rDNA technology

Assistant teacher .Zahraa Aljanabi
Second stage /medical lab technology/molecular biology
Mustaqbal college

rDNA

* the two DNA molecules of different origin are combined, the resulting DNA is known as recombinant DNA molecule. The term “gene cloning,” “DNA cloning,” “molecular cloning,” and “recombinant DNA technology” all refer to same technique: Insertion of DNA fragment of interest from one organism into a vector which is a self- replicating genetic element inside a living cell. Gene cloning processes include removal of DNA from the cell, carrying out the DNA manipulations in test vial and, transformation of constructed DNA molecule back into the cells.

Cloning

- Cloning :is making of identical copies. DNA cloning is process of making several identical copy of a gene or gene fragment.
* Vectors: are generally double stranded closed circular DNA which has origin of replication through which they can replicate in the host cell. Vectors also have a selectable marker (generally antibiotics resistance gene)

cloning

* Cloning is a natural process in biology where genetically identical individuals are produced by asexually reproducing organisms such as bacteria, insects or plants. In biotechnology, the process of producing multiple identical copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms is referred to as cloning

cloning

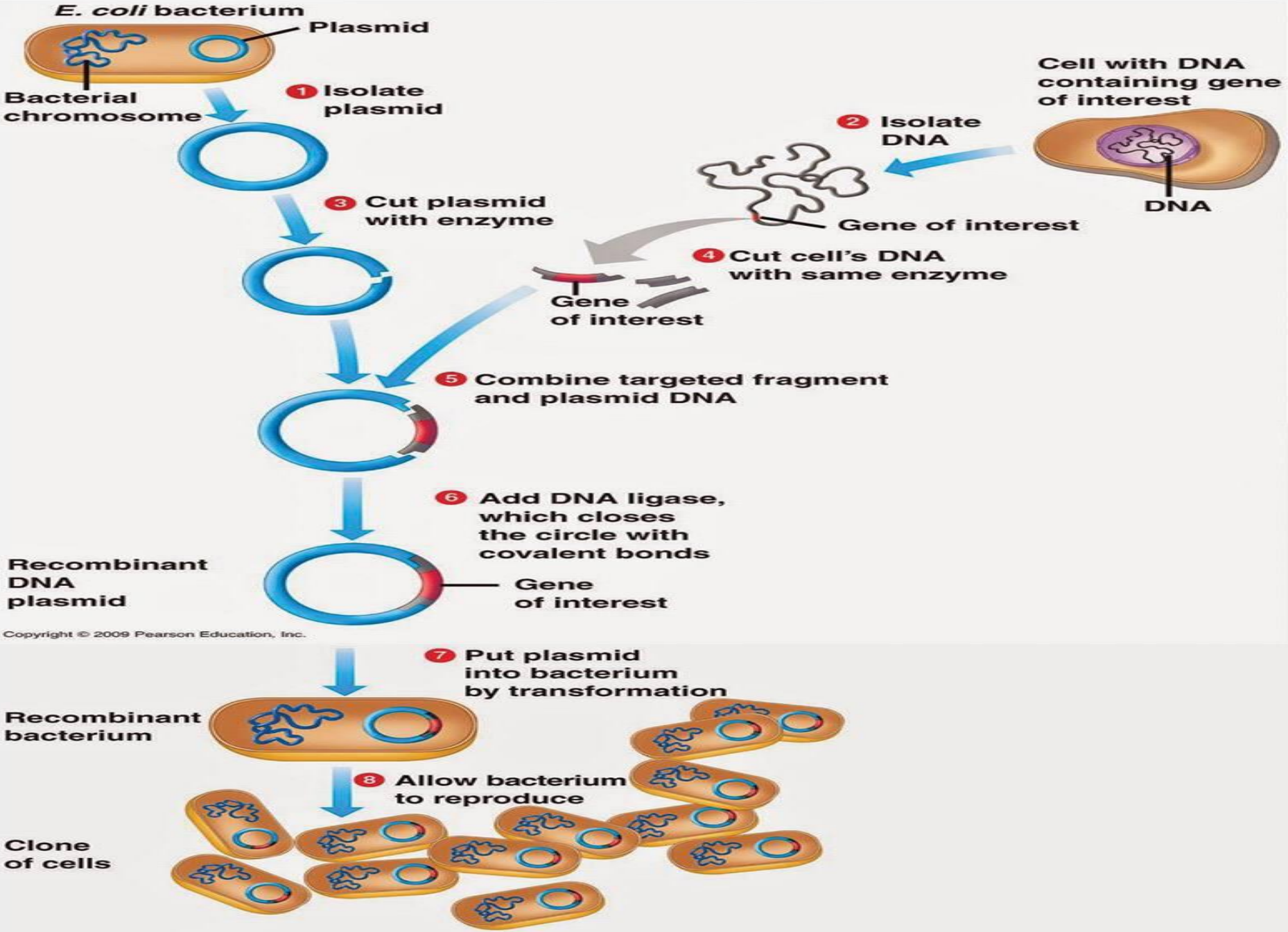
* A clone has an exact genetic imprint as that of the original cell, tissue or organism. There are different types of cloning technologies used for various purposes besides producing the genetic copy of an organism. Basically the cloning technology can be divided into three types as reproductive cloning, therapeutic cloning and recombinant DNA technology or DNA cloning.

