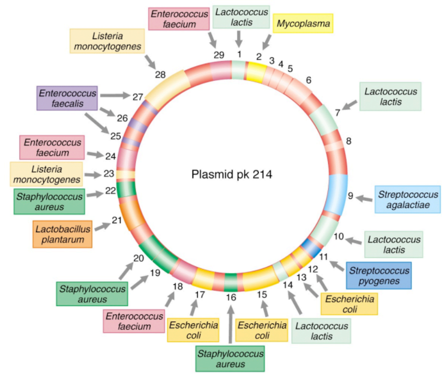
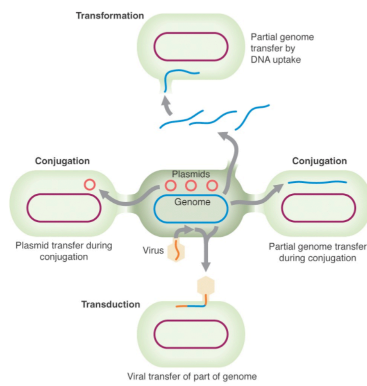
Chapter 7

Recombination in Bacteria and their Viruses

Conjugation, transformation, transduction



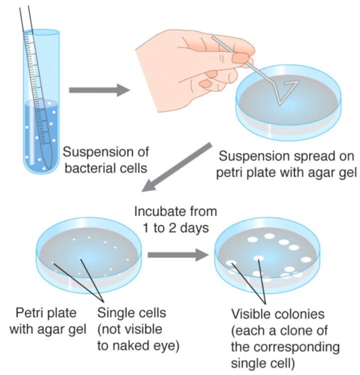


Genetic "exchange"in bacteria

- Conjugation
  - transfer of plasmids
  - transfer of genome when plasmid is integrated
- Transduction
  - bacteriophage can "pick up" fragment of genome and deliver it to another cell
- Transformation
  - uptake of DNA fragments from extracellular medium (environment)
- All are essentially one-way transfers from donor to recipient

Bacteria

- Can be rapidly grown in large quantities
- Cells divide by binary fission
- Growth medium
  - liquid
  - solid, such as nutrient agar
- Colony: asexual descendents of single cell
- Prototroph: wild-type bacterium that produces colonies on minimal medium
- Auxotroph: mutant that requires one or more nutrients in addition to minimal medium



Gene nomenclature

- Usually 2-3 letters with + or – symbol
- Prototrophy/auxotrophy
  - *met*⁺: can synthesize methionine, a wild-type prototroph
  - *met*⁻: requires methionine, a mutant auxotroph
- Energy extraction
  - *gal*⁺: can utilize galactose, wild-type
  - *gal*⁻: can not utilize galactose, a mutant
- Drug resistance
  - *str*ʳ: resistant to streptomycin in medium
  - *str*ˢ: sensitive to streptomycin in medium

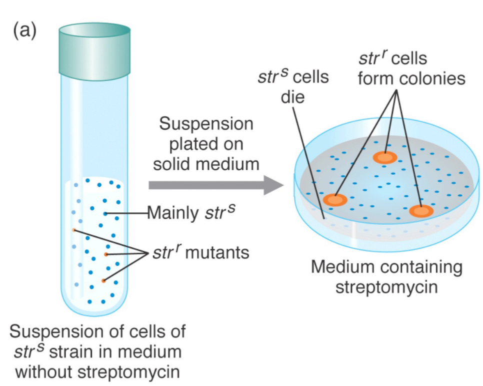
Selective systems

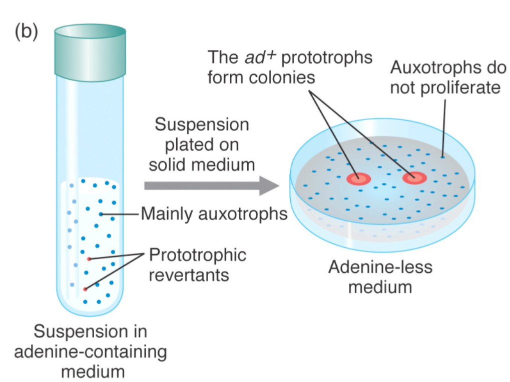
- Allows the desired mutant to reproduce but not wild-type genotypes
  - antibiotic resistance
  - minimal medium supplemented with specific nutrient
- Revertant: reverse change from mutant to wild-type
  - similar selection regimens

TABLE 7-1 Some Genotypic Symbols Used in Bacterial Genetics

Symbol	Character or Phenotype Associated with Symbol
bio⁻	Requires biotin added as a supplement to minimal medium
arg⁻	Requires arginine added as a supplement to minimal medium
met⁻	Requires methionine added as a supplement to minimal medium
lac⁻	Cannot utilize lactose as a carbon source
gal⁻	Cannot utilize galactose as a carbon source
*str*ʳ	Resistant to the antibiotic streptomycin
*str*ˢ	Sensitive to the antibiotic streptomycin

NOTE: Minimal medium is the basic synthetic medium for bacterial growth without nutrient supplements.



