Systems Microbiology

Monday Oct 16 - Ch 10 -Brock

Genetic Exchange in Bacteria

- Homologous recombination
- Transformation
- Plasmids and conjugation
- Transposable elements
- Transduction (virus mediated xchange)

Gene exchange in bacteria

- Transfer of DNA from one bacterium to another is a common means of gene dispersal. It has a big effect on bacterial evolution, and tremendous practical implications. For example, lateral transfer is responsible for the spread drug resistance determinants between bacterial species.

- Three common mechanisms of lateral gene exchange :
  - **Transformation (extracellular DNA uptake)**
  - **Conjugation (bacterial mating systems)**
  - **Transduction (viral mediated gene exchange)**

RecA mediated Homologous recombination

Images removed due to copyright restrictions.
See Figures 10-9 and 10-10 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms.*11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Gene exchange in bacteriaTransformation

- Discovered by Griffith in 1928 during the course of his studies of virulence in *Streptococcus pneumoniae*.
- **S**=smooth colony morphotype
- R=rough colony morphotype

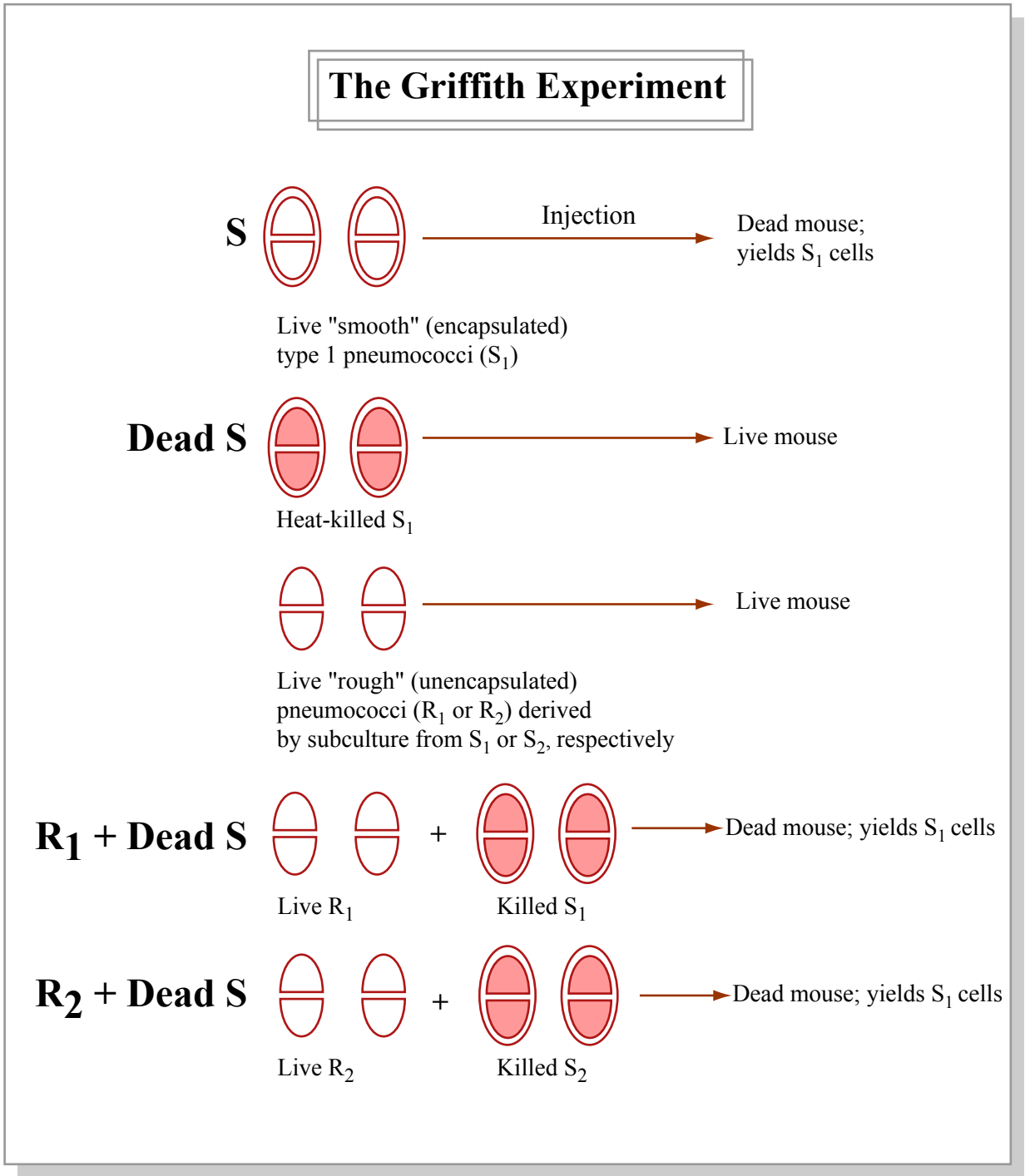
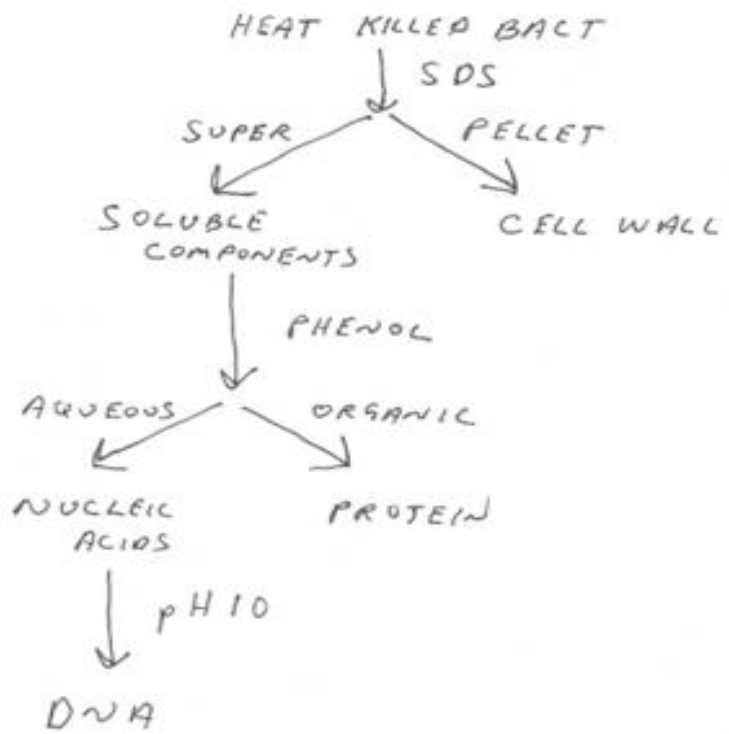


Figure by MIT OCW.

Gene exchange mechanisms in bacteria **Transformation**

Avery, MacLeod, and McCarthy (1944) fractionation studies led to conclusion that transformation principle is DNA.



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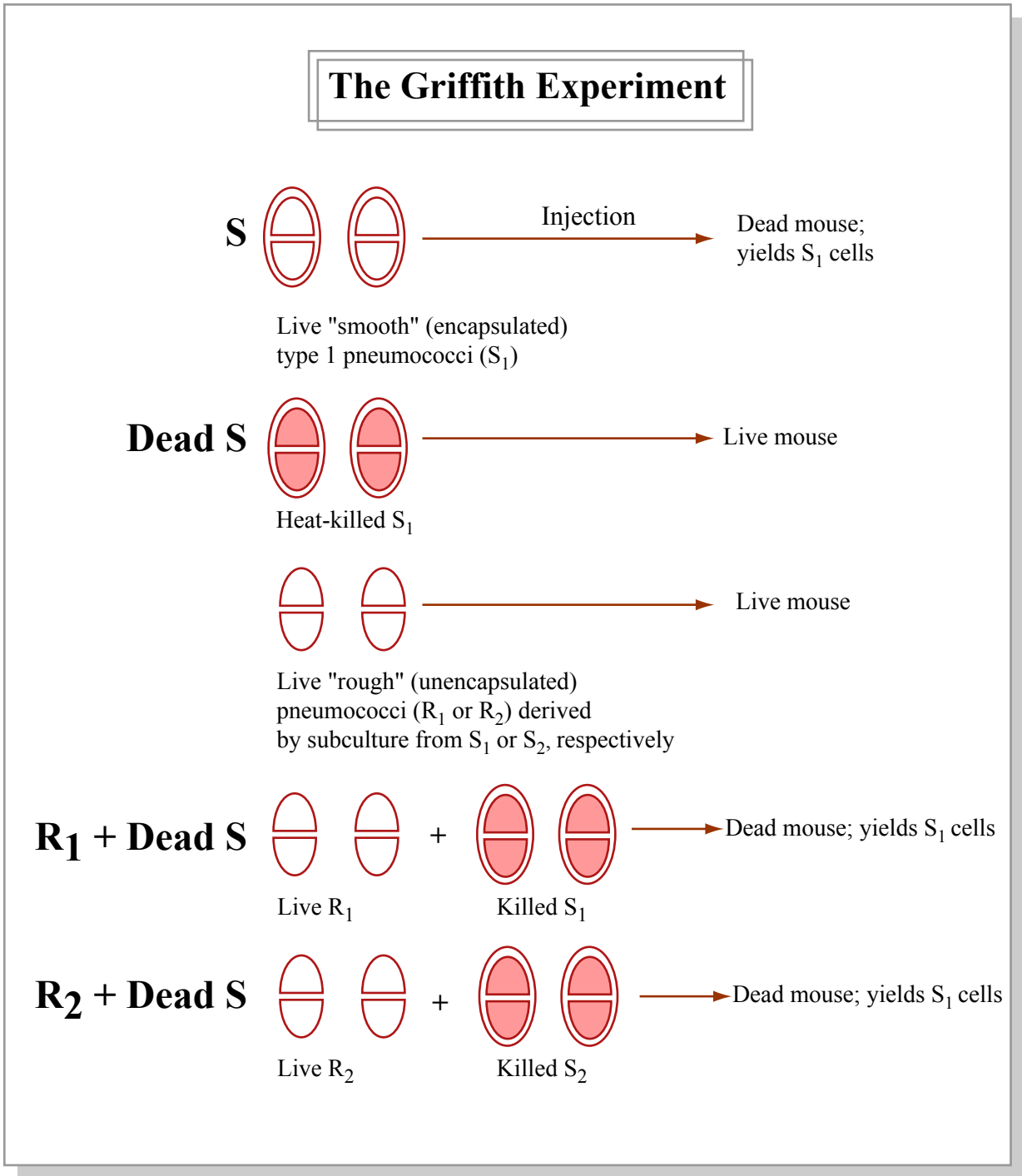


Gene exchange mechanisms in bacteria

Transformation (uptake of exogenous DNA)

- Physiological transformation occurs in nature in a wide variety of genera which include:

- 1) *Streptococcus*
- 2) *Staphylococcus*
- 3) *Bacillus*
- 4) *Acinetobacter*
- 5) *Hemophilus*
- 6) *Neisseria*

Diagram showing the genetic interconnections demonstrated between bacterial groups removed due to copyright restrictions.

