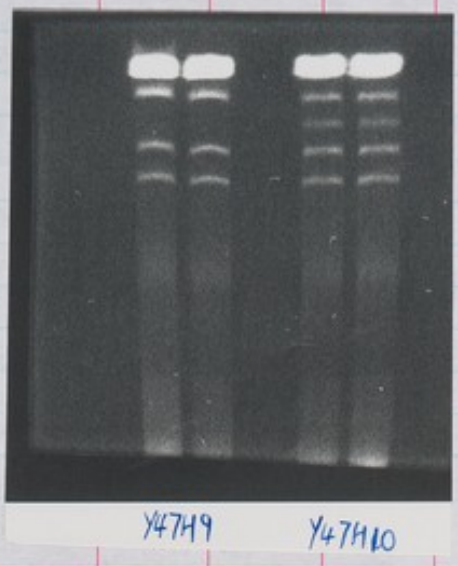
Y63D3 Y40H7 Y7A9

Y63D3 : ~~starting~~ YAC may be bait, but really needs transfer into non-reformed W1.

Y40H7 : starting YAC prb seperable

Y7A9 : W1A - W1C prb good : YAC = bottom band.

To resolve possible muddle



2/12

Streaked for window transfer

Y1797	II	230	W1
Y18010	I	280	W2
Y26910	IV	230	W1
Y4703	III	350	W2
(Y47H9	III	400	W3 – W3 grew v. poorly – suspected)
Y56f9	I	280	W2

	Y1797	Y18010	Y26910	Y4703	Y56F9	W1	W2
10×D 550	297	270	144	245	177	350	273
cells/ml ×5×10⁴	1.4×10⁸	1.3×10⁸	7×10⁷	1.2×10⁸	9×10⁷	1.7×10⁸	1.3×10⁸
5×10⁶cell	35	40	80	45	70	30	40

No luge after mixing YACs/window strains + 1ml YPD + cystine (W1) or YPD (W2's)

(W1 was restreak from original 2° glycerol – couldn't get colonies from glycerol made directly from cotton bud as supplied).

10.30 → 4.30 (6 hr.)

3×200µl each transfer -ura -trp + cycloheximide (+ cysteine W1's)

1×200µl YACs/windows/o.

35° 5/12 →

6/12

	Y47HR	W3
10×D 550	136	214
	7×10⁷	1×10⁸
5×10⁶cell	80	50

As above; 9.00 → 3.00

9/12

Y17Q1	W7	~6	medium, white
Y18D10	W2	4	medium; pinkish
Y26G10	W1	~20	various, some pinkish
Y47D3	W2	~6	" "
Y47H9	W3	5 large, ~10	medium (smaller pinkish)
Y56F9	W2	6	medium, pinkish

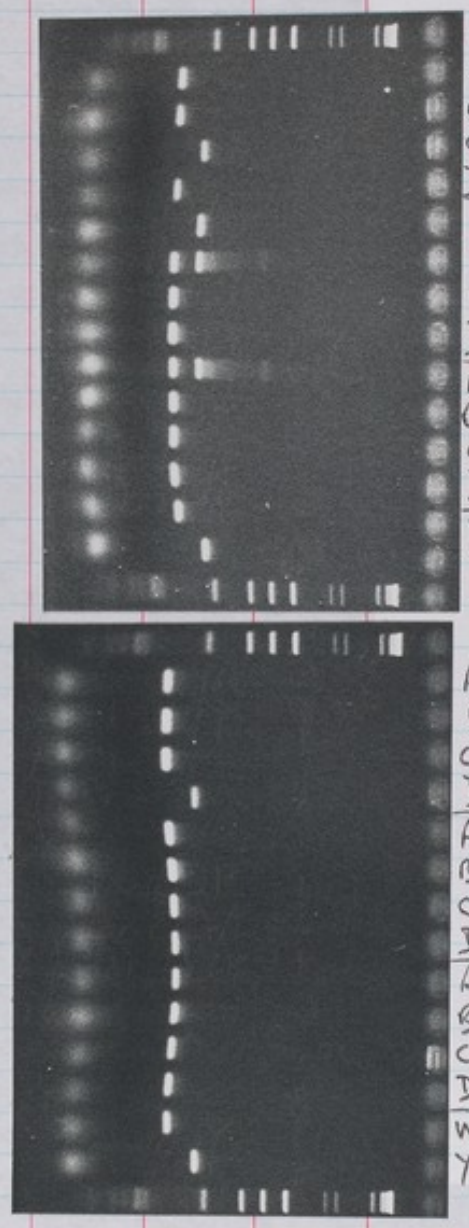
control:

Y17Q7	0
Y18D10	0
Y26G10	0
Y47D3	0
Y47H9	0
Y56F9	0
W1	0
W2	0
W3	0
0	0

Up to 6 colonies/transfer restreaked on -Ura -Trp + cycloHex (+ cysteine).
(4 colonies for Y18D10).
(For PCR assay / miniglycerol).

11/12 4 colonies of each for PCR assay. (A-D)

12/12



Y17G7W1

Y18D10W2

Y26G10W1

Y4703W2

Y47H9W3

Y56F9W2

Window test:

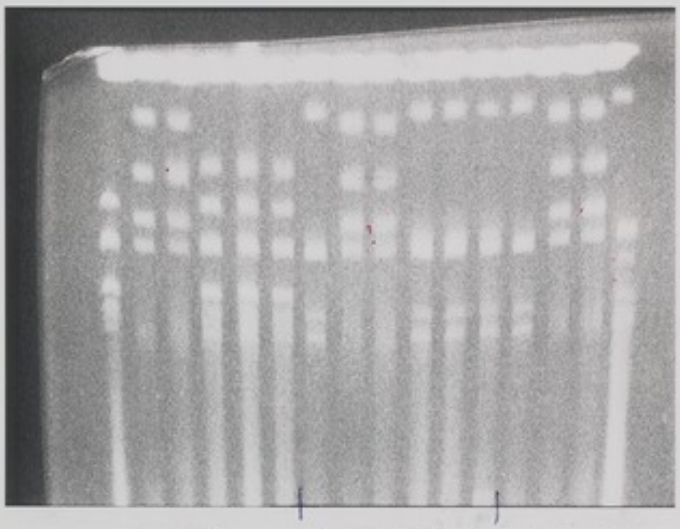
Y17G7W1 A, B, D
Y18D10W2 C, D
Y26G10W1 B, C, D ← Y41C4W2A
Y4703W2 A, B, C
Y47H9W3 A, B, C
Y56F9W2 A, B, C (18)

29 tubes

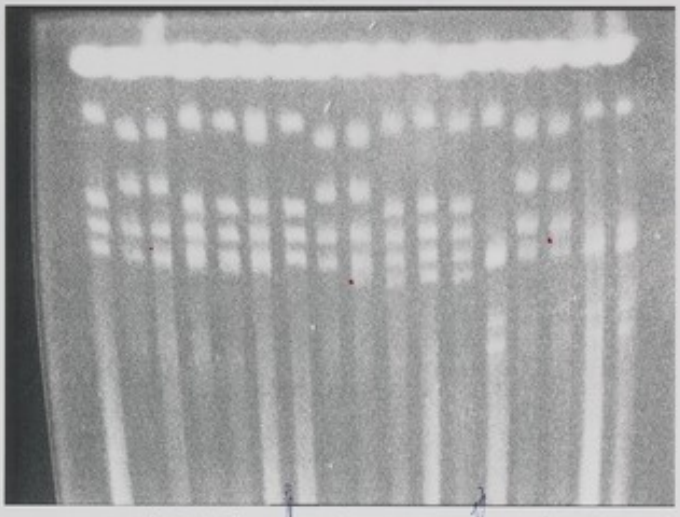
Also grow W1, W2, W3, AB1380 and relevant YACs

Started 13/12 pm

24/12/96



Y47H9 Y56F9 Y41C4



Y17G7 Y26G10 Y18D10

1	YLBW3
2	AB1380
3	Y47H9 (400 kb)
4	" W3A
5	" W3B
6	" W3C
7	YLBW2
8	AB1380
9	Y56F9 (280 kb)
10	" W2A
11	" W2B
12	" W2C
13	YLBW2
14	AB1380
15	Y41C4 (300 kb)
16	" W2A (29/10)
1	YLBW1
2	AB1380
3	Y17G7 (230 kb)
4	" W1A
5	" W1B
6	" W1D
7	YLBW1
8	AB1380
9	Y26G10 (230 kb)
10	" W1B
11	" W1C
12	" W1D
13	YLBW2
14	AB1380
15	Y18D10 (280 kb)
16	" W2C
1	" W2D

Y47D3 omitted - see 14/1/96

8

Conclusions from 24/12 gel.

Y47H9 W3A, B, C all good transfers

Y56F9 May be OK; need trial gel of YAC and W2C expanding 230-280. – below

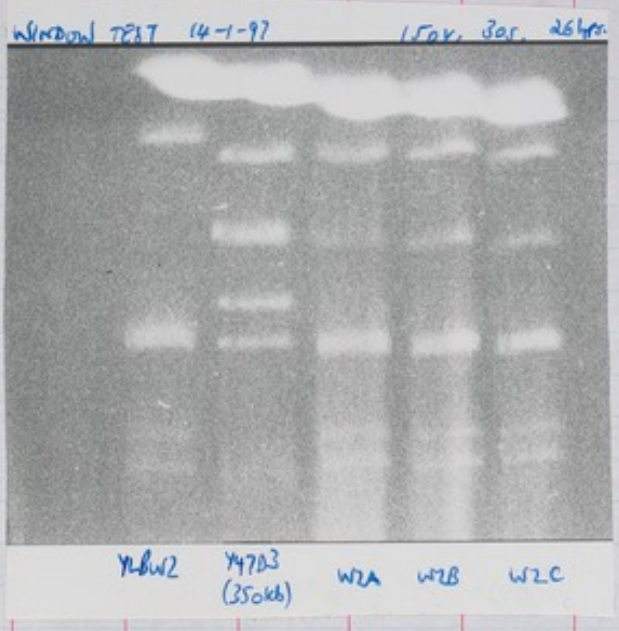
Y41C4 Prob deletions or similar?

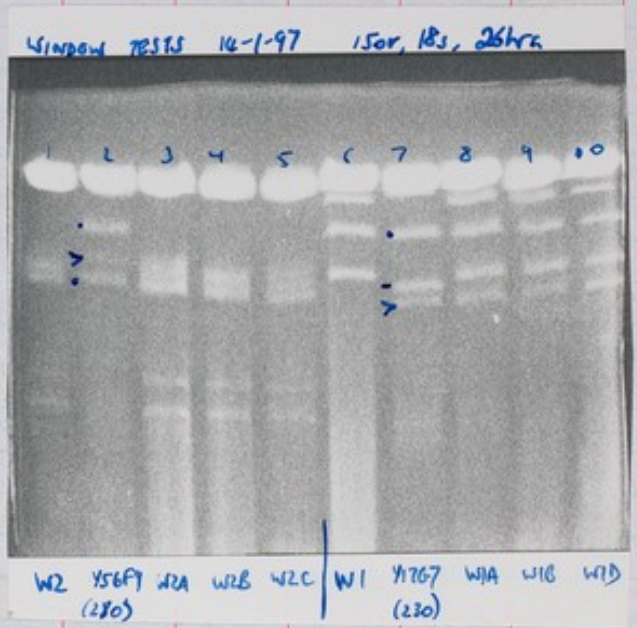
Y17G7 May be OK; need trial gel of YAC and W1A expanding 230-280 (as Y56F9) – below

Y26G10 Transfer poss not necessary, but B, C, D all look good.

Y18D10 Transfers C & D prob. OK. Prep D.

14/1/96





Y47D3 (350kb) W2A, B, C all good

Y56F9 (280kb) running fast; non-transfered YAC good

Y17G7 (230kb) Non-transfer OK, but W1A, B, D better & all OK

10/1/97

Streaked for transfer

1 Y39A1 → W2
2 Y41C4 → W2
3 Y52D3 → W2
4 Y105C5 → W6
5 Y106S6 → W6
6 Y111B2 → W4
7 YLBW2 (single colony only).
8 4
9 6

10/1

YACs → 10 ml -ura -trp

YLBWs → 10 ml YPD 4:00 →

		Y39A1	Y41C4	Y52D3	Y105C5	Y106S6	Y111B2	YLBW2	4	6
10x0	559	261	149	207	249	247	229	241	255	153
cells/ml x10^4		1.3x10^8	7.5x10^7	1x10^8	1.2x10^8	1.2x10^8	1.1x10^8	1.2x10^8	1.3x10^8	7.5x10^7
5x10^6 cells		40	80	50	40	40	45	40	40	80

15/1

19/1

1	Y39A1/W2	14, 11, 9
2	Y41C4/W2	1, 0, 0
3	Y5203/W2	9, 2, 7
4	Y105C5/W6	7, 7 (1 mouldy).
5	Y1069G/W6	8, 3 (")
6	Y111B2/W4	25, 20 (")
7	Y39A1	0
8	Y41C4	0
9	Y5203	0
10	Y105C5	0
11	Y1069G	0
12	Y111B2	0
13	W2	0
14	W4	0
15	W6	0
16	0	0

10.45 – 4.45

3 x 200 µl 1-6, 1 x 200 µl 7-16 plated.
(Plates ~1 month old, some cracked).

