April 8, 1977

SCIENCE

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

8 April 1977, Volume 196, No. 4286

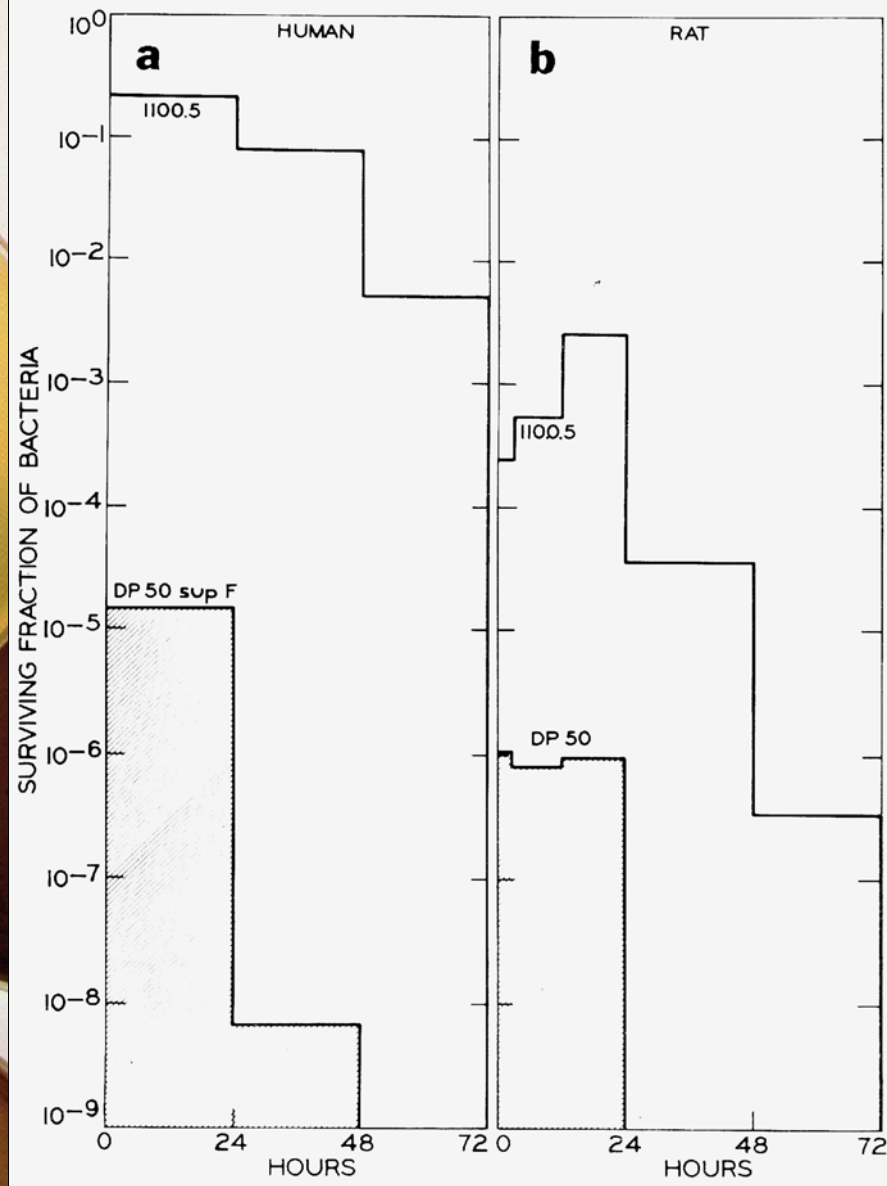
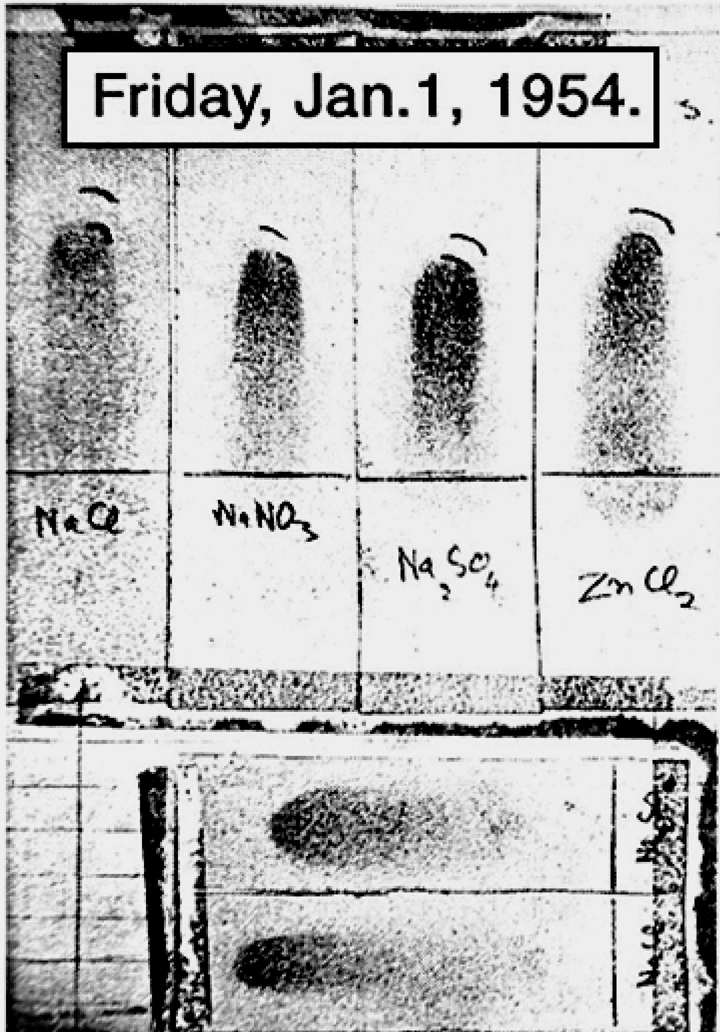


Fig. 4. Survival of host and vector in natural environments. Survival i
debilitated laboratory hosts DP50 and DP50SupF and, for comparison
strains are resistant to nalidixic acid, and titers can be determined on e
free Casamino acids and containing nalidixic acid (100 μg/ml). Such plat
yeast extract, and permit discrimination between Gal$^+$ (1100.5) and Ga
9.4×10^8 1100.5 was fed in 250 ml of milk to three humans. Bar heights



Fri Jan 1st 1954

Set up .01 M NaCl, Na_2SO_4,

$K_3Fe(CN)_6$, $K_4Fe(CN)_6$

12.45 ~~Reacted chemically~~

2.25 Ferro oxidising to ferri

Off at 3.55 (3 1/6 hrs) The ferri(o)cyanide does not move at all – chemical action. Result other?

At present stage may have to use a frontal type of paper e.g. as more [illegible] substance

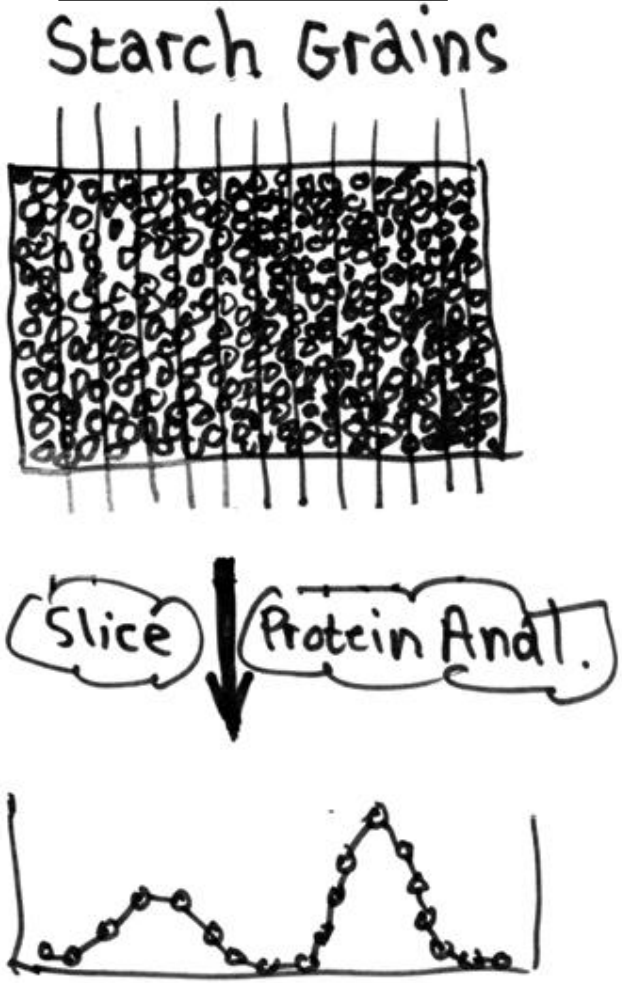
reduces tail much as [illegible] a longer times.

Try Al''' or Al'''' if can find.

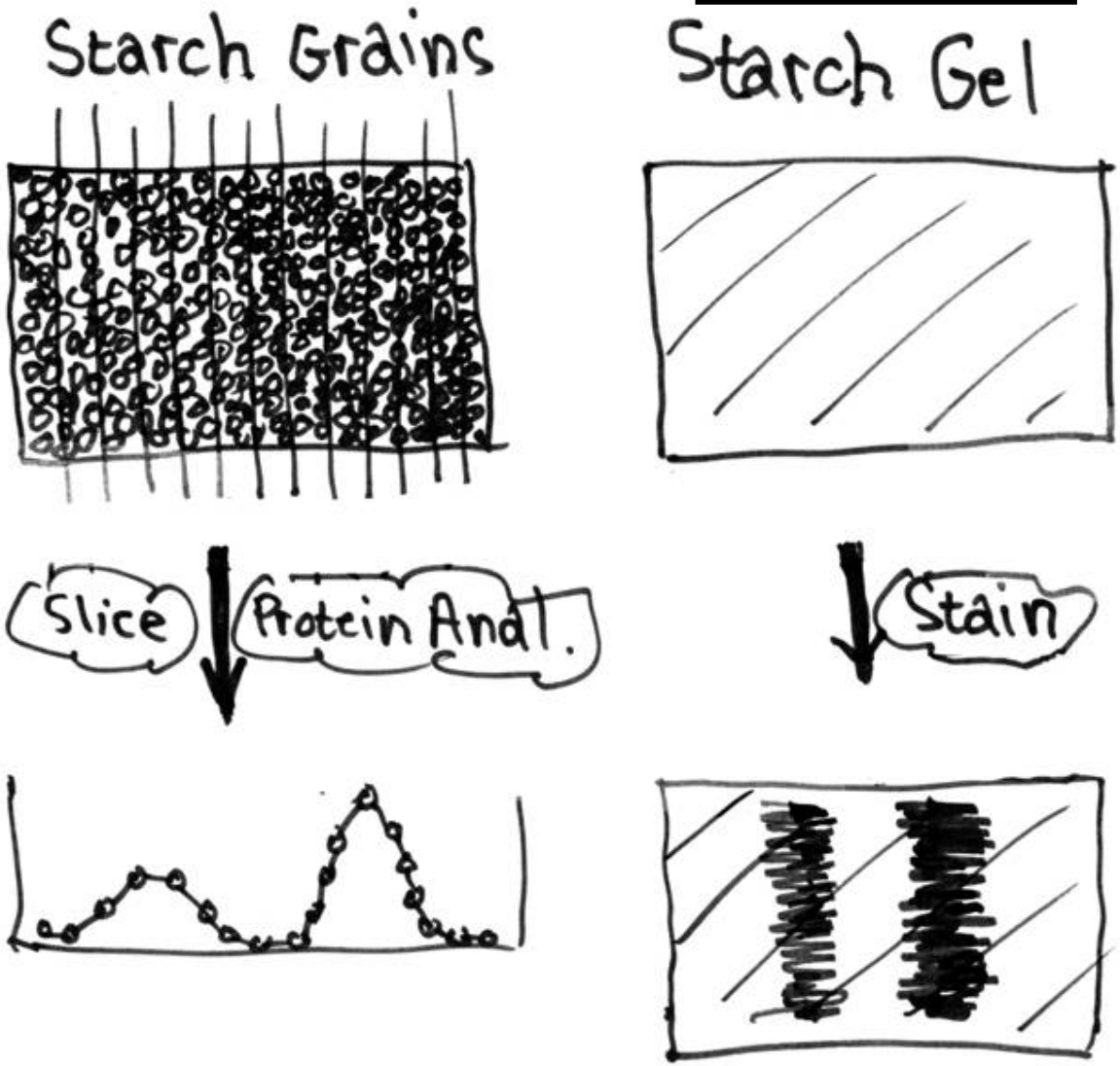
Better go over at alkaline side of I.E.P. if $MgCl_2$ or $AlCl_3$ no good ? try an amino acid which binds hard as #3

Try other papers before

Saturday am,
Jan.23, 1954



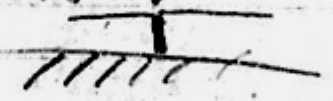
Saturday pm,
Jan.23, 1954



Jan 23rd

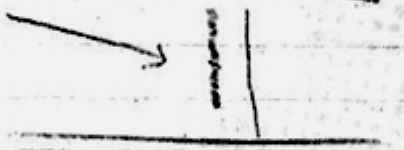
Saturday, Jan. 23, 1954.

Set up with dilute soluble starch jelly containing 0.1 M Ac + 0.01 M $MgCl_2$ (? rec'd). Potato slit in jelly → introduced 1/0 ins. in 0.1 M Ac →

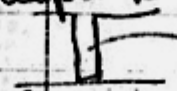


Run ~ 3/4 hr. with ~ 220 V.

Result v. promising dough only surface staining poss



∴ 12.0 am reset up for longer test.
Current 17 ma 3.30 current ~ 0 due to drying out [redacted].
Reset up → made much larger hole indeeper → more viscous jelly → covered ~ 1/32 across whole with liquid paraffin.



On at 3.45 220 V ~ 14 ma if d works clearly stiffness of jelly unimportant.
Current ~ 20 ma at 4.0. Off at 7.30 —

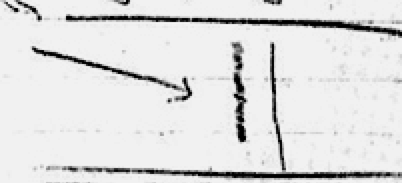
Saturday,
Jan. 23, 1954.

Jan 23rd

Set up with dilute soluble starch jelly containing 0.1 M HAc + 0.01 M $MgCl_2$ (? rec'd). Narrow slit in jelly & introduced 1% ins. in 0.1 M HAc ?

Ran ~$\frac{3}{4}$ hr. with 220V.

Result v. promising though only surface staining poss.



∴ 12.0 am reset up for longer test

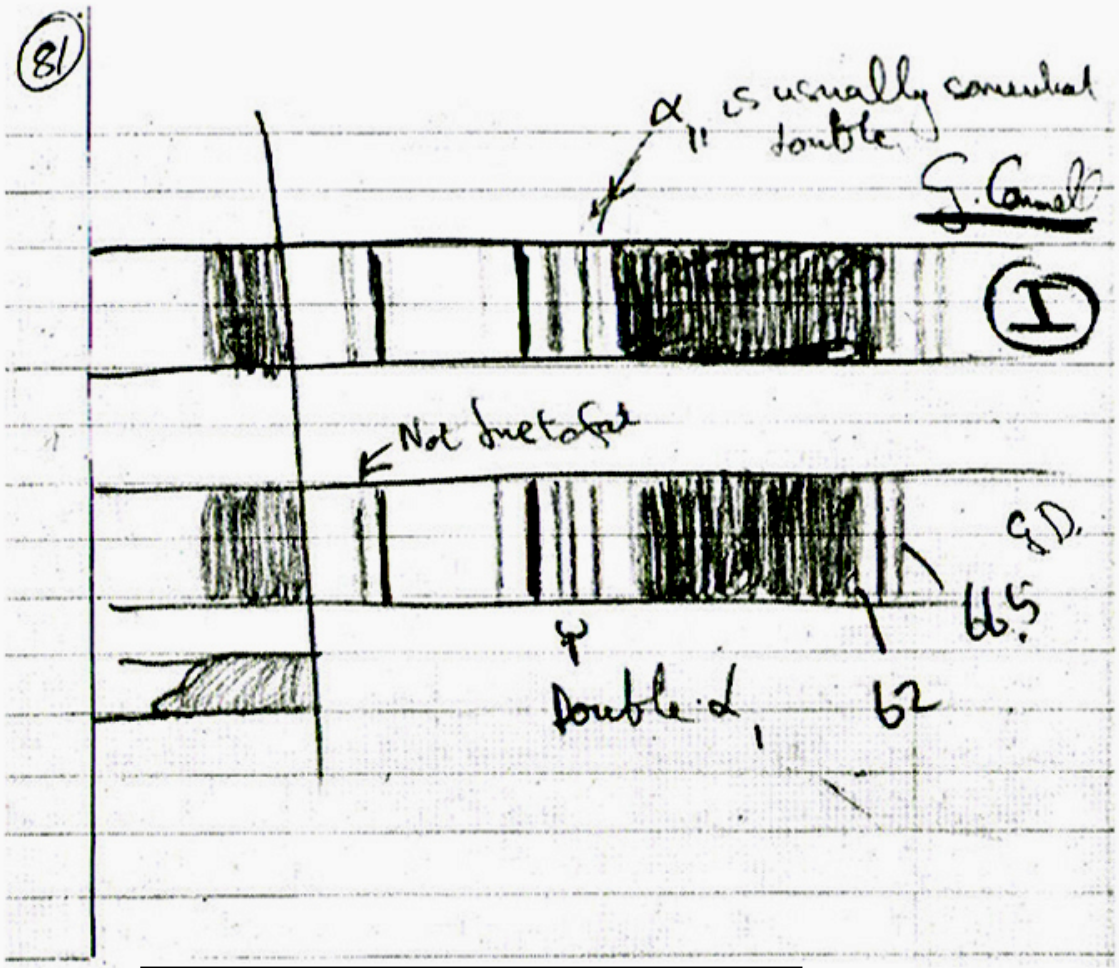
Current 17 ma   3.30 current ~0 due to drying out.

Reset up & made much larger hole indeeper & more viscous jelly

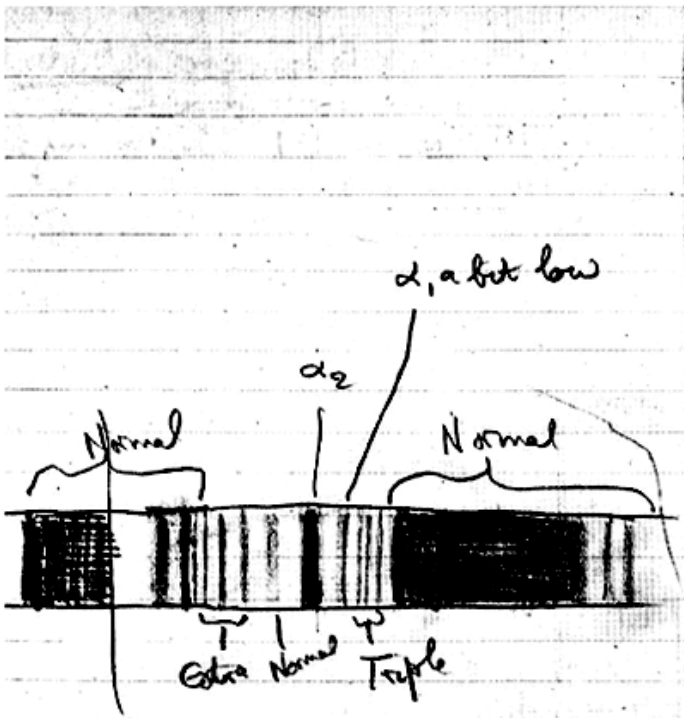
~$\frac{1}{32}$ across & covered whole with liquid paraffin.

On at 3.45 220v ~14 ma. If it works clearly stiffness of jelly unimportant.

Current ~20 ma at 4.0.   Off at 7.30



Friday, Oct. 16, 1954.



Tuesday, Oct. 26, 1954.

Tues. Oct 26

W. serum prepared. (II B)

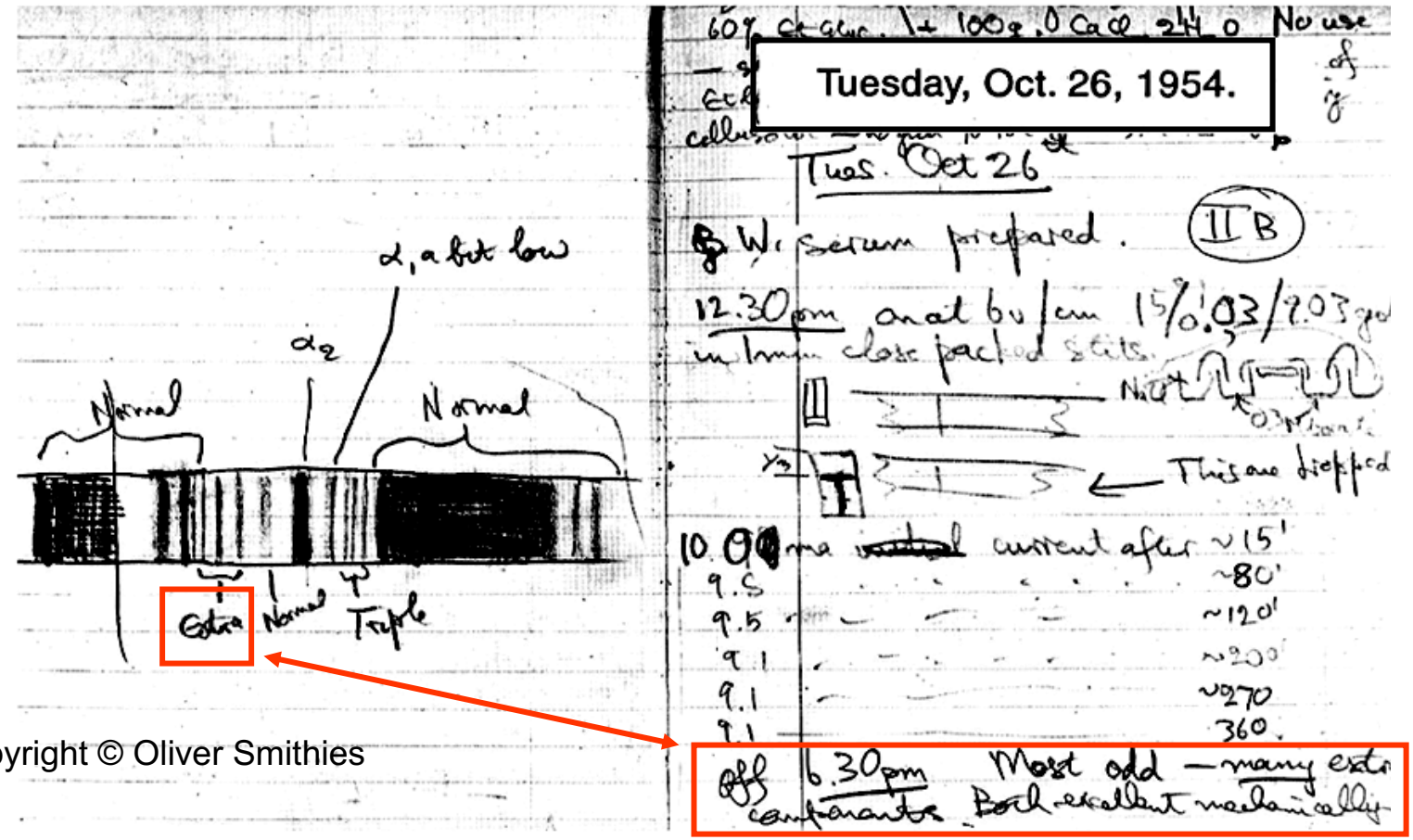
12.30 pm anal bx/cm 15/0.03/9.03 gm
in 1mm close packed stits.

