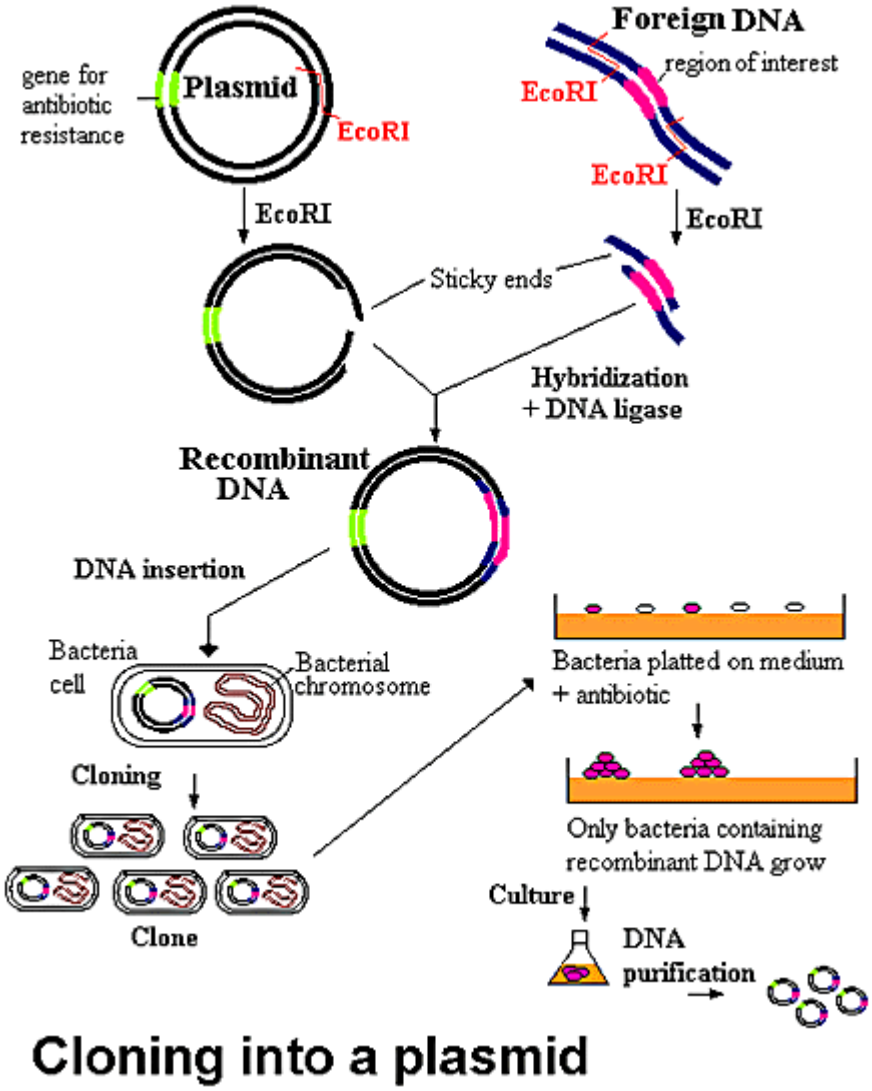
Andrea Leite Camargo Santana

A guide to understanding how molecular cloning works

If by reading the term "molecular cloning" you instantly associate with animal cloning like Dolly the sheep, I'm sorry to inform you, it's not about cell cloning that I'm going to write about here. Molecular cloning, which I am referring to, encompasses a set of techniques that allow amplification (obtaining multiple copies) of a given DNA fragment in a host cell. In summary, the whole cloning process can be divided into 3 steps: 1 - binding of the gene of interest to a cloning vector generating the recombinant vector, 2 - insertion by transformation and multiplication of the recombinant vector into a host cell and 3 - multiplication of the recombinant cell clones, in solid medium, forming the transforming colonies (Figure 1). A clone is a set of cells or molecules identical to an initial cell/molecule.

