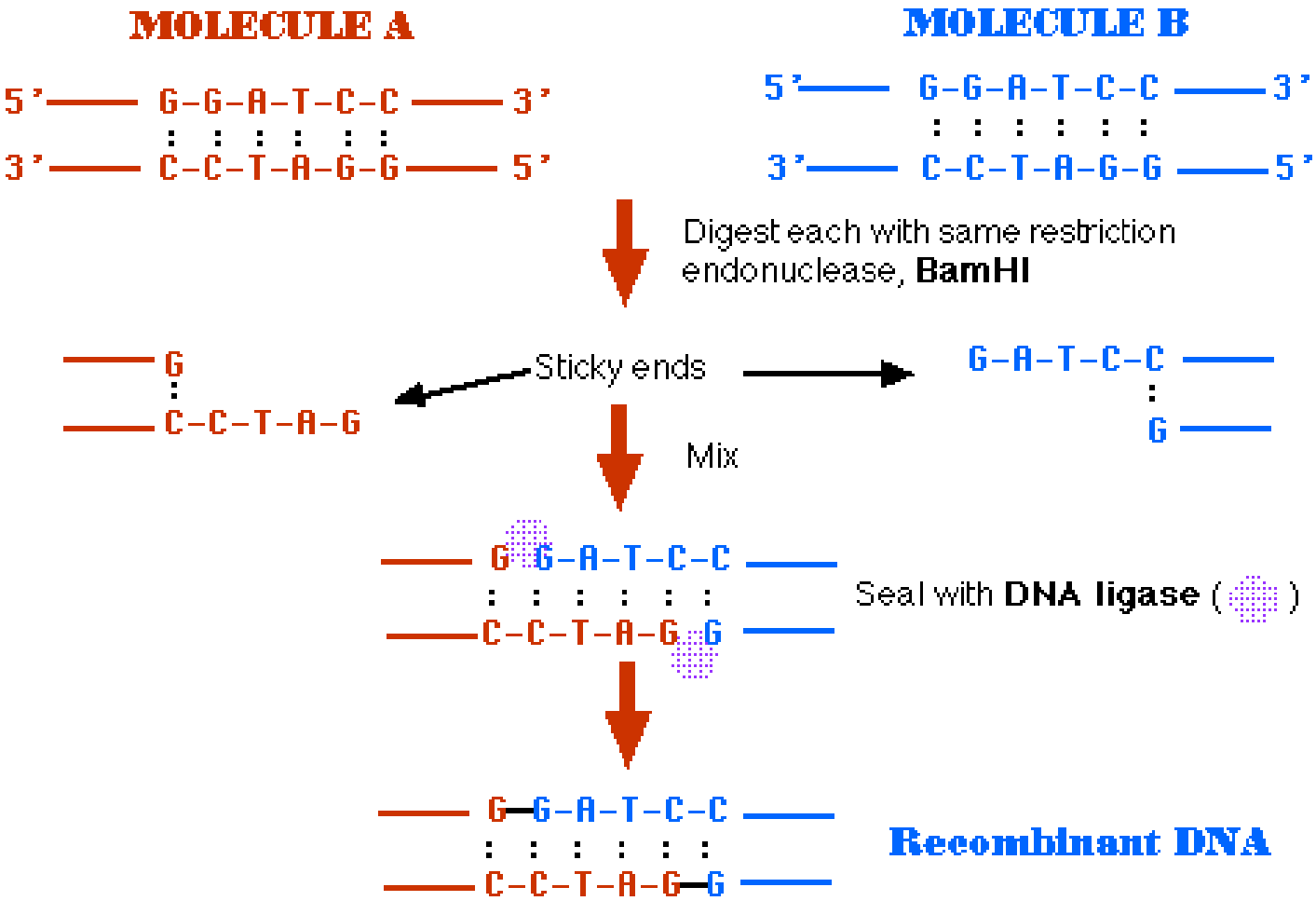
Ligation Strategies

Ligating Compatible Sticky Ends- Easy
Eg. BamHI cut fragment with BamHI cut fragment



