# *Molecular Genetics*

- **Control Of Gene Expression In Eukaryotes**
- **Application Of Genetic Technologies; Recombinant DNA**

# Control of Gene Expression in Eukaryotes:

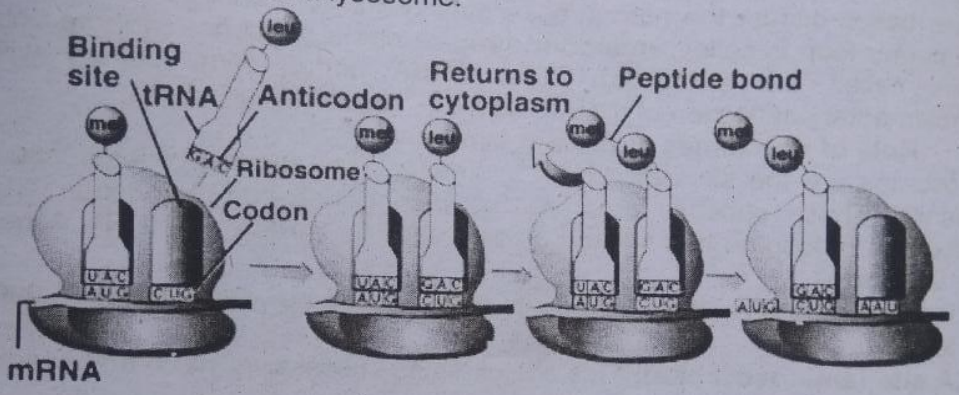


**3. Termination**

The ribosome reaches at the end of the mRNA. The polypeptide chain has been synthesized. Termination codon like UAA comes and translation ends.

**4. Export of protein**

Protein synthesis occurs on ribosomes. Most of the ribosomes are attached on the surface of the rough endoplasmic reticulum. The newly synthesized protein moves into the ER. The protein then moves into the Golgi apparatus. It is packed into a secretary vesicle or a lysosome.



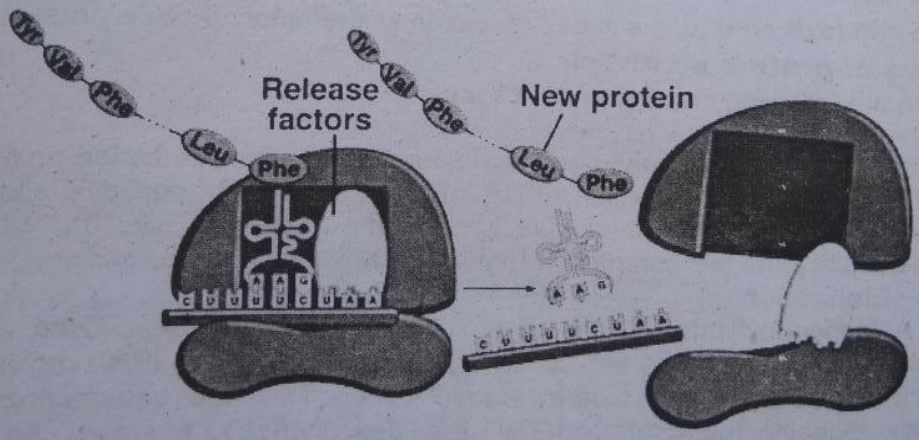




Fig: Termination

## CONTROL OF GENE EXPRESSION IN EUKARYOTES

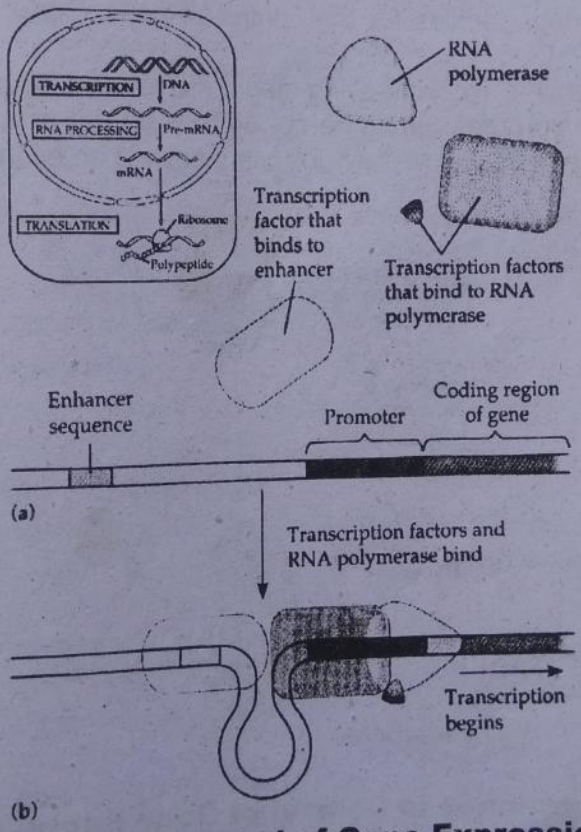
The expression of gene can be controlled at different levels in the eukaryotes.

