Hello everyone and welcome to

this presentation on Maxam and

Gilbert's method of DNA sequencing.

In this presentation we will be

studying Maxam & Gilbert's technique

of DNA sequencing which is also called

as the chemical cleavage method.

At the end of this presentation

you will understand the chemical

cleavage method for determining

the sequence of a DNA fragment.

Let us begin with the definition

of DNA sequencing. DNA sequencing.

Is the process of determining the

order of the four nitrogenous bases,

namely adenine, guanine, cytosine,

and thymine in the DNA strand.

Now why is DNA sequencing done?

DNA sequencing tells the kind of

genetic information that is

carried in a particular DNA segment.

And knowing the DNA sequence helps in

identification and cataloguing of organisms,

it helps in the identification

of pathogenic strains.

It is used in medical diagnosis.

It helps in drug discovery and

it is indispensable in all basic

biological research.

How is DNA sequencing carried out?

Several techniques were developed

for DNA sequencing.

However, only two were widely used.

One the chain termination method,

which was developed by Frederick Sanger

and the second one is the chemical

cleavage method or the chemical

degradation method that was developed

by Alan Maxam and Walter Gilbert in 1977.

In this presentation we will be studying

the chemical cleavage method or what is

also called as the Maxam & Gilberts method.

This method of DNA sequencing is

based on a chemical degradation

reaction that is specific for each

type of nitrogenous base followed by

gel separation and autoradiography,

wherein the order of the bands on the

AUTORADIOGRAPH determines the sequence.

Let's have a look at the main steps

in Maxam and Gilbert's method.

So there are four main steps.

The first step is end labeling

of the DNA fragment.

Second, chemical cleavage.

Third,

electrophoresis and autoradiography and 4th

is the final step of deducing the sequence.

The advantage of Maxam & Gilberts

method is that whatever fragment

needs to be sequenced once purified

can be directly taken for sequencing

without any preparatory procedures.

So the DNA fragment which is to be

sequenced is cloned to obtain multiple

copies and then the four steps begin.

So the first step is the end

labeling of the DNA fragment.

So the DNA fragment which is to be

sequenced is a double stranded fragment.

OK so it has two 5’ ends and these two

five prime ends are the phosphate ends.

OK,

five prime phosphate ends.

By treatment with alkaline phosphatase,

the phosphate groups at the five

prime ends are removed.

And then the double stranded fragment

is treated with polynucleotide

kinase in the presence of ATP that

contains radioactive phosphorus

32. Kinase adds the radioactive

phosphate group to the five prime

terminus of the double stranded DNA,

thus giving rise to an end labelled

double stranded DNA fragment.

OK,

so the double stranded DNA fragment

is now labelled at the five prime

ends with radioactive phosphorus 32.

The double stranded DNA fragments

next are denatured to give

rise to single strands.

The single strands are

separated by electrophoresis,

where the two strands separate

based on heavy and light strands.

The one with more purines

is the heavy strand.

2 bands are obtained on the gel.

Anyone can be eluted

and taken for sequencing.

So at this step,

now we have a suspension of single

stranded DNA which is radioactively

labeled with phosphorus 32 that

is ready for sequencing. the second

step and the most important step

in this sequencing technique

is the chemical cleavage step.

So our suspension or our reaction tube

has single stranded DNA fragments

which are radioactively labeled.

This suspension,

or the contents of this tube

are now divided into four aliquots, into four portions.

OK,

and each portion is treated with

a set of chemicals wherein the

chemicals used are specific for

a particular nitrogenous base.

Have a look at the slide, in the

slide you see the four reaction

tubes OK containing the strand

to be sequenced in the form of a

single strand that is radioactively

labelled. To the first tube.

Dimethyl sulfate and piperidine are added.

this is for treatment of guanine

residues in the DNA strand.

The second tube is treated with

dimethyl sulfate in the presence

of formic acid,

followed by piperidine treatment.

