

Feb 10, 1954

W-2394 x W-2344 (P2) 1:1;5 6:30 - 8:00 PM. Then diluted 1:100 (Refr. for later

renewal. *Note, in several previous manip, micropipette is used to transfer cells to preplaced larger drops. This allows possibility of contamination by cells sticking to*

Set I (8:15-10:00) = ABCD Set II = HFG, H6 *contamination by cells sticking to (original notation: ABC, G5) outside of pipette.*

Set A (Previous evening, same cross?) H (originally D).

As this run and in future, have small drops (observed) only, and explained by micropipette in short interval.

couple
see D1

Green
A1
A2
3x cont.
A4x lot

| Lac | Gal/THTal | H ₂ L Xyl | S |
|-----|-----------|----------------------|---|
| - | + S - | - - | R |
| ± | - R + | + + | S |
| ± | (+ S) - | + - | R |

pair

B1-2 X
3
4

| | | | |
|---|-------|-----|---|
| - | + S - | - - | R |
| - | + S - | - - | R |

pair

C1
2

| | | | |
|---|-------|-----|---|
| - | + S - | - - | R |
| - | + S - | - - | R |

dump.
D

3
4
5
3
4
5

| | | | |
|---|-------|-----|---|
| - | + S - | - - | R |
| - | + S - | - - | R |
| - | + S - | - - | R |
| - | + S - | - - | R |
| ± | - R + | + + | S |
| ± | - R + | + + | S |
| ± | - R + | + + | S |

(contain? for Gal-ucan?)

see A2 (nb)

D1

| | | | |
|---|-------|-----|---|
| - | + S - | - - | R |
|---|-------|-----|---|

OK.

single

D2

| | | | |
|---|-------|-----|---|
| - | + S - | - - | R |
|---|-------|-----|---|

∴ 25, 27 only near-parentals.

[lot

E1]

E

A1
A2

| | | | |
|---|-------|-----|---|
| - | + S - | - - | R |
| - | + S - | - - | R |

O

3
4
5

| | | | |
|---|-------|-----|---|
| - | + S - | - - | R |
| - | + S - | - - | R |

F

B1
2
3
4
5

| | | | |
|---|-------|-----|---|
| - | + S - | - - | R |
| - | + S - | - - | R |
| - | + S - | - - | R |
| ± | - R + | + + | S |
| ± | - R + | + + | S |

G

C
2
3
5

| | | | |
|---|-------|-----|---|
| - | + S - | - - | R |
|---|-------|-----|---|

single
H6
H1-2
H3-4

DRY DRY
G5
C

| | | | |
|---|---------|-----|--------------|
| - | + S - | - - | R |
| - | + S - | - - | R |
| ± | (+ S) - | - - | R (lot, -) |
| ± | + S - | - - | R |
| ± | + S - | - - | R (all lot-) |

DATE: Feb. 11, 1954.

REF:

1 2 3 4 5 6 7 8 9 10

H1-2. 1 = "volunteer" 1 = P2.
 H2 = "large pair" ϕ = P1 + K1. (V, R)

H ~~4~~ = couple. ϕ -5 \rightarrow H4: P1
 \searrow H5: P1 + P2.

10 In retrospect, I would not rely absolutely on purity of H4 isolate in view of remote possibility of contamination from outside of pipette. In any event, the coupling is trivial.

20

All Restricts H2, H5:

H2 = $\left. \begin{matrix} \text{lac} - V_1^S \\ \text{lac} + V_1^R \end{matrix} \right\}$ of each tested.

30

H5 = parents.

