

Hello students.

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I am here to present a module

from the programme Bachelor of

Science in the subject of Botany,

from the course Molecular Biology

and Genetic Engineering. Title

of the unit is methods of gene

transfer and the name of the module

is selection of transformants.

In this module you will be able to

learn about direct selection method,

Insertional inactivation selection method,

Blue white screening method,

Colony hybridization technique

and immunological test.

At the end of this module you will be

able to describe the direct selection method,

explain the insertional
inactivation selection method,
describe blue white screening method,
describe colony hybridization technique
and explain immunological
test selection method.

After introducing recombinant
DNA into the host cell,
it is important to identify those
cells which receive recombinant DNA.

This process is called as
selection or screening.

It depends on expression or
non expression of characters.

The vector of foreign DNA expresses
certain characters or traits.

And the non-recombinants do not
express the traits in the cells.

There are various methods used for
this selection of transformants.

The first method is direct

selection of recombinants.

Here the desired gene specifies

antibiotic resistance.

Consider an experiment to clone the gene

for ampicillin resistance from plasmid.

If the cloned DNA itself quotes

for resistance to the antibiotic,

i.e. *amp^R*,

the recombinants are allowed to grow on

minimal medium containing ampicillin.

The recombinants which contain

ampicillin resistant gene that is

amp^R on its plasmid vector will grow

and produce colony on the medium.

Now let us study the second

method that is insertional

inactivation selection method.

Insertional inactivation is the

inactivation of a gene after

insertion of another gene in its

place or within its coding sequence.

In this method,

one of the genetic traits is

disrupted by inserting foreign DNA.

Antibiotic resistant gene

Serve as a good insertion system.

Plasmid pBR322

contains two antibiotic resistant genes,

one for ampicillin and other for tetracycline.

If the target DNA is inserted into

tetracycline resistant gene using an enzyme,

*Bam*HI, the property of resistance

against

tetracycline will be lost. When

recombinants containing target DNA

in tetracycline resistant gene are

grown on the medium containing tetracycline,

they will not grow.

because their tetracycline

gene has been inactivated.

But these recombinants will

be resistant to ampicillin,

because ampicillin resistance gene *amp^R* is functional.

Self-ligated recombinants show resistance against tetracycline and ampicillin and therefore they can grow on medium containing tetracycline as well as ampicillin. In order to confirm the presence or absence of tetracycline resistant gene in the inserted DNA fragment in plasmid a replica plating is done from the master plate.

Bacterial colonies on master plate are gently pressed with sterile velvet, a few cells of each colony may adhere on it, which is then pressed on the other plate containing the nutrient medium amended with tetracycline.

Plates are then incubated for the growth of bacterial colonies.

The appearance of colonies is

compared with the master plate and the colonies which fail to grow on replica plate is considered to possess a plasmid which contains insert DNA in the tetracycline resistant gene *tet^R* of plasmid which destroyed tetracycline resistant gene and therefore this tetracycline resistant became non-functional.

Now let us study the third method of selection that is blue white screening method. It is a powerful method for screening recombinants.

In this method, a reporter gene *lac Z* is inserted in the vector.

The *lac Z* encodes enzyme β -galactosidase which contains several recognition sites for restriction enzyme. β -galactosidase breaks X-gal (5-bromo-4-chloro-indodyl- β -D-galactopyranoside) into an insoluble blue coloured product.

If a foreign gene is inserted into *lac Z*,

this gene will be inactivated.

Therefore no blue color will develop as

β -galactosidase is not synthesized

due to inactivation of *lac Z*.

The host cells containing recombinant

DNA will form white coloured colonies

on the medium containing X-gal.

The host cells which contain non

recombinant DNA will form the

blue coloured colonies. On the basis

of colony colour

the recombinants can be selected

from the agar plate.

The fourth method is colony

hybridization technique.

It was developed by M.

Grustein and S. Hogness in 1975.

The transformed cells grow on nutrient medium,

but it is possible that among several

thousand cells very few cells may

have specific DNA as desired.

