Basics of DNA Cloning

DNA cloning is process of making several identical copy of a gene or gene fragment.

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.

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).

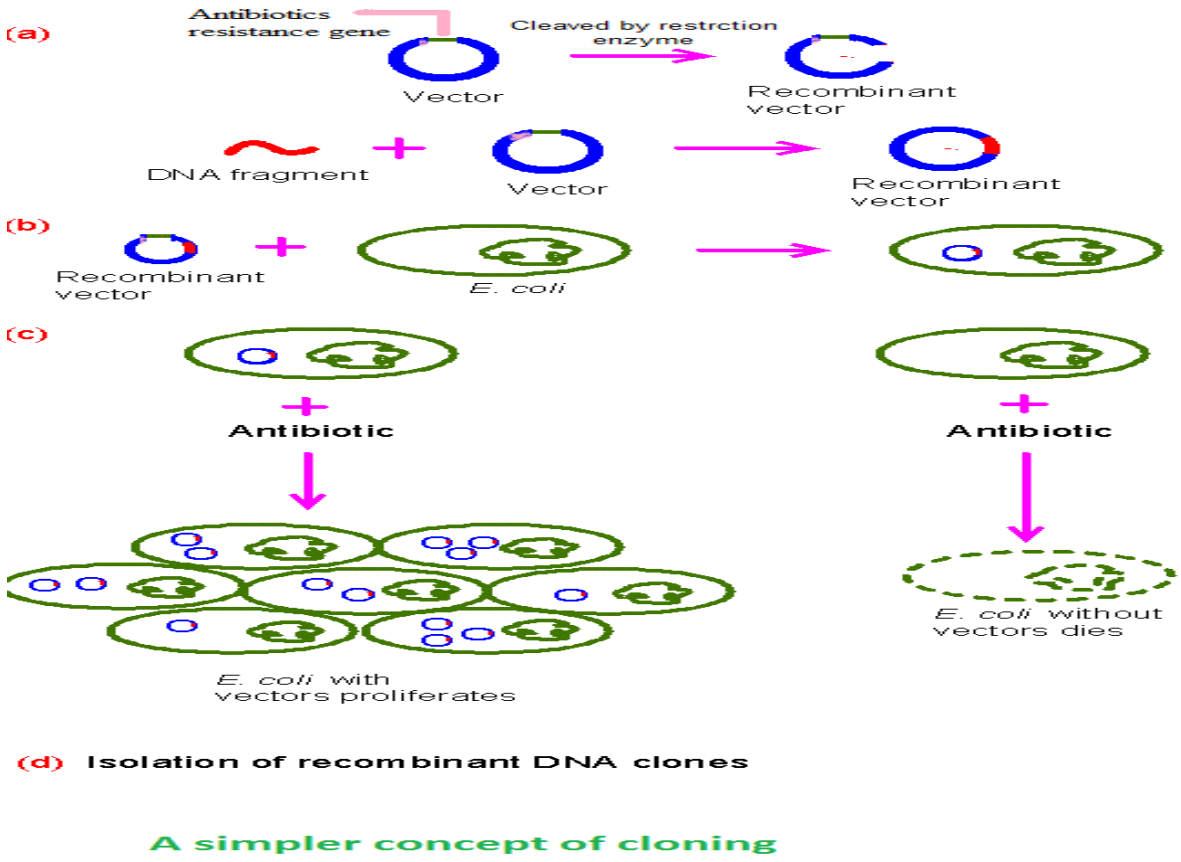
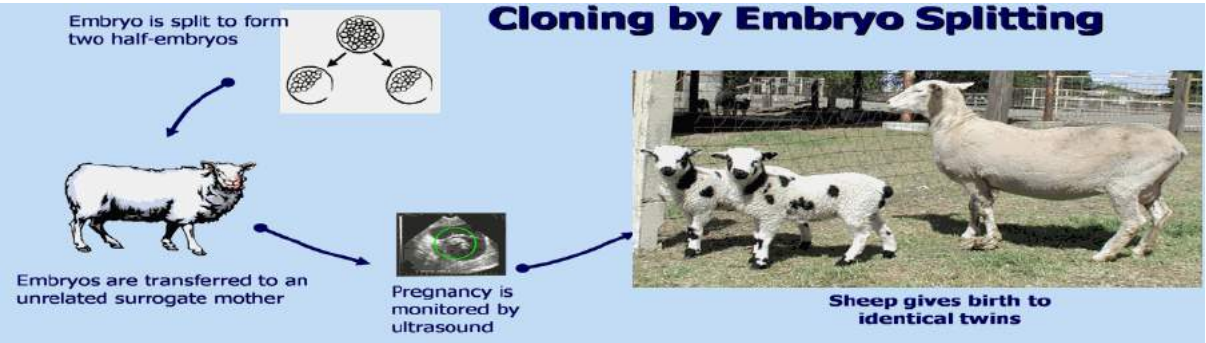


Figure 1: A simpler concept of cloning.

