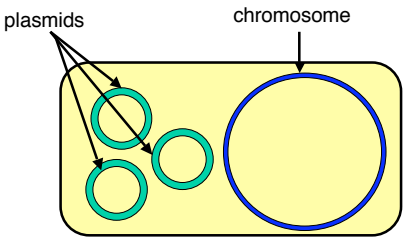
Genetics II:

Transfer of Genetic Material

and

Genetic Engineering

Bacterial genetic material



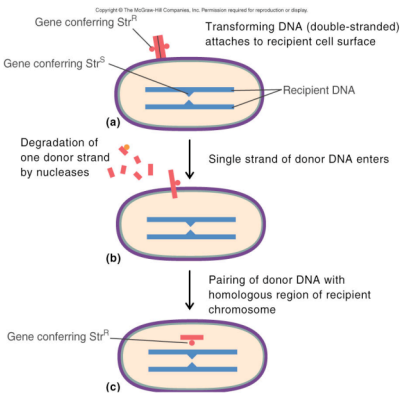
Gene Transfer in Bacteria

- Transformation
- Transduction
- Conjugation

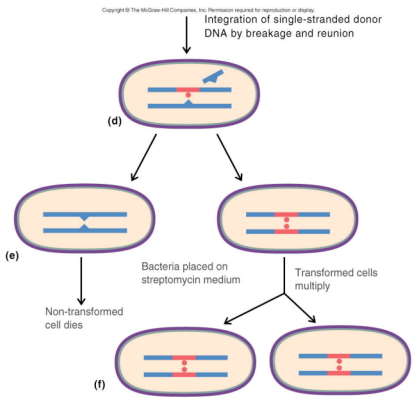
Transformation

- Griffith's experiment
- Uptake of "naked" DNA
  - Competence
    - competent factor
  - Recombination

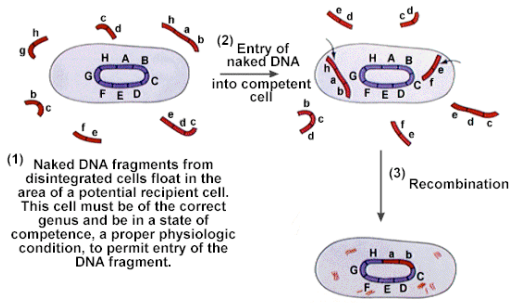
Transformation



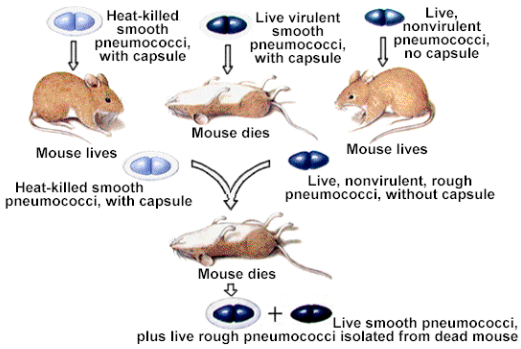
Recombination



Transformation with recombination



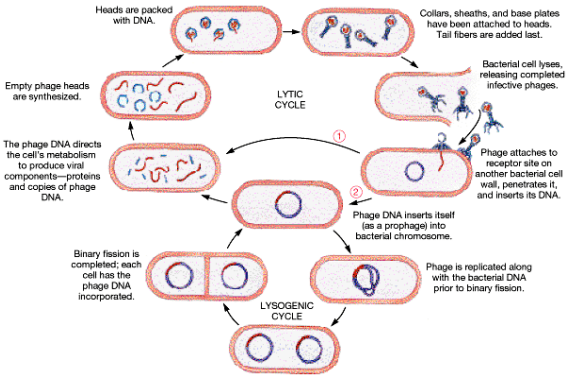
Griffith's experiment



Transduction

- Transfer of DNA by a bacteriophage.
  - Virulent phage are capable of causing infection, death, destruction of bacterial cell. (LYTIC INFECTION)
  - Temperate phage ordinarily do not cause a disruptive infection, but DNA is incorporated into bacterial chromosome (forming a prophage).
    (LYSOGENIC INFECTION)