**1. Transcriptional Control of Gene Expression**

The RNA synthesis depends on RNA polymerase enzymes. Numerous proteins called **transcription factors** help in the action of these enzymes. The RNA

polymerase and transcription factor bind to specific sequences of the promoter. This **promoter** is present at the start of the coding sequence of a gene. Then the polymerase moves along the DNA template. It produces complementary strand of RNA. The transcription factors bind selectively to the **enhancer** regions of DNA. This enhancer region of the DNA is present thousands of nucleotides away from the promoter.

The specific associations between transcription factors and enhancer sites in the genome play an important role in the control of gene expression in eukaryotes. According to one hypothesis, a **hairpin loop** is formed in DNA. This hairpin loop brings the transcription factor attached to the enhancer in contact with the transcription factors. It activates the polymerase at the promoter to start transcription. Over 100 transcription factors have been discovered in eukaryotes.




## 2. Posttranscriptional Control of Gene Expression

Gene expression may be blocked or stimulated at any posttranscriptional step. Gene expression can be controlled during RNA Processing and export of mRNA. The cell must process its initial transcripts before they can act as mRNA, tRNA, or rRNA. An mRNA transcript must receive a 5' cap and a poly-A tail. Similarly, the introns of the RNA segments must he removed and the exons (coding

segments) spliced together. Then this RNA passes from the nucleus to the cytoplasm through a nuclear pore. In the cytoplasm, the mRNA interacts with a number of specific proteins and may associate with ribosomes to undergo translation. Each step in RNA processing provides an opportunity for control of expression.

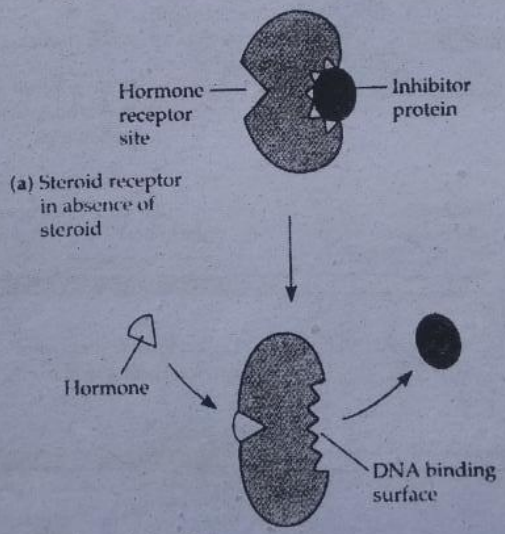
**Regulation of mRNA Degradation**

The life span an mRNA molecule in the cytoplasm is also an important factor in controlling the pattern of protein synthesis in a cell. The mRNA molecules of eukaryotes can have lifetimes of hours, or even weeks. The mRNAs for hemoglobin are translated repeatedly in the developing blood cells of most vertebrate species. The egg cell can store a large number of mRNA.

### 3. Translational and Posttranslational control

Translation in eukaryotic cells involves many protein factors like initiation factors. Thus, there are opportunities for the control of gene expression at the level of translation.

The last opportunities for controlling gene expression occur after translation. Often, eukaryotic polyptides must he cut up to yield the active final protein. The posttranslational processing of the hormone insulin is an example of this control.




### 4. Role of hormones in Control of Gene Expression:

**(a) Action of hormones in invertebrates:** Giant (polytene) chromosomes are present in the salivary glands in certain insect larvae. The presence of these chromosomes provides an evidence for the control of gene expression at the transcriptional level. The chromosome puffs appear at specific sites of the polytene chromosomes. It makes the DNA in that region more accessible to

RNA polymerase. The formation of puff is controlled by a hormone **ecdysone**. It causes molting in insects.