There are different types of cloning technologies used for various purposes. Basically the cloning technology can be divided into **three** types as:

1-Reproductive cloning
2-Therapeutic cloning
3-Recombinant DNA technology or **DNA cloning**.

1-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.



2-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.

3-Recombinant DNA technology: In biotechnology, 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**.

Cloning vectors:

The first step in cloning is to prepare large amount of the vector and chromosomal DNA. To carry the gene or the desired DNA fragment to the cell there is a need of a vector molecule. All cloning vectors have few common features in general such as; all vectors are [1]self replicating in the cell, [2]they contain a number of unique restriction enzyme cleaving sites that are present only once in the vector, they [3]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 size

and the application of the insert the suitable vector is selected. The different types of vectors available for cloning are :

1- 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 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.

2- 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.

3- Cosmids: A **cosmid** vector is produced by inserting the **cos sequence** from **λ-phage DNA** into a small ***E. coli*** **plasmid vector** about **5** kb long. Cosmid vectors contain all the essential components found in plasmids. The cosmid can incorporate foreign DNA inserts that are between **35** and **45** kb in length.
Cells containing cosmid molecules can be selected using antibiotics as described for ordinary plasmid cloning.

4- 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 (antibiotic resistance genes) and a very stable origin of replication (ori). BACs are basically used in sequencing the genome of organisms in genome projects (example: BACs were used in human genome project).

5- Yeast artificial chromosomes (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.

6- Human artificial chromosomes (HACs): or mammalian artificial chromosomes (MACs) are still under development. HACs range in size from 6 to 10 Mb that carry new genes introduced by human researchers.

Major steps of DNA Cloning : Molecular cloning using a plasmid vector involves **five** major steps :

Step 1: **Isolation of DNA (gene of interest and vector):**

The first initial step in cloning a DNA fragment is to isolate foreign DNA containing gene of interest and bacterial plasmid. If the sequence of the gene of interest is known it is isolated by PCR amplification using gene specific primers which include restriction sites selected from the multiple cloning site of the plasmid selected for cloning (Figure 1). When the sequence of the gene is not known degenerate primers are used for PCR amplification. Most of the time people generate genomic DNA library and screen for the gene using southern hybridization technique. According to the result of southern hybridization, the DNA is sequenced and the gene was confirmed by BLAST analysis(see page). Now the gene is amplified by PCR and cloned. There are many plasmids available commercially for cloning.