Natural Bacterial Transformation

Image removed due to copyright restrictions.

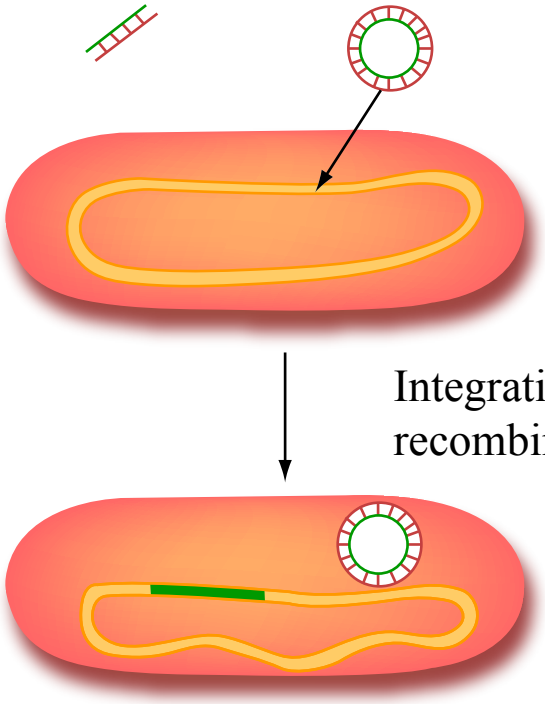
Closely Linked Genes will Tend to Transform Together More Frequently than More Distal Genes

Gene exchange mechanisms in bacteria

Transformation

- **Competence**. The ability to take up DNA varies regularly during the cell cycle. In *Streptococcus* competence is highest shortly after cell division.
- Entry & integration. Cell components required for uptake.
- Heteroduplex formation with homologous recipient DNA.

Artificial Transformation



Double-stranded DNA forced through cytoplasmic membrane

Integration of linear fragments by homologous recombination or replication of plasmid

Figure by MIT OCW.

Bacterial Conjugation

Image of bacterial conjugation, showing the donor (F+), pilus, and recipient (F-), removed due to copyright restrictions.

PLASMIDS

- Extrachromosomal DNA, usually circular
- Usually encode ancillary functions for in vitro growth
- Can be essential for specific environments: virulence, antibiotics resistance, use of unusual nutrients, production of bacteriocins (colicins)
- Must be a replicon - self-replicating genetic unit

Plasmid Replication

- Plasmid DNA must replicate each time cell divides or it will be lost
- Host cells do not "spit out" plasmid DNA
- Two functions required in replication
  - DNA replication
  - Partitioning (distributing plasmid to progeny cells)
- High copy (>20) and low copy (<5) plasmids

Plasmid Replication

- High copy plasmids are usually small; low copy plasmids can be large
- Partitioning is strictly controlled for low copy, but loose for high copy
- Plasmid replication requires host cell functions (DNA polymerase, etc.)
- Copy number is regulated by initiation of plasmid replication
- Plasmids are incompatible when they cannot be stably maintained in the same cell because they interfere with each other's replication.

Confers resistance :
sulfonamide
chloramphenicol
mercury ions
streptomycin
tetracycline

Image removed due to copyright restrictions.
See Figure 10-20 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Table of some phenotypes conferred by plasmids in prokaryotes removed due to copyright restrictions. See Figure 10-3 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

ROLLING-CIRCLE MODEL OF BACTERIOPHAGE λ DNA REPLICATION
FOR THE SYNTHESIS OF **DOUBLE-STRANDED DNA daughters**

ColEI plasmid

- small (6.6 kb)
- medium copy #/cell (20 copies/cell)
- non-self-transmissible
- does not require de novo protein synthesis for replication (chloramphenicol amplifiable)
- RNA-II is transcribed through the origin of replication, gets cut by RNaseH and serves as the primer for DNA replication
- RNA-I is transcribed in the opposite orientation and is complementary to RNA-II.
- binding of RNA-II and RNA-I prevents initiation of replication (RNA-I is a negative regulator)
- the Rom/Rop protein made by the *rom/rop* gene stabilize the binding of RNA-I and RNA-II (also negative regulator)

F plasmid

- large (100 kb)
- low copy #/cell (1-2 copies/cell)
- self transmissible (*tra* genes)
- requires protein synthesis (chloramphenicol-sensitive)
- *repE* gene encodes RepE protein
- RepE protein binds to origin of replication (*oriS*) and initiates DNA replication
- RepE binds to the *repE* promoter and activates transcription
- RepE binds to the *copA/incC* locus and is titrated away from *oriS* and *repE* (negative regulation of replication)

Image removed due to copyright restrictions.
See Figure 10-18 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms.* 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

:rial Conjugation

Diagram showing the process of bacterial conjugation removed due to copyright restrictions.
See Figure 10-22 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*.
11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

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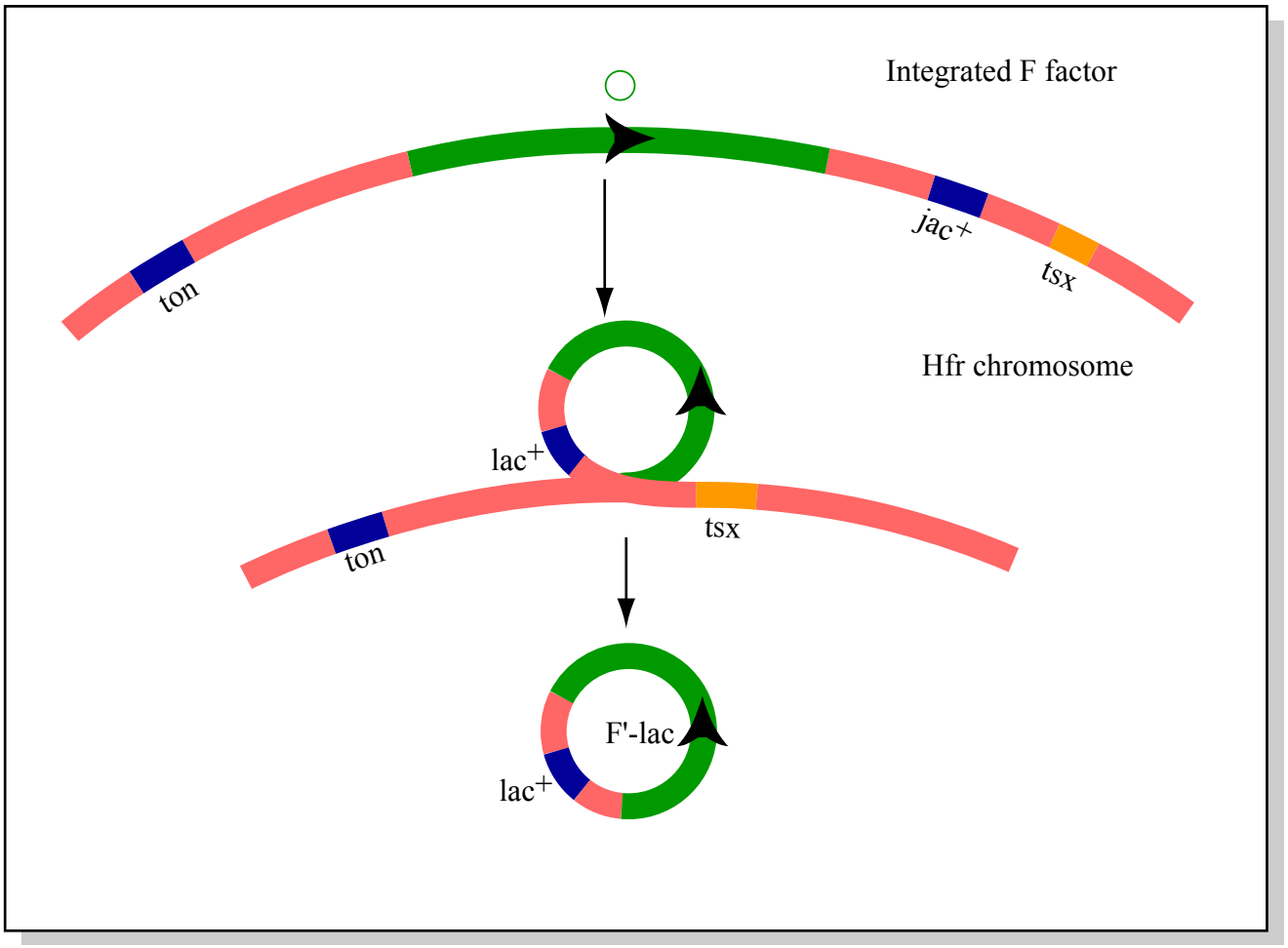
Creation of an F' Strain

