Gene Cloning

- The production of exact copies of a particular gene or DNA sequence using genetic engineering techniques is called gene cloning.

- The term “gene cloning,” “DNA cloning,” “molecular cloning,” and “recombinant DNA technology” all refer to same technique.

- In gene (DNA) cloning a particular gene is copied forming “clones”.

DNA cloning can be achieved by two different methods:

1. Cell based DNA cloning
2. Cell-free DNA cloning (PCR)

Requirements for Cell Based Gene Cloning

1. DNA fragment containing the desired genes to be cloned.
2. Restriction enzymes and ligase enzymes.
3. Vectors – to carry, maintain and replicate cloned gene in host cell.
4. Host cell– in which recombinant DNA can replicate.

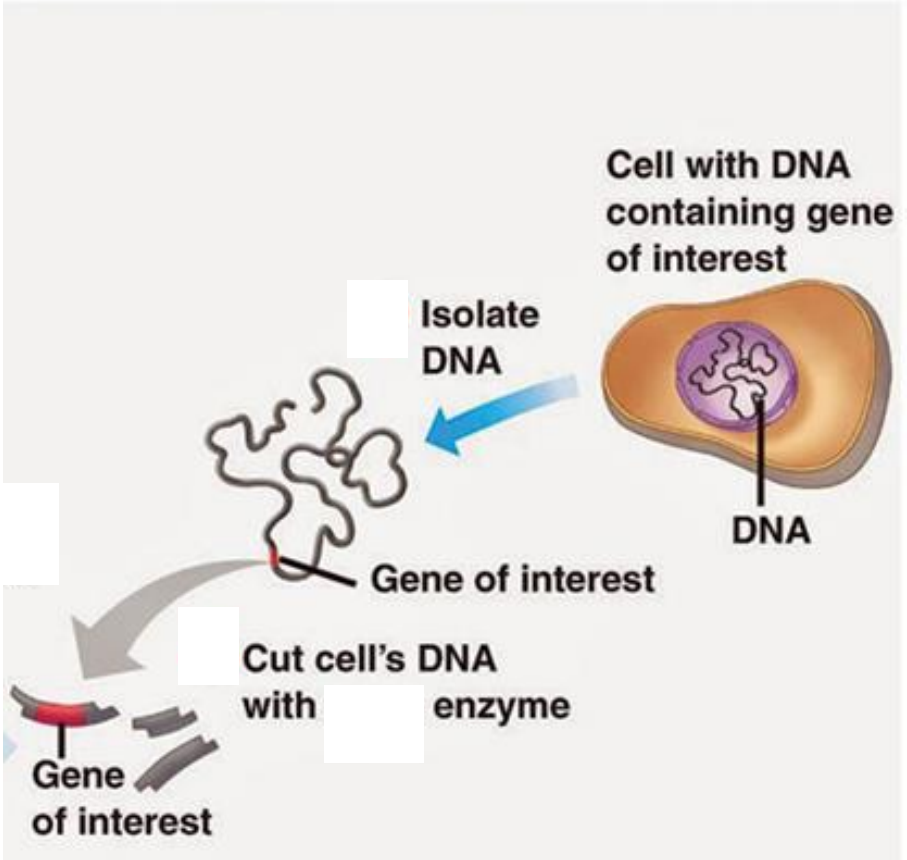
Steps in Gene Cloning

The basic 7 steps involved in gene cloning are;

1. Isolation of DNA (gene of interest - GI) fragments to be cloned.
2. Insertion of isolated DNA into a suitable vector to form recombinant DNA.
3. Introduction of recombinant DNA into a suitable organism known as host.
4. Selection of transformed host cells and identification of the clone containing the gene of interest.
5. Multiplication/Expression of the introduced Gene in the host.
6. Isolation of multiple gene copies/Protein expressed by the gene.
7. Purification of the isolated gene copy/protein

Isolation of the DNA fragment or gene

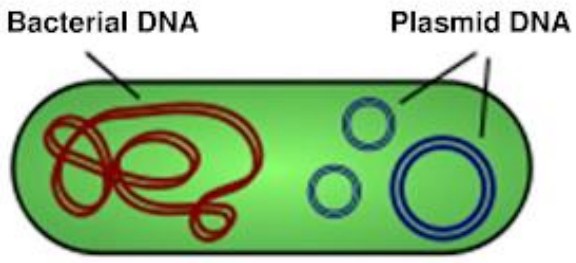
- The target DNA or gene to be cloned must be first isolated. A gene of interest is a fragment of gene whose product (a protein, enzyme or a hormone) interests us. For example, gene encoding for the hormone insulin.
- The desired gene may be isolated by using restriction endonuclease (RE) enzyme, which cut DNA at specific recognition nucleotide sequences known as restriction sites producing blunt or sticky ends.



