FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

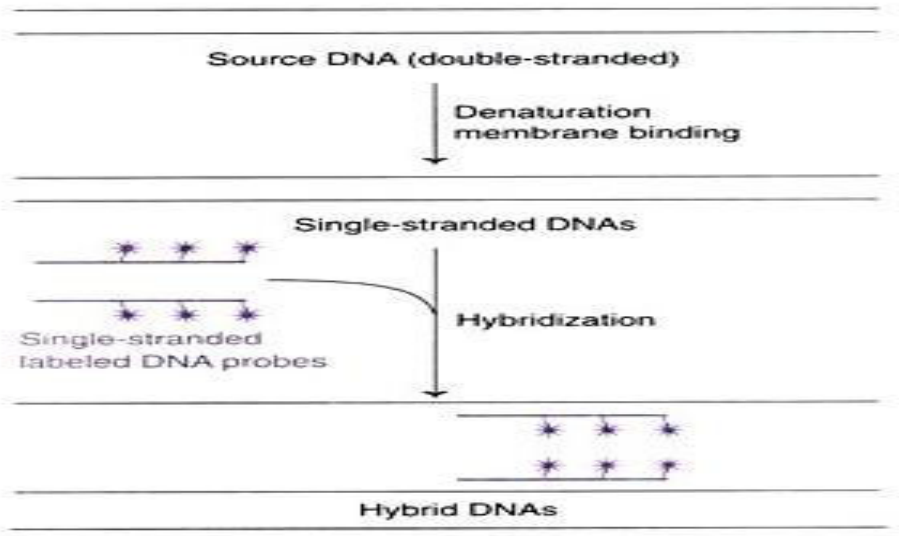
GENE LIBRARY SCREENING STRATEGIES

There are five main screening methods;

1. Screening by DNA Hybridization
2. Screening by Colony Hybridization
3. Screening by PCR
4. Screening by Immunological Assay
5. Screening by Protein Function.

1. DNA hybridization Method:

- The target sequence in a DNA can be determined with a DNA probe
- The double-stranded DNA of interest is converted into single strands by heat or alkali (denaturation).
- The two DNA strands are kept apart by binding to solid matrix such as nitrocellulose or nylon membrane.
- The single strands of DNA probe (100-1,000 bp) labeled with radioisotope are added.
- Hybridization occurs between the complementary nucleotide sequences of the target DNA and the probe.
- The hybridized DNA can be detected by autoradiography.



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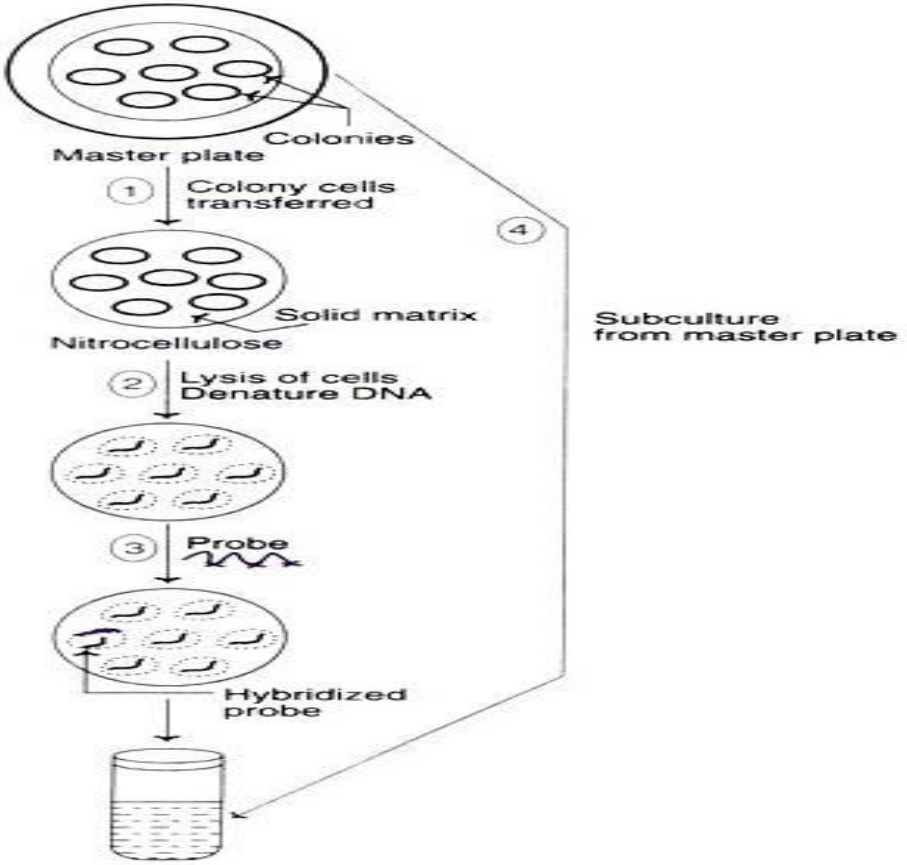
Screening by Colony Hybridization:

- The DNA sequence in the transformed colonies can be detected by hybridization with radioactive DNA probes (sometimes labeled RNA probes can also be used).
- Colony hybridization technique is also referred to as replica plating by some authors.



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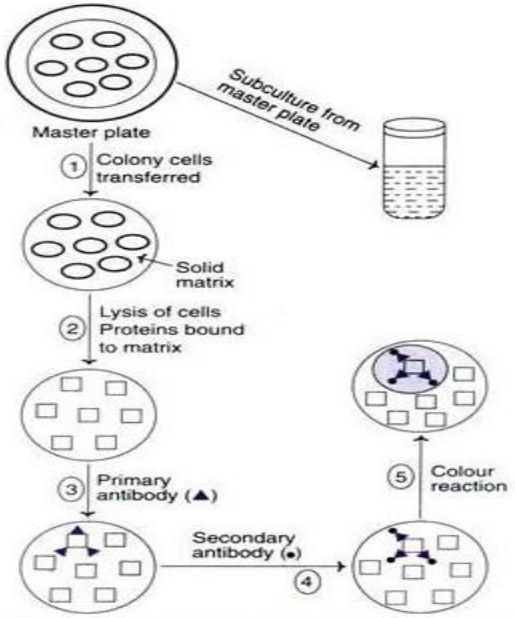
Screening by PCR:

➢Polymerase chain reaction (PCR) is as good as hybridization technique for screening DNA libraries.
➢But adequate information (on the franking sequences of target DNA) must be available to prepare primers for this method.
➢The colonies are maintained in multiwall plates, each well is screened by PCR and the positive wells are identified.

Screening by Immunological Assay:

➢Immunological techniques can be used for the detection of a protein or a polypeptide, synthesized by a gene (through transcription followed by translation).
➢The procedure adopted for immunological assay and hybridization technique (described already) are quite comparable. Screening procedure by immunological assay is depicted in and briefly described hereunder.



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Screening by Protein Function:

- If the target DNA of the gene library is capable of synthesizing a protein (particularly an enzyme) that is not normally produced by the host cell, the protein activity can be used for screening.

- A specific substrate is used, and its utilization by a colony of cells indicates the presence of an enzyme that acts on the substrate.

- The genes coding for enzymes α-amylase and β-glucosidase can be identified by this technique.