

Experimental Biotechnology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology – Guwahati

Lecture – 45
Designing Experiments

Hello, everybody listen Doctor Vishal Trivedi from Department Of Biosciences And Bioengineering IIT Guwahati and in the course experimental biotechnology. So far we have discussed the different types of techniques and the aspects where you can be able to utilize these techniques and how you can be able to design the different types of experiments to resolve the different types of scientific questions.

So, now it is the time to review whatever we have discussed so far in this course and try to refresh our memories. Because this is very important that you should recall what you have discussed or whatever we have discussed in this course. So that in case you have any queries or whatever you have not been ask during the previous module. You can be able to ask me those questions are in case something which you could not be able to understand.

While we were going to review the content you can be able to again get the opportunity to understand that. So in this particular lecture we are going to summarise what we have discussed so far and in this process will try to understand how the different techniques can be exploited and can be used even to answer the single questions or the similar kind of scientific questions.

(Refer Slide Time: 02:26)

Good Lab Practices (GLP)

An internationally recognized definition of GLP goes like this: *Good Laboratory Practice (GLP) embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported, and archived.*

New Zealand and Denmark were the first to introduce GLP in 1972. United States was the next to introduce GLP in response to the poor scientific practices prevalent in US around that time.

Why ???

An international economic organization, the Organisation for Economic Co-operation and Development (OECD) published the principles of GLP in 1981 under the name 'OECD Guidelines for the Testing of Chemicals' that were internationally accepted.



<http://www.oecd.org/chem/testing/oecdguidelinesfortestingchemicals.htm>

So we started the course with a good lab practices. So where we have discuss about the what are the what are the things are allowed and what are the things are not allowed what are the different types of the quotient you have to take while you are in going to be enter in to the lab. In that particular type of module we have discuss about how to prepare the different types of buffers are how to even operate the different types of instruments.

We have also discuss different types of precautions. So what we have discussed is that the good lab practices are actually been very, very important for you to understand that the; it is not important that you are going to perform the experiment. It is also important that you should perform the payment as per the prescribed guidelines. So that is why the people have come up with the idea of the good lab practice.

And the major advantage of the; if you follow the good lab practices that whatever you are going to report or whatever you are going to make the discovery through the different types of experiments can actually be able to advance the ongoing literature and as well as it is actually going to Help others to utilise that particular information. Because when you are going to follow the good lab practices, you are actually going to document each and every observations.

You are actually going to use the complete list of material and methods and experimental procedures in your notebook. So this aspect also we have discussed in detail in this particular

type of module. Where we said that it is important that you should also maintain a very neat and clean notebook where you should start it with the objectives. Following the objectives you have to list out the all the reagents and experiment and all the details of the experimental protocol and then subsequent to that you are actually going to write each and every step what are the modifications you have done?

And how you how long you have incubated the samples and all those kind of technical informations followed by that. You are also going to write the observation what you have gone and then ultimately you are going to get the results. Of these results could be of the raw result what you are going to directly get from the machine or you can have the calculated results like the interpreted results.

In that particular context we also discuss that. Suppose you got the protein Estimation and suppose you are doing a protein estimation. So one is the raw data that the any particular type of absorbent what you are going to get from the spectrophotometer. The other is the background signal what you are going to get from the spectrophotometer. So if you subtract the background values from your experimental value then you are actually going to get calculated value.

And these calculated values are the derived data what you got from your raw data. So it is important that you should note down the raw data as well as the calculated data or the experimental data. So that you can be able to cross verify or if the people who want to repeat the experiment or the people who want to reduce the data. They should be able to go through with your notebook that they should be able to understand each and every aspect.

(Refer Slide Time: 06:06)

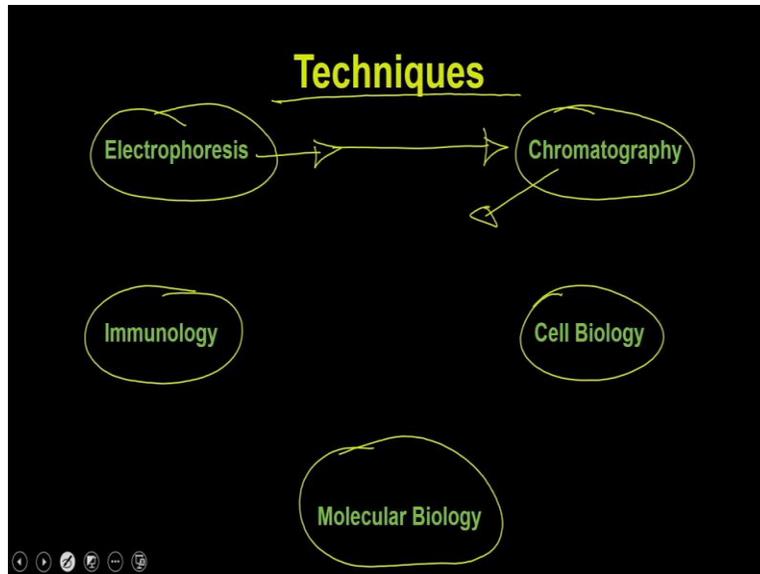
- Good Lab Practices
- Precautions
- To do
- Not Recommended Action.
- Data Register

So at the end of this module we have summarised that you have to follow the good lab practices, which means you have to follow the particular type of disciplines. You have to in a should not eat or drink into the labs. Because the lab is a full of bacteria, viruses and all those kind of infectious organism. So that is why you should not consume food into the labs as well. As you cannot just to as per your wish.

So you have to always adhere the safety of instruments like you have to use the apperance you have to use safety goggles. You have to use the that kind of the lead Shields and all that kind of thing. And then if you are handling the infectious organisms then you have to appropriately use the different types of the biosafety cabinets. Like either you use the laminar or to the biosafety cabinets and that also we have discussed in detail.

That what are the different types of bio-safety level are possible and how you should be able to handle the bio-safety levels? This is what we are discussed the precautions, what you should do and what should not do. And what is recommended and what is not recommended and at the end we have also discuss about how you can be able to design the data register so that you can be able to document each and every detail of the experiment that you have performed. And so that it should help the other people to follow the content.

(Refer Slide Time: 07:35)



After that we directly jumped onto the different types of techniques, but you should use to advance the experimental work. So what we have discussed we have discussed about the experimental electrophoresis, we were discussed about the chromatography, we discuss about the immunological techniques. We discuss about the cell biology techniques and at the end we have also about the molecular biology techniques.