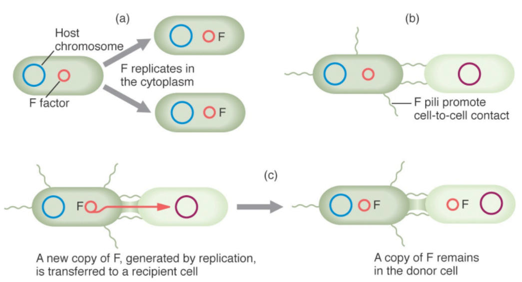


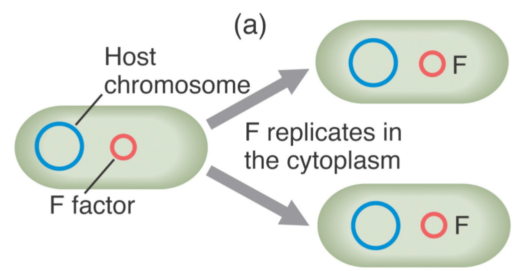
Detecting recombination

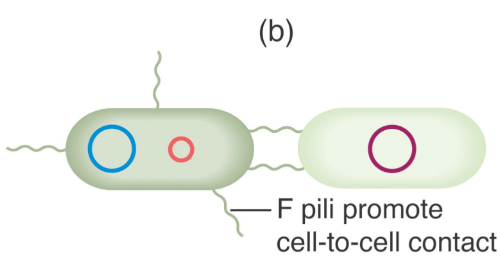
- In bacteria, gene transfer is partial and unidirectional, unlike in eukaryotes
- Donor: contributes DNA; exogenote
- Recipient: receives contributed DNA (has own complete genome); endogenote
- Merozygote
  - contains endogenote and exogenote
  - partial diploid
  - opportunity for recombination

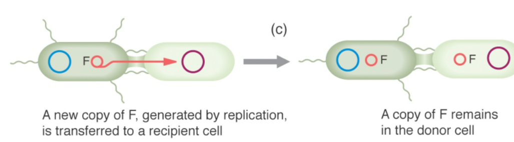
F plasmid

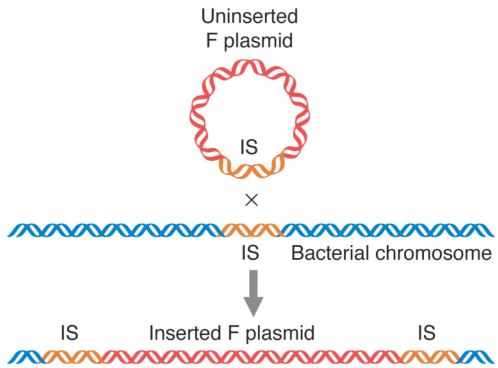
- Replicates inside host cell
- Contains genes encoding synthesis of pili which allow cell contact
- F^+ cells conjugate with F^-
  - F^+ donates single-stranded copy of F to F^- cell (rolling circle)
  - F^+ retains copy of plasmid
- F carries one or more insertion sequence elements (IS)
  - permit homologous recombination with same IS in genome
  - results in integration of F into genome to form **Hfr** strain (high frequency of recombination)
  - F may leave genome, taking copies of some genes (F')

