April 8, 1977

SCIENCE

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

8 April 1977, Volume 196, No. 4286

a HUMAN

b RAT

1100.5

DP 50 sup F

DP 50

SURVIVING FRACTION OF BACTERIA

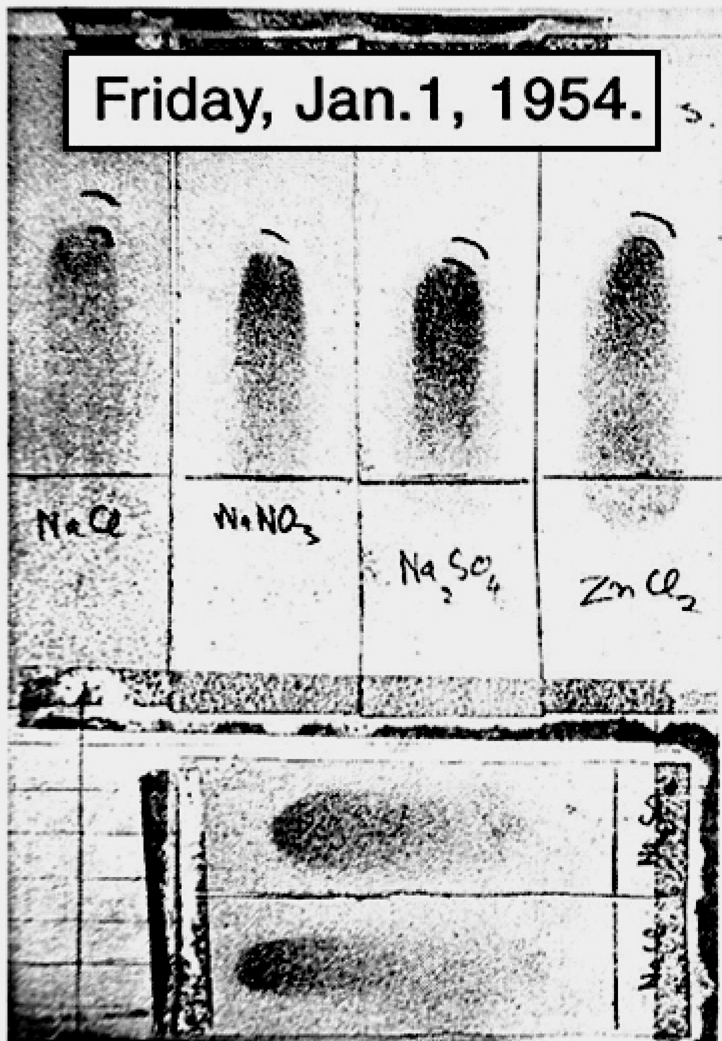
10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}

0 24 48 72 HOURS

Fig. 4. Survival of host and vector in natural environments. Survival i
debilitated laboratory hosts DP50 and DP50SupF and, for compariso
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

Copyright © Oliver Smithies

Copyright © Oliver Smithies



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 ($3\frac{1}{6}$ 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 of those substances reduces tail much as [illegible] for a longer times

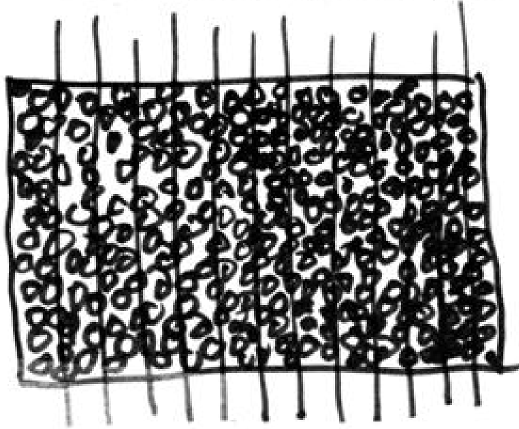
Try Al''' or Al'''' if can find.

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

Try other papers before.

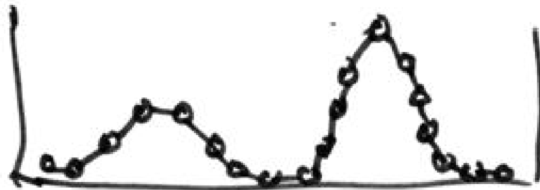
Saturday am, Jan.23, 1954

Starch Grains



Slice

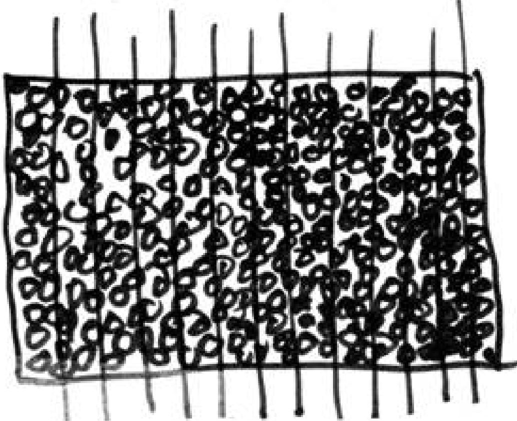
Protein Anal.



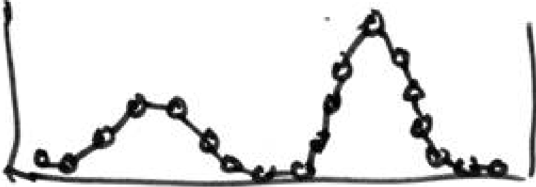
Copyright © Oliver Smithies

Saturday pm, Jan.23, 1954

Starch Grains

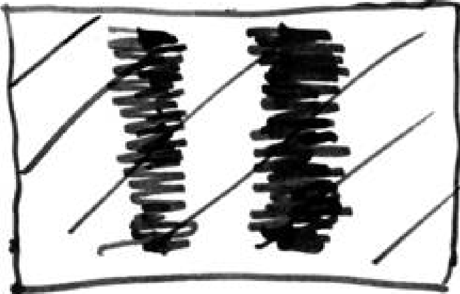


Slice | Protein Anal.



Starch Gel

Stain



Copyright © Oliver Smithies

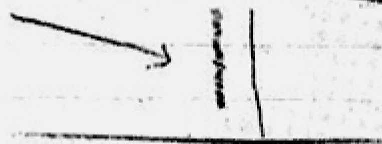
Copyright © Oliver Smithies

Saturday, Jan. 23, 1954.

Jan 23rd

Set up with dilute soluble starch jelly containing 0.1M HAc + 0.01M $MgCl_2$ (? rec'd). Narrow slit in jelly + introduced 1/10 ins. in 0.1M 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. ~~[illegible]~~
Reset up + made much larger hole in deeper + more viscous jelly ~ $\frac{1}{32}$" across + covered whole with liquid paraffin.
On at 3.45 220v ~ 16 ma if it works clearly stiffness of jelly unimportant.
Current ~ 20 ma at 4.0. Off at 7.30

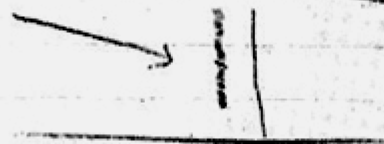
Copyright © Oliver Smithies

Saturday,
Jan. 23, 1954.

Jan 23rd

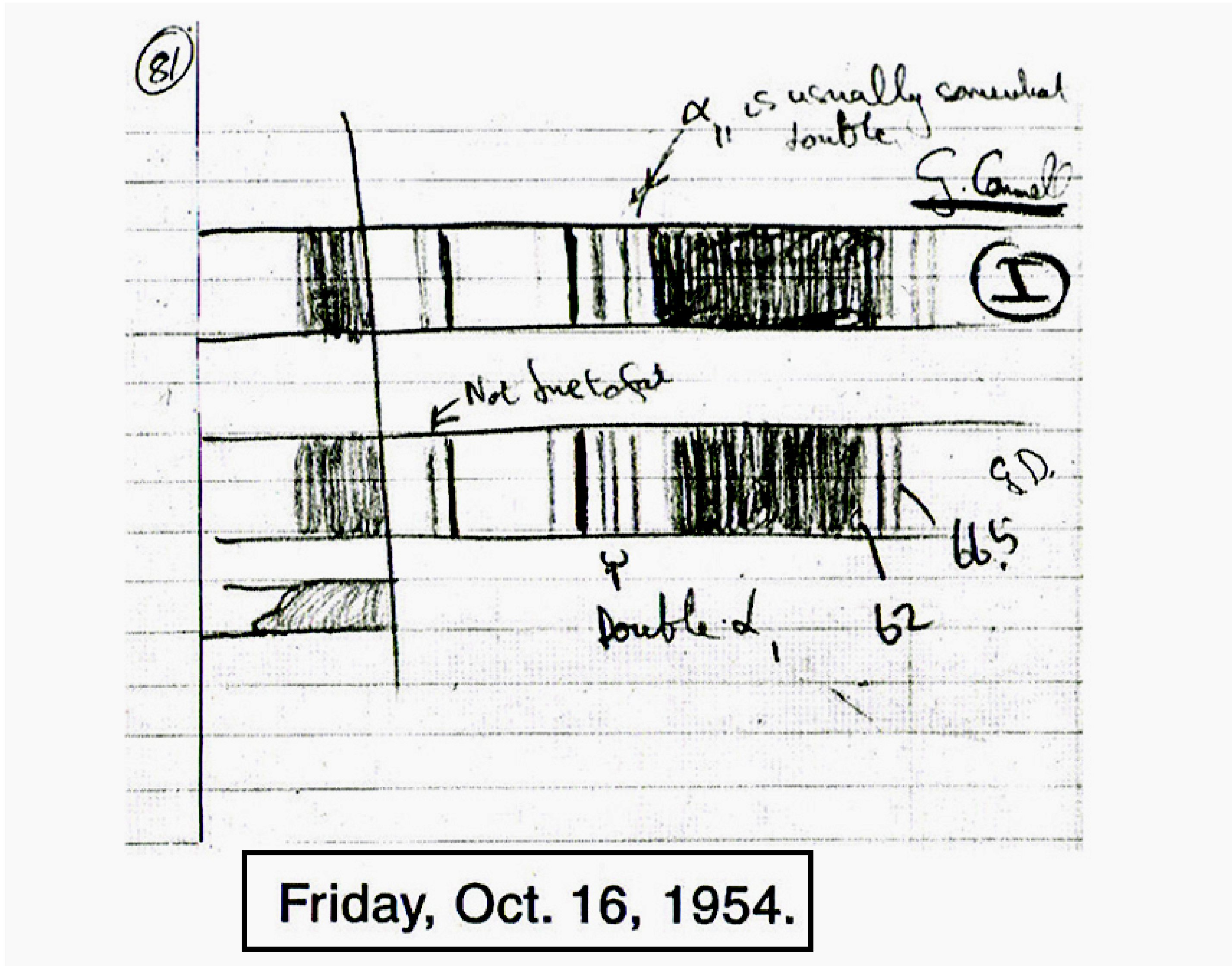
Set up with dilute soluble starch jelly containing 0.1 M HAc + 0.01 M MgCl₂ (? fec?). Na tc slit in jelly + introduced 1/10 ins. in 0.1 M HAc ?
Ran ~ $\frac{3}{4}$ hr. with 220V.

Result v. promising though only surface staining poss.



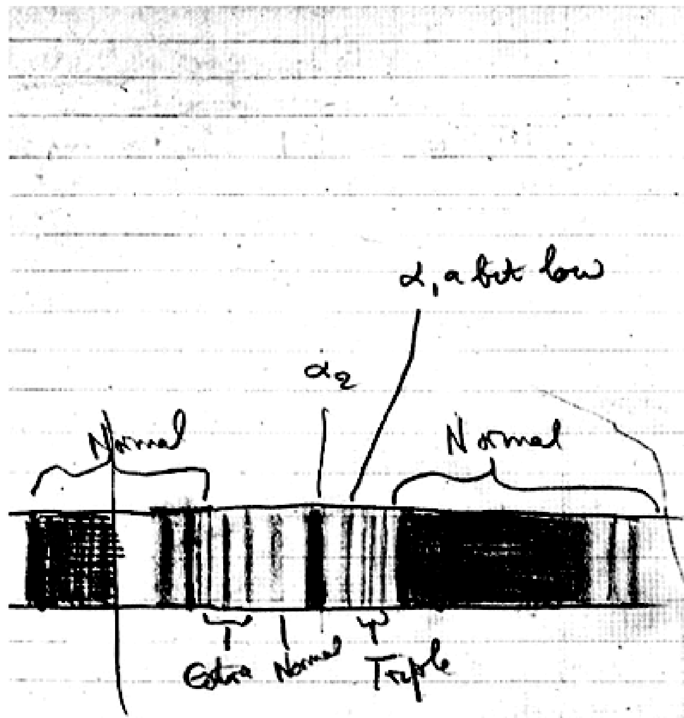
12.0 am reset up for larger test
Current 17 ma 3.30 current ~ 0
due to drying out.
Reset up + made much larger hole in deeper + more viscous jelly ~ $\frac{1}{32}$" across + covered whole with liquid paraffin.
On at 3.45 220v ~ 11 ma if it works clearly stiffness of jelly unimportant.
Current ~ 20 ma at 4.0. Off at 7.30

Copyright © Oliver Smithies



Friday, Oct. 16, 1954.

Tuesday, Oct. 26, 1954.

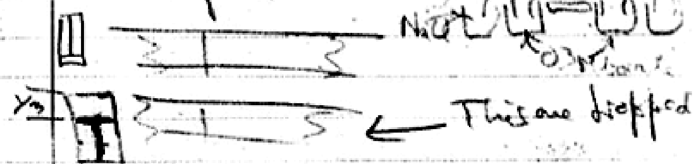


60% ... + 100g ... CaCl₂ 2H₂O No use of

Tues. Oct 26

W. serum prepared. (II B)

12.30pm anat 6v/cm 15%.03/9.03 ... in 1mm close packed slits.

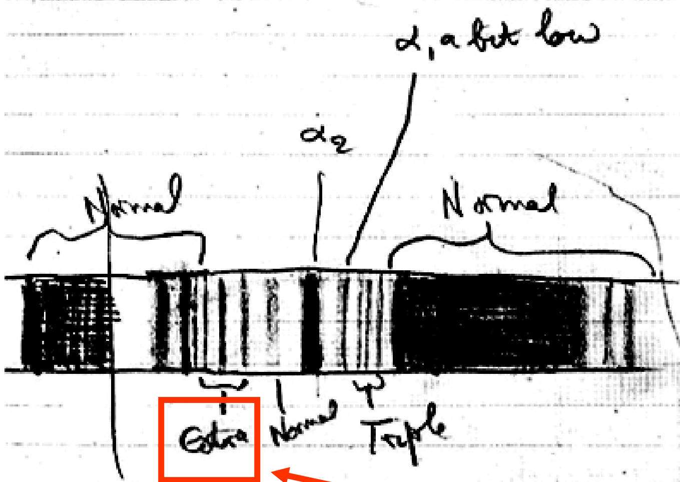


10.0 ma current after ~15'
9.5 ~80'
9.5 ~120'
9.1 ~200'
9.1 ~270
9.1 360.

