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Comparison of genetic apparatus of eukaryotic and prokaryotic cells

- The most fundamental difference is that eukaryotes do have "true" nuclei containing their DNA, whereas the genetic material in prokaryotes is not membrane-bound.
- Prokaryotes also differ from eukaryotes in that they contain only a single haploid chromosome in an area named the nucleoid (exceptions – *Vibrio cholerae* and *Brucella melitensis* have 2 circular chromosomes), while eukaryotic DNA is found on tightly bound and organized in several (more than one) chromosomes.
- Consequently, any gene in prokaryotic cell is dominant. Prokaryotic cell lacks recessive genes.
- Bacterial DNA can be circular (for most microorganisms) or linear (rare exceptions - for *Borrelia burgdorferi* and several *Streptomyces* species).
Comparison of genetic apparatus of eukaryotic and prokaryotic cells

(continuation)

• Nucleoid lacks histone proteins (but histone-like proteins are present in bacteria and presumably play the role similar to that of histones of eukaryotes).

• Nucleolus is present in eukaryotic cell, but absent in prokaryotic cell.

• Telomeres are present (linear DNA) in eukaryotic cell but circular DNA of prokaryotes doesn't need telomeres.

• Plasmids are generally regarded as a prokaryotic feature and many important genes in prokaryotes are stored on plasmids (several eukaryotes can possess plasmids, but they are not typical for Eucarya).

• Genetic recombination in eukaryotes is mitosis and fusion of gametes, but genetic recombination in prokaryotes is partial, undirectional transfers DNA.
Comparison of eukaryotic and prokaryotic genes

Prokaryotes also differ from eukaryotes in the structure, packing, density, and arrangement of their genes on the chromosome.

1. Prokaryotic genes lack introns and large non-coding regions between each gene (nearly 98% of the human genome does not code for proteins or RNA or includes a gene promoter), nearly all of the prokaryote genome codes or controls something.

2. Prokaryotic genes are also expressed in groups, known as operons, instead of individually, as in eukaryotes.

3. In a prokaryotic cell, all genes in an operon (three in the case of the famous lac operon) are transcribed on the same piece of RNA and then made into separate proteins, whereas if these genes were native to eukaryotes, they each would have their own promoter and be transcribed on their own strand of mRNA. This lesser degree of control over gene expression contributes to the simplicity of the prokaryotes as compared to the eukaryotes.
DNA Structure
Figure 11.6  DNA Structure

A schematic, nonhelical model of DNA structure. The two phosphodiester bonds form a backbone between the sugar-phosphate units. The nitrogenous base pairs are held together by hydrogen bonds.
Expression of genetic information

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<td>AAA</td>
<td>Lysine</td>
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*The codons in DNA are complementary to those given here. Thus, U here is complementary to the A in DNA, C is complementary to G, G to C, and A to T. The nucleotide on the left is at the 5'-end of the triplet.
†AUG codes for N-formylmethionine at the beginning of mRNAs of bacteria.
Expression of genetic information

Figure 2-5  Overview of gene expression components; transcription for production of mRNA and translation for production of polypeptide (protein).
The Bacterial Genome is the total collection of genes carried by a bacterium both on its chromosome and on its extrachromosomal genetic elements (plasmids).
Structure of Bacterial Chromosome

The total length of *E. coli* chromosome is about 1 mm. The bacterium itself is only several micrometers in length. The DNA is about 1,000 times longer than bacterium and must be condensed (supercoiling). Super coiling helps to pack the circular molecule into a compact structure. *Escherichia coli* nucleoid has 40 - 50 supercoiled loops but because of a presence of a break in one segment it suffers from loss of super coiling. The protein component of the nucleoid includes DNA gyrase and DNA topoisomerase I, which are the enzymes responsible for maintaining supercoiled state.

The molecular weight of DNA molecule ranges from about $10^9$ - $10^{10}$ Da. The typical bacterial chromosome contain about $4 \times 10^6$ base pairs, thus, it is considerably smaller than the size of eukaryotic chromosomes.
Chromosomal DNA is compacted ~ 1000 fold to fit within cell
DNA shapes

a) The DNA double helix of most prokaryotes has the shape of a closed circle

b) The circular DNA strands, already coiled in a double helix, are twisted a second time to produce supercoils
Enzymes in Bacterial Chromosome

**Topoisomerases** are enzymes that alter the supercoiling of double-stranded DNA.

The topoisomerases act by transiently cutting one or both strands of the DNA to relax the coil and extend the DNA molecule.

Because bacterial topoisomerases are essential and unique, they are targets of antibiotics (DNA-gyrase or topoisomerase II is target for fluoroquinolones).
Electron micrograph of a DNA of *Micrococcus lysodeikticus*
Replication of DNA

DNA replication of bacteria is rapid and continues throughout the life cycle of cell division, in contrast to replication of DNA by eukaryotic cells, which takes place during one distinct stage of cell cycle. The replication of DNA within the circular bacterial chromosome begins at a single specific site on the bacterial chromosome, termed oriC for the origin of replication. The DNA is unwound, and the double-stranded DNA is separated into two single-stranded templates, and synthesis proceeds from this point in the opposite directions. The bidirectional replication mechanism requires the two sites of presence of the new DNA. Each of these two sites, termed a replication fork, comprise a complex of proteins that incorporate complementary nucleotides, accurately copying the template of the parent cell.
DNA Replication

**Semiconservative replication of DNA**

The replication fork of DNA showing the synthesis of two progeny strands. Each copy contain one new and one old strand.