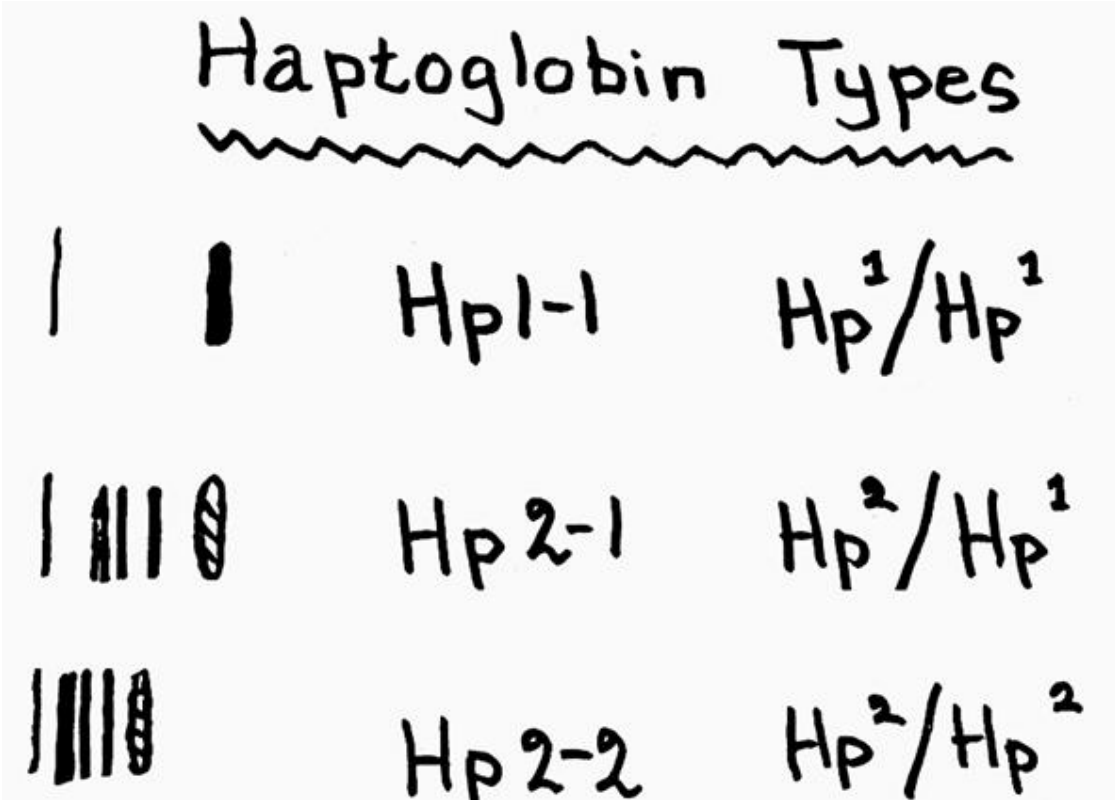
Nat

This one dropped

10.0 ma ~~initial~~ current after ~15'
9.5 ~80'
9.5 ~120'
9.1 ~200'
9.1 ~270
9.1 360.