Off 6.30pm Most odd — many extra components. Both excellent mechanically

Copyright © Oliver Smithies

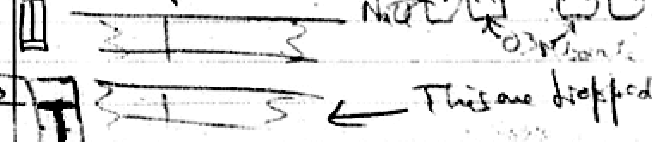
Tuesday, Oct. 26, 1954.



Tues. Oct 26

W. serum prepared. (IIB)

12.30pm anal 6v/cm 15%/0.03/9.03 in 1mm close packed slits.



10.0 ma ~~initial~~ current after ~15'

9.5 ~80'

9.5 ~120'

9.1 ~200'

9.1 ~270

9.1 360.

Off 6.30pm Most odd — many extra components. Both excellent mechanically.

Copyright © Oliver Smithies

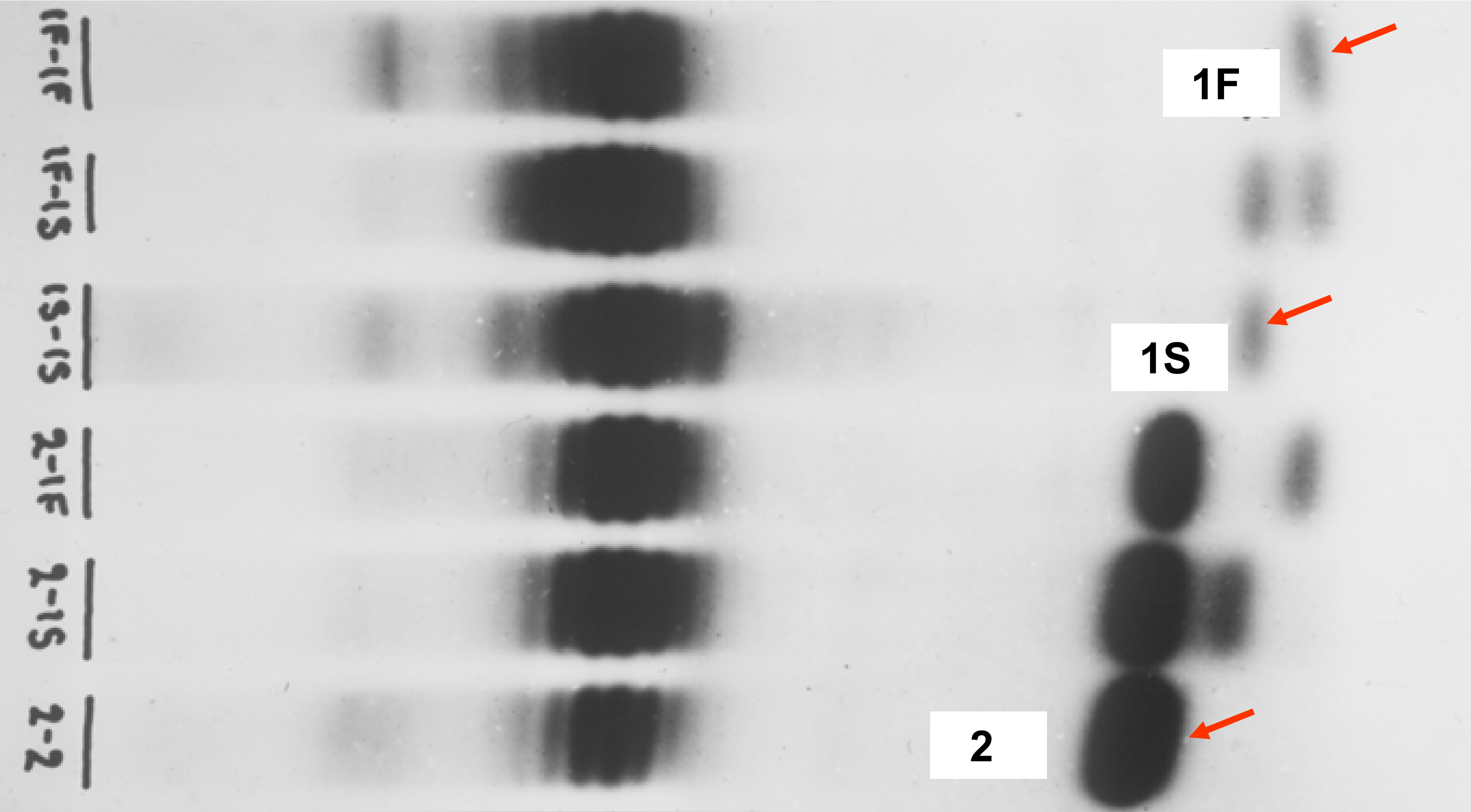
Copyright © Oliver Smithies

Haptoglobin Types

	Hp1-1	Hp^1/Hp^1
	Hp 2-1	Hp^2/Hp^1
	Hp 2-2	Hp^2/Hp^2

With Norma Ford-Walker, 1955

Beta Subunit **Alpha Subunits**



1962

Copyright © Oliver Smithies

Haptoglobin Genes

Hp^{1F} ABCDEFGHI

X

Hp^{1S} ABCDESGHI

1962

Copyright © Oliver Smithies

Haptoglobin Genes

Hp^{1F} ABCDEFGHI

Hp^{1S} ABCDESGHI

Recombination

Haptoglobin Genes

Hp^{1F} ABCDEFGHI

Hp^{1S} ABCDESGHI

↓ Recombination

Hp^{2} ABCDEFGCDESGHI

Duplication

Copyright © Oliver Smithies

Haptoglobin Genes

Hp^{1F} ABCDEFGHI

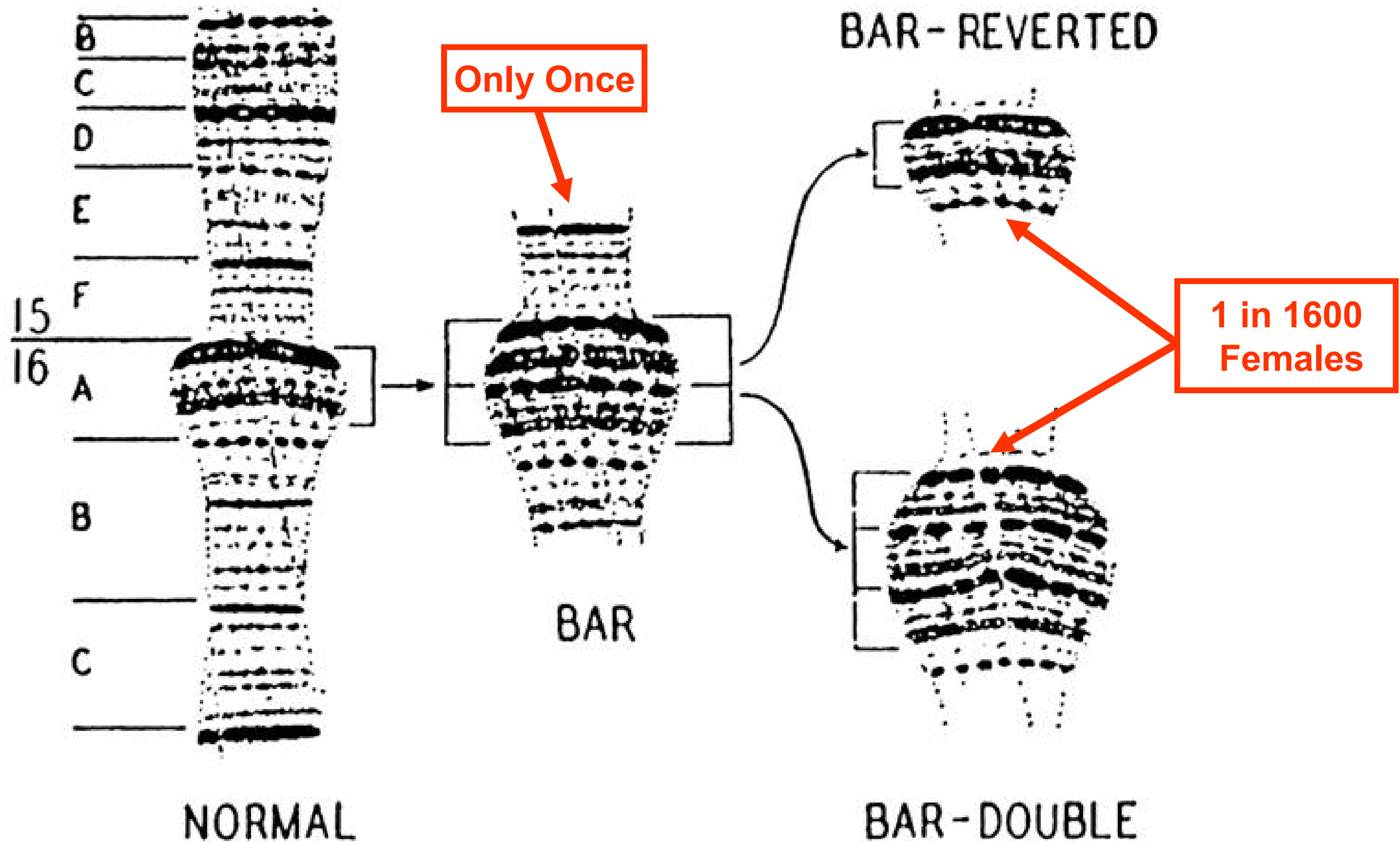
Hp^{1S} ABCDESGHI

Non-homologous Recombination

Hp^{2} ABCDEFGCDESGHI

Duplication

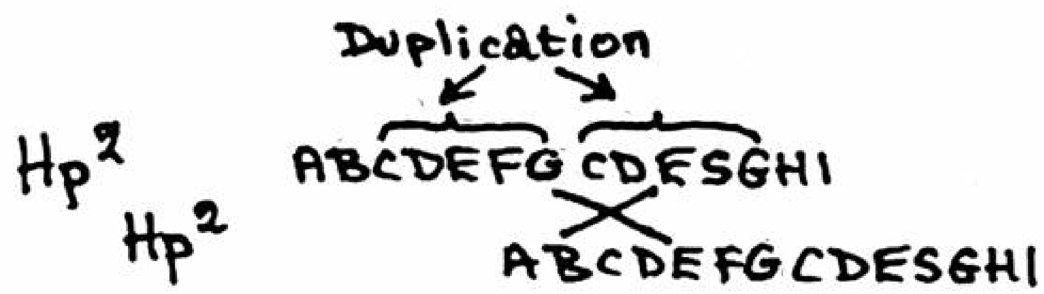
Copyright © Oliver Smithies



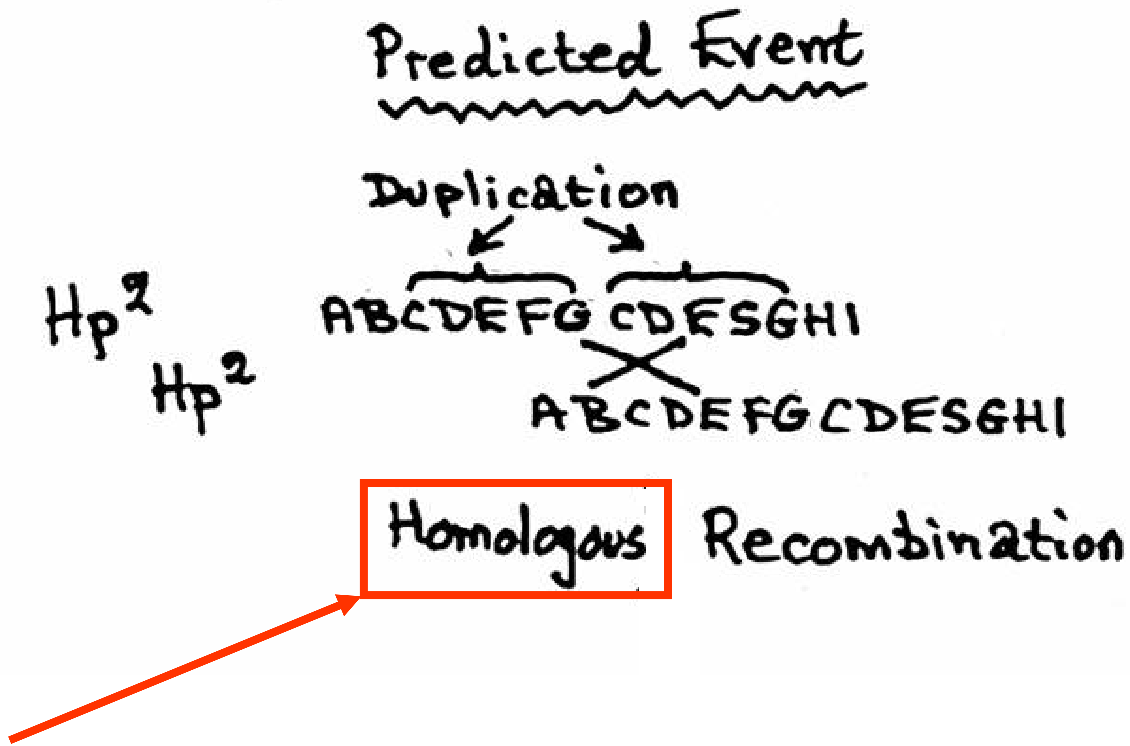
Tice 1914; Zeleny 1919; Sturtevant 1925; Bridges, 1936.

Copyright © Oliver Smithies

Predicted Event



Copyright © Oliver Smithies



Predicted Event

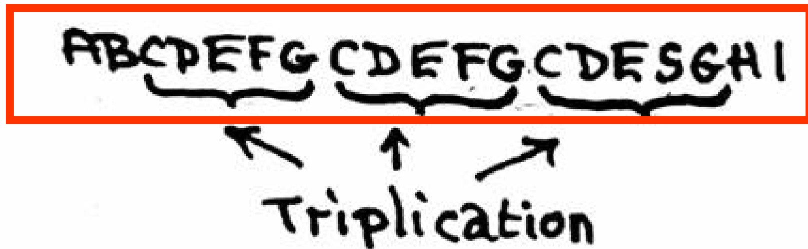
Duplication

Hp²

Hp²

Homologous Recombination

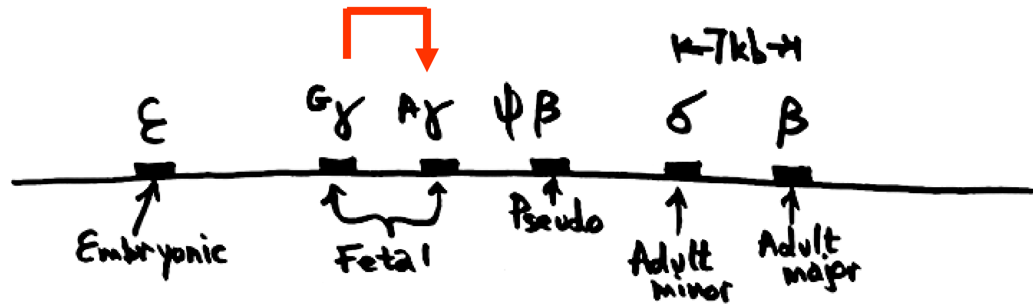
Hp³



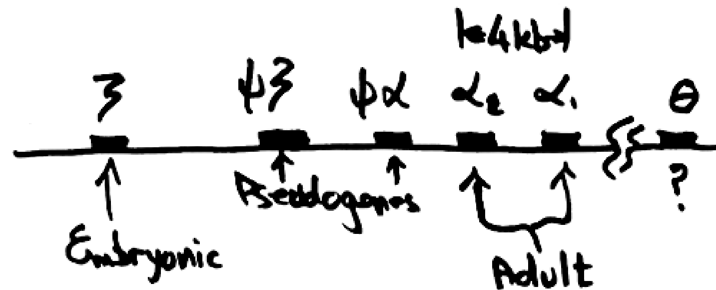
Copyright © Oliver Smithies

Human Globin Genes

β



α



Slightom et al.,1980

Copyright © Oliver Smithies

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

© 1982 Macmillan Journals Ltd

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

© 1982 Macmillan Journals Ltd

Copyright © Oliver Smithies

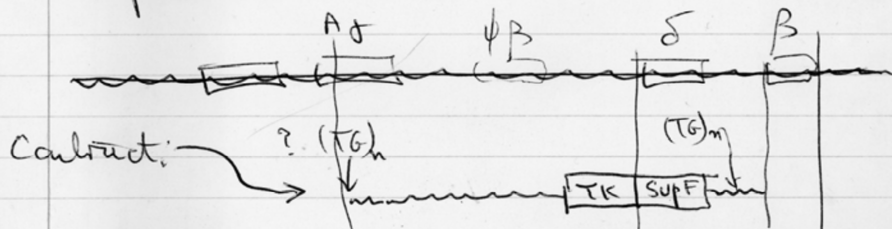
Thurs. April 22nd

Assay for gene placement

Aim: to place corrective DNA in the right place.

