
Test type of 407 in BMT B (L) with very light inocula of 100 cc

Streak out colonies of 407 in EMB. Lactose test in:

<table>
<thead>
<tr>
<th>No.</th>
<th>407-</th>
<th>+5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>+5</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>+5</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
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<tr>
<td>5</td>
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<td>6</td>
<td>7</td>
<td>+5</td>
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<tr>
<td>7</td>
<td>10</td>
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<tr>
<td>8</td>
<td>11</td>
<td>+5</td>
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<td>9</td>
<td>12</td>
<td>+5</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>+R</td>
</tr>
<tr>
<td>11</td>
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<td>-R</td>
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<tr>
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<td>15</td>
<td>+R</td>
</tr>
<tr>
<td>16</td>
<td>16</td>
<td>-R</td>
</tr>
<tr>
<td>17</td>
<td>16</td>
<td>+R</td>
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<tr>
<td>18</td>
<td>18</td>
<td>+R</td>
</tr>
<tr>
<td>19</td>
<td>20</td>
<td>+R</td>
</tr>
</tbody>
</table>

Transfer 10, 11, 12 to Shotts + test further.

16, 17
January 27, 1947

1. \( \text{BM} + R < TLB, -R \quad \text{Y64} \rightarrow -R_1 + R \)

\( \text{Y40} \quad \text{TLB}, +S \quad \text{Y10} \rightarrow +R, +S \)

\(-S \quad -R \quad +S \quad +R \quad 0 \quad 16 \quad 7 \quad 28 \quad 751 \)

Therefore one can assume that an error was made in the previous experiment.

See 368, 398.

\( 58/161 \rightarrow \text{Y10/1} \rightarrow 38 -S \quad 16 +R \quad 0 +S (\text{raw!}) \quad 0 -R \)
January 27, 1987

581-161. TLB, +R Y46 → +S, +R.

547 + S TLB, -S Y53 → -S, +S

-S - R + S + R

3 0 0 0 0.
January 27, 1972

1. Plate Y40 + Y53 with 0.1 agar.
   (Use colonies of Y07) stand in cold room.

2. Streak out on EMB agar. a. plate remainder of colonies in T(0) agar.
   Use unduplicated colonies
   
   +       -
   1       2
   3       3
   4       1
   5       0
   6       0
   7       0
   8       0
   9       0
   10      0
   11      0
   12      0
   13      0
   14      0
   15      0
   16      0

   b. Streak one other colony on EMB, looking for variation.

A

1 +
2 -
3 -
4 -
5 -
6 -
7 0
8 0
9 0

Total: 6 + 31 = 37%
only heterogeneous.

B
January 27, 1947

1. Y57 x Y68 (baccillus communis secretum) No colonies

2. Y64 x Y68 m B. No colonies
   Y53 1/ x 58-161 M

3. Y53 x Y68 (test for recombination). No colonies

4. Y67 x Y40
   Y53 M x 58-161/ M

5. Y68 x Y53.
   Y53 x 58-161/ M

6. Y67 x Y68
   allel

7. Y67 x Y69
   allel

1. Y53 x 68 n.g.

2. Y57 x Y68 n.g.

3. Y64 x Y68 n.g.

4. Y69 x Y53
   Y40 x Y53

5. Y40 x Y67
   Y40 x Y53

7. Y67 x Y69 ok, but poor

Y67 = Y53 M
Y68 = 58-161 M
Y69 = Y40 M
January 30, 1947.

Received from Dr. Max Zell, P.H.H. Bethesda, Md.

\[ \text{bar} \quad V_i \]

\[ \text{B} \quad - \quad s \]

\[ \text{C} \quad - \quad s \]
\[ - \quad s \]
\[ - \quad s \]

\[ \text{D} \quad - \quad s \]
\[ - \quad s \]
\[ - \quad s \]
\[ - \quad s \]
\[ - \quad s \]
An analysis of male theory:

<table>
<thead>
<tr>
<th>Gene</th>
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<th>25</th>
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<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>a</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>b</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

1. Plectinae or 0.5 types or 0.5 types. By the cross: Bp x Y64:

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

The most frequent plectinae class, by far, should be - R.

