Cloning vectors for *Escherichia coli*-II

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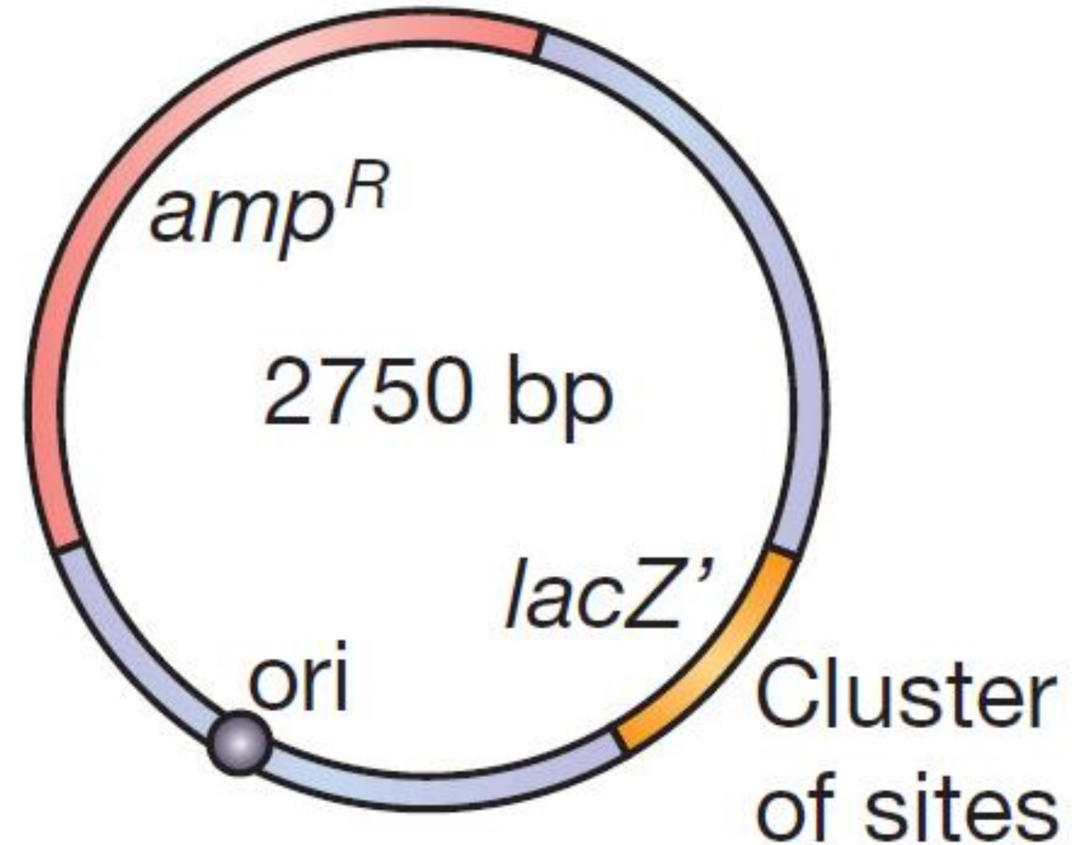
Cloning vectors based on *E. coli* plasmids

- The simplest cloning vectors used in gene cloning are based on small bacterial plasmids.
- A large number of different plasmid vectors are available for use with E. coli.