2. Selection of suitable cloning vector

- The vector is a carrier molecule which can carry the gene of interest (GI) into a host, replicate there along with the GI making its multiple copies.
- The cloning vectors are limited to the size of insert that they can carry.
- Depending on the size and the application of the insert the suitable vector is selected.
- The different types of vectors available for cloning are plasmids, bacteriophages, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs) and mammalian artificial chromosomes (MACs).
- However, the most commonly used cloning vectors include plasmids and bacteriophages (phage λ) beside all the other available vectors.

Essentially, plasmids are small, circular molecules of DNA that are capable of replicating independently. As such, they do not rely on chromosomal DNA of the organism for replication. Because of this characteristic, they are also referred to as extra-chromosomal DNA.

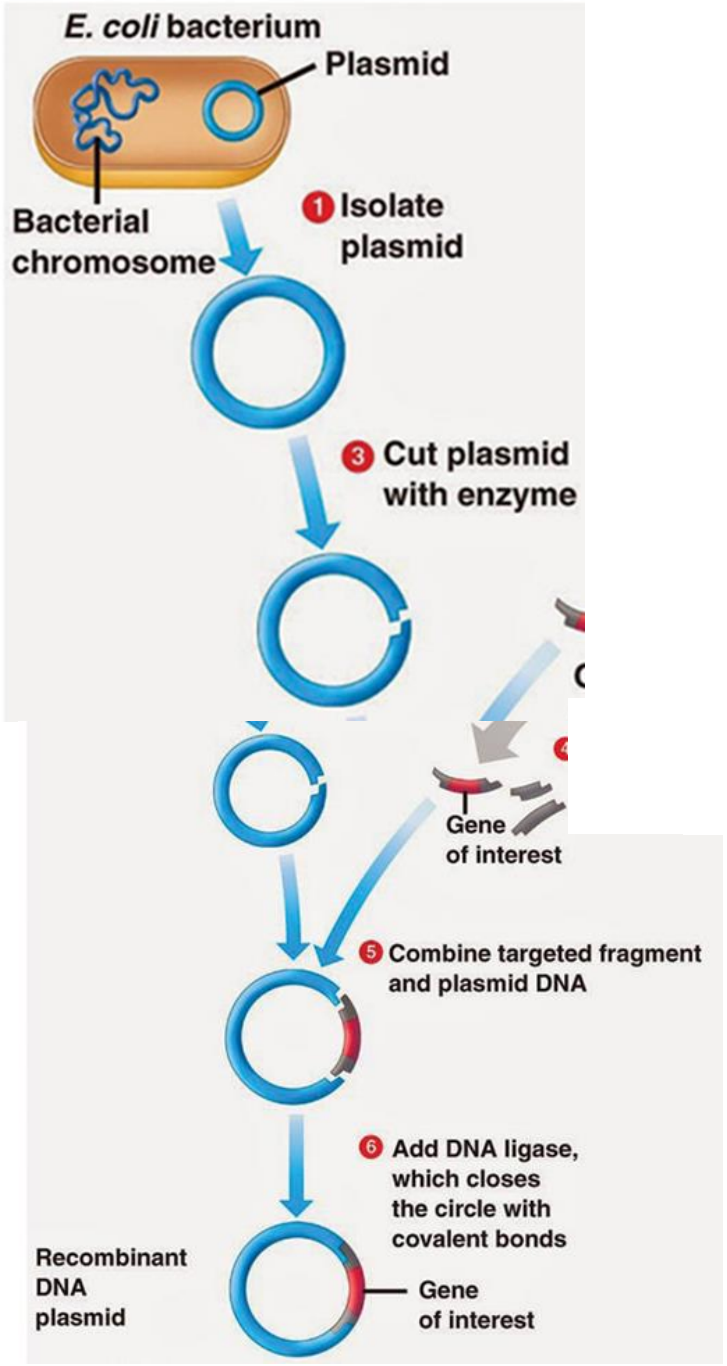


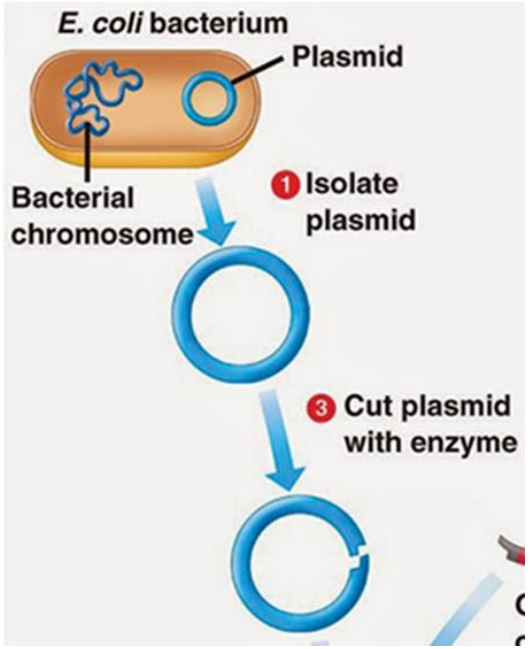
Essential Characteristics of Cloning Vectors

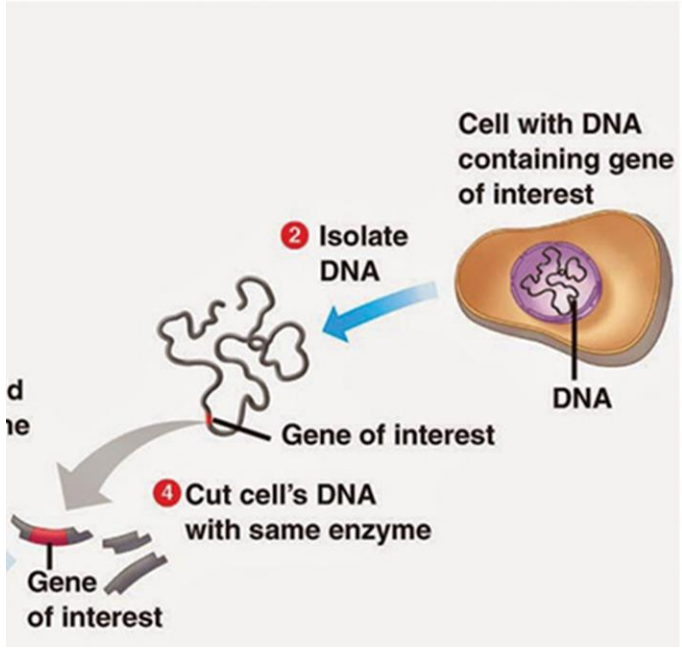
1. It must be self-replicating inside host cell.
2. It must possess a unique restriction site for RE enzymes.
3. Introduction of donor DNA fragment must not interfere with replication property of the vector.
4. It must possess some marker gene such that it can be used for later identification of recombinant cell (usually an antibiotic resistance gene that is absent in the host cell).
5. They should be easily isolated from host cell.