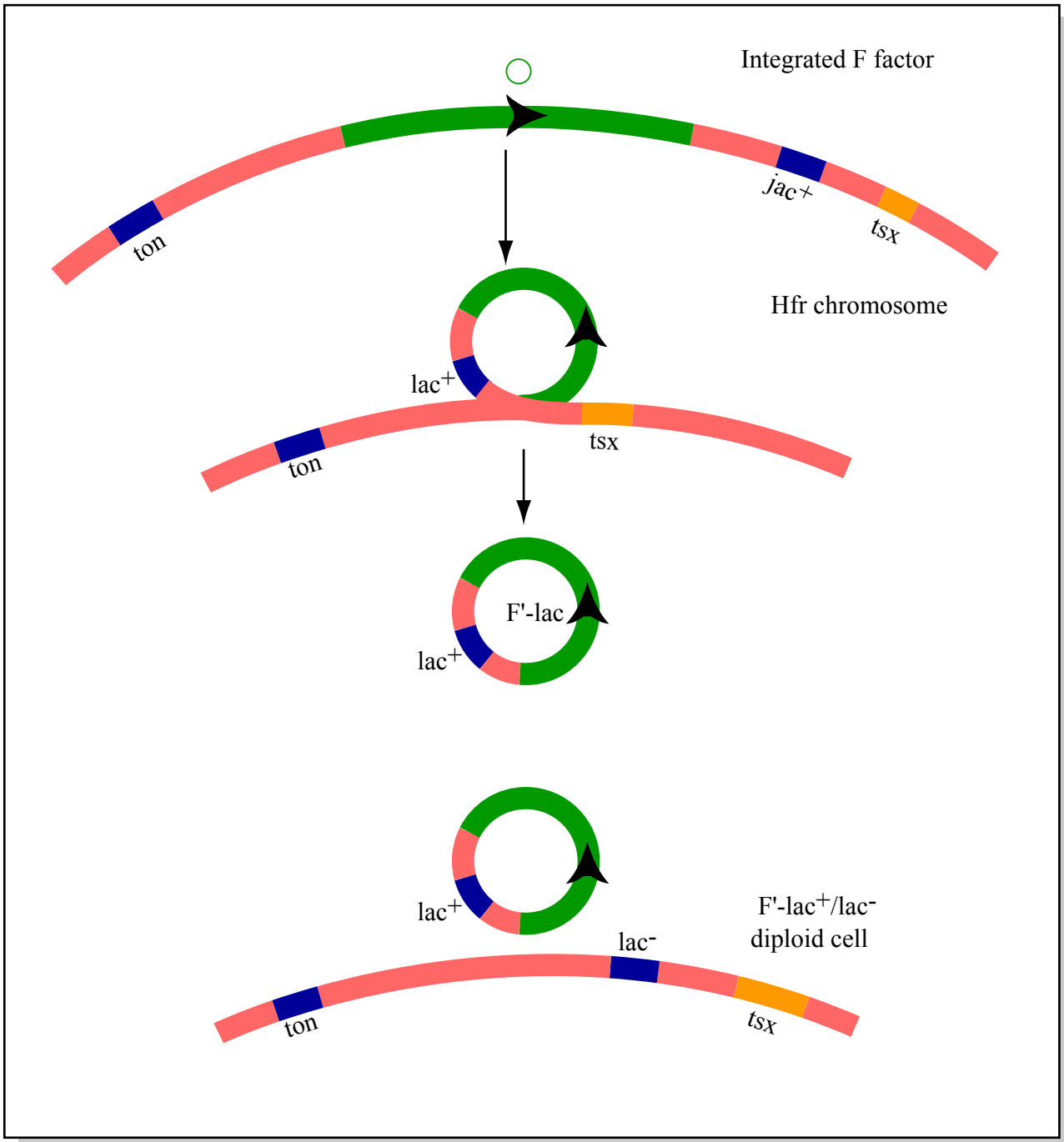


E. coli chromosome

Will transfer Lac+ frequently

Figure by MIT OCW.

Creation of an F' Strain



Lac merozygote
(can assess dominance)

Figure by MIT OCW.

Hfr Strains

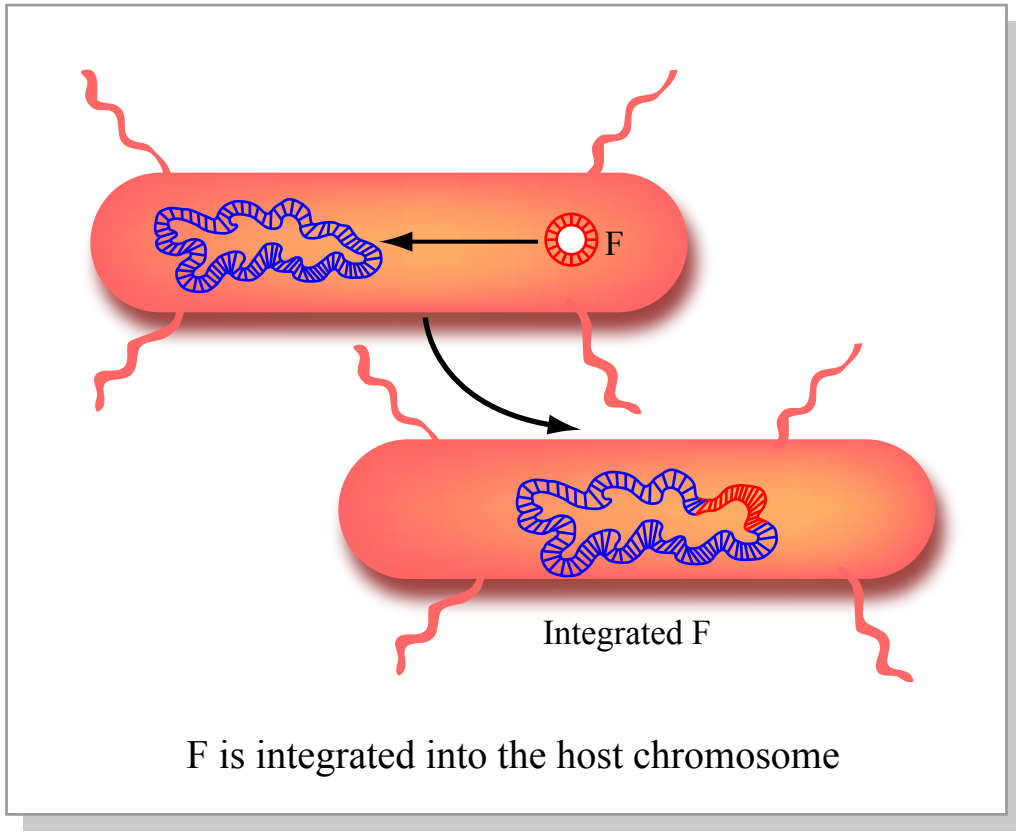
• The F plasmid can integrate into the chromosome (many sites – directed by transposon homology). This creates a high frequency of recombination (Hfr) strain.

• The integrated F plasmid directs transfer of the chromosome, starting from the origin. Genes close to the site of integration will be transferred first.

• Transfer continues, with the order of transfer matching the order of genes along the chromosome, until it is interrupted.

(interrupted mating experiments for chromosomal mapping…)

Creation of an Hfr Strains



Hfr strain

DNA Transfer in an Hfr Strain

Diagram removed due to copyright restrictions.

Image removed due to copyright restrictions.
See Figure 10-24 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Order of Gene Transfer in an Hfr Strain