Need: an assay for developing techniques.

Proposal:



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

Prepare DNA from TK^+ cells

Cut with restn. enz. & size to

Clone in an amber phage — SupF ~ β

Plate on su^o & screen with β specific probe

Vary $(TG)_n$ or single stranded ends or UV or BUdR etc. to try to ↑ # once ones 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

Copyright © Oliver Smithies

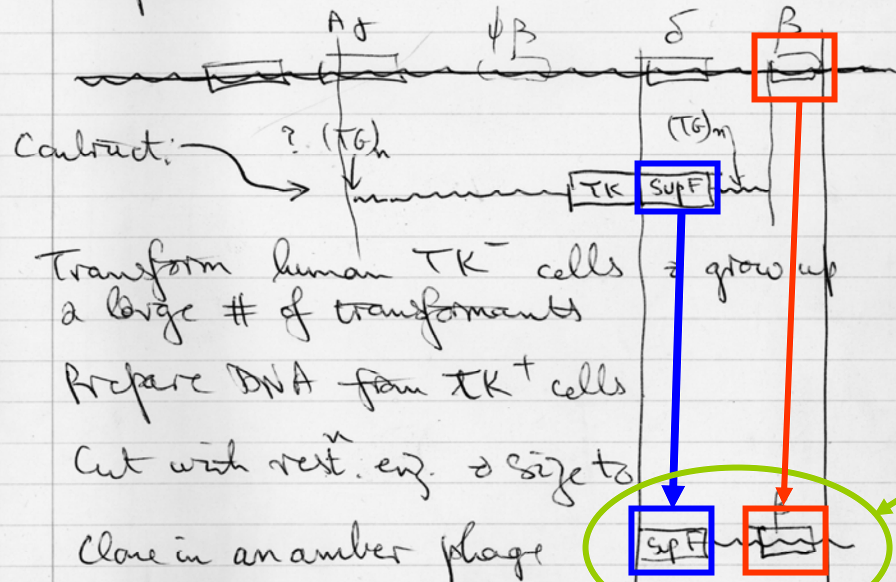
Thurs. April 22nd

Assay for gene placement

Aim: to place corrective DNA in the right place.

Need: an assay for developing techniques.

Proposal:



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

Prepare DNA from TK⁺ cells

Cut with restn. 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 tries 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

Copyright © Oliver Smithies

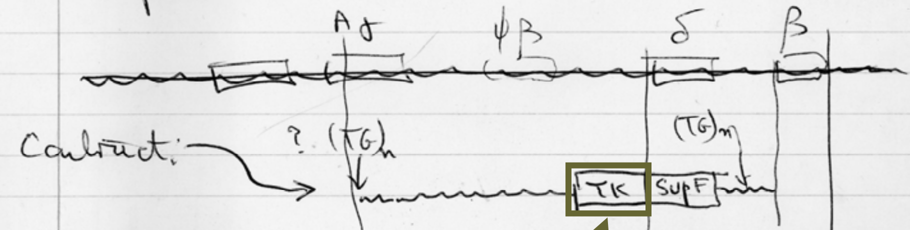
Thurs. April 22nd

Assay for gene placement

Aim: to place corrective DNA in the right place.

Need: an assay for developing techniques.

Proposal:



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

Prepare DNA from TK⁺ cells

Cut with restn. 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 true 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

Copyright © Oliver Smithies

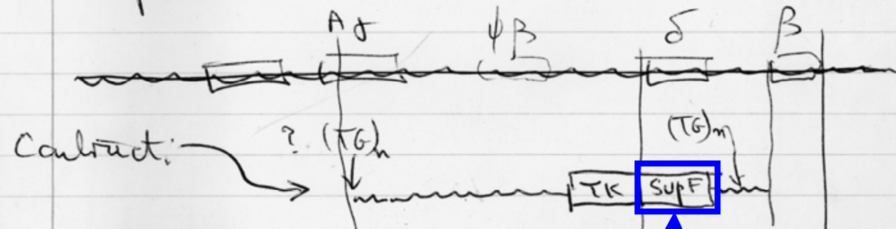
Thurs. April 22nd

Assay for gene placement –

Aim: to place corrective DNA in the right place.

Need: an assay for developing techniques.

Proposal:



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

Prepare DNA from TK^+ cells

Cut with restn. enz. & size to

Clone in an amber phage

Plate on su^o & screen with β specific probe

Vary $(TG)_n$ or single stranded ends or UV or BUdR etc. to try to ↑ # correct ones

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

Copyright © Oliver Smithies

Can accept non-linear → Duplication → Elimination & correction

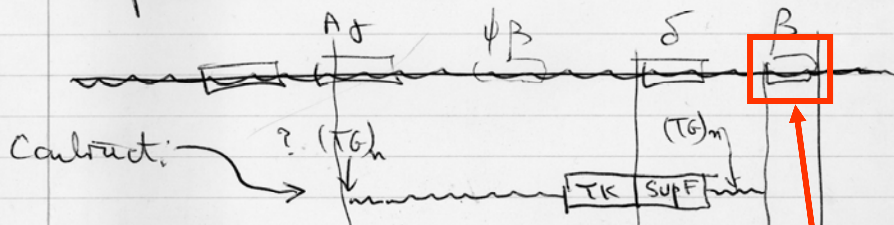
Thurs. April 22nd

Assay for gene placement

Aim: to place corrective DNA in the right place.

Need: an assay for developing techniques.

Proposal:



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

Prepare DNA from TK^+ cells

Cut with restn. enz. & size to

Clone in an amber phage SupF β

Plate on su° & screen with β specific probe

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

Selection in eukaryote × selection in prokaryote × β probe selection

1982

Copyright © Oliver Smithies

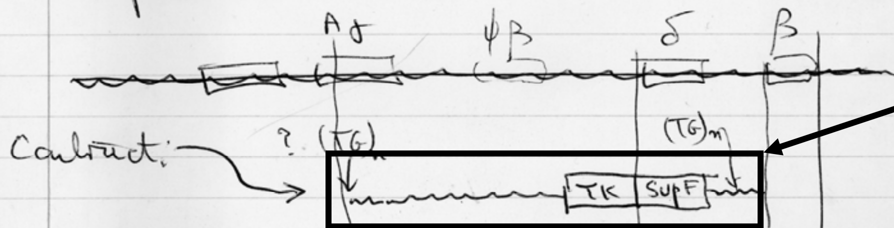
Thurs. April 22nd

Assay for gene placement

Aim: to place corrective DNA in the right place.

Need: an assay for developing techniques.

Proposal:



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

Prepare DNA from TK⁺ cells

Cut with restⁿ. 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 ?ves 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

Wed. Nov 3rd

Orientation of site by Sal & Kpn

(12)

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

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

Copyright © Oliver Smithies

1982

91

Tues. May 17th

Cosos 17 transformed DNA
– the real thing!

5/16/83

RK41 from Raju

Human cell line (E.J) transfected 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

Copyright © Oliver Smithies

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

Copyright © Oliver Smithies

1983

Thurs. June 23rd

ligation trial (near full scale)

Take ✓100 μl RK41/Xba (Cent^d) (≅ 20μg) ζ141

+ ✓200 μl ζ137 Ch 3A.ΔΔX/Xba (Cent^d) (≅ 25μg)

✓+ 30 μl 8M NH_4A

✓+ 660 μl EtOH

↑ I doubt this

Copyright © Oliver Smithies

Friday June 24th

ζ143 packaging

Total volume of ζ143 lig^n is ~100 μl ≅ 5-10 μg φ

∴ take ✓ 140 μl buffer A BDKB 4/13/82 ζ145 PP
✓ 20 μl MI buffer ζ145
Whole of ζ143 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 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) (Cont'd) 141
+ ✓200 μl 137 @3A.ΔΔX/Xba (Cont'd)(≅ 25 μg)
✓+ 30 μl 8M NH_4A
✓+ 660 μl EtOH

↑ I doubt this

My 58th Birthday

Copyright © Oliver Smithies

Friday June 24th

143 packaging

Total volume of 143 lig^n is ~100 μl ≅ 5 - 10 μg φ

∴ take ✓140 μl buffer A BDKB 4/13/82 PP
✓20 μl MI buffer 145 145
Whole of 143 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 INS2 +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) (cont'd) ζ141
+ ✓ 200 µl ζ137 @ 3A.ΔΔX/Xba (Cont'd) (≅ 25 µg)
✓ + 30 µl 8M NH_4A
✓ + 660 µl EtOH

↑ I doubt this

Copyright © Oliver Smithies

Friday June 24th

ζ143 packaging

Total volume of ζ143 lign is ~100 µl ≅ 5-10 µg φ

∴ take ✓ 140 µl buffer A BDKB 4/13/82 PP ζ145
✓ 20 µl MI buffer ζ145
Whole of ζ143 lign ~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

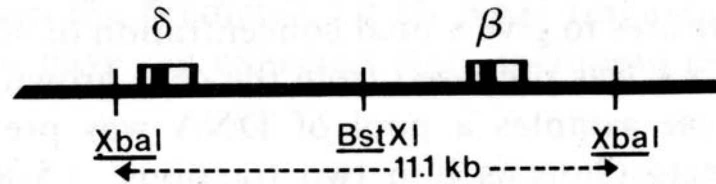
(Preliminary results) Thurs. June 30th

~ 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

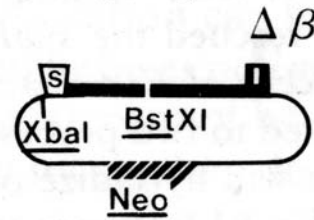
Normal Locus



b

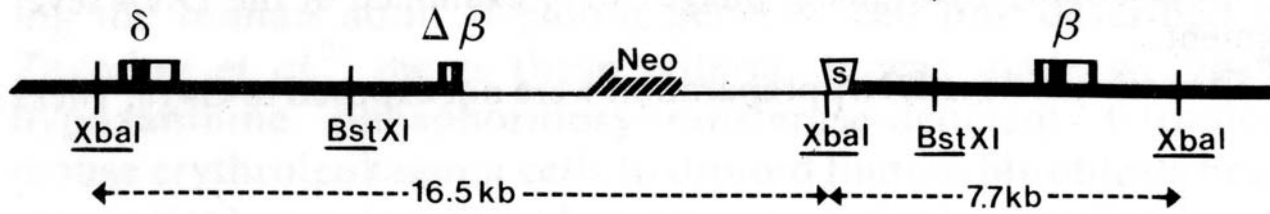
Test Plasmid, pΔβ117

(13.1kb)



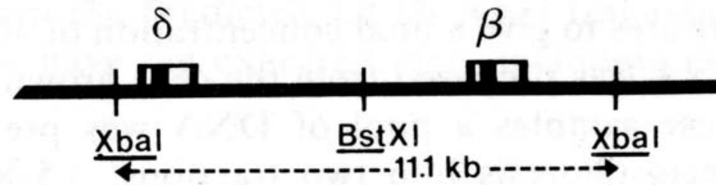
c

Modified Locus



a

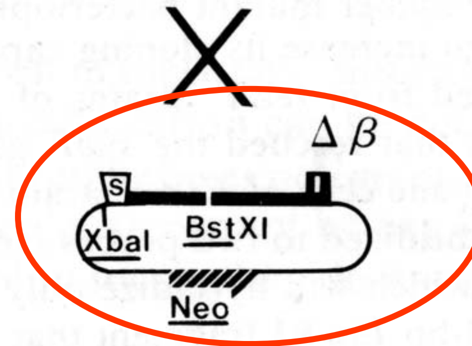
Normal Locus



b

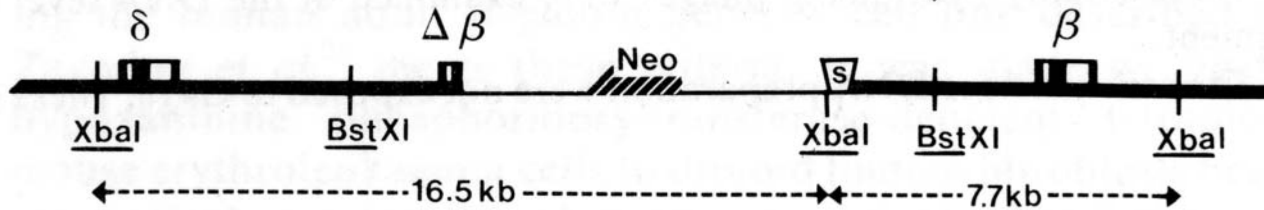
Test Plasmid, pΔβ117

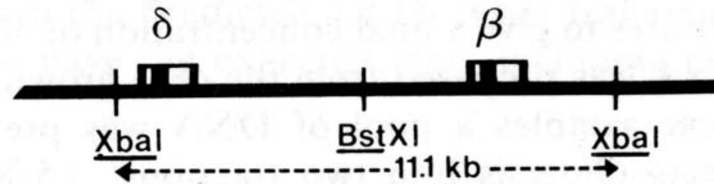
(13.1kb)

