

## Nucleic acid

### Learning Objectives

This unit gives an account of the structure and functions of nucleic acids present in cells. The phenomenon of replication and recombinant DNA synthesis are also described. Further, the processes involved in the transcription and translation of RNA are also described.

### Structure of Nucleic acids

#### Nucleic acid

A "nucleic acid" is a polymer in which the monomer units are nucleotides. Nucleic acids fall into two classes according to the nature of the sugar they contain: the **deoxyribonucleic acid** (DNA) and the ribose containing **ribonucleic acid** (RNA). DNA is found primarily in cell nuclei and certain viruses, but it may also occur in other portions of cell, e.g. mitochondria.

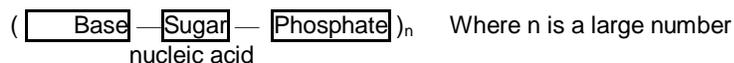
#### Function of nucleic acids

**DNA** is the carrier of genetic information in all cells and in many viruses. It stores the complete genetic information required to specify the structure of all the proteins and RNAs of each species of organisms. It programmes in time and space the orderly biosynthesis of cell and tissue components determines the activities of an organism and it determines the individuality of a given organism.

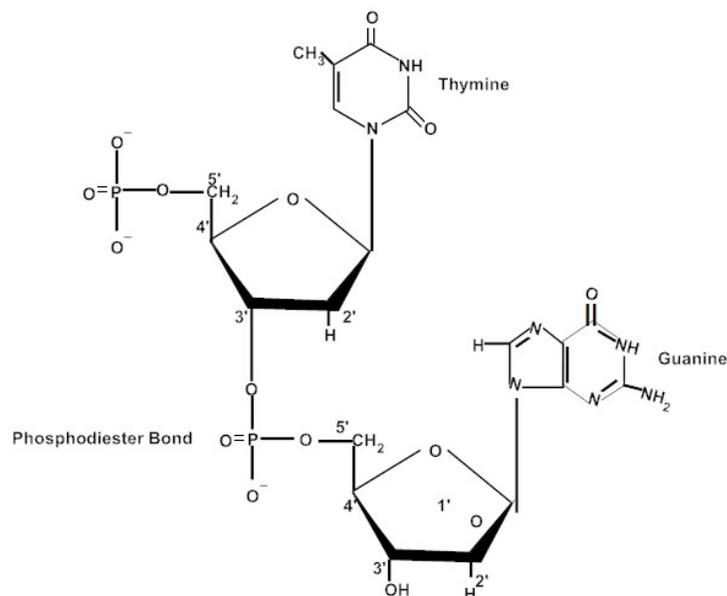
**RNA** is the link between DNA and enzymes. It is associated with the process of protein synthesis which expresses genetic information. It is found in cytoplasm, both in soluble form (sRNA) and associated with ribosomes. It also occurs in the nucleolus.

#### Structure of Nucleic acids

DNA and RNA are polymers of nucleotides composed of nitrogenous bases, sugars, and phosphoric acid thus,

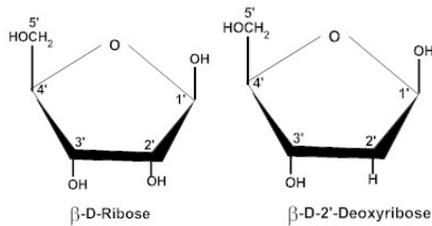


They serve as monomeric precursors of RNA and DNA. The link between the polymer units is the phosphate diester bond



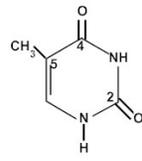
#### Pentose sugars

The sugar unit of a nucleotide is either the pentose ribose or deoxyribose. The sugar unit present in RNA is ribose and that in DNA is deoxyribose.