20/1

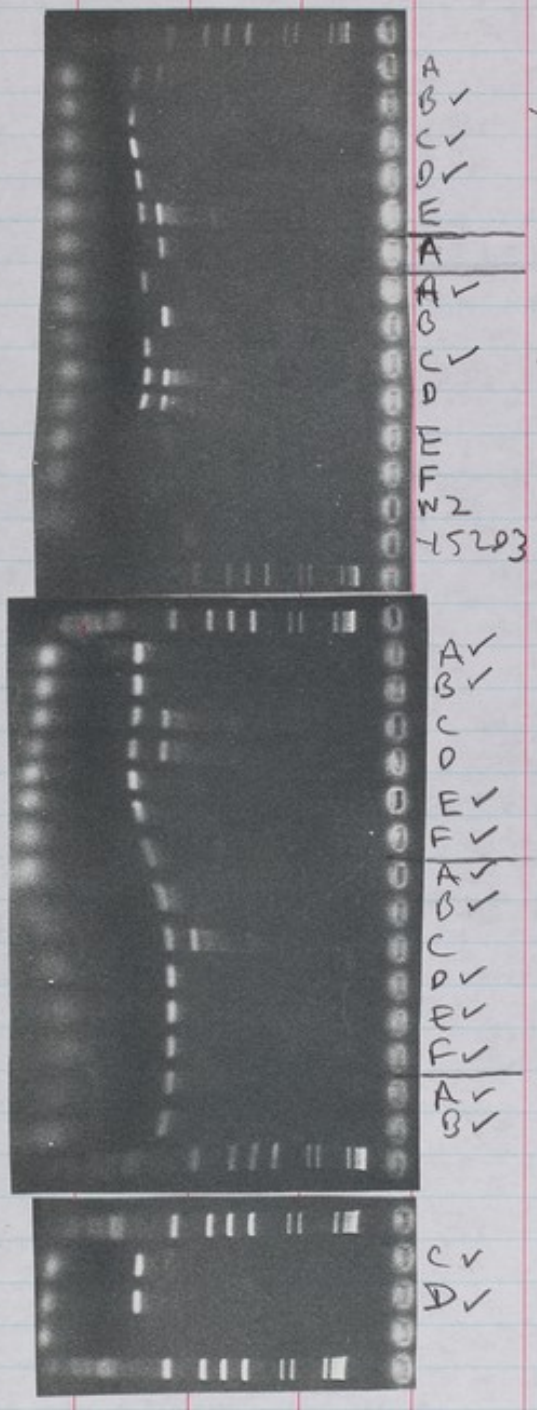
Miniglycerol:

Y39A1 W2	A-E
Y41C4 W2	A
Y5203 W2	A-F
Y105C5 W6	A-F
Y1069G W6	A-F
Y111B2 W4	A-D

26/1

PCR assays of above (1-28)

+ W2 (29)
Y5203 (30)
0 (31).



Y39A1W2

Y41C4W2 failed again (3rd)

Y52D3W2

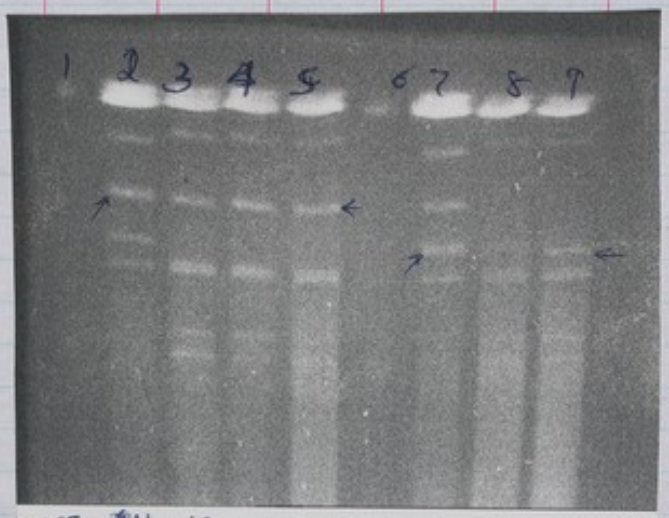
Y105C5W6

Y106G6W6

Y111B2W4

TO Bijay for PFG assay: Y39A1W2 B, C, D
Y52D3W2 A, C
Y105C6W6 A, B, E
Y106G6W6 A, B, D
Y111B2W4 A, B, C

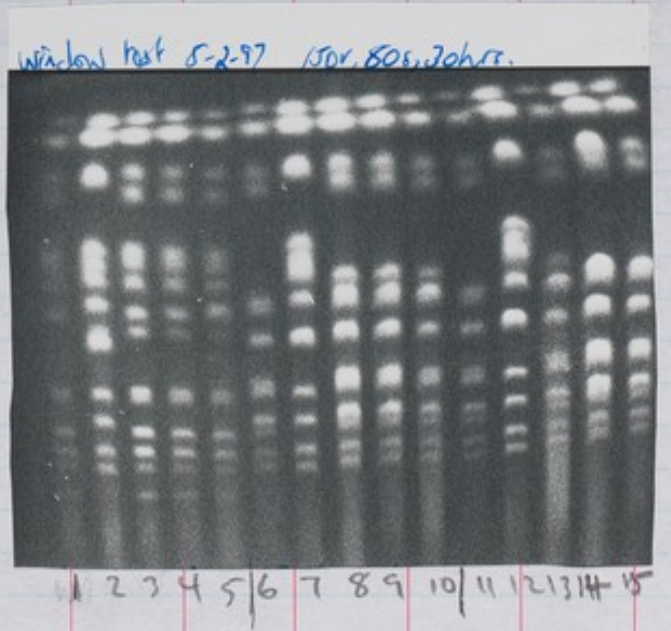
3/2/97



W2 [illegible] W2B W2C W2D W2 S2 D3 W2A W2C

Y39A1 | Y5203

W2 marker failed, but A transfers good: Y39A1W2C, Y5203W2C



1	YLBW4	
2	Y111B2	
3	"	W4A
4	"	W4B
5	"	W4C
6	YLBW6	
7	Y105C5	
8	"	W6A
9	"	W6B
10	"	W6E
11	YLBW6	
12	Y106G6	
13	"	W6A
14	"	W6B
15	"	W6D

All good; to prep: Y111B2W4A
Y105C5W6A
Y106G6W6A

17/2/97

Platings for window transfers:

Y41C4 (W2)
Y45F3 (W5)
Y63D3 (W1)
Y10293 (W5)
MTT4B12 (W6) (plate from Alton colony)

W2
W5
W6

(W1 plated earlier on YPD/tcys)

19/2

10 ml O/N's

Y41C4
Y45F3
Y63D3
Y10293
MTT4B12
} -ura

W1 - YPD + W1
W2
W5
W6
} YPD

	Y41C4	Y45F3	Y63D3	Y10293	MTT4B12	W1	W2	W5	W6
10+0 5gr	240	245	255	260	261	244	230	200	260
~ 5x10^6 cell	40	40	40	40	40	40	40	50	40

21/2

1	Y41C4	/W2	
2	Y45F3	/W2	
3	Y6303	/W1	3 colonies streaked from +cys plate
4	Y10293	/W5	6 colonies streaked
5	M774B12	/W6	
6	Y41C4		
7	Y45F3		
8	Y6303		
9	Y10293		
10	M774B12		
11	W1		
12	W2		
13	W5		
14	W6		
15	0		

22/2

9.15 → 3.15 6hr.

3 × 200 µl plated -trp-ura+cyclohex.

except 3 and 8 3: 2 × 200 µl -trp-ura+cyclo +cys.

2 × 200 µl no cys.

8: 1 × 200 µl +cysteine

1 × 200 µl no cys

23/2

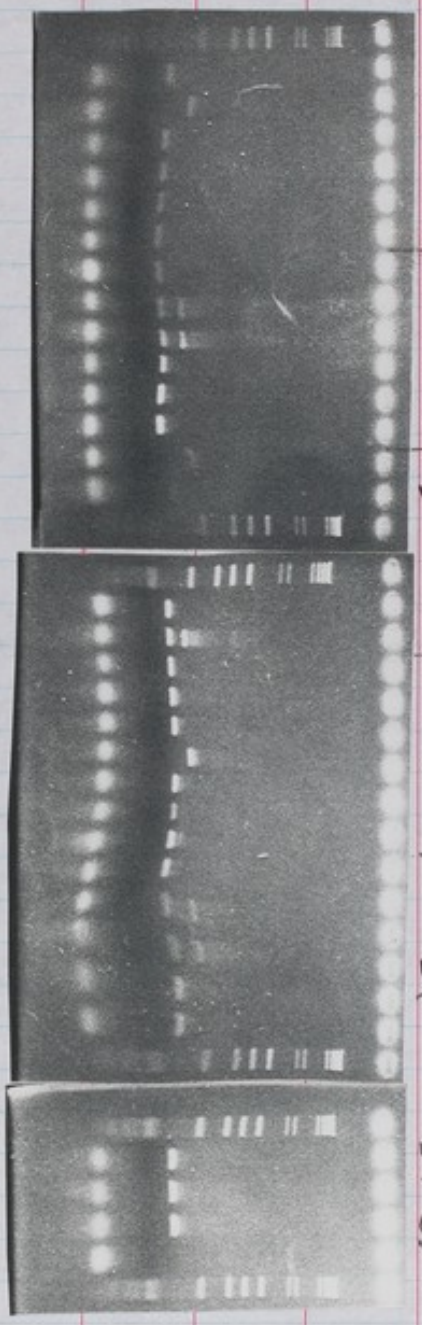
Bad contamination: big blobby colonies on most plates.

24/2 Colonies restreaked:

			PCR
Y45F3	1-6		1-6
Y6303	1-2	then 25/2 1-6	7-14
Y10293	1-6		15-20
M774B12	1-3	then 25/2 1-6	21-29

Y5491 needs W2.

3/3/97
PCR assays



Y45F3 W2

Y63D3 W1

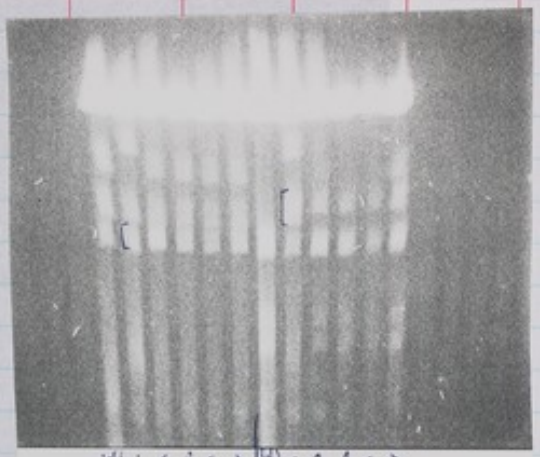
Y10293 W5

M774B12 W6

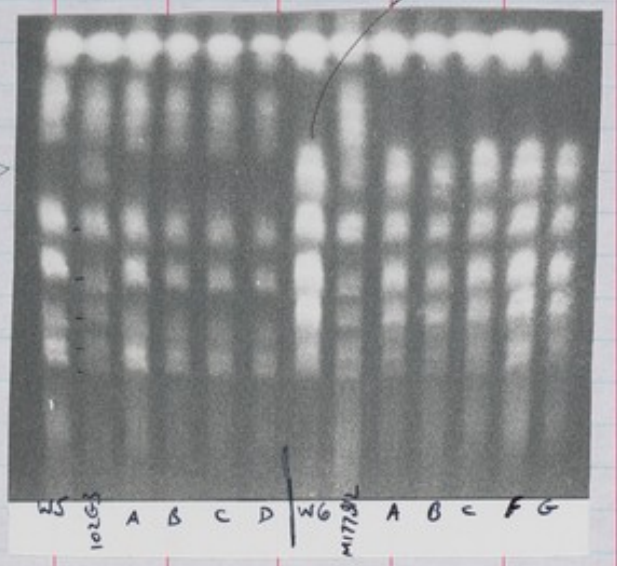
To Bijay for gel assay 4/3.

W 160
Y 226

8/4/97



Ywc3w reformed again, but prob worth trying any of these.
Y45F3 A-C OK (D not OK).



?>

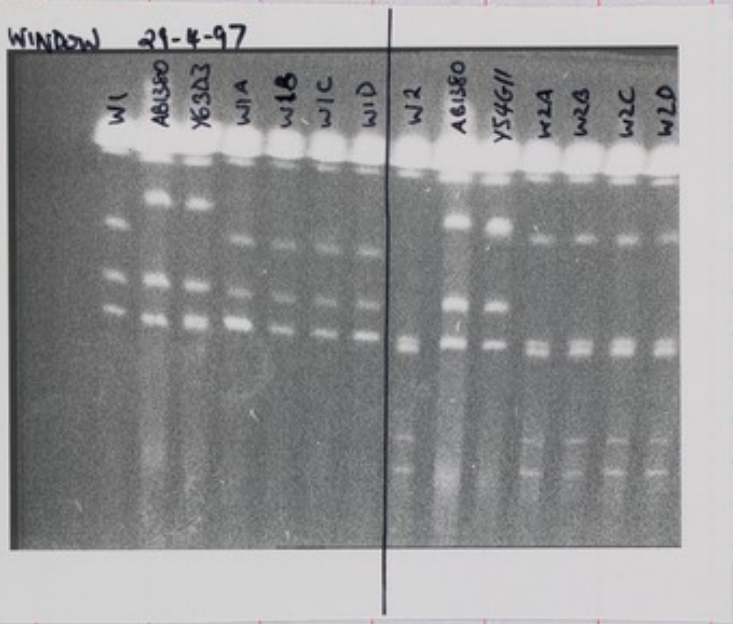
<?