Off 6.30 pm Most odd — many extra
contraction Both excellent mechanically

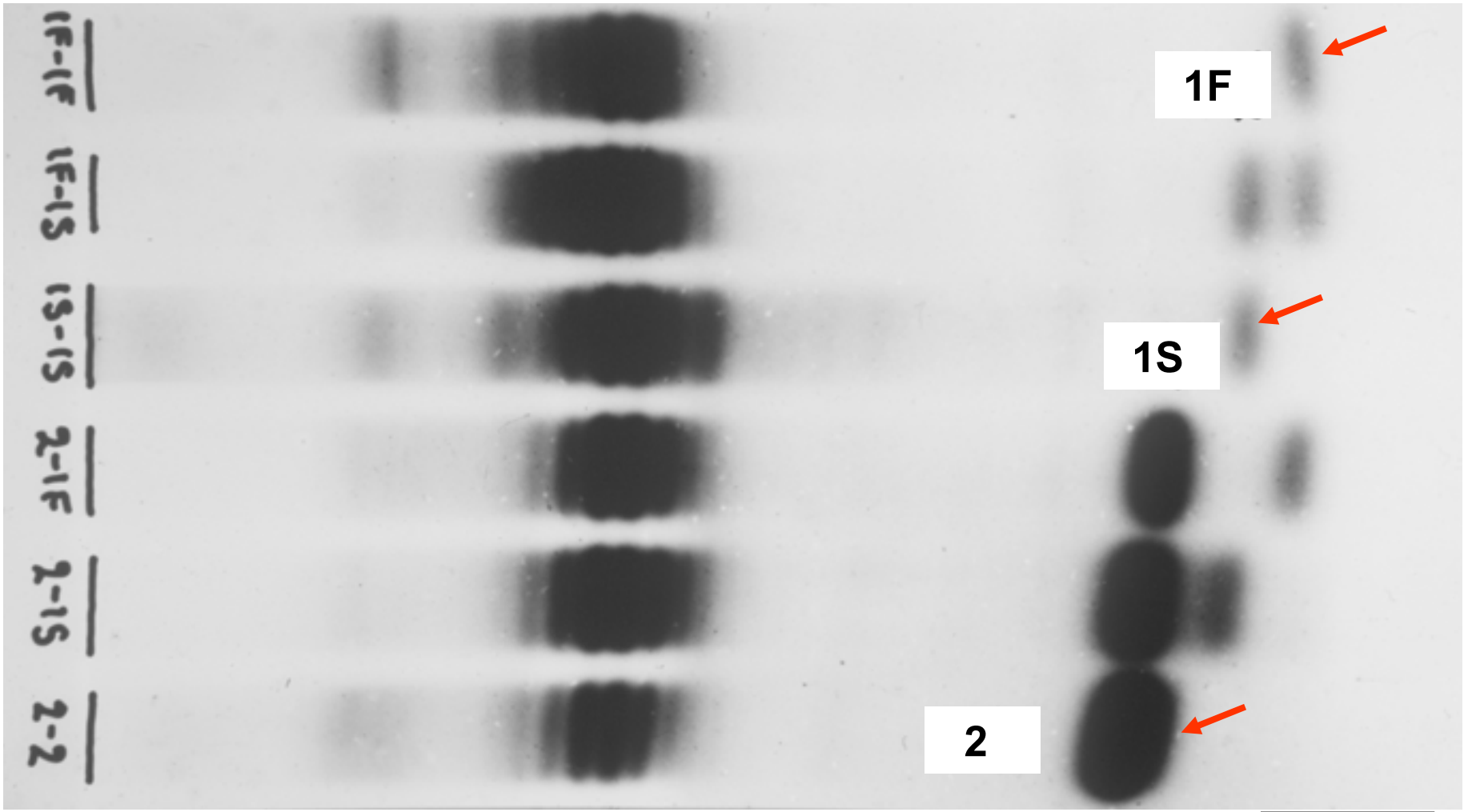




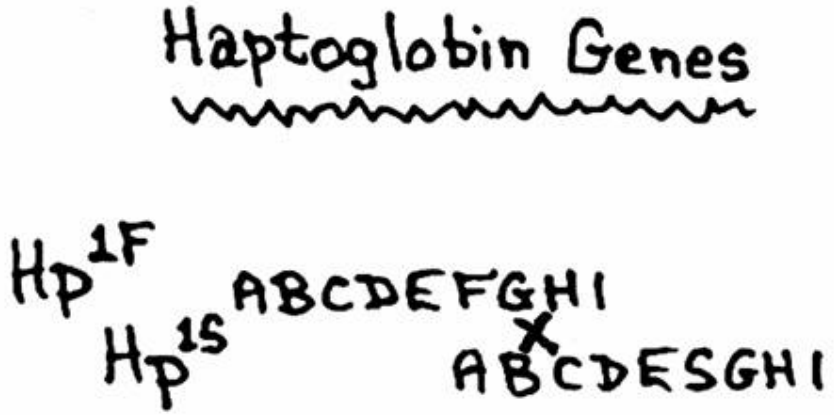
With Norma Ford-Walker, 1955

Beta Subunit

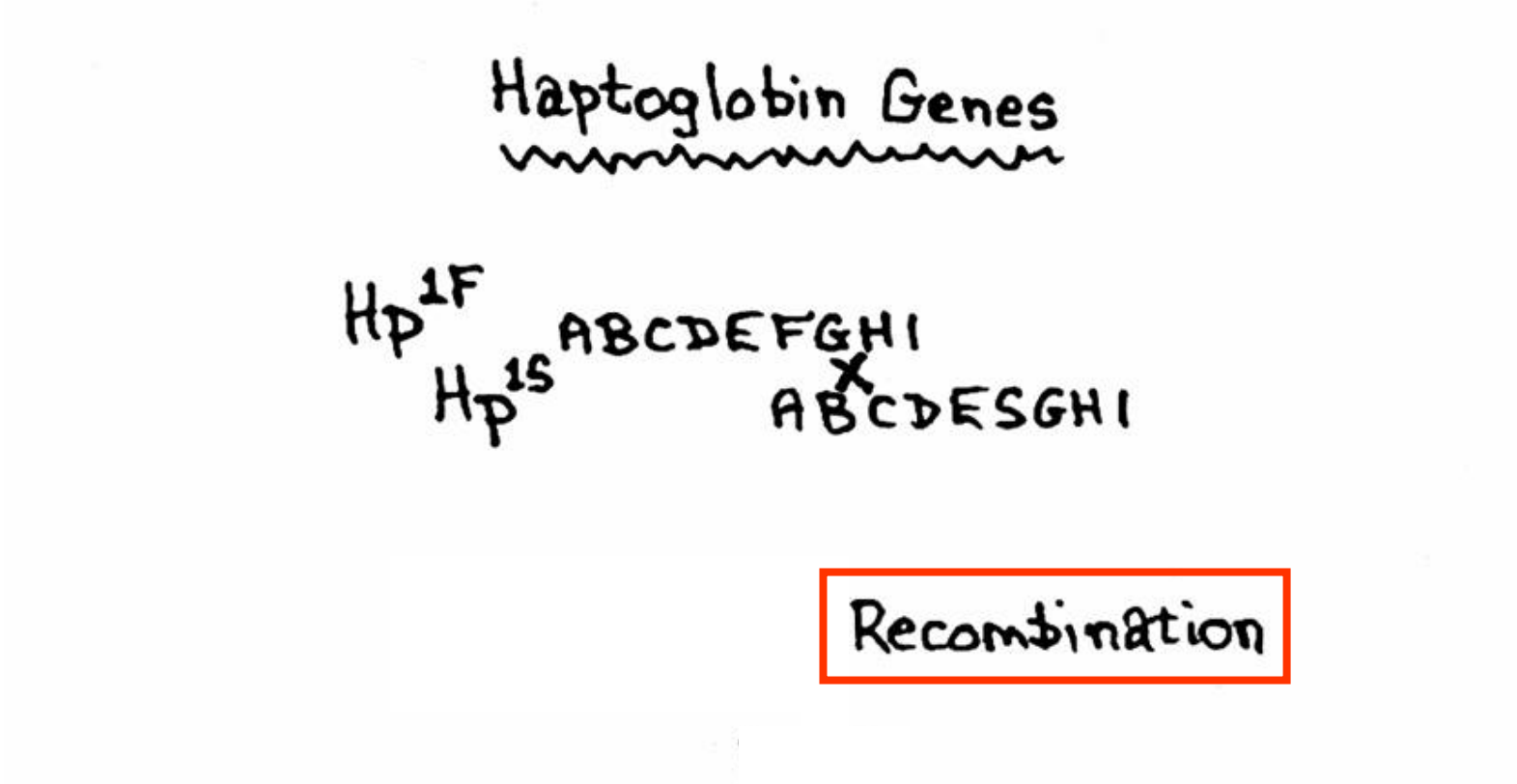
Alpha Subunits

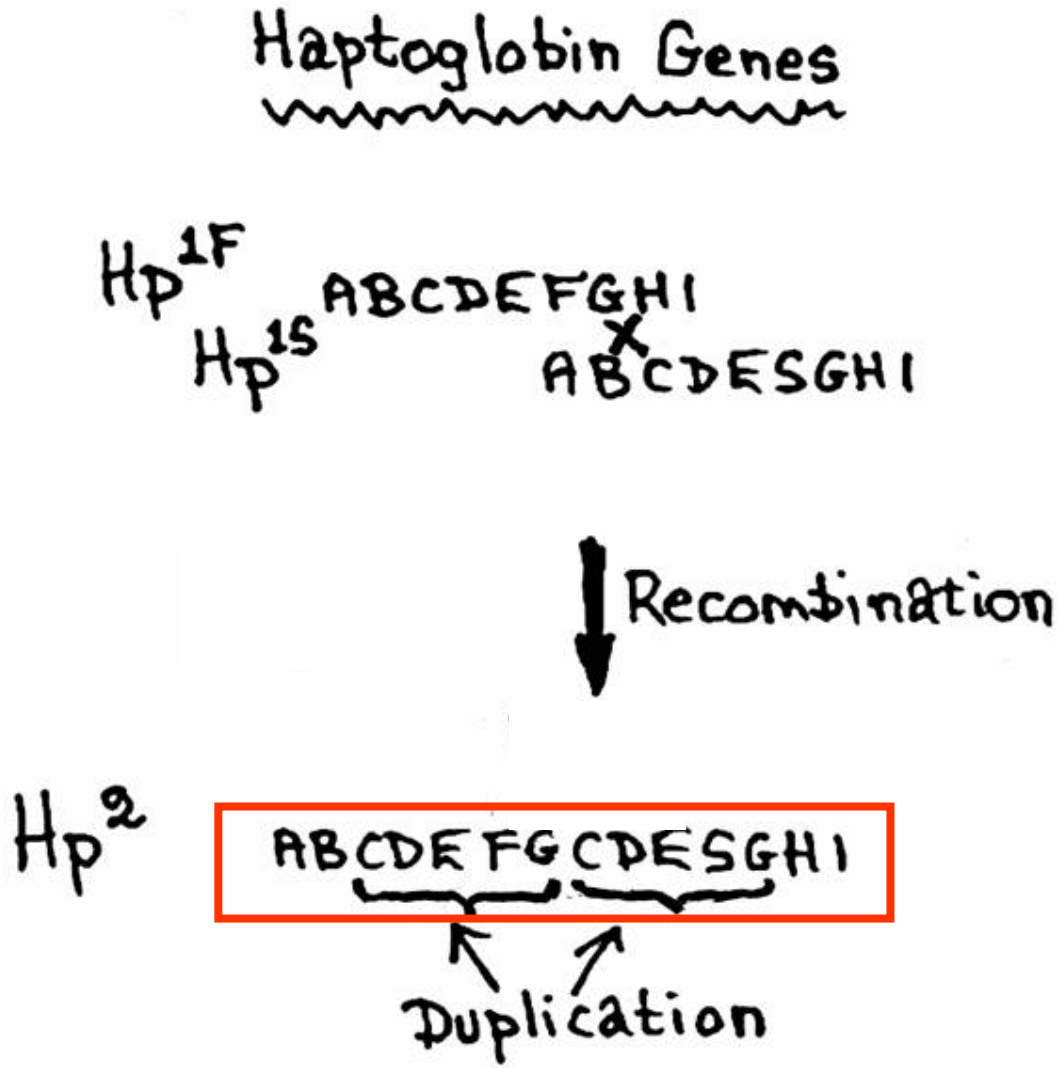


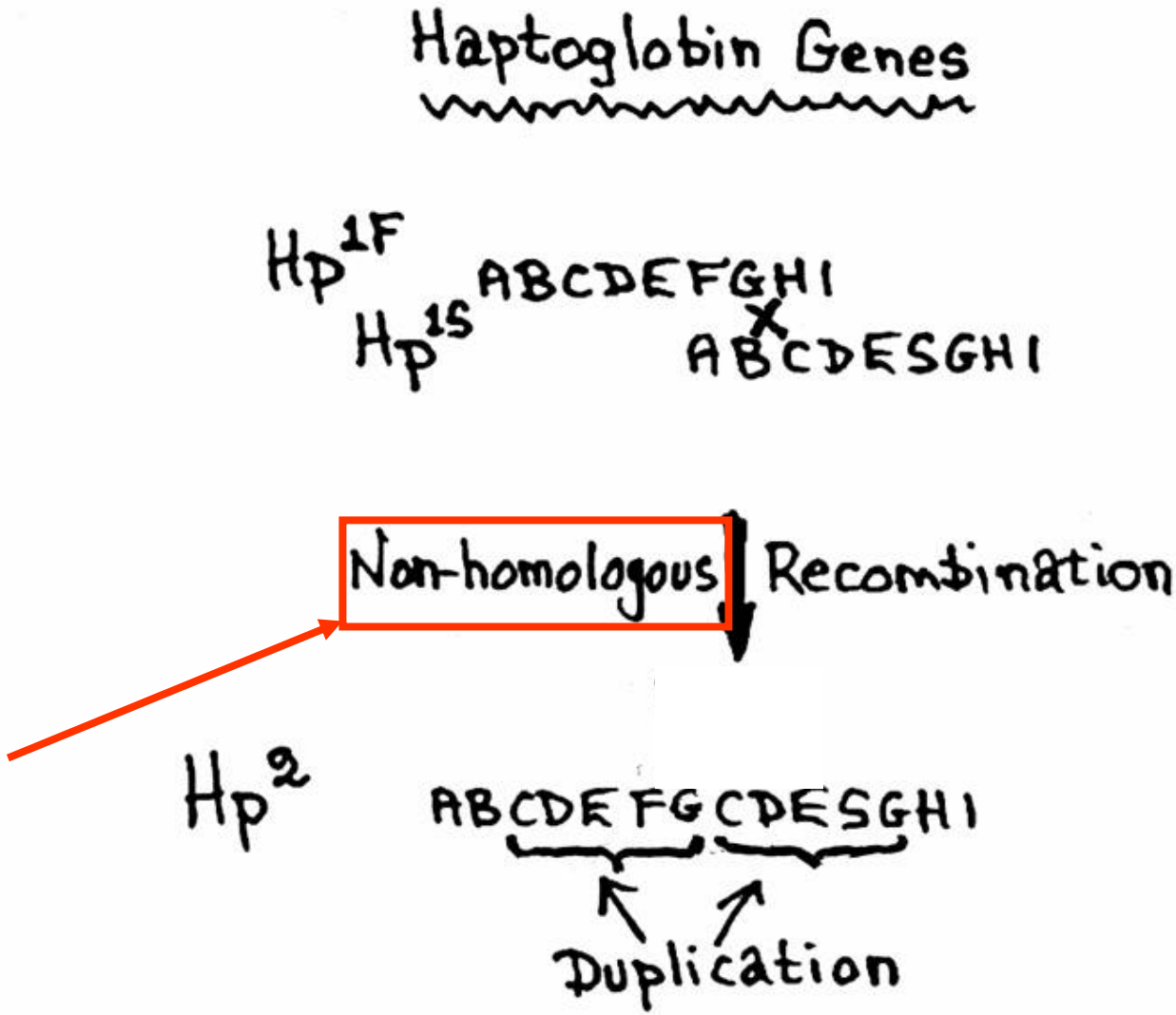
1962

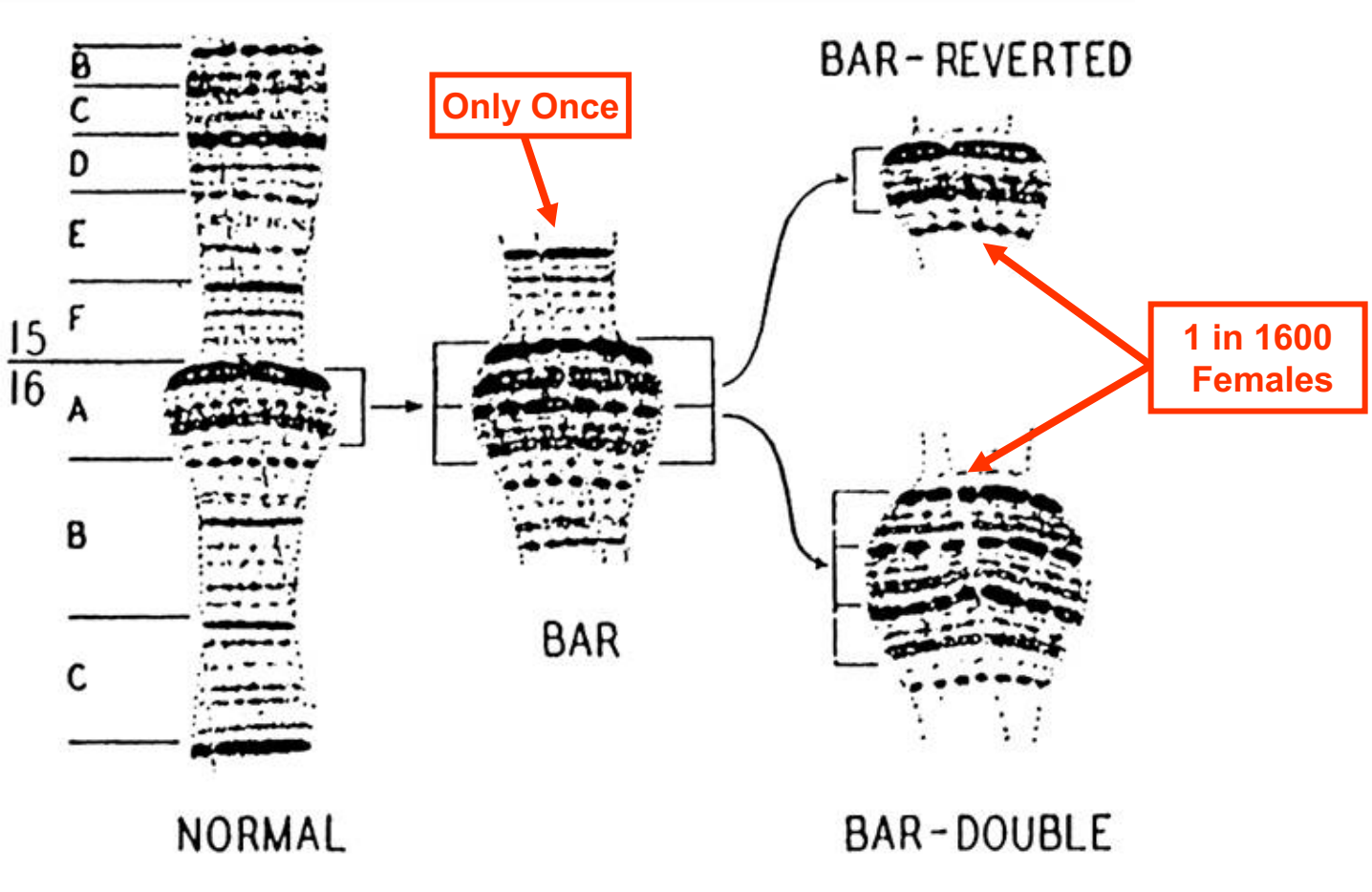


1962

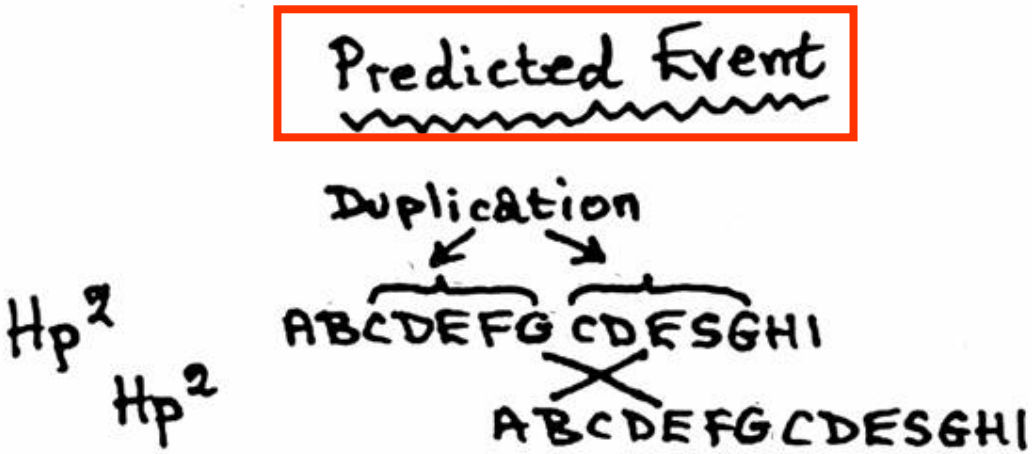


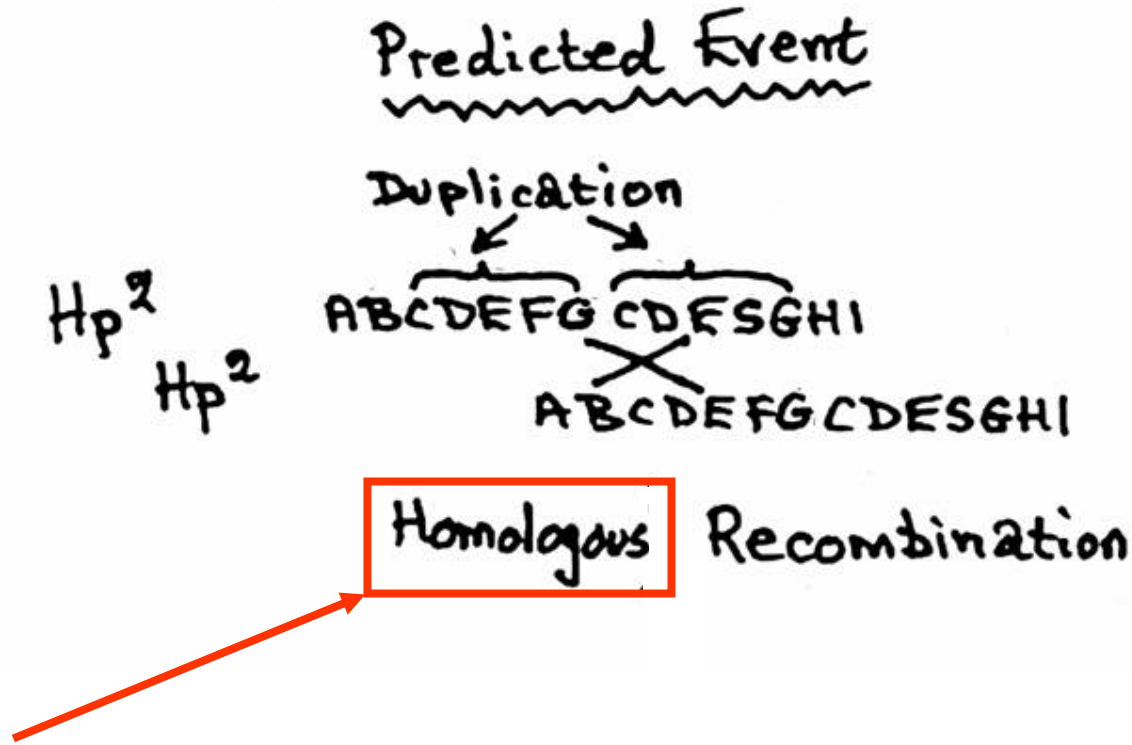


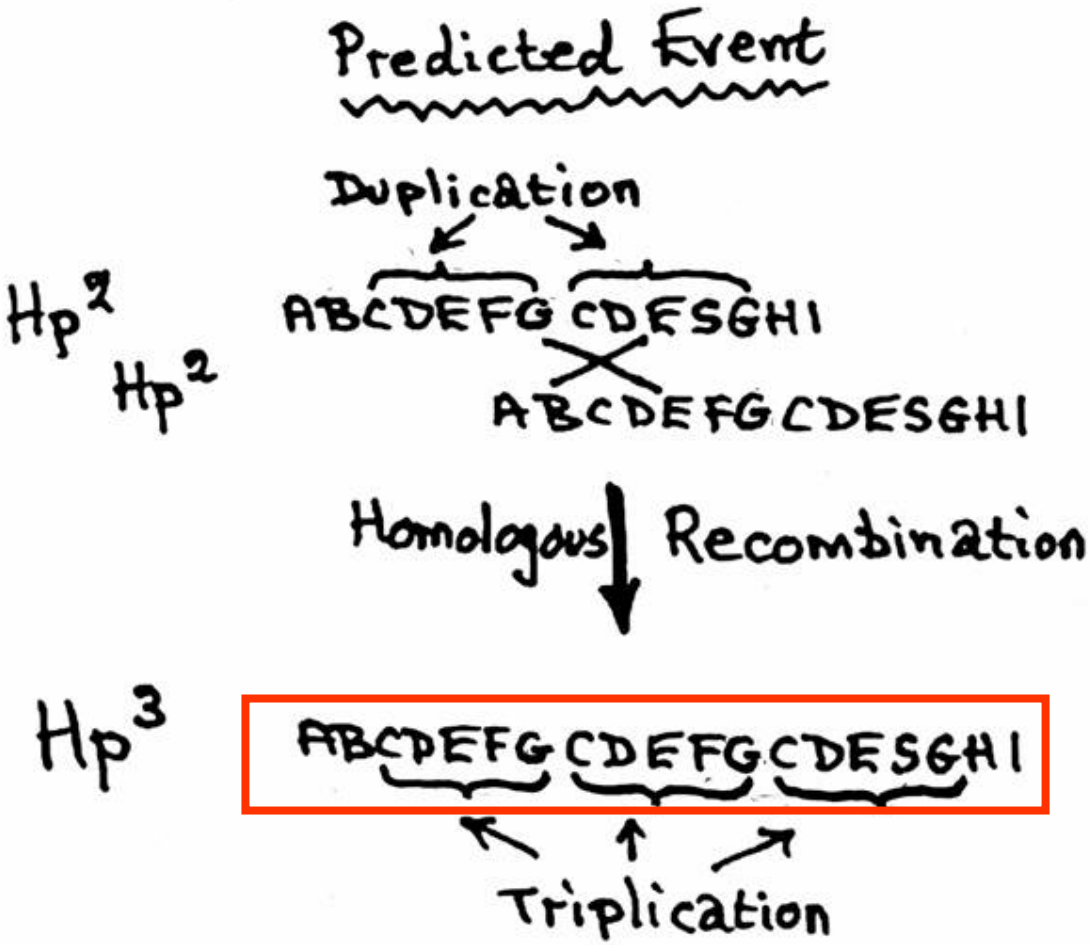


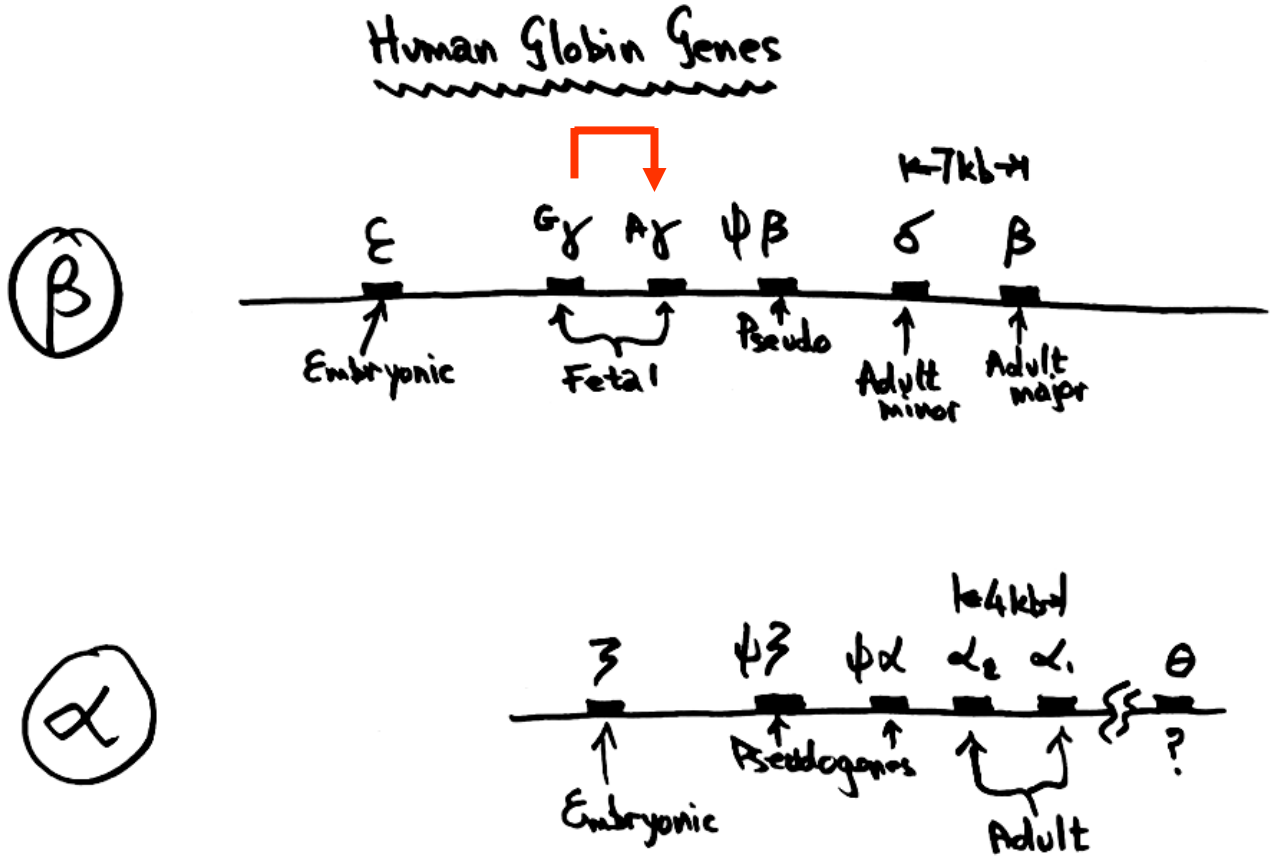


Tice 1914; Zeleny 1919; Sturtevant 1925; Bridges, 1936.









Slightom et al.,1980

Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells

Mitchell Goldfarb, Kenji Shimizu, Manuel Perucho & Michael Wigler

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

DNA from T24, a cell line derived from a human bladder carcinoma, can induce the morphological transformation of NIH 3T3 cells. Using techniques of gene rescue to clone the gene responsible for this transformation, we have found that it is human in origin, <5 kilobase pairs in size and is homologous to a 1,100-base polyadenylated RNA species found in T24 and HeLa cells. Blot analysis indicates extensive restriction endonuclease polymorphism near this gene in human DNAs.

THE progression of a cell lineage from normalcy to malignancy may involve the mutation or activation of one or more genes. The genomes of retroviruses contain candidates for such 'oncogenes'. Certain retroviruses capable of inducing neoplasia *in vivo* and cell transformation *in vitro* contain transduced cellular genes which entirely encode the oncogenic proteins of these viruses[1,2]. If these or other oncogenes are expressed in tumours of viral or nonviral origin, the introduction of these genes into cultured cells might transform the recipients and render them tumorigenic. Indeed, DNA from some chemically transformed mouse cells can morphologically transform NIH 3T3 mouse fibroblasts following DNA-mediated gene transfer[3]. More recently, it has been reported that DNA from certain human tumour cell lines can also morphologically transform NIH 3T3 cells[4,5]. We have detected transforming activity in DNA from 5 of 21 human tumour cell lines[6]; the resulting

0028-0836/82/130404—06$01.00

Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells

Mitchell Goldfarb, Kenji Shimizu, Manuel Perucho & Michael Wigler

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

DNA from T24, a cell line derived from a human bladder carcinoma, can induce the morphological transformation of NIH 3T3 cells. Using techniques of gene rescue to clone the gene responsible for this transformation, we have found that it is human in origin, <5 kilobase pairs in size and is homologous to a 1,100-base polyadenylated RNA species found in T24 and HeLa cells. Blot analysis indicates extensive restriction endonuclease polymorphism near this gene in human DNAs.

THE progression of a cell lineage from normalcy to malignancy may involve the mutation or activation of one or more genes. The genomes of retroviruses contain candidates for such 'oncogenes'. Certain retroviruses capable of inducing neoplasia *in vivo* and cell transformation *in vitro* contain transduced cellular genes which entirely encode the oncogenic proteins of these viruses[1,2]. If these or other oncogenes are expressed in tumours of viral or nonviral origin, the introduction of these genes into cultured cells might transform the recipients and render them tumorigenic. Indeed, DNA from some chemically transformed mouse cells can morphologically transform NIH 3T3 mouse fibroblasts following DNA-mediated gene transfer[3]. More recently, it has been reported that DNA from certain human tumour cell lines can also morphologically transform NIH 3T3 cells[4,5]. We have detected transforming activity in DNA from 5 of 21 human tumour cell lines[6]; the resulting

0028-0836/82/130404—06$01.00

Can accept non-linear → Duplication → Elimination ± correction

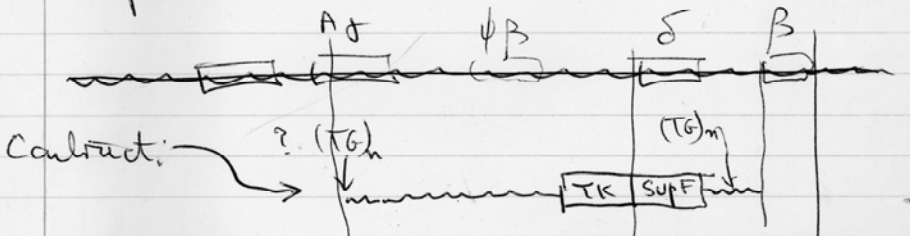
Thurs. April 22nd

Assay for gene placement -

Aim: to place corrective DNA in the right place.

Need: as assay for developing techniques.

Proposal:



Transform human TK^- cells → grow up a large # of transformants

Prepare DNA from TK^+ cells

Cut with rest. enz. → size to

Clone in an amber phage

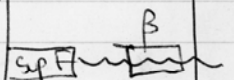


Plate on su^0 → screen with β specific probe

Vary $(TG)_n$ or single stranded ends or UV or BUdR etc. to try to ↑ # once-trues are found - Can also treat recipient cells with agents to ↑ SCE etc.

Selection in eukaryote × selection in prokaryote × β probe selection

1982

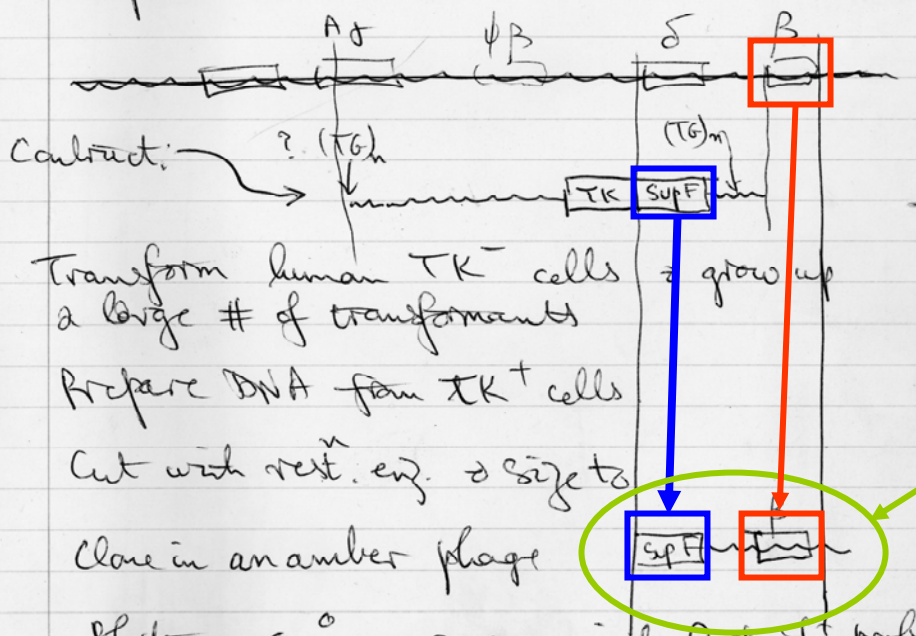
Thurs. April 22nd

Assay for gene placement -

Aim: to place corrective DNA in the right place.

Need: as assay for developing techniques.

Proposal:



Transform human TK⁻ cells → grow up a large # of transformants

Prepare DNA from TK⁺ cells

Cut with rest. enz. → size to

Clone in an amber phage

Plate on su° → screen with β specific probe

Vary $(TG)_n$ or single stranded ends or UV or BUdR etc. to try to ↑ # once-tves are found - Can also treat recipient cells with agents to ↑ SCE etc.

Selection in eukaryote × selection in prokaryote × β probe selection

Can accept non-linear → Duplication → Elimination → correction

1982

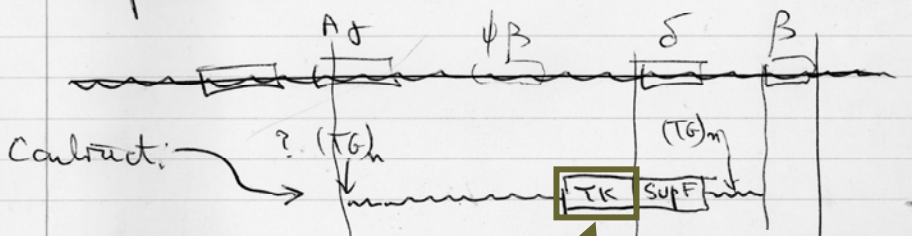
Thurs. April 22nd

Assay for gene placement

Aim: to place corrective DNA in the right place.

Need: as assay for developing techniques.

Proposal:



Transform human TK⁻ cells → grow up a large # of transformants

Prepare DNA from TK⁺ cells

Cut with rest. enz. → size to

Clone in an amber phage

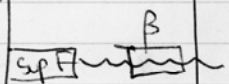


Plate on su° → screen with β specific probe

Vary $(TG)_n$ or single stranded ends or UV or BUdR etc. to try to ↑ # once-trfxs are found. Can also treat recipient cells with agents to ↑ SCE etc.

Selection in eukaryote × selection in prokaryote × β probe selection

Can accept non-linear → Duplication → Elimination → correction

1982

Can accept non-linear → Duplication → Elimination ± correction

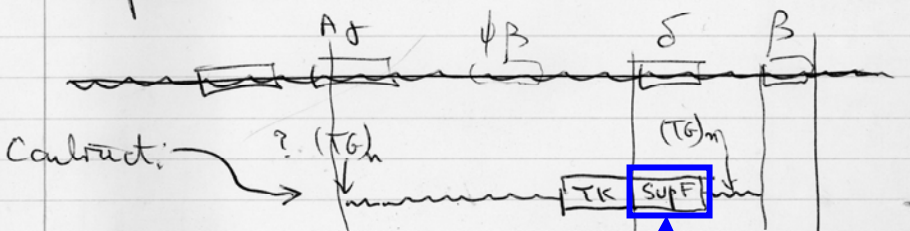
Thurs. April 22nd

Assay for gene placement -

Aim: to place corrective DNA in the right place.

Need: as assay for developing techniques.

Proposal:



Transform human TK⁻ cells ± grow up a large # of transformants

Prepare DNA from TK⁺ cells

Cut with rest. enz. ± size to

Clone in an amber phage

Plate on su° ± screen with β specific probe

Vary $(TG)_n$ or single stranded ends or UV or BUdR etc. to try to ↑ # ones that are found - Can also treat recipient cells with agents to ↑ SCE etc.

Selection in eukaryote × selection in prokaryote × β probe selection

1982

Can accept non-linear → Duplication → Elimination ± correction

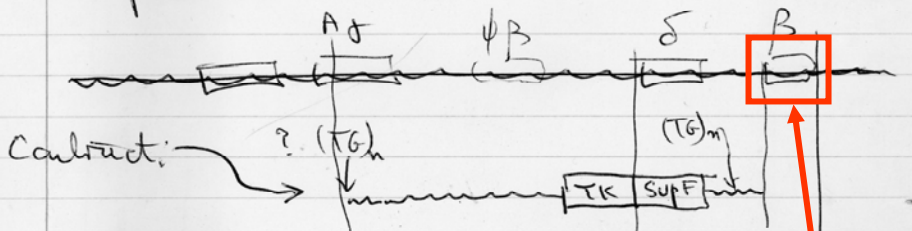
Thurs. April 22nd

Assay for gene placement -

Aim: to place corrective DNA in the right place.

Need: as assay for developing techniques.

Proposal:



Transform human TK⁻ cells ± grow up a large # of transformants

Prepare DNA from TK⁺ cells

Cut with rest. enz. ± size to

Clone in an amber phage

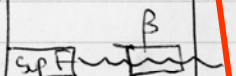


Plate on Su° ± screen with β specific probe

Vary $(TG)_n$ or single stranded ends or UV or BUdR etc. to try to ↑ # once-tives are found - Can also treat recipient cells with agents to ↑ SCE etc.

Selection in eukaryote × selection in prokaryote × β probe selection

1982

Can accept non-linear → Duplication → Elimination ± correction

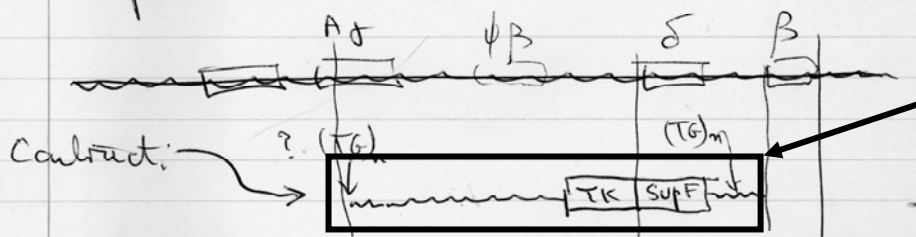
Thurs. April 22nd

Assay for gene placement -

Aim: to place corrective DNA in the right place.

Need: as assay for developing techniques.

Proposal:



Targeting Construct

Transform human TK⁻ cells → grow up a large # of transformants

Prepare DNA from TK⁺ cells

Cut with rest. enz. → size to

Clone in an amber phage

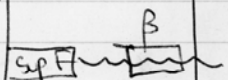
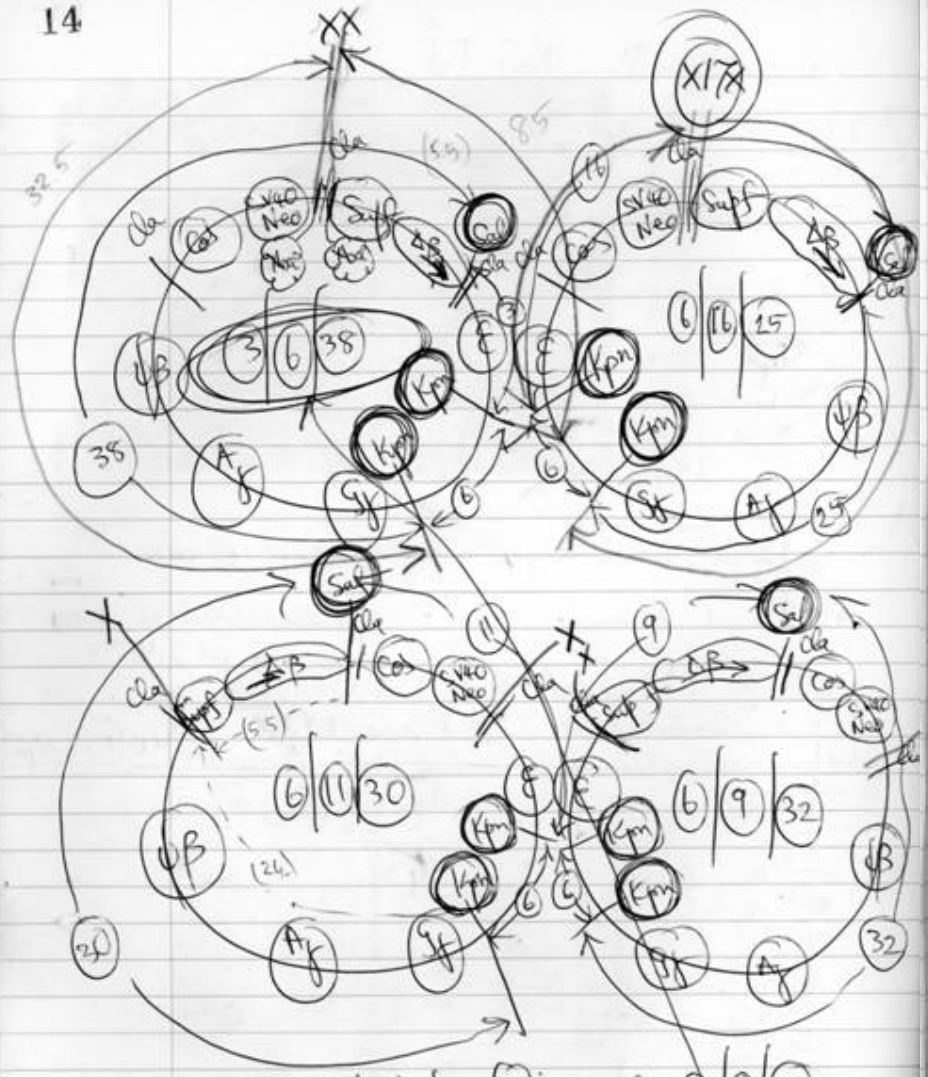


Plate on su° → screen with β specific probe

Vary $(TG)_n$ or single stranded ends or UV or BUdR etc. to try to ↑ # once-trves are found - Can also treat recipient cells with agents to ↑ SCE etc.

