Recombinant DNA Technology

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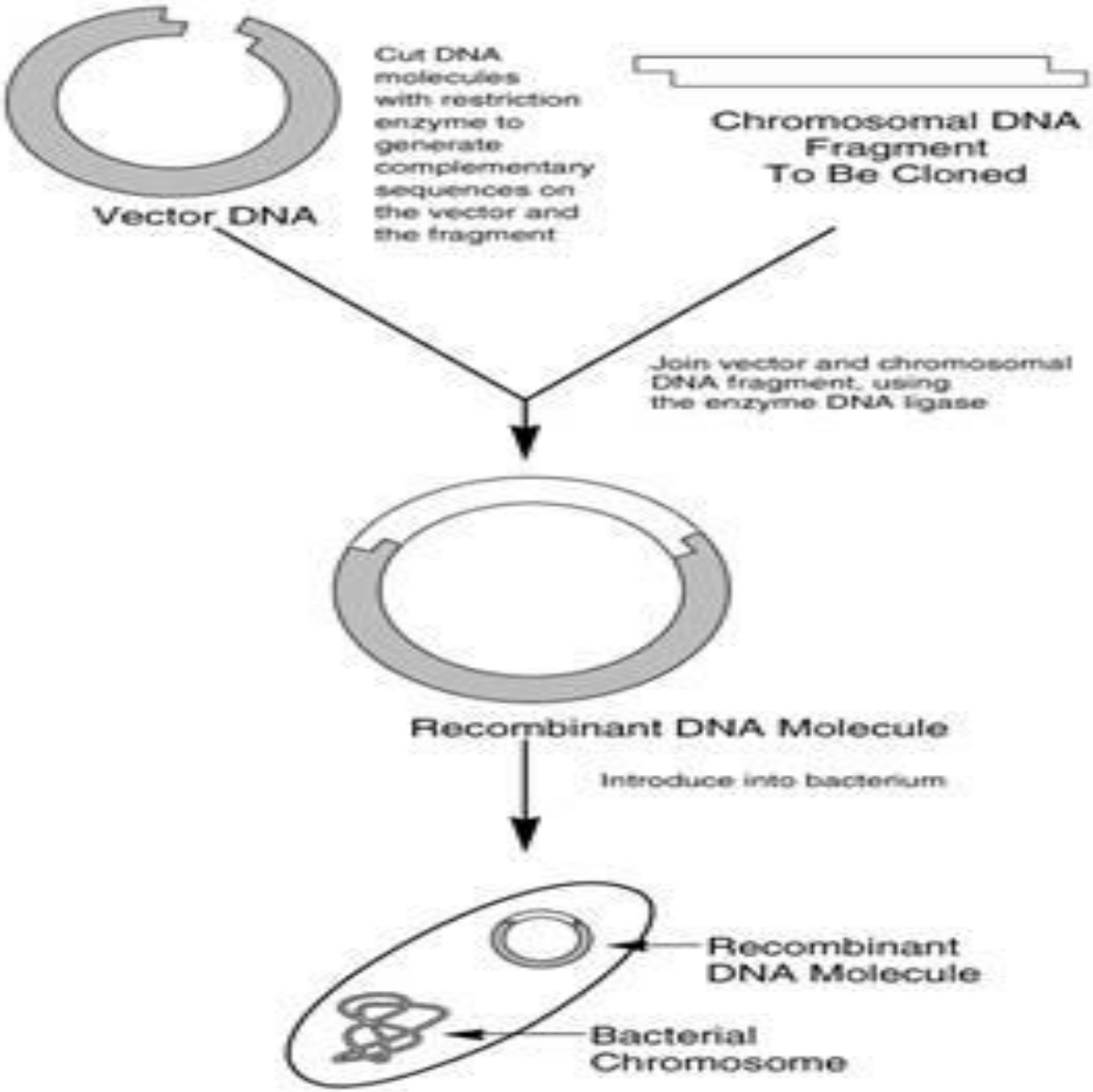
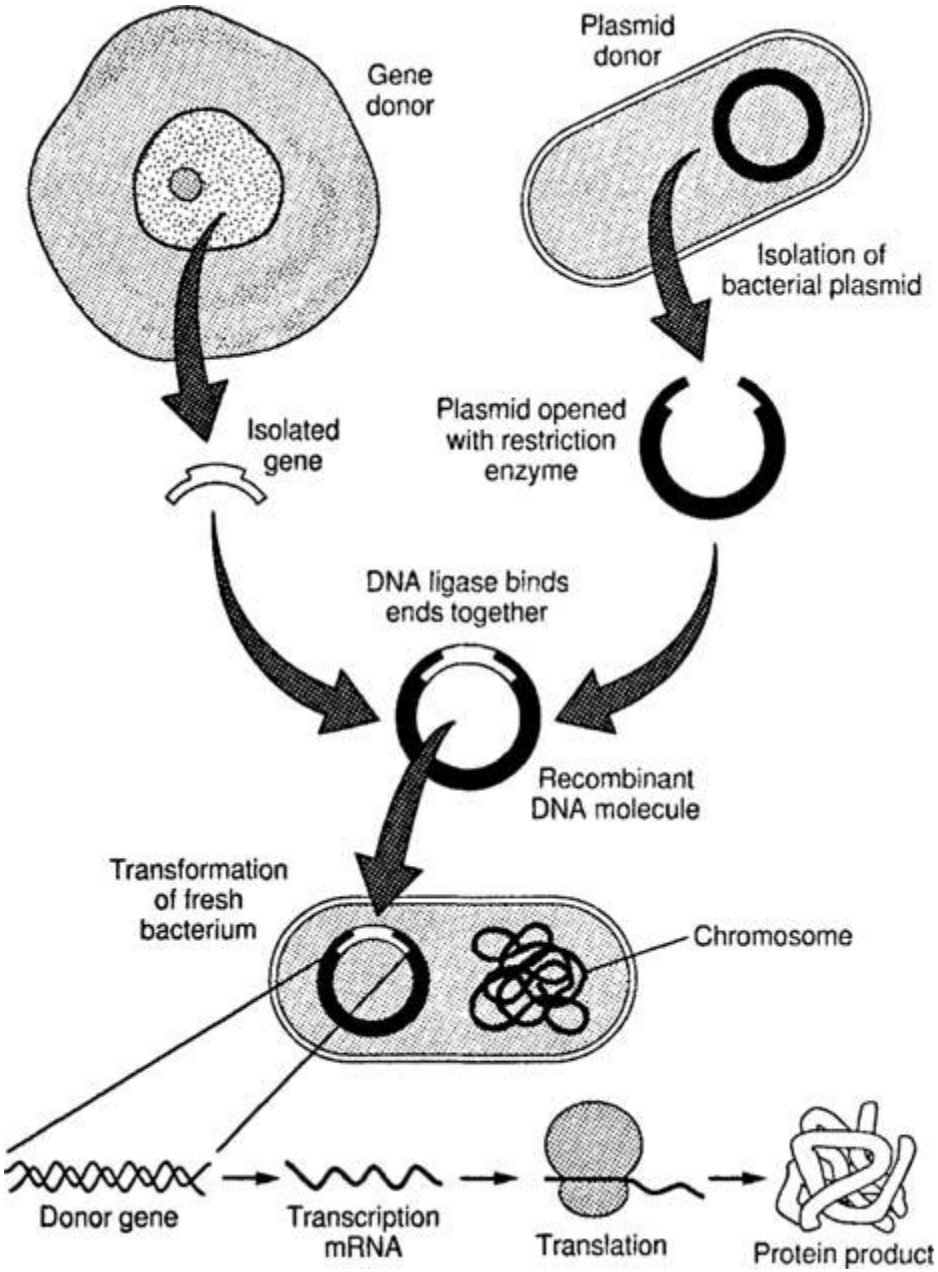
What is RDT