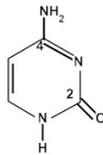


### Nitrogen-containing heterocyclic base

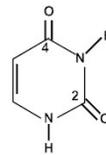
Five nitrogen-containing heterocyclic bases form the nucleotide components. The three pyrimidine derivatives found in nucleic acids are uracil (U) thymine (T), [cytosine](#) (C). The remaining two are derivatives of purine viz. [Adenine](#) (A), and [guanine](#) (G). [Cytosine](#), [guanine](#), [adenine](#) are found in both DNA and RNA. Thymine is found only in DNA and uracil only in RNA.



Thymine(T)

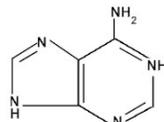


Cytosine(C)

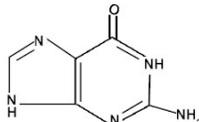


Uracil (U)

### Pyrimidine derivatives



Adenine

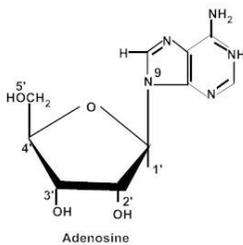


Guanine

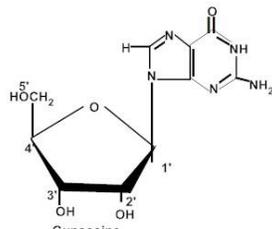
### Purine derivatives

### Structure of Nucleosides

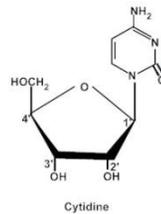
The nucleotide base is covalently bound to the 1' carbon of a ribose or deoxyribose and is called a nucleoside, and a nucleoside with one or more phosphate groups attached at the 5' carbon is called a nucleotide.



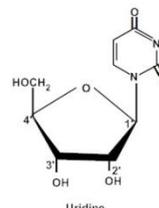
Adenosine



Guanosine



Cytidine



Uridine

### Structure of nucleotides

There are six classes of nutrients that contribute to an adequate diet. Nucleotides are the nucleosides phosphorylated one or more of the hydroxyl groups of the sugar. The nucleotides present in nucleic acids are

DNA = [Adenine](#) | + 2-deoxyribose +phosphate

[Guanine](#) |

[Cytosine](#) |

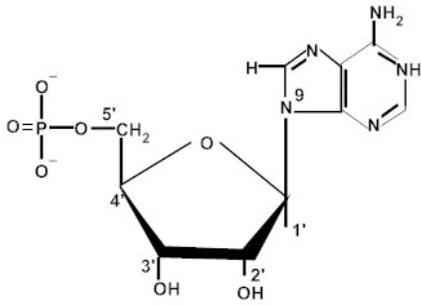
Thymine |

RNA = [Adenine](#) | +ribose +phosphate

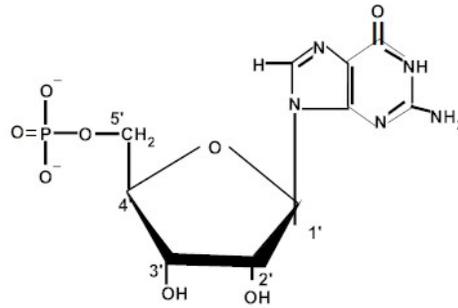
[Guanine](#) |

[Cytosine](#) |

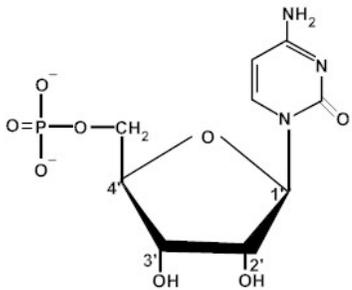
Uridine |  
Nucleotides of RNA



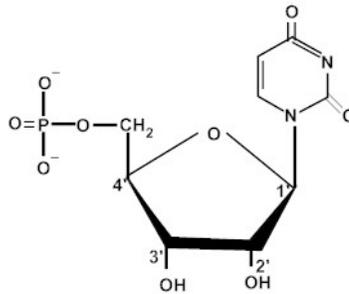
Adenosine mono phosphate (AMP)