Selection in eukaryote × selection in prokaryote × β probe selection

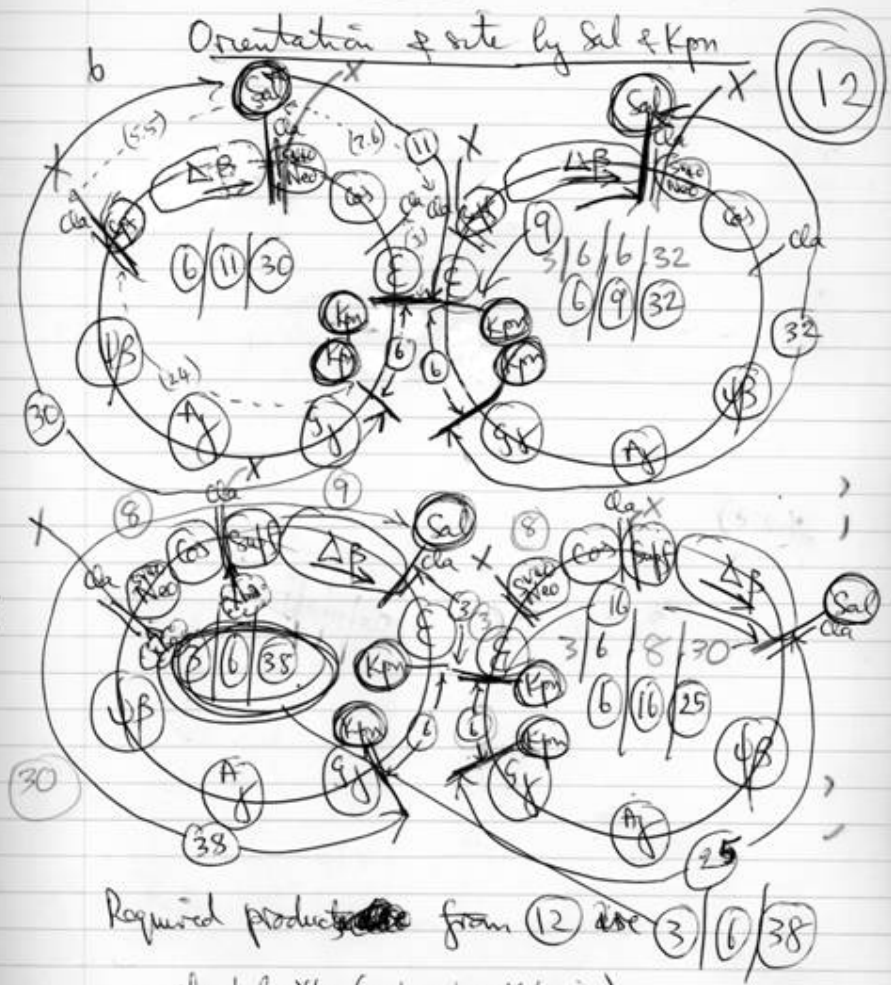
1982



Required products from (17) are also (3)|(6)|(38)
– check by Xba (pseudo single cut)

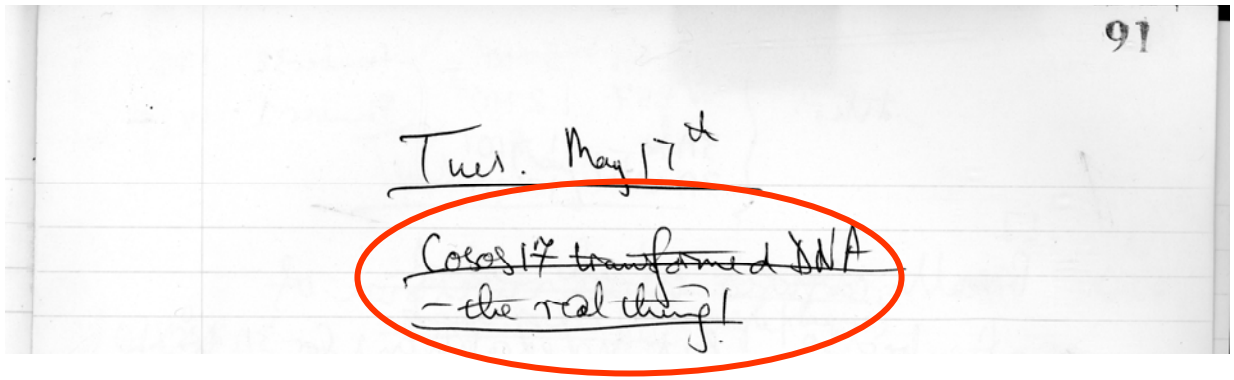
Wed. Nov 3rd

Orientation of site by Sal & Kpn



Required products from (12) are (3)|(6)|(38)
– check by Xba (cuts out a 7.6 piece).

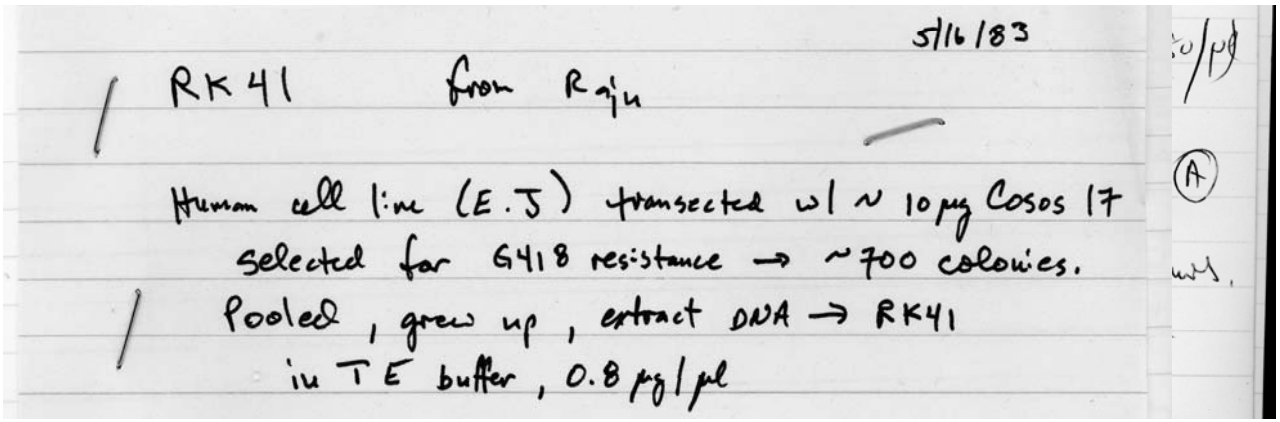
1982



91

Tues. May 17th

Cosos 17 ~~transformed DNA~~
– the real thing!



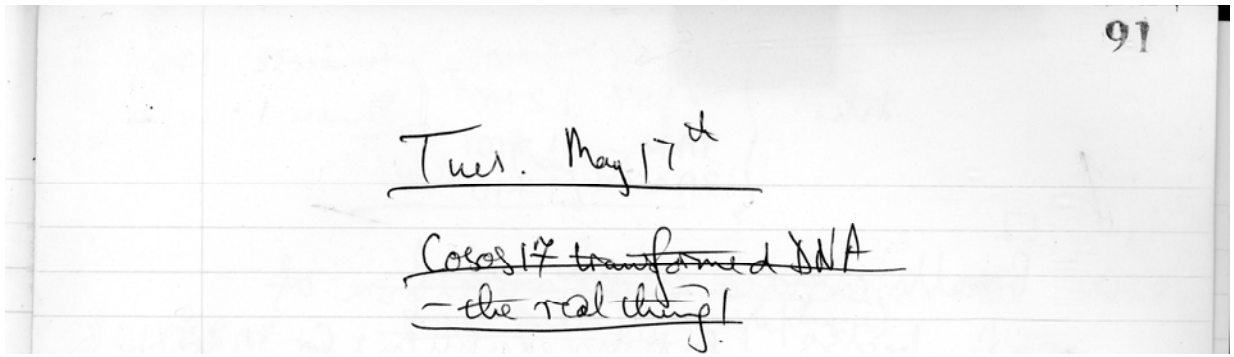
5/16/83

RK41 from Raju

Human cell line (E.J) transected w/ ~ 10 μg Cosos 17
selected for G418 resistance → ~700 colonies.
Pooled, grew up, extract DNA → RK41
in TE buffer, 0.8 μg/μl

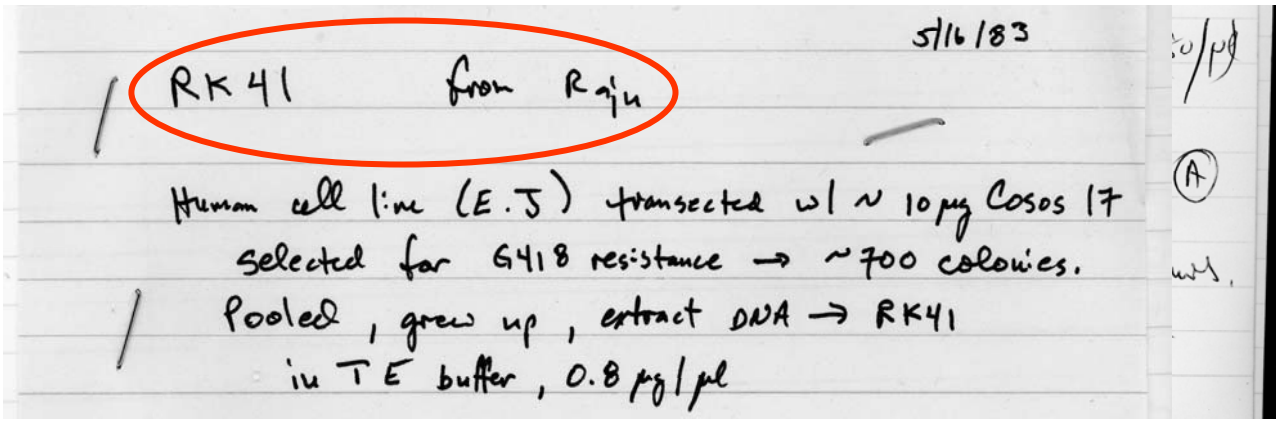
(A)

1983


91

Tues. May 17th

Cosos 17 ~~transformed~~ DNA
– the real thing!


5/16/83

RK 41 from Raju

Human cell line (E.J) transected w/ ~ 10 µg Cosos 17
selected for G418 resistance → ~700 colonies.
Pooled, grew up, extract DNA → RK41
in TE buffer, 0.8 µg/µl

1983

Thurs. June 23rd

ligation trial (near full scale)

Take ✓100 μl RK41/Xba (Cent.d) (≅ 20μg) ʒ141
+ ✓200 μl ʒ137 @ 3A.ΔΔX/Xba (Cent.d) (≅ 25μg)
✓+ 30 μl 8M NH_4A
✓+ 660 μl EtOH

↑ I doubt this

Friday June 24th

ʒ143 packaging

Total volume of ʒ143 lig.n is ~100 μl ≅ 5-10 μg ϕ

∴ take ✓ 140 μl buffer A BDKB 4/13/82 ʒ145 PP
✓ 20 μl MI buffer ʒ145
Whole of ʒ143 lig.n ~100 μl ✓
✓ 20 μl SE Y
✓ 80 μl FTL DM-B49
} on ice

Room temp. ~1 pm to 2.15 pm
(140 μl) Add ϕ storage buffer to give 500 μl
Titrate on C600.SF8 at 2.1 — 6.01
Plate 2 μl, 4 μl, 8 μl, 16 μl, 32 μl, 64 μl on C1A

(Preliminary results) Thurs. June 30th

a 20-30% of C1A +ve cells are 5.2K+
None are yet IVS 2 +ve. Total checked 288 on C1A
104 are 5.2K
(i.e. 36%)
↑ Not bad

1983

Thurs. June 23rd

ligation trial (near full scale)

Take ✓100 μl RK41/Xba (≅ 20μg) (Cont'd) p141
+ ✓200 μl p137 Ch 3A.ΔΔX/Xba (Cont'd)(≅ 25μg)
✓+ 30 μl 8M NH_4A
✓+ 660 μl EtOH

↑ I doubt this

My 58th Birthday

Friday June 24th

p143 packaging

Total volume of p143 lig^n is ~100 μl ≅ 5–10 μg φ

∴ take ✓140 μl buffer A BDKB 4/13/82 PP
✓20 μl MI buffer p145 p145
Whole of p143 lig^n ~100 μl ✓ on ice
✓ 20 μl SE Y
✓ 80 μl FTL DM-B49

Room temp. ~1 pm to 2.15 pm
(140 μl) Add φ storage buffer to give 500 μl
Titrate on C600.SF8 at 2.1 — 6.01
Plate 2 μl, 4 μl, 8 μl, 16 μl, 32 μl, 64 μl on C1A

(Preliminary results) Thurs. June 30th

~ 20–30% of C1A +ve cells are 5.2K+
None are yet IVS 2 +ve. Total checked 288 on C1A
104 are 5.2K
i.e. 36%

Not bad

1983

Thurs. June 23rd

ligation trial (near full scale)

Take ✓100 μl RK41/Xba (≅ 20 μg) ξ141
(Cent^d)
+ ✓200 μl ξ137 Ch3A.ΔΔX/Xba (Cent^d)(≅ 25 μg)
✓+ 30 μl 8M NH_4A
✓+ 660 μl EtOH

↑ I doubt this

Friday June 24th

ξ143 packaging

Total volume of ξ143 lig^n. is ~100 μl ≅ 5-10 μg ϕ

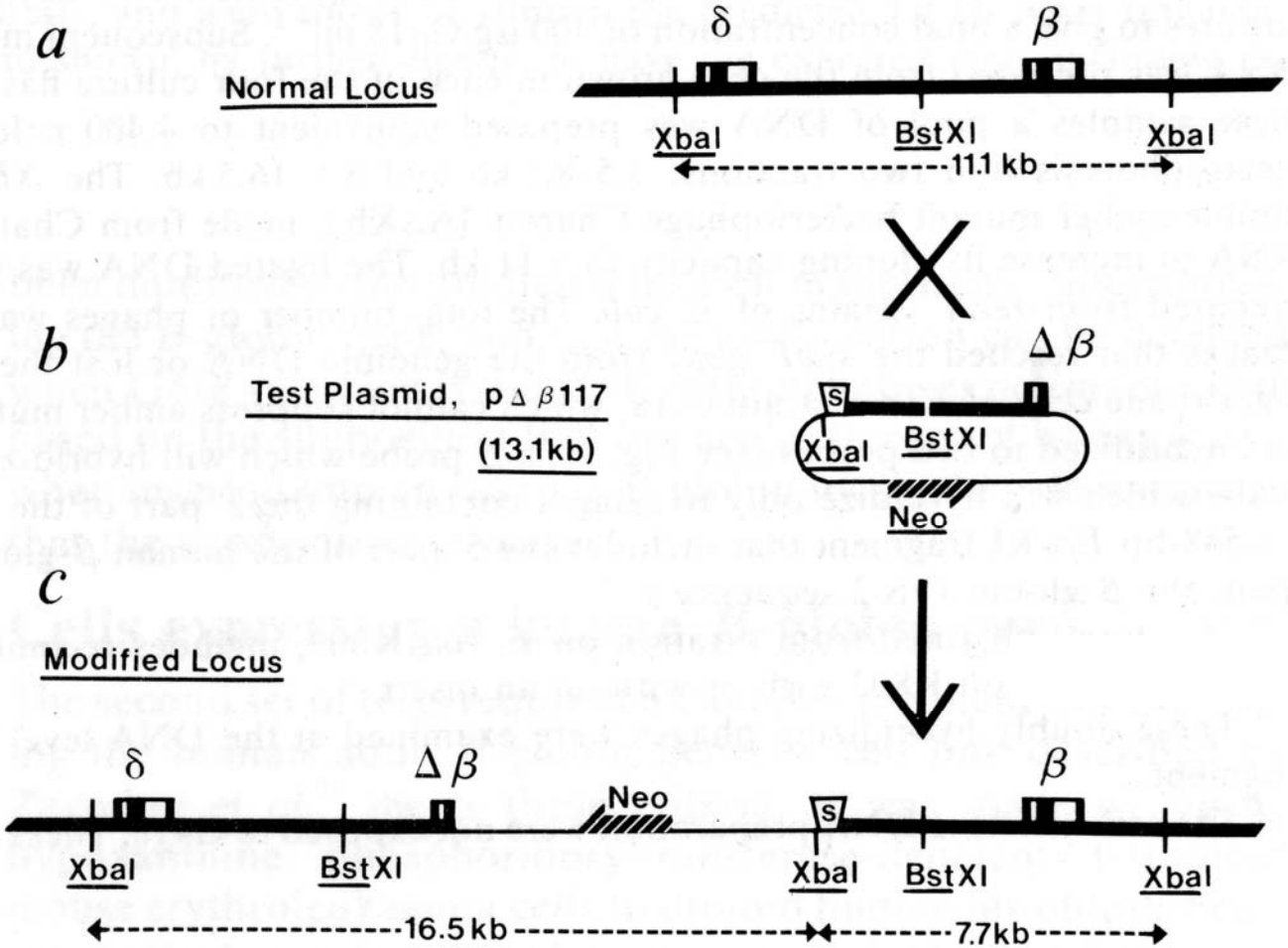
∴ take ✓140 μl buffer A   BDKB 4/13/82 PP ξ145
✓20 μl MI buffer ξ145
Whole of ξ143 lig^n. ~100 μl ✓
✓ 20 μl SE   Y
✓ 80 μl FTL   DM-B49
} on ice

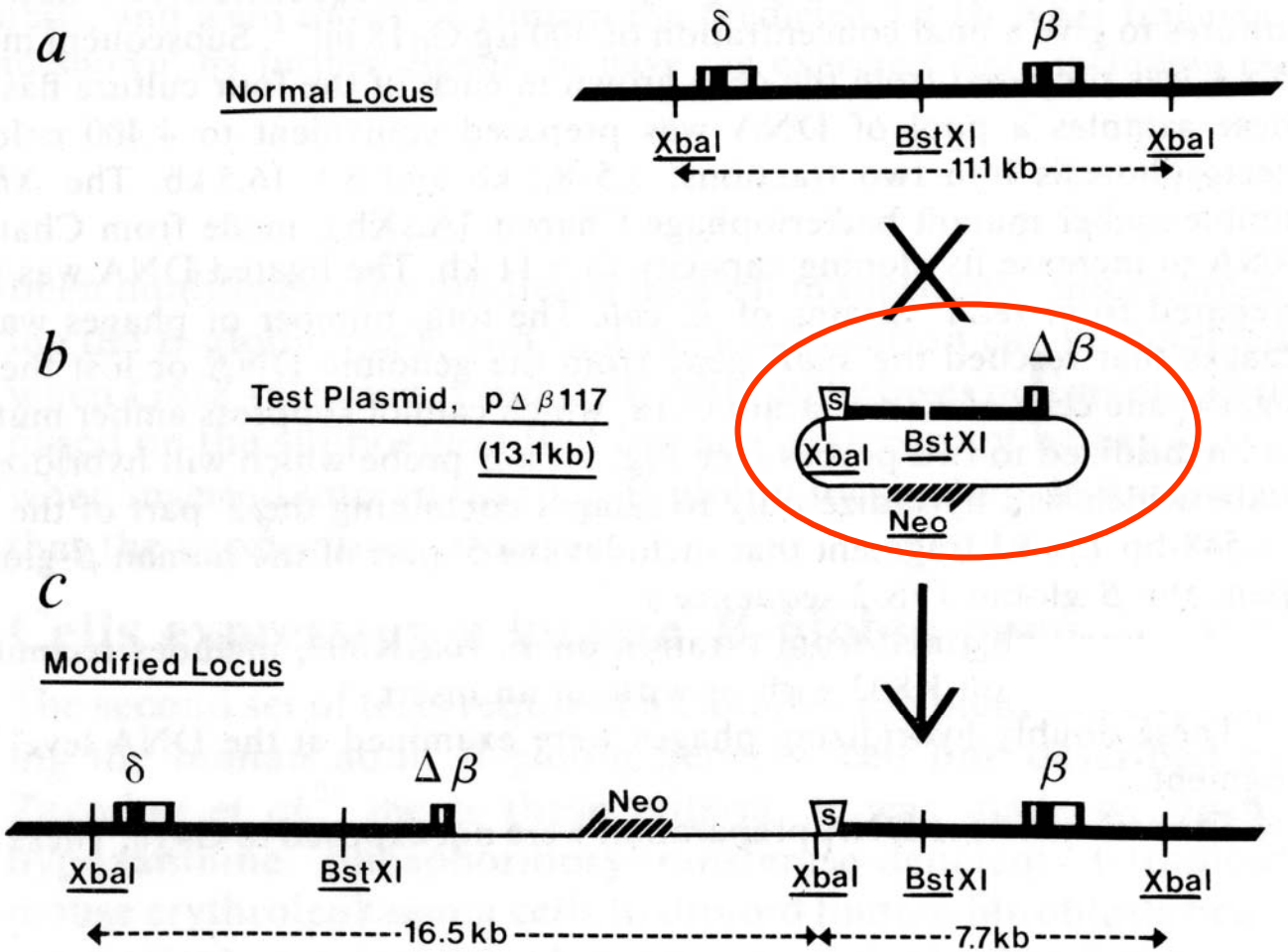
Room temp. ~1 pm to 2.15 pm
(140 μl) Add ϕ storage buffer to give 500 μl
Titrate on C600.SF8 at 2.1 — 6.01
Plate 2 μl, 4 μl, 8 μl, 16 μl, 32 μl, 64 μl on C1A