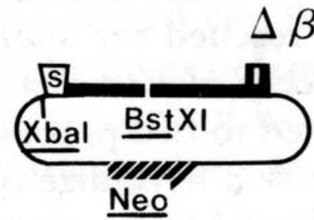


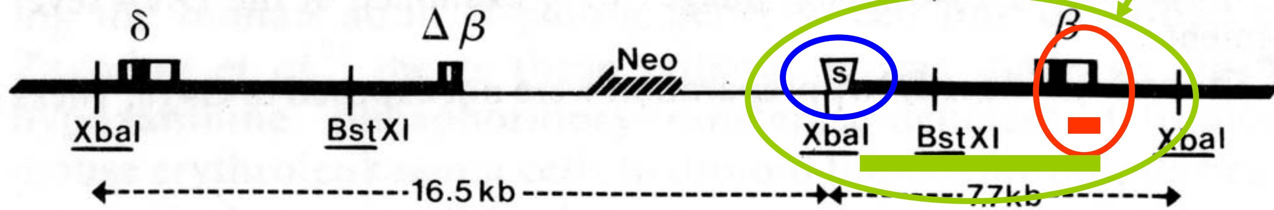
c

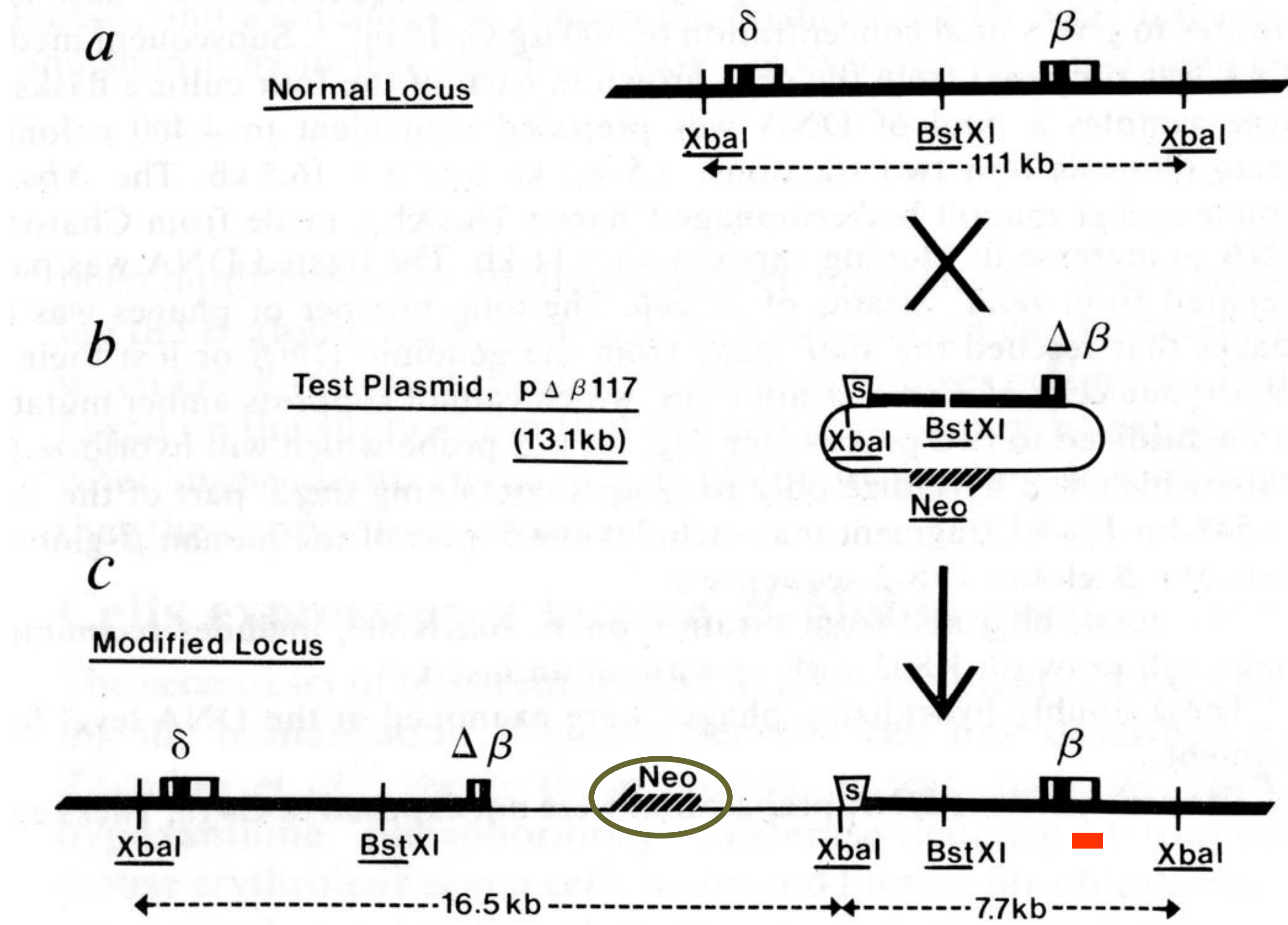
Modified Locus




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
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

Copyright © Oliver Smithies

Thurs. Aug. 23rd

(See MK/F24)

Unselected DNA

RK 57 was DNA from EJ cells (9 dishes of 5 x 10^5 + 1µg each of ΔB117 BstX1 cut) unselected in G418. (1µg gave 45 on G418)
~250µg DNA digested & cloned into ΔΔXba. (100µg of it was)

Total φ 6.3 x 10^8 on K802
2520 CIa plaques
125 +ve for β 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+Bam 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 SF8

1984

Copyright © Oliver Smithies

Mike Koralewski

Thurs. Aug. 23rd

See MK/F24

Unselected DNA

RK 57 was DNA from EJ cells (9 dishes of 5 x 10^5 + 1 μg each of Δβ117 BstX1 cut) unselected in G418. (1 μg gave 45 on G418)

~ 250 μg DNA digested & cloned into ΔΔ Xba. 100 μg of it was

Total φ 6.3 x 10^8 on K802

2520 CIa plaques

125 +ve for β probe

1 +ve for IVS 2 probe (c'd be more on longer exposure).

K802 recA⁻ Minis made after plaque purification but trouble with Xba I (Eco RI & Eco + Bam 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 SF8

1984

Copyright © Oliver Smithies

Thurs. Aug. 23rd

Unselected DNA

See MK/F24

RK 57 was DNA from EJ cells (9 dishes of 5×10^5 + 1 μg each of ΔB117 BstX1 cut) unselected in G418. (1 μg gave 45 on G418)
~ 250 μg DNA digested & cloned into ΔΔXba.
100 μg of it was

Total φ 6.3×10^8 on K802
2520 CIa plaques
125 +ve for β 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 & Eco+Bam were okay) ... set up four more minis, on thought that modification may have occurred (Kim had similar trouble with K802 recA⁻).

CIA
K802
DP50supF
C600SF8

1984

Copyright © Oliver Smithies

Thurs. Aug. 23rd

Unselected DNA

See MK/F24

RK 57 was DNA from EJ cells (9 dishes of 5×10^5 + 1 μg each of ΔB117 BstX1 cut) unselected in G418. (1 μg gave 45 on G418)
~250 μg DNA digested & cloned into ΔΔXba. 100 μg of it was

Total φ 6.3×10^8 on K802
2520 C1a plaques
125 +ve for β 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 (EcoRI & Eco+Bam 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 SF8

1984

Copyright © Oliver Smithies

Thurs. Aug. 23rd

Unselected DNA

See MK/F24

RK 57 was DNA from EJ cells (9 dishes of 5 × 10^5 + 1 μg each of ΔβII7 Bst XI cut) unselected in G418. (1 μg gave 45 on G418)
~ 250 μg DNA digested & cloned into ΔΔXba. 100 μg of it was

Total φ 6.3 × 10^8 on K802
2520 CIa plaques
125 +ve for β 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 + Bam 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 SF8

1984

Copyright © Oliver Smithies

Tues June 12th

Transformation of β-globin-producing cells

Huntlotter, L. Weir & P. Lefer 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

ASF2-1 cells are hybrid between MEL cells & human diploid fibroblast with X-11 translocation. (Hprt⁻)
Under HAT the X-11 is retained. Making Hb at low level – can be induced with DMSO – but differentiation ...
Stationary cultures are ok. in bottles in:–

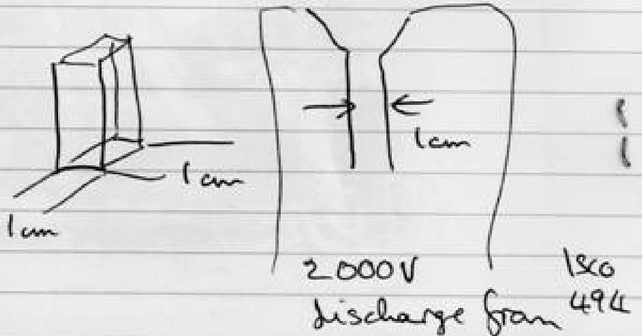
Gibco

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 10 fold dilution.

Cell



1984

Copyright © Oliver Smithies

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.

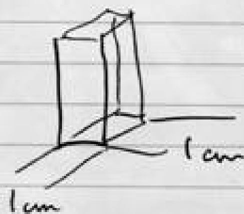
originally from single cell

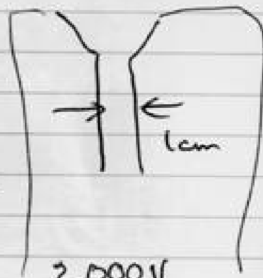
ASF2-1 cells are hybrid between MEL cells & human diploid fibroblast with X-11 translocation. (3? chromosomes – normal 40) Hprt⁻
Under HAT the X-11 is retained. Making Hb at low level – can be induced with DMSO – but differentiation ? bad
Stationary cultures are ok. in bottles in:–

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.

Cell





2000 V discharge from Isco 494

1984

Copyright © Oliver Smithies

Wed. Jan 23rd

Size fractionation using DEAE paper

DNA available from Zapping +Neo expts.:–

RG B31.1B → RG B31.4B (used Δβ117)
RG B31.1C → RG B31.4C (used Cosos 17)

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

[1 more C to go ≡ 67 colonies]

1B – 4B ≅ 1131 colonies / flask i.e. tube.
~200 μg 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 others to same

Aim to get 40 μg of size fractionated product with 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 requires 400 μg of DNA, or 100 μg / tube

Ron had digested with Xba I (large lot) 4 μl for 20 μg in 200 μl (i.e. 4 units/μg)
using 1/10 10 X RS 1.5 M NaCl
≥ 2 hrs at 37°

Copyright © Oliver Smithies

Wed. Jan 23rd

Size fractionation using DEAE paper

DNA available from Zapping + Neo expts.:—

RG B31.1B → RG B31.4B (used ΔB117)
RG B31.1C → RG B31.4C (used Cosos 17)

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

1 more C to go ≡ 67 colonies

1B — 4B ≅ 1131 colonies / flask i.e. tube.
~200 μg 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 others to same

Aim to get 40 μg of size fractionated product with 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 requires 400 μg of DNA, or 100 μg / tube

Ron had digested with XbaI (large lot) 4 μl for 20 μg in 200 μl i.e. 4 units/μg
using 1/10 10×RS 1.5 M NaCl
≥ 2 hrs at 37°

1985

Copyright © Oliver Smithies

Wed. Jan 23rd

Size fractionation using DEAE paper

DNA available from Zapping + Neo expts.:—

RG B31.1B → RG B31.4B (used ΔB117)
RG B31.1C → RG B31.4C (used Cosos 17)

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

1 more C to go ≡ 67 colonies

1B — 4B ≅ 1131 colonies / flask i.e. tube. ~200 μg 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 others to same

Aim to get 40 μg of size fractionated product with 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 requires 400 μg of DNA, or 100 μg / tube

Ron had digested with Xba I (large lot) 4 μl for 20 μg in 200 μl (i.e. 4 units/μg) using 1/10 10 X RS 1.5 M NaCl ≥ 2 hrs at 37°

Recombinant Fragment

1985

Copyright © Oliver Smithies

Wed. Jan 23rd

Size fractionation using DEAE paper

DNA available from Zapping + Neo expts.:–

RG B31.1B → RG B31.4B (used Δβ117)
RG B31.1C → RG B31.4C (used Cosos 17)

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

1 more C to go ≡ 67 colonies

1B – 4B ≡ 1131 colonies / flask i.e. tube.
~200 μg 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 others to same

Aim to get 40 μg of size fractionated product with 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 requires 400 μg of DNA, or 100 μg / tube

Ron had digested with Xba I (large lot) 4 μl for 20 μg in 200 μl i.e. 4 units/μg
using 1/10 10 X RS 1.5 M NaCl
≥ 2 hrs at 37°

1985

	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

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
	4 µl	✓ 4 µl	i142 Charon 3AΔlac 4 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]

1 µg ≅ DNA

Number titration or plates should be divided by

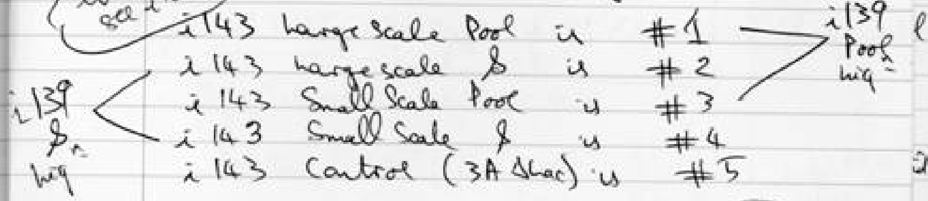
Set for 40 min → to 37° at 5 pm

Copyright © Oliver Smithies

Wed. Jan 30th (1985) i139

2 positive on large #4 Pool & 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

i139 Pool Mg²⁺ ; i139 S Mg²⁺

Total Kcoc = 2.4×10^8

Take out (4 µl of i139 Pool Mg²⁺ #3) → (4 µl of i139 S Mg²⁺ to make #4)

Total Kcoc 1.4×10^8

Add:—	#1 large Pool Mg²⁺ (~400 µl)	#2 large S Mg²⁺ (~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 2.40 pm	✓ 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 $CaCl_2$ $MgCl_2$ 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°

Copyright © Oliver Smithies

	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 β	#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 of titration on plates should be divided by ??

Set for 40 min → to 37° at 5 pm

Wed. Jan 30th (1985) i139

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

large scale packaging → plating

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

(i139 Pool hig⁻ → #1, #2; i139 β hig⁻ → #3, #4)

Take out 4 µl of i139 Pool hig⁻ #3 → 4 µl of i139 β to make hig⁻ #4

Total 2.4×10^8 — Total 1.4×10^8

Add:— #1 large Pool hig⁻ (~400 µl) — #2 large β hig⁻ (~400 µl)

{Buffer A 7 parts + Buffer M1 1 part} ✓ 800 µl — ✓ 800 µl
{Sonic Extract MK102 F} ✓ 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 CaMgCl₂ 10 mM 10 mM
plus 3.6 ml of C1A (grown 28-29th out 1/4 dil^d today at ~1 pm with NZY & regrown) 15 min at 37°

1985

Copyright © Oliver Smithies

Maxi To 37° at 3.0 pm

Titrations (C.B.) on K802

	F	G	H
4.1			
4.01	55	121	183
6.1	3	[illegible]	[illegible]

These are titres/μg

Average by Charlie was 1×10^8/μg

1.5×10^8/μg

These are titres/ml ×1.9 = total φ

Top agar was not completely solubilized!

