INTRODUCTORY BIOLOGY AND MICROBIOLOGY

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Genetic Recombination

- **Genetics** is a branch of biology that involves the study of genes, genetic variation, and heredity in living organisms.
- While the chromosomes are aligned, a bit of the maternal and paternal chromosomes crosses over and gets reconnected to the opposite chromosome.
- The DNA from the mother and the father has been recombined on each daughter chromosome thus the term recombination.

- Therefore genetic recombination is the exchange of genetic material either between multiple chromosomes or between different regions of the same chromosome.
- This process is generally mediated by homology; that is, homologous regions of chromosomes line up in preparation for exchange, and some degree of sequence identity is required. Various cases of non-homologous recombination do exist, however.

- One important instance of recombination in diploid eukaryotic organisms is the exchange of genetic information between newly duplicated chromosomes during the process of meiosis.
- In this instance, the outcome of recombination is to ensure that each gamete includes both maternally and paternally derived genetic information, such that the resulting offspring will inherit genes from all four of its grandparents, thereby acquiring a maximum amount of genetic diversity.

- Recombination is also used in DNA repair (particularly in the repair of double-stranded breaks), as well as during DNA replication to assist in filling gaps and preventing stalling of the replication fork.
- In these cases, a sister chromatid serves as the donor of missing material via recombination followed by DNA synthesis.
- Recombination also occurs in prokaryotic cells, and it has been especially well characterized in *E. coli*.

- Although bacteria do not undergo meiosis, they do engage in a type of sexual reproduction called conjugation, during which genetic material is transferred from one bacterium to another and may be recombined in the recipient cell. As in eukaryotes, recombination also plays important roles in DNA repair and replication in prokaryotic organisms.
- Gene transfer can also occur through transformation and transduction (*We will discuss conjugation, transformation and transduction later* under this topic)

Models of Recombination

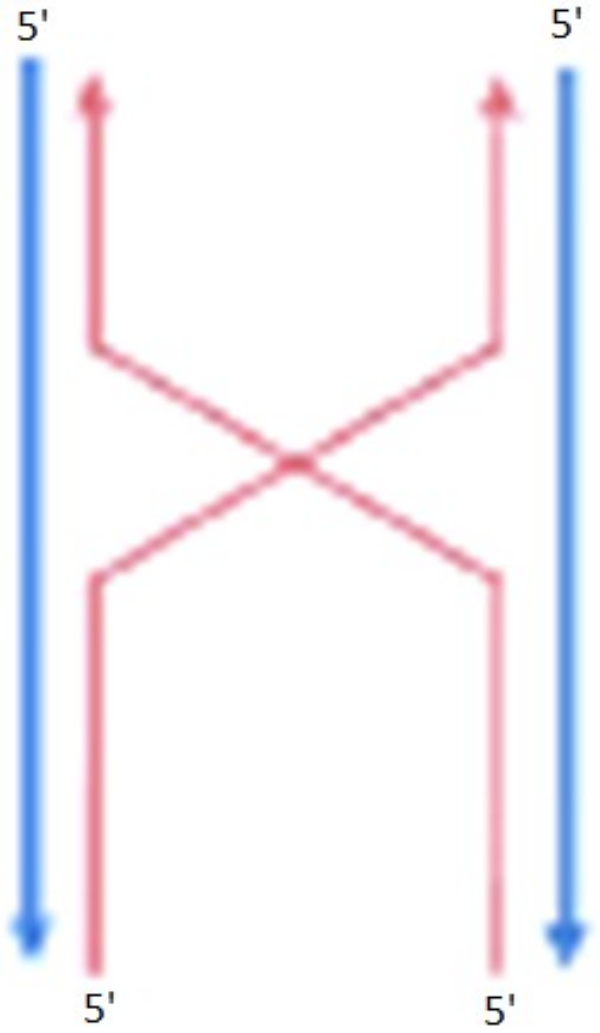
- Although common, genetic recombination is a highly complex process. It involves the alignment of two homologous DNA strands.
- The requirement for homology suggests that this occurs through complementary base-pairing, however, this has not been definitively shown:
  - the precise breakage of each strand,
  - exchange between the strands, and
  - sealing of the resulting recombined molecules.