The other types all require double crossovers. The relative frequencies of types should be of the order of:

- R 0.87
+ R 0.08
+ S 0.03
- S 0.04

2) \( b^- > b^+ \) from influence on segregation.

\( b^- > b^+ \) following distribution (ca.)

- R 0.30
+ R 0.50
+ S 0.15
- S 0.05

BLT, BIP, LT, should be usually recoverable.
To demonstrate genetic as well as biochemical distinctions of pseudoklebs (glut -) and pseudoklebs (glut +).

58-5255 x 679-662  Rather small moieties (<10^8)

8 colonies found.
1. Plate 5255 in O, Biotin alone for B + 24 hr. no colonies.

2. Plate into O, B1, B.

B, B1 > O, (5-10X)

B ca. 2.3 x 0.

\[ \chi^2_{3/2} B/B_1 = 11.1 \]

of which 8.3 is +R, 3 IS classes. B is essentially modifier of the lect.

An basis of map B, B is a V in TL, the phenotypic distribution should have been dominantly -R, while B - should have B + R, which is not suggested by these data. Also wouldeae better data as the frequency of B - B +. Their distribution suggests the map order:

B, B1, B V, TL.
Reed. 200,000 units of streptomycin HCl, Mead, Lot 277.
Potency 250U/mg, unoffically from H. Robinson.

A. Dissolve 100,000 units in 10 ml H₂O for stock solution: 10⁴/10³ dilute serially for stocks of 10⁻³ + 10⁻²/ml.

Use 10⁴, 10³, 10² + u. plate & controls on washed YS3 (standing lyo. 1 hour in H₂O per.), + + + T.

10⁴
10³
10²
ca. 100, very small "resistant" colonies at 18 hours, incubate further.

Rec. types - see 407


Test types of 407 in YMTB, [−L] at 14 days light/medium of the 30-

Streak and colonies of 407 in EMB-lactose. Test for:

AMB, AMTL.

1. 2 + 5
2. + +
3. 3 + 5
4. use a colony 4 + 5 + R in luminescent streak (maybe mutant form 5 R) + +
5. 6 + 5 do.
6. 7 + 5
7. 6 + 5 do.
8. 10 + 5
9. 11 + 5

P
Degrade 10 mg DNase (gift of Arne McCarty) in 10 ml 2x coli minimal. Streak filter - filtered well. Store in cold. Freeze refrigeration.

Plan: Add 1 ml of DNase (1 mg/ml) to 1 ml of cell suspension, separately. Mix cell suspension + plate 1 ml. Also, add cells in DNase, in minimal medium.

A. 0.1 ml Total 200 µl 1 plate: 17, 8, 19, 15 15

B. 0.5 ml ca. 2 µl 4 plates: 6, 11, 12, 33 15.5

C. control 9, 7, 23, 13 13

In this exp., DNase has had no appreciable influence on recombination.
2/10/47

BTL x B, L.

\[
\begin{array}{cccc}
B_1 & B_2 & V & T \\
+ & + & S & + \\
- & + & R & - \\
42 & 16 \\
\end{array}
\]

Plate mixture into 0, T, L.

0: 1/4
T 1
L 1

m.v. at recombination.
February 10, 1947

Y64 x Y10 x Y40.

Yield very poor. Do not use for testing.

cf. Other experiments. Reisdate !!!! — preliminary results??
February 10, 1947

Repeat part B and controls of 420.  Y40 x Y53.

B: 1/2 ml cells + 1/2 ml DNase separately + mix cells. Plate onto minimal + B, again. (1/2 ml of mixture).

B: 0  20
8, ca 150.

C: 0  2
8, 8

controls did not grow well here!
(agar base cloudy!)
Test various polynucleotides against Y41, etc. for use in homology.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Y41.86</th>
<th>No. prot. 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Feb. 10 '47</td>
<td>58-3214 x Y41.86</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2. Feb. 13</td>
<td>6177</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3232</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6049</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6317</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5450</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5235</td>
<td>0, 0</td>
<td></td>
</tr>
</tbody>
</table>

679-440 x 5355 0
February 10, 1947.

