BIOLOGY CAPSULE GENETIC ENGINEERING

ENZYMES produced as **Genetic Engineering** Products.

1. **Glucose Oxidase** Converts glucose to gluconic acid. It is used as a diagnostic enzyme for blood sugar detection.
2. **Renin** For cheese preparation.
3. **Papainain** Used as Meat tenderizer.
4. **Urokinase** Antithrobotic agent in treatment of pulmonary embolism.
5. **Asperaginase** Used in Chemotherapy.
6. **Tyrosine hydroxylase** - Converts tvrosine to DOPA – used in Parkinsons disease.

ENZYMES used in Genetic Engineering

POLYMERASES

1. **DNA dependant DNA polymerase** – Require a DNA primer for DNA synthesis.
2. **RNA dependant DNA polymerase** – It is the *Reverse Transcriptase*.
3. **T4 DNA polymerase** – Obtained from T4 phage. It cannot utilize nicked DNA since it lacks 5′-3′ exonuclease activity.
4. **T7 DNA polymerase** – Only *common DNA polymerase.*
5. **Taq polymerase** – From *Thermus aquaticus* or from thermophilic bacterium *Thermus thermophilus*. It is used in PCR since it can tolerate high temperature.

REVERSE TRANSCRIPTASES

Key enzyme used for the first time in cloning. Discovered in 1970 and used in 1971 for DNA cloning. c DNA Cloning using RT was done in 1976.

AMURT – *Avian Myeloblastosis Virus Reverse Transcriptase.* Used extensively in genetic engineering.

Tdt – Teminal deoxyribonucleotidyl Transferase. – Add triphosphates to 3′ end.

Ligase – Joins nucleotides by forming Phospho diester bonds between terminals .

NUCLEASES

BAL 31 Nuclease – Extra cellular nuclease obtained from Marine bacterium Pseudomonas BAL31.

Mung – Bean Nuclease 1 – From Mung bean purified by Sung and Laskowski in 1962. Preference for Single stranded nucleic acids

Pronase – Proteolytic enzyme.

RESTRICTION ENDONUCLEASES

Term coined by ***Lederberg Meselson*** in 1964.

3 types of RE are present.

1. **Type I.** React with unmodified recognition sequences in double stranded DNA. Cleave only one DNA strand randomly. It is not site specific and cannot be used for DNA manipulation.
2. **Type II**. Widly used RE. Recognize particular sequences of double stranded DNA.
3. **Type III**. Cleave dsDNA at well defined sites. Require ATP and Mg .Intermediate property between Type I and Type II.
4. **Iso schizomers** – RE Recognize identical base sequences of DNA

G | G T AC C
C C A TG | G

Recombinant DNA

Insert DNA (Foreign DNA carrying desired sequences) plus Vector DNA (plasmid). It is also called **Hybrid DNA** or **Chimaeric DNA**

Transformation – Bacterial plasmids are used for gene transfer.

Transfection – Viral DNA is used for gene transfer

Marker gene – Genes of vector DNA that help to Select host cells.

Vectors – Virus, Bacteriophage, Plasmids, Synthetic Chromosomes.

Cosmid – Artificial Plasmids carrying COS (Cohesion) Gene of Lambda phage.

PLASMIDS

Consitute 5% of the total bacterial DNA. It is Circular. Used as Vectors for DNA up to 20kb. Absent in Eukaryotes. Provide resistance against heavy metals and Antibiotics.