So we did the experiment electrophoresis technique, we discuss about the basics of the electrophoresis how the electrophoresis actually separate the molecules? And how the electrophoretic mobility is directly proportional to the charge and the inversely proportional to the mass and what are the different factors which actually can regulate the resolution or the separation of the molecule during the electrophoresis.

And then following that we also discuss about the different variants of the electrophoresis, like we discuss about the vertical electrophoresis, the horizontal electrophoresis. Within the vertical gel electrophoresis discuss about the SDS page on the denaturing gel electrophoresis or the native gel electrophoresis, as well as the urea page whereas in the horizontal gel electrophoresis we discuss about the agro gel electrophoresis or the high resolution horizontal gel electrophoresis.

While we were discussing about all these techniques at the end of the electrophoresis we have also discuss about how the electrophoresis technique can be utilised to answer some of the scientific questions. And all those questions we have taken In such a way so that you should actually not going to help you to understand the potentials of the vertical gel electrophoresis or to the horizontal gel electrophoresis.

It is also also going to help you to design your own experiment? Because once you know that this particular technique can be used in all these different types of ways then you can actually make a combination of those techniques and you can be able to answer some of the and resolved question. So at the end of the electrophoresis we also discuss about the some of the staining techniques.

Like we discuss about the commercial building staining techniques of week discuss about the silver staining. And we also discuss once you have got the images how you can be able to utilize different types of softwares to analyse the image data. How you can be able to determine the molecular weight of the unknown proteins or how you can be able to determine concentration of unknown protein if you have.

So if you if you can be able to utilise the softwares you can be able to get this information. So we concluded the electrophoresis module and then we directly jumped onto the chromatography. Within the chromatography with discuss about different types of chromatography techniques to initially we started with the basic principle of the separation where we have taken very simple examples of different types of molecules.

How they are actually wearing in their physical and the chemical as well as the biological properties and how you can be able to utilise the physical chemical and biological properties to separate them. Because using these properties you can be able to distribute the molecules into the different phases. And during that discussion we discuss about how even the simple technique like distillation can be used to separate the two molecule like the benzene and the alanine.

And how the benzene is actually distributing themselves within the liquid phase as well as the vapour phase compared to that it is aniline is distributing themselves from the liquid phase to vapour phase. And these distribution of the benzene and alanine within the liquid versus the vapour phase can be modulated in such a way that the benzene is going to be collected separately, where as alanine is going to be separated separately.

So that is just simple example of showing you the potential of distributing the molecule between the two different phases that can be even further amplified or for the enhanced when you use the chromatography techniques because in the chromatography technique you are going to use the different types of beads. And all these beads for example in a particular chromatography column if you have the 100 layers of the beads.

All these 100 layers of beads are actually going to give you the 100 different types of distribution planes and because of that in compared to that when we were talking about the distillation, you are only having the two phases. The liquid as well as the vapour phase whereas in the case of chromatography if you have the chromatography column with the 100 distribution planes then you are actually going to distinguish the molecule utilising these 100 distribution planes.

And because of that the molecules are going to be get separated more efficiently into a chromatography column. Following that we also discussed in detail about the chromatography systems, whether it is a low pressure systems or middle pressure system or the high pressure systems and then during that process I have taken you to my lab also where some of my students have also given you a demo about how to operate 3 purification systems and how you can be able to utilise the purification system.

So what we have given? We have given you a demo of the at tap your M but as I said in the lecture itself that it does not matter whether you have this particular instrument in your lab or not. Because more or less the procedure remains the same except that you might have to use the different patterns and different things. So that information you can easily get from the manual of that particular chromatography system.

And then with the short span of time, you could be able to train yourself to a this particular instrument what you have in your lab and that is how you can be able to utilise that instrument very efficiently. Following that we have also discuss about the different types of chromatography technique. So that we have discussed we discussed about the Ion exchange chromatography. We discussed about the hydrophobic interaction chromatography.

We discuss about the gel filtration chromatography and we also discuss about the affinity chromatography. And within this chromatography techniques we have also discuss about what is the basic principle of the Ion exchange chromatography? What is the basic principle of hydrophobic interaction chromatography or gel filtration chromatography on the affinity chromatography?

How these individual chromatography techniques are actually distributing the molecule of separating the molecules. And how what are the different parameters as well as the factors what is governing their potential to separate the molecules into the different distribution planes. And because of that we have in depth taken how Ion exchange chromatography, hydrophobic interaction chromatography, gel filtration chromatography as well as the affinity chromatography can be used to design the different types of experiments to answer some of the questions and while we were discussing about the affinity chromatography.

We have also discussed how you can be able to generate affinity column. How you can be able to utilise that to purify the antibodies or whether you can use the antibodies to purify the antigen. How we can be able to utilise the metal affinity columns? How we can be able to utilise this pseudo affinity columns and all that. So with that we have taken up the complete detail of the chromatography procedures.

How you can be able to use the chromatography to design the new experiments and how you can be able to use that to answer some of the question. Ultimately and after that; we jumped onto the immunological techniques. So in the immunological technique we started with the immunological technique with the first the basic understanding what is the immunology? How

the different candidates are playing together and how they are actually giving you the immune responses.

And then subsequently we also discuss about the antigens how you can process the antigen so that you can be able to use that to immunize the animals and how that can actually give you the antibodies. We discuss about the production of the monoclonal antibody as well as the polyclonal antibody. And in that particular context we have also shown you a very detailed demo how you can be able to immunize a rabbit.

And how you can be able to what are the different steps are required to prepare the emulsions and how you can be able to check the quality of the emulsion. And how you can inject a emulsion into the rabbit. And then how you can be able to collect the blood and how you can be able to separate the plasma. And ultimately we have also shown you how to purify the antibodies from that particular plasma.

And apart from that we also talked about the monoclonal antibodies. We have also discuss about how you can be able to separate the B cell and Myeloma cells. And all how you can be able to create generate and how you can be able to do the fusion reactions and how you can be able to generate the hybridomas. How you can be able to screen the hybridomas and all that. And after that we have taken few of the classical techniques like the agglutination reaction, precipitation reactions.

Or the in-depth we have taken the Elisa also and while we were discussing about the Eliza about the indirect Elisa, direct Elisa, sandwich Elisa. And as well as we have also discuss about the cytokines arrays. So in the cytokines arrays while we were discussing about the cytokine array we have taken you to my lab. How you can be able to perform the cytokine array to measure the different types of cytokines in a single reactions.