1. pBR322
2. pUC8
3. Pgem3z



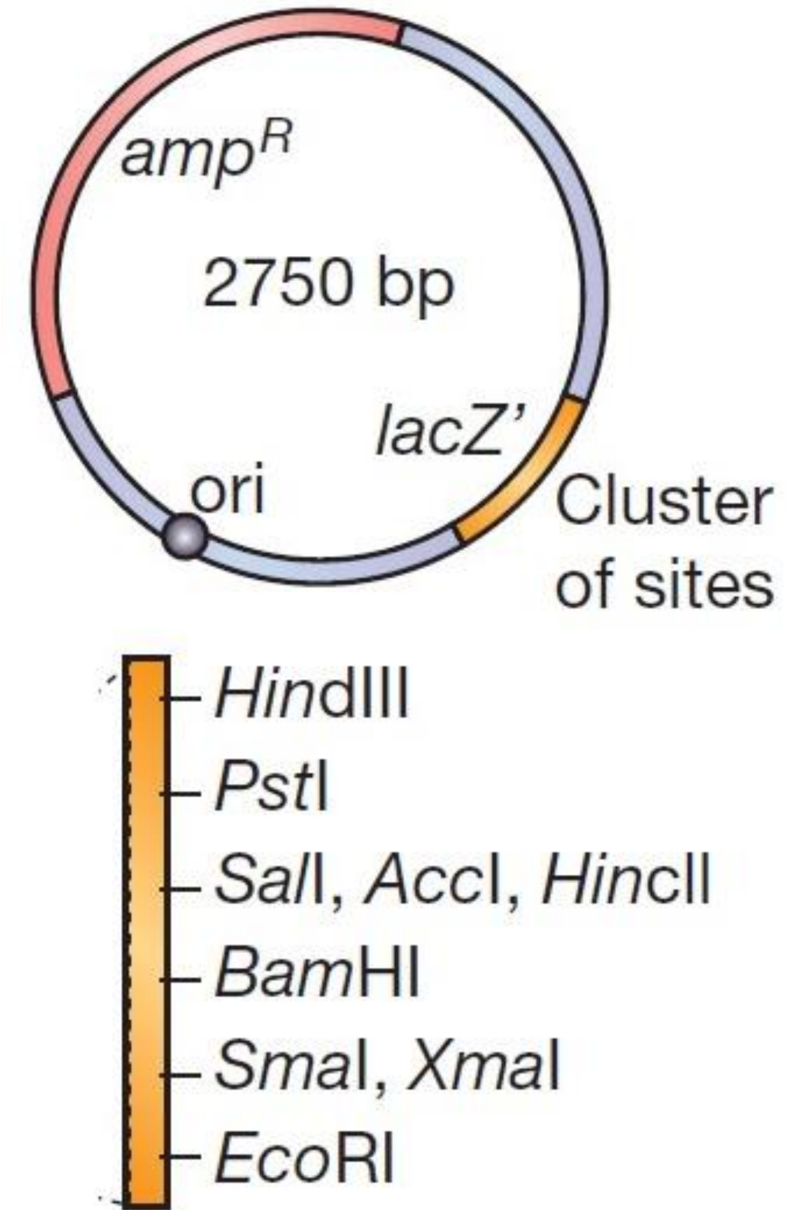
#pUC8: A Lac selection plasmid#

Nomenclature of pUC8:

- 'p' indicates a plasmid.
- 'UC' identifies University of California where the vector was originally constructed
- '8' distinguishes this plasmid from others developed in the same laboratory (there are also plasmids called pUC9, Puc18 etc.