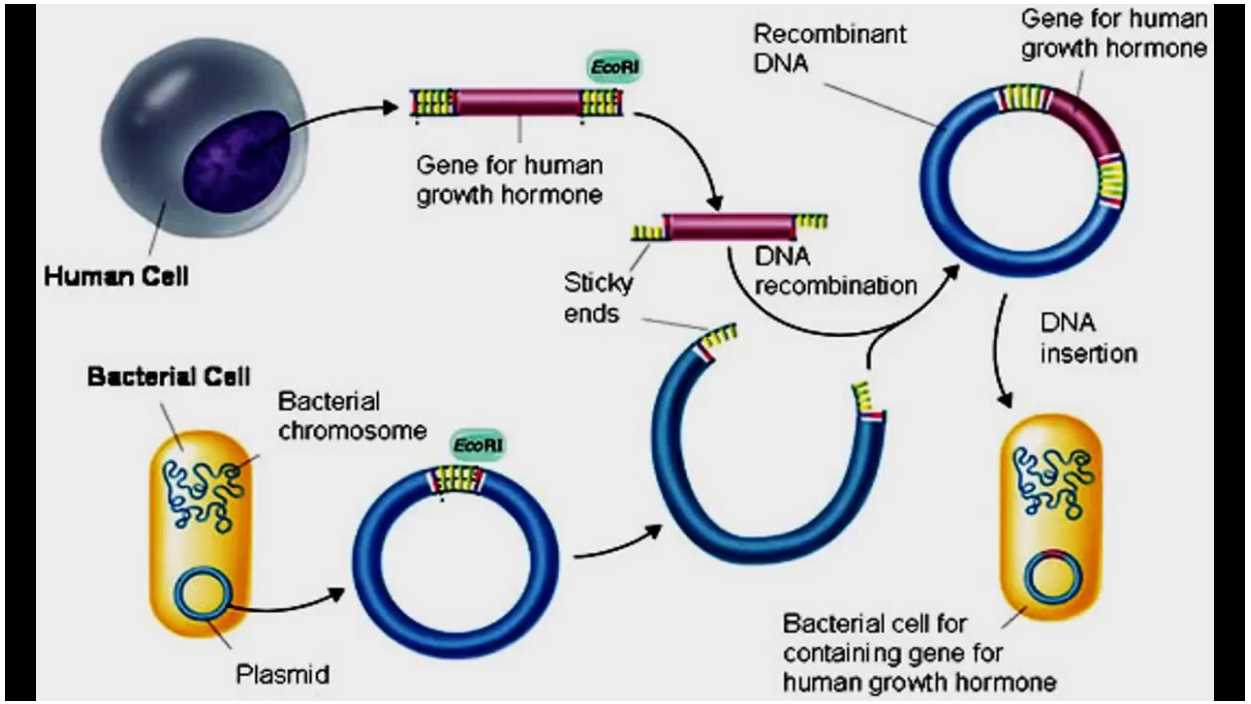
Recombinant DNA technology, joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry.


