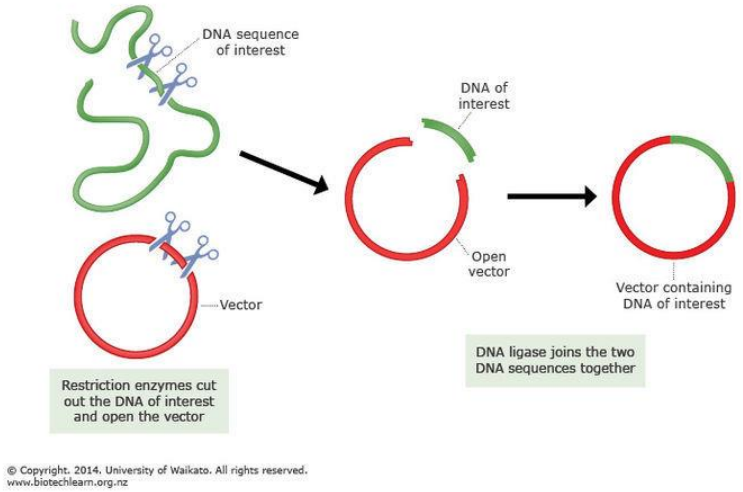
Cloning



DNA cloning is the starting point for many genetic engineering approaches to biotechnology research.

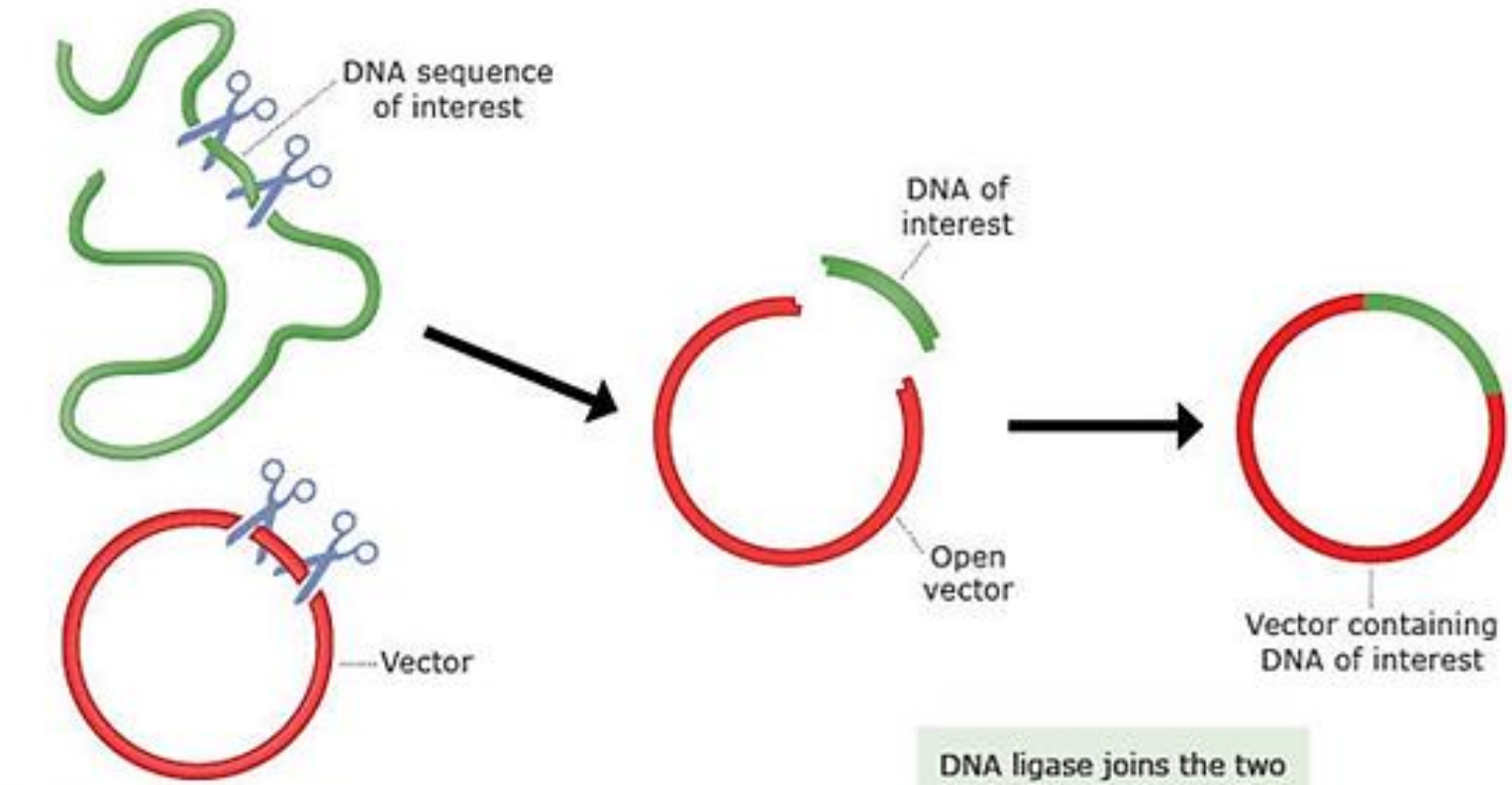
To get multiple copies of a gene or other piece of DNA you must isolate, or ‘cut’, the DNA from its source and then ‘paste’ it into a DNA vector that can replicate (or copy) itself.

