The distances are described as minutes on the chromosome as the matings were disrupted at various times (minutes) following the mating.

CHOICE OF CLONING VECTORS

Vectors must be small molecules for convenient manipulation.

They must be capable of prolific replication in a living cell in order to amplify the inserted donor fragment.

They must also have convenient restriction sites at which the DNA to be cloned may be inserted. Ideally, the restriction site should be present only once in the vector because then restriction fragments of donor DNA will insert only at that one location in the vector.

It is also important that there be a way to identify and recover the recombinant molecule quickly.

Numerous cloning vectors are in current use, suitable for different sizes of DNA insert or for different uses of the clone.

Good plasmid cloning vehicles share a number of desirable features

An ideal cloning vehicle would have the following three properties:

- low molecular weight;
- ability to confer readily selectable phenotypic traits on host cells;
- single sites for a large number of restriction endonucleases, preferably in genes with a readily scorable phenotype.

Ability to replicate within the host cell, so that numerous copies of the recombinant DNA molecule can be produced and passed to daughter cells.

A DNA fragment of interest is covalently joined to a DNA *vector.*

The essential feature of a vector is that it can replicate autonomously in an appropriate host.

Plasmids *(naturally occurring circles of DNA that act as* accessory chromosomes in bacteria) and **bacteriophage** lambda (phage), a virus, are choice vectors for cloning in *E. coli.*

Plasmids are widely used as cloning vehicles. They are stably inherited in an extrachromosomal state.

Most plasmids exist as double-stranded circular DNA molecules.

The plasmids used as vectors carry genes for drug resistance.

These drug-resistance genes provide a convenient way to select for cells transformed by plasmids: those cells still alive after exposure to the drug must carry the plasmid vectors containing the DNA insert.

Plasmids are also an efficient means of amplifying cloned DNA because there are many copies per cell, as many as several hundred for some plasmids.

General-purpose cloning vectors. Cloning of foreign DNA fragments in general-purpose cloning vectors (e.g., pBR322 [11]) selectively inactivates one of the markers (insertional inactivation) or derepresses a silent marker (positive selection) so as to differentiate the recombinants from the native phenotype of the vector.

Expression vectors. In expression vectors (e.g., pUC18 [123]), DNA to be cloned and expressed is inserted downstream of a strong promoter present in the vector.

The vector can be prepared for accepting a new DNA fragment by cleaving it at a single specific site with a restriction enzyme. For example, the plasmid pSC101, a 9.9-kb double-helical circular DNA molecule, is split at a unique site by the *EcoRI restriction enzyme.*

The staggered cuts made by this enzyme produce *complementary single-stranded ends, which have specific affinity* for each other and hence are known as *cohesive or sticky ends.*

Any DNA fragment can be inserted into this plasmid if it has the same cohesive ends.

Such a fragment can be prepared from a larger piece of DNA by using the same restriction enzyme as was used to open the plasmid DNA

