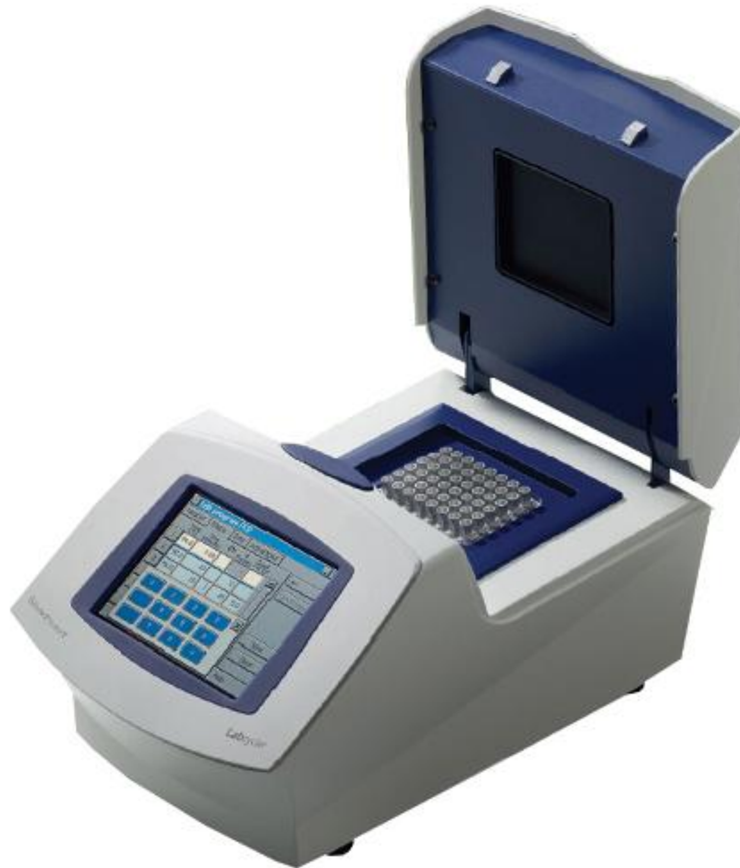
Tools and techniques of genetic engineering:

Each DNA strand contains thousands of genes. Gene cloning is the process of replicating a specific set of genes from a strand of DNA. Genetic engineering is the process of using **gene cloning** and other **genetic manipulations** to isolate specific genes and use it for research and other purposes.

Important Molecular Tools In Genetic Engineering

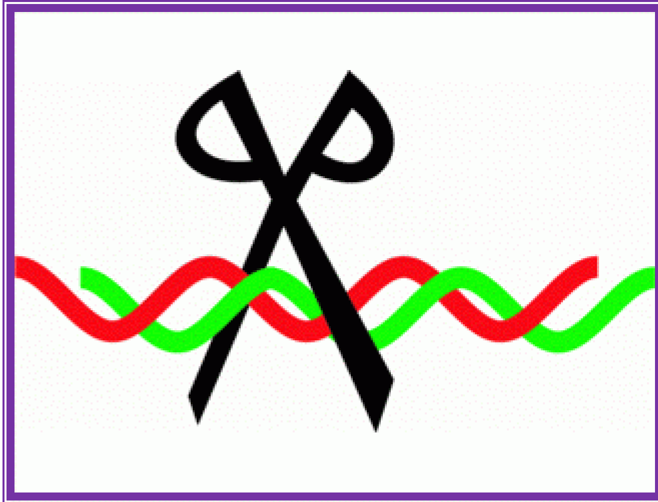
Here is a list of a genetic engineer's molecular tools/enzymes most commonly used in genetic engineering experiments:

1. Polymerase Chain Reaction (PCR)



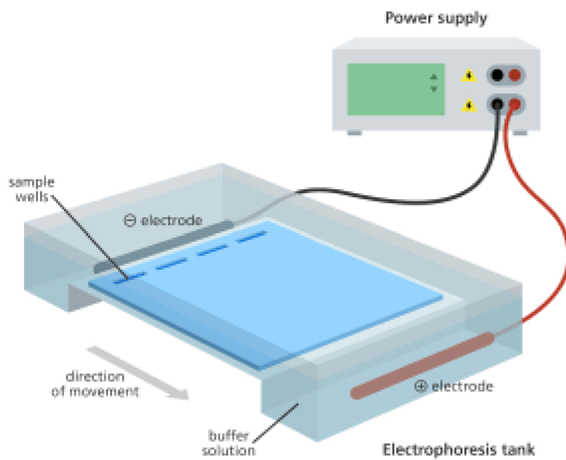
Polymerase Chain Reaction (PCR) is the process of replicating multiple copies of the genes of interest. The discovery of thermostable DNA polymerases, such as *Taq* Polymerase, has made it possible to manipulate DNA replication in the laboratory. It amplifies the quantities of DNA segments. Primers are used to identify the gene of interest and replicate them. These copies can then be separated and purified using gel electrophoresis.

2. Restriction Enzymes (Molecular Scissor)



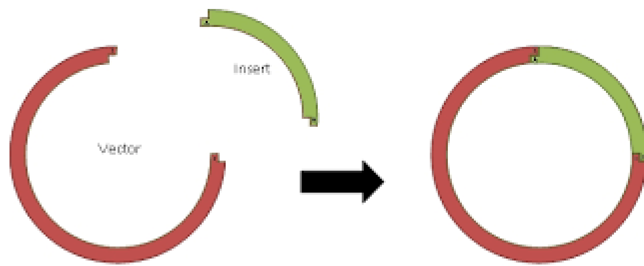
The discovery of enzymes known as restriction endonucleases has been essential to protein engineering. Based on the nucleotide sequence, these enzymes cut DNA at specific locations. DNA cut with a restriction enzyme produces many smaller fragments of varying sizes. These can be separated using gel electrophoresis or chromatography.

3. Gel Electrophoresis



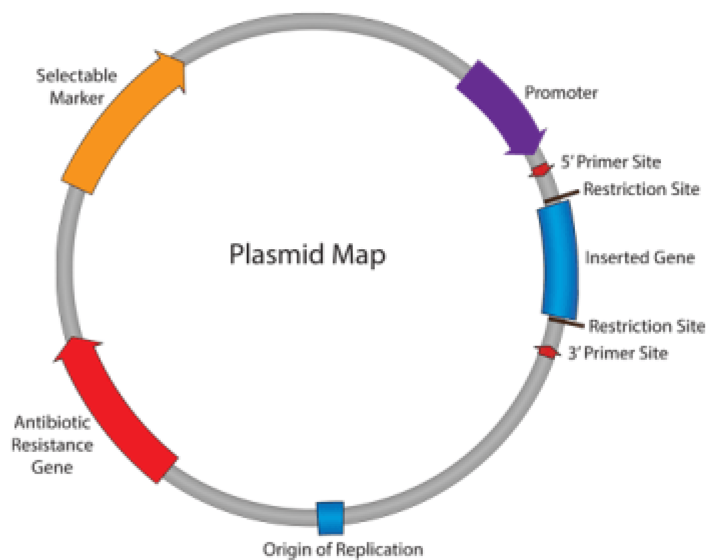
Purifying DNA from cell culture, or cutting it using restriction enzymes wouldn't be of much use if we couldn't visualize the DNA. Gel electrophoresis helps visualize the size and type of DNA extracted using PCR and restriction enzymes. It is also used to detect DNA inserts and knockouts.