- This process occurs with a high degree of accuracy at high frequency in both eukaryotic and prokaryotic cells.
- The basic steps of recombination can occur in two pathways, according to whether the initial break is single or double stranded.
- In the single-stranded model, following the alignment of homologous chromosomes, a break is introduced into one DNA strand on each chromosome, leaving two free ends.

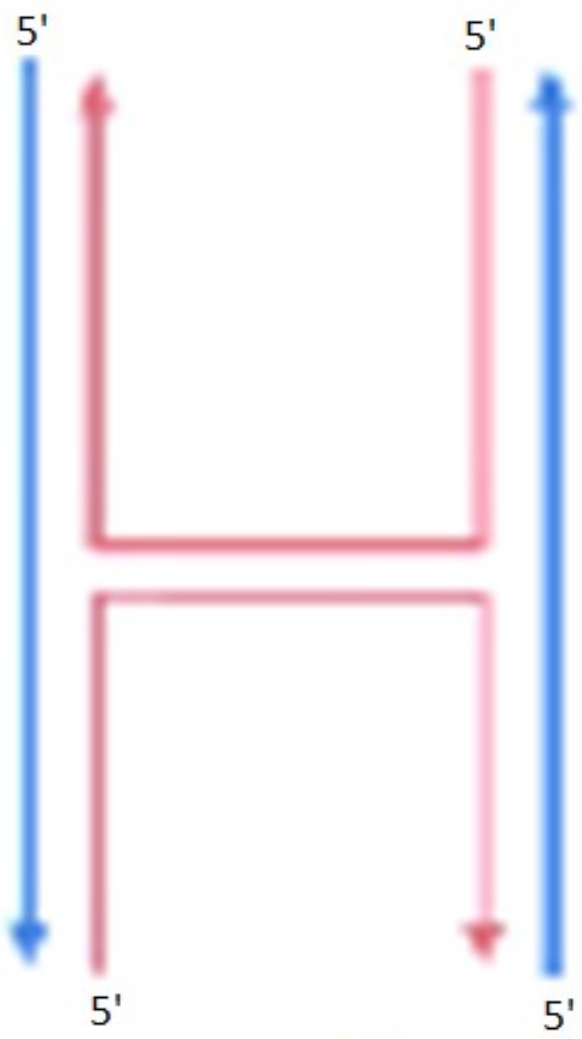
Single-stranded Model

- In the single-stranded model, following the alignment of homologous chromosomes, a break is introduced into one DNA strand on each chromosome, leaving two free ends.
- Each end then crosses over and invades the other chromosome, forming a structure called a Holliday junction.

- Two possible configurations for the Holliday junction, with the DNA shown in the parallel (left) or antiparallel configuration (right).
- The next step, called branch migration, takes place as the junction travels down the DNA.
- The junction is then resolved either horizontally, which produces no recombination, or vertically, which results in an exchange of DNA.