Y10293's look to have failed.

M177B12's could do with repeat gel, inc. AB1380. Could be OK.

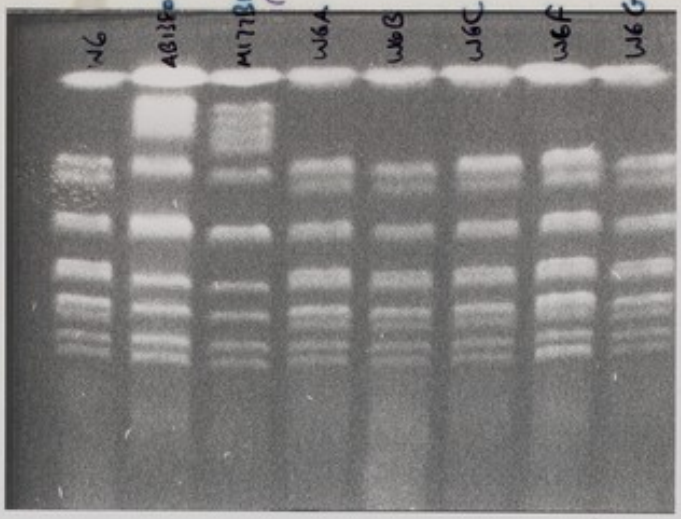
670

29/4/97
Repeat window assay



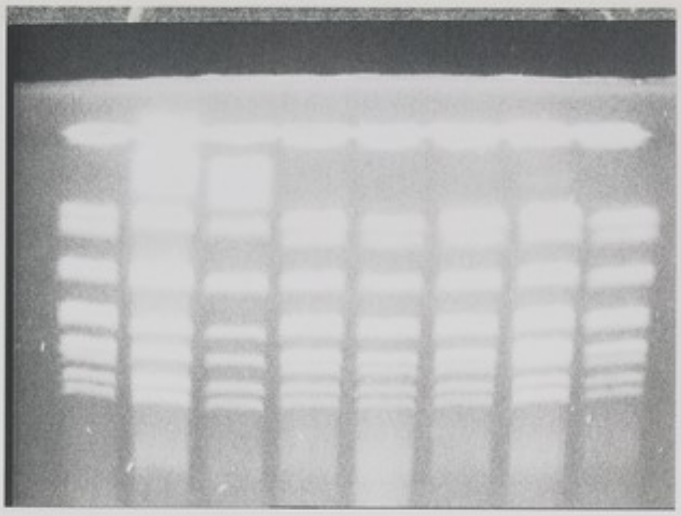
Y6303 : window worse than wildtype.
original may be separable. Prep

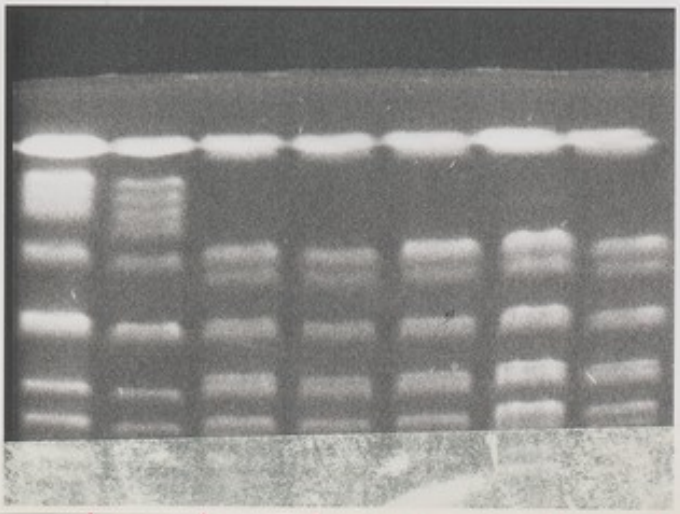
Y54G11 : Good ; prep W2A



WINDOW TEST 29-4-97 150v, 70s, 28hrs.

<? Poss band in W6F?





<?

3/4/97

Plating for window transfers.

Y54911
4L BaO2

6/4

10 ml o/n's

7/4

	Y54911	W2
10+0 sig	226	160
~5x10^6 cell	40 µl	60

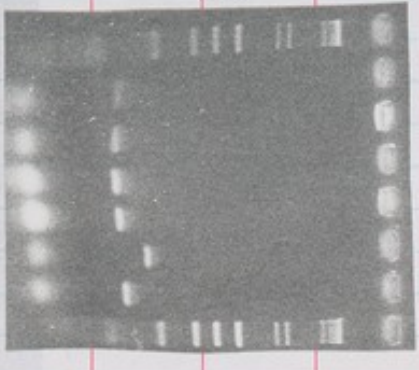
1	Y54911	W2	20 + colonies
2	Y54911		0
3	W2		0
4	0		0

9.45 → 3.45 30°

3x200µl Y54911W2 -wa-trp+cyclohex.
1x200µl control

6 colonies restreaked

Only 4 grew. PCR assays.



1 A
2 B Y54911W2
3 C
4 D
Y
W

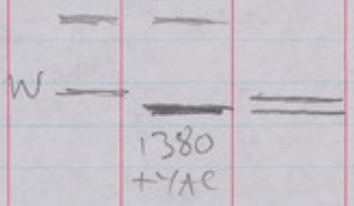
PFG assay previous page

12/5/97

Window transfers

Y48G10
+cyc. W1/4

Y102AT5
W3



Y48G10	Y102AT	W1/4	W3
215	176	227	216
50µl	60µl	45µl	40µl

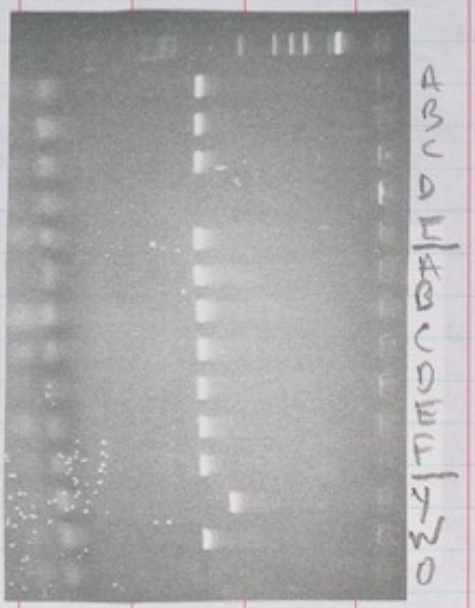
30° 9.30 → 3.30

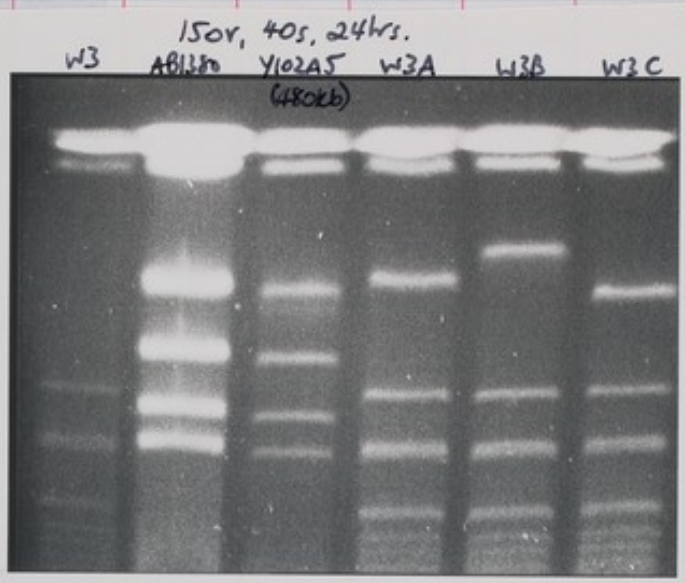
19/5/97

PCR assays of above

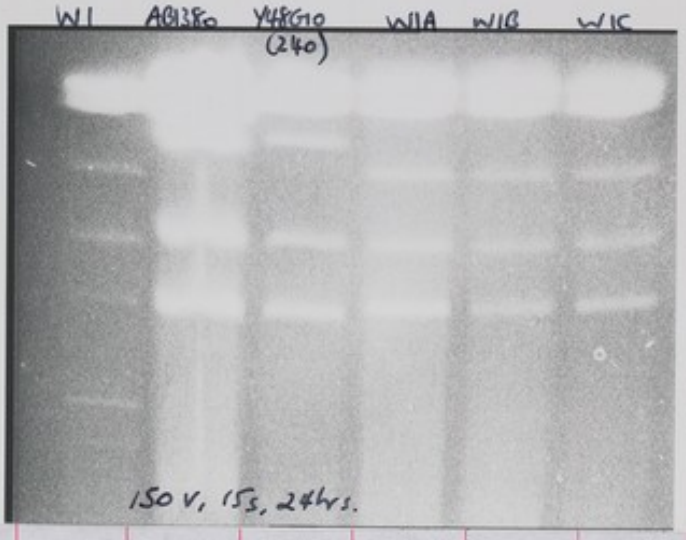
1-5 Y48G10W1 A-E
6-11 Y102AT W3 A-F
Y
W
O

All OK except Y48G10W1D.





4-6-97

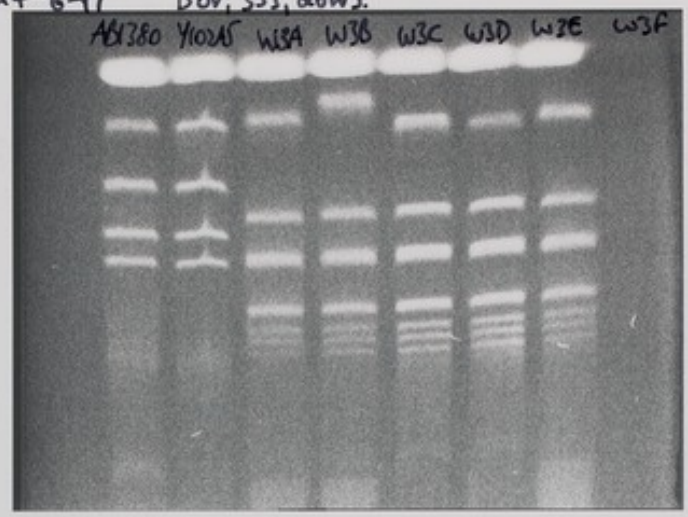


Y102A5 : variety of sizes may be due to deletions of ~~15kb~~ tandem repeats (~100 x 1kb). Prep W3A-F for assay of ~~extent~~ of tandem repeat by ~~Sma I~~ digest.

OVER

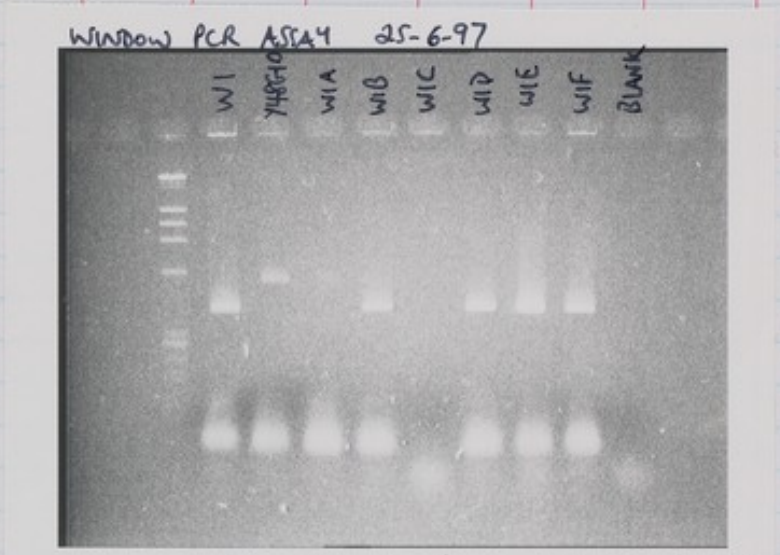
Y48G10 : Looks like failure - reformation of 230kb W1 chromosome and not obvious where YAC is.

24-6-97 150V, 35s, 26hrs.

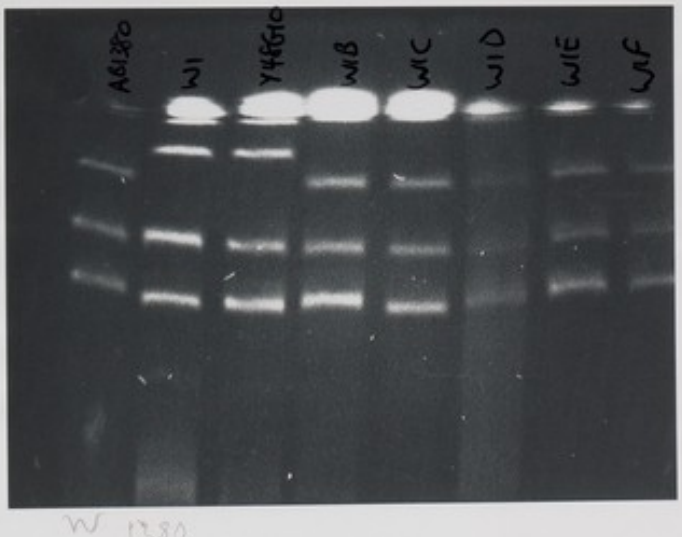


25/6.