4. DNA Ligase



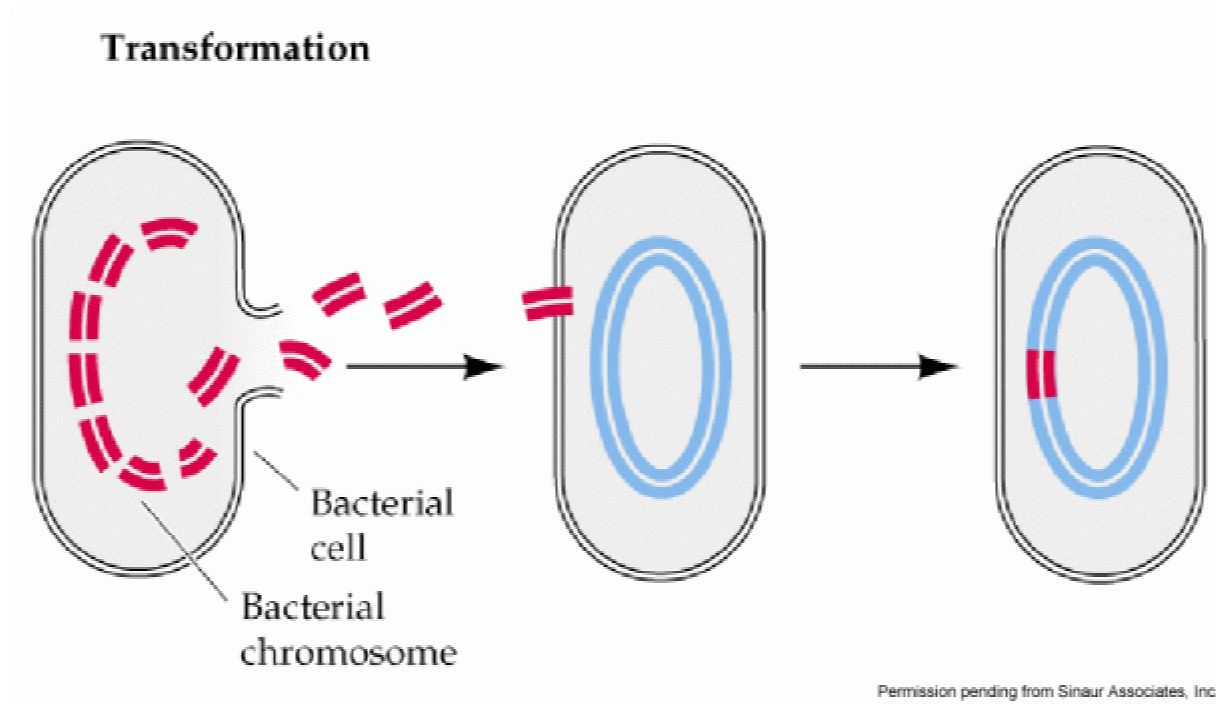
DNA ligase can create covalent bonds between nucleotide chains. This is done to create recombinant strands or close a circular strand that has been cut by restriction enzymes. The enzymes DNA polymerase I and polynucleotide kinase are also important for filling in gaps or phosphorylating the 5′ ends, respectively.