40

50

Feb 11, 1954

W-2397 x W-2344

1:1:4 3:35-4:25. Dilute 1:20 (Kefr. sero)
Also plate 6:20

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | |
|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|--|
| A | 1 | 2 | 3 | 4 | 5 | | | | | | | | | | | | | | | | | | |
| B | 1 | 2 | 3 | 4 | 5 | | | | | | | | | | | | | | | | | | |
| C | 1 | 2 | 3 | 4 | 5 | | | | | | | | | | | | | | | | | | |
| D | 1 | 2 | 3 | 4 | 5 | | | | | | | | | | | | | | | | | | |
| E | 1 | 2 | 3 | 4 | 5 | | | | | | | | | | | | | | | | | | |

lac *S* *Gal* *T1* *MH* *Xyl* *Mal*
 ✓ ✓ ✓ ✓ ✓ ✓
no Xyl
no Xyl
no Xyl (reverse)
ult.
no Xyl
no Xyl

∴ all parentals except D3. B5 was doubly mated with P1, P2 as recorded in notes. When picked, this diplet had two separate clones, and since moderately well separated.

Phage reaction of D3 not clear enough to analyze total content, though only *Gal*⁺, *V*³ are seen. cf. S44 which showed only *lac*-*S*^R, no *lac*⁺.

D3: P1-P2? ✓ *lac*-*S*^R { *Xyl* *MH* *Mal* - }
lac-*S*^R { + }
 Remaining are P1 16
 P2 5

Xyl, lac recombination:

1129

random?

DATE: 2/13/54.

REF: 1125 CD.

- A W2394 } x W2344 1:1:5 12 hours overnight.
- B W2397 }
- C. W2394 SH- mutants from EML x W2344 2:45 PM -

A, B - Yields of Xyl+ Δ^R about same in both ($\approx 10\%$ of Lac+ SR).
10 Picked to EMB lac. (most still Xyl+).

C: Crossed overnight. Plate out N14. See EML. Choose #16 = W2400 up best.

A, B. Picked to EMB lac.

A5, A6, B3, B5, B6, B15 had lac+.

20

Tested directly to EMB M^H, T5.

lac, v. of M^H (or Xyl+) camp

| | lac | T5 (M ^H) | M ^H [⊕ = papillate] | Notes |
|------|------------|----------------------|--------------------------------|--|
| A. 1 | S. | S. | ⊕ | M ^H +, - all lac - v ^s |
| 2 | S | S | ⊖ | X + L - v ^s , - - S ⁱ |
| 3 | S, R(-) | S, R(-) | ⊕ | as A1. M ^H - v ^s must also be plus. |
| 4 | S, R(+) | S, R(+) | ⊕ | M ^H + lac - v ^s , R; M ^H - lac - v ^s , S |
| 5 | S, R(+, -) | S, R(+, -) | ⊕ | Xyl + lac - v ^s ; Xyl - lac - v ^s ; Xyl - lac + v ^s . |
| 6 | S, R(+) | S, R(+) | ⊕ | X + L + R; X - lac + S; X - lac - S |
| 7 | S | S | ⊖ | X +, - S |
| 8 | S, R | S, R | ⊖ | M ^H +, M ^H - } L - S. |
| B. 1 | S | S | ⊖ | Xyl +, - S |
| 2 | S, R | S, R | ⊖ | No M ^H and - R (Xyl+) purified |
| 3 | S, R | S, R | ⊕ | X + L + R; X - L + R |
| 4 | S, R | S, R | ⊖ | X +, - S and X - - R |
| 5 | S, R | S, R | ⊕ | X + L + S; X - L + S |
| 6 | S, R | S, R | ⊖ | + - S, - - S |
| 7 | S, R | S, R | ⊕ | M ^H + M ^H - } S - S |
| 8 | S, R | S, R | ⊖ | So. |
| 9 | S, R | S, R | ⊕ | + - S, - - S No M ^H + |
| 10 | S, R | S, R | ⊖ | " |
| 11 | S, R | S, R | ⊕ | M + L - R, M - L - S |
| 12 | S, R(+) | S, R(+) | ⊕ | |
| 13 | S, R(-) | S, R(-) | ⊖ | |
| 14 | S, R(+) | S, R(+) | ⊕ | |

∴ of M^H +
induced,
17 - S 8
2 - R 3
1 + S 1
2 + R 3

6+, 7-! 12S 11R(S) | 11-, 12⊕.
Xyl - M^H correlation? at least partial
But suspicious: Xyl+ not always recovered.
• had lac+

1-cell.

