



COURSE BSc (BIOTECHNOLOGY) III YEAR

PAPER CODE: BBT-301

PAPER TITLE: RECOMBINANT DNA TECHNOLOGY

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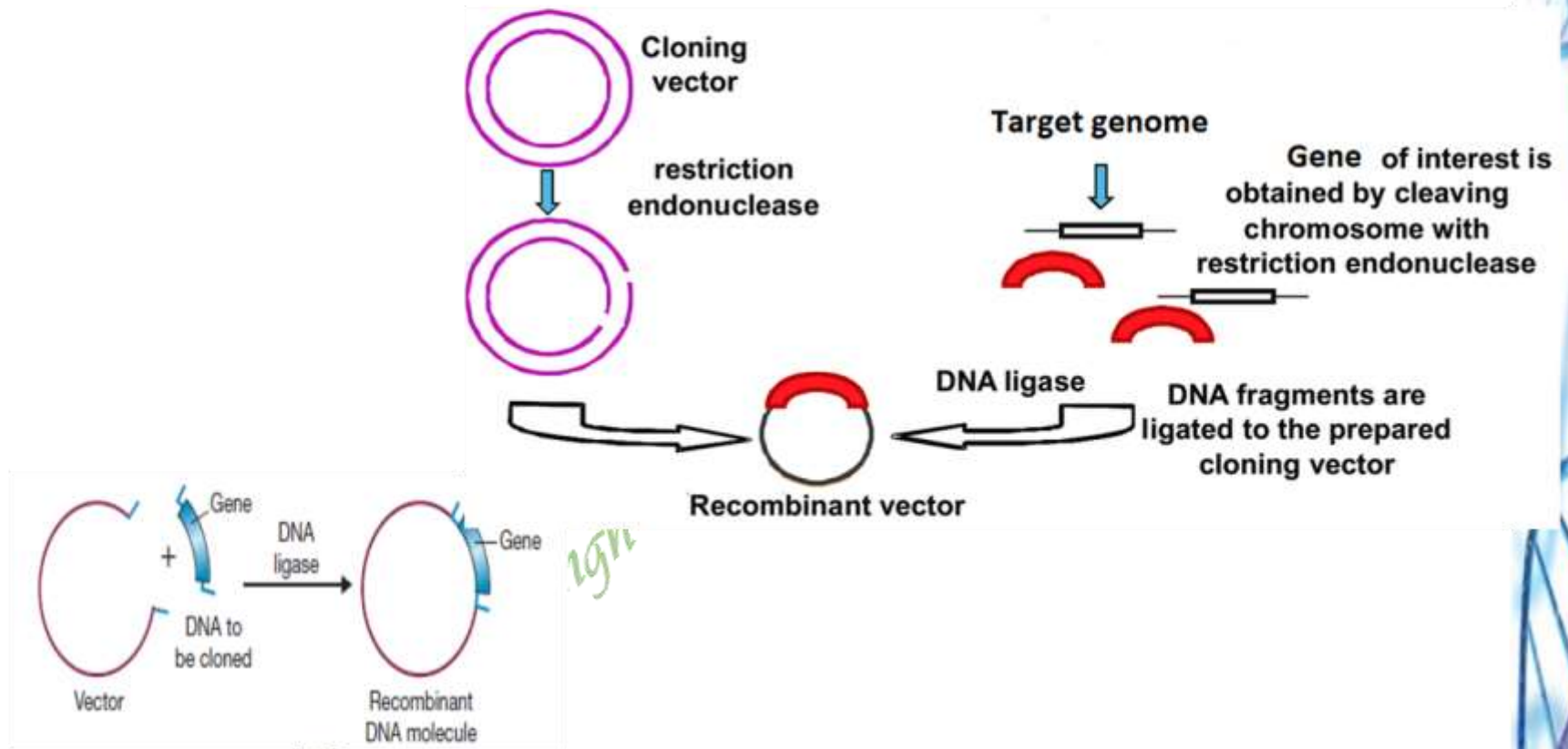
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LIGATION



- The final step in the construction of a recombinant DNA molecule is joining of the vector molecule and the DNA to be cloned **i.e. ligation**, and the process is catalysed by DNA ligase.