Guanosine mono phosphate (GMP)



Cytidine mono phosphate (CMP)

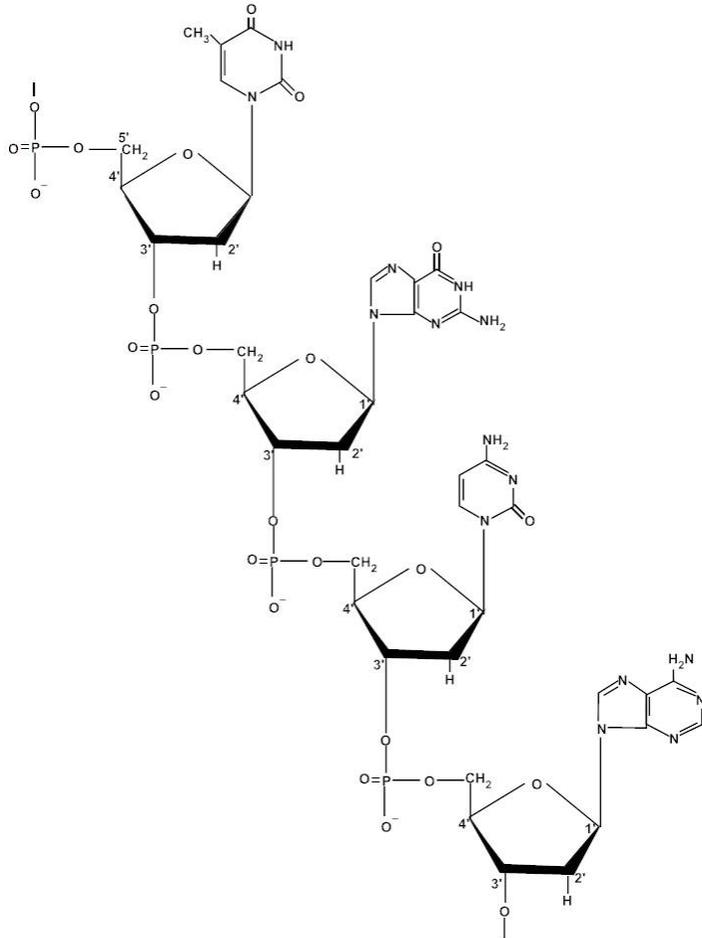


Uridine mono phosphate (UMP)

### Structure of nucleic acids

Nucleic acids are polynucleotides. There are two types of nucleic acids –DNA and RNA.

**Deoxyribonucleic acids (DNA):** DNA exists as a double-stranded structure, with both strands coiled together to form the characteristic double-helix. Each single strand of DNA is a chain of four types of nucleotides: [adenine](#), [cytosine](#), [guanine](#), and thymine. The monomeric units are held in polymeric form by **3', 5' phosphodiester linkages**.



DNA Segment

### The Waston-Crick model of DNA

1. Two polynucleotide chains wind in a right handed manner around a common axis to produce a 20Å -diameter double helix.
2. The two strands of DNA are antiparallel (run in opposite directions), but each forms at right-handed helix.
3. The bases occupy the core of the helix and sugar-phosphate chains run along the periphery, thereby minimizing the repulsions between charges phosphate groups. The surface of the double helix contains two grooves of unequal width; the major and minor grooves.
4. Each base is hydrogen bonded to a base in the opposite strand to form a plane base pair. The Waston-Crick structure can accommodate only two types of base pairs. Each [adenine](#) residue must pair with a thymine residue and vice versa, and each [guanine](#) residue must pair with a [cytosine](#) residue and vice versa. These hydrogen-bonding interactions, a phenomenon known as complementary base pairing, result in the specific association of the two chains of the double helix.
5. The hydrogen bonding between base pairs is an important factor in stabilizing the DNA double helix structure. The pairing of bases in DNA through hydrogen bonding means that the information contained within each strand is redundant.
6. The nucleotides on a single strand can be used to reconstruct nucleotides on a newly synthesized partner strand. DNA strands have a directionality, and the different ends of a single strand are called the 3' end and the 5' end. These terms refer to the carbon atom in ribose to which the next phosphate in the chain attaches.
7. In addition to being complementary, the two strands of DNA are antiparallel: they are orientated in opposite directions. This directionality has consequences in DNA synthesis, because [DNA polymerase](#) can only synthesize DNA in one direction by (5'-3') adding nucleotides to the 3' end of a DNA strand. Deoxyribonucleic acid contains the genetic instructions used in the development and functioning of all known living organisms.
8. The main role of DNA molecules is the long-term storage of information and it contains the instructions needed to construct other components of cells, such as proteins and RNA molecules. The DNA segments that carry this genetic information are called genes or cistrons, but other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information.

