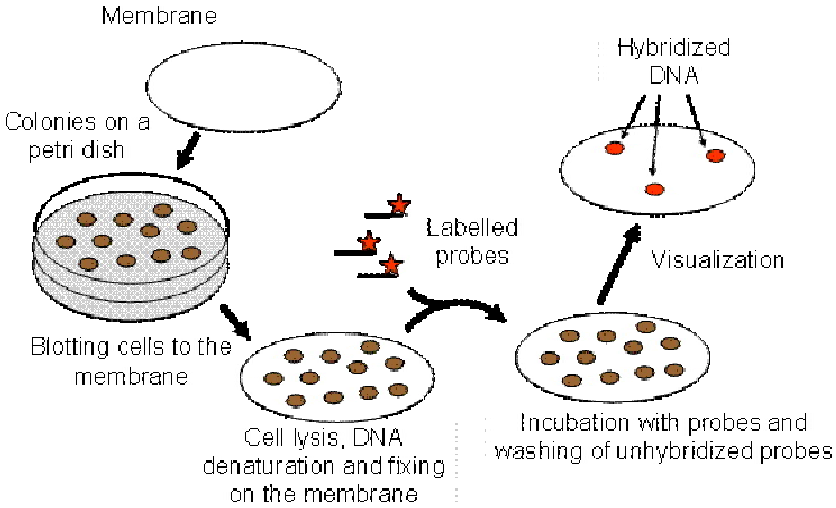
University of Duhok
College of Science
Department of Biology
4th Year Class
Biotechnology

Lecture8. Clonal Screening and Selection methods

Lecture outlines:

- Introduction
- A classic Screening Methods
- Negative selection system (Blue-white screening)
- Positive selection system (lethal gene)
- Screening based on detecting a DNA sequence
  - Screening by hybridization
  - Screening by PCR
- Screening methods based on gene expression
  - Immunological screening



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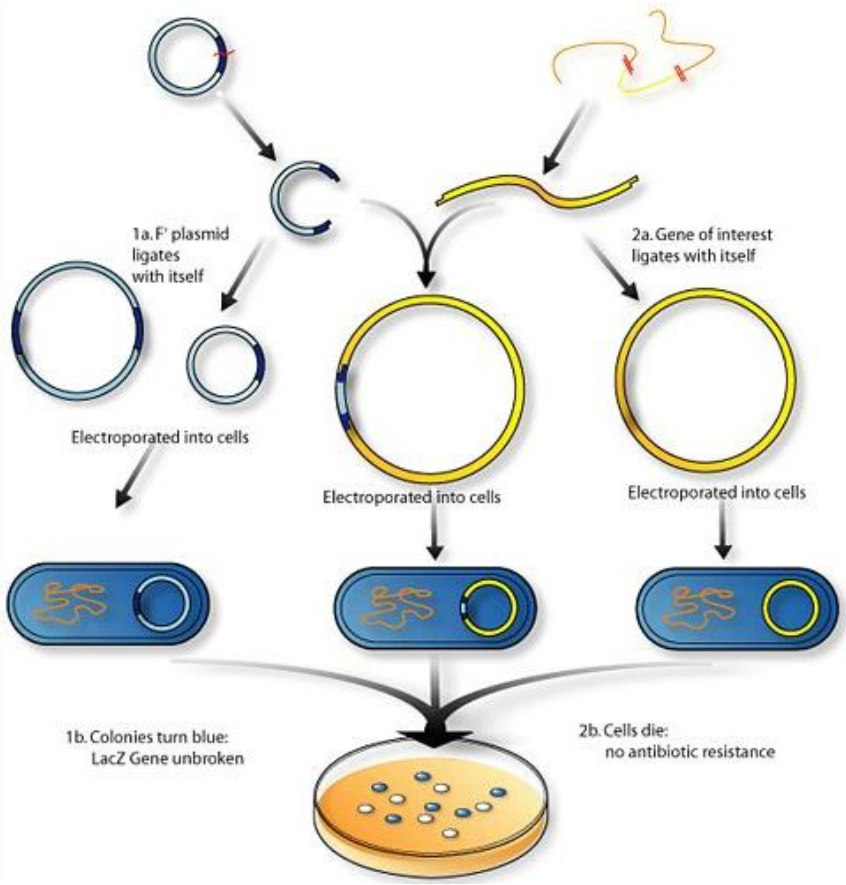
Introduction