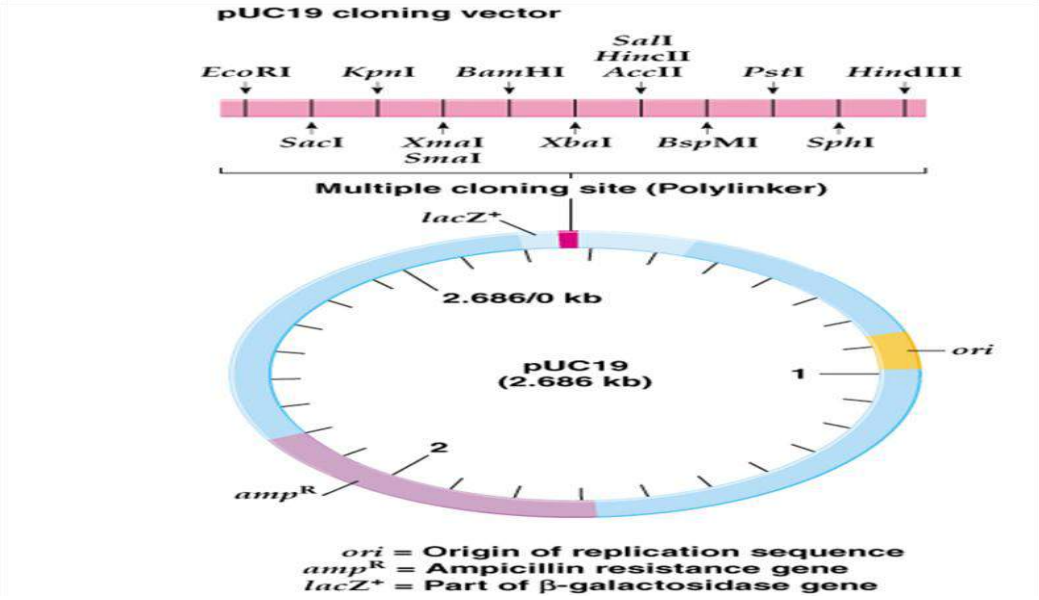


Figure 1: Plasmid pUC 19, The commonly used plasmid pUC19 (“puck 19”) is a small plasmid with the essential elements for a vector: An origin of DNA replication A dominant selectable marked (resistance to an antibiotic, ampicillin) And a cloning site, usually a polylinker with recognition sites for numerous restriction enzymes.

Step 2: Treatment of plasmid and foreign DNA with the same restriction enzyme and ligation