Window transfer Y48G10 → W1 (original) by Bijay



B, D, E, F for analytical PFG



W 1370

27/7/97

Y41C4 → W2 (5th attempt?)
Y75B8 → W2

o/n 30°

	Y41C4	Y75B8	W2
10^4	160	157	300
5x10^6	75	75	31

1	Y41C4	a	75	Y41C4	31	W2
2	"	b	200	"	"	
3	"	c	500	"	"	} spin before addn of YPD
4	"	d	500		100	"
5	Y75B8		75	Y75B8	31	W2
6	Y41C4		75			
7	Y75B8		75			
8	W2		31			
9	0					

30° 10:30 → 4:30

3x200 µl 1-5 plated -ura-MPT cyclohex
1x200 µl 6-9 " " "
(Mon-Fri 30°)

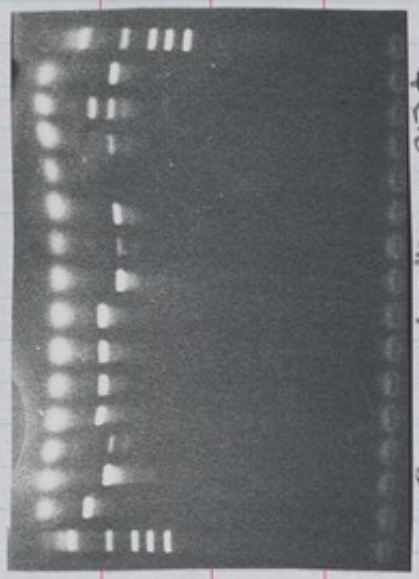
1/8 restreaking Y41C4, reincubated
Y41C4W2 a 1 colony Fri →
b 3 colonies
c bad contamin; no colonies picked
d 2 colonies

Y75B8W2 6 colonies

4/8/97

PCR assay:

1-6	Y41C4W2 A-F
7-12	47588W2 A-F
13	Y41C4
14	W2
15	0



Y41C4 failed again

47588 B-E for gel assay.

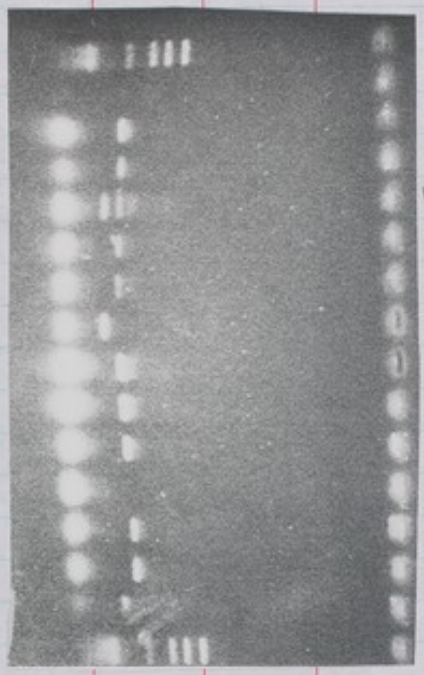
5/8

12 small colonies from Y41C4W2 re-incubation streaked for PCR assay.

6/8

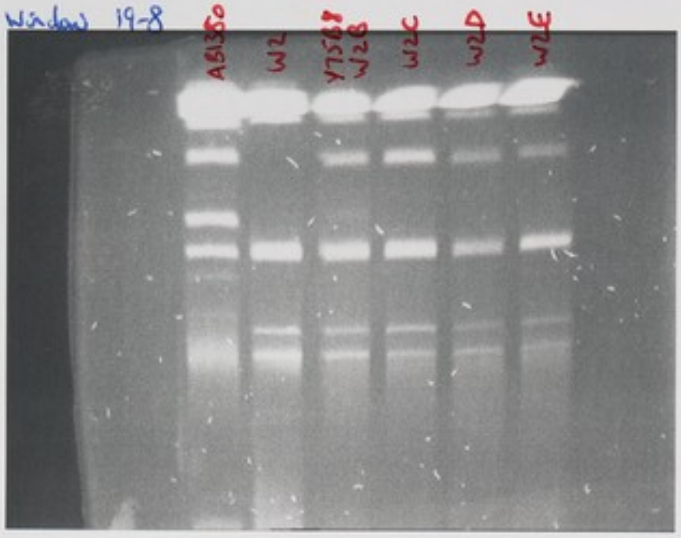
Y41C4W2 7-18 PCR assays.

OVER



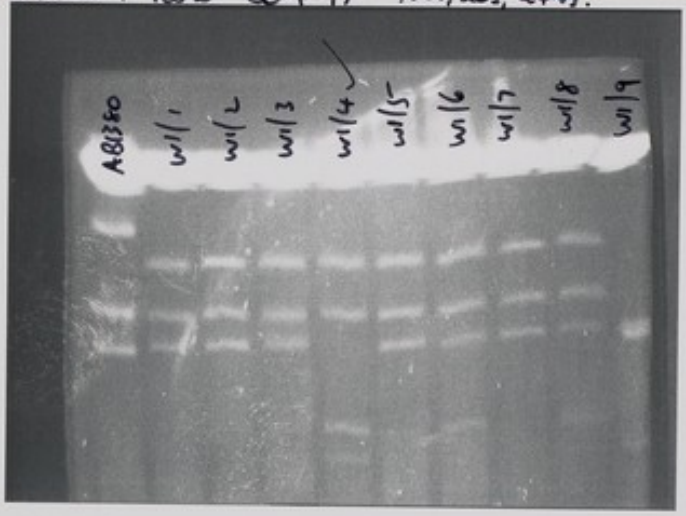
Y41C4W2

#13 possible: V. slow grower.
Make miniglycerol. — Y41C4W2 4/8
To prep for PFG assay.
(Disposed of Y41C4 A-F miniglycerols)



Alan's copy

WINDOW 1 TESTS 30-4-97 15or, 20s, 24hrs.



Also W10 reformed

^ ^ ^ Lweird
? ?

15/9/97

Window transfer

O/N growths from colonies:

Y66A7 (→ W1)
Y8003 (→ W3)
W1
W3

-ura -trp

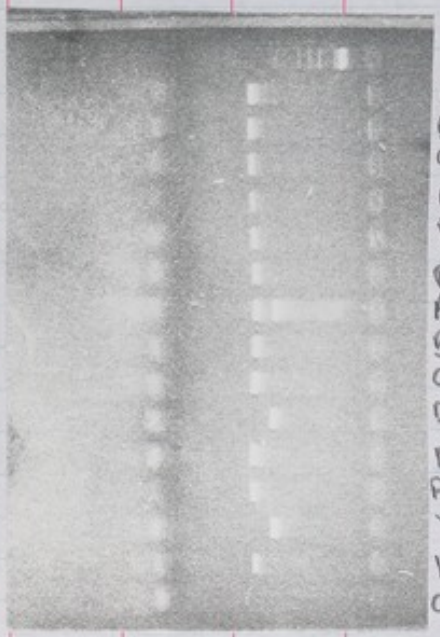
YPD + cyh
YPD

	Y66A7	Y8003	W1	W3
10x1)	136	195	234	260
5x10⁶	90	60	45	40

30° 9.00 → 3.30 (6.5 hr)

17/9 3x200 µl plated -trp -ura + cyclohex

Plenty colonies of each transfer Controls Q.
6 of each streaked for PCR and glycerols.



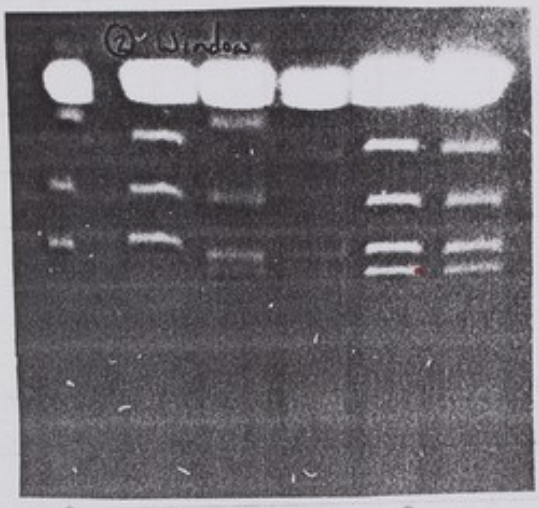
Y66A7W1

Y8003 W3

Y66A7 A-C
Y8003 B, C, E for PFG

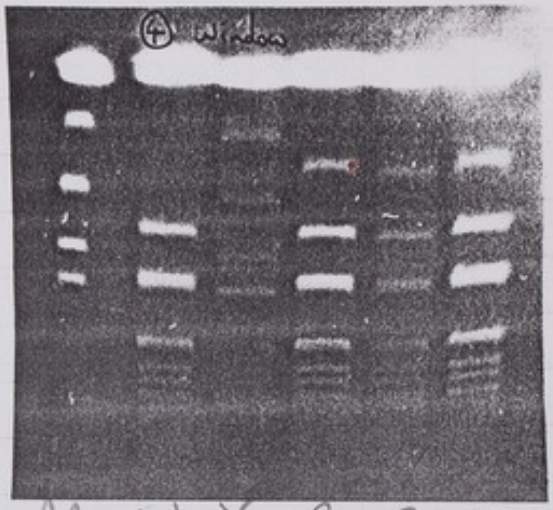
16/10 rjd gel

Y66A7



AB W Y A B C
Y66A7

Y80D3



AM W Y B C E
Y80D3

Y66A7W1B To prep
Y80D3W3B To prep

3/10/97

Y113B8 / W2

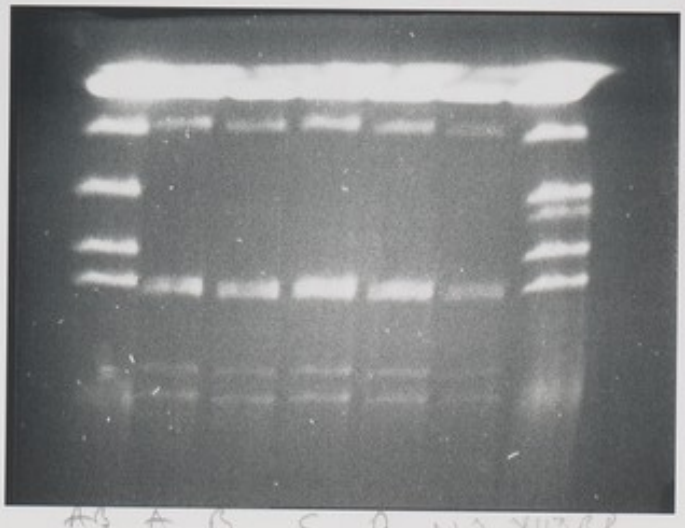
24 growths from colony
o/n 4°

	Y113B8	W2
10⁷	167	229
5×10^6	60	45

9.15 — 3.15

New -trp -ura + cyclo plates turned out to be gooey after spotting 20µl of transfer. Remaining 300µl spread on 2 old plates.
But expt may need repeating.

→ 6 colonies (all) restreaked (controls = 0)



A5 A B C D W2 Y113B8
Y11768

Window not necessary!

29/10

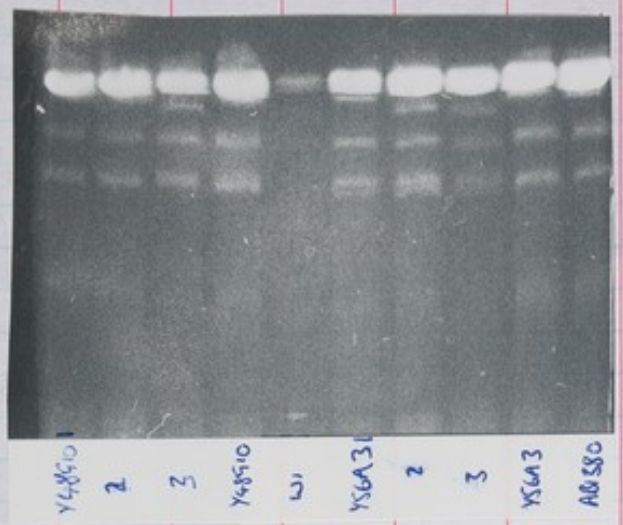
Y48910
Y18A3 } W1

	Y48910	Y18A3	W1
10x10	276	204	239
	315	510	475

30° 9.15 → 3.15

3x200µl plated.

→ PFG assay 10/11. (No PCR assay)



Y56A3W1A to prep for seq (19/11)

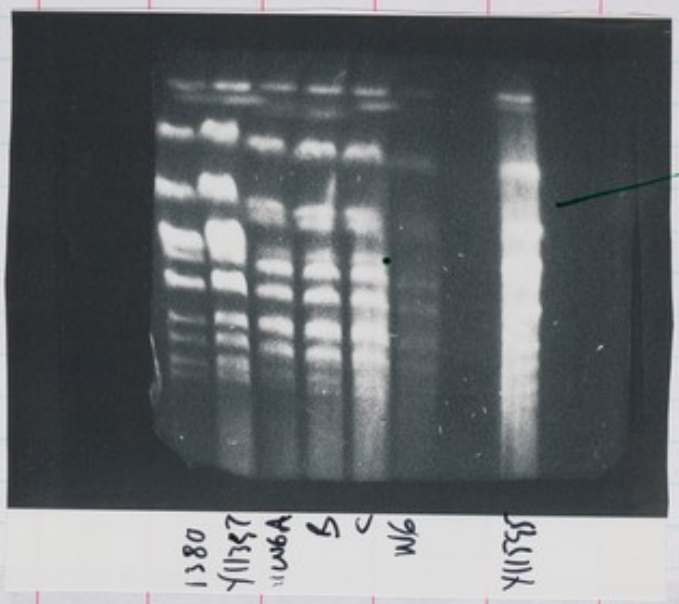
16/11

Y11347 / w6

Y11347 = 800kb
(See 15/10 Southern)

	Y11347	w6
10xD	124	152
	9µl	60µl
	30	10 - 4

~40 colonies from 3 x 200µl
3 for PFG assay.