Ligating Blunt Ends

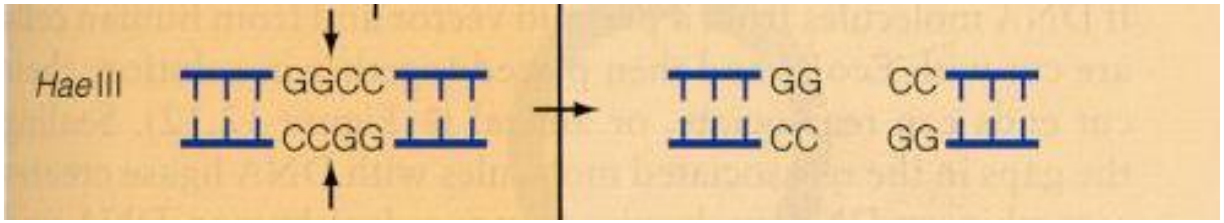
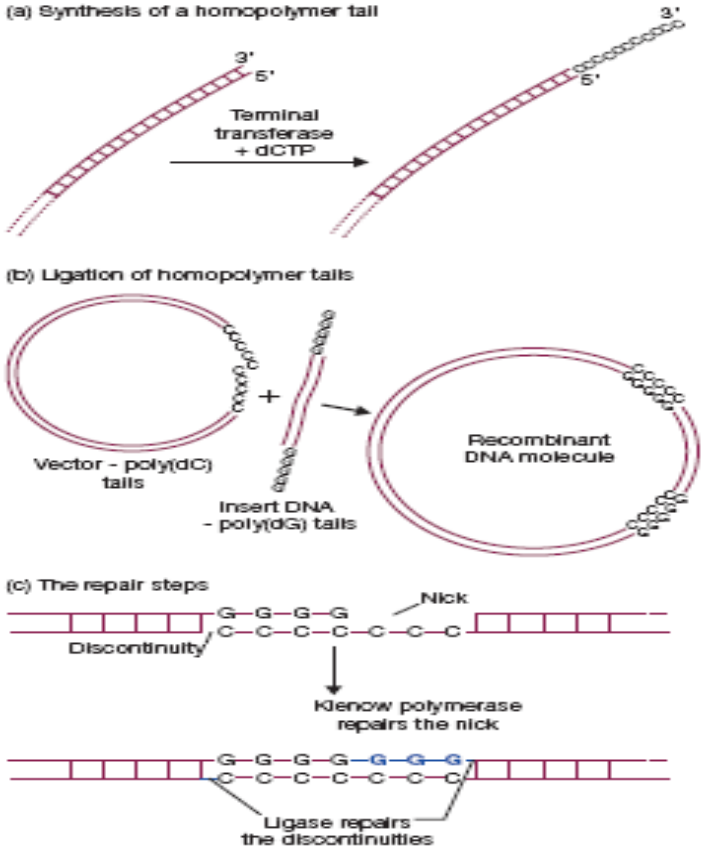
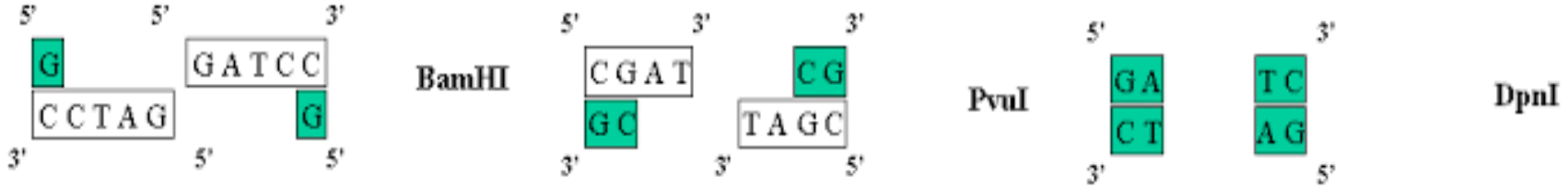


Figure 4.26
Homopolymer tailing: (a) synthesis of a homopolymer tail; (b) construction of a recombinant DNA molecule from a tailed vector plus tailed insert DNA; (c) repair of the recombinant DNA molecule.

Option- Add compatible terminal nucleotides



Ligating Blunt Ends with sticky ends (5’ or 3’ overhangs)



Option 1- Removal of 5’ or 3’ overhangs or fill-in of 5’ overhangs

Disadvantage- You will lose some bases and RE site (in case you remove, also no directionality