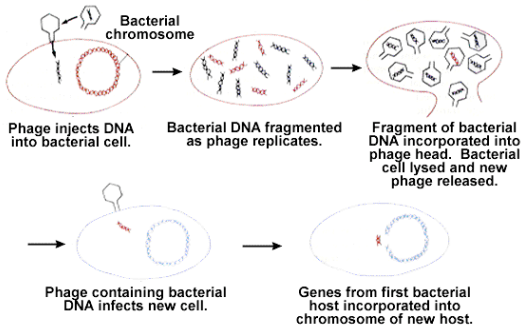
Lytic and lysogenic phages



Generalized Transduction

- DNA fragments of differing length are packaged into virus.
  - Phage infects cell (injecting in DNA)
  - Host DNA is cut into fragments as viral DNA replicates in lytic cycle
  - Newly made virus particles incorporate viral DNA
  - Host cell lyses, viruses released to infect again

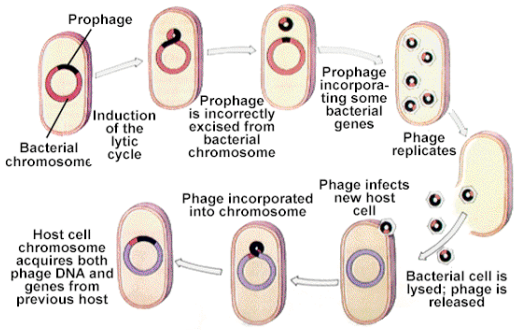
Generalized transduction



Specialized Transduction

- Genes near the prophage can be transferred to another organism.
  - Lytic cycle starts
  - Prophage + extra genes is excised
  - Phage replicates
  - Cell lyses releasing phage
  - Phage infects new host
  - Phage inserts into host chromosome

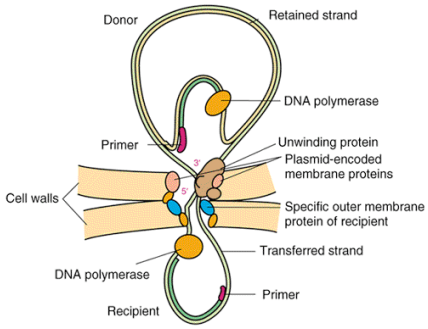
Specialized transduction



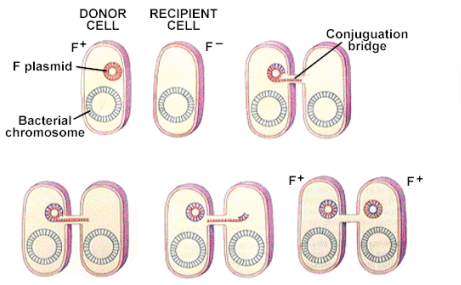
Conjugation

- Transfer of plasmids
  - Plasmids are circular, small, self-replicating extra-chromosomes.

Conjugation



Conjugation (fertility plasmids)



F plasmids

- F^+ donor cells containing F plasmids
- F^- recipient cells lacking F plasmids

Hfr strains

- Hfr=high frequency of recombination
- Arise from F+ cells when F plasmid is incorporated into bacterial chromosome.
  - Only the initiating segment from F plasmid is transferred, together with some adjacent chromosomal genes.

F' plasmids

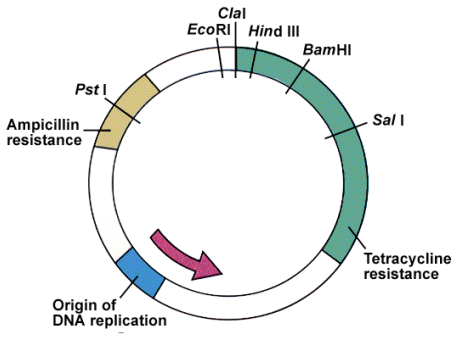
- When F plasmid excises from host chromosome, it can be imprecise and carry with it extra DNA from the host. Cells of the new host are termed F' strains.