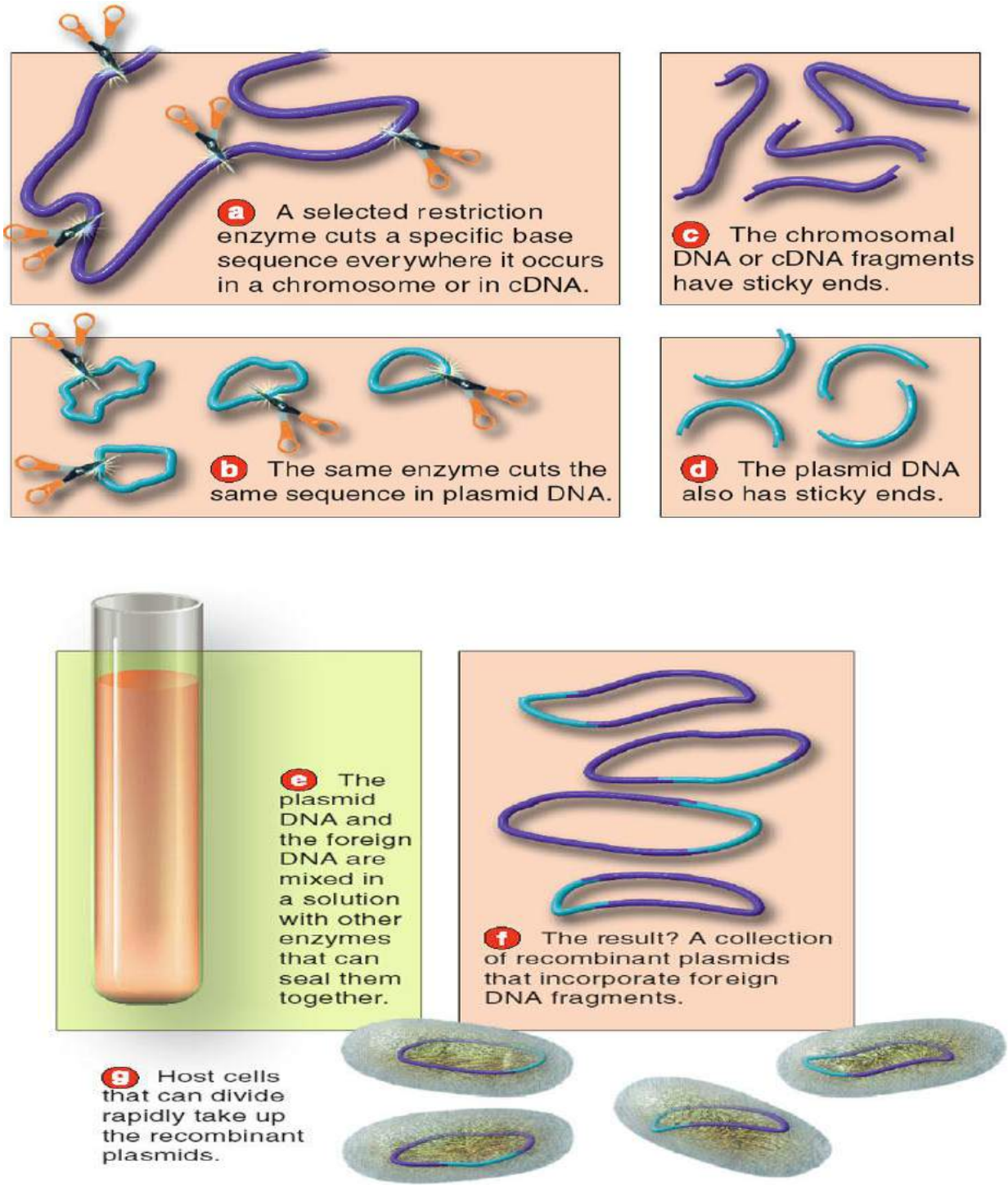


Figure 2: Treatment of plasmid and foreign DNA with the same restriction enzyme and ligation.**(الرسم للاطلاع)**

Step 3: Transformation: transfer of recombinant plasmid DNA to a suitable host:

There are basically two general methods for transforming bacteria:

The first is a **chemical method** utilizing CaCl2 and heat shock to promote DNA entry into cells.The traditional method to prepare cells for transformation process is to incubate the cells in a concentrated calcium salt solution to neutralize the negative charge of membrane (due to salicylic acid), so that the negatively charged DNA molecules can come close to bacterial membrane and during heat shock can easily enter in the cells. These "competent" cells are then mixed with ligation product to allow entry of the DNA into the bacterial cell (Figure 3).

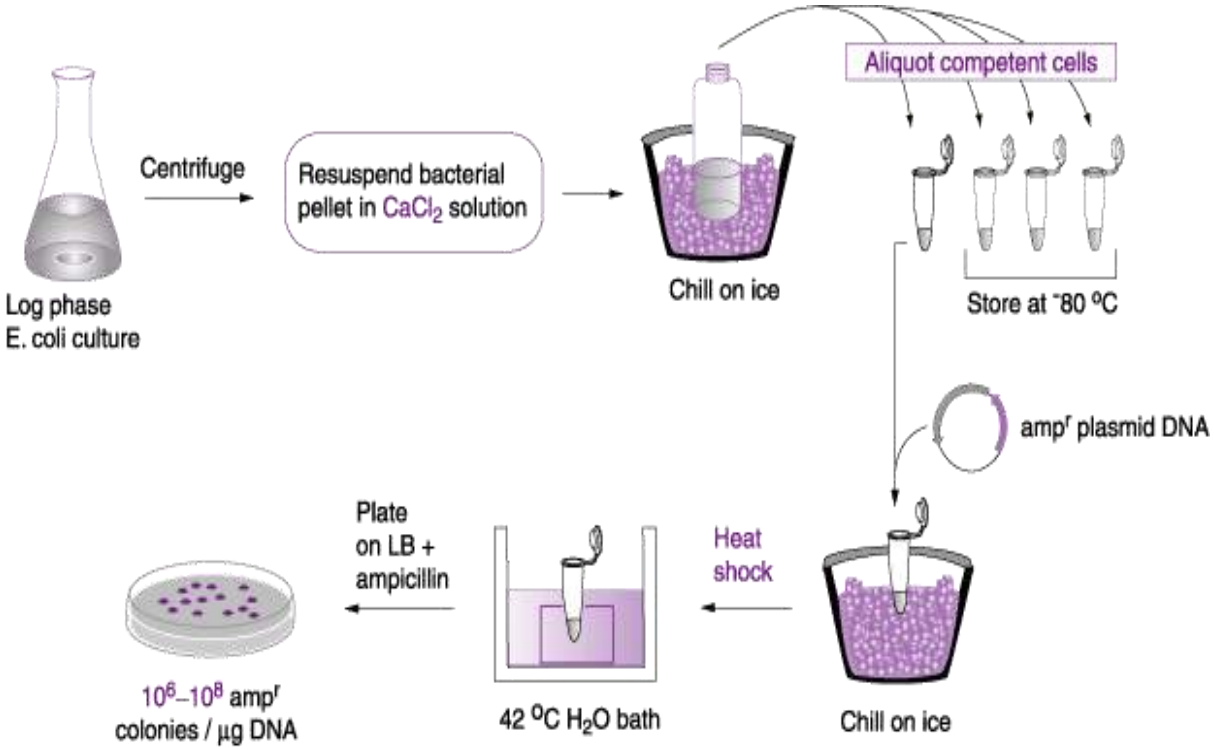


Figure 3: Chemical transformation with calcium chloride

A second method is called **electroporation** based on a short pulse of electric charge to facilitate DNA uptake. Electroporation method is an alternative mode of transformation used to drive DNA (comparatively larger size) into cells by a strong electric current. This method is not very common due to less percentage of survival of transformed cells (Figure 4).