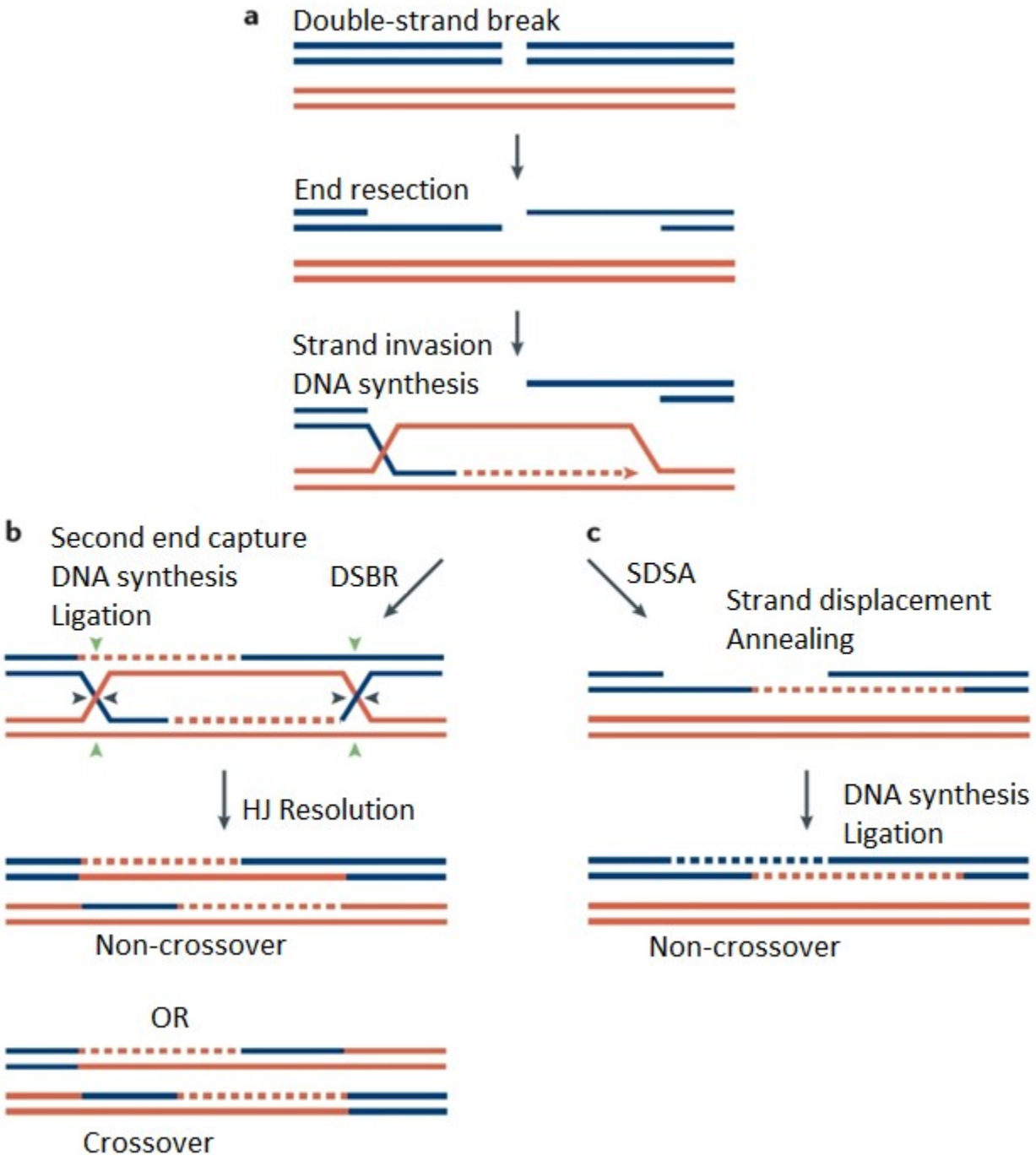
Parallel



Anti-parallel

Double-stranded Model

- In the double stranded model, which is initiated by double-stranded breaks, the ends at the breakpoints are converted into single strands by the addition of 3' tails.
- These ends can then perform strand invasion, producing two Holliday junctions.
- From that point forward, resolution proceeds as in the single-stranded model


a
Double-strand break
End resection
Strand invasion
DNA synthesis
b
Second end capture
DNA synthesis
Ligation
DSBR
HJ Resolution
Non-crossover
OR
Crossover
c
SDSA
Strand displacement
Annealing
DNA synthesis
Ligation
Non-crossover

- Double-strand breaks (DSBs) can be repaired by several homologous recombination (HR)-mediated pathways, including double-strand break repair (DSBR) and synthesis-dependent strand annealing (SDSA).
  - a) In both pathways, repair is initiated by resection of a DSB to provide 3' single-stranded DNA (ssDNA) overhangs. Strand invasion by these 3' ssDNA overhangs into a homologous sequence is followed by DNA synthesis at the invading end.

- b) After strand invasion and synthesis, the second DSB end can be captured to form an intermediate with two Holliday junctions (HJs).
- After gap-repair DNA synthesis and ligation, the structure is resolved at the HJs in a non-crossover (black arrow heads at both HJs) or crossover mode (green arrow heads at one HJ and black arrow heads at the other HJ).

- c) Note that a third model of recombination, synthesis-dependent strand annealing [SDSA], can also occur in the lack of crossover typical of recombination in mitotic cells and observed in some meiotic cells to a lesser degree.
- This proceeds via strand displacement and annealing of the extended single-strand end to the ssDNA on the other break end, followed by gap-filling DNA synthesis and ligation. The repair product from SDSA is always non-crossover.