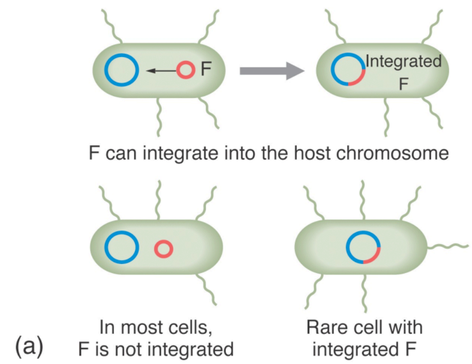






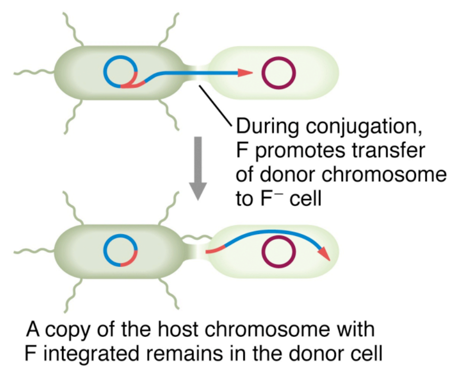


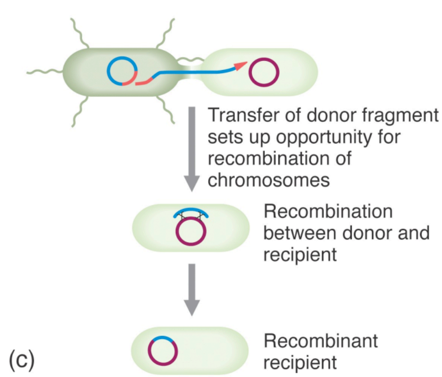


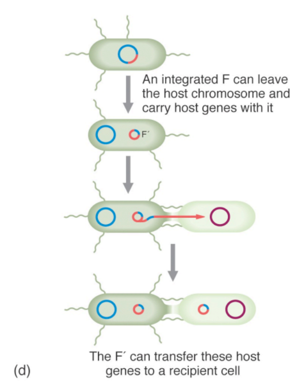


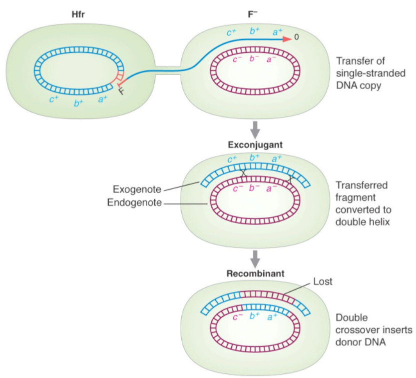
Conjugation in *Escherichia coli*

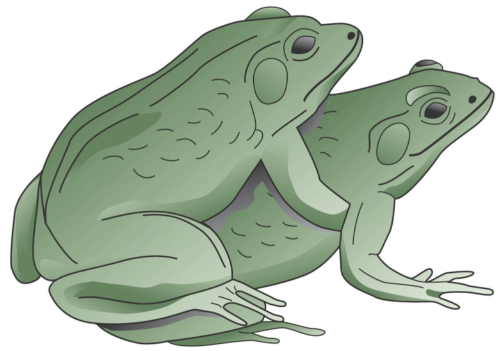
- Temporary cytoplasmic bridge between two cells
  - driven by plasmid called the fertility factor or F
    - found in some but not all *E. coli*
    - one of several different types of plasmid
- Mating only between cell with F (F^+) and cell without F (F^-)
- Transfer of information is one-way from donor to recipient

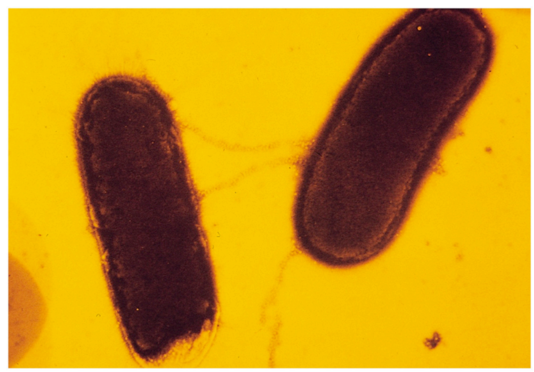






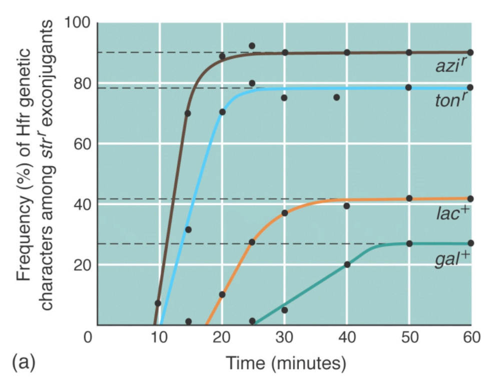


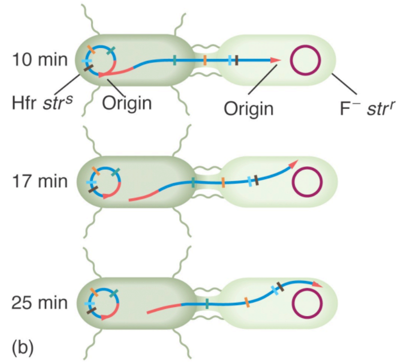


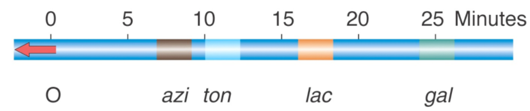


Mapping by interrupted conjugation

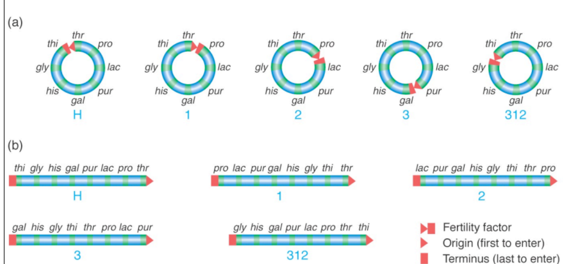
- Hfr strains transmit host chromosome to F⁻ in linear manner, about 1% of chromosome per minute
- After timed intervals, mating is interrupted and cells are plated on selective medium to recover recombinants
- Genes are mapped according to time of appearance of recombinants
- Circular, low resolution map is made by combining maps from different Hfr donors
- Higher resolution map is made by RF analysis