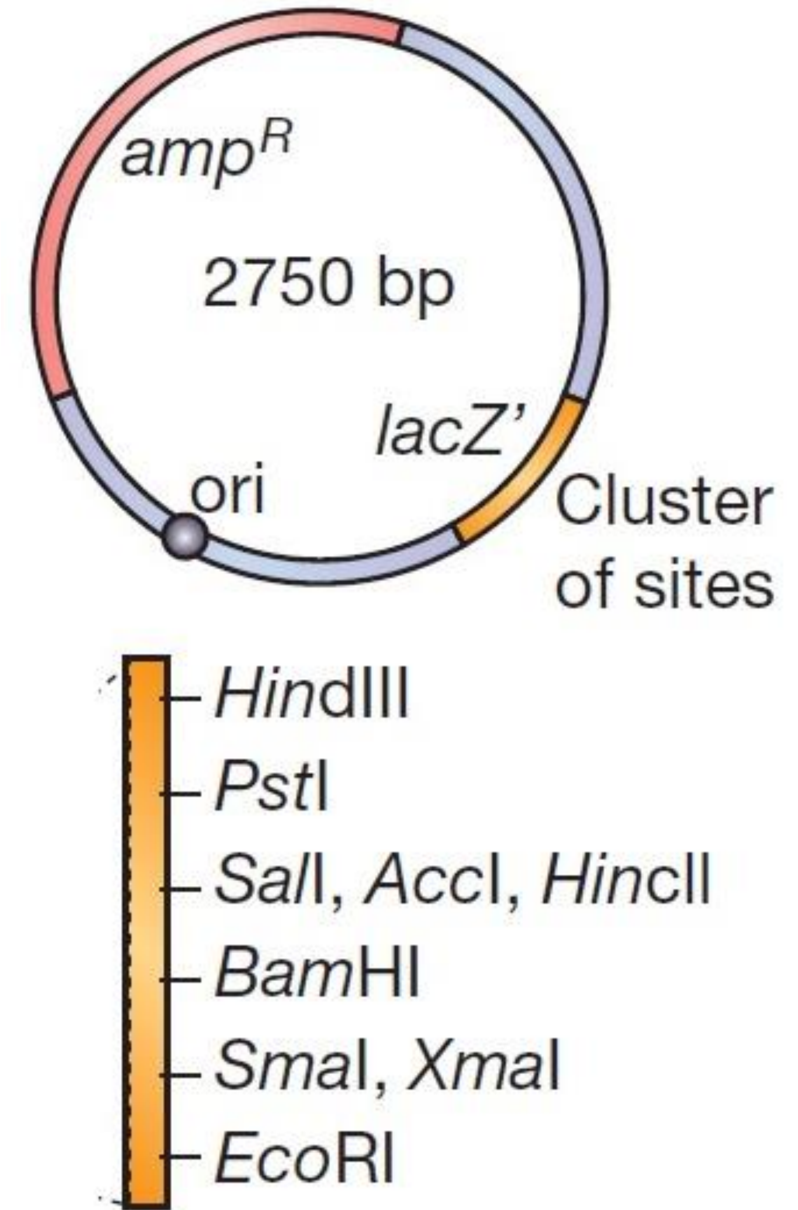
Properties of pUC8:

- pUC8 has two important advantages that have led to it becoming one of the most popular E. coli cloning vectors.
- The manipulations involved in constructing pUC8 were accompanied by a chance mutation, within the origin of replication, which results in the plasmid having a copy number of 500–700, even before amplification.
- This has a significant effect on the yield of cloned DNA obtainable from E. coli cells transformed with recombinant pUC8 plasmids.
- The second advantage is that identification of recombinant cells can be achieved by a single-step process, by plating onto agar medium containing ampicillin plus X-gal.
- With both pBR322 and pBR327, selection of recombinants is a two-step procedure, requiring replica plating from one antibiotic medium to another.

