Detection of the Cloned Gene (Recombinants):

Cells with recombinant DNA (rDNA) are selected on the expression or non-expression of some traits like resistance to antibiotic chloramphenicol. Direct selection of recombinants is made due to encoding of these traits by vector or cloned DNA sequence.

Various methods for identification of recombinants are:

1. Transformants (host cells with foreign DNA) can be selected by:

(i) Host cells transformed with plasmid having ampicillin resistant gene are grown on medium having antibiotic ampicillin, only those cells bearing the above plasmid will be able to grow on it.

(ii) But one is not able to know that which colony bear recombinant plasmid and which bear relegated vector plasmid.

2. Insertional Inactivation Method:

It is based on basic principle that cloned DNA fragment disrupts the coding sequence of gene.

To identify recombinants, one of the important approaches is to use DNA probe. In a DNA molecule, the two complementary strands are held together by hydrogen bonds. If two similar DNA pieces are mixed together and hydrogen bonds broken (by heating) the strands will separate.

Upon lowering the temperature, the hydrogen bonds are formed again. Some of the resultant double-stranded DNA will be hybrids i.e., composed of one strand of one type and one strand of the other type. This concept of DNA hybridization has been exploited for utilizing the DNA molecules as probes (Fig. 11.22).

The transformed colonies are replica plated to a nitrocellulose filter and are lysed to release the DNA. This DNA is denatured (by raising the temperature) and fixed to the nitrocellulose so as to produce a DNA print corresponding exactly to the position of the colonies on the original plate.

The DNA print is then hybridized with the probe which has been previously radioactively labelled. After washing off unhybridized DNA, the position of the radioactive spots on the filter is indicated by autoradiography in order to identify the presence of the required DNA.

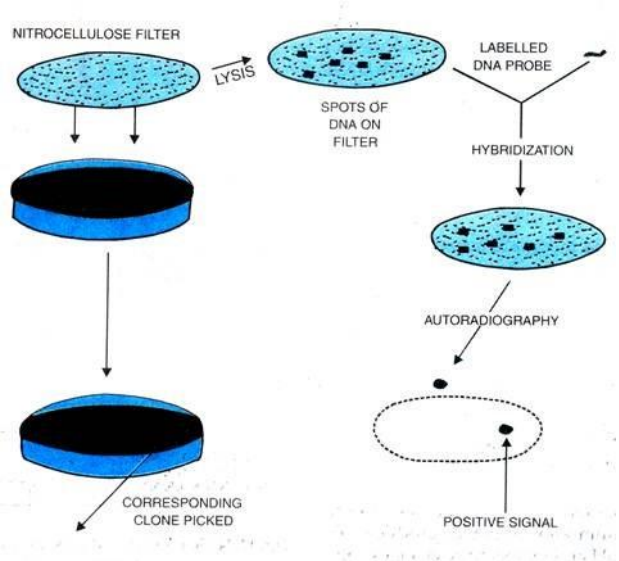

Fig. 11.22. Use of DNA probe to detect recombinant clones.

Direct Selection of Recombinant Clones:

A recombinant clone can be selected with the help of marker genes present in the vector as well as in the donor DNA. An example of direct selection can be cloning of threonine synthesis gene (thr^+) in a thr^-amp^s host through a vector containing an ampicillin-resistance gene (amp^r). In this case, the objective would be to select a clone containing the thr^+ gene.

Transformation with recombinant DNA will form some host cells which have the thr^+-gene. The non-trans-formant host cells fail to grow in a medium which contains ampicillin but no threonine. Also, those host cells which take up the vector DNA other than thr^+ gene fail to grow in such a medium, because threonine is absent, though such host cells possess the amp' gene carried by the vector.

Only those trans-formants are able to form colonies on such a selective medium which have taken up the thr^+ gene from the donor DNA fragment and amp' gene of the vector. The presence of these genes in a recombinant host enables it to synthesise threonine and to destroy ampicillin. As a result, such a trans-formant can form a clone of cells.

This is diagrammatically represented in Fig. 9.136:

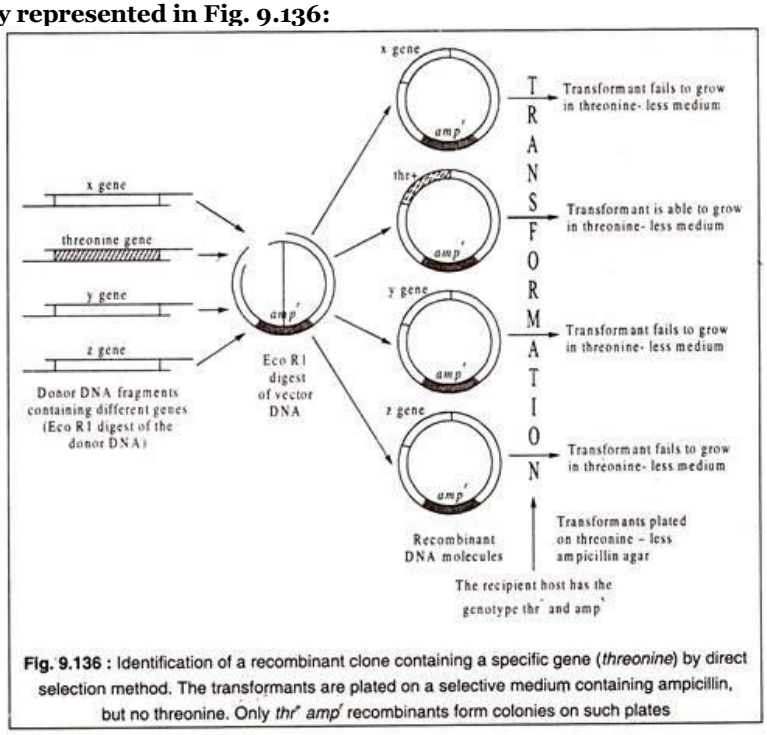

Fig. 9.136 : Identification of a recombinant clone containing a specific gene (*threonine*) by direct selection method. The transformants are plated on a selective medium containing ampicillin, but no threonine. Only thr^+ amp^r recombinants form colonies on such plates

(b) Colony Hybridization:

By this procedure, it is possible to detect the colonies of recombinants which have taken up a desired DNA segment containing a gene of choice. For detection of such colonies a sample of the DNA containing the particular gene which has been labelled with radioactivity must be available. Alternatively, a sample of radioactive m-RNA of that gene may also be used.

After the host cells are transformed by the recombinant vector DNA, they are plated on agar to allow formation of individual discrete colonies. Among these colonies, the majorities are produced by trans-formants without the desired gene, and only very few might contain it. An impression of the colonies is next transferred to a nitrocellulose filter.

They are then treated with alkali (sodium hydroxide) which causes lysis of the cells. At the same time, the DNA molecules liberated from the cells are denatured in situ to produce single-strands. At the next step, the radioactive DNA probe, denatured to produce single-strands or the radioactive m-RNA is added to the filter.

The single-stranded probe DNA or RNA is allowed to hybridize with the single stranded DNA of the colonies. After removing the unbound radioactive nucleic acid by washing, the filter is kept in contact with an X-ray film for a few weeks to detect the presence of colonies which have taken up the desired gene (auto-radiographic technique).

The procedure is schematically shown in Fig. 9.137:

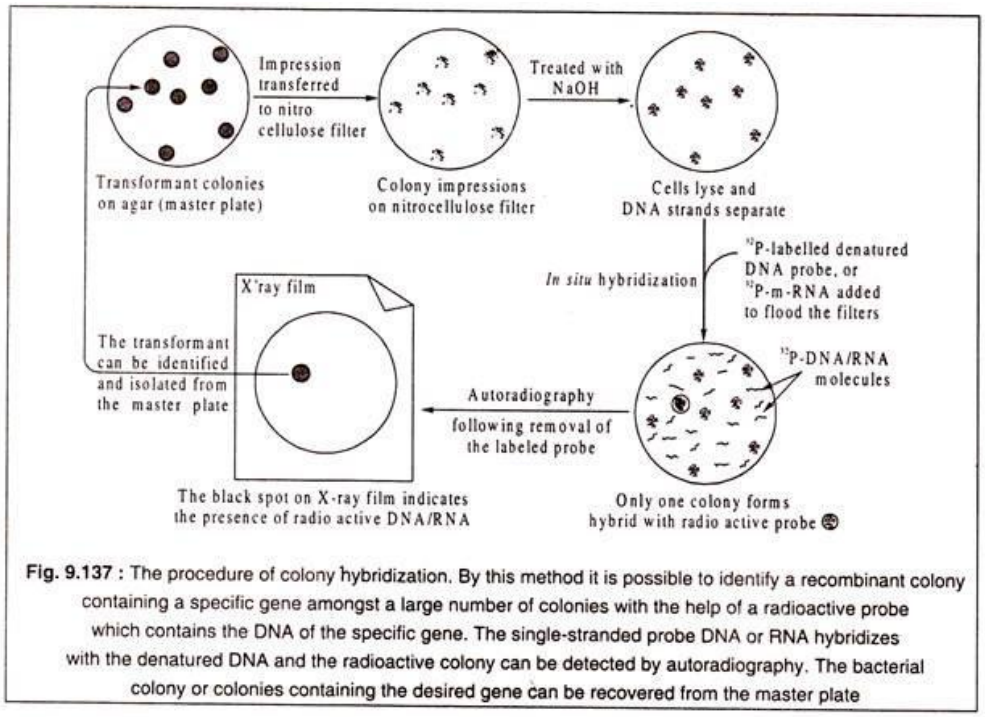

Fig. 9.137 : The procedure of colony hybridization. By this method it is possible to identify a recombinant colony containing a specific gene amongst a large number of colonies with the help of a radioactive probe which contains the DNA of the specific gene. The single-stranded probe DNA or RNA hybridizes with the denatured DNA and the radioactive colony can be detected by autoradiography. The bacterial colony or colonies containing the desired gene can be recovered from the master plate