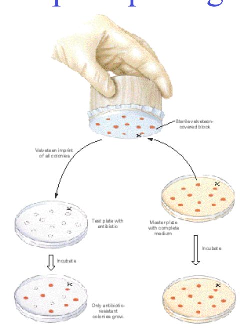


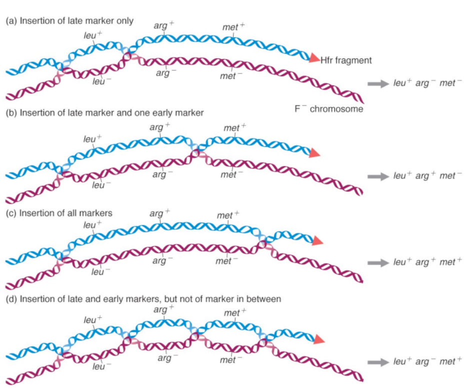


F plasmid inserted in different places and directions

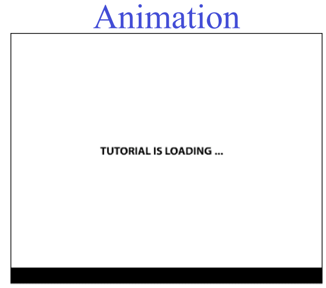


Replica plating

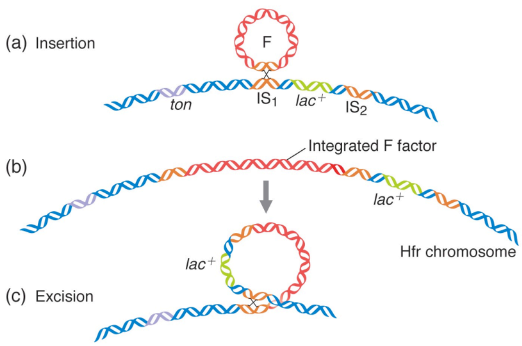




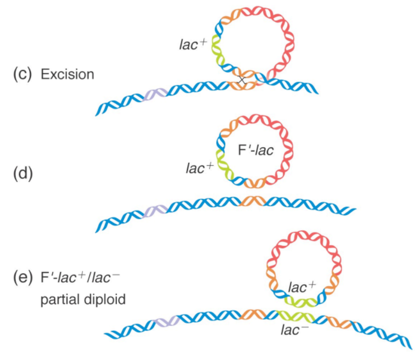
Animation



Sexduction (partial diploid)



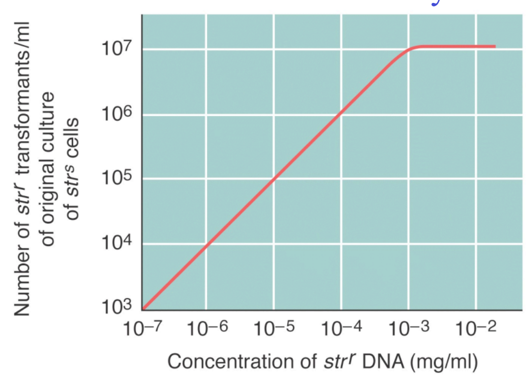
Sexduction (partial diploid)



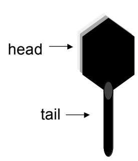
Transformation

- Uptake of DNA from environment, e.g., culture medium
- Useful for determining tightness of linkage
  - closely linked genes will be cotransformed
  - not-so-closely linked genes will be on separate fragments, requiring that *both* be taken up
    - a rare event by product rule
- RF used to estimate gene order and distances
  - Estimate for linkage $(a^+b^+)/T > (a^+b^-)/T * (a^-b^+)/T$
- Used in recombinant DNA technology