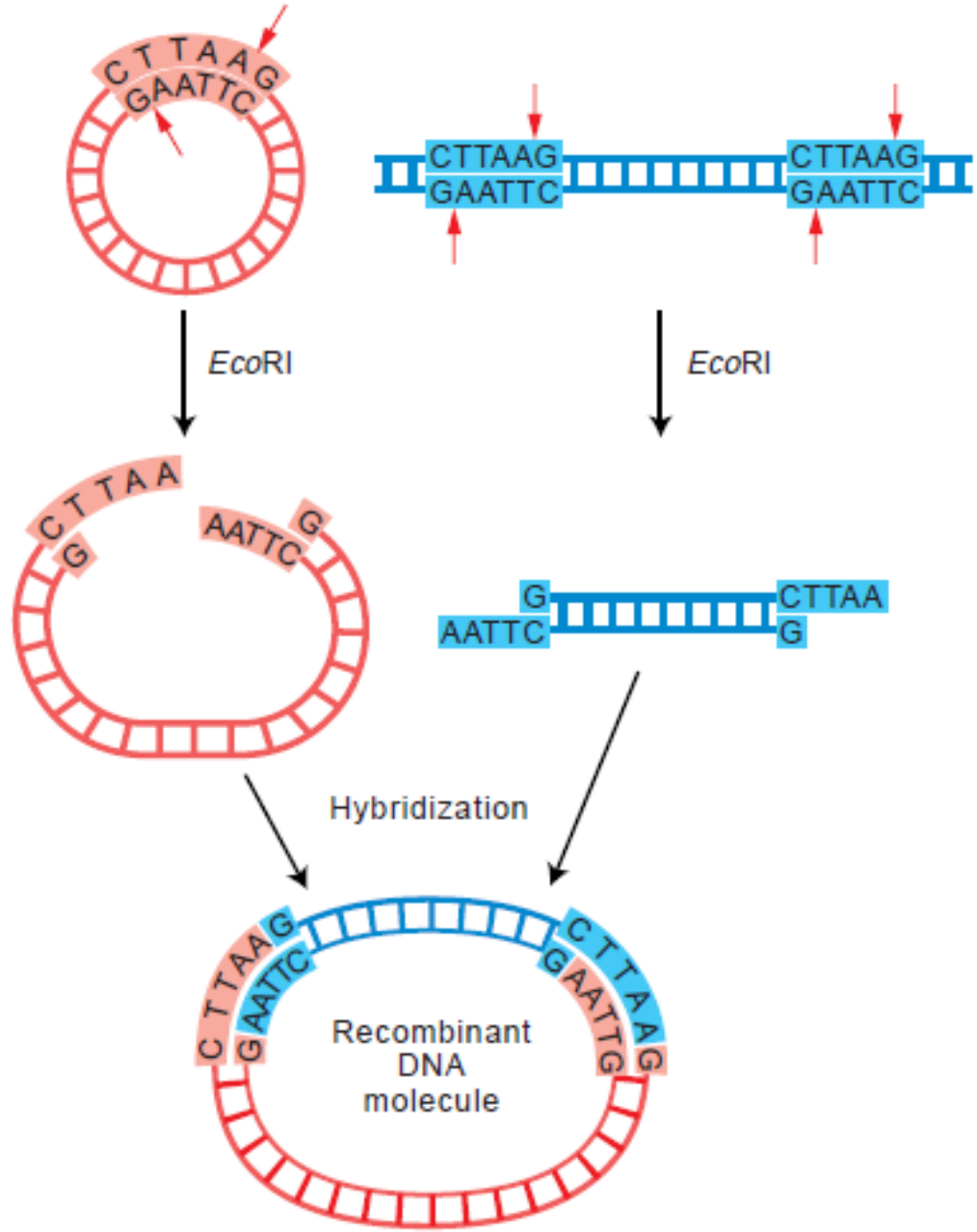


Figure 11-3 Formation of a recombinant DNA molecule.

