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FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

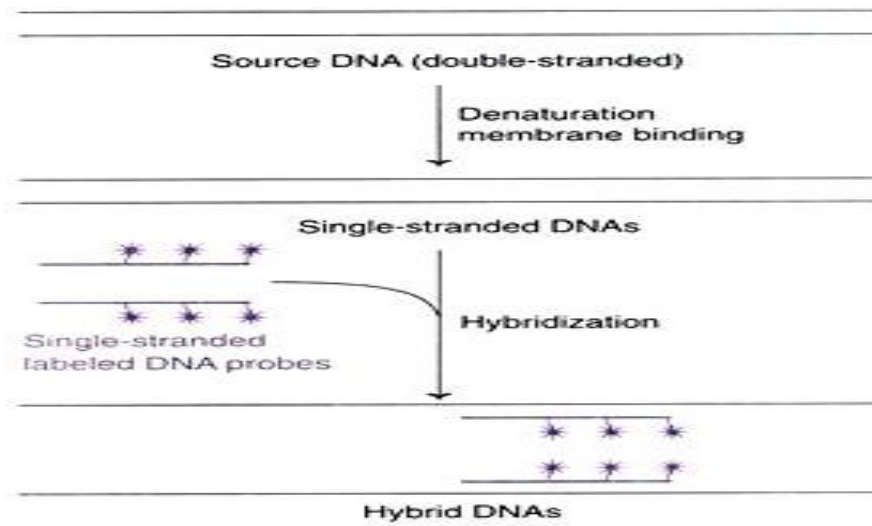
GENE LIBRARY SCREENING STRATEGIES

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:

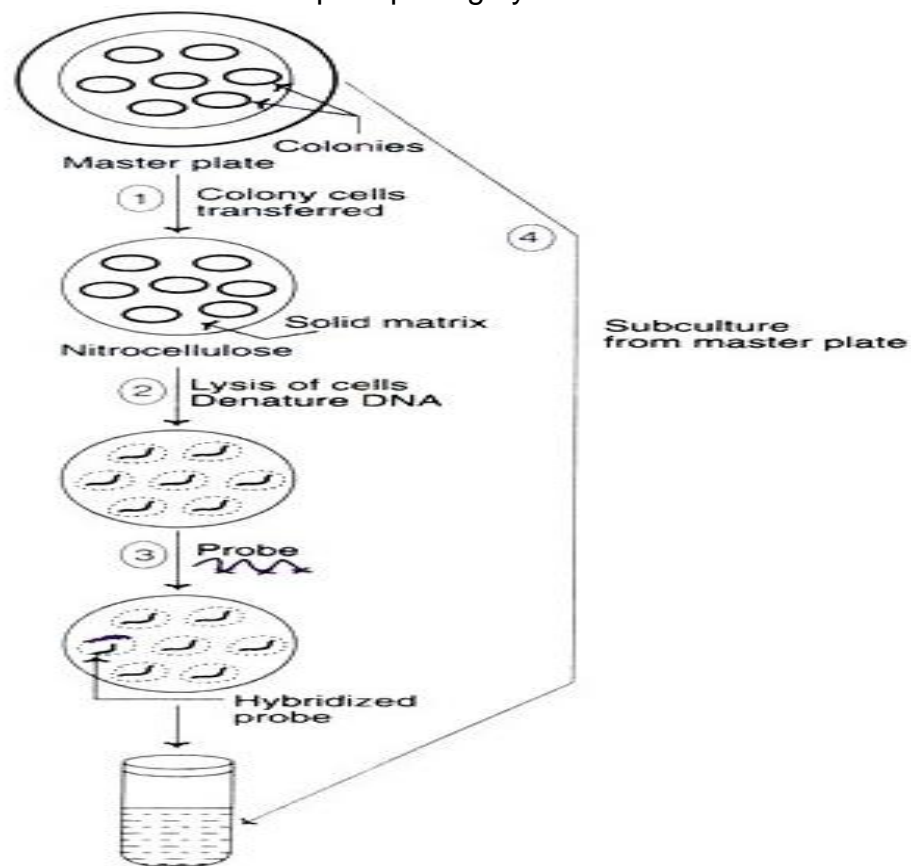
- ❖ The target sequence in a DNA can be determined with a DNA probe
- ❖ The double-stranded DNA of interest is converted into single strands by heat or alkali (denaturation).
- ❖ The two DNA strands are kept apart by binding to solid matrix such as nitrocellulose or nylon membrane.
- ❖ The single strands 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.



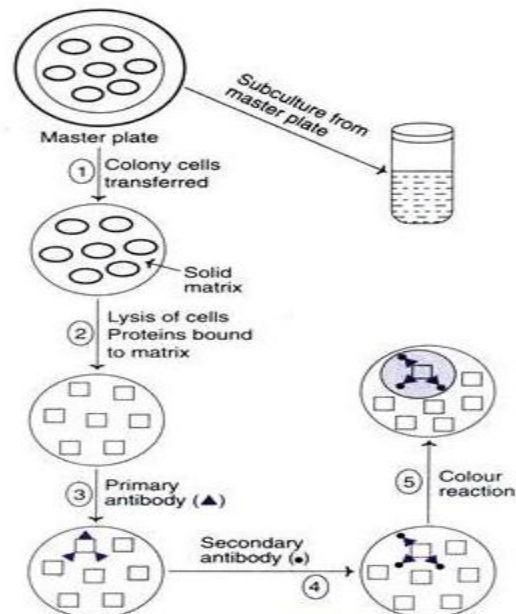
GENE LIBRARY SCREENING STRATEGIES

Screening by PCR:

- Polymerase chain reaction (PCR) is as good as hybridization technique for screening DNA libraries.
- But adequate information (on the flanking sequences of target DNA) must be available to prepare primers for this method.
- The colonies are maintained in multiwell 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.



GENE LIBRARY SCREENING STRATEGIES

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.

