第三章 细菌和噬菌体遗传学

The Genetics Of Bacteria And Their Viruses

Live in a bacterial world

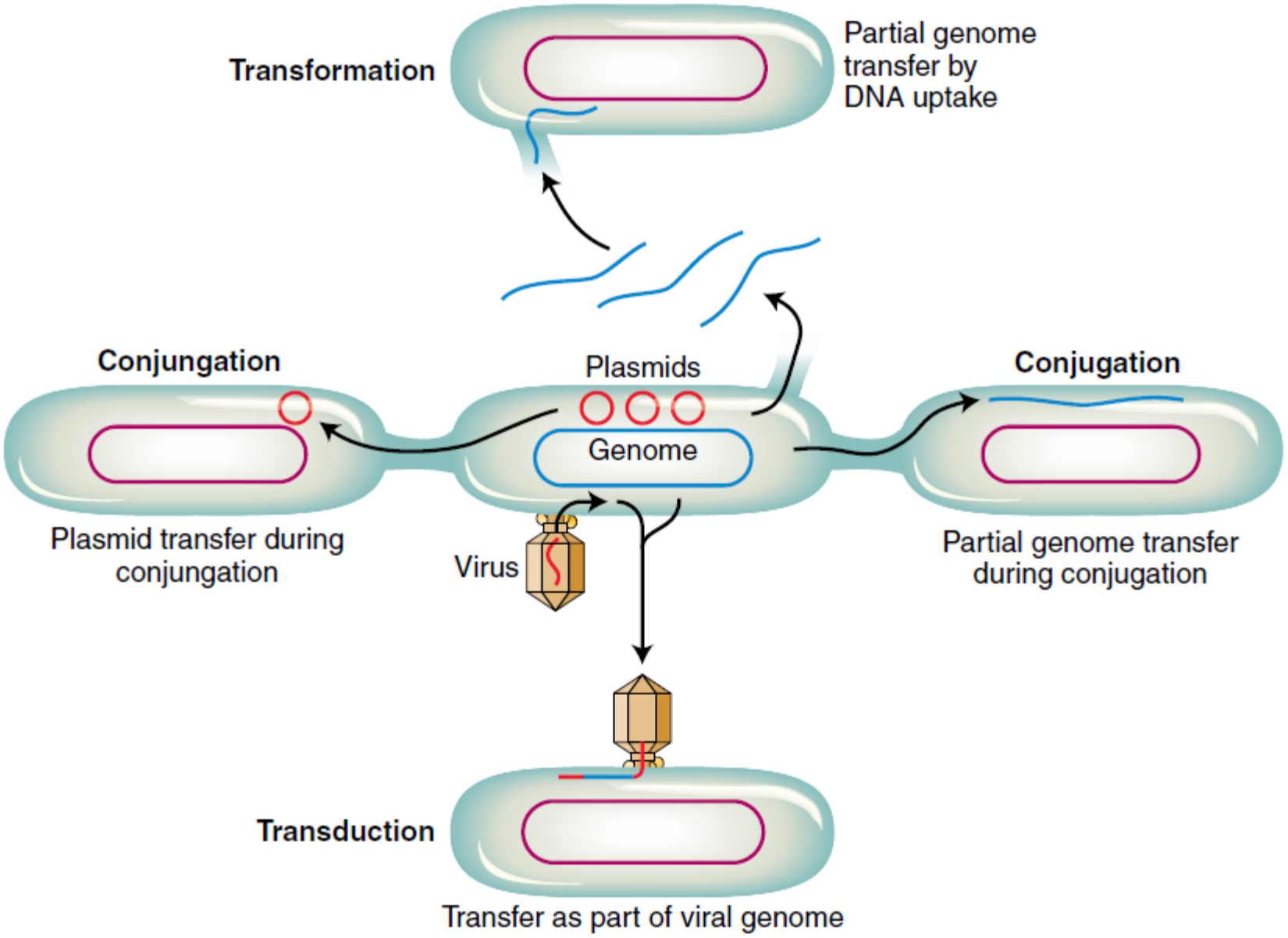
Table 8.1 Advantages of using bacteria and viruses for genetic studies

1. Reproduction is rapid.
2. Many progeny are produced.
3. The haploid genome allows all mutations to be expressed directly.
4. Asexual reproduction simplifies the isolation of genetically pure strains.
5. Growth in the laboratory is easy and requires little space.
6. Genomes are small.
7. Techniques are available for isolating and manipulating their genes.
8. They have medical importance.
9. They can be genetically engineered to produce substances of commercial value.

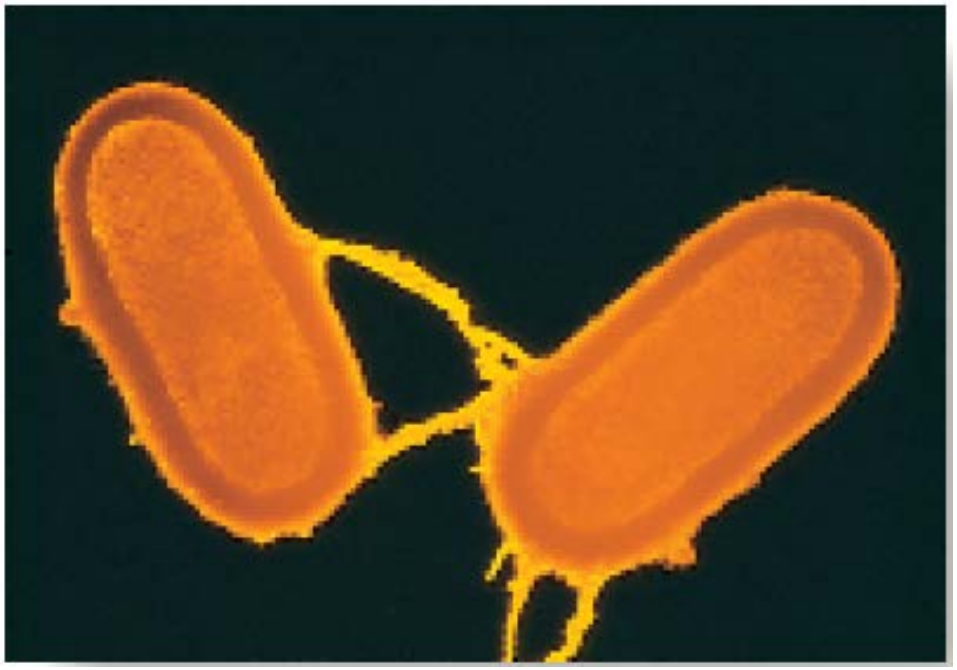
第一节 细菌的遗传分析与作图

Genetic Analysis and Mapping in Bacteria

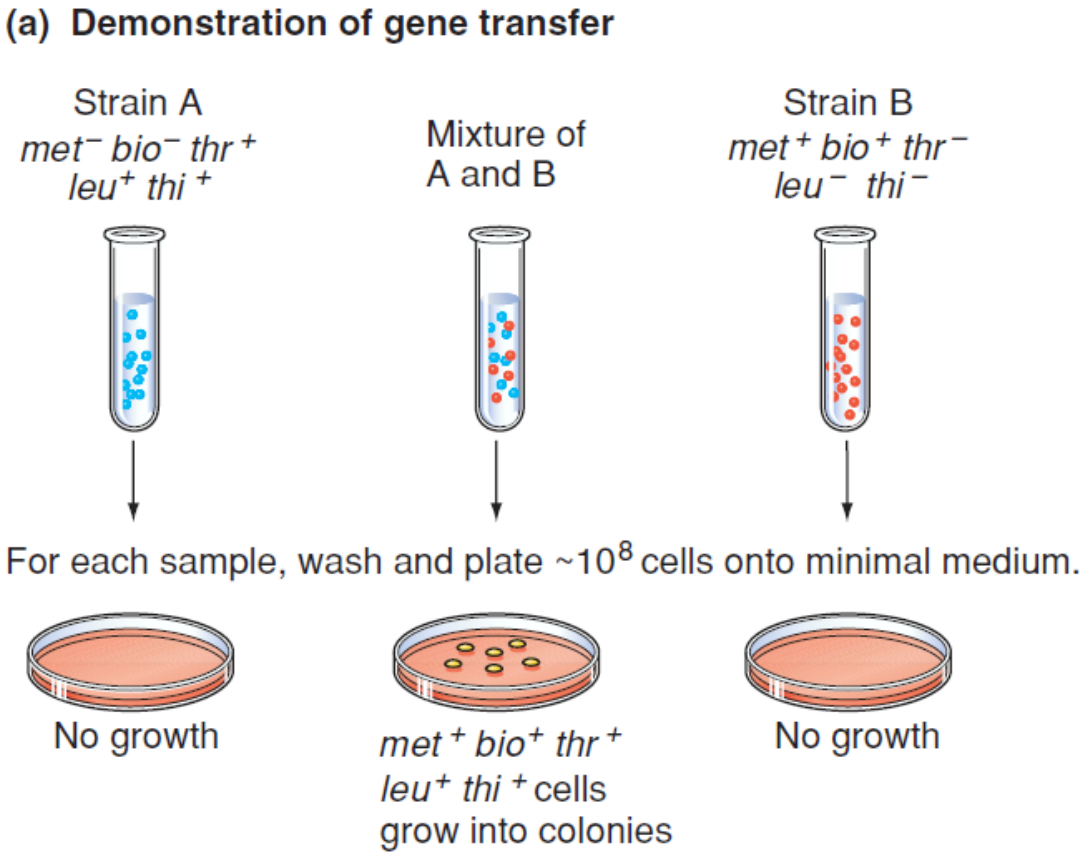
Bacterial exchange DNA by several processes

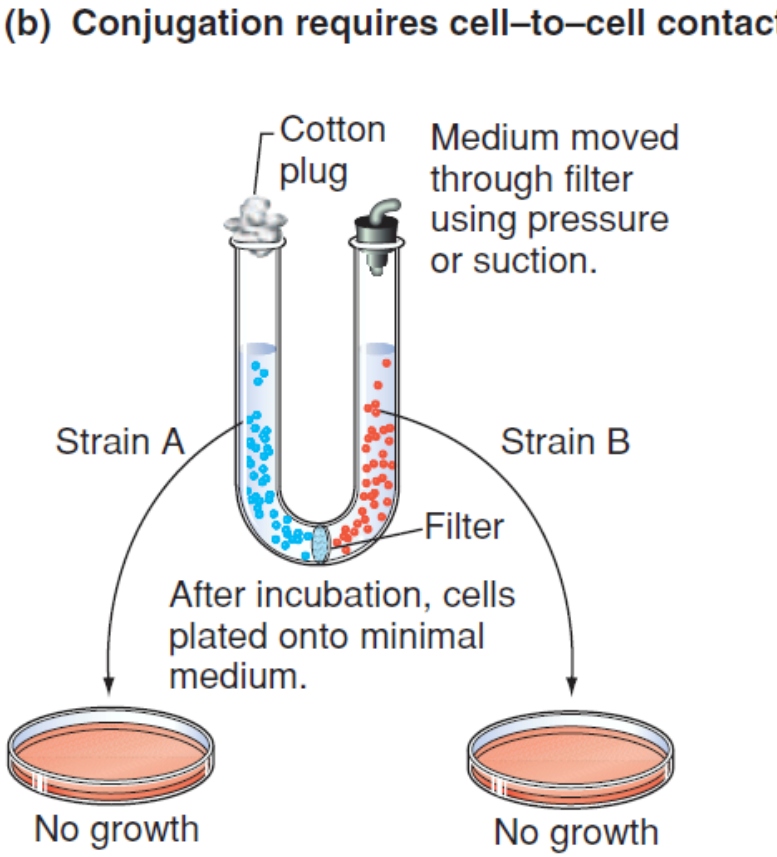


I. Bacteria Conjugation



The Discovery of Conjugation





Lederberg and Tatum, 1946

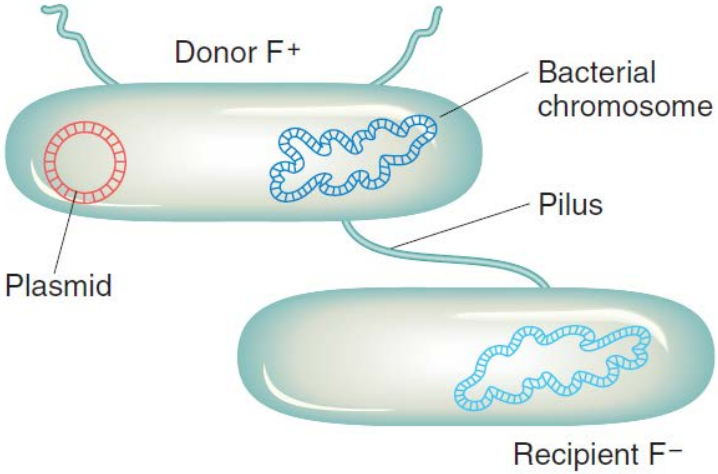
Bernard Davis

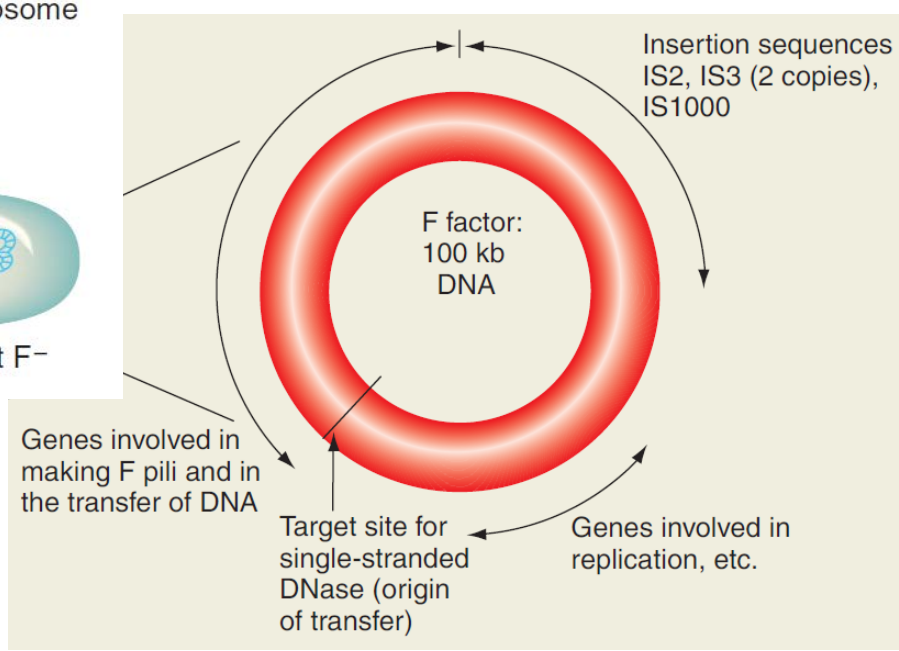
The gene transfer requires cell-to-cell contact, is unidirectional

Donor: F^+ cells (F for "fertility"); Recipient: F^- cells

1. The F plasmid and conjugation (F质粒与接合)

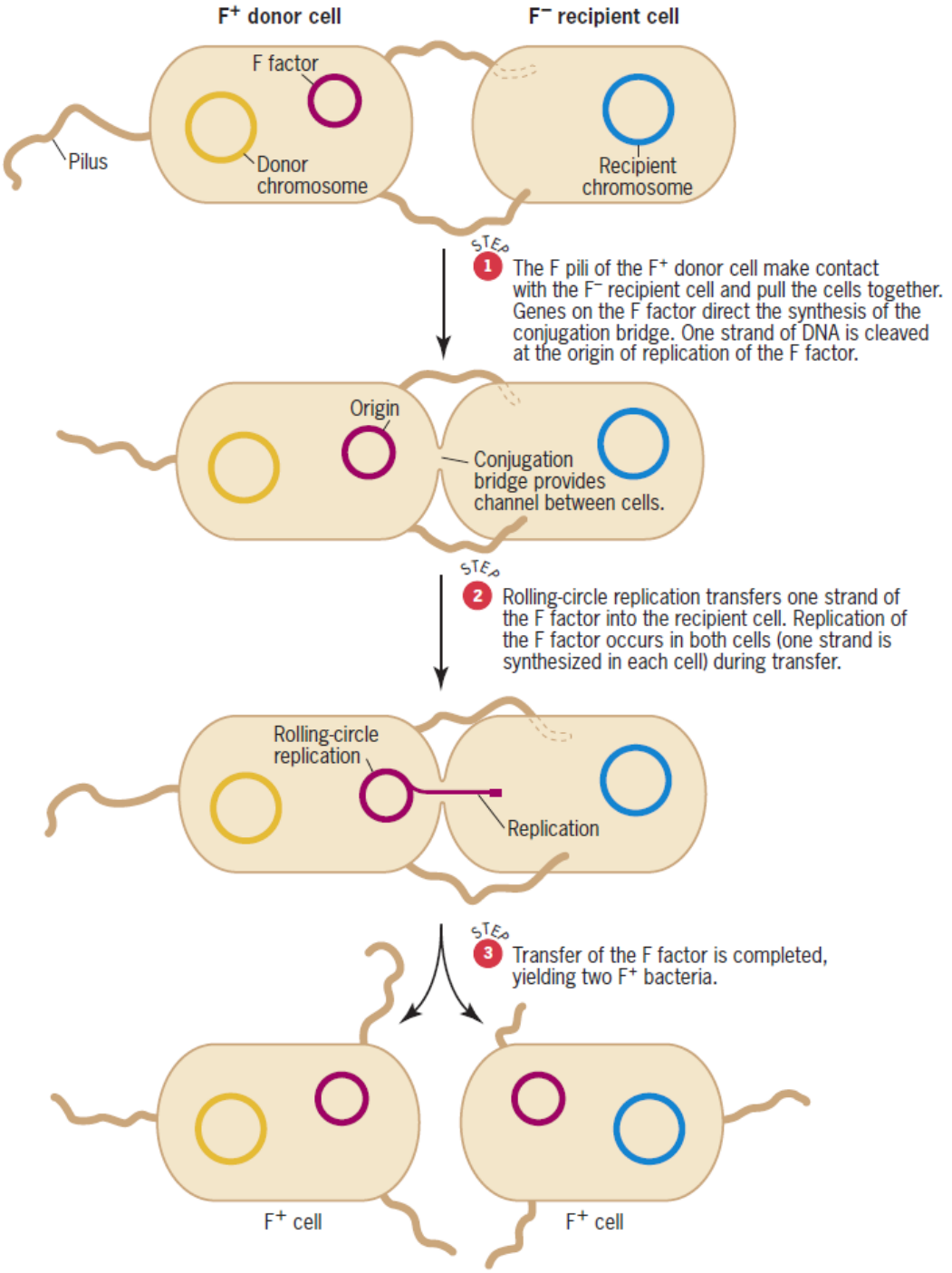
F plasmid (sex factor, fertility factor)