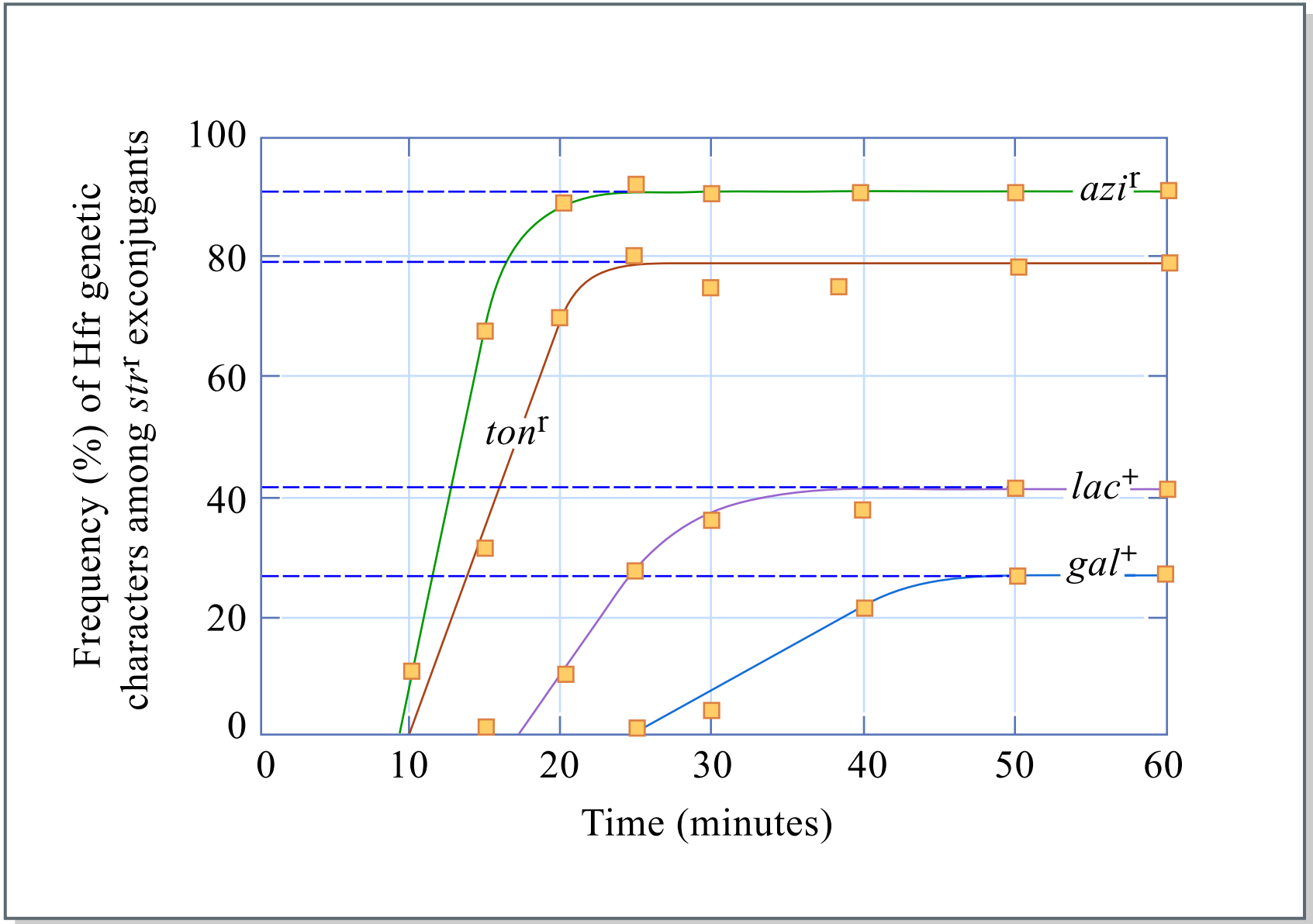
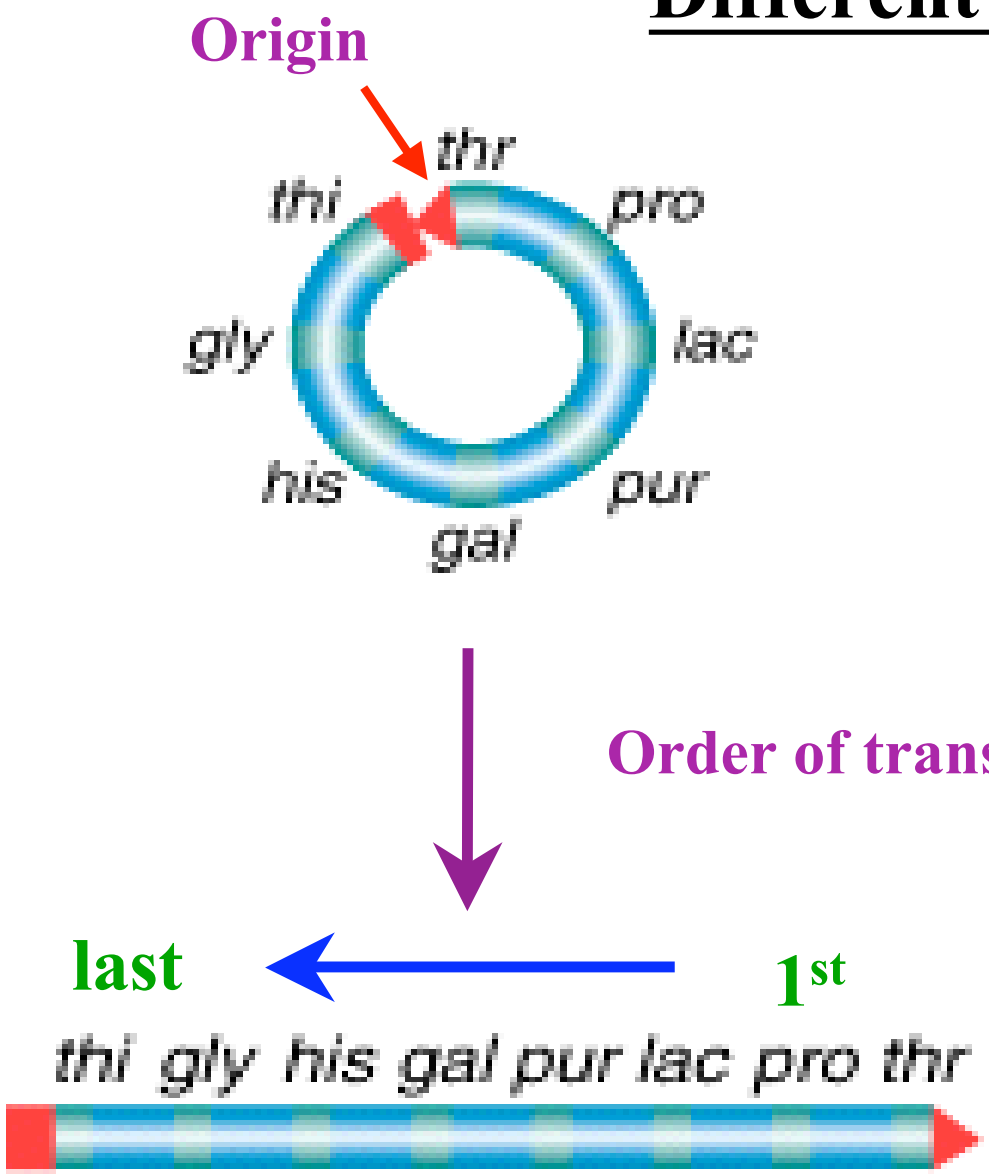


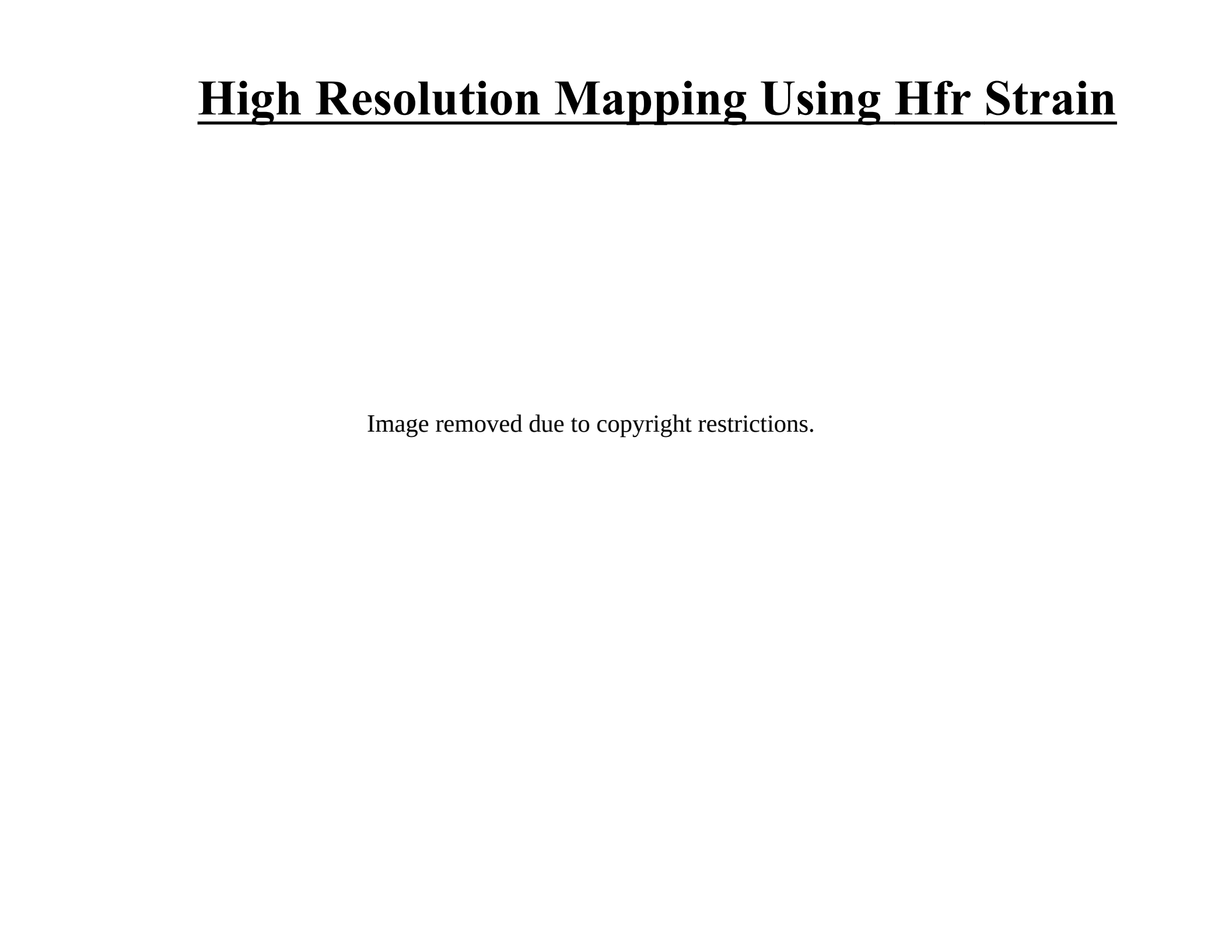
Figure by MIT OCW.