Lifted moderately well → baked 11.40 am to 1.40

Then 6×SSC Denh. from 2.00 to 3.00 pm
(put filters 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 × 70 ml total	60,000 cpm/ml × 70 ml total
∴ 220 μl RG C33 BINS2 ~ now 95,000/μl	∴ 75 μl K27 5.2K
+44 μl 2M NaOH	+15 μl 2M NaOH
+44 μl 2M HCl	+ 15 μl 2M HCl
+70 ml	+70 ml

BINS

5.2K

68°C Set 3.50 pm

Total φ 1.05×10^8

Fri. Feb 22nd

13-25 + 5.2K K21F Packages

0 on IVS2

On C1A hard to count but >125

	F	G	H
DNA	Whole of K21F lig^n^ ~400 μl (≃100 μg DNA)	4 μl Ch3AΔlac i142	4 μl Ch3AΔlac i142
Buffer A	700 μl SM 1-12-84	7 μl SM 1-12-84	7 μl K19
Buffer M-1	100 μl MK D-24	1 μl MK D-24	1 μl K19
Sonic extract K F102	100 μl	1 μl	1 μl
FTL BB42	600 μl	6 μl	6 μl
Total volume	1.9 ml		

800 μl; 8 μl; 8 μl

1 hr at 37° — 12.35 pm → 1.35 pm

	F	G	H
	10 μl to 1 ml φ80 + Mg	1 ml φ80 + Mg	1 ml φ80 + Mg
	3.6 ml Ca/Mg → transfer to 50 ml; 3.6 ml C1A overnite; 15' at 37° Plate	Titrate at 4.1, 4.01	Take these as 0 → titrate at 4.1, 4.01

1985

Copyright © Oliver Smithies

20 min. each way.

9.05 am Plates cracked – look fine

Controls were messed up somehow. 4.01 is nearly confluent & may have been 2.01 (control)

K33F 4.01	56	5.6 × 10^7
K33F 6.1	?	

= Total φ 1.1 × 10^8

100 on C1A

Lifted & baked 2 hrs at 68°C

Hybridized: K33F.2 / K33F.1

	βIVS 2	5.2 K
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 45,000 cpm/μl ∴ need 135 μl for 100 ml
2M NaOH	80 μl ✓	27 μl ✓
	Wait 10 min	
2M HCl	80 μl ✓	27 μl ✓
Add	100 ml 6×SSC Denhardts ✓	100 ml ✓

10 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 hig.n ✓	4 μl Ch3AΔlac/i 142 (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

Copyright © Oliver Smithies

	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

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 δ	#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]

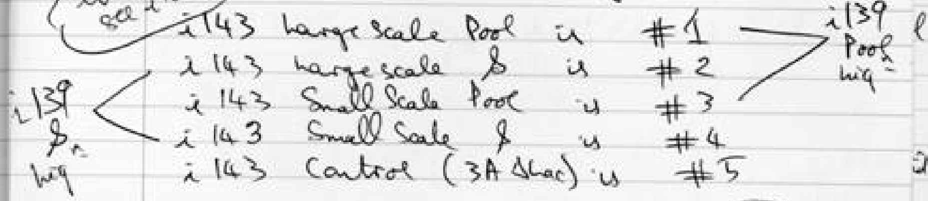
Number of titration or plates should be divided by [?]

Set for 40 min → to 37° at 5 pm

Wed. Jan 30th (1985) i139

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

Large scale packaging → plating



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

i139 Pool lig^n → #1, #2; i139 δ lig^n → #3, #4

Total 2.4×10^8

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

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 M1 1 part}	✓ 800 μl	✓ 800 μl
{Sonic Extract MK102 F}	✓ 100 μl	✓ 100 μl
{Freeze Thaw lysate LB-12}	✓ 400 μl 2.40 pm	✓ 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 1/4 dil^d today at ~1 pm with NZY + regrown) 15 min at 37°

1985

Copyright © Oliver Smithies

	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

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 titrated or plated 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, 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

i139 Pool lig^n; i139 S_n lig

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

Total 2.4×10^8 — Total 1.4×10^8

Add:—	#1 large Pool lig^n (~400 μl)	#2 large S 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 CaMgCl₂ 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

Copyright © Oliver Smithies

Mon. April 15th

Ran will clone individual cells of RG B11.6 & give them to me in DNA pools of 20, or 50 or 100. Expect positive in less than 5 × 20!

1985

Copyright © Oliver Smithies

In NZY presumably

(Tues) from ~6am
Fresh colony to 0.55 by 10am
- diluted to 0.23 & back up to 1.045 by 12 noon

Fresh maxi plates.

144A	144B	Pool 1	3B/M	Control	
4.01 (27)	4.01 (40)	4.01 (47)	4.01 (47)	60 4.01 (35)	51 4.01 (65)
Total	Total	Total	Total	1 µg	1 µg
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 concn – insufficient inserts. Changed mind after & lift other 3.

60,000 cpm /ml

300,000 cpm/ml

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

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

This is it! ≥ 8 positive / ~30

144B 2 incorrectly labelled 3B/M 2

144A 144B 3B/M

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

68° at 5.30 Fri pm
On film 2.30 pm Sat

Tues. May 7th

Packaging

C1A 5.2 / IVS	≈1×10⁻⁶ 43	≈1×10⁻⁶ 62	≈1.5×10⁻⁶ 115	≈0.5×10⁻⁶ 53	V. odd Control	
	7+(2) / 0	15+(1) / 0	28+(7) / (8)	0+(3) / 0	(60)	(57)
	K115 144A whole	K115 144B whole	K115 Pool 1 whole	K115 3B/M whole	4 µl Ch3A Δlac K77	0.25 µg/µl
3.15 ml I A/M1 1	800 µl	800 µl	800 µl	800 µl	8 µl	8 µl
450 µl SE CB57	100 µl	100 µl	100 µl	100 µl	1 µl	1 µl
FTL	LB60 ~400 µl	LB60 ~400 (3 tubes)	LB60 ~400 µl	LB51 ~800 µl 2 tubes	LB60 4 µl	LB51 8 µl

20 tubes — closer to 300

~2pm to 3pm — 1 hr @ 37°

10 µl to 1 ml φ80 + Mg ($\equiv 10^{-2}$) +1 ml +1 ml

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 @ 3.45 pm

C1A 3.6 ml Ca/Mg
3.6 ml O.D. 1.05
15 min
50 ml NZTA agar

3.45 pm to 37°

Try ↑ DNA concn. by using 200 µl p95

1985

Copyright © Oliver Smithies

In NZY presumably

(Tues) Fresh colony / from ~ 8am to 0.55 by 10 am
– diluted to 0.23 & back up to 1.045 by 12 noon

Fresh maxi plates.

144A	144B	Pool 1	3B/M	Control 60	Control 51
4.01 (27)	4.01 (40)	4.01 (47)	4.01 (47)	4.01 (35)	4.01 (68)
Total	Total	Total	Total	1 μg	1 μg
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 concn – insufficient inserts. Changed mind after → lift other 3.

60,000 cpm / ml

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

300,000 cpm/ml

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

This is it! ≥ 8 positive / ~30

144B 2 incorrectly labelled 3B/M. 2

144A 144B 3B/M

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

68° at 5.30 pm Fri. On film 2.30 pm Sat

Tues. May 7th

Packaging

C1A / 5.2 / IVS	43	62	115	53	Control 60	Control 57
	≃ 1×10^{-6}	≃ 1×10^{-6}	~ 1.5×10^{-6}	≃ 0.5×10^{-6}	N. odd	
	7+(2) / 0	15+(1) / 0	28+(?) (8)		0+(3) / 0	
	K115 144A whole	K115 144B whole	K115 Pool 1 whole	K115 3B/M whole	4 μl Ch3A Δlac K77	0.25 μg/μl
3.15 ml A / 1 MI	800 μl	800 μl	800 μl	800 μl	8 μl	8 μl
450 μl SE CB57	100 μl	100 μl	100 μl	100 μl	1 μl	1 μl
FTL	LB60 ~400 μl	LB60 ~400	LB60 ~400 μl	LB51 ~800 μl 2 tubes	LB60 4 μl	LB51 8 μl
~2 pm to 3 pm	1 hr @ 37°		closer to 300			
	10 μl to 1 ml		φ80 + Mg ($\equiv 10^{-2}$)		+1 ml	+1 ml
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

(3 tubes; 20 tubes)

C1A 3.6 ml Ca/Mg

3.6 ml O.D. 1.05

15 m

50 ml NZY Agar

3.45 pm to 37°

To 37°C @ 3.45 pm

Try ↑ DNA concn by using 200 μl p95

1985

Copyright © Oliver Smithies

(In NZY presumably)

(Tues) Fresh colony / from ~9am to 0.55 by 10 am — diluted to 0.23 & back up to 1.045 by 12 noon

Fresh maxi 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	1μg	1μg
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 ... lift 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 bag	✓ 30ml 6×SSC bag

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

This is it! ≥ 8 positive /~30

144B 2 incorrectly labelled 3B/M 2

(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
90 ml 6×SSC	90 ml

68° at 5.30 Fri pm. On film 2.30 pm Sat

Tues. May 7th

Packaging

C1A	43 (≃1×10⁻⁶)	62 (≃1×10⁻⁶)	115 (≃1.5×10⁻⁶)	53 (≃0.5×10⁻⁶)	Control (N. odd)	
5.2 / IVS	7+(2) / 0	15+(1) / 0	28+(7) (8)	0+(3) / 0	60	57
	K115 144A whole	K115 144B whole	K115 Pool 1 whole	K115 3B/M whole	4μl Ch3A Δlac K77	0.25μg/μl
3.15 ml T A 1 M1	800μl	800μl	800μl	800μl	8μl	8μl
450μl SE CB57	100μl	100μl	100μl	100μl	1μl	1μl
FTL	LB60 ~400μl 3 tubes	LB60 ~400 (20 tubes)	LB60 ~400μl	LB51 ~800μl 2 tubes	LB60 4μl	LB51 8μl
~2pm to 3pm	1 hr @ 37°	closer to 3.00				
	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

C1A 3.6ml Ca/Mg
3.6ml O.D. 1.05
15 m
50 ml NZYTA jar

3.45pm to 37°

Try ↑ DNA conc^n by using 200μl p95

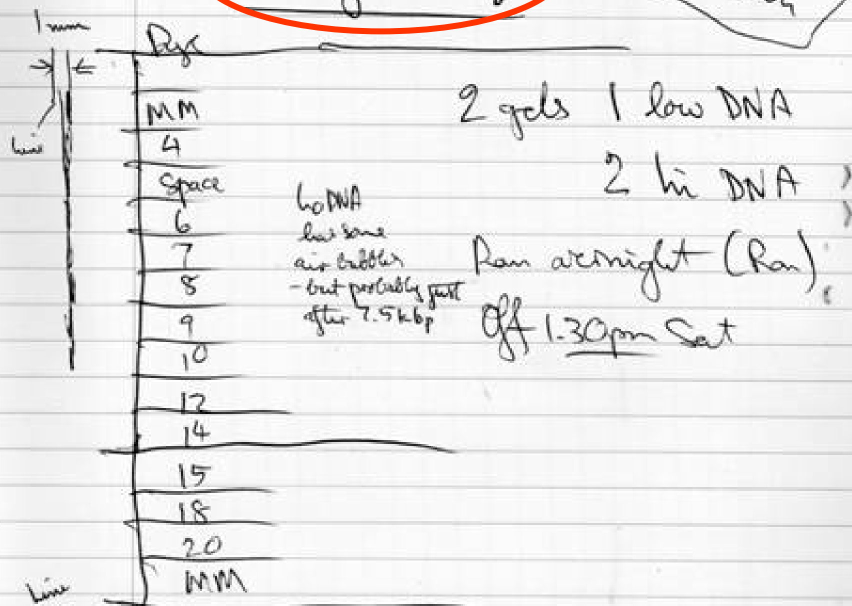
1985

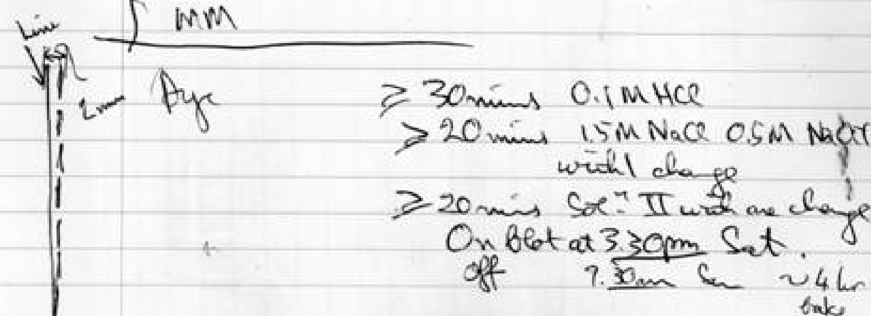
Copyright © Oliver Smithies

Sat. May 18th

See p 13
3 years
1 month

Colony Blots!





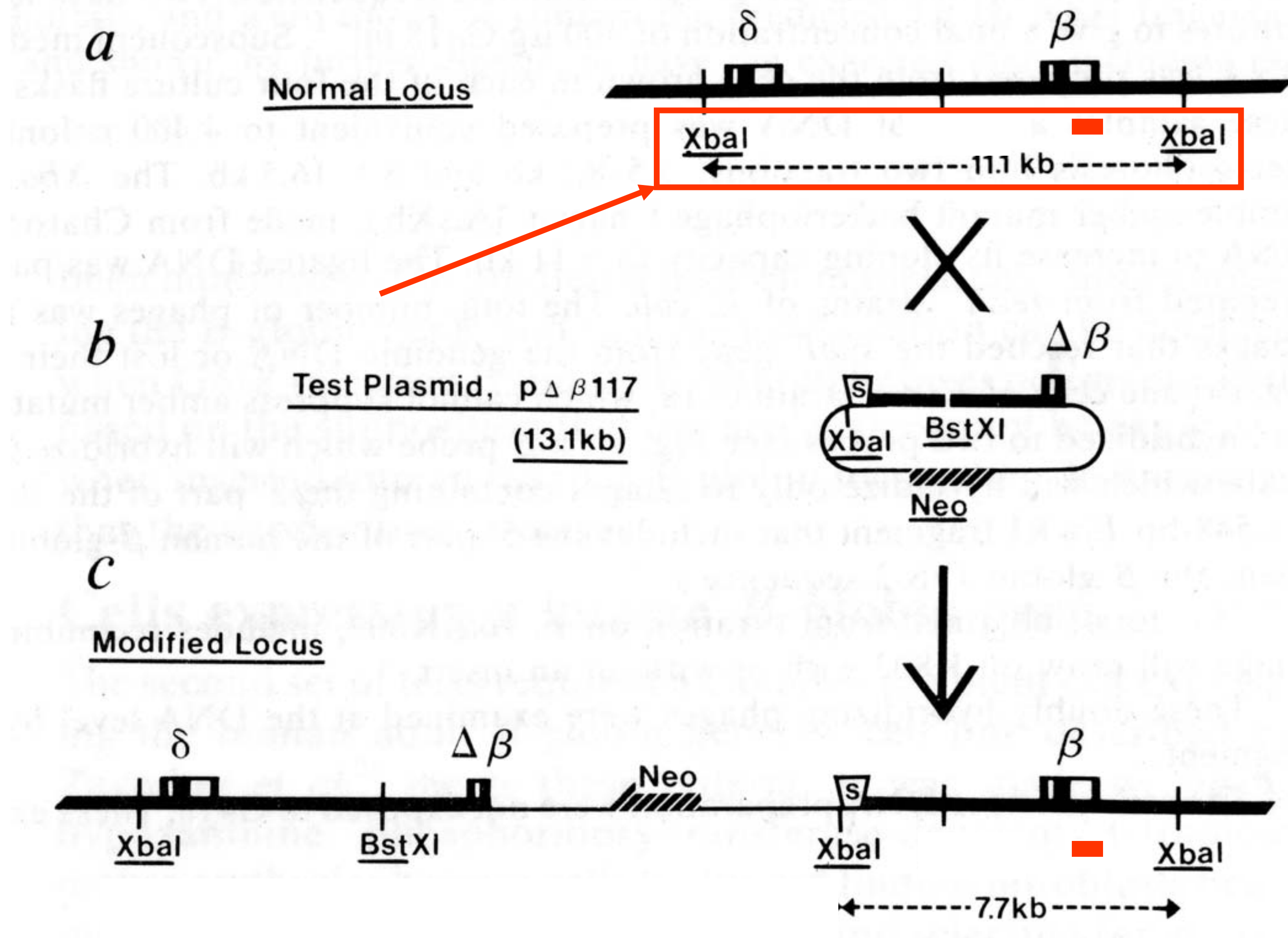
Flavell's prehybridize using

~17 ml of

10 ml 5x Flavell's
2.5 ml 4% BSA
250 μl poly rA
37 ml ddw

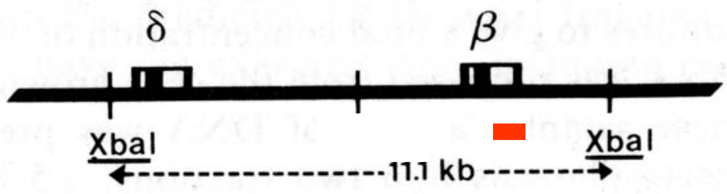
3.50pm
4.50pm

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
S
β
XbaI
BstXI
XbaI
XbaI
7.7kb