(b) **Action of steroid hormones in vertebrates:** Sex hormones and other steroids alter gene expression in target cells of vertebrates. Steroids are soluble in lipids. When a cell is exposed to a steroid, the hormone diffuses across the plasma membrane. It crosses the cytoplasm. It then enters the nucleus, where it combines with a soluble receptor protein. In the absence of the steroid, the receptor protein is associated with an inhibitory protein. Binding of the steroid hormone to the receptor causes the release of the inhibitory protein. Therefore, the activated receptor protein can now attach on the specific sites on the DNA. These sites are within enhancer regions that control steroid-responsive genes. Thus steroid acts as a chemical signal to switch on specific genes in certain cells.

# MUTATIONS

**The changes in the genetic makeup of a cell are called mutations.** The mutations alter the structure of chromosomes. Sometimes, a point mutation occurs in a gamete, or in a cell. This cell gives rise to gametes. Thus the mutation is transmitted to offspring and to future generations. Sometimes, the mutation has an adverse effect on the phenotype. In this case the mutant condition is taken as a **genetic disorder**, or hereditary disease. For example, sickle-cell anemia affects a single nucleotide in the gene. This gene codes for one of the polypeptides of haemoglobin.

## Types of Mutations

Point mutations within a gene can be divided in to two general categories: base-pair substitutions and base pair insertions or deletions.

### (a) A base-pair substitution

The replacement of one nucleotide and its partner from the complementary DNA strand with another pair of nucleotides is called a base-pair substitution. There may be following types of base pair substitution:

**(1) Missense mutation:**

In this case altered codons still code for amino acids and thus make sense. But these sense may not be the right sense. There are two types of missense mutations:

(a) **Harmless missense mutation:** There is redundancy of the genetic code. So some substitution mutations have no effect on the protein coded. In other words, a change in a base pair may transform one codon into another. The new codon is translated into the same amino acid.

(b) **Lethal missense mutations:** The alteration of a single amino acid in important area of a protein is called lethal missense mutation. This alteration significantly alters protein. Such mutations are dangerous. They create

useless or less active proteins that impair cellular function.

**(2) Nonsense mutation**

**Alterations in change of codon of an amino acid to a stop signal are called nonsense mutation.** In this case a point mutation changes a code for an amino acid into a codon that signals termination.

**(b) Base pair Insertions and Deletions or frame shift mutations**

Insertions and deletions are additions or losses of one or more nucleotide pair in a gene. These mutations have a more dangerous effect. The mRNA is read as a series of nucleotide triple during translation. Therefore, the insertion or deletion of nucleotides may alter the reading frame of the genetic message. Such mutations are called **frames shift mutation**. These mutations occur whenever the number of nucleotides inserted or deleted is not a multiple of 3.

## Mutagenesis

**The creation of mutations is called mutagenesis.** In the 1920s, Hermann Muller discovered X-rays causes genetic changes in the drosophila. Muller obtained mutant Drosophila. He used this drosophila in his genetic studies. But he also found dangerous aspects of his discovery. X-rays and other forms of radiation cause many lethal effects.

Mutagenesis can occur in a number of ways. Errors during DNA replication, repair, or recombination can lead to base-pair substitutions, insertions, or deletions. Mutations resulting from such errors are called **spontaneous mutations**.

**Mutagen is a chemical or physical agent that causes mutation.** Mutagens interact with DNA to cause mutations. There are two categories of mutagens:

1. **X rays and ultraviolet (UV):** X rays and ultraviolet (UV) light are examples of physical mutagens. The UV of sunlight can produce mutations in DNA.
2. **Chemical mutagens:** Chemical mutagens have several categories. Some of these are base analogues. Base analogues are chemicals that are similar to normal DNA bases. But they pair incorrectly.

## Recombinant DNA and Recombinant DNA Technology

**The DNA which contains DNA from two different sources is called Recombinant DNA and the technology for the formation of recombinant DNA is called DNA technology.**

Following materials are required for producing recombinant DNA:

1. **Gene of interest:** The genes are to be cloned.
2. **Molecular scissors:** These are used to cut out the gene of interest.
3. **Molecular carrier or vector:** The genes of interest can be placed on it for transport.
4. **Expression system:** The gene of interest with the vector is introduced into an expression system. Thus a specific product is made.

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### (a) Gene of interest

There are three possible ways to get the gene of interest.

