### A. Y40 × Y53

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Σ 10 8 10 0 28

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Σ 73 46 50 4 173

See summaries of data.

### B. Y64 × 58-161

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</table>

Σ 37 55 5 19 116

Σ 109 125 8 70 312

See next page.
April 20, 1947

\[ Y_0 \times 58 - 16/8 \]
\[ Y_40 \times Y_{53} \]

\[ A : \begin{bmatrix} -k & -s & +k & +s \\ 7 & 9 & 3 & 0 \\ 4 & 5 & 3 & 1 \\ 20 & 22 & 10 & 3 \end{bmatrix} \]

In previous crypts, \(+k > s\) occasionally.

\[ A(B_i) : \begin{bmatrix} 9 & 4 & 4 & 1 \\ 8 & 8 & 4 & 0 \\ 11 & 4 & 3 & 1 \\ 16 & 10 & 8 & 0 \end{bmatrix} + 18 \]

\[ 59 & 34 & 31 & 3 \]

\[ B : \begin{bmatrix} 8 & 9 & 1 & 2 \end{bmatrix} \]

\[ B(B_i) : \begin{bmatrix} 6 & 7 & 0 & 6 \\ 4 & 9 & 1 & 2 \end{bmatrix} \]

\[ 10 & 16 & 1 & 8 \\ 10 & 5 & 0 & 1 \]

\[ 20 & 21 & 1 & 9 \]

\[ 2 & 2 & 11 \]

\[ \text{A} \]

\[ A(B_i) \]

\[ 11 \]
\[
\begin{align*}
\text{Y}4a \times \text{Y}5b &\rightarrow \text{Y}161 \quad T(13,1) \\
-6R &\rightarrow -6S & +6R &\rightarrow +6S \\
1a. &\quad 7 & 4 & 1 & 7 \\
2a. &\quad 5 & 7 & 1 & 7 \\
b. &\quad 8 & 10 & 1 & 4 \\
c. &\quad 9 & 5 & 0 & 4 \\
3a. &\quad 4 & 14 & 1 & 1 \\
b. &\quad 6 & 17 & 1 & 5 \\
4a. &\quad 5 & 12 & 0 & 3 \\
b. &\quad 6 & 8 & 1 & 4 \\
5a. &\quad 9 & 10 & 0 & 2 \\
b. & & & & \\
6. &\quad 11 & 6 & 6 & 10 \\
7a. &\quad 10 & 6 & 0 & 0 \\
b. &\quad 5 & 8 & 0 & 6 \\
8a. &\quad 9 & 5 & 1 & 5 \\
b. &\quad 2 & 10 & 0 & 7 \\
9a. &\quad 7 & 10 & 0 & 2 \\
b. &\quad 10 & 3 & 0 & 5 \\
10a. &\quad 9 & 12 & 0 & 3 \\
b. &\quad 7 & 4 & 1 & 7
\end{align*}
\]

- Numbers designate separate recombinant plates. Letters are testing plates.
- Rest - plasm. 13-R: 6 + R.
- Appearance very poor!
April 15, 1947.

FHM (7% glucose).

HC 7%: ++

N2Case 7%: +++

1. Glycerine: ++
2. Glucose: ++
3. Serine: ++
4. Aspartic acid: +
5. Asparagine: +
6. Glutamic acid: +
7. Proline: +
8. Hydroxyproline: ++
9. Cysteine: ++H. S. +
10. Alanine: +
11. Tyrosine: ++
12. O.: ++

1mg/10cc.

The production of gas in the minimal medium is at odds with previous results, and perhaps requires some correction.

Repeat: OK, - gas produced on FHM (0) by K-12 in 36-48 h.

abandon maneuver of experiments which seems to be present in N2 case.
Phytonomes - Recombination

April 1947

Strains:
B. A6 / Pro / MG.
A. A6 / 5th / Sm.

One separately and together:

A growth on 50 plants, into cystic medium.
- 4 days. Schmidt reverse plates.
1: count 2: streak.

For transconjugation:

A: 1AB / Nih. Agar plate! "rough" colony noted.

For 1AB / Nih. Plate:

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<th>1A/B</th>
<th>2A</th>
<th>2B</th>
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For series P 21: also on NA plates for series 3.

Streak on plates in order:

1, 2, 3, 4, 5, 6, 7, 8, 9, 10.

Read at 36 hours.

No evidence of recombination. Leftover + H5. Apparently internal.

See CD. But 3 and 4 don't suggest recombination.

R - individual resistant colonies (1-10)

Use ACD 2 -> B in combination.
20 min. 15'

Y40 x Y88.

