MSc III Sem – Biotechnology

Course – Principles of Genetic Engineering

Linkers, Adaptors, Homopolymeric Tailing

Ligation efficiency depends on the ends of DNA in the reaction, the two types of end:

1. "sticky" ends: Ligation is efficient annealing of complementary overhangs brings 5'P and 3'OH into close proximity.
2. "Blunt" ends: Ligation is less efficient need high concentrations of ligase and DNA

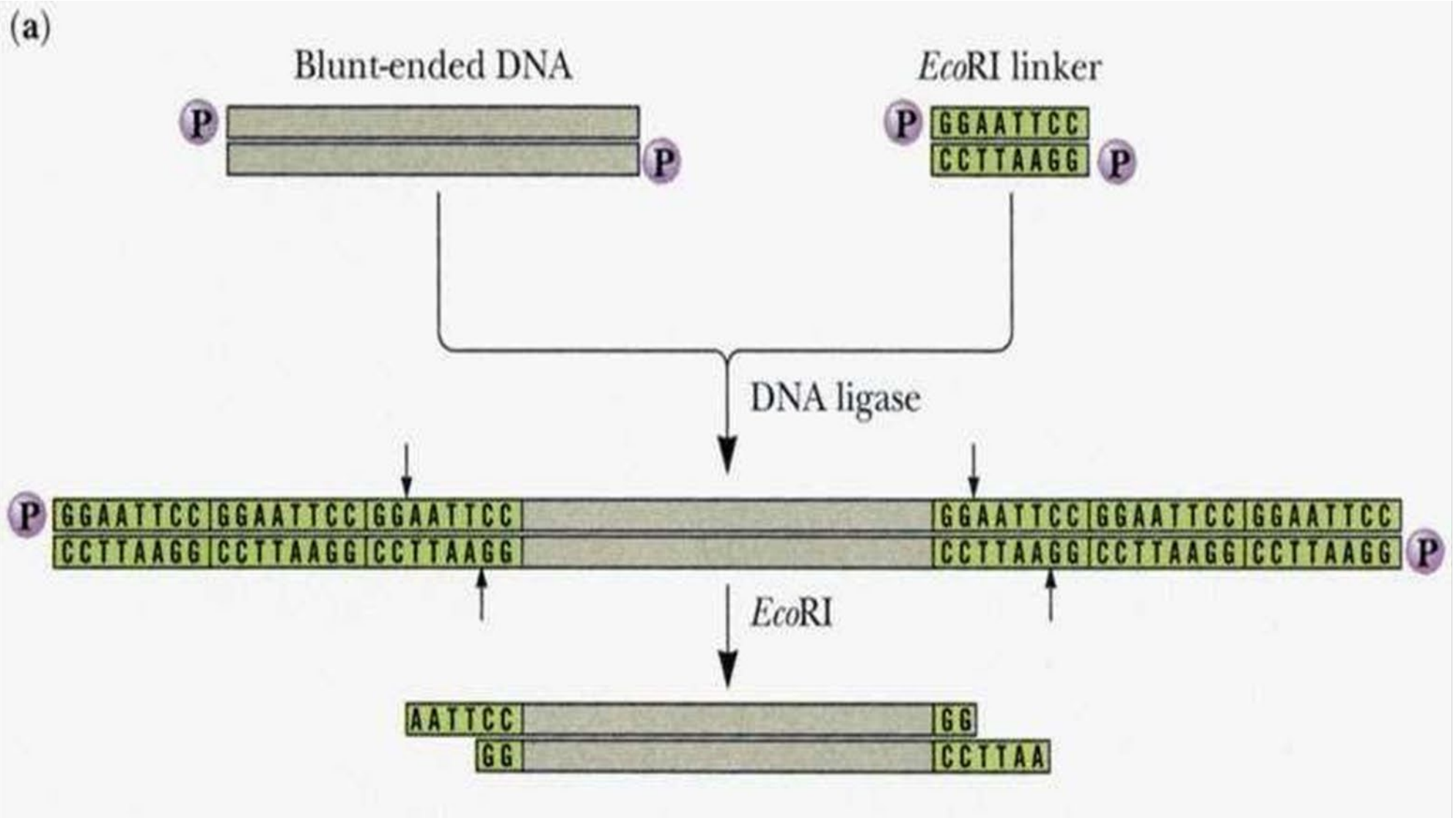
Blunt End Ligation

Mainly three methods can be used to put the correct sticky ends onto the DNA fragments-

1. Cloning foreign DNA by adding linkers
2. Cloning foreign DNA by adding adaptors
3. Homopolymeric tail adding by using Terminal transferase enzyme.