## Ribonucleic acid (RNA)

**Ribonucleic acid, or RNA**, is a nucleic acid polymer consisting of nucleotide monomers, which plays several important roles in the processes of transcribing genetic information from deoxyribonucleic acid (DNA) into proteins.

RNA acts as a messenger between DNA and the protein synthesis complexes known as ribosomes, forms vital portions of ribosomes, and serves as an essential carrier molecule for amino acids to be used in protein synthesis.

### Important types of RNA

1. Transfer RNA-tRNA
2. Ribosomal RNA-rRNA
3. Messenger RNA-mRNA

## Transfer RNA or tRNA

The molecules that translate the codons into the amino acid sequence of a protein are the transfer RNA (tRNA) molecules. They function as intermediates that deliver amino acids to the mRNA.

20 distinct tRNA transfer each amino acid to the site of protein synthesis. All tRNA molecules have the same general shape, a “**cloverleaf**” and this shape is crucial to how they function.

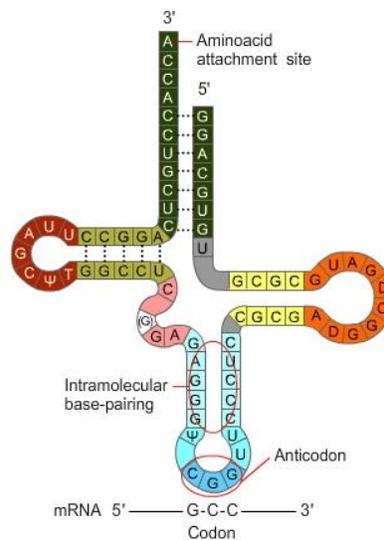
The **3'** end of the open part of the cloverleaf structure is where an **amino acid** becomes **covalently bonded** to the tRNA molecule. The carboxyl groups of amino acids are attached to the 3-hydroxyl group of the adenosyl moiety through an ester bond.

The **anticodon** loop at the end of a base paired stem recognizes the **triplet nucleotide or codon of the template mRNA**. The interaction of the codon on the mRNA and the anticodon on the tRNA leads to the proper placement of an amino acid on the growing peptide chain during protein synthesis.

In all tRNA molecule, there is a **loop** containing the **nucleotides** of **ribothymine** and **pseudouridine** for binding amino acyl tRNA by **ribosome** and another loop containing the minor base **dihydrouracil** for recognition of tRNA.

Each of the different tRNA molecules is specifically recognized by an **aminoacyl tRNA synthetase** enzyme. These enzymes recognize the kind of amino acid that belongs to the particular tRNA and facilitates its bonding to the tRNA.

## Structure of tRNA



## Ribosomal RNA (rRNA)

**Ribosomal RNA** is the RNA that combines with specific proteins to form ribosomes, the sites for protein synthesis. On the ribosome, the [mRNA](#) and tRNA molecules interact to translate information transcribed from the gene in to specific protein molecule.

**Ribosomes:** A ribosome is a cytoplasmic nucleoprotein structure that acts as the machinery for the synthesis of proteins from the [mRNA](#) templates. The mammalian ribosome contains two major nucleoprotein subunits, a larger one (**60S**) and a smaller one (**40S**).