1. The bid - 5
2. meth x 1000 - 10^3.5
3. lys - 0
4. ped - 0
5. meth x 100 - 3 - 1
   Latin 3/4 plate!

6. The - 0
7. ind - 5
8. meth - 2
9. aux x 1000 - 10^3.5
10. lys - 40
11. leuc - 1 (probably contain!) - 45
12. ped - 100
13. cite - none 0 (substituted)

No evidence of recombination.
Threon + leuc seem to be most stable type in this series.

Treat cells suspension of Y53, 1/2 0.1% HN2 (bio-β-naphthyl
methylamine, HCl) in phosphate-citrate buffer, pH 4.0 for 1 hour
at room temperature. Terminate treatment by diluting with
broth, centrifuge & wash into fresh YB broth. Incubate over-
night.

A. Streak out EMB agar after 4-hour incubation.
Y53 has proliferated considerably; Y40 has not!
Take isolated colonies to YB liquid. 

B. Treatment cultures after 20-hour incubation.
Also, take slants from entire population.

P13. Cross 10 cultures from A, each, to Y40 + Y76 resp. in

<table>
<thead>
<tr>
<th>0 + B1 agar</th>
<th>0 + B1 agar</th>
<th>0 + B1 agar</th>
<th>0 + B1 agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.10 150 11</td>
<td>21 20 100 31</td>
<td>22 10 100 32</td>
<td></td>
</tr>
<tr>
<td>2.14 150 12</td>
<td>23 60 300 33</td>
<td>24 20 500 34</td>
<td></td>
</tr>
<tr>
<td>3 2.5 150 13</td>
<td>25 8 150 35</td>
<td>26 14 50 36</td>
<td></td>
</tr>
<tr>
<td>4 15 150 14</td>
<td>27 20 150 37</td>
<td>28 40 500 38</td>
<td></td>
</tr>
<tr>
<td>5 20 150 15</td>
<td>29 50 500 39</td>
<td>30 10 500 40</td>
<td></td>
</tr>
</tbody>
</table>

all OK.

Request 6, 7, 8 +

[Compare E & appropriate controls]

Y76 x 58-161. m T(0) + T(12).

\[ B_i - 44 \text{ lac+} \]
\[ B_i + 9 \text{ lac-} \]
\[ = 53 \text{ lac-} \]
\[ \text{add to 418: 45 tests.} \]
\[ = 98 \text{ tests.} \]

This tests for only 49 recombination, since \( \frac{1}{2} \) would be 5 lac-.
Feb. 13, 1947

1/2 ml eq. 446. on plate: NSA.

*Streptomycin*:
- 10 U. turbid plate.
- 50 U. as below.
- 100 U. ca. 10^2 small resistant colonies. (difficult mix; adenomatous, again).
- 1000 U. med > 5 u/ml.

*Brilliant Green (1:1000)*
- 1 ml -
- 0.5 ml - no resistant found!
- 0.1 ml turbid.

HyCl2
- 10 mg -
- 1 mg not well diffused; evidence of resistance in some regions.

*Tyrodein* (in alcohol)
- 500 U. turbid (ca. 10^3 colonies) = 50 u/ml no inhibition.
- 200 U. do.
- 100 U. do.

*Tyrodein mg.*
- B.S. OK at ca +/1: 800,000
- Mg OK at ca 5-10 /mil.
- Allopurinol OK at 5-10 u/ml.
February 17, 1947.

Repeat - [used unshaker-treated cultures].

streak out Y40, Y53 on sugar-EMB media.

<table>
<thead>
<tr>
<th></th>
<th>Y40</th>
<th>Y53</th>
</tr>
</thead>
<tbody>
<tr>
<td>lactose</td>
<td>+ + +</td>
<td>-</td>
</tr>
<tr>
<td>melrose</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

Mannitol variable, variable; predominantly -; lactose -; peed +
Glycerol - (2+), variable, +! Note diff. Y53 + Y40.

