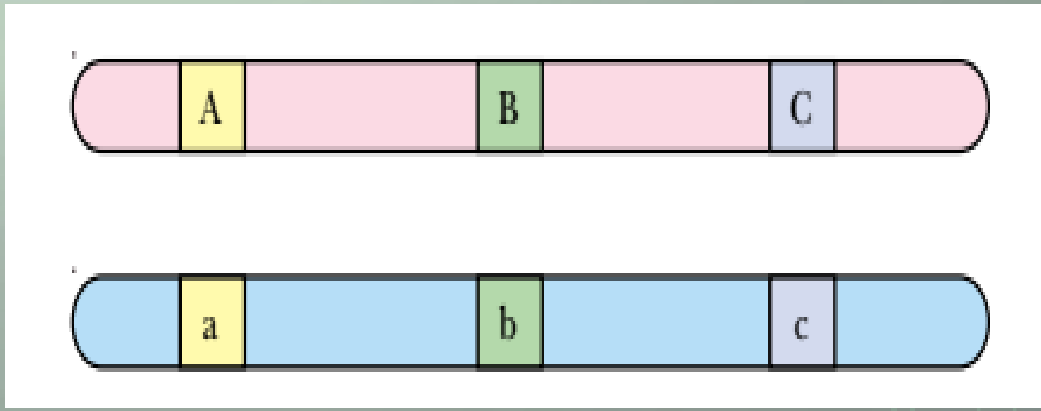
RECOMBINATION AND LINKAGE

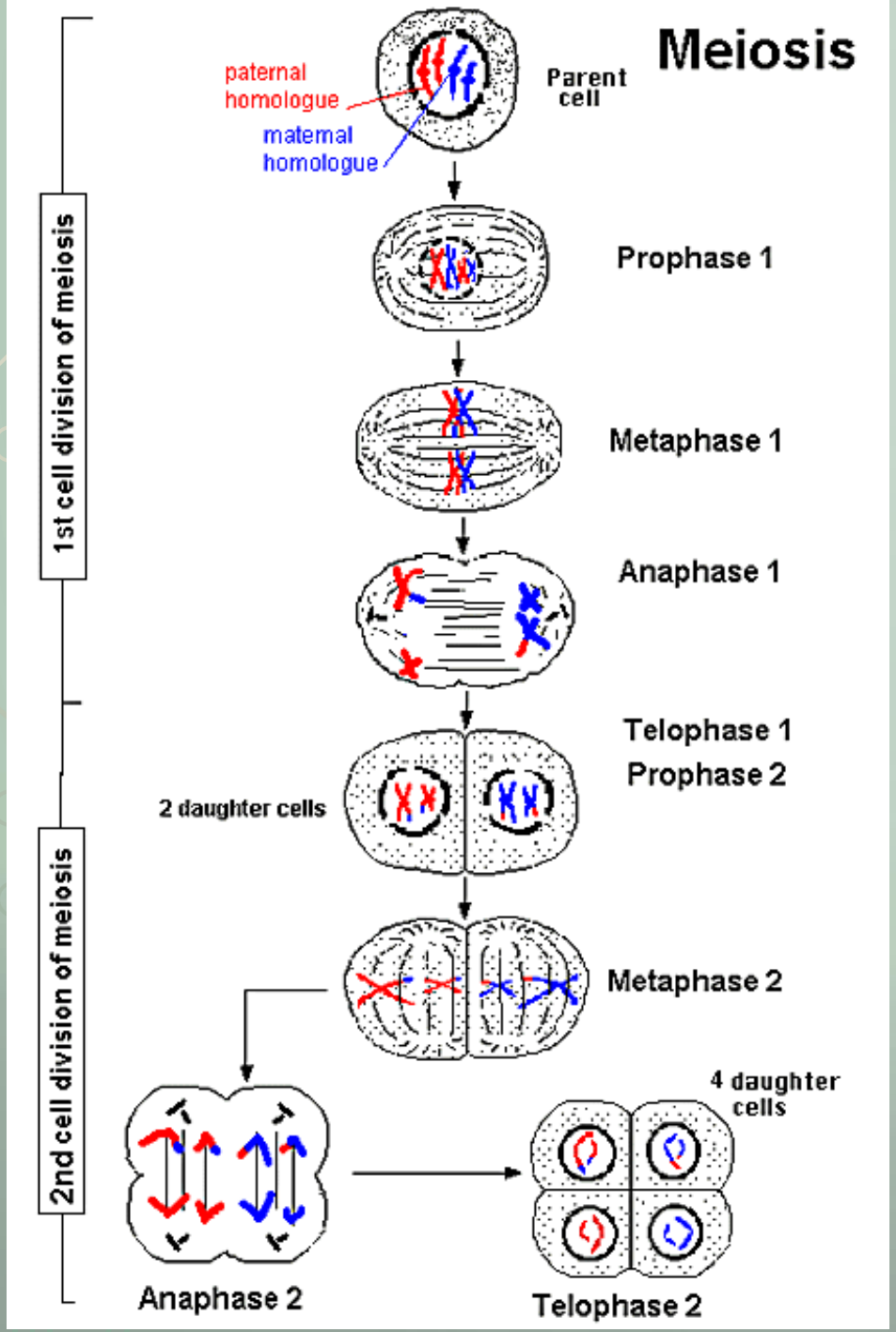
LINKAGE

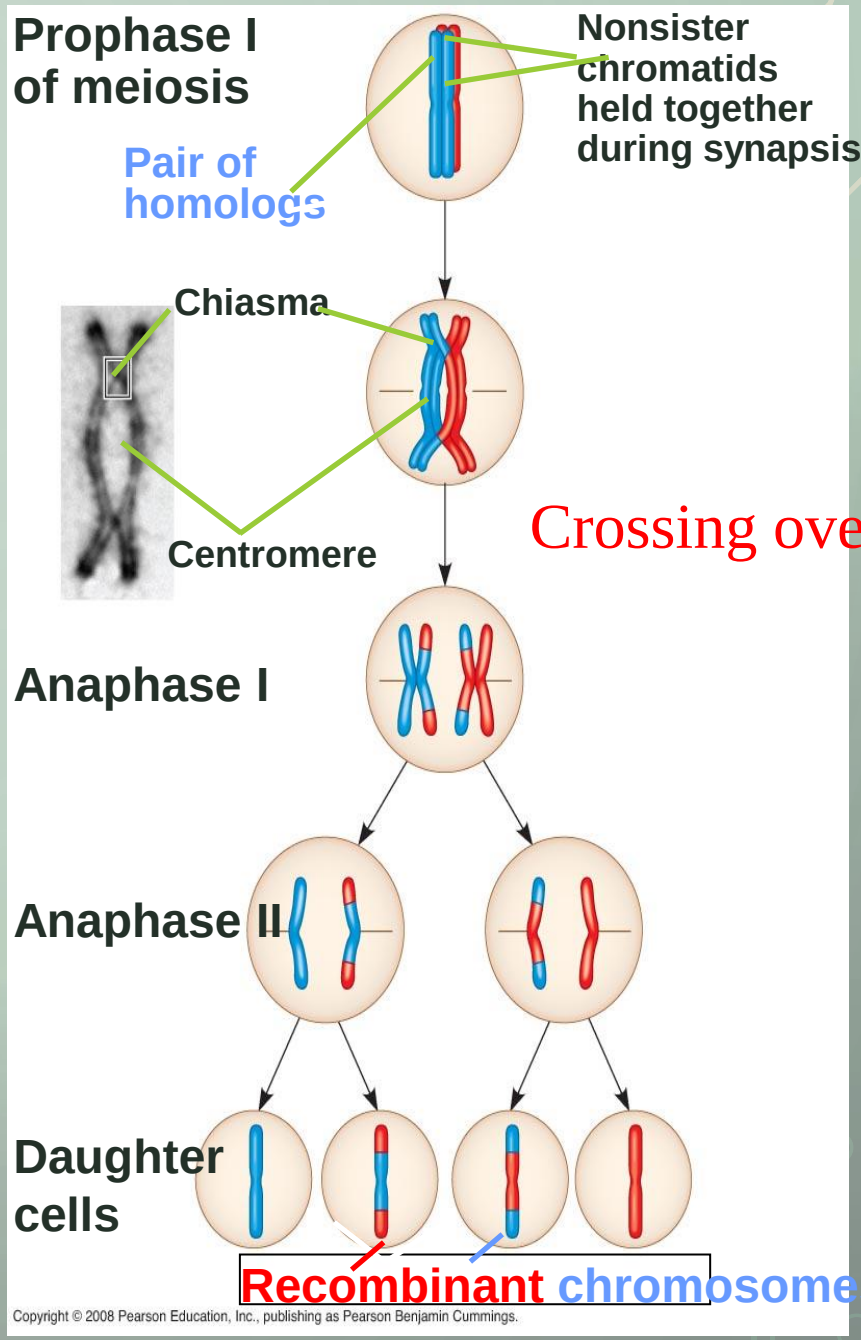
- When the two members of a chromosome pair are shown, each carrying different alleles. Because the three alleles A, B, and C are on the same molecule of DNA, they will tend to stay together.
- So if the offspring inherits allele A from one parent, it will usually get alleles B and C, rather than b and c. The genes A, B and C are said to be linked and the phenomenon is termed linkage.
- Genetic linkage is the tendency of alleles carried by the same DNA molecule to be inherited together.