The four main steps in DNA cloning are:

Step 1. The chosen piece of DNA is ‘cut’ from the source organism using restriction enzymes.

Step 2. The piece of DNA is ‘pasted’ into a vector and the ends of the DNA are joined with the vector DNA by ligation.

Step 3. The vector is introduced into a host cell, often a bacterium or yeast, by a process called transformation. The host cells copy the vector DNA along with their own DNA, creating multiple copies of the inserted DNA.



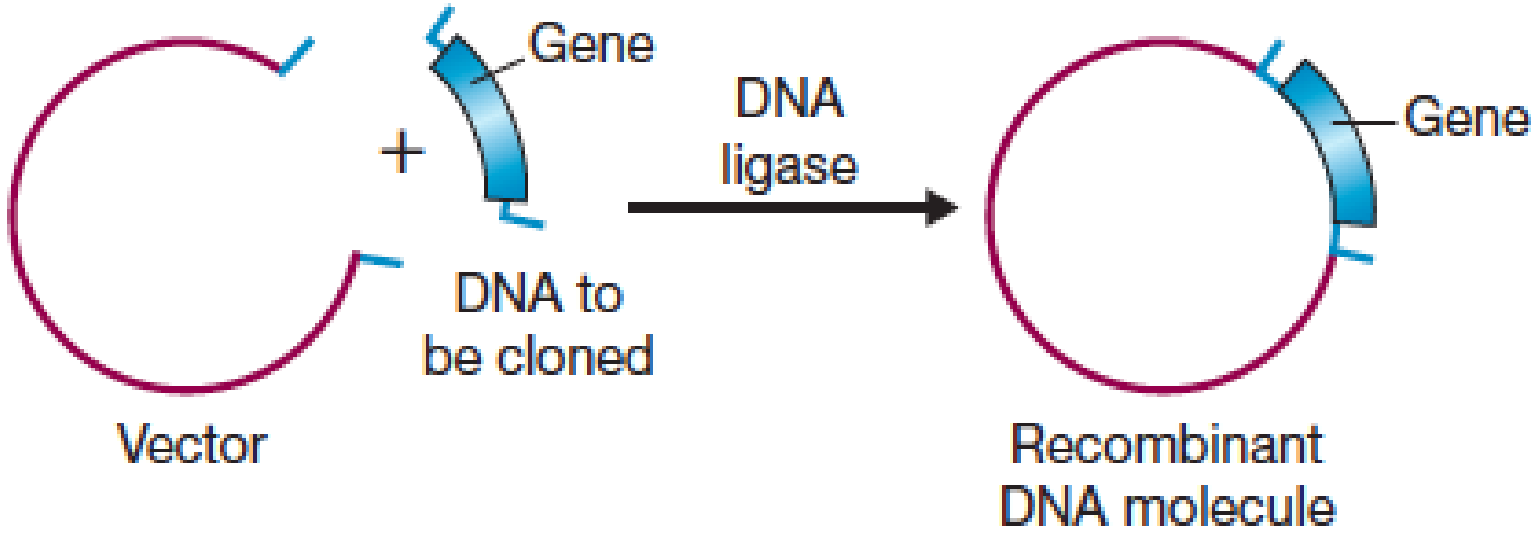
What is cloned DNA used for?