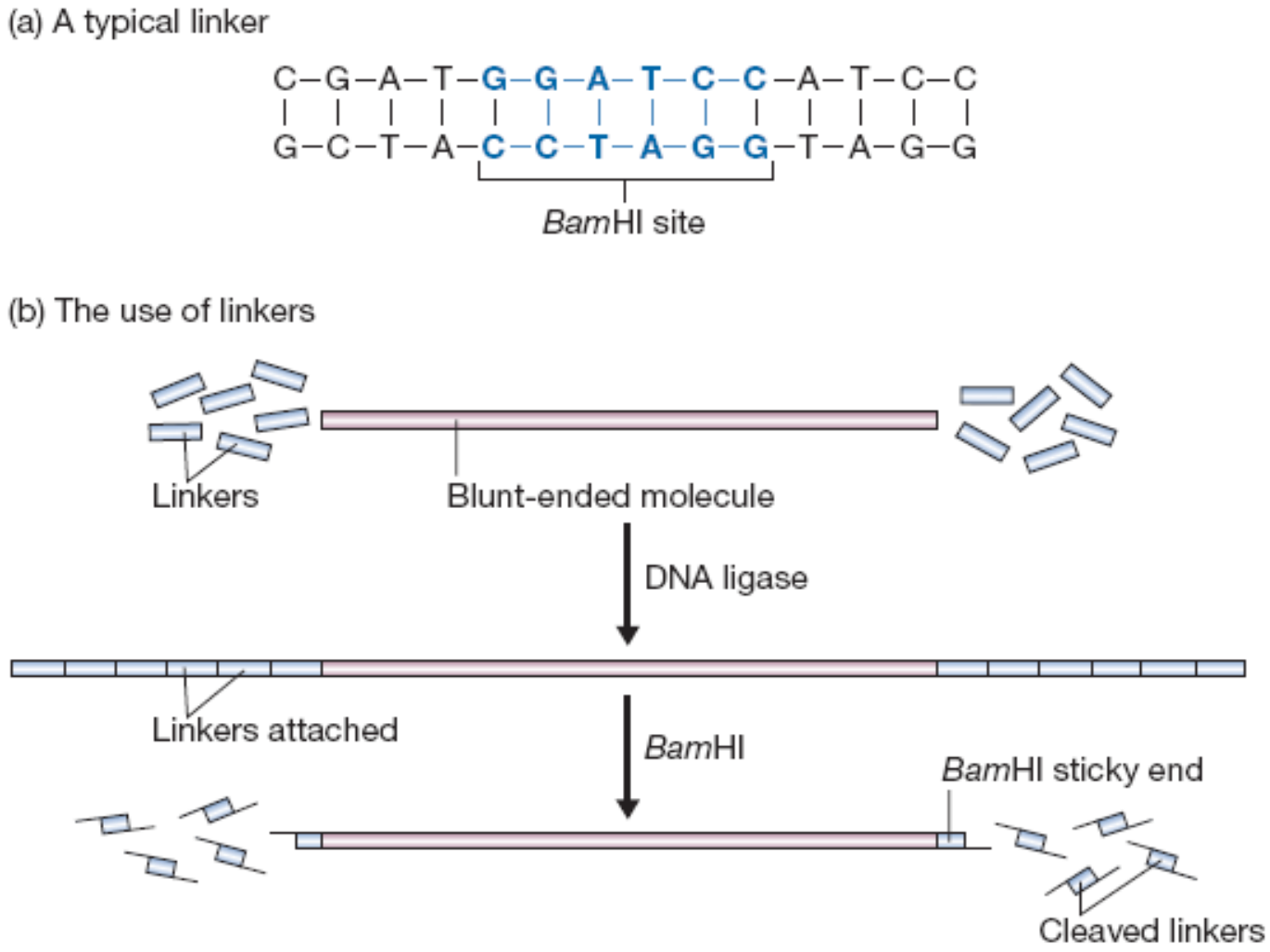
Blunting Selection Chart

	T4 DNA Polymerase* (NEB #M0203)	DNA Polymerase I, Large (Klenow) Fragment (NEB #M0210)	Quick Blunting Kit (NEB #E1201)	Mung Bean Nuclease (NEB #M0250)
APPLICATION				
Removal of 3′ overhangs	√	√	√	√
Removal of 5′ overhangs				√
Fill in of 5′ overhangs	√	√	√	

** T4 DNA Polymerase has a strong 3′→ 5′ exo activity*

Ligating Blunt Ends to Sticky Ends- Use of Linkers

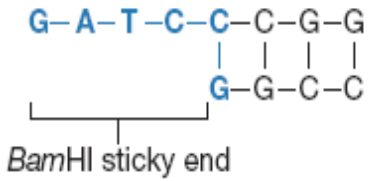
Option 2- Add linkers with RE sites to the blunt end molecule



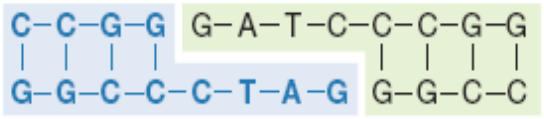
Ligating Blunt Ends to Sticky Ends- Use of Adaptors

Option 3- Add Adaptors with compatible overhangs to the blunt end molecule

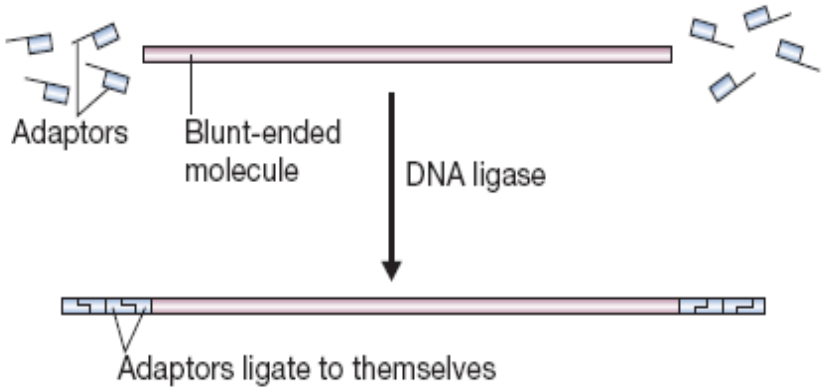
(a) A typical adaptor



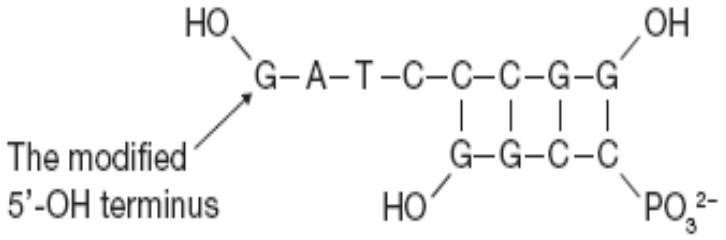
(b) Adaptors could ligate to one another



