A study material for M.Sc. Biochemistry (Semester: III) Students
on the topic (CC-13; Unit I)

Ligase Enzyme in RDT

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Ligation In rDNA technology

- Cut the DNA
- Joining of the DNA fragment
- Currently 3re methods to join two different fragments
- 1. ***E.coli* DNA ligase** for sticky end ligation
- 2. **T_4 DNA ligase** to join blunt end ligation
- 3. **Terminal nucleotidyl transferase (TTase):** TTase synthesizes homopolymeric 3' tails at the ends of the fragments.

E.coli DNA Ligase

- *E.coli* coded enzyme seals ss nick between adjacent nucleotides in a duplex DNA Chain (Olivera et. al., 1968, Gumport and Lehman 1971)
- *E.coli* DNA ligase utilizes NAD for ligation reaction

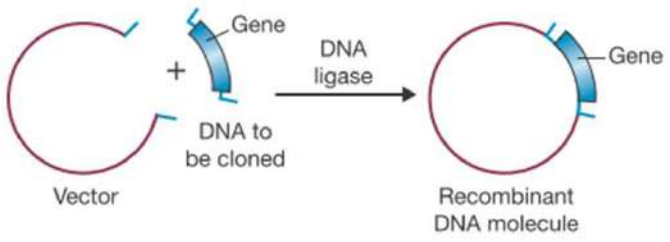

Figure 4.19
Ligation: the final step in construction of a recombinant DNA molecule.

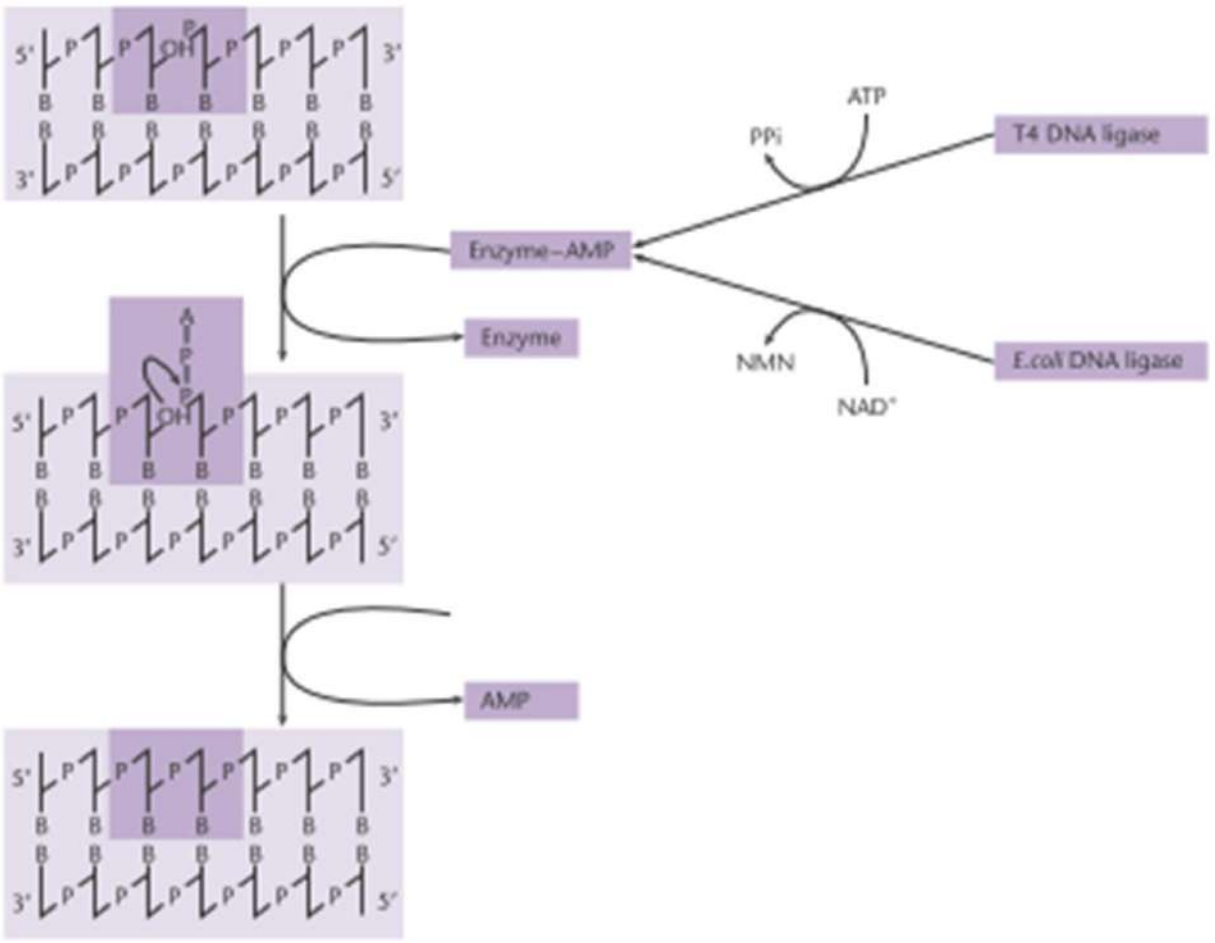

Fig. 3.6 Action of DNA ligase. An enzyme–AMP complex binds to a nick bearing 3′ OH and 5′ P groups. The AMP reacts with the phosphate group. Attack by the 3′ OH group on this moiety generates a new phosphodiester bond, which seals the nick.