DNA cloning is used to create a large number of copies of a gene or other piece of DNA. The cloned DNA can be used to:

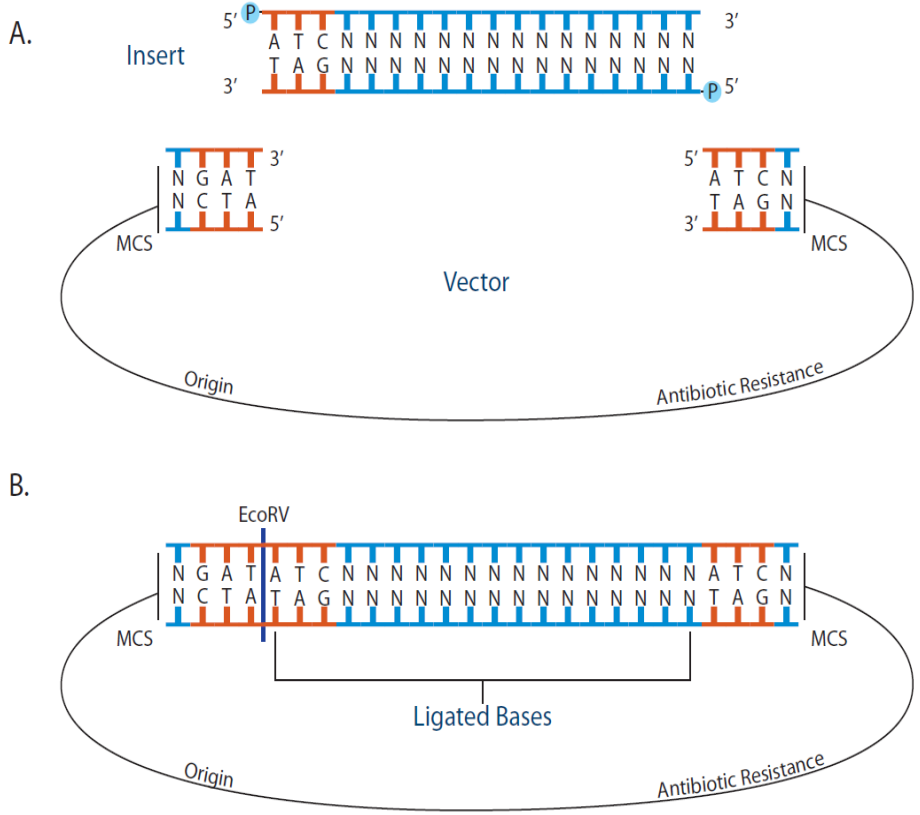
- Work out the function of the gene
- Investigate a gene's characteristics (size, expression, tissue distribution)
- Look at how mutations may affect a gene's function
- Make large concentrations of the protein coded for by the gene