LIGATION

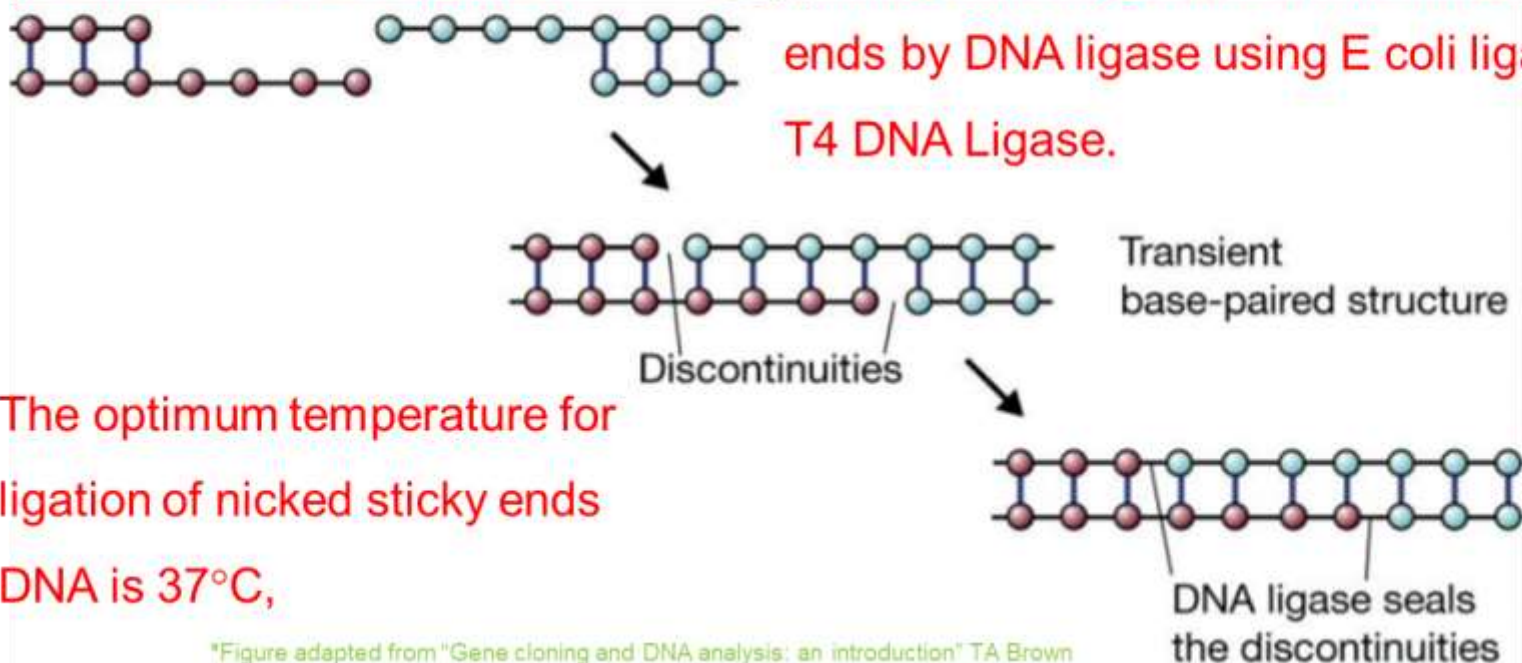
(A) Blunt Ligation • Joining DNA fragments with blunt-ended DNA molecules by DNA ligase using T4 DNA Ligase

- Procedure depends upon the ability of T4 DNA ligase to join blunt-ended DNA molecules.



(B) Cohesive Ends Ligation

- Joining DNA fragments with cohesive ends by DNA ligase using E coli ligase or T4 DNA Ligase.



