Genetic Engineering: Theory & Applications
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Module XII
Summary and Conclusions
Lecture No 39
Summary and Conclusions (Part – II)

Hello everybody, this is doctor Vishal Trivedi from department of biosciences and bioengineering IIT, Guwahati and in this last module what we were doing, we were discussing about the, we were discussing about what we have discussed so far in different modules. So so far we have discussed about up to the module 6 and in today's lecture, we are going to discuss about the remaining course and the purpose of this revision is that it should refresh your memory and as well as it will help you to prepare for your exam in due course.

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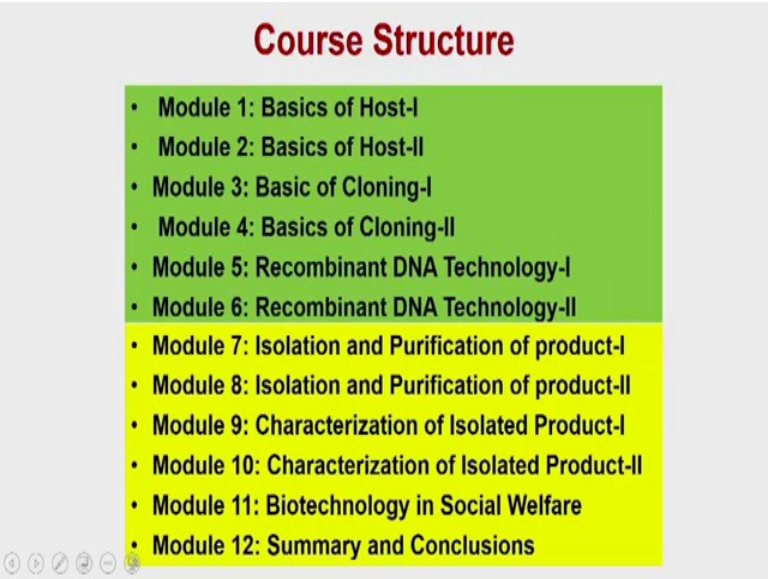

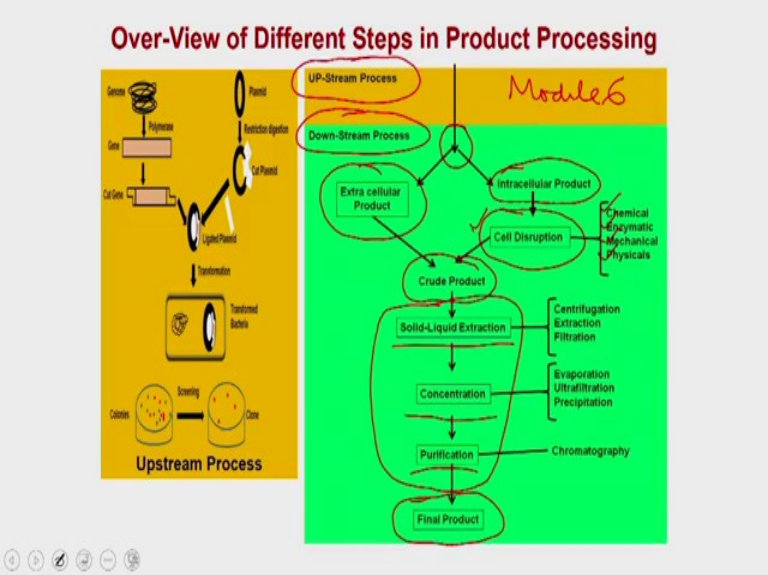

So what we have discussed so far up to the up to the module 6 is that you have isolated a protein, we have first understood about the basics of the host so that you will be able to choose the appropriate host for your protein productions. Then we have discussed about the strategies, how you can isolate the different types of genes, different genes from the different sources. Either you use the genomic library, CDNA library or the you can use the PCR as a approach to isolate your gene of your interest. Then we have also discussed about the different types of molecular tools which are available for manipulating the DNA, whether it is restriction enzymes or DNA ligases or alkaline phosphatases or we have also discussed about the molecular tools like linkers or the adapters.

The by the use of these tools, you could be able to integrate the recombinant your genes of interest into the vector of your choice. So we have also discussed about the different types vectors whether it is a vector for the cloning purposes or the expression purposes and then we have also discussed about the different types of vector, whether the vector for we have the plasmids for the bacterial expression system or we have the vectors or the yeast expression system or we have the vector for the bacteriophage expression system, insects align expression system or the mammalian expression system.

And in all these system we have also discussed about how you can have the expression in a (constitute) using their constitutive promoters or the inducible promoters and once you have the cloned and over express the protein into the these hosts cells, all these procedures are coming

under the preview of the up-stream processes and once you protein is present inside the overexpression inside the host's cells then you have to get this protein out or the other secondary metabolites of out from the cells and then you have to processes these cells so that you will be get the purified product for your downstream applications and that is how these processes comes under the preview of the down-stream processes.

So once you have the protein or the factor what you are over expressing in your hosts cells. You have 2 conditions, in condition number 1 it could be extracellular products or it could be a intercellular product. For extra cellular product you do not need to do anything except that will do clarifications and then you are going to get a crude product which is going to have the some more contaminants and then you can go for different types of procedures. If it is intracellular product, then the first step is that you have to do a cell disruption and we have discuss about different types of cell disruption whether it is the chemical method, enzymatic method, mechanical method or the physical method.

After you have done the cell disruption you are going to get the crude (pro) crude product either from the extracellular product or the intracellular product. Once you got the crude product then you are going to employ different types of fractionation techniques, whether it is the liquid-liquid, soli-liquid extractions or concentrations or purifications and finally you are going to get the purified product which you are going to use in down-stream applications. So following the down-stream processes, we have discussed many of these steps. So we started with the cell disruption methods.

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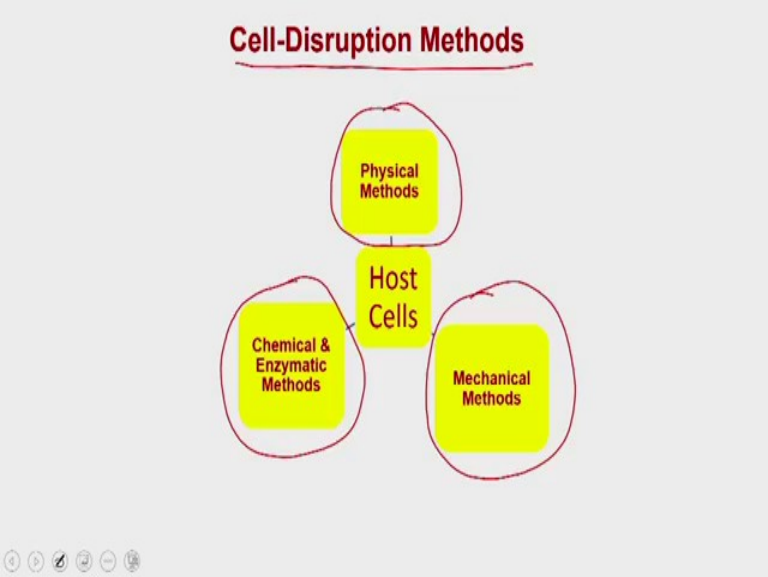

So in the cell disruption method, we discuss about the 3 different types of cell disruption method. Whether it is the physical method, chemical or the enzymatic method or the mechanical method and all these methods are being used depending on the type of host cells you are using or host cells you are using for overexpression of your protein. If it is a plant cell, you are going to use 1 set of cell disruption methods. If it is a bacterial cell, then you are going to use a separate set of cell disruption methods or if it is a animal cells then you are going to use the other method of cell disruption method.

In addition to that it also depends the kind of product what you are expressing in yours in your host cells. If the product is thermostable then you have a different set of cell disruption methods. If it is temperature sensitive, then some of the methods may not be suitable for cell disruptions. On on the other hand, when you use the cell disruption method and suppose your product is present inside the mitochondria then you also have to see that the product should not get damage. So the purpose of cell disruption, all the cell disruption method is that it should disrupt the cells, it should release your product but it should not denature or it should not damage the products.