Vector DNA
Cut DNA molecules with restriction enzyme to generate complementary sequences on the vector and the fragment
Chromosomal DNA Fragment To Be Cloned
Join vector and chromosomal DNA fragment, using the enzyme DNA ligase
Recombinant DNA Molecule
Introduce into bacterium
Recombinant DNA Molecule
Bacterial Chromosome


Gene donor
Plasmid donor
Isolation of bacterial plasmid
Isolated gene
Plasmid opened with restriction enzyme
DNA ligase binds ends together
Recombinant DNA molecule
Transformation of fresh bacterium
Chromosome
Donor gene
Transcription mRNA
Translation
Protein product

Construction of Recombinant DNA

To produce this recombinant molecule, the Vector as well as the DNA to be cloned must be cut in specific points and then joined together in a controller manner.



DNA Manipulative Technique

It involves :

- Cut and Join
- DNA molecule can be shorten or lengthen
- Copied in RNA
- Copied into new DNA molecule etc

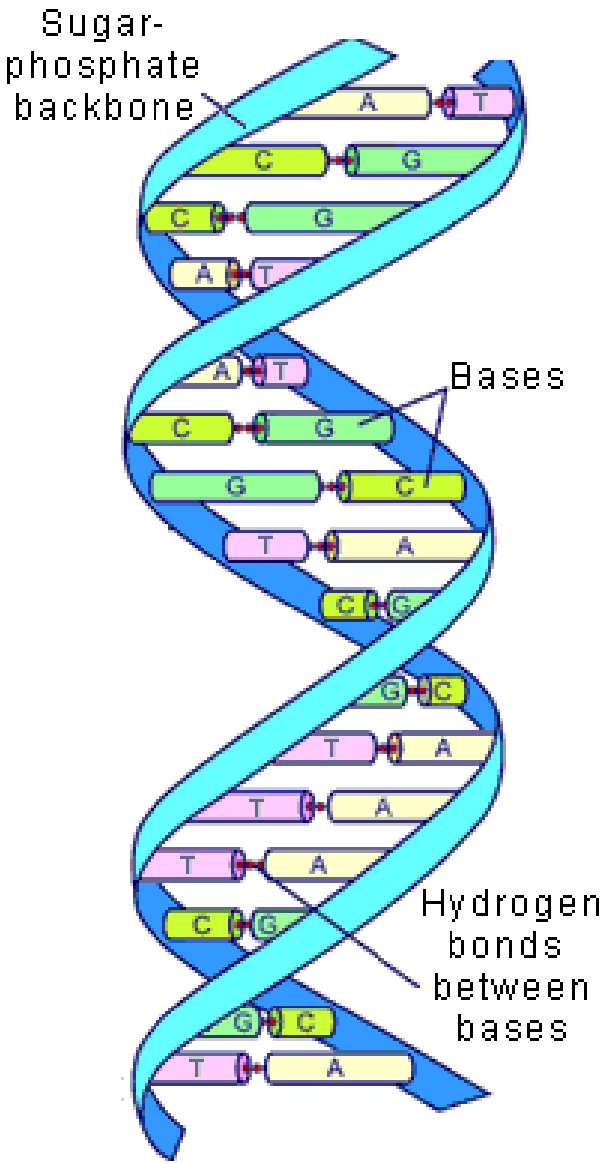
The Range of DNA manipulative Enzyme

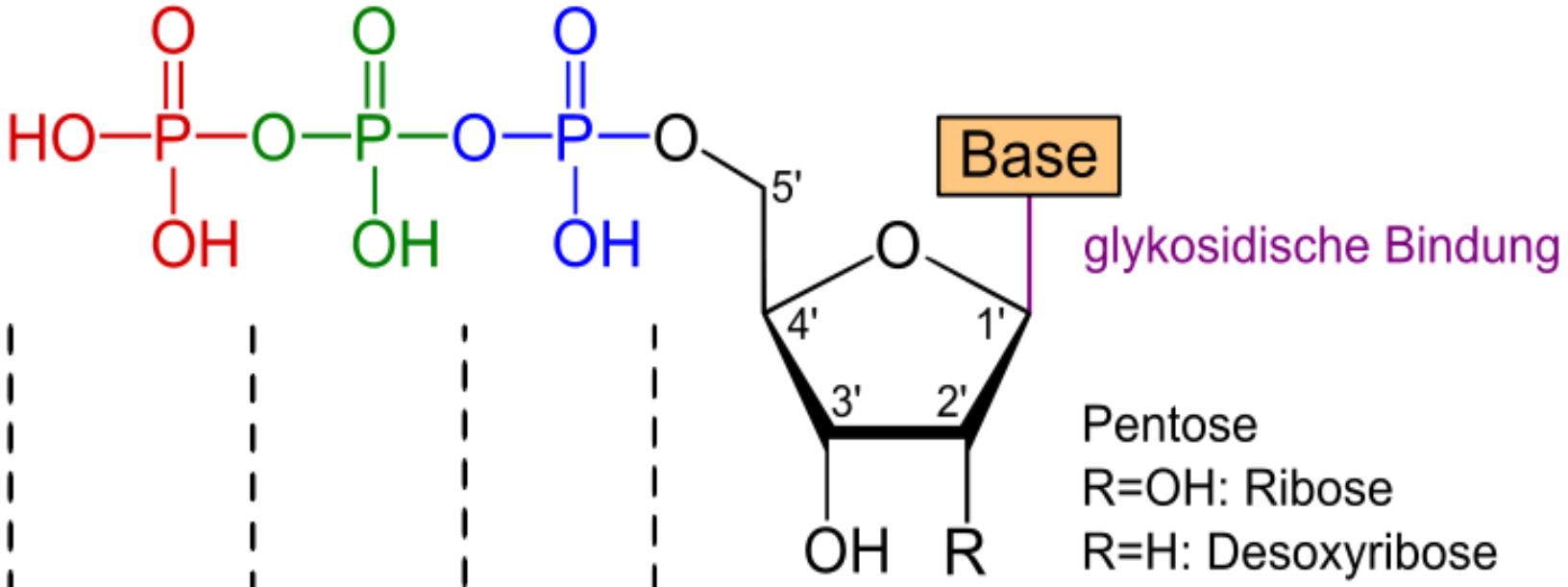
4 broad classes depending on the type of reaction that they catalyze :

- **Nucleases** : are enzymes that cut, shorten or degrade nucleic acid molecules.
- **Ligases :** Join nucleic acid molecules together.
- **Polymerase** : make copies of molecules.
- **Modifying enzymes** : remove or add chemical groups

Nuclease

Nucleases degrade DNA molecules by breaking the phosphodiester bonds that link one nucleotide to the next in a DNA strand