**Replicon** is a part of the genome that contains an origin site and is replicated as a whole unit. Closed DNA circles contain necessary information for their own replication. Bacterial chromosome is called **replicon**
Replication of DNA

An autoradiograph of a replicating E.coli chromosome; about one-third of the chromosome has been replicated.
When the replication forks meet at approximately the opposite side of the circular DNA duplex, each strand has been copied into double-stranded helix, which can be partitioned into daughter cells.

The replication of this enormous molecule is very rapid; replication forks proceed at a rate of 1,000 base pairs per second, resulting in replication of the total 1-mm-long chromosome in about 40 min. This fact doesn’t explain how certain rapidly growing bacteria can divide in 20 min (S.aureus and E.coli). To take advantage of optimal growing conditions, DNA replication is reinitiated before one round of replication is completed. The genomes partitioned into a new daughter cells will already replicated, enabling them to divide again more quickly. A concomitant effect is that in these rapidly dividing cells, genes near oriC will present in more than one copy.
Replication of a circular bacterial chromosome.

Replicons have an origin of replication and can replicate autonomously. The ori site is about 400 base pairs long.

A replication “bubble” forms at the unique ori site.

The two complete circles separate by breaking and rejoining.

Replication proceeds bidirectionally until the entire circle is copied.
Replication of Bacterial DNA

Features:
1. Semiconservative
2. Multiple growing forks
3. Bidirectional
4. Proofreading (DNA polymerase)
Figure 2-4  Bacterial DNA replication depicting bidirectional movement of two replication forks from origin of replication. Each parent strand serves as a template for production of a complementary daughter strand and, eventually, two identical chromosomes.
Replication of circular DNA

Mechanism used by some bacterial viruses, plasmids and in chromosomal transfer
Elements and functions of prokaryotic genome

The primary function of the prokaryotic genome is to store its hereditary information.

Elements of prokaryotic genome:
- The bacterial chromosome
- Transposable elements
- Prophages
- Plasmids

In some prokaryotes genetic material can be transformed from one m/o to another m/o.
Transposable elements

Transposons (transposable elements or "jumping genes" are small pieces of DNA that encode enzymes that transpose the transposon, that is, move it from one DNA location to another, either on the same molecule of DNA or on a different molecule. Transposons may be found as part of a bacterium's nucleoid (conjugal transposons) or in plasmids and are usually between one and twelve genes long. A transposon contains a number of genes, coding for antibiotic resistance or other traits, flanked at both ends by insertion sequences coding for an enzyme called transposase. Transposase is the enzyme that catalyzes the cutting and resealing of the DNA during transposition. Thus, such transposons are able to cut themselves out of a bacterial nucleoid or a plasmid and insert themselves into another nucleoid or plasmid and contribute in the transmission of antibiotic resistance among a population of bacteria.
Transposable elements

Insertion sequences are the smallest and simplest “jumping genes”
Transposable elements

- Transposon at this original site can jump to other sites, leaving behind a copy of itself.
- Transposon can jump into and out of a plasmid.

<table>
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<tr>
<th>abc</th>
<th>Transposase</th>
<th>Resolvase</th>
<th>Antibiotic Resistance</th>
<th>cba</th>
</tr>
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</table>

These two genes control the movement of the transposon.
**Transposable elements**

**Functions**

1. Cause deletions and inversions of DNA sequences (internal or biological mutagenic agents)
2. Insert into genes and inactivate those genes
3. Spread of antibiotic resistance genes
4. Mobile genetic elements are responsible for the major part of genetic variability in natural bacterial populations
5. Transposons may enter other genera of bacteria during transfer of plasmids or via transducing phage
Insertion Sequences (Is)

The simplest transposons, insertion sequences (Is), are short sequences of DNA bounded at both ends by identical sequences of nucleotides in reverse orientation (inverted repeats). Insertion sequences can insert within a gene and cause a rearrangement mutation of the genetic material. If the sequence carries a stop codon, it may block transcription of the DNA during protein synthesis. It may also encourage the movement of drug-resistance genes between plasmids and chromosomes.
**Pathogenicity islands**

Some bacterial species are efficient at causing disease in higher organisms because they possess specific genes for pathogenic determinants.

These genes are often clustered together in the DNA and are referred to as **pathogenicity islands**. These genes encode adhesins, invasins, exotoxins…

**Pathogenicity islands** are a special class of mobile genetic elements containing groups of coordinately controlled virulence genes, often with IS, direct repeat sequences at their ends.
Pathogenicity islands
**Plasmids** are a small extrachromosomal circular molecules, but some are linear. They are double-stranded DNA molecule. Plasmids are variable in size. Size ranges from 1 to 2 kbp up to 120 kbp or more (Bacterial chromosome ranges from 580 kbp up to 5220 kbp). The number ranging from 1-1000 copies/cell. Plasmids do not carry genetic material which is essential to the growth of an organism, although they contain features that may enhance the survivability of the m/o in a particular environment.
The bacterial chromosome and bacterial plasmids, as shown in the electron microscope. The plasmids (arrow) are the circular structures, much smaller than the main chromosomal DNA.
Structure of Plasmids

The plasmids typically have three important elements:

1. An origin of replication
2. A selectable marker gene (e.g. resistance to ampicillin)
3. A cloning site (a place to insert foreign DNAs)

Plasmid is (by definition) an extrachromosomal element, thus it cannot make use of any origin of DNA replication in a chromosome. DNA synthesis within (i.e. copying of) a plasmid depends on its having an origin of DNA synthesis of its own.