Selected conjugations

Donor	Recipient	Product
F+	F-	F+
Hfr	F-	F- + some chromosomal DNA
F'	F-	F' + some chromosomal DNA

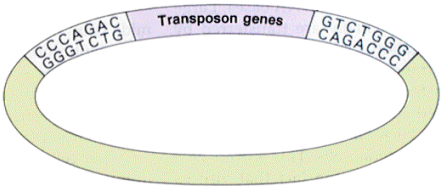
Resistance plasmids

- R plasmids or R factors
  - Carry genes for resistance to one or more antibiotics or to inorganic substances, such as heavy metals.
- RTF (Resistance Transfer Factor) mediates transfer of plasmid.

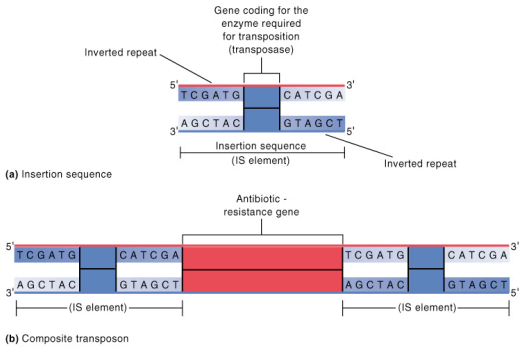


Transposons

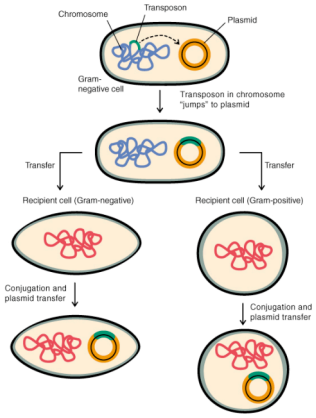
- Genetic elements that can "hop" around in an organism--from plasmid to plasmid, or from plasmid to chromosome.



Transposable elements



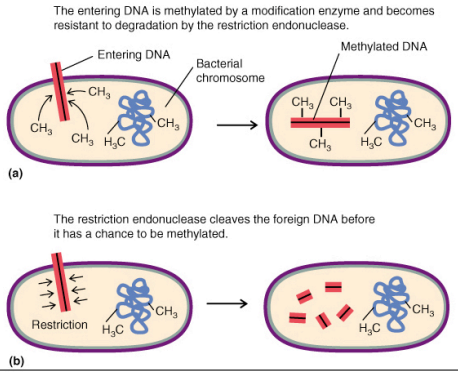
Movement of transposon



Bacteriocinogens

- Responsible for production of colicins or bacteriocins.
  - A class of growth-inhibiting proteins
  - Inhibit growth of closely-related bacteria or strains of the same species

Restriction enzymes



Genetic engineering

- The purposeful manipulation of genetic material to alter the genetic characteristics of an organism.

Recombinant DNA Technology

- Formation of DNA molecules that are a hybrid of the DNA of two species of organism
  - Vector DNA molecules
- Forms transgenic or recombinant organisms

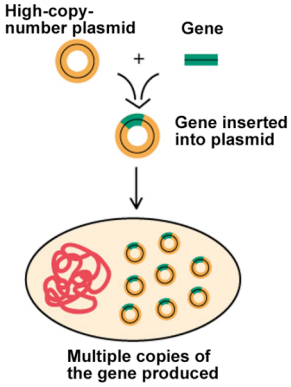
Restriction endonucleases

- Recognize specific sites in DNA
- Cleave the DNA sugar-phosphate backbone.

