

Lec. 11 Recombinant DNA Technology and basics of DNA Cloning

assist by- Safaa Abbass Abd Al-kahdum

Introduction

Cloning is making of identical copies. DNA cloning is process of making several identical copy of a gene or gene fragment. DNA fragment from an organism is cleaved or amplified and inserted in a DNA carrier called vector. Vectors are generally double stranded closed circular DNA which has origin of replication through which they can replicate in the host system. Vectors also have a selectable marker (generally antibiotics resistance gene) for screening of recombinant colonies.

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.

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. This way the first recombinant DNA molecule was created. Later on, in 1980, Paul Berg shared Nobel prize in Chemistry for the work. A simplified concept of cloning is given in Fig. 1.

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. 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.

Lec. 11 Recombinant DNA Technology and basics of DNA Cloning

assist by- Safaa Abbass Abd Al-kahdum

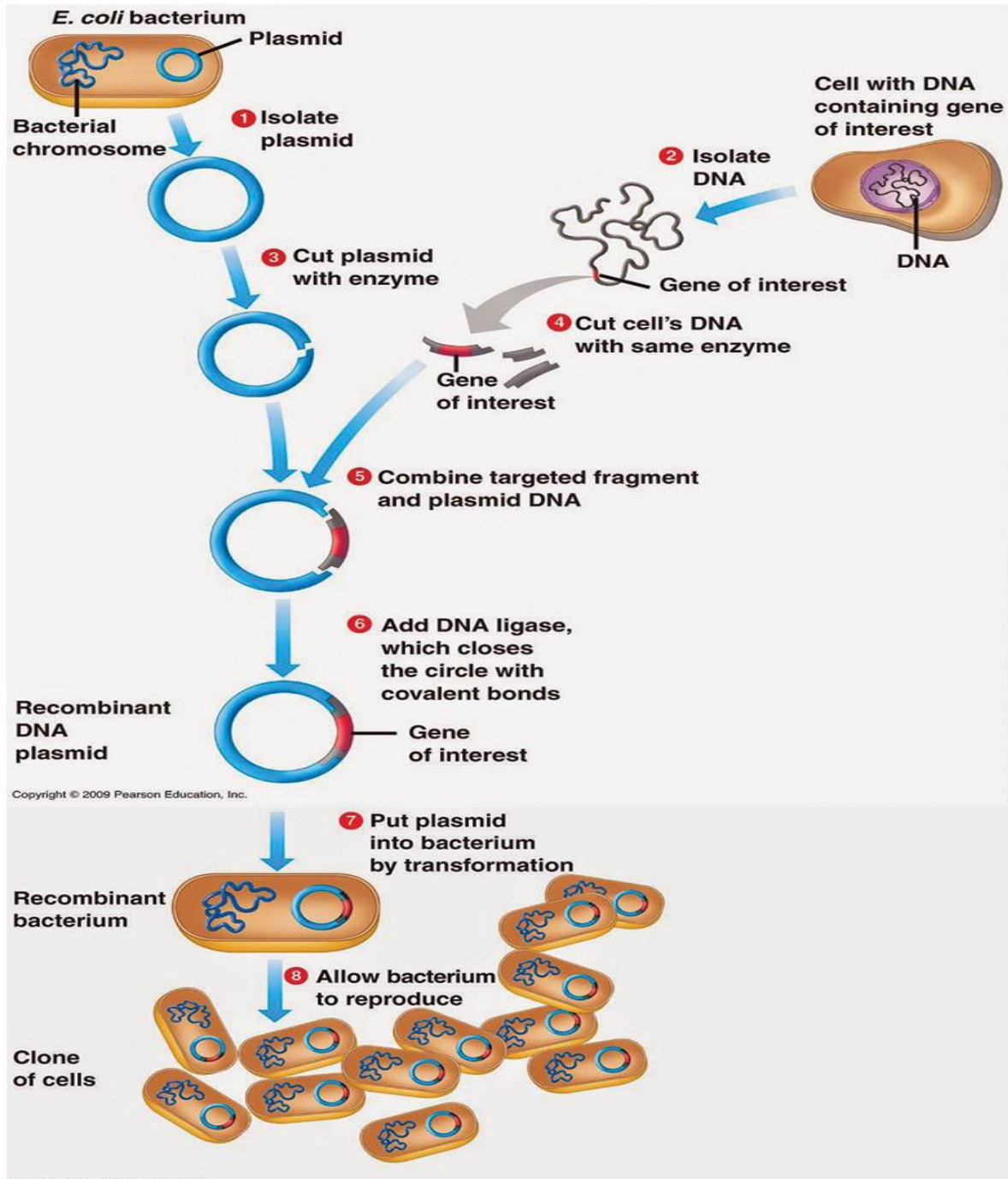


Figure 1: A simplified concept of cloning

Lec. 11 Recombinant DNA Technology and basics of DNA Cloning

assist by- Safaa Abbass Abd Al-kahdum

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.

In Biotechnology the gene is the cornerstone of most molecular biology studies. The study of genes can be facilitated by isolation and amplification of gene of interest. Cloning is one method used for isolation and amplification of gene of interest. The gene is cloned by inserting it into another DNA molecule which acts as vehicle or vector that will replicate in living cells. As 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.

The first step in cloning is to prepare large amount of the vector and chromosomal DNAs. To carry the gene or the desired DNA fragment to the cell there is a need of a vector molecule. All cloning vectors are carrier DNA molecules. These carrier molecules host few common features in general such as; all vectors are self-replicating in the cell, they contain a number of unique restriction enzyme cleaving sites that are present only once in the vector, they carry the selectable marker gene which is useful in selection of clone (usually an antibiotic resistance gene that is absent in the host cell) and, they can be very easily isolated from host cell. Depending on the purpose of cloning there are many vectors available. For use in the bacterial host *E. coli* system a greatest variety of cloning vectors have been developed. Thus, the first thing in cloning that a molecular biologist requires is to grow pure culture and isolate the cloning vector from the cells.