So in that case we give you a complete detail of how you can be able to measure all the 50, 60 cytokines what is present in to a cell supernatant simultaneously with the help of this cytokine array. Apart from that we have also tried our level best to show you some of the demos that is

been related to the immunological techniques. Like we have shown you how to perform the Elisa, how you can be able to measure the antibody level and so on.

After that we moved on and gave you a full discussion about the cell biology techniques. So we started with the very basic that the cell biology techniques started with the cell culturing. So you can actually have the different types of media. So we have given you the information about the media. How you can be able to prepare the cell culture media? How and what are the precautions you should take while you are preparing the cell culture media.

How you can be able to in autoclave or how you can be able to do the sterilization of the media whether it is a microbiology media or the cell culture media. And then we moved on to the sum of the techniques like we have shown you are the microscopic techniques like the simple bright field microscopy or the fluorescence microscopy and as well as we also show you the scanning electron microscopy or that transmission electron microscope.

Not only had we discussed about the microscopy. We also discuss in detail about how to process the samples so that it should actually give you a very detailed insight, whether you how to process the sample when you are actually going to design the experiment. So these are not the only the theoretical aspects what we discussed. We also discuss about the experimental aspects like how to perform the protocol for different precautions you should take.

And how you can be able to modulate the sum of the factors that it should actually going to give you the troubleshooting or some of the factors how you can be able to government so that is actually going to make you more better images and all that. And at the end of the particular cell biology techniques we have also discuss about the different types of the experiment what you can actually explore.

Where we were talking about the microscopic techniques or cell biology techniques some of the places we have also discuss about the flow cytometry. So in detail we have discussed about the flow cytometry. How you can be able to utilise the flow cytometry. What are the basic principles

of the flow cytometry. How you can be able to utilise that to answer some of the questions. What are the potential of a flow cytometer and all that?

After that we directly jumped onto the molecular biology techniques. So within the molecular biology techniques we started with a very basic that the polymerization reactions and we discuss about PCR. What are the best, how the PCR is being developed, how the technique is being developed. The idea of discussing the development of a particular technique from a very crude status to a very refined level is that actually give you idea.

How the people are actually developing a technique when they are going through a different stages. Like for example, when the people were initially starting the PCR they are actually using the non thermostable enzyme. So, that because of that they were using the thermolabile enzyme for because of that because there was no discovery of the tag DNA polymerases because there was not a thermally stable enzymes.

They were using the thermostable enzyme. They were supposed to add the enzyme after every action because of that it was actually causing a lot of hindrance. Apart from that the people were also having the very crude kind of thermal cycler. And so in the crude type of thermal cycling they were supposed to handle or they were supposed to transfer the tubes from one compartment to another compartment.

And that is all and that is how the PCR actually started. But when the people have developed the Philadel based systems the ramping speed as well as all the other kind of temperature changes were very, very rapid. And because of that the PCR was becoming a very, very popular and useful technique. And at the end of that we have also discuss about how the PCR can be used for answering some of the experimental questions.

How the PCR can be used to event detect the different types of the infectious organism or the contamination in a particular sample. So we have taken an example from the agriculture Biology, we have taken example from the plant science cell, we are taken an example from the infectious

diseases like HIV, we have taken example from the hepatitis B, we taken an example from the event the some of the other viral diseases.

And how you can be able to detect viral diseases and how you can be able to event detect the infectious organism that is been present into the fruits and vegetables as well. And how the PCR is a very, very potential technique and robust technique even to answer some of the Genetics related questions like who will be the potential criminal and all those kind of thing. And after that within the molecular biology techniques we have also discuss about the some of the basic blotting techniques.

Like we have discussed about the southern blotting, we discuss about the northern blotting and at the end, we also discuss about the Sequencing techniques. So we were discuss about the DNA Sequencing as well as the protein Sequencing. So this is what we have discussed so far as a whole and when we were discussing about the individual module. We were not considering the other act other experiments.

Like when you are talking about electrophoreses we were not discussing about the chromatography. While we were discussing about the chromatography we were not discussing about the cell biology or immunology or the molecular biology. In totality when you are designing an experiment, you actually require the submission of all the techniques what you are can do. Because when you are actually designing an experiment you actually require the help from the electrophoresis required and help from the chromatography.

Sometimes you require the help from the immunology and sometimes you might have to required even the assistance as well as the feedback from the molecular biology. So all these are actually separate techniques but they are integrative in nature because these techniques have to be come together, then only you can be able to perform the experiment. You can be able to designed the experiments with a more fine control.

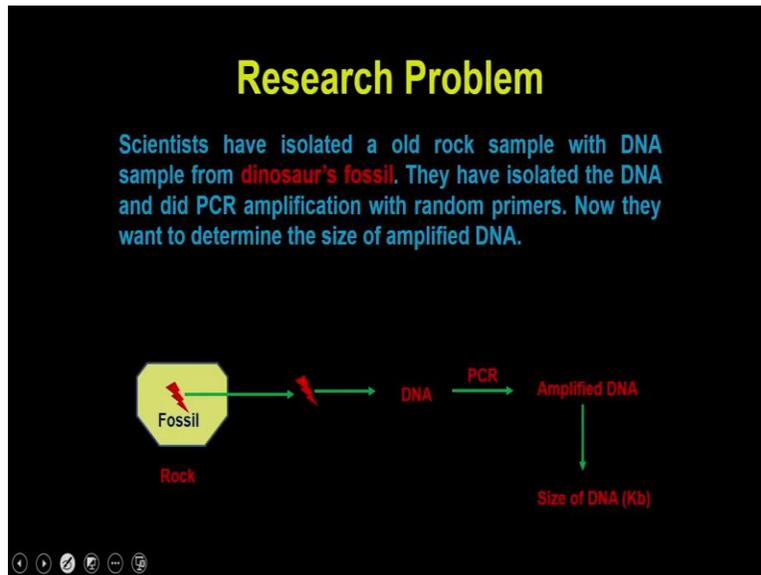
You cannot design an experiment simply with the electrophoresis or simply with the chromatography that is one aspect. The other aspect is that in some cases when you are actually

trying to answer the questions, but you do not have the electrophoresis. So what you can do you cannot just answer the questions. No, that is not the case when you have a question you have to actually answer that particular scientific question by designing an experiment.

What means because you do not have the electrophoresis system in your lab does not mean that you cannot be able to design an experiment that you can do simply by the chromatography or the with the help of immunology. That is what we are going to discuss now where we are going to take the similar problems from what we have discussed so far. But we are going to tell you that the same problem can be even handle with the multiple techniques.

So that you can be able to utilise these multiple techniques and you can be able to get the answer. So irrespective of whether you have the electrophoresis is in your lab or whether the chromatography in lab or not. You can still be able to design the experiments.