Ligation – joining DNA molecules together

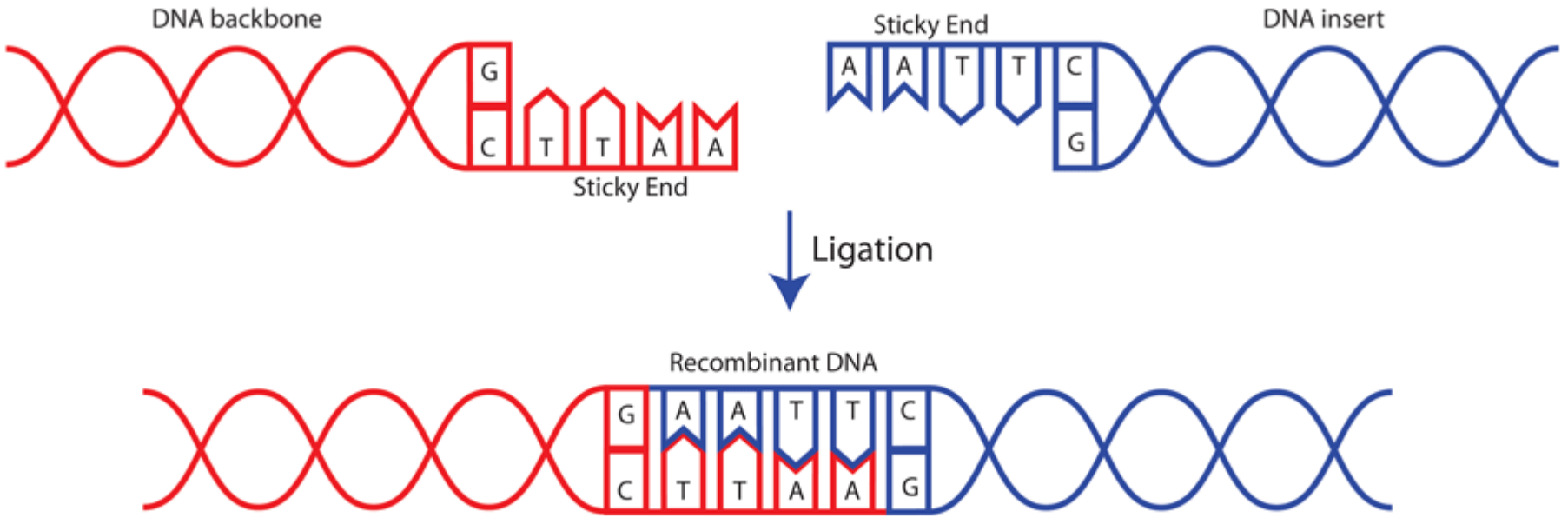
The final step in construction of a recombinant DNA molecule is the joining together of the vector molecule and the DNA to be cloned). This process is referred to as ligation, and the enzyme that catalyses the reaction is called DNA ligase.



The ligation reaction in **Figure** shows two blunt-ended fragments being joined together. Although this reaction can be carried out in the test tube, it is not very efficient. This is because the ligase is unable to “catch hold” of the molecule to be ligated, and has to wait for chance associations to bring the ends together. If possible, blunt end ligation should be performed at high DNA concentrations, to increase the chances of the ends of the molecules coming together in the correct way.



In contrast, ligation of complementary sticky ends is much more efficient. This is because compatible sticky ends can base pair with one another by hydrogen bonding (Figure), forming a relatively stable structure for the enzyme to work on. If the phosphodiester bonds are not synthesized fairly quickly then the sticky ends fall apart again. These transient, base-paired structures do, however, increase the efficiency of ligation by increasing the length of time the ends are in contact with one another.



Putting sticky ends onto a blunt-ended molecule

A : Linkers

The first of these methods involves the use of linkers. These are short pieces of double-stranded DNA, of known nucleotide sequence, that are synthesised in the test tube.