This tube is for reaction with

adenine residues in the DNA strand.

The third tube is treated with hydrazine,

followed by piperidine,

and this treatment is specific

for thymine is meant at least to

be specific for thymine in this

reaction and the last tube is treated

With hydrazine in the presence of

sodium chloride in the presence of salt,

followed by piperidine,

and this reaction is specific for

cytosine residues in the DNA strand.

Before we proceed,

I'd like to draw your attention.

Please note that each reaction

tube has several copies of that

single stranded DNA,

which we need to sequence.

And each single stranded DNA is

radioactively labeled ,

so now let's see what happens

inside each of these tubes.

Individual reactions inside each

of these tubes.

Let's take the first tube that is

treatment for G treatment for guanine.

So this reaction tube contains dimethyl

sulfate and piperidine. Dimethyl sulfate.

acts by methylating the guanine

residues in the DNA strand.

So the glycosidic bond of the

methylated guanine residues

becomes susceptible to hydrolysis.

Then when Alkali or the piperidine is added,

the phospho diester bond of the

DNA strand is broken at the

methylated guanine residues.

So let's have a look at how it happens here.

So we are referring to the

first reaction tube.

To understand this better,

let us take an example wherein we

have the target strand in our sample,

and let's assume that this target

strand has a sequence as given

in the slide from the five prime

to three prime end as.

TTCA GCCGAT.

Let's take this sequence as

an example for our study.

The five prime end of this target strand

is radioactively labeled with phosphorus 32.

OK, so all our reaction tubes here

have got this target strand. In the

first reaction tube, dimethyl sulfate,

and piperidine is added.

So we said that dimethyl sulfate methylates

the guanine residues in the DNA strand.

In our example, which we have taken,

we have two guanine residues.

Right ? one is at the fifth position

in the strand and the other one is at

the eighth position in the Strand.

So now any of these guanine residues

could get methylated at random if the

5th guanine residue got methylated,

then the strand breaks at the methylated

guanine residue to give rise to a fragment,

which is 4 nucleotides long.

As shown in the slide below,

at number one,

we see the guanine in the fifth

position that has got methylated

and the strand has cleaved at

this methylated guanine residue,

giving rise to a fragment of

four nucleotides in length.

Now, this fragment has five

prime i.e. at its five prime end,

the radioactive phosphorus 32.

In another strand in the same reaction tube,

the guanine present at the eighth

position could have got methylated.

OK, so if the guanine in the

eighth position got methylated,

then strand cleavage happens

at this guanine residue,

giving rise to a DNA fragment

that is 7 nucleotides long.

so in example number two we see a

fragment that is 7 nucleotides long,

and again this fragment is

labeled at the five prime end

with radioactive phosphorus 32.

So in this way,

all the strands which are

present in the reaction tube.

Please note there are multiple

strands in the reaction tube,

so all the reaction strands will get

methylated at one guanine residue at a time,

and the strand breaks.

To give rise to.

Cleaved strands of unequal length,

or of different lengths.

Depending upon where the cleavage has taken.

Place.

So if you take this mixture and

run it on gel electrophoresis,

that is,

if you take the contents of this first

tube and run it on gel electrophoresis.

Each of these strands will separate

out on the gel based on their size

based on their length with the smallest

strand migrating the farthest in the gel.

so this is what happens to reaction number

one in this chemical cleavage method.

Next,

let's go to Tube #2.

This reaction mixture contains dimethyl

sulfate with formic acid and piperidine.

This treatment is supposedly

meant for adenine.

OK, now in the presence of formic acid,

the DNA gets protonated.

And here both the purine residues,

namely adenine and guanine,

get protonated at equal rates.

So the protonated glycosidic bond

becomes susceptible to hydrolysis,

and upon piperidine treatment the

strand breaks at both residues.

That is protonated at adenine residues,

as well as protonated guanine residues.

Let's take a look with our example.

So we had a tube number 2.