(Refer Slide Time: 26:08)



So let see this is the old technique where old such problems where the search problem is that the scientists have isolated old Rock sample with DNA sample from the dinosaur. They have isolated the DNA and did the PCR amplification with the random primers. Now, they want to determine the size of the amplified DNA. So if you remember while we were discussing about electrophoresis, we have taken this problem where what is the problem?

Problem is that the scientists have discovered a rock sample where they could find the dinosaur Fossil and when they extracted the dinosaur Fossil they could be able to get some amount of DNA as well. What they have done? They have done the PCR and then they got the amplified DNA and then this amplified DNA they are trying to identify the size of this DNA. Now you understand that this is actually one of the parts of this problem that you are actually simply going to run a molecular marker.

And it is actually going to give you a calibration curve between the size of the DNA versus The distance what it covered and then you can be able to utilise the amplified DNA you can actually calculate the RF values and it is actually going to give you the size of that particular DNA. But what I am trying to say is that in this particular problem you are simply using the one of the molecular biology technique which is called as the PCR.

So you are using a Molecular Biology technique, then only you are getting this amplified DNA then only you can be able to utilise the electrophoresis to answer the question. Apart from that you can also incorporate many other techniques so that you can be able to understand more about this particular DNA. For example, you got the amplified DNA first thing you have actually added the a tool from the molecular biology.

The second is once you got this amplified DNA then you can be able to do a sequencing with the help of either the Sanger's method or the Maxwell Gilbert method for you can actually get the sequence. Once you got the sequence then you can be able to in a clone this DNA when you got the sequence you can be able to do some different kinds of the reaction. So once you have done the sequencing what will happen you are actually going to get the sequence.

Now, once you got the sequence you can actually use the some of the Bioinformatics techniques. Like you can use bio informatics techniques and that is how you can actually take this DNA sequence blasted into the genome at that is actually going to tell you whether this sequence is actually; how much is it is close to some of the organism. It is actually going to give you closeness to its relative.

So that you can be able to identify what kind of dinosaur is this because that information is still missing because you got the dinosaur DNA and but that DNA you do not know what kind of dinosaur whether it is Archosauria or whether it is a dinosaur which was closer to bird whether it is a dinosaur which is closer to lizards or whether it is a dinosaur close to; those kind of information you will get when you are actually going to do a Bioinformatics analysis to test the DNA.

Apart from that once you got the sequence you can easily use and clone the sequence into expression vector. So you can use some of the molecular biology techniques you can do a PCR with the primers, The site specific primers and then you just clone it and that actually is going to give you the protein. Once you got the protein than what you can do is you can used immunological technique to generate the antibodies.

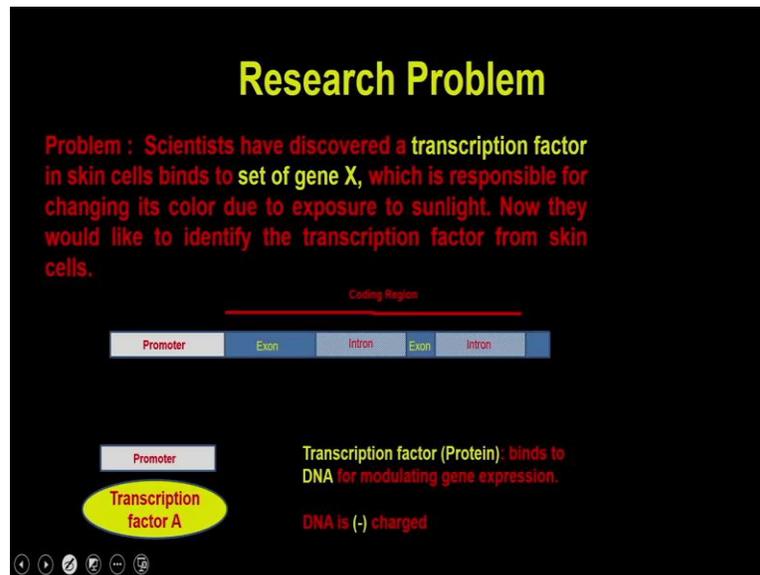
And once you got antibodies you are now going to have another kind of an tool which you actually can use. Apart from that once you got the protein. This protein can be asked from any other kind of experiments. Like you can usually ask whether this enzyme, this is the enzyme weather what could be the activity of the particular protein. Whether it is a structural protein or whether it is a functional protein like the enzyme and so on.

So that is actually going to tell you the potential of the different types of techniques what you can actually integrate in your experiment and that is how it is actually going to give you the bigger picture of what you have actually. So earlier we were just using the electrophoresis technique just simply doing the RF versus molecular weight analysis and you are just trying to identify the size of that particular DNA.

But now you have the complete information about that particular dinosaur what you actually having the DNA from. That is actually going to give you lot of informations and lot of insight into what kind of dinosaur and is apart from that if you have the Rock sample, also, you can easily calculate the age of that particular Rock and it is actually going to tell you the at what era of in which this particular dinosaur was existing.

So that is actually the way you should actually planned experiment integrate all other techniques and that is how you can going to get global picture and the full picture about the particular type of problems.

(Refer Slide Time: 31:40)

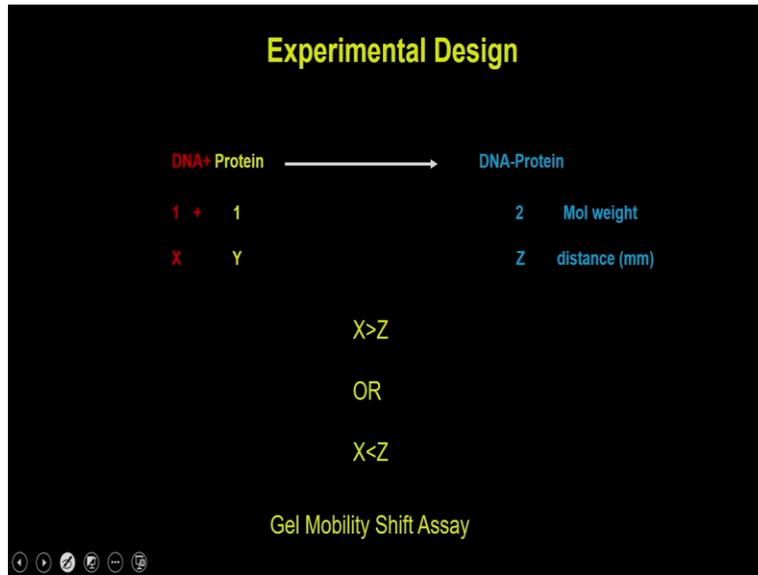


Now we have the other problem that is scientists have discovered a transcription factor and a skin cell by the set of gene X which is responsible for changing its colour due to the exposure of light so that to the sunlight. Now, they would like to identify the transcription factor from the skin cells. So these are things we have discussed when we were discussing about the electrophoresis.