Transformation efficiency

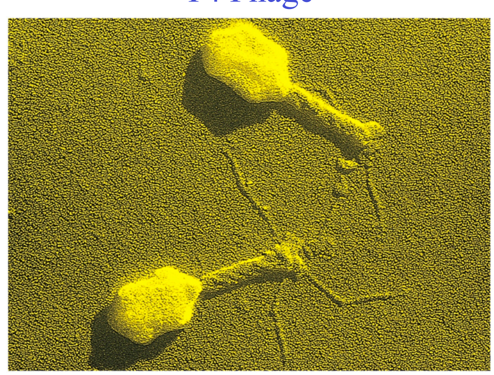


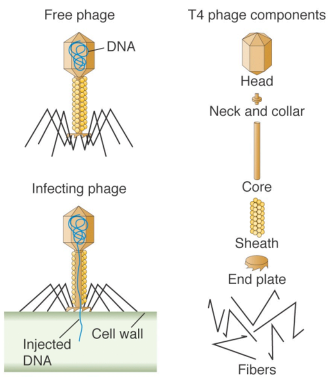
Bacteriophage

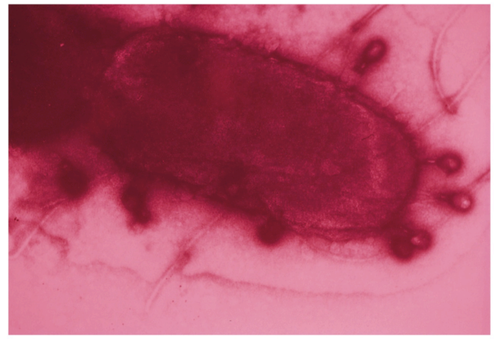


- “Eater of bacteria”
  - in reality, kill and lyse bacterial cells
  - sometimes simply called “phage”
- Two major parts
  - protein coat (e.g., head, tail)
  - capsid (head) with DNA or RNA
- Two distinct phage genotypes can be analyzed in crosses, allowing mapping the viral genome
- Phage can be used to introduce genes into bacterial cells by transduction
- Also used in recombinant DNA technology

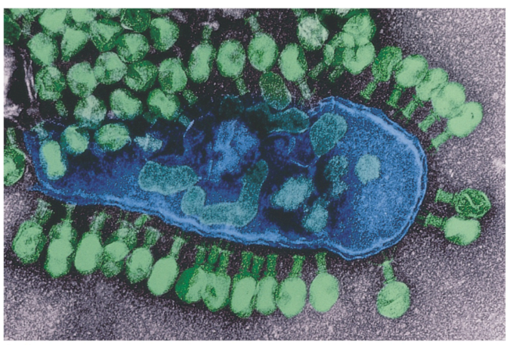
T4 Phage





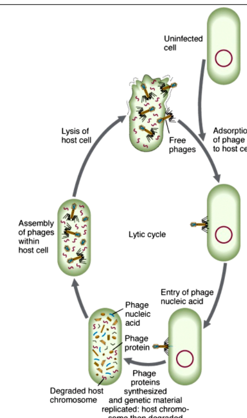


(a)



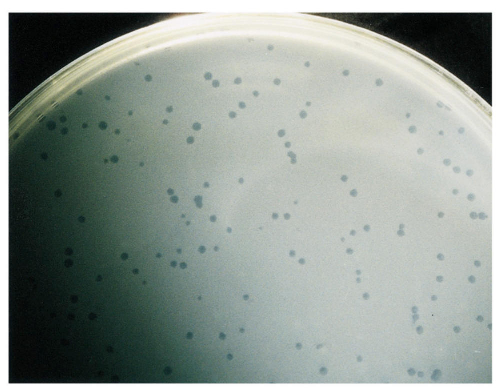
(b)

Lytic cycle



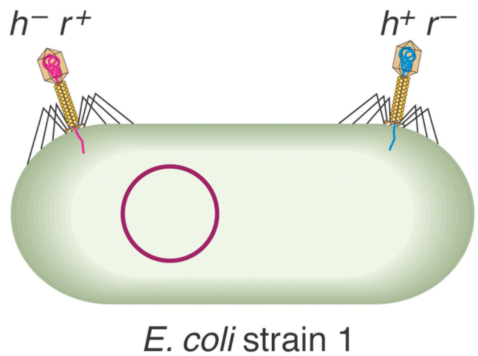
Lytic phage life cycle (virulent)

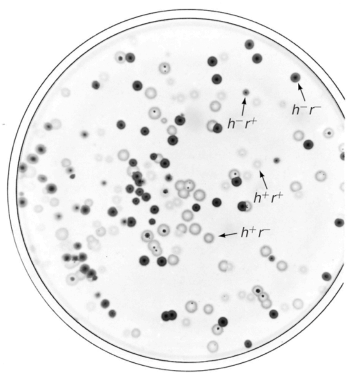
- Phage consists of nucleic acid and protein
- DNA or RNA is injected by phage into host cell
- Phage genetic information is expressed, taking over host cell and redirecting machinery to phage replication
- Upon completion of replication, bacterial cell lyses, releasing phage (lysis)
- When this happens on a lawn of agar-cultured bacteria, a plaque is formed