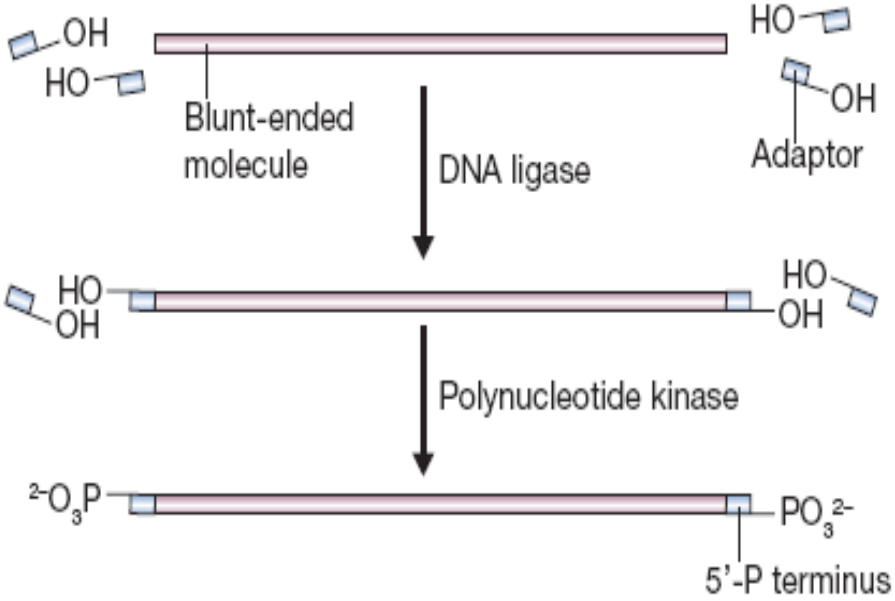
(c) The new DNA molecule is still blunt-ended



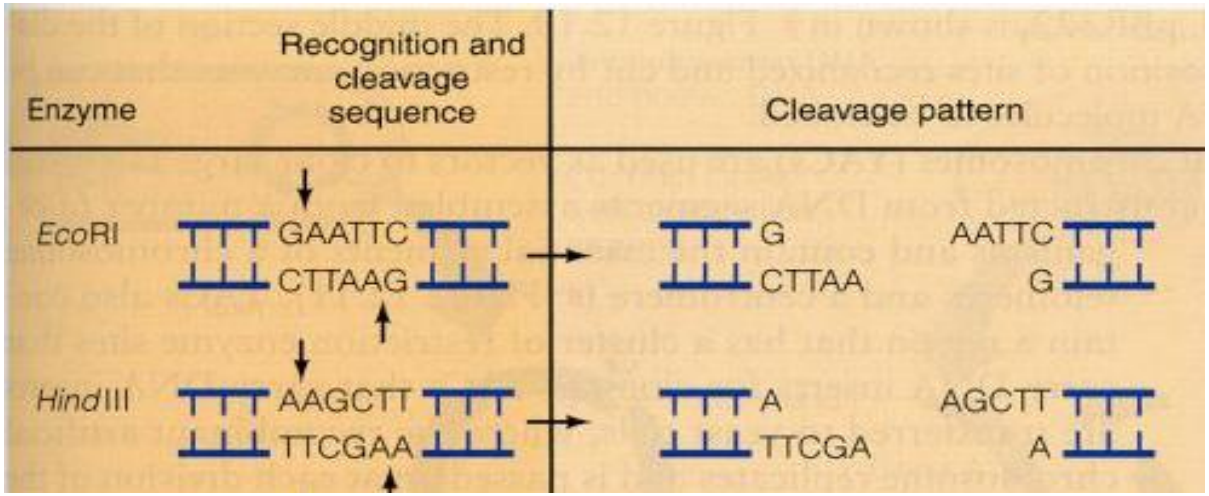
(a) The precise structure of an adaptor



(b) Ligation using adaptors



Ligating Incompatible Sticky Ends
Eg. EcoRI cut fragment with HindIII cut fragment



Options:

1) Make both molecules blunt by using

a) Mungbean nuclease to remove overhangs- Disadvantage (lose the bases/RE sites)