And what we said is that you have actually a promoter region. So if you talk about gene. The gene has two region 1 is the regulatory region which is called as the promoter. The other one is as the coding region, which is actually been coding for a particular type of protein. So if it is a functional protein if it could be a structural protein for sometime the gene does not code for any other protein as well.

So this transcription factor is going to bind this regulatory region and that is how it is actually changing the activity of the production of this particular enzyme. So what you are supposed to do if you are actually going to do the interaction analysis of the DNA region with the protein and that you can do with simple assay which is called as the gel shift assay.

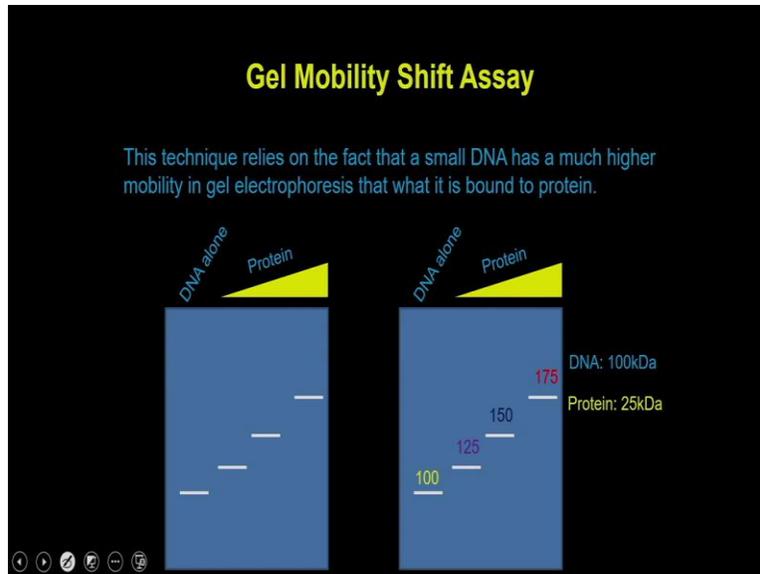
(Refer Slide Time: 32:55)



We discuss that in detail about the gel shift assay how what is the basic principle of the gel shift essay how the DNA and protein when they are present in a ratio of 1 is to 1. How that is actually going to give you the resultant molecular weight, which is 2 and how that resultant Molecular weight is actually going to reduce its electrophoretic migration on to the gel. So you can imagine that if the DNA is X Molecular weight or Y molecular weight.

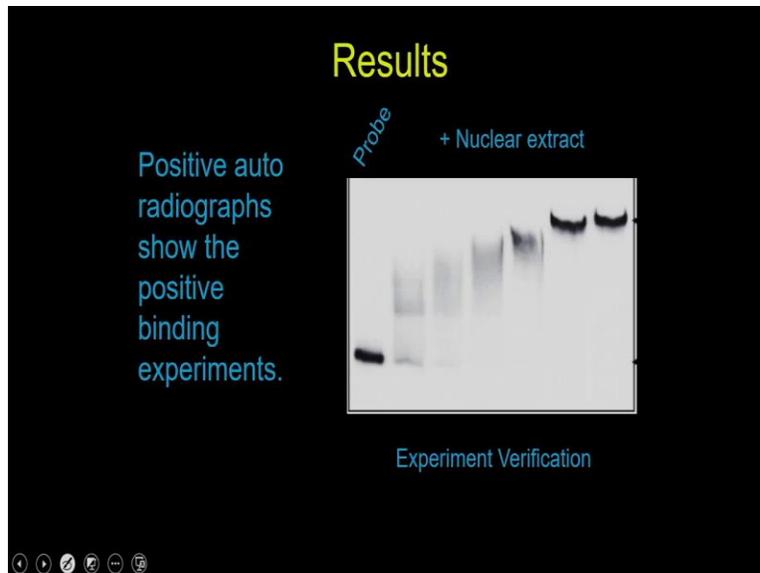
So the DNA will run for the X distance the protein will run for the Y distance and the DNA protein complex is going to run for the Z distance then in that case the access going to be the bigger on the smaller to the Z. In this case the X is going to be bigger to the Z because Z is going to be of high molecular weight because instead of 2 units where as the DNA is going to be of 1 unit. So the Z is going to is going to be less electrophoretic mobility compared to the DNA alone.

(Refer Slide Time: 34:02)



And this is what we were doing when running the DNA alone or we are incubating the DNA with the different amount of protein. So what you see is the DNA alone is going to have the higher electrophoretic mobility. And when you are adding the protein electrophoretic mobility is reducing and that is how you are actually going to have the shift in the electrophoretic mobility.

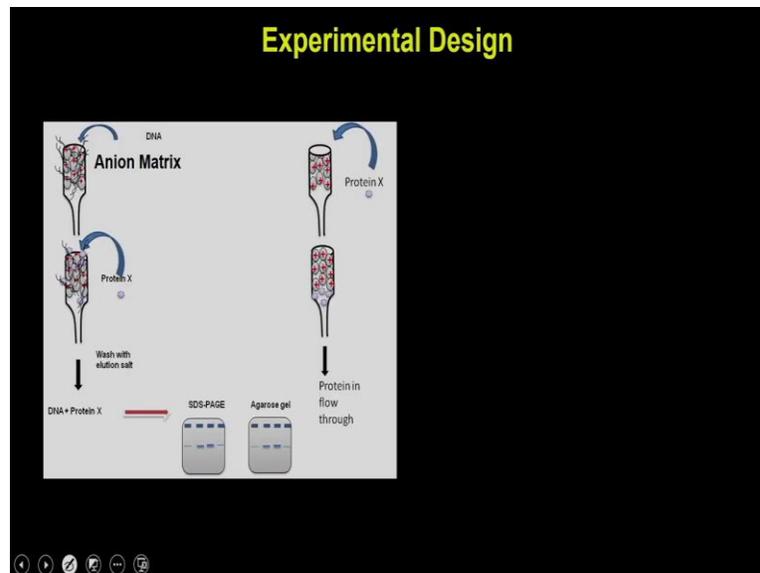
(Refer Slide Time: 34:25)



Results what you see is something like this where you have the DNA alone and then in if you are adding the protein it is the actually the shift of the DNA from the; so this is actually going to show you the electrophoretic mobility. And this is actually going to give you the less electrophoretic mobility. And if you require you can actually do some of the verification experiments were you can add the unlabeled probe and that it is actually going to competitor for

the labelled probe. And that is how you are actually going to labelled probe coming out from the reactions.