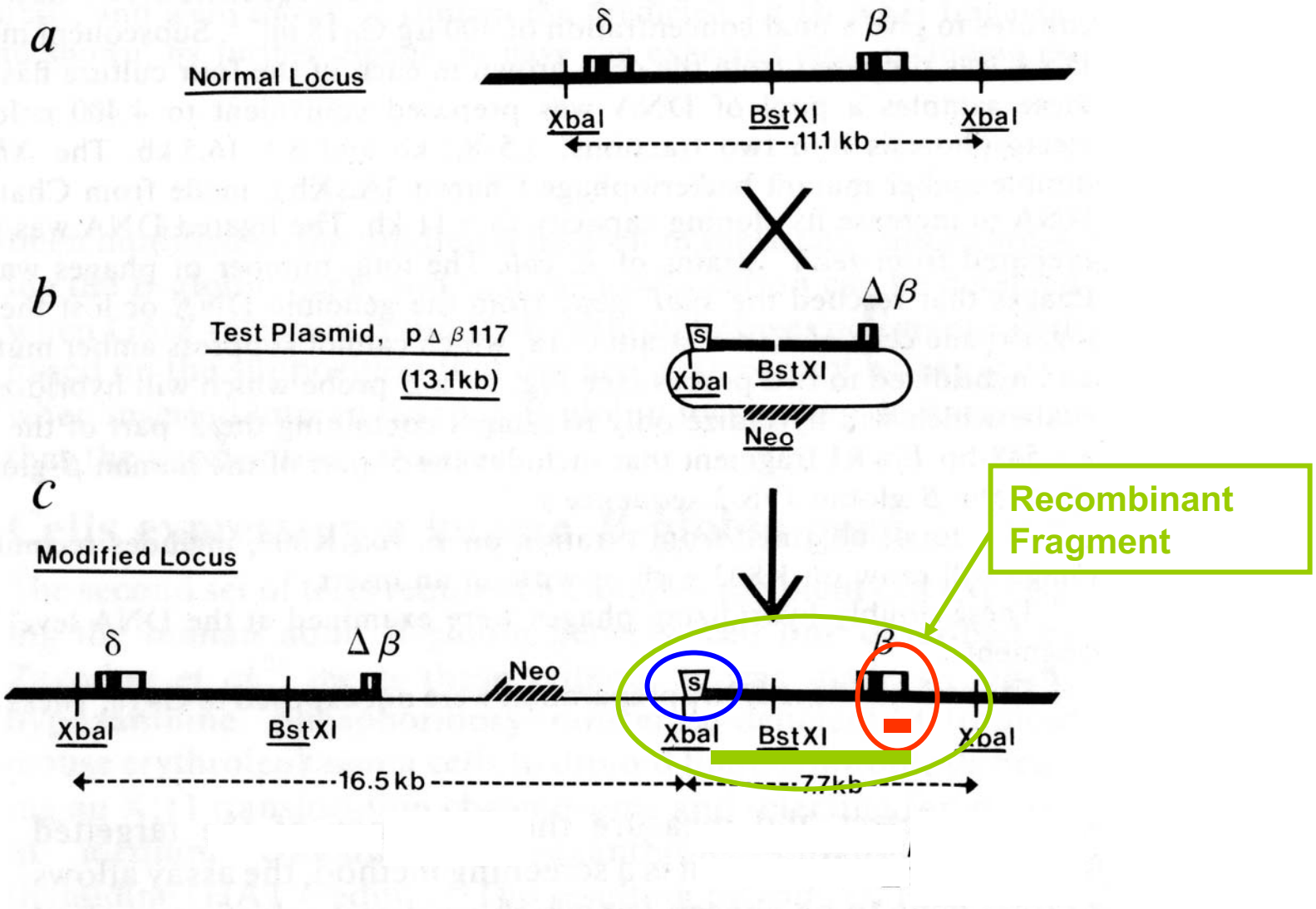
(Preliminary results) Thurs. June 30th

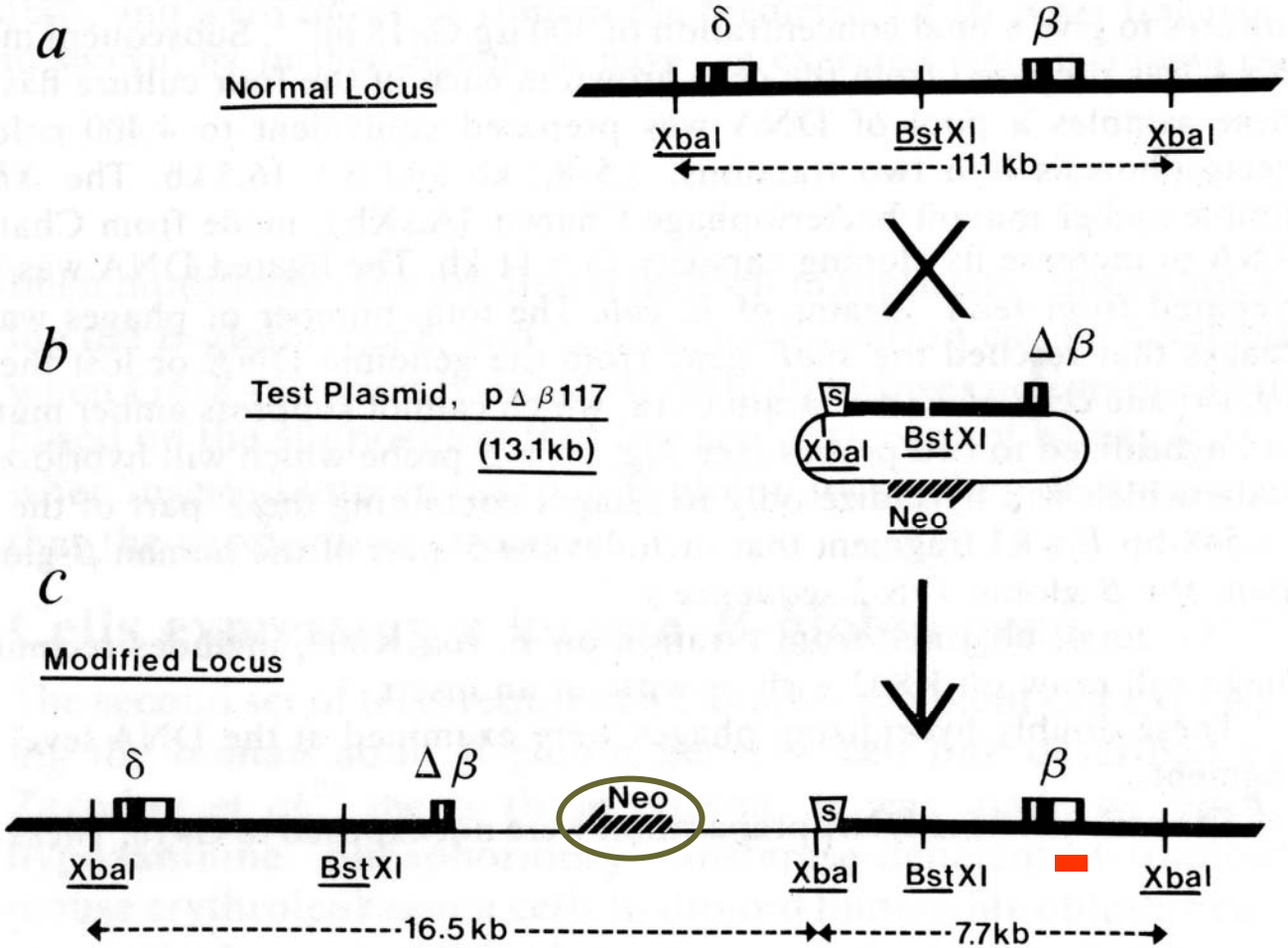
~ 20–30% of C1A +ve cells are 5.2K +
None are yet INS 2 +ve   Total checked 288 on C1A
104 are 5.2K
i.e. 36%
↑ Not bad

1983


a
δ
β
Normal Locus
XbaI
BstXI
XbaI
11.1 kb
b
Δβ
Test Plasmid, pΔβ117
(13.1kb)
S
XbaI
BstXI
Neo
c
Modified Locus
δ
Δβ
Neo
β
S
XbaI
BstXI
XbaI
BstXI
XbaI
16.5 kb
7.7kb


a
Normal Locus
δ
β
XbaI
BstXI
XbaI
11.1 kb
b
Test Plasmid, pΔβ117
(13.1kb)
Δβ
S
XbaI
BstXI
Neo
c
Modified Locus
δ
Δβ
Neo
S
β
XbaI
BstXI
XbaI
BstXI
XbaI
16.5 kb
7.7kb


a
Normal Locus
δ
β
XbaI
BstXI
XbaI
11.1 kb
b
Test Plasmid, pΔβ117
(13.1kb)
Δβ
S
XbaI
BstXI
Neo
c
Modified Locus
δ
Δβ
Neo
β
S
XbaI
BstXI
XbaI
XbaI
BstXI
16.5 kb
7.7kb
Recombinant Fragment


a
Normal Locus
δ
β
XbaI
BstXI
XbaI
11.1 kb
b
Test Plasmid, pΔβ117
(13.1kb)
Δβ
S
XbaI
BstXI
Neo
c
Modified Locus
δ
Δβ
Neo
S
β
XbaI
BstXI
XbaI
BstXI
XbaI
16.5 kb
7.7kb

Thurs. Aug 23rd

See MK/F24

Unselected DNA

RK 57 was DNA from EJ cells (9 dishes of $5 \times 10^5 + 1\mu g$ each of ΔB117 Bst XI cut) unselected in G418. (1μg gave 45 on G418)
~ 250μg DNA digested → cloned into ΔΔXba.
100μg of it used

Total φ 6.3×10^8 / K802

2520 CIa plaques
125 +ve for B probe
1 +ve for IVS2 probe (c'd be more on longer exposure).

K802 recA⁻ Minis made after plaque purification but trouble with XbaI (Eco RI → Eco+Bam were okay) . . . set up four more minis, & thought that modification may have occurred (Kim had similar trouble with K802 recA⁻).

CIA
K802
DP50SupF
C600 S#8

1984

Mike Koralewski

See MK/F24

Thurs. Aug 23rd

Unselected DNA

RK 57 was DNA from EJ cells (9 dishes of 5×10^5 + 1μg each of ΔB117 Bst XI cut) unselected in G418. (1μg gave 45 on G418)
~ 250μg DNA digested → cloned into ΔΔXba.
100μg of it used

Total ϕ 6.3×10^8 in KS02

2520 CIa plaques
125 +ve for B probe
1 +ve for IVS2 probe (c'd be more on longer exposure).

KS02 recA⁻ Minis made after plaque purification but trouble with XbaI (Eco RI → Eco+Bam were okay) . . . set up four more minis, on thought that modification may have occurred (Kim had similar trouble with KS02 recA⁻).

CIA
KS02
DP50SupF
C600 S#8

1984

Thurs. Aug 23rd

See MK/F24

Unselected DNA

RK 57 was DNA from EJ cells (9 dishes of $5 \times 10^5 + 1\mu g$ each of ΔB117 Bst XI cut) unselected in G418. (1μg gave 45 on G418)
~ 250μg DNA digested → cloned into ΔΔXba. (100μg of it was)

Total ϕ 6.3×10^8 in K802

2520 CIa plaques
125 +ve for B probe
1 +ve for IVS2 probe (c'd be more on longer exposure).

K802 recA⁻ Minis made after plaque purification but trouble with Xba I (Eco RI → Eco tBam were okay) . . . set up four more minis, on thought that modification may have occurred (Kim had similar trouble with K802 recA⁻).

CIA
K802
DP50 SupF
C600 S+8

1984

Thurs. Aug 23rd

See MK F24

Unselected DNA

RK 57 was DNA from EJ cells (9 dishes of 5×10^5 + 1μg each of ΔB117 BstXI cut) unselected in G418. (1μg gave 45 on G418)
~250μg DNA digested → cloned into ΔΔXba.
100μg of it used

Total φ 6.3×10^8 at K802

2580 CIA plaques

125 +ve for B probe

1 +ve for IVS2 probe (c'd be more on longer exposure).

K802 recA⁻ Minis made after plaque purification but trouble with XbaI (EcoRI → EcoRI+BamHI were okay) . . . set up four more minis, as thought that modification may have occurred (Kim had similar trouble with K802 recA⁻).

CIA
K802
DP50SupF
C600SF8

1984

Thurs. Aug 23rd

Unselected DNA

See MK/F24

RK 57 was DNA from EJ cells (9 dishes of 5×10^5 + 1μg each of ΔB117 Bst XI cut) unselected in G418. (1μg gave 45 on G418)
~ 250μg DNA digested → cloned into ΔΔXba.
100μg of it used

Total φ 6.3×10^8 on K802

2520 CIa plaques

125 +ve for B probe

1 +ve for IVS2 probe (c'd be more on longer exposure).

K802 recA⁻ Minis made after plaque purification but trouble with XbaI (Eco RI → Eco tBam were okay) . . . set up four more minis, on thought that modification may have occurred (Kim had similar trouble with K802 recA⁻).

CIA
K802
DP50SupF
C600 S#8

1984

Tues. June 12th

Transformation of β-globin-producing cells

Hunt Taber, L. Weir & P. Leder In press PNAS

E. Neumann, Schaefer-Ridder, Wang & Hofschneider EMBO J 1 (1982) 841

Zimmerman & Vienken J. Membrane Biol. 67 (1982) 165

- use hi-voltage pulse across cells + DNA to get transformation.

Originally from single cell

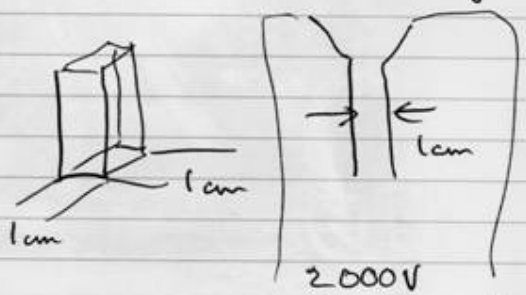
ASF2-1 cells are hybrid between MEL cells & human hybrid fibroblast with X-11 translocation. (3 chromosomes - normal 40) Under HAT the X-11 is retained. Making Hb at low level - can be induced with DMSO - but differentiation → halt. Stationary cultures are ok. in Bottles in:-

Gibco

Dulbecco's modified Eagle (high gluc.) | Pregassed with 10% CO_2 or in 10% CO_2 incubator
+10% F.C.S.
+HAT

Change medium at 2×10^5/ml by 10fold dilution.

Cell



2000V discharge from Iso 494

1984

Tues. June 12th

Transformation of β-globin-producing cells

Hunt Potter, L. Weir & P. Leder In press PNAS

E. Neumann, Schaefer-Ridder, Wang & Hofschneider EMBO J 1 (1982) 841

Zimmerman & Vienken J. Membrane Biol. 67 (1982) 165

- use hi-voltage pulse across cells + DNA to get transformation.

(39 chromosomes - normal 40)

Originally from single cell

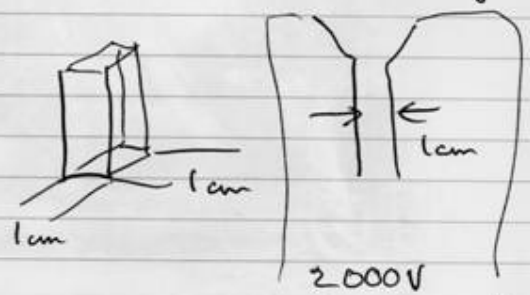
ASF 2-1 cells are hybrid between MEL cells (Hprt-) & human hybrid fibroblast with X-11 translocation. Under HAT the X-11 is retained. Making Hb at low level - can be induced with DMSO - but differentiation → halt. Stationary cultures are ok. in Bottles in:-

Gibco

Dulbecco's modified Eagle (hi gluc.) + 10% F.C.S. + HAT

Pregassed with 10% CO_2 or in 10% CO_2 incubator

Change medium at 2×10^5/ml by 10fold dilution.



1984


1984

Wed. Jan 23rd

Size fractionation using DEAE paper

DNA available from Zapping + Neo expts.:-

RG B31.1B → RG B31.4B (used αβ117)
RG B31.1C → RG B31.4C (used Cos617)

1C – 4C were equivalent to 740 colonies ~ equally divided.

1 more (to go) ≡ 67 colonies

1B – 4B ≅ 1131 [redacted] colonies / flask i.e. tube.

~200μg available of each

2 more B 98+287 colonies ≡503 when corrected

		100 μg in	~150 μg in
1B	272 μg/ml	370 μl	580 μl ✓
2B	173 μg/ml	580 μl	909 μl ✓
3B	286 μg/ml	350 μl	549 μl ✓
4B	275 μg/ml	370 μl	580 μl (by mistake) ✓

So corrected output to same

Aim to get 40 μg of size fractionated product with [redacted] range 5 – 10 kbp (to include 7.6 kbp target).
Assume yield is 100% (with this paper it is close) ⇒ that fraction comprises 10% of total, this required 400 μg of DNA, or 100 μg/tube

i.e. 4 units/μg

Ran had digest with XbaI (large lot) 4μl for 20 μg in 200 μl using 1/10 [redacted] 10×RS 1.5M NaCl ≥2 hrs at 37°

1985

Wed. Jan 23rd

Size fractionation using DEAE paper

DNA available from Zapping + Neo expts.:-

RG B31.1B → RG B31.4B (used αβ117)
RG B31.1C → RG B31.4C (used Cos 17)

1C – 4C were equivalent to 740 colonies ~ equally divided.

1 more (to go ≡ 67 colonies

1B – 4B ≅ 1131 colonies / flask i.e. tube.

~200μg available of each

2 more B 98+287 colonies ≡503 when corrected

		100 μg in	~150 μg in
1B	272 μg/ml	370 μl	580 μl ✓
2B	173 μg/ml	580 μl	909 μl ✓
3B	286 μg/ml	350 μl	549 μl ✓
4B	275 μg/ml	370 μl	580 μl (by mistake) ✓

So corrected output to same

Aim to get 40μg of size fractionated product with range 5 – 10 kbp (to include 7.6 kbp target).

Assume yield is 100% (with this paper it is close) ⇒ that fraction comprises 10% of total, this required 400μg of DNA, or 100μg/tube

i.e. 4 units/μg

Ran had digest with XbaI (large lot) 4μl for 20μg in 200μl using 1/10 [redacted] 10×RS 1.5M NaCl ≥2 hrs at 37°

1985

Wed. Jan 23rd

Size fractionation using DEAE paper

DNA available from Zapping + Neo expts.:-

RG B31.1B → RG B31.4B (used αβ117)
RG B31.1C → RG B31.4C (used Cos 17)

1C – 4C were equivalent to 740 colonies
∿ equally divided.

1 more (to go ≡ 67 colonies

1B – 4B ≅ 1131 [redacted] colonies / flask i.e. tube.
∿200μg available of each

2 more B 98+287 colonies ≡503 when corrected

		100 μg in	~~150 μg~~ in
1B	272 μg/ml	370 μl	580 μl ✓
2B	173 μg/ml	580 μl	909 μl ✓
3B	286 μg/ml	350 μl	549 μl ✓
4B	275 μg/ml	370 μl	580 μl (by mistake) ✓

So corrected extract to same

Aim to get 40 μg of size fractionated product with [redacted] range 5 – 10 kbp (to include 7.6 kbp target).
Assuming yield is 100% (with this paper it is close) → that fraction comprises 10% of total, this required 400 μg of DNA, or 100 μg/tube

i.e. 4 units/μg

Ran had digested with XbaI (large lot) 4μl for 20 μg in 200 μl using 1/10 [redacted] 10×RS 1.5M NaCl
≥2 hrs at 37°

Recombinant Fragment

1985

Wed. Jan 23rd

Size fractionation using DEAE paper

DNA available from Zapping + Neo expts.:-

RG B31.1B → RG B31.4B (used αβ117)
RG B31.1C → RG B31.4C (used Cos 17)

1C – 4C were equivalent to 740 colonies
∴ equally divided.

1 more (to go) ≡ 67 colonies

1B – 4B ≡ [redacted] colonies / flask i.e. tube.
∼200μg available of each

2 more B 98+287 colonies ≡503 when corrected

		100 μg in	∼150 μg in
1B	272 μg/ml	370 μl	580 μl ✓
2B	173 μg/ml	580 μl	909 μl ✓
3B	286 μg/ml	350 μl	549 μl ✓
4B	275 μg/ml	370 μl	580 μl (by mistake) ✓

So corrected output to same

Aim to get 40 μg of size fractionated product with [redacted] range 5 – 10 kbp (to include 7.6 kbp target).
Assuming yield is 100% (with this paper it is close) ∴ that fraction comprises 10% of total, this required 400 μg of DNA, or 100 μg/tube

i.e. 4 units/μg

Ran had digested with XbaI (large lot) 4 μl for 20 μg in 200 μl using 1/10 [redacted] 10×RS 1.5 M NaCl
≥2 hrs at 37°

1985

	Titrate	
#1	1.4×10⁸	≡ 2.4×10⁸/1.7 ml
#2	0.848×10⁸	≡ 1.4×10⁸/1.7 ml
#3	4×10⁹	≡ 2.4×10⁹/ml ×1.7 (≡ 4×10⁹/1.7 ml)
#4	4×10⁹	≡ 2.4×10⁹/ml ×1.7 (≡ 4×10⁹/1.7 ml)
#5	1.5×10⁶	≡ 1.5×10⁶/μg

1/100th of total   1/100th of total

i142 Ch3AΔlac 0.25 μg/μl from 810 μg/ml PH diluted 45 μl + 100 μl DNA dial. buffer

	#3 Small Pool	#4 Small S	#5 Control
1 μg ≅ DNA	4 μl	✓ 4 μl	i142 Charon 3AΔlac 0.25 μg/μl 4 μl
	✓ 8 μl	✓ 8 μl	8 μl
	✓ 1 μl	✓ 1 μl	✓ 1 μl
	✓ 4 μl	✓ 4 μl	✓ 4 μl
	✓ 83 μl	✓ 83 μl	Add 83 μl φ80+Mg [2]

Number on titration or plates should be divided by 4

Set for 40 min → to 37° at 5 pm

Wed. Jan 30th (1985) i139

? positive on large (#4) (#1) Pool lig eventually see i160-162

Large scale packaging → plating

i143 Large scale Pool is #1
i143 Large scale S is #2
i143 Small Scale Pool is #3
i143 Small Scale S is #4
i143 Control (3AΔlac) is #5

(#1, #3 → i139 Pool lig.; #2, #4 → i139 Sn lig)

Total 2.4×10⁸

Take out (4 μl of i139 Pool lign #3) → (4 μl of i139 S to make lign #4)

Total 1.4×10⁸

Add:—	#1 Large Pool lign (~400 μl)	#2 Large S lign (~400 μl)
{Buffer A 7 parts + Buffer M1 1 part}	✓ 800 μl	✓ 800 μl
{Sonic Extract MK102}	✓ 100 μl	✓ 100 μl
{Freeze Thaw lysate LB-12} 2.40 pm	✓ 400 μl	✓ 400 μl

37°C for 1 hr. (2.45 pm → 3.45 pm)
→ plate out
Titrate 10 μl into 1 ml φ80 + Mg [2]   Titrate 10 μl into 1 ml φ80 + Mg [2]

Each plus 3.6 ml Ca Mg Cl₂ 10 mM 10 mM

plus 3.6 ml of C1A (grown 28-29th but 1/4 dil.ᵈ today at ~1 pm with NZY & regrown)
15 min at 37°

1985

	Titrate	
#1	1.4×10⁸	≡ 2.4×10⁸/1.7 ml
#2	0.848×10⁸	≡ 1.4×10⁸/1.7 ml
#3	4×10⁹	≡ 2.4×10⁸/ml ×1.7 (≡ 4×10⁸/1.7 ml)
#4	4×10⁹	≡ 2.4×10⁸/ml ×1.7 (≡ 4×10⁸/1.7 ml)
#5	1.5×10⁶	≡ 1.5×10⁶/μg

1/100th of total — 1/100th of total

i142 Ch 3AΔlac 0.25 μg/μl

from 810 μg/ml PH diluted 45 μl + 100 μl DNA dil buffer

	#3 Small Pool	#4 Small ♁	#5 Control
1 μg ≅ DNA	4 μl	4 μl	i142 Charon 3AΔlac 0.25 μg/μl
	✓ 8 μl	✓ 8 μl	8 μl
	✓ 1 μl	✓ 1 μl	✓ 1 μl
	✓ 4 μl	✓ 4 μl	✓ 4 μl
	✓ 83 μl	✓ 83 μl	Add 83 μl φ80+Mg [2]

Set for 40 min → to 37° at 5 pm

Number titration on plates should be divided by [illegible]

Wed. Jan 30th (1985) i139

2 positive on large #4 #2 Pool ♁ eventually see i160-162

Large scale packaging → Plating

- i143 Large Scale Pool is #1
- i143 Large scale ♁ is #2
- i143 Small Scale Pool is #3
- i143 Small Scale ♁ is #4
- i143 Control (3AΔlac) is #5

(i139 Pool lig^n → #1, #2; i139 ♁ lig^n → #3, #4)

Total recov = 2.4×10⁸

Take out 4 μl of i139 Pool lig^n #3 → 4 μl of i139 ♁ lig^n to make #4

Total recov = 1.4×10⁸

Add:—	#1 large Pool lig^n (~400 μl)	#2 large ♁ lig^n (~400 μl)
{Buffer A 7 parts + Buffer M1 1 part}	✓ 800 μl	✓ 800 μl
{Sonic Extract MK102}	✓ 100 μl	✓ 100 μl
{Freeze Thaw lysate LB-12}	✓ 400 μl	✓ 400 μl

2.40 pm

37°C for 1 hr. (2.45 pm → 3.45 pm)

→ plate out

Titrate 10 μl into 1 ml φ80 + Mg [2] — Titrate 10 μl into 1 ml φ80+Mg [2]

Each plus 3.6 ml CaCl₂ MgCl₂ 10mM 10mM

plus 3.6 ml of C1A (grown 28-29th but 1/4 dil^d today at ~1 pm with NZY & regrown) 15 min at 37°

1985

Maxi To 37° at 3.01 pm

Titrations (C.B.) on K802

F	G	H
4.1	4.1	4.1
4.01 55	4.01 121	4.01 183
6.1 3		

2.5×10⁸/μg

Average by Clostie was 1×10⁸/μg

These are titres/μg

There are titres/ml ×1.9 = total ϕ

Top agar was not completely solubilized!

lifted moderately well → baked 11.40am to 1.40

Then 6×SSC Denh. from 2.00 to 3.00 pm
(put fetters into hot sol^n - tried to avoid bubbles but did not succeed. Cold may be cleaner)

Hybridized:—

	K21F.2	K21F.1
	300,000 cpm/ml ×70ml total	60,000 cpm/ml ×70 ml total
BIVS	220μl RG C33 BIVS2 raw 95,000/μl	75μl K29 5.2K
68°C Sat 3.50pm	+44μl 2M NaOH	5.2K +15μl 2M NaOH
	+44μl 2M HCl	+15μl 2M HCl
	+70ml	+70ml

Total ϕ 1.05 ×10⁸ Exp. Feb 22nd

13-25 + 52K K21F Packages

0 on IVS2

On C1A hard to count but >125

	F	G	H
DNA	Whole of K21F liq^n ~400μl ≅100μg DNA	4μl Ch3AΔlac i142	4μl Ch3AΔlac i142
Buffer A	700μl SM 1-12-84	7μl SM 1-12-84	7μl K19
Buffer M-1	100μl MK D-24	1μl MK D-24	1μl K19
Sonic extract K F102	100μl	1μl	1μl
FTL BB42	600μl	6μl	6μl
Total volume	1.9ml 1 hr	at 37°	12.35pm 1.35pm
	10μl to 1ml ϕ80+Mg	1ml ϕ80+Mg	1ml ϕ80+Mg
	3.6ml Ca/Mg → transfer to 50ml	Titrate at 4.1 → 4.01	Take titre as titrate at 4.1 → 4.01
	3.6ml C1A overnite		
	15 at 37° Plate		

1985

20 min. each way.