Recombination Enzymes

- No matter which pathway is used, a number of enzymes are required to complete the steps of recombination. The genes that code for these enzymes were first identified in *E. coli* by the isolation of mutant cells that were deficient in recombination.
- A gene simply known as *recA* gene encodes a protein necessary for strand invasion. Meanwhile, the *recB*, *recC*, and *recD* genes code for three polypeptides that join together to form a protein complex known as *Rec*BCD.

- This complex has the capacity to unwind double-stranded DNA and cleave strands.
- Two other genes, *ruvA* and *ruvB*, encode enzymes that catalyze branch migration, while Holliday structures are resolved by the protein resolvase, which is product of the *ruvC* gene.
- Several enzymes involved in DNA replication, such as ligase and DNA polymerase, also contribute to recombination.

- In eukaryotes, recombination has been perhaps most thoroughly studied in the budding yeast *Saccharomyces cerevisiae*.
- Many of the enzymes identified in this yeast have also been found in other organisms, including mammalian cells.
- Studies have revealed that the *Rad* genes (named for the fact that their activity was found to be sensitive to radiation) play a key role in eukaryotic recombination. In particular, the *Rad51* gene, which is homologous to *recA*, encodes a protein (called Rad51) that has recombinase activity.

- This gene is highly conserved, but the accessory proteins that assist *Rad*51 appear to vary among organisms. For example, the *Rad*52 protein is found in both yeast and humans, but it is missing in Drosophila melanogaster and *Caenorhabditis elegans* (A transparent round worm that lives in temperate soil environments).
- In eukaryotic cells, single-stranded DNA (ssDNA) becomes rapidly coated with the protein RPA (replication protein A).

- RPA has a higher affinity for ssDNA than Rad51, and it therefore can inhibit recombination by blocking Rad51's access to the single strand needed for invasion.
- In yeast, however, binding of *Rad*51 to ssDNA is enhanced by the proteins Rad52 and the complex Rad55-*Rad*57.
- Once access has been gained, Rad51 polymerizes on the DNA strand to form what is called a presynaptic filament, which is a right-handed helical filament containing six *Rad*51 molecules and 18 nucleotides per helical repeat.

- In addition to proteins that assist Rad51 activity, there are also some proteins that inhibit it. In yeast, for instance, the helicase Srs2 dismantles the Rad51-ssDNA complex, while the proteins Sgs1 and BLM inhibit the complex.
- It is thought that these proteins play a role in preventing recombination during DNA replication when it is not needed.

- In humans, the tumor suppressor genes *BRCA1* and *BRCA2* also play a role in regulating recombination.
- Individuals who are heterozygous for *BRCA2* are subject to increased risk for breast and ovarian cancer; loss of both alleles causes Fanconi's anemia, a genetic disease characterized by predisposition to cancer, among other defects. *BRCA2* appears to promote *Rad51* binding to ssDNA.

Coming Together of Homologous Sequences

- Not so well understood is the question of how homologous sequences come to be in proximity so that recombination can proceed.
- Two hypotheses have tried to explain this phenomenon:
  - null model,

Null Model

- This model proposes that homologues find one another through a passive process of diffusion, in which the DNA sequence at the broken end of a strand is sequentially compared to all of the other potential end sequences in the genome.
- In order for diffusion to account for the rapid repair of double-stranded breaks observed in yeast, Barzel and Kupiec (2008) calculated that each homology search would have to proceed at a speed 40 times faster than the rate at which DNA polymerase adds a single nucleotide to a replicating DNA chain, which seems unlikely.

Homologous chromosomes reside in constitutive pairs

- An alternate hypothesis proposes that homologous chromosomes reside in pairs constitutively.
- Acting against this hypothesis is the finding that in induced recombination experiments, the broken ends of strands recombine with what are called ectopic homologues (areas of fortuitous sequence identity) as frequently as they recombine with their true homologous chromosomes.

- Furthermore, although homologous pairing has been observed in somatic cells of some organisms (e.g., *Drosophila*, *Neurospora*), it is not widely seen in the cells of other organisms, including mammals.
- Barzel and Kupiec (2008) also point out, the absence of general homologous pairing does not necessarily mean random assortment. Instead, discrete sections of chromosomes may be required for homology. The use of subdomains for homology searches would reduce the time it takes to find a homologous partner.