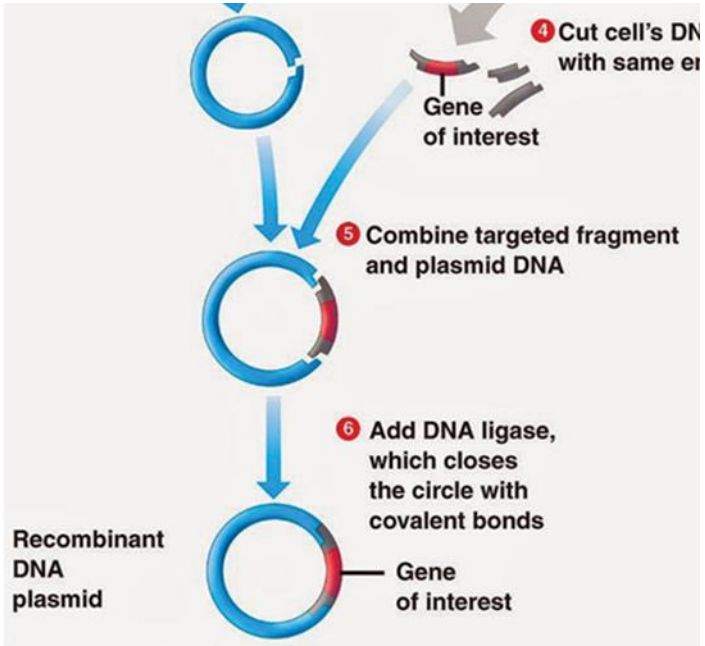
Formation of Recombinant DNA

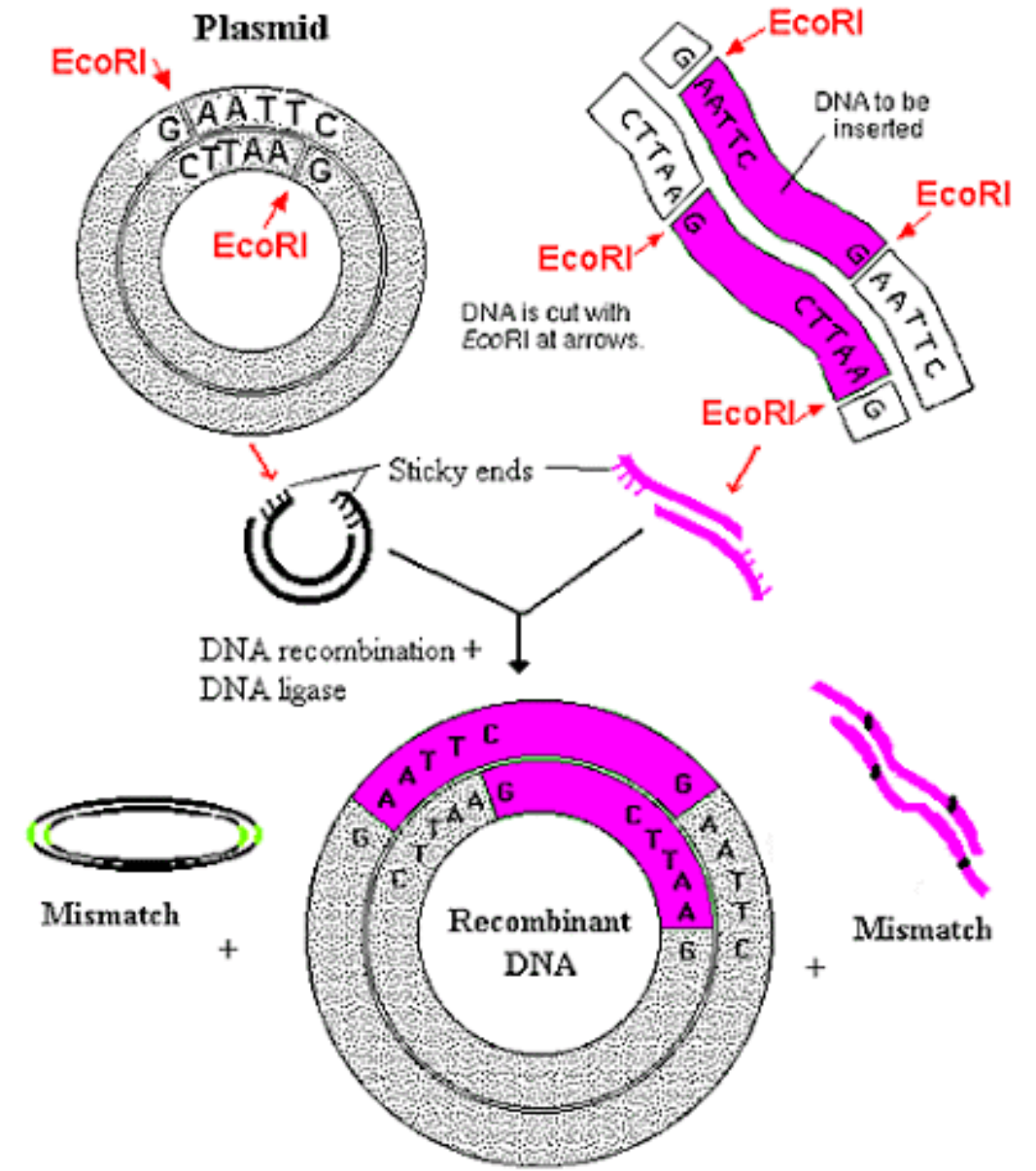
- The plasmid vector is cut open by the RE enzyme used for isolation of donor DNA fragment.
- The mixture of donor DNA fragment and plasmid vector are mixed together.
- In the presence of DNA ligase, base pairing of donor DNA fragment and plasmid vector occurs.
- The resulting DNA molecule is a hybrid of two DNA molecules – the GI and the vector.
- In the terminology of genetics this intermixing of different DNA strands is called recombination.
- Hence, this new hybrid DNA molecule is also called a recombinant DNA molecule and the technology is referred to as the recombinant DNA technology.