vectors

- Vector with desired DNA insert is called recombinant DNA. This can be transferred to suitable host system (generally E.Coli) where it finds machinery for replication and makes several copies of it (may also express protein). The process is also called recombinant DNA technology or genetic engineering. **Choice of vector is dependent on** insert size and application

The scientist

* Recombinant DNA technology is largely based on the work of Paul Berg, Herbert W. Boyer and Stanley N. Cohen although many other scientists have also made important contributions.
* Paul Berg in 1972, isolated a gene from a human cancer- causing monkey virus (SV40) using a restriction enzyme and joined this virus DNA with a molecule of DNA from the bacterial virus lambda using an enzyme called DNA ligase.


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

cloning

* Reproductive cloning is a technology used to generate a twin of an animal that is genetically same as another currently or previously existing animal. The best example for reproductive cloning is Dolly, the first cloned sheep. Therapeutic cloning which is also known as "embryo cloning," is production of human embryos for use in research and treatment of diseases. The aim of this technique is not human cloning, but rather to harvest stem cells that are used for research studies and to treat diseases. The last and most widely used cloning technique in biotechnology is recombinant DNA technology.

* The different types(5) of vectors available for cloning are plasmids, bacteriophages, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs) and mammalian artificial chromosomes (MACs)

plasmid

* **Plasmids**: Plasmids are extra chromosomal circular double stranded DNA replicating elements present in bacterial cells. Plasmids show the size ranging from 5.0 kb to 400 kb. Plasmids are inserted into bacterial calls by a process called transformation. Plasmids can accommodate an insert size 10 kb DNA fragment.

Bacteriophage

* **Bacteriophage**: The viruses that infect bacteria are called bacteriophage. These are intracellular obligate parasites that multiply inside bacterial cell by making use of some or all of the host enzymes. Bacteriophages have a very high significant mechanism for delivering its genome into bacterial cell. Hence it can be used as a cloning vector to deliver larger DNA segments. Most of the bacteriophage genome is non-essential and can be replaced with foreign DNA. Using bacteriophage as a vector, a DNA fragment of size up to 20 kb can be transformed.

Bacterial artificial chromosomes (BACs)

* **Bacterial artificial chromosomes (BACs):** Bacterial artificial chromosomes (BACs) are simple plasmid which is designed to clone very large DNA fragments ranging in size from 75 to 300 kb. BACs basically have marker like sights such as antibiotic resistance genes and a very stable origin of replication (ori) that promotes the distribution of plasmid after bacterial cell division and maintaining the plasmid copy number to one or two per cell. BACs are basically used in sequencing the genome of organisms in genome projects (example: BACs were used in human genome project). Several hundred thousand base pair DNA fragments can be cloned using BACs

Yeast artificial chromosomes

* **Yeast artificial chromosomes (YACs):**

  YACs are yeast expression vectors. A very large DNA fragments whose sizes ranging from 100 kb to 3000 kb can be cloned using YACs. Mostly YACs are used for cloning very large DNA fragments and for the physical mapping of complex genomes. YACs have an advantage over BACs in expressing eukaryotic proteins that require post translational modifications. But, YACs are known to produce chimeric effects which make them less stable compared to BACs

Human artificial chromosomes

* **Human artificial chromosomes (HACs):** Human artificial chromosomes (HACs) or mammalian artificial chromosomes (MACs) are still under development. HACs are micro chromosomes that can act as a new chromosome in a population of human cells. HACs range in size from 6 to 10 Mb that carry new genes introduced by human researchers. HACs can be used as vectors in transfer of new genes, studying their expression and mammalian chromosomal function can also be elucidated using these micro chrosomes in mammalian system.

Thank you