Choice of vector is dependent on insert size and application The most commonly used cloning vectors include plasmids and bacteriophages (phage λ) beside all the other

Lec. 11 Recombinant DNA Technology and basics of DNA Cloning

assist by- Safaa Abbass Abd Al-kahdum

available vectors (Table 1). 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).

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 cells by a process called transformation. Plasmids can accommodate an insert size of upto 10 kb DNA fragment. Generally plasmid vectors carry a marker gene which is mostly a gene for antibiotic resistance; thereby making any cell that contains the plasmid will grow in presence of the selectable corresponding antibiotic supplied in the media.

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) 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 sites 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 (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

Lec. 11 Recombinant DNA Technology and basics of DNA Cloning

assist by- Safaa Abbass Abd Al-kahdum

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 (HACs): Human artificial chromosomes (HACs) or mammalian artificial chromosomes (MACs) are still under development. HACs are microchromosomes 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 microchromosomes in mammalian system. Different types of vectors are summarized in Table 1.

Table 1: Different types of vector with their properties.

Vector	Basis	Size limit of insert	Major application
Plasmid	Naturally occurring multi copy plasmids	≤ 10 kb	Subcloning and gene manipulation, cDNA cloning and expression studies.
Phage	Bacteriophage λ	10- 20 kb	Genomic DNA cloning, cDNA and expression libraries.
Cosmid	Plasmid containing a bacteriophage λ <i>cos</i> site	35- 45 kb	Genomic library construction.
BACs	<i>Escherichia coli</i> F factor plasmid	75- 300 kb	Analysis of large genomes.
YACs	Saccharomyces cerevisiae centromere, telomere, and autonomously replicating sequence	100- 3000 kb	Analysis of large genomes, YAC transgenic mice.
MACs	Mammalian centromere, telomere, and origin of replication	4> 10 mb	Still in budding stage for use in animal biotechnology and human gene therapy.