Lysogenic phage life cycle

- Also called temperate phage
- Capable of either lytic cycle or lysogenic cycle
- Lysogen
  - phage genome is integrated into host genome as silent prophage
  - host cell is resistant to further phage infection
- Prophage may spontaneously excise and enter lytic cycle





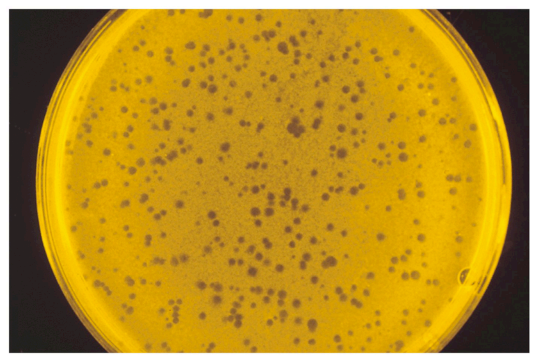


TABLE 7-2 Progeny Phage Plaque Types from Cross $h^- r^+ \times h^+ r^-$

Phenotype	Inferred Genotype
Clear, small	$h^- r^+$
Cloudy, large	$h^+ r^-$
Cloudy, small	$h^+ r^+$
Clear, large	$h^- r^-$

NOTE: Clearness is produced by the h^- allele, which allows infection of *both* bacterial strains in the lawn; cloudiness is produced by the h^+ allele, which limits its infection to the cells of strain 1.

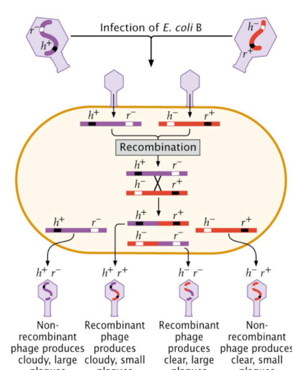
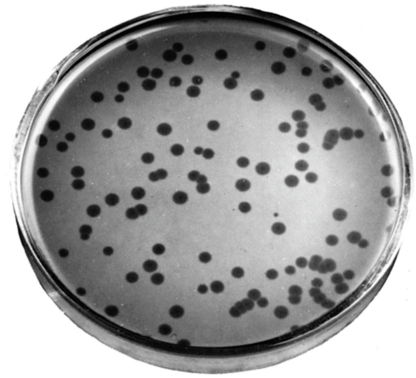


Table 8.4 Progeny phage produced from $h^- r^+ \times h^+ r^-$

Phenotype	Genotype
Clear and small	$h^- r^+$
Cloudy and large	$h^+ r^-$
Cloudy and small	$h^+ r^+$
Clear and large	$h^- r^-$



Results of a cross for the *h* and *r* genes in phage T2 ($h r^+ \times h^+ r$)

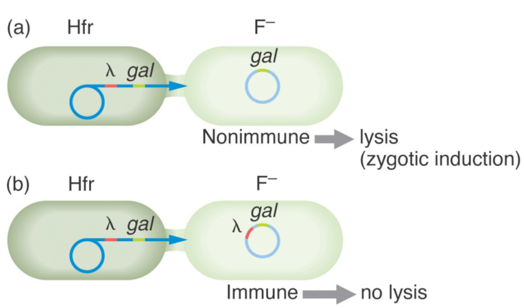
Genotype	Plaques	Designation
$h^- r^+$	42	Parental progeny 76%
$h^+ r^-$	34	
$h^+ r^+$	12	Recombinant 24%
$h^- r^-$	12	

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

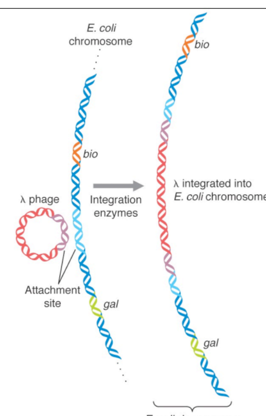
Phage lamba (λ)

- A temperate phage of *E. coli*
- Upon entering cell, λ DNA circularizes. It can align with specific region of genome, the λ attachment site, and recombine with host genome
- Upon integration, it can become silent prophage, synthesizing inhibitor of its further replication
- Upon exit, it may pick up adjacent host genes which can be transduced to next host cell
- If Hfr donates λ prophage to λ-free cell, prophage begins lytic cycle
  - called zygotic induction
  - owing to absence of inhibitor synthesized in Hfr

