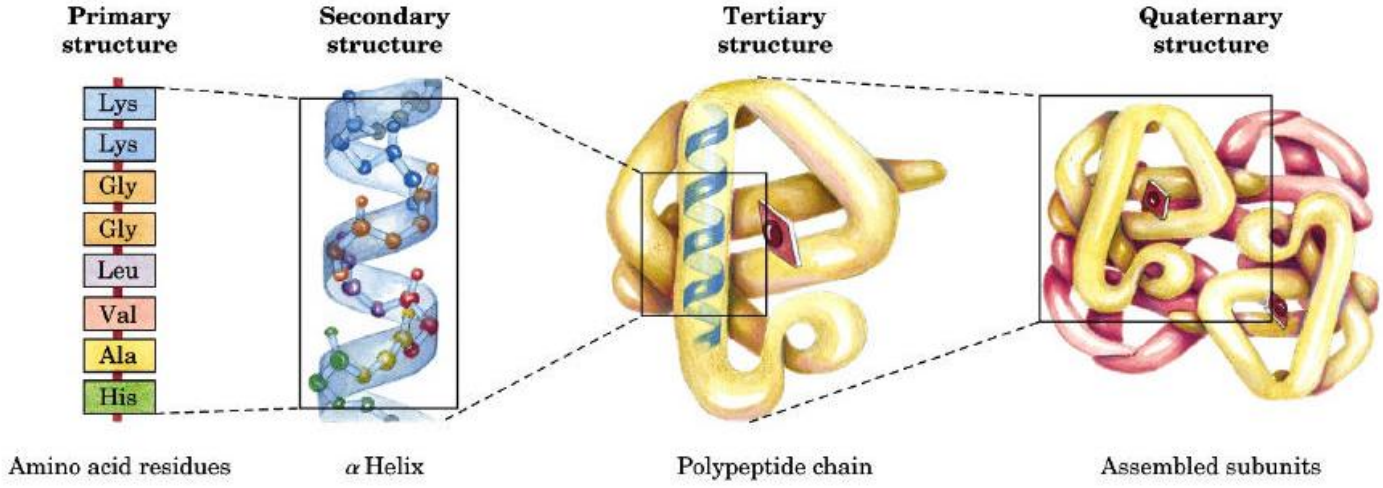
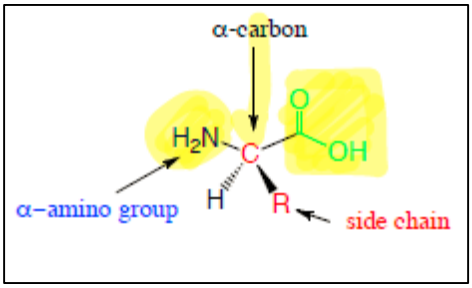
DNA, Gene and Protein:

Deoxyribonucleic acid (DNA) stores genetic information. Its components, two types of nucleotide base (purines and pyrimidines), deoxyribose, and a phosphate group, are arranged in a specific chemical relationship. They determine the three-dimensional structure of DNA, from which it derives its functional consequences. The gene is the basic unit of heredity. A gene is a sequence of nucleotides in DNA that codes for the sequence of the primary structure of a protein. Single gene sequences within the genome, ultimately work together to produce the quaternary structure of a functioning protein. The tasks of bio-engineers are easily outlined - they localize the genes that code for new enzymes and introduce them into a suitable host; i.e. the introduction of the new genes interfere with the already existing pathway by forcing the organism to generate substitute products; by doing so they assign that organism a totally new set of characteristics, besides creating species with new properties.



Levels of structure in proteins

Primary structure: Linking of amino acid.
Secondary structure: Stable arrangement of amino acid gives rise to structural patterns.
Tertiary structure: 3-dimensional folding of polypeptides.
Quaternary structure: Arrangement in space of 2 or more polypeptide subunits.



Basic amino acid structure

Example of proteins

For structure	For function
Collagen Found in bone and skin	Hormones Control body function
Keratin Makes hair and nails	Antibodies Fight infection
Fibrin Helps clot blood	Enzymes Help speed up chemical reactions in the body
Elastin Major part of ligaments	Hemoglobin Carries oxygen in the blood

Genetic Engineering

In the early 1970's restriction enzymes were discovered. These are naturally occurring enzymes that are part of the natural defense mechanisms that a bacteria use to defend themselves. When a bacteriophage (virus) infiltrates a bacterial cell, the bacteria release restrictions enzymes that cut the DNA/RNA of the invader in small fragments; consequently the viral DNA is not able to launch the reverse transcriptase in order to use the bacteria's own enzymatic machinery to produce more viruses. So far, many different restriction enzymes have been identified. Each restriction enzyme "cuts" at specific sites within the genome of an intruding DNA; Gene technology uses practical modification of these enzymes. The basic tool to modify an organisms genome is achieved with recombinant DNA.