5. Plasmids



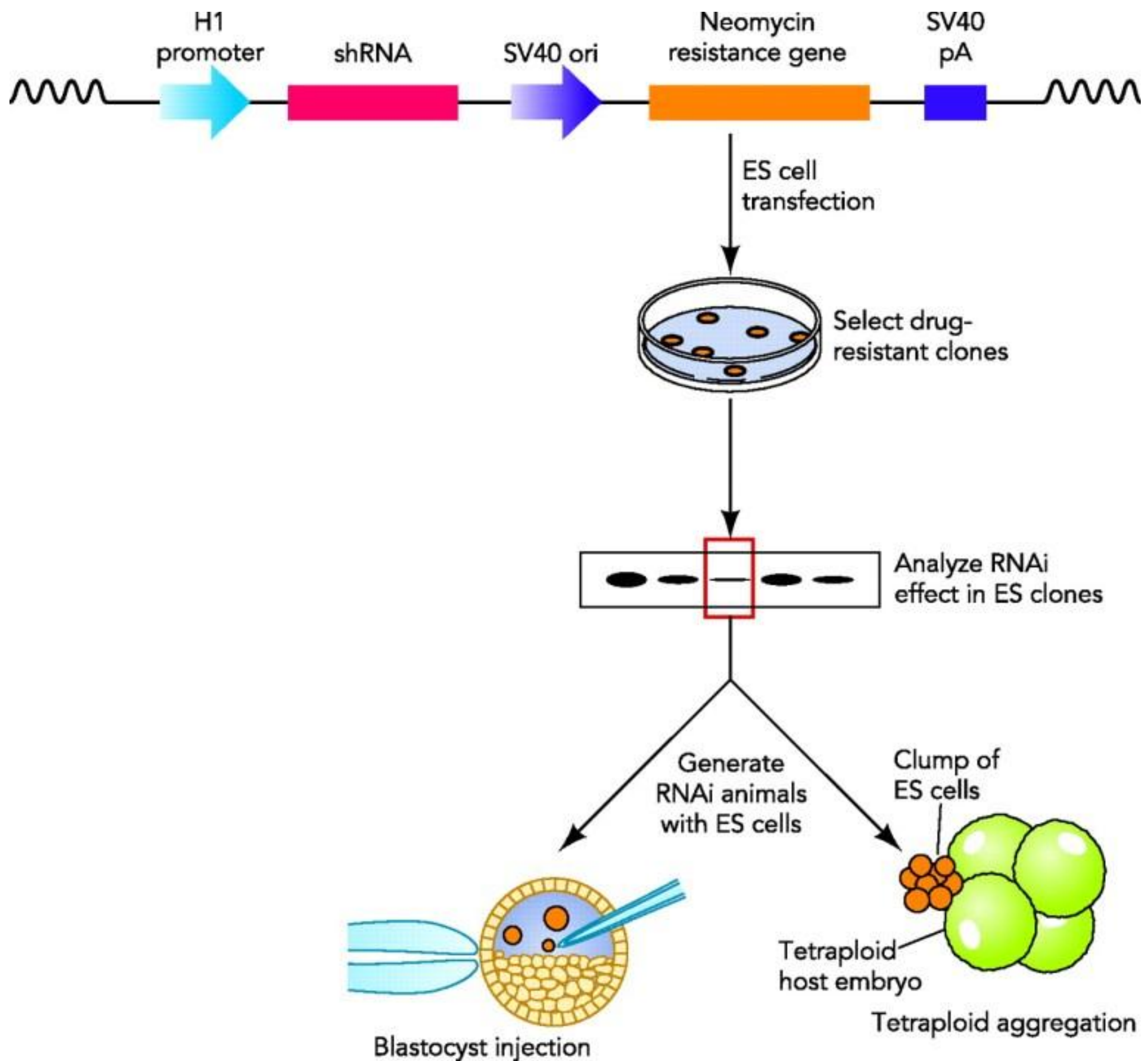
Plasmids are small, circular pieces of DNA that are not part of the bacterial genome but are capable of self-replication. It is used as vectors to transport genes between microorganisms. Once the gene of interest has been amplified with PCR, the gene and plasmid are cut by restriction enzymes and ligated together. The resulting combination is known as Recombinant DNA. Viral (bacteriophage) DNA can also be used as a vector, as can cosmids, which are recombinant plasmids containing bacteriophage genes.

6. Transformation/Transduction



Transformation is the process of transferring genetic material in a vector, such as a plasmid, into host cells. The host cells are exposed to an environmental change, such as electroporation, which makes them "competent" or temporarily permeable to the vector. The larger the plasmid, the lower the efficiency with which it is taken up by cells.
Larger DNA segments are more easily cloned using bacteriophage, retrovirus, or other viral vectors or cosmids in a method called **Transduction**. Phage or viral vectors are often used in regenerative medicine but may cause the insertion of DNA in parts of our chromosomes where we don't want it, causing complications and even cancer.

7. Identifying Transgenic Organisms



Not all cells will take up DNA during transformation. Therefore, it is essential to identify the cells that undergo a transformation and those that have not. Generally, plasmids carry genes for antibiotic resistance, and transgenic cells can be selected based on the expression of those genes and their ability to grow on media containing that antibiotic. Alternative methods of selection depend on the presence of other reporter proteins such as the x-gal/*lacZ* system, or green fluorescence protein, which allow selection based on color and fluorescence, respectively.