Inserting a DNA Sample into a Plasmid

Transformation of recombinant vector into suitable host

- The recombinant vector is transformed into suitable host cell mostly, a bacterial cell.
- This is done either for one or both of the following reasons:

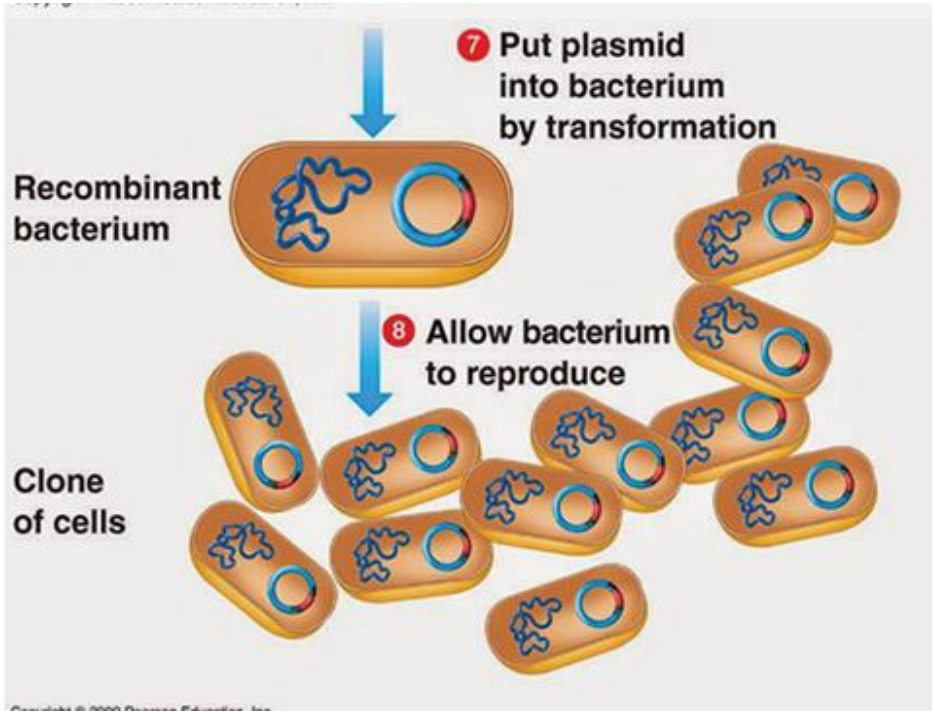
✓ To replicate the recombinant DNA molecule in order to get the multiple copies of the GI.

✓ To allow the expression of the GI such that it produces its needed protein product.

- Some bacteria are naturally transformable; they take up the recombinant vector automatically.

✓ For example: Bacillus, Haemophillus, Helicobacter pylori, which are naturally competent.

- Some other bacteria, on the other hand require the incorporation by artificial methods such as Ca++ ion treatment, electroporation, etc.