- Despite such theories, the exact mechanism responsible for locating and lining up homologous segments remains to be determined.

Gene transfer

- Causes in a change in the genome either due to mutation or as a result of acquisition of a new DNA from an external source.
- DNA may be transferred between bacteria by:
  - Transformation
  - Conjugation and
  - Transduction

Transformation

- This is taking up of DNA that is either extracted artificially or released by lysis from cells of another bacterial strain.
- Examples of strains of bacteria that are able to do this are pneumococci; *Haemophilus influenza* and certain *Bacillus*.
- Cells that are able to take up and incorporate into their genetic make up such foreign DNA are called *competent cells*.

- Cells are *competent* for transformation in the late log phase or during sporulation as is the case in *Bacillus spp*.
- Bacterial cells can also be made competent by artificial treatment e.g. treatment by $CaCl_2$ and heat shock treatment as well as electroporation.
- Once the DNA has entered the cell through transformation then the DNA is incorporated into the cell by recombination (Recombinant DNA technology).

- There must be a high degree of nucleic acid similarity (homology) in order for recombination to be successful.
- DNA used in transformation are relatively short and contains only a few number of genes as such transformation is not widely used in gene mapping (organization of genes in relation to one another).

Conjugation

- This is the process by which one cell (donor/male cell) makes contact with another (the recipient/female cell) and DNA is transferred from the donor into the recipient.
- There is need for a plasmid to carry the genetic information so that conjugation can occur.
- Only cells with plasmid containing a desired *gene* acts as a donor whereas those that lack the corresponding plasmid will act as the recipient (female).

- Plasmids that help in conjugation carry a 1 – 2 µm long protein appendage called the *pillus*, which must only be on the surface of the donor/male cell.
- Tip of the pillus attaches on the surface of the recipient cell and hold the two cells together so that DNA can pass into the recipient cell.
- It is hypothesized that the gene transfer is through the pillus or that the pillus only act to bring the two cells together.

- During conjugation one strand of the circular plasmid DNA is nicked open at a specific site and the free end is passed into the recipient cell.
- DNA is replicated during transfer so that each cell receives a copy.
- The process converts a recipient into a donor and will continue to transfer the DNA to other recipients a process called infectious spread of a plasmid.

Mobilization of genes by Conjugation

- Some plasmids have the ability to mobilize the chromosomal genes of bacteria.
- Example of such a plasmids is F-factor (fertility factor) of *E. coli*.
- The F factor is a plasmid that contains the basic genetic information for extrachromosomal existence and for self-transfer.

- Cells that have F plasmid (F+ cells) have the ability to produce F pilli and can transfer the F plasmid to F- cells by conjugation.
- Once F plasmid is inserted into the chromosome of the recipient cells, the entire chromosome will behave like the plasmid and chromosomal genes can then be transferred to the recipient cells at a higher frequency.
- Such cultures are called high-frequency recombination (Hfr) strains.

- The F plasmid system is confined to *E. coli and* close related enteric bacteria.
- However, other plasmids such as plasmid RP4 and its relatives are capable of mediating conjugation and gene mobilization.

Transduction

- This is the transfer of DNA between bacterial cells by bacteriophages.
- The phage genome exist as a length of double stranded DNA coiled up inside a protein coat.
- Other phages also have their DNA as a ssDNA or RNA, however, all transducing phages exist as dsDNA.

Types of Transduction

- There are generalized and specialized transductions.
- In these two types of transductions, microbial genes are occasionally and accidentally incorporated into new phage particles.
- Infection of another bacterial cell by such kind of a phage, the DNA + a short segment of chromosome from the original bacteria enters the second bacteria.

- The genes from the first bacteria is said to have been transduced by phage into the second bacterial cell.
- Transduction can only take place between two bacterial strains that are closely related.
- Transducing bacteriophages can also pick and transfer plasmid DNA for instance the penicillinase gene in Staphylococci is usually located on a plasmid and can be transferred to other staphylococci strains by transduction.