Compare to T(B).

A. \( T(0) \) is \( T(B) \).

Student on CHA-minimal or CHA-biotin agar.

A. 1R/65. = \( \frac{1}{7} \)  

B. 1R/65. = \( \frac{1}{7} \).

2/16 = R.

Muddled in migration to B.

Use solution of CHA \( R \) on B plates, & reverse vials to establish conclusion.

4/16 Res.
(A) Plate Y40 x Y88 on B1-Acetate medium. This should result in
B1- la^5 segregates. Replating these colonies into B1-Acetate medium
should allow the elimination of parental type B1-Acetate sensitivity.
1-4 x 8, acquired. Check for any complementary appropriate.
Growth in meth- eaten, suggesting phage is probably not.

(B) Plate Y40 x Y88 on B1 medium, spreading vigorously to avoid contamination.
Pick 100 colonies carefully to minimal (ca 20 colonies/plate).
and test: streakout original isolates of those only a B+ component
to find any possible B- types.
Scoring as B+ or B- not very clear cut. Group A. more definitely B+, Group
B doubtful.

A - 15,  B - 17. Streakout on the plates.

1 2  3  4  5  6  7  8  9  10 11 12 13 14 15
all - all+ + - - -
+ - - -
+ - - - ( - )
+ - - - ( - )
- - - -
- no cols.

1 2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17
100+ 11 - 100+ 11 - all -
- - -
- - -
- - -
- - -
- - -
- no colonies all -
- all -

Streak more plates & 4 cols. each plate.
A) mostly \(+R\) \(\mathcal{E}_{k,n}^R\)

\[
E_R = \mathcal{E}_{k,n}^R
\]

\(1 - S\) \(\mathcal{T_{LB}}\)

B) mostly \(-R\) \(\mathcal{E}_{k,n}^R (R,?)\)

\[
1 + R \quad \mathcal{A}_{n,s} \quad \text{(big)}
\]

no other combinations aside from main component in these columns.
A. $YH0 \times Y86$. (to compare $V^R$ loci)

$B_i$. (to reduce mutation required). $B - M^{-} Lac + V_{IA}^R Nuc^{-} x T - L - B_i - Lac + V_{IB}^R Nuc^+$

- $S_{Sm}$.

$V^R M^+ Lac^-$ \hspace{1cm} 16 \hspace{1cm} V_i^+ V_{IA}^R$

$V^R M^+ Lac^+$ \hspace{1cm} 3 \hspace{1cm} V_{IA}^R$

$V^R M^- Lac^-$ \hspace{1cm} 1

488-1 \hspace{1cm} $-V^S M^- Lac^-$ \hspace{1cm} 1

488-2 \hspace{1cm} $V^R M^- Lac^+$ \hspace{1cm} 1

$\frac{26}{2} \hspace{1cm} V_{IB}^R = 75\%$

B. $Y537 - 161 \times Y86$. (to test complexity of $Nuc^+ - V^R$). $Lac + V_{IA}^R M^- x Lac + V_{IB}^R M^+$

- $R_{Sm}$.

$V^R M^+ Lac^-$ \hspace{1cm} 13 \hspace{1cm} Nuc^+ + V_{IB}^R$

$V^R M^+ Lac^+$ \hspace{1cm} 3 \hspace{1cm} Nuc^+ + V_{IA}^R$

$V^S M^- Lac^-$ \hspace{1cm} 1

488-3 \hspace{1cm} $V^R M^- Lac^-$. \hspace{1cm} 1

This recombination suggests that

$Nuc^+ + V_{IB}$

(compared to all plaque. contain? $I$ 'diplomy')

Compare the resistance patterns of 488-1; 2; 3; $Y40; Y86$

Compare recombination values of $Nuc^+ / Lac$ & standard $V_i^+ / Lac$.

From accumulated data:

45.79

$Y40 \times Y53$. 846 546. \hspace{1cm} 1392 = 60.6\% \cdot 37\%$. 846 546. \hspace{1cm} 1392 = 60.6\% \cdot 37\%$

$Y40 \times Y88$. 56 15 \hspace{1cm} 71 = 79\% \cdot 21\%$

$V_{IA}^R$ is $\underline{\text{incorrect}}$. 
April 23, 1947

Allelism of V^n

Allelism of V^n: Y40 x Y64. 