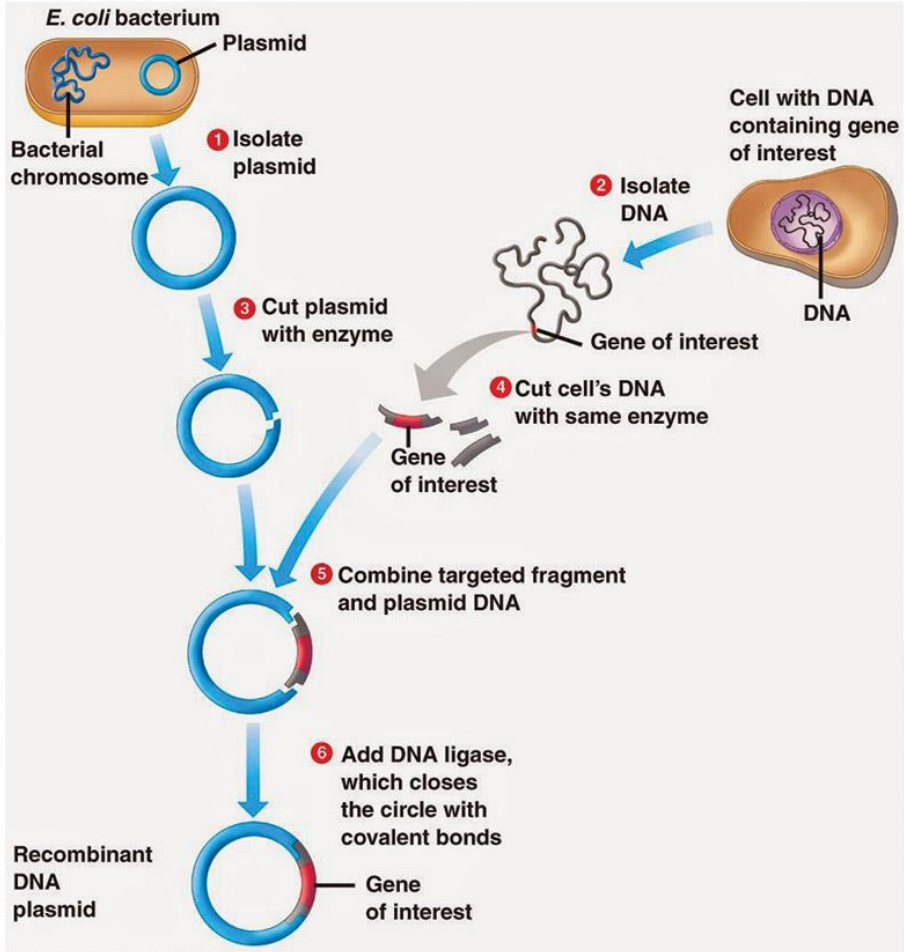
Isolation of Recombinant Cells

- The transformation process generates a mixed population of transformed and non-transformed host cells.
- The selection process involves filtering the transformed host cells only.
- For isolation of recombinant cell from non-recombinant cell, marker gene of plasmid vector is employed.

✓ For examples, PBR322 plasmid vector contains different marker gene (Ampicillin resistant gene and Tetracycline resistant gene. When pst1 RE is used it knock out Ampicillin resistant gene from the plasmid, so that the recombinant cell become sensitive to Ampicillin)

Multiplication of Selected Host Cells

- Once transformed host cells are separated by the screening process; It is necessary to provide them optimum parameters to grow and multiply.
- In this step the transformed host cells are introduced into fresh culture media .
- At this stage the host cells divide and re-divide along with the replication of the recombinant DNA carried by them.
- If the aim is obtaining numerous copies of GI, then simply replication of the host cell is allowed.
- But for obtaining the product of interest, favorable conditions must be provided such that the GI in the vector expresses the product of interest.