RECOMBINATION DURING MEIOSIS

- However, the alleles A, B, and C (or a, b, and c) **do not *always* stay together during reproduction.**
- **Crossing over** occurs during the process of meiosis when the gametes are formed.
  - First, the chromosome carrying a specific sequence of genes lines up next to the homologous chromosome with allele sites matching.
  - Second, swapping of segments of the chromosomes can now occur by breaking and rejoining of the neighboring DNA strands.
- Note that the breaking and joining occurs in equivalent regions of the two chromosomes and neither chromosome gains or loses any genes overall.
- The genetic result of such crossing over, the shuffling of different alleles between the two members of a chromosomal pair, is called **recombination** or **crossing over** .
- **recombination** occurs at many sites along a pair of chromosomes.


Meiosis
paternal homologue
maternal homologue
Parent cell
Prophase 1
Metaphase 1
Anaphase 1
Telophase 1
Prophase 2
2 daughter cells
Metaphase 2
4 daughter cells
Anaphase 2
Telophase 2
1st cell division of meiosis
2nd cell division of meiosis


Prophase I of meiosis
Nonsister chromatids held together during synapsis
Pair of homologs
Chiasma
Centromere
Crossing over
Anaphase I
Anaphase II
Daughter cells
Recombinant chromosomes
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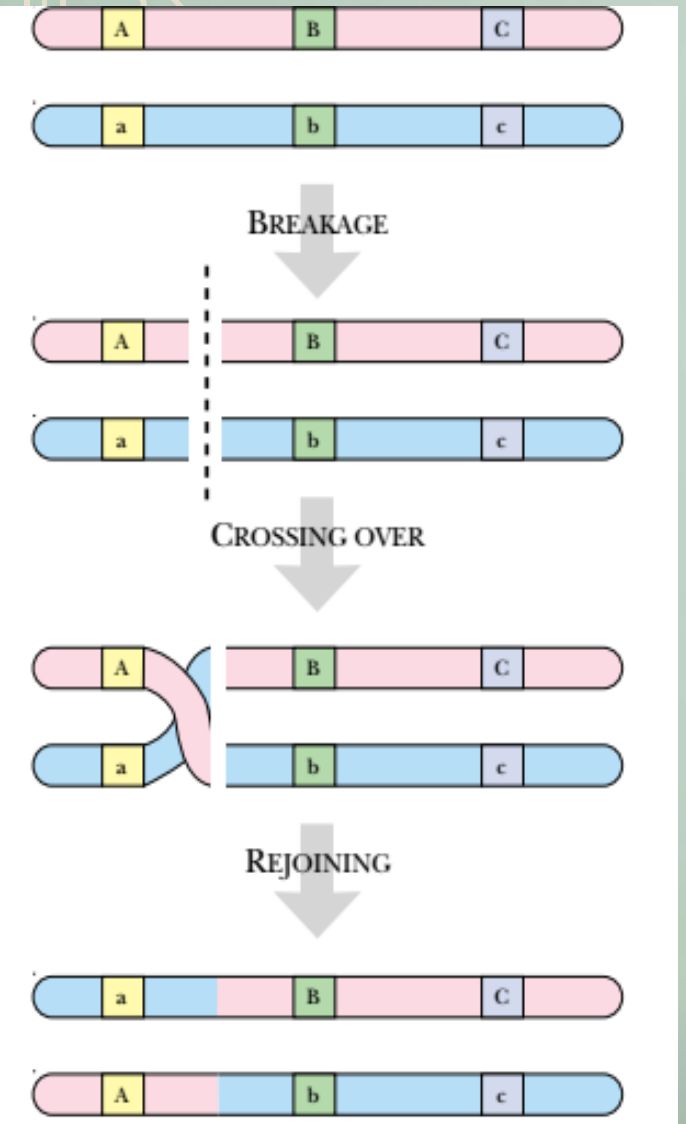
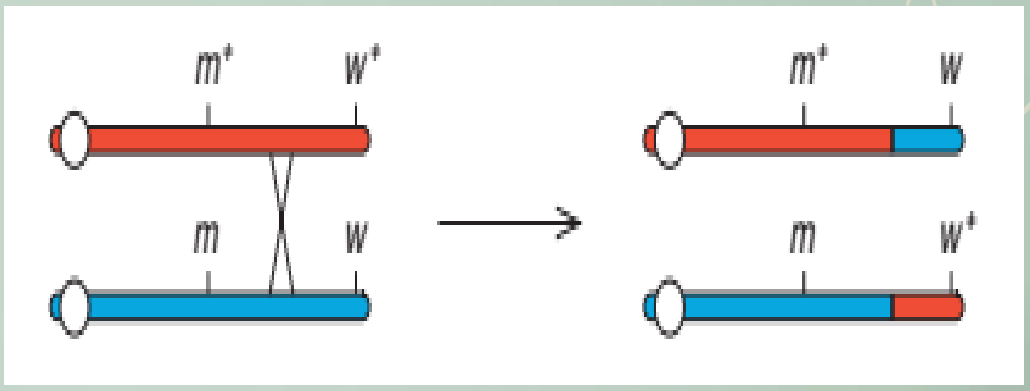
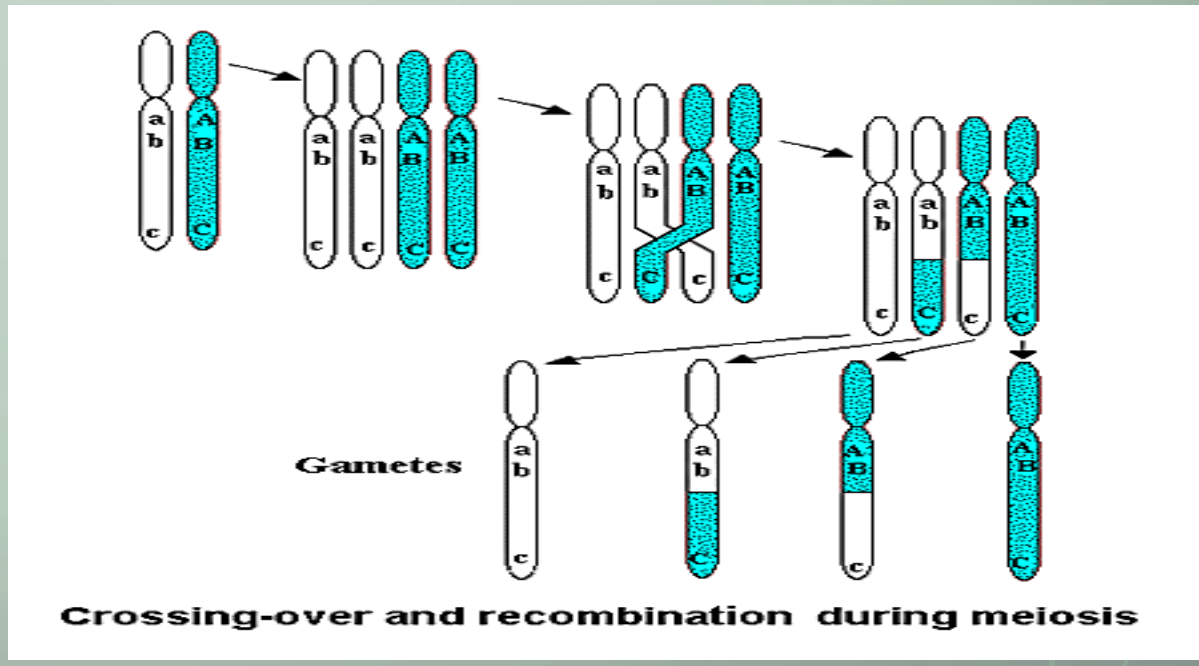






