Bacterial colonies growing on Petri dish
Evidence for recombination in bacteria

**A**
- $met$ bio $thr^+$
- $leu^+$ $thi^+$

Mixture of A and B

B
- $met^+$ bio $thr$ leu thi

**Steps:**
- Wash and plate $\approx 10^8$ cells
- Wash and plate $\approx 10^8$ cells
- Wash and plate $\approx 10^8$ cells

- **Minimal medium**
  - No colonies (auxotrophic cells)
  - Prototrophic colonies

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Evidence for the requirement of contact in conjugation

F⁺ (strain A)  F⁻ (strain B)

Plate on minimal medium and incubate

Medium passes back and forth across filter; cells do not

Pressure/suction alternately applied

Plate on minimal medium and incubate

No growth

No growth
Bacterial conjugation
An $F^+ \times F^-$ mating

1. Conjugation occurs between $F^+$ and $F^-$ cell.

2. One strand of the $F$ factor is nicked by an endonuclease and moves across the conjugation tube.

3. The DNA complement is synthesized on both single strands.

4. Movement across conjugation tube is completed; DNA synthesis is completed.

5. Ligase closes circles; conjugants separate.

Exconjugants
Hfr bacteria and chromosome mapping

Step 1. F factor is integrated into the bacterial chromosome, and the cell becomes an Hfr cell.

Step 2. Conjugation occurs between an Hfr and F⁻ cell. The F factor is nicked by an enzyme, creating the origin of transfer of the chromosome (O).


Step 4. Replication begins on both strands as chromosome transfer continues. The F factor is now on the end of the chromosome adjacent to the origin.

Step 5. Conjugation is usually interrupted before the chromosome transfer is complete. Here, only the A and B genes have been transferred.

Exconjugants
Mapping by interrupted mating

Hfr H \((thr^+ \ leu^+ \ azi^R \ ton^S \ lac^+ \ gal^+)\)
\[ \times \]
F\(^-\) \((thr^- \ leu^- \ azi^S \ ton^R \ lac^- \ gal^-)\)

azi\(^R\) = resistant to sodium azide

ton\(^R\) = resistant to bacteriophage \(T\)\(_{1}\)

**Figure 8-7 Essentials of Genetics, 6/e © 2007 Pearson Prentice Hall, Inc.**
Genetic evidence for a circular chromosome

(a) Genetic evidence of chromosome transfer in Hfr strains:

<table>
<thead>
<tr>
<th>Hfr strain</th>
<th>(earliest)</th>
<th>Order of transfer</th>
<th>(latest)</th>
</tr>
</thead>
</table>

(b) Diagrams illustrating the order of transfer in different Hfr strains:

- **Hfr strain H**
- **Hfr strain 1**
- **Hfr strain 2**
- **Hfr strain 7**
Evidence for circular map

a) Orders of gene transfer

_Hfr_ strains:

H  origin-thr-pro-lac-pur-gal
1  origin-thr-thi-gly-his
2  origin-his-gly-thi-thr-pro-lac
3  origin-gly-his-gal-pur-lac-pro

b) Alignment of gene transfer for the _Hfr_ strains

H  thr-pro-lac-pur-gal
1  his-gly-thi-thr
2  his-gly-thi-thr-pro-lac
3  pro-lac-pur-gal-his-gly

c) Circular _E. coli_ chromosome map derived from _Hfr_ gene transfer data
F’ and the generation of partial diploids (merozygotes)

Step 1. Excision of the F factor from the chromosome begins. During excision, the F factor sometimes carries with it part of the chromosome (the A and E regions).

Step 2. Excision is complete. During excision, the A and E regions of the chromosome are retained in the F’ factor. The cell is converted to F’.

Step 3. The F’ cell is a modified F’ cell and may undergo conjugation with an F’ cell.

Step 4. The F factor replicates as one strand is transferred.

Step 5. Replication and transfer of the F factor is complete. The F’ recipient has become partially diploid (for the A and E regions) and is called a merozygote. It is also F’.
Transformation

**Competent bacterium**
- Receptor site
- Bacterial chromosome
- DNA entry initiated

**Step 1.** Extracellular DNA binds to the competent cell at a receptor site.

**Step 2.** DNA enters the cell, and the strands separate.

**Step 3.** One strand of transforming DNA is degraded; the other strand pairs homologously with the host cell DNA.

**Step 4.** The transforming DNA recombines with the host chromosome, replacing its homologous region, forming a heteroduplex.

**Step 5.** After one round of cell division, a transformed and a nontransformed cell are produced.

**Transformed cell**

**Untransformed cell**
Determining gene order by co-transformation

Donor bacteria

Extract DNA from population of donor bacteria; DNA breaks into fragments

Donor DNA

Transformation genotypes:
- $p^+ q o$
- $p^+ q^+ o$
- $p q^+ o^+$
- $p q o^+$

DNA fragments used to transform $p q o$ recipient bacteria

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Bacteriophage genetics

Mature T4 phage
Plaques of bacteriophage T4 in *E. coli*
Life cycle of bacteriophage T4

Step 1. Phage is adsorbed to bacterial host cell.

Step 2. Phage DNA is injected; host DNA is degraded.

Step 3. Phage DNA is replicated; phage protein components are synthesized.

Step 4. Mature phages are assembled.