Order: Hfr – Azi – Ton – Lac – Gal

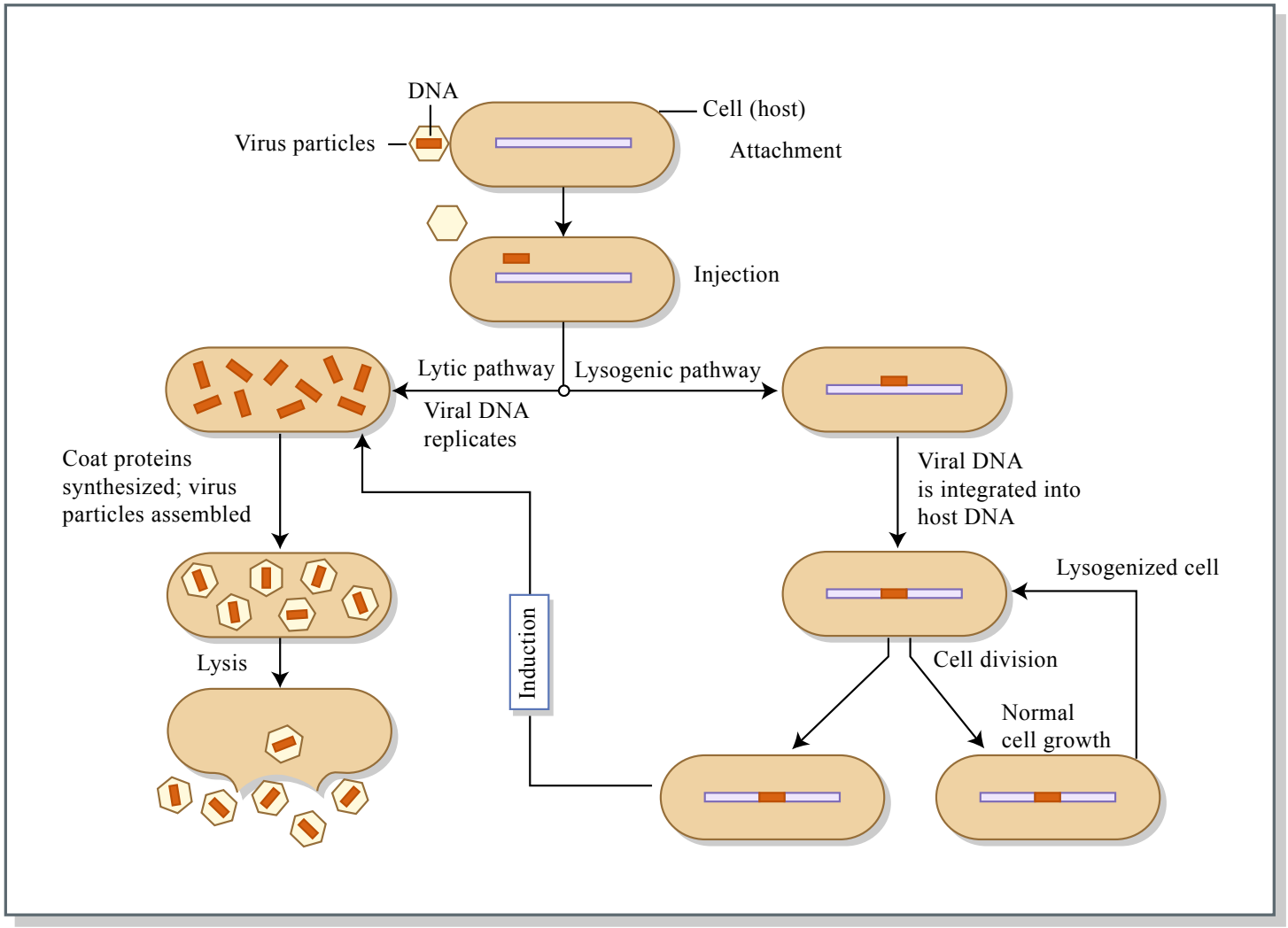
Different Hfr Strains



High Resolution Mapping Using Hfr Strain



Temperate Phage and Lysogeny



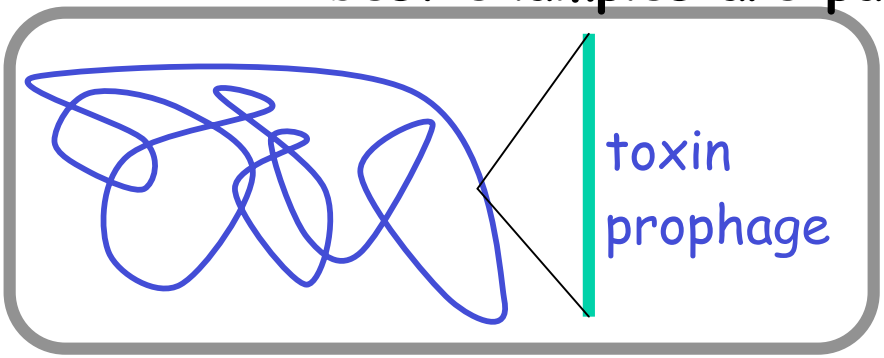
Microscopic photograph removed due to copyright restrictions.

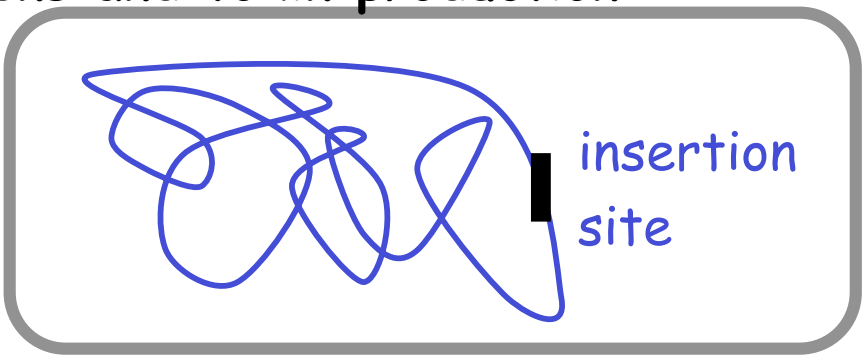
Figure by MIT OCW.

Phage conversion

Dormant prophage – integrated bacteriophage – carries genes that alter the phenotype of the microbe

- best examples are pathogens and toxin production





Corynebacterium diptheriaea

Phage produces diptheria toxin

This is what makes people sick

C.diptheriaea

without phage strain produces no toxin

Does not cause diptheria

The lysogenic pathway of bacteriophage infection

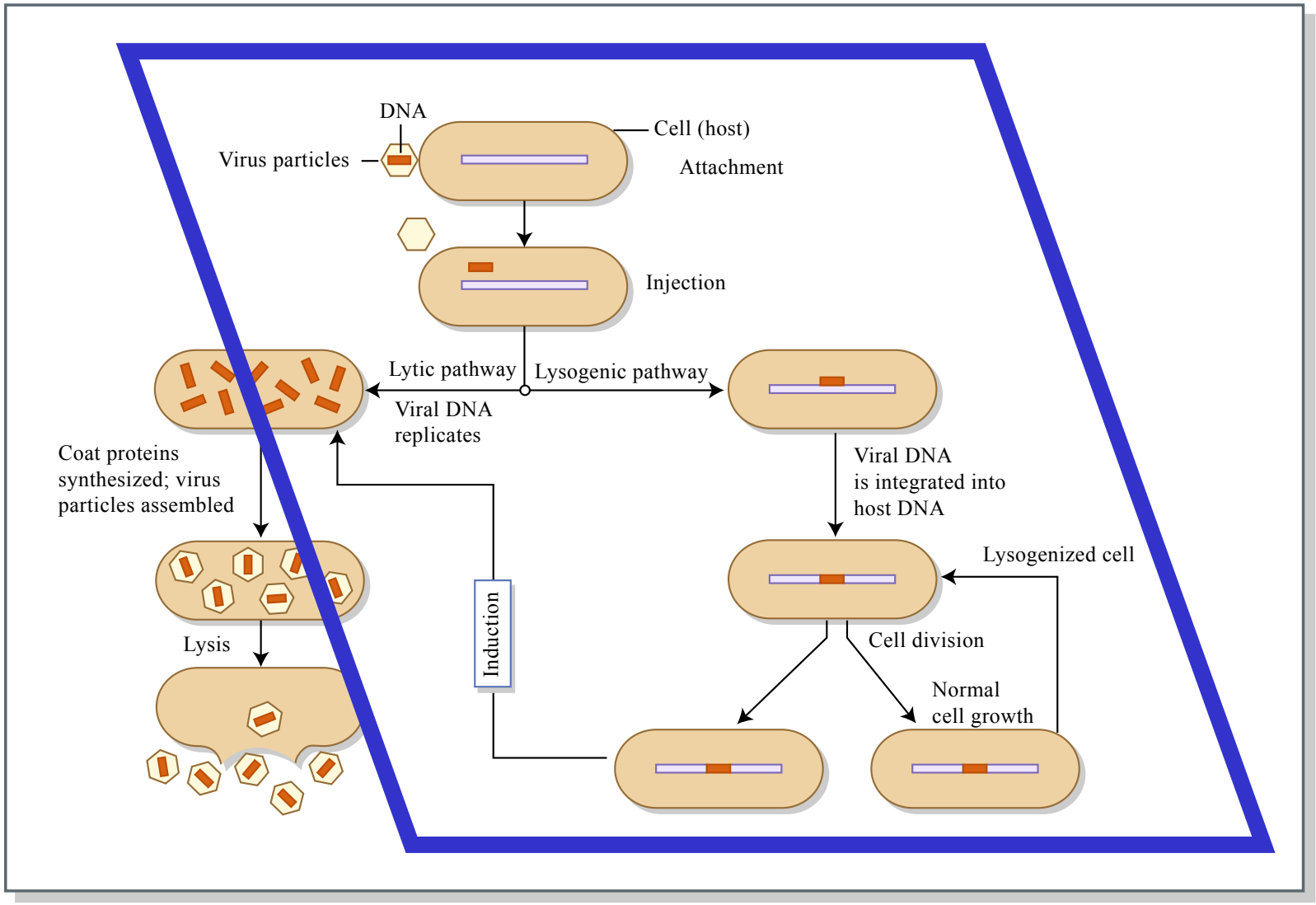


Figure by MIT OCW.

