Hello students, today we will start

our session on genetic engineering.

The course code for this is M IC110

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Today we will be doing in Unit

2 methods in molecular cloning,

the name of the module is northern blotting.

In this particular session,

we will be learning about the

principle of northern blotting,

the technique of northern blotting,

and the applications and

limitations of northern blotting.

The learning outcomes are as follows.

You will be able to understand

the significance,

the principle and the technique

of northern blotting.

Now, before going to northern blotting,

what is blotting?

A blot in molecular biology is a method

of transferring biomolecules such as DNA,

RNA or proteins onto a carrier.

Now, from where are we transferring

these on to the carrier?

We are transferring these molecules

from the separating matrix that

is the gel onto the carrier.

The different carriers which we use

are nitrocellulose polyvinylidene

nylon or any other membrane.

So what is the purpose of blotting and

why do we need to carry out blotting?

We need to carry out blotting in order

to transfer the separated bands of

the biomolecules from the fragile

gel matrix onto a sturdy carrier,

and this is necessary in order to

maintain a permanent record of the

results of gel electrophoresis.

The fragile gel matrix cannot

remain for long periods of time

and it gets dehydrated,

and this in turn would lead

to loss of the biomolecules.

The principle of northern

blotting is as follows.

It states that in northern blotting

the RNA molecules are separated

by size and they are detected on

a membrane using a hybridization

probe with the base sequence which

is complementary to all or part of

the sequence of the target M RNA.

Now we have to remember that in

northern blotting the targeted

molecule is the RNA molecule.

To be more specific from amongst

the three RNA molecules,

that is the rRNA ,t RNA, and m RNA.

We choose the m RNA molecules.

So from the entire sample of the

RNA molecules,

the m RNA molecules are selected

then separated based on size,

and then they are detected .The

required or the targeted m RNA

molecule is detected by using a probe.

Now the probe can be anything.

It can either be a single stranded DNA

probe or it can also be a RNA probe.

The normal technique was developed

in the year 1979 by James Alwine.

David Camp and George Stark at

the Stanford University.

In fact,

this was not the first blotting

technique that was developed.

The first blotting technique

developed was the southern blotting,

developed by Edwin Southern,

and as a result this particular

technique is named as northern blotting.

What are the steps involved

in northern blotting?

These are the different steps we have.

Electrophoresis followed by blotting,

followed by probing and then finally

detection in electrophoresis.

That RNA is separated in a

gel matrix on the

basis of size in an electrical field in

blotting the RNA bands are transferred

from the gel matrix onto the membrane.

In probing hybridization of complementary

labeled single stranded DNA probe is

carried out and finally in detection

based on the label on the probe.

We will either use autoradiography

or chemiluminescence.

Take a look at the schematic flow

sheet of this northern blotting.

As you can see,

the sample is extracted and this

particular sample will contain m RNA,

rRNA, and tRNA.

We selectively look for m RNA molecule

because it has got a Poly A tail.

Later on, this particular RNA is

separated on a gel electrophoresis,

a special type of gel electrophoresis,

that is the denaturing gel electrophoresis,

wherein the mRNA are separated

by size and at the same time the

RNA molecules are also opening out

from their secondary structure.

You may remember that RNA

molecules are never linear,

they will always attain

a secondary structure,

and if they are in the secondary structure

than the probe cannot hybridize to it.

Therefore,

denaturing gel electrophoresis

is carried out in order to

linearize the RNA molecules.

Then blotting procedure is carried

out in which they are separated.

RNA bands are transferred onto a membrane.

Now the membrane,

which is specifically used over here

is amino benzyloxy methyl filter paper.

Now you can also use Nitro cellulose

or nylon membrane,

but best results are seen on

this particular membrane.

That is the amino benzyloxy methyl filter paper membrane.

Once this is done then the membrane is

subjected to treatment by the labeled.

Probes now wherever there is a

complementarity between the labeled

probe and the m RNA molecule,

the probe will go and bind.

Later on washing is carried out

in order to remove the excess

amount of unbound probes.

And finally,

based on the labeling visualization is

carried out in this particular scheme,

you can see that the labeled probe

is labeled with radioactive tag and

hence we carry out autoradiography

on an X ray film.

In order to visualize the RNA molecule,

the hybridized RNA molecule.

Applications of northern blotting.

There are various applications

of northern blotting.

The primary,

one amongst them being

gene expression studies.

Since we're targeting M RNA,

you know very well that in gene expression

that is through transcription.

The first molecule which is

produced will be the m RNA molecule.

So the higher the number of m RNA molecules,

the larger will be the expression or

the greater will be the expression.

So this is used to observe

overexpression of cancer causing genes.

It is also used in diagnosis of

several diseases, example, crohnes,

disease.

It is used in detection of viral

micro RNA's that play key roles in

viral infections and it is also used

to screen recombinants by detecting

the m RNA which is formed by the transgene.

The northern blot technique

also has certain limitations.

The limitations are as follows.

Lower sensitivity as compared to that of RT.

PCR and nucleus protection essays.

A large amount of target RNA

sample is required over here.

The technique is very sensitive to even

slight degradation of the RNA samples.

Hence one should be very

careful by using the glassware.

The glassware should be thoroughly washed

such that any amount of RNase enzyme.

If present on it should be removed and

as far as possible use new glassware.

Multiple probes cannot

be used simultaneously.

If you want to carry out multiple

probing that you have to first

carry out single probing.

Again, wash the membrane and then

carry out the second probing.

Sometimes what happens is that when

you carry out multiple probing

in this manner sequentially,

the amount of RNA which is present in

the bands goes on reducing and as a result,

the sensitivity of the technique decreases.

This technique can also only be used to

measure steady state m RNA accumulation.

And not RNA stability or transcription rates.

So these are some of the limitations

of that northern blot.

I would like to state over here

that there is another technique,

which is a takeaway on this

particular technique.

Northern Blot,

which is called as the reverse,

northern blot. In the reverse

northern blot

What happens is that instead

of using the the DNA probe,

we use the RNA sample itself as a probe.

So what we do is we take the RNA

sample and we tag it with either a

radioisotope or we tag it with an enzyme.

And that is used as the probe and

we treat it to be subjected to

hybridization of a DNA microarray

in the DNA microarray there will be

different sequences of DNA which will

be present in a different quadrants

and will hybridize with the reverse northern blot.

We can try to find out what are the

different types of RNAs which are

present in a particular sample.

So in order to summarize this

particular technique,

the northern blotting technique is.

The technique is widely used in molecular biology

and it basically detects the m RNA.

The targeted m RNA by using a

hybridization with the DNA probe

and the detection is either carried

out by autoradiography or by

chemiluminescence based on the

label which is used.

So this is the session of northern blotting.

These are my references, thank you.