↖ This for southern sizing.
Prep'd from plate scrape.

Prep Y11347 w6A

24/11

Y51H4 → W2
Y60A9 → W1
Y79H2 → W2
Y87Q2 → W2

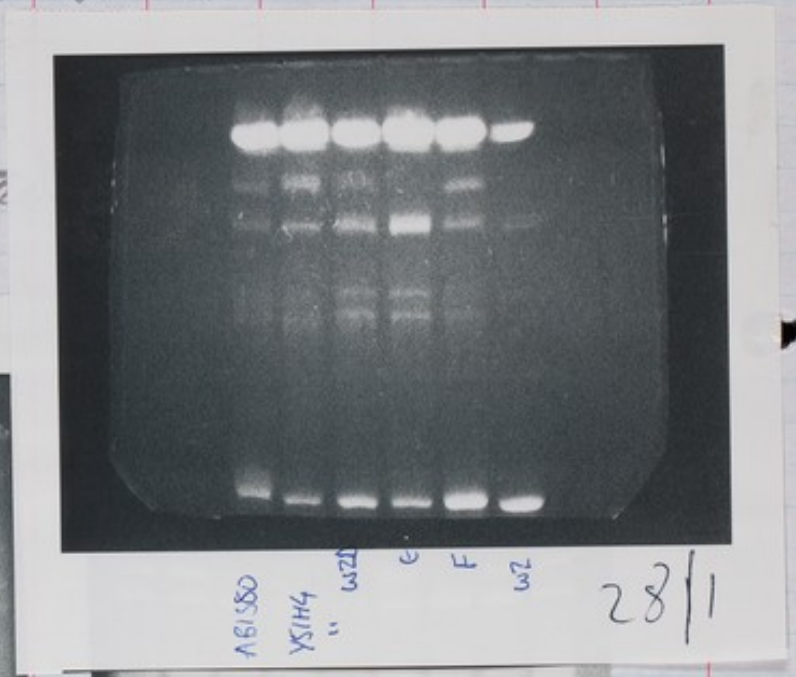
(Y79H2 - 2 x O/N)

	Y51H4	Y60A9	Y79H2	Y87Q2	W1	W2
10xD	194	266	179	232	148	327
5x10⁶	50	40	60	41	80	30

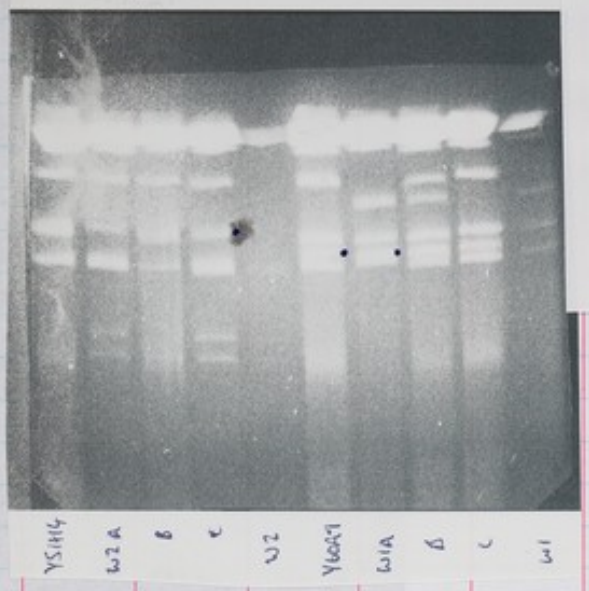
35° 10 → 4.00

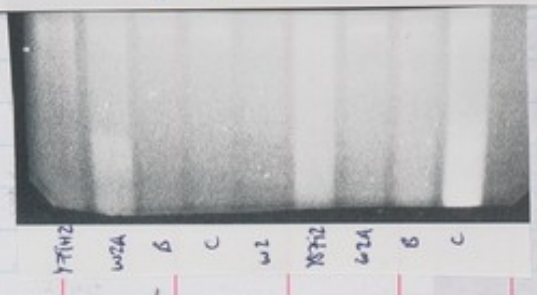
3x200µl plate

17/12 Gel assays:



28/1



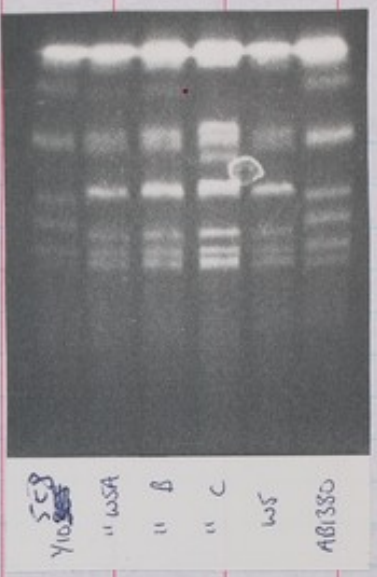


Y51H4 : needs W1 transfer
Y60A9 : untransformed strain OK for seq prep
Y79H2 : W2 C for seq prep
Y87Q2 : needs W1 transfer

21/12

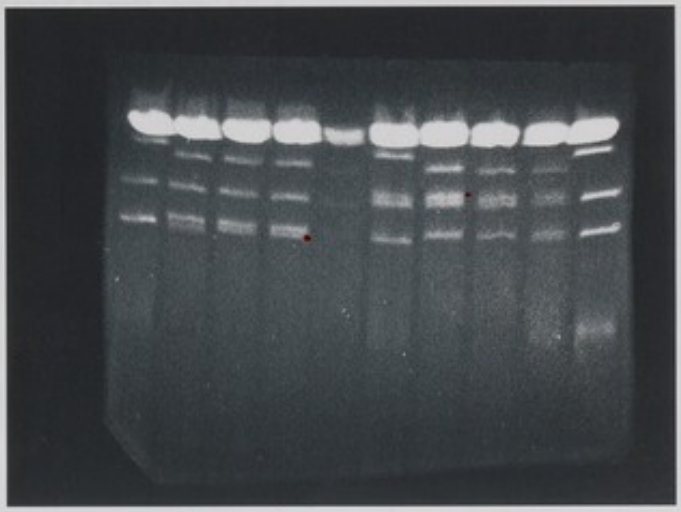
Transfer 1.

Y5 1H4 → W1
Y8752 → W1
Y10E8 → W5

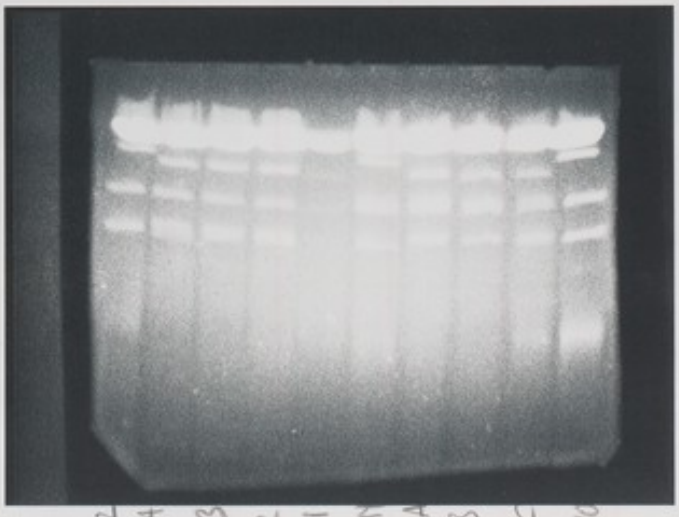


	W1	W5	Y5 1H4	Y8752	Y10E8
10×D	196	214	130	141	118
	50	50	80	80	100

9/1/98



Y8752W1 Y5 1H4 W1



Y8752W1B
⊕ prep.

Repeat gel assay of Y5 1H4 W2 D-F!

Y10E8W5B
⊕ prep.

12/1/98

Y116F11 → W6

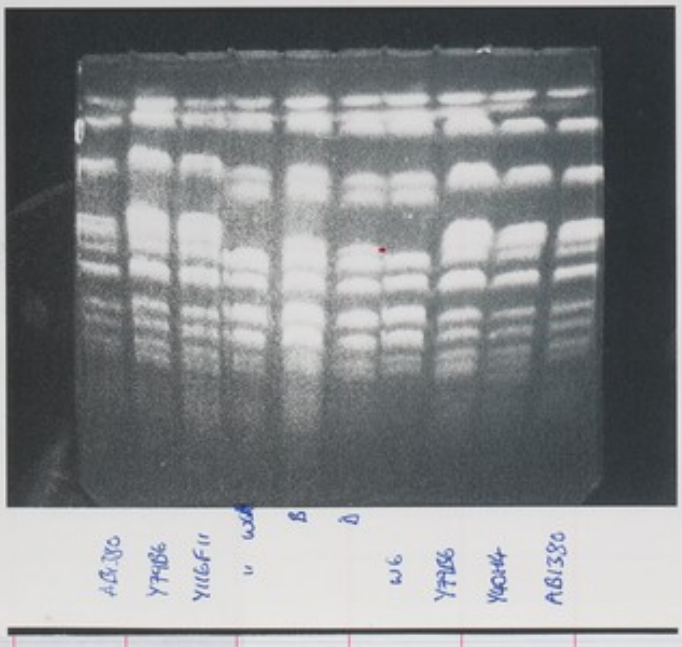
	Y116F11	W6
10×D	137	162
	80	60

10 - 4.00

1:1
Lots of colonies on W6 control!
Few on Y116F11/W6.

A, C, D for gel assay.

(replated 12 colonies on -ura -trp cycloser — no growth).



Y116F11W6D
to prep.

20/1/98.

Y109H1 → W6, W7

	Y109H1	W6	W7
10x0	204	193	198
vol	15µl	50	50

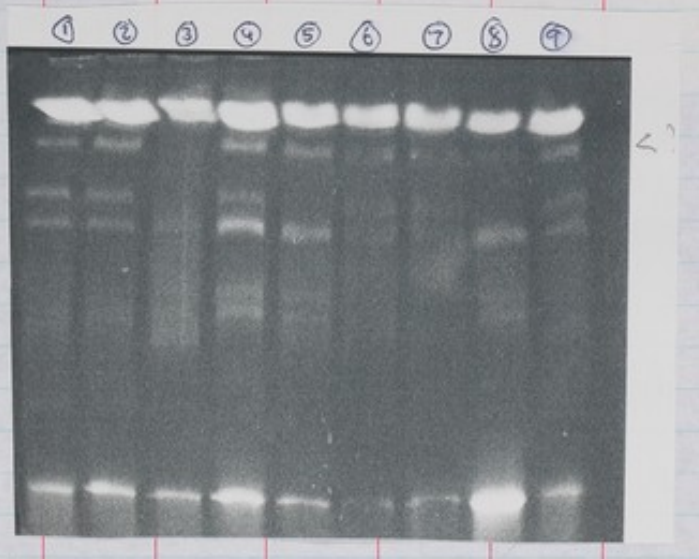
8.45 →

Abandoned.

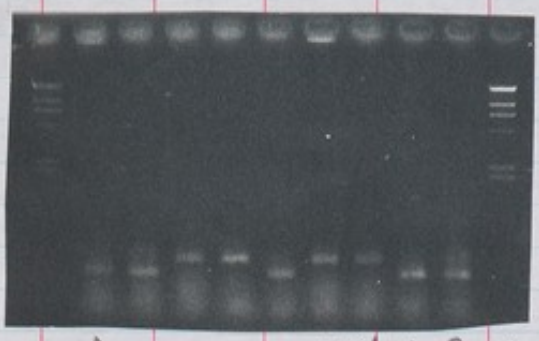
Probing results show not required

9/98

Y4304 → W2



1 Y503 (100)
2 Y4304
3 W2
4 Y4304W2 A
5 -
6 - C
7 - D
8 - E
9 - F



A FYW B repeat

sl. doubtful about streak above band in B (and others).

