FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

LIST OF ENZYMES USED IN GENETIC ENGINEERING

1. Restriction Enzymes (Already discussed in the previous slides)
2. DNA Ligase (Already discussed in the previous slides)
3. DNA Polymerase I
4. Klenow Fragment
5. T_4 DNA Polymerase
6. Thermostable DNA Polymerases
7. Terminal Deoxinucleotidyl transferase
8. Reverse Transcriptase

DNA Polymerase I

The source of DNA polymerase I is *E.coli*

Function of DNA polymerase

- ❖Exonuclease activity 5'-3'
- ❖Exonuclease activity 3'-5'
- ❖Polymerase activity 5'-3': addition of dNTP's at 3'-OH termini.
- ❖Fills gaps in ds DNA

KLENOW FRAGMENT

- ❖Klenow Fragment can be produce by treating with protease subtilisin.

Function of Klenow Fragment:

- ❖Klenow fragment don't have 5' -3' Exonuclese activity. But has 3'-5' Exonuclese activity, and 5'-3' Polymerase activity.

LIGASE ENZYME

➢The term Ligase is taken from the Latin verb ligāre, meaning of Ligare is "to bind" or to glue together.

➢Ligase is an enzyme that can catalyze the joining of two large molecules by forming a new chemical bond, by hydrolysis of a small chemical group dependent to one of the larger molecules or the enzyme catalyzing the linking together of two compounds.

➢In general, a ligase enzyme catalyzes the reaction:

Ab + C → A–C + b

or

Ab + cD → A–D + b + c ……

➢Ligase can join two complementary fragments of nucleic acid by catalyzing the formation of a phosphodiester bond .

➢DNA Ligase play role in

1. DNA replication
2. DNA repair

MECHANISM:

➢Adenylation (addition of AMP) of a lysine residue in the active center of the enzyme takes place and pyrophosphate is released.

➢Transfer of AMP to the 5' phosphate and pyrophosphate bond formation takes place.

➢Formation of a phosphodiester bond between the 5' phosphate of the donor and the 3' hydroxyl of the acceptor.

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T4 DNA Polymerase

T4 bacteriophage is the souce of T4 DNA Polymerase.

Functions of T4 DNA Polymerase:

- ❖It has 3'-5' exonuclease activity.
- ❖It has 5'- 3' Polymerase activity.
- ❖It catalyze template directed DNA synthesis.
- ❖It has processivity (400 nucleotides/second).

Thermostable DNA Polymerases:

Thermostable DNA polymrase usually isolated from thermophillic bacteria (like *Thermus aquaticus*).

Functions:

- ❖The function of Thermostable DNA polymerae is to catalize template directed synthesis from free 3'-OH and bound to prime.
- ❖It can synthesizes DNA at high temperature (in case of PCR).
- ❖For example: *Taq* DNA Polymerase, *pfu* DNA polymerase.

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Terminal Deoxynucleotidyl Transferase:

Terminal deoxynucleotidyl transferase enzymes isolated form Immature, pre-B, pre-T lymphoid cells and acute lymphoblastic leukemia/lymphoma cells and commercially available terminal deoxynucleotidyl transferase enzyme is purified from recombinant *E. coli* cells expressing calf / rat / mouse thymus gene.

Functions:

- ❖It adds a specific nucleotide to the 3'-end of a DNA strand
- ❖It do not require a template
- ❖It required protruding 3' overhang

Reverse Transcriptase:

The source of Reverse transcriptase is retrovirus.
For example: 1. Moloney murine leukemia virus (Mo-MLV)
2. Avian myeloblastosis virus (AMV)

Functions:
Reverse Transcriptase enzymes have two types of activities
1. DNA Polymerase Activity
2. RNase H activity