Recombination during Meiosis Ensures Genetic Diversity

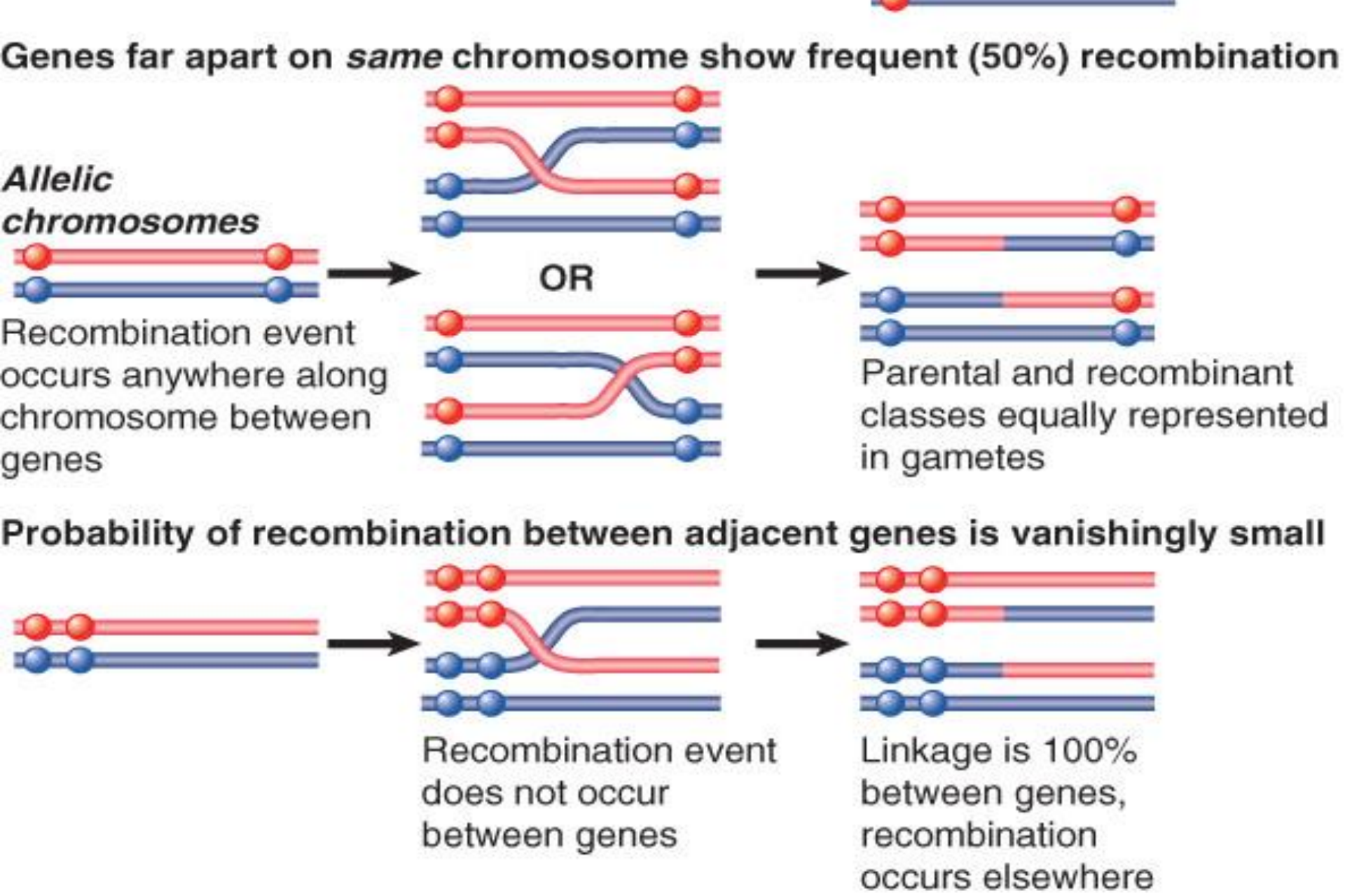


Figure : Genes on different chromosomes segregate independently so that all possible combinations of alleles are produced in equal proportions.

- Causes of genetic variation:
  - Independent orientation of chromosomes in meiosis
  - Crossing over of chromosomes in meiosis
  - Random fertilization
- Definition of recombination:

Breaking and rejoining of two parental DNA molecules to produce new DNA molecules

TYPES OF RECOMBINATION

1. Homologous - occurs between sequences that are nearly identical (during meiosis)

The exchange of homologous regions between two DNA molecules occurs extensively in eukaryotes during meiosis.

In prokaryotes, *recA*-dependent recombination

2. Site-Specific - occurs between sequences with a limited stretch of similarity; involves specific sites.

This involves the exchange of nonhomologous but specific pieces of DNA and **recombination** is mediated by proteins that recognize specific DNA sequences.

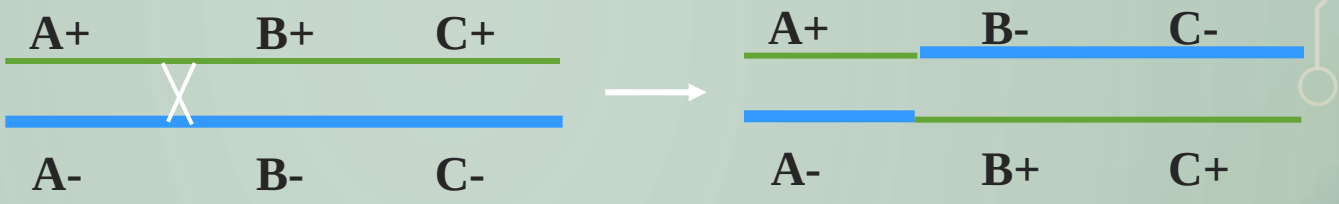
- Bacteriophage has the ability to insert its genome into a specific site on the *E. coli* chromosome. The attachment site on the DNA shares an identical 15 bp sequence with the attachment site on the *E. coli* DNA.

3. Transposition – DNA element moves from one site to another, usually little sequence similarity involved .

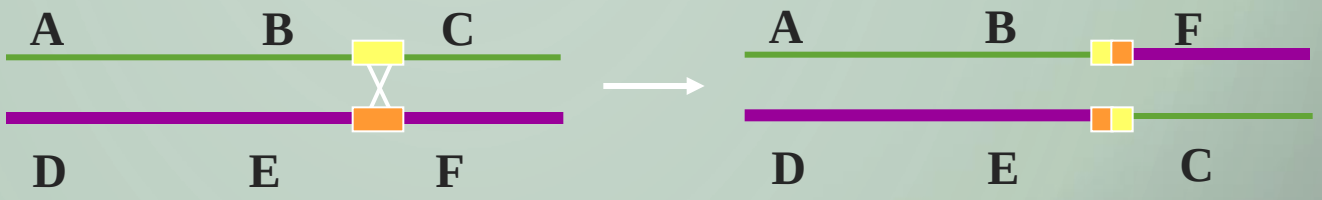
Transposons-DNA sequence that moves by a mechanism that cuts the sequence from its original location in the genome and inserts it in a different site