Step 5. Host cell is lysed; phages are released.
Generalized transduction

Step 1. Phage infection.

Step 2. Destruction of host DNA and replication synthesis of phage DNA occurs.

Step 3. Phage protein components are assembled.

Step 4. Mature phages are assembled and released.

Step 5. Subsequent infection of another cell with defective phage occurs; bacterial DNA is injected by phage.

Step 6. Bacterial DNA is integrated into recipient chromosome.
<table>
<thead>
<tr>
<th>Selected Marker</th>
<th>Unselected Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>$leu^+$</td>
<td>50% $= azi^R$</td>
</tr>
<tr>
<td></td>
<td>2% $= thr^+$</td>
</tr>
<tr>
<td>$thr^+$</td>
<td>3% $= leu^+$</td>
</tr>
<tr>
<td></td>
<td>0% $= azi^R$</td>
</tr>
</tbody>
</table>

Table 18.1 Transduction Data for Deducing Gene Order

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Order of genes from generalized transduction data

thr  leu  azi

leu⁺ + aziᴿ = 50%
leu⁺ + thr⁺ = 2%
thr⁺ + leu⁺ = 3%
thr⁺ + aziᴿ = 0%
Life cycle of a temperate phage

1. Infection
   - Phage DNA enters the host cell
2. Lysogenic response
   - Integration of λ chromosome into host chromosome
3. Establishment of lysogeny
4. Lytic response
   - Lytic replication of λ chromosome
5. Release of progeny λ phages
6. Assembly of progeny λ phages

Additional steps:
- Multiple divisions
- Induction of lytic cycle
- Excision of λ chromosome
- Enters lytic cycle
- Replicated λ chromosomes
- Lysogenic response
- Integration of λ chromosome into host chromosome
- Lytic response
- Lytic cycle
Specialized transduction by λ

a) Production of lysogen

Circularized phage chromosome (λ)

Region of homology between chromosomes

Bacterial chromosome

λ chromosome

b) Production of initial lysate

(1) Normal outlooping

(2) Rare abnormal outlooping

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Plaques of bacteriophage T2 in *E. coli*
Mapping Bacteriophage genes
Looking for recombination

Phage = T2

Genes=
\( h^+ \), h  host range
\( h^+ \) can lyse B not B/2
h can lyse both

\( r^+ \), r  rapid lysis mutant
\( r^+ \) small plaques
r large plaques
Mapping Bacteriophage genes

$h^{+} r^{+}$ X $h^{+} r$

parentals

$h^{+} r^{+}$

$h^{+} r$

recombinants

$h^{+} r^{+}$

Recombinant phages growing on B/B2 lawn

Distance = $\frac{\text{number of recombinant plaques}}{\text{total number of plaques}}$
Learning more about phage genetics

Fine structure analysis of bacteriophage genes

T4 rII mutants
Seymour Benzer

Two questions to be answered:
1. Are all rII mutations in the same gene?
   Complementation testing
2. Are mutations in the same gene capable of recombination?
rII mutants of bacteriophage T4

wild-type phage - produce small plaques in both *E. coli* strain B and *E. coli* strain K12

rII mutants – produce large plaques and are able to grow on *E. coli* strain B but not on *E. coli* strain K12(λ)
Complementation testing

(a) Complementation (two mutations, in different cistrons)

During simultaneous infection, complementation occurs because both functional A and B products are present

(b) No complementation (two mutations, in same cistron)

During simultaneous infection, no complementation occurs because no functional A products are present
A geneticist is working with a new bacteriophage, Y3, that infects *E. coli*. He isolated eight mutations that fail to produce plaques in strain K. To determine if these are mutations of the same functional gene, he infects K cells with paired combinations of his mutants to see if plaques are formed. Here are the results. + = plaques, - = no plaques.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>+</td>
<td>+</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>+</td>
<td></td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>+</td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

To how many functional genes (cistrons) do these mutations belong? Which mutations belong to each functional gene?
How recombination within a gene could generate wild-type

Simultaneous infection of *E. coli* B and recombination

Recombinants

Gene bearing two mutations

Resultant phage will grow on *E. coli* B but not on K12 ($\lambda$)

Wild type gene restored

Resultant phage will grow on *E. coli* B and K12 ($\lambda$)
Protocol for recombination studies

Simultaneous infection with two rIIA or two rIIIB mutations

E. coli B

Recombinant (wild-type) phages infect E. coli K12(λ)

Serial dilutions and plaque assay

This plate allows the determination of the number of recombinants: 4 x 10^3 recombinant phages/mL

E. coli K12(λ)

plaques

10^{-3}

Non-recombinant (rII mutants) phages infect E. coli B

This plate allows the determination of the total number of phages/mL: 8 x 10^8 rII phages/mL

E. coli B

10^{-9}
Benzer’s method for identifying recombinants of two \( rII \) mutants of T4.
How deletions are used for rapid mapping

Area of deletion
Deletion mutation

Since recombination cannot occur in the area of the deletion, no wild-type recombinants of the A cistron can be produced

Point mutation

While the B product remains normal, the lack of a functional A product prevents wild type phage from being produced
Deletions for rapid mapping of point mutations to a region of the chromosome

- **Point mutation within deletion limits**
  - Overlapping deletions: Cannot produce wild-type progeny by recombination
  - Nonoverlapping deletions: Produce wild-type progeny by recombination