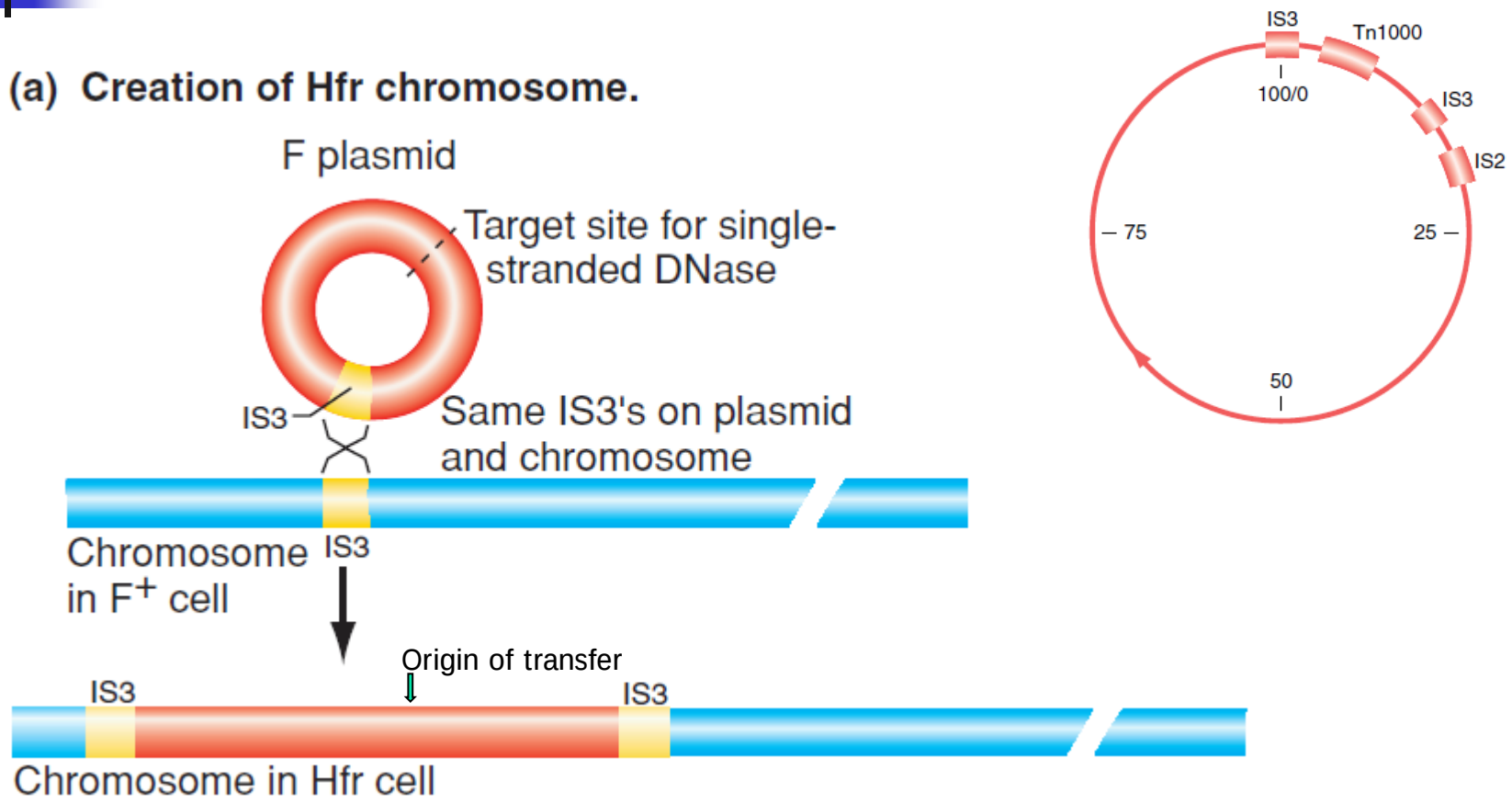


F plasmid transfer during conjugation

- **$F^+ \times F^-$接合，95%的F^-转变为F^+，染色体上基因转移频率极低**
- **TraS和TraT编码“表面排斥”蛋白，使F^+与F^+细胞不能结合**

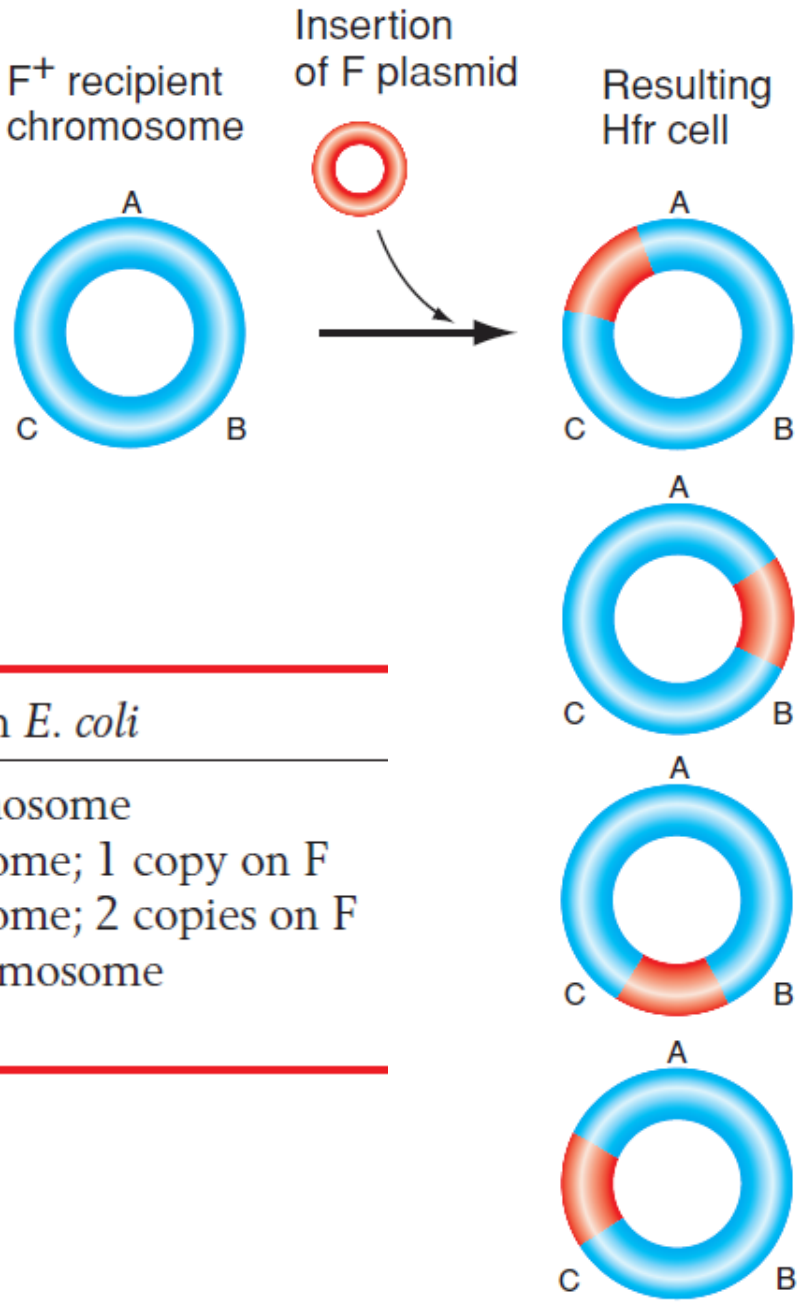


2. Integration of F plasmid and Hfr cells



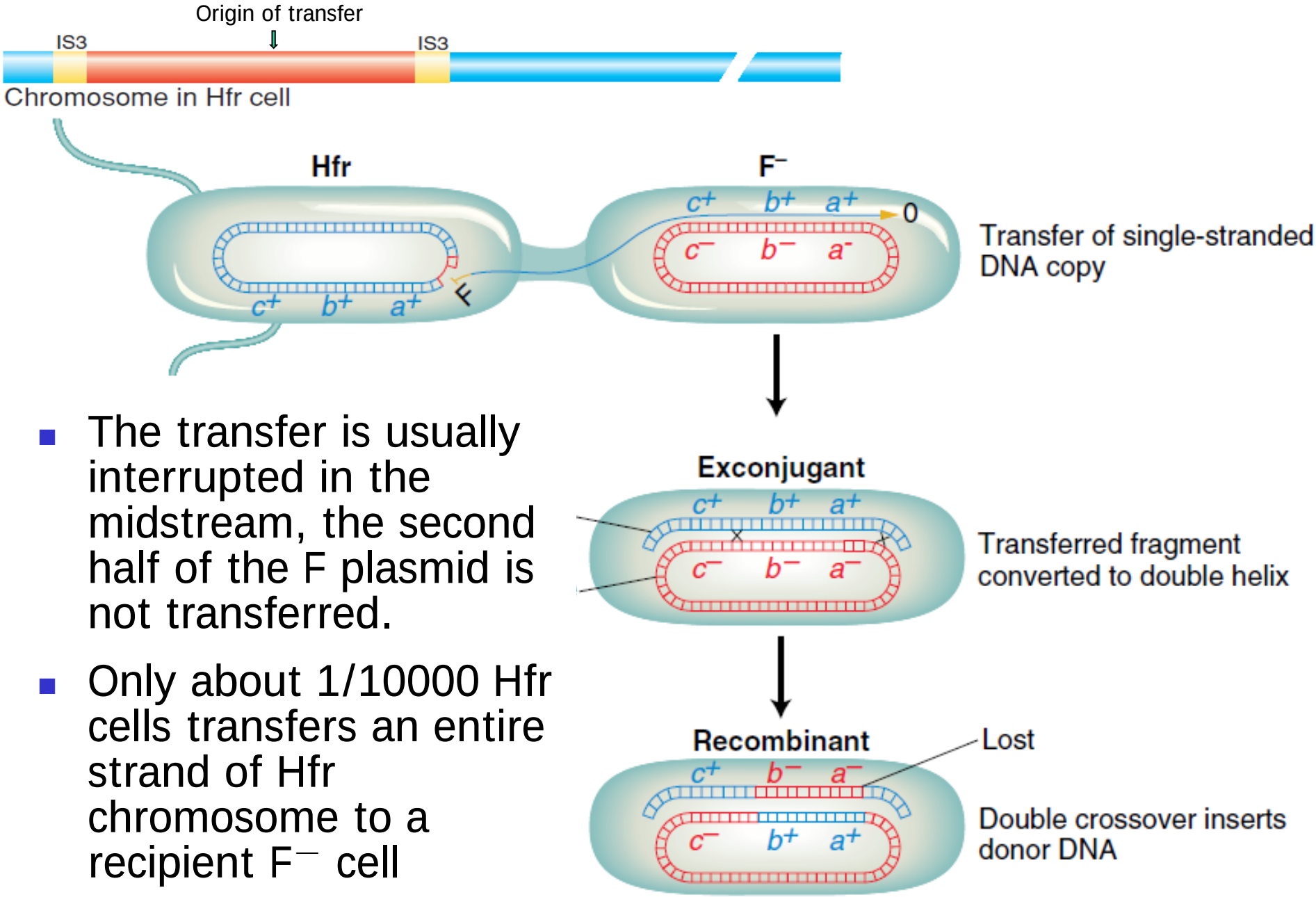
Hfr: High frequency recombination

(b) Many different Hfr strains can form.



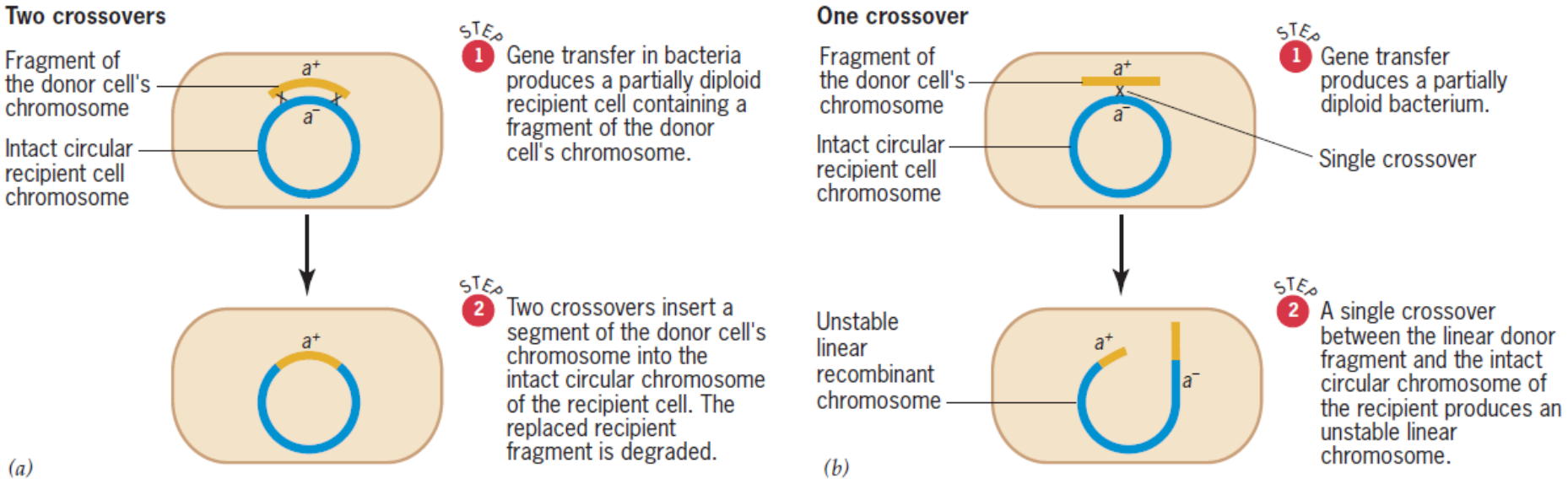
Insertion sequence	Normal occurrence in *E. coli*
IS1	5–8 copies on chromosome
IS2	5 copies on chromosome; 1 copy on F
IS3	5 copies on chromosome; 2 copies on F
IS4	1 or 2 copies on chromosome
IS5	Unknown

Hfr conjugational transfer of chromosomal genes



- The transfer is usually interrupted in the midstream, the second half of the F plasmid is not transferred.
- Only about 1/10000 Hfr cells transfers an entire strand of Hfr chromosome to a recipient F^- cell

Property of bacterial recombination

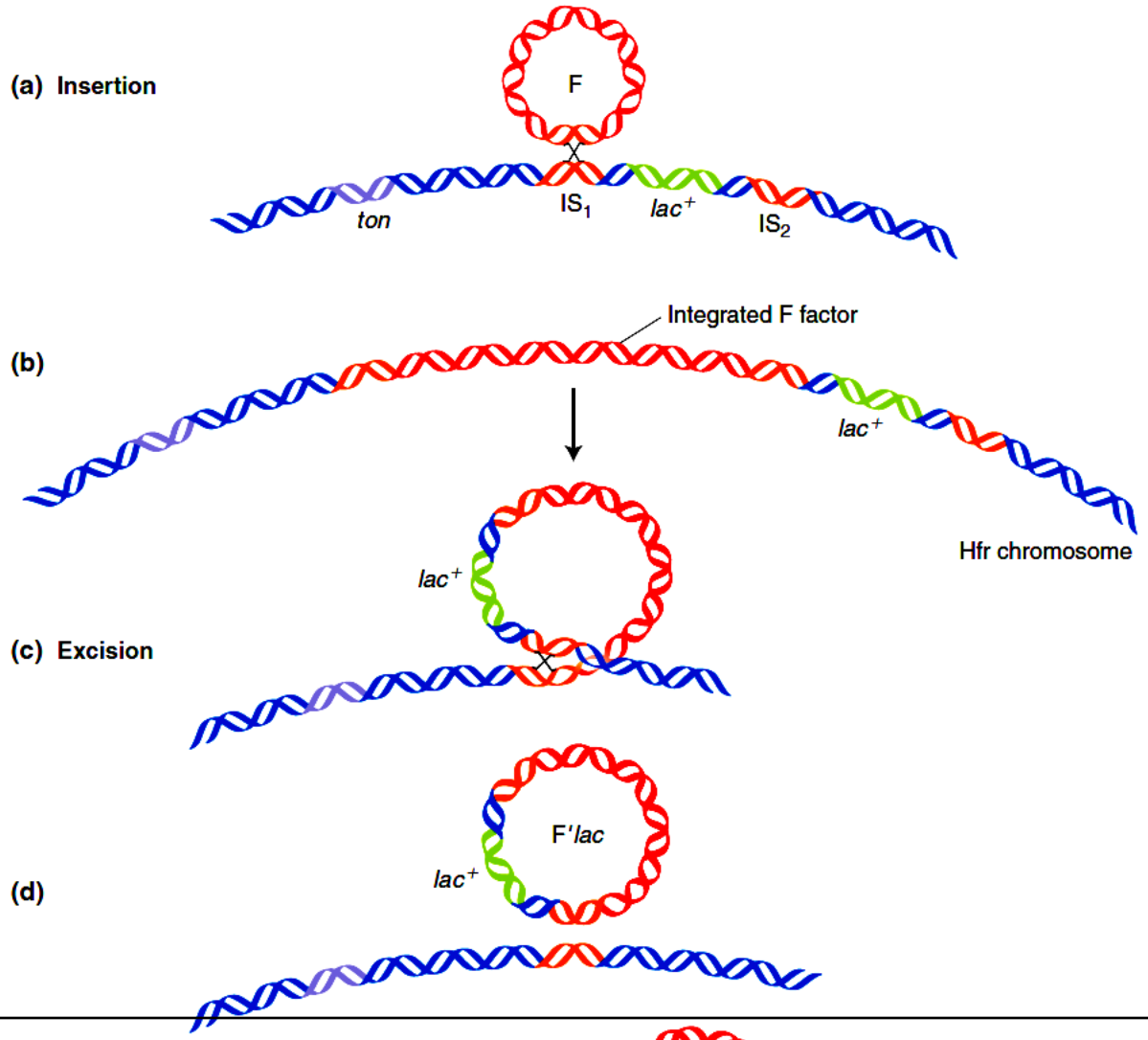


- A **single crossover**(or odd number) would be lethal because the ring is broken to produce a strange, partly diploid linear chromosome
- To produce recombination cells, there must be an **even number of crossovers**
- Only **one product** of recombination survives. The reciprocal exchange product is generally lost in subsequent cell growth