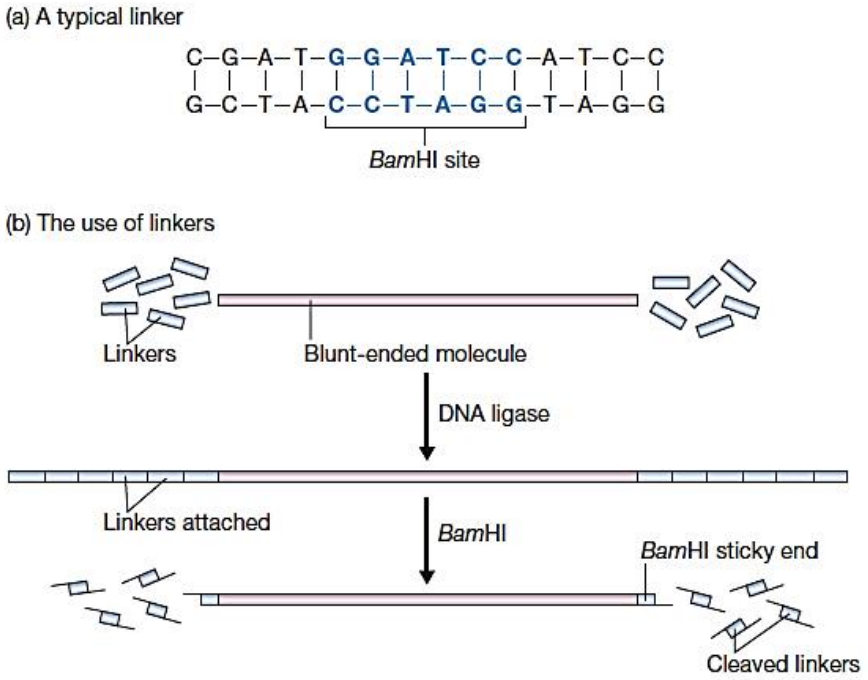
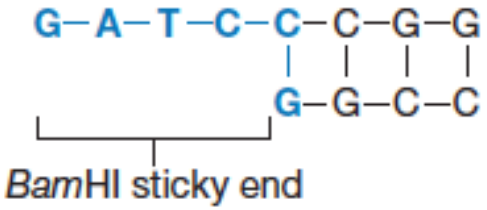


Figure 4.21

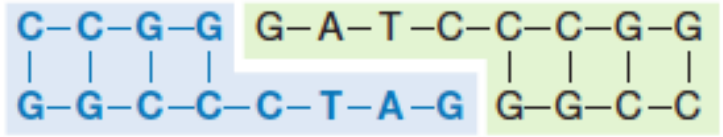
Linkers and their use: (a) the structure of a typical linker; (b) the attachment of linkers to a blunt-ended molecule.