GENETIC ENGINEERING

- Microbial genetic engineering is basically involved with transformation of bacterial genes.
- This involves isolation and amplification of microbial DNA and expressing such amplified DNA at higher levels.
- Specific gene fragments are cleaved by restriction enzymes and such gene fragments are covalently bound to plasmids (“vectors”), which can then be inserted into bacterial hosts.

- Bacterial colonies or clones carrying such inserted genes can be identified by hybridization of DNA or RNA with chemical or radiochemical probes.
- Alternatively protein products encoded by such genes can be recognized either by enzyme activity or by immunologic techniques.
- The isolated genes can also be used for site directed mutagenesis leading to an altered DNA sequence of a gene.

- Hybridization techniques can use DNA as a probe to help recognize nucleic acids corresponding to complementary sequence of a latent pathogen present in a human system.

Restriction of DNA Fragments

- Bacteria have a varying range of restriction enzymes.
- These enzymes have the ability to recognize specific regions of a DNA for cleavage.
- Such recognizable DNA sites are said to be palindromic i.e. areas with inverted sequence repeti tions.
- A typical sequence palindrome, recognized by the frequently used restriction enzyme EcoR1, is GAATTC.

- The length of DNA fragments produced by restriction enzymes varies and is determined largely by the number of specific bases recognized by an enzyme.
- Most restriction enzymes recognize four, six, or eight base sequences; however, other restriction enzymes can recognize 10, 11, 12, or 15 base sequences.

Separation of Restricted DNA Fragments

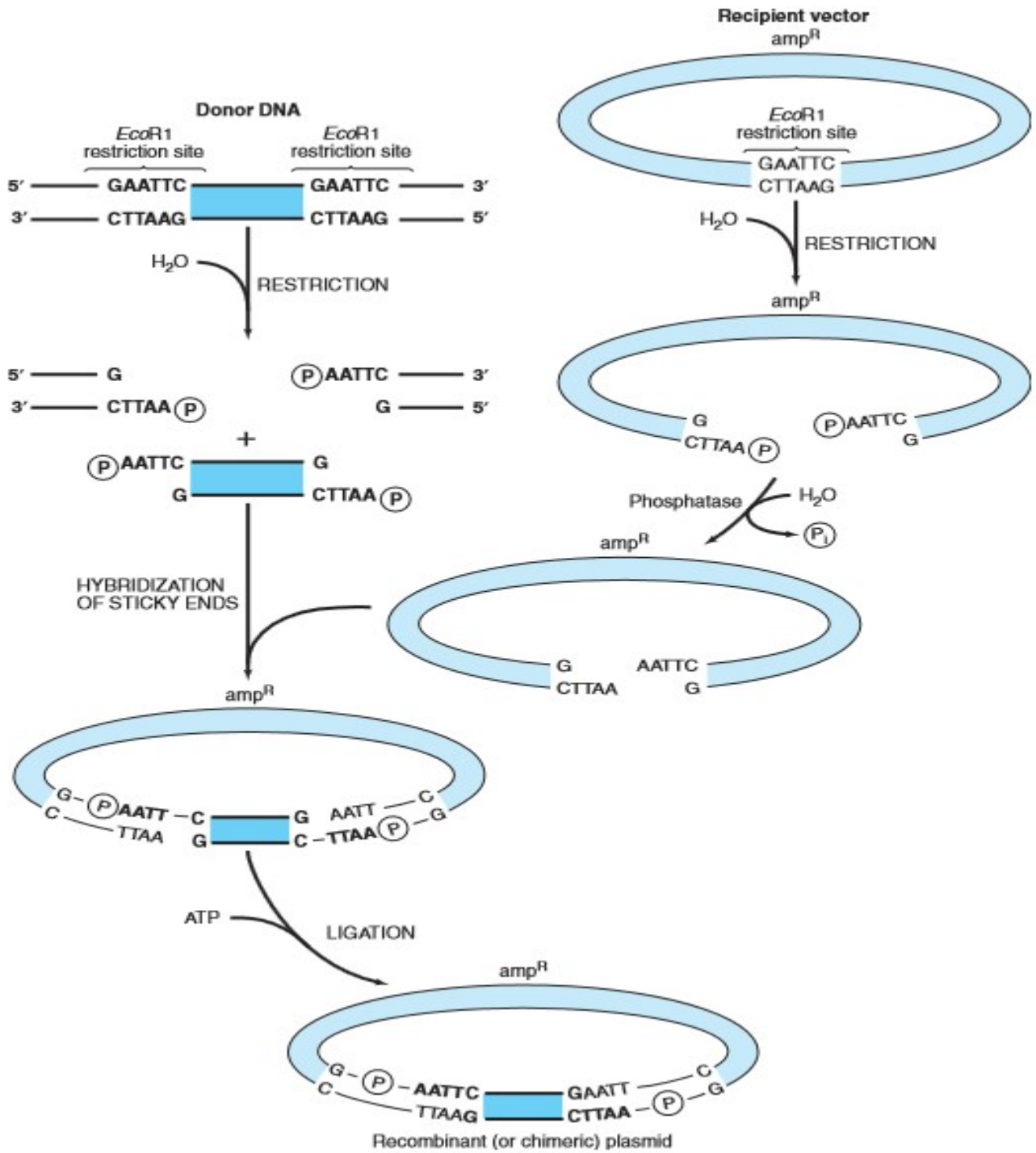
- This can be achieved through gel electrophoresis.
- Gel electrophoresis permits DNA fragments to be separated on the basis of size.
- The smaller the fragment, the more rapid the rate of migration.
- Dye ethidium bromide forms a brightly fluorescent adduct as it binds to DNA, so that small amounts of separated DNA fragments can be photographed on gels.