Linker

- Linkers are the chemically synthesized double stranded DNA oligonucleotides containing on it one or more restriction sites for cleavage by restriction enzymes, e.g. Eco RI, Hind III, Bam HI, etc.
- Linkers are ligated to blunt end DNA by using DNA ligase.
- Both the vector and DNA are treated with restriction enzyme to develop sticky ends.
- The staggered cuts i.e. sticky ends are then ligated with T4 DNA ligase with very high efficiency to the termini of the vector and recombinant plasmid DNA molecules are produced.



Before cloning of a particular sequence in PCR, a primer, associated with a linker is used. This type of primer is called as linker-primer. Now-a-days, two different linkers are used which has different RE sites with F/R primer. This strategy helps in directional cloning.

Adaptors

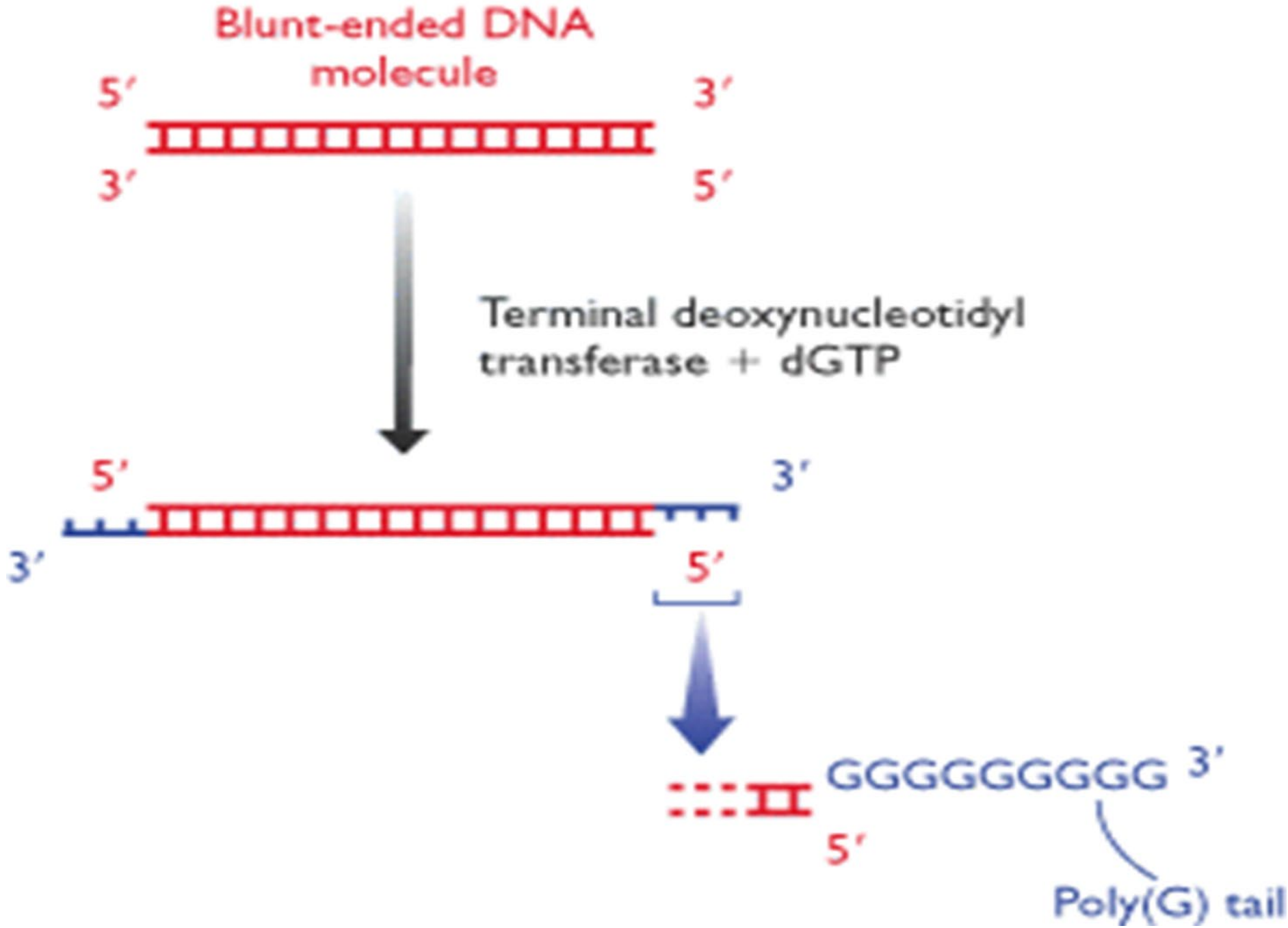
- They are also short double stranded oligonucleotides that carry an internal RE sites and single stranded tails at one or both ends.
- This protruding sequences can be ligated to DNA fragments containing a complementary single stranded terminus.
- After ligation, the DNA can be cleaved with appropriate RE to create new protruding terminus.
- Adaptors are available in two basic designs and a variety of specifications.

