Recombinant Cell Selection

- **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.**

Direct Selection

- **The simplest example of Direct Selection occurs when the desired gene specifies resistance to an antibiotic. As an example, consider an experiment to clone the gene for kanamcycin resistance from plasmid R6-5.**

- **The organism which is transformed with the desired gene will clearly and directly exhibit resistance to the antibiotic, in this case the desired gene itself serves as a marker.**

Indirect Selection

INSERTIONAL INACTIVATION

- **The desired gene is inserted in the vector carrying atleast two marker genes.**

Method 1 for screening recombinants

- **For example the vector may contain two genes resistant to two different antibiotics**

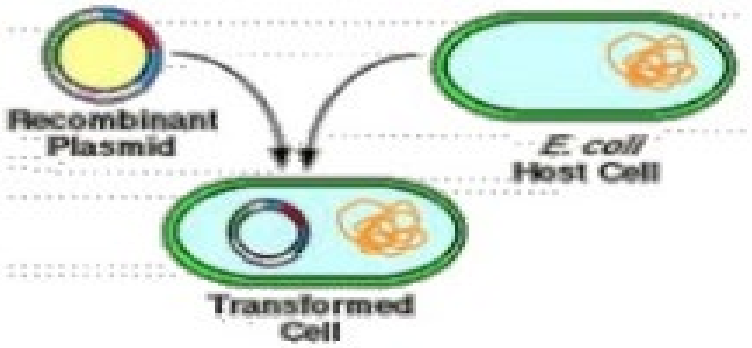
- **The desired gene and one of the antibiotic resistance genes is digested with the same restrict enzyme and ligated**

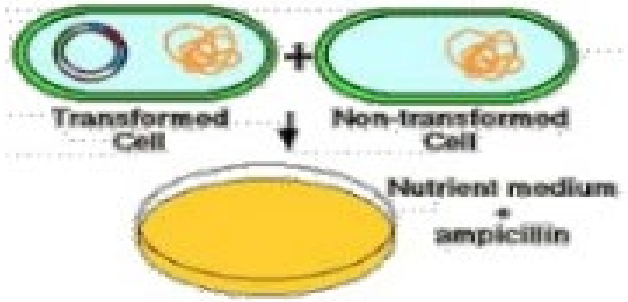
- **The vector when mixed with the desired genes may religate to its own cohesive ends giving back the vector carrying two resistance genes**
- **The vector may ligate with the desired gene in the marker gene to give rise to the recombinant vector**
- **The cells transformed with the vectors will grow in the presence of the antibiotics**
- **The cells transformed with the recombinant vector will be able to grow in the presence of one antibiotic but will be unable to grow in the presence of the chosen marker gene, thus allowing selection using replica plating method**

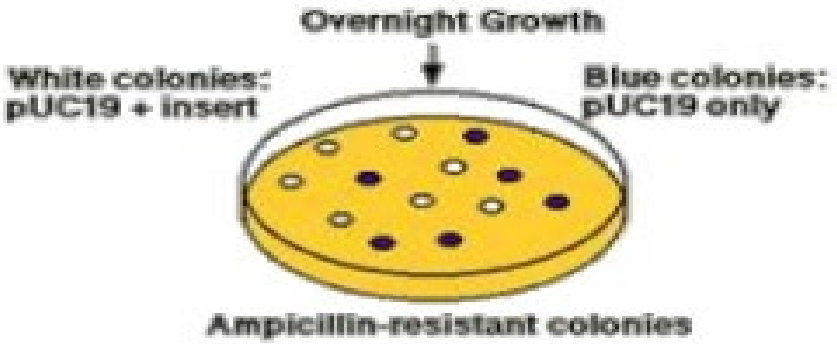
Method 2 for screening recombinants

- In this method a reporter gene lacZ is inserted in the vector (encodes β –balactosidase)
- If a foreign gene is inserted into lacZ, this gene will be inactivated; therefore no blue colour will develop.
- The host cells containing recombinants will turn blue in colour.
- On the basis of colony colour the recombinants can be selected.

Selection of recombinant cells







Method 3 for screening recombinants

Colony blot hybridization is applied to DNA or RNA released from blotted microbial colonies

- Colony blot hybridization is applied to DNA or RNA released from blotted microbial colonies

- The microbial colonies are transferred (blotted) to a membrane
- The RNA or DNA (after denaturation) is fixed to the filter and hybridized with a labeled probe.

