DNA Cloning

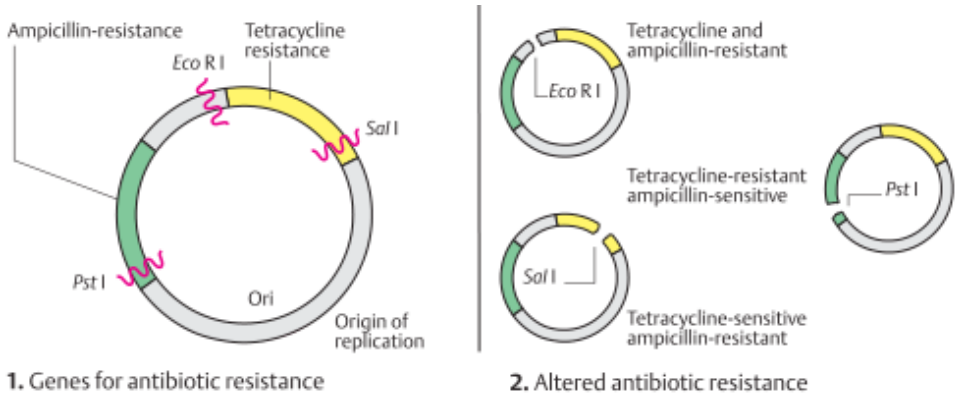
To obtain sufficient amounts of a specific DNA sequence (e.g., a gene of interest) for study, it must be selectively amplified. This is accomplished by DNA cloning, which produces a homogeneous population of DNA fragments from a mixture of very different DNA molecules or from all the DNA of the genome. Here procedures are required to identify DNA from the correct region in the genome, to separate it from other DNA, and to multiply (clone) it selectively. Identification of the correct DNA fragment utilizes the specific hybridization of complementary single-stranded DNA (molecular hybridization). A short segment of single stranded DNA, a probe, originating from the sequence to be studied, will hybridize to its complementary sequences after these have been denatured (made single-stranded, see Southern blot analysis). After the hybridized sequence has been separated from other DNA, it can be cloned. The selected DNA sequences can be amplified in two basic ways: in cells (cell-based cloning) or by cell-free cloning.

Cell-based DNA cloning

Cell-based DNA cloning requires four initial steps. First, a collection of different DNA fragments are obtained from the desired DNA (target DNA) by cleaving it with a restriction enzyme. Since fragments resulting from restriction enzyme cleavage have a short single-stranded end of a specific sequence at both ends, they can be ligated to other DNA fragments that have been cleaved with the same enzyme. The fragments produced in step 1 are joined to DNA fragments containing the origin of replication (OR) of a replicon, which enables them to replicate. In addition, a fragment may be joined to a selectable marker, e.g., a DNA sequence containing an antibiotic resistance gene. The recombinant DNA molecules are transferred into host cells (bacterial or yeast cells).

Here the recombinant DNA molecules can replicate independently of the host cell genome. Usually the host cell takes up only one (although occasionally more than one) foreign DNA molecule. The host cells transformed by recombinant (foreign) DNA are grown in culture and multiplied (propagation, 4). Selective growth of one of the cell clones allows isolation of one type of recombinant DNA molecule (5). After further propagation, a homogeneous population of recombinant DNA molecules is obtained (6). A collection of different fragments of cloned DNA is called a clone library (7, see DNA libraries). In cell-based cloning, the replicon-containing DNA molecules are referred to as vector molecules.

A plasmid vector for cloning



A plasmid vector for cloning

Many different vector systems exist for cloning DNA fragments of different sizes. Plasmid vectors are used to clone small fragments. The experiment is designed in such a way that incorporation of the fragment to be cloned changes the plasmid's antibiotic resistance to allow selection for these recombinant plasmids. A formerly frequently used plasma vector (pBR322) is presented. This plasmid contains recognition sites for the restriction enzymes PstI, EcoRI, and SalI in addition to genes for ampicillin and tetracycline resistance. If a foreign DNA fragment is incorporated into the plasmid at the site of the EcoRI recognition sequence, then tetracycline and

ampicillin resistance will be retained (2). If the enzyme PstI is used to incorporate the fragment to be used, ampicillin resistance is lost (the bacterium becomes ampicillin sensitive), but tetracycline resistance is retained. If the enzyme SalI is used to incorporate the fragment, tetracycline resistance disappears (the bacterium becomes tetracycline sensitive), but ampicillin resistance is retained. Thus, depending on how the fragment has been incorporated, recombinant plasmids containing the DNA fragment to be cloned can be distinguished from non recombinant plasmids by altered antibiotic resistance. Cloning in plasmids (bacteria) has become less important since yeast artificial chromosomes (YACs) have become available for cloning relatively large DNA fragments.