In this reaction cofactor split and forms an enzyme-AMP complex
This complex binds to nick (5’P-3’OH) and makes a covalent bond in the phosphodiester as shown in figure.

Use of DNA ligase to create the rDNA molecule

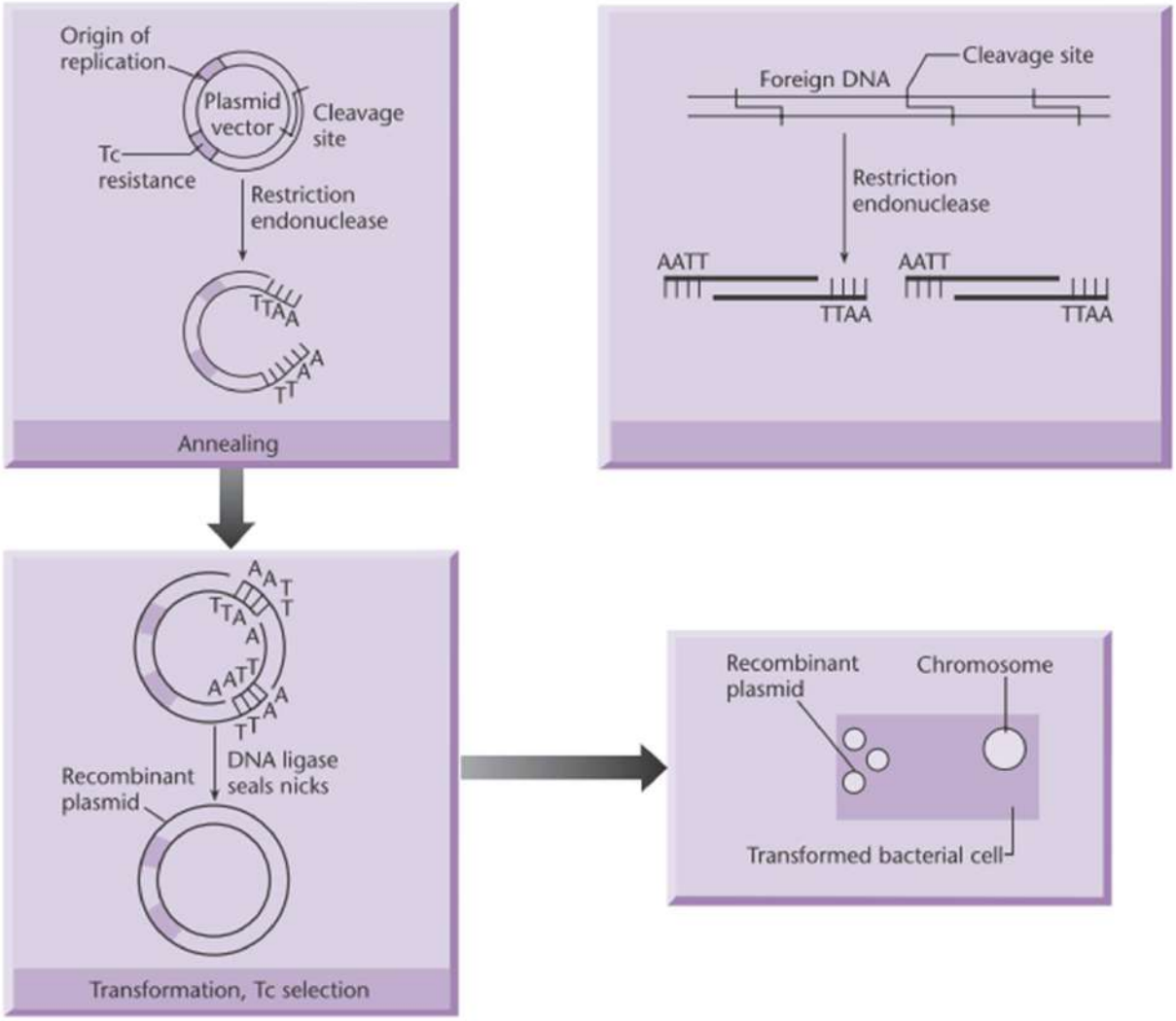

Fig. 3.7 Use of DNA ligase to create a covalent DNA recombinant joined through association of termini generated by *Eco*RI.