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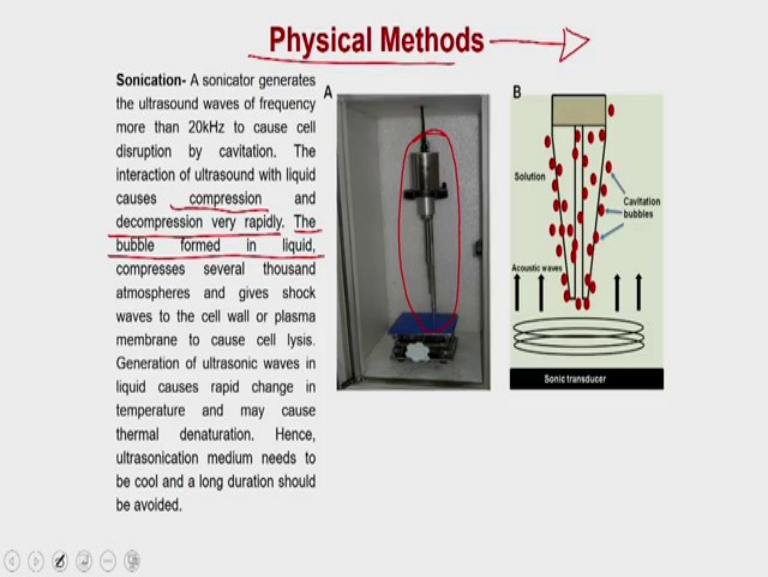

So we, we we studied many of the physical method. We have discussed about the thermolysis, we have discussed about the osmoticlysis, we have discussed about the sonications and so on. So in the sonication we we have given you we have even also even shown you a demo we have where we we have we have I have I have taken you to my laboratory and we have shown you how to a sonications. So sonication is a very very powerful cell disruption method and it actually generate the sonic waves and because the sonic waves heats the cells, it actually generates pressure difference within the cell and that actually actually lyse the cells.

So you can see that you have a sonic probe in sonic probe you can actually put your sample into this sonic probe and then the sonic probes are going to generate the sonic waves and these sonic waves are actually because of the compression and decompression of your liquid very rapidly because the sonic waves are going to heat the liquid and that actually will cause the compression and decompression very rapidly. The bubbles are going to be formed in the liquid and because there is a compression and decompression there will be a there will be a change in pressure within the liquid and that change in pressure is actually going to lyse the cells.

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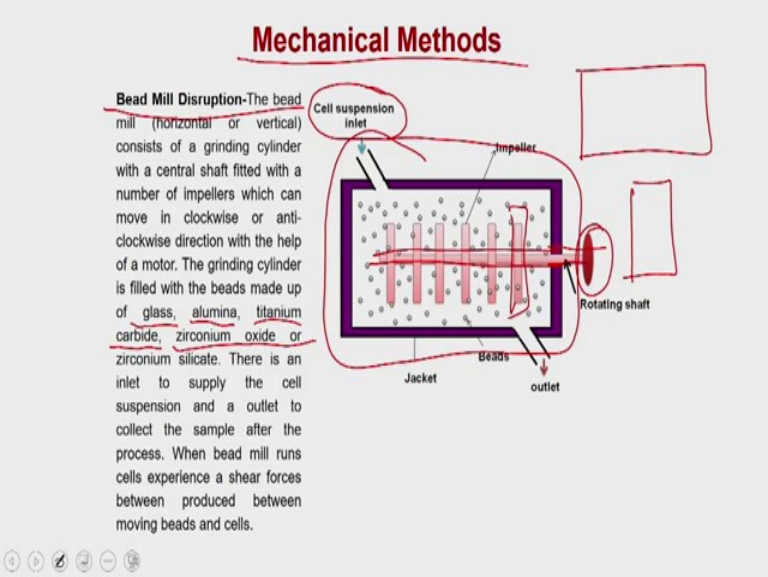

Then we have also discussed about the mechanical methods. So we, we have discussed about the high pressure homogenizer and we have also discussed about the Bead Mill (homo) Bead Mill disrupters. So Bead Mill disrupter or the high pressure homogenizers are being used in in those conditions where the host cell is very sturdy. For example, you can use them for the yeast cells or you can use them for the plants but mostly people do not use the use the mechanical methods for the, for the animal cells or for the bacterial cells.

So in the typical Bead Mill disrupter what you have is it is it could be in the vertical direction or it could be in the horizontal direction or in the vertical direction and one of the classical example which closely resembles to the (blead) Bead Mill disruption is that you might have seen the cement mixer. So it is actually exactly the similar kind of thing and what you have seen is that it has jacket and within the jacket you have a shaft, on this shaft you the propellers are been attached. So these propellers when they moves, they actually mix the beads so you can add the different types of beads and then you put the cell suspension. This is exactly what you might see when you the when you see a (cell) the cement mixer in in any of the construction site, so it, so the process is also same.

Then this shift shaft is connected to a motor so you can have the flexibility of adding running this into the clockwise or anti clockwise and once it runs, actually it gives the motion to these beads and then these beads actually runs over the cell. And when the cell passes through these beads it

the the beads actually creates a pressure difference because of that the cells are going to be disrupted. Depending on the type of cell, you can use the beads of glass, alumina, titanium, zirconium and so on and so depending on the type of cell you can use the beads of different sizes, you can use the different material and so on.

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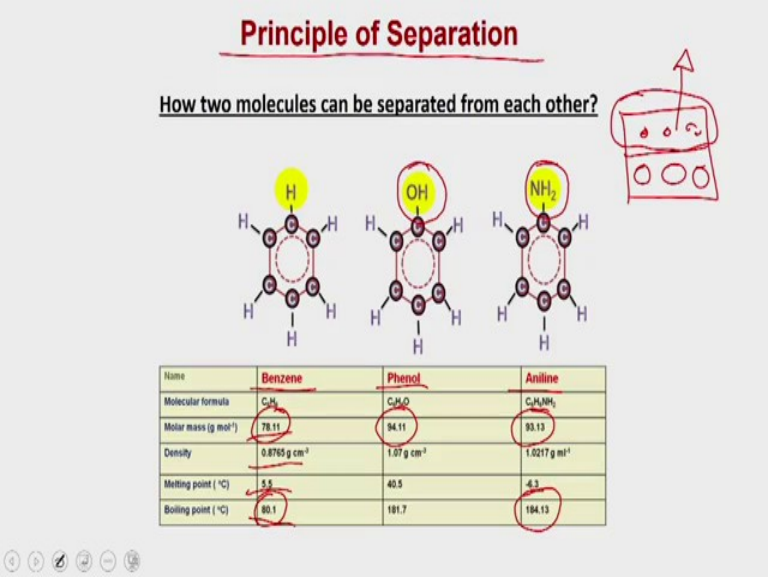

So once your product is coming out, whether you are producing the product into a extracellular media or whether the product is intracellular, once you have done the cell disruption method, the product is going to be present in the extracellular media and then you will do a clarifications and then you are going to have a clear cell lysate or clear extract of your bacterial cells or the hosts cells and that actually going to contain your, your factors, whatever you want to purify. So subsequently to that module we have also discuss about the different types of purification techniques. But before getting into detail of purifications we have discussed about how the separation works and what is a principal behind the separation techniques.

So for explaining the principle of separation techniques, we have take an example of 3 molecules. One is called Benzene, Phenol and Aniline and what were what we have discussed is that what you see is that all these 3 molecules are very different from each other in terms of the many properties. For example, their their mass is very different. Benzene is 78.11, phenol is 94.11 and the aniline is 93. Their densities are very different, their melting point is very different and their boiling point is also very different. Apart from that, the all these 3 molecules are having

the different functional groups which are attached to the core benzene ring. So if you use or you if you exploit these physical, chemical methods, for example in the case of protein it becomes even the more more complex because there you can use even the more properties associated with the biological properties as well.

So if you use the chemical methods, if chemical properties, physical properties or biological properties you could be able to (separ) you could be able to distribute the molecules between the two phases and that is the basic principal of separation which means if you have the 2 material, within a small beaker or something, then what your purpose is that you do some kind of changes in this environment, in such a way that one material should remain in one phase, the other material should go into the separate phase. If you if you could be able to achieve that, then you are actually going to achieve the purification.

Because in this particular phase, your X molecule is going to be present, in the other phase it is Y molecule is present. And if you remember, following to this we have also taken an example of the distillation. How the benzene is being separated from the aniline, simply by the distillation because their vapor pressure is different. So the benzene is tend to be more, so because the boiling point of benzene is only the 80 degree whereas the boiling point of aniline is 184. So when we have taken an example of distillation, we have shown that the benzene is, if you heat the, this solution which actually contains the benzene and aniline.

The benzene has a more tendency or more affinity towards the vapor phase. Whereas the aniline actually wants to remain in the liquid phase. So if you do a distillation, what will happen is the benzene will evaporate and it will be get condensed into a separate flask, whereas the aniline will remain into the original flask. So if you repeat the distillation multiple times, you will be able to separate that these kind of 2 molecules which are liquid-liquid but they are having a difference in terms of their boiling points.

