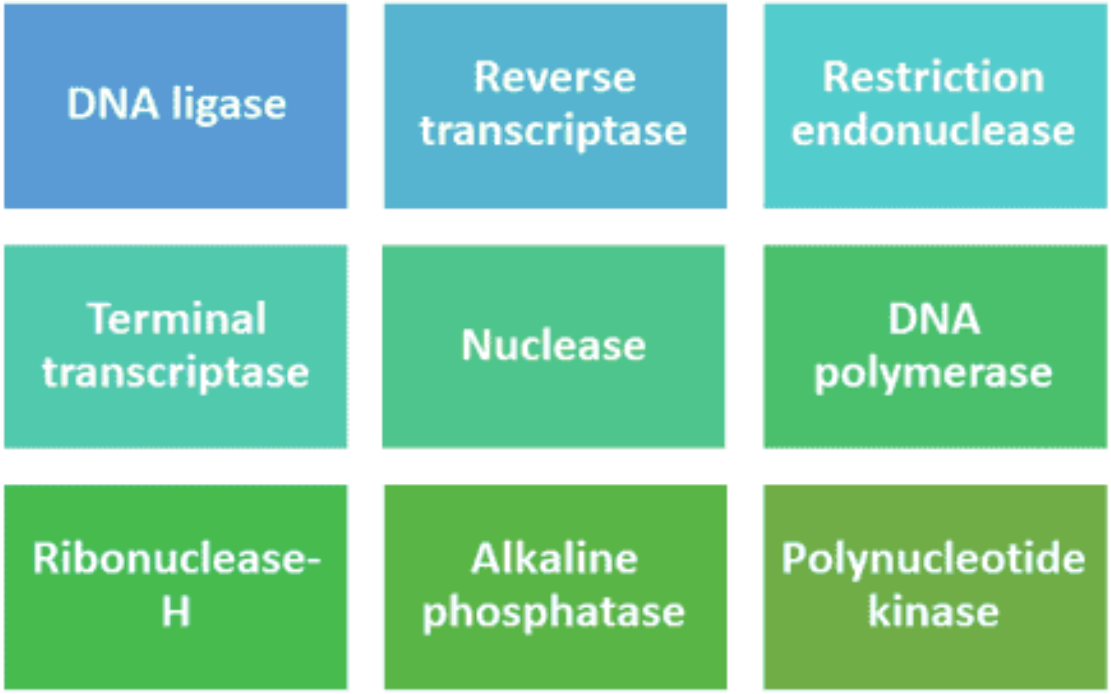
CSIR NET Life Science Unit 13

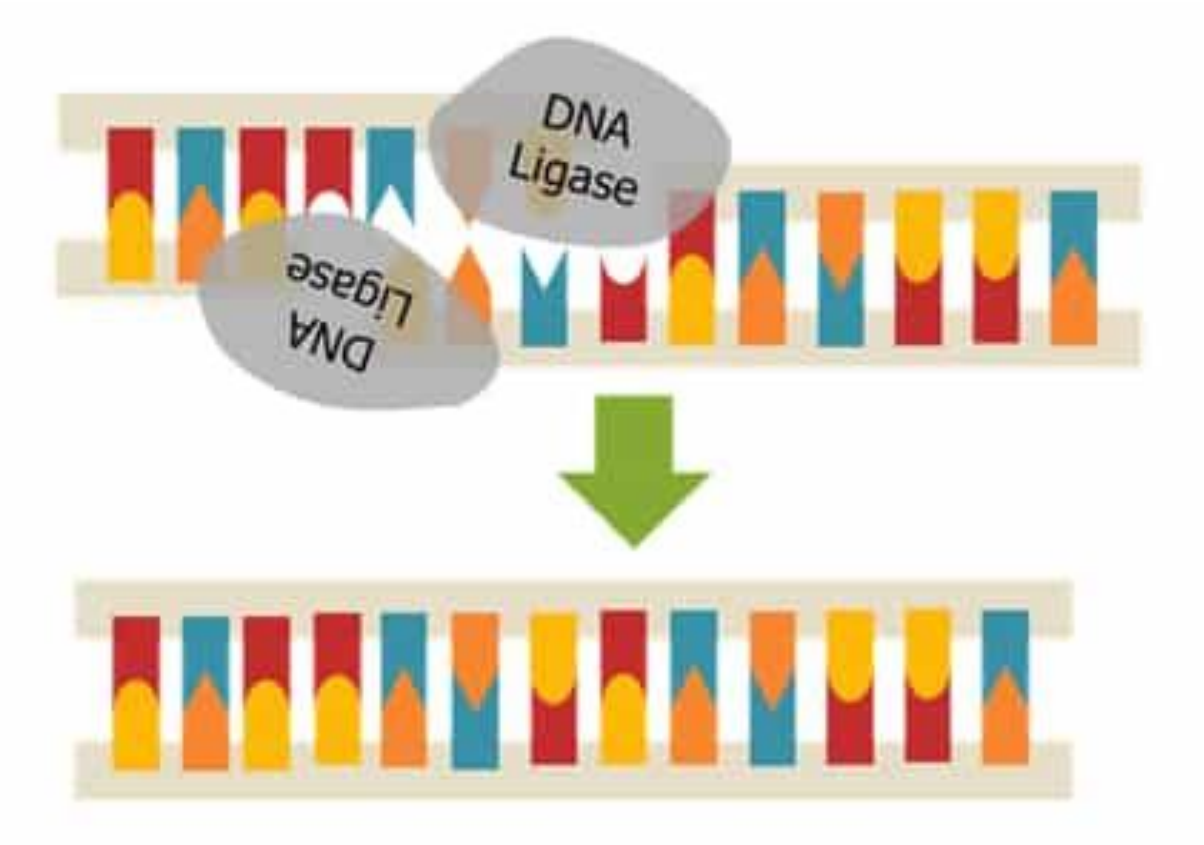
Enzymes of rDNA Technology

Enzymes used in rDNA Technology



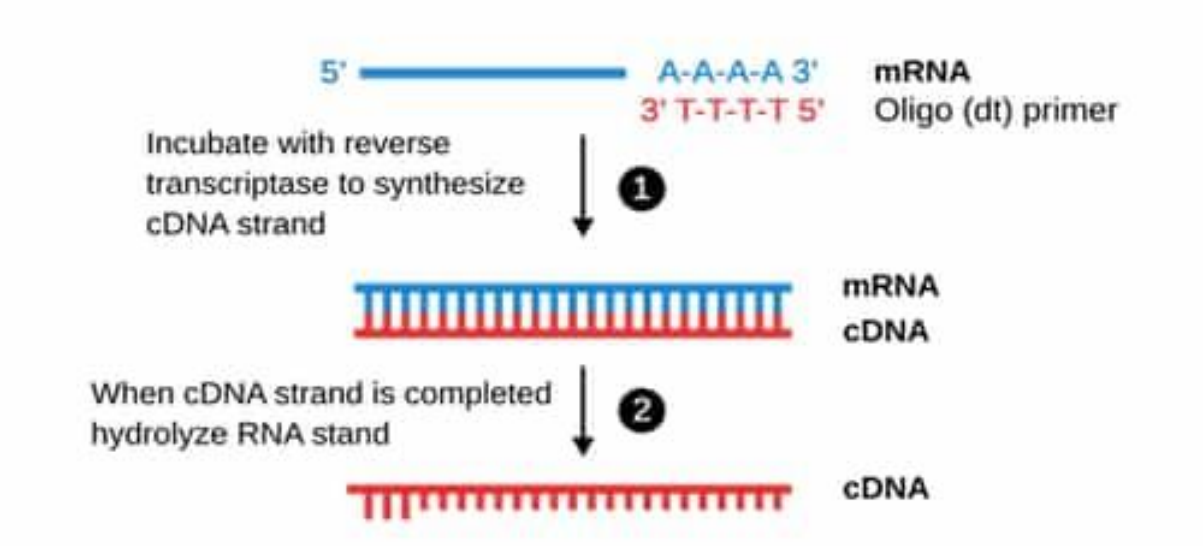
DNA ligase

DNA ligase is isolated from *E.coli* and Bacteriophage commercially and used in recombinant DNA technology. The enzyme DNA ligase joins the DNA fragments with a cloning vector. DNA ligases can form a phosphodiester bond at a single-strand break in DNA, a reaction between a 3'-OH group and a 5'-monophosphate.



Reverse transcriptase

RT is used to synthesize complementary strands (cDNA) from mRNA template. It is also known as RNA-dependent DNA polymerase. It is isolated from a retrovirus.



Restriction endonuclease