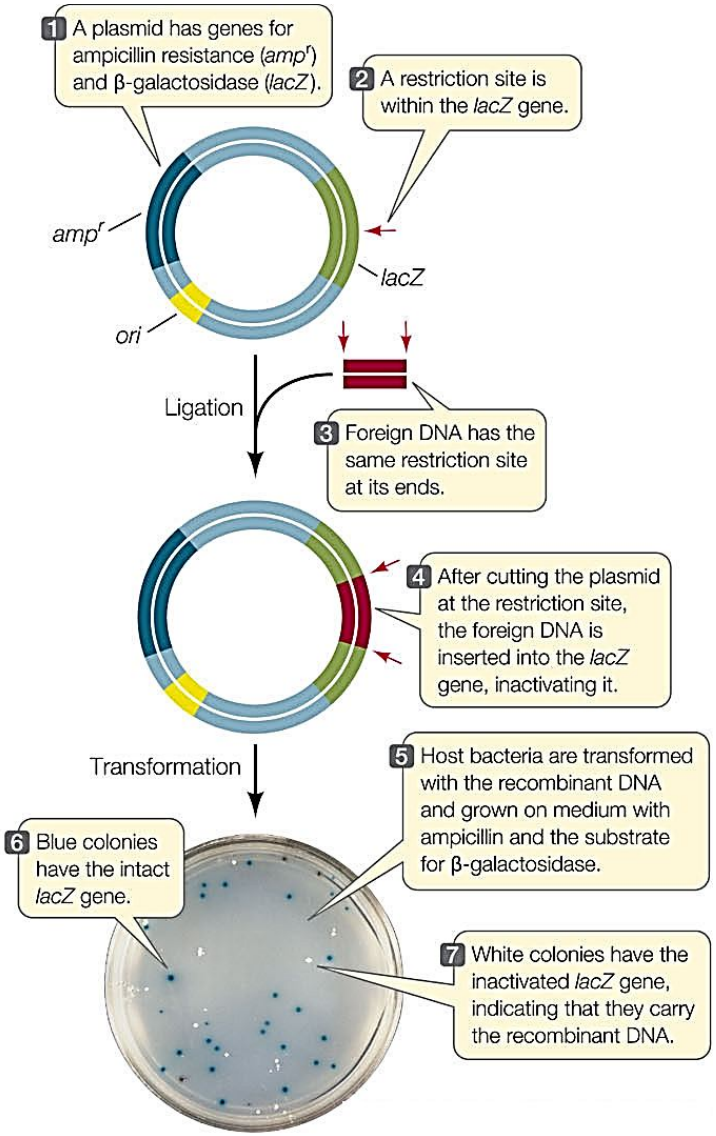
- After the introduction of recombinant DNA into the host cells, it is essential to identify those cells which received rDNA molecule - screening (or) selection.
- The vector or foreign DNA present in the recombinant cells expresses certain characters or traits, while non-recombinants do not express the traits.
- Following the methods for screening or selection of recombinant clones.



Classic Screening Methods

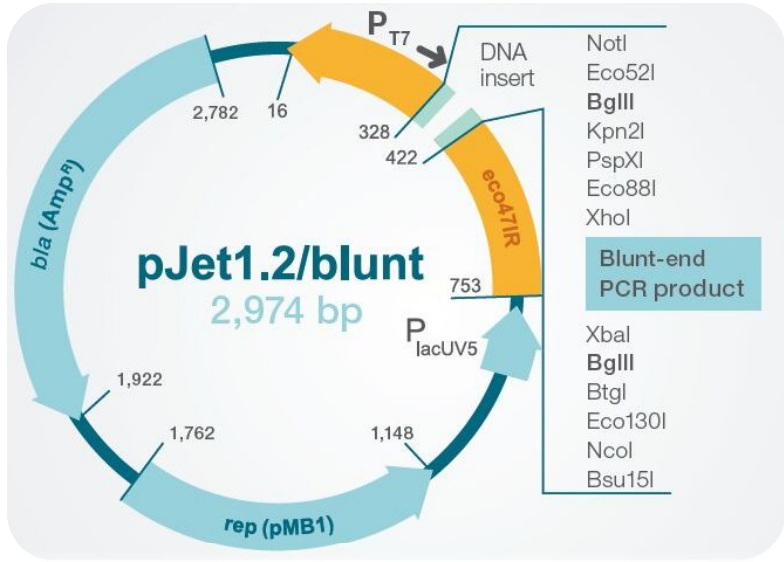
A. Negative selection system (Blue-white screening)