TYPES OF RECOMBINATION

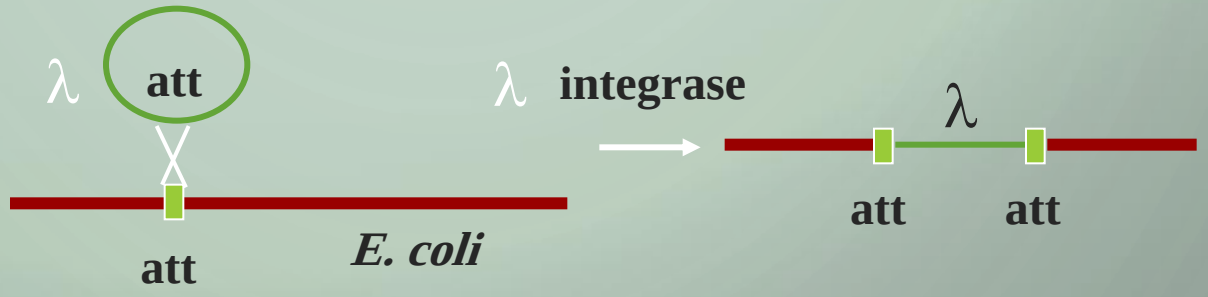
Homologous or general



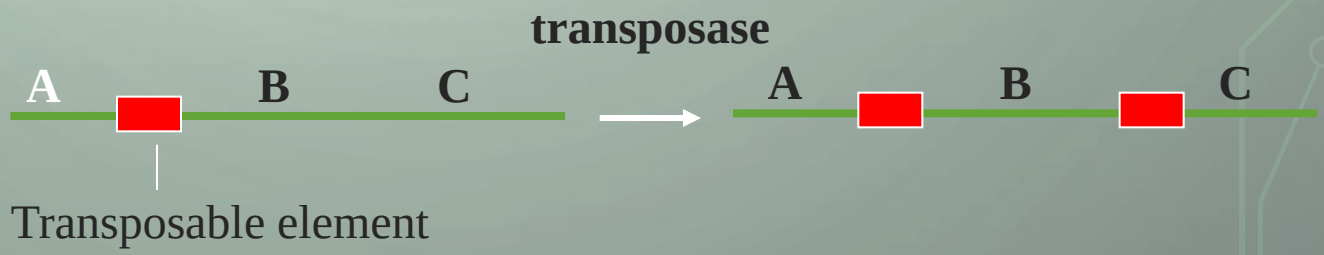
Nonhomologous or illigitimate

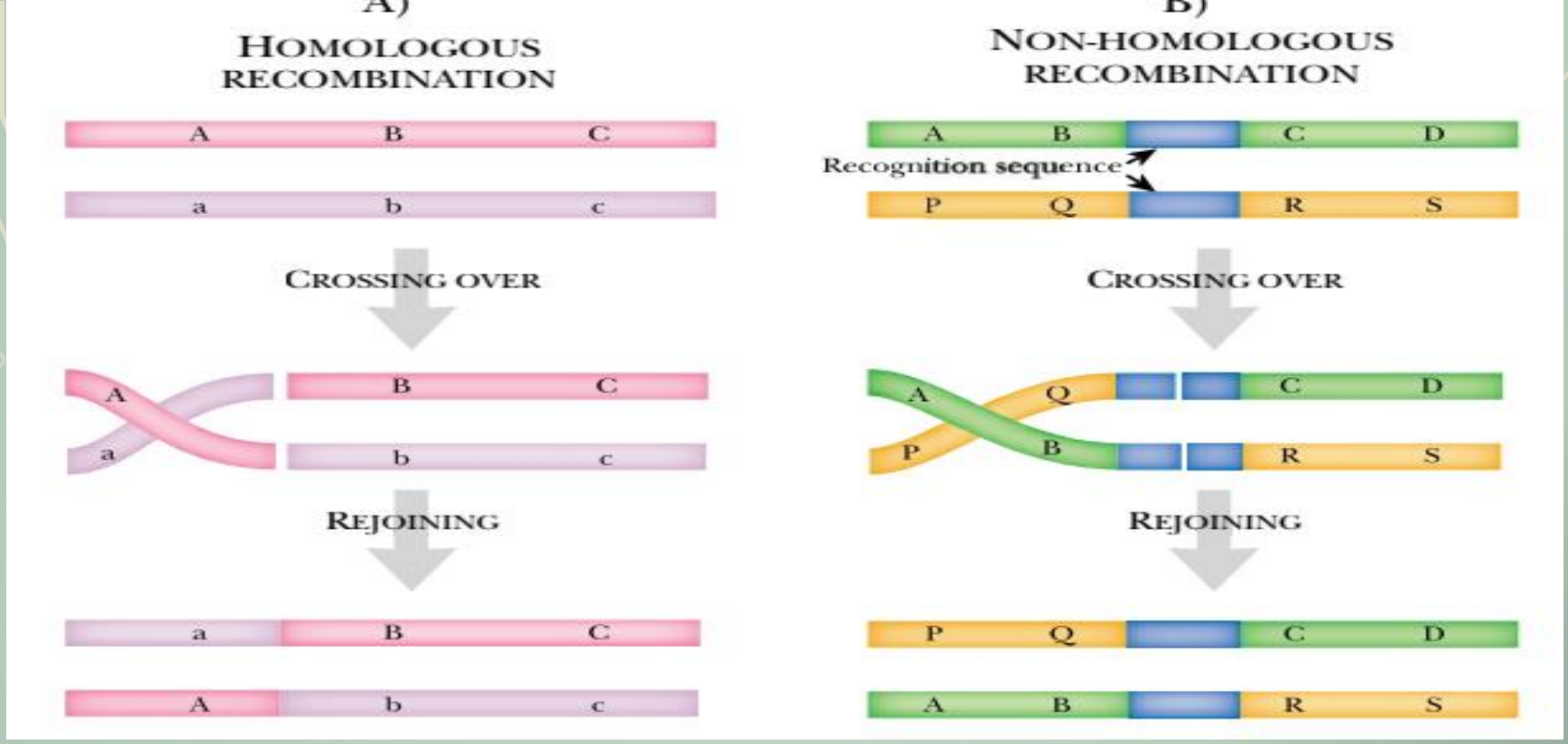


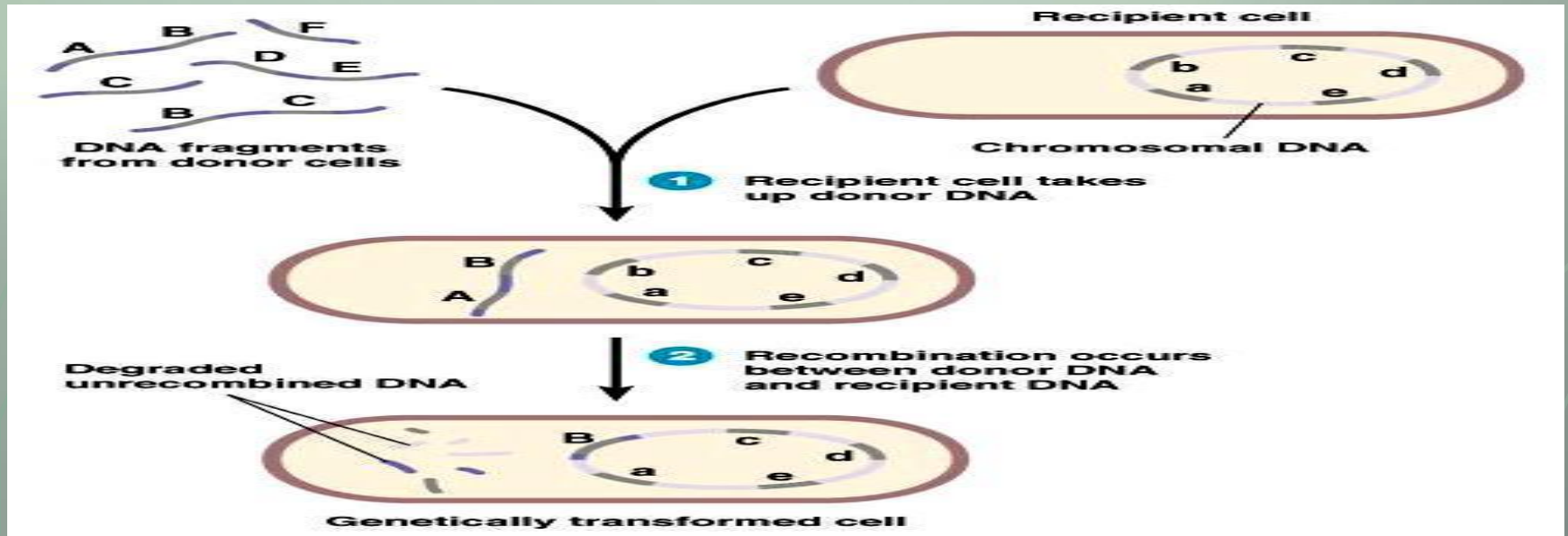
Site-specific

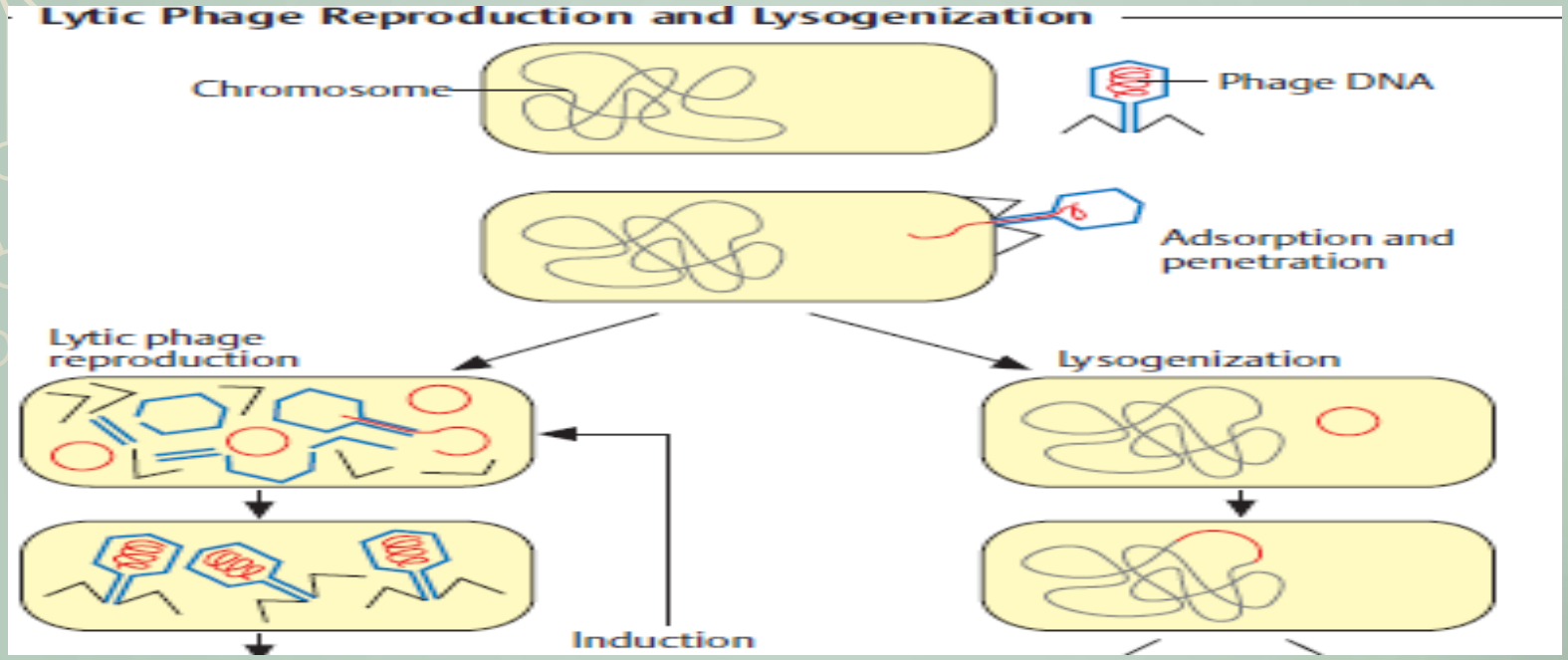


Replicative recombination, transposition




A)
HOMOLOGOUS RECOMBINATION
A B C
a b c
CROSSING OVER
A B C
a b c
REJOINING
a B C
A b c
B)
NON-HOMOLOGOUS RECOMBINATION
A B C D
Recognition sequence
P Q R S
CROSSING OVER
A Q C D
P B R S
REJOINING
P Q C D
A B R S


A B F
C D E
B C
DNA fragments from donor cells
Recipient cell
b c d
a e
Chromosomal DNA
1 Recipient cell takes up donor DNA
B A
b c d