W2B to prep. 10/9/98

25/8/98

Window transfer Y143F9 → W2

O/N single colony inoculation

/10 W2 241 45ul } + 1 ml YPD
Y143F9 149 80ul } 10 - 4.00 30°

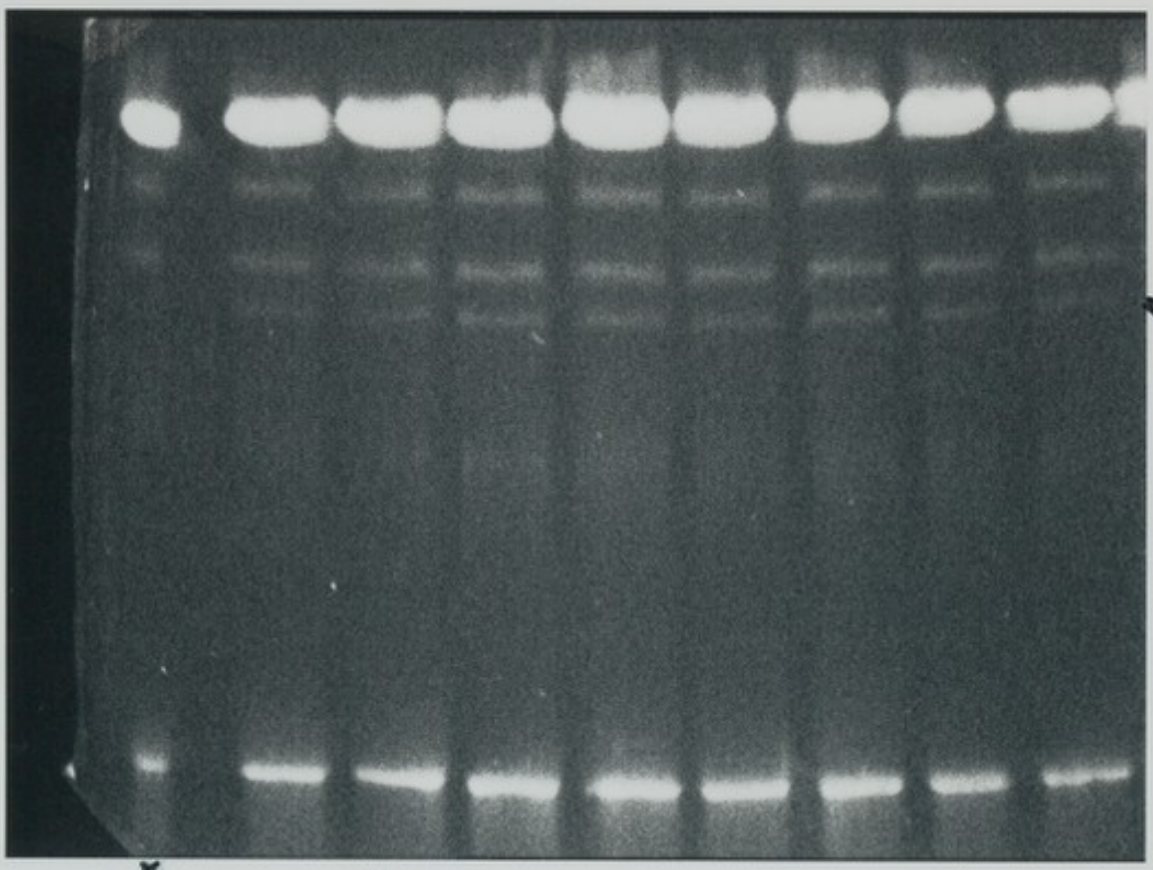
Y143P4W2B.

46.2 % good quality 133

38.9 % yeast

288 reads 112

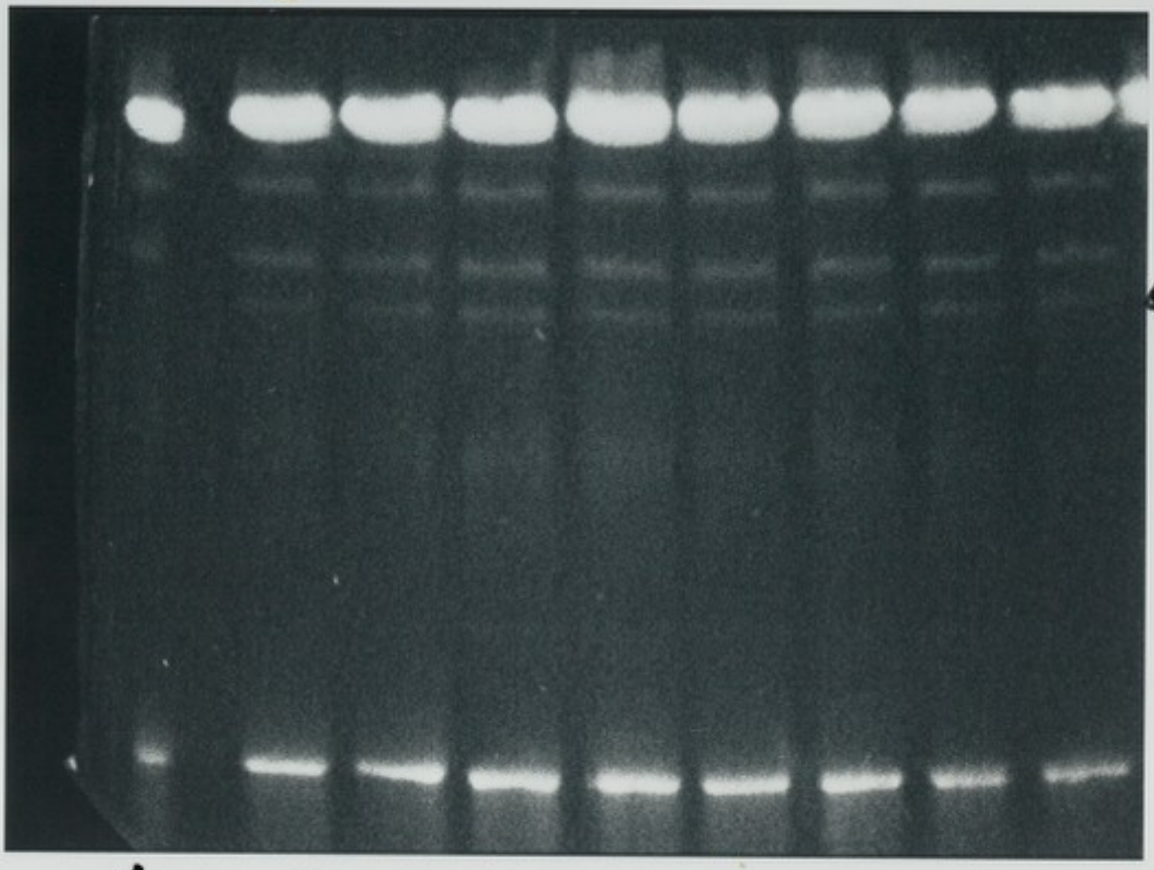
45 passed. 88 repeated prep



4 YAC

4
ABI 377

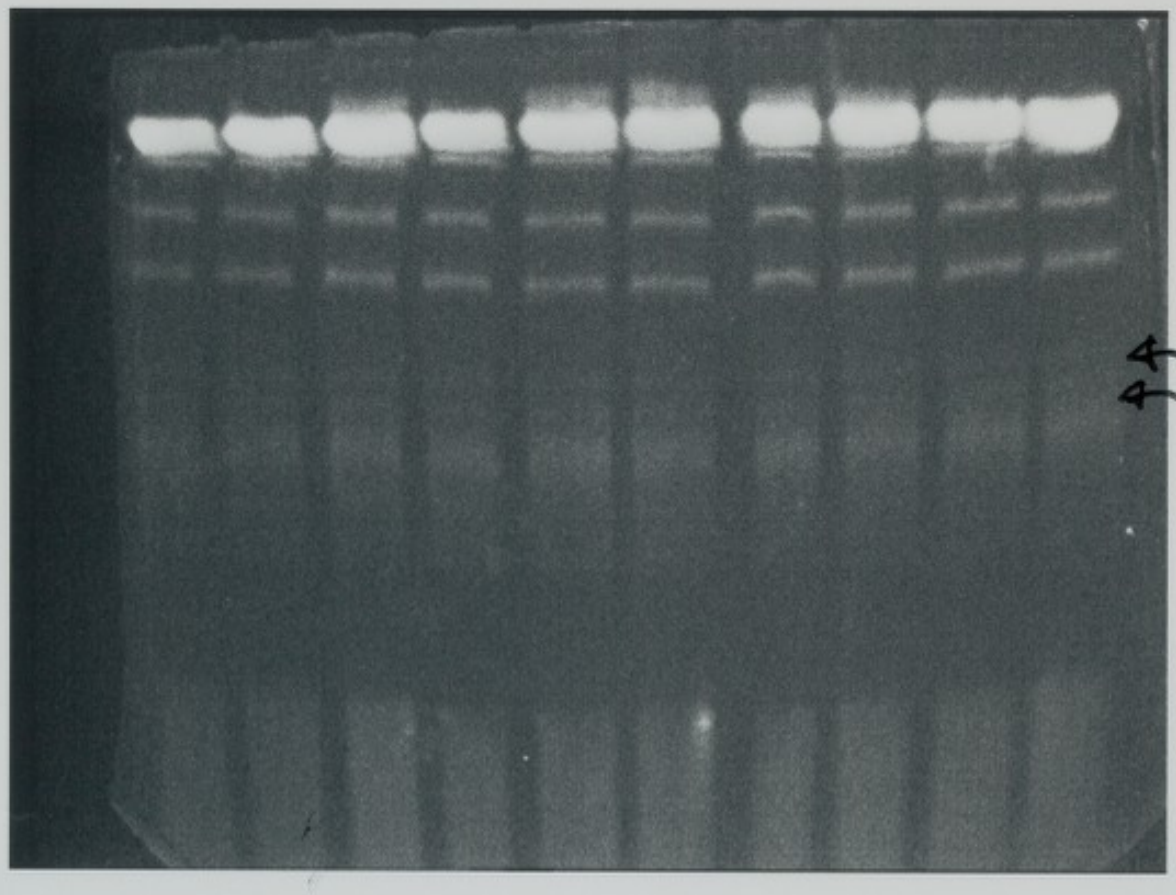
R7CC1



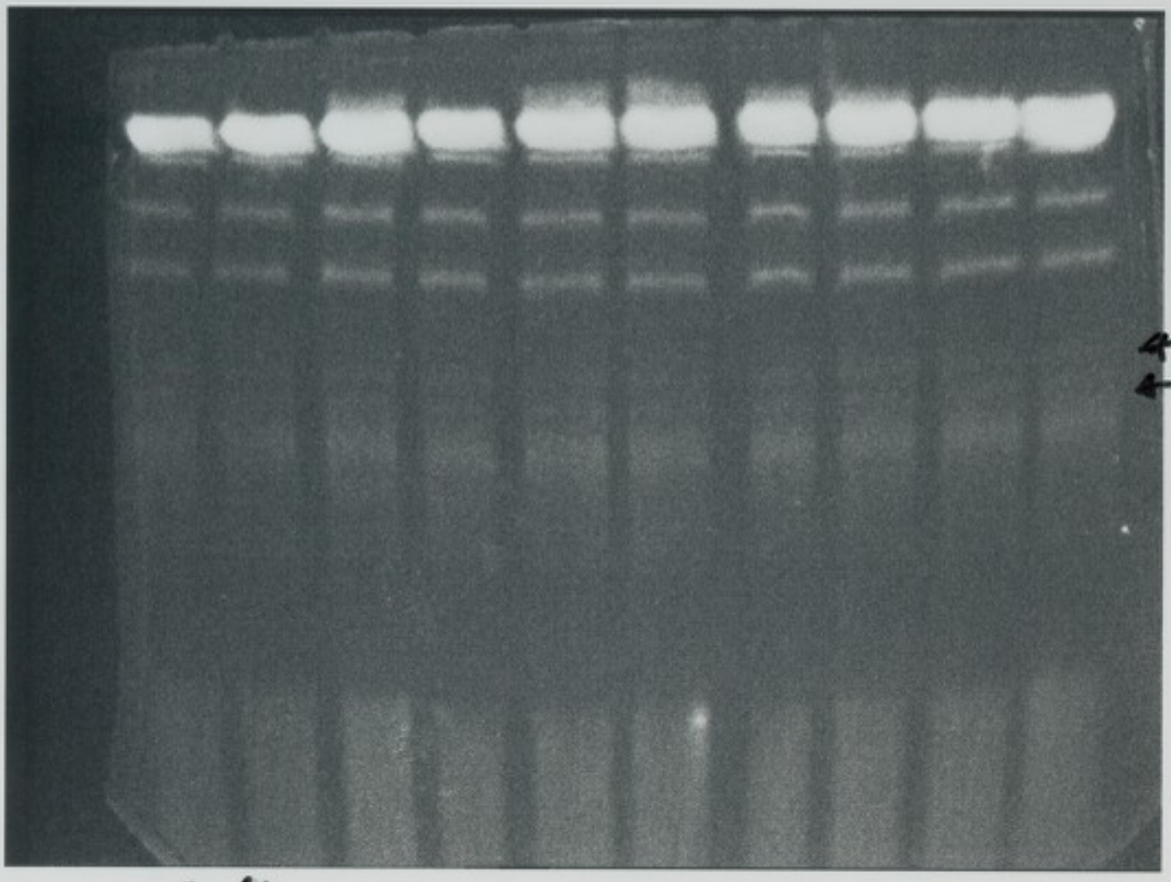
4 YAC

4
ABI 377

R7CC1



M736A3



M736A3

X-Authentication-Warning: fes11.sanger.ac.uk: mq1 owned process doing -bs
Date: Tue, 20 Apr 1999 12:03:07 +0100 (BST)
From: Michael Quail <mq1@sanger.ac.uk>
To: Alan Coulson <alan@sanger.ac.uk>
Subject: window strains
Content-Type: TEXT/PLAIN; charset=US-ASCII

Dear Alan,

The list of clones I know of that require window transfer are:

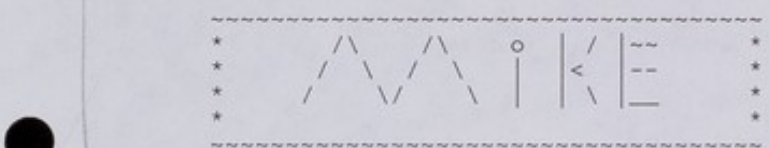
D.discoideum
DY3567 (800kb)
DY3689 (260kb)
DY3180 (225kb)

malaria ch.5
MY227
MY415 (250kb)

Dave may well know of one or two more by now, and he will have the gel pictures for the above, so you should chat to him.