Recombinant DNA:

Recombinant DNA is made by splicing a foreign DNA fragment into a small replicating molecule (such as a plasmid), which will then amplify the fragment along with itself resulting in a molecular "clones" of the inserted DNA fragment.

Making Recombinant DNA:

The organism under study, which will be used to donate DNA for the analysis, is called the donor organism. The basic procedure is to extract and cut up DNA from a donor genome into fragments containing one to several genes and allow these fragments to insert themselves individually into opened-up small autonomously replicating DNA molecules such as bacterial plasmids. These small molecules act as carriers, or vectors, for the DNA fragments. The vector molecules with their inserts are called recombinant DNA because they represent novel combinations of DNA from the donor genome (which can be from any organism) with vector DNA from a completely different source (generally a bacterial plasmid or a virus). The recombinant DNA mixture is then used to transform bacterial cells, and it is common for single

recombinant vector molecules to find their way into individual bacterial cells. Bacterial cells are plated and allowed to grow into colonies. An individual transformed cell with a single recombinant vector will divide into a colony with millions of cells, all carrying the same recombinant vector. Therefore, an individual colony represents a very large population of identical DNA inserts, and this population is called a DNA clone.

Clone*: A plant or animal that has same genes as original from which it was produced.*

Cloning: *allows the amplification and recovery of a specific DNA segment from large, complex DNA sample such as a genome.*

Isolating DNA:

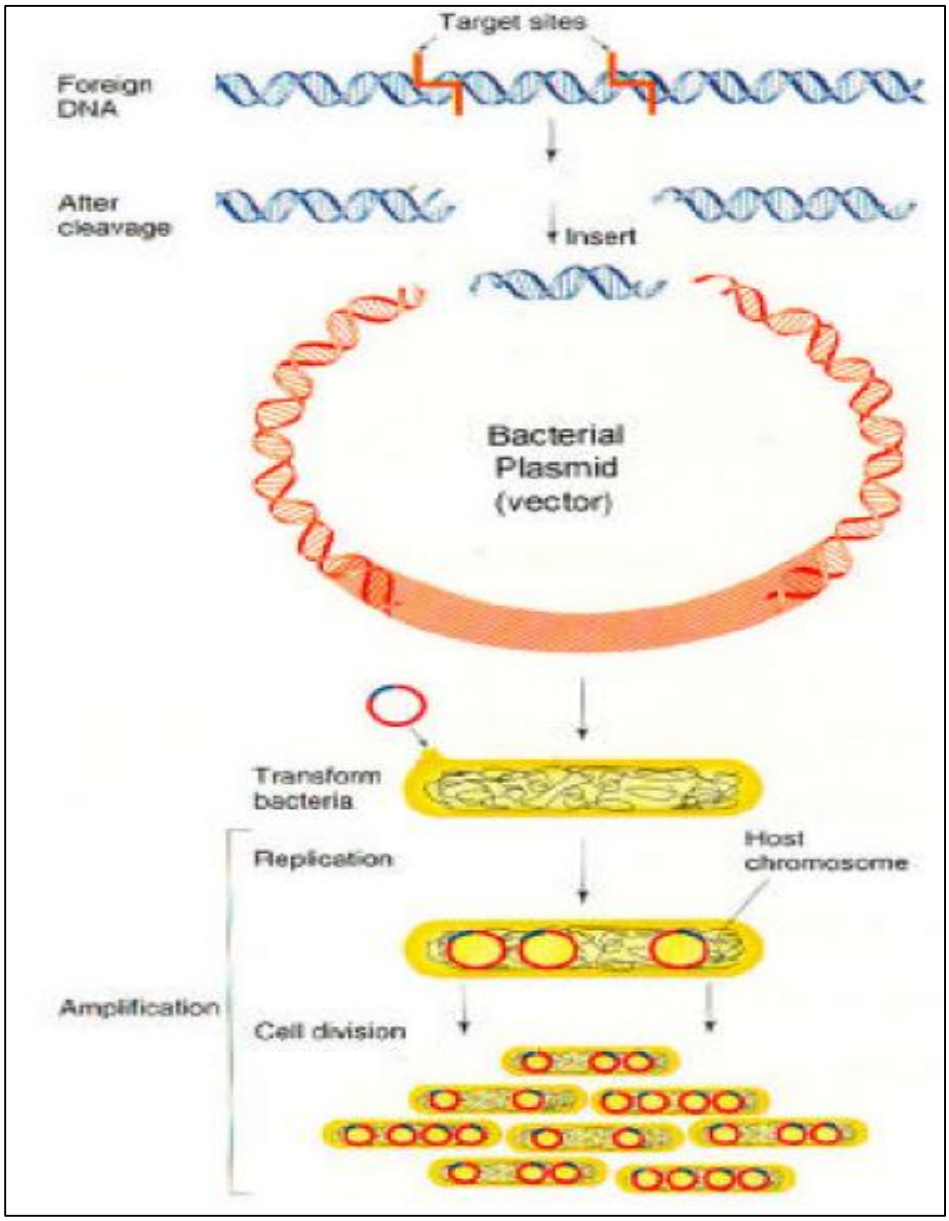
The first step of making recombinant DNA is to isolate a donor and a vector DNA. Generalprotocols for DNA isolation were available many decades before the advent of recombinant DNA technology. Using such methods, the bulk of DNA extracted from the donor will be genomic DNA, and this generally is the type required for analysis. The procedure used for obtaining vector DNA depends on the nature of the vector.