B : Adaptor

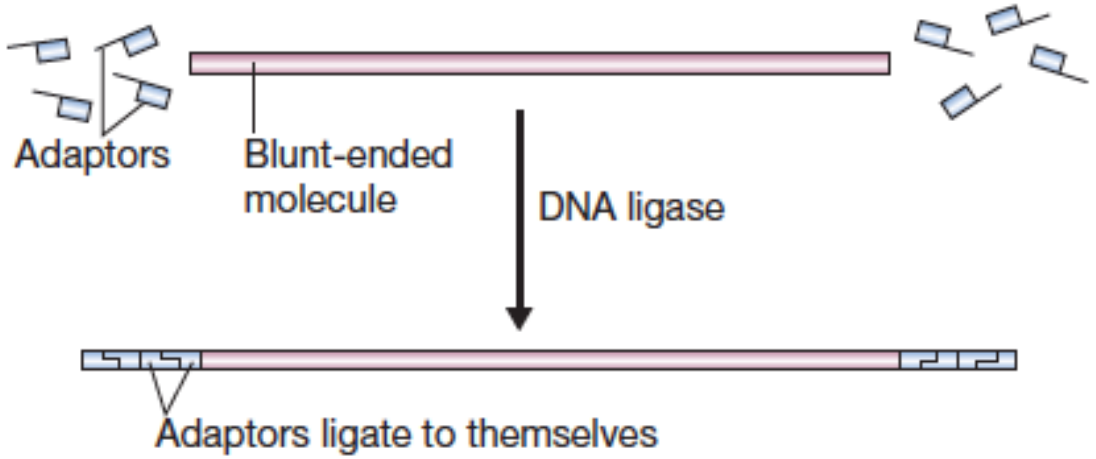
(a) A typical adaptor



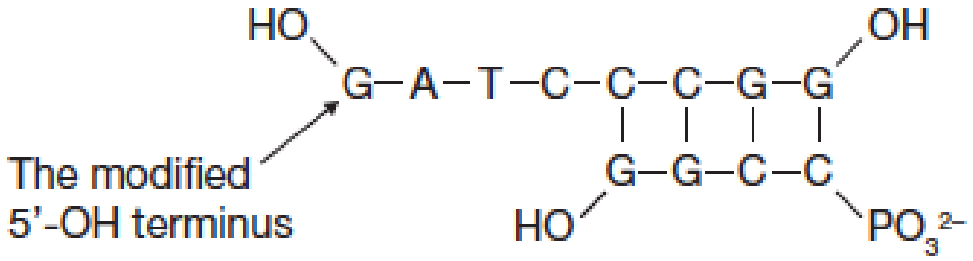
(b) Adaptors could ligate to one another



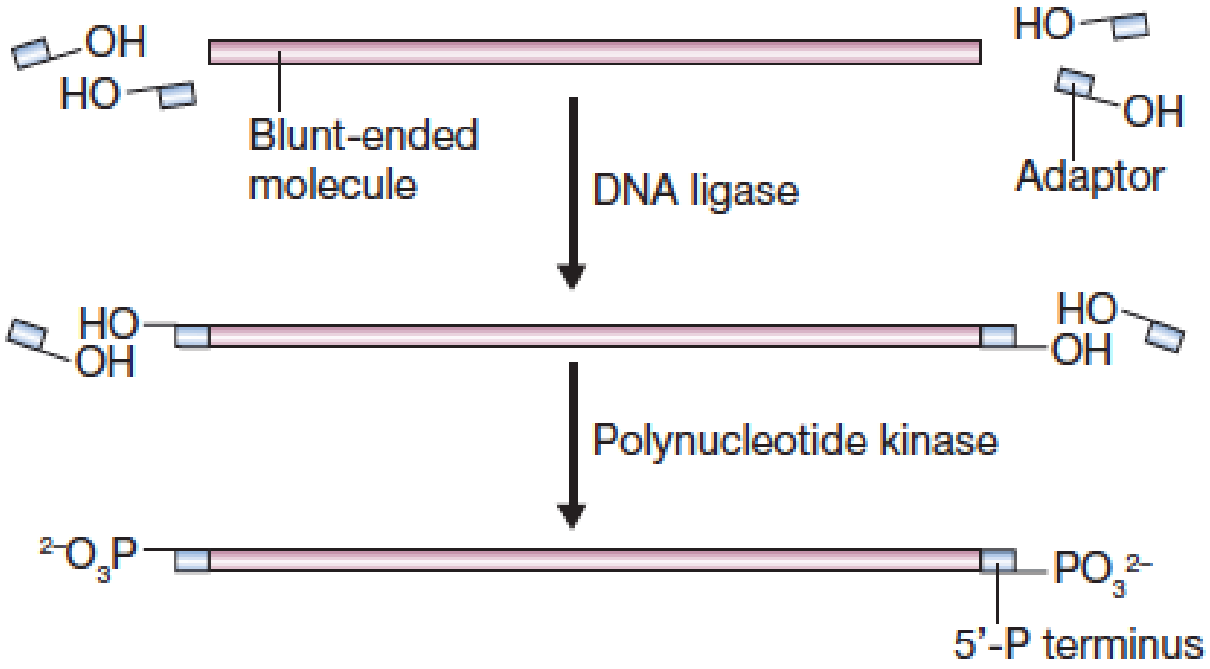
(c) The new DNA molecule is still blunt-ended