*If a plasmid couldn't be copied, it would be rapidly diluted out in a population of dividing cells because it couldn't be passed on to daughter cells.*
Classification of Plasmids by Transfer Properties

1. **Conjugative plasmids**
   Conjugative plasmids are those that mediated conjugation. These plasmids are usually large and have all the genes necessary for autonomous replication and for transfer of DNA to a recipient (e.g. genes for sex pilus).

2. **Non-conjugative plasmids**
   Non-conjugative plasmids are those that cannot mediate conjugation. They are usually smaller than conjugative plasmids and they lack one or more of the genes needed for transfer of DNA. A non-conjugative plasmid can be transferred by conjugation if the cell also harbors a conjugative plasmid.
Classification of Plasmids by Compatibility Properties

1. **Compatible plasmids** are categorized into different groups and can coexist in one bacterial cell simultaneously.

2. **Incompatible plasmids** are unable to coexist stably in the same bacterial cell because they are dependent on the same replication and maintenance functions.
Classification of Plasmids by Phenotypic Effects

Plasmids made their presence by conferring phenotypes of cell harboring them.

- **F** - fertility factor
- **R** - antibiotic resistance
- **Col** - colicin production

- **Virulence plasmids:**
  - **Ent** - enterotoxin production
  - **Hly** - hemolysin production
  - **CFA-I; CFA-II** - adhesin production
Fig. 18.2. A map of F plasmid. The transfer operon (tra operon) has genes A to T. Insertion sequences IS2, IS3, γ-δ provide the sites of integration into the chromosome of various Hfr strains. The entry during conjugation with an F- cell is in the order of ori T - inc - frp - ori V etc., the tra operon entering at the end.
Plasmids can carry virulence genes

Plasmids may encode toxins and other proteins that increase the virulence of microorganisms:
in *Shigella spp.* adherence factors necessary for mucosal invasion;
in *Bacillus anthracis* both capsule (on one plasmid) and edema factor, lethal factor and protective antigen (on another plasmid);
the virulent enterotoxinogenic strains of *E.coli* that cause diarrhea produce one of two different types of plasmid-encoded enterotoxin (*Ent* plasmid);
in *Staphylococcus aureus* both an enterotoxin and a number of enzymes involved in bacterial virulence (*hemolysin*, fibrinolysin) are encoded by plasmid genes (*Hly* plasmid).
Bacteriophages

- **Bacteriophages** are viruses that infect bacteria.
- **Virulent bacteriophage** is a bacteriophage which always causes the lytic cycle, resulting in a death of a cell and production of new phage particles.
- **Temperate (or lysogenic) bacteriophage** is a bacteriophage whose DNA integrates into bacterial chromosome.
- Replication of phages nucleic acid is linked to replication of host cell DNA.
- Temperate bacteriophage doesn’t cause the lyses of the host cell and production new phage particles.
- The integrative phage’s DNA is called **prophage**.
- A bacterial cell with prophage is called **lysogenic cell**.
Historical classification of bacteriophages

History: Discovery and investigation of phages had been induced by problems in food industry (milk products). Phages cause lysis of lactic acid bacteria.
Main Families of Bacteriophages

(more than 2000 species of phages)
Schemes and electron photograph of bacteriophages

From: Chopin et al. (2002)
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The life cycle of bacteriophages

**Figure 2.13** The life cycle of bacteriophages.

- **bacterial cell**
  - **bacterial chromosome**
  - **bacteriophage**
    - **viral attachment and injection of DNA**
  - **viral integration into chromosome**
  - **lysogenic cell prophage**
  - **continued cell division**
  - **prophage induction**
  - **bacteriophage stably replicates with bacterial chromosome**
  - **lysogenic pathway**
  - **viral protein and DNA synthesis**
  - **destruction of bacterial chromosome**
  - **packaging and assembly of complete viruses**
  - **cell lysis**
  - **releases many bacteriophage particles**
  - **virulent pathway**
Bacteriophages

Lytic cycle

1. Attachment of phage to cell surface receptor
2. Injection of phage DNA into cell
3. Synthesis of new phage DNA and phage proteins
4. Assembly of new phage particles
5. Lysis of cell and release of progeny phage

Lysogenic cycle

1. Phage attaches to bacterial cell wall and injects its DNA.
2. Phage DNA circularizes.
3. Phage DNA integrates into bacterial chromosome to become a prophage.
4. Cell division
5. Dormant prophage is propagated indefinitely in progeny lysogenic cells.
Bacteriophages