a e
2 Recombination occurs between donor DNA and recipient DNA
Degraded unrecombined DNA
B c d
a e
Genetically transformed cell



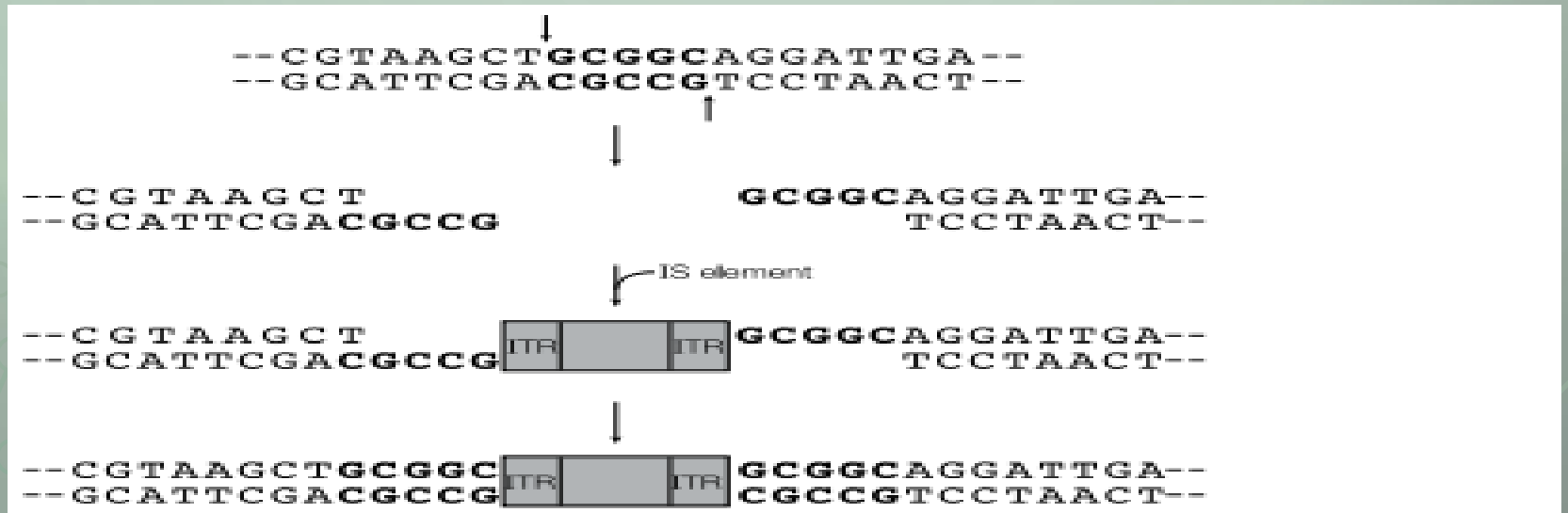


Fig. 2. Transposition of an insertion sequence (IS) element into a host DNA with duplication of the target site (shown in bold). ITR, inverted terminal repeat sequence.

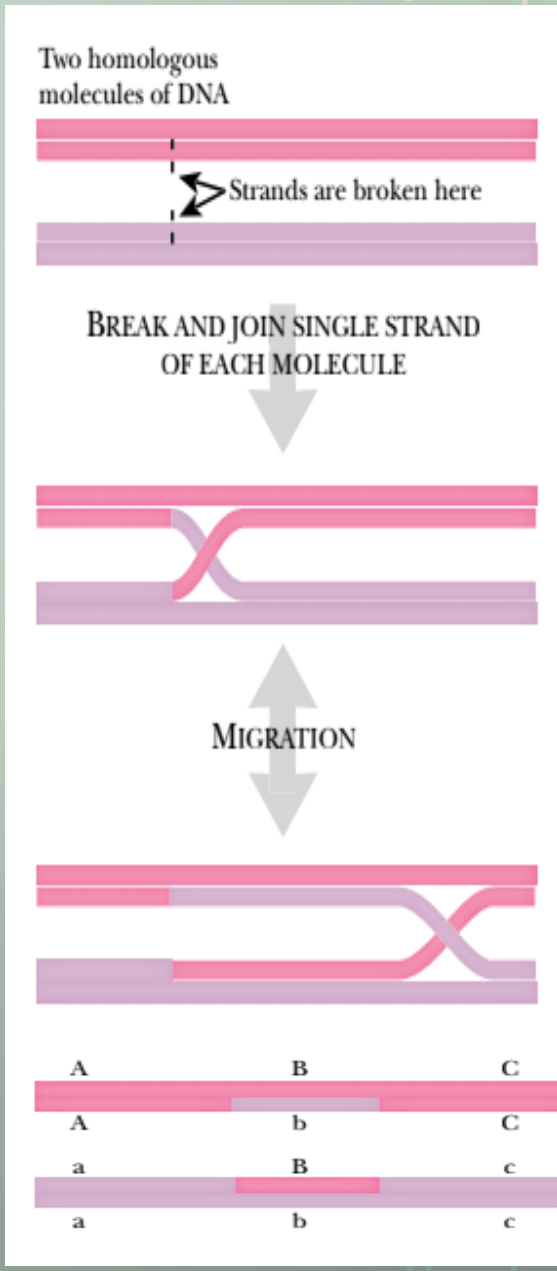
4 REQUIREMENTS FOR RECOMBINATION

1. Identical or very similar DNA sequences in the cross over region.
2. Complementary base pairing between double stranded DNA molecules. This ensures that recombination will occur only at these sequences.
3. Recombination enzymes-the machinery of the recombination complex.