Bacterial plasmids:

Are commonly used vectors, and these must be purified away from the bacterial genomic DNA. Plasmids such as those carrying genes for resistance to the antibiotic can be separated from the bacterial chromosomal DNA. Because differential binding of ethidium bromide by the two DNA species makes the circular plasmid DNA denser than the chromosomal DNA, the plasmids form a distinct band on centrifugation in a cesium chloride gradient and can be separated easily. They can then be introduced into bacterial cells by transformation.

Restriction enzymes:

Have two properties useful in recombinant DNA technology. First they cut DNA into fragments of a size suitable for cloning. Second, many restriction enzymes make staggered cuts generating single-stranded sticky ends conducive to the formation of recombinant DNA. This type of segment is called a DNA palindrome, which means that both strands have the same nucleotide sequence but in antiparallel orientation. The enzyme cuts within this sequence, but in a pair of staggered cuts between the G and the A nucleotides, which means that fragments produced from a double strand DNA-helix have "sticky ends"; i.e. single stranded DNA with sticky ends have a sequence which enables self-recognition to pair with one another.



General steps in recombinant DNA technology

For example: The restriction enzyme EcoR1 recognizes the base sequence G*AATT*C; it cuts the double stranded DNA into an AATT and TTAA HindIII is another restriction enzyme; it acts specifically on AAGCTT yielding single stranded fragments with AGCT and TCGA (of the mirror like strand) ends Upon bringing these spliced strands together, they spontaneously join and form a double stranded DNA-segment. DNA Ligase ("sealant") is important in the cutting of the same enzyme. Therefore, nature has been using this tool and uses them well all the time (in prokaryotes, eukaryotes, etc.); the methodology is not new only the endpoints.

Joining DNA:

Donor DNA (sometimes called Foreign DNA) and vector DNA are digested with restriction enzymes and mixed in a test tube in order to allow the ends to join to each other and form recombinant DNA. There are several ways of joining the donor to the vector to create a recombinant DNA molecule. Cleave DNA at a specific sequence and make single-stranded sticky tails. Such strands in the donor DNA then anneal to sticky ends in the vector, which has been cleaved by the same restriction enzyme.

Amplifying Recombinant DNA:

Recombinant plasmid DNA is introduced into host cells by transformation. Once in the host cell, the vector will replicate in the normal way, but now that the donor DNA insert is part of its length, the donor DNA is automatically replicated along with the vector. Each recombinant plasmid that enters a cell will form multiple copies of itself in that cell. Subsequently many cycles of cell division will occur, and the recombinant vectors will undergo more rounds of replication. The resulting colony of bacterial will contain billions of copies of the single donor DNA insert. This set of amplified copies of the single donor DNA fragment is the DNA clone. This gene cloning is a reproduction process (in DNA culture) that yields many copies of a single gene sequence. A clone is a group of cells, organisms, genes, etc. that are an exact copy of each other. Recombinant DNA is put together to generate many clones of a particular sequence. The key element in gene cloning is the plasmid. It is a circular strand of DNA, that is separated from the bacterial chromosome – it is a natural vehicle that is often used to introduce a foreign gene into another cell - plasmids are a commonly used in transferring resistance genes against antibiotics from one cell to the other - this process is natural. As bacteria are R-strategists, they yield immediate results as they multiply quickly - the only task the researcher has to do is to find the cell with the wanted for properties.

References:

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Biotechnology unzipped promises and realities (2006) by Eric S. Grace, 2nd edition