- **Lysogenic (prophage) conversion** is a state when bacterial cell exhibit new properties, that are coded by the prophage genes.
- **Prophage presence may increase bacterial virulence**

**Examples:**
- Toxigenic (tox+) and nontoxigenic strains of *Corynebacterium diphtheriae*
- Production of erythrogenic toxin by *Streptococcus pyogenes* (scarlet fever)
- Production botulinum toxin of *Clostridium botulinum*
The mechanisms by which genetic information is changed and exchanged among bacteria

- Change: Mutation

- Exchange: Genetic recombination

  Exchange is most efficient between cells of the same species

  These processes result in progeny with phenotypic characteristics that may differ from those of the parent, e.g., acquisition of new characteristics (antigens, toxins, antibiotic resistance, etc.).
Genetic Variation

Mutations are stable hereditary changes in the coding sequence of DNA. They occur spontaneously (result of errors in the DNA replication process) or are induced by different chemical or physical factors (mutagens).

Various kinds of mutations are illustrated in the diagram.
Genetic Variation

**Genetic recombination** at the molecular level is the process by which DNA from a donor cell and DNA from a recipient cell combine to yield a new genome containing information from both sources.

**Molecular Mechanisms of Recombination:**

1. Homologous (legitimate)
2. Nonhomologous (illegitimate)
Genetic Variation

• **Homologous recombination** occurs between closely related DNA sequences and generally substitutes one sequence to another. The process requires a set of enzymes produced by the group “rec” genes, and homology in 100-200 nucleotide pairs.

• **Nonhomologous recombination** occurs between dissimilar DNA sequences and generally produces insertions or deletions or both. This process usually requires specialized (**site-specific**) recombination enzymes, such as those produced by many transposons and lysogenic bacteriophages.
Integrons are mobile genetic elements that are able to use site-specific recombination to acquire new genes in “cassette-like” fashion (independent open reading frames) and express them in a coordinated manner.
Gene Transfer

- The transfer of genetic material between prokaryotes is called *horizontal (or lateral)* gene transfer.
- It takes place by one of three ways:
  1. Transformation
  2. Conjugation
  3. Transduction
Gene Transfer

For prokaryotic cells recombination plays more important role than mutation in the process of changing in DNA. Recombination give material for evolution of the bacteria.

**Gene transfer is one-way process:** a piece of genetic material (the exogenote) is donated to the chromosome of a recipient cell (the endogenote) and integrated into it. Such forms temporarily diploid which called merozygote.
Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell. Transformation is typical only for certain bacteria (e.g., Streptococcus pneumoniae, Haemophilus influenzae, Bacillus spp., Neisseria spp.).
Gene Transfer

**Transformation**
was the first mechanism of genetic transfer to be discovered in bacteria.

Was discovered by Griffits in 1928 during the experiments with Streptococcus pneumoniae. Later Avery, MacLeod and McCarty identified the DNA as the **transforming agent** and as the **hereditary genetic material**

**Transformation** is the process by which bacteria take up fragment of **free DNA** and incorporate this molecule into recipient chromosome in a heritable form.
Classical series of experiments by Frederick Griffith

HYPOTHESIS: Material in dead bacterial cells can genetically transform living bacterial cells.

METHOD:
- Living S strain (virulent)
- Living R strain (nonvirulent)
- Kill the virulent S strain bacteria by heating.
- Mix dead S strain cells with living, nonvirulent R strain bacteria.

RESULTS:
- Mouse dies: Living S strain cells found in heart
- Mouse healthy: No bacterial cells found in heart
- Mouse healthy: No bacterial cells found in heart
- Mouse dies: Living S strain cells found in heart

CONCLUSION: A chemical substance from one cell is capable of genetically transforming another cell.
Factors affecting transformation

a. DNA size state
Double stranded DNA of at least $5 \times 10^5$ daltons works best. Thus, transformation is sensitive to nucleases in the environment.

b. Competence of the recipient develops during the end of logarithmic growth.
Some bacteria are able to take up DNA naturally. At this stage the bacteria are said to be competent. Other bacteria are not able to take up DNA naturally (E.coli). However, in these bacteria competence can be induced in vitro by electroporation (the use of high-voltage pulses) or treatment with chemicals (e.g. CaCl$_2$).
Steps in transformation

Uptake of DNA by Gram+ and Gram- bacteria differs. In Gram + bacteria the DNA is taken up as a single stranded molecule and the complementary strand is made in the recipient. In contrast, Gram- bacteria take up double stranded DNA.

*Legitimate/Homologous/General Recombination*

After the donor DNA is taken up, a reciprocal recombination event occurs between the chromosome and the donor DNA. This recombination requires homology between the donor DNA and the chromosome and results in the substitution of DNA between the recipient and the donor as illustrated in scheme.
Steps in transformation (continuation)

Recombination requires the bacterial recombination genes (recA, B and C) and homology between the DNA's involved. This type of recombination is called legitimate or homologous or general recombination. Because of the requirement for homology between the donor and host DNA, only DNA from closely related bacteria would be expected to successfully transform, although in rare instances gene transfer between distantly related bacteria has been shown to occur.

Significance

Transformation occurs in nature and it can lead to increased virulence. In addition transformation is widely used in recombinant DNA technology.
Transduction

Transduction was discovered by Zinder and Lederberg in 1951-1953.