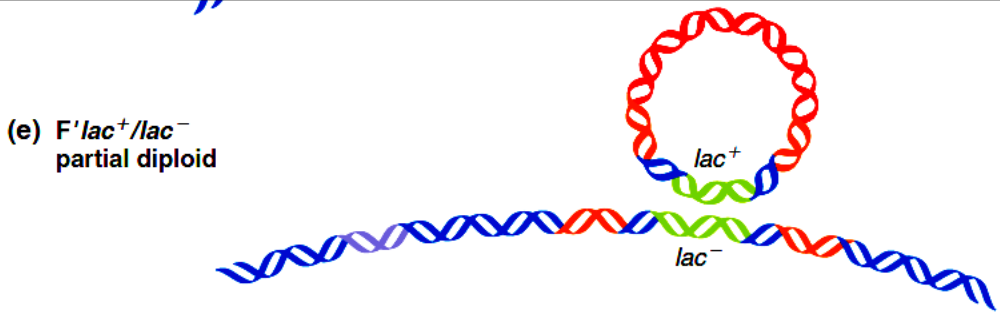
3. **F’ plasmid and sex conduction**

F’ 质粒和性导

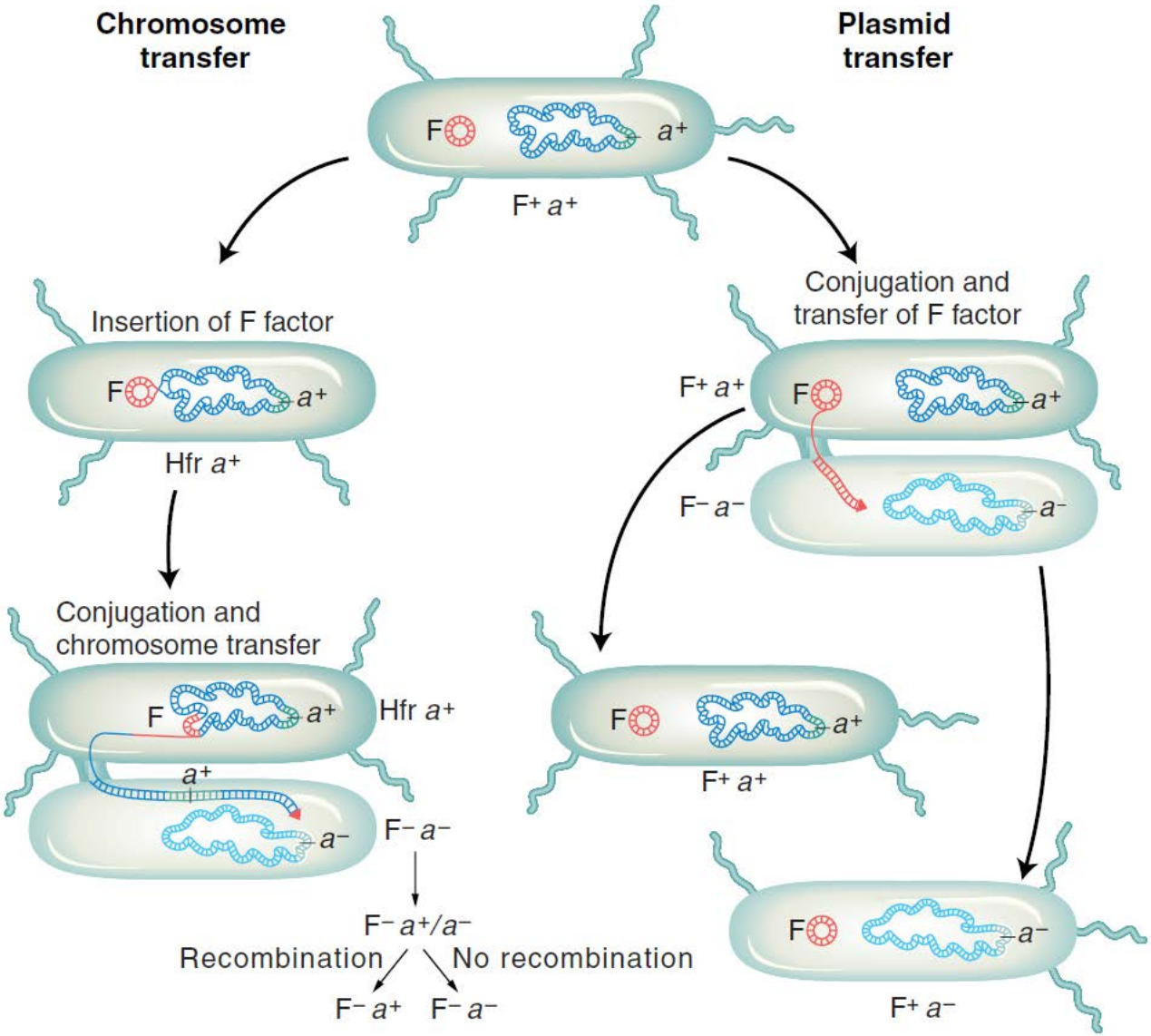
Formation of an F' plasmid



Sex conduction



Summary of conjugation in *E.coli*



see movie

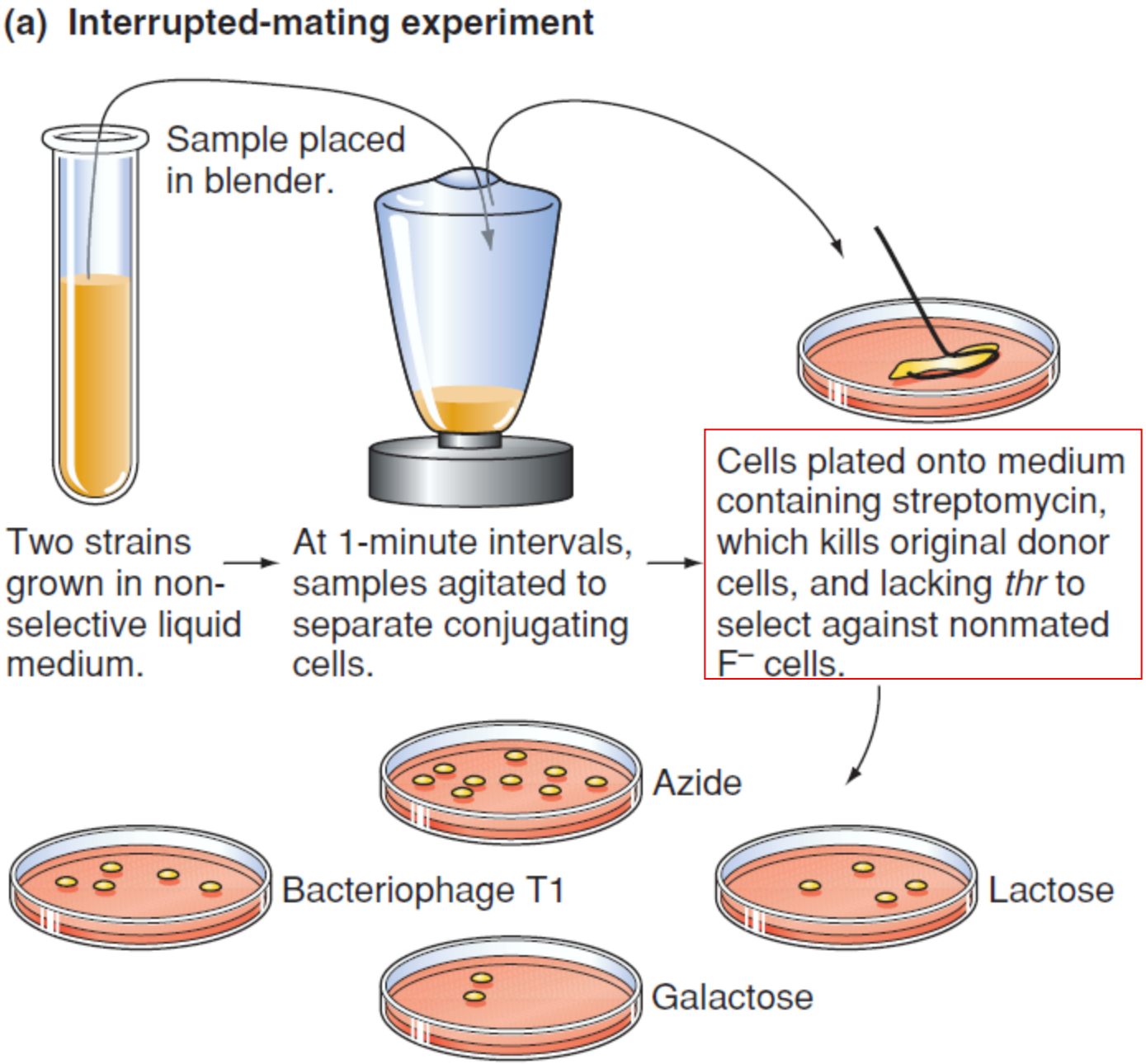
4. Mapping bacterial genes with interrupted mating experiment

In the mid-1950s

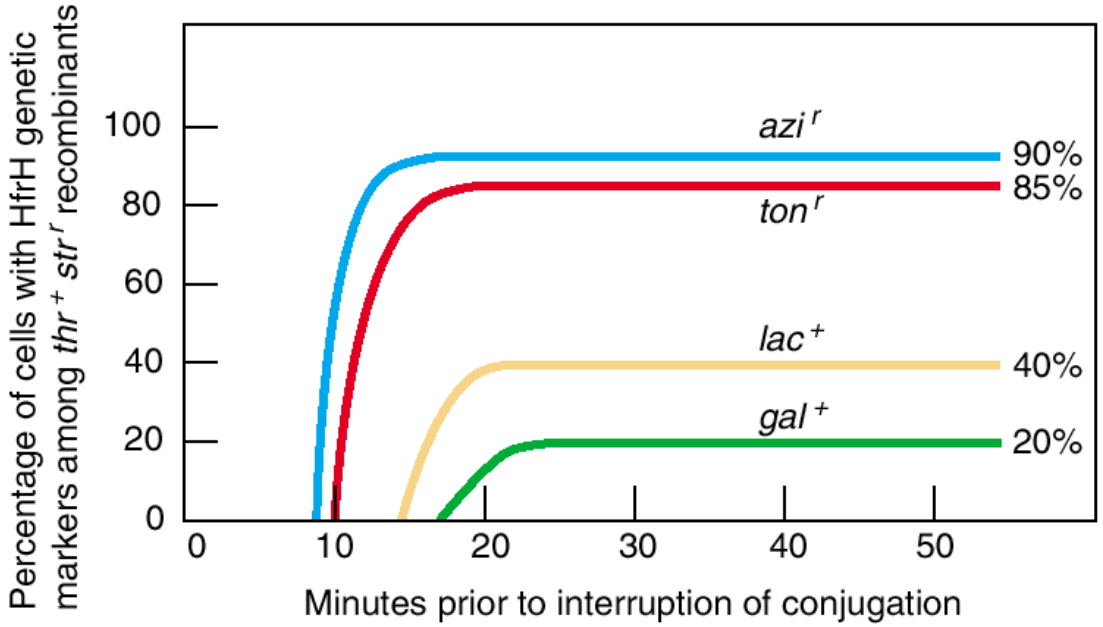
Ellie Wollman & Francois Jacob

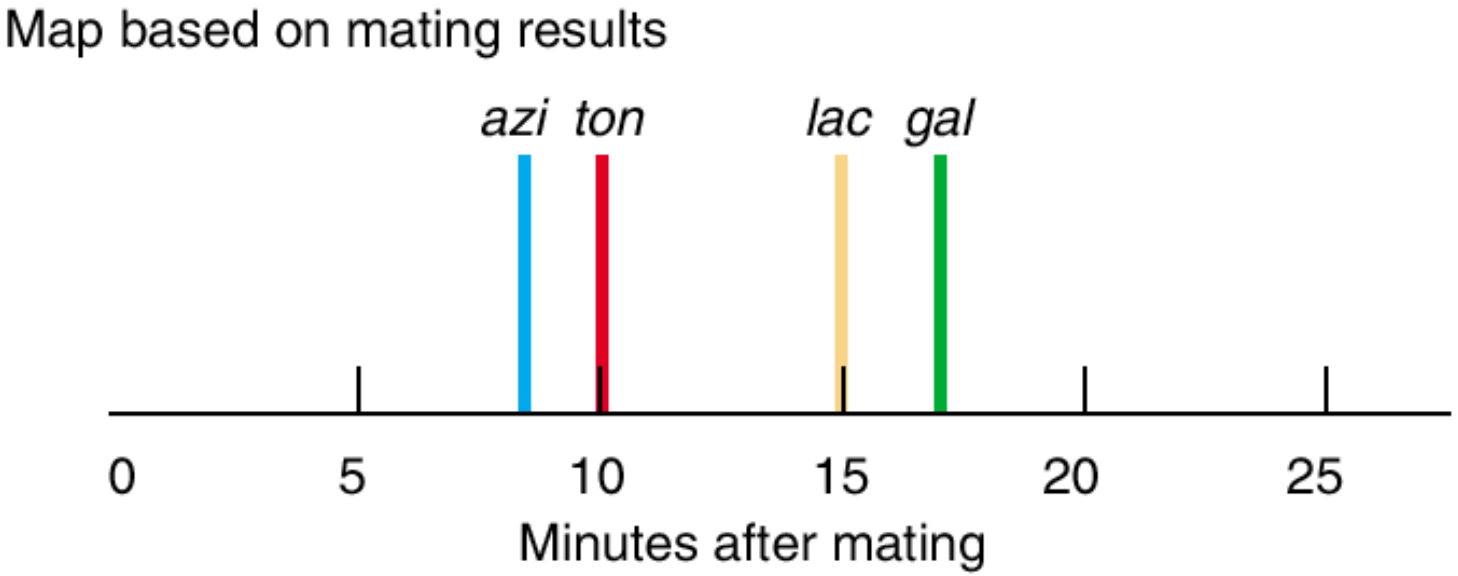
Interrupted mating experiment

HfrH	×	F^-
str^s (sensitive to streptomycin)		str^r (resistant to streptomycin)
thr^+ (able to synthesize the amino acid threonine)		thr^- (threonine auxotroph)
azi^r (resistant to sodium azide)		azi^s (sensitive to sodium azide)
ton^r (resistant to bacteriophage T1)		ton^s (sensitive to phage T1)
lac^+ (able to grow with lactose as sole source of carbon)		lac^- (unable to grow on lactose)
gal^+ (able to grow with galactose as sole source of carbon)		gal^- (unable to grow on galactose)


(a) Interrupted-mating experiment
Sample placed in blender.
Two strains grown in non-selective liquid medium.
At 1-minute intervals, samples agitated to separate conjugating cells.
Cells plated onto medium containing streptomycin, which kills original donor cells, and lacking thr to select against nonmated F⁻ cells.
Azide
Bacteriophage T1
Lactose
Galactose
Replica plating transfers each colony to media that select for four donor markers other than streptomycin.

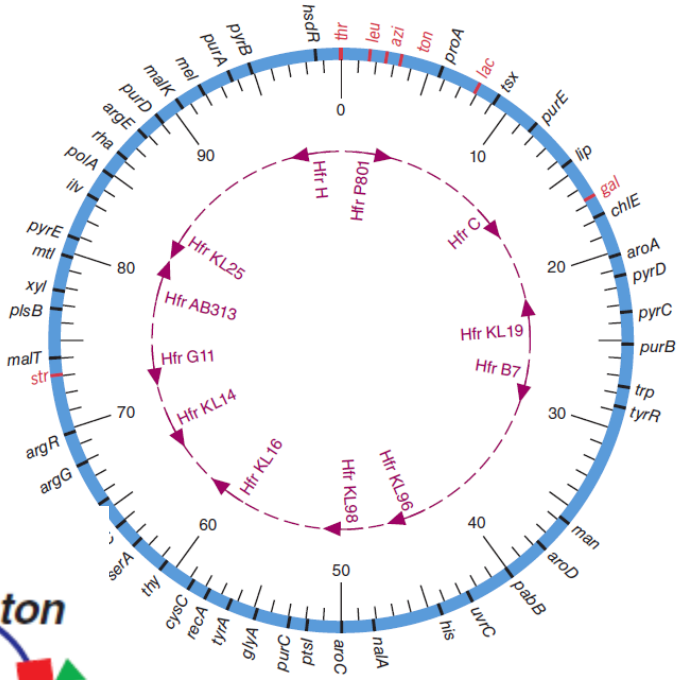
Time (min)	Transferred genes
< 9	
9	azi^r
11	azi^r ton^r
18	azi^r ton^r lac$^+$
24	azi^r ton^r lac$^+$ gal$^+$



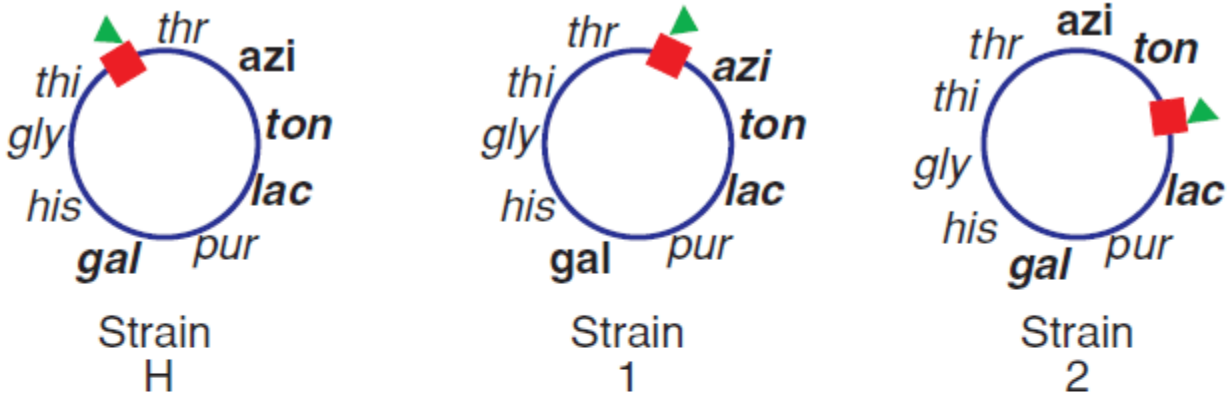


(a) Gene transfer in different Hfrs

Hfr strain	Order of transfer →
H	*thr* ***azi*** ***ton*** ***lac*** *pur* ***gal*** *his* *gly* *thi*
1	*thr* *thi* *gly* *his* ***gal*** *pur* ***lac*** ***ton*** ***azi***
2	***lac*** *pur* ***gal*** *his* *gly* *thi* *thr* ***azi*** ***ton***
3	***gal*** *pur* ***lac*** ***ton*** ***azi*** *thr* *thi* *gly* *his*



(b) Data interpretation

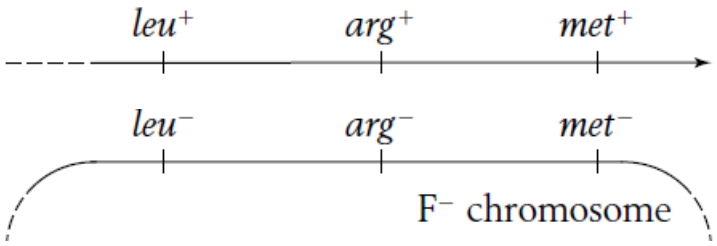


Comparisons of interrupted mating studies using different Hfr strains confirm that the bacterial chromosome is a circle

5. Mapping closely linked genes

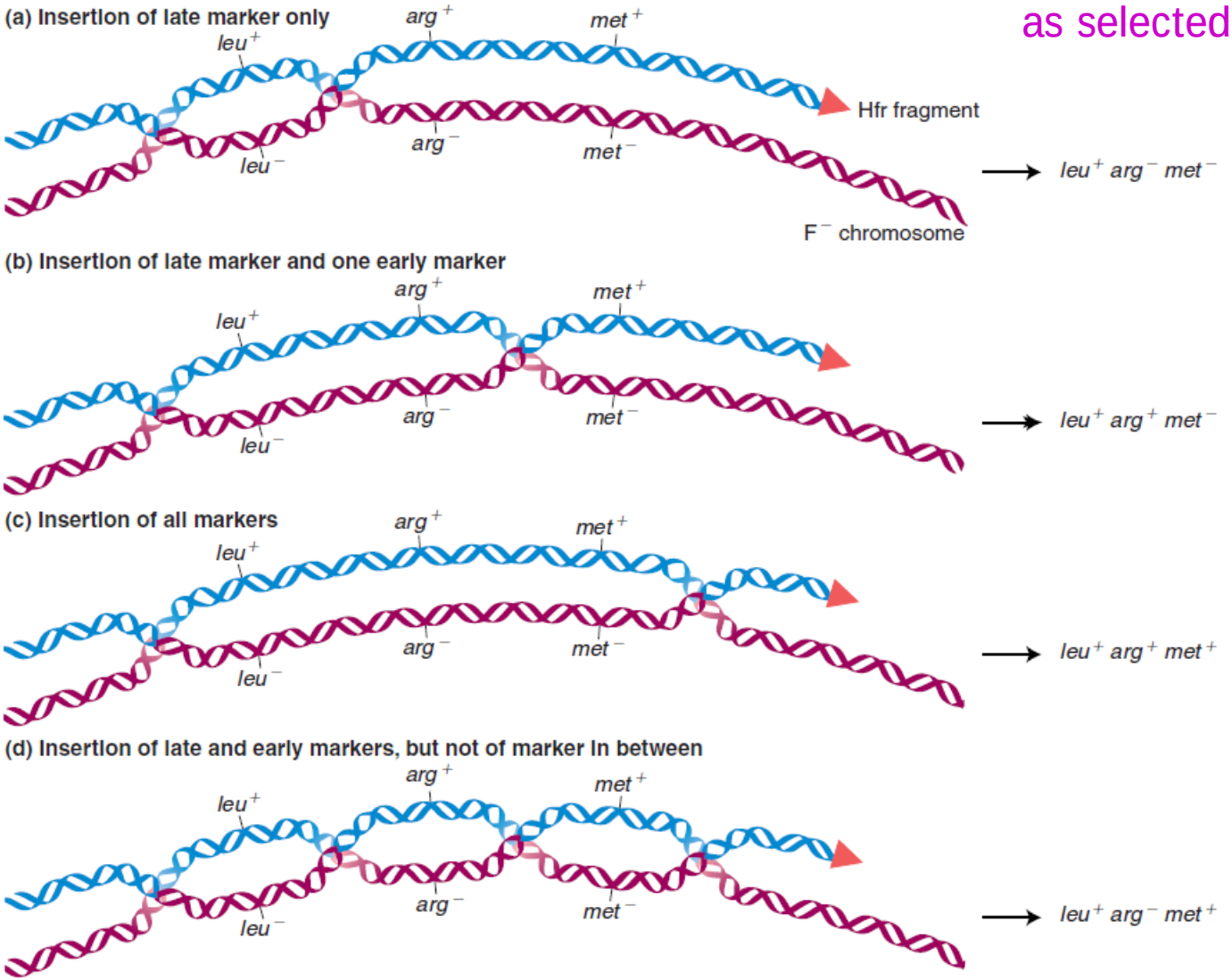
- Interrupted mating experiments cannot distinguish the relative positions of genes **within about 2 minutes** of each other and thus give only a crude idea of gene location
- **Recombination analyses** of Hfr crosses improve mapping accuracy

Hfr fragment



$leu^+\ arg^-\ met^-$	4%
$leu^+\ arg^+\ met^-$	9%
$leu^+\ arg^+\ met^+$	87%

Use the last donor allele, leu+, as selected marker gene.