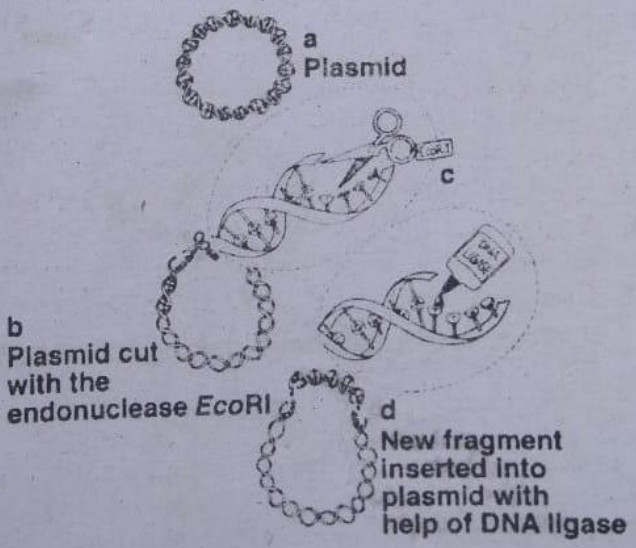
1. **Isolation of gene from the chromosome:** Genes can be isolated from the chromosomes by cutting the chromosomes on the flanking sites of the gene. Special enzymes **restriction endonucleases** are used to cut the genes.
2. **Chemical synthesis of genes:** Small genes can be synthesized in the laboratory.
3. **Making gene from mRNA:** It is a very common method of getting the gene. In this case, gene is synthesized in the laboratory from messenger RNA by reverse transcriptase enzyme.

### (b) Molecular Scissors: Restriction Endonucleases

The restriction enzymes are present in bacteria. The bacteria use these enzymes for their own protection against viruses. The restriction enzyme cuts down the viral DNA. But they do not harm the bacterial chromosome. They restrict the growth of viruses. So they are called restriction enzymes.

Hamilton O. Smith isolated the first restriction enzyme in 1970. These enzymes cut the DNA at very specific sites. These sites have specific sequence of four or six nucleotides.

**EcoRI** is a commonly used restriction enzyme. It cuts double-stranded DNA at the specific sequence of bases. So a gap is produced in this DNA. A piece of foreign DNA with complementary ends can be placed in this gape. The single stranded DNA with complementary ends of the two DNA molecules is called **"sticky ends"** Thus they can bind by complementary base pairing. Therefore, the restriction enzymes help in the insertion of foreign DNA into vector DNA.




### (c) Molecular Carrier: Vector

**The means by which recombinant DNA is introduced into a host cell is called vector.** A vector is selected to make recombinant DNA.

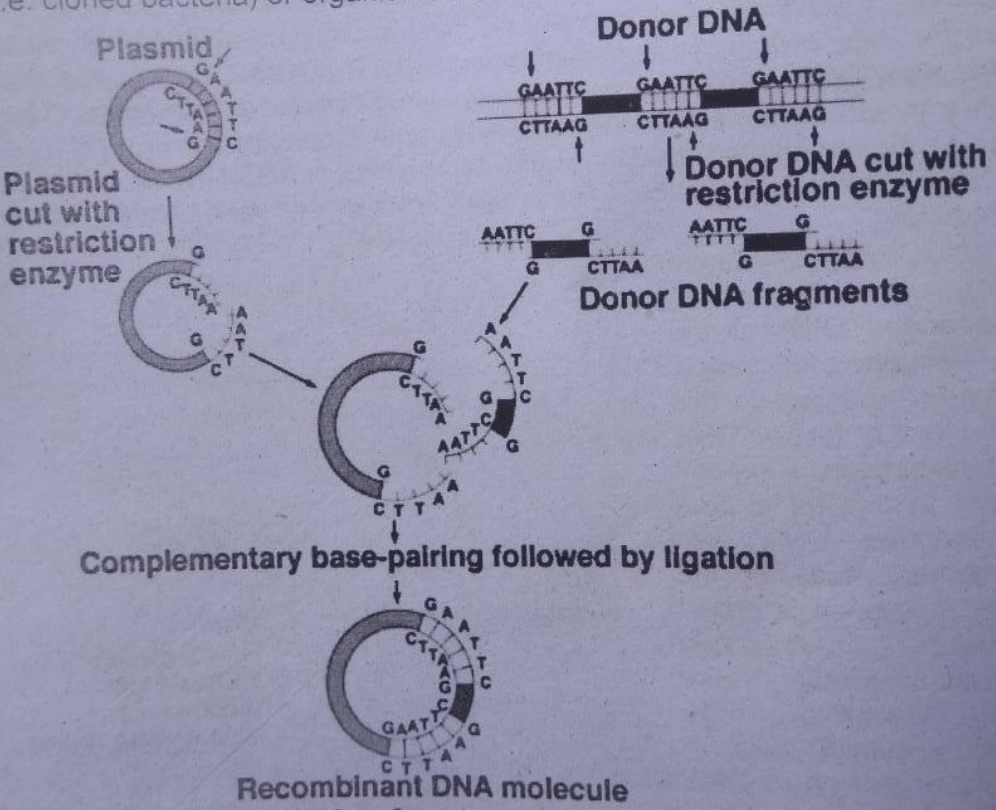
1. **Plasmids: Plasmids are natural extra chromosomal circular DNA molecules.** Plasmid is common type of vector. The investigators discovered Plasmids during their study of the sex life of the intestinal bacterium,