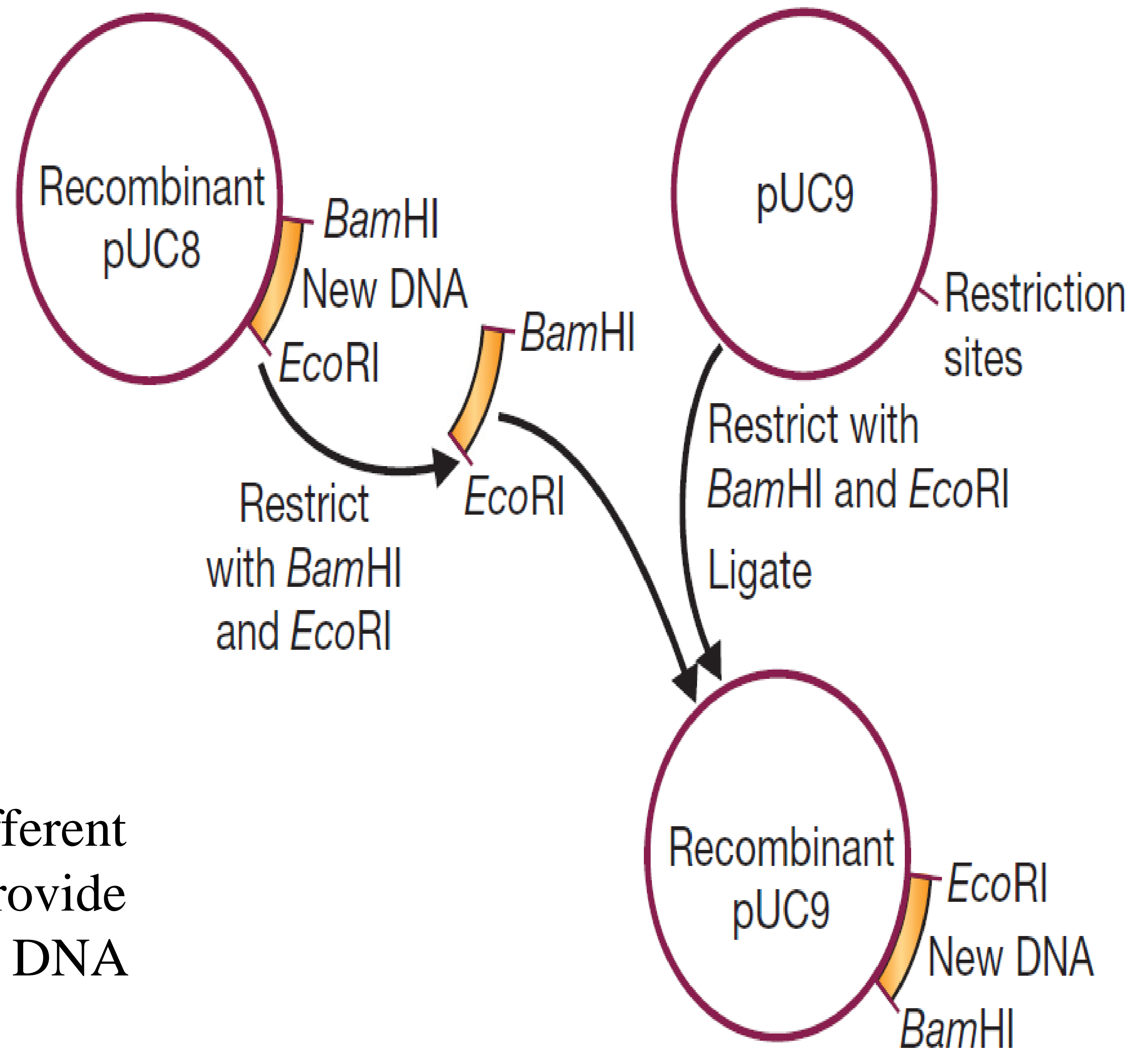


Properties of pUC8:

- A cloning experiment with pUC8 can therefore be carried out in half the time needed with pBR322 or pBR327.
- The cluster of cloning sites are contained in a short artificial oligonucleotide, called a polylinker, which was inserted into the lacZ′ gene when the first pUC8 plasmid was created.
- The polylinker is designed so that it does not totally disrupt the lacZ′ gene, the reading frame being maintained throughout the polylinker, so that a functional (though altered) β-galactosidase enzyme is still produced.
- The polylinker in pUC8 contains nine unique restriction sites, ones that are not found elsewhere in the vector.
- Because these sites are clustered, a DNA fragment with two different sticky ends (say EcoRI at one end and BamHI at the other) can be cloned without resorting to additional manipulations such as linker attachment.



- pUC8 is one of a family of vectors, differing only in the identity of the cloning sites.
- A second member of the family, pUC9, has the same polylinker as pUC8, but inserted into the lacZ′ gene in the opposite orientation.
- This pair of vectors can therefore be used to clone a DNA fragment in both forward and reverse directions, which enables antisense RNA to be prepared.

