Selection of transformed cells

Selection of host cells/ organism containing vector sequences:

Selection of the transformed cells from the non-transformed population is done by using selectable marker genes that confers resistance to antibiotics. Hence, cells only having the vector with the resistance gene for the antibiotic would grow in the selection media containing the antibiotic (ampicillin, tetracycline etc.); while the non-transformed cells would die.

Screening clones having desired DNA inserts with the help of biological properties:

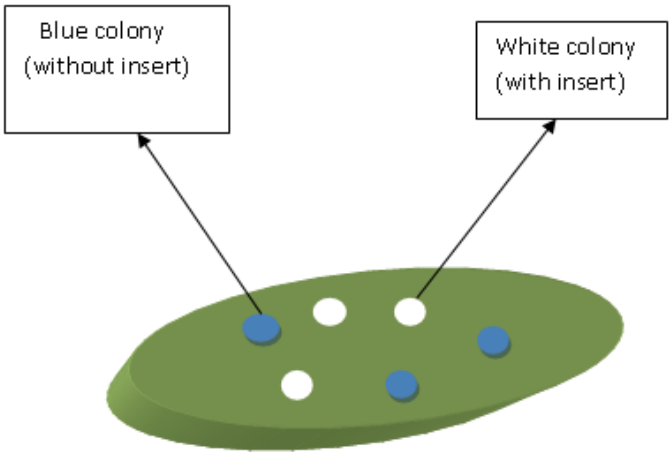
• After selection of colonies having the vector, the next step is to screen the colonies having the recombinant vector (vector containing foreign DNA insert).

• Bacterial cloning vectors (e.g. pUC19, pGEM vectors) use the blue-white screening system based on *lacZ* system to distinguish transgenic cells from those that contain the parental vector (i.e. vector DNA with no recombinant sequence inserted). The recombinant colonies are grown in presence of X-gal.

• In these vectors, foreign DNA is inserted into a sequence that encodes an essential part of beta-galactosidase (an enzyme which cleaves galactose). Its activity results in formation of a blue-colour colony on the culture medium.

Insertion of the foreign DNA into the beta-galactosidase coding sequence disrupts the function of the enzyme, and colonies containing recombinant plasmids give no blue colour (white).

• Using this colour phenotype, transgenic bacterial clones can be easily identified from those that do not contain recombinant DNA.



Screening for clones with desired DNA inserts

Insertional inactivation of antibiotic gene can also be used for the selection of recombinant cells.

• A vector is chosen where restriction sites are available for cloning within the antibiotic gene. Insertion of a foreign gene in the restriction site will lead to the loss of activity of the selectable marker (antibiotic) gene. For example-pBR322 have several restriction sites. *BamH1* cuts at a one position within genes that code for tetracycline resistance. Thus recombinant pBR322 carrying foreign DNA at *BamH1* site will not confer resistance to tetracycline, but are still resistant to ampicillin, which remains elsewhere.

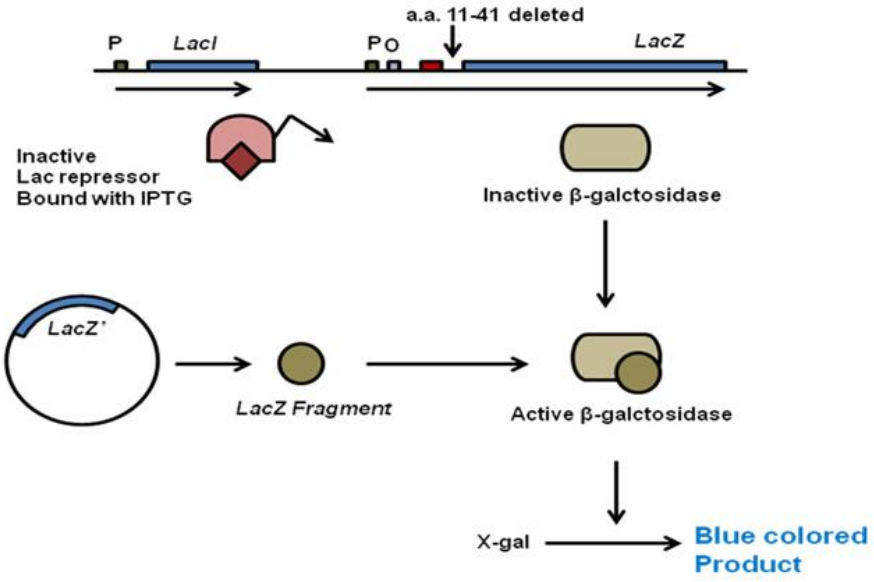
• These recombinant cells are selected by replica plating method. The transformed cells are first plated on ampicillin containing medium and after the selection of transformed from non-transformed; the colonies are replica plated on medium containing tetracycline for screening of recombinant clones. After incubation, the viable colonies carrying pBR322 without DNA insert will appear and the positions in plate where the non-viable recombinant clones are present can be easily identified. Using the original master plate, these recombinant clones are picked up and subcultured using the same procedure to obtain a pure recombinant clone.