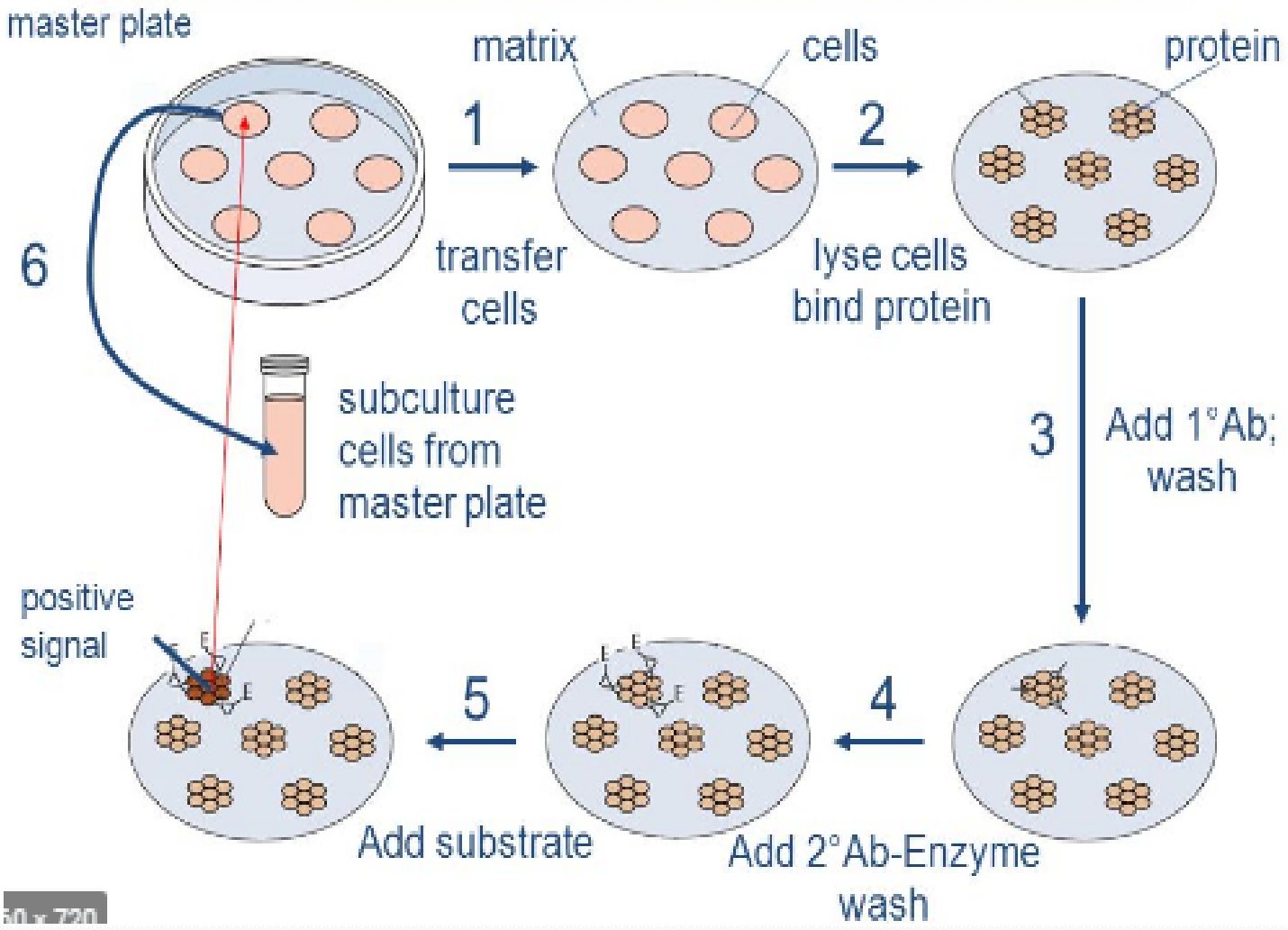
- **Blocking reagent may be added prior to the probe to prevent unspecific binding**
- **Excess probe is washed away and the membrane is visualized by UV or autoradiography**
- **Colony blot hybridization can be used for screening clones or bacterial isolates.**

Immunological Screening

- **Antibodies are used to identify the colonies developed that synthesize antigens encoded by the foreign DNA present in plasmids of the bacterial clones**
- **Replica plating**
- **Lysis of cells using chloroform vapour/high temp**
- **Making gentle contact with a solid support (cellulose filter paper)**

- **Detection of antigen antibody complex by incubating the cellulose filter paper with a radio labeled second antibody**
- **The antibodies which do not react are washed off**
- **The determination of antigen antibody complex is determined by passing through x ray**

Immunological Screen




Thank
You