(a) The precise structure of an adaptor

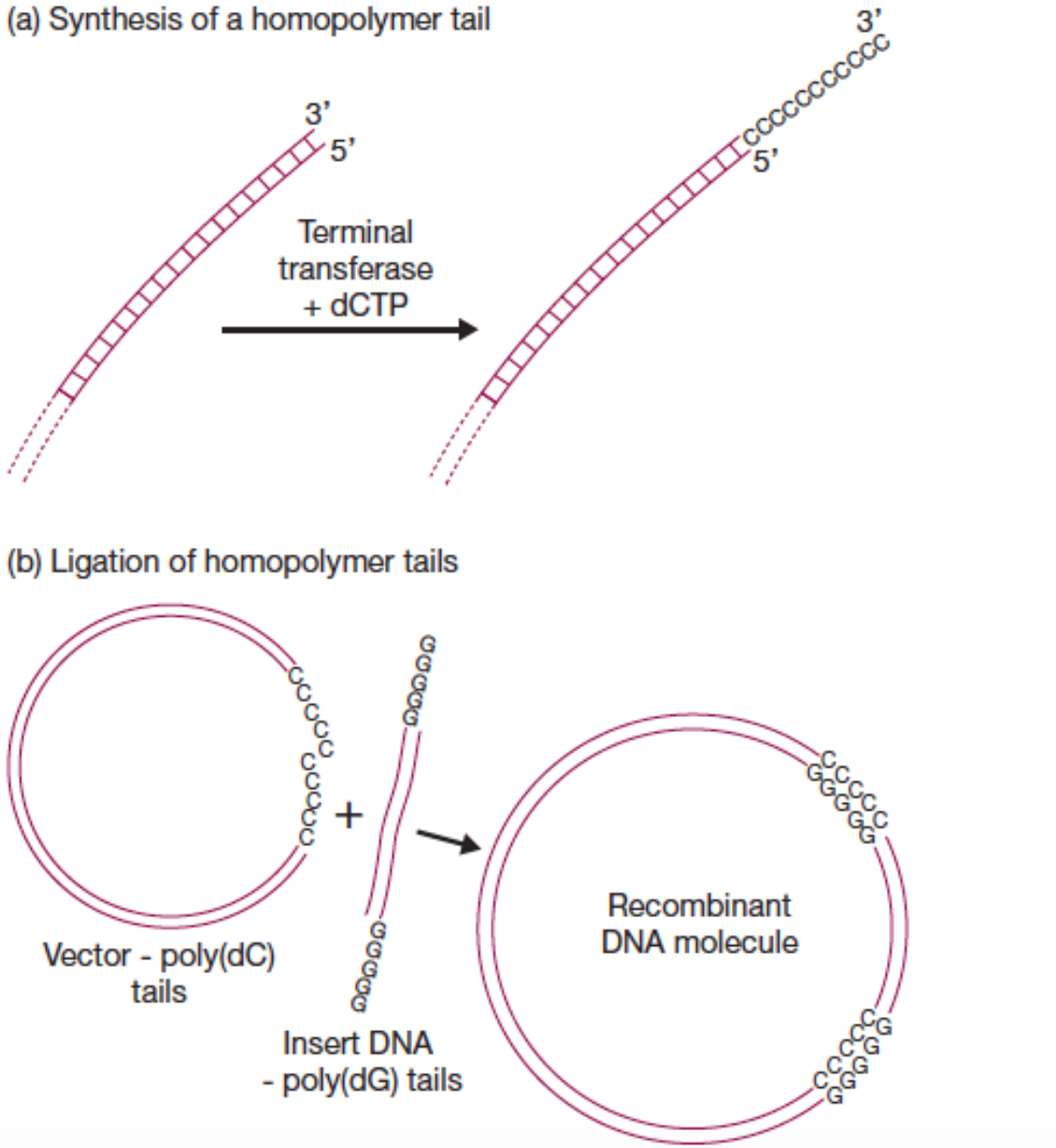


(b) Ligation using adaptors



Producing sticky ends by homopolymer tailing

❖ The technique of homopolymer tailing offers a radically different approach to the production of sticky ends on a blunt-ended DNA molecule. A homopolymer is simply a polymer in which all the subunits are the same. A DNA strand made up entirely of, say, deoxyguanosine is an example of a homopolymer, and is referred to as polydeoxyguanosine or poly(dG).


(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