leu-arg:
4 map unit

arg-met:
9 map unit

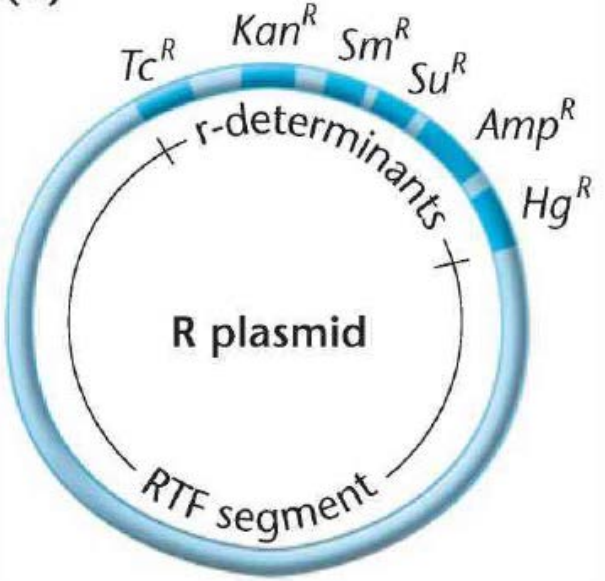
The relationship of interrupted mating mapping and recombination mapping

- 中断杂交图是以基因转移的先后顺序为依据，基因间距离以转移时间（**min**）为单位绘制的，对于紧密连锁的基因无法定位
- 一般选择距离在**2min**内的基因进行重组作图
- 中断杂交图**1min**的图距相当于重组图中的**20 m.u.**，**1 m.u.**相当于**1600bp**

6. Conjugational Gene Transfer and Antibiotic Resistance

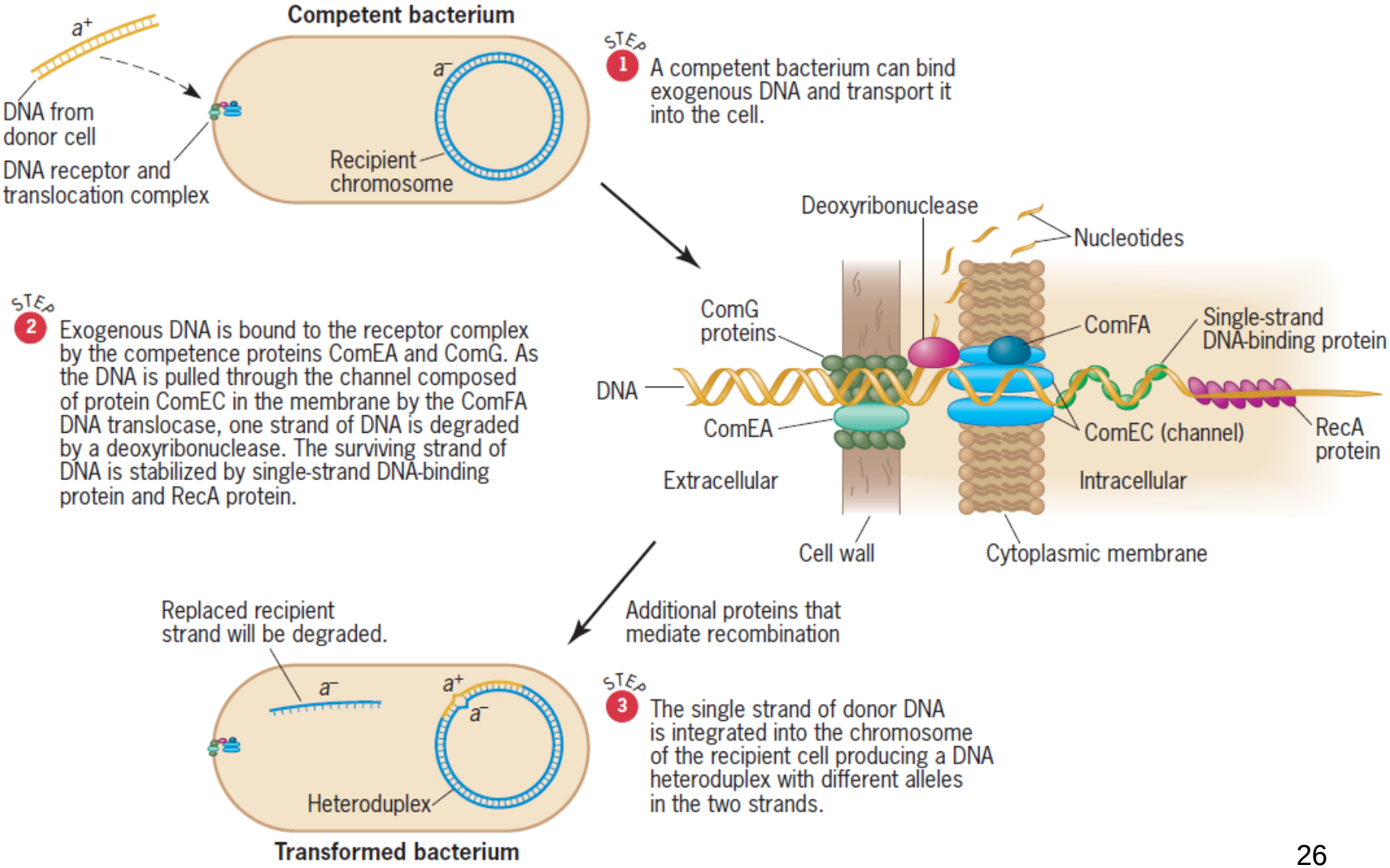
- 1943, streptomycin was discovered
- In 1950s, antibiotics-resistant *Shigella*, Japan,
  - 1953, 0.2% were resistant
  - 1965, 58% were resistant, multi-drug-resistant (**MDR**)
- The antibiotics-resistant genes often are present on plasmids----**R plasmid**

R plasmid



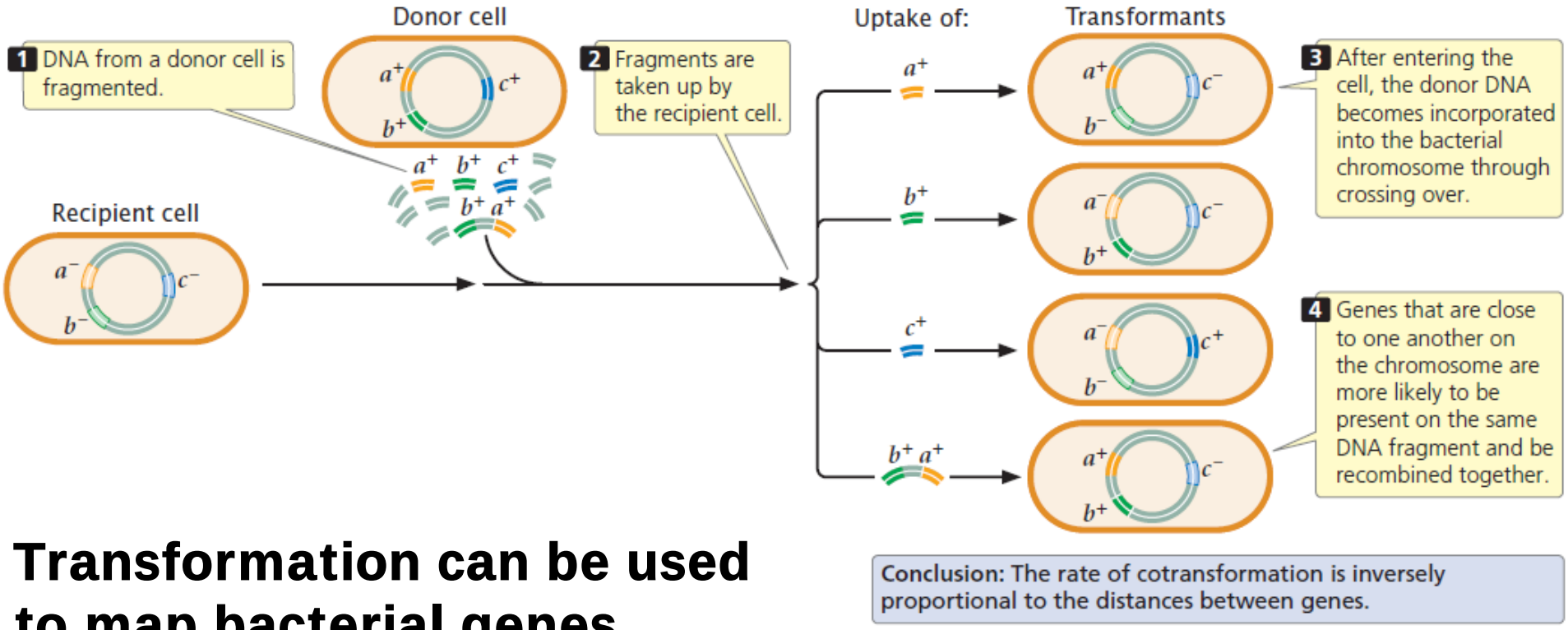
- Most R plasmids consist of two components: **the resistance transfer factor (RTF)** and one or more **r-determinants**
- R plasmids can be transferred from one bacterium to another, sometimes even across species
- Overuses of antibiotics accelerate the antibiotics-resistant bacteria spread
  - *Further Reading*: "Antibiotic resistant bacteria"
    "Get pigs off antibiotics"

II. Bacterial Transformation



Transformation and gene mapping

- The size of DNA that is effective in transformation is between **10~20kb**, about 1/200 of the *E.coli* chromosome. The DNA thus contains several genes.
- **Cotransformation** is the simultaneous transformation of two or more genes.
- Genes that are close enough to each other to be cotransformation are said to be linked.
- If two genes are not linked, the probability of them to be transformed simultaneously equals to the product of the individual probabilities, which is much lower than they are linked.



Transformation can be used to map bacterial genes

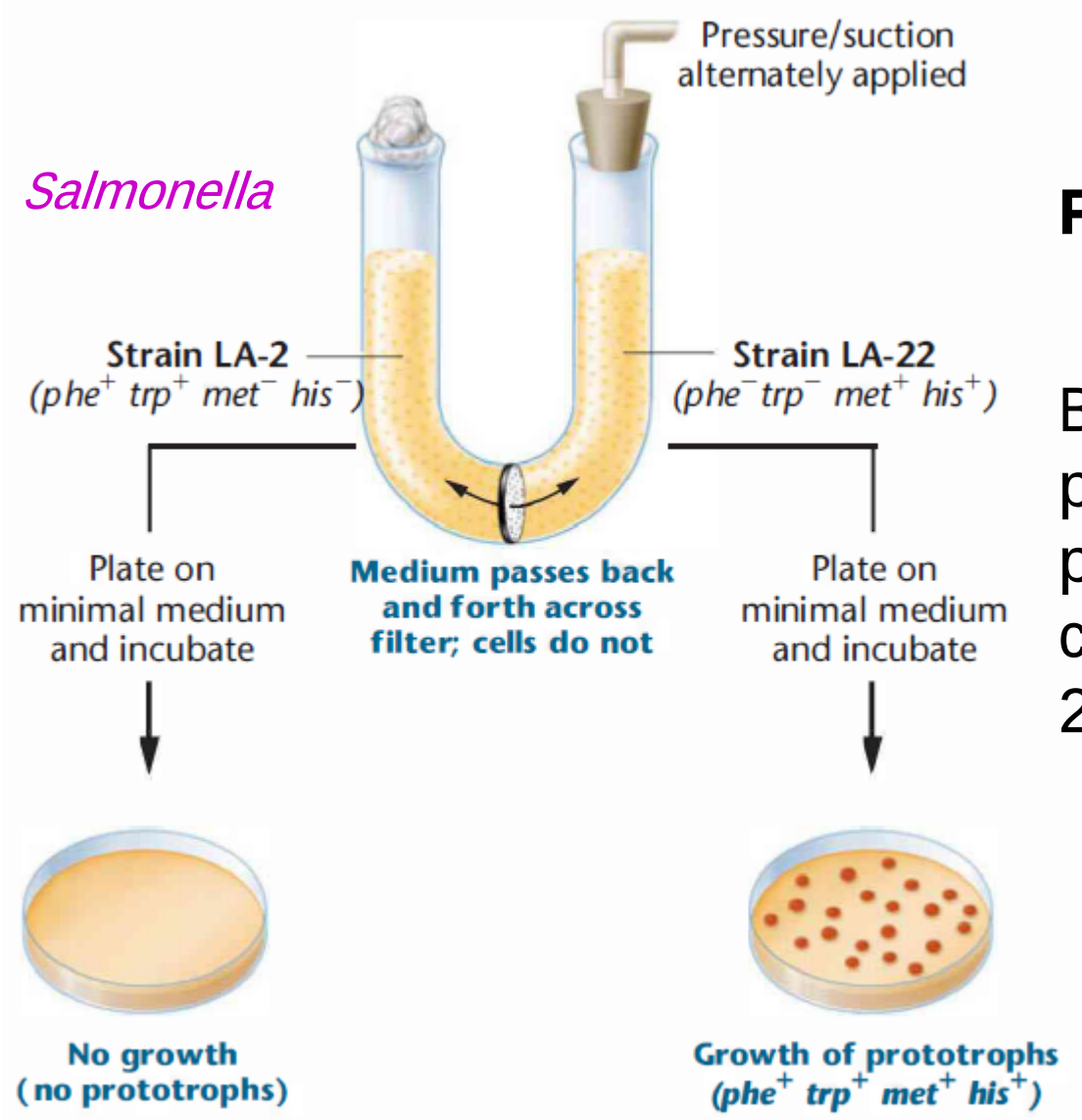
Confirm whether the genes were linked or unlinked

Donor DNA	Recipient Cell Genotype	Transformed Genotypes(%)		
		$str^r mtl^-$	$str^s mtl^+$	$str^r mtl^+$
$str^r mtl^+$	$str^s mtl^-$	4.3	0.40	0.17
$Str^r mtl^-$ and $str^s mtl^+$		2.8	0.85	0.0066

III. Transduction and mapping

- Transduction is virus-mediated bacterial DNA transfer

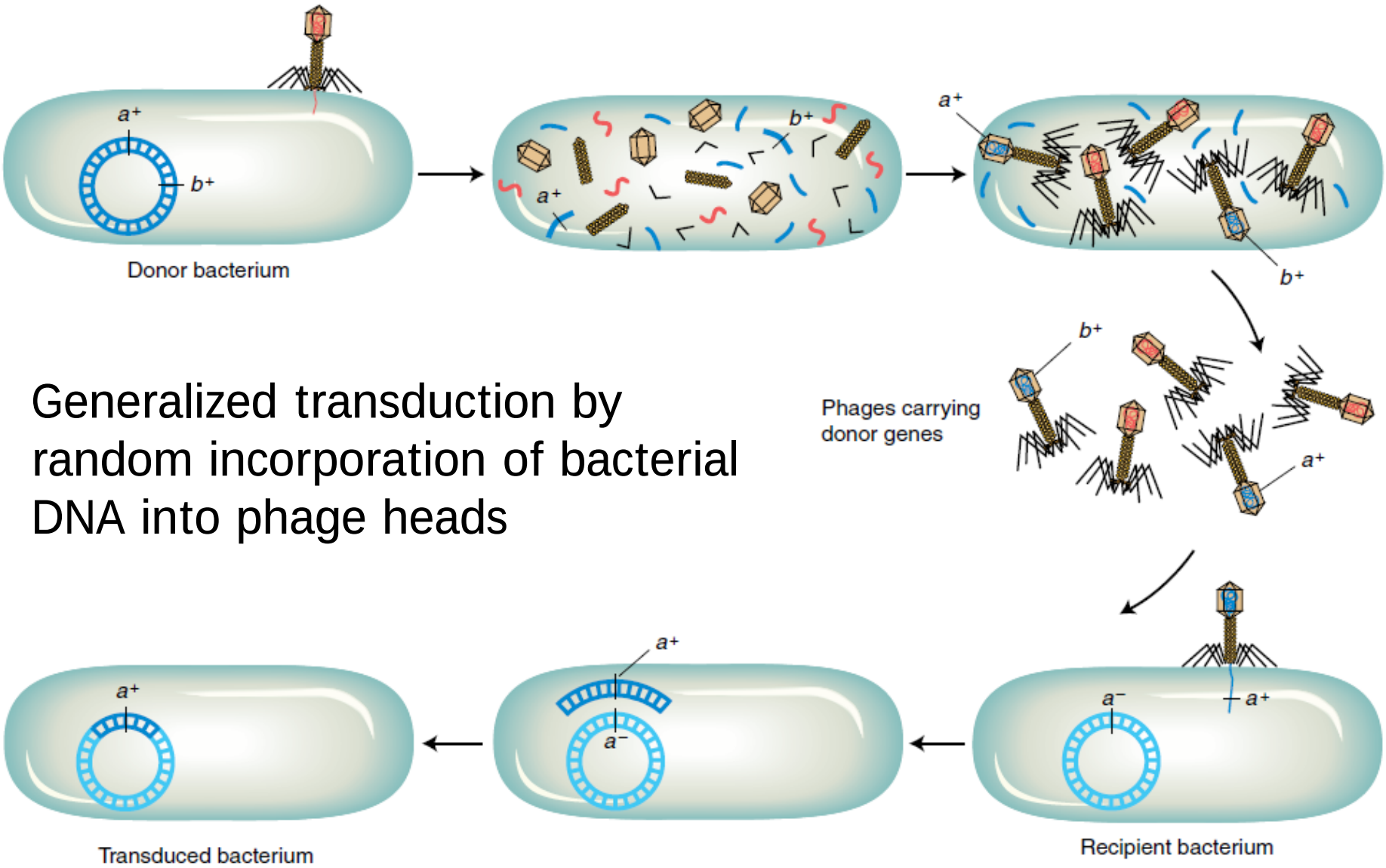
The Lederberg-Zinder Experiment, 1952



Filterable agent (FA)

Bacteriophage P22, present initially as a prophage in the chromosome of the LA-22 *Salmonella* cells.

1. Generalized transduction (普遍性转导)



Generalized transduction by random incorporation of bacterial DNA into phage heads

Cotransduction

- The closer two genes are, the more likely they are appear on the same short DNA fragment and be packaged into the same transducing phage and be **cotransduced**.