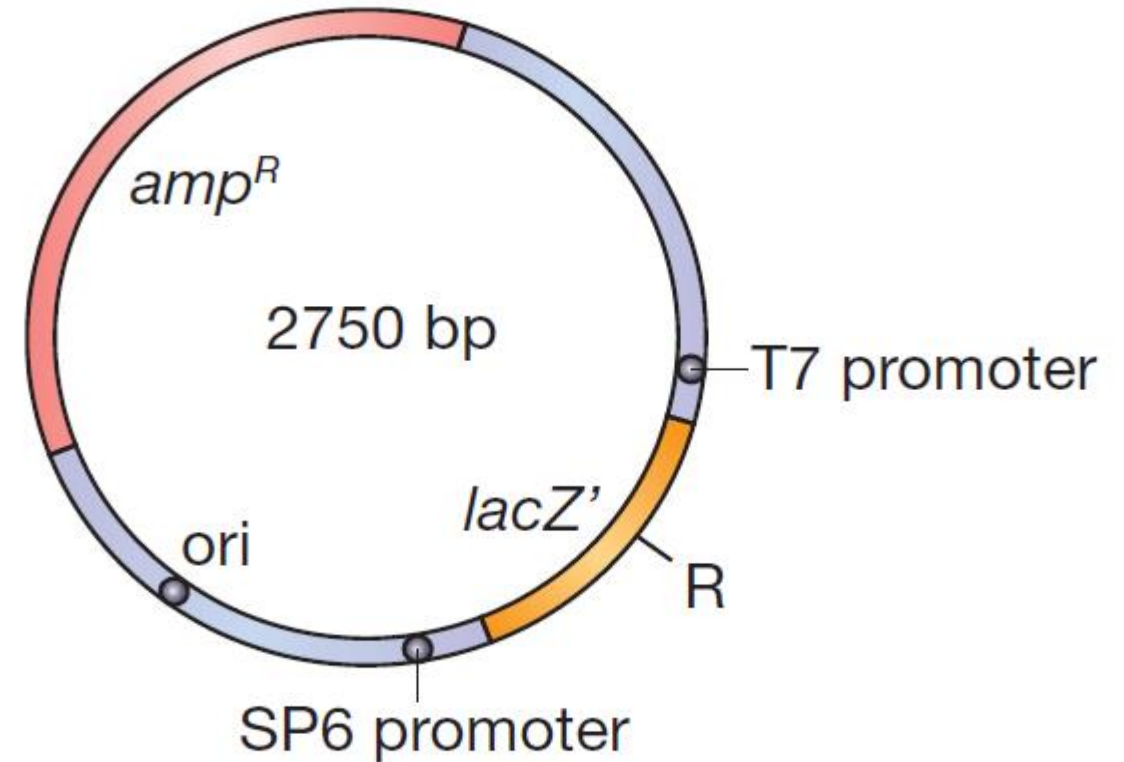


- Other pUC vectors carry different combinations of restriction sites and provide even greater flexibility in the types of DNA fragment that can be cloned.