E. coli bacterium
Plasmid
Bacterial chromosome
1 Isolate plasmid
Cell with DNA containing gene of interest
2 Isolate DNA
DNA
Gene of interest
3 Cut plasmid with enzyme
4 Cut cell's DNA with same enzyme
Gene of interest
5 Combine targeted fragment and plasmid DNA
6 Add DNA ligase, which closes the circle with covalent bonds
Recombinant DNA plasmid
Gene of interest
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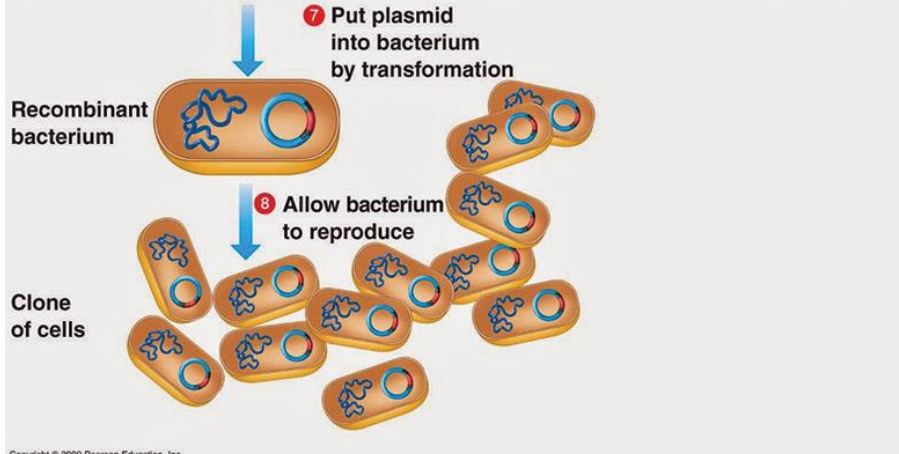
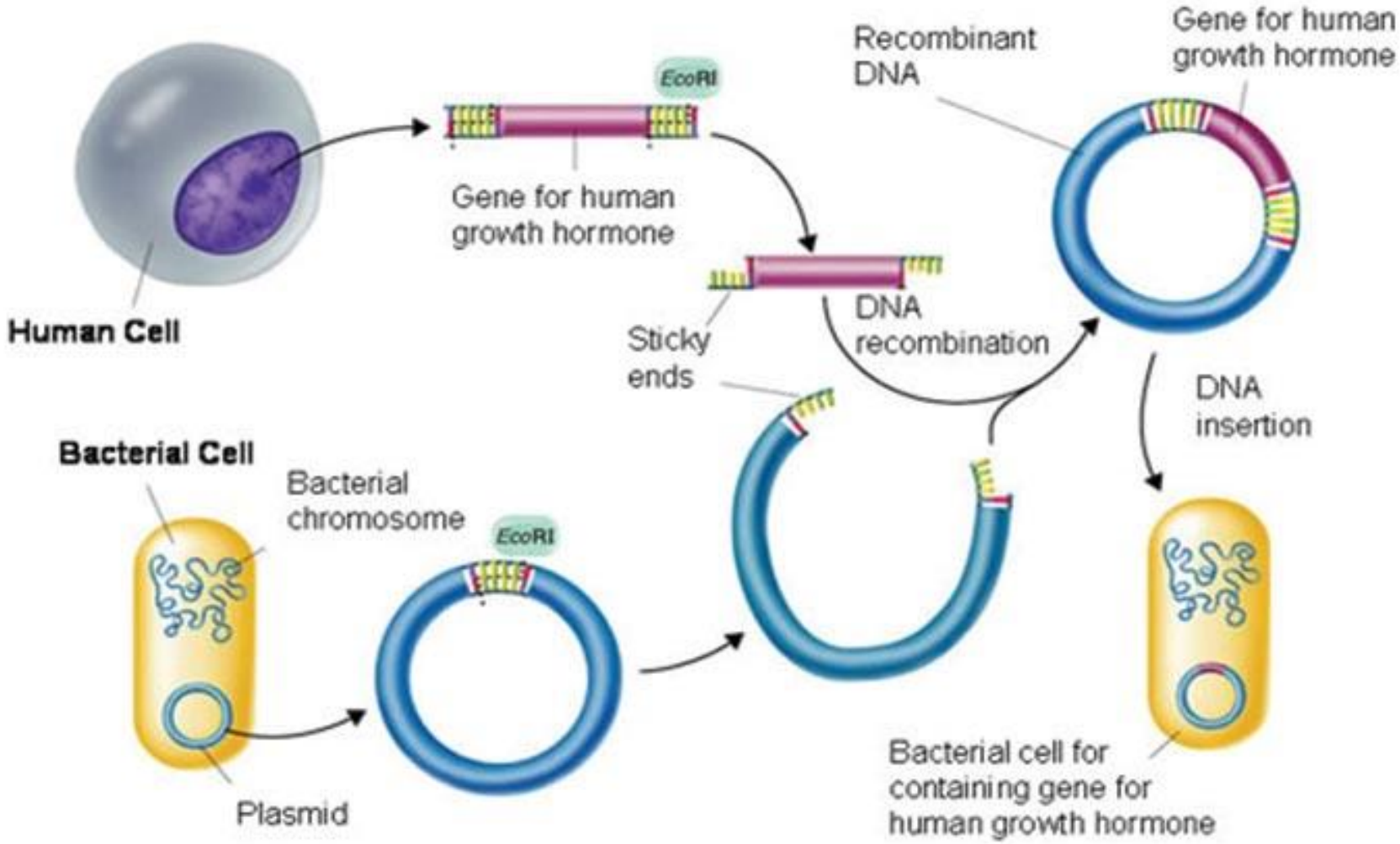

7 Put plasmid into bacterium by transformation
Recombinant bacterium
8 Allow bacterium to reproduce
Clone of cells


Human Cell
EcoRI
Gene for human growth hormone
Recombinant DNA
Gene for human growth hormone
Sticky ends
DNA recombination
DNA insertion
Bacterial Cell
Bacterial chromosome
EcoRI
Plasmid
Bacterial cell for containing gene for human growth hormone