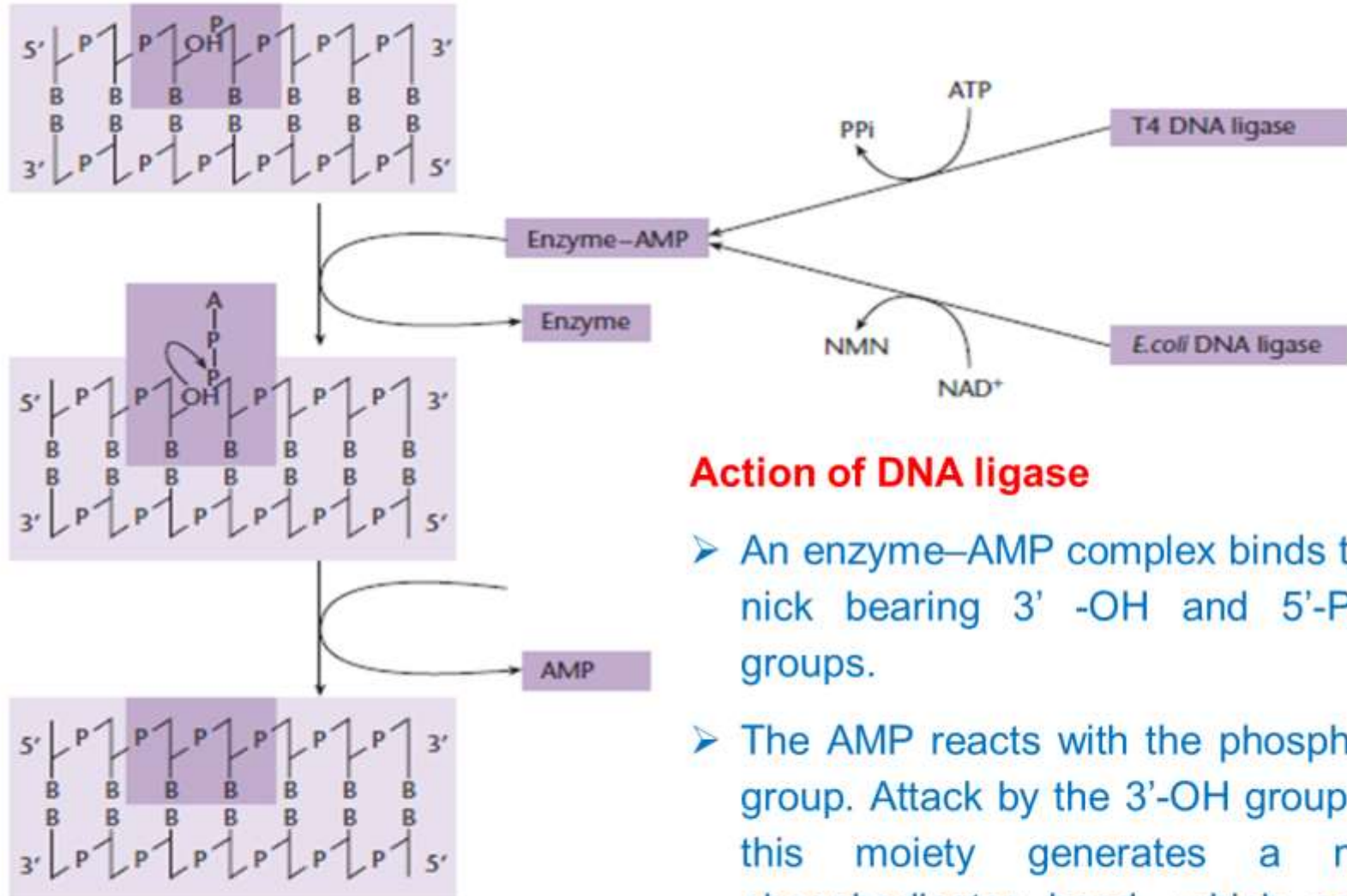
- The optimum temperature for ligation of nicked sticky ends DNA is 37°C,



LIGASES

- DNA ligases catalyse the formation of phosphodiester bonds at single-strand breaks in double-stranded DNA
- Required for the repair, replication and recombination of DNA.
- Enzymes categorized into two groups, based on their cofactor specificity, those requiring NAD^+ for activity and those requiring ATP.
- The eukaryotic, viral and archaeal bacteria ligases require ATP.
- NAD^+ -requiring DNA ligases have only been found in prokaryotic organisms
- *E. coli* and phage T4 both encode DNA ligase, which seals single-stranded nicks between adjacent nucleotides in a duplex DNA chain

LIGASES



Action of DNA ligase

- An enzyme-AMP complex binds to a nick bearing 3' -OH and 5'-PO₄ groups.
- The AMP reacts with the phosphate group. Attack by the 3'-OH group on this moiety generates a new phosphodiester bond, which seals the nick.