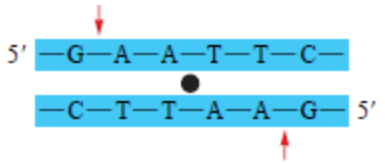
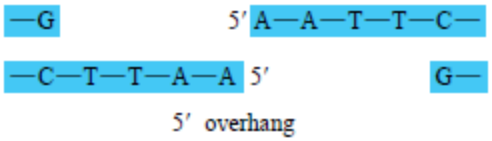
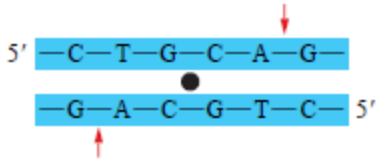
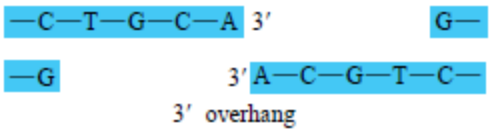
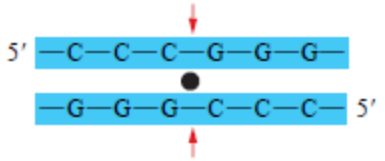
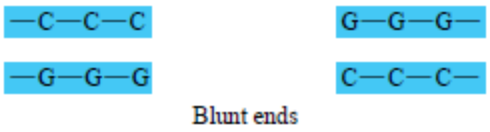
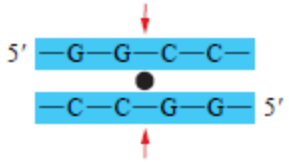
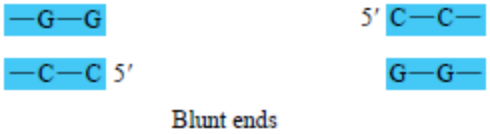
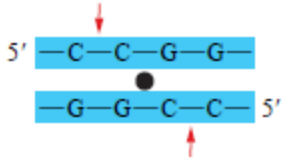
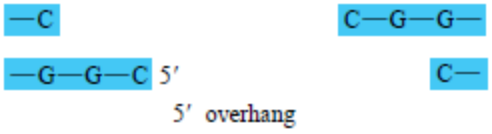
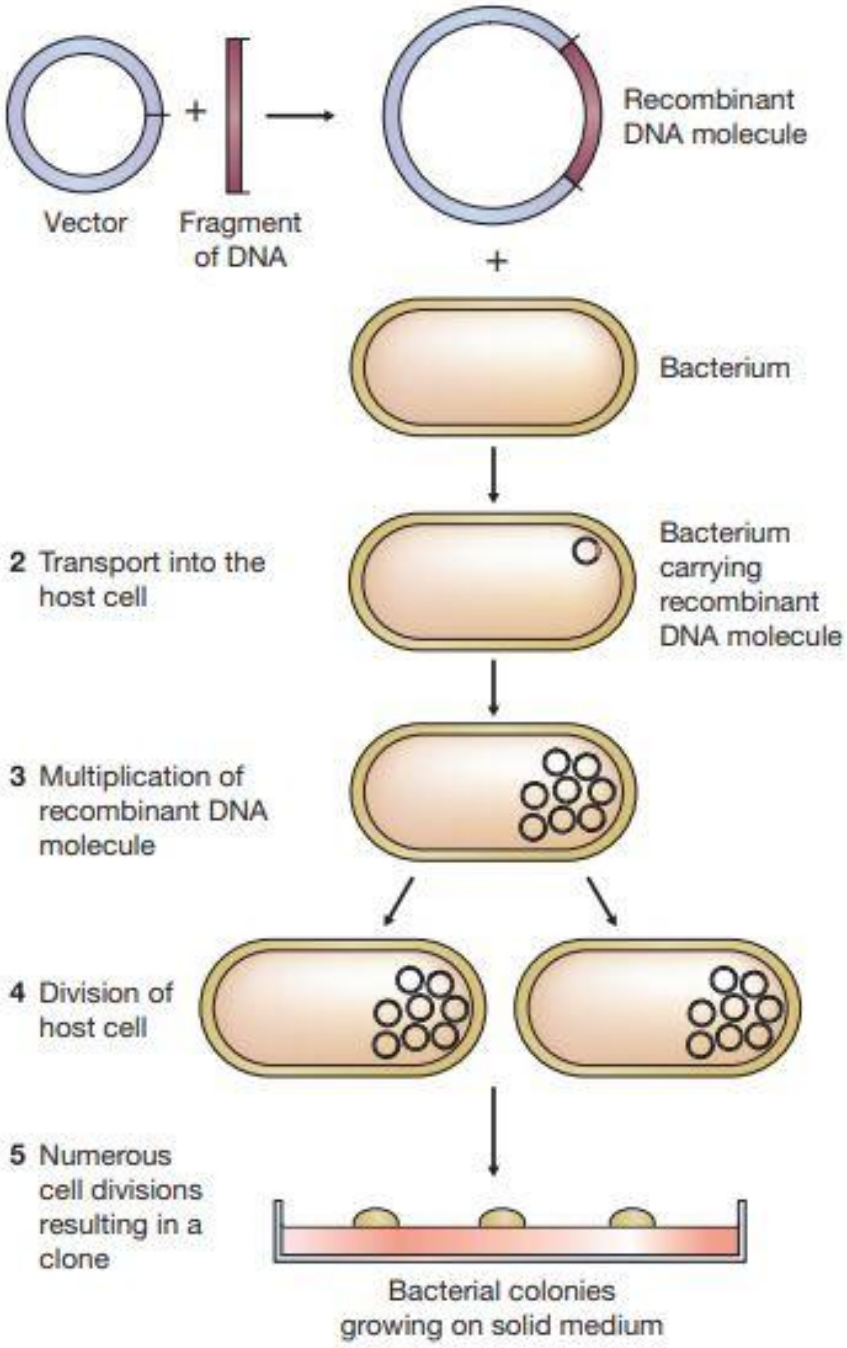
Enzyme	Source organism	Restriction recognition site in double-stranded DNA	Structure of the cleaved products
(a)			
EcoRI	*Escherichia coli*		
PstI	*Providencia stuartii*		
SmaI	*Serratia marcescens*		
(b)			
HaeIII	*Haemophilus aegyptius*		
HpaII	*Haemophilus parainfluenzae*		