Gene mapping by cotransduction

(a)

Donor: *thyA*$^+$ *lysA*$^+$ *cysC*$^+$

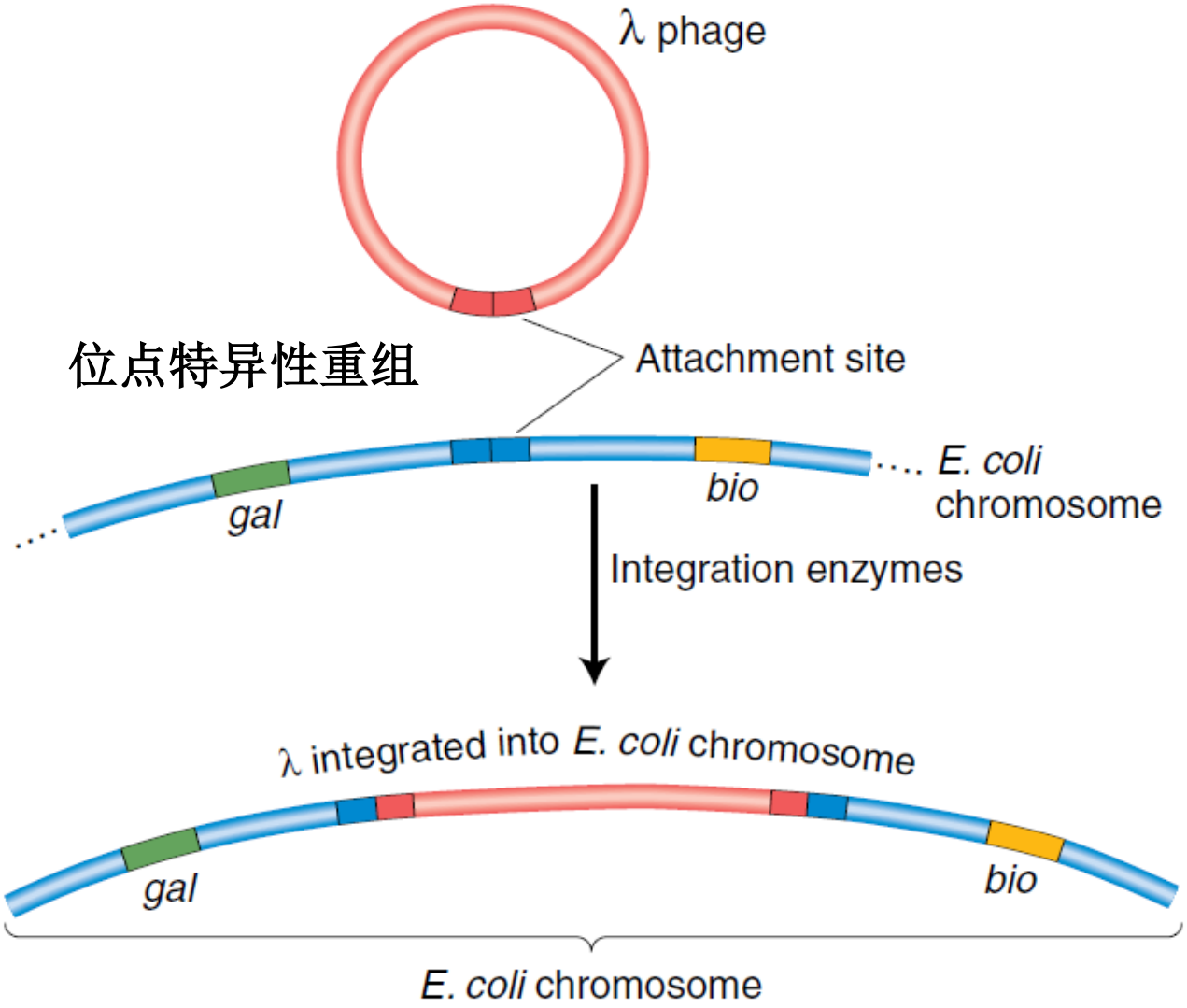
make P1 lysate infect recipient

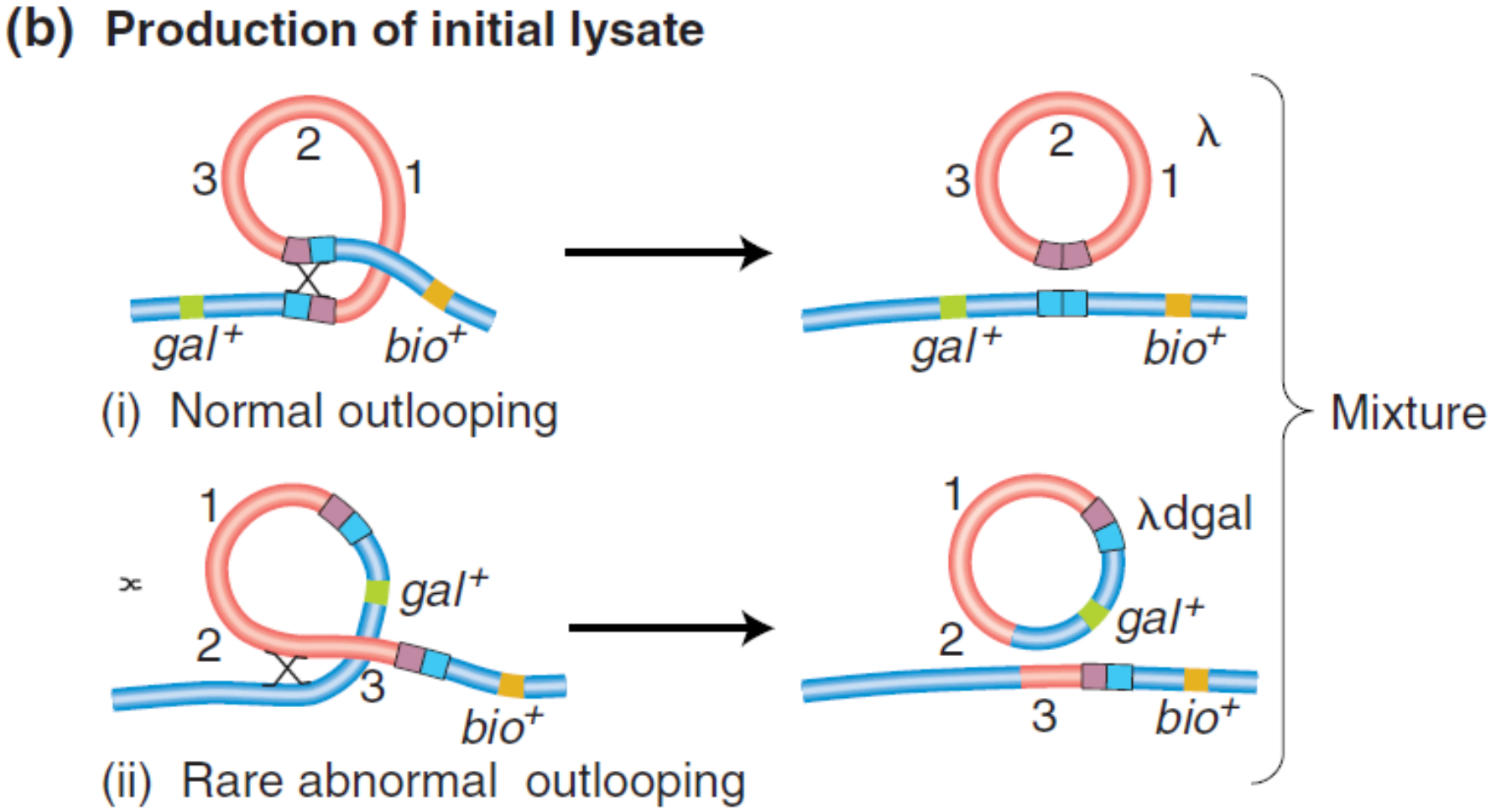
Recipient: *thyA*$^-$ *lysA*$^-$ *cysC*$^-$

Selected marker	Unselected marker
thy$^+$	47% *lys*$^+$; 2% *cys*$^+$
lys$^+$	50% *thy*$^+$; 0% *cys*$^+$

(b)

2. Specialized transduction（局限性转导）

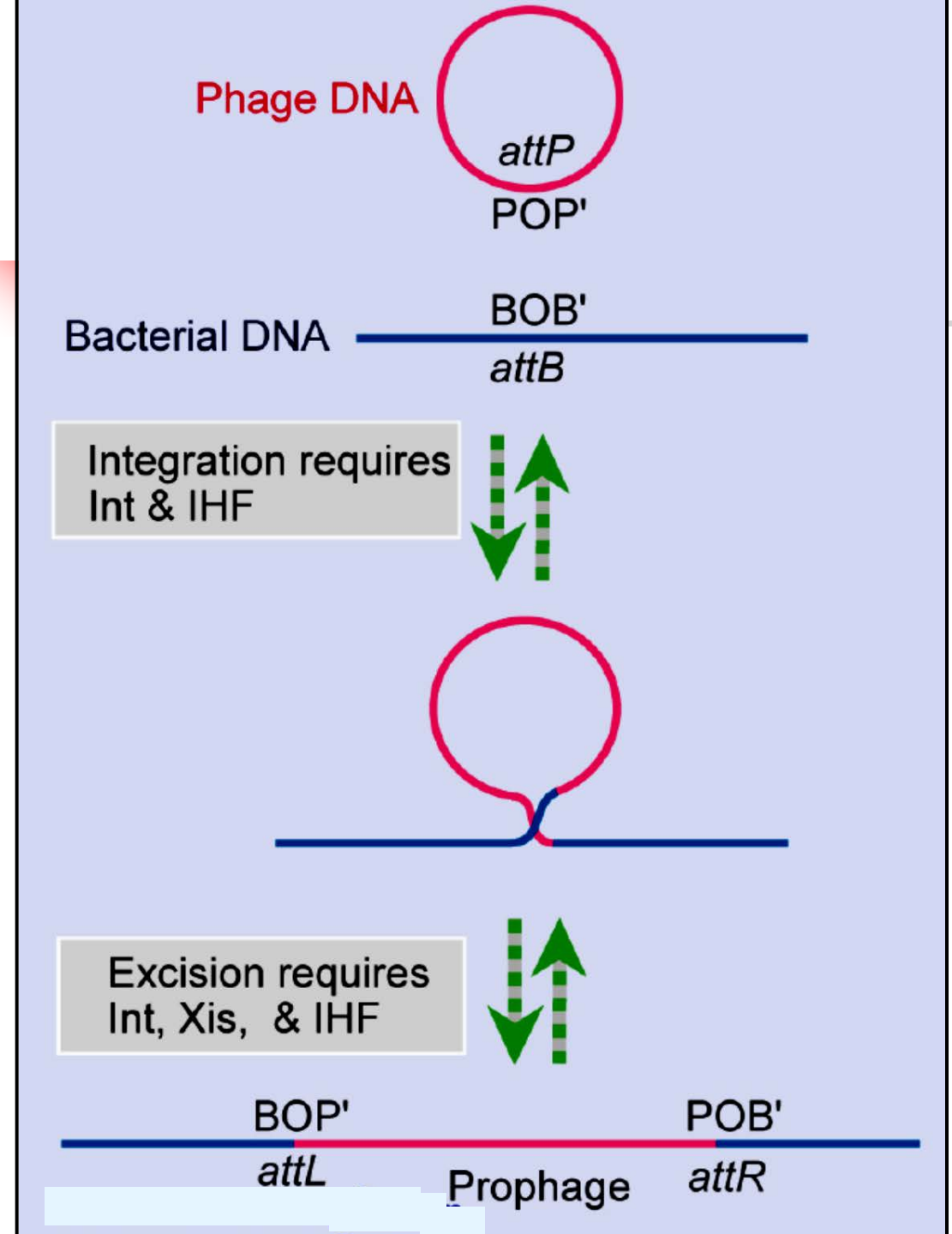




特异性转导只能转导位于原噬菌体附近的宿主遗传标记

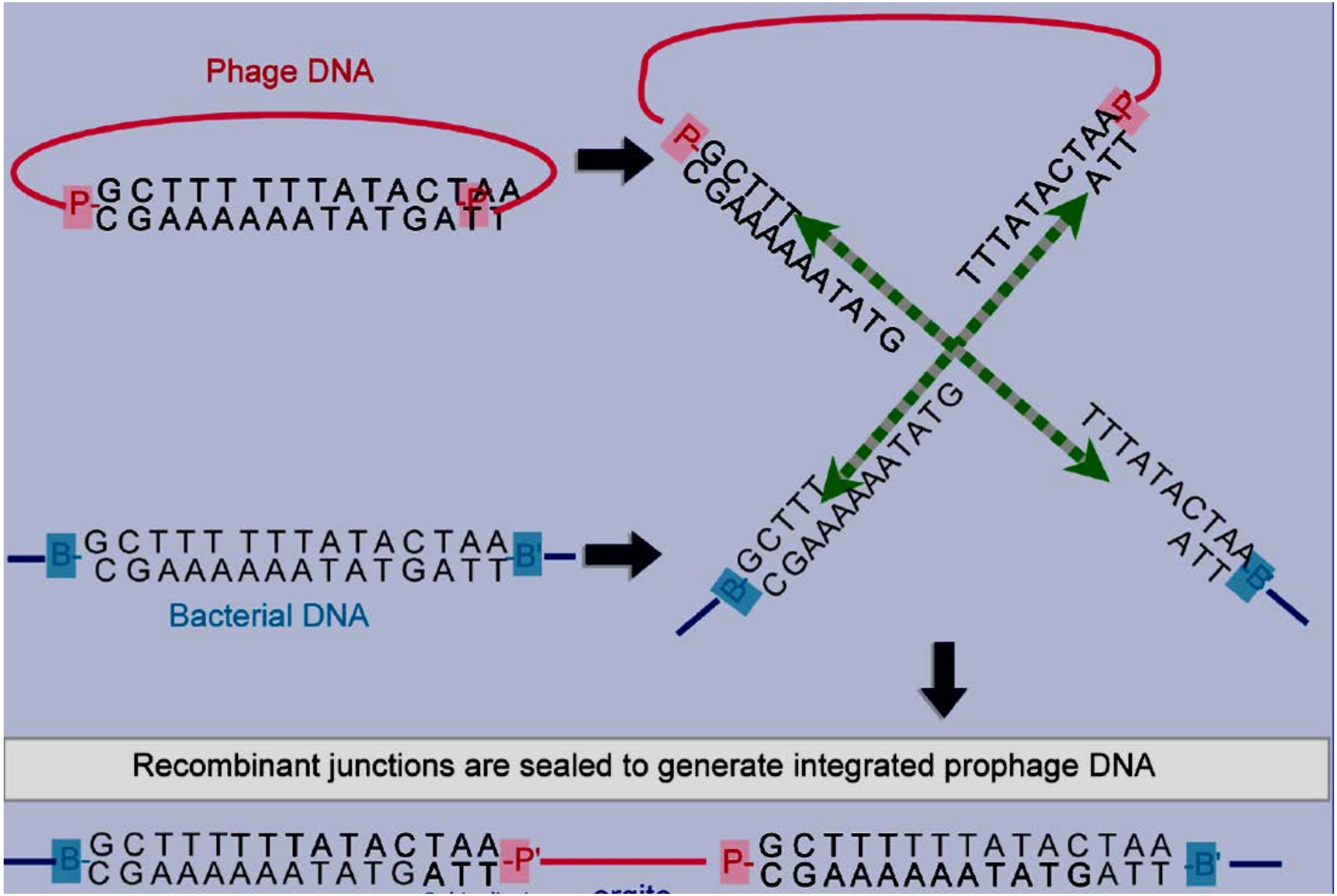
λ噬菌体位点特异性重组
Site-specific recombination

- The integration and excision of λDNA involves site specific recombination
- Attachment sites (att) are the loci on a phage and the bacterial chromosome at which site-specific recombination occurs
- When the *att*$^{\lambda}$ site is deleted from the *E. coli* chromosome, λ phage can integrate elsewhere, although the efficiency is <0.1% of the frequency of integration at *att*$^{\lambda}$. This inefficient integration occurs at *secondary attachment sites.*

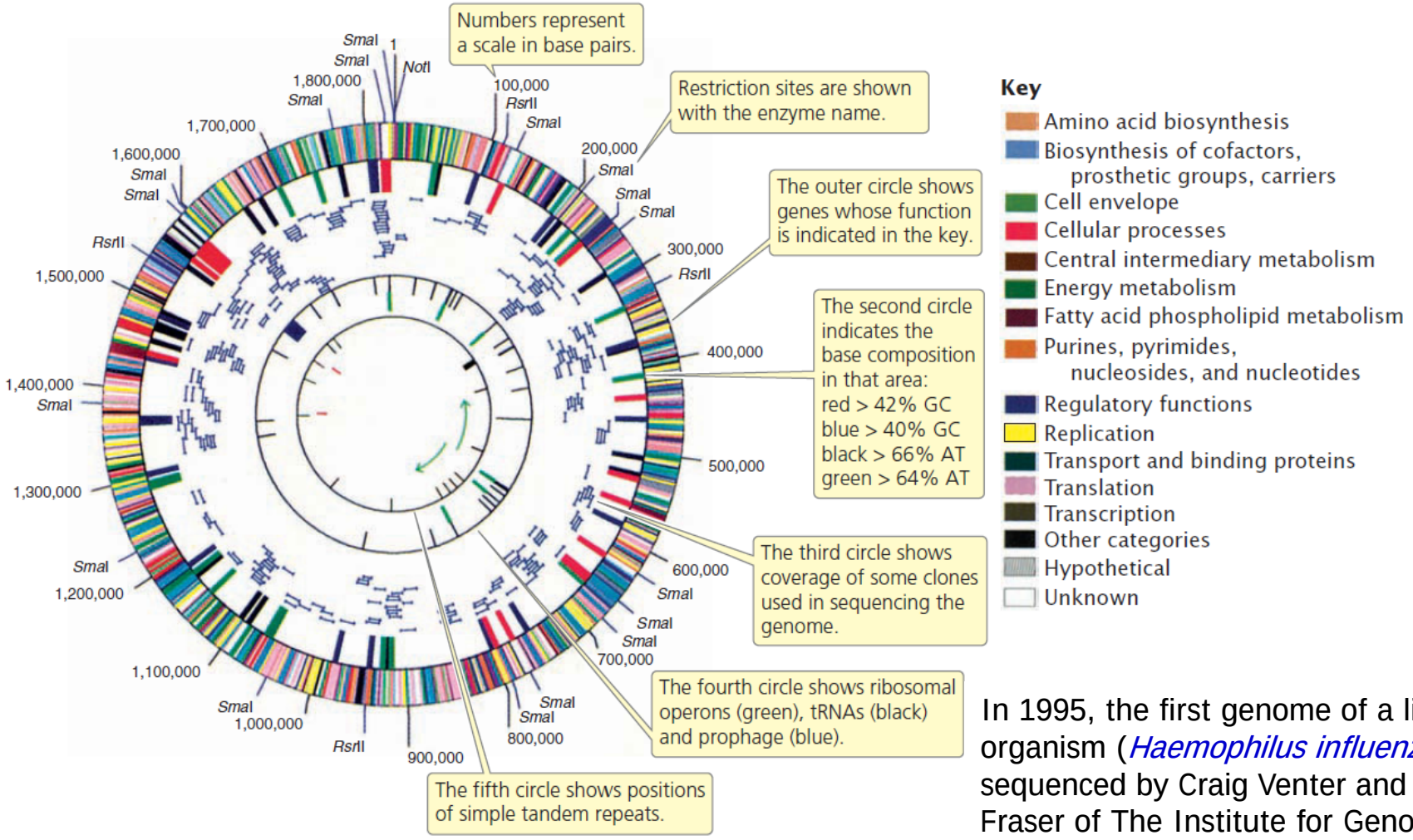


Integration (*attB*×*attP*) requires the product of the phage gene int, which codes for an integrase enzyme, and a bacterial protein called integration host factor (IHF)

The sequence O is common to *attB* and *attP*. It is called the *core sequence*; and the recombination event occurs within it.



IV. Bacterial Genome Sequences

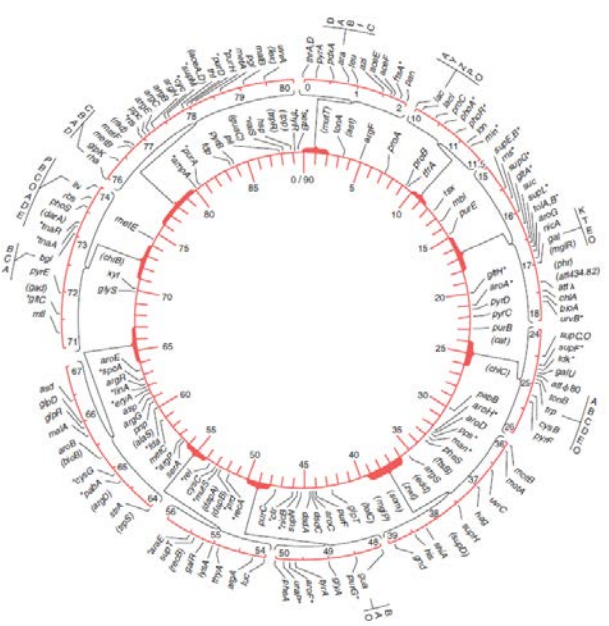


In 1995, the first genome of a living organism (*Haemophilus influenzae*) was sequenced by Craig Venter and Claire Fraser of The Institute for Genomic Research (TIGR) and Hamilton Smith of Johns Hopkins University.