The **60S subunit** contains a 5S ribosomal RNA (rRNA), a 5.8S rRNA, and a 28S rRNA. There are also probably more than 50 specific polypeptides. The smaller **40S subunit** contains a single 18S rRNA and approximately 30 polypeptide chains.

The highly methylated ribosomal RNA molecules are packaged in the nucleolus with the specific ribosomal proteins. In the cytoplasm, the ribosomes remain quite stable and capable of many translation cycles.

The functions of the ribosomal RNA molecules in the ribosomal particle are not fully understood, but they are necessary for ribobinding of [mRNA](#) to ribosome and its translation.

### **Messenger RNA (mRNA)**

**Messenger RNAs** are single stranded molecules that vary greatly in length whose nucleotide sequences direct protein synthesis.

In **prokaryotes** a single [mRNA](#) molecule may code for a single polypeptide or for two or more polypeptide chains. If it codes for a single polypeptide, the [mRNA](#) is **monogenic** or **monocistronic**; if it codes for two or more different polypeptides, the [mRNA](#) is **polygenic** or **polycistronic**. The minimum length of an [mRNA](#) is set by the length of the polypeptide chain for which it codes.

mRNAs formed in bacterial transcription are always longer than needed for the polypeptides which they code, since they have a noncoding polynucleotide '**leader**' at the 5'end with 25 to 150 bases long.

Polygenic mRNAs may have nontranslated intergenic or spacer regions, which separate the segments coding the individual polypeptide chains and appear to help regulate the rate of translation.

## **Replication of DNA molecules**

### **DNA Replication**

The two strands of the DNA double helix are a pair of templates, or patterns. During replication, the strands separate. Each can then act as a template for the synthesis of a new, complementary strand. The result is two daughter DNA molecules with base sequences identical to those of the parent double helix.

#### **The details of replication**

Under the influence of the enzyme DNA helicase, the DNA double helix unwinds, and the hydrogen bonds between complementary bases are broken. The base of the separated strands is no longer connected by hydrogen bonds. They can pair with free individual nucleotides present in the cell's nucleus. The base pairing always involves C pairing with G and A pairing with T. This pairing process occurs one nucleotide at a time.

After a free nucleotide has formed hydrogen bonds with a base of the old strand(the template), the enzyme [DNA polymerase](#) verifies that the base pairing is correct and then catalyzes the formation of a new phosphodiester linkage between the nucleotide and the growing stand.

The [DNA polymerase](#) then slides down the strand to the next unpaired base of the template, and the same process is repeated.

Each of the two daughter molecules of double-stranded DNA formed in the DNA replication process contains one strand from the original parent molecule and one newly formed strand.

DNA replication is the process by which new DNA molecules are generated. It is the process by which DNA molecules produce exact duplicates of themselves. DNA molecules are the carriers of genetic information within a cell. Each time a cell divided, an exact copy of the DNA of the parent cell is needed for the new daughter cell

### **The Replication process**

DNA is replicated by enzymes known as DNA-directed [DNA polymerase](#) or [DNA polymerase](#).

**DNA polymerase:** These enzymes use single stranded DNA as templates on which to catalyse the synthesis of the complementary strand from the appropriate deoxynucleoside triphosphates.

All the DNA polymerases can add a nucleotide only to the free 3' -OH group of a base-paired polynucleotide so that DNA chains are extended only in the **5' to 3' direction**.

Because the two strands of parent DNA run in opposite directions (one in 5' to 3' and the other 3' to 5'), only one strand can grow continuously in the 5'-to-3' direction (Lagging strand).

The other strand must be formed in short segments, called **Okazaki fragments** (after their discoverer, Reiji Okazaki), as the DNA unwinds (Lagging strand).

The breaks or gaps in this daughter strand are called nicks.

To complete the formation of this strand, the Okazaki fragments are connected by action of the **enzyme DNA ligase**.