Where the reaction is meant for adenine.

However, the chemicals used.

act on both the purines,

adenine and guanine to equal extents.

That means both adenine and guanine

are equally susceptible

to the chemical treatment.

So if you see in the example taken there,

both adenine and guanine will get

protonated upon treatment with

formic acid and dimethyl sulfate.

However,

only one purine per strand

will get protonated.

That's because once one

purine gets protonated,

the strand breaks at that protonated purine.

So in our example we have

adenine at position four.

so if adenine is protonated,

the strand breaks at position four

and therefore you will have a

fragment which is 3 nucleotides long,

and that carries the radioactive phosphorus

label at the five prime end. The other.

Portion of the cleaved fragment

is also there, however,

it does not carry any radioactive label.

Next you have guanine.

At the fifth position,

Guanine also gets protonated

by this treatment,

and therefore the strand will

break at the guanine residue.

Now,

giving rise to a DNA fragment that is

4 nucleotides long and carries the

FIVEPRIME label of phosphorus 32 with it.

So on and so forth.

The remaining guanine and adenine

residues also will get protonated,

and the strand will be degraded

to give rise to fragments of

different lengths in this tube.

Next is the portion for

treatment for thymine.

This tube is treated with

hydrazine followed by piperidine

Now,

treatment with hydrazine makes

both cytosine and thymine

susceptible to hydrolysis.

Hydrazinolysis of both cytosine

and thymine take place.

upon treatment with piperidine

therefore the strand will

break at both cytosine residues

as well as thymine residues.

Let's take a look at our

example in the illustration.

So here.

Both thymine and cytosine are subjected

to hydrolysis of the glycosidic bond.

Once piperidine is added,

the strands will break depending

upon whether a thymine got

hydrolyzed or whether a cytosine got hydrolysed

in a given strand,

only one pyrimidine will get hydrolyzed,

so that's how you get these fragments.

Look at the examples and the different

fragments shown here in the slide,

so the first fragment that is

produced has only one nucleotide.

This is because the thymine at

position two got hydrolyzed and

broke. the strand.

cleaved at this time in position 2.

Therefore a very small fragment

of only one nucleotide got

produced which contains the

radioactive phosphorus 32 label.

OK then you have cytosine

which got hydrolysed.

And the strand breaks at

the cytosine residue.

So in our second example we have

a fragment produced which

is now made up of two nucleotides

and that carries the phosphorus

32 label at the five prime end.

As I mentioned earlier,

the other portion of the fragment

of the cleaved fragment is also

there in the reaction mixture,

but will not show up because it does

not carry any radioactive label.

OK, so this is the third reaction

tube wherein the fragments cleave

or the strands cleave at every

thymine or cytosine residue

present. one pyrimidine per strand.

And the last aliquot is treatment

for cytosine wherein the reaction tube

contains hydrazine in the presence of salt,

followed by piperidine treatment.

In this reaction tube.

This treatment affects only cytosine,

so although hydrazine affected

both pyrimidines equally in

the presence of salt,

only cytosine residues get affected.

That is,

the glycosidic bonds at cytosine residue are

Hydrolyzed so therefore on piperidine

treatment the strand breaks

at the cytosine residues.

Let's take a look at the illustration.

So back again the same example.

Treatment with hydrazine salt and piperidine.

The treatment affects only

cytosine as we said,

so in our example cytosine is present

at position number three in the

Strand at position #6 and position #7.

OK,

so depending upon where hydrazinolysis

took place which cytosine got affected,

we will have fragments produced

upon cleavage giving rise to single

strands of different lengths.

One strand which is made up of

two nucleotides.

Another strand which is made up of

five nucleotides and another strand

that is made up of 6 nucleotides.

I hope you understand how

these lengths of fragments are.

Produced. OK?

it's the same manner of cleavage as

happened in the earlier three tubes.