Introduction- The different vectors are used in cloning techniques to produce recombinant DNA or clone. Transformation of recombinant DNA into the suitable host gives colonies and

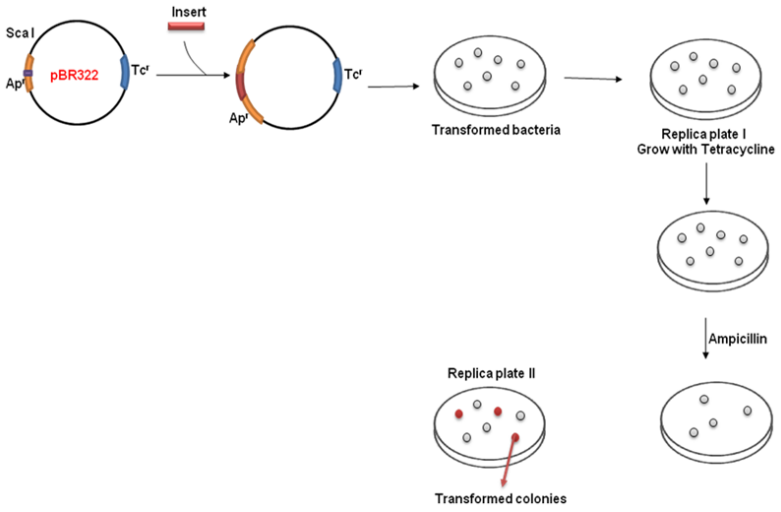
screening of the clone containing desired gene fragment is required for down-stream applications.

Chromogenic Substrate- The use of chromogenic substrate to detect a particular enzymatic activity is the basis to screen the desired clone. The most popular system to exploit this feature is **"Blue white screening"** where a coloroless substrate is processed to a colored compound. The colorless compound X-gal or 5-bromo-4-chloro-3-indolyl-β-D-galactoside used in this screening method is a substrate for β-galactosidase (Figure 22.1). The enzyme β-galactosidase is the product of lacZ gene of the lac operon. It is a tetrameric protein and an initial N-terminal region (11-41) of the protein is important for activity of the protein. In this system, host contains lacZ gene without the initial region where as vector contains α-peptide to complement the defect to form active enzyme. As a result, if a vector containing α-peptide will be transformed into the host containing remaining lacz, the two fragment will reconstitute to form active enzyme. In addition, the α-peptide region in vector contains multiple cloning site and as a result of insertion of gene fragment, consequently α-peptide will not be synthesized to give fully active β-galactosidase. The enzyme β-galactosidase oxidizes x-gal to form 5-bromo-4-chloro-indoxyl and galactose. The indoxyl derivative is oxidized in air to give a blue colored dibromo-dichloro derivative (Figure 22.2). Hence, blue colored colonies indicate the presence of an active enzyme or absence of insert where as colorless colonies indicate presence of an insert.

Insertional inactivation- In this approach a foreign DNA is cloned within the coding gene responsible for a phenotype. As a result of insertion, the gene product is not available to modulate the phenotype of the host. This approach is known as insertional inactivation, and it can be used with a suitable genetic system.



(1) Insertional Inactivation of antibiotic resistance gene- As discussed in an earlier lecture, bacterial plasmid PBR322 has two antibiotic resistance gene, Ap^r and Tc^r. If a gene fragment will be cloned in ScaI, it will disrupt the Ap^r gene. As a result, the clone will be ampicillin sensitive and Tc^r. where as the original plasmid will be Ap^r and Tc^r. To select the clone, first the transformed *e.coli* is plated on tetracycline containing media. Subsequently, a replica plate will be made on ampicillin containing media to identify the clone growing on tetracycline media but not on ampicillin media. This approach is schematically depicted in Figure 22.3.