Figure 1: Scheme of a molecular cloning into a plasmid



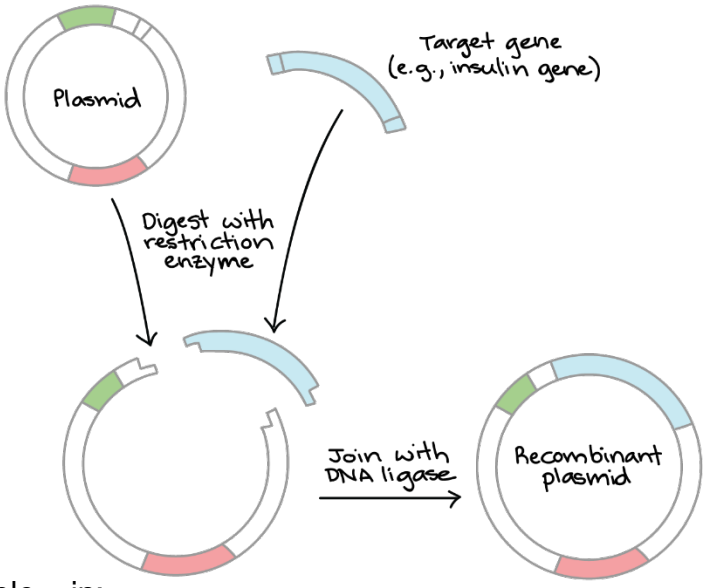
Cloning into a plasmid

Available at: http://sivabio.50webs.com/dnacloning.htm

More specifically, the technique begins by obtaining the DNA of interest, usually by PCR (polymerase chain reaction - another technique that allows us to simulate in the laboratory the DNA duplication that occurs inside cells by obtaining at the end of the process several copies of the DNA of interest). This DNA obtained by PCR must then be linked to a vector, usually a plasmid (circular DNA found in bacteria). The chosen plasmid (there are several different types) must be linearized and, for this, restriction enzymes are used which are capable of cutting the DNA in specific sequences of bases. Once the plasmid is opened, the DNA fragment obtained by PCR must be digested with the same enzymes utilized to digest the plasmid and after that can be ligated to the vector using an enzyme capable of performing this task called DNA ligase (Figure 2). This enzyme catalyzes the formation of the phosphodiester bond between adjacent 3'-OH and 5'-PO4 ends intramolecularly in cut regions performed by the restriction enzymes (Figure

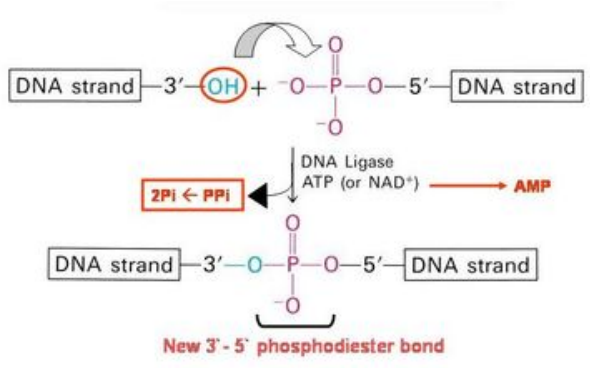
3). To do this, the DNA fragment must have at its ends sequences complementary to the ends of the plasmid to which it will be ligated. Such strategy can be obtained by adding the restriction sites of the enzymes to the primers constructed for use during the PCR reaction, prior to the digest reaction.

Figure 2: Synthesis of the recombinant plasmid



Available in: https://pt.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/overview-dna-cloning

