Medium supplements
+ Histidine
+ Biotin

Plate 1

Plate 2
+ Histidine
– Biotin

Plate 3
– Histidine
+ Biotin

Figure 3.1
<table>
<thead>
<tr>
<th>Substance</th>
<th>Toxicity</th>
<th>Resistance mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteriophage T1</td>
<td>Infects and kills</td>
<td>Inactivates tonB outer membrane protein; phage cannot absorb</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Binds to ribosomes; inhibits translation</td>
<td>Changes ribosomal protein S12 so that it no longer binds</td>
</tr>
<tr>
<td>Chlorate</td>
<td>Converted to chlorite, which is toxic</td>
<td>Inactivates nitrate reductase, which converts chlorate to chlorite</td>
</tr>
<tr>
<td>High concentrations of valine, no isoleucine</td>
<td>Feedback inhibits acetolactate synthetase; starves for isoleucine</td>
<td>Activates a valine-insensitive acetolactate synthetase</td>
</tr>
</tbody>
</table>
Figure 3.2

A

1. Plate
2. Incubate

E. coli → Agar medium with streptomycin → No growth

B

1. Inoculate with E. coli Str<sup>+</sup>
2. Add streptomycin
3. Incubate
4. Plate

Agar medium with streptomycin → Growth Str<sup>r</sup>
Figure 3.3

Culture 1

Culture 2

Generation

1st

2nd

3rd

4th
Figure 3.4

Wild type
Protein receptor
Bacteriophage T1

E. coli

Mutation

T1r mutant
Mutant receptor

E. coli survives

T1 cannot bind
Experiment 1

1. Measured aliquots taken
2. Spread on plates previously spread with phage T1
3. Number of resistant mutants in each aliquot determined

Experiment 2

1. Several cultures inoculated simultaneously
2. Spread on plates previously spread with phage T1
3. Entire culture is spread on a T1-containing plate

Total number of resistant mutants in each culture determined.
<table>
<thead>
<tr>
<th>Aliquot no.</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of resistant bacteria</td>
<td>Culture no.</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>6</td>
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<tr>
<td>7</td>
<td>26</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>8</td>
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<tr>
<td>9</td>
<td>20</td>
<td>9</td>
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<td>10</td>
<td>13</td>
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<td></td>
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<td></td>
<td>12</td>
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<tr>
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<td>14</td>
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<td>15</td>
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<td></td>
<td>16</td>
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<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>
Spread bacteria

Spray virus on plate

Respread bacteria; then spray virus

Scrape off bacteria; then dilute and plate to determine total number

Count phage-resistant colonies

Figure 3.6
### Table 3.3

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>No. of bacteria plated</th>
<th>Ending no. of bacteria</th>
<th>No. of resistant colonies$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>$5.1 \times 10^4$</td>
<td>$2.6 \times 10^8$ (plate 3)</td>
<td>8 (plate 1)</td>
</tr>
<tr>
<td>6</td>
<td>$5.1 \times 10^4$</td>
<td>$2.8 \times 10^9$ (plate 6)</td>
<td>49 (plate 4)</td>
</tr>
</tbody>
</table>

$^a$unsp, unspread; sp, spread.
Figure 3.8

Slope = $a$ (mutation rate)
Figure 3.9

Transitions

Transversions
Figure 3.10

(a) Wild type

(b) Mutant

(c) Transition

(d) Transversion

B

Deoxyribose

Guanine

Thymine (enol form)
Figure 3.11

Mutation and Genetic Analysis

Cytosine is deaminated

URACIL N-glycosylase removes uracil

Repair synthesis and ligation

AT → GC

TA → GC
Figure 3.13

Wild type
DNA 5’ AAA CAA CCC GGG TAA 3’
3’ TTT GGT GGG CCC ATT 5’

mRNA AAA CAA CCC GGG 3’
GAA UAA 3’

Polypeptide Lys Gln Pro Gly COOH

Mutant
DNA 5’ AAA TAA CCC GGG TAA 3’
3’ TTT ATT GGG CCC ATT 5’

mRNA AAA UAA CAA CCC GGG 3’

Truncated polypeptide Lys
Figure 3.14

Wild type

5’-CAAUCUCGG

Gln Ser Arg
downstream

Mutant

5’-CAAUAUCGG

Gln Ile Pro
All amino acids downstream are changed
Slippage of DNA occurs during replication.

Extra base is inserted.

Next round of replication.