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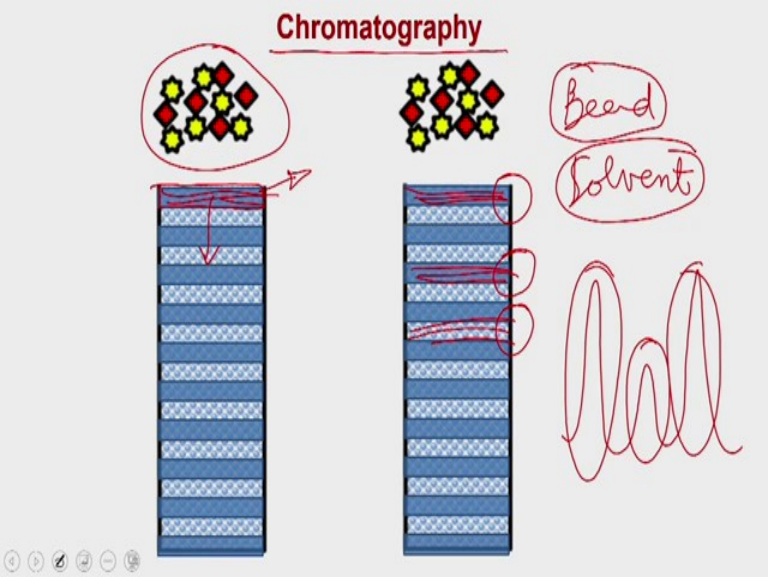

Following to this, I have also discussed about how the separation works even in the in the in the case of chromatography. So you can imagine that these are the 3 different types of molecule which you have loaded onto that column and column is nothing but a chamber where you have the beads of different sizes or beads of the functional groups present. So we can imagine that, the each block is actually one layer of beads which is present. So what will happen is when these molecules will interact to these beads they are actually going to distribute themselves either to the bead phase either to the bead phase or to the solvent phase.

So the molecule which will remain toward the solvent phase it will be keep migrating towards the lower side, whereas the molecule which will have the more affinity for the beads will is going to immobilize on to the first layer. So if you lower these molecules, the molecule will distribute whether they want to remain with the bead fraction or whether they want to remain with the liquid fraction. So if they want to remain with the bead fraction, they will go and bind to the first layer. If they want to remain with the liquid phase, they will go and bind they will remain in the liquid until they will find the place where they will like the bead fraction.

And that is how what you will what will happen at the end is that some molecules are going to be immobilize here, some molecules are going to be immobilize into the second layer or third layer and some molecules are going to be immobilize into another layer and that actual actually going to happen because they have the different affinities for these beads. And when you will do the

when you will change the conditions the, this molecule is going to be eluded first, this molecule is going to be eluded second and this molecule is going to be eluded separate. And that is how you are going to have the 3 individual peaks which is corresponding to these molecules which are present, which are being immobilized onto the beads but at different positions. And that is how you are going to achieve the purification of X, Y, and Z and that is what we have discussed in detail when we were talking about when we were when we were talking up the how the distribution phenomenon is working within the chromatography.

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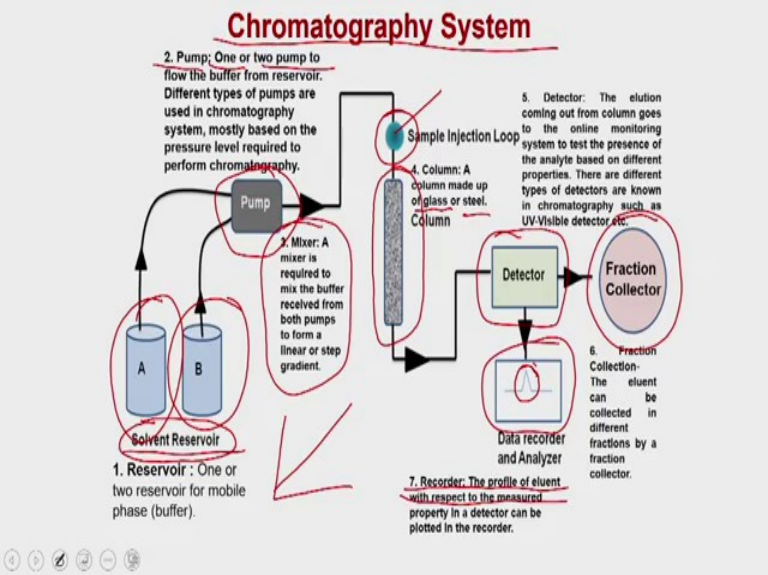

Following to this we have also discussed about the different types of chromatography systems. So we started very simple with the solvents reservoirs. So for a typical chromatography system you need these are the following components. You need the solvent reservoirs which means you need to have the reservoir for A pump, you need reservoir for B pump. Then these are the 2 reservoirs are going to be connected to a pump and the pump either it will be 1 pump or 2 pump are going to be present onto the purification system depending on the kind cost you are going to invest in to this purification system, the kind of the purification you require from these systems or the sensitivity.

And the pressure of these pumps are also going to vary whether you are looking for, whether you would like to perform the chromatography of a low pressure chromatography, middle pressure chromatography or the high pressure chromatography. Depending on that requirement, the

pumps are going to be of different types. Then next to the pump you are going to have the mixture which is actually going to mix these 2 components so that you will be able to make a gradient between A and B

Either it will be the linear upward gradient or the downward gradient which means either you will increase the (()) (18:05) of the B or you will decrease the (()) (18:08) of the B. And in many of the chromatography techniques both are these procedures are been followed. Following to this mixture you are going to have the sample injection ports. Through this sample injection port you are going to inject your crude mixture. Once the you will inject the crude mixture that is going to be applied on to the column and the column depending on the pressure system, the column could be of made up of glass or the steel.

And next to the column you are going to have the detector. The detectors job is to detect the flow of the biomolecules. The detector could be of different types and there are different types of detectors in the chromatography system depending on the what kind of properties you would like to monitor. Either you would like to monitor only the UV visible (spectro) UV visible phenomenon, so then you can have the UV visible detector. If you want to detect the fluorescence the you can have the fluorescence detector. If you want to detect the the molecular weight, then you can have the mass spectrometer and so on. So depending on the detector the cost of these purification system also will go up. The detectors job is to give you the pattern of the elusion of these molecules from the column and that will be present that will be going to show you in the form of recorders.

So recorder is nothing but a computer screen which is going to show you the pattern of that particular purification profile. And depending on which profile, suppose you want to collect this particular peak then you can ask the system and it has going to have a fraction collector. So fraction collector is going to start collecting this particular peak in the in the form of different fractions. So you can it can you can give the instruction that I want to have 1ml fractions. So it will start collecting 1ml, 1ml fractions and collect all this hill peak. So this is all about the chromatography system.

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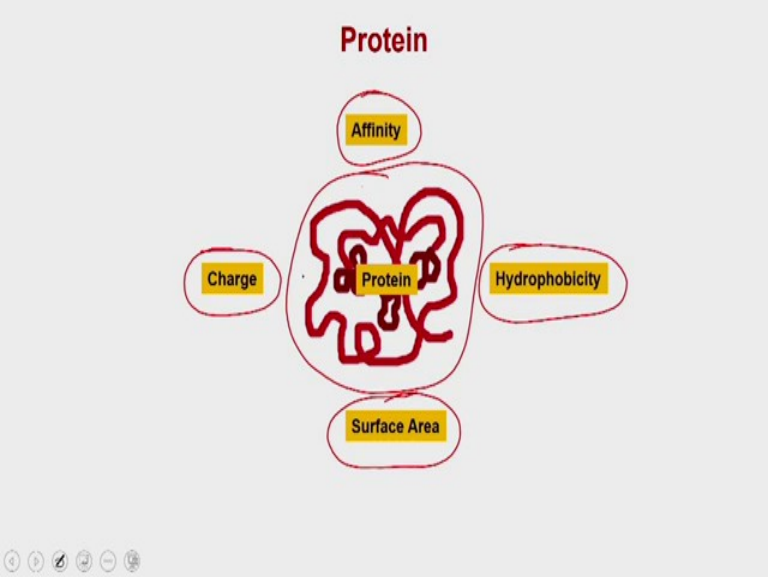

After discussing the chromatography system, we moved on to the different types of chromatography. Different types of chromatographic. So if you if you working with the complex protein mixture or the different types of proteins they will be going to vary in different properties. They are going to have different level of charge present on their surfaces. Because the proteins are made up of amino acids. So they can have different types of charge, they can have different types of hydrophobicity, they can have the different types of surface area and they can also have the affinity which is actually going to be exploited in different types of chromatography techniques.

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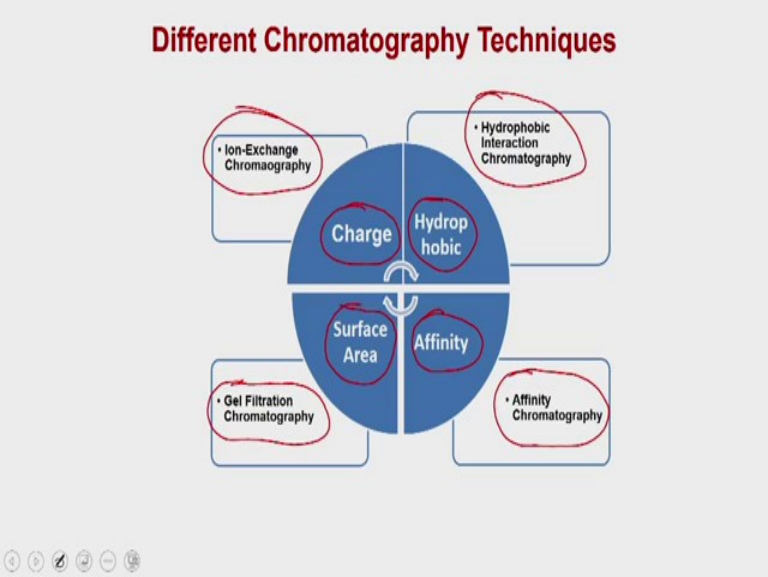

So that is what we have decided. So if the protein is having a charge you can actually use the chromatography technique that is called as the Ion Exchange Chromatography. The protein has the hydrophobic patches. Then you can use the chromatography technique that is called as the Hydrophobic Interaction Chromatography. If the protein is having a different surface area or the hydro dynamics volumes then you can use the Gel Filtration Chromatography.