\( \frac{1}{10} \): lac - lac\+

\( \frac{4}{1} \) (Hae coil)

\( \frac{1}{10} \): lac + lac -

\( \frac{1}{10} \): 159/159

\( T(\theta) \)

\[
\begin{array}{cccc}
11 & 4 \\
15 & 4 \\
13 & 4 \\
18 & 5 \\
15 & 3 \\
13 & 5 \\
\end{array}
\]

\( \frac{122}{154} = 79\% \) lac-

This distribution fits Y64 x 58-161 better than Y53 x Y54

Empirical fit to accumulated data.

\[ a) \]

\[
\begin{array}{ccc}
106 & 48 & \checkmark \\
122 & 32 & 154 \\
562 & 573 & 1784 \\
1528 & 610 & 1938 \\
\end{array}
\]

\[ \chi^2 = \frac{16^2}{1} = 2.5 \]

\[ \chi^2 = 2.42 \]

\[ \chi^2 = 5.32 \]

\[ \chi^2 = 8.44 \]

\[ \chi^2 = 15 \]

\[ \chi^2 = 32 \]

\[ \chi^2 = 696 \]

\[ \chi^2 = 659 \]

\[ \chi^2 = 191 \]

\[ \chi^2 = 850 \]
Plate $\frac{4}{10} \times 1\frac{8}{8}$ into Biurei - Acetate agar. Isolate for $Ac^+ = Cla^-$ and compare segregation of $B^-$ and $B^-_1$ in the Cla class. I.

H. G. see 489.
1. Cf. 490. 1 drop of Y 40 x Y 64 mixture in C; mix well and incubate, to compare e 90 for rate. (grown in Y 10, plate in HW) 0.

2. Y 40 x Y 53. grow in M-W: 00 Plate do.

3. Y 40 x Y 53 grow in M-W Plate ni T(0) \[ \text{adjusted} \] \[ \text{same medium} \] \[ y \ 200. \]

4. Y 40 x Y 53 grow in YB Plate T(0) \[ 39; 35; \text{ca} \ 40 \]

5. do. do. Plate M-W 0.

The medium is N.Y. for plating, but may be OK, or better than YB for growing cells (especially). Try 5 buffer, 5 KNO3. Difference is within range of normal variation, except to another.

Medium: per liter

\[ \text{KNO}_3 \text{ 1} \]
\[ \text{glucose} \text{ 10} \]
\[ \text{NaCl} \text{ 5} \]
\[ \text{KH}_2\text{PO}_4 \text{ 3} \]
\[ \text{Na}_2\text{PO}_4 \text{ 1} \]
\[ \text{MgSO}_4 \text{ 0.1} \]
\[ \text{trace + CaCO}_3 \]

1 (N2-base 5)
\[ \text{Yeast extract 2.5} \] for 20.
April 26, 1947.

A. Y87 (B-M-V, R, Lec-) x Y10 (T-L-B, V, 5, lac+). for segregation of Lec-. (prediction: +R +S = -R -S.)

B. Y87 x Y64 (B-M-V, 8, Lec- x T-L-B, V, 8, lac-). fastest gal+leu-.

B). 13 14 tests of prototrophs all Lec- . . . loci are allelic.

A) Segregation:

\[
\begin{array}{cccc}
- \bar{R} & -S & +\bar{R} & +S \\
7      & 1     & 7     & 6     \\
5      & 3     & 9     & 9     \\
3      & 1     & 6     & 7     \\
4      & 6     & 11    & 16    \\
\hline
28     & 6     & 46    & 37    \\
\end{array}
\]

\[
\begin{array}{cccc}
9      & 1     & 16    & 9     \\
7      & 10    & 13    & 10    \\
5      & 0     & 10    & 3     \\
4      & 0     & 12    & 1     \\
3      & 1     & 12    & 5     \\
4      & 0     & 14    & 4     \\
1      & 0     & 17    & 3     \\
4      & 0     & 9     & 4     \\
6      & 0     & 7     & 3     \\
4      & 0     & 10    & 3     \\
\hline
102    & 7     & 201   & 91/401 \\
\end{array}
\]
Y91 x Y53

(Y91 x Y53) (B-M-Cl+V 1\textsuperscript{R} x B 1-T-L-2ae - V 1\textsuperscript{5}Cl \textsuperscript{5})

Minimal plating too crowded.

B 2 - screenings B 2-Cl+ or B 2-Cl+ to classify mutants/muse

4/56 (4/56 Plasmid Asistant) Using plating on B. Agar for

Plasmid Assists.
April 26, 1947

Strain on indicated plates.

Y64  Cla 1  + + +
58-161 Cla 1  ++ -
Y90  ++ + + +

4/27.