Escherichia coli. They carry genes for antibiotic resistance and fertility etc.

2. **DNA of bacterial viruses:** The DNA of bacterial virus can also be used as a vector. For example **lambda phage**. Lambda phage attaches to a host bacterium. The recombinant DNA is released from the virus and enters the bacterium. The recombinant DNA replicates and many copies of the viruses are formed. Each virus in bacteriophage clone contains a copy of the gene of choice.

**(d) Expression System**

Bacteria are mostly used as expression system. The clone of bacteria is prepared. A clone can be a large number of molecules (i.e. cloned genes) or cell (i.e. cloned bacteria) or organisms that are identical to an original specimen.




**Process of synthesis of recombinant DNA**

1. The bacterial cells are treated with calcium chloride. It makes the bacterial membrane more permeable. Now the bacterial cells take up recombinant plasmid.
2. These bacteria reproduce and bacterial clones are formed. Each new cell contains at least one plasmid. Therefore, each clone of bacteria contains the

gene of interest,
3. The clone bacteria express themselves and make a product.
4. The protein product can be separated from the clone bacteria.
5. The cloned gene can be isolated from this bacterial clone for further analysis.

## APPLICATIONS OF GENETIC TECHNOLOGIES

Today biotechnology products are produced from genetically engineered bacteria, plants and animals. **Organisms that have a foreign gene inserted into them are called transgenic organisms.**

### (a) Transgenic Bacteria

**The bacteria with foreign DNA are called transgenic bacteria.** Recombinant DNA technology is used to produce transgenic bacteria. These bacteria have following uses:

1. **Synthesis of pharmaceutical products:** A foreign gene is replicated and expressed in these bacteria. Thus a large amount of protein product is obtained. Many biotechnology products are produced by bacteria. These products are now available in markets. Some of these products are: **Insulin,** Human growth hormone, Tissue plasminogen activator, Haemophilia factor VIII, Hepatitis B vaccine
2. **Promoting health in plants:** Transgenic bacteria are used to promote health of plants. For example: A bacterium normally forms colonies in the roots of corn plants. Some genes from another bacterium have been inserted into these bacteria. These genes code for an insect toxin. The toxin protects the roots from insects.
3. **Biodegradation:** Bacteria can degrade a particular substance. The ability of degradation of bacteria can be enhanced by genetic engineering.
4. **Biofilters:** The transgenic bacteria can be used as biofilter in industries.
5. **Synthesis of organic compounds:** The catalysts act on precursor molecules during synthesis of organic chemicals. Bacteria can be used in place of these catalysts. These bacteria carry out the synthesis of these compounds. For example, aspartame is dipeptide sweetener. It is known as **Nutrasweet**. It is prepared by transgenic bacteria.
6. **Use in Mining industry:** Many major mining companies are using bacteria to obtain various metals. Genetic engineering enhances the ability of bacteria to extract copper, uranium and gold from low grade sources.

### (b) Transgenic Plants

**The plants with foreign DNA are called transgenic plants.** There following uses of transgenic plants:

1. **Pest and herb resistance:** Foreign genes are transferred to cotton, corn and potato. The cell of these transgenic plants produces an insect toxin. So these plants become resistant to pests.

# Applications of Genetic Technologies:



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2. **Transgenic crops:** In 1999, these transgenic crops were planted on more than 70 million acres worldwide. Their acreage (total acre) is expected to become triple in about five years.
3. **Increasing production of wheat, corn and rice:** Agribusiness companies are also developing transgenic varieties of wheat, rice and corn.