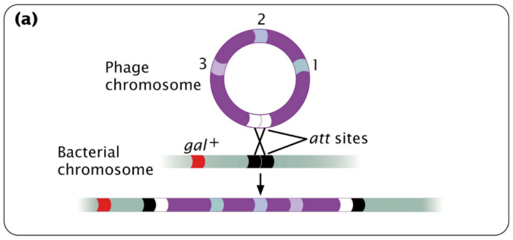
Zygotic induction



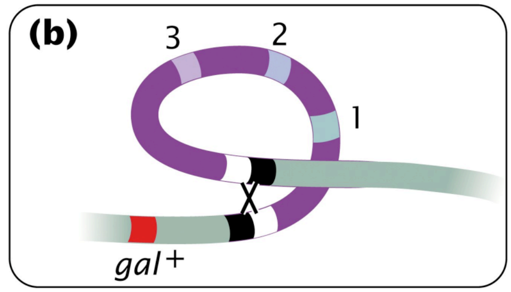
Lambda integration



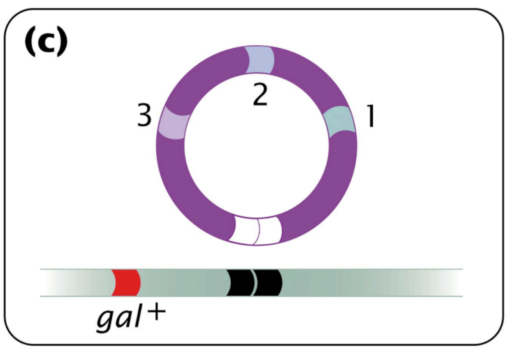
Phage integration at the *att* site



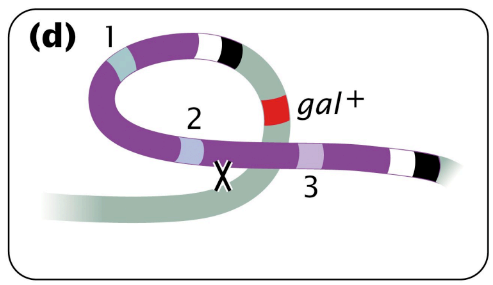
Normal excision through crossover



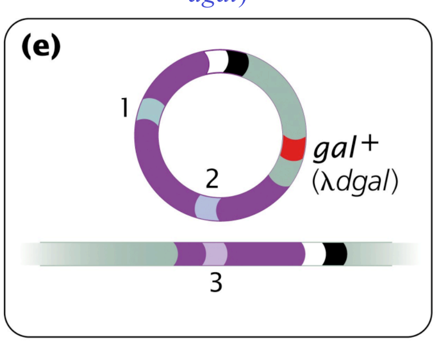
Norma λ phage product



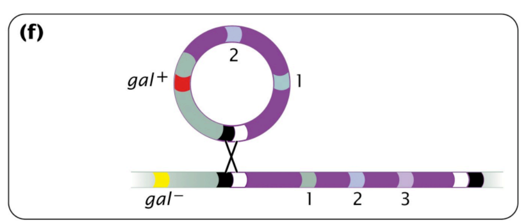
Error in excision



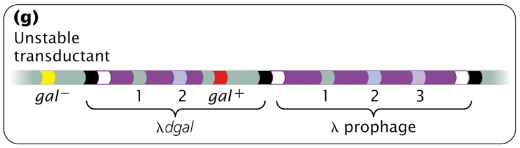
Phage with *gal*[+] called λ gal defective (λ *dgal*)



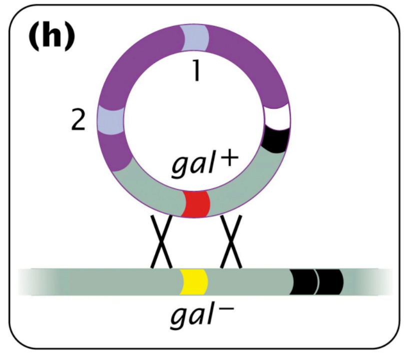
λdgal may integrate



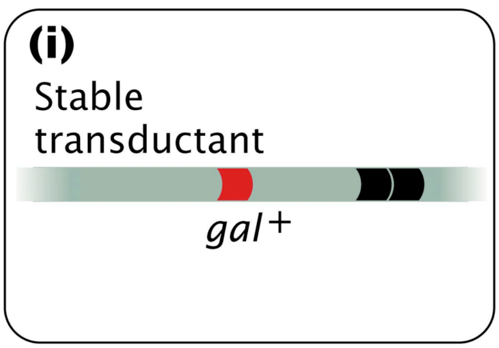
Two copies of lambda



May integrate at the *gal* gene



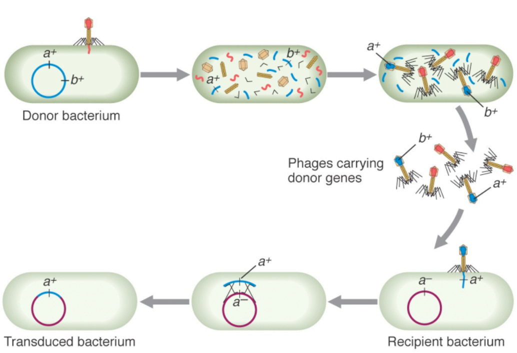
gal gene is exchanged -stable transducent