a. **Endonuclease nicks at corresponding regions of the same strands of homologous chromosomes.**

b. **Ligase seals nicks to form a joint molecule.**

4. Heteroduplex formation- complementary base pairing between two DNA molecules in a synapse.



BIOLOGICAL ROLES FOR RECOMBINATION

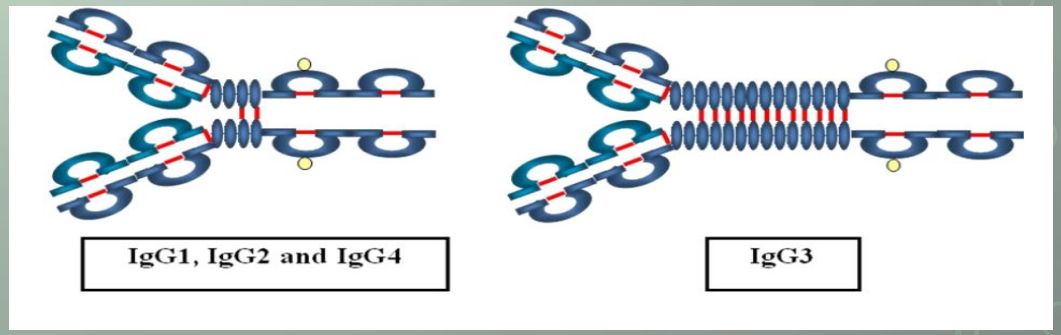
1. Generating new gene/allele combinations (crossing over during meiosis)
2. Generating new genes (e.g., Immuno- globulin rearrangement)

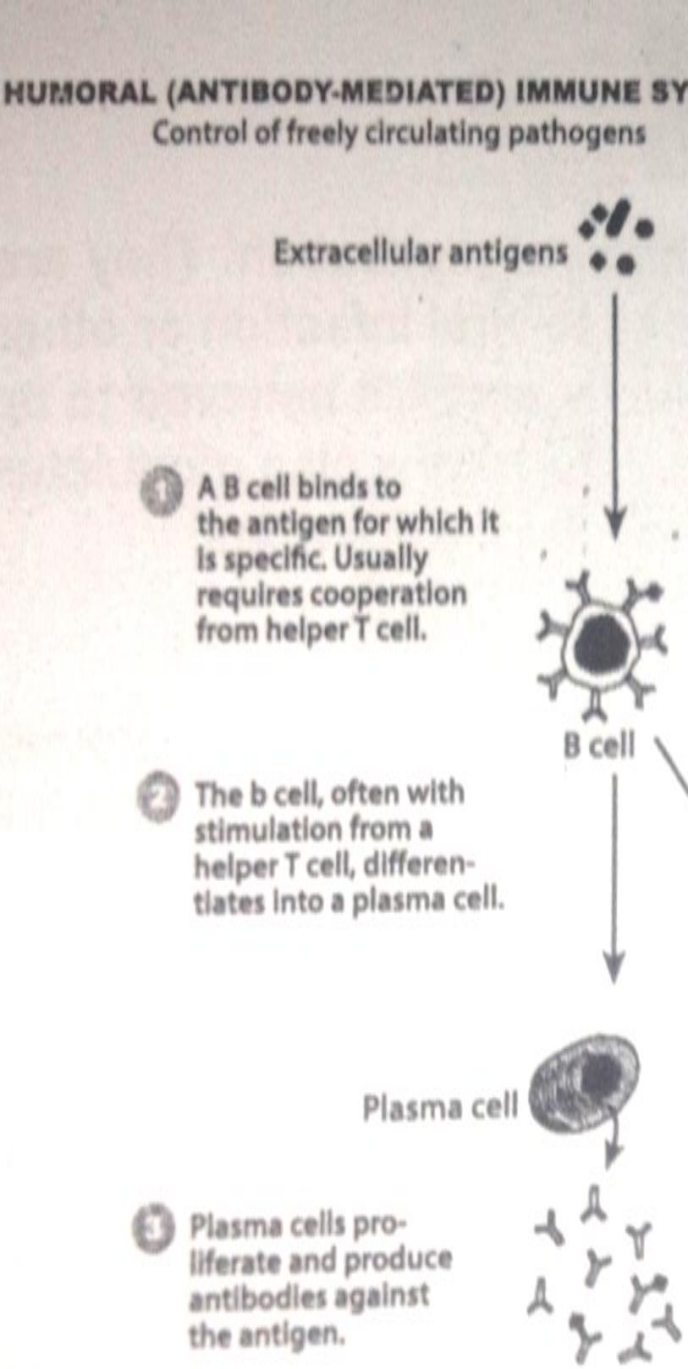
: the generation of antibody diversity in animals.

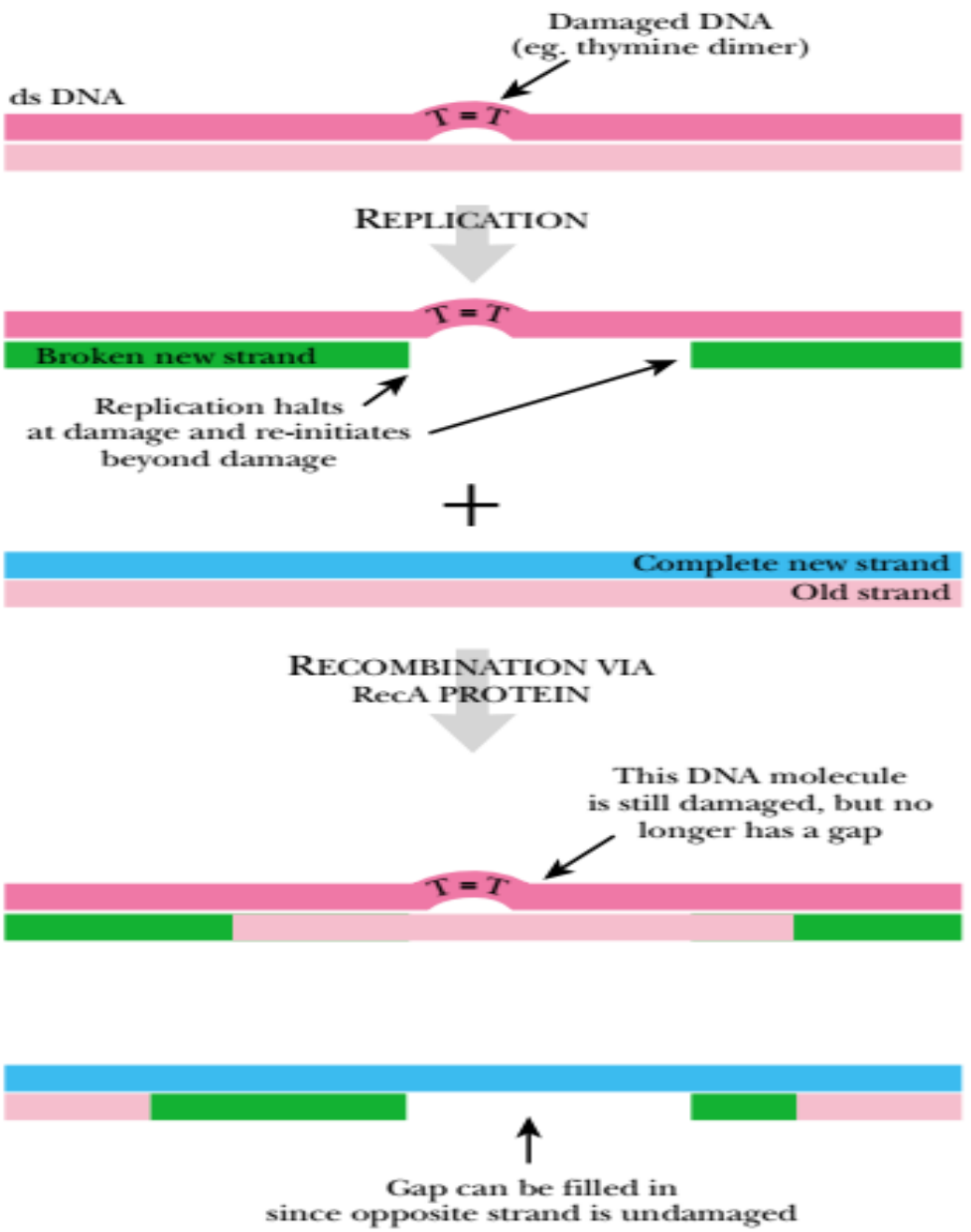
- Site-specific recombination is responsible for the generation of antibody diversity during differentiation of antibody-producing cells.