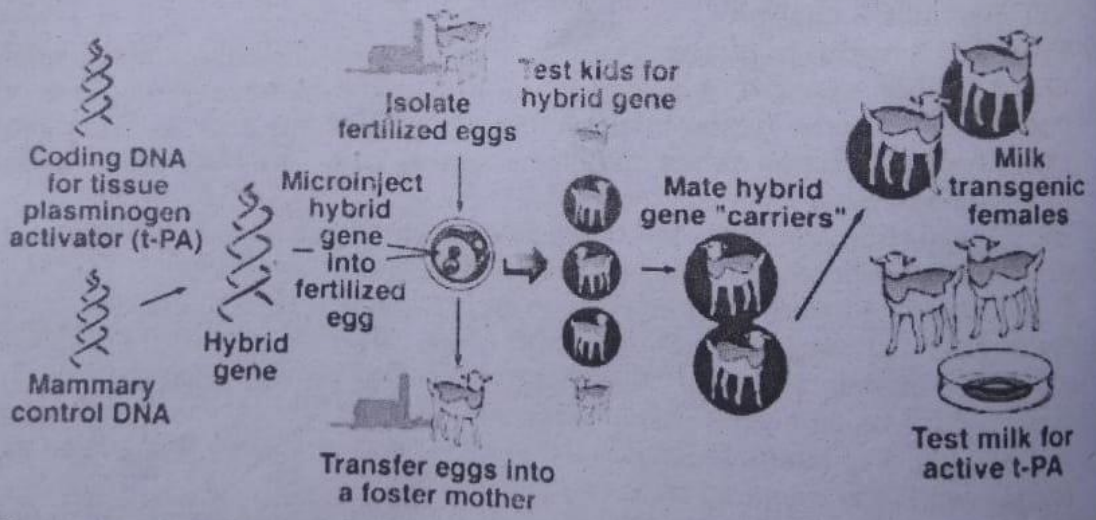
### (c) Synthesis of human products

Plants are engineered to produce human hormones, clotting factors, and antibodies in their seeds. For example: an antibody made by corn can deliver radio isotopes to tumor cells.

### (d) Transgenic Animals

There are following uses of transgenic plants:

1. **Higher growth rate of animals:** The genes for bovine (cattle) growth hormone are injected by this technique into many types of animal eggs. Thus many larger fishes, cows, pigs, rabbits and sheep are produced by this procedure.
2. **Gene pharming:** The use of transgenic farm animals to produce pharmaceuticals is called gene pharming. Many antibiotics, hormones are produced by these techniques.




### (f) GENE THERAPY

**The insertion of genetic material into human cells for the treatment of a disorder is called gene therapy.** Gene therapy is used for two purposes:

There are two main methods used for gene therapy i.e. Ex-vivo and in Vivo.

1. **Ex Vivo gene therapy:** The gene therapy in which genes are inserted into the cell outside the body is called Ex Vivo gene therapy. Following diseases are treated by ex vivo gene therapy: SCID, hypercholesterolemia,
2. **In Vivo gene therapy:** The gene therapy in which genes are inserted in the

cells within the body is called In Vivo gene therapy. Following diseases are treated by these techniques: Cystic fibrosis, cancer Coronary artery angioplasty hemophilia, diabetes Parkinson disease and AIDS.

### (g) DNA finger printing

DNA analysis can be used for following purposes:

1. **Diagnosis:** DNA analysis are used to diagnose viral infections, genetic disorders, and cancer
2. **Use in forensic laboratories:** DNA analysis is used to identify criminals.
3. **Parentage:** The DNA is inherited. Thus the finger print of offspring resembles the finger prints of one's parents. So it can be used to establish parentage.

## DEFINITIONS AND KEY POINTS

| TERMS | DEFINITIONS |
|---|---|
| Primer | This chain of nucleotides is called a primer. |
| Gene | A sequence of bases in DNA that codes for the synthesis of one polypeptide is called gene. |
| Genetic code | The genetic code is a sequence of three bases |
| Transcription | The synthesis of messenger RNA from DNA is called transcription. |
| Primary transcript | The newly transcribed mRNA is called the primary transcript. |
| Translation | The synthesis of protein at the ribosomes form mRNA is called translation. |
| Mutation | The changes in the genetic makeup of a cell are called mutations. |
| Nonsense mutation | Alterations in change of codon of an amino acid to a stop signal are called nonsense mutation. |
| Mutagenesis | The creation of mutations is called mutagenesis. |
| Recombinant DNA | The DNA which contains DNA from two different sources is called Recombinant DNA. |
| Recombinant DNA technology | The technology for the formation of recombinant DNA is called DNA technology. |
| Vector | The means by which recombinant DNA is introduced into a host cell is called vector. |
| Gene therapy | The insertion of genetic material into human cells for the treatment of a disorder is called gene therapy. |