*Transduction is the transfer of genetic information from a donor to a recipient by way of a bacteriophage.*

The phage coat protects the DNA in the environment so that transduction, unlike transformation, is not affected by nucleases in the environment. Not all phages can mediate transduction. In most cases gene transfer is between members of the same bacterial species. However, if a particular phage has a wide host range then transfer between species can occur. The ability of a phage to mediated transduction is related to the life cycle of the phage.
The ability of a phage to mediated transduction is related to the life cycle of the phage.
Types of transduction

- **Generalized**
  - any gene of the host bacterium can be transferred and doesn’t require lysogeny (Salmonella enterica – P-22)

- **Specialized (restricted)**
  - only specific genes near the attachment sites of a lysogenic phage in the host chromosome can be transferred (E.coli- λ)

- **Abortive**
  - the transferred DNA is not integrated but often is able to temporary survive and express. The fragment inherits linearly and lost in progeny
Generalized transduction

Any gene of the host bacterium can be transferred. Phages that mediate generalized transduction generally breakdown host DNA into smaller pieces and package their DNA into the phage particle by a "head-full" mechanism. Occasionally one of the pieces of host DNA is randomly packaged into a phage coat. Thus, any donor gene can be potentially transferred but only enough DNA as can fit into a phage head can be transferred. If a recipient cell is infected by a phage that contains donor DNA, donor DNA enters the recipient. In the recipient a generalized recombination event can occur which substitutes the donor DNA and recipient DNA.
Specialized transduction

Specialized (restricted) transduction is transduction in which only certain donor genes can be transferred to the recipient. Different phages may transfer different genes, but an individual phage can only transfer certain genes. Specialized transduction is mediated by lysogenic or temperate phage and the genes that get transferred will depend on where the prophage has inserted in the chromosome.

During excision of the prophage, occasionally an error occurs where some of the host DNA is excised with the phage DNA. Only host DNA on either side of where the prophage has inserted can be transferred (i.e. specialized transduction). After replication and release of phage and infection of a recipient, lysogenization of recipient can occur resulting in the stable transfer of donor genes. The recipient will now have two copies of the gene(s) that were transferred. Legitimate recombination between the donor and recipient genes is also possible.
Specialized transduction

- only specific genes near the attachment sites of a lysogenic phage in the host chromosome can be transferred
Transduction

A Generalized Transduction
- Infecting phage
- Bacterial chromosome
- Phage DNA
- a^+ gene
- Lysis
- These are normal, nontransducing phage.

B Specialized Transduction
- Integrated prophage
- s^+ gene
- UV induction
- Normal excision of prophage
- Rare abnormal excision of prophage that picks up the adjacent s^+ gene
- Lysis
- These are normal, nontransducing phage.

This rare phage, which has accidentally packaged the a^+ gene, can transduce an s^- cell to s^+.

The Bacterial Genome
Conjugation

Conjugation is the transfer of genetic material between two living bacteria that are in physical contact with one another. Was discovered in 1946 by Lederberg and Tatum.

In 1952 Hayes demonstrated that the gene transfer was polar. Polarity is mediated by plasmid, known as F-factor.
Conjugation

Donor
The ability of a bacterium to be a donor is a consequence of the presence in the cell of an extra piece of DNA called the **F factor** or fertility factor or sex factor. The F factor is a circular piece of DNA that can replicate autonomously in the cell; it is an independent replicon. Extrachromosomal pieces of DNA that can replicate autonomously are given the general name of plasmids. The F factor has genes on it that are needed for its replication and for its ability to transfer DNA to a recipient. One of the things the F factor codes for is the ability to produce a sex pilus (F pilus) on the surface of the bacterium. This pilus is important in the conjugation process. The F factor is not the only plasmid that can mediated conjugation but it is generally used as the model.

Recipient
The ability to act as a recipient is a consequence of the lack of the F factor.
The Bacterial Genome
Physiological states of the F factor

a. Autonomous (F+)  In this state the F factor carries only those genes necessary for its replication and for DNA transfer. There are no chromosomal genes associated with the F factor in F+ strains. In crosses of the type F+ x F- the F- becomes F+ in 100% of cases, while F+ remains F+. Thus, the F factor is infectious. No bacterial genes are transferred.

b. Integrated (High frequency of recombination (Hfr)  In this state the F factor has integrated into the bacterial chromosome via a recombination event. In crosses of the type Hfr x F- the F- rarely becomes Hfr and Hfr remains Hfr. In addition, there is a high frequency of transfer of donor chromosomal genes.

c. Autonomous with chromosomal genes (F')  In this state the F factor is autonomous but it now carries some chromosomal genes. F' (F prime) factors are produced by excision of the F factor from an Hfr. Occasionally, when the F factor is excising from the Hfr chromosome, donor genes on either side of the F factor can be excised with the F factor generating an F'. F' factors are named depending on the chromosomal genes that they carry. In crosses of the type F' x F- the F- becomes F' while F' remains F'. In addition there is high frequency of transfer of those chromosomal genes on the F'.
Donor types

1. F^+ cells with free F-plasmid:
   \[ F^+ \times F^- \rightarrow F^+ \]

2. Hfr cells with integrated plasmid:
   \[ Hfr \times F^- : \text{mating leads to acquisition of a few new genes by recipient}, \text{which usually doesn’t become } Hfr \]

3. F’ free F-plasmid with a portion of chromosomal genes
   \[ F' \times F^- \rightarrow F' \]
F+ x F- crosses

1) Pair formation
The tip of the sex pilus comes in contact with the recipient and a conjugation bridge is formed between the two cells. This bridge as a tube ensures that DNA transfer from the donor to the recipient. Thus, the DNA is protected from environmental nucleases. The mating pairs can be separated by shear forces and conjugation can be interrupted. Consequently, the mating pairs remain associated for only a short time.