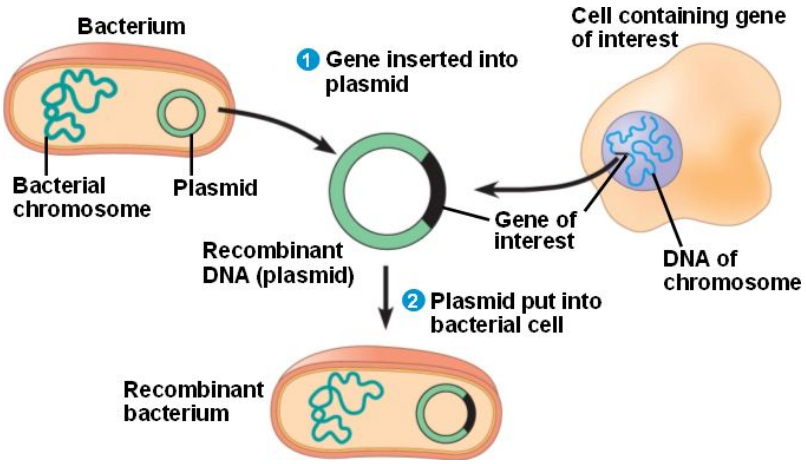
Figure 3: Ligase Reaction



Available in: http://uvmgg.wikia.com/wiki/DNA_Ligase

The plasmid, now with the DNA of interest attached thereto, is referred to as “recombinant plasmid” since the DNA thereon is derived from an organism different from the organism from which the plasmid was taken. Such a recombinant plasmid can then be put by transformation into a host organism (Figure 4), such as the *Escherichia coli* bacterium used in the vast majority of experiments originating a recombinant bacterium.

Figure 4: Transformation of the recombinant plasmid into the host bacterium



Available in: https://step1.medbullets.com/biochemistry/102099/cloning

The transformation is a natural ability that some bacteria possess to internalize dispersed DNA in the medium. In the laboratory we can simulate this process in two different ways, using electric shock or heat shock capable of destabilizing the plasma membrane of the cells of the organism of choice and thus allowing the entry of the recombinant plasmid (Figure 5).

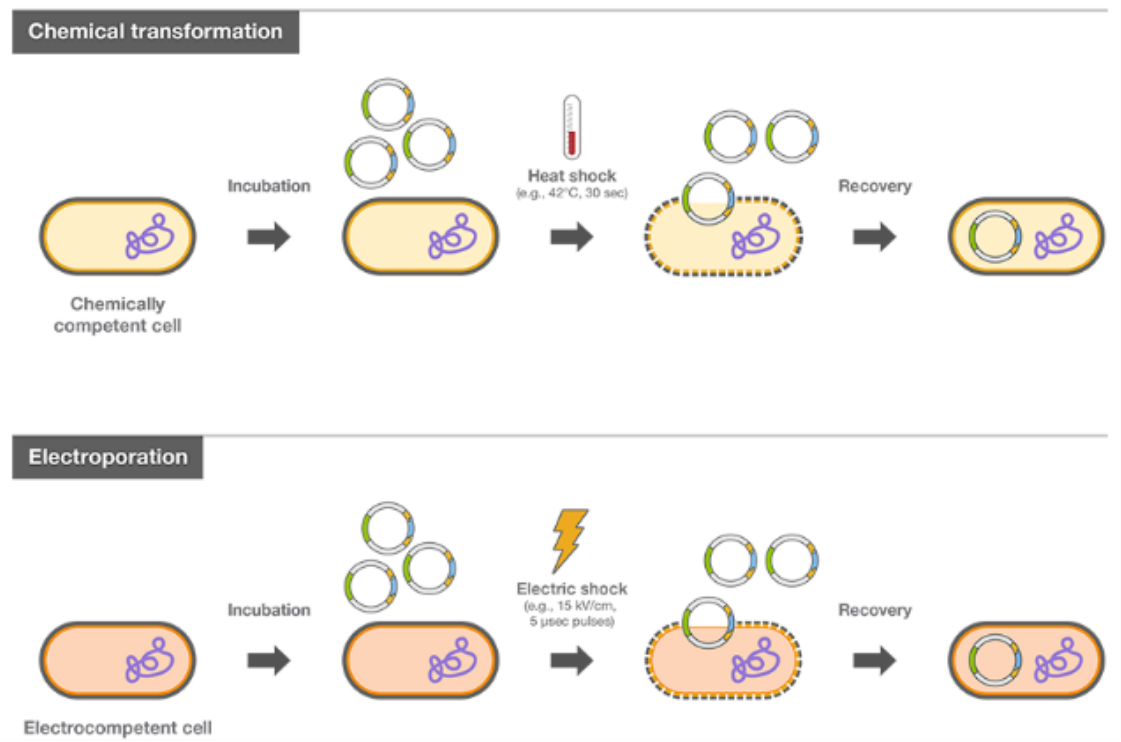


Figure 5: Thermal transformation and electroporation

Available in: https://www.thermofisher.com/br/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/molecular-cloning/transformation/competent-cell-basics.html