(Refer Slide Time: 35:00)



Now, if you want to do the same experiment, you can also use the Ion exchange chromatography and that also be discussed. In Ion exchange chromatography what you are going to do if you just take this promoter region or you take the promoter of this particular gene and then you use you know you a couple it and to the Anion matrix and Anion matrix is going to bind the DNA. And then what you are going to do? if you are going to put the protein X.

In such a way that the protein X itself is going to have the zero charges, which means the you have to add the protein X onto its PI value, which means if you add the protein X to its PI value the protein X is going to have the net zero charge because that is very important that protien X should not bind to the column directly. So in that case what you are going to do your first going to bind the DNA onto the column.

And then you are actually going to flow the protein X at a place where the protein X should not have its own charge. And then what will happen the protein X will go and bind to DNA not to the column and ultimately you are going to elude. If you are going elude that any of you analyse the profile what you can see is that the protein is going to follow a pattern the DNA is going to follow the path and onto the horizontal gene.

And if these two patterns are matching with each other then you are going to say that these gene proteins are matching with each other and actually the protein is binding to the DNA because the protein itself does not have any affinity for the matrix. So the protein can only bind to the matrix if it is interacting with the DNA. As a verification experiment, you can still do some of the verification experiment, like you can add some of the DNA degrading enzymes or you can actually change the pH and all that.

This is what you have to do. If you have to do like gene promoter you have to clone then you have to do the Ion exchange chromatography. You have to do the Agarose gel electrophoresis, and you have to do the SDS-Page. What you can see is that we are using the electrophoresis approaches. We discuss that how you can use the electrophoretic approaches to answer the questions. How you can be used in Chromatography techniques and how the same approach can be used even to answer the same questions.

Now what the way it is going to be different. In the electrophoretic technique you are only going to get the semi quantitative values. Like you are going to see whether the protein is binding to DNA or not whether that is causing a shift into the band or not but you could not be able to get an quantitative information. So it is a semi quantitative or the qualitative information what you are going to get from the electrophoresis.

Whereas from the Ion exchange chromatography what you can also do if you can also get information whether the DNA and protein are interacting with scissors or not. On the other hand you can also get the some of the quantitative information. Like you can get the distribution at the binding constants you can be able to do all those kind of analysis as well. As you can see even when we are doing the Ion exchange chromatography, you still require the other techniques like SDS page as well as the Agarose gel electrophoresis to complement your experiment.

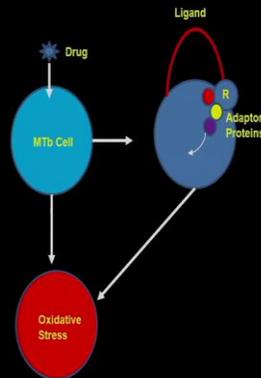
So that is why I just said not a single technique is on its own it requires the assistance from the other techniques to complete the experiments.

(Refer Slide Time: 38:35)

Research Problem

Mycobacterium Tuberculosis

H37Rv was treated with drug and it causes generation of oxidative stress inside the bacterial cells. The ligand responsible for this effect was isolated and now PhD student wants to identify the adaptor proteins from *Mycobacterium tuberculosis H37Rv* to understand the signaling events and associated molecular components.

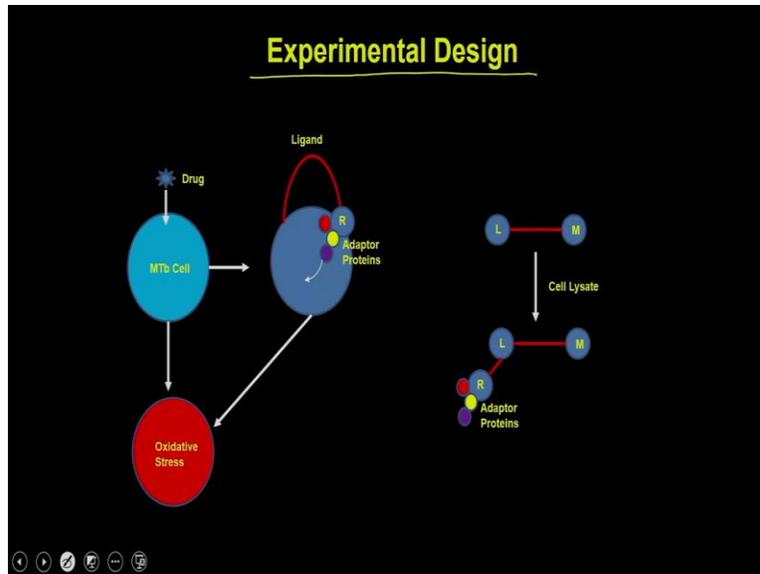


Now we have the third experiment which is actually mycobacterium tuberculosis was treated with the drug and it causes the generation of the oxidative stress inside the bacterial cell. Now the ligand responsible for this effect was isolated and now the Ph.D students want to identify the adaptor protein from the mycobacterium tuberculosis to understand the signaling events and the associated molecular components.

Now, what is the problem? Problem is that you treated and mycobacterium tuberculosis the infectious organism, which is responsible for TB and once you done that it is actually generating a ligand and that ligand is going out and then it is binding to it is binding to receptor this receptor. Once it is binding to its receptor this receptors is bringing proteins and then because of these adaptor proteins it is actually driving a downstream signaling.

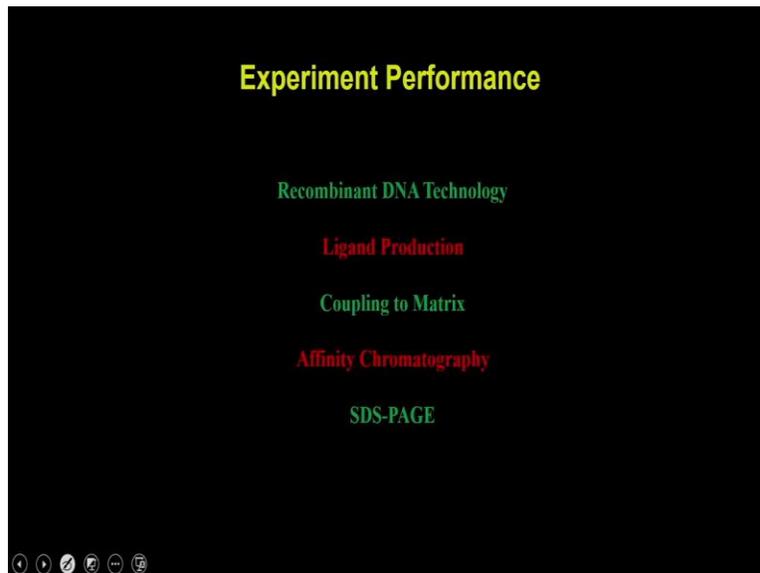
So what the student wants, he wants to identify this adaptor proteins. And if you remember we have used some of the techniques where you can be able to answer this questions.