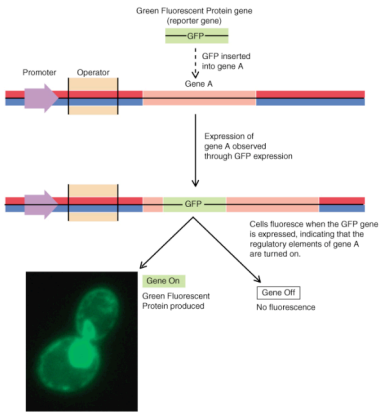
Plasmid vectors

- Contain unique restriction sites within genes for:
  - Antibiotic or heavy metal resistance
  - Metabolism of lactose
- These are called “marker genes.”

Cloning a gene



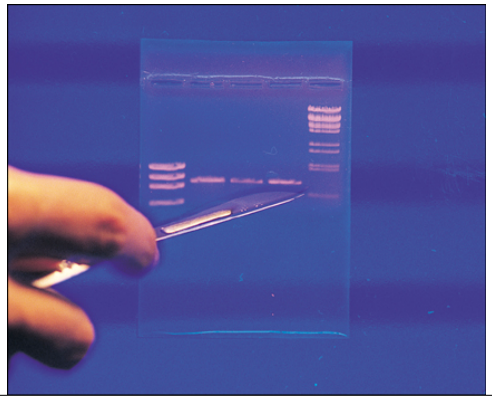
Reporter gene



Insertional inactivation

- If foreign DNA is inserted into a unique restriction site within a marker gene, that gene is then inactivated.

DNA stained with Ethidium bromide



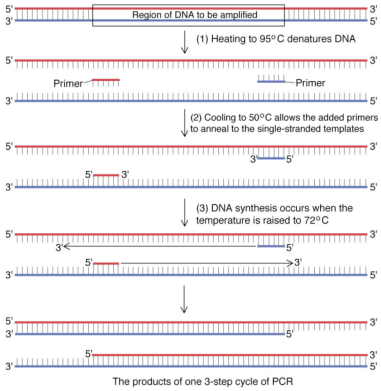
DNA ligase

- When plasmid vectors anneal with foreign DNA, the sugar-phosphate backbone of the DNA molecule can be repaired with DNA ligase.
- This forms a recombinant plasmid.

Transformation

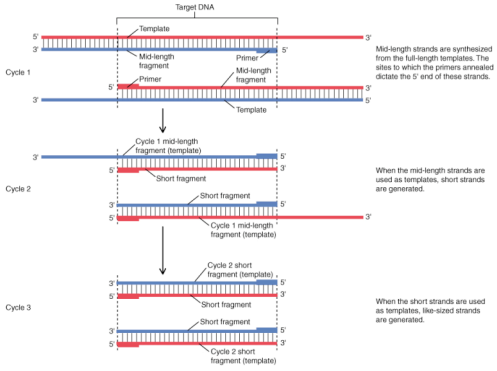
- Recombinant vectors can be used to transform recipient bacterial cells.
- Clones containing the foreign DNA are then grown on media that enable the biologist to determine which clones contain foreign DNA.

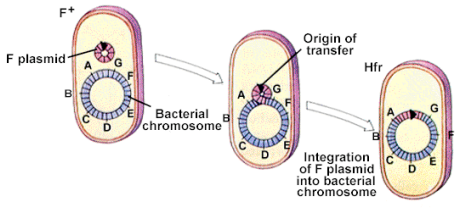
PCR amplification of DNA

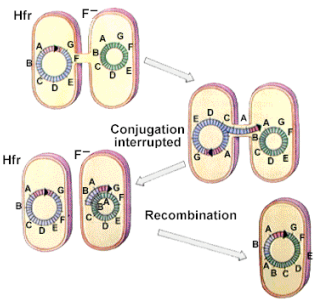


The products of one 3-step cycle of PCR

PCR amplification







DONOR	RECI-PIENT	MOLECULE(S) TRANSFERRED	PRODUCT
F^+	F^-	F plasmid	F^+ cells
Hfr	F^-	Initiating segment of F plasmid and variable quantity of chromosomal DNA	F^- with variable quantity of chromosomal DNA
F'	F^-	F plasmid and some chromosomal genes it carries with it	F plasmid and some duplicated chromosomal genes