b) Use T4 DNA polymerase- Will fill in the 5'overhangs and remove 3' overhangs

Or

2) Add compatible adaptors to each molecule and then ligate

Ligation using DNA Topoisomerases

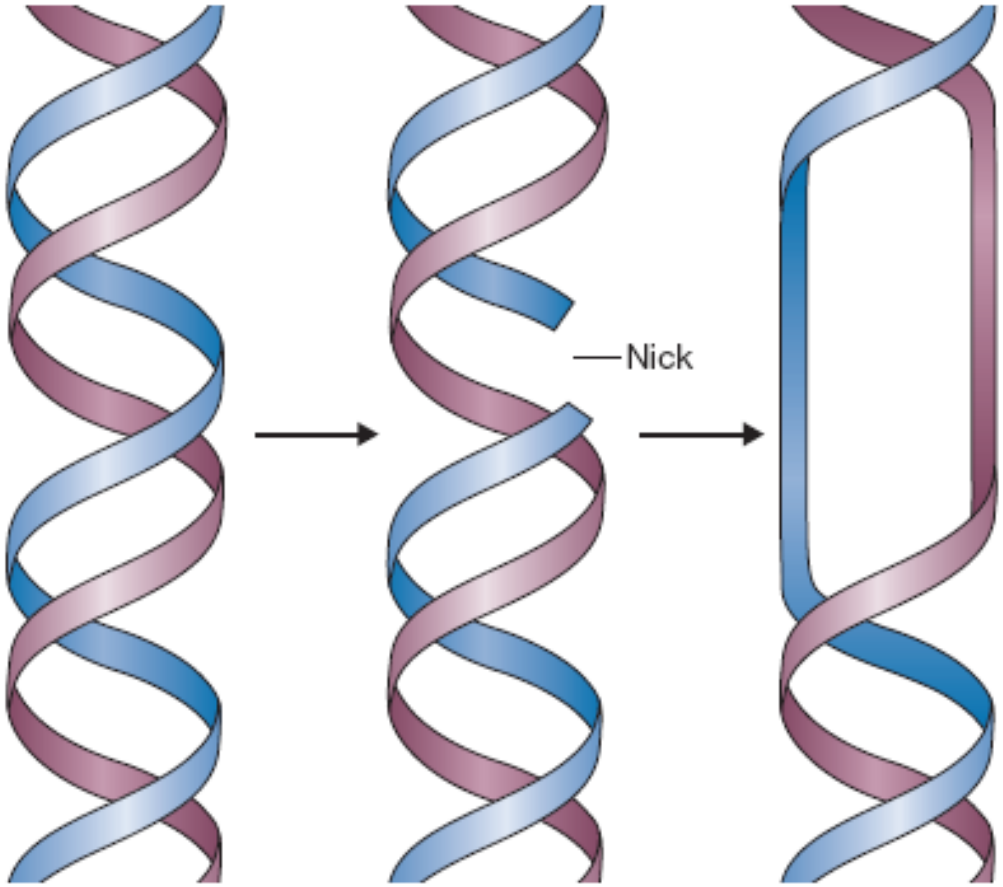
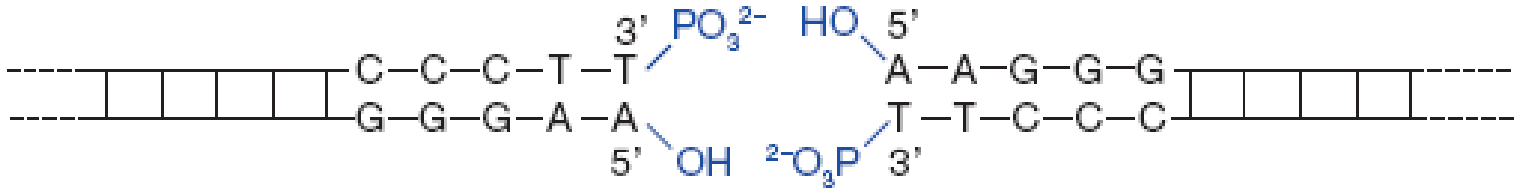


Figure 4.27

The mode of action of a Type 1 DNA topoisomerase, which removes or adds turns to a double helix by making a transient break in one of the strands.