(Refer Slide Time: 39:46)



But in this technique we have discussed with said that you can just simply make a column where you can actually put the like, Onto the matrix and then these used as this as a scaffolding protein and I knew if you in Kuwait with the cell lysate, it is actually going to bind the receptor as well as the adaptor protein and then you can actually isolate these components and you can be able to answer the questions. What are the adaptor proteins are present? And that is actually going to give you the complete information about the adaptor proteins.

(Refer Slide Time: 40:19)



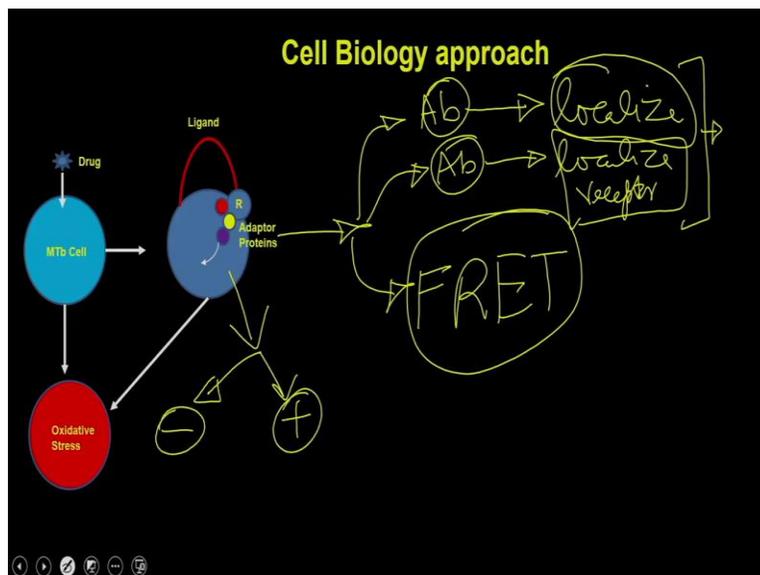
This are the tricks what you require the Recombinant DNA technology, you can have ligand production, you required the coupling to the matrix, then you require the affinity chromatography and then you require the SDS page to answer the question. Now, let us see a

different angle through which you can be able to even answer the similar questions, utilising the cell biology techniques. In the Cell Biology techniques what we can do if we can easily look for two things.

One when the ligand is binding you can actually first characterized that and also once the adaptor proteins are known like if you hard doing that experiment, it is actually going to tell you the information about the adaptor proteins. But they are false positive what is actually going to come because when you are processing the samples and you are processing the whole cell lysate the some of the protein which may not be adopted protein are actually also going to unofficially bind to your bead.

So, how to get information about this? So what you can do if you can simply take the adaptor proteins and you can actually do a Cell Biology techniques are you can actually first utilise the immunological techniques to generate antibodies if antibodies are already available from the commercial vendor. Then it is fine. Otherwise you can generate the antibodies and process the things like that.

(Refer Slide Time: 41:43)



So imagine that you know the adaptor proteins now, what you can do if you can simply use the adaptor proteins and generate the antibodies. Now these antibodies you can use to localise the adaptor proteins and you can also use the antibodies to localise **the** your receptor. Now once you

do that and they are actually going to be a adaptor protein of that particular receptor. They are actually going to give you the colocalization which means the signal is going to come out from the same point.

That is one thing. The second is once you have the adaptor proteins and once you have the you know the receptors. You can also plan an experiment and suppose you have the antibodies for the adaptor protein as well as the antibodies for the receptor then you can also design an experiment which is called as the fluorescence resonance energy transfer. In the FRET experiment it is actually going to tell you the interaction between the different types of proteins what is present within the cell?

And that information is going to be more and more authentic and more and more accurate compare to that when you break open the cells and trying to purify the protein or trying to verify the factors with the help of the sum of the affinity chromatography technique that is actually going to give you the opportunity even for the non specifically bound protein to interact. Third is you can also use some of the proteomic based approach as well to answer the same kind of questions.

Because what will happen if that what you have 2 condition one you have the minus drug conditions you have the plus drug conditions. So what you can do if you can simply take the to cells. One, it is control cell the second is that drug treated cell and then you what you do is to just break open the cell lysate and you prepare the; you can just simply take out the membranes from these two bacteria. And then I had ask for what are the differential different proteins are present.

For example in the control cells suppose you are isolated the membrane and you got the 40 spots ok and in this one suppose you got the 47 spots. So what you can do if you can simply do a image analysis and see what are the separate 7 spot are coming up and whether those 7 spots are could be a potential adaptor proteins. So than what you can do is you can just exclusively identify those 7 proteins or 7 spots trying to identify the proteins with the help of the proteomic approaches and you extract and get the information about those 7 protein.

And you can be able to utilise that information to cross verify whether those additional 7 proteins could be adaptor protein for the particular receptor or not. So this the way we should actually integrate multiple approaches multiple techniques so that you can be able to answer different questions and you can be able to understand the whole process in detail.