Site-specific integration of λ

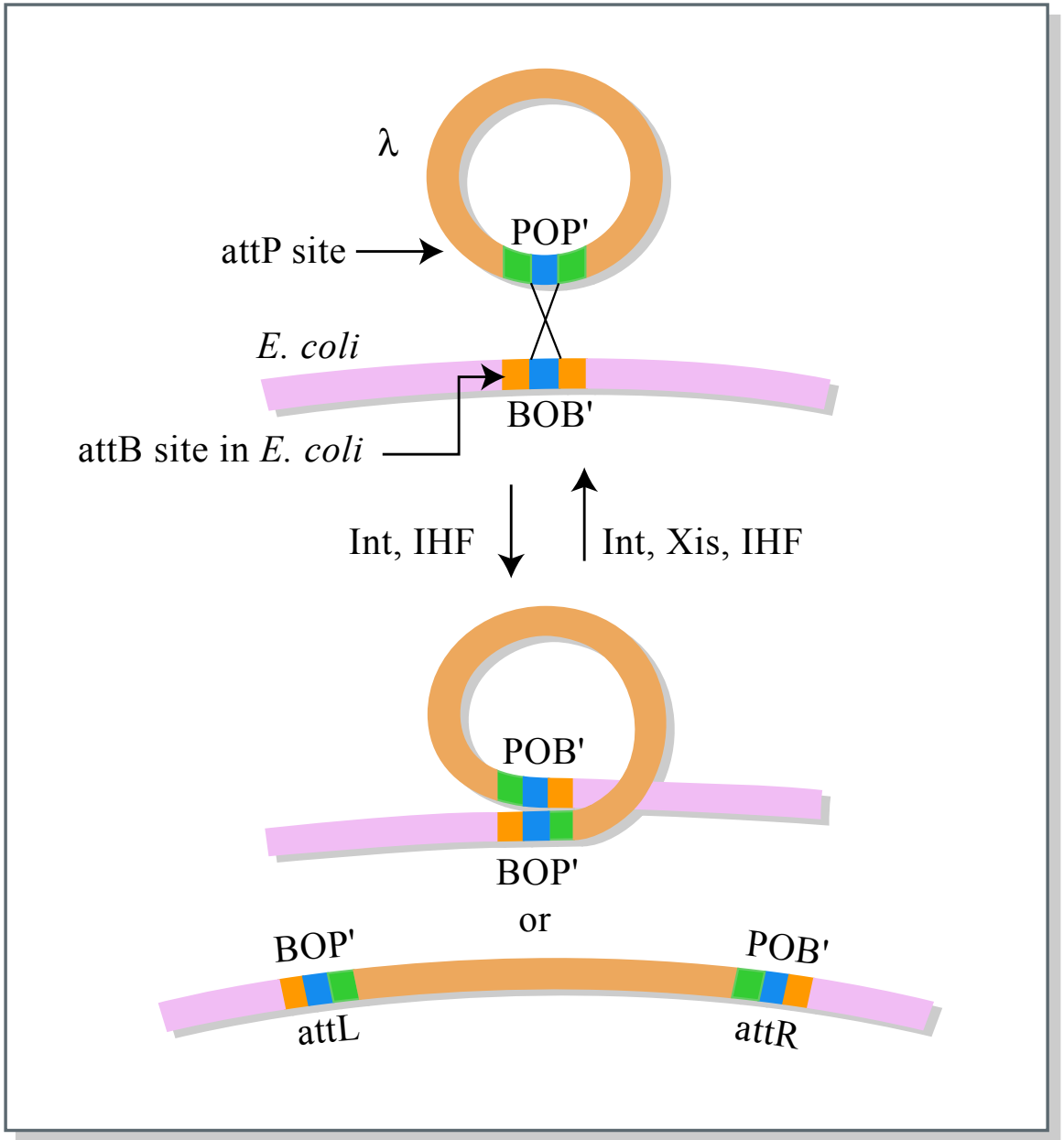


Figure by MIT OCW.

- attP binds Integrase, IntHostFactor
- complex binds attB
- Int recombines the two molecules using the match “O” sequence
- Xis removes “lysogenic” phage in response to environmental stress

Invitrogen image of Phage lambda recombination in *E. coli* removed due to copyright restrictions.

Mechanism of Integrase action

Diagram showing the mechanism of integrase action removed due to copyright restrictions.

- ATP independent process
- 5’ OH and 3’ phosphates
- Covalent **enzyme-tyrosine-integrase** attachment -akin to topoisomerases

Specialized transsduction
(in phage lambda)

Specialized transduction is site specific, and so results in transfer only of specific genes.

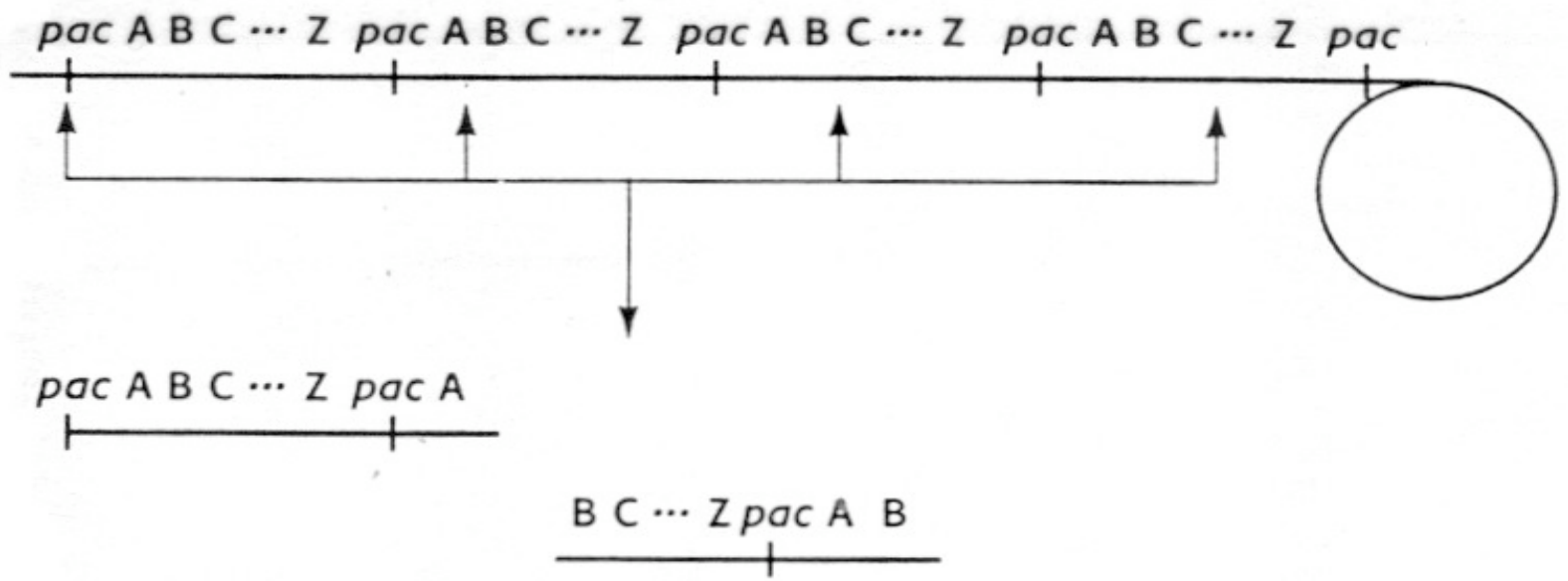
Eg, genes next to the attB site for lambda Infecting E. coli

Diagram removed due to copyright restrictions.
See Figure 10-16 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Visible effects on DNA during viral infection

Images showing DNA and T4 phage in a pre-infection and post-infection cell removed due to copyright restrictions.

The “headfull” mechanism utilized by some bacteriophage lends itself to mispackaging – there are no specific sequences recognized by the packaging machinery



Molecular Genetics of Pathogenic Bacteria, 1996, Stewart and Maloy.

Packaging mechanism of Phage P22 of *Salmonella typhimurium*

Generalized transduction

This happens when host DNA, instead of phage DNA is accidentally packaged.

Generalized transduction is more or less random, and so can result in the transfer of almost any gene.

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See Figure 10-15 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

An example – P22 phage transduction of *Salmonella typhimurium*

P22 HT is a efficient generalized transducer
- its sloppy – 50% of the viral particles contain host cell DNA (ie are transducing particles or TPs)

Each transducing particle (TP) carries 44 kb of DNA – the *Salmonella* genome is app. 4400 kb in size

Therefore, if the process is random 100 different transducing particles should represent the entire genome.

$(0.5)(10^{11}$ viruses/ml)/(100 TP [1 genome]) = $5x10^8$ copies of the genome/ml of lysate

Generalized transduction is a useful way to exchange genes between bacteria

Also extremely useful for mapping of genetic markers relative to each other

Image removed due to copyright restrictions.

Mobile genetic elements

DNA transposition

- Movement of DNA sequences from a "donor site" to a new "target site" within the genome
- Discovered by Barbara McClintock "jumping genes"
- Takes place in virtually all organisms
- Potentially mutagenic (transposon mutagenesis)
- Rare infrequent events (tightly regulated)

- **Donor site**

  contains a transposable element (transposon)

- **Target site**

  in general is random

  hot spots: preferred sequences that are targeted

Mobile genetic elements

Insertion sequences (I.S. elements, Class I transposons). Small discrete segments of DNA ranging in size from 750 bp to 1600 bp.