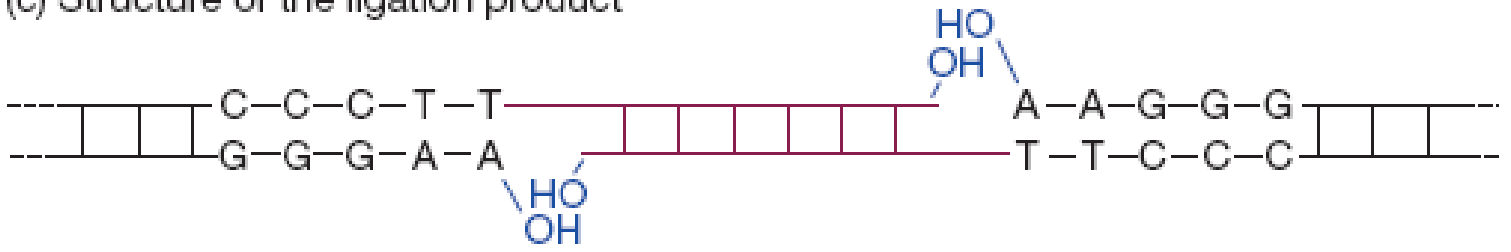
Ligation by DNA topoisomerase

(a) The ends of the vector resulting from topoisomerase cleavage



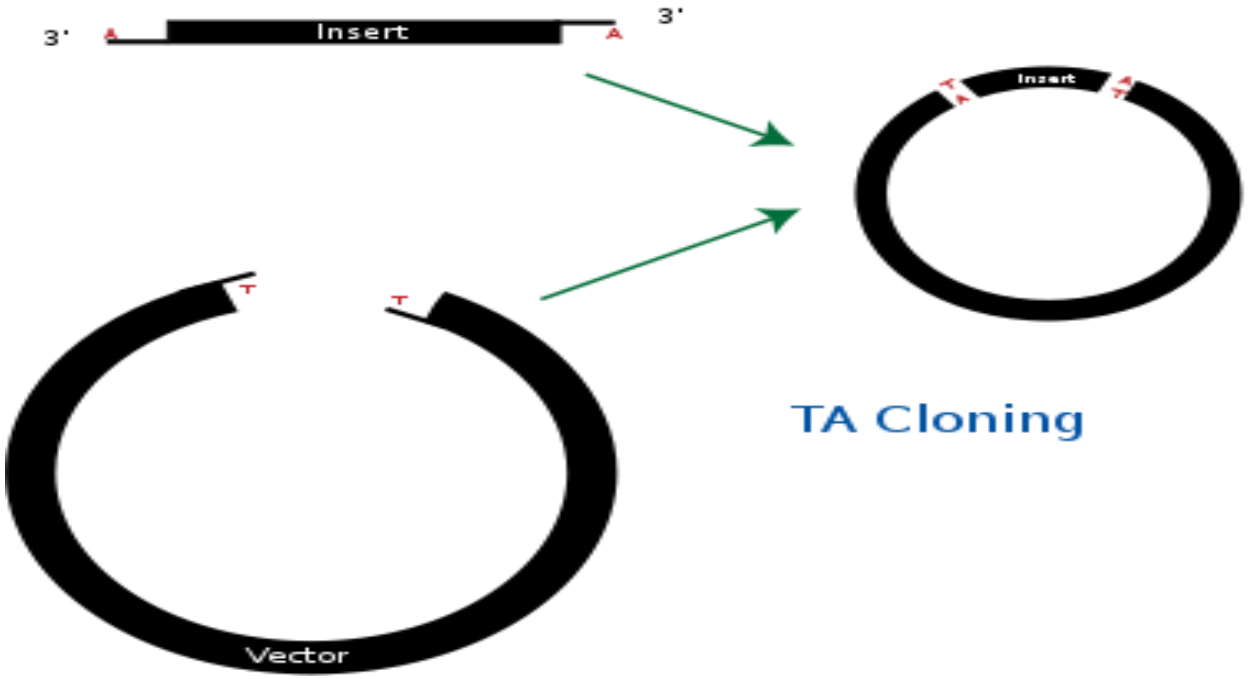
(b) Removal of terminal phosphates from the molecule to be cloned