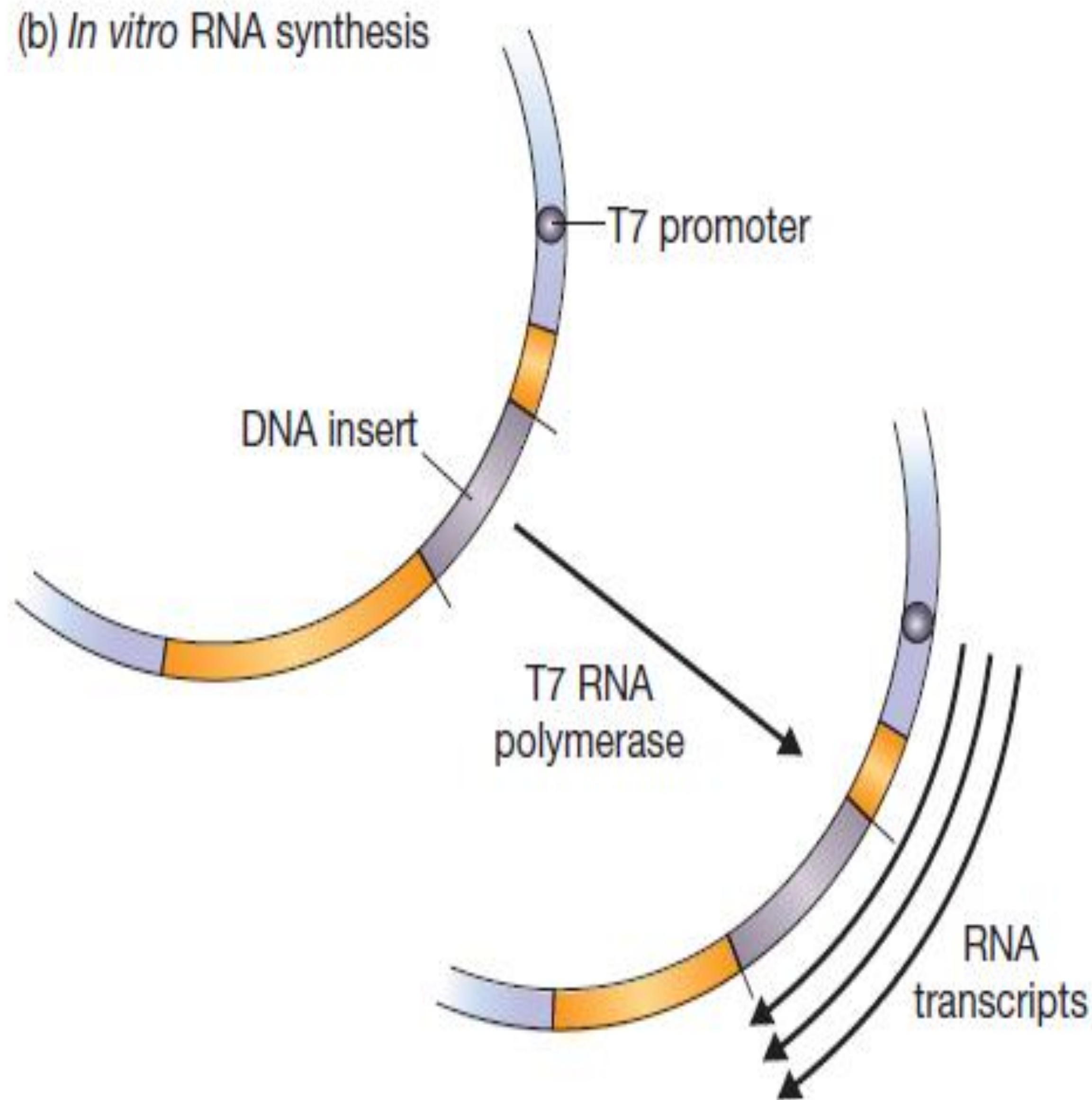
pGEM3Z
In vitro transcription of cloned DNA

•Pgem3z is very similar to a pUC vector in that it carries the ampR and lacZ′ genes, the latter containing a cluster of restriction sites, and it is almost exactly the same size.