2) DNA transfer
The plasmid DNA is nicked at a specific site called the origin of transfer and is replicated by a rolling circle mechanism. A single strand of DNA passes through the conjugation bridge and enters the recipient where the second strand is replicated.

3) This process explains the characteristics of F+ x F- crosses. The recipient becomes F+, the donor remains F+. Indeed, there is no transfer of donor chromosomal genes.
1. Pilus of donor cell attaches to recipient cell. Pilus contracts, drawing cells together to make contact with one another.

2. One strand of F plasmid DNA transfers from donor cell to recipient cell.

3. Donor synthesizes complementary strand to restore plasmid. Recipient synthesizes complementary strand to become F+ cell with pilus.
Conjugation

\[ F^+ \times F^- \]
Formation of Hfr

F+ → F+ → Hfr → Hfr → F'
Hfr x F- crosses

1) Pair Formation

2) DNA transfer
   The DNA is nicked at the origin of transfer and is replicated by a rolling circle mechanism. But the DNA that is transferred first is the chromosome. Depending upon where in the chromosome the F factor has integrated and in what orientation, different chromosomal genes will be transferred at different times. However, the relative order and distances of the genes will always remain the same. *Only when the entire chromosome is transferred will the F factor be transferred.* Since shearing forces separate the mating pairs it is rare that the entire chromosome will be transferred. Thus, usually the recipient does not receive the F factor in a Hfr x F- cross.

3) Legitimate recombination
   Recombination between the transferred DNA and the chromosome results in the exchange of genetic material between the donor and recipient.

4) This mechanism explains the characteristics of Hfr x F- crosses. The recipient remains F-, the donor remains Hfr and there is a high frequency of transfer of donor chromosomal genes.
Conjugation

**Hfr x F-**

![Diagram showing the process of conjugation between Hfr and F- bacteria](image)
F' x F- crosses

- 1) Pair formation
- 2) DNA transfer
   This process is similar to F+ x F- crosses. However, since the F' has some chromosomal genes on it these will also be transferred.
- 3) Homologous recombination is not necessary although it may occur.
- 4) This mechanism explains the characteristics of F' x F- crosses. The F- becomes F', the F' remains F' and there is high frequency transfer of donor genes on the F'.
Conjugation

F’ x F"
Gene Transfer

Cell-to-cell transfer of a conjugative plasmid

Integration of conjugative plasmid into the bacterial chromosome an Hfr

The order of genes on the bacterial chromosome can be determined by the time of entry of the genes into a recipient cell
Significance of conjugation

Among the G- bacteria this is the major way that bacterial genes are transferred. Transfer can occur between different species of bacteria. Transfer of multiple antibiotic resistance by conjugation has become a major problem in the treatment of certain bacterial diseases. Since the recipient cell becomes a donor after transfer of a plasmid it is easy to see why an antibiotic resistance gene carried on a plasmid can quickly convert a sensitive population of cells to a resistant one.

G+ bacteria also have plasmids that carry multiple antibiotic resistance genes, in some cases these plasmids are transferred by conjugation while in others they are transferred by transduction.
Mapping of bacteria

- In genetic map distances between loci are measured in number of minutes required for transfer in conjugation.
Gene Transfer

Conjugation is very useful for genetic mapping of bacteria

A circular genetic map of E. coli K12 with the location of selected genes. The map is divided into 100 minutes.
Figure 13.38  An Interrupted Mating Experiment. An interrupted mating experiment using Hfr × F− conjugation. (a) The linear transfer of genes is stopped by breaking the conjugation bridge to study the sequence of gene entry into the recipient cell. (b) An example of the results obtained by an interrupted mating experiment. The gene order is lac-tsx-gal-trp.
Gene Transfer

**A. Recombination**
- Rec A protein
- Recipient DNA
- Uptake of donor DNA ("foreign") DNA
- Alignment of donor DNA with homologous recipient DNA
- Recombined DNA fragment (blue)

**B. Transformation**
- Donor
- Cell lysis and release of free DNA
- Recipient
- Uptake and recombination

**C. Transduction**
- Donor cell DNA packaged in bacteriophage
- Release of bacteriophage from donor cell
- Bacteriophage infects and releases donor DNA

**D. Conjugation: Chromosome transfer**
- Donor
- Transfer of newly synthesized chromosomal DNA mobilized through intercellular bridge
- Recipient

**E. Conjugation: Plasmid transfer**
- Donor
- Chromosome
- Plasmid
- Transfer of newly synthesized plasmid DNA through intercellular bridge
- Recipient

Figure 2-8 Genetic recombination (A). The mechanisms of gene exchange between bacteria: transformation (B), transduction (C), and conjugation (D).
Genetic Engineering

- Transformation, conjugation and transduction are natural recombination processes.