And at the end, if the protein has any kind of affinity to the biological molecule or to the non biological molecules which you can use then you can use the Affinity Chromatography. So in this particular module we have taken up each and every affinity (chromat) each and every chromatography techniques. We have discussed each and every chromatography. How to perform that particular chromatography, what is the application of that chromatography technique and so on.

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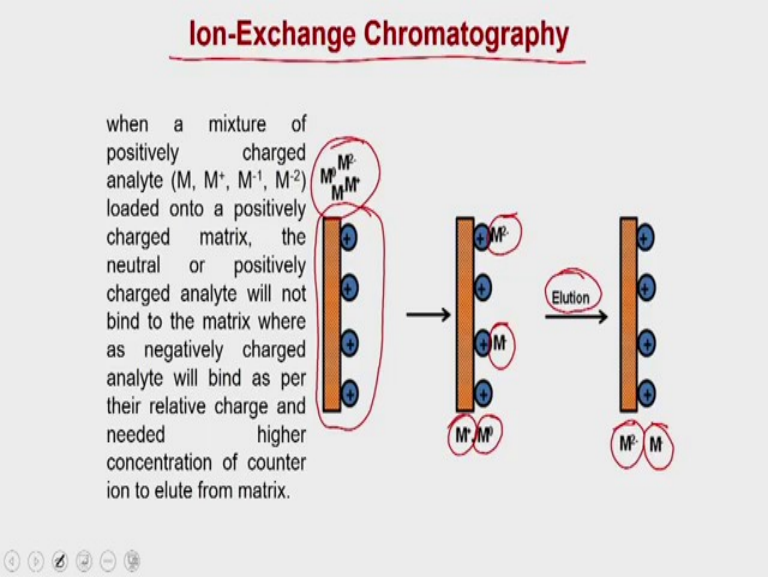

So what we have discussed that. So we started with the ion-exchange chromatography. So in the ion-exchange chromatography we took a simple example that suppose you have a mixture of the 4 proteins containing a charge of M0, M2 minus M plus and M minus and suppose you have used a matrix which is positively charge. So what will happen is all these 4 molecules when you inject the matrix, what will happen is M2 minus will going to bind to the first layer whereas M minus is going to bind to a lower level, whereas M plus will not going to bind because already the matrix is also positively charge.

Whereas the M0 which is does not contain any charge is not going to bind this particular matrix. And after that what you will do is, you will do a elusion which means you change the condition in such a way so that and in that case what will happen is the M minus will come out first and the M2 minus will come out later because M2 minus has 2 negative charges so it will have a higher affinity to this particular matrix compare to the M minus which is actually going to have 1 unit of negative charge.

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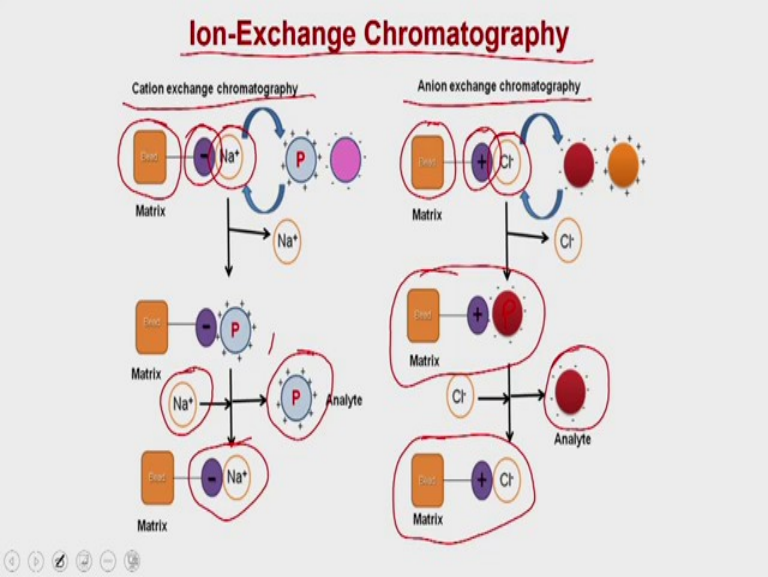

So ion-exchange chromatography can be of 2 types and whether it is a cation exchange chromatography or to the anion exchange chromatography. Now for the cation exchange chromatography what you are going to have? You have a matrix, you have a negatively charge functional group onto that it has the sodium immobilize which is positively charge. So what will happen is, you have to keep all the positively charge protein are going to replace the sodium and in that process the protein is going to bind to these particular beads.

And then what you do is, you supply the very high concentration of sodium or different concentration of sodium and as a result what will happen is the protein is going to be eluded from the matrix and ultimately the sodium will go and bind to the, this functional group which is present on the beads. So that is the cation exchange chromatography. Whereas in the anion exchange chromatography, the bead is going to have a functional group which is actually positively charge and it is going to have the chloride as a immobilize ion.

And what will happen is that your protein is going to be exchanged with this chloride and as a result the protein is going to bind to the to the matrix and when you will do the elusion with the help of the chloride ions, the chloride ion are going to elude the analyte from the column and

ultimately the chloride ion will again bind. So in this, why the ion exchange chromatography is called as ion exchange?

Because you are either using the cation as a exchanger or the anion as an exchanger and that is why the protein either it is positively charge or the negatively charge can be purified using the ion-exchange chromatography. If you remember when we were discussing about the ion-exchange chromatography, we have also discussed bout the iso-electric point and how the iso-electric point and the running buffer conditions could be modulated to run cation exchange or the anion exchange chromatography simultaneously for a single protein.

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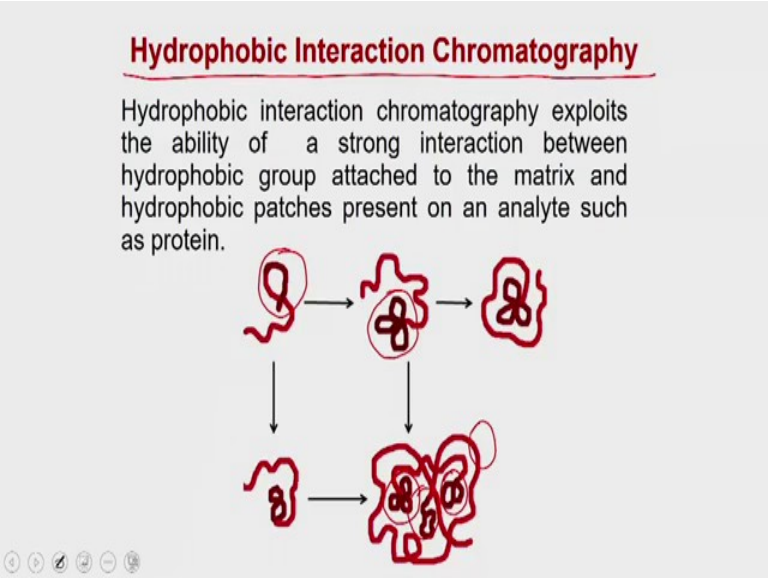

Then we moved on to discuss about the Hydrophobic Interaction chromatography. Hydrophobic Interaction chromatography exploits the presence of the hydrophobic patches which are actually going to be formed when the protein is actually folding. So when you can imagine that is has a hydrophobic patch and when the protein folds it actually keeps all these hydrophobic patches into the interior core whereas outside it is actually hydrophilics. So the purpose, so the question is how you can actually get the hydrophobic patches out of the protein so that you will be able to exploit this into the purification.