1. Some consists of a partial duplex formed between two oligonucleotides of different length; for example, the EcoR1-Not1 adaptor.

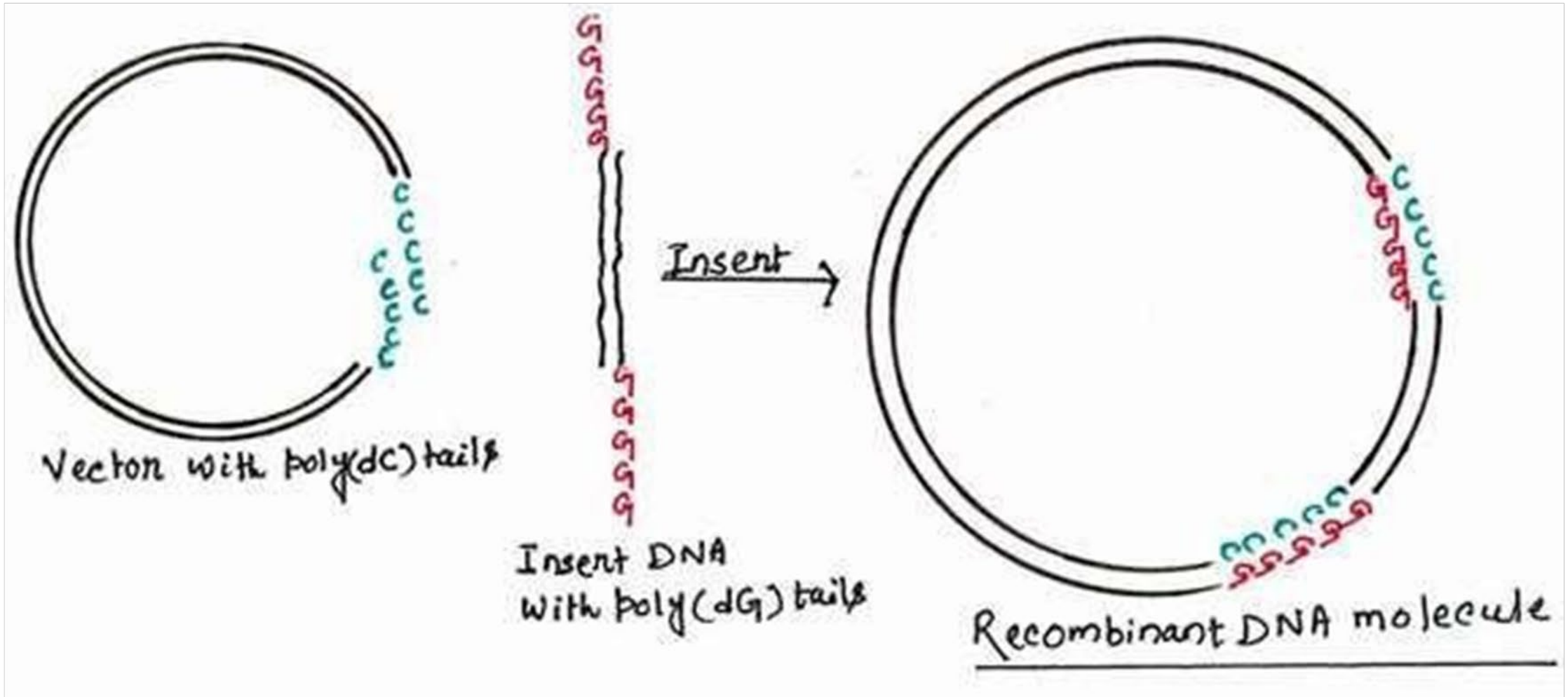
2. Another class of adaptor is supplied as an unphosphorylated single oligonucleotide whose sequence is partially self complementary. As an example- EcoR1-Pst1 adaptor.