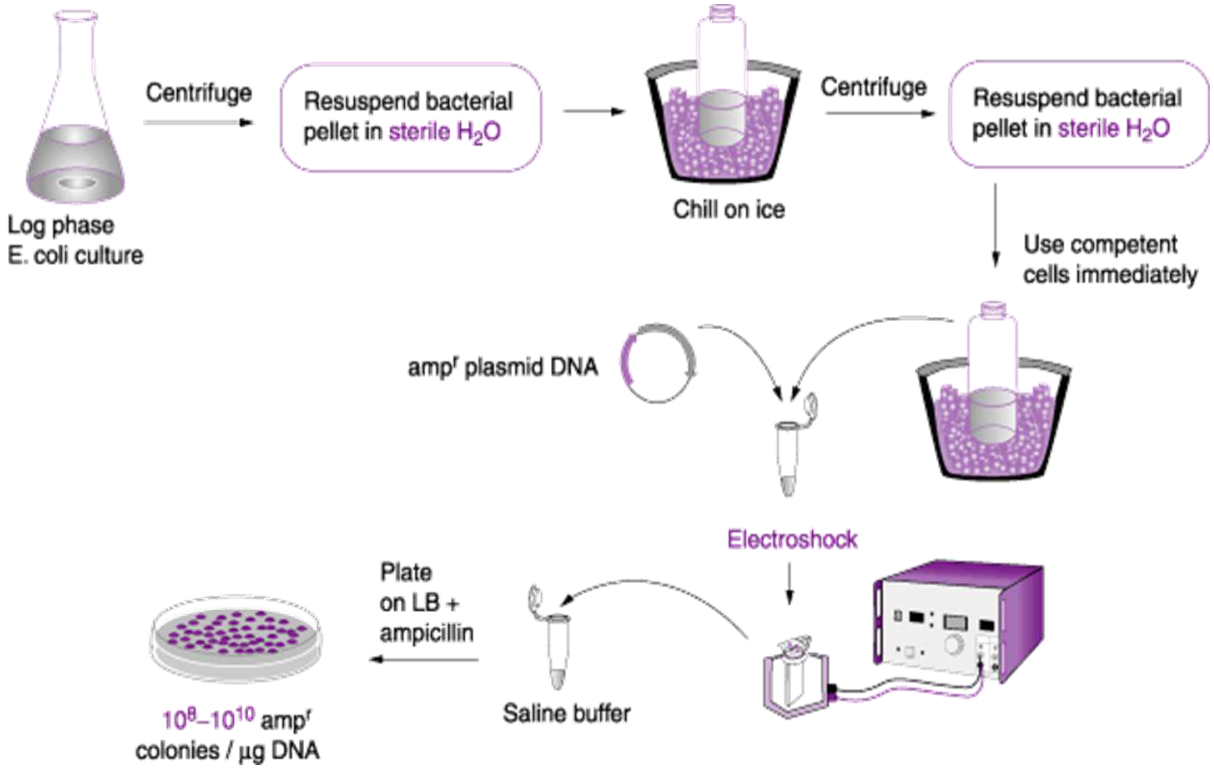


Figure 4: Transformation by electroformation. (الرسم للاطلاع)

Step 4: Screening for transformed cells:

To avoid the growth of the untransformed bacterial cells, plasmid vectors are engineered with selectable marker gene for resistance to the antibiotics. The media in which the transformed bacterial cells are grown is supplied with that antibiotic whose resistance gene is present in the plasmid. Due to this only transformed cells show antibiotic resistance will grow in the media supplied with antibiotic and untransformed cells cannot grow as they do not carry antibiotic resistance gene. Transformed bacterial cells may contain either recombinant plasmid DNA (vector containing foreign DNA insert) or non-recombinant plasmid DNA (self ligated vector only). Both type of transformed bacterial cells will show antibiotic resistance and grow on the agar media plate.