3. Integration of a specific DNA element (or virus) : Integration of bacteriophage into the *E. coli* genome

4. DNA repair







ingle-stranded DNA

Recombinant DNA(rDNA)

- DNA molecules formed by laboratory methods of genetic recombination to bring together genetic material from multiple sources, creating sequence that would not otherwise be found in the genome.

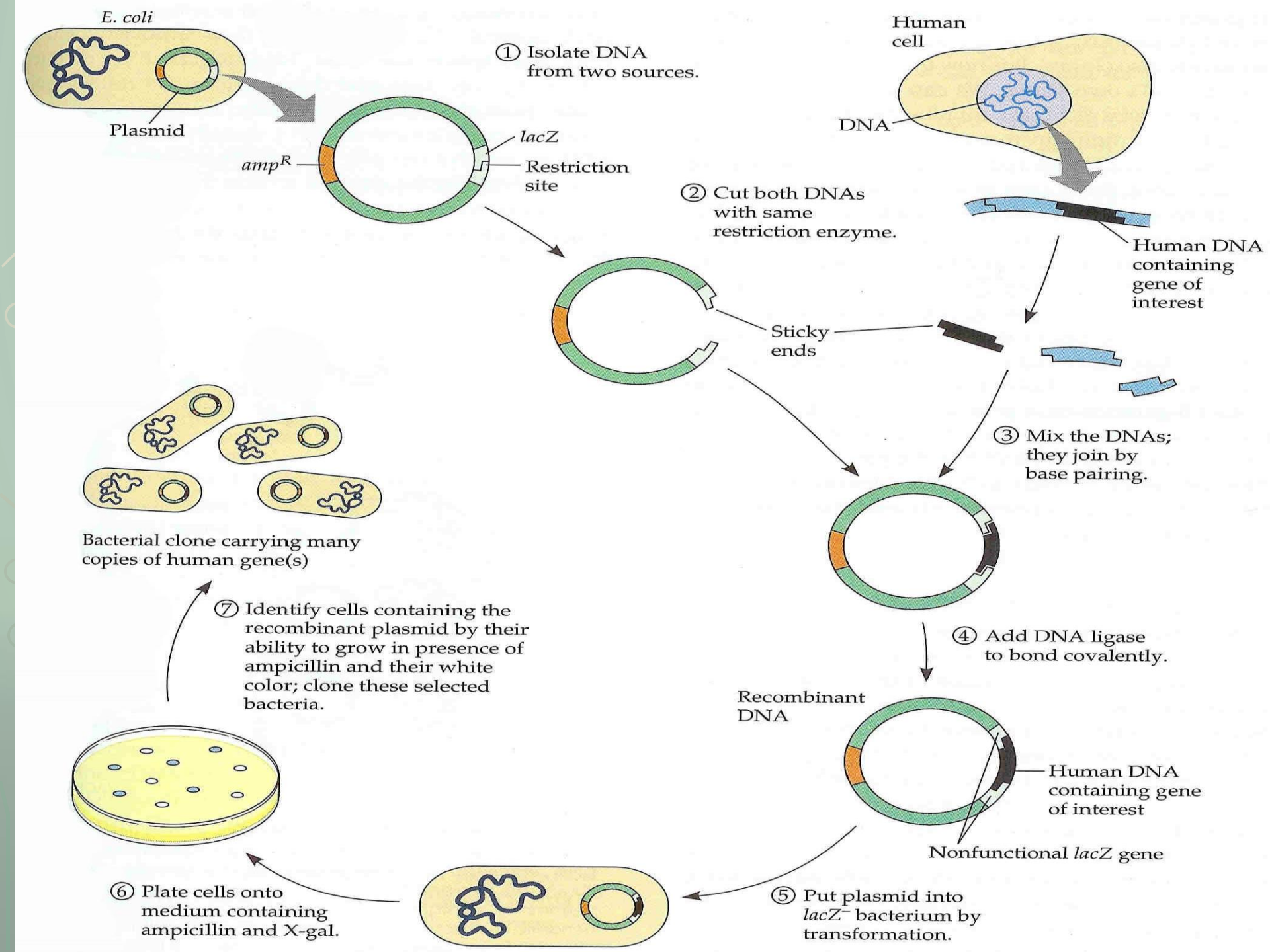
Recombinant DNA Technology

isolate and clone single copy of a gene or a DNA segment into an indefinite number of copies, all identical.

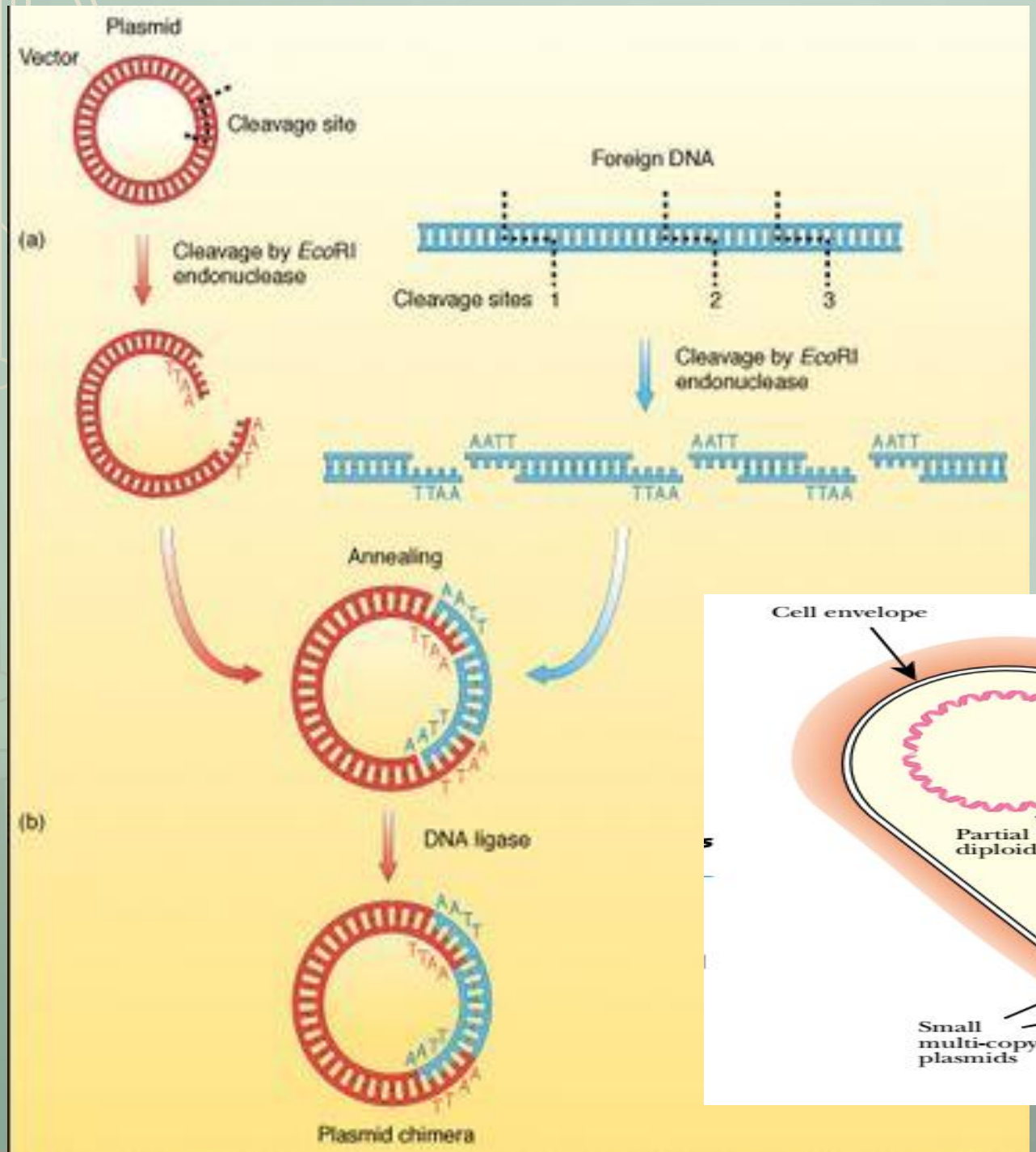
These new combinations (Recombinant DNA (rDNA) are introduced into the host cells, where they propagate and multiply.