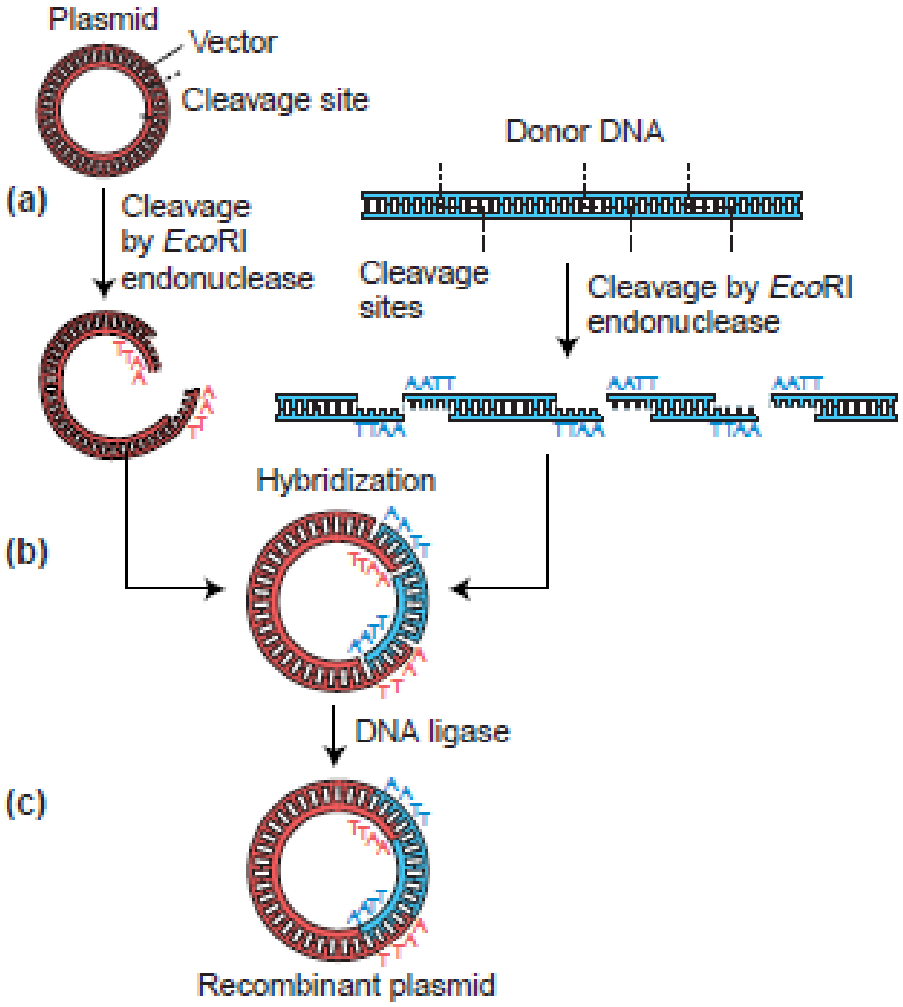
Figure 11-4 The specificity and results of restriction enzyme cleavage. The 5′ end of

Attaching donor and vector DNA

Most commonly, both donor and vector DNA are digested
by a restriction enzyme that produces complementary
sticky ends and are then mixed in a test tube to allow the sticky ends of vector and donor DNA to bind
to each other and form recombinant molecules.