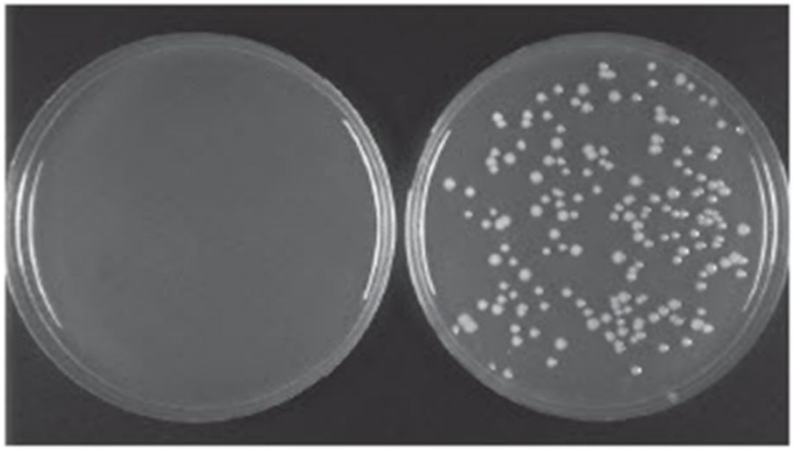
- Blue-white screening is a negative selection system using bacterial lactose metabolism as an indicator of successful cloning.
- This technique relies on the *lacZ* gene which encodes the enzyme *β*-galactosidase that can hydrolyse X-gal (a lactose analog) present in the media into an insoluble blue pigment and the bacterial colony becomes blue in color.
- When your DNA of interest is inserted into the plasmid in transformed *E. coli*, the gene is disrupted, the enzyme *β*-galactosidase won't be produced, X-gal won't be hydrolyzed, and the bacterial colony becomes white in color.



B. Positive selection system (lethal gene)

- An effective method to simplify screening is to use a positive selection system.
- Positive selection vectors conditionally express a lethal gene (e.g. pJET1.2/blunt cloning vector), such as a restriction enzyme that digests the genomic DNA of the bacterial host.
- When your DNA is successfully inserted in the plasmid, the lethal gene is disrupted and can no longer be expressed.
- As a result, only cells with recombinant plasmids are able to grow.
- This positive selection drastically accelerates the process of colony screening and eliminates additional costs required for blue/white selection.





Screening methods based on detecting a DNA sequence

1. Screening by hybridization

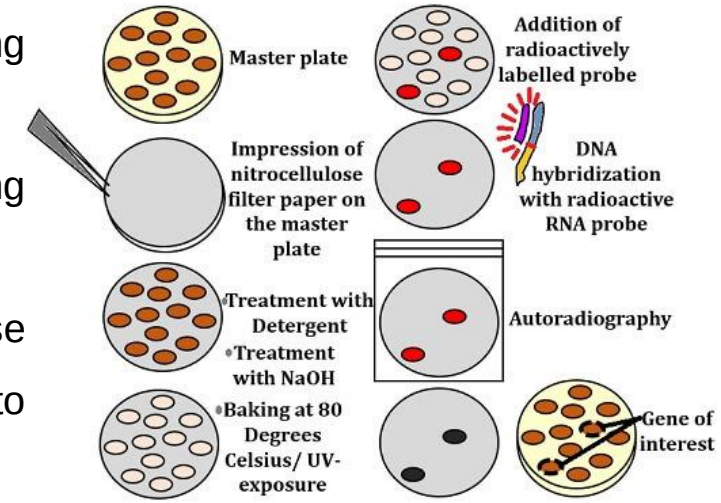
- Nucleic acid hybridization is the most commonly used method of clonal screening.
- First developed by Grunstein and Hogness in1975 to detect DNA sequences in transformed colonies using radioactive RNA probes.
- It relies on the fact that a single-stranded DNA molecule, used as a probe can hybridize to its complementary sequence and identify the specific sequences.
- The commonly used methods of hybridization are:

  A. Colony hybridization.

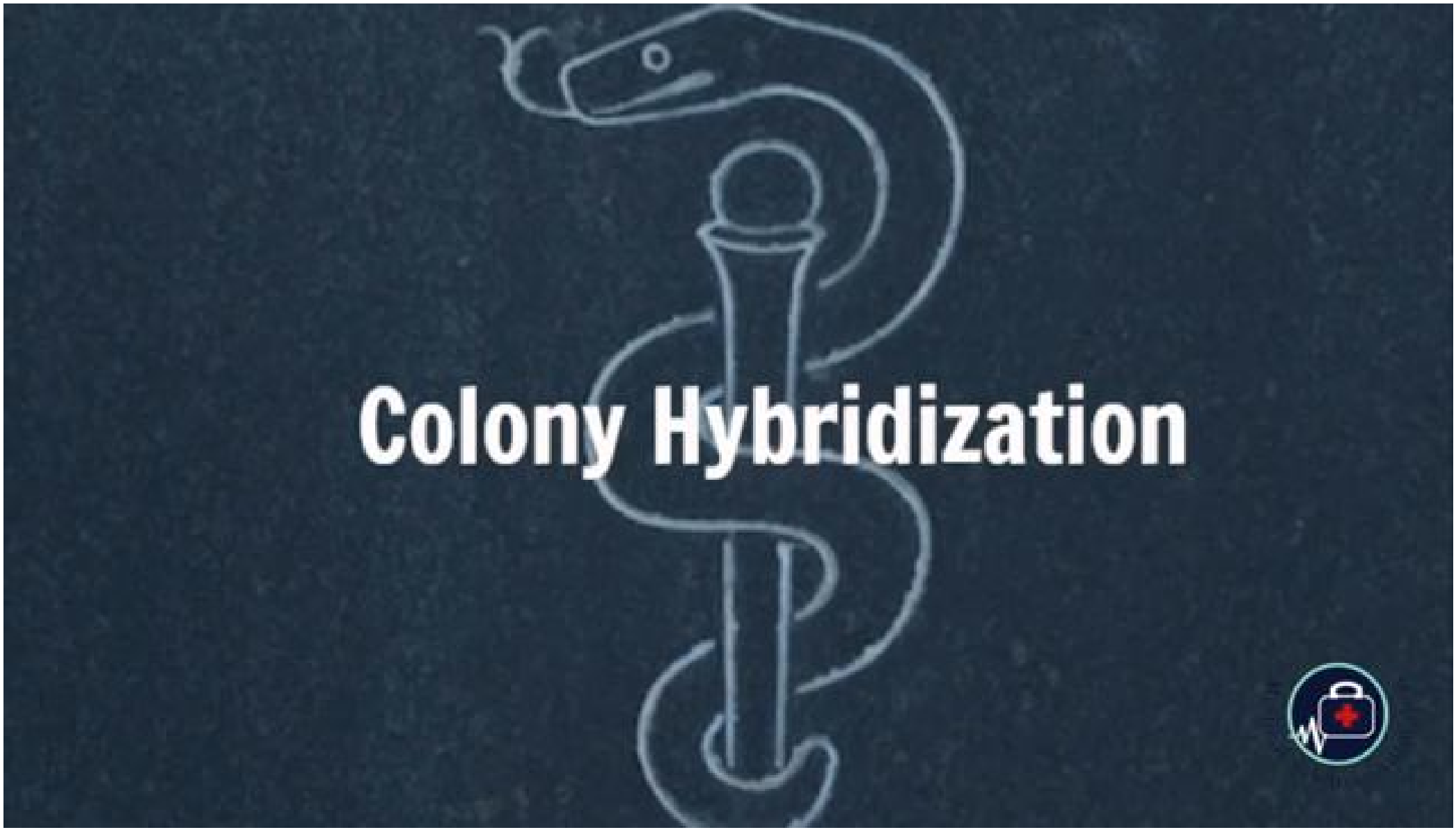
  B. Plaque hybridization.