Sugar-
phosphate
backbone
Bases
Hydrogen
bonds
between
bases



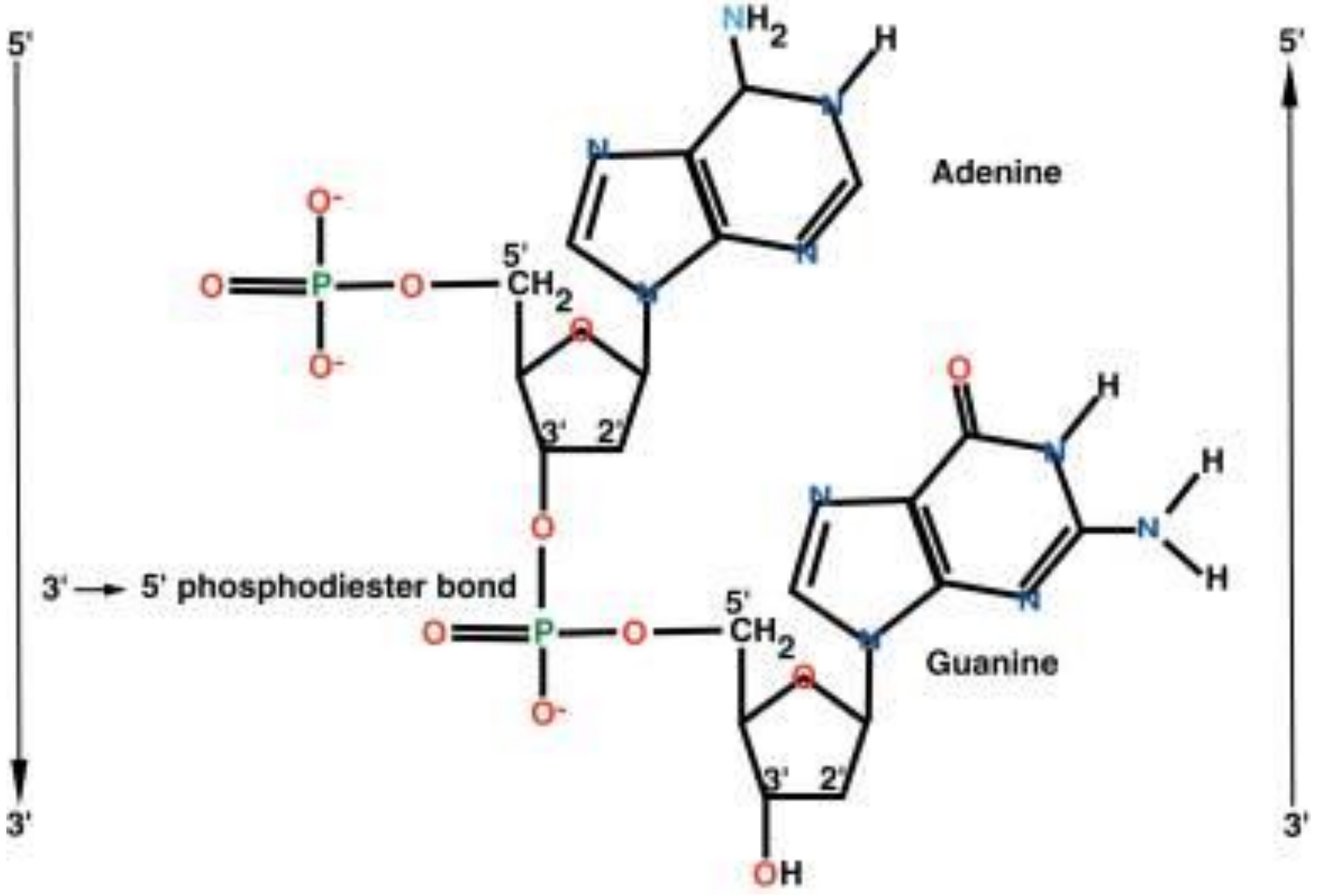
Nukleosid

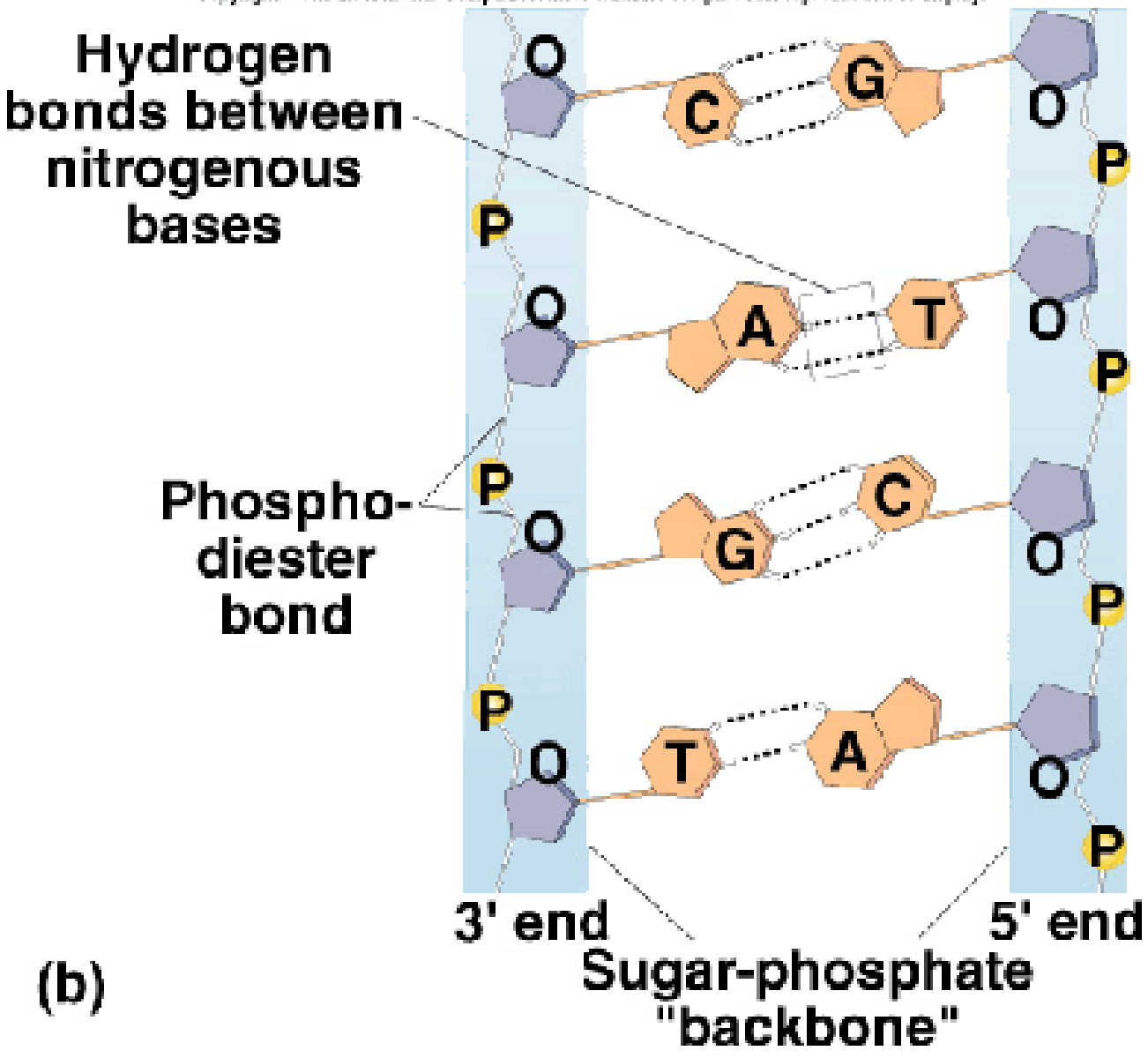
Nukleosid*mono*phosphat

Nukleosid*di*phosphat

Nukleosid*tri*phosphat

Nukleotid


5'
3'
5'
3'
Adenine
Guanine
3' → 5' phosphodiester bond



Two types of Nucleases:

1. Exonuclease : **Exonucleases** are enzymes that work by cleaving nucleotides one at a time from the end (exo) of a polynucleotide chain.

2. Endonuclease : **Endonucleases** are enzymes that cleave the phosphodiester bond within a polynucleotide chain.

Example of Exonuclease

Exonuclease III : degrade just one strand of a double stranded molecules leaving single-stranded DNA as the product.

Endonuclease

S1 endonuclease : (*Aspergillus)* only cleaves single strands

Dnase 1: cut both single and double stranded molecules

Restriction Endonucleases

A special class is called **Restriction Endonucleases**, group of enzymes cleave double stranded DNA only at a limited number of specific recognition sites.

Ligases

Ligase is to repair single stranded breaks (discontinuities) and that facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond.