Y64  Cla 2  ± ± ++ + + Y94  Salmon. 20  ± ± + +
58-161 Cla 2  ± ± ++ Y96  Salmon. 21  ± ± + +
Y90  Cla 2  ± ± + + Y95  -
Y92  A2 50  +++ -
Y92  A2 100.  ++ -
Y53  A2 50  ++ -
Y53  A2 100  - ++ -
Y40  IA 25  ++ -
Y93  IA 25  ++ -
Y40  IA 50  - ++ Y97  -
Y93  IA 50  ++ -

Y95:  Cla  IA  Cla + IA.

Interaction??

Sce about all mutants on N.A.
Test on inhibitor and transfer to plants.

Note: fermentation: unless otherwise stated, figures are %/ml. Undetermined figures are N/A.
### Virus Resistance Pattern

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<tr>
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<th>T1</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
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<td>S</td>
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<td>S</td>
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<tr>
<td>488-2</td>
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<td>R</td>
<td>S</td>
<td>S</td>
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**Note:**
- "V₃₄, λc=+ 488-1 ~ V₅₄ λc=+ 488-2"
- "R" indicates resistance.

---

**Experiments:**

- **Y40 V₁ R V₃ S V₅ S:** compare original livestock.
- k12 = S...
- 488-1 = S...
- 488-2 = V₁ R... S...
- 486 = V₅ S! (unstable; retrieve?)
- Y65 = R... S...
- Y68 = S...

---

**Explanations:**
- **Repeat T3, T1.**
- **Unstable resistant??** Less mucoid on this plate.

---

**Observations:**
- Y86 is predominantly mucoid; a few smooth colonies.
- Y65 and Y68 are smooth colonies, predominantly smooth; a few mucoid colonies.
Camphor Polyplody

April 25, 1977.

Add varying amounts of 30% camphor to plates to find following "concentrations" of camphor. Incubate 2 days. Shp. 95.

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Recover 4, 5, 8, 9 to test for polyplody.
Utilization of Acetyl-Glycine

April 27, 1947
Sel 480, Glycine Glycine Acetate

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<th>C.</th>
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ACETATE GLYCINE

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+ + + + +

F: 1. Acetyl-Glycine .5%
2. " + glucose.
Both + in 11 hours.
all others -.

End: Glycine is not utilized; not inhibitory.
Acetyl glycine is utilized by both.
Acetate is not utilized by mutant camp/wild.
Staining in zone of lysis.

April 27, 1947.

Compare V53 lyzed by T/10:

- E516
- E513
- E512
- E511
- E510
- E509

all show odoration nile cut zone, suggesting that it is mostly staining of debris.
April 28, 1947

A. Y90 x Y53  B. Y92 x Y53
(Y40/Ia)       (Y40/A2)

A.

Reading on
complete are unreliable.

Technique factors on
synthetic should be
developed.

<table>
<thead>
<tr>
<th>B: lac vi</th>
<th>R</th>
<th>S</th>
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<tr>
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<td></td>
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</tr>
<tr>
<td>-S</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>-R</td>
<td>8</td>
<td>1</td>
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<tr>
<td>+R</td>
<td>12</td>
<td>1</td>
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<tr>
<td>49</td>
<td>4</td>
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Use 100v/ml NaN₃ in T(0)+B₁.  Immunostat too
conc.²

<table>
<thead>
<tr>
<th>B.</th>
<th>BM</th>
<th>Lac</th>
<th>V</th>
<th>TL</th>
<th>A₂</th>
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<td>+</td>
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<td>+</td>
<td>R</td>
<td>++</td>
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</tbody>
</table>

Heavily R. A₂ is near TL.
ca 8% recombinant.

either beyond or between T-L
Use sections to locate
April 28, 1947.

A) Y86 x 58-161
B) Y86 x Y40

not useful. Interesting types could be merely mutants. [Accelerate mutation?]

T(10) - no colonies (even did ??)
T(13) -

Mucoid character too poorly expressed, although, many of the colonies picked looked as if they should be muc. Do these progressive "attenuation" of this character ??

A1. 1. Strain out Y86 stock on EMB- lactose;
34 Muc: 31 smooth.

P2 2. Strain out: A. Muc from 1.
B. Mix pop. from 1.
A: "all mucoid"
B: 19 Muc: 70 smooth.

P4 3. A - mucoid from 2 A.
all mucoid.

B. Mix pop. from 2 B.
ca 100:1 smooth: mucoid

A6 4. A - mucoid from 3 A.
al mucoid.