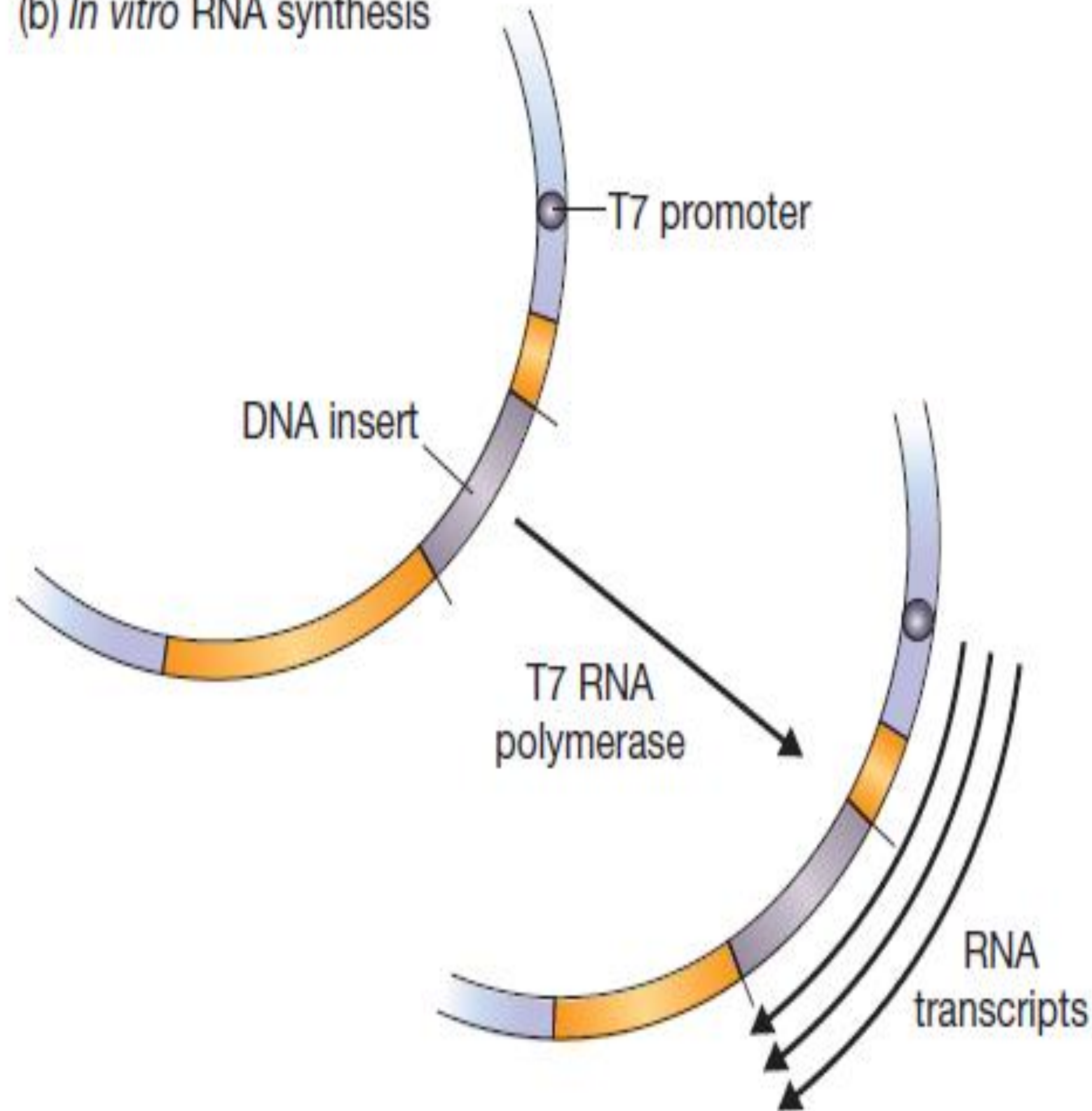
•The distinction is that pGEM3Z has two additional short pieces of DNA, each of which acts as the recognition site for attachment of an RNA polymerase enzyme.



- These two promoter sequences lie on either side of the cluster of restriction sites used for the introduction of new DNA into the pGEM3Z molecule.
- This means that if a recombinant pGEM3Z molecule is mixed with purified RNA polymerase in the test tube, transcription occurs and RNA copies of the cloned fragment are synthesized.



- The RNA that is produced could be used as a hybridization probe, or it might be required for experiments aimed at studying RNA processing (e.g., the removal of introns) or protein synthesis.
- The promoters carried by pGEM3Z and other vectors of this type are not the standard sequences recognized by the E. coli RNA polymerase.
- Instead, one of the promoters is specific for the RNA polymerase coded by T7 bacteriophage and the other for the RNA polymerase of SP6 phage.
- These RNA polymerases are synthesized during the infection of E. coli with one or other of the phages, and are responsible for transcribing the phage genes.



- They are chosen for in vitro transcription as they are very active enzymes (remember that the entire lytic infection cycle takes only 20 min, so the phage genes must be transcribed very quickly).
- These polymerases are able to synthesize 1–2 mg of RNA per minute, which is substantially more than can be produced by the standard E. coli enzyme.
- ‘R’ indicates a polylinker containing restriction sites for EcoRI, SacI, KpnI, AvaI, SmaI, BamHI, XbaI, SalI, AccI, HincII, PstI, SphI, and HindIII.