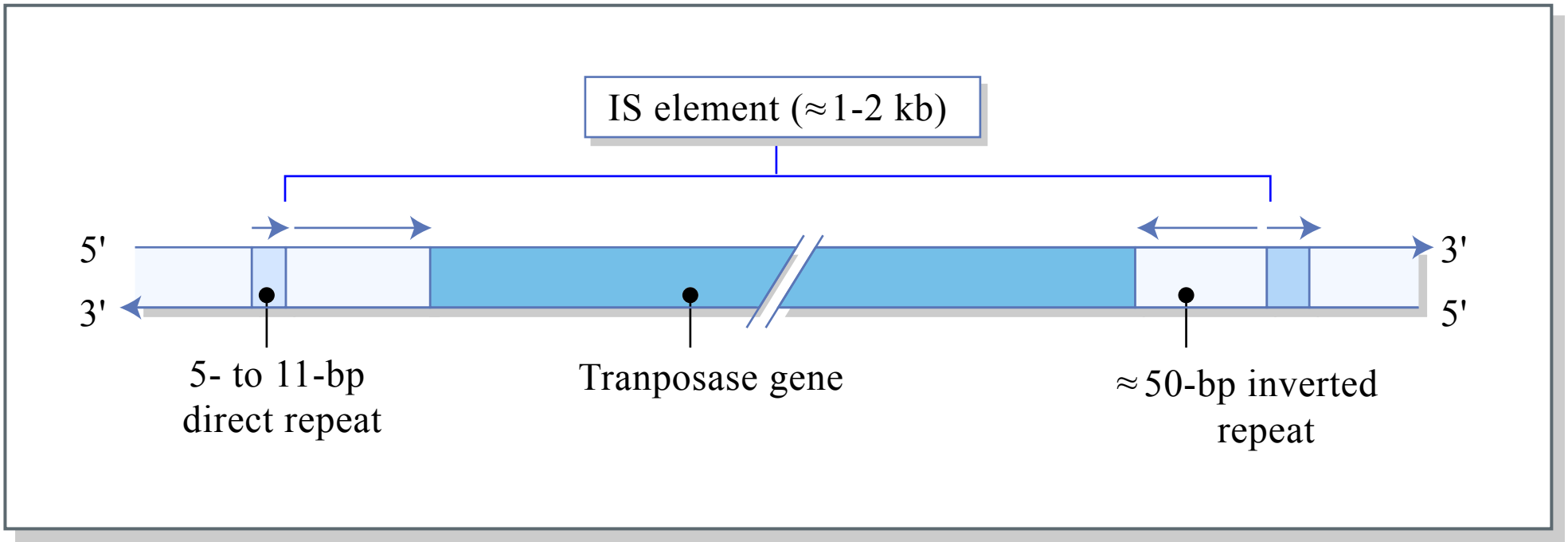
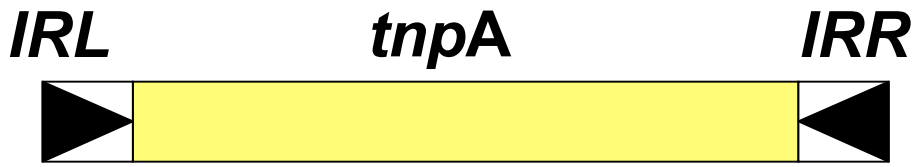


Figure by MIT OCW.

Bacterial transposable elements

Class I transposons (insertion sequences)

- Relatively small (~ 750 – 1600 bp)
- Flanked by terminal inverted repeats (IRs)
- Generally only 1 gene
- transposase (*tnpA*) = ~ 37 Kda
- "Hop" from one part of the genome to another.
- Sometimes have an outward facing promoter !



Mobile genetic elements

I.S. elements can act in pairs to mobilize intervening DNA.

I.S. elements can mobilize important determinants such as antibiotic resistance genes, genes for lactose utilization, or genes for bacterial enterotoxins.

In *E. coli* the ST enterotoxin gene is encoded by a transposon and is sometimes found on plasmids and sometimes on temperate phages.

Mobile genetic elements

Transposon formation.

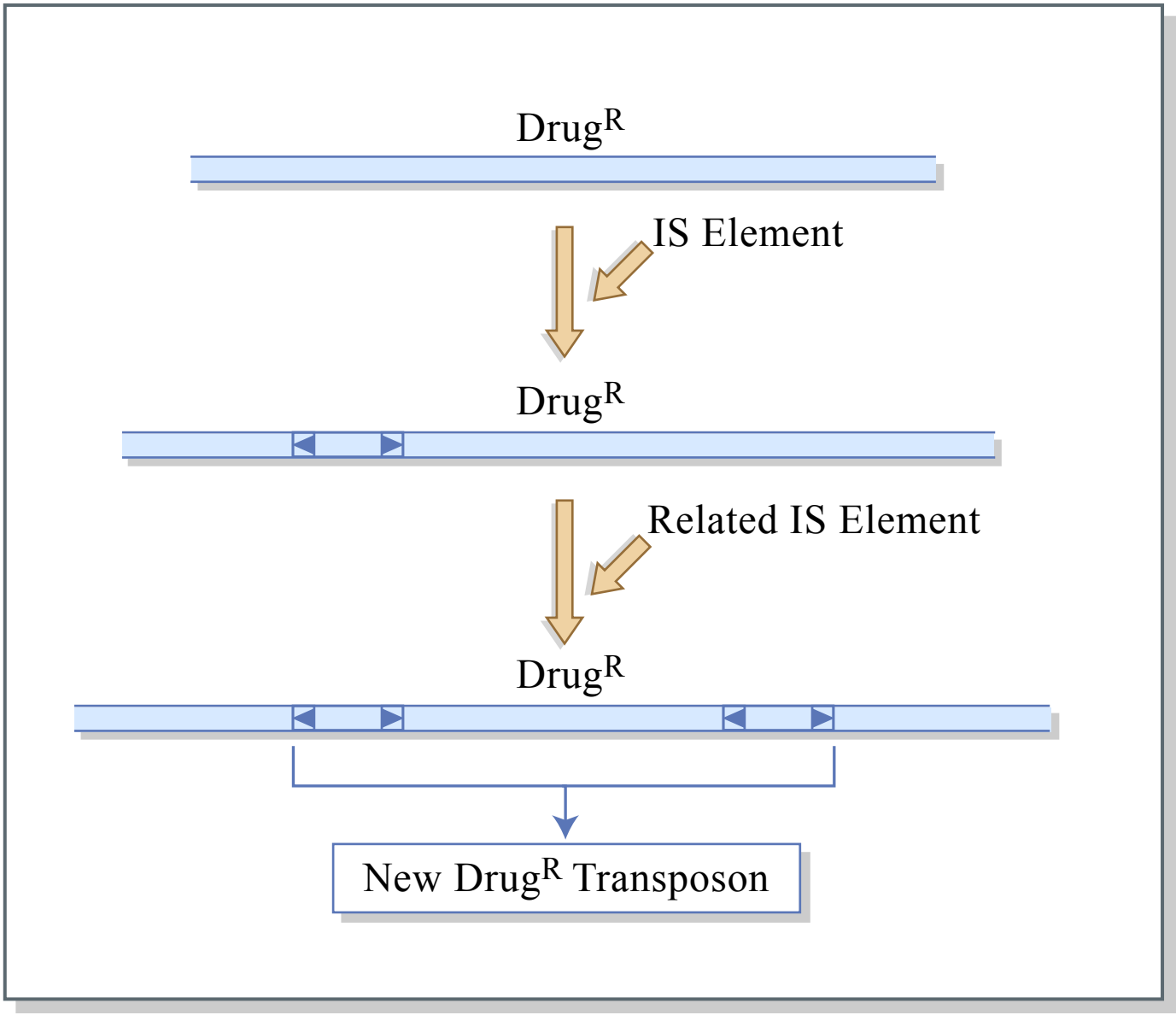
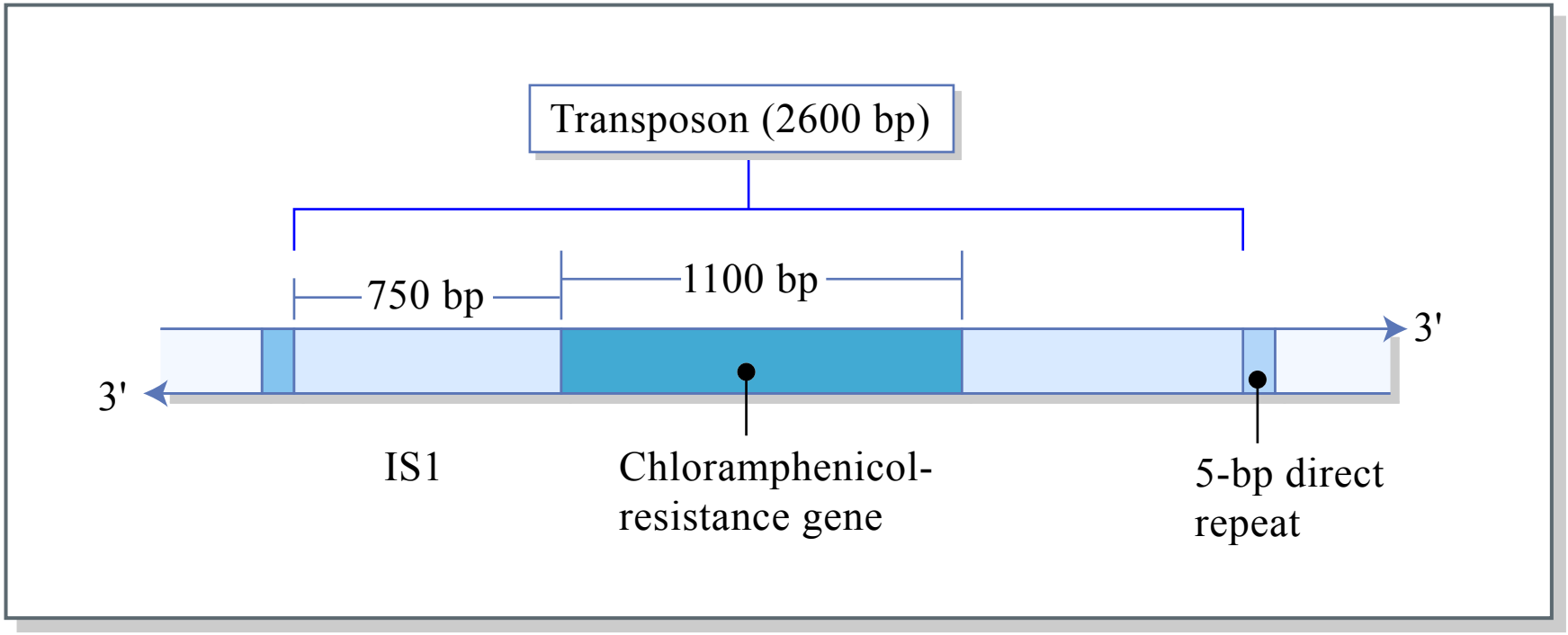


Figure by MIT OCW.