a

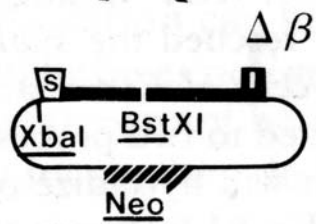
Normal Locus



b

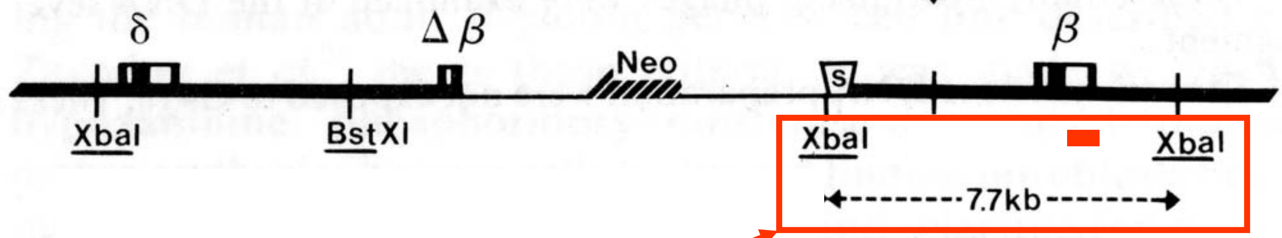
Test Plasmid, pΔβ117

(13.1kb)



c

Modified Locus



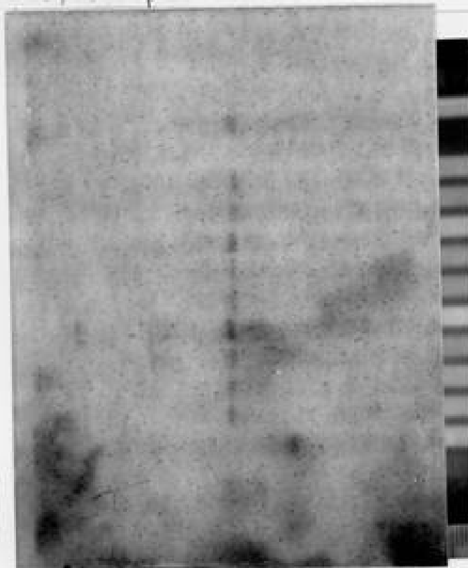
Copyright © Oliver Smithies

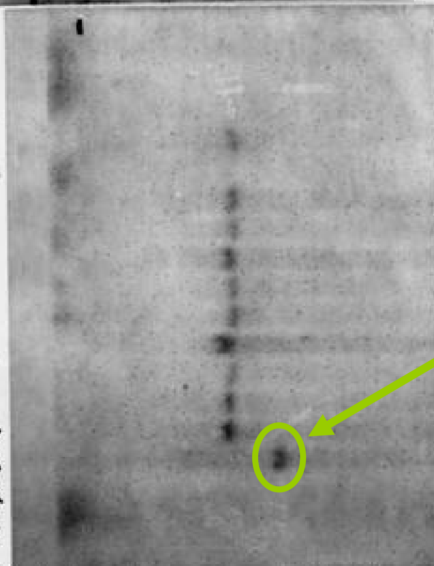
Hybridized to + 85 μl Sonicated Salmon Sperm DNA
750 μl K133IVS2 probe 64×10^6 cpm
150 μl 2M NaOH
150 μl 2M HCl
17 ml Flavell's 1x
(i.e. 3.6×10^6/ml — nearly 2x)
½ to each bag
On at 5.50 pm Sun
Off at 10.20am Mon (17 hr)
3x rinsed
2x wash — total ~1 to 1½ hr
On film 12 noon Mon. May 20th
Off 9.15 Tues

20 is it!

Back on film 9.45am May 21st Tues
↓
8.30pm Sun May 26th
Same result
Equivalent phage

Rescued to get 7.6 kb phage ...

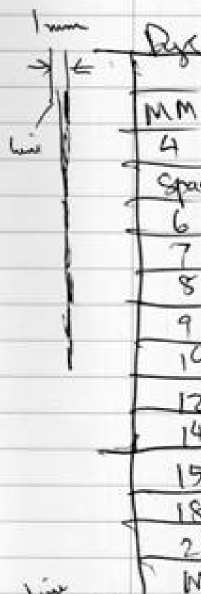




Sat. May 18th

Colony Blots!

See p. 13
3 years 1 month



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

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 1.30pm Sat

Line
2 mm
Dye

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

Flavell's prehybridize using
~17 ml of
10 ml 5× Flavell's
2.5 ml 4% BSA
250 μl poly rA
3.7 ml H₂O

3.50pm
4.50pm

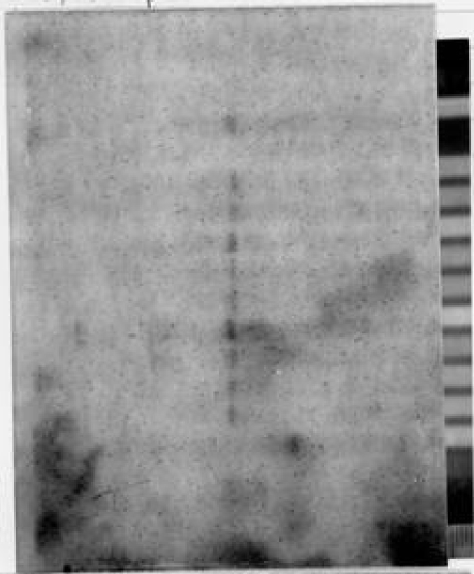
1985

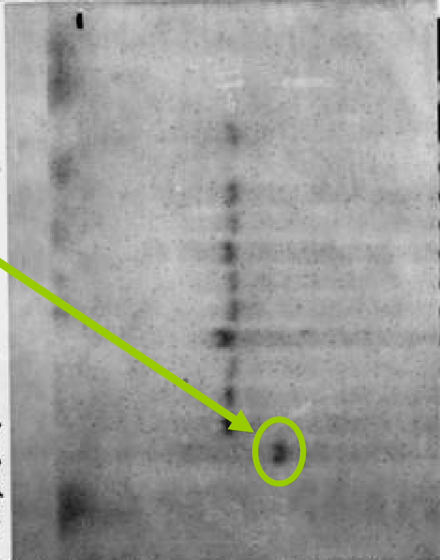
Copyright © Oliver Smithies

Hybridized to ✓ Sonicated Salmon Sperm DNA
+85 μl   64 × 10^6 cpm
750 μl K133IVS2 probe
150 μl 2M NaOH
150 μl 2M HCl
17 ml Flavell's 1x
(i.e. 3.6 × 10^6/ml – nearly 2x)
½ to each bag
On at 5^20 pm Sun
Off at 10.20 am Mo (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



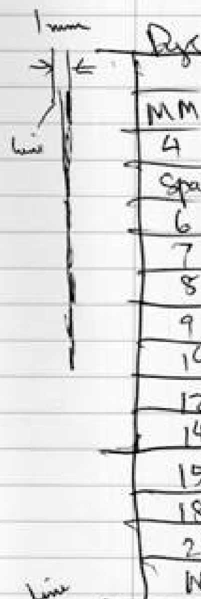


Rescued to get 7.6 kb phage ... mini

See 813
3 years
1 month

Sat. May 18th

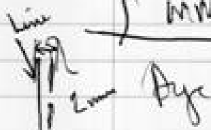
Colony Blots!



2 gels 1 lo DNA
2 hi DNA

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

Ran overnight (Ran)
Off 1.30 pm Sat



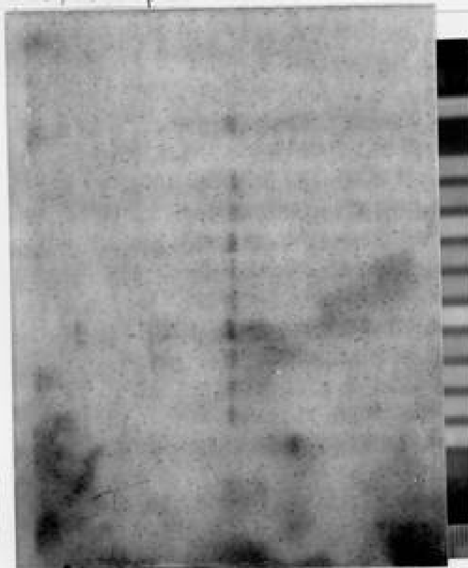
≥ 30 mins 0.1 M HCl
≥ 20 mins 1.5 M NaCl 0.5 M NaOH with 1 change
≥ 20 mins Sol^n II with one change
On blot at 3.30 pm Sat.
Off 7.30 am Sun ~4 hr bake

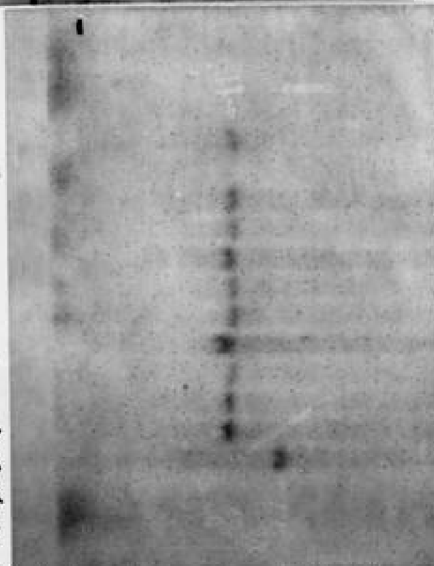
Flavell's prehybridize using
~17 ml of
{10 ml 5x Flavell's
2.5 ml 4% BSA
250 μl poly rA
3.7 ml ddw}
3.50 pm
4.50 pm

1985

Copyright © Oliver Smithies

Hybridized to ↙ + 85 µl sonicated salmon sperm DNA
64 × 10⁶ cpm
750 µl K133 IVS2 probe
150 µl 2M NaOH
150 µl 2M HCl
17 ml Flavell's 1x
(i.e. 3.6 × 10⁶/ml – nearly 2×)
½ to each bag
On at 5 pm Sun
Off at 10.20 am Mon (17 hr)
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
9.45 am May 21st Tues
↓
8.30 pm Sun May 26th
Same result
equivalent phage
Rescued to get 7.6 kb phage ... mini





Sat. May 18th

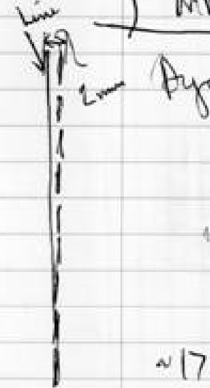
See 113
3 years 1 month

Colony Blots!

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

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

2 gels 1 low DNA
2 hi DNA
Ran overnight (Ron)
Off 1.30 pm Sat



Line
2 mm
Dye

≥ 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 5× Flavell's
2.5 ml 4% BSA
250 µl poly rA 10 mg/ml
37 ml ddw
3.50 pm
4.50 pm
Then

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

Copyright © Oliver Smithies

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 [colonies] by pipette onto P60 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[inatio]n & get chimaeras & germ line by blastocyst route.

1985

Copyright © Oliver Smithies

Mon Dec 30th

EK cell system

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

EK cells (CC 1.2)

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 colonies by pipette onto P60 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 chimaeras & germ line by blastocyst route.

Brought personally by Martin Evans

1985

Copyright © Oliver Smithies

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 ~~fly~~ colonies by pipette onto P60 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°

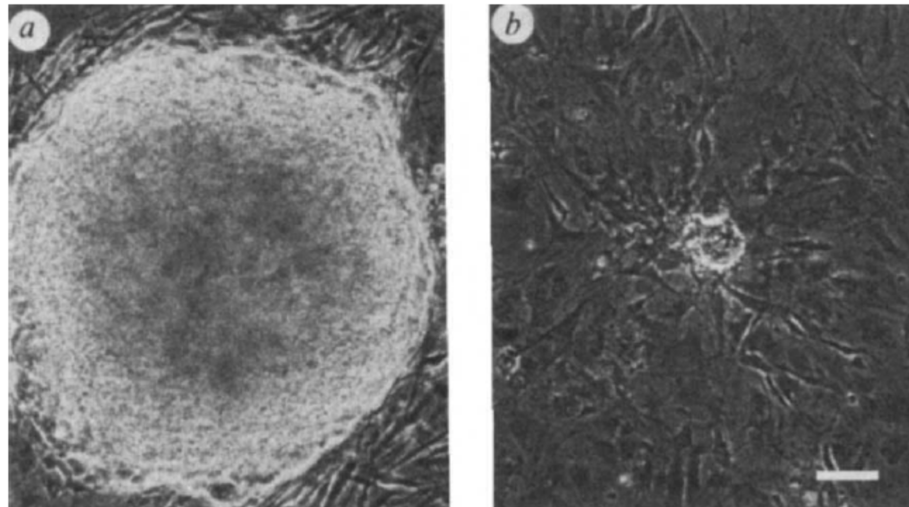
The cells are fine — but we must improve freezing.