1130.
labelled 1129

DATE: 2/12/54.

REF:

n993

Fresh cells. Do not mix before manipulation. ϕ clumping alone and together. Ca 1:5 dilution of the cells

Setup 2:55 PM - ca 5 PM.

Gal TI Lac β galactosidase: Gal GalTS Mal Xyl MHLSTL SPI

A2
A3
A4

+ S -
+ S -
- R ±

B

B11
B12
B13
B15
B20

+ S -
+ S -
+ S -
- R ±
- R ±

✓ ✓ ✓ ✓ ✓ ✓
accordant.

all parents SPI 3P2 No R.

30

2/17 improved media to avoid clumping. Test P2c W2400 (3 hours) in ① NB case 10% glucose 0.2% ② = ① + galactose 10% ③ Nutrient broth, no salt. clumping (macro) is largely averted.

streak out lac sm:

① < 1% ② ditto ③ 2-3% O.K. Use NB for crosses if less microscopically clumping than Penassay.

50

W 2401-2 X P2.
 Inbred components: lac, V₁

Pick from the screen; test: Lac TS
 % Lac+ among Ara+
 17/19:
 21/29:

①

| | lact+ | lact- | | + | - |
|----|-------|---------|--|---|---|
| 1 | S | R | | R | S |
| 2 | R | | | S | S |
| 3 | R | | | S | S |
| 4 | | S | | R | R |
| 5 | | R | | R | R |
| 6 | S | | | R | R |
| 7 | R | | | R | R |
| 8 | R | [S S ?] | | R | R |
| 9 | R | [S S ?] | | R | R |
| 10 | R | [S S ?] | | R | R |

lact+ < R 12 12
 S 5 4

lac- (whenever) < R 1 5
 S 1 3

②

| | + | - | | + | - | | + | - |
|----|---|---|--|---|---|--|---|---|
| 1 | | S | | R | | | R | S |
| 2 | R | S | | R | | | R | S |
| 3 | | R | | R | | | R | S |
| 4 | S | R | | R | | | R | S |
| 5 | R | R | | R | | | R | S |
| 6 | R | R | | R | | | R | S |
| 7 | R | R | | R | | | R | S |
| 8 | R | R | | R | | | R | S |
| 9 | R | R | | R | | | R | S |
| 10 | R | R | | R | | | R | S |

what are Ara's screen under this heading?

Ara almost certainly linked to lac. Confirmed by Ara ratios among
 lac+ S^R: (Ara, TS): par = Ara - V₁^S, +R

| | | |
|-------|----|----|
| -R | | -R |
| -S | 18 | -S |
| +R | 4 | +R |
| +S | | +S |
| +R -S | | +R |
| -S -R | 3 | -R |
| +S -S | 1 | +S |
| <hr/> | | |
| 37 | | |

∴ 14/37 have Ara+ (< 1/2).
 definite linkage to V₁ is apparent.

| Ara ⁺ V ₁ ^S | V ₁ ^R |
|--|-----------------------------|
| 1 | 13 |
| 18 | 5 |
| Ara ⁻ V ₁ ^S | V ₁ ^R |

Not clear whether Ara/lac linked. Use V₁/S crosses.

Pending:
comprehensive linkage tests
V of $lac - V_1 - Ara.$

The independence of $lac - V_1 - Ara$
has not been settled, partly on account
of short supply of substrate (phage stock).
Probably not substantiated

2/15/54

~~W1122 x W1895 3-999. E1715 L22 m. (for H^S/R)~~

3/8/54. W2407 x W1895. For Gal⁺V^R recomb.
possibly Hfr!

all 10± Gal⁺ isolated probable recombinants (see OCA notes)