Mobile genetic elements

Class II transposon structure.



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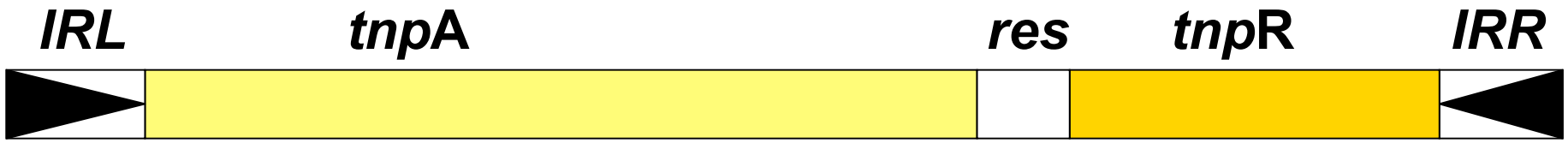
See Figure 10-29b in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Bacterial transposable elements

Class II transposons (complex transposons)

- *tnpA* (transposase) ~ 120 Kda
- *tnp*R (site-specific recombinase) ~ 21 Kda
- TnpR acts on resolutions site (*res*)
- long terminal inverted repeats (35 – 40 bp) (LTRs)
- **duplicate a ~ 5- bp target site** upon transposition
- often carry genetic markers (antibiotic resistance genes)
- Families: ***Tn*3** & *Tn*501

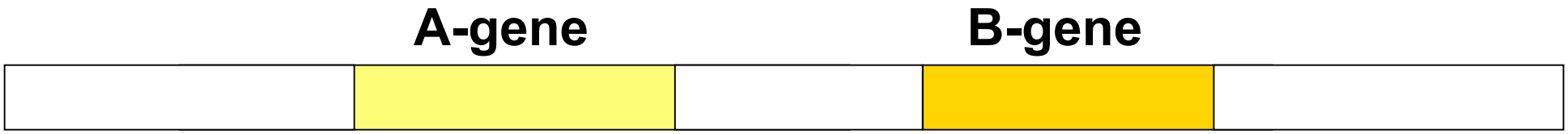


Bacterial transposable elements

Class III transposons (Mu and others)

Bacteriophage Mu

- ~ 38 Kb linear DNA molecule
- transposition results in duplication of target site
- lacks terminal inverted repeats
- A-gene (transposase)
- B- gene (replication and transposition)



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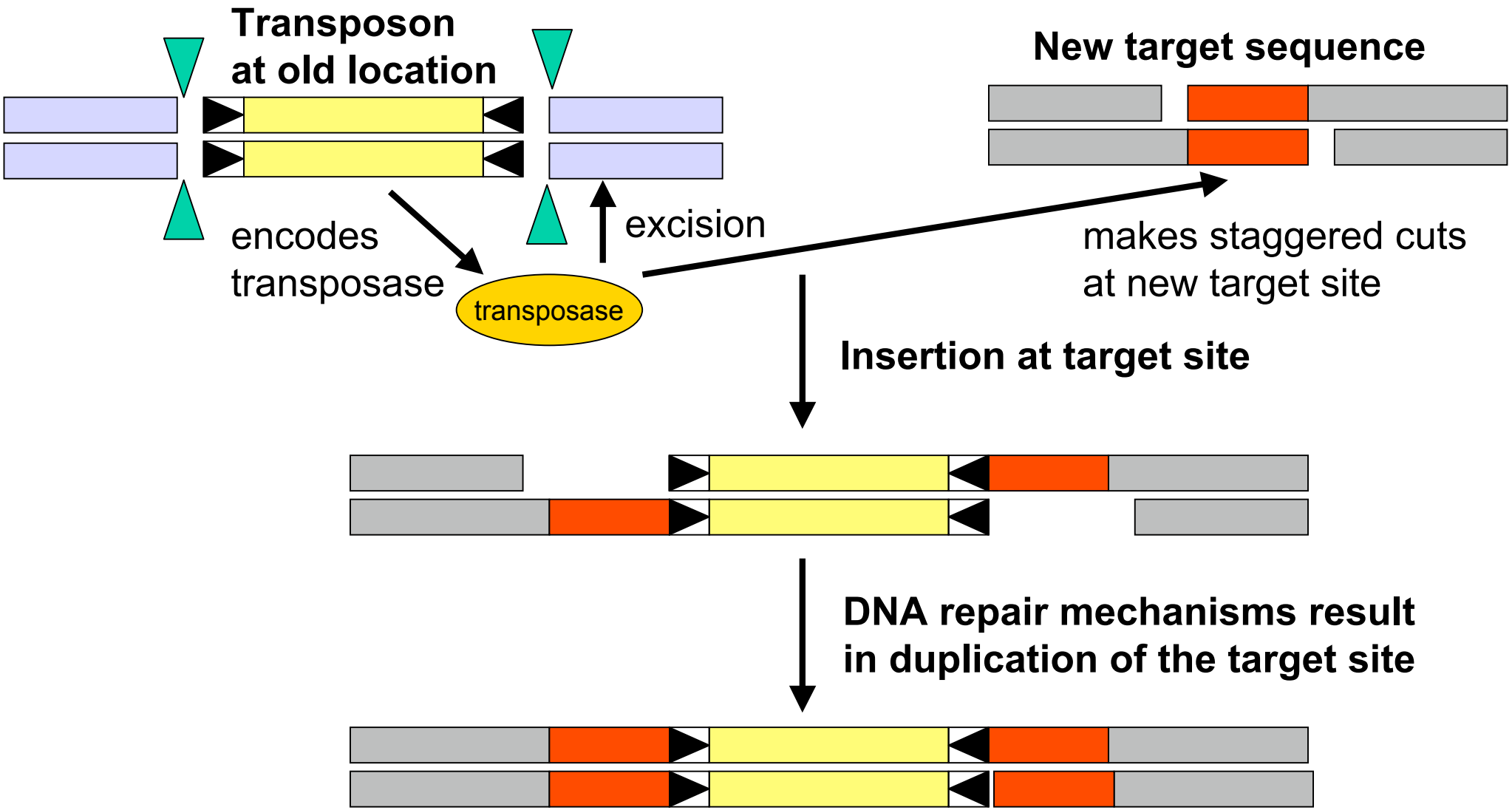
See Figure 10-31 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Direct transposition (conservative)



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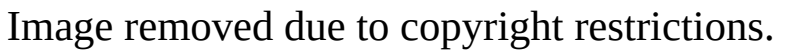
See Figure 10-32 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Replication-dependent transposition



Strategy for transposon mutagenesis

Diagram showing the process of transposon mutagenesis removed due to copyright restrictions.

In vivo Tn mutation

Generating mutants templates in vitro

Mutagenesis of bacteria

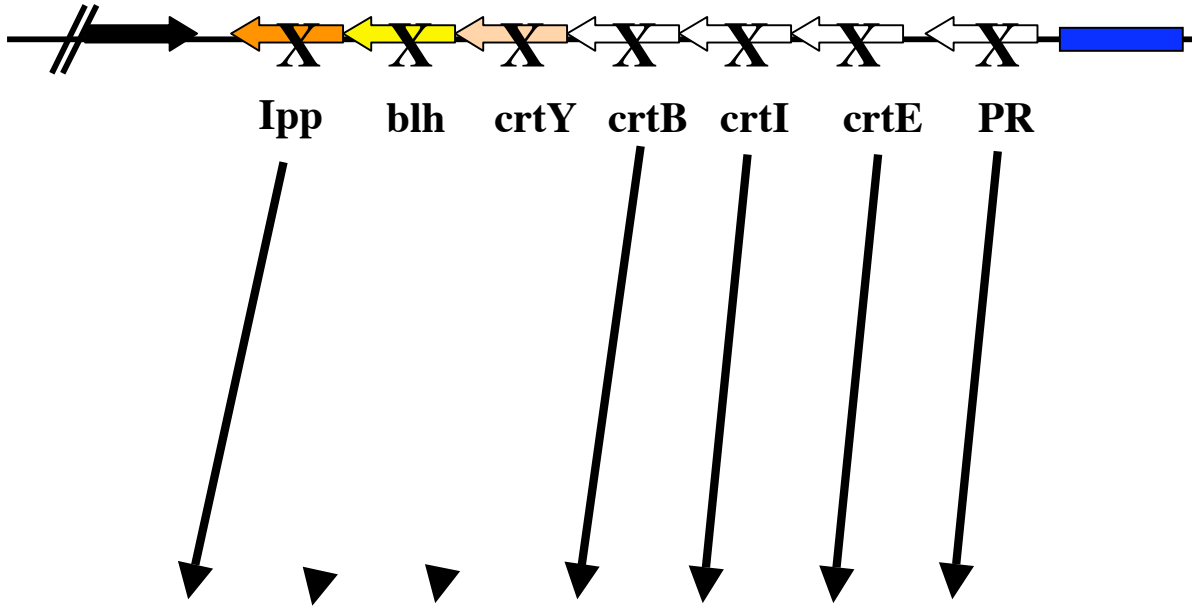
Diagrams removed due to copyright restrictions.

Epicentre Biotechnologies Website

Gene expression on cloned operons from environmental libraries

Proteorhodopsin/retinal +

β -carotene/retinal/proteorhodopsin operon



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