Restriction endonuclease enzymes recognize and cut DNA strands at specific sequences called restriction sites. These enzymes are isolated from a wide variety of microorganisms. Endonuclease enzymes degrade foreign genomes

when they enter a microbial cell but the host cell's own DNA is protected from its endonuclease by methylation of bases at the restriction site. There are 3 types of restriction endonuclease:

1. **Type I Restriction endonuclease -** It has both methylation and endonuclease activity. It requires ATP to cut the DNA. It cuts DNA about 1000bp away from its restriction site. For example *Eco*KI.

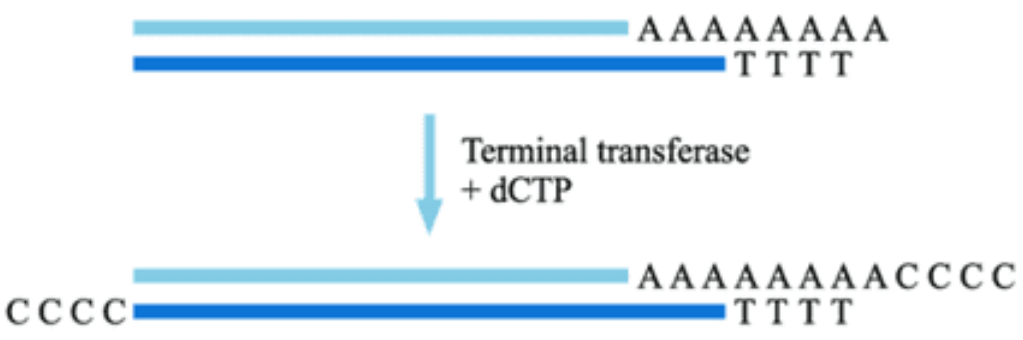
2. **Type II Restriction endonuclease -** It does not require ATP to cut DNA. It cuts DNA at the restriction site itself. For example, *Eco*RI, *Hind* III