Alcohol ±
Sucrose - 5± - 5± (fault blue coloration; not a + reaction).
Citrate pH too low

Note 3/18: Xylose: K12 is + +

Melrose is definitely +.

Sucrose seems distinctly - - select for + reactants??
Mannitol + glycerol may be too variable to be useful.

Sucrose - E. coli communis.
February 18, 1977.

Cross 426-6, 7, 8 with Y53, Y76 to +5 B.

<table>
<thead>
<tr>
<th></th>
<th>426-6</th>
<th>0</th>
<th>Y76</th>
<th></th>
<th>0</th>
<th>Y53</th>
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<td>1</td>
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<td>50</td>
<td>200</td>
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<td>426-7</td>
<td>50</td>
<td>600</td>
<td></td>
<td>50</td>
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<td>3</td>
<td>426-8</td>
<td>150</td>
<td>4+T;</td>
<td>150</td>
<td>4+T;</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5P-161</td>
<td>200</td>
<td>4+T</td>
<td>200</td>
<td>4+T</td>
<td></td>
</tr>
</tbody>
</table>

Note: Y4000.
February 18, 1947.

per "20" ml plate NSA.

Y64  Hg(100V)  "resistant colony. not too well diffused"
Y46  do. clear. - "secondary colony!!"

Y64  Staphylococcus saline turbid 100u. clear in parts. Not killed.

Y46  58-161 50u. turbid 100u. as above.

Y64  Brilliant Green 5ml 1/1000 clean! (1:10,000) S. pick expressed

"  Methylene Blue 1/5000 1ml clean! (1:10,000) C. & B. large; 40mm. 20mm.

1ml 1/100,000) very difficult. clear where B is visible

Y46  Sodium Aride 10V turbid 1mp. irregular cleaning.

Y64  "Quercus" (1/20) 1/10 no ind.

Y64  tri-carbomycin (1/20) 1/10

Y64  Hg Cl2, 1mp. autolysed with 100ml N.A. - not indistinct.

Y64  Bi. 312, 2mg/100ml N.A. - moderate only faintly indistinct.

= 1:50,000. (more!) Y64  Staphylococcus 5u/ml  OK.

Y64  NaCl, 2ml/100 1/10 no ind. - later grows uniformly.

Y64  58-161. Hg 732, 2mg/100 N.A. - not enough!
Sex in L15 mutants?

February 17, 1947.

Grow separately, plate together.

Y5  2
"them.  0
Y5 x them  0.
Y5 x YYY  0
Y5 x SP-161  0

no evidence for sexuality under these conditions. Try growing together!
2/21/47

Mix Y40 + Y53 in water till to agar, mixing. Add 5 ml. aliquots ≈ 10 μl. to various suppl. plates. (see Y33 for note on media).

<table>
<thead>
<tr>
<th>5 μl.</th>
<th>0</th>
<th>10</th>
<th>10</th>
<th>10</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>10</td>
<td>4</td>
<td>15</td>
<td>10</td>
</tr>
</tbody>
</table>

B1: >25 T. T. T. T.

This expr. illustrates influence of conditions on detection of recombinants.

10 ml - 8 sublayers:

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
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<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>B. T.</td>
<td>B. 12</td>
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</tbody>
</table>
Tests for inversion in β.-Brucella.

2/4/1/47.

Cross mustard treated (126) isolates of Y40, Y53 =

Y53 x Y40 resp vi o, B, medium resp

Plate in medium lacking NH4NO3

(medium for inversion).

Y40 test:

<p>| | | |</p>
<table>
<thead>
<tr>
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<td>T ++</td>
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<tr>
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<td>T ++</td>
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</tr>
<tr>
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</table>

Y53 test:

<p>| | | |</p>
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<td>T ++</td>
</tr>
<tr>
<td>30</td>
<td>++</td>
<td>T</td>
</tr>
</tbody>
</table>

Note: no evidence of invasion in any of these isolates —

17 + 20 = 37 tests.

Number of prototrophs in this minimal seems quite unusually high.

In test-tube tests, Y40 x Y53 mix resp. inhibited by anaerobic conditions.