Polymerase

DNA polymerase are the enzymes
that synthesize a new strand of DNA complementary to an existing DNA or RNA template

Mainly 3 types of DNA polymerase have been used in genetic engineering

TABLE 28.1. Properties of DNA polymerases I, II and III of *E. coli*

	DNA polymerase		
	I	***II***	***III***
Molecular weight (Daltons)	1,09,000	1,20,000	>2,50,000
Constitution	Monomer	Not known	Heteromultimer
Molecules/cell	400	Not known	10-20
Nucleotides polymerised at 37° C/min/molecule	Upto 1,000	Upto 50	Upto 15,000
Affinity for 5′-triphosphates of deoxyribonucleosides	Low	Low	High
Activities:			
5′ → 3′ polymerase	present	present	present
3′ → 5′ exonuclease	present	present	present
5′ → 3′ exonuclease	present	absent	absent
Functions in	DNA repair; excision of RNA primers	DNA repair	***DNA replication; it is the real replicase***
Mutant loci	*PolA*	*PolB*	*dnaE (polC), dnaN, dnaX, dnaQ, dnaT*

DNA POL I :

This enzyme is attaches to a short single stranded region (or nick) in a mainly double-stranded DNA molecule and then synthesizing a completely new strand, degrading the existing strand as it proceeds.