(Refer Slide Time: 45:15)

Research Problems

Protease PFI1625c was cloned from Plasmodium Falciparum 3D7 and PhD student wants to identify the Substrate peptide sequence to understand its role in parasite metabolism and to design potent inhibitors.

peptide

PFI1625c

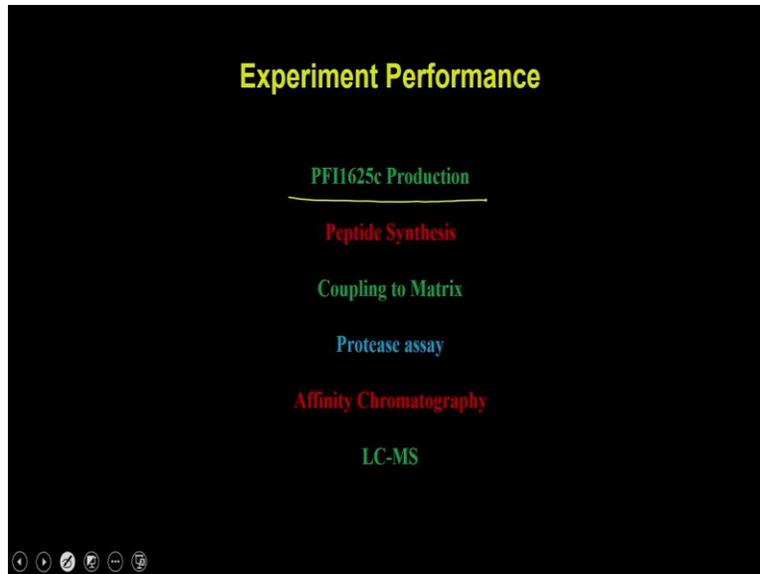
peptide

P4 P3 P2 P1 P1 P2 P3 P4

Then we also discuss about this problem where we said that the protease PFI 1625 was cloned from the plasmodium Falciparum and all that and you want to identify the substrate sequence and he said that you can actually use a peptide coupled and coupled to the beads and then if the process will digest it is actually going to generate the different peptide sequences and then you can actually do this peptide sequence you can do a multi mass.

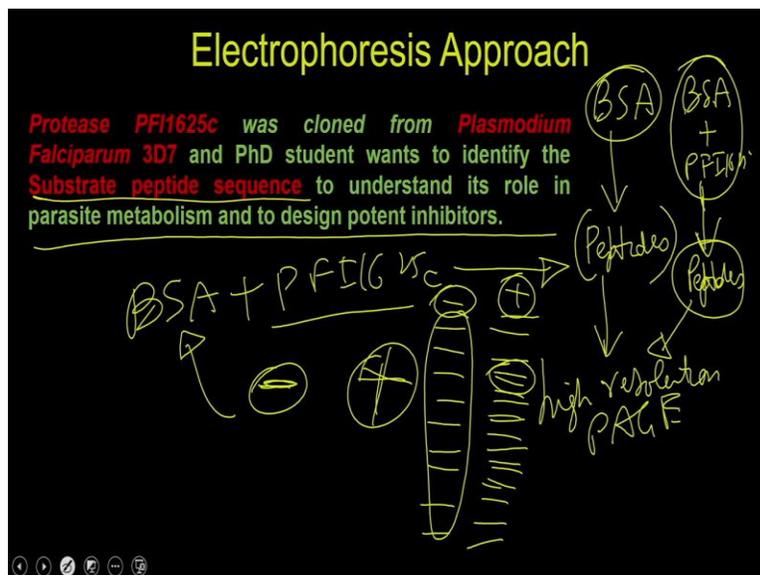
And that actually is going to tell you what is the left over onto this beads and because of that analysis you can be able to do the peptide sequence.

(Refer Slide Time: 45:54)



So in this one you are going to do a protein production, peptide synthesis, coupling to the matrix, protein assay, affinity chromatography and ultimately you are going to do the LC-MS and then what you are going to get? You are going to get the peptide or the peptide sequence what this protein is cutting but at the end it is not going to tell you the potential substrate what is present in the cell.

(Refer Slide Time: 46:21)



Now if you want to do the same experiment and you want to do with the help of the electrophoretic approaches what you can do also is? Suppose because this one it is only going to tell you the peptide sequence it is not going to tell you the potential substrates. So what you can do is also or what alternatively you can do also is. Suppose. I take the BSA ok and I will

incubator BSA with PFI 1625 C which is a protease present in the plasmodium Falciparum and then what I do is I am going to have to reactions when place where the protease is absent the place where the protease is present.

And now what I am doing to do is? I am going to do the incubation with the BSA in the presence and absence. So you are going to have to reactions. One the place where you have the BSA other the place you are where you are going to have the BSA plus PFI 16256 C. Now, what I will do is? I will allow these two reactions to run for different amount of time, for example every 3 hours for 4 hours I will start taking out the reactions.

So what will happen is it is actually going to start giving you the peptides because irrespective of that you are not adding a protease into a BSA structure or sequence since you keep the protein into the water. It is actually going to go through with the autocatalysis or auto lysis. So because of that some protein is going to be degraded in due course of time. So you are going to get the peptides from here. Also you are going to get the peptides.

Now what you are supposed to do? You can take these two sequential order is two samples and then you separate them into using the high resolution page. And what will happen when you do that you are going to get the peptide sequences present in both the structures and eventually definitely when you are having the no protease versus the presence of protease the number of band what you are going to see is more compared to this one.

So you are going to if you do a image analysis and if you do a subtractive analysis and also if you are going to see that some of the bands are actually common between them where as the some of the bands are actually reappearing and the new bands. Now once you see that these bands are actually reappearing and the new bands then what you can do if you can simply take out these bands from the gel with the help of the isoelectric illusions or you can be able to use some other technique to extract this protein bands.

And then you can simply go with the proteomics approaches at identifying the peptide sequence. Now this peptide sequence, so if you support this is a protein right if it has been chewed up for

multiple places by the PFI 16256C. This is the peptide what you are going to see into the sample. So once you have the ends of this particular peptide. So if you use the protein Sequencing method for the proteomics approaches, you are actually going to get the sequence of this peptide.

So once you get the peptide sequence and you are not going to get one peptide sequence you are going to get the multiple peptide sequences, you will be able to deduce from where this particular peptide is coming off because you already know the sequence of the BSA. So, from there you can say ok this is the place I am getting this is the place I am getting this means you are going to get not only one peptide you are going to get the multiple peptides.

And that so if you do all that analysis, it is actually going to tell you the preferences of this particular protease or the sick site what it actually required to cut the proteins and that you can be able to deduce not only the Peptide sequence which is reported which is actually prefers to cut the protease by the protease or it also going to tell you whether the BSA is a standard substrate or whether BSA is going to be a substrate for this protease or not.

I am just giving example with BSA but if you require you can actually use some other proteins also from the host cells or from the parasites cells. So, this is all about the course experimental biotechnology so far what we have discussed we discuss about the different types of techniques. We have also summarised you good lab practices what we should not do and what should do as well. At the end we also discuss about the some of the different experiments and how the multiple approaches can be used to answer these questions.

So with this I would like to conclude my lecture here and I hope you will actually understand the content and I hope that you will be able to follow these experiments. And I hope that the discourse is going to advance your work. So with this I would like to conclude my lecture here. Thank you.