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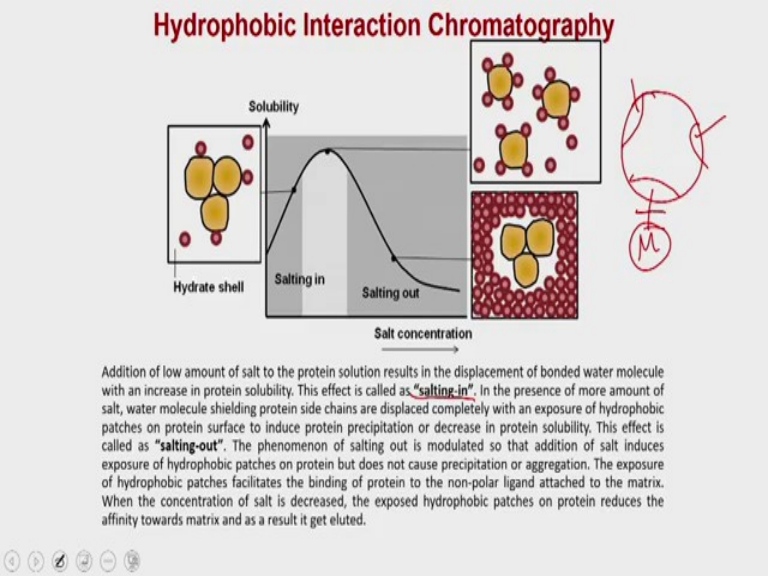

So what happen is that, if you protein, if you expose the protein to a small amount of salt that actually removes the the water molecules which are present into the hydration shells and because of that, it actually increases the more space for a protein to get solubilized and as a result it actually increases its protein solubility. And that effect is called as the Salting-in effect. But if you increase the salt beyond that point then it starts removing the (pro) water which is actually protecting the hydrophobic patches which is present into the protein.

And as a result the hydrophobic patches which are present onto the protein are also going to be exposed partially or fully and then in these conditions, you can be able to use the hydrophobic interactions chromatography because now the hydrophobic patches are exposed and you can use a matrix which is actually having the hydrophobic groups attached to it and then it will actually going to bind. And how you going to elude, you actually going to bring the hydration shells back. And once you bring the hydration shell back, the interaction between this matrix and the functional group is going to be broken down.

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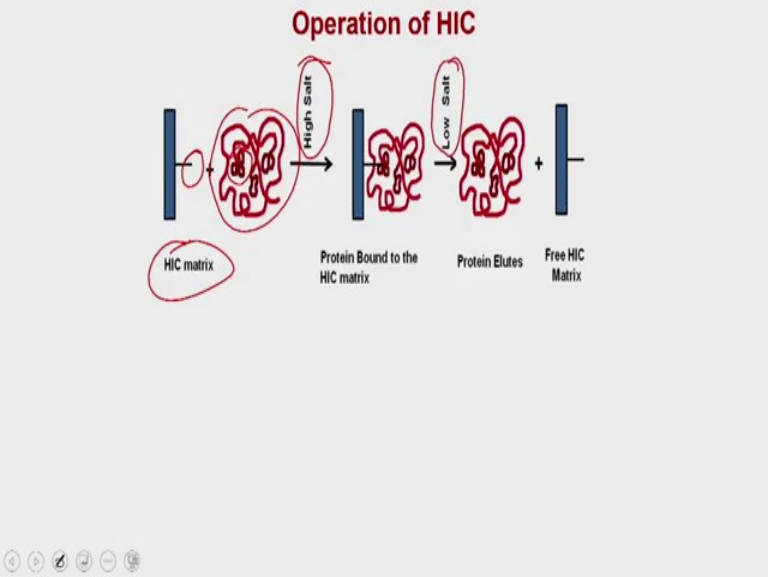

So this is what is exactly happen. Most of the HIC matrix are going to have a functional group, mostly the aromatic functional group such as benzene or phenyl group. And these phenyl groups are going to have a very high strong (interact) affinity for these hydrophobic groups which are present onto the protein. But the protein will not bind until you keep the high salt concentration. So in the high salt concentration, this functional group is going to interact with the hydrophobic patches which are present onto the protein and once it bounds then you wash and then you elude with a low salt solution.

And once you change the condition to the low salt, the hydrophobic patches are again going to be masked by the water molecules and that is how it is actually going to bring, break the interaction between the matrix functional group as well as the aromatic hydrophobic patches present on to the protein. So if you remember, I was telling you that the gradient mixture is actually allowing you to make a upward gradient which means increase in B as well as in decrease in B.

So this is an classical example where you are actually going to reduce the salt concentration, which means you are actually going to load the protein in the condition of 100 percent B and then you are going to reduce the B in such a way so that it is actually going to elude the protein from the column. Whereas exactly the reverse you will do when you are going to have the ion-exchange chromatography.

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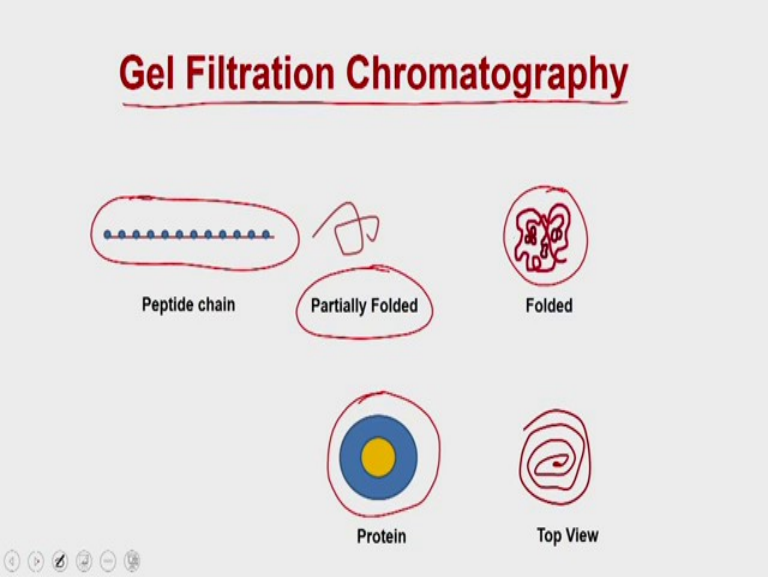

Next to that we discussed the Gel Filtration Chromatography. So in the gel filtration chromatography we explained you how the protein is actually folding. So protein is being produced as an extended peptide chain, then it gets partially folded then it get completely folded and as a tendency the protein actually keeps the hydrophobic core into the center and the hydrophilic molecules on the corners. And as a result if you see a protein molecules it actually arranges it is amino acid around a particular center and because of that most of the globular proteins are actually forming a balls of different sizes.

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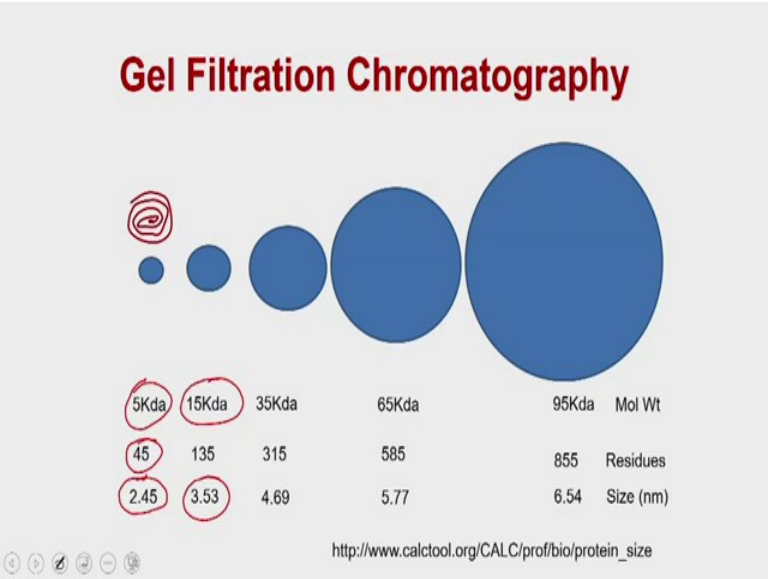

And if you want to see how it happens that if you have a protein of 5 kilo Dalton, you are going to which is actually going to have 45 amino acid residues. It is actually going to have the hydro dynamic size or the diameter of that particular ball should be of 2.45 nanometer. Whereas if it is a 15 kilo Dalton protein, the size would be 3.35 centimeter. So what you will see is, that as the protein size is increasing the volume or the diameter of this ball is also increasing. So this is actually mostly applicable only for the globular protein not for the fibrous protein. If you want to know the examples of the globular or the fibrous protein, the globular protein classical example is hemoglobin. The fibrous protein the classical example is our hairs.

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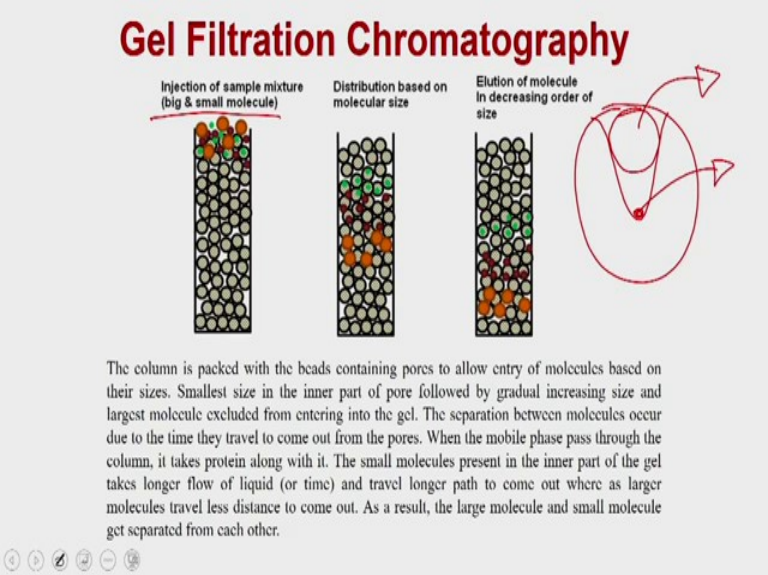

So because of this if you inject a big or the small molecules into a gel filtration matrix, what will happen is that this matrix are having a funnel shape pores onto the surface and because of that the small molecules are going to sit at the bottom of this pore. Whereas the big molecules are going to sit onto the top of this pore and as a result when they will elude, this molecule is going to be elude first and this molecule is going to be elude at the end. So because of that, the small molecule and the large molecule are going to be separated from each other and it is going to give you the the small molecule later on, (small) big molecule first.