Plan is to use these to get HPRT⁻ by recomb^n & get chimaeras & germ line 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)

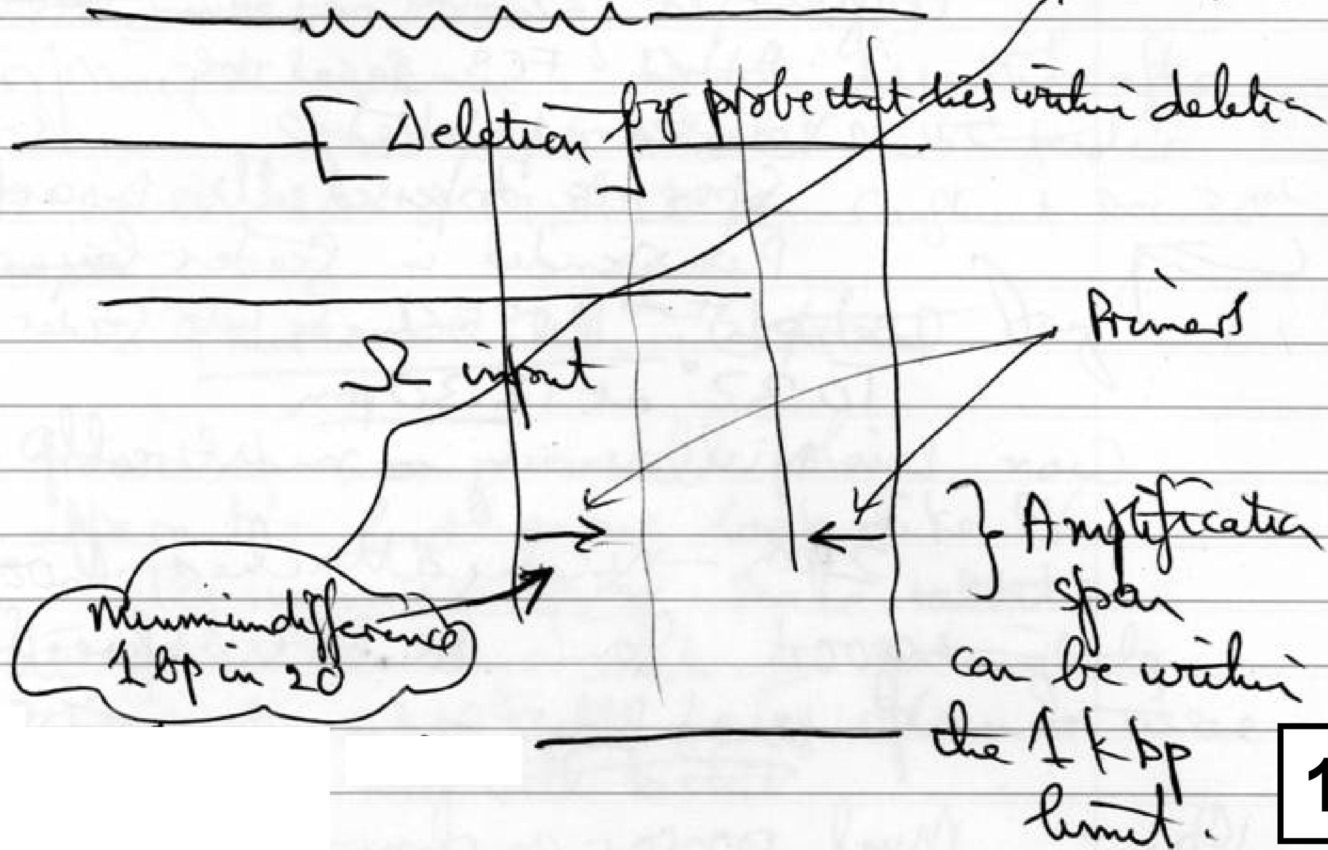
Copyright © Oliver Smithies

Wed Dec 17th

New idea for recombinants using amplification

PCR

Substitution by probe that covers region of diff



1986

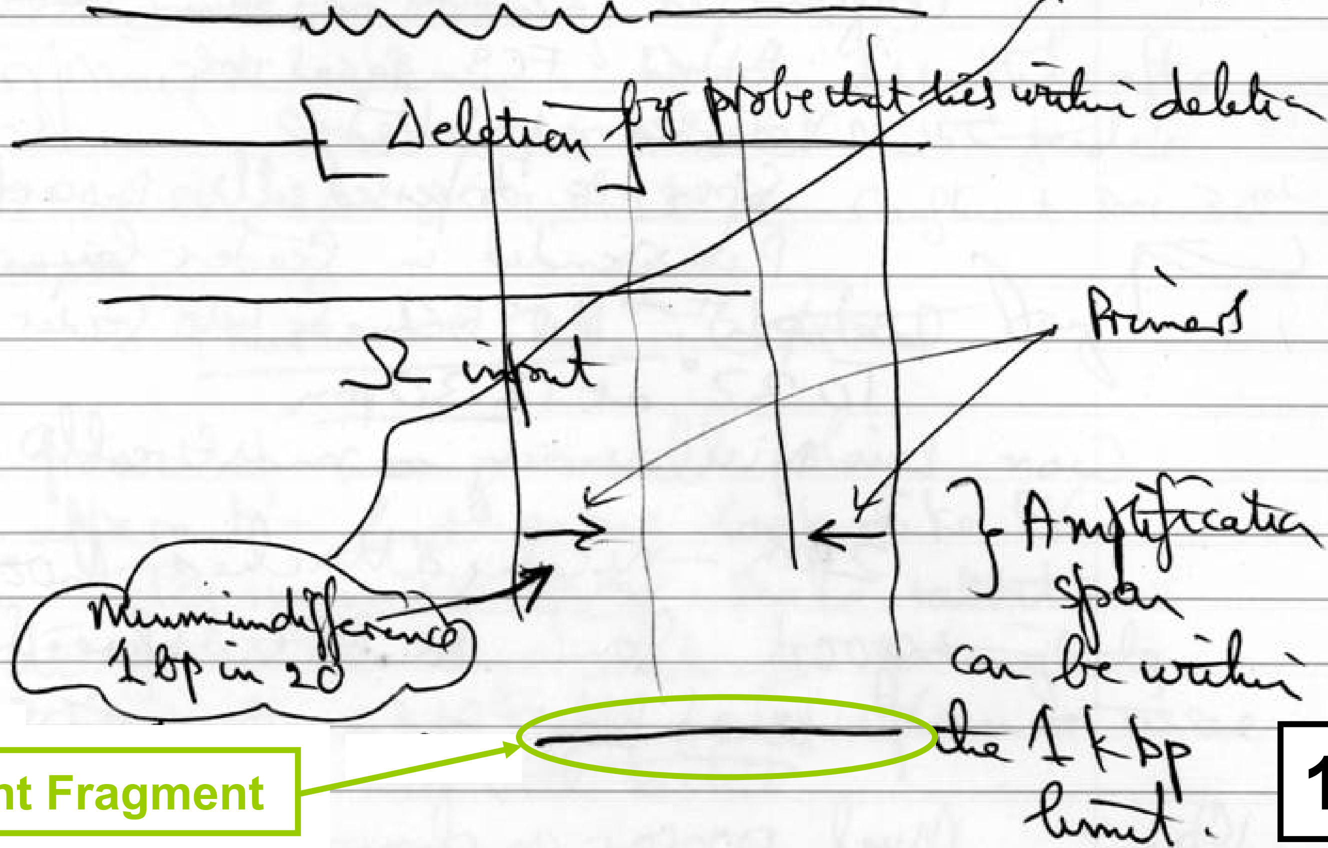
Copyright © Oliver Smithies

Wed Dec 17th

New idea for recombinants using amplification

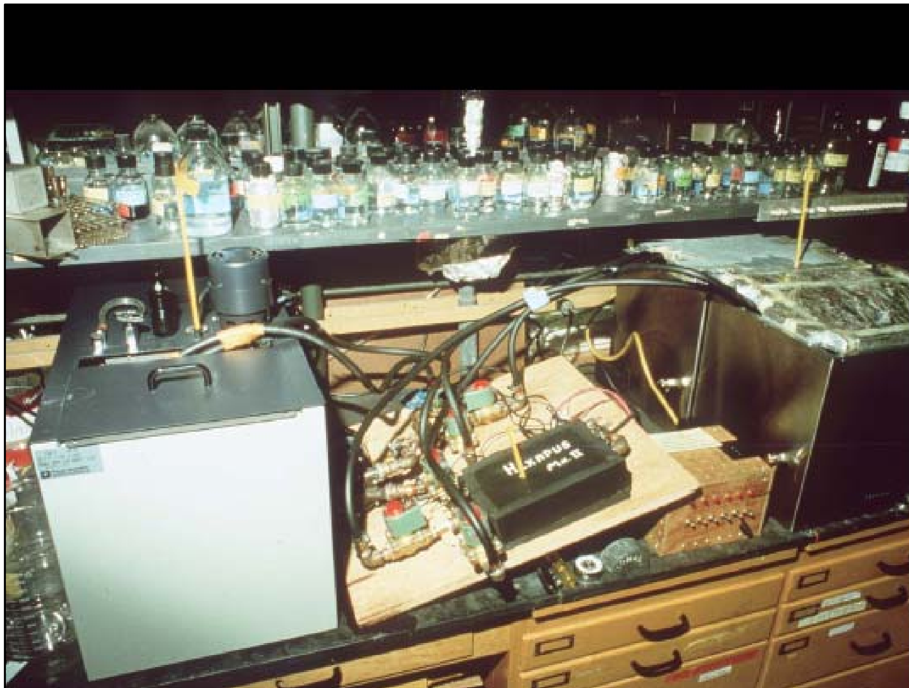
PCR

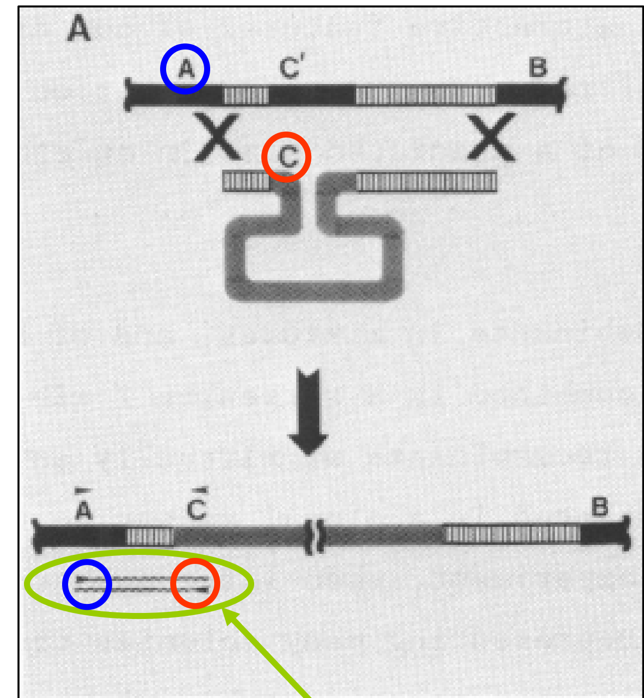
Substitution by probe that covers region of diff



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

Hyung-Suk Kim and Oliver Smithies



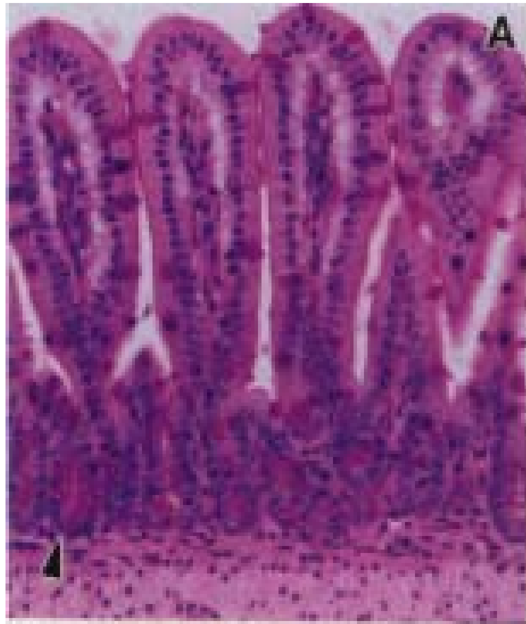


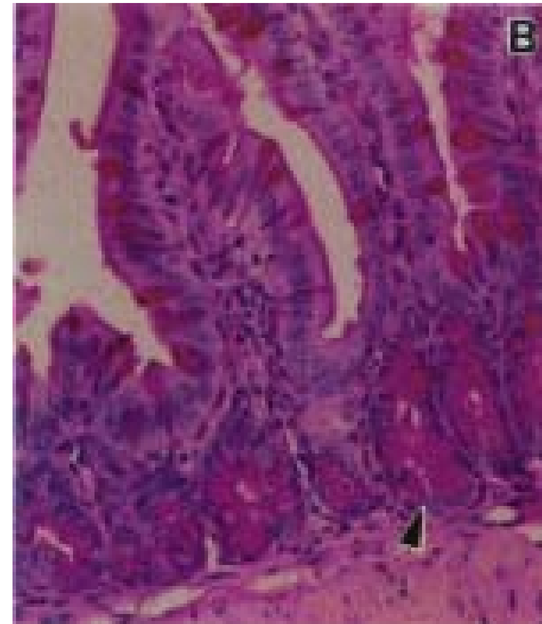
Recombinant Fragment

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*

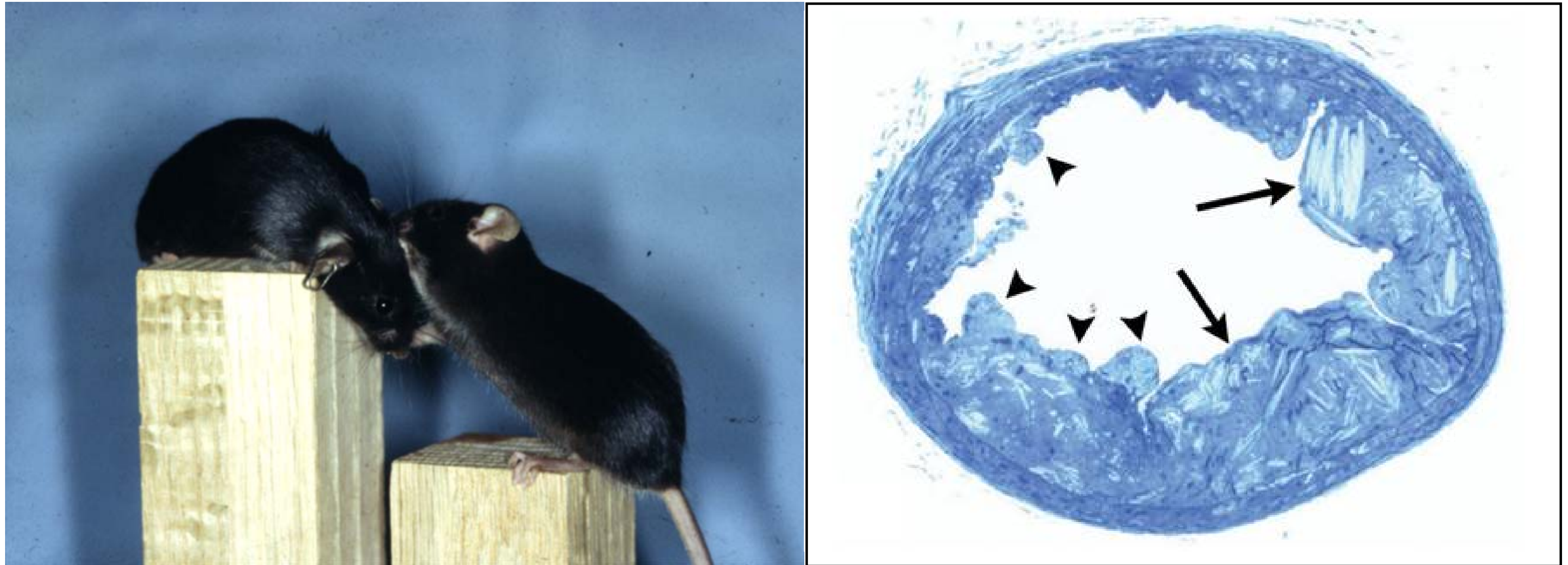




Science *257* 1083-6 (1992)

Spontaneous Hypercholesterolemia and Arterial Lesions in Mice Lacking Apolipoprotein E