Cloning of DNA Restriction Fragments

- Many restriction enzymes cleave and produce DNA fragments with sticky ends that may hybridize with one another.
- For example, cleavage of DNA with EcoR1 produces DNA containing the 5′ tail sequence AATT and the complementary 3′ tail sequence TTAA.
- Cleavage of a plasmid (a circular piece of DNA) with the same restriction enzyme produces a linear fragment with cohesive ends that are identical to one another.

- Ligation of cleavage plasmid (a circular piece of DNA) with the EcoR1 cleaved DNA fragment produces a recombinant plasmid, or chimeric plasmid, which contain DNA fragments as insert in covalently closed circular DNA.
- Plasmids must be in a circular form in order to replicate in a bacterial host. Recombinant plasmids may be introduced into a bacterial host, frequently E coli, by transformation.

- The resulting bacterial population contains a library of recombinant plasmids carrying various cloned inserted restriction fragments derived from the donor DNA.
- Electroporation is a recently developed procedure for introduction of DNA into bacteria.


Donor DNA
EcoR1 restriction site
EcoR1 restriction site
5′ GAATTC GAATTC 3′
3′ CTTAAG CTTAAG 5′
H2O
RESTRICTION
5′ G
3′ CTTAA P
P AATTC 3′
G 5′
+
P AATTC G
G CTTAA P
HYBRIDIZATION OF STICKY ENDS
ampR
Recipient vector
ampR
EcoR1 restriction site
GAATTC
CTTAAG
H2O
RESTRICTION
ampR
G
CTTAA P
P AATTC
G
Phosphatase
H2O
Pi
ampR
G AATTC
CTTAA G
ampR
ATP
LIGATION
ampR
Recombinant (or chimeric) plasmid

Application of Genetic Recombination

- Hybridization using gene probes have been used to identify bacterial pathogens that cannot be cultured.
- Probes have been used to identify specific antibiotic resistance genes so that antimicrobial susceptibility of an infecting bacteria/pathogen can be identified directly without isolation and growth.

- Recombinant DNA (rDNA) is widely used in biotechnology, medicine and research. Proteins and other products that result from the use of rDNA technology are found in essentially every western pharmacy, doctor's or veterinarian's office, medical testing laboratory, and biological research laboratory.
- Organisms that have been manipulated using recombinant DNA technology, and products derived from those organisms have found their way into many farms, supermarkets, home medicine cabinets, and even pet shops.

- Biochemical products of recombinant DNA technology in medicine and research include: human recombinant insulin, growth hormone, blood clotting factors, hepatitis B vaccine, and diagnosis of HIV infection.
- Biochemical products of recombinant DNA technology in agriculture include: golden rice, herbicide-resistant crops, and insect-resistant crops.

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