There are three different methods used under this colony hybridization technique.

The first one is DNA probes method, second is

Colony or Plaque Hybridization Technique

and third is Plaque Lifting Method.

First method is DNA probes method.

This method is based on the

availability of radiolabelled DNA probe.

DNA probe is about 20 - 40 bp and it is labelled with

$^{32}\text{PO}_4$ or ^{125}I .

Here you can see a portion of host DNA.

First the separation of strain is

carried out and then a single strain is

allowed to hybridize with the DNA probe.

DNA probe recognizes ATG and C nucleotides

and combines with the complementary

sequences of the target DNA.

A and T of the probe combined with T and A

of the target DNA and *vice versa*.

Similarly, G and C of probe combine with

C and G of target DNA and vice versa.

Next is Colony or Plaque

Hybridization Technique.

Colony hybridization is applied to DNA or

RNA released from blotted microbial colonies.

In this technique, master plates of

microbial colonies are prepared.

Replica plating of colonies is done

onto a nitrocellulose filter disc which

is placed on the gelled nutrient medium.

Master plate and replica plates

are incubated to develop colonies.

Cells growing on nitrocellulose filter

disc gets nourishment through diffusion

of nutrients from the medium.

The filter disc is removed and put

on blotting paper soaked with 0.5N NaOH solution.

The cells are lysed to release the

nucleic acid. Then it is neutralised by tris

aminomethane-HCl buffer by

maintaining high salt concentrations.

Then the filter is dipped

in proteinase K solution which

removes protein from the filter.

Due to this, the binding of DNA with

nitrocellulose disc takes place in the

same pattern as the bacterial colonies.

In order to fix the cDNA properly,

the disc is baked at 80° C.

After that it is incubated with a solution

containing radioactive chemical labelled

probe (P^{32} DNA) at suitable conditions.

The probe will hybridise any bound

DNA which contains sequences

complementary to the probe.

Excess probe is washed away and the

membrane is visualized by autoradiography

of the nitrocellulose filter disc.

Colonies which develop positive X-ray

images are compared with the master

plate and picked up and they are

multiplied on the nutrient medium.

Next is Plaque lifting method.

In this method, the recombinant phage particles are isolated and used for construction of library.

A lawn of *E.*

coli is prepared and they are allowed to get infected by recombinant phage particles.

They infect *E. coli*,

multiply inside the cells and causes lysis

leading to the formation of plaque.

Replica plates are prepared from

the master plate containing plaques by

using nitrocellulose filter paper.

The filter is then treated with

alkali solution to denature phage DNA.

Then filter is baked at 80° C to fix denatured phage

DNA and it is transferred in a

solution containing ³²P-DNA probe.

The probe hybridizes the desired DNA

when complementary bases are present.

The filter paper is then washed with

standard saline citrate solution

to remove unhybridized probe and it is autoradiographed.

Dark spots will be formed where probes

have hybridized the DNA.

Then the recombinant phage particles

can be isolated from the plaque.

Next method is immunological test.

Here instead of radio-labelling of

DNA molecules,

antibodies are used to identify

the colonies or plaques developed

on the master plate,

which synthesize antigen encoded

by the foreign DNA present in

plasmids of the bacterial clones.

A special vector, known as

expression vector is designed where

foreign DNA is transcribed and

translated within bacterial cell.

The growth medium containing

specific anti-serum help in detection

of viable immunoprecipitated.

This method consists of following

steps:

replica plating of bacterial

colonies which is followed by lysis

of cells after their growth by

exposing them to chloroform vapour or

treatment with high temperature.

Then making gentle contact with a solid

support containing immobilized antibody

to solid support with the lysed cells

within the colonies

to allow the absorption of antigen to antibody.

The antibodies which do not react are

washed off, and then determination

of position of antigen antibody

complex by passing the filter paper

through X-ray is carried out.

It gives the signal of those

bacterial cells which synthesize

antigen on the master plate.

This is the reference used for
the preparation of this module.

This is an additional reference. Thank you.