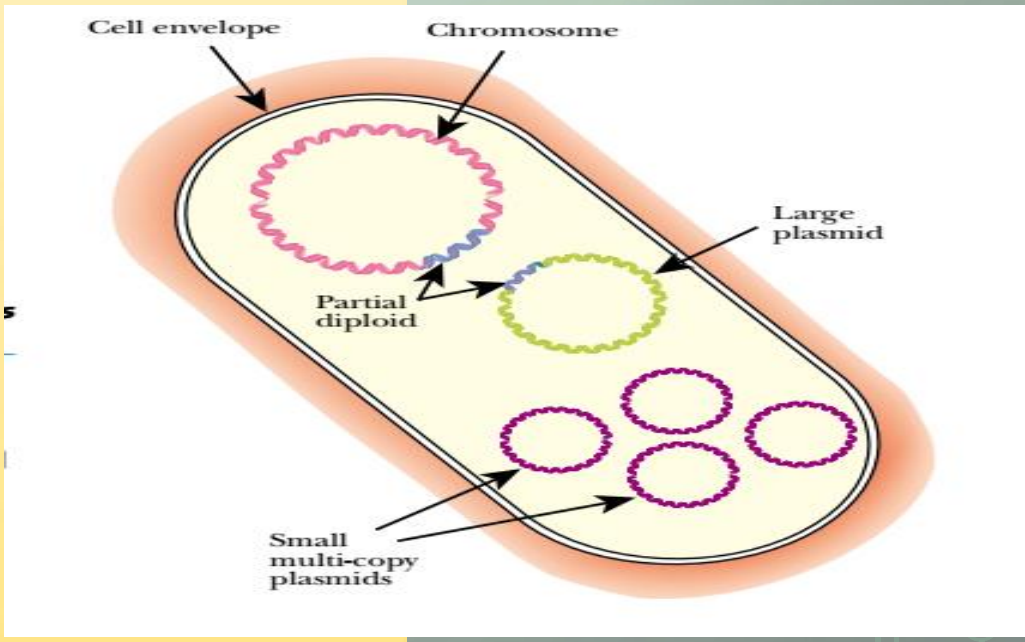
Basic Steps of Genetic Modification

1. Isolating a gene to be inserted
2. Inserting the gene in a Vector(Agent used to carry foreign gene)
3. Inserting Vector into the host.
4. Multiplication of host cells by cloning.
5. Extraction of desired product.

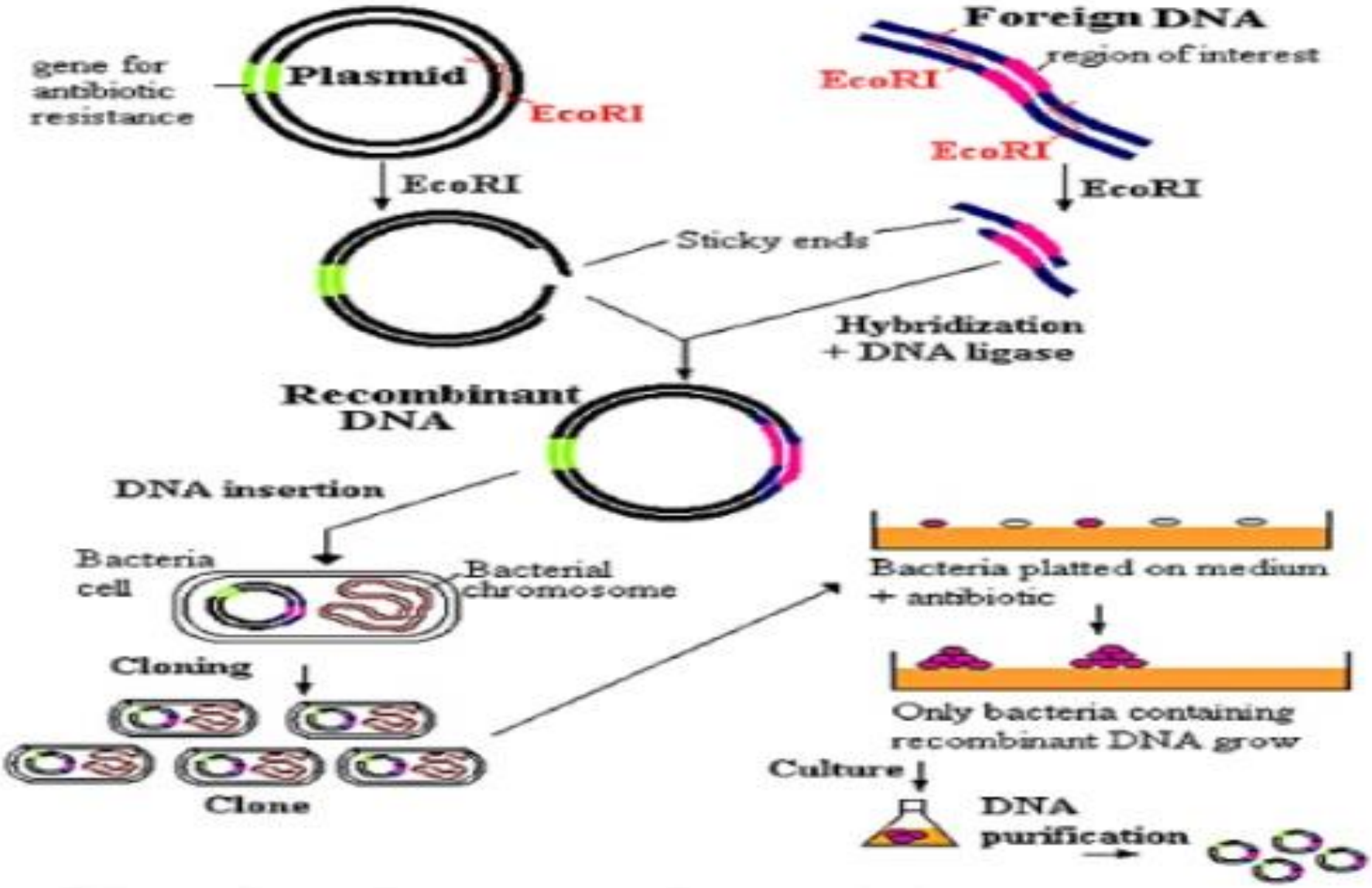

E. coli
Plasmid
① Isolate DNA from two sources.
Human cell
DNA
lacZ
ampR
Restriction site
② Cut both DNAs with same restriction enzyme.
Human DNA containing gene of interest
Sticky ends
③ Mix the DNAs; they join by base pairing.
Bacterial clone carrying many copies of human gene(s)
⑦ Identify cells containing the recombinant plasmid by their ability to grow in presence of ampicillin and their white color; clone these selected bacteria.
④ Add DNA ligase to bond covalently.
Recombinant DNA
Human DNA containing gene of interest
Nonfunctional lacZ gene
⑥ Plate cells onto medium containing ampicillin and X-gal.
⑤ Put plasmid into lacZ⁻ bacterium by transformation.

Joining DNA


Plasmid
Vector
Cleavage site
Foreign DNA
(a)
Cleavage by EcoRI endonuclease
Cleavage sites 1 2 3
Cleavage by EcoRI endonuclease
AATT
TTAA
Annealing
(b)
DNA ligase
Plasmid chimera


Cell envelope
Chromosome
Large plasmid
Partial diploid
Small multi-copy plasmids

Cloning into a Plasmid



EXAMPLES OF USE OF RECOMBINANT DNA TECHNOLOGY

- **DNA ligase enzyme splices (joins) together the plasmid DNA and the insulin DNA.**
- **The plasmid (now genetically modified) is inserted back into the bacterium.**
- **The bacterium host cell, divides and produces copies of the plasmid.**
- **The Bacterium makes human insulin using the gene in the plasmid.**
- **The insulin is extracted from the bacterial culture.**

Production of an Insulin