E.coli genome (1997)

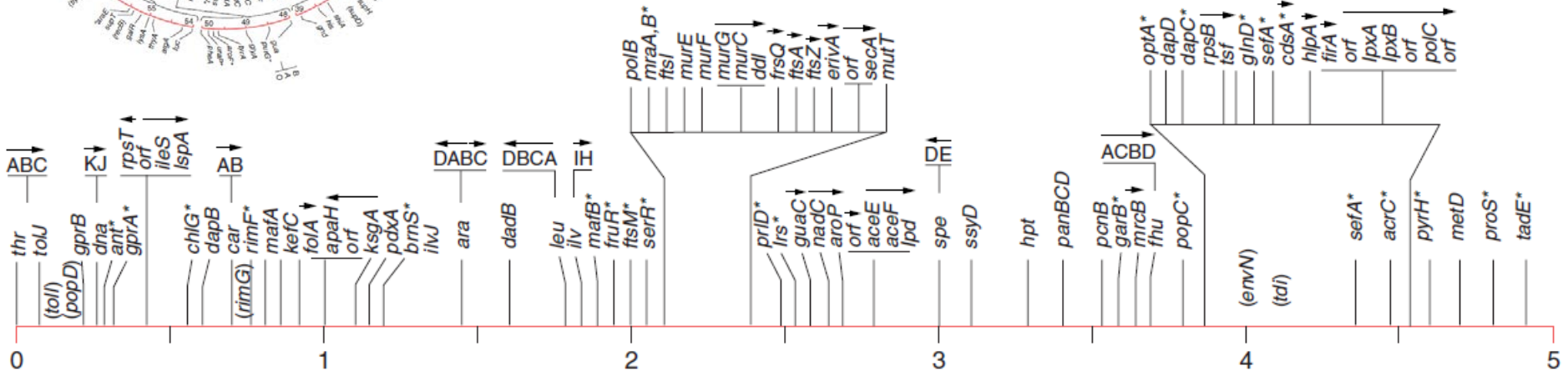
- Close to 90% of *E. coli* DNA encodes proteins
- 4288 genes, with 40% of the genes remains unknown
- Existence in eight different locations of remnants of bacteriophage genomes

Genetic maps and Physical maps



The 1963 genetic map of *E. coli* (100 genes)

Part of the genetic map
obtained in 1990 (1400 genes)



1997, *E. coli* genome of 4,632,221 bp (4288 genes)

Table 20.2 Characteristics of some completely sequenced representative prokaryotic genomes

Species	Size (millions of base pairs)	Number of Predicted Genes
Archaea		
Archaeoglobus fulgidus	2.18	2407
Methanobacterium thermoautotrophicum	1.75	1869
Methanococcus jannaschii	1.66	1715
Nanoarchaeum equitans	0.490	536
Eubacteria		
Bacillus subtilis	4.21	4100
Bradyrhizobium japonicum	9.11	8317
Buchnera species	0.64	564
Escherichia coli	4.64	4289
Haemophilus influenzae	1.83	1709
Mesorhizobium loti	7.04	6752
Mycobacterium tuberculosis	4.41	3918
Mycoplasma genitalium	0.58	480
Staphylococcus aureus	2.88	2697
Vibrio cholerae	4.03	3828

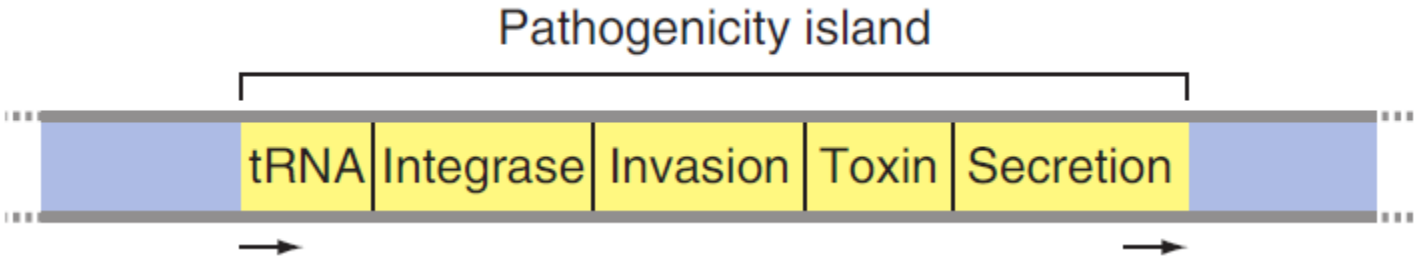
Comparative Genome Studies How Bacteria Evolve

- The availability of genome sequences has provided evidence that many bacteria have acquired genetic information from other species of bacteria—and sometimes even from eukaryotic organisms—in a process called **Horizontal Gene Transfer (HGT)**
- **HGT** has taken place repeatedly among bacteria through transformation, conjugation and transduction
  - 17% of *E.coli*'s genome has been acquired from other bacteria

Genomic islands

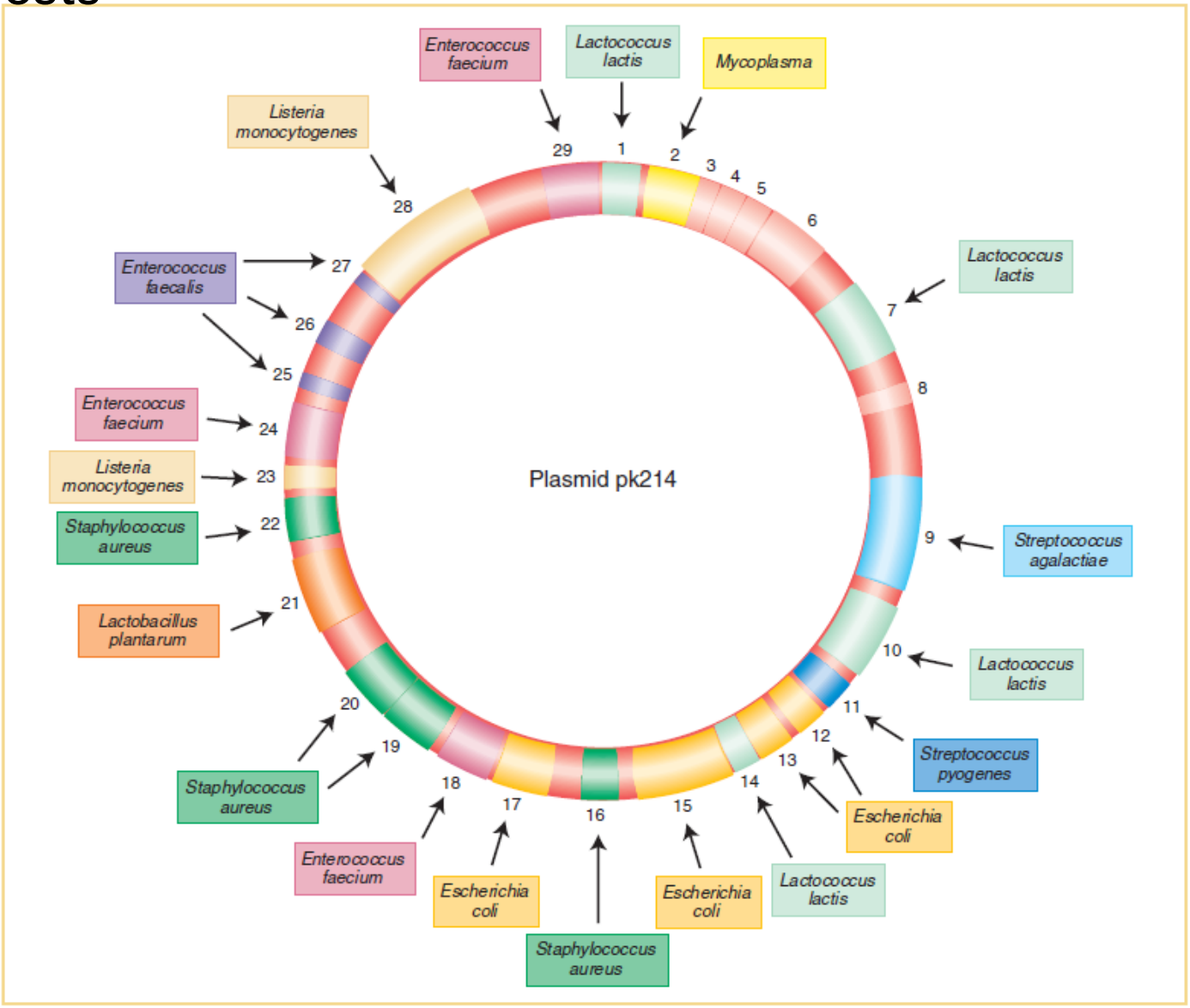
- Large segments of DNA (10–200 kb)
  - The G+C content of the DNA in the island is different
  - Direct repeats of DNA are present at each end
  - Islands are found at the sites where tRNA genes are located
  - Islands encode enzymes for integration and sites at which these integrase enzymes act
- Genomic islands carry many different types of genes involved in newly derived functions
  - New metabolic enzymes, antibiotic resistance, toxins, or enzymes to degrade poisonous substances

In pathogenic bacteria, the pathogenic determinants are often clustered in a subtype of genomic islands, called **pathogenicity islands**.



- **The pathogenicity island of *E. coli* O157:H7**
  - This island encodes proteins that facilitate attachment to epithelial cells, and secretion systems and proteins that cause cytoskeletal changes and loss of fluid.
  - A toxin from the bacterium *Shigella* that targets the rRNA of the host cells, stopping protein synthesis in these cells----bloody diarrhea (出血性痢疾)
  - Additional smaller pathogenicity islands

A *Lactococcus lactis* plasmid with segments from many former bacterial hosts



Nature 389,1997, 801–802

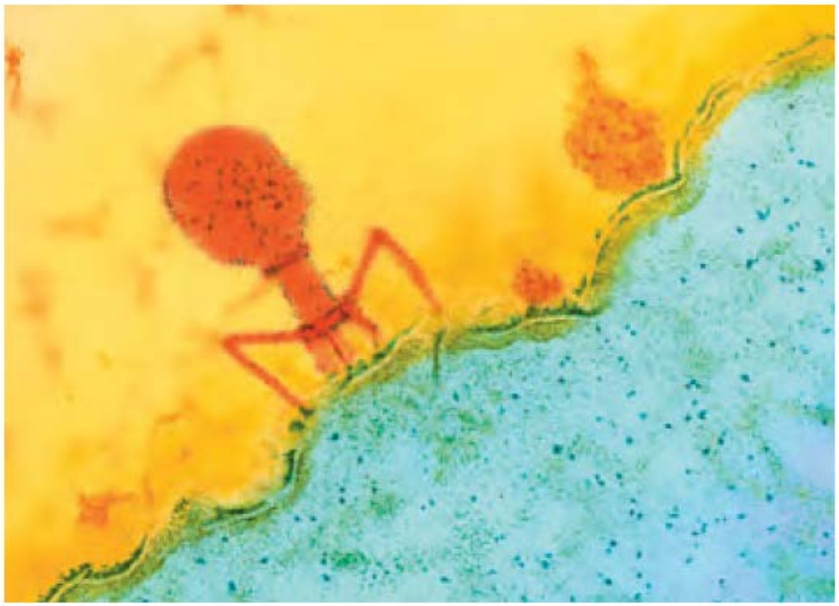
Evolutionary implications of HGT

- The glimpse into prokaryotic history made possible by comparative genome analysis has altered our view of evolution.
- Bacterial genomes have picked up DNA from several different sources during the course of their evolution, that is crucial for rapid adaptation of bacteria to a changing environment
- **HGT** is a significant evolutionary factor in pathogenicity and many other bacterial functions.

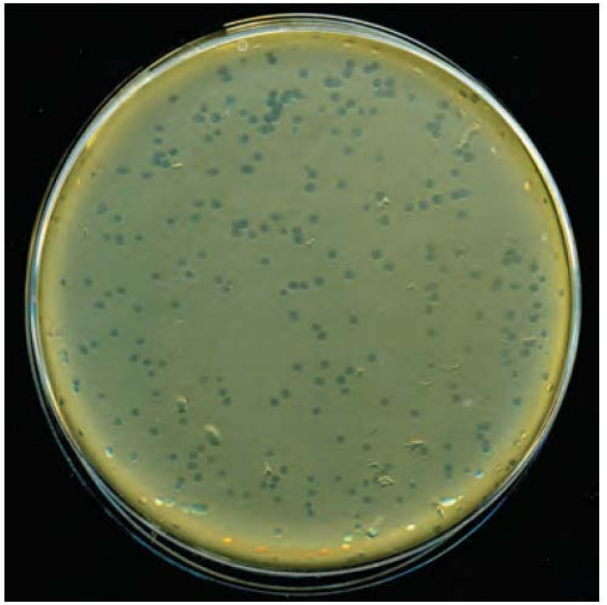
Further reading

- Antibiotic-resistant bacteria
- Get pigs off antibiotics

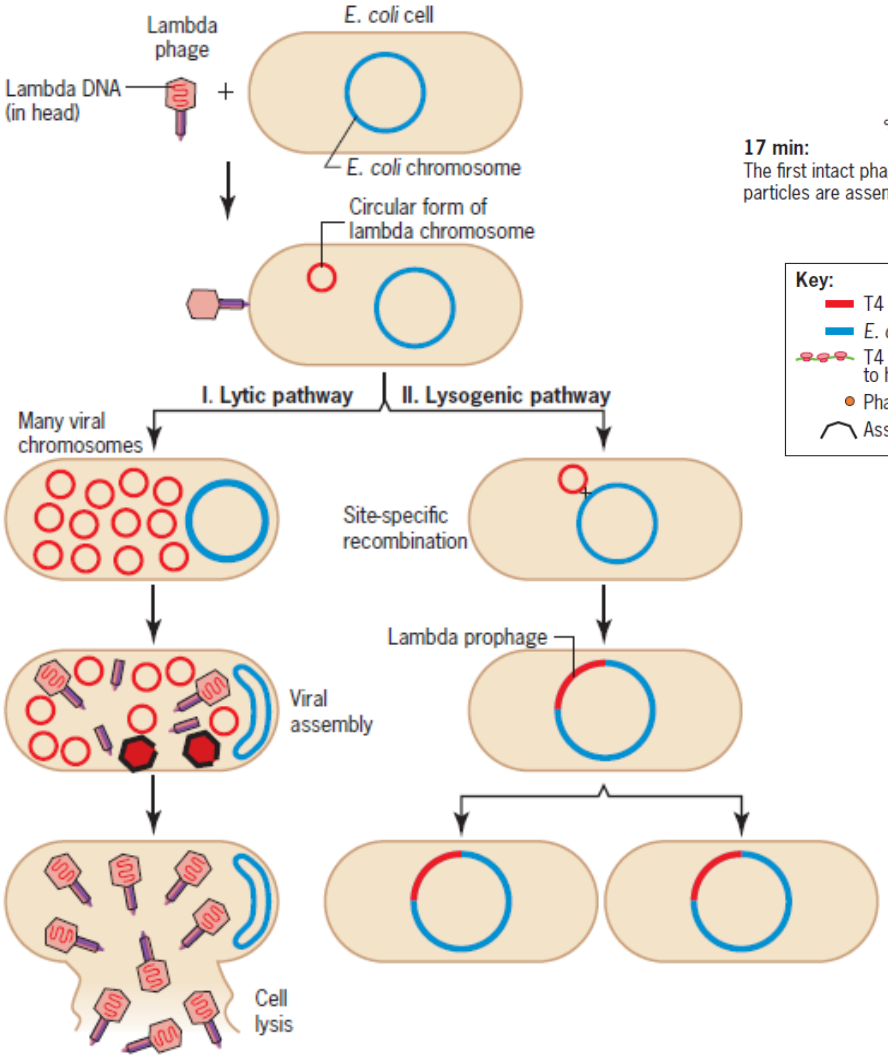
第二节　噬菌体的遗传分析
Bacteriophage Genetics

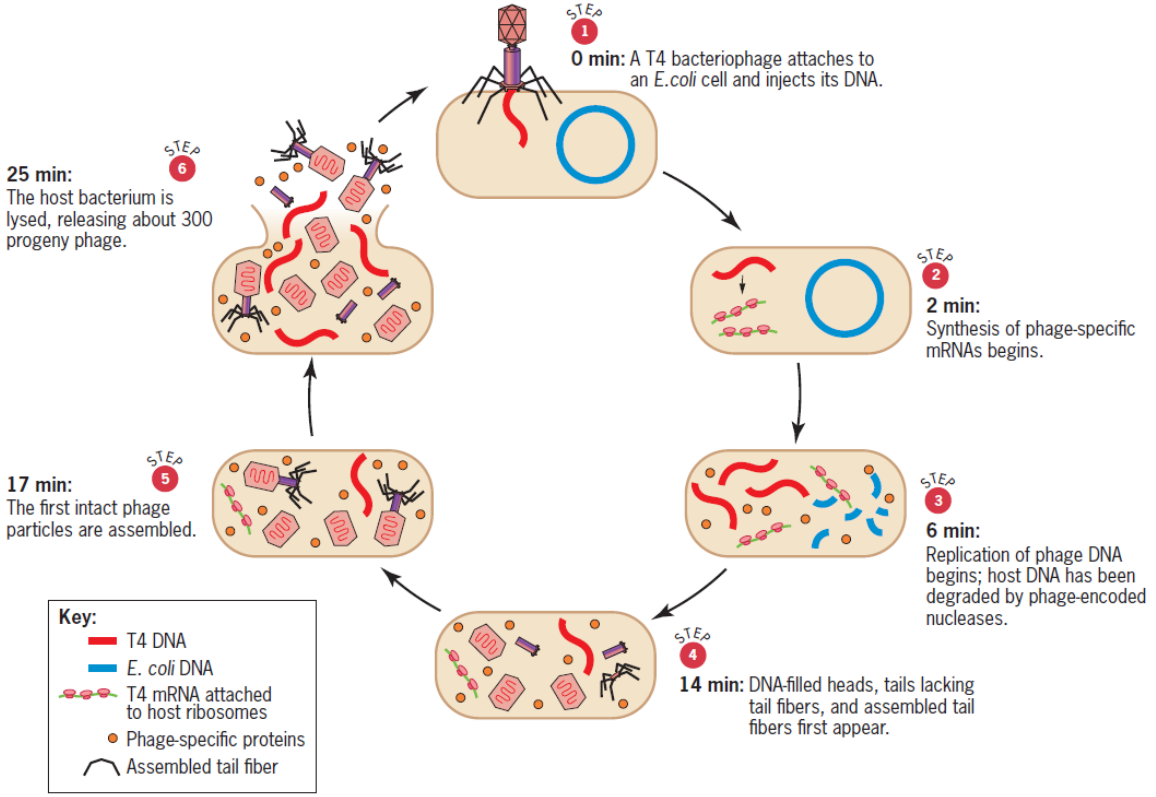


T4 bacteriophage



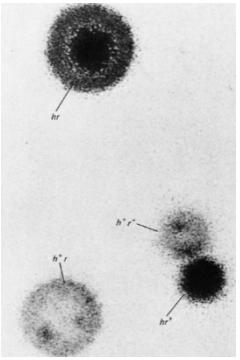
Plaques are clear patches of lysed cells on a lawn of bacteria





Bacteriophages have two life cycles

I. Bacteriophage Mutations



- Plaque morphology（噬菌斑形态突变型）
- Altered host range（宿主范围突变型）
- Conditional lethal（条件致死突变型）
  - ◇ **temperature sensitive mutations （ts）**
    （温度敏感突变）
  - ◇ **suppressor-sensitive mutations（sus）**
    （抑制因子敏感突变）

Suppressor-sensitive mutations (sus)

- **Nonsense mutation**（无义突变）

琥珀型（**sus amber**）：**UAG**
赭石型（**sus ocher**）：**UAA**
乳白型（**sus opal**）：**UGA**

Nonsense mutation is conditional lethal mutation, because the host has **nonsense suppressor**

Nonsense suppressor (su^+)

The nonsense suppressors encoded by **altered tRNA genes** can insert a specific amino acid at the position of stop codon.

Nonsense mutation and nonsense suppressors

a, b, c

Phage genotype	Bacteria genotype			
	su^-	su^+amb	su^+och	su^+op
wildtype	+	+	+	+
sus amber	−	+	−	−
sus ochre	−	−	+	−
sus opal	−	−	−	+

+: produce progeny phage **−: no progeny phage**

II. Mapping phage genes by mixed infection experiments

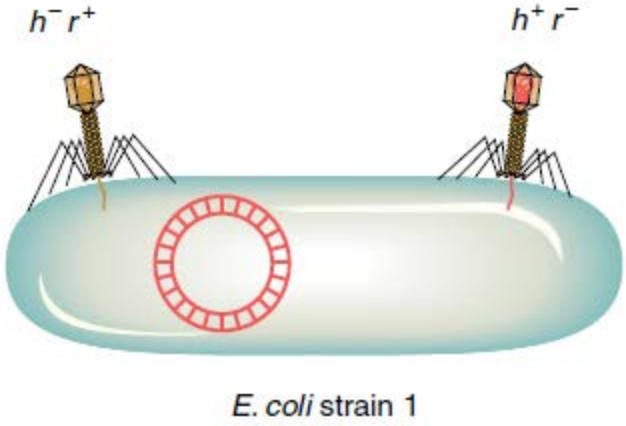
Two-point cross of phage T2

- **h$^+$** can infect only *E. coli* B
- **h$^-$** can infect two *E. coli* strains (B and B-2)

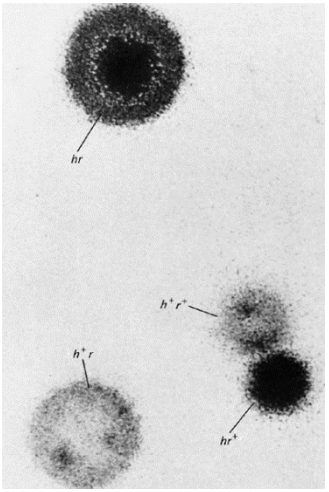
when infect a mixture of *E. coli* B and B-2, plaque of h$^-$ is distinct, while plaque of h$^+$ is fuzzy.