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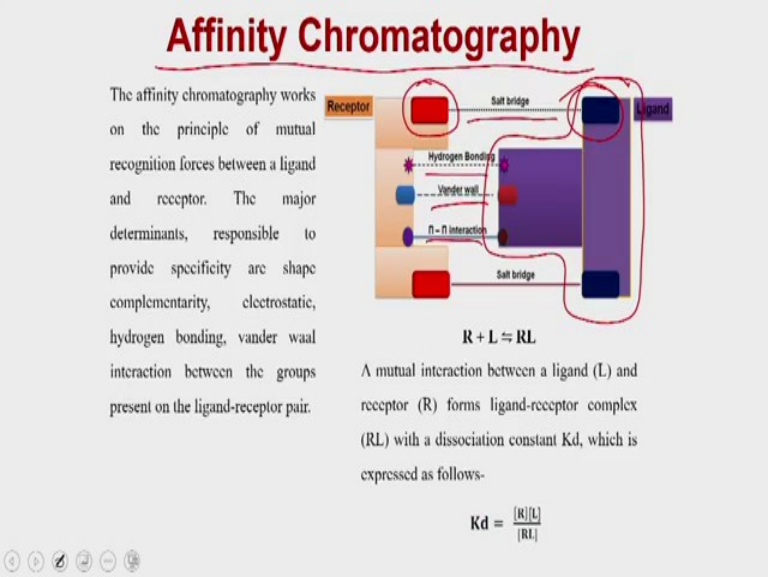

After that we have also discuss about the affinity chromatography. We have discuss that affinity chromatography means the the interaction between the ligand and the receptor. So affinity chromatography is based on the principle of the receptor and ligand. It believes that there is a receptor then there is a ligand. And all these receptor and ligand interactions are very specific. They are very specific simply because you have the shape complementarity playing in a between the receptor and the ligand.

Then you have the different types of functional group which are present onto the receptor and the ligand which is actually be not going to present in any other combination of receptor and ligand. So suppose you have receptor for insulin, then it is insulin actually going to satisfy all these interactions, whether it is the salt bridge interactions, hydrogen bonding interaction, vander wall, pi-pi interactions. All these interactions are going to be met by the cognate ligand not by the any other ligand. And because of that the receptor and the ligand are going to be very very specific.

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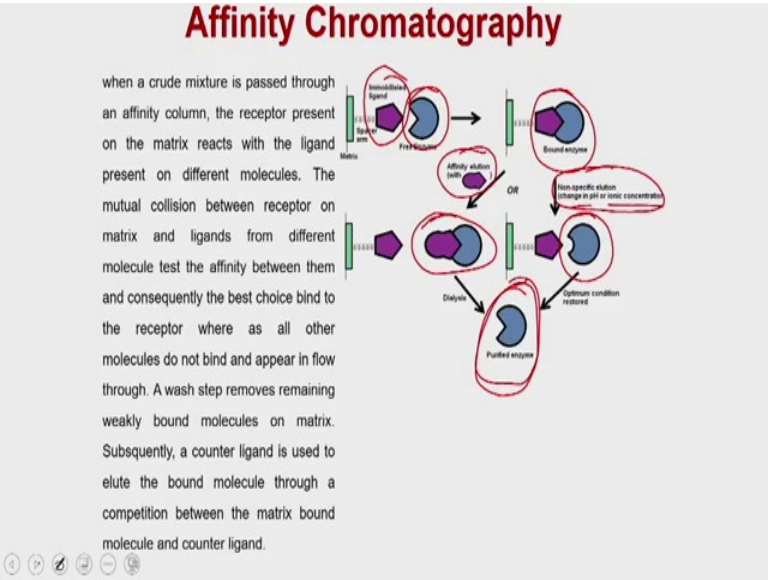

So exactly what is this? So you have an option of immobilizing either the ligand or the receptor. So if you immobilize the ligand, suppose this is a receptor so this is an enzyme which has an affinity for this particular ligand. So what will happen is, you have coupled the ligand to the matrix and the enzyme will go and bind to this particular ligand. Then you you can add the competitors which means you can add another molecule which will have a higher affinity for this particular enzyme.

And as a result what will happen is the enzyme will go and bind to this particular ligand, which is in the solution compared to the ligand which is present onto the matrix. That is called as the specific elusions but if you want to do a non-specific elusion then you can actually play with the interactions which are playing crucial role in making the interaction between this ligand and the receptor. And you can change the ph or you can increase the ionic strength and ultimately either you use the specific affinity elusions or you will use the non-specific elusions, you are going to get the purified enzyme.

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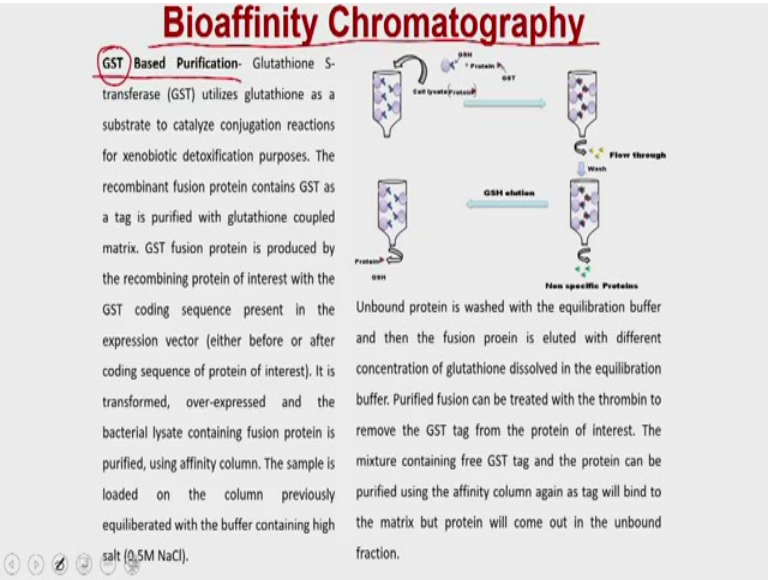

We have discussed different types of chromatography (tech), different types of affinity chromatography we have also discussed, how you can be able to produce the receptor or the ligand, we have also discussed about that how you can produce the antibodies and you can purify the antibodies, utilizing the same affinity chromatography technique only and then we have taken couple examples of bioaffinity chromatography.

We have taken Pseudo affinity chromatography examples and so on. We have also shown you how to perform these chromatography techniques. So for that purpose I took you to my laboratory, we have shown couple of demos of how to do the gel filtration chromatography, how to do the nickel NTA chromatography and we have also take you to the our laboratory to show you how a protein purification system look like and so on.

So in this is this is a classical examples of bioaffinity chromatography, where we have taken an example of GST, GSH complex and where the GST is the receptor and the GSH is the substrate. So in this case we are talking, we are taking an example of the enzyme substrate as a pair instead of the receptor and ligand. And you can have the you you couple GST to your recombinant protein and that is how you can be able you use the this particular bioaffinity chromatography to purify the protein of your interest.

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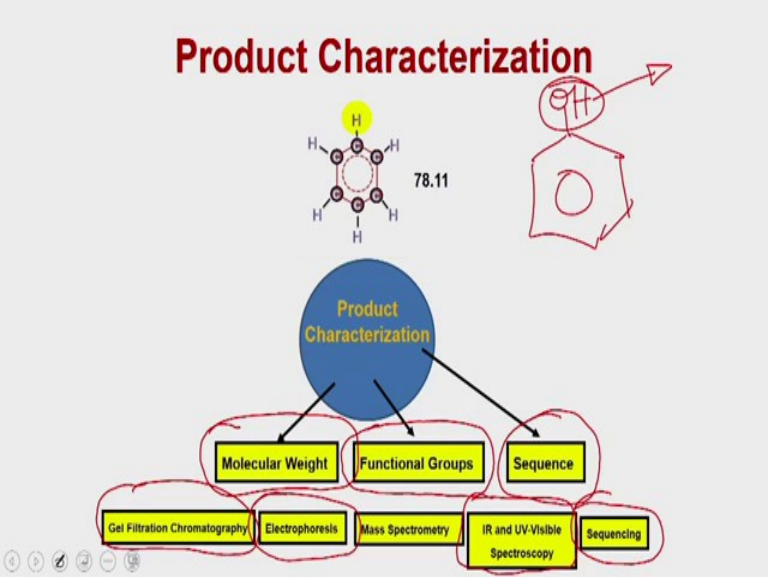

Once your product is purified then you are going to have the product. But the product is useless until it is not going to be characterized. Which means you have to do a lot of quality testing to know that this product what I am purifying is of high standard, it is the same product what it should be and so on. And product characterization is always being done by a set of the parameters. For example, once as soon as you produce the product, you are going to calculate the molecular weight. Then you are going to calculate or you want to see the functional groups.