LIGASES

- The function of DNA ligase inside the cell is to repair single-stranded breaks that arise in double-stranded DNA molecules during, DNA replication.
- The T4 enzyme requires ATP, while the *E. coli* enzyme requires NAD⁺.
- The *E. coli* DNA ligase will not catalyze blunt ligation except under special reaction conditions of macromolecular crowding, while T4-DNA Ligase does.

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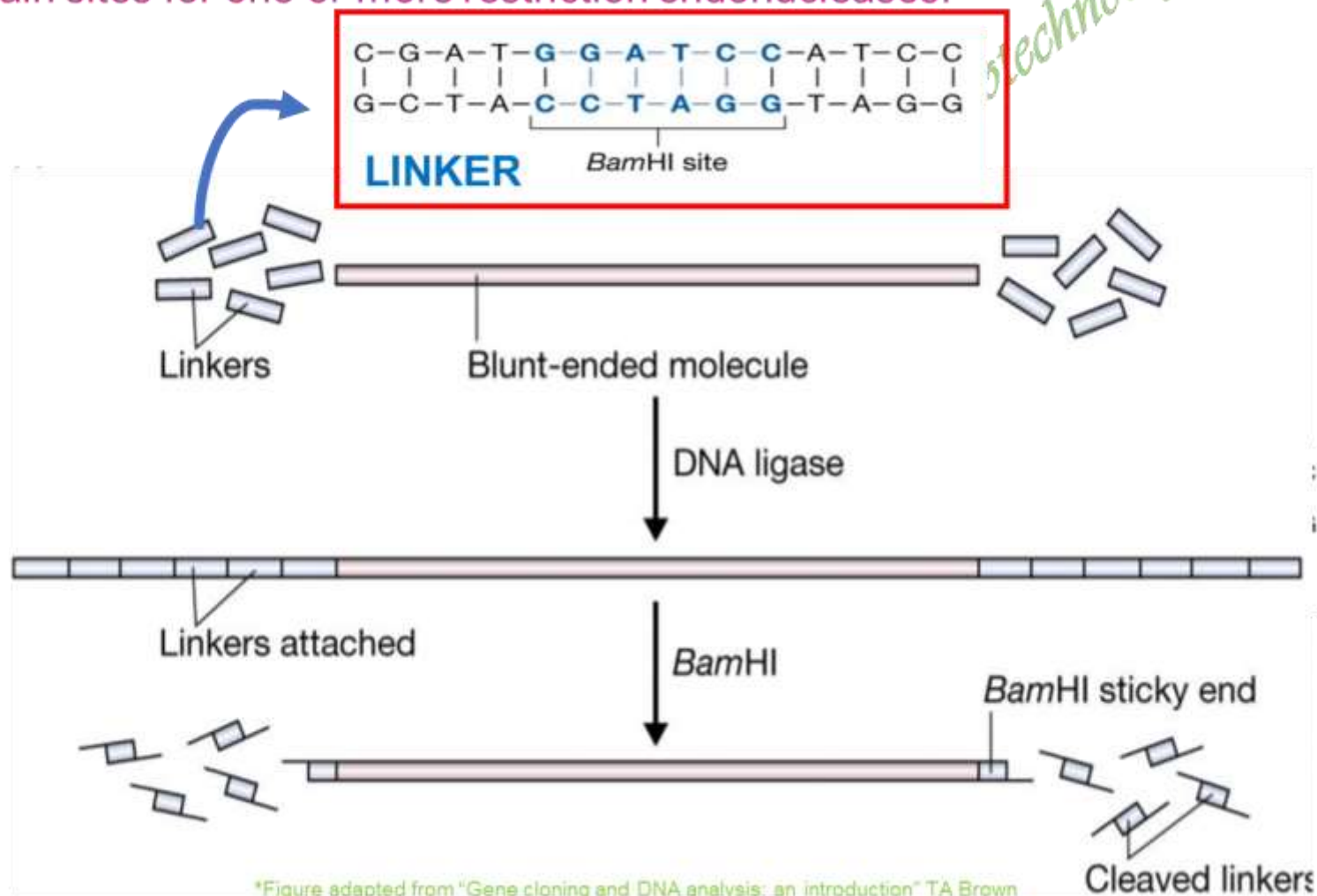
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LINKERS AND ADAPTORS

LINKER MOLECULES: self-complementary decameric oligonucleotides, which contain sites for one or more restriction endonucleases.