- **r$^+$** slowly lyses cells, producing small plaques
- **r$^-$** rapidly lyses cells, producing large plaques

$h^- r^+ \times h^+ r^-$



- Firstly, *E. coli* B is infected with both parental T2 phages
- The progeny phage is then spread onto a bacterial lawn composed of a mixture of *E. coli* B and B-2
- Four plaque types appear



$h^- r^+$: distinct, small

$h^+ r^-$: fuzzy,large

$h^+ r^+$: fuzzy, small

$h^- r^-$: distinct, large

Genotype	Plaques	Designation
$h r^+$	42	Parental progeny
$h^+ r$	34	76%
$h^+ r^+$	12	Recombinants
$h r$	12	24%

Source: Data derived from Hershey and Rotman (1949).

$$\mathbf{RF} = \frac{(\mathbf{h}^+ \mathbf{r}^+) + (\mathbf{h}^- \mathbf{r}^-)}{\text{total plaques}}$$

Three-point cross of phage T4

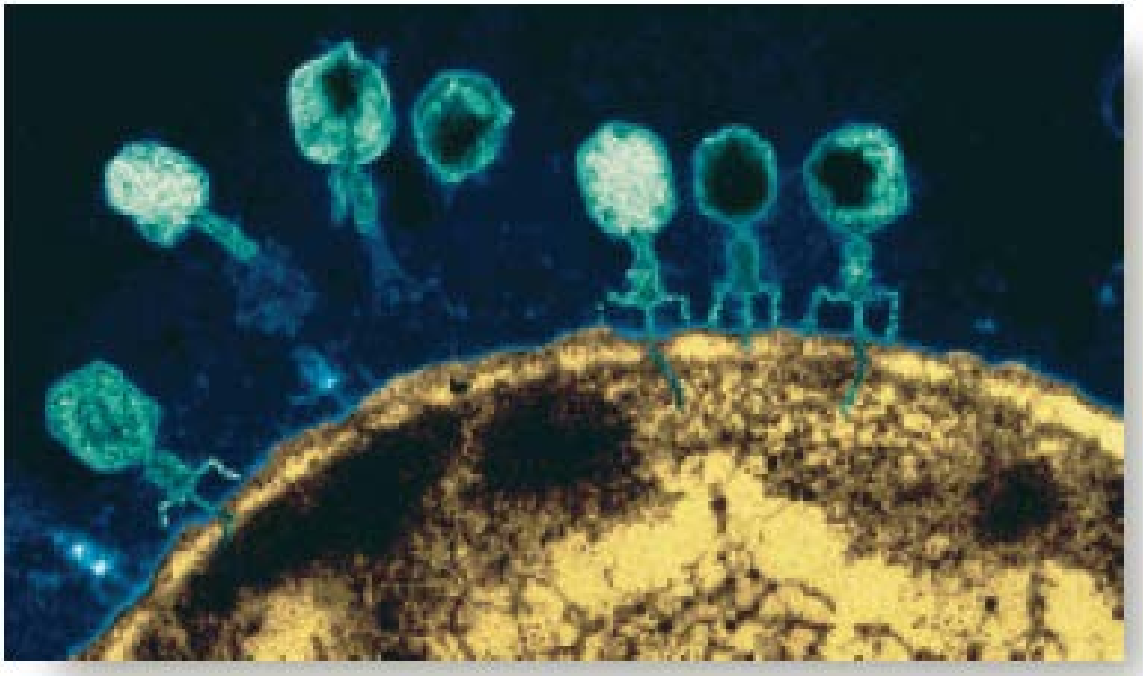
m r tu × + + +

Types	No. of plaques	Percentage %	Recombination frequency (%)		
			m-r	r-tu	m-tu
m r tu	3467	69.6			
+ + +	3729				
m + +	520	9.6	√		√
+ r tu	474				
m r +	853	17.5		√	√
+ + tu	965				
m + tu	162	3.3	√	√	
+ r tu	172				
total	10342		12.9	20.8	27.1

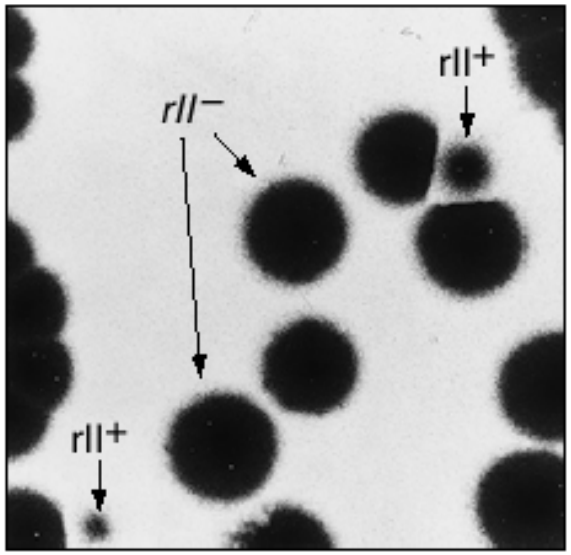
负干扰（negative interference）

- In phage cross, negative interference often occurs
- Genetic exchange between phage chromosomes will occurs before, during, and after replication
- Recombination is not restricted to exchange between two chromosomes----three or more may be involved simultaneously

III. Intragenic Recombination Occurs in Phage T4



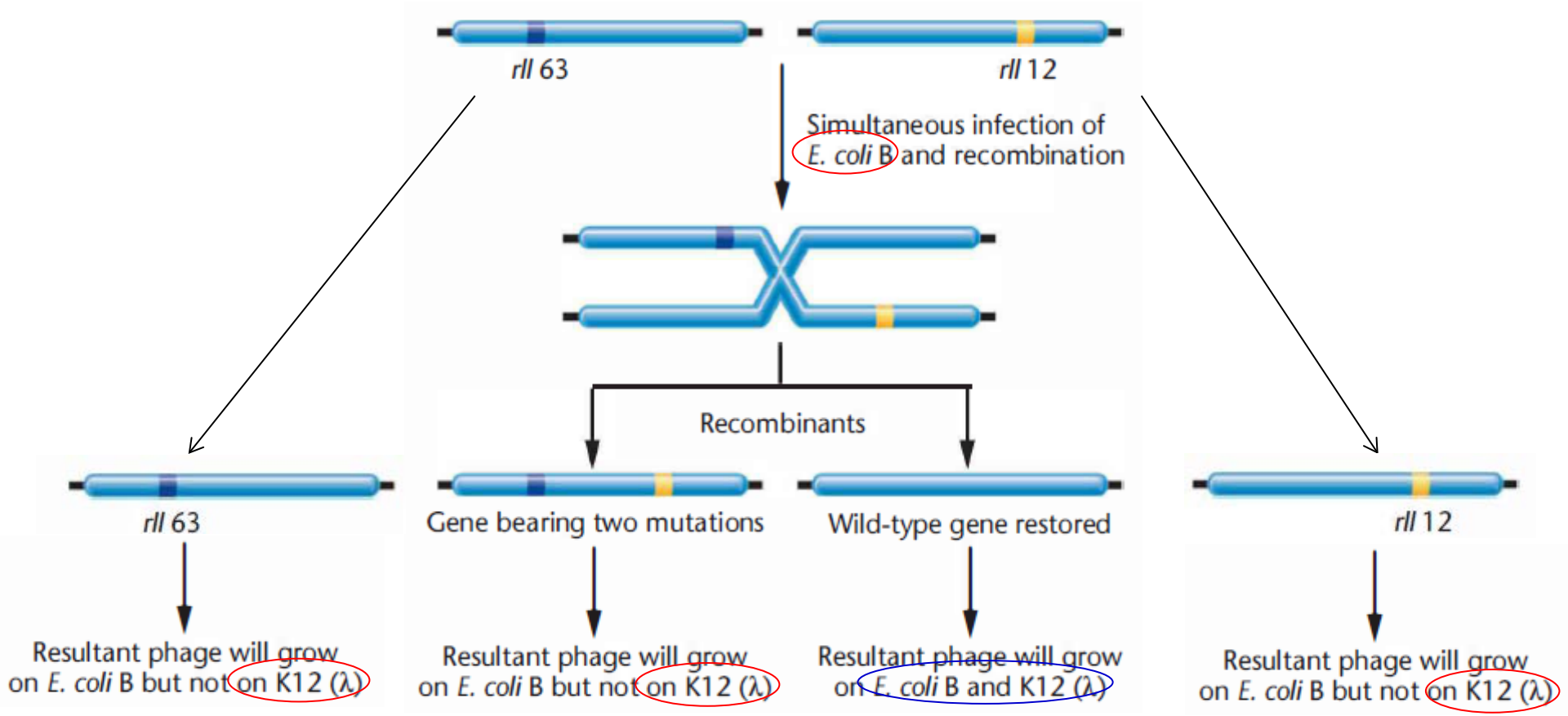
Bacteriophage T4 *rII*$^-$ mutation



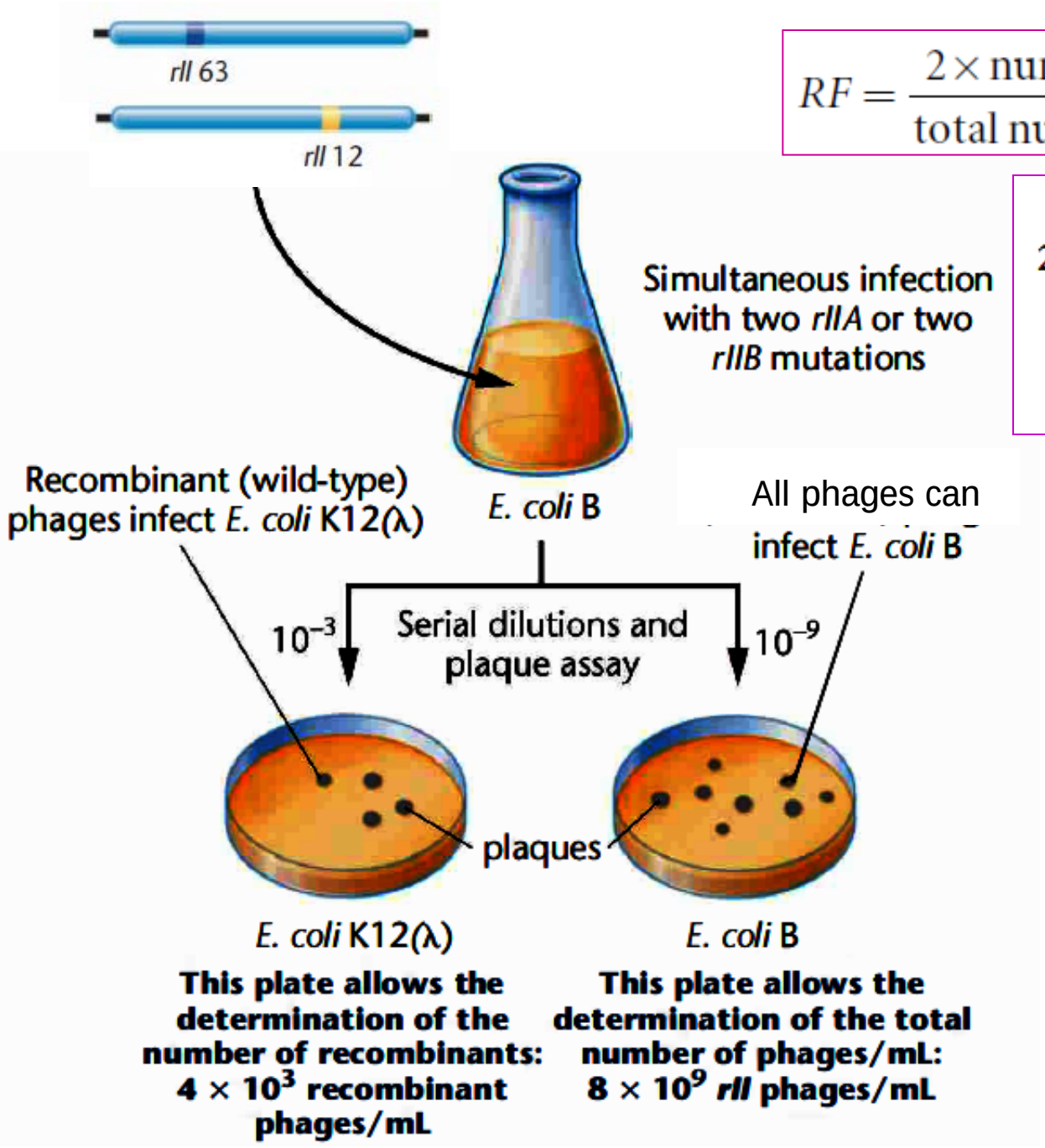
T4 strain	*E. coli* strain B	*E. coli* strain K(λ)
rII$^-$	Large, distinct	No plaques
rII$^+$	Small, fuzzy	Small, fuzzy

- Benzer obtained about 20,000 independent *rII* mutants
- Benzer assumed that most of these mutations, because they were randomly isolated, would represent different locations within the *rII* locus
- Benzer performed recombinational studies on these mutants so as to produce a genetic map of this locus
- The key to Benzer's analysis was that mutant phages could not successfully lyse strain *E.coli* K (λ)

Recombinational Analysis



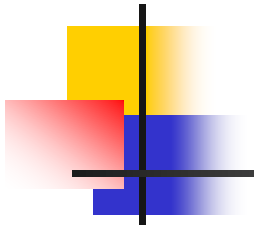
Detect revertants?



$$RF = \frac{2 \times \text{number of plaques on } E.coli \text{ K}}{\text{total number of plaques on } E.coli \text{ B}}$$

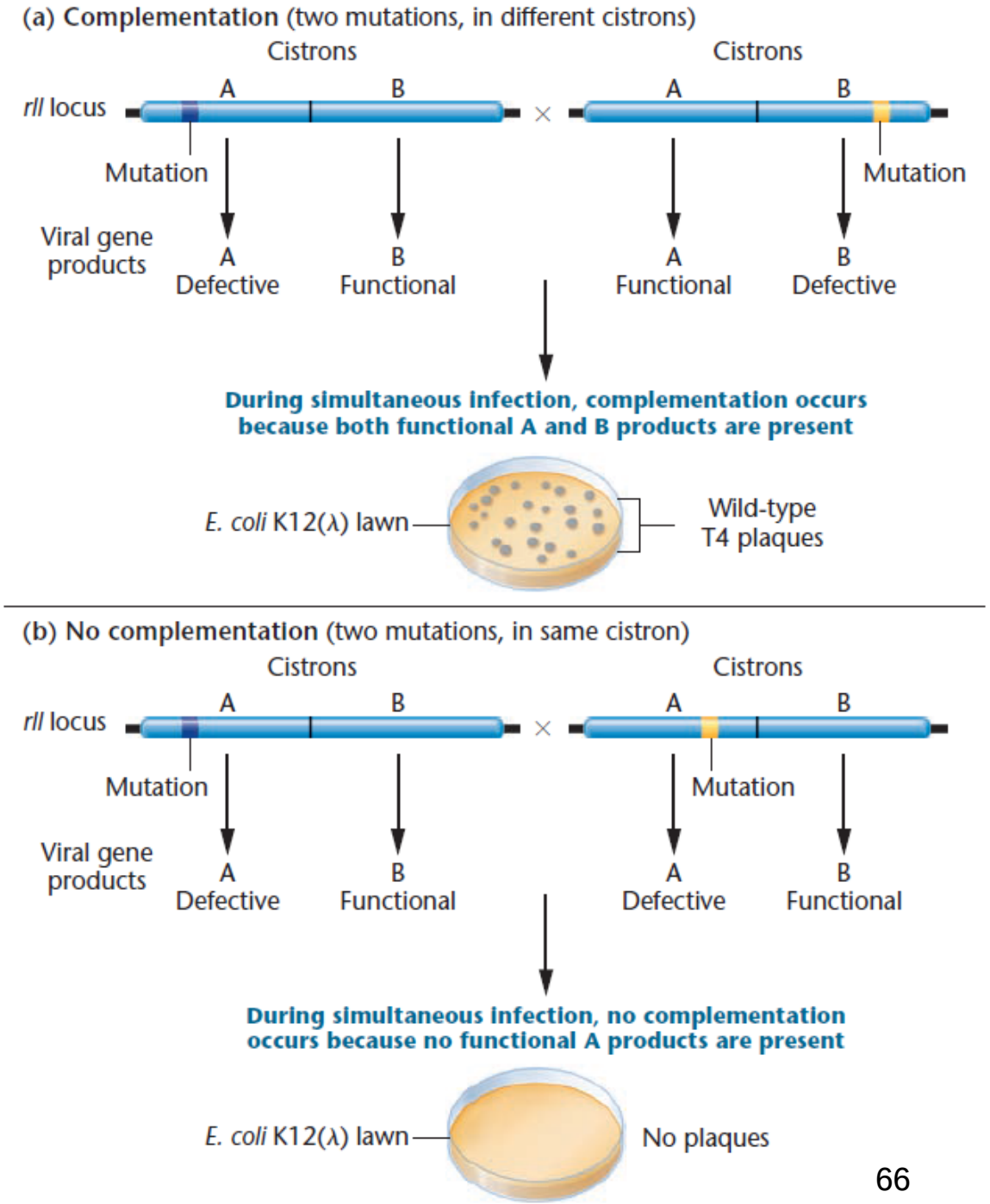
$$2\left(\frac{4 \times 10^3}{8 \times 10^9}\right) = 2(0.5 \times 10^{-6})$$
$$= 10^{-6}$$
$$= 0.000001$$

This experiment was extraordinarily sensitive to detect as few as one recombinant wild-type phage among 100 million mutant phages



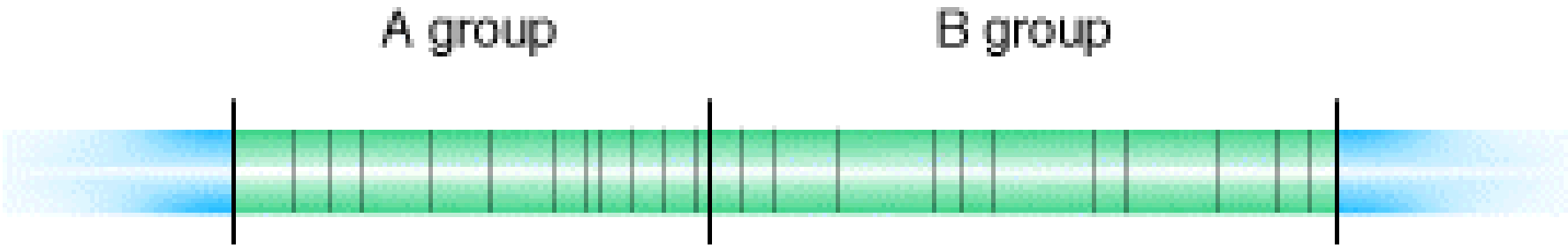
- Recombination analysis revealed the positions of the different *r*// mutations along the phage chromosome.
- Eventually, Benzer mapped more than 2400 *r*// mutations, and some *r*// mutations were very closely linked.
- This finding raised the question: *whether these mutations were at the same locus or at different loci?*

Complementation experiments



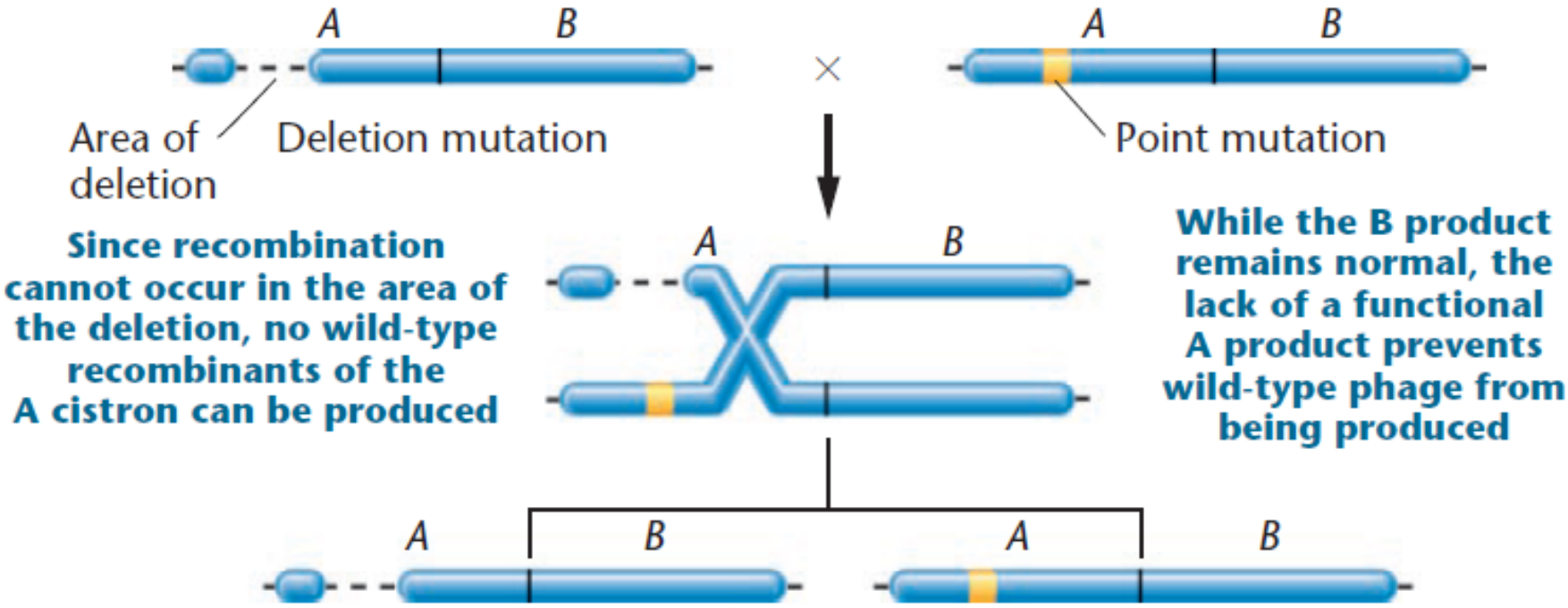
For complementation test, the mutations must be recessive.