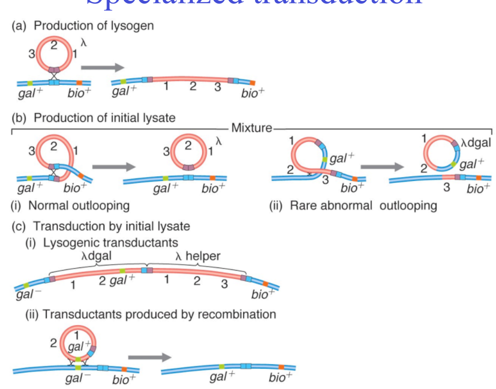
Transduction

- Generalized
  - phage incorporates random fragments of host DNA for transfer to another host
  - e.g., phages P1 and P22, temperate phages which accidentally stuff host DNA into phage head
- Specialized
  - specific genes are transferred
  - e.g., phage λ which transduces only adjacent genes
- Frequencies of *cotransduction* can be used to map genes; inversely proportional to distance

Generalized transduction

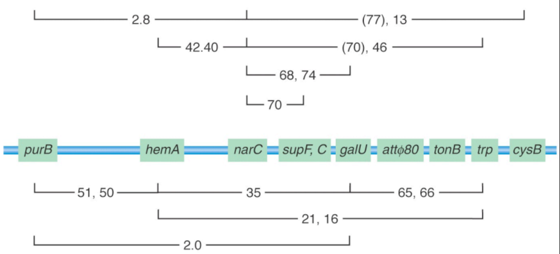


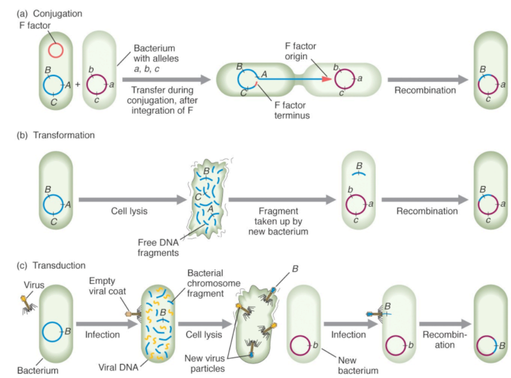
Specialized transduction



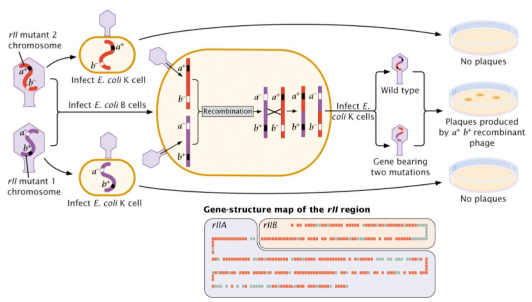
Mapping with cotransduction

Numbers indicate percent recombinants

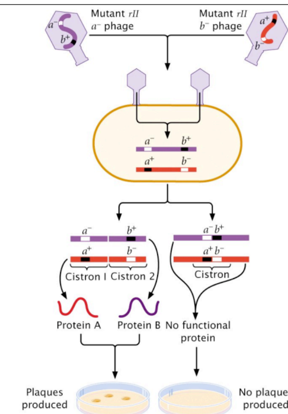




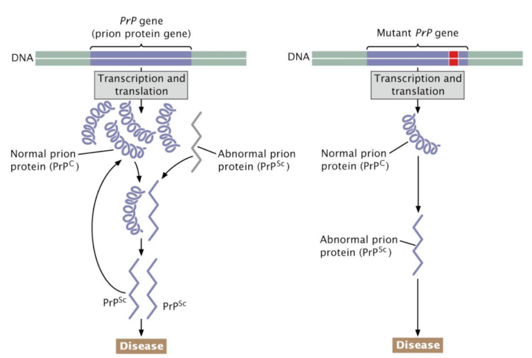
Mappping rII mutants



Complementation



Prion disease



Overview

- The F factor (sex factor) is a circular plasmid that may exist free in the cytoplasm or integrated into the chromosome of *E. coli*.
- Free F in F^+ cells passes a copy of itself to F^- cells in conjugation, whereas integrated F (Hfr) transfers chromosomal DNA.
- Bacteriophages can transduce bacterial genes from one cell to another.
- In transformation, DNA from the environment can enter bacterial cells and integrate into the chromosome.
- These methods of gene transfer generate partial diploids that allow study of genes.