DNA unwinding during replication process

The process of DNA unwinding does not have to begin at an end of the DNA molecule. It may occur at any location within the molecule. Unwinding usually occurs at several interior locations simultaneously and that DNA replication is bidirectional for those locations; that is, it proceeds in both directions from the unwinding sites.

The result of this multiple-site replication process is formation of "bubbles" of newly synthesized DNA. The bubbles grow larger and eventually coalesce, giving rise to two complete daughter DNAs. Multiple-site replication enables large DNA molecules to be replicated rapidly.

1. DNA is replicated by enzymes known as DNA-directed DNA polymerases or simply [DNA polymerase](#). These enzymes use single-stranded DNA (ssDNA) as templates on which to catalyze the synthesis of the complementary strand from the appropriate deoxynucleoside triphosphates. The reaction occurs through the nucleophilic attack of the growing DNA chain's 3'-OH group on the alpha-phosphoryl of an incoming nucleoside triphosphate.
2. One strand of the preformed DNA serves as the primer. [DNA polymerase](#) adds nucleotides sequentially to the 3' end of one strand, the primer. Therefore, the synthesis of the new DNA strand is in the 5' → 3' direction. The [DNA polymerase](#) cannot start a new DNA strand by itself; it can only add to a preexisting strand and even then can add new nucleotide residues only if a template strand is available.
3. The other strand of the preformed DNA serves as a [Template](#)

Nucleotides are added to the primer strand in accordance with the base sequence of the template strand, following Watson-Crick base pairing.

Wherever a thymine residue occurs in the template strand, an [adenine](#) residue is added to the daughter strand, and vice versa.

Similarly, wherever a [guanine](#) residue is present in the template strand, a [cytosine](#) residue is inserted in the daughter strand and vice versa.

The product of DNA Polymerase is thus a base-paired [duplex](#).

### **Differences between RNA and DNA**

For major differences exist between RNA molecules and DNA molecules.

1. The sugar unit in the backbone of RNA is ribose; it is deoxyribose in DNA.
2. The base thymine found in DNA is replaced by uracil in RNA. Uracil, instead of thymine, pairs with (forms hydrogen bonds with) [adenine](#) in RNA.

3. RNA is a single-stranded molecule; DNA is double-stranded (double helix). Thus RNA, unlike DNA, does not contain equal amounts of specific bases.

4. RNA molecules are much smaller than DNA molecules, ranging from 75 nucleotides to a few thousand nucleotides.

- **Chapter 3: Recombinant DNA and Genetic Engineering**

### **Recombinant DNA and Genetic Engineering**

Increased knowledge about how DNA molecules function under various chemical conditions has opened the door to the field of technology called genetic engineering or biotechnology. Techniques now exist whereby a “foreign” gene can be added to an organism, and the organism will produce the protein associated with the added gene.

The example of benefits that can come from genetic engineering is the production of human insulin. For many years, because of the very limited availability of human insulin, the insulin used by diabetics was obtained from the pancreases of slaughterhouse animals. Such insulin is structurally very similar to human insulin and can be substituted for it. Today, diabetics can also choose to use “real” human insulin produced by genetically altered bacteria. Such “genetically engineered” bacteria are grown in large numbers, and the insulin they produce is harvested in a manner similar to the way some antibiotics are obtained from cultured microorganisms. Human growth hormone is another substance that is now produced by genetically altered bacteria.

Genetic engineering procedure involves a type of DNA called recombinant DNA. [Recombinant DNA](#) is DNA that contains genetic material from two different organisms.

The bacterium *E.coli* which is found in the intestinal tract of humans and animals is the organism most often used in recombinant DNA experiments. Yeast cells are also used, with increasing frequency, in this research.

In addition to their chromosomal DNA, *E.coli* (and other bacteria) contains DNA in the form of small, circular, double-stranded molecules called plasmids. These plasmids, which carry only a few genes, replicate independently of the chromosome. Also, they are transferred relatively easily from one cell to another. Hence plasmids from *E. coli* are used in recombinant DNA work.