Ligation reaction temperature

- Why ligation is done at low temperature?
- The optimal temperature of ligation is 37°C. Hydrogen bonds are unstable at 37°C temperature. *Eco*RI cuts produces AT overhangs. At 37°C temperature thermal disruption takes place. So, the compromise temperature is 4°C-15°C (Dugaicyzk et. al., 1975)

The ligation reaction can be performed so as to favors the recombinants formation

- 1, high concentration of DNA is required because low concentration of DNA favors recircularization of vector
- 2, Treatment of vector with alkaline phosphatase
- -removes phosphate group from 5’ end
- -prevents self circularization
- -prevent dimer formation

Application of alkaline phosphatase

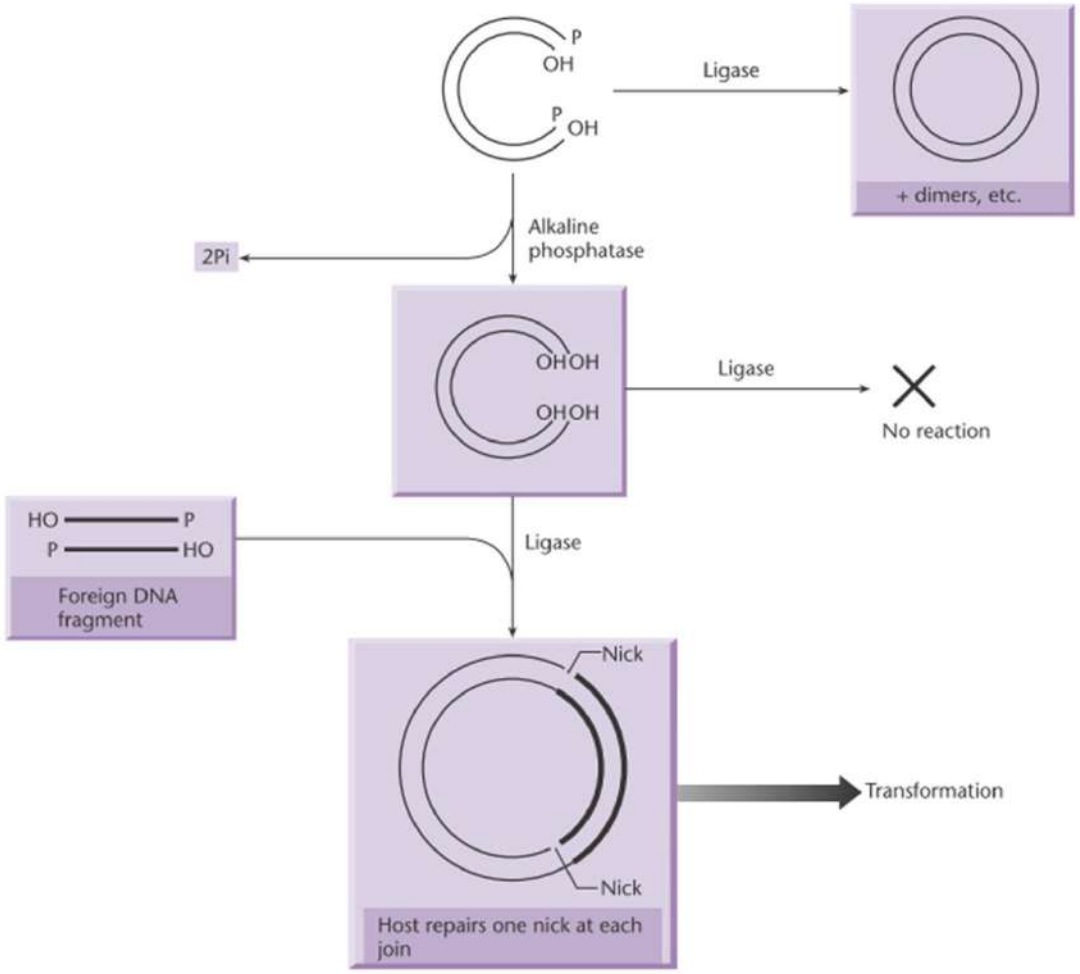

Fig. 3.8 Application of alkaline phosphatase treatment to prevent recircularization of vector plasmid without insertion of foreign DNA.

T_4 DNA ligase

- T4 lambda phage coded
- Uses ATP as a cofactor
- Both cohesive and blunt end ligation

Acknowledgement and Suggested Readings:

1. Gene Cloning and DNA Analysis: An Introduction; Sixth Edition ; T. A. Brown; Wiley – Blackwell Publications
2. Principles of Gene Manipulation; Sixth Edition; Sandy B Primrose, Richard M Twyman and Robert W. Old; Wiley – Blackwell Publications
3. Biotechnology: Applying the Genetic Revolution; David P. Clark and Nanette J. Pazdernik; Academic Press (Elsevier)

Thanks