Best of luck

Mike



From: Dr Michael A. Quail
Room D3-29
The Sanger Centre
Wellcome Trust Genome Campus
Hinxton
Cambridgeshire CB10 1SA
UK

Tel: (01223) 494837 office
(01223) 494892 lab
Fax: (01223) 494919

email: mq1@sanger.ac.uk

24/5

Malaria and dicty without transfers.

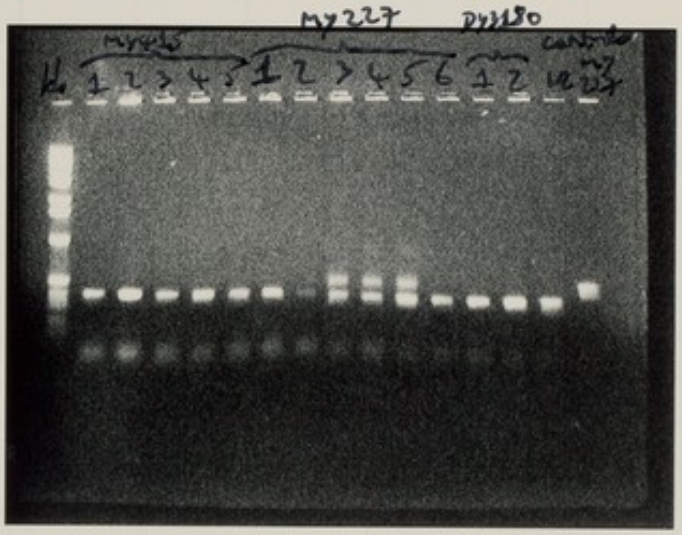
		10x0 cell count	µl
O/N cultures	W1	262	40
	W2	325	30
	W6	299	31
	MY108	364	21
	MY227	302	31
	MY415	280	33.5
	DY3567	314	31
	DY3689	283	31
	DY3180	195	15

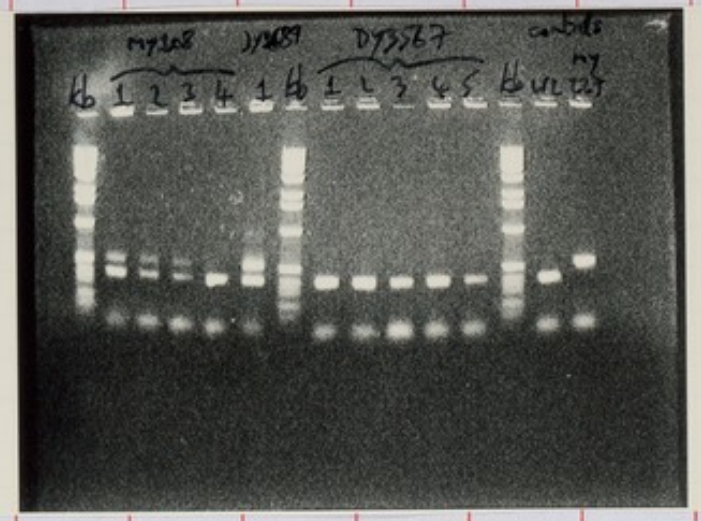
colonies picked to -ura 31/5 - streaked

1	MY108 → W2	4	
2	MY227 → W1	6	
3	MY415 → W1	5	
4	DY3567 → W6	5	for PCR assay.
5	DY3689 → W2	1	
6	DY3180 → W1	2	- passed to M.Q. for PCR and miniprep sequencing.
7	W1		
8	W2		
9	W6		
10	MY108		NB These had colonies, but different batch of plates.
11	MY227		
12	MY415		
13	DY3567		
14	DY3689		
15	DY3180		
16	0		

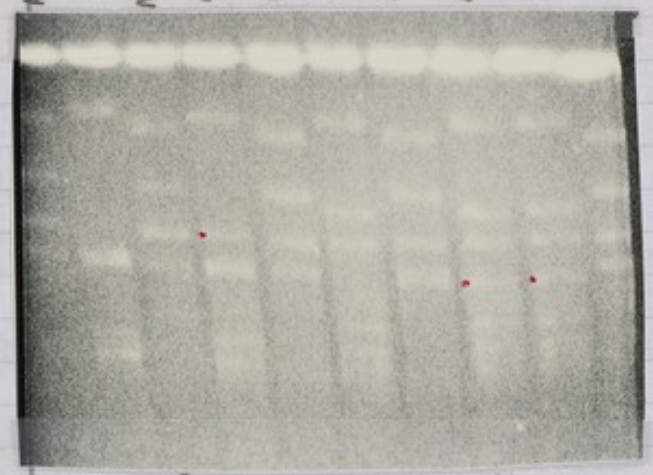
Vols as above.
6W 30°

1x200µl each plated on cycloheximide.


My227
Dy150
Kb 1 2 3 4 5 1 2 3 4 5 6 1 2 Kb My 227

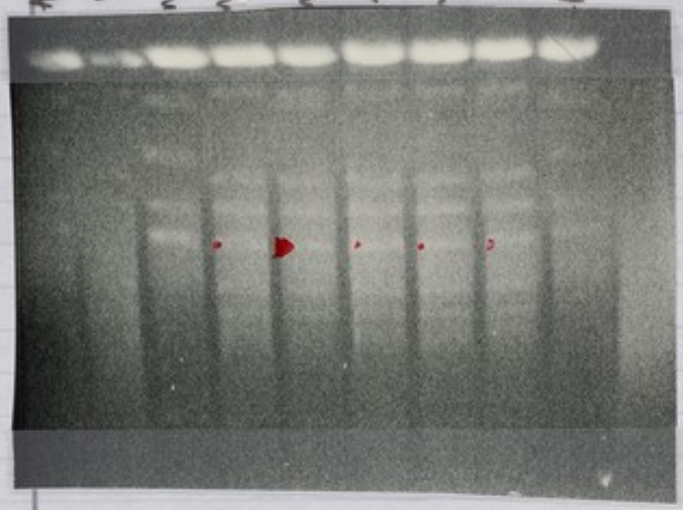

My208
Dy389
Dy387
Kb 1 2 3 4 1 Kb 1 2 3 4 5 Kb My 227

8/7

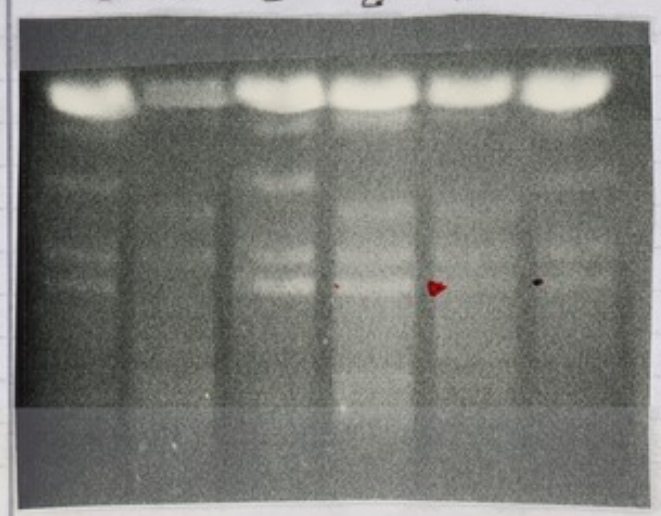


* named initially MY227-W1(1)... etc. on plates. So that
MY227-W1a = MY227W1(1)
W1b = (2)...

ran at 30s, 150v, 23hrs

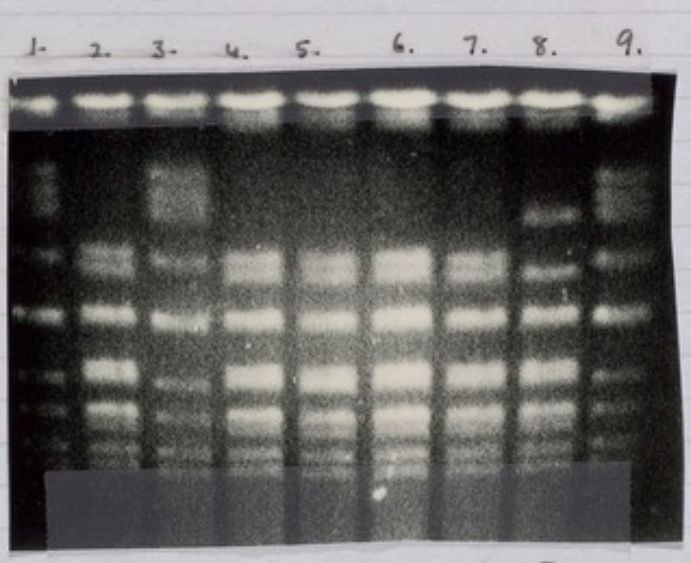


ran at 30s, 150v 23hrs.



ran at 30s 150v 23hrs.

8.7.99



1. AB1380
2. W6
3. DY3567 - AB1380
4. DY3567 - W6-a
5. " - W6-b
6. " W6-c
7. " W6-d
8. " W6-e
9. AB1380

?

The current complete list for 1° gels is:

Y116F11 – staining
Y71A12 W2B - done
Y59A8 - problem
(Y73F8
Y24F12)

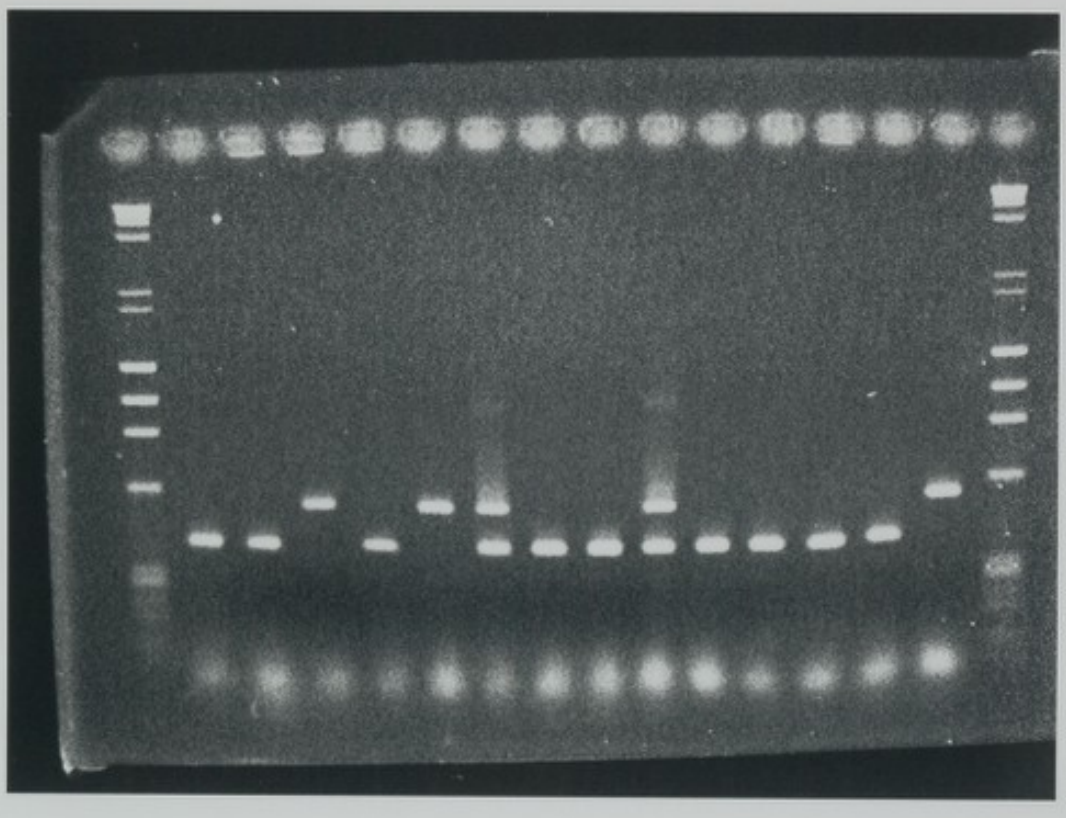
single primary gel please :-

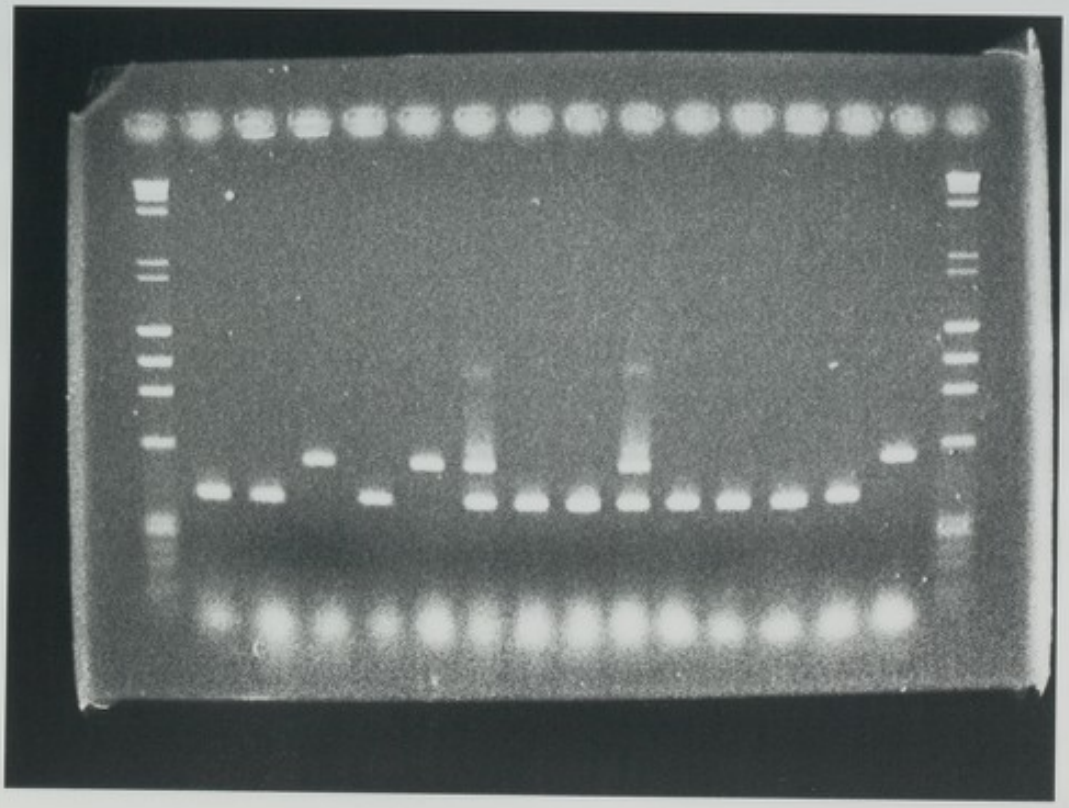
Y73F8 440
Y24F12 150.
Y59A8 (empty well - mistake.)
Thanks