Plasmids can also be categorized on the basis of their being maintained as multiple copies per cell *(**relaxed plasmids**) or as a limited number of copies per cell (**stringent plasmids**).*

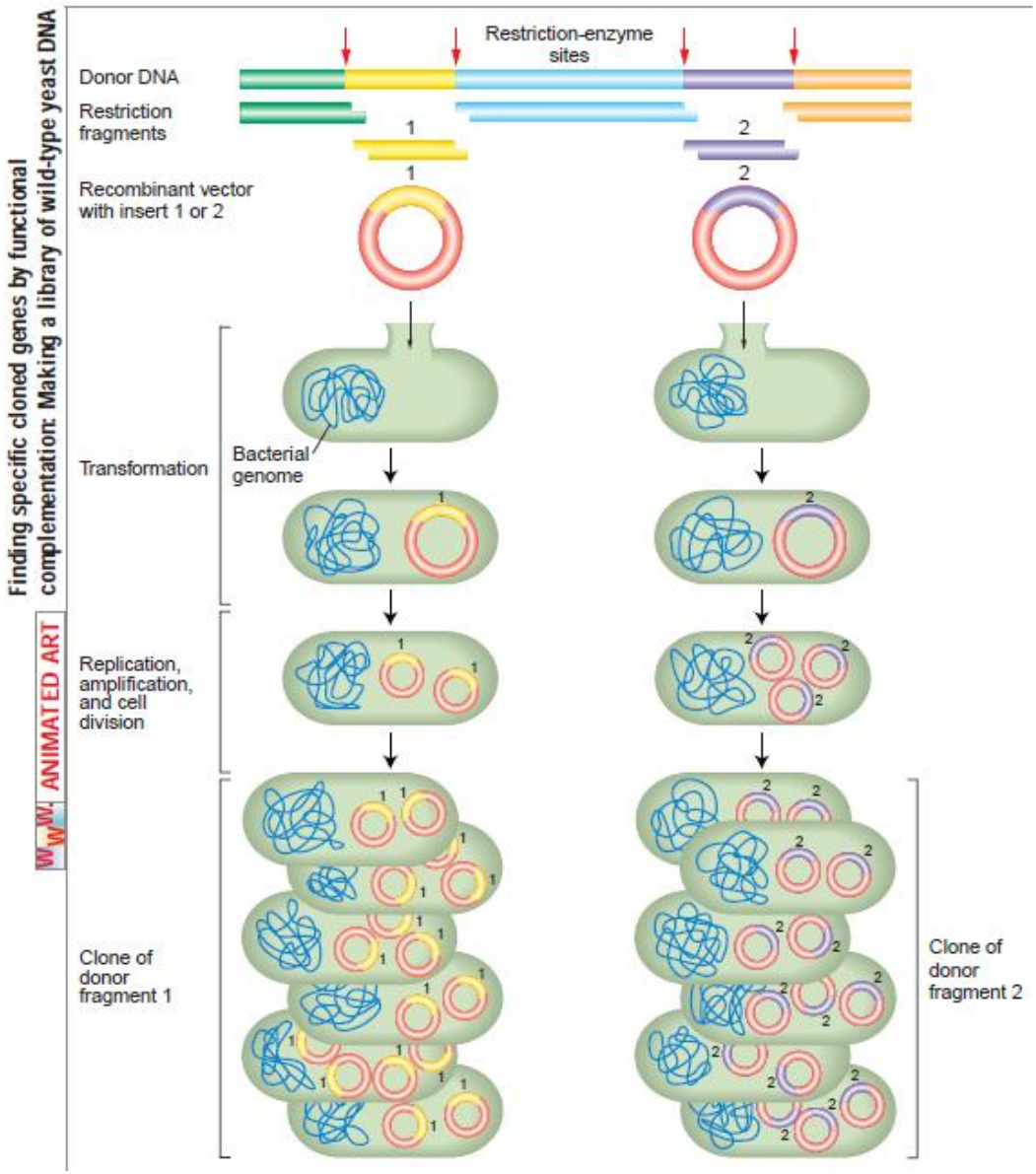


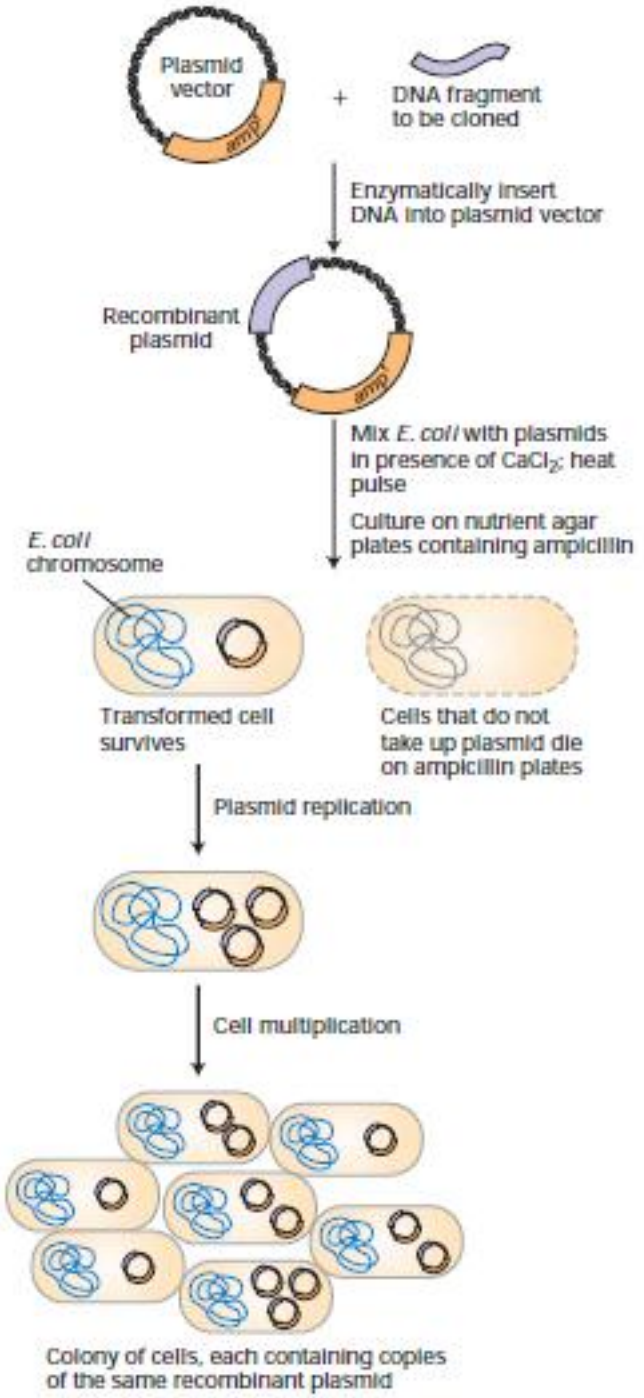
After a piece of foreign DNA is inserted into a vector, the resulting chimeric molecules have to be transformed into a suitable recipient.

Since the efficiency of transformation is so low, it is essential that the chimeras have some readily scorable phenotype.

Usually this results from some gene, e.g. antibiotic resistance, carried on the vector, but could also be produced by a gene carried on the inserted DNA.

How amplification works. Restriction-enzyme treatment of donor DNA and vector allows the insertion of single fragments into vectors. A single vector enters a bacterial host, where replication and cell division result in a large number of copies of the donor fragment.





Vector + DNA fragment

↓

Recombinant DNA

↓

Replication of recombinant DNA within host cells

↓

Isolation, sequencing, and manipulation of purified DNA fragment

- The judicious choice of markers on cloning vectors can greatly simplify the selection and analysis of recombinant clones.

- A key step in any cloning procedure is the selection of transformants carrying the desired recombinant plasmid.

- Because transformation efficiencies are so low it is essential to be able to select positively those rare cells that have been transformed.

- The commonest selectable markers are ones encoding resistance to antibiotics such as ampicillin (ApR), chloramphenicol (CmR), tetracycline (TcR), streptomycin (SmR), and kanamycin (KmR).

- Another type of positive selection is reversal of auxotrophy. For example, if the *hisB+gene is cloned in a vector then it is easy to select recombinants by transforming a hisB auxotroph and growing it in a medium lacking histidine.*

- **Reporter genes** are ones whose phenotype can be discerned by visual examination of colonies growing on a plate and/or ones that can be used to measure levels of gene expression. **In terms of analysis of recombinants, the most widely used reporter gene is the *lacZ gene encoding β-galactosidase.***

The pUC vectors also incorporate a DNA sequence that permits rapid visual detection of an insert.

The MCS is inserted into the *lacZ sequence,which encodes the promoter and the a-peptide of ~-galactosidase.*

The insertion of the MCS into the lacZ' fragment does not affect the abilityof the a-peptide to mediate complementation, but cloning DNA fragments into the MCS does.

Therefore,recombinants can be detected by blue/white screening on growth medium containing Xgal.