Homopolymeric Tailing

- It is a technique by which sticky ends can be produced on a blunt-ended DNA molecule.
- In a homopolymer, all the subunits are same. A DNA strand made up entirely of deoxyguanosine is an example of homopolymer, and is referred to as polydeoxyguanosine or poly(dG).
- Tailing involves using the enzyme terminal deoxynucleotidyl transferase to add a series of nucleotides on to the 3'-OH termini of a double-stranded DNA molecule.
- The reaction when carried out in the presence of just one deoxynucleotide, then a homopolymer tail will be produced.


Blunt-ended DNA molecule
5′
3′
3′
5′
Terminal deoxynucleotidyl transferase + dGTP
5′
3′
3′
5′
GGGGGGGGGG 3′
5′
Poly(G) tail

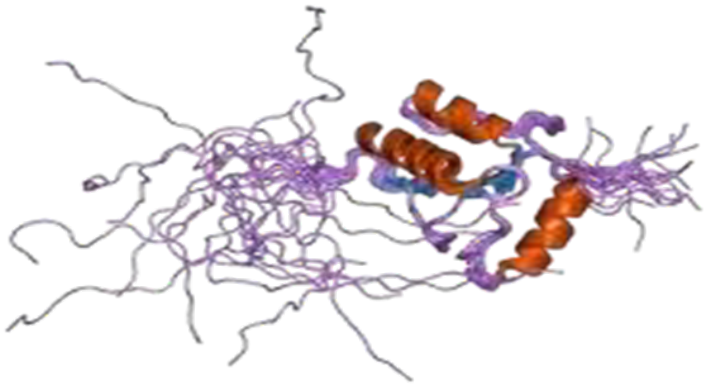
For ligation of two tailed molecules, the homopolymers must be complementary. Frequently poly(dc) tails are attached to the vector and poly(dg) to the DNA to be cloned.



Terminal Transferase

- Terminal deoxynucleotidyl transferase (TdT) is a template independent DNA polymerase.
- It is expressed in immature, pre-B, pre-T lymphoid cells, and acute lymphoblastic leukemia/lymphoma cells.
- In humans, terminal transferase is encoded by the *DNTT* gene.

Structure



Monomeric

Mol. Wt. 58000 Da

Amino acids 508 to529 (depending upon source)