Blue-white screening or "*lac* selection" (also called α-complementation) can be used to distinguish between recombinant transformants and non- recombinant transformants. Bacterial colonies are allowed to grow on selective media containing antibiotic and X-gal (5-bromo-4-chloro-indolyl-β-D-galactopyranoside), a colorless chromogenic compound. Not all plasmid vectors are engineered for "*lac* selection"; the plasmid that are engineered for blue-white screening carry a MCS site in between gene that encodes for amino acids

for enzyme β-galactosidase which cleaves β-glycosidic bond in D- lactose. X-gal mimic D-lactose and β-galactosidase enzyme acts on X- gal and produces a blue color complex (**Figure 5**).

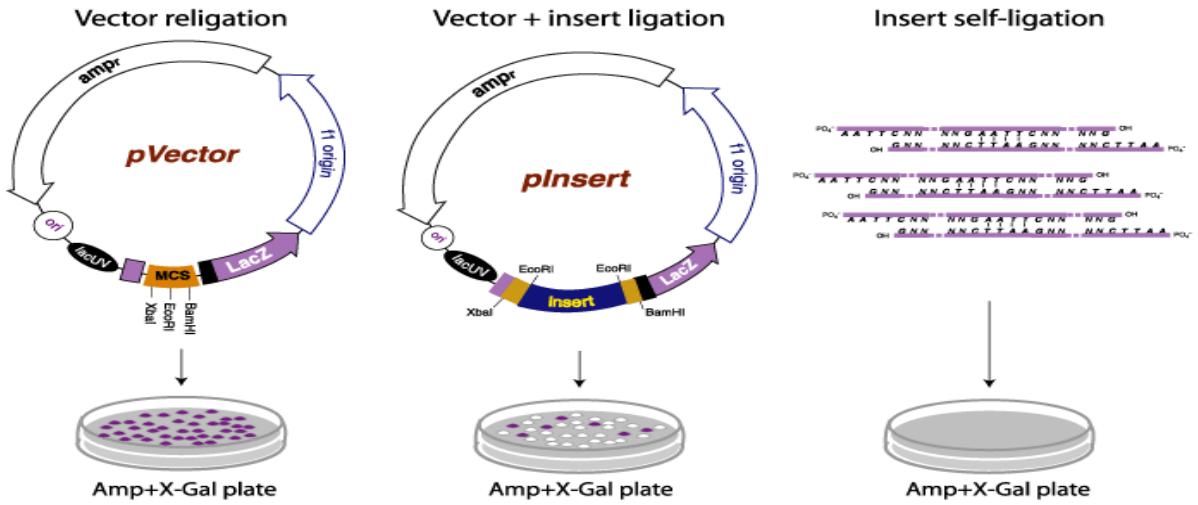


Figure 5: Growth on agar plates. Blue colonies represent Ampicillin-resistant bacteria that contain pVector and express a functional alpha fragment from an intact LacZ alpha coding sequence. White colonies represent Ampicillin-resistant bacteria that contain pInsert and do not produce LacZ alpha fragment.

Explanation of the colony selection: finding the rare bacterium with recombinant DNA
Only *E. coli* cells with resistant plasmids grow on antibiotic medium
Only plasmids with functional lacZ gene can grow on Xgal
lacZ(+) = blue colonies
lacZ functional = polylinker intact = nothing inserted, no clone
lacZ(-) = white colonies polylinker disrupted = successful insertion & recombination.

Step 5: Amplification and purification of recombinant plasmid DNA

The final step in DNA cloning is the isolation of the cloned recombinant DNA. A positive colony containing recombinant plasmid is identified and it is aseptically transferred to liquid medium and cell are allowed to grow exponentially overnight. A fully grown culture contains trillions of identical cells, which is harvested for the isolation of the plasmid DNA. The plasmid DNA is purified from harvested bacterial cell lysates. The purified plasmid DNA is dissolved in an appropriate buffer solution and can be used for further confirmation of the clone by restriction digestion and sequencing the plasmid DNA.

Additional information about The BLAST Sequence Analysis Tool

(فقط التعريف مطلوب)

BLAST (**B**asic **L**ocal **A**lignment **S**earch **T**ool) is one of the most widely used bioinformatics programs for sequence searching. BLAST is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of proteins or the nucleotides of DNA sequences.

The BLAST program is available online at several servers including the one at NCBI: http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Note: NCBI (**N**ational **C**enter for **B**iotechnology **I**nformation) is part of the United States National Library of Medicine , a branch of the National Institutes of Health (NIH). The NCBI is located in Bethesda, Maryland and was founded in 1988 through legislation sponsored by Senator Claude Pepper.