DNA pol I is an example of an enzyme of Dual Activity

1. Polymerization

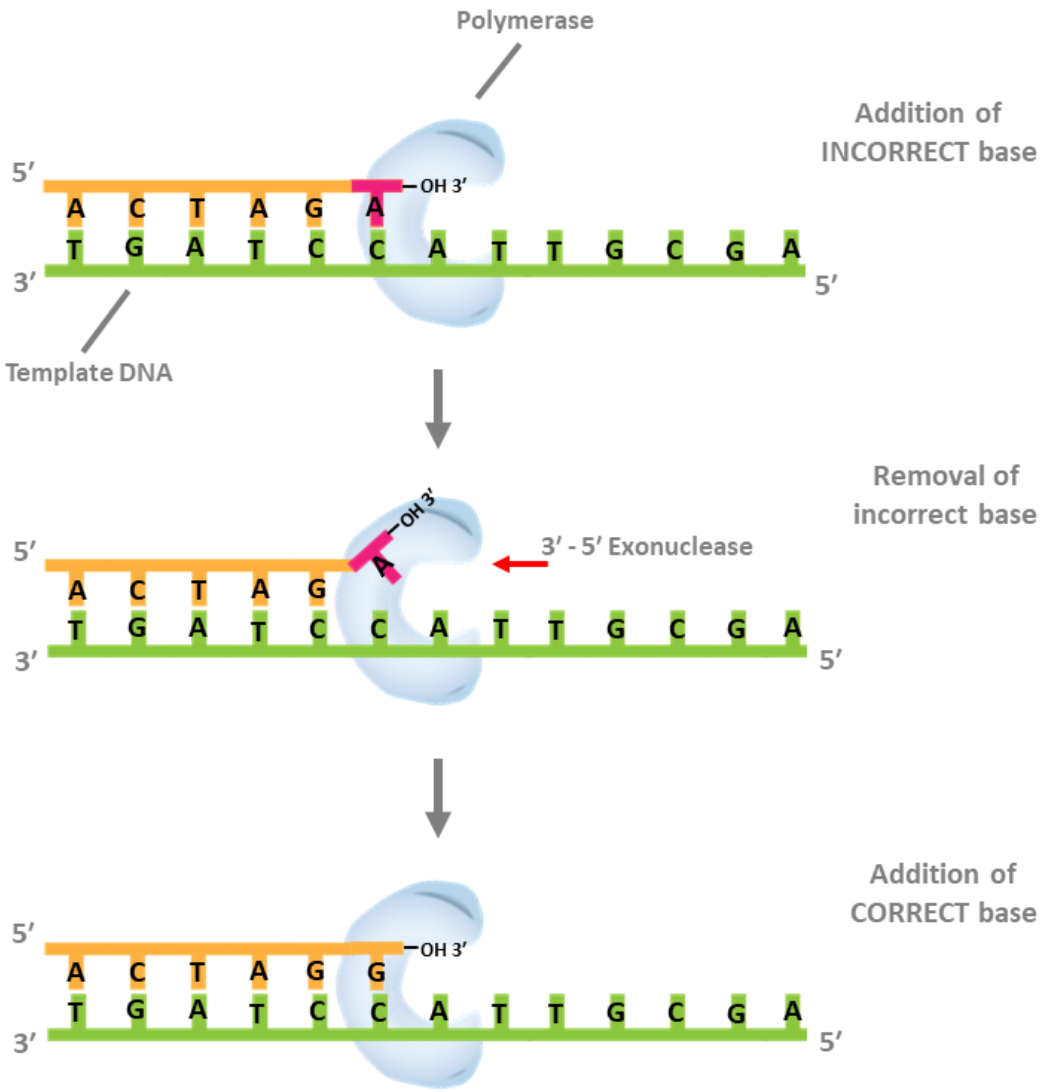
2. Degradation

1. 5'-to-3' DNA Polymerase activity
2. 3'-to-5' exonuclease (Proofreading activity)

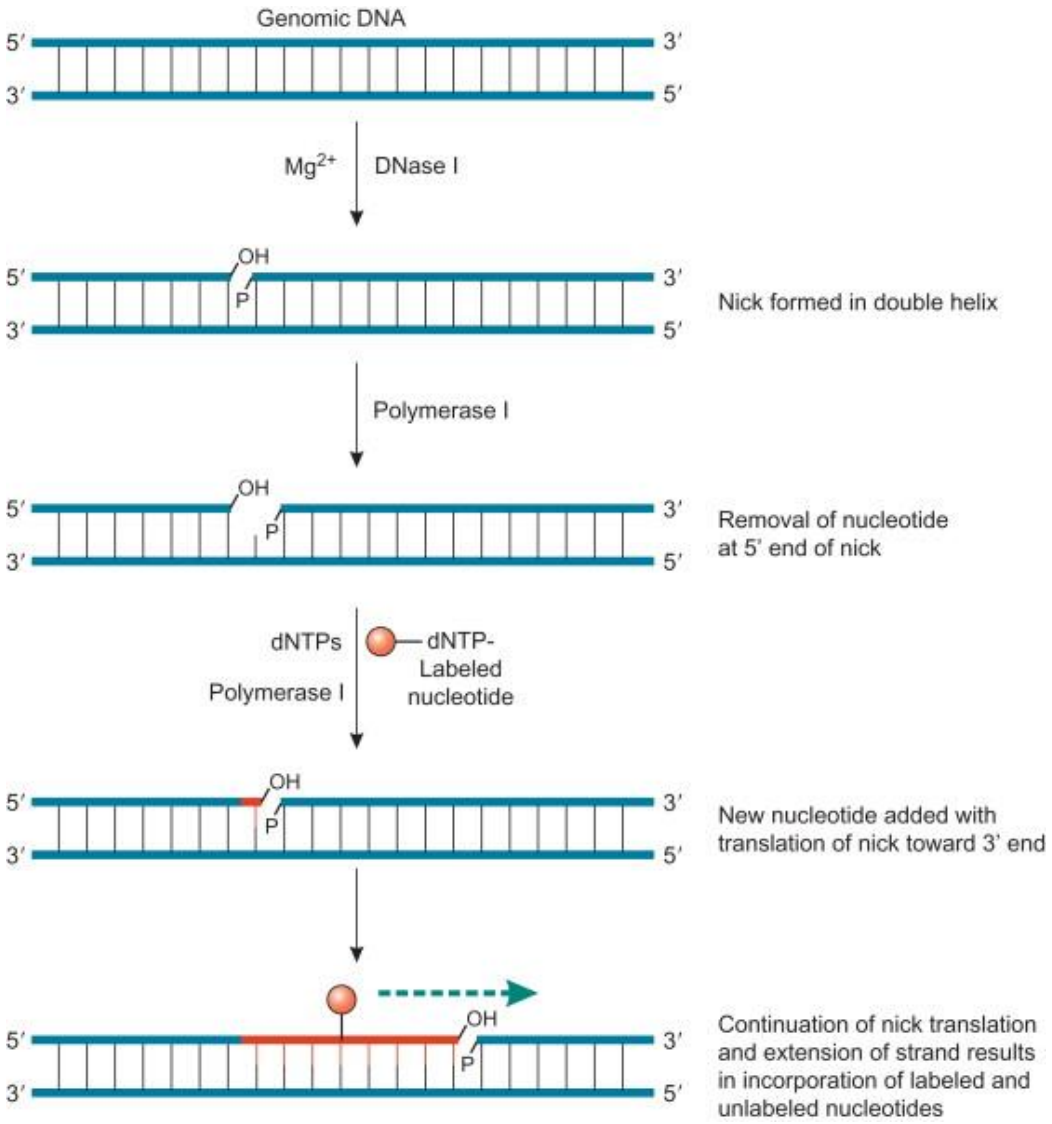
using 3'->5' exonuclease activity. When an incorrect base pair is recognized, DNA polymerase reverses its direction by one base pair of DNA and excises the mismatched base. Following base excision, the polymerase can re-insert the correct base and replication can continue.