The procedure used to obtain *E.coli* cells that contain recombinant DNA involved the following steps

### **The procedure of Recombinant DNA**

**Step1.Cell membrane dissolution:** *E.coli* cells of a specific strain are placed in a solution that dissolves cell membranes, thus releasing the contents of the cells.

**Step2. Isolation of plasmid fraction:** The released cell components are separated into fractions, one fraction being the plasmids. The isolated plasmid fraction is the material used in further steps.

**Step3. Cleavage of plasmid DNA:** A special enzyme, called a restriction enzyme, is used to cleave the double-stranded DNA of a circular plasmid. The result is a linear (noncircular) DNA molecule.

**Step4. [Gene](#) removal from another organism:** The same restriction enzyme is then used to remove a desired gene from a chromosome of another organism.

**Step5. [Gene](#)-plasmid splicing:** The gene (from Step 3) and the opened plasmid (from Step3) are mixed in the presence of the enzyme DNA [ligase](#), which spliced the two together. This splicing, which attaches one end of the gene to one end of the opened plasmid and attaches the other end of the gene to one end of the plasmid, results in an altered circular plasmid(the recombinant DNA).

**Step6. Uptake a recombinant DNA:** The altered plasmids (recombinant DNA) are placed in a live *E.coli* culture, where they are taken up by the *E.coli* bacteria. The *E.coli* culture into which the plasmids are placed need not be identical to that from which the plasmids were originally obtained.

It can be noted from step 3 that the conversion of a circular plasmid into a linear DNA molecule requires a restriction enzyme. A restriction enzyme is an enzyme that recognizes specific base sequences in DNA and cleaves the DNA in a predictable manner at the sequences. The discovery of restriction enzymes made genetic engineering possible.

Restriction enzymes occur naturally in numerous types of bacterial cells. Their functions are to protect the bacteria from invasion by foreign DNA by catalyzing the cleavage of the invading DNA. The term restriction relates to such enzymes placing a "Restriction" on the type of DNA allowed into the bacterial cells.

The restriction enzyme works in the following way. Consider one restriction enzyme that cleaves DNA between G and A bases in the 5'-to-3' direction in the sequence G-A-A-T-T-C. This enzyme will cleave the double-helix structure of a DNA molecule in the manner shown in Figure .

During this process the double helix is not cut straight across but the individual strands are cut at different points, giving a staircase cut. (Both cuts must be between G and A in the 5'-to-3' direction). This staircase cut leaves unpaired bases on each cut strand. These ends with unpaired bases are called "sticky ends" because they are ready to "sticky to"(pair up with) a complementary section of DNA if they can find one.

If the same restriction enzyme used to cut a plasmid is also used to cut a gene from another DNA molecule, the sticky ends of the gene will be complementary to those of the plasmid. This enables the plasmid and gene to combine readily, forming a new, modified plasmid molecule.

This modified plasmid molecule is called recombinant DNA. In addition to the newly spliced gene, the recombinant DNA plasmid contains all of the genes and characteristics of the original plasmid. Step 6 involves inserting the recombinant DNA(modified plasmids)back into *E.coli* cells. The process is called transformation. Transformation is the process of incorporating recombinant DNA into a host cell.

The transformed cells then reproduce, resulting in large number of identical cells called clones. [Clones](#) are cells with identical DNA that have descended from a single cell. Within a few hours, a single genetically altered bacterial cell can give rise to thousands of clones. Each clone has the capacity to synthesize the protein directed by the foreign gene it carries.

Researches are not limited to selection of naturally occurring genes for transforming bacteria. Chemists have developed nonenzymatic methods of linking nucleotides together such that they can construct artificial genes of any sequence they desire. Instruments are now available that can be programmed by a microprocessor to synthesize any DNA base sequence automatically. The operator merely enters a sequence of desired bases, starts the instrument, and returns later to obtain the product. Such flexibility in manufacturing DNA has opened many doors, accelerated the pace of recombinant DNA research, and redefined the term designer genes.