Transgenic Organisms

- An organism made from taking the gene(s) of one organism, and incorporating that gene(s) into another
- The DNA of a transgenic organism is called recombinant DNA, which is DNA produced by combining DNA from different sources
- This means that the DNA has been "recombined" by adding genes from elsewhere, creating a transgenic organism

Spider Goat

- Strong, flexible spider silk is one of the most valuable materials in nature, and it could be used to make an array of products — from artificial ligaments to parachute cords — if we could just produce it on a commercial scale.
- Researchers inserted a spiders' dragline silk gene into the goats' DNA in such a way that the goats would make the silk protein only in their milk. This "silk milk" could then be used to manufacture a web-like material called Biosteel

Glo Fish

- World's first commercially available transgenic animal
- Have had fluorescent proteins extracted from jellyfish inserted into their DNA to make them glow green, orange, or red
- Originally for studying water pollutants - insert genes for proteins that would fluoresce in the presence of certain contaminants

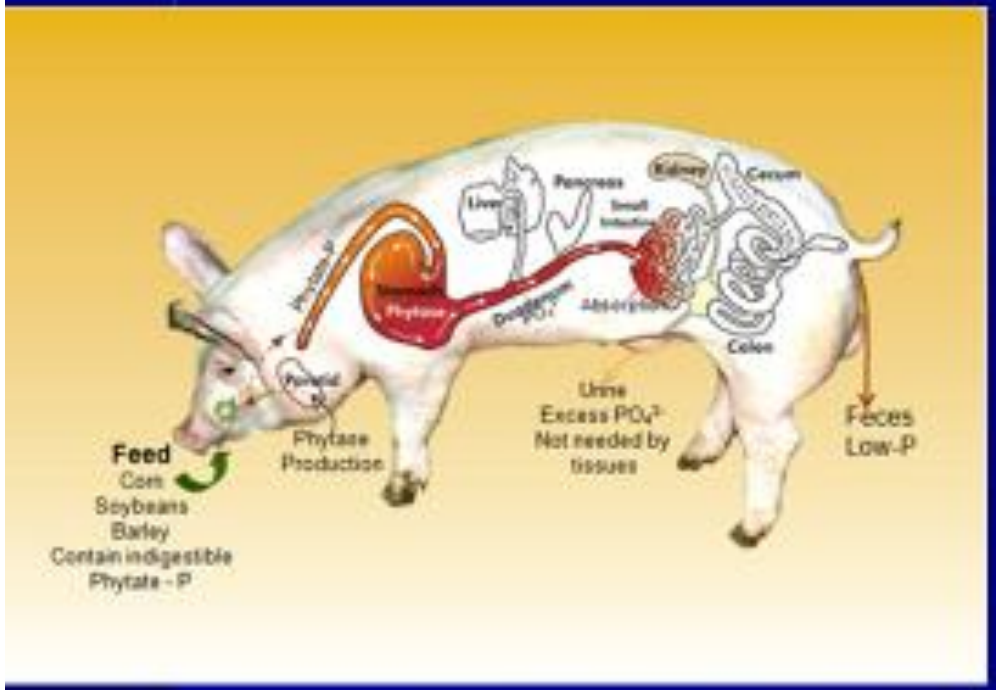
Drug-Producing Chicken

- Chickens are simpler and more efficient factories than synthetic methods for producing pharmaceutical proteins (the proteins are extracted from the egg white).
- Next aim is to produce an egg that contains an antibody against a virus of the gut. "The idea is that children will eat the egg white and the antibody will protect them from developing a diarrhoeal disease."

Medicinal Eggs

- Breed of genetically modified hens that produce cancer-fighting medicines in their eggs. The animals have had human genes added to their DNA so that human proteins are secreted into the whites of their eggs, along with complex medicinal proteins similar to drugs used to treat skin cancer and other diseases

Top 10 Transgenic Animals: *Enviropig*



- Pig manure has lots of Phosphorus which kills marine species and generates greenhouse gases
- Transgenic pigs produce *phytase* that breaks down phosphorus efficiently.
- Phosphorus is the major nutrient causing algal growth leading to fish kills and reduced water quality
- The low phosphorus manure from Enviropigs has a reduced environmental impact (These pigs produce 75% less phosphorus).