Welcome to this presentation.

On mode of action,

nomenclature and applications of Type 2

restriction enzymes in genetic engineering.

In this module we will discuss nomenclature

of Type 2 restriction enzymes.

The mode of action of these

Type 2 restriction enzymes.

And a few applications of Type 2

restriction enzymes in genetic engineering.

At the end of this presentation you will

be able to name restriction endonucleases

and explain their nomenclature.

You will be able to illustrate

blunt end and sticky end cleavage

by type 2 restriction enzymes.

And you will be able to explain

the role of Type 2 restriction

enzymes in genetic engineering.

So from the previous modules we have

come across the properties of Type 2

restriction endonucleases and we know

that they recognize palindromic sequences.

They cleave the DNA within

the recognition sequences.

Their cleavage is very specific.

More than 3500 species of Type 2 restriction

endonucleases have been discovered.

And since there are so many, a

nomenclature , essentially a

system of nomenclature was required.

This system of nomenclature was put

forth by Smith and Nathans in 1973.

Where they named restriction

endonucleases with a three letter code.

The name was derived from the

name of the genus, the species,

and the strain of the bacteria

that produced them.

Each enzyme was given a three letter code.

The first letter came from the genus name.

The second and third letter

from the species name.

This was followed by the strain number

and then Roman numerals were given to

identify if there was more than one

restriction enzyme in that bacterial strain.

Let's take an example to understand

the system of nomenclature.

Consider the restriction enzyme

named Eco R1.

So the name is a 3 letter word E c o.

E comes from the genus name Escherichia.

co comes from the species name coli.

R comes from the strain.

This particular strain is R I.

Roman number one denotes the first

enzyme that was isolated from this bacterium.

So that's how you have the name Eco R I..

Similarly,

I have examples of other names

here of restriction endonucleases.

Hind III derived from Haemophilus

influenzae, Pst I from Providencia stuartii

Taq I from Thermus,

aquaticus , Bam HI from Bacillus amyloliquefaciens strain H.

And Sau 3A from Staphylococcus aureus 3A.

OK.

Next,

let us move on to the mode of action

of these Type 2 restriction enzymes.

So these restriction enzymes

recognize specific sequences and

these recognition sequences range

from 4 to 8 base pairs in length.

Usually 4 to 6.

The sequences are palindromic in nature.

OK,

a palindrome is a word that reads the

same from the forward and backward direction.

For example, the word “Madam”.

It reads the same in the forward

as well as the backward direction.

Similarly,

DNA sequences exist which has

sequences that read the same from

the five prime to three prime

direction in one strand and the

same from 5 prime to three

prime direction in the opposite strand.

Complementary strand.

So such sequences in DNA are called

Palindromic sequences.

One sequence is shown here below

in the slide, so you have GG ATCC.

From five prime to three prime direction

in the top part of this strand,

the bottom strand reads the same from

5 prime to three prime direction.

So these sequences are palindromic

and such palindromic sequences

serve as recognition sites for

Type 2 restriction endonucleases.

These recognition sites or palindromic

sequences have an exact two fold

rotational symmetry as shown in the slide.

The axis of symmetry is shown

in a dotted line.

Some examples here,

for example, Eco RI.

The palindrome is shown here GA ATTC.

And the cleavage sites are between G&A.

You have Hind III.

You have Kpn I and Sma I.

These are examples of some restriction

endonucleases and the cutting sites.

How these restriction endonucleases

recognize those palindromic sequences and

the arrowheads show the cutting sites,

the cleavage sites of these

restriction endonucleases.

Now talking about cleavage sites

restriction endonuclease type 2 have two

types of cleavage or they make two types

of cuts in the recognition sequence.

One wherein the restriction enzyme

or the restriction endonuclease

cuts both the strands at the axis

of the two fold symmetry.

OK, so if you see in the example here.

The restriction enzyme Alu I,.

recognizes the sequence,

palindromic sequence, AGCT.

The axis of symmetry is in between G&C.

And the cleavage site is also

at the axis of symmetry. OK,

the red arrows show you the cleavage site.

If cleavage occurs at the axis of symmetry,

then the cutting gives rise to what are

called as blunt ended DNA fragments.

It gives rise to blunt ends or flush ends.