(c) Structure of the ligation product

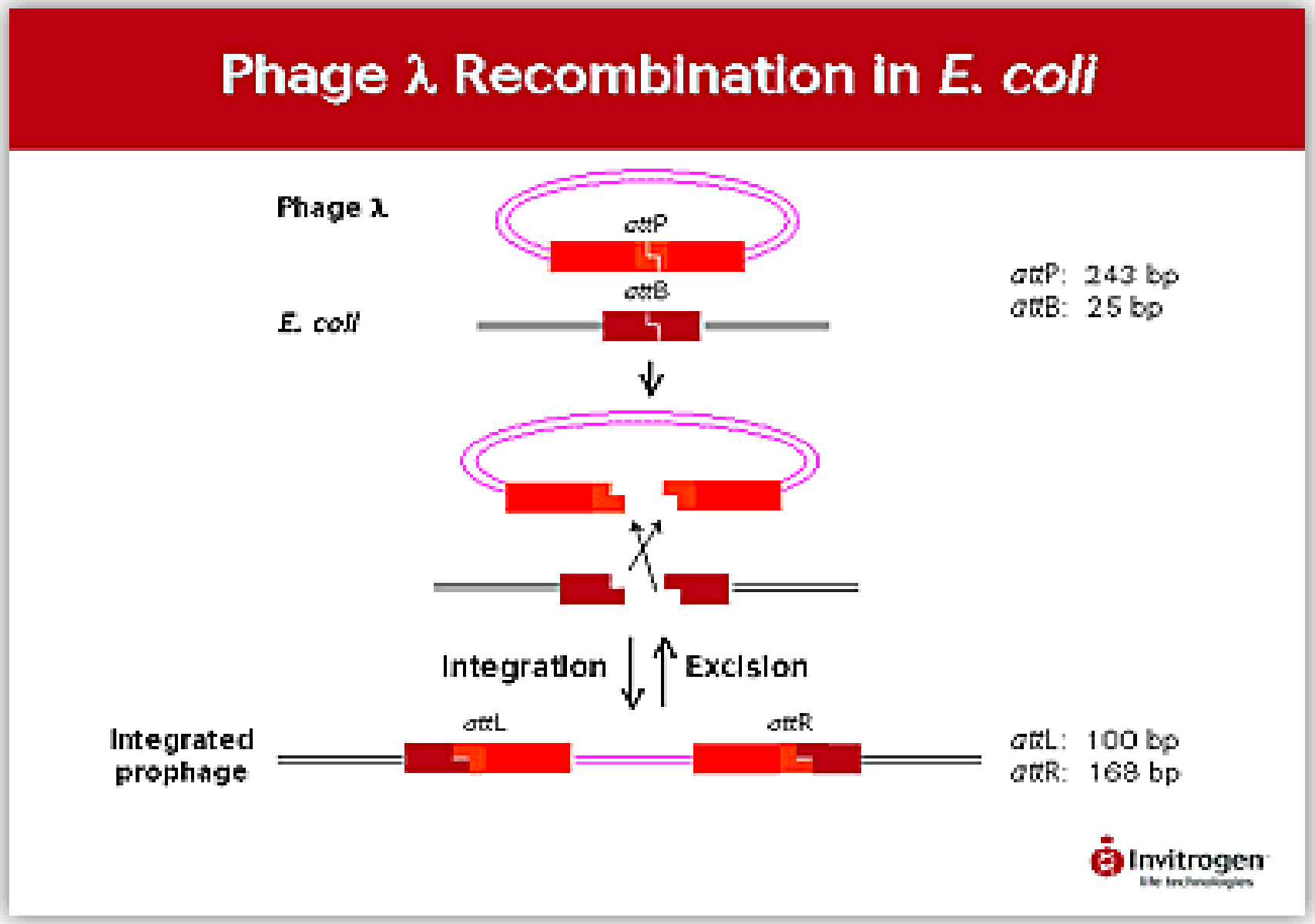


TA vectors

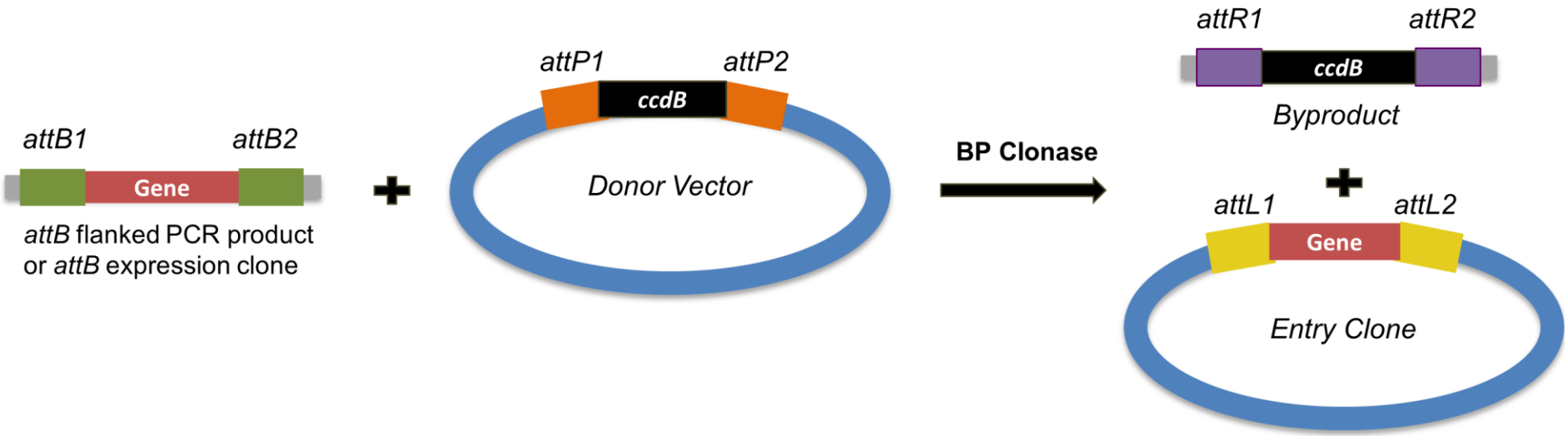
TA cloning (also known as rapid cloning or T cloning) is a subcloning technique that avoids the use of restriction enzymes[1] and is easier and quicker than traditional subcloning. The technique relies on the ability of adenine (A) and thymine (T) (complementary basepairs) on different DNA fragments to hybridize and, in the presence of ligase, become ligated together. PCR products are usually amplified using Taq DNA polymerase which preferentially adds an adenine to the 3' end of the product. Such PCR amplified inserts are cloned into linearized vectors that have complementary 3' thymine overhangs.[2]