9.05 am Plates cracked – look fine

Controls were messed up somehow. 4.01 is nearly confluent so may have been 2.01 control

K33F 4.01	56	5.6 × 10^7	= Total ϕ 1.1 × 10^8
K33F 6.1	7		

100 on C1A

Lifted & baked 2 hrs at 68°C

Hybridized: K33F.2 / K33F.1

	βIVS 2	5.2K
Counts	300,000 cpm/ml × 100 ml RG C33 now 75,000 cpm/µl ∴ need 400 µl for 100 ml	60,000 cpm/ml × 100 ml K29 5.2K, now 44,000 cpm/µl ∴ need 136 µl for 100 ml
2M NaOH	80 µl ✓	27 µl ✓
	Wait 10 min	
2M HCl	80 µl ✓	27 µl ✓
Add	100 ml 6×SSC Denhardt	100 ml

0 on 5.2K !
0 on IVS 2

To 68° at 3.30 pm Off ~9.30 am

On film by 12 noon

Charlie repeated K27 hig^n expt. & got 91 on C1A

5 +ve 5.2K 0 IVS 2

P117

MAKE NEW PROBES

C1A 5.2 IVS — 100

Wed. Feb 27th

K33F Packaging

	K33F	K37 Control
DNA	~400 µl (~100 µg) K33F lig^n ✓	4 µl Ch3AΔlac/λ142 (1 µg) ✓
Buffer A (K19)	700 µl ✓	7 µl ✓
Buffer M1/K19	100 µl ✓	1 µl
SE MKF102	(~90 µl) Rest of 100 µl tube ✓	1 µl ✓
FTL LB BL2	600 µl ✓ ~1900 µl total	6 µl ✓

1 hr at 37°

For titration on KP802: 10 µl to 1 ml ϕ80+Mg (K33F 10^-2) ✓	+ 1 ml ϕ80+Mg (K37 Control Undil^d) for titration on KP802
Plate with 3.6 ml Ca/Mg 3.6 ml C1A. Wait 15 to 20 min at 37° before 50 ml plating	Titrate K33F at 4.1, 4.01 & 6.1. Titrate K37 control at 4.1, 4.01

Both to 37° at 4.30 pm

1985

	Titrate	
#1	1.4×10^8	$\equiv 2.4\times10^8$/1.7 ml
#2	0.848×10^8	$\equiv 1.4\times10^8$/1.7 ml
#3	$\sim4\times10^9$	$\equiv 2.4\times10^8$/ml ×1.7 ($\equiv 4\times10^8$/1.7 ml)
#4	$\sim4\times10^9$	$\equiv 2.4\times10^8$/ml ×1.7 ($\equiv 4\times10^8$/1.7 ml)
#5	1.5×10^6	$\equiv$ [redacted] 1.5×10^6/μg

i142 clu 3A Δlac 0.25 mg/μl

from 810 μg/ml PH diluted 45 μl + 100 μl DNA dilut. buffer

1/100th of total — 1/100th of total

#3 Small Pool
- 4 μl
- 8 μl
- 1 μl
- 4 μl
- 83 μl

#4 Small Σ
- 4 μl
- 8 μl
- 1 μl
- 4 μl
- 83 μl

#5 Control
- i142 clu 3A Δlac 0.25 mg/μl 8 μl
- 1 μl
- 4 μl
- Add 83 μl Φ80+Mg [2]

1 μg ≡ DNA

Numbers on titration plate should be divided by 4

Set for 40 min → to 37° at 5 pm

Wed. Jan 30th (1985)

i139

2 positive from large #4 #7 pooled eventually see i160-162

large scale packaging → plating

- i143 large scale Pool is #1 → i139 Pool hiq
- i143 large scale Σ is #2 → i139 Σ hiq
- i143 Small Scale Pool is #3
- i143 Small Scale Σ is #4
- i143 Control (3A Δlac) is #5

Total $\sim2.4\times10^8$ = 9

Take out 4 μl hiq of i139 Pool #3 → 4 μl of i139 Σ hiq to make #4

Add:—

	#1 large Pool hiq (~400 μl)	Total $\sim1.4\times10^8$	#2 large Σ hiq (~400 μl)
Buffer A 3 parts + Buffer M 1 part	800 μl		800 μl
Sonic Extract MK102	100 μl		100 μl
Freeze Thaw lysate LB-12	400 μl		400 μl
	2.40 pm		

37°C for 1 hr. (2.45 pm → 3.65 pm)

→ plate out

Titrate 10 μl into 1 ml Φ80+Mg [2] — Titrate 10 μl into 1 ml Φ80+Mg [2]

Each plus 3.6 ml Ca Mg Cl2 10 mM 10 mM

plus 3.6 ml of C1A (grown 28-29th out 1 in 4 dil.ⁿ today at ~1 pm with NZY to regrow)

15 min at 37

	Titrate	
#1	1.4×10^8	≡ 2.4×10^8 / 1.7 ml
#2	0.848×10^8	≡ 1.4×10^8 / 1.7 ml
#3	~4×10^9	≡ 2.4×10^8/ml × 1.7 (≡ 4×10^8 / 1.7 ml)
#4	~4×10^9	≡ 2.4×10^8/ml × 1.7 (≡ 4×10^8 / 1.7 ml)
#5	1.5×10^6	≡ 1.5×10^6 / μg

i142 Ch3AΔlac 0.25 μg/μl

from 810 μg/ml PH diluted 45 μl + 100 μl DNA dial. buffer

1/100th of total — 1/100th of total

	#3 Small Pool	#4 Small δ	#5 Control
	4 μl	4 μl	i142 Charon 3AΔlac 0.25 μg/μl 8 μl
1 μg DNA ≅	✓ 8 μl	✓ 8 μl	
	✓ 1 μl	✓ 1 μl	✓ 1 μl
	✓ 4 μl	✓ 4 μl	✓ 4 μl
	✓ 83 μl	✓ 83 μl	Add 83 μl φ80+Mg [2]

Number of titration or plates should be divided by 1/4

Set for 40 min → to 37° at 5 pm

2 positive on large #4 #1 Pool & eventually see i160-162

Wed. Jan 30th (1985) i139

Large scale packaging → plating

i143 Large scale Pool is #1 → i139 Pool lig^n
i143 Large scale δ is #2
i143 Small Scale Pool is #3
i143 Small Scale δ is #4 ← i139 δ lig^n
i143 Control (3AΔlac) is #5

Take out (4 μl of i139 Pool lig^n #3) → (4 μl of i139 δ lig^n #4) to make

Total 2.4×10^8

Total 1.4×10^8

Add:—	#1 large Pool lig^n (~400 μl)	#2 large δ lig^n (~400 μl)
{Buffer A 7 parts + Buffer M 1 part}	✓ 800 μl	✓ 800 μl
{Sonic Extract MK102}	✓ 100 μl	✓ 100 μl
{Freeze Thaw lysate LB-12} 2.40 pm	✓ 400 μl	✓ 400 μl

37°C for 1 hr. (2.45 pm → 3.45 pm)
→ plate out

Titrate 10 μl into 1 ml φ80 + Mg [2] — Titrate 10 μl into 1 ml φ80+Mg [2]

Each plus 3.6 ml CaMgCl₂ 10 mM 10 mM

plus 3.6 ml of C1A (grown 28-29th but 1 dil^d today at ~1 pm with NZY & regrown)
15 min at 37°

1985

Mon. April 15th

Ran will done ~~industrial cells~~ of RG B11.6 & give them to me in ~~DNA~~ pools of 20 or 50 or 100. Expect positive in less than 5 x20!

1985

(Tues) from nbarn 10 am
Fresh colony / to 0.55 by a.m.
– diluted to 0.23 → back up to 1.045 by 12 noon

In NZY/ programatically

Fresh mini plates.

144A	144B	Pool 1	3B/M	Control	
4.01 (27)	4.01 (40)	4.01 (47)	4.01 (47)	60 4.01 (55)	51 4.01 (68)
Total	Total	Total	Total	1mg	1mg
4.6×10^7	6.8×10^7	7.5×10^7	9.9×10^7	3.5×10^7	6.8×10^7

Decided to lift only Pool 1 → retest

144A → 144B at 4× DNA conc^n – insufficient inserts. Changed mind after 3 lifts other 3.

60,000 cpm/ml

300,000 cpm/ml

5.2K	IVS 2
✓ 100µl K102 probe	✓ 280µl K102 probe
✓ 18µl 2M NaOH	✓ 50µl 2M NaOH
✓ 18µl 2M HCl	✓ 50µl 2M HCl
✓ 30ml 6×SSC pen	30ml 6×SSC pen

Pool 1 To 68° @ 9.50pm Wed On film Thurs. 5.45pm

This is it! ≥ 8 positive / ~30

144A 144B 3B/M

5.2K	IVS 2
✓ 300µl K102 probe	✓ 840µl K102 probe
✓ 54µl 2M NaOH	✓ 150µl 2M NaOH
✓ 54µl 2M HCl	150µl 2M HCl
90ml 6×SSC	90ml

68° at 5.30pm Fri On film 2.30

144B 2 incorrectly labelled 3B/M. 2

Tues. May 7th

Package

	~1×10^{-6}	~1×10^{-6}	~1.5×10^{-6}	~0.5×10^{-6}	V. odd	
CIA	43	62	115	53	0 (3)	Control 60 57
5.2 IVS	7+(2) 0	15+1 0	28+(7) (8)	0		
	K115 144A lysate	K115 144B lysate	K115 Pool 1 lysate	K115 3B/M lysate	4µl ch3A dlac K77	0.25µg/µl
3.15ml I A MK1	800µl	800µl	800µl	800µl	8µl	8µl
SE CB57 450µl	100µl	100µl	100µl	100µl	1µl	1µl
		20 litres				
FTL	LB60 ~400µl	LB60 ~400	LB60 ~400µl	LB51 ~800µl 2 litres	LB60 4µl	LB51 8µl
		3 litres				
	~2pm to 3pm	1 hr @ 37°	Closer to 30°			
	10µl to 1ml	ϕ80 + mg (≡ 10^{-2})			+1ml	+1ml
K802 titrate	4.1, 4.01 6.1	4.1, 4.01 6.1	4.1, 4.01 6.1	4.1, 4.01 6.1	4.1 4.01	4.1 4.01

To 37°C @ 3.45pm

CIA 3.6ml Ca/Mg
3.6ml O.D. 1.05
15m
50ml NZTA jar
3.45pm to 37°

Sat → Try ↑ DNA conc^n by using 200µl, p95

(Tues) from 9am

Fresh colony / to 0.55 by 10 a.m.
- diluted to 0.23 → back up to 1.045 by 12 noon

In NZY overnight

Fresh mini plates.

144A	144B	Pool 1	3B/M	Control	
4.01 (27)	4.01 (40)	4.01 (47)	4.01 (47)	60 4.01 (55)	51 4.01 (68)
Total	Total	Total	Total	1/mg	1/mg
4.6×10^7	6.8×10^7	7.5×10^7	9.9×10^7	3.5×10^7	6.8×10^7

Decided to lift only Pool 1 → retest

144A → 144B at 4× DNA conc^n — insufficient inserts. Changed mind after 3 lifts other 3.

60,000 cpm/ml

5.2K	IVS 2
✓ 100µl K102 probe	✓ 280µl K102 probe
✓ 18µl 2M NaOH	✓ 50µl 2M NaOH
✓ 18µl 2M HCl	✓ 50µl 2M HCl
✓ 30ml 6×SSC per	30ml 6×SSC per

300,000 cpm/ml

Pool 1 To 68° @ 9.50pm Wed On film Thurs. 5.45pm

This is it! ≥ 8 positive / ~30

144A 144B 3B/M

5.2K	IVS 2
✓ 300µl K102 probe	✓ 840µl K102 probe
✓ 54µl 2M NaOH	✓ 150µl 2M NaOH
✓ 54µl 2M HCl	150µl 2M HCl
90ml 6×SSC	90ml

68° at 5.30pm Fri On film 2.30pm

144B 2 incorrectly labelled 3B/M. 2

Tues. May 7th

	~1×10^{-6}	~1×10^{-6}	~1.5×10^{-6}	~0.5×10^{-6}	V. odd	
CIA	43	62	115 (284) (8)	53	0+(3)	Control 60 57
5.2 IVS	7+(2) 0	15+1 0			0	
	K115 144A whole	K115 144B whole	K115 Pool 1 whole	K115 3B/M whole		4µl ch3A&Lac K77 0.25µg/µl
3.15 ml I A MM1	800µl	800µl	800µl	800µl		8µl 8µl
SE CB57 450µl	100µl	100µl	100µl	100µl		1µl 1µl
FTL	LB60 ~400µl	LB60 ~400	LB60 ~400µl	LB51 ~800µl 2 tubes	LB60 4µl	LB51 8µl
		20 tubes	3 tubes			
	~2pm to 3pm	1 hr @ 37°	closer to 300			
	10µl to 1ml	ϕ80 + mg (≡ 10^{-2})			+1ml	+1ml
K802 titration	4.1, 4.01 6.1	4.1, 4.01 6.1	4.1, 4.01 6.1	4.1, 4.01 6.1	4.1 4.01	4.1 4.01

To 37°C @ 3.45pm

CIA 3.6ml Ca/Mg
3.6ml O.D. 1.05
15m
50ml NZTA jar
3.45pm to 37°

Sat Try ↑ DNA conc^n by using 200µl, p95

(Tues) from νbam

Fresh colony / to 0.55 by 10 a.m.
– diluted to 0.23 → back up to 1.045 by 12 noon

In NZY overnight

Fresh mini plates.

144A	144B	Pool 1	3B/M	Control	
4.01 (27)	4.01 (40)	4.01 (47)	4.01 (47)	60 4.01 (55)	51 4.01 (68)
Total	Total	Total	Total	1mg	1mg
4.6×10^7	6.8×10^7	7.5×10^7	9.9×10^7	3.5×10^7	6.8×10^7

Decided to lift only Pool 1 → retest

144A → 144B at 4× DNA conc^n – insufficient inserts. Changed mind after 3 lifts other 3.

60,000 cpm/ml

300,000 cpm/ml

5.2K	IVS 2
✓ 100μl K102 probe	✓ 280μl K102 probe
✓ 18μl 2M NaOH	✓ 50μl 2M NaOH
✓ 18μl 2M HCl	✓ 50μl 2M HCl
✓ 30ml 6×SSC pen	30ml 6×SSC pen

Pool 1 To 68° @ 950pm (after) Fri film Thurs. 5.45pm

This is it! ≥ 8 positive /~30

144A 144B 3B/M

5.2K	IVS 2
✓ 300μl K102 probe	✓ 840μl K102 probe
✓ 54μl 2M NaOH	✓ 150μl 2M NaOH
✓ 54μl 2M HCl	✓ 150μl 2M HCl
90ml 6×SSC	90ml

68° at 5.30pm Fri on film 2.30

144B 2 incorrectly labelled 3B/M. 2

Tues. May 7th

	≈ 1×10^{-6}	≈ 1×10^{-6}	≈ 1.5×10^{-6}	≈ 0.5×10^{-6}	V. odd	
CIA	43	62	115	53	0+(3)	Control 60 57
5.2 IVS	7+(2) / 0	15+(1) / 0	28+(7) (8)	0	0	
	K115 144A whole	K115 144B whole	K115 Pool 1 whole	K115 3B/M whole	4μl ch3A dlac K77	0.25μg/μl
3.15 ml I / A / MK1	800μl	800μl	800μl	800μl	8μl	8μl
SE CB57 450μl	100μl	100μl	100μl	100μl	1μl	1μl
		20 litres				
FTL	LB60 ~400μl	LB60 ~400	LB60 ~400μl	LB51 ~800μl 2 litres	LB60 4μl	LB51 8μl
		3 litres				
~2pm	to 3pm	1 hr @ 37°	Closer to 30s			
	10μl to 1ml	ϕ80 + mg (≡ 10^{-2})			+1ml	+1ml
K802 titration	4.1, 4.01 / 6.1	4.1, 4.01 / 6.1	4.1, 4.01 / 6.1	4.1, 4.01 / 6.1	4.1 4.01	4.1 4.01

To 37°C @ 3.45pm

CIA 3.6ml Ca/Mg
3.6ml O.D. 1.05
15m
50ml NZTA jar

3.45pm to 37°

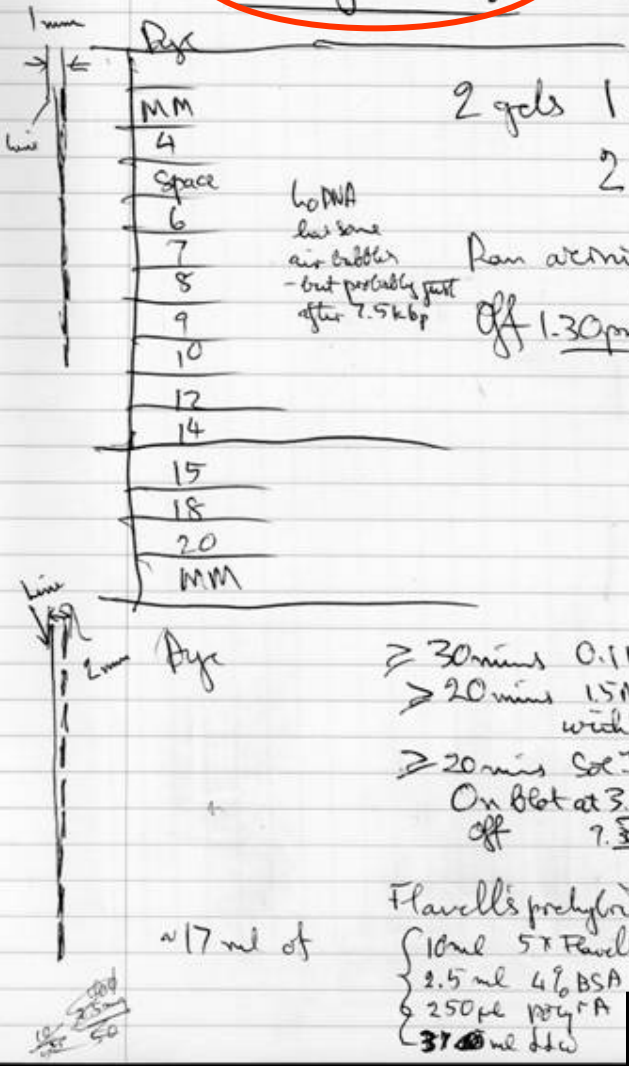
Sat Try ↑ DNA conc^n by using 200μl, p95

See p 13

3 years
1 month

Sat. May 18th

Colony Blots!



2 gels 1 low DNA
2 hi DNA

Run overnight (Ran)

Off 1.30pm Sat

lo DNA had some air bubbles - but probably just after 7.5 kbp

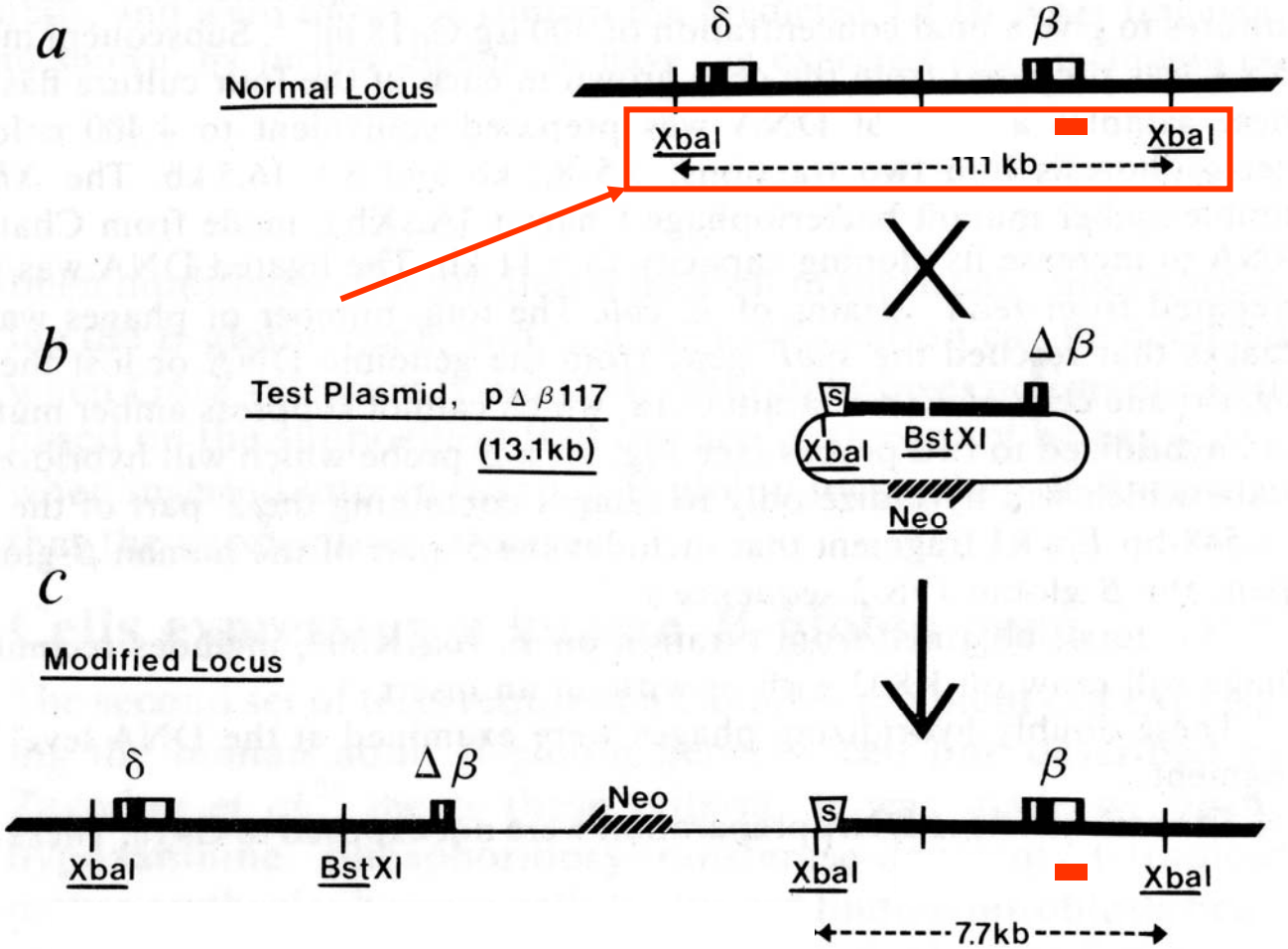
≥ 30 mins 0.1 M HCl
≥ 20 mins 1.5 M NaCl 0.5 M NaOH with 1 change
≥ 20 mins Sol. II with one change
On blot at 3.30pm Sat.
off 7.30am Sun ~4 hr bake