For example, if I am making a phenol then I will definitely want to see that it is phenol, it is not benzene. So I will go for and see whether there is a there is a phenyl group present onto this or not or there is a OH group present onto the phenol or not. And then ultimately if you are talking about the protein or the DNA, I would I also want to know that it is not only important that it has a functional group, it also should have the sequence in which these molecules or the monomeric units are being attached.

Because the protein may have the same amino acids but if you change the sequences it is going to change the overall protein. So for the molecular weight determinations, we discuss about the gel filtration chromatography, we discuss about the electrophoresis and for the functional groups, we discuss about the UV visible and IR spectroscopy. Whereas for the protein sequencing we discuss about the sequencing techniques, Edman degradation methods or and other methods.

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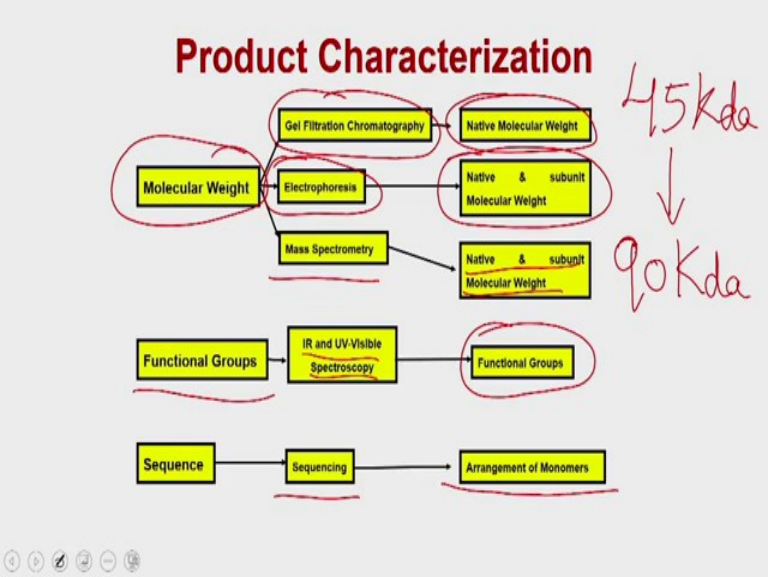

So this is the for example the molecular weight. We discuss about the gel filtration chromatography which is actually going to give you the native molecular weight. Electrophoresis, you have the choices, it will give you the native or the sub unit molecular weight. So if you do not understand which means what is mean by the native or the sub unit molecular weight, for example you have a protein which is of 40 kilo Dalton or 45 kilo Dalton but if this protein is being formed by 2 chains of the same protein, which means it is a dimer.

Then under the native condition, the same protein is going to give you a molecular weight of 90 kilo Dalton. Then the mass spectrometry that actually will give you the native or the sub unit molecular weight depending on the type of ionization source you will use. Then for the functional groups you can use the IR or the UV visible spectroscopy and that will give you the information about the functional group. It will tell you whether this particular molecule has the characteristic absorption spectra or not because that characteristic absorption spectra only will come when the molecule will have the sequence of molecules or sequence attach or the different monomeric amino acids been attached in a particular sequence.

And then we also discuss about the sequencing techniques, so that will actually will tell you the arrangement of monomer because that is very much relevant in terms of the biological molecules such as protein or DNA. Because the protein and DNA are the polymer of amino acid or the

nucleotide but the sequence of amino acid or the sequence of nucleotide is very very important for the functional outcomes of that particular proteins or the DNA.

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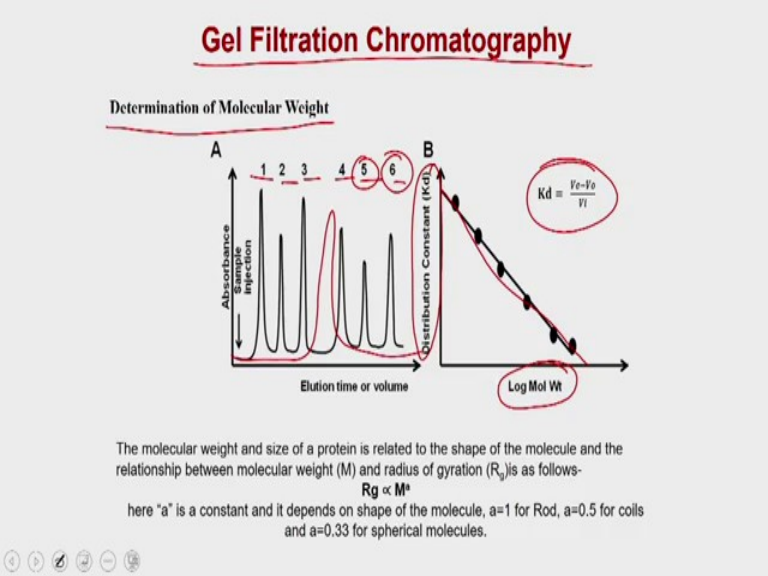

So we discuss about the gel how you can use the gel filtration chromatography to determine the (cal) molecular weight. In this example what we have said is that you run the first the proteins of known molecular weight like for example you run all these 6 proteins for which you know the molecular weight and you calculate the elusions volumes and taking the elusion volume and the molecular weight, you calculate the distribution coefficient and then you plot the distribution coefficient along with the log molecular weight and that will actually will going to give you a calibration curve and then you will do the your unknown protein and exactly the same exercise you will do. You will going to calculate the distribution coefficient for your unknown protein as well. And using the distribution coefficient and using the, this calibration curve you could be able to calculate the native molecular weight using the gel filtration chromatography.

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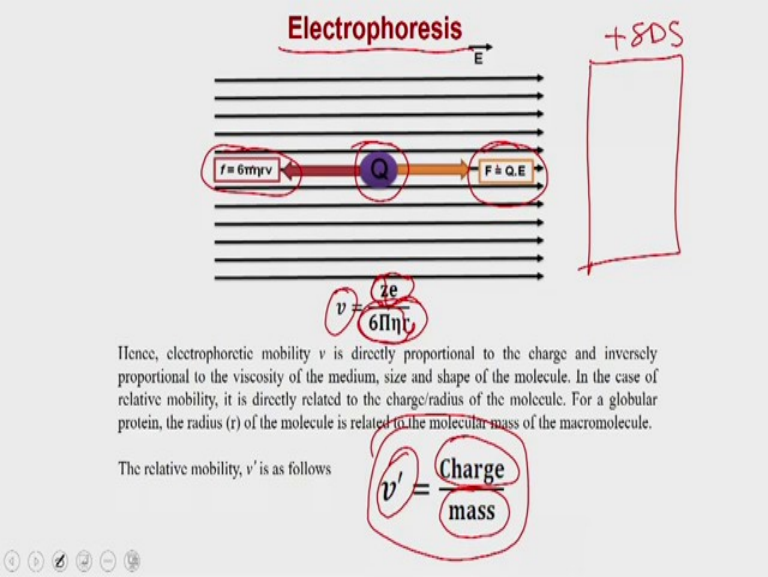

And then we have also discuss in detail about the Electrophoresis. What is the basic principle of electrophoresis and so you can imagine that you have a charge molecule which actually is running into a electro electric field and if suppose this electric field is also having a jelly like material, so in the presence of electric field it is going to have the electro motive force which is equivalent to Q E, whereas because there is a gel present or there is a viscous material present, it is going to have the frictional forces which is equivalent to 6 pi eta r v.

And the molecule is going to be immobilized or it will not going to migrate at a place where this force is equivalent to this force and under these circumstances the electrophoretic mobility is going to be proportional to ze by 6 pi eta r and if you see that this particular value is constant which means the relative electrophoretic mobility is directly proportional to the charge and inversely proportional to the mass.

So using this particular type of equation you can be able to design the electrophoresis in 2 different mode. In in one mode where you are not going to play with the intrinsic charge of that particular molecules and in those cases the electrophoretic mobility will be at the basis of charge by mass ratio. But if you keep the charge constant, then the electrophoretic mobility will be inversely proportional to the mass of the charge. Which means, if you are going to run the protein under the presence of the SDS which is actually going to make the charges equivalent, the small molecule is going to run faster, the large molecule is going to run slower.