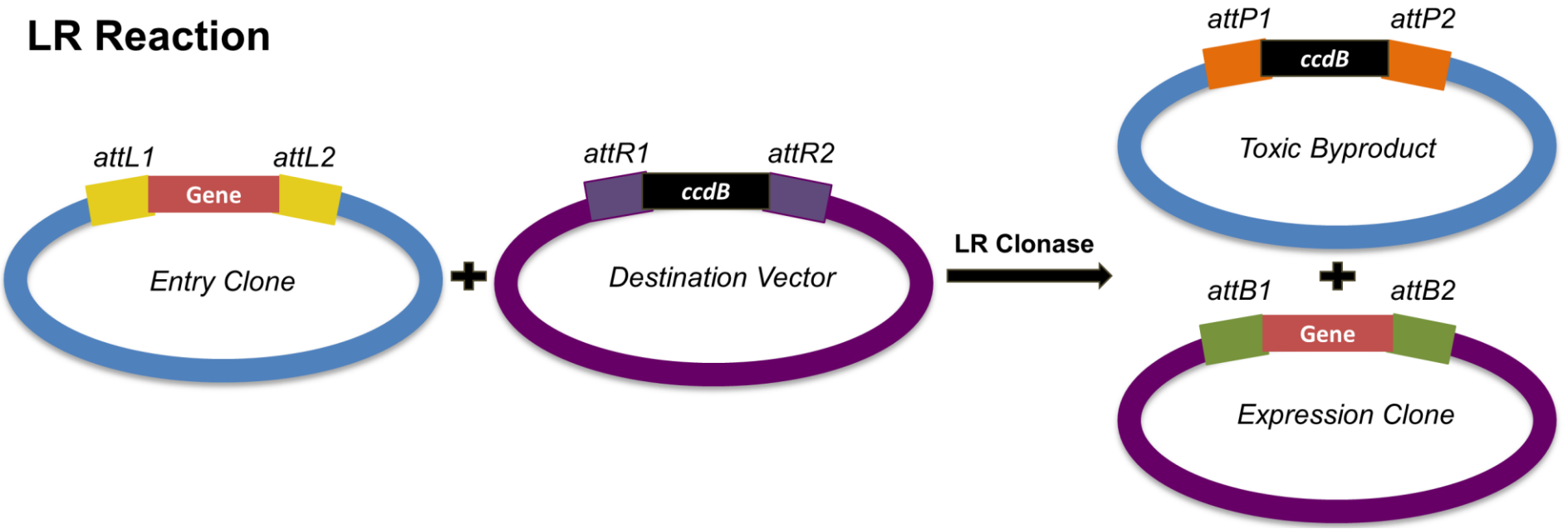
GATEWAY TECHNOLOGY



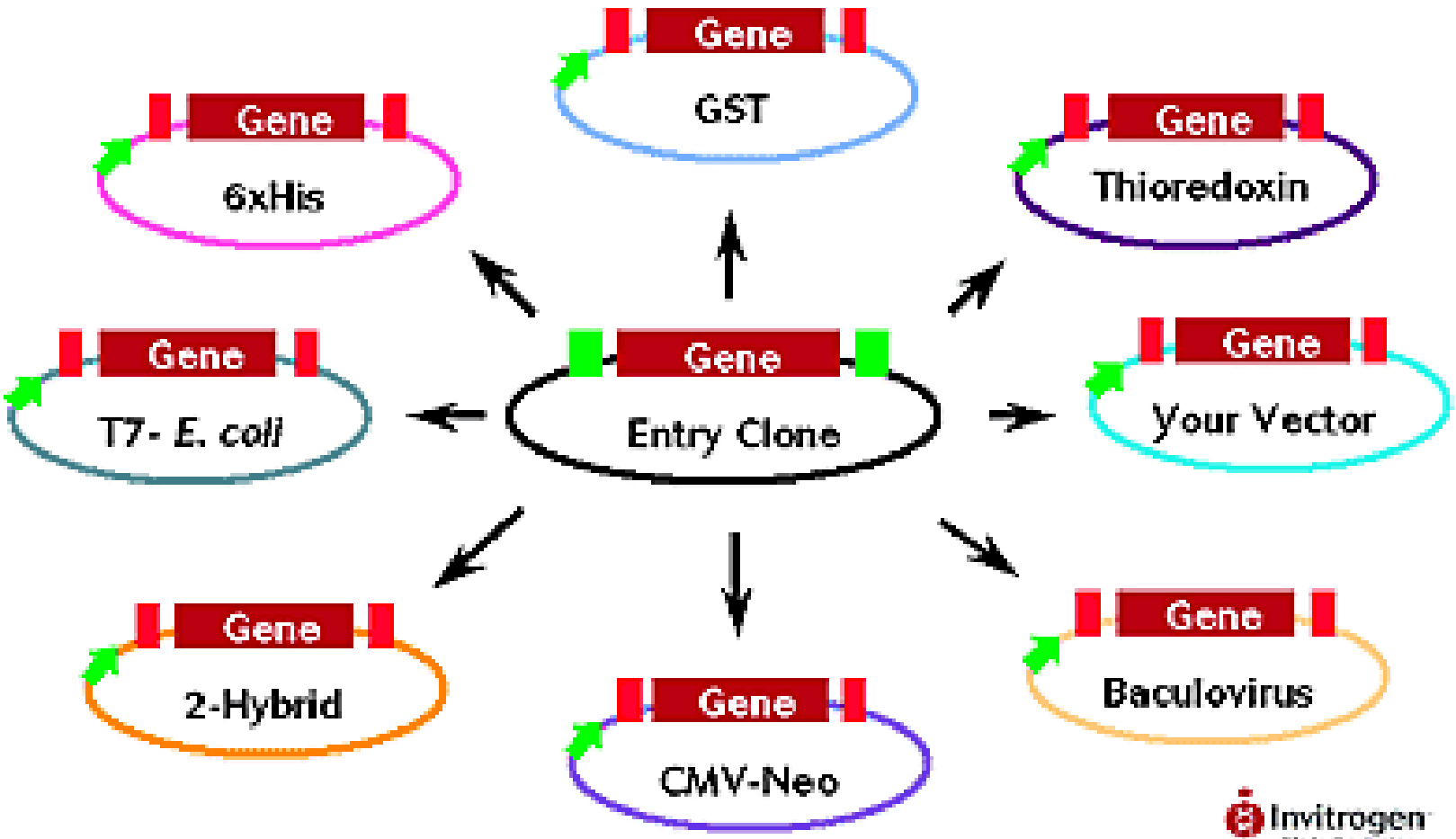
BP Reaction



LR Reaction



Subcloning an Entry Clone into Multiple Destination Vectors



Gateway cloning

- https://www.youtube.com/watch?v=qGSI5qxPDM0
- https://www.embl.de/pepcore/pepcore_services/cloning/cloning_methods/recombination/gateway/
- https://blog.addgene.org/plasmids-101-gateway-cloning