http://library.thinkquest.org/C004367/be9.shtml

“Recently, we have begun to learn how to take evolution into our own hands through genetic engineering, which involves altering or manipulating an organism's genome to create a new and useful result. The methods often used by genetic engineers are many and varied, but generally fall under one of **three** categories: the plasmid method, the vector method, and the biolistic method.

The Plasmid Method

The first technique of genetic engineering, the plasmid method, is the most familiar technique of the three, and is generally used for altering microorganisms such as bacteria. In the plasmid method, a small ring of DNA called a plasmid (generally found in bacteria) is placed in a container with special restriction enzymes that cut the DNA at a certain recognizable sequence. The same enzyme is then used to treat the DNA sequence to be engineered into the bacteria; this procedure creates "sticky ends" that will fuse together if given the opportunity.

Next, the two separate cut-up DNA sequences are introduced into the same container, where the sticky ends allow them to fuse, thus forming a ring of DNA with additional content. New enzymes are added to help cement the new linkages, and the culture is then separated by molecular weight. Those molecules that weigh the most have successfully incorporated the new DNA, and they are to be preserved.

The next step involves adding the newly formed plasmids to a culture of live bacteria with known genomes, some of which will take up the free-floating plasmids and begin to express them. In general, the DNA introduced into the plasmid will include not only instructions for making a protein, but also antibiotic-resistance genes. These resistance genes can then be used to separate the bacteria which have taken up the plasmid from those that have not. The scientist simply adds the appropriate antibiotic, and the survivors are virtually guaranteed (barring spontaneous mutations) to possess the new genes.

Next, the scientist allows the successfully altered bacteria to grow and reproduce. They can now be used in experiments or put to work in industry. Furthermore, the bacteria can be allowed to evolve on their own, with a "selection pressure" provided by the scientist for producing more protein. Because of the power of natural selection, the bacteria produced after many generations will outperform the best of the early generations.

Many people strongly object to the plasmid method of genetic engineering because they fear that the engineered plasmids will be transferred into other bacteria which would cause problems if they expressed the gene. Lateral gene transfer of this type is indeed quite common in bacteria, but in general the bacteria engineered by this method do not come in contact with natural bacteria except in controlled laboratory conditions. Those bacteria that will be used in the wild - for example, those that could clean up oil spills - are generally released for a specific purpose and in a specific area, and they are carefully supervised by scientists.

The Vector Method

The second method of genetic engineering is called the vector method. It is similar to the plasmid method, but its products are inserted directly into the genome via a viral vector. The preliminary steps are almost exactly the same: cut the viral DNA and the DNA to be inserted with the same enzyme, combine the two DNA sequences, and separate those that fuse successfully. The only major difference is that portions of the viral DNA, such as those that cause its virulence, must first be removed or the organism to be re-engineered would become ill. This does yield an advantage - removal of large portions of the viral genome allows additional "space" in which to insert new genes.

Once the new viral genomes have been created, they are allowed to synthesize protein coats and then reproduce. Then the viruses are released into the target organism or a specific cellular subset (for example, they may be released into a bacterium via a bacteriophage, or into human lung cells as is hoped can be done for cystic fibrosis patients). The virus infects the target cells, inserting its genome - with the newly engineered portion - into the genome of the target cell, which then begins to express the new sequence.

With vectors as well, marker genes such as genes for antibiotic resistance are often used, giving scientists the ability to test for successful uptake and expression of the new genes. Once again, the engineered organisms can then be used in experiments or in industry. This technique is also being studied as a possible way to cure genetic diseases.

Many people object to this type of genetic engineering as well, citing the unpredictability of the insertion of the new DNA. This could interfere with existing genes' function. In addition, many people are uncomfortable with the idea of deliberately infecting someone with a virus, even a disabled one.

The Biolistic Method

The biolistic method, also known as the gene-gun method, is a technique that is most commonly used in engineering plants - for example, when trying to add pesticide resistance to a crop. In this technique, pellets of metal (usually tungsten) coated with the desirable DNA are fired at plant cells. Those cells that take up the DNA (again, this is confirmed with a marker gene) are then allowed to grow into new plants, and may also be cloned to produce more genetically identical crop. Though this technique has less finesse than the others, it has proven quite effective in plant engineering.

Objections to this method arise for many of the same reasons: the DNA could be inserted in a working gene, and the newly inserted gene might be transferred to wild plants. Additionally, this technique is commonly opposed because of its association with genetically modified foods, which many people dislike.”