- The artificial, laboratory-controlled DNA recombination of bacteria is known as gene manipulation, also called genetic engineering or recombinant DNA technology.

- Specified DNA fragments can be isolated and amplified, and their genes can be expressed at high levels.
**Genetic Engineering** – a combination of methods which allows to conduct artificial recombination of DNA and produce chimerical molecules, non-typical for nature.
Medical Significance of Genetic Engineering

1. The production of **hormones** (insulin, human growth hormone, etc.)
2. The production of **cytokines** (interferons, interleukin-2, tumor necrosis factors, etc.)
3. **Recombinant vaccines** (hepatitis B vaccine, papillomavirus vaccine, vaccine against Lyme disease)
4. **Genetically engineered antigens** which are used in diagnostic purposes.
Genetic Engineering

The basic tools of Genetic Engineering:

1. **Restriction enzymes** which are used to cleave DNA at defined sequences
2. **Cloning vectors** which can be used to deliver the DNA sequences into receptive bacteria
3. **DNA – ligase** – the enzyme that links the fragment to the cloning vector
Types of vectors:

1. **Plasmids** (pUC, pBR322, pGEM) are used for DNA fragments up to 20Kb

2. **Bacteriophages** (λ, T7) are used for larger fragments up to 25Kb

3. **Cosmid** (combination of plasmid and phage genes, pJC720) for fragments up to 45Kb

4. **Viruses** (smallpox vaccine)
The specific properties of plasmid cloning vectors:

1. Small size to be easy inserted into bacteria
2. High number of copies to be easily purified in sufficient quantities
3. Ability to replicate within a host cell
4. Selectable traits (resistance to an antibiotic)
5. One or few sites for restriction endonucleases which cut DNA and allow the insertion of foreign DNA
Steps in Cloning a Gene:

- Isolation a DNA to be cloned:
  a. short genes can be synthesized chemically, or
  b. use a restriction enzyme to generate necessary fragments of DNA from prokaryotic genome, or
  c. use mRNA of eukaryotes and reverse transcriptase for synthesis of gene sequence (elimination of introns of eukaryotic DNA)

- Generate a recombinant molecule by inserting DNA fragments into a cloning vector

- Introduce recombinant molecule into new host

- Select bacterial clones carrying specific genes
Principles of laboratory diagnosis of infectious diseases

**FIGURE 4-15. DNA probe hybridization.**

**A.** A single-stranded (denatured) target nucleic acid is bound to a membrane. A DNA probe with attached enzyme (E) is also employed. **B.** If the probe finds complementary sequences, it hybridizes to the target DNA forming a double-stranded hybrid. **C.** A colorless substrate is added, which in the presence of the enzyme is converted to a colored substrate. Measuring the color development quantitates the amount of probe bound to the original target. (Reproduced with permission from Wiley J, Sherwood L, Woolverton C (eds). Prescott’s Principles of Microbiology. New York: McGraw-Hill; 2008.)
Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is an *in vitro* method for enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands and flanks the region of interest in the target DNA. Following a series of repetitive cycle that involve template denaturation, primer annealing, and the extension of annealed primers by *Taq* polymerase, there is an exponential accumulation of the specific target DNA.

*Taq* polymerase is thermostable enzyme, which is isolated from the bacteria *Thermus aquaticus*. Due the stability of the enzyme, fresh enzyme no longer had to be added after each amplification cycle.
Procedure of PCR

The PCR requires two synthetic oligonucleotide primers that are complementary to the region on opposite strands of a target piece of DNA, a target sequence in a DNA sample that occurs between the pair of primers, a thermostable DNA polymerase and the four deoxyribonucleotides. The procedure, performed in termocycle, usually consists of three basic steps and is completed within minutes.

The first step is **denaturation**, which increases the temperature within the sample vial to about 94-96°C, causing the double-stranded DNA within the sample to separate into two pieces.

The second step is renaturation (**annealing**). The step is completed by dropping the temperature within the thermocycler to about 50-55°C, which allows primers to anneal with the complementary sequences in the source of DNA.

The third (final) step (**extension, elongation**) is the synthesis portion on the reaction, wherein the temperature is raised to about 72-74°C, the optimum temperature for the catalytic functioning of Taq DNA polymerase. Target DNA is extended, replicating to the form additional copies of the target DNA.

Within 30 cycles, over a million copies of the original target DNA can be reproduced.
Three Basic Steps of the PCR Cycle

- DNA denaturation
- Primer annealing
- Primer extension
Polymerase chain reaction - PCR

1. Denaturation at 94-96°C
2. Annealing at ~68°C
3. Elongation at ca. 72°C
Principle of PCR (polymerase chain reaction)

One cycle of PCR

1. Denaturation
2. Annealing with primer
3. Extension

Taq polymerase

Template

After 1 cycle

$2^n$ copies

After 3 cycles

Template

After n cycles
Significance of PCR

1. PCR allows to obtain the results faster in comparison with classical methods of cultivation and identification of m/o.