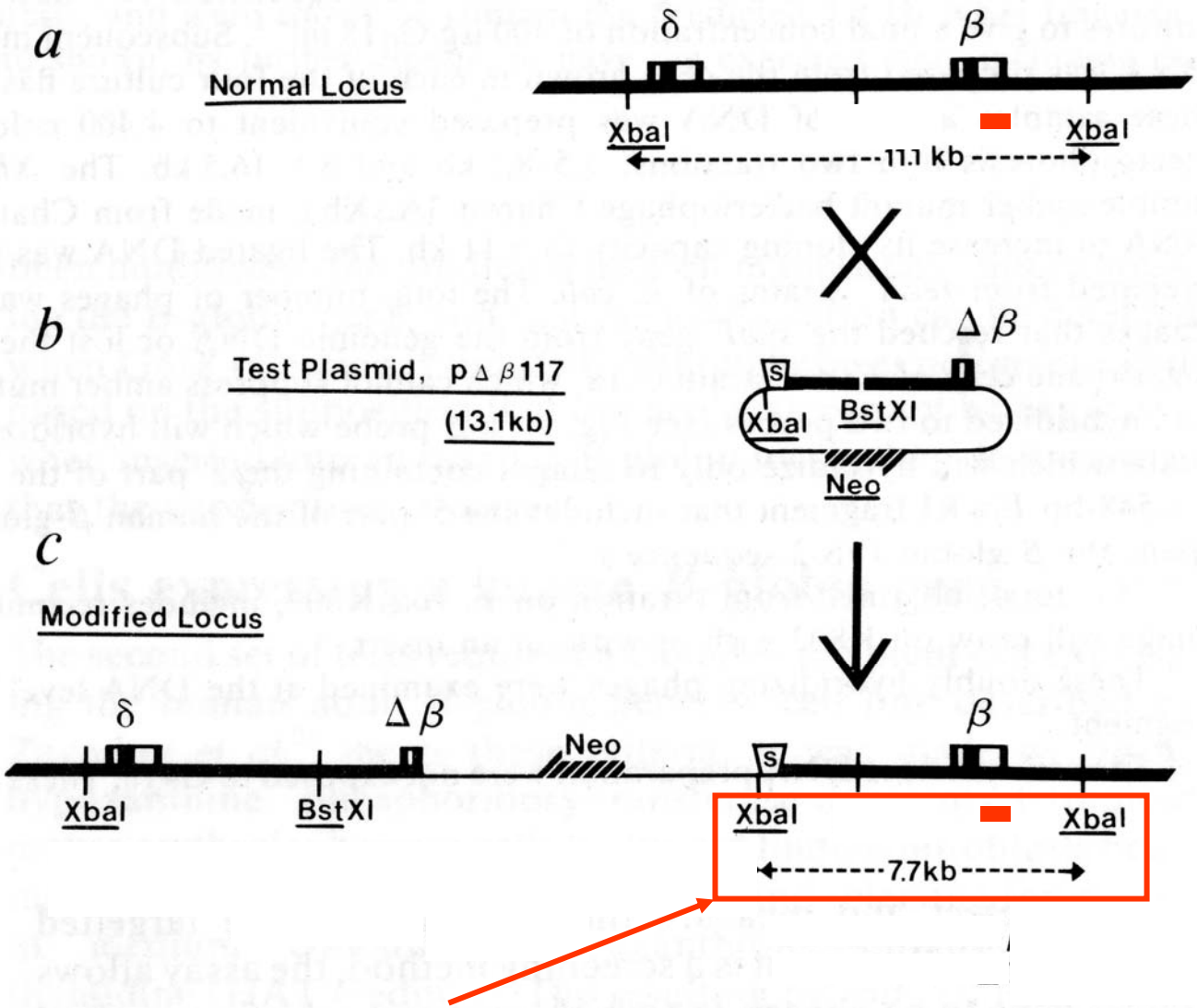
Flavell's prehybridize using
10 ml 5× Flavell's
2.5 ml 4% BSA
250 µl 10 mg/ml tRNA
37 ml H₂O

3.50pm
4.50pm

~17 ml of

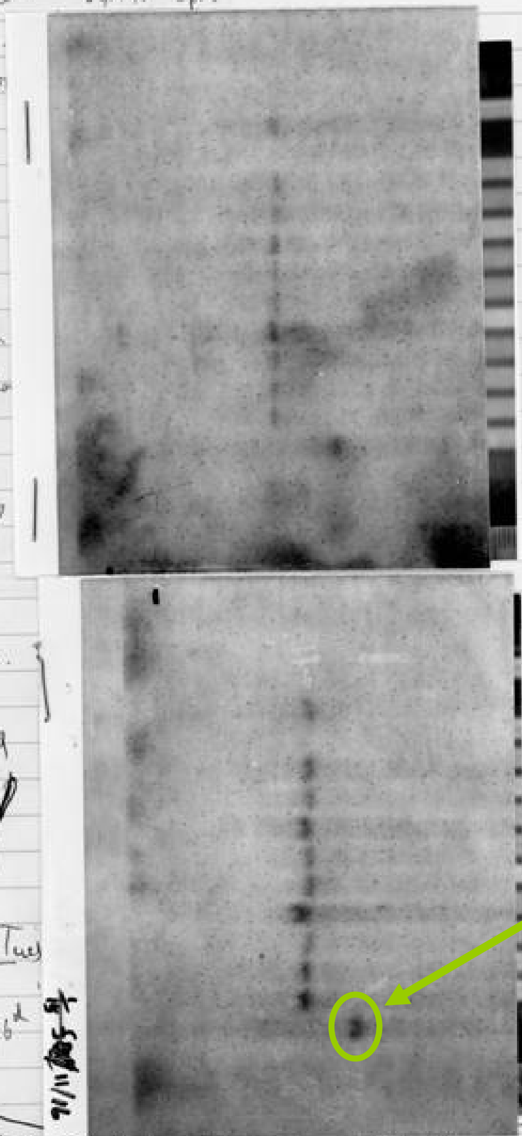
1985


a
Normal Locus
δ
β
XbaI
XbaI
11.1 kb
b
Test Plasmid, pΔβ117
(13.1kb)
Δβ
S
XbaI
BstXI
Neo
c
Modified Locus
δ
Δβ
Neo
β
S
XbaI
BstXI
XbaI
XbaI
7.7kb


a
Normal Locus
δ
β
XbaI
XbaI
11.1 kb
b
Test Plasmid, pΔβ117
(13.1kb)
Δβ
S
XbaI
BstXI
Neo
c
Modified Locus
δ
Δβ
Neo
β
S
XbaI
BstXI
XbaI
XbaI
7.7kb

Copyright © Oliver Smithies

Hybridized to ✓ + 85 μl Sonicated Salmon Sperm DNA
750 μl K133IVS2 probe 64 × 10⁶ cpm
150 μl 2M NaOH
150 μl 2M HCl
17 ml Flavells 1x
(i.e. 3.6 × 10⁶/ml
~ nearly 2x)
½ to each bag
On at 5.40 pm Sun
Off at 10.20 am Mon
(17 hr)
3x rinsed
2x wash – total
~1 to 1½ hr
On film
12 noon Mon.
May 20th
Off 9.15 Tues
#20 is it!
Back on film
9.45 am May 21st Tues
↓
8.30 pm Sun May 26th
Same result
Equivalent phage
Rescued to get 7.6 kb phage as minimum



Recombinant Fragment

Sat. May 18th

See p 13
3 years 1 month

Colony Blots!

1 mm
line
Dye
MM
4
space
6
7
8
9
10
12
14
15
18
20
MM
line
2 mm
Dye

2 gels 1 low DNA
2 hi DNA

lo DNA
has some
air bubbles
– but probably past
after 7.5 kbp

Ran overnight (Ran)
Off 1.30 pm Sat

≥ 30 mins 0.1 M HCl
≥ 20 mins 1.5 M NaCl 0.5 M NaOH
with 1 change
≥ 20 mins Solⁿ II with one change
On blot at 3.30 pm Sat.
off 7.30 am Sun ~4 hr
bake

Flavell's prehybridize using
~17 ml of
{ 10 ml 5x Flavells
2.5 ml 4% BSA
250 μl poly rA
3.7 ml ddw }
3.50 pm
4.50 pm

1985

Hybridised to ✓ Sonicated Salmon Sperm DNA
64 × 10⁶ cpm
+55 µl
750 µl K133IVS2 probe
150 µl 2M NaOH
150 µl 2M HCl
17 ml Flavells 1×
(i.e. 3.6 × 10⁶/ml
- nearly 2×)
½ to each bag
On at 5.30pm Sun
Off at 10.20am Mon
(17hr)
3× rinsed
2× wash - total
~1 to 1½ hr
On film
12 noon Mon.
May 20th
Off 9.15 Tues

#20 is it!

Back on film
9.45am May 21st Tues
↓
8.30pm Sun May 26th

Same result
Equivalent phage

Rescued to get 7.6kb phage

K135 Bgl II

Sat. May 18th

Coloring Blots!

See p 13
3 years
1 month

1mm
Bgl
MM
4
Space
6
7
8
9
10
12
14
15
18
20
MM
Line
2mm Bgl

2 gels 1 low DNA
2 hi DNA

Lo DNA had some air bubbles - but probably past after 7.5kbp

Ran overnight (Ran)
Off 1.30pm Sat

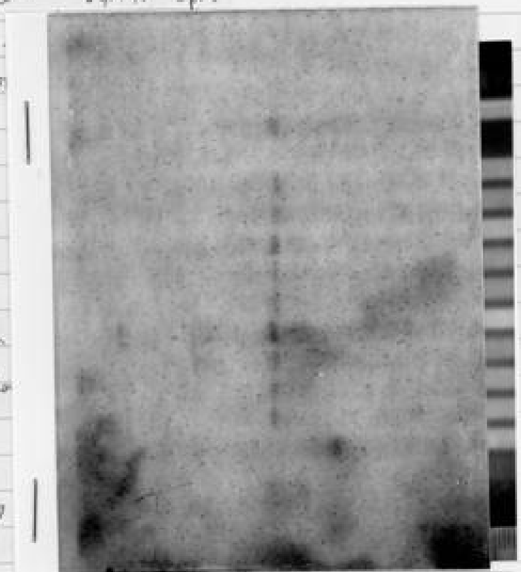
≥ 30 mins 0.1M HCl
≥ 20 mins 1.5M NaCl 0.5M NaOH with 1 change
≥ 20 mins Sol. II with one change
On blot at 3.30pm Sat.
Off 7.30am Sun ~4hr bake

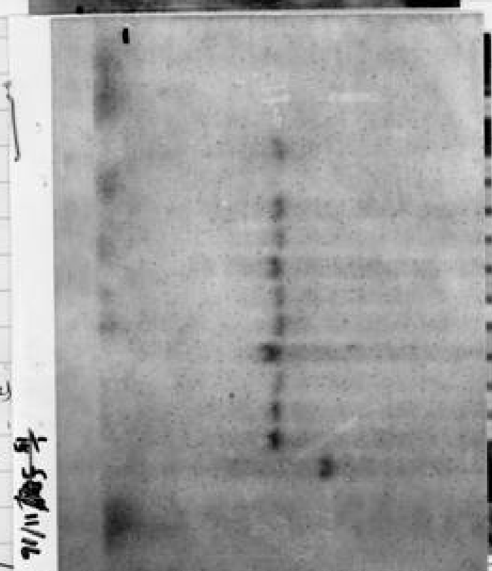
~17 ml of

Flavell's prehybridise using
10ml 5× Flavell's
2.5 ml 4% BSA
250 µl Poly rA
37 ml H₂O
3.50pm
4.50pm

1985

Hybridized to ← + 85µl Sonicated Salmon Sperm DNA
750µl K133IVS2 probe 64×10^6 cpm
150µl 2M NaOH
150µl 2M HCl
17 ml Flavell's 1x
(i.e. 3.6×10^6/ml
– nearly 2×)
½ to each bag
On at 5pm Sun
Off at 10.20am Mon
(17hr)
3× rinsed
2× wash – total
~1 to 1½ hr
On film
12 noon Mon.
May 20th
Off 9.15 Tues
#20 is it!
Back on film
9.45am May 21st Tues
↓
8.30pm Sun May 26th
Same result
Equivalent phage
Rescued to get 7.6kb phage ...





Sat. May 18th

See p 13
3 years 1 month

Colony Blots!

1mm
Dye
line
MM
4
Space
6
7
8
9
10
12
14
15
18
20
MM
line
2mm
Dye

2 gels 1 low DNA
2 hi DNA

Lo DNA had some air bubbles – but probably just after 7.5kbp

Ran overnight (Ran)
Off 1.30pm Sat

≥ 30 mins 0.1M HCl
≥ 20 mins 1.5M NaCl 0.5M NaOH with 1 change
≥ 20 mins Soln II with one change
On blot at 3.30pm Sat.
Off 7.30am Sun ~4 hr bake

Flavell's prehybridize using ~17 ml of
10ml 5× Flavell's
2.5 ml 4% BSA
250µl poly rA 10mg/ml
37 ml ddw
3.50pm
4.50pm
Then

With Ron Gregg, Sallie Boggs, Mike Koralewski & Raju Kucherlapati, 1985

Mon Dec 30th

EK cell system

Feeder layer STO fibroblasts (HPRT i.e. thioguanine & ouabain resistant)
Subcultured & later frozen

EK cells CC1.2
Originally received end of Nov. 1985.
One flask was contaminated.
One was trypsinized & frozen in 8 ampoules

One ampoule was thawed & onto P100. From ~9-10th Dec. to Dec. 23rd to get ~8 colonies.

Trypsinized & removed a few by pipette onto P10 of STO
Remainder dispersed & onto P100 of STO. (2°)
Also thawed out 3 new vials – 2 onto P100, 1 onto P60. (1°)
Dec. 30th Several 100 to 1000 colonies on P100 } 2°
& comparable density on P60 }
A few colonies on P100 & P60 1°

The cells are fine – but we must improve freezing.

Plan it to use these to get HPRT by recomb.
& get chimeras & germline by blastocyst route.

1985

Mon Dec 30th

EK cell system

Feeder layer (STO fibroblasts) (HPRT⁻ i.e. thioguanine & ouabain resistant)
Subcultured & later frozen

EK cells CC1.2

Originally received end of Nov. 1985.
One flask was contaminated
One was trypsinized & frozen in 8 ampoules

One ampoule was thawed & onto P100. From ~9-10th Dec. to Dec. 23rd to get ~8 colonies.

Trypsinized & removed a few by pipette onto P10 of STO
Remainder dispersed & onto P100 of STO. (2°)
Also thawed out 3 new vials – 2 onto P100, 1 onto P60. (1°)
Dec. 30th Several 100 to 1000 colonies on P100 } 2°
& comparable density on P60 }
A few colonies on P100 & P60 1°

The cells are fine – but we must improve freezing.

Plan is to use these to get HPRT⁻ by recomb.ⁿ & get chimeras & germline by blastocyst route.

Brought personally by Martin Evans

1985

Mon Dec 30th

EK cell system

Feeder layer STO fibroblasts (HPRT⁻ i.e. thioguanine & ouabain resistant)
Subcultured & later frozen

EK cells CC1.2
Originally received end of Nov. 1985.
One flask was contaminated.
One was trypsinized & frozen in 8 ampoules

One ampoule was thawed & onto P100. From ~9-10th Dec. to Dec. 23rd to get ~8 colonies.

Trypsinized & removed a few by pipette onto P10 of STO
Remainder dispersed & onto P100 of STO. (2°)
Also thawed out 3 new vials – 2 onto P100, 1 onto P60. (1°)
Dec. 30th Several 100 to 1000 colonies on P100 } 2°
& comparable density on P60 }
A few colonies on P100 & P60 1°

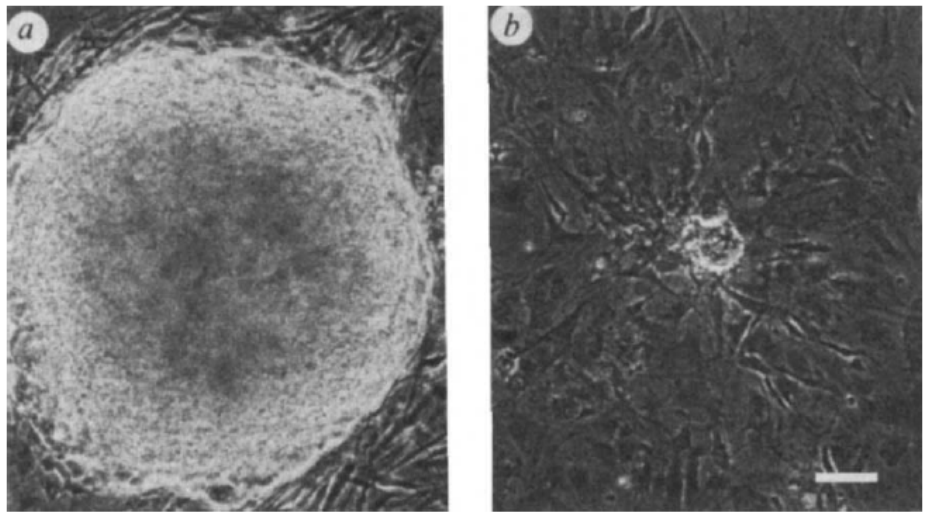
The cells are fine – but we must improve freezing.

Plan is to use these to get HPRT⁻ by recomb.ⁿ & get chimeras & germline by blastocyst route.

1985

Targetted correction of a mutant HPRT gene in mouse embryonic stem cells

Thomas Doetschman*, Ronald G. Gregg*,
Nobuyo Maeda*, Martin L. Hooper†,
David W. Melton‡, Simon Thompson‡
& Oliver Smithies*§



Nature *330* 576-578 (1987)

(By HAT Selection)

Wed Dec 17th

New idea for recombinants using amplification

PCR

Substitution by probe that covers region of diff

[Deletion by probe that lies within deletion]

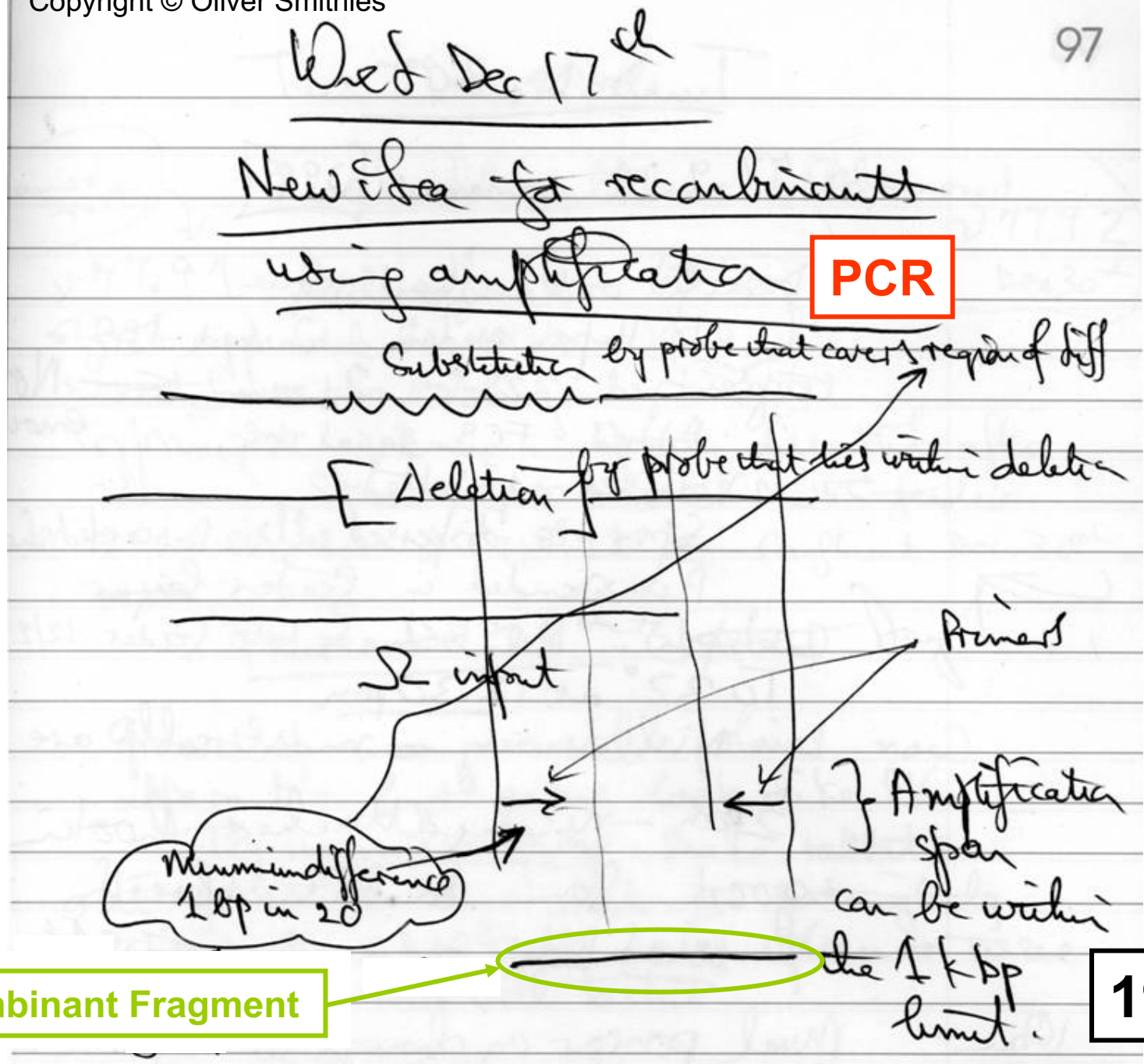
Primers

Ω input

Minimum difference 1 bp in 20

} Amplification span can be within the 1 kbp limit.