After *E. coli* is transformed it will replicate and each new cell will be a copy (clone) of the original cell displaying the recombinant plasmid therein. The plasmids used for cloning have some specific features such as the presence of a multiple cloning site containing the restriction sites (where the genes to be cloned will be inserted into the vector), an origin of replication (which allows them to multiply) and genes that confer resistance to certain antibiotics (Figure 6). Since the plasmid used for cloning has a resistance gene for a given antibiotic in its DNA, this allows the selection of the transforming organisms, once in the presence of the antibiotic to which it is resistant only the cells containing the recombinant plasmid will survive and grow in plates containing solid medium forming colonies, however, those cells that have not been transformed with the recombinant plasmid (and therefore do not have the resistance gene) will die in the presence of the antibiotic (Figure 7). Simple, isn´t it?

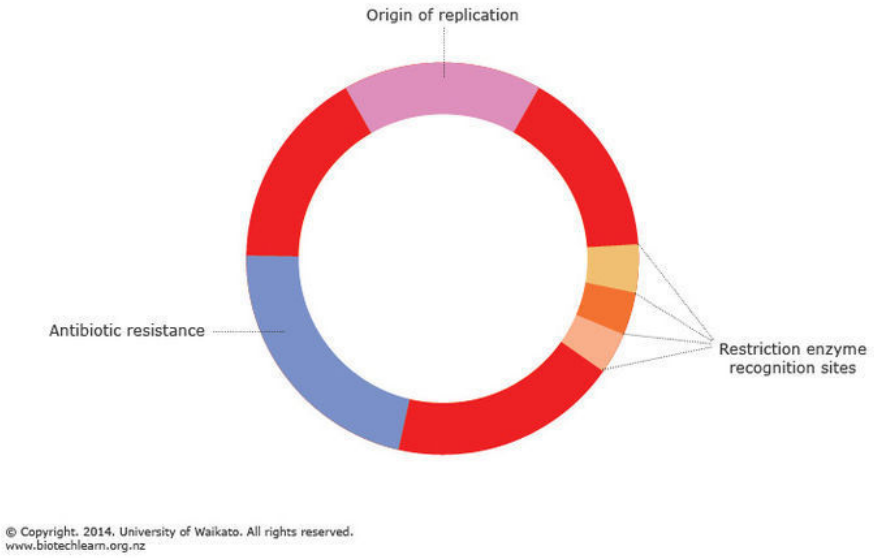
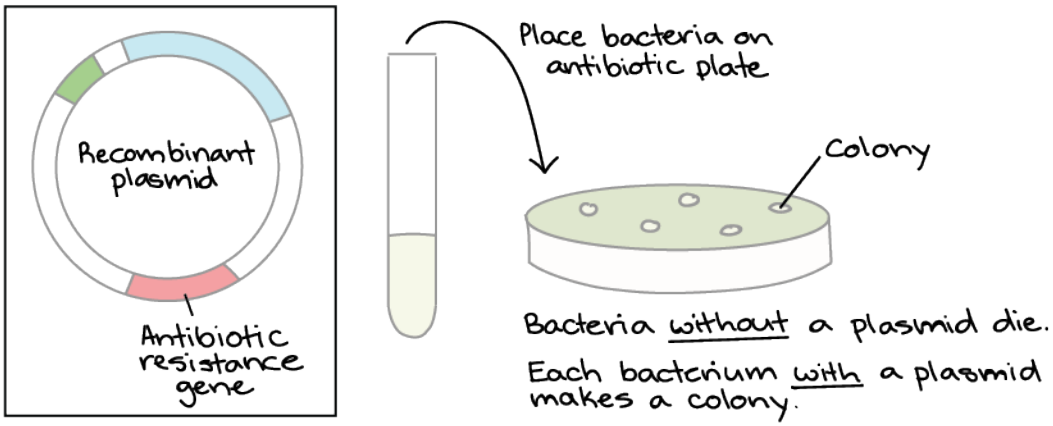


Figure 6: Map of a plasmid vector

Available in: https://www.sciencelearn.org.nz/images/2331-plasmid-vector

Figure 7: Selection of transformants clones by antibiotic resistance



Available in: https://pt.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/overview-dna-cloning