8.4: Cloning DNA - Plasmid Vectors

8.4.1 Plasmids are Naturally Present in Some Bacteria

Many bacteria contain extra-chromosomal DNA elements called plasmids. These are usually small (a few 1000 bp), circular, double stranded molecules that replicate independently of the chromosome and can be present in high copy numbers within a cell. In the wild, plasmids can be transferred between individuals during bacterial mating and are sometimes even transferred between different species. Plasmids are particularly important in medicine because they often carry genes for pathogenicity and drug-resistance. In the lab, plasmids can be inserted into bacteria in a process called transformation.

8.4.2 Using Plasmids as Cloning Vectors

To insert a DNA fragment into a plasmid, both the fragment and the circular plasmid are cut using a restriction enzyme that produces compatible ends (Figure 8.9). Given the large number of restriction enzymes that are currently available, it is usually not too difficult to find an enzyme for which corresponding recognition sequences are present in both the plasmid and the DNA fragment, particularly because most plasmid vectors used in molecular biology have been engineered to contain recognition sites for a large number of restriction endonucleases.
Figure 8.9: Cloning of a DNA fragment (red) into a plasmid vector. The vector already contains a selectable marker gene (blue) such as an antibiotic resistance gene. (Original-Deyholos-CC:AN)

After restriction digestion, the desired fragments may be further purified or selected before they are mixed together with ligase to join them together. Following a short incubation, the newly ligated plasmids, containing the gene of interest are **transformed** into *E. coli*. Transformation is accomplished by mixing the ligated DNA with *E. coli* cells that have been specially prepared (i.e. made **competent**) to uptake DNA. Competent cells can be made by exposure to compounds such as CaCl$_2$ or to electrical fields (**electroporation**). Because only a small fraction of cells that are mixed with DNA will actually be transformed, a **selectable marker**, such as a gene for antibiotic resistance, is usually also present on the plasmid. After transformation (combining DNA with competent cells), bacteria are spread on a bacterial agar plate containing an appropriate antibiotic so that only those cells that have actually incorporated the plasmid will be able to grow and form colonies. This can then be picked and used for further study.

Molecular biologists use plasmids as **vectors** to contain, amplify, transfer, and sometimes express genes of interest that are present in the cloned DNA. Often, the first step in a molecular biology experiment is to **clone** (i.e. copy) a gene into a plasmid, then transform this recombinant plasmid back into bacteria so that essentially unlimited copies of the gene (and the plasmid that carries it) can be made as the bacteria reproduce. This is a practical necessity for further manipulations of the DNA, since most techniques of molecular biology are not sensitive enough to work with just a single molecule at a time. Many molecular cloning and recombination experiments are therefore iterative processes in which:

0. a DNA fragment (usually isolated by PCR and/or restriction digestion) is cloned into a plasmid cut with a compatible restriction enzyme
0. the recombinant plasmid is transformed into bacteria
0. the bacteria are allowed to multiply, usually in liquid culture
0. a large quantity of the recombinant plasmid DNA is isolated from the bacterial culture
0. further manipulations (such as site directed mutagenesis or the introduction of another piece of DNA) are conducted on the recombinant plasmid
0. the modified plasmid is again transformed into bacteria, prior to further manipulations, or for expression

An Application of Molecular Cloning: Production of Recombinant Insulin

Purified insulin protein is critical to the treatment of diabetes. Prior to ~1980, insulin for clinical use was isolated from human cadavers or from slaughtered animals such as pigs. Human-derived insulin generally had better pharmacological properties, but was in limited supply and carried risks of disease transmission. By cloning the human insulin gene and expressing it in *E. coli*, large quantities of insulin identical to the human hormone could be produced in fermenters, safely and efficiently. Production of recombinant insulin also allows specialized variants of the protein to be produced: for example, by changing a few amino acids, longer-acting forms of the hormone can be made. The active insulin hormone contains two peptide fragments of 21 and 30 amino acids, respectively. Today, essentially all insulin is produced from recombinant sources (Figure 8.10), i.e. human genes and their derivatives expressed in bacteria or yeast.

![Figure 8.10: A vial of insulin. Note that the label lists the origin as “rDNA”, which stands for recombinant DNA.](Flickr-DeathByBokeh-CC:AN)

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