A. Colony hybridization

- Colony hybridization, also known as replica plating, allows the screening of colonies plated at high density using radioactive DNA probes.
- It is a rapid method of isolating a colony containing a plasmid harboring a particular sequence or a gene from a mixed population.
- The colonies to be screened are first replica-plated on to a nitrocellulose filter disc that has been placed on the surface of an agar plate prior to inoculation.

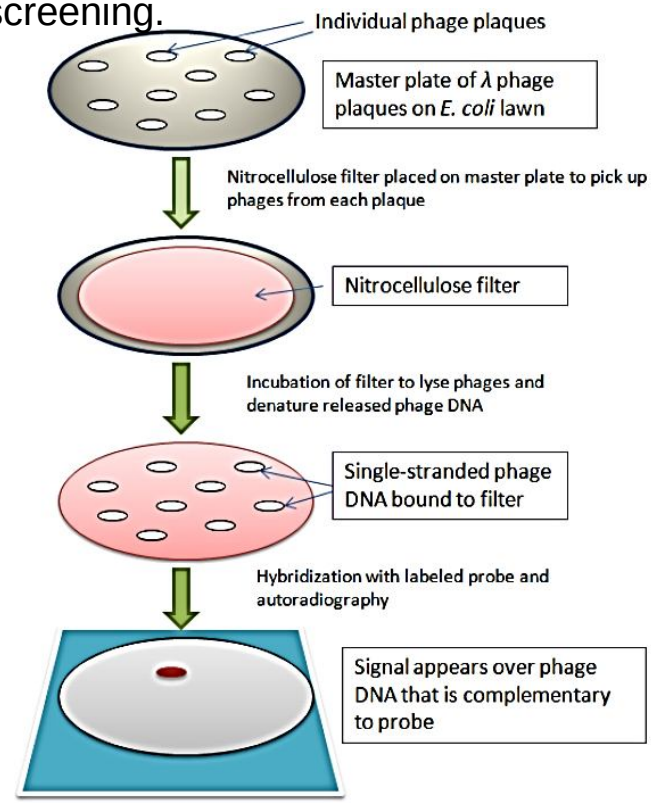


- The filter bearing the colonies is removed and treated with alkali (NaOH) so that the bacterial colonies are lysed and the DNA they contain is denatured.
- The filter is then treated with proteinase K to remove protein and leave denatured DNA bound to the nitrocellulose. The DNA is fixed firmly by baking the filter at 80°C.
- A labeled probe is hybridized to this DNA which is monitored by autoradiography. A colony whose DNA print gives a positive auto radiographic result on X-ray film can then be picked from the reference plate.
- This method can be used to screen plasmid or cosmid based clones.