Wild type

Mutant

Figure 3.15
Figure 3.16

I

GCAT  GCAT

CGTA  CGTA

“Unequal” crossing over

A  GCAT  GCAT  GCAT

CGTA  CGTA  CGTA

B  GCAT

CGTA

II

“Looping out”
Pairing of homologous sequences

Figure 3.17
Box 3.2

Inversion Box 3.2
Figure 3.18

Direct repeats

“Mistaken” pairing and recombination

Duplication

Deletion
Figure 3.19

Pathway

1. Galactose + ATP $\xrightarrow{\text{GalK}}$ Galactose-1-PO$_4$ + ADP

2. Galactose-1-PO$_4$ + UDPglucose $\xrightarrow{\text{GalT}}$ UDPgalactose + glucose

3. UDPgalactose $\xrightarrow{\text{GalE}}$ UDPglucose
Mutations and Genetic Analysis

Figure 3.20

A. **Wild type**

Polypeptide will incorporate Gln

B. **Mutant** with nonsense mutation in gene X

Truncated polypeptide

No tRNA to pair with nonsense codon; ribosome dissociates

C. **Mutant** with nonsense mutation in gene X and nonsense suppressor mutation in gene for tRNA

Mutant anticodon pairs with nonsense codon
### Table 3.4

<table>
<thead>
<tr>
<th>Suppressor name</th>
<th>tRNA</th>
<th>Anticodon change</th>
<th>Suppressor type</th>
</tr>
</thead>
<tbody>
<tr>
<td>supE</td>
<td>tRNA^{Gln}</td>
<td>CUG-CUA</td>
<td>Amber</td>
</tr>
<tr>
<td>supF</td>
<td>tRNA^{Tyr}</td>
<td>GUA-CUA</td>
<td>Amber</td>
</tr>
<tr>
<td>supB</td>
<td>tRNA^{Gln}</td>
<td>UUG-UUA</td>
<td>Ochre/amber</td>
</tr>
<tr>
<td>supL</td>
<td>tRNA^{Lys}</td>
<td>UUU-UUA</td>
<td>Ochre/amber</td>
</tr>
</tbody>
</table>
Culture of His<sup>-</sup> bacteria in medium containing histidine

Spread on plates containing all growth supplements except histidine

Incubate

Only His<sup>+</sup> revertants can multiply to form colonies
Figure 3.22

A

Nonselective plate; colonies transferred to cloth

B

Selective plate pressed down on cloth to transfer colonies

C

Incubate

Nonselective plate

Selective plate
Figure 3.23

A

hisA1  hisB+

(+)

hisA+  hisB3

Phenotype

His+

B

hisA1  hisB+

(+)

hisA2  hisB+

Phenotype

His−
### Table 3.5
Interpretation of complementation tests

<table>
<thead>
<tr>
<th>Test result</th>
<th>Possible explanations</th>
</tr>
</thead>
<tbody>
<tr>
<td>x and y complement</td>
<td>Mutations are in different genes</td>
</tr>
<tr>
<td></td>
<td>Intragenic complementation has occurred(^a)</td>
</tr>
<tr>
<td>x and y do not complement</td>
<td>Mutations are in the same gene</td>
</tr>
<tr>
<td></td>
<td>One of the mutations is dominant</td>
</tr>
<tr>
<td></td>
<td>One of the mutations affects a regulatory site or is polar</td>
</tr>
</tbody>
</table>

\(^a\)See the text for an explanation of intragenic complementation. This is a less likely explanation than the mutations being in different genes.
A

Step 1  Transform \textit{thyA} mutant \textit{E. coli} with library made from \textit{thyA}^{+} \textit{E. coli}

Step 2  Spread transformed \textit{E. coli} on plate without thymine

Thy^{+} colonies have complementing clone

B

\textit{thyA}^{+} → Thymidylate synthetase

\textit{thyA}
DNAs break at and pair

Nicks are ligated at * to form recombinant molecules

Figure 3.25
Figure 3.26

A

Wild type crossed with Revertant strain

Recombinant progeny

Phenotypes

His⁺

B

Wild type crossed with Suppressed strain

Recombinant progeny

Phenotypes

His⁻

His⁺
Figure 3.27

A

Replicate onto plate without thymine

Library of thyA+ E. coli DNA in thyA mutant E. coli

Colonies with many thy+ recombinants contain clone with at least part of thyA gene

B

Plasmid with clone

Chromosome

thyA

thyA+ Chromosome

Chromosome
Figure 3.28

A

```
<table>
<thead>
<tr>
<th>HindIII</th>
<th>Pstl</th>
<th>PvuII</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>400</td>
<td>600</td>
</tr>
<tr>
<td>N1</td>
<td>N2</td>
<td>N3</td>
</tr>
<tr>
<td>N4</td>
<td>N5</td>
<td>N6</td>
</tr>
<tr>
<td>N8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```

B

- **C3**: 757
- **C4**: 401
- **C5**: 509
- **C6**: 569
- **C7**: 620
- **C8**: 778
- **N1**: 971
- **N2**: 108
- **N3**: 83
- **N4**: ∆2
- **N5**: ∆64
- **N6**: 7
- **N7**: D6
- **N8**: NH2 (60) (120) (180) (240) COOH
Mutations and Genetic Analysis

A

I. One crossover breaks the chromosome

II. Two crossovers replace the chromosomal region

B

I. First crossover produces duplication of Y

II. Second crossover can replace Y

Figure 3.29
Figure 3.30

A

B
Figure 3.31

Mutations and Genetic Analysis

pSC101

42°C + tetracycline

30°C

Tet' repA(Ts) ori

Die

(Tet') ori repA(Ts) (Live)

(Tet') ori repA(Ts) (Live)