2. Application of PCR to infectious diseases focused on m/o that were impossible or slow to grow in culture, were difficult to cultivate, or posed significant health hazards using standard recovery techniques (Mycobacterium tuberculosis, Helicobacter pylori, Chlamydia trachomatis, and HIV).

3. Clinicians use the PCR method to monitor the effect of antimicrobial therapy.

4. PCR is used for retrospective analysis of samples from outbreaks or deaths caused by previously unidentified etiological agent. Sometimes the diagnosis is absolutely changed retrospectively.

5. Several diagnostic techniques needed very rare and expensive reagents for the ability to perform the methods. PCR has provided a solution to this problem.

6. PCR is less stressful to the patient, because serial serum samples are not required and results can be obtained in less than a day.
Reverse Transcriphiase 
Polymerase Chain Reaction 
(RT-PCR)

A technique similar to conventional PCR, except that the starting material is RNA rather than DNA. Because of this, a DNA copy first must be made from RNA, using an enzyme known as reverse transcriptase. Once a copy of DNA is made, the PCR proceeds as usual.
Scheme of RT-PCR

Reverse transcription:
- M-MuLV or AMV Reverse Transcriptase or Tth DNA Polymerase,
- Titan One Tube RT-PCR System/Kit,
- C. therm. Polymerase One-Step RT-PCR System or C. therm. Polymerase for Reverse Transcription in Two-Step RT-PCR

Primer: Oligo (dT)_{12-20} or hexamers or site specific primer

cDNA
- Primer: site specific primers
- Polymerase: Taq DNA Polymerase or the Expand PCR Systems or Tth DNA Polymerase from RT reaction

Amplified DNA

Reverse Transcription PCR flow
Quantitative, or Real-Time PCR

Quantitative, or real-time, PCR is standard PCR with the advantage of detecting the amount of DNA formed after each cycle with either dyes or fluorescently-tagged oligonucleotide probes. Real-time PCR results can be obtained faster and with less variability than standard PCR due to sensitive fluorescent chemistry and elimination of post-PCR detection procedures.

All real-time PCR instruments combine a thermal cycler to drive DNA amplification, an optical system to excite fluorophores and capture emitted fluorescence from the detection chemistry, and specialized software to collect and analyze the quantitative data generated. The only difference between real-time and regular PCR machines is the fluorescence detection system.

Real-time PCR is useful for the investigation of gene expression, viral load, pathogen detection, etc.
Optional: Perform dilution series of samples

Add DNA templates to all PCR tube strips

Add IQ SYBR Green supermix containing primers to all samples

Place PCR tube strips with optical flat caps in real-time thermal cycler and amplify target DNA sequence

Set up PCR reactions and amplify using a real-time thermal cycler

Use dilution series to optimize real-time PCR conditions. Determine starting quantities using real-time PCR results

Perform melt-curve analysis to distinguish specific PCR products from non-specific products such as primer dimers

Analyze the results

Optional: Electrophoresis of PCR products and gel staining

Compare and contrast data obtained from real-time PCR to data obtained from conventional PCR
SYBR Green Chemistry

SYBR Green, a non-specific chemistry, is the simplest and least costly approaches to real-time PCR that utilizes DNA-binding fluorophores for nonspecific detection of target DNA sequences. In this type of chemistry, the sensitivity and specificity of the assay is determined only by the primers. SYBR Green is a naturally fluorescing dye that can intercalate between DNA bases. SYBR Green exhibits low fluorescence when unbound in solution, but starts to fluoresce brightly when associated with double stranded DNA (dsDNA) and exposed to a suitable wavelength of light: the fluorescence increases exponentially as the DNA target sequence is amplified.
A) DNA is denatured and SYBR Green molecules are free in the reaction mix. B) Primers anneal and SYBR Green molecules bind to the dsDNA. C) DNA polymerase elongates the template and more SYBR Green molecules bind to the product formed resulting in exponential increase in the fluorescence level.
Evaluation of Real-time PCR
Multiplex polymerase chain reaction

Multiplex-PCR consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. By targeting multiple sequences at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes, i.e., their base pair length, should be different enough to form distinct bands when visualized by gel electrophoresis. Alternatively, if amplicon sizes overlap, the different amplicons may be differentiated and visualised using primers that have been dyed with different colour fluorescent dyes.
Scheme of Multiplex Polymerase Chain Reaction
Multiplex PCR (process overview)

1) Multiplex PCR with plasmid-specific primers
   - Primer Pair 1
   - Replicon 1
   - PCR Product 1

2) Asymmetric Primer Extension with Biotin-dCTP
   - Primer Pair 2
   - Replicon 2
   - PCR Product 2
   - No Product

3) Hybridization with Luminex® xTAG beads
   - Addition of SA-PE

A. Reverse gene-specific primer with universal sequences (non-labeled)
B. Forward gene-specific primer with universal sequences (non-labeled)
C. Multiplex PCR with labeled universal forward sequences
D. Multiplex PCR product
E. Multiplex PCR product analyzed by capillary electrophoresis
Scheme of Multiplex Polymerase Chain Reaction
Distinct bands are visualized by gel electrophoresis