Shown in the slide are blunt

ends which are produced.

OK, to illustrate this further,

have a look at this slide.

So let's assume the two blue

strands are double stranded DNA and

you have the palindrome AGCT.

Alu cuts AGCT between G&C ; after cutting

the fragments are shown below,

so you will have two pieces produced,

two fragments produced,

each one ending in those blunt

ends or flush ends.

So this is blunt end style cutting.

The second type of cutting is where the

enzyme can make cuts or make breaks

symmetrically around the axis

of the two fold symmetry.

That means, as shown in the example here.

If you have a palindromic sequence GG ATCC.

The axis of symmetry is between A&T.

However,

the cutting sites or the cleavage

sites are symmetrically placed on

both sides of the axis of symmetry,

so the cutting sites are between

G&G on both the strands.

OK,

so this type of cutting generates

DNA fragments with unequal length.

If you notice in the slide,

the product of cutting is shown below,

you have one strand which is

shorter than the other strand?

OK,

so these type of ends which are

produced are protruding ends.

And the two fragments which are generated

can easily join back together

because the protruding ends/ the

overlapping ends will easily base

pair with each other because they are

complementary so they can join back

again and therefore they are called

as sticky ends or cohesive ends.

So this type of cutting produced by Bam HI , in this sequence yields

sticky ends or cohesive ends.

This is the second cutting style.

As illustrated here, again,

double stranded DNA BamHI

cuts to give rise to

two fragments of DNA.

Each fragment has strands of unequal length.

They have protruding ends

which are sticky and which can

join back again, hence their called as

cohesive ends or sticky ends.

The sticky ends can be of two types

depending upon where the enzyme cleaves.

If the enzyme cleaves to the

left of the axis of symmetry,

you have five prime overhangs produced.

As produced by Eco RI,

have a look at the illustration shown below.

Eco RI cuts, GAA TTC to give

rise to five prime overhangs.

While Pst I cuts to the right

of the axis of symmetry and it

produces three prime overhangs.

Let's next move on to the applications

of Type 2 restriction endonucleases.

We have just a few applications listed here.

The 1st and most common application of

restriction endonuclease Type 2 is to

obtain DNA fragments so DNA can be cut or

broken into fragments by making use of

restriction endonucleases and these DNA

fragments are used for various studies.

They are used as reference markers,

ladders to determine the size of DNA

fragments by gel electrophoresis.

They are used in DNA fingerprinting

in southern blotting and so on.

In this slide you can see here

a gel, picture of a gel where in the

ladder, DNA ladder is created that has

DNA fragments of various sizes that are

produced by restriction endonucleases

that is used as a reference to compare

with other samples put in the wells such

that you can obtain the size of other

samples of DNA with reference to the

marker or with reference to the ladder.

The second application of restriction

endonucleases is in restriction mapping.

This involves digesting the DNA with

two or more restriction enzymes,

separating them on agarose gel and

the distance between the restriction

sites is determined by the pattern of

fragments that are produced on the gel.

An example here.

So you have a DNA fragment which

is cut by Eco RI.

The first gel shows two bands produced

the size of which is given; the second

gel you have a mixture of fragments

which are produced when the DNA fragments

are treated with Eco RI and Hind III.

So in this gel you get four

bands denoting four fragments.

If you compare the size of these

Four fragments with the two fragments

obtained in the first gel,

you can deduce the arrangement of the

strands/ of fragments on the entire DNA

strand and thus obtain a restriction map

as shown in the diagram in the slide.

The third application spoken of here

is the application of restriction

endonucleases to produce recombinant

DNA molecules and cloning wherein

DNA fragments are produced and

vectors plasmids are cut also with

restriction endonucleases. DNA is cut

with restriction endonucleases

to produce recombinant molecules,

and these recombinant molecules are

then used for cloning and various other

procedures in genetic engineering.

So these are the applications of

restriction endonucleases type 2..

To summarize,

the ability of Type 2 restriction

endonucleases to cut DNA molecules

at defined sequences, their

cutting is very precise actually.

This ability of them gave rise to

the development of recombinant DNA

technology or gene manipulation.

Type 2 restriction enzymes are

indispensable tools in genetic engineering.

Thank you.