Colony Hybridization

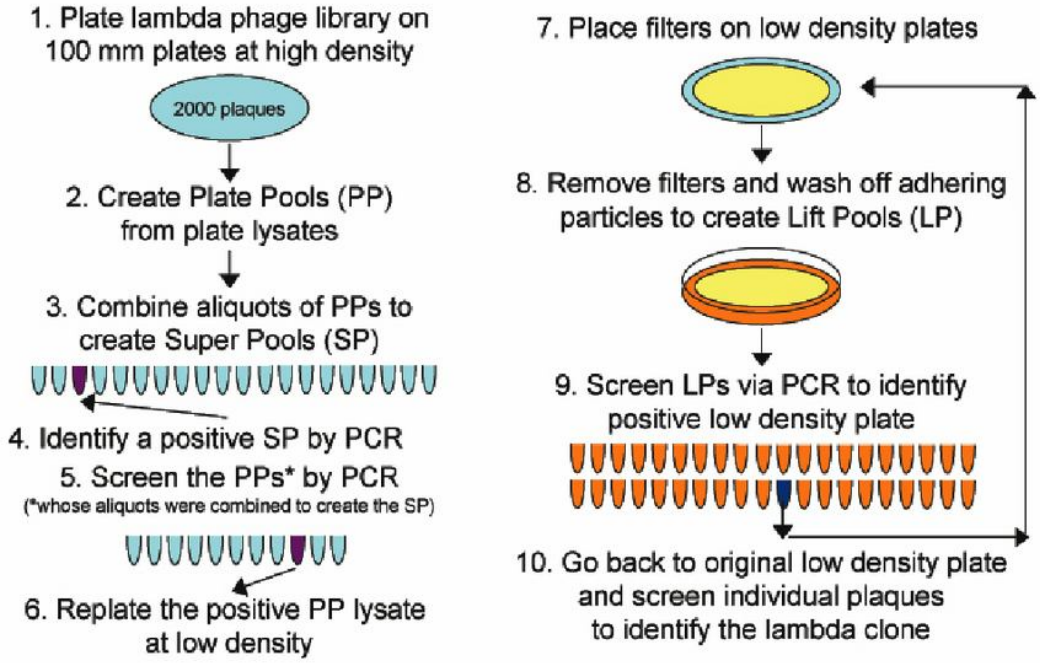
B. Plaque hybridization

- Plaque hybridization, also known as *Plaque lift*, was developed by Benton and Davis in 1977 and employs a filter lift method applied to phage plaques.
- This procedure is successfully applied to the isolation of recombinant phage by nucleic acid hybridization and probably is the most widely applied method of recombinant clone screening.
- The method of screening library by plaque hybridization is described below:
  - The nitrocellulose filter is applied to the upper surface of agar plates, making a direct contact between plaques and filter.
  - The plaques contain phage particles, as well as a considerable amount of unpackaged recombinant DNA which bind to the filter.
  - The DNA is denatured, fixed to the filter, hybridized with radioactive probes and assayed by autoradiography.



2. Screening by PCR

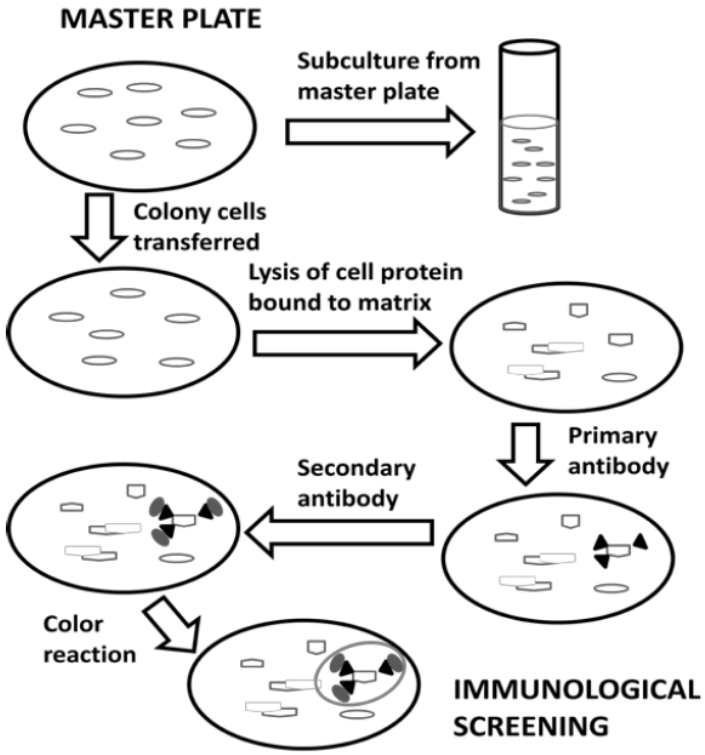
- PCR screening is employed for the identification of rare DNA sequences in complex mixtures of molecular clones by increasing the abundance of a particular sequence.
- It is possible to identify any clone by PCR only if there is available information about its sequence to design suitable primers.



Screening methods based on gene expression

Immunological screening

- In this technique, the cells are grown as colonies on master plates and transferred to a solid matrix.
- These colonies are subjected to lysis releasing the proteins which bind to the matrix.
- These proteins are treated with a primary antibody which specifically binds to the protein (acts as antigen), encoded by the target DNA. The unbound antibodies are removed by washing.
- A secondary antibody is added which specifically binds to the primary antibody removing the unbound antibodies by washing.



- The secondary antibody carries an enzyme label (e.g., horseradish peroxidase or alkaline phosphatase) bound to it which converts colorless substrate to colored product. The colonies with positive results (i.e. colored spots) are identified and sub-cultured from the master plate.


TIME FOR QUESTIONS
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