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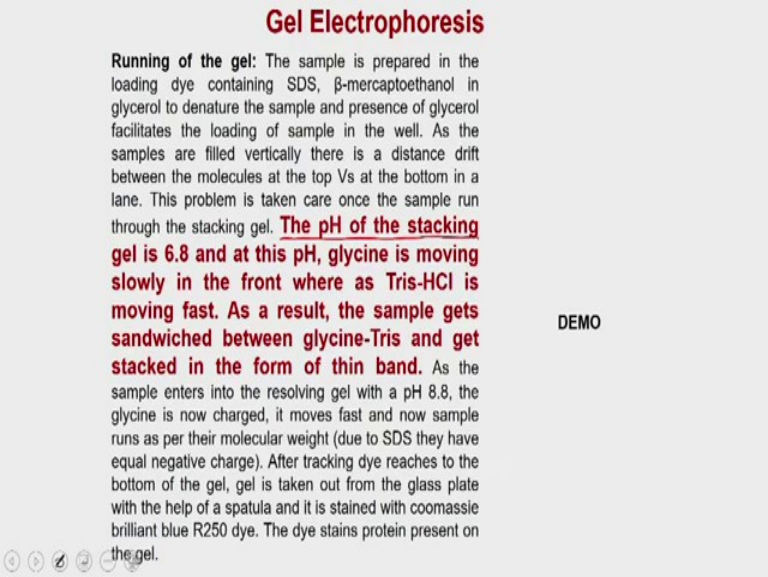

Apart from that we also discussed about the many types of technical details. For example, why you need a stacking gel, why you need a dissolving gel, what is the purpose of different reagents in the stacking gels and why you keep the ph of the 6.8 when you are running a stacking gel and so on. And we have given you a very very in depth and detailed demo how to perform the electrophoresis technique while we took you to our laboratory, where we have shown how to cast the gels, how to resolve the samples, how to prepare the samples, how to stain it and how to distain and then afterwards we have also discuss about the image analysis as well.

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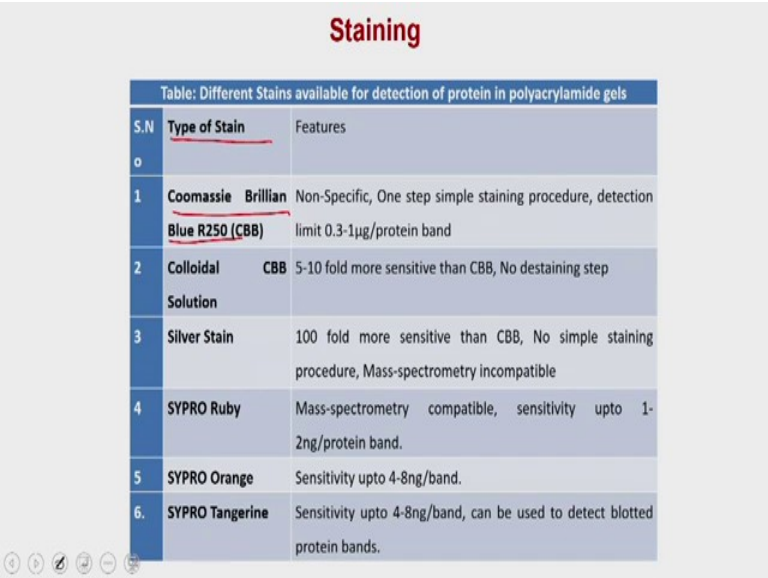

So then we discuss about different types of stain which you will can use. So you can use the Coomassie Brillian Blue R250 or all other stains and they, the purpose of these stains are very very different. And depending on the type application you would like to explore, you can use any of these stains.

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So this is just a classical example of what will happen if you stain a gel, dye, gel with the Coomassie Brilliant Blue. It is going to be a black sheet. But once you done with the de-staining step, what will happen is the dye is going to be removed from all those places where the protein is not present and as a result you are going to get a very nice contrast and as a result you are going to see a protein bands onto the SDS page.

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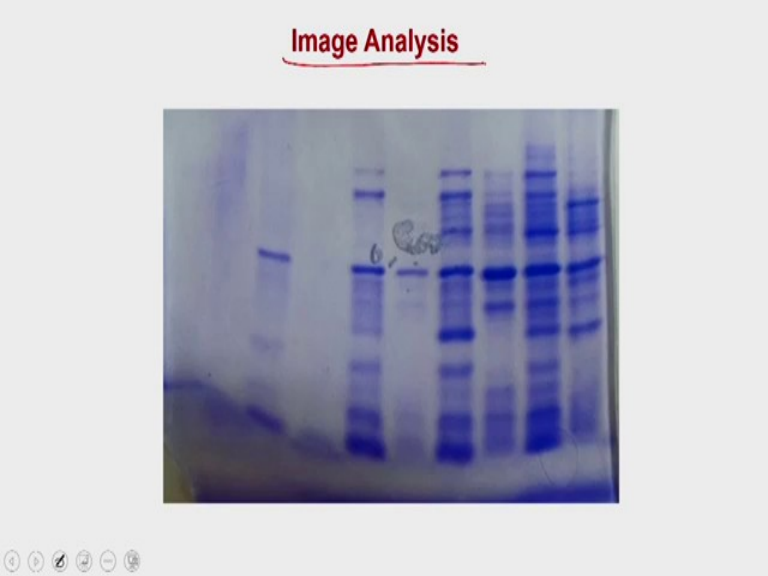

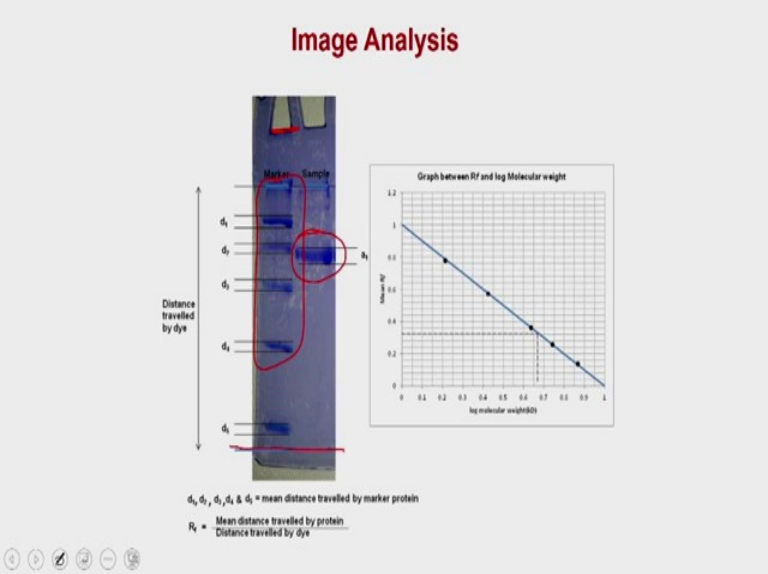

So then we also discuss about different types of (dep) in depth analysis of image analysis using different softwares and as well and the point that we have covered while we were discussing about the image analysis is that how you can use the image analysis to calculate the molecular weight of the unknown proteins. So what for that what you are going to do is, you are going to run a molecular weight marker where you are going to have know the molecular weight of these proteins or the you will run the known molecular weight proteins. Then based on the distances from the well and the dye front you can be able to calculate the RF values of all these different

proteins. And then the same exercise you will do for your unknown protein and using the calibration curve, you could be able to calculate the molecular weight.

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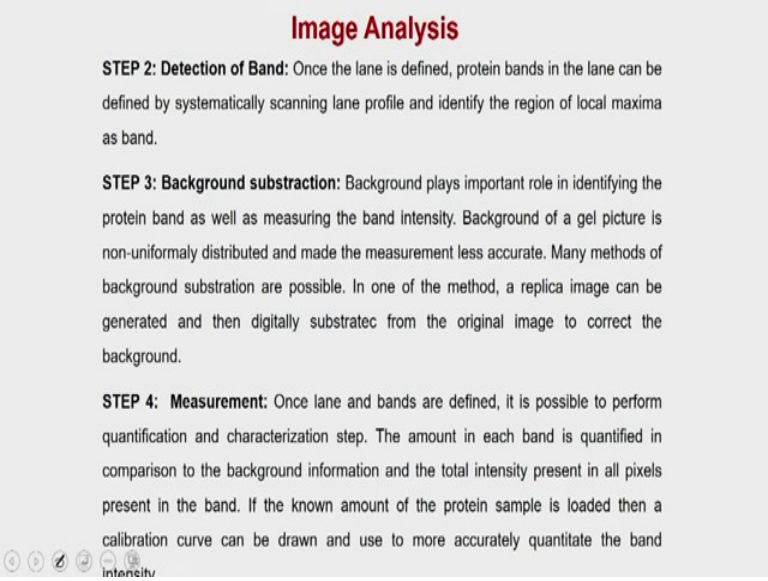

So apart from calculating the molecular weight, you can be able to do a lot of things when we are doing the image analysis. You can be able to detect the bands, you can be able to calculate their intensity, you can be able to (cal) subtract the background so that there will be more uniformity while you are doing the image analysis and so on.

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