A high degree of sequence homology(>80%)in TdT between different species

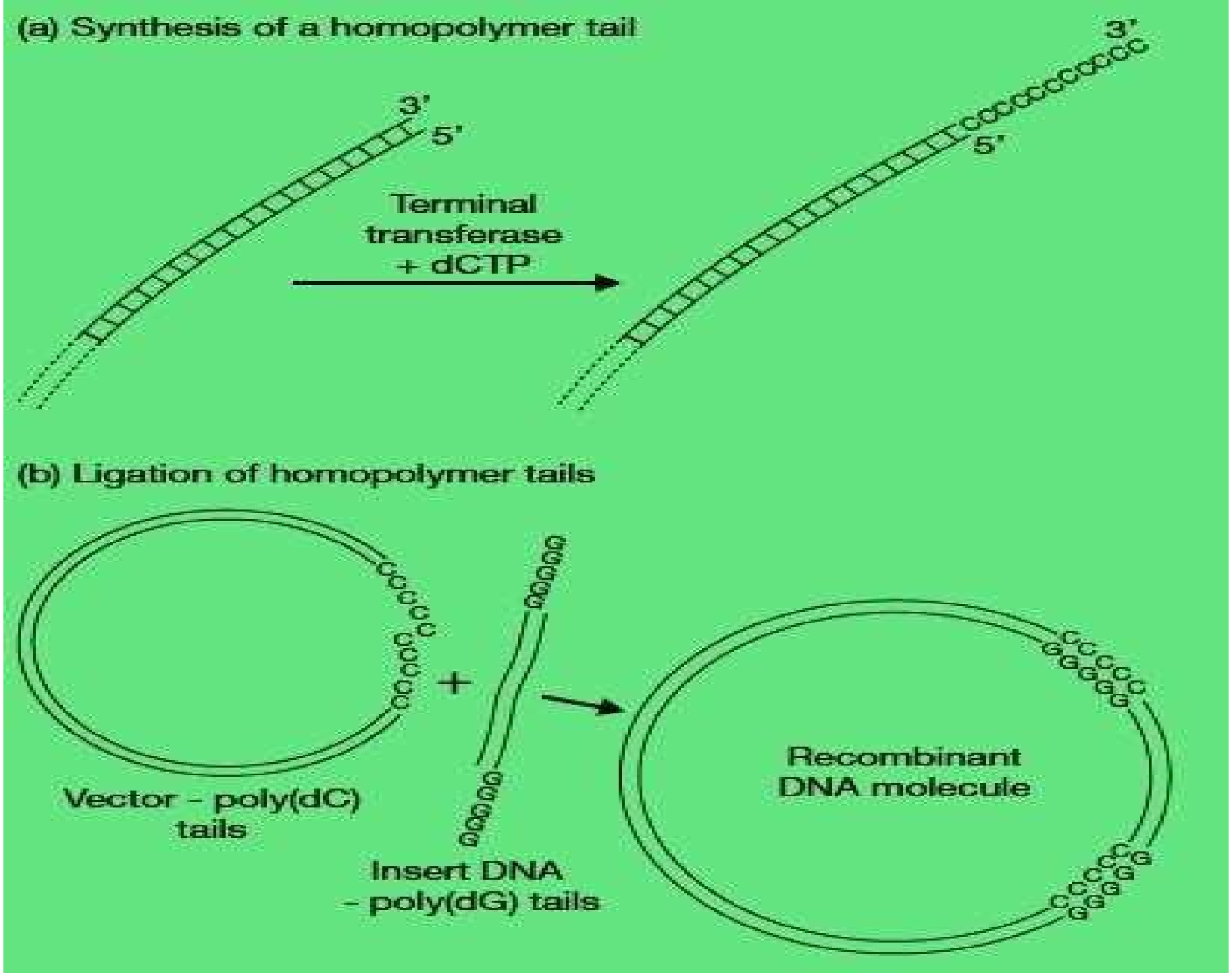
TdT Function and Regulation

- TdT catalyses the addition of nucleotides to the 3' terminus of a DNA molecule. Unlike most DNA polymerases, it does not require a template.
- The preferred substrate of this enzyme is a 3'- overhang but it can also add nucleotides to blunt or recessed 3' ends.
- Cobalt is a necessary cofactor, however the enzyme catalyzes reaction upon Mg and Mn administration *in vitro*.

- TdT is expressed mostly in the primary lymphoid organs, like the thymus and bone marrow.
- Regulation of its expression occurs via multiple pathways. These include protein-protein interactions, like those with TdIF1.
- TdIF1 is another protein that interacts with TdT to inhibit its function by masking the DNA binding region of the TdT polymerase

Applications

- Addition of homopolymeric tails to plasmid DNA and to cDNA.
- Double- or single-stranded DNA 3´-termini labeling with radioactively labeled or non-radioactively labeled nucleotides.
- Addition of single nucleotides to the 3´ ends of DNA for in vitro mutagenesis.
- Production of synthetic homo- and heteropolymers.
- RACE (Rapid Amplification of cDNA Ends).
- TUNEL assay (*in situ* localization of apoptosis).


(a) Synthesis of a homopolymer tail
3'
5'
Terminal
transferase
+ dCTP
3'
5'
(b) Ligation of homopolymer tails
Vector - poly(dC)
tails
+
Insert DNA
- poly(dG) tails
Recombinant
DNA molecule

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