B. Mix from 3 B.
ca 200:1 smooth: mucoid

P7 5. B. (mix) from strain of 4.
all mucoid

Selection and mutation of $V_{ri}$

May 15, 1947.

A15. 7. Scale from mass-strike of 6.

c. 45:20 M:Sm.

A17. 8. Scale from mass-strike of 7.

c. 23:43 M:Sm.

P18. 9. do. 9:21 M:Sm.


P22. 11. do.
Acetyl utilization

April 29, 1947

Ac. Glucose Glycine 12-24 h. 36-48 h. 60 h.
K-12 Y89. K-12 Y89

1. 1/2%  
2. 1/4% 1/4%  
3. 1/4% 1/4%  
4. 1/2%  
5. 1/2% 1/3%  
6. 1/2%  
7. 1/4%  
8. Acetyl Glycine 1/2%.  
9. — — — — — ±  ±  

Add sterile separately from medium. Adjust acetate to pH 6.8 ± AcOH before adding.

The differential between K-12 and Y89 on acetate is not complete; there is a definite residual growth. Stimulation by glycine (not used by itself) accentuates the difference.

(Add sterile acetate anaerobic conditions)

8 h: K-12 Y89.

1. Ac.  ++  +  
3. 8  ++  ±  ! eventually the bug does better in acetate thanon Ac gly!
8  +  +  

Acetyl-diketopiperazine ½% wetted K-12 nor Y89. Shown as very responsive!
May 1, 1947.

See 499.

become presumptive thans.

a) streak again on CBA agar.  b) Cross to Y90.  c) streak set on EMBLacks plate soaked with M67 auxotroph 1.45, 8 finally three of these mutants 9 only 2.

b. 92. 1 ml mixtures into B1, plain agar respectively.


Discrepancies between 0 and B, plates are only ca 3 fold rather than 10 fold.

<table>
<thead>
<tr>
<th></th>
<th>Smooth</th>
<th>Hairy</th>
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<tr>
<td>1-C0</td>
<td>+R -R +S -S</td>
<td>+R -R +S -S</td>
</tr>
<tr>
<td>①</td>
<td>170</td>
<td>2</td>
</tr>
<tr>
<td>②</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>③</td>
<td>13</td>
<td>1</td>
</tr>
</tbody>
</table>

T(0) | 14 | 32 | 1 | 11 | Typical segregation.

T/(b) | 2 | 8 | 2 | 10 |

T/(b) | 16 | 21 | 2 | 16 | 2

The Y53 2 x doubled is not a good test; better would be Y90 which carries more dominant alleles.
Resistance Patterns

<table>
<thead>
<tr>
<th>T-1</th>
<th>T3A</th>
<th>T3 B</th>
<th>T3 2</th>
<th>T5</th>
<th>T5frey 2</th>
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<tbody>
<tr>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
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</table>

Viruses stocks which have varied, not original cultures since Y40 = Y40 now in all respects. T3A must be fallacious. T5 - batch 2 behaves like T1, and is similar to previous responses. Could it be contaminated??

Phages and purification! Recheck T3A. Present indicator’s favor the interpretation that the results of last fall were due to gross contamination of T3 and T5 & T1.

Program: Purify T5 - batch and isolate components.

T-5 from original culture (December) was plated with K-12 and grew uniformly, e.g. several identical, appeared, first these with T1, etc. Use this to reinoculate T5 stock.
1. Start new T5 stock from 1) lysate using original T5 on K-12 2) small colony picked from existing T5.

2. @thus far ok. Renew T1 on K-12.

3. Test a large plaque component of old T5 on K-12, Y40, K/5.

4. Test T1 on K-12, Y40, K/5 (from 526 R5).

<table>
<thead>
<tr>
<th>T1</th>
<th>&quot;T5&quot; large</th>
<th>&quot;T5&quot; small</th>
<th>&quot;T5&quot;</th>
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<tbody>
<tr>
<td>K-12</td>
<td>S</td>
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<td>R</td>
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<tr>
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</table>

"T5" from original!

flaccus indicus

from 526 - 0 which, previously, lysed Y40.
May 2, 1947.

Plate 10^9/mL on middle glycerol-plates and count the colonies in 2500x/min 20 min = 25,000 x. Harvest 600 x 25 mm.

1. After shaking agar step in H_2O ca. 300, streak out on EMB.

2. Streak out original sample on EMB. (YY only.)

3. Streak out depleted cultures on EMB.

Isolate 14 colonies each from YY0X and Y53X and streak across each other. Plate mixed growth on T(0) agar. (28 tests). No crossover suppression here!

<table>
<thead>
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