*Figure adapted from "Gene cloning and DNA analysis: an introduction" TA Brown



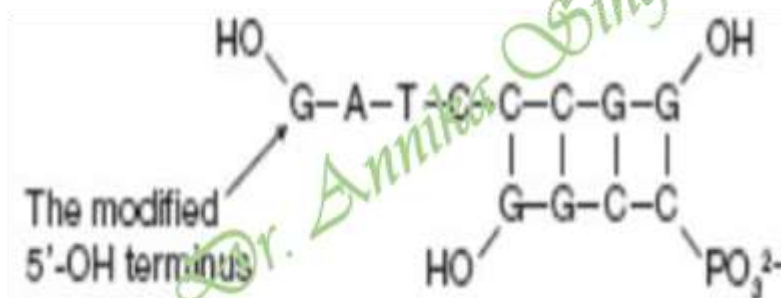
LINKERS AND ADAPTORS

ADAPTORS: An adaptor, like a linker, is a short synthetic oligonucleotide with one end blunt and another one is sticky end.

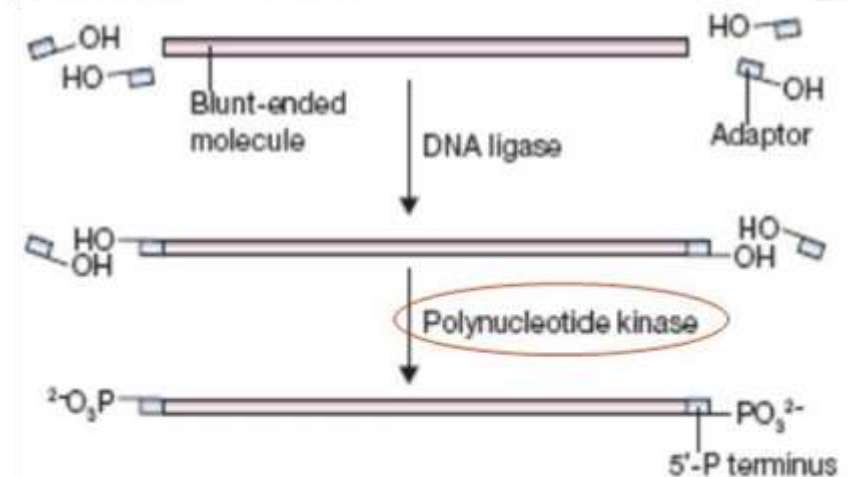
The blunt end of the adaptor ligates to the blunt ends of the DNA fragment, to produce a new molecule with sticky ends.

The sticky ends of individual adaptor molecules could base pair with each other to form dimers

(a) Adaptor



(b) Use of adaptors



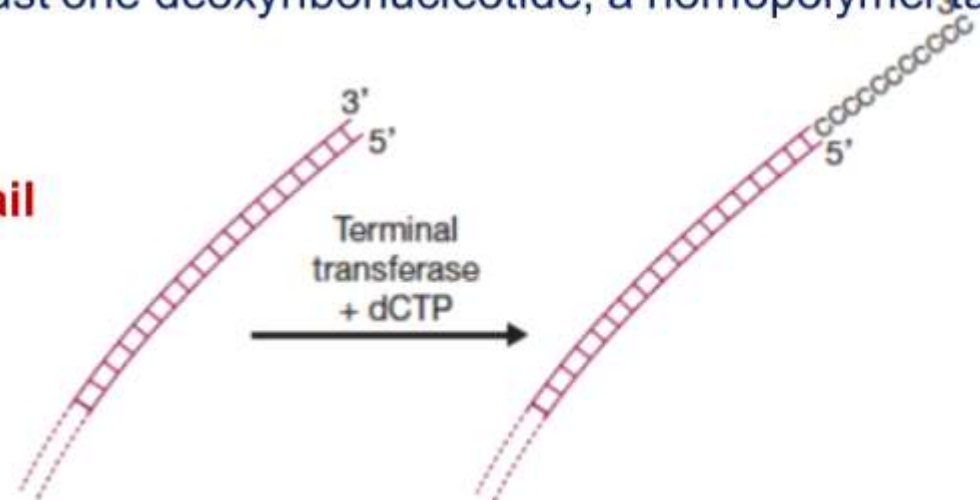


LIGATION

HOMOPOLYMER TAILING:

- The technique of homopolymer tailing offers a different approach to the production of sticky ends on a blunt-ended DNA molecule.
- A homopolymer is a polymer in which all the subunits are the same, e.g. deoxyguanosine and is referred to as polydeoxyguanosine or poly(dG).
- Tailing involves using the **enzyme terminal deoxynucleotidyl transferase** to add a series of nucleotides onto the 3'-OH termini of a double-stranded DNA molecule in the presence of just one deoxyribonucleotide, a homopolymer tail is produced

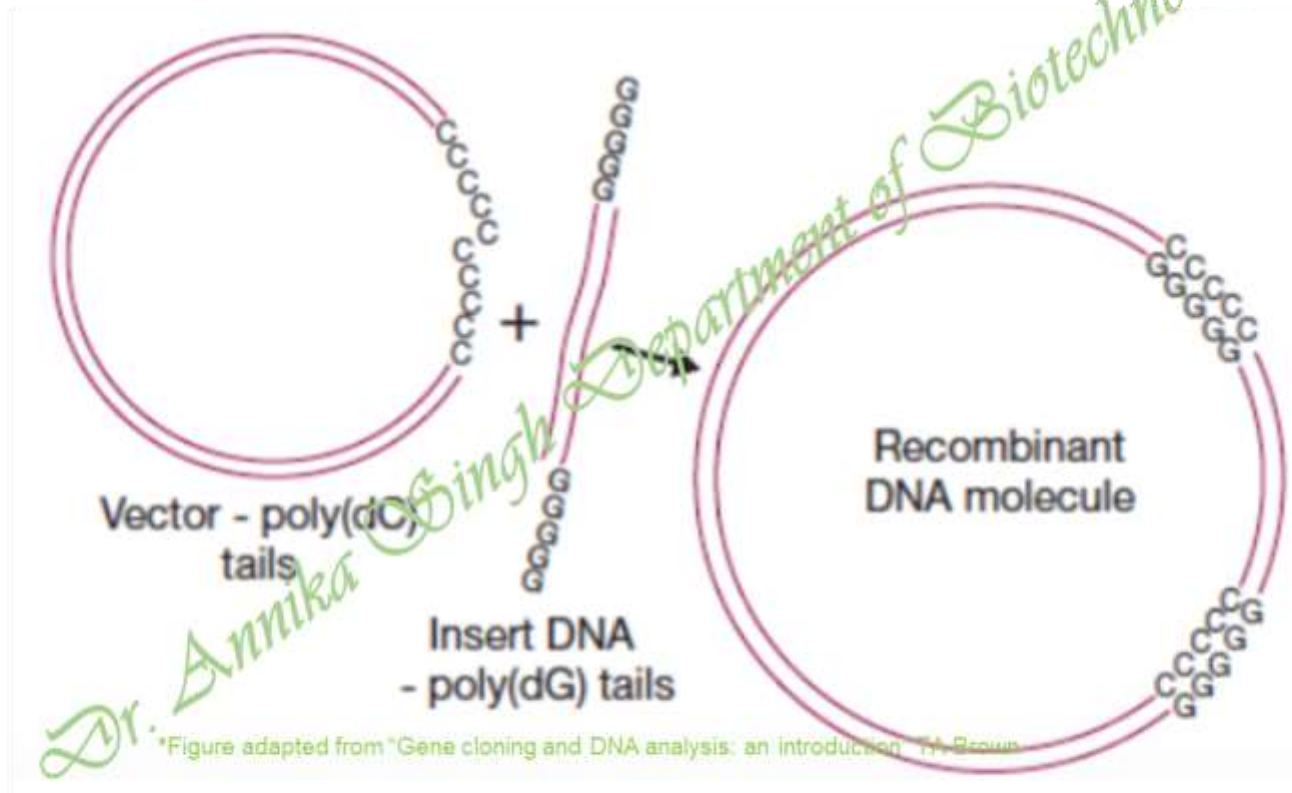
Synthesis of homopolymer tail





LIGATION

Ligation of homopolymer tail



References

1. S.B. Primrose, R.M. Twyman and R.W.Old; Principles of Gene Manipulation, S.B.University Press.
2. J. Sambrook and D.W. Russel; Molecular Cloning: A Laboratory Manual, Vols 1-3.
3. Brown TA, Genomes
5. Technical Literature from Promega, and NEB