Thank you

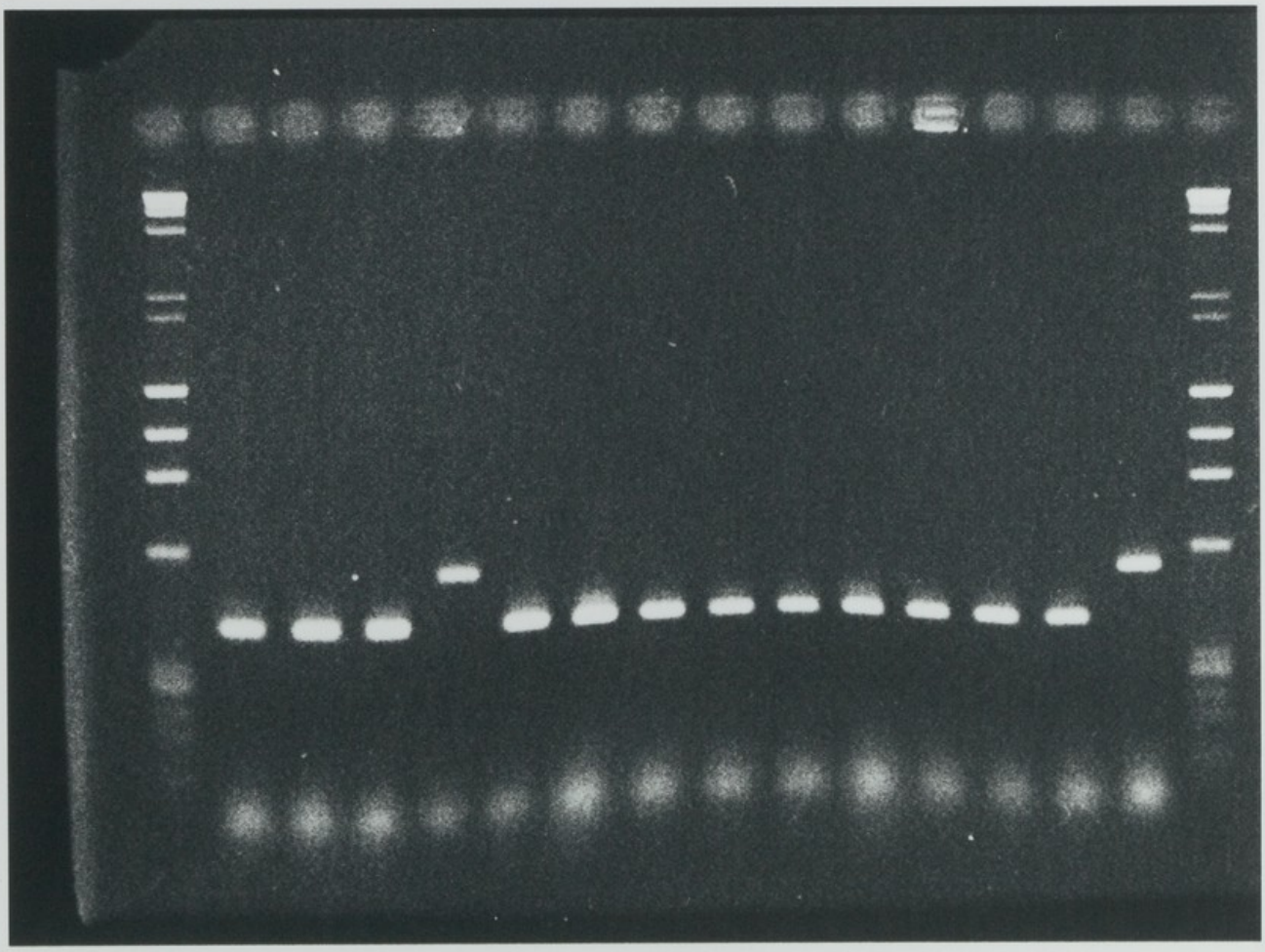
SUMMARY DATA

b





d



7/11/96

III

445	F3	280	
439	A1	350	early Mg++ titr; repeat? window transfer?
453	B3	300	
448	A6	300	
447	D3	350	window transfer? do prelim
441	C4	300	attempting window transfer
449	E10	150	
411	B2	620	
437	D8	420	
439	E4	350	window transfer awaiting PFG assay
443	F4	300	
476	A2	280	Sof- OK

7/11/96

11

Y17	G7	230		needs window transfer
Y52	D6	200		
Y38	E10	350		window transfer awaiting PFG assay
Y46	G5	340		
Y38	F1	170		
Y81	G3	320		in library prepⁿ.
Y48	C3	280		good window transfer
Y48	E1	330		in sequence Tm 11
Y54	G9	310		
Y36	C4	150	?	Hold for Y51H1 data (may o/l R06A)
Y51	H1	130		in sequence Tm 12
Y39	G8	230		good window transfer; WIA in lib prepⁿ
Y48	B6	280		possible good window transfer, but prob. OK without transfer
Y54	G11	370		
Y17	B7	90		in library prepⁿ
Y54	E2	330		in library prepⁿ
Y53	F4	430		in library prepⁿ

Cluster

Y53	C12	280		in library prepⁿ. mab-3; Zark says separates.

7/11/96

IV

438	H8	210	
445	F10	450	awaiting window transfer PFG
437	A1	350	
451	H5	190	lib, but high yeast; repeat
464	G10	210	in library prep
467	A10	200	in library prep
457	G11	520	in sequence, TM 13
441	E3	280	for window transfer
440	H4	230	for window transfer
473	F8	440	
410	C5	790	may need window transfer (do prelim)
465	A5	210	in library prep
47	A9	230	for window transfer

hip-1 contig: examine ends etc

7/11/96

V

Y6E2	210	} 'clusterish'; try other methods first
Y32B12	200	
Y70C1	150	
Y102A5	480	
Y6 98	230	Good library available. Odd prelim. analysis
Y26 G10	230	may need window. Do prelim?
Y59A8	350	in library prep
Y37H2	460	awaiting window transfer PFG
Y51A2	180	
Y69H2	170	
Y17D7	210	enzymic lib in sequencing TM14
Y33A10?	200	
Y112C10 or Y119E4 or Y119H2?		being sized

may be displaced due to Y698 preliminary analysis oddity, not yet resolved.

//

Y59C12	350	seq- OK (Bijay 6/14/96).
Y43F8	480	
Y39B6	310	high yeast lib; repeat
Y102G3	670	awaiting window transfer PFG

//

Y44A6	390	
Y38H6	280	may need window; do prelim?

7/11/96

I

Y119E10	?	being sized
Y53H11	450	
Y47H9	400	
Y53C10	350	for window transfer
Y47H10	350	prob separable; do prelim?
Y91F4	200	
Y56F9	280	may need window transfer; do prelim
Y19D10	280	may need window transfer; do prelim
Y41E12?	380	big YAC for small gap; try other ways first
Y50B1	280	looks separable, but do prelim.

//

Y6B3	230	Seq^n OK previously.
Y66F9	310	
Y71A12	340	awaiting window transfer PFG
Y63D3	230	for window transfer
Y112D2 or Y120H4		being sized.
Y54E5	290	for window transfer

Non-cluster YAC selections

NCIII

Y45F3	280kb	
Y39A1	350	sepn should be OK
Y52D3	300	sepn should be OK at 20sec.
Y48A6	300	in library prepn (18/11); for Tm11
Y47D3	350	for window transfer >W2
Y41C4	300	window transfer failed at 1st stage 2x.
Y49E10	150	
Y111B2	620	for window transfer >W4 for Tm11
Y37D8	420	in library prepn (25/11)
Y39E4	350	good window transfer (W2A)
Y43F4	300	
Y76A2	280	sepn OK; in library prepn (18/11);for Tm11

NCII

Y17G7	230	needs window transfer
Y52D6	200	in library prepn (25/11)
Y38E10	350	good window transfer (W2A)
Y46G5	340	
Y38F1	170	
Y81G3	320	in library prepn (22/10):v.high yeast (excision error?);
Y48C3	280	good window transfer
Y48E1	330	in sequence, Tm11
Y54G9	350	
Y36C4	150	? hold for Y51H1 data, which may o/l R06B9
Y51H1	130	in sequence, Tm12
Y39G8	230	good window transfer; W1A in library prepn.(28/10);wrong
Y48B6	280	possible good window transfer, but prob OK without.
Y54G11	370	
Y17B7	90	in library prepn (5/11); for Tm12
Y54E2	330	in library prepn (5/11); for Tm12
Y53F4	430	in library prepn (5/11); for Tm12

ClusterII

Y53C12	280	library available(21/11)(mab-3 bridge; Zarkower says est

NCIV

Y38H8	210	
Y45F10	450	good window transfer (W3A)
Y37A1	350	
Y51H5	190	in library prepn (25/11); earlier library high yeast
Y64G10	250	in library prepn (28/10)
Y67A10	200	library available (21/11)
Y57G11	520	in sequence, Tm13
Y41E3	280	window transfer awaiting PFG assay (22/11)
Y40H7	230	window transfer awaiting PFG assay (22/11)
Y73F8	440	
Y105C5	790	for window transfer >W6
Y65A5	210	library available (26/11)
Y7A9	230	window transfer awaiting PFG assay (22/11)

(hsp-1 contig requires further analysis)

NCV

Y6E2	210	clusterish; try other approaches first?
Y32B12	200	
Y70C5	150	

Y102A5 480
Y6G8 230 good library available, but odd preliminary analysis. Tm
Y26G10 230 for window transfer >W1
Y40B3 350 hold
Y59A8 350 in library prepn (28/10)
Y37H2 460 good window transfer (W3A)
Y51A2 180
Y69H2 170
Y17D7 210 in sequence, Tm14 (enzymatic library)
Y33A10 200 may be displaced due to Y6G8 paradoxical preliminary ana
Y112C10 or Y119E4 or Y119H2? being sized

Y59C12 350 sepn OK
Y43F8 480
Y39B6 310 in library prepn (25/11); earlier library high yeast
Y102G3 670 window transfer may be OK, but size discrepancy; Y102G3W

Y44A6 390
Y38H6 280 may need window transfer (preliminary PFG)

NCI
Y119E10 being sized
Y53H1 450 in library prepn (13/11); for Tm14
Y47H9 400 needs window transfer (>W2)
Y53C10 350 window transfer awaiting PFG assay (22/11)
Y47H10 350 sepn prob OK (but preliminary PFG)
Y91F4 200 in library prepn (13/11); for Tm14
Y56F9 280 for window transfer >W2
Y18D10 280 for window transfer >W2
Y47E12 380 ? big YAC for poss small gap; try other methods first?
Y50B1 230 sepn may be OK (but preliminary PFG)

Y6B3 230 sepn OK
Y66F9 310
Y71A12 340 window transfer good (but unnecessary): use Y71A12W2B
Y63D3 230 window transfer awaiting PFG assay (22/11)
Y112D2 or Y120H4 being sized
Y54E5 290 window transfer awaiting PFG assay (22/11)

7/11/96

Window transfer summary.

Y39	G8	→ 1	good			
Y48	B6	2	good			
Y48	C3	2	good			
Y71	A12	2	under assay			
Y102	G3	4	" "			
Y102	G3	5	" "			
Y39	E4	2	" "			
Y41	C4	2	needs repeat			
Y45	F10	3	under assay			
Y37	H2	3	" "			
Y38	E10	2	" "			
Y40	H7	230	do	do	→	W1
Y71	A9	230	do	do		W1
Y41	E3	280	do	do		W2
Y53	C10	350	do	do		W2
Y63	D3	230	do	do		W1
Y54	E5	290	do	do		W2

For preliminary assay if necessity for window transfer:

Y18	D10	280	
Y26	G10	230	
Y38	H6	280	
Y47	D3	350	
Y47	H10	350	
Y50	B1	230	
Y56	F9	280	
Y105	C5	790	