1986

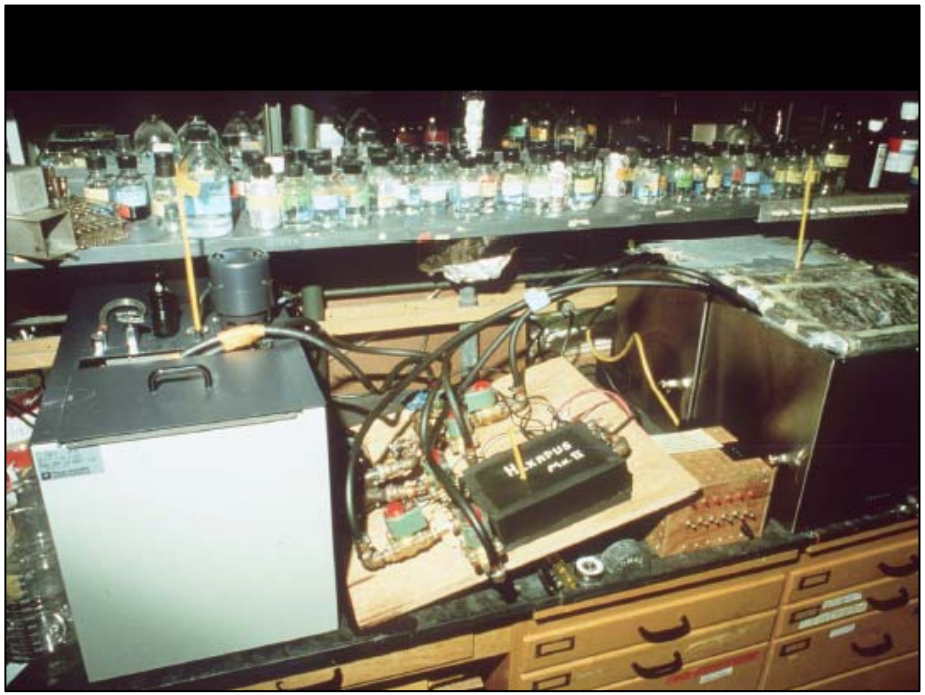


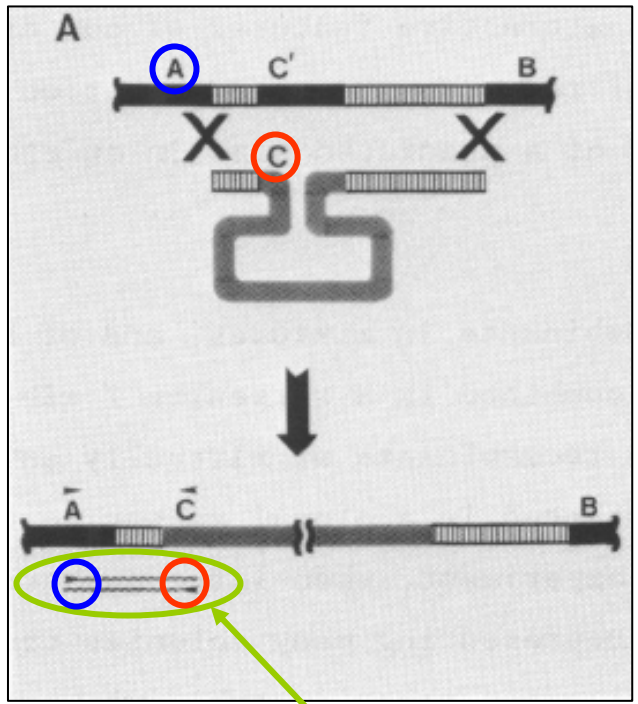
Recombinant Fragment

1986

Recombinant fragment assay for gene targetting based on the polymerase chain reaction

Hyung-Suk Kim and Oliver Smithies

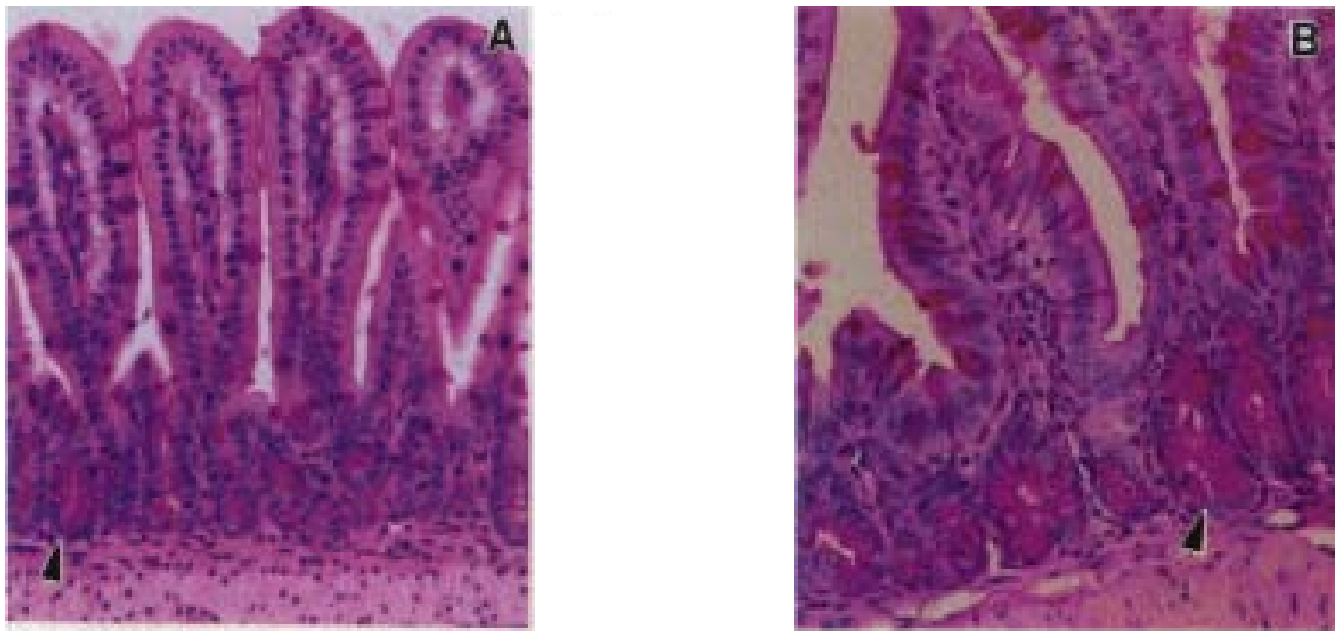




Nucleic Acids Research *16* 8887-8903 (1988)

An Animal Model for Cystic Fibrosis Made by Gene Targeting

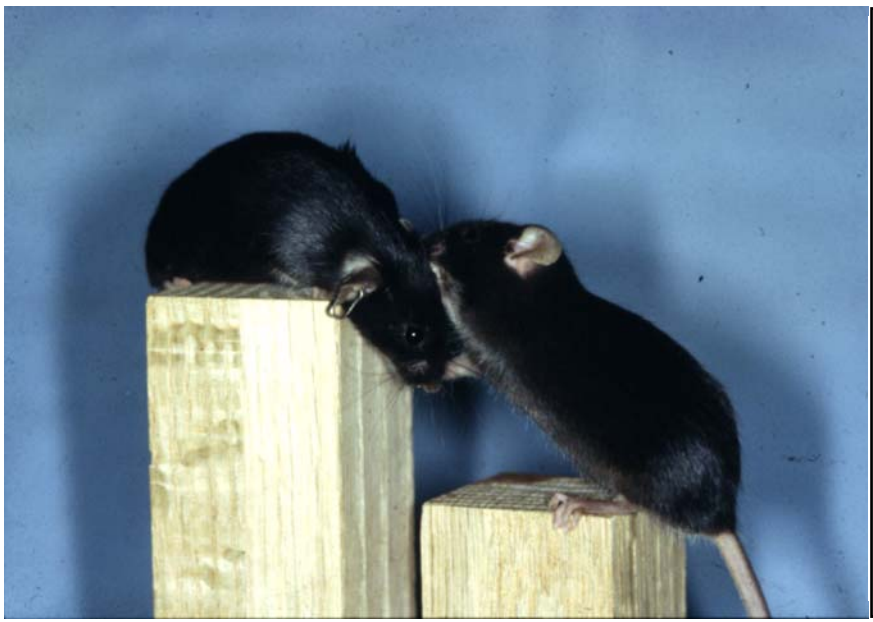
John N. Snouwaert, Kristen K. Brigman, Anne M. Latour, Nadia N. Malouf, Richard C. Boucher, Oliver Smithies, Beverly H. Koller*

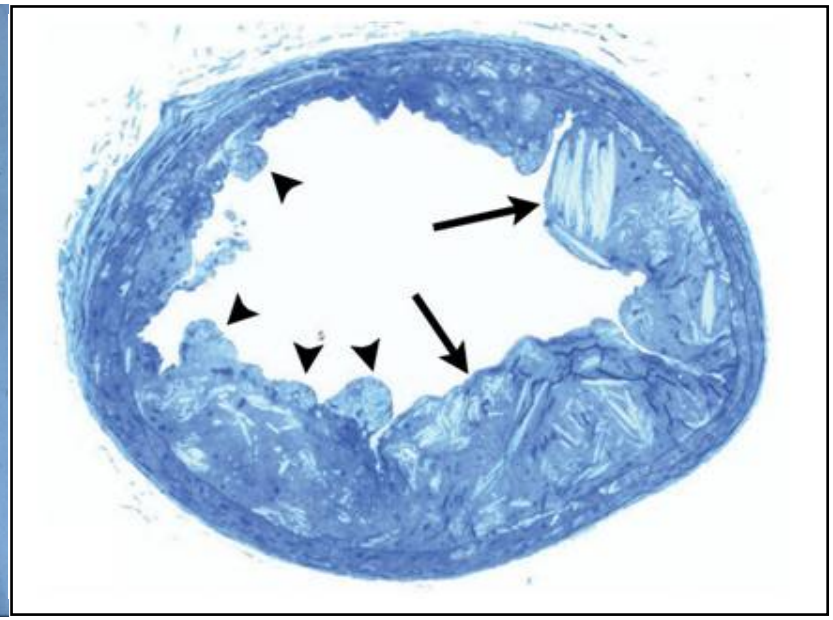


Science *257* 1083-6 (1992)

Spontaneous Hypercholesterolemia and Arterial Lesions in Mice Lacking Apolipoprotein E

Sunny H. Zhang, Robert L. Reddick, Jorge A. Piedrahita, Nobuyo Maeda*

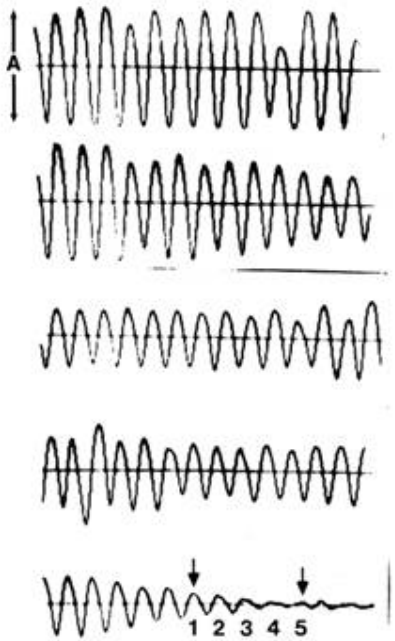




Science *258* 468-471 (1992)

Angiotensin-Converting Enzyme Gene Mutations, Blood Pressures, and Cardiovascular Homeostasis

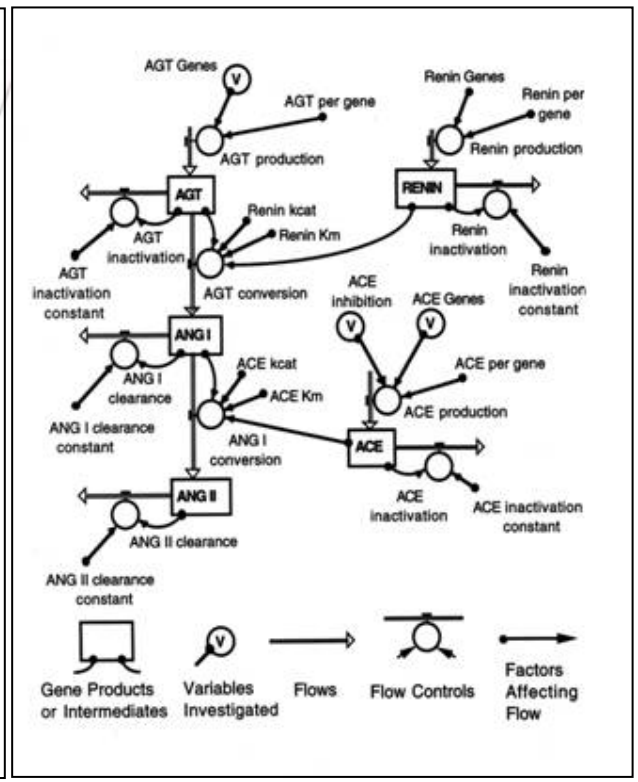
John H. Krege, Hyung-Suk Kim, Jeffrey S. Moyer, J. Charles Jennette, Li Peng, Sylvia K. Hiller, Oliver Smithies



Hypertension *29* 150-157 (1997)

Importance of quantitative genetic variations in the etiology of hypertension

OLIVER SMITHIES, HYUNG-SUK KIM, NOBUYUKI TAKAHASHI, and MARSHALL H. EDGELL



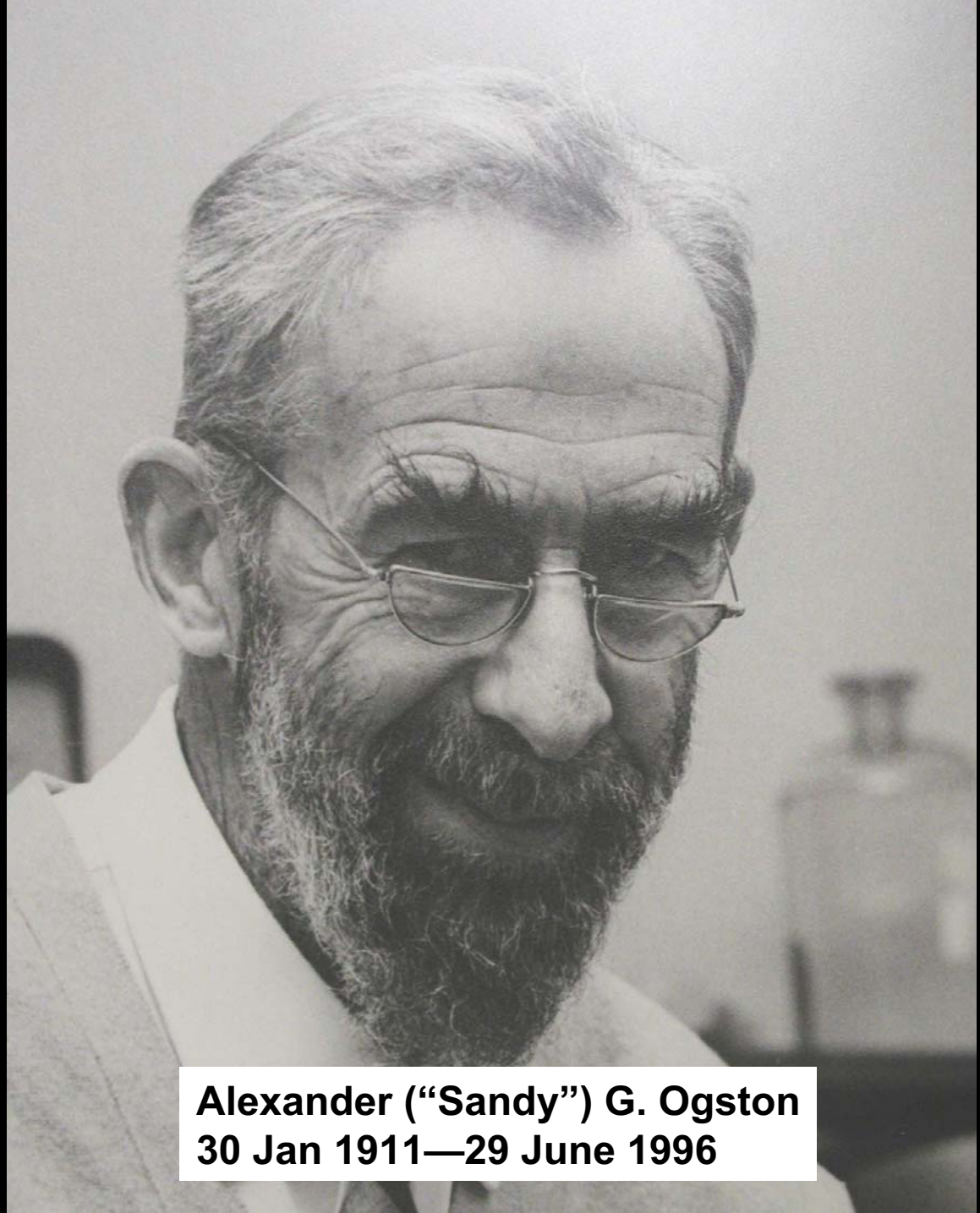
Kidney International *58* 2265-80 (2000)

Frontiers in Nephrology Research – Applications to the Clinic

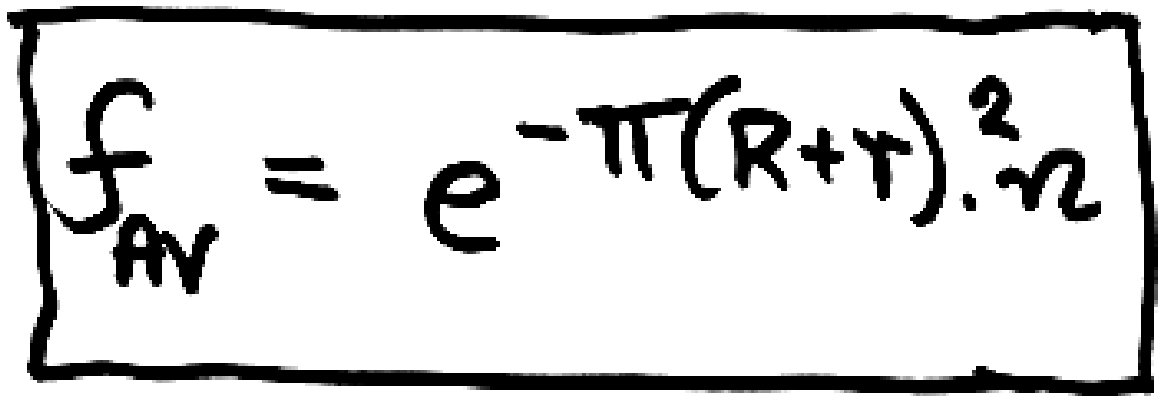
Friday, September 6, 2002

Nobel Forum, Stockholm, Sweden

Organizers:	**Anita Aperia and Karl Tryggvason**
	Chairs: A. Erik G. Persson and Anita Aperia
09.00-09.45	**Andrew McMahon** (Harvard University, USA) *Cell signaling in mammalian kidney development*
09.45-10.30	**Oliver Smithies** (University of North Carolina at Chapel Hill, USA) *Mouse solutions to human problems*
10.30-11.00	COFFEE
11.00-11.45	**Richard Lifton** (Yale University, USA) *Targets for the rational treatment of hypertension: Insights from human genetics*
11.45-12.30	**Karl Tryggvason** (Karolinska Institutet, Sweden) *Molecular make-up of the renal filter*



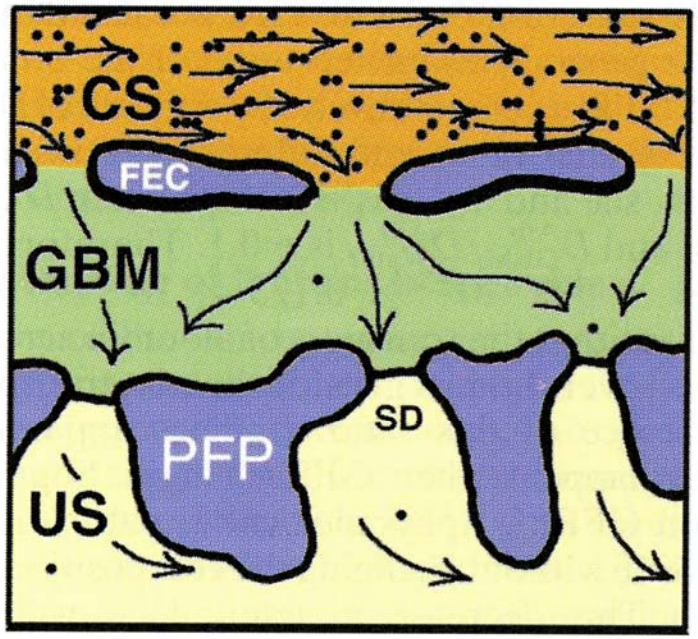
Alexander (“Sandy”) G. Ogston
30 Jan 1911—29 June 1996

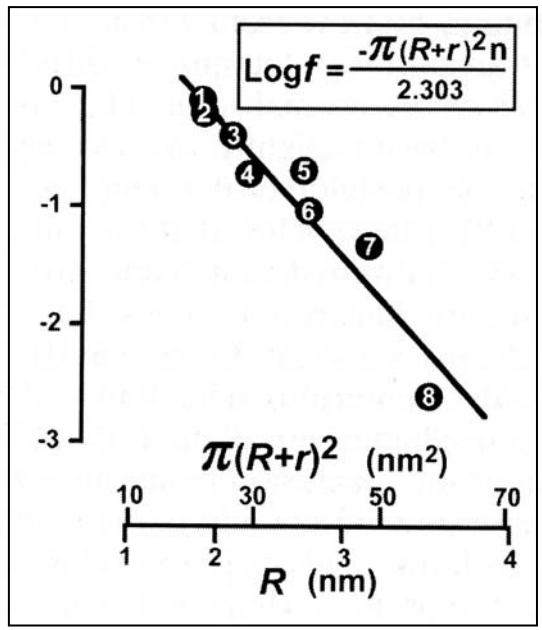


Ogston, 1958.

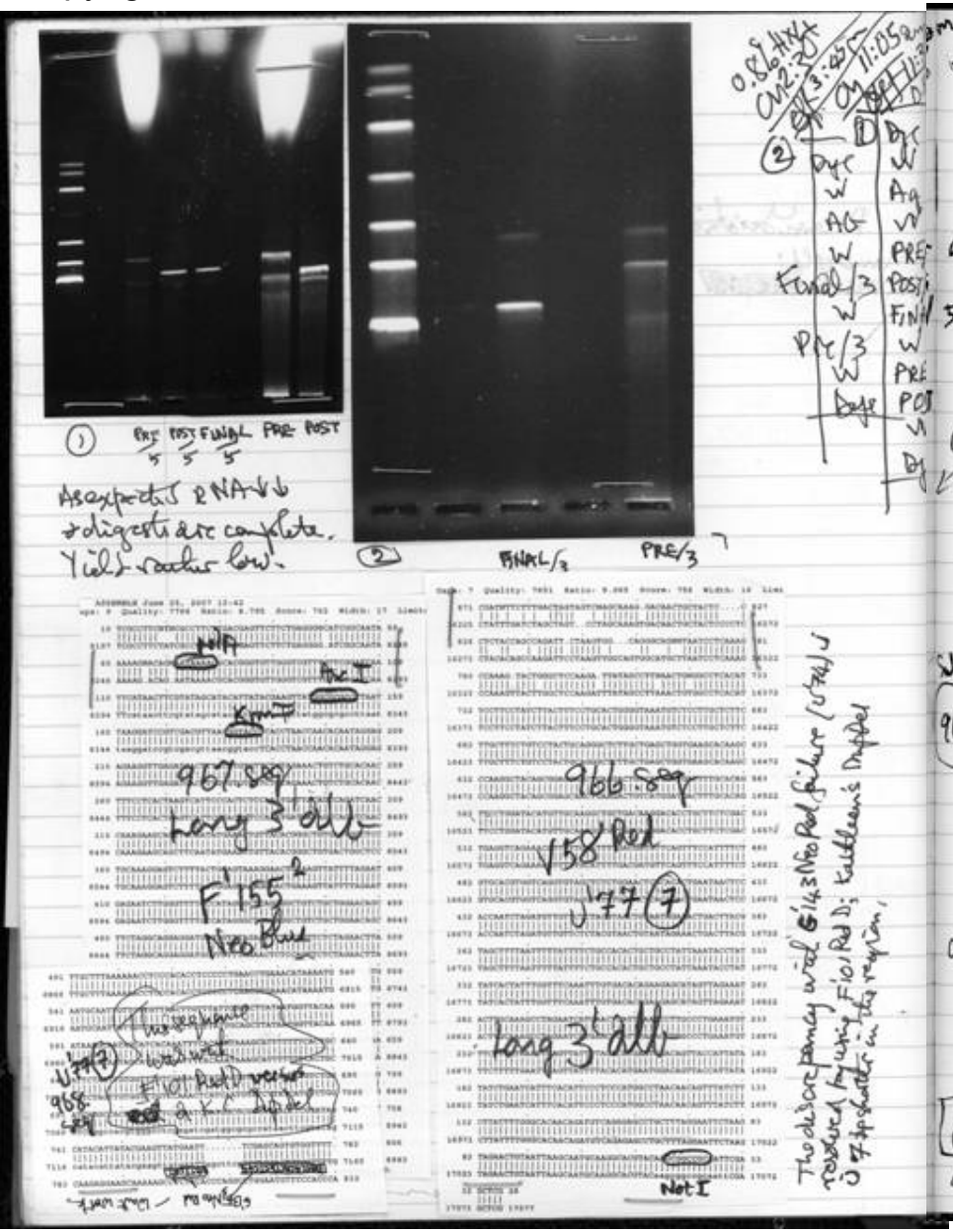
Why the kidney glomerulus does not clog: A gel permeation/diffusion hypothesis of renal function

Oliver Smithies*





PNAS *100* 4108-13 (2003)



Sun. July 29th

<5 weeks!

Bulk sequences of final candidate

Sun. July 29, 2007

What's on the next page?

I don't know!

But that's what makes
Science exciting!


G 109 B
N84KS

A. G. OGSTON, 1911-1996

"For science is more than the search for truth, more than a challenging game, more than a profession. It is a life that a diversity of people lead together, in the closest proximity, a school for social living. We are members one of another."

A.G.Ogston,

Australian Biochem. Soc. Annual Lecture,

***Search*, Vol.1, No.2, August, 1970.**