In this slide you have all the

four reaction tubes at a glance.

so looking at this slide you will now

realize that in each reaction mixture.

The strands were subjected to

chemical degradation. they were

subjected to chemical treatment

followed by strand cleavage,

giving rise to fragments of the

DNA strand of different lengths.

Since they differ in length,

these fragments can be separated

by gel electrophoresis.

So the contents of each tube.

are loaded onto a polyacrylamide

gel and electrophoresis is allowed

to take place or they are allowed

to electrophores in parallel lanes.

So we are at step three,

which involves electrophoresis

and autoradiography.

Have a look at the picture of

the gel shown here.

So four lanes are there,

one for guanine,

the second one for adenine and guanine.

Because the second treatment

hydrolyzed the strand at both

adenine and guanine residues.

The third lane is for the reaction

mixture for cytosine and thymine.

Again,

this treatment hydrolyzed the strand

at both cytosine and thymine

residues in the final lane.

The last lane is for cytosine.

After gel electrophoresis,

autoradiography is carried out

. Now each of these strands separate out

or come out as bands on the gel and on the

autoradiograph they will show up as dark

spots because each of these strands have a

radioactive label at the five prime end.

Looking at the autoradiograph the order

of bands on the gel can be identified.

From the order of bands on the gel,

the sequence can be deduced.

So we read the sequence

of the strand from the order

of the bands on the gel.

So reading of the sequence is done

From the bottom of the gel to the top.

Have a look at this slide.

So this is the gel.

The bands are there. now

we proceed to read the sequence looking

at the order of the bands in the gel.

So the band which has traveled

the farthest is in the C + T lane.

OK, so this band is taken as T.

The next band in order.

Is a band in the C + T lane

as well as in the C lane.

So when you have bands like

this in both the lanes.

The band or the Strand base is red as C.

OK.

So wherever you have bands in the

C + T lane and in the C lane,

That band is read as a sequence

pertaining to C pertaining to cytosine.

Likewise,

you will have bands in the G Lane

traveled to the same distance

as the bands in the A+G Lane.

Have a look at the slide.

Have a look at the gel.

You have in the fourth position. if you

go by the DNA bands that are produced.

In the gel, if you go from bottom to top.

Go to the 4th row in the fourth row we

see a band in the guanine lane in the G Lane,

as well as in the A + G lane.

So when you have bands in both the lanes,

the strand is read as a sequence of G.

But when you have a band only

in the A + G lane.

Like the band seen in the third row.

Then that band refers to A.

that is adenine.

So following this convention,

the sequence is deduced.

So let's read the sequence from

the bottom of the gel to the top.

That is,

from the five prime end to

the three prime end we have.

TCAGCCGAT, so this is the

sequence of the target strand.

Now, if you notice at the end

of this sequencing process,

the first nucleotide is not identified.

OK, this is how Maxam Gilberts method is.

That is, the first nucleotide cannot be

read from a single run. To overcome this.

Sequencing is carried out of

the complementary strand

where in this missing nucleotide will

feature in the three prime position,

and therefore you can identify

this missing nucleotide.

So this is the manner in which

DNA sequencing is performed by

the chemical cleavage method

or the Maxam Gilbert method.

This technique has a few limitations.

It is time consuming,

labor intensive,

and more importantly it makes use

of toxic chemicals and therefore

it is not very popular.

Other sequencing techniques

are nowadays used.

So to summarize the Maxam

Gilberts method of DNA sequencing

involves chemical modification

of a nitrogenous base followed by

cleavage of the sugar phosphate

backbone of the DNA fragment.

Partially cleaved fragments of DNA

are produced of different sizes.

The DNA is subjected to different

digestion to deduce and interpret

the sequence of nucleotides.

A complete set is obtained of the

cleavage product wherein each

fragment is short by 1 nucleotide and

electrophoresis and autoradiography

determines the order of the fragments.

That's all about Maxam and

Gilbert's method of DNA sequencing,

thank you.