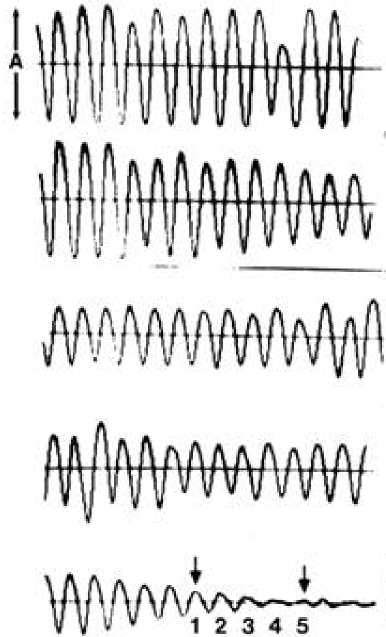
Sunny H. Zhang, Robert L. Reddick, Jorge A. Piedrahita, Nobuyo Maeda*



Science *258* 468-471 (1992)

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

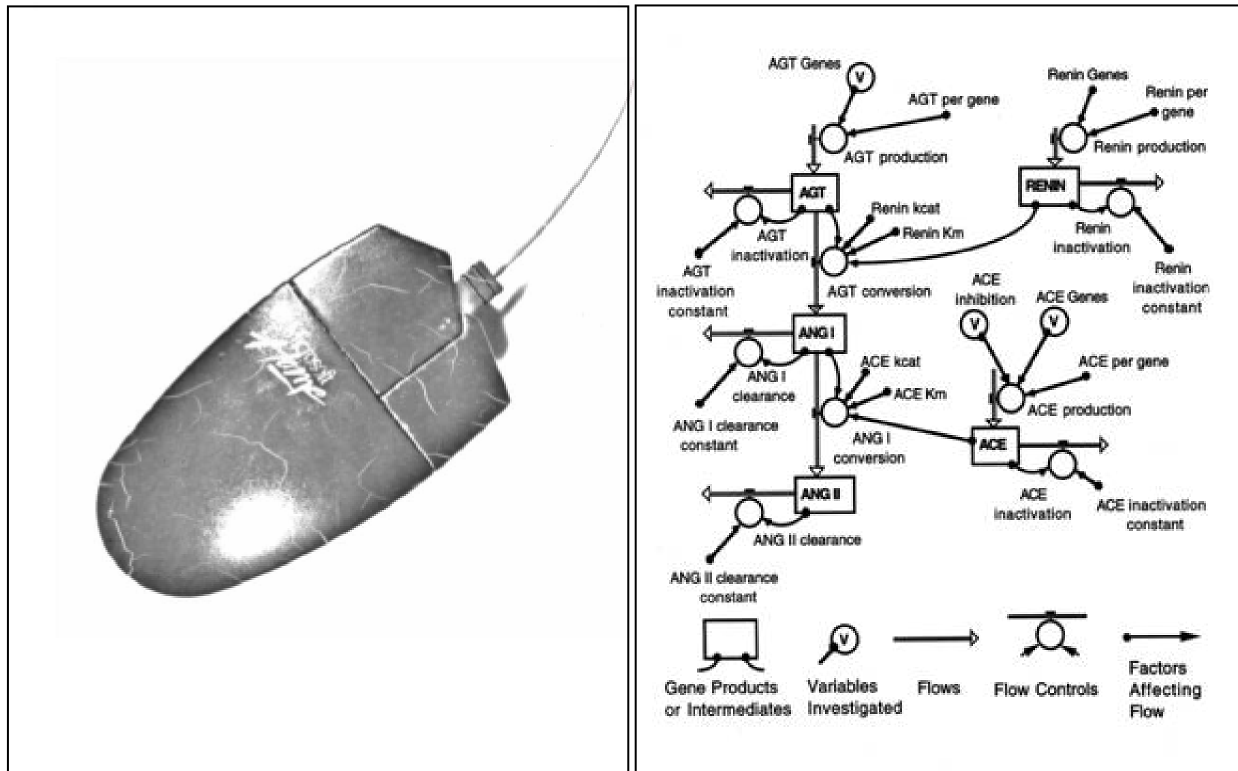
John H. Krege, Hyung-Suk Kim, Jeffrey S. Moyer, J. Charles Jennette, Li Peng, Sylvia K. Hiller, Oliver Smithies



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

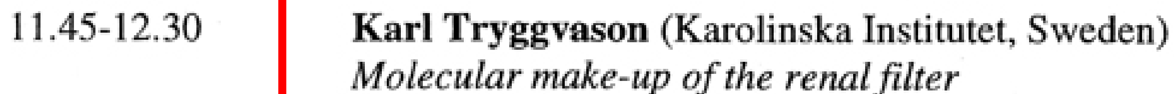


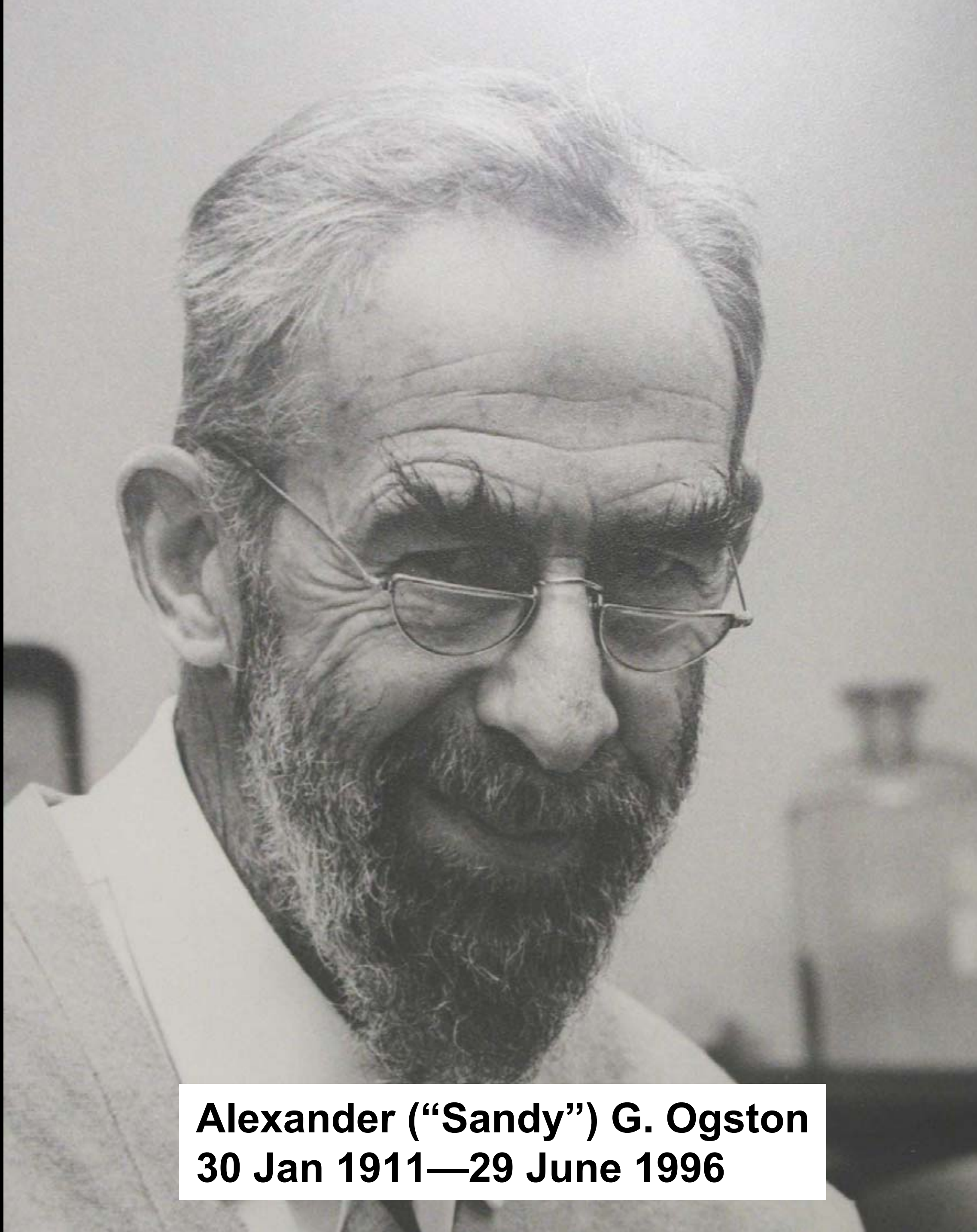
Kidney International *58* 2265-80 (2000)

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*





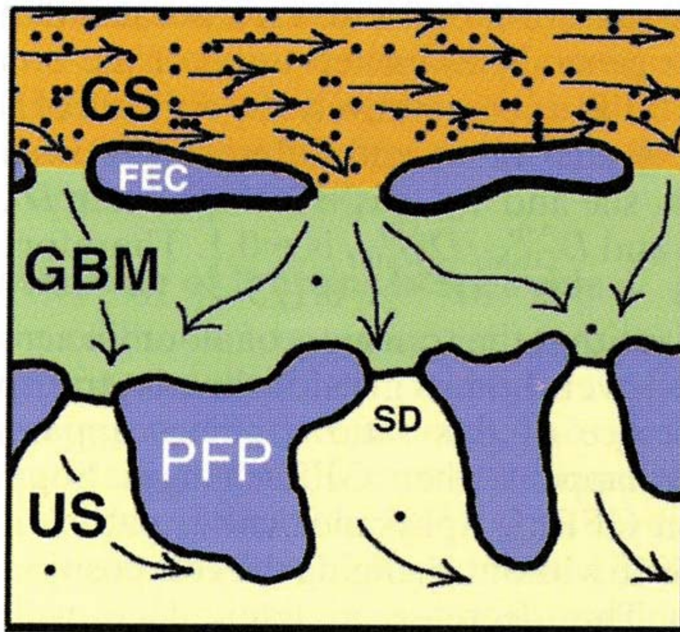
Alexander (“Sandy”) G. Ogston
30 Jan 1911—29 June 1996

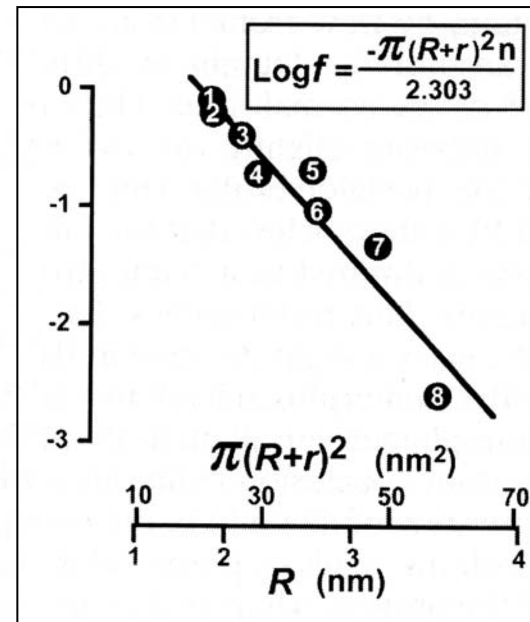
$$f_{AV} = e^{-\pi (R+r)^2 \cdot n}$$

Ogston, 1958.

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

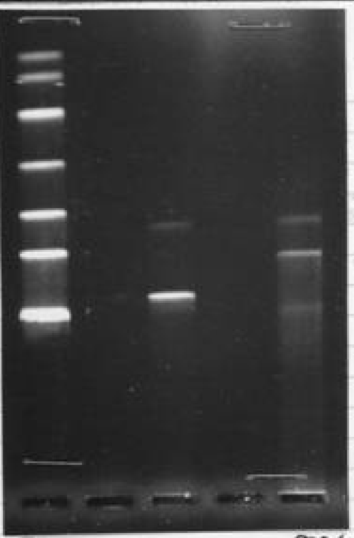
Oliver Smithies*





PNAS *100* 4108-13 (2003)

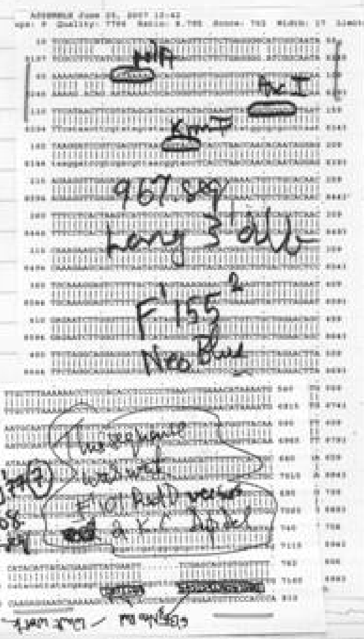
Copyright © Oliver Smithies



As expected RNA ✓✓ & digests are complete. Yield rather low.

0.86 HX4
OVN 2:30
B 3:05 pm
D 11:05 am

2	1
Dye	Dye
W	W
AG	Ag
W	W
Final/3	PRE
W	POST
Pre/3	FINAL 5
W	W
Dye	PRE
	POST
	W
	Dye



The discrepancy with G143 NeoRed failure (U'74)J resolved by using F101 RedD; Kathleen's Mrp del is ? 4 nucleotides in this region.

Sun. July 29th

<5 weeks!

Bulk of sequences of final candidate

U80 O.K.

966	5 μl U'77 7 1/10	1 μl V58 Red G135	14
967	5 μl U'77 7 1/100	F'155[2] Neo Blue 1 μl	14

O.K. 967

Transform U'81 D#5α U'77 7 — 4/26/7 (S.H.)

80 μl of 1/10 → 3 μl

0° 4:45 to 5:12 / 4:33 4:45 pm ~5.0 pm / 90 sec 42°

1 X AMP NZY
3 x 5/16 9 x 20/16 37° 5:20 pm

Good proportion again. ~3 pm

Mon. July 30th

To 2 tubes. Processed as 4 minis/tube to ~400 μl 0.01 M EDTA as U'81 7 Bulk

Not I digestion. Take 150 μl U'81 7 Bulk + 90 μl ddw + 30 μl 10x NEB #3 ✓ + 30 μl 10x BSA

+15 μl (150 u) Not I lot 50 (10 u/μl)
+1.5 μl (1.5 g) lab stock RNAse (1 μg/μl)

Submitted for Seq

968	5 μl 1/10 U'77 7	1 μl F101 RedD	14 μl

15 μl PRE U'81

37° ~5:30 pm U'81 7 RNAse — 9:30 am

Tues. July 31st — 20 μl POST U'81

1 x φOH/HOQ 2 x CIAA (sample 250 μl)
+ 1/10 vol 5 M NH_4Ac + 3 vols. EtOH — visible RNA-like ppt.
Washed 75% EtOH/MTris/then 70% EtOH
Back into 250 μl as U'81 Not 7 RNAse ... TBE

Not enough. Repeat growth, ~~...~~

#2 ~~...~~ Processed as 8 minis/tube x 2 tubes.
Back into ~~...~~ as U'81 7 BULK[2]
2 x 0.4 ml

U'81 Not 7 RNAse

One tube + 0.4 ml ddw + 0.1 ml 10 x NEB #3 + 0.1 ml 10 x BSA + 4 μl (4 μg) lab RNAse + 20 μl (200 u) Not I 37° 7:07 pm

20 μl U'81 PRE

Sun. July 29, 2007

What's on the <u>next</u> 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.