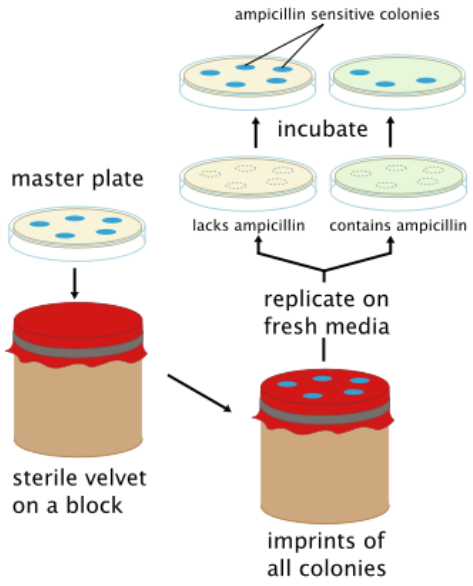


Figure: Insertional Inactivation of antibiotic resistance gene in pBR322 to screen recombinant clone.

(2) Insertional Inactivation of LacZ gene- LacZ is a part of lac operon and responsible for synthesis of β-galactosidase. As discussed earlier, X-gal system can be used to detect the insertional inactivation of LacZ gene to screen the cloned fragment. If the gene is inserted into the lacz, the clone will not be able to produce a functional β-galactosidase. Hence, blue colored colonies indicate the presence of an active enzyme or absence of insert where as colorless colonies indicate presence of an insert. This approach is schematically depicted in Figure 22.4.

TABLE 22.1: ANTIBIOTICS RESISTANCE GENE AND THEIR MODE OF MECHANISM.

Antibiotic	Gene product	Mechanism
Ampicillin	β-lactamase	Degradation of ampicillin
Kanamycin	Neomycin phosphotransferase II	Covalent modification of kanamycin
Tetracycline	Ribosomal protection proteins	Efflux of tetracycline outside of the bacteria
Chloramphenicol	Chloramphenicol acetyl transferase	Chloramphenicol to acetyl Chloramphenicol

Negative selection through replica plating to screen for ampicillin sensitive colonies

Replica plating is a microbiological technique in which one or more secondary Petri plates containing different solid (agar-based) selective growth media(lacking nutrients or containing chemical growth inhibitors such as antibiotics) are inoculated with the same colonies of microorganisms from a primary plate (or master dish), reproducing the original spatial pattern of colonies. The technique involves pressing a velveteen-covered disk, and then imprinting secondary plates with cells in colonies removed from the original plate by the material. Generally, large numbers of colonies (roughly 30-300) are replica plated due to the difficulty in streaking each out individually onto a separate plate.

A sterile velvet on a plastic block for replica plating

The purpose of replica plating is to be able to compare the master plate and any secondary plates, typically to screen for a desired phenotype. For example, when a colony that was present on the primary plate (or master dish), fails to appear on a secondary plate, it shows that the colony was sensitive to a substance on that particular secondary plate. Common screenable phenotypes include auxotrophy and antibiotic resistance.

Replica plating is especially useful for "negative selection". However, it is more correct to refer to "negative screening" instead of using the term 'selection'. For example, if one wanted to select colonies that were sensitive to ampicillin, the primary plate could be replica plated on a secondary Amp^+ agar plate. The sensitive colonies on the secondary plate would die but the colonies could still be deduced from the primary plate since the two have the same spatial patterns from ampicillin resistant colonies. The sensitive colonies could then be picked off from

the primary plate. Frequently the last plate will be non-selective. In the figure, a nonselective plate will be replica plated after the Amp+ plate to confirm that the absence of growth on the selective plate is due to the selection itself and not a problem with transferring cells. If one sees growth on the third (nonselective) plate but not the second one, the selective agent is responsible for the lack of growth. If the non-selective plate shows no growth, one cannot say whether viable cells were transferred at all, and no conclusions can be made about the presence or absence of growth on selective media. This is particularly useful if there are questions about the age or viability of the cells on the original plate.

By increasing the variety of secondary plates with different selective growth media, it is possible to rapidly screen a large number of individual isolated colonies for as many phenotypes as there are secondary plates.

The development of replica plating required two steps. The first step was to define the problem: a method of identifiably duplicating colonies. The second step was to devise a means to reliably implement the first step. Replica plating was first described by Esther Lederberg and Joshua Lederberg in 1952.