3. **Type III Restriction endonuclease -** It requires ATP to cut DNA. It cuts DNA about 25 bp away from the restriction site. For example, *Sty*

Restriction Endonuclease	Structure	Recognition Site	Restriction and Methylation	Cleavage Site
Type I	Bifunctional enzyme (3 subunits)	Bipartite and asymmetric	Naturally exclusive	Nonspecific >1000 bp from recognition site
Type II	Separate endonuclease and methylase	4–6 bp sequence, often palindromic	Separate reaction	Same as or close to recognition site
Type III	Bifunctional enzyme (2 subuints)	5–7 bp asymmetric sequence	Simultaneous	24–26 bp Downstream of recognition site

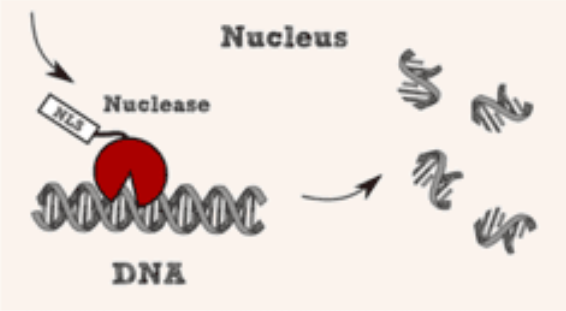
Terminal transferase

It is the enzyme that converts the blunt end of DNA fragments into a sticky end. If the restriction enzyme cuts DNA forming blunt ends, then efficiency of ligation is very low. So the enzyme terminal transferase converts the blunt end into a sticky end. Terminal transferase enzymes synthesize short sequences of complementary nucleotides at free ends of DNA, so that the blunt end is converted into a sticky end.



Nuclease

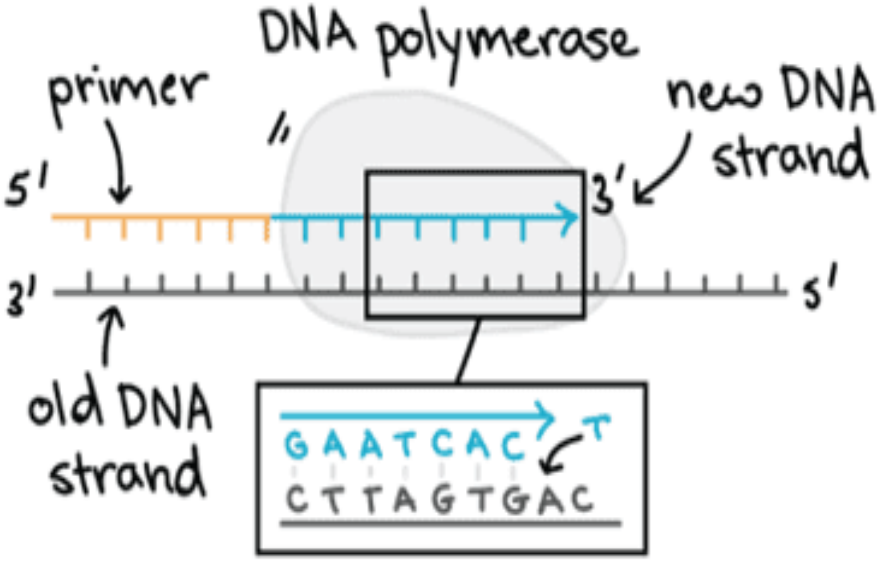
The enzyme nucleases hydrolyze the phosphodiester bond on the DNA strand creating the **3'-OH** group and **5'-P** group. It usually cut DNA on either side of distortion caused by thymine dimers or intercalating agents. The gap is filled by DNA polymerase and the strand is joined by DNA ligase. Nucleases are of two types; endonuclease and exonuclease.



Exonuclease	Endonuclease
These enzymes cleave the phosphodiester bonds which are present **internal** in the polypeptide.	These enzymes cleave the phosphodiester bonds which are present **at the end** of the polypeptide.
These are usually **sequence specific**.	These are usually **non-specific** when it comes to sequences.
It does not require a free 3' or 5' end for its action.	It requires a free 3' or 5' end for its action.

DNA polymerase

DNA polymerase is a complex enzyme that synthesizes nucleotides complementary to template strands. It adds nucleotide to free the 3' OH end and help in the elongation of the strand. It also helps to fill gaps in double-stranded DNA. DNA polymerase-I isolated from E. coli is commonly used in gene cloning. Taq polymerase isolated from *Thermus aquaticus* is used in PCR.



Ribonuclease-H (RNase H)

RNase-H removes mRNA from DNA-RNA heteroduplex and that mRNA is used to synthesize cDNA. It is isolated from retrovirus.