3. 5'-to-3' exonuclease (Nick translation activity)

3'-to-5' exonuclease (Proofreading activity)

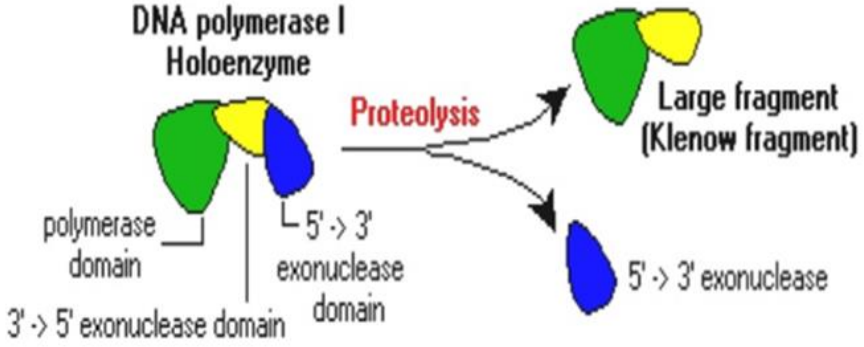


5'-to-3' exonuclease (Nick translation activity)



The polymerase and nuclease activities of DNA Pol I are controlled by different parts of enzyme molecules.

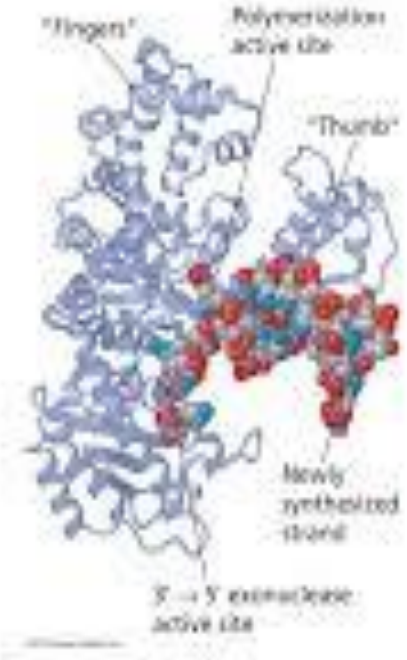
The nuclease activity is contained in the first 323 amino acids of the polypeptides.



The removal of the segment leaves a modified enzymes that retains the polymerase function but is unable to degrade DNA.

This modified enzyme called **Klenow Fragment**

Klenow Fragment



- DNA polymerase I was first discovered
  - Kornberg Nobel prize
- Can be cut into two pieces that retain activity
- Klenow fragment
  - Bigger piece with 5'→3' exonuclease removed
  - "Usable" form of DNA polymerase III

The **Taq DNA polymerase** used in PCR (*Thermus aquaticus)*

Reverse Transcriptase :an enzyme involved in the replication of several kinds of virus. It is unique in that it uses as a template not DNA but RNA.