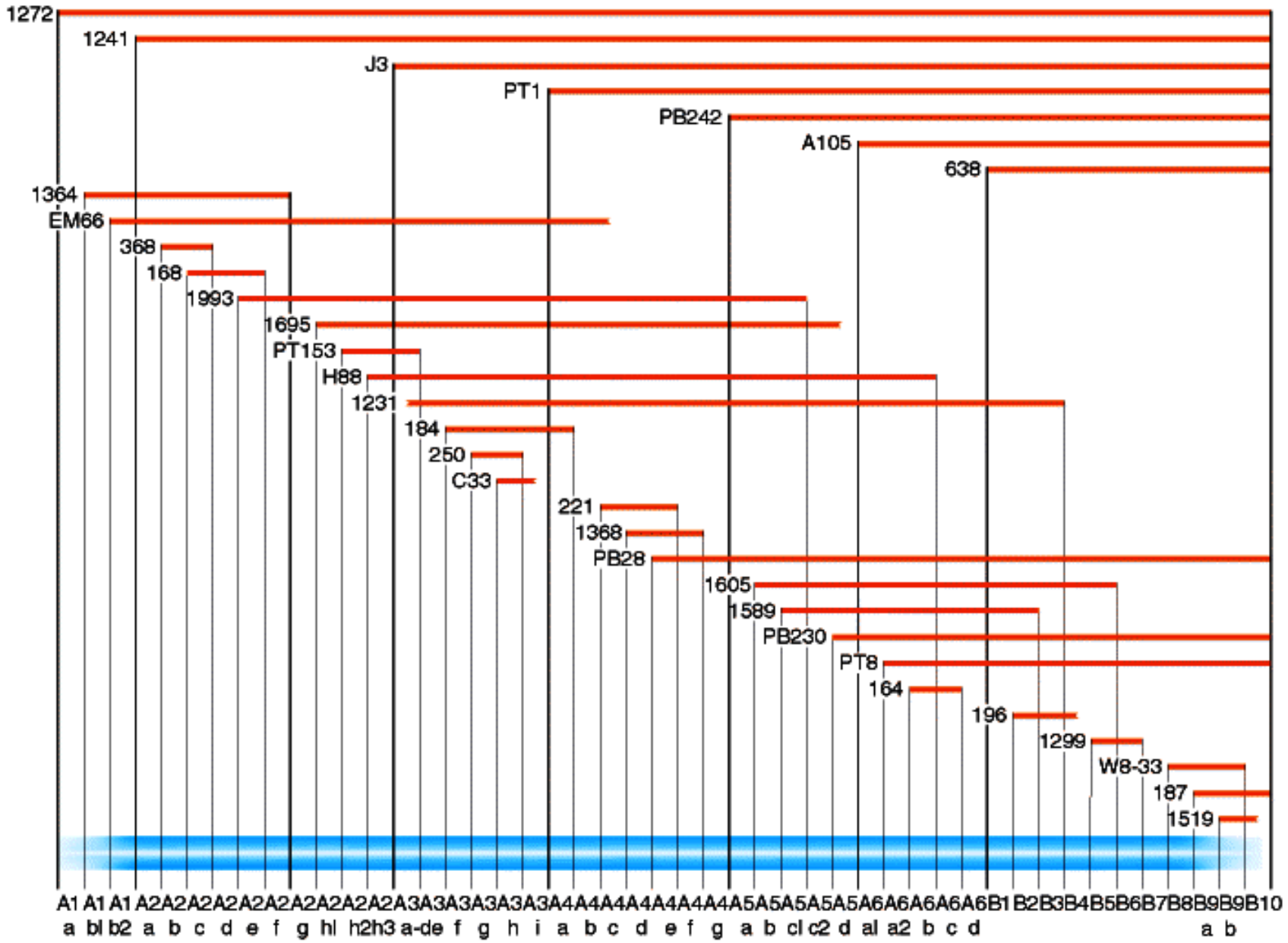
Gene map of *r//*

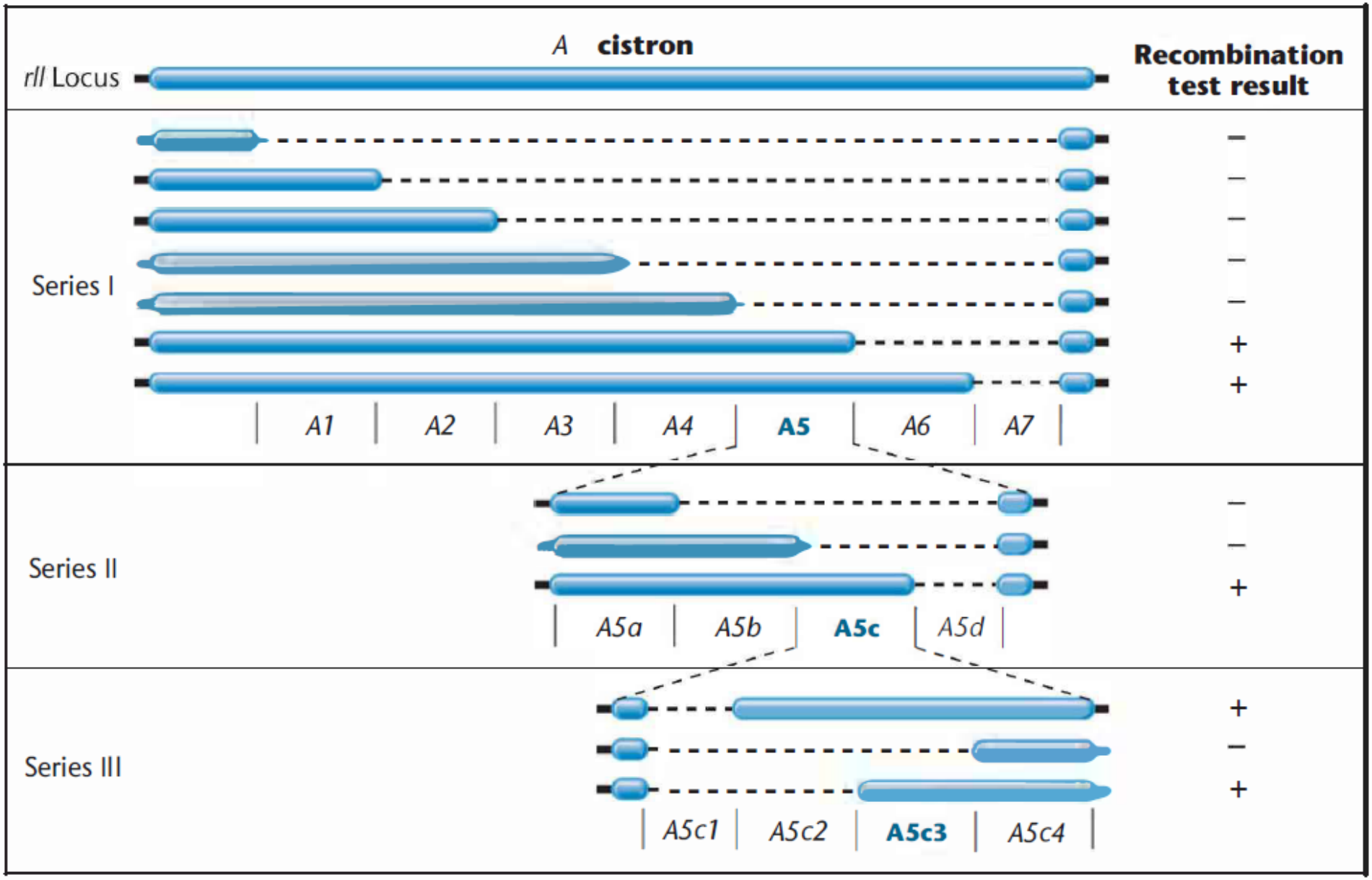


Recombination could take place within a single gene---- **Intragenic Recombination**

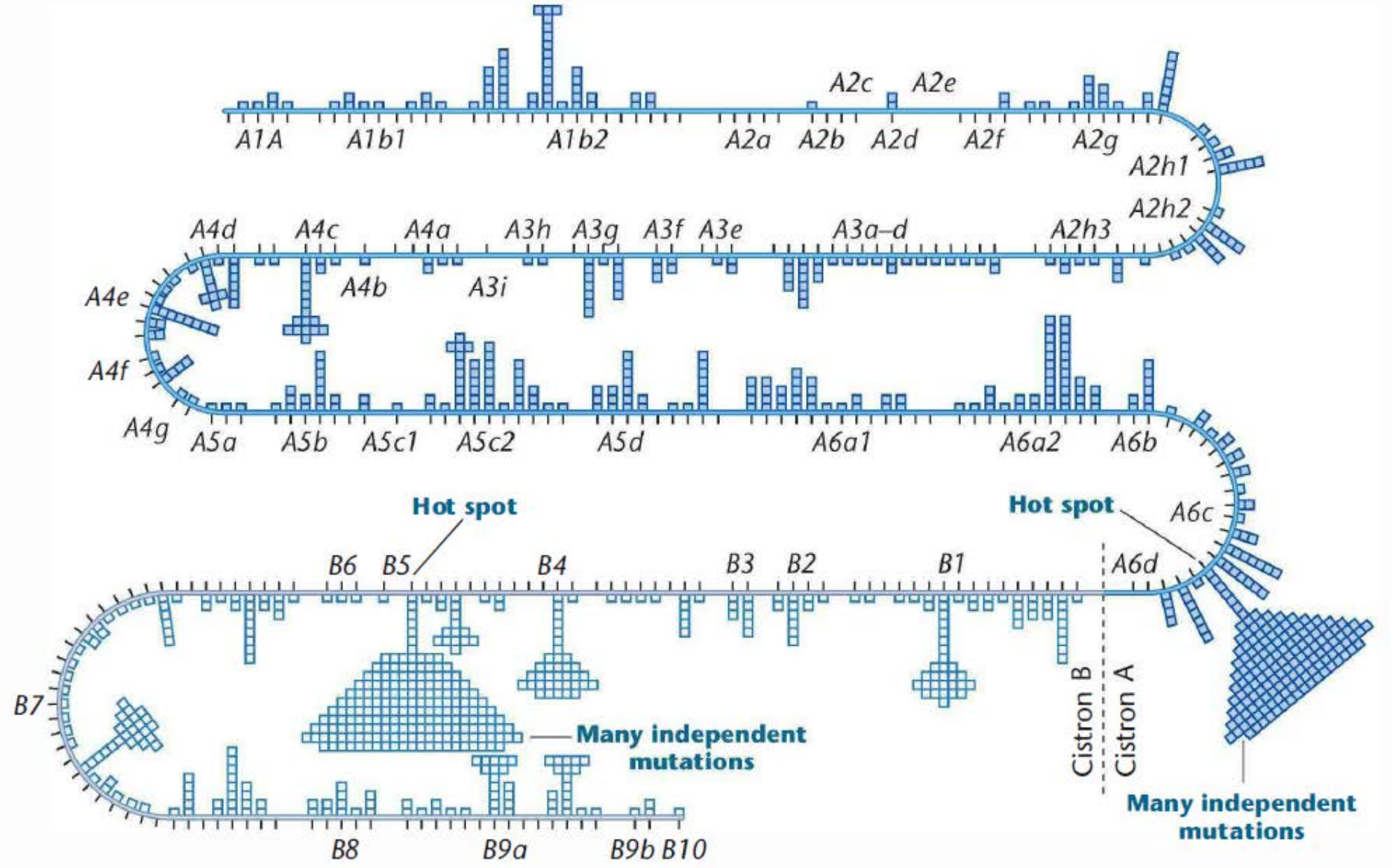
IV. Deletion testing of the *r*// locus




1272
1241
J3
PT1
PB242
A105
638
1364
EM66
368
168
1993
1695
PT153
H88
1231
184
250
C33
221
1368
PB28
1605
1589
PB230
PT8
164
196
1299
W8-33
187
1519
A1 A1 A1 A2 A2 A2 A2 A2 A2 A2 A2 A2 A2 A3 A3 A3 A3 A3 A3 A4 A4 A4 A4 A4 A4 A4 A4 A5 A5 A5 A5 A5 A6 A6 A6 A6 A6 B1 B2 B3 B4 B5 B6 B7 B8 B9 B9 B10
a b1 b2 a b c d e f g h1 h2 h3 a-de f g h i a b c d e f g a b c1 c2 d a1 a2 b c d a b

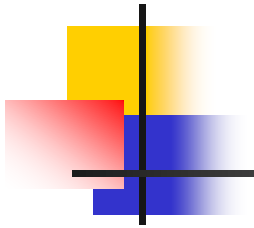

A cistron
rII Locus
Recombination test result
Series I
A1
A2
A3
A4
A5
A6
A7
Series II
A5a
A5b
A5c
A5d
Series III
A5c1
A5c2
A5c3
A5c4

A partial map of mutations in the A and B cistrons of the *rII* locus of phage T4



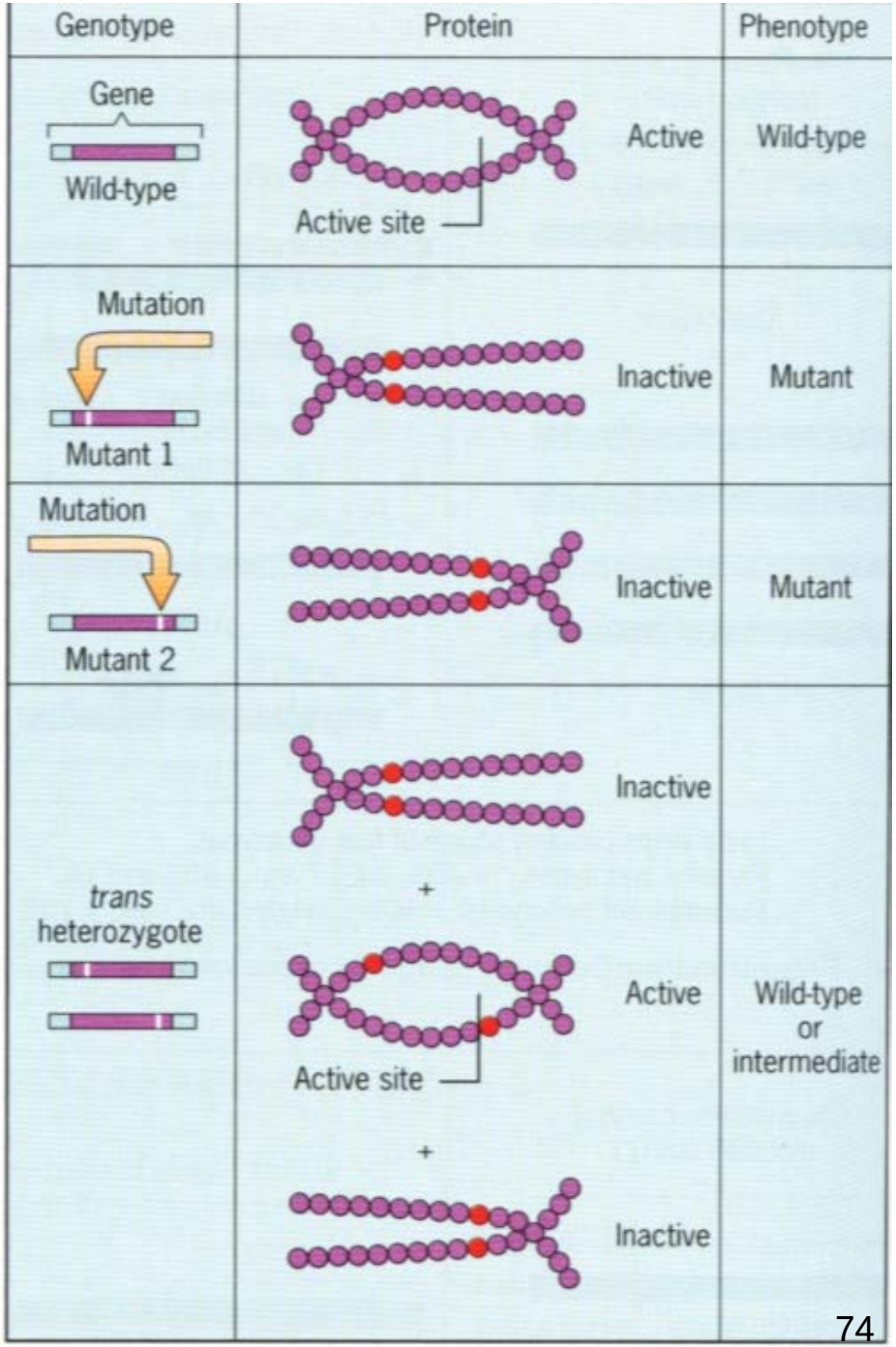
The Significance of Benzer's Work

- At the time of Benzer’s research, the relation between genes and DNA structure was unknown. A gene was defined as a functional unit of heredity that encoded a phenotype.
- Benzer demonstrated in 1955 that a gene is not an indivisible particle, but instead consists of mutational and recombinational units that are arranged in a specific order.



A gene is a linear sequence of nucleotide pairs that can mutate independently and recombine with each other

- Intragenic complementation
（基因内互补）



The figures and tables are cited from:

- **Genetics (From genes to genomes)**, Leland Hartwell, Mcgraw-Hill Companies, Inc
- **Concept of Genetics**, William S.Klug, Prentice Hall, Inc
- **Introduction to Genetics Analysis**, Anthony J.F. Griffiths, W.H.Freeman, Inc
- **Principle of Genetics**, D.Peter Snustad, John Wiley & Sons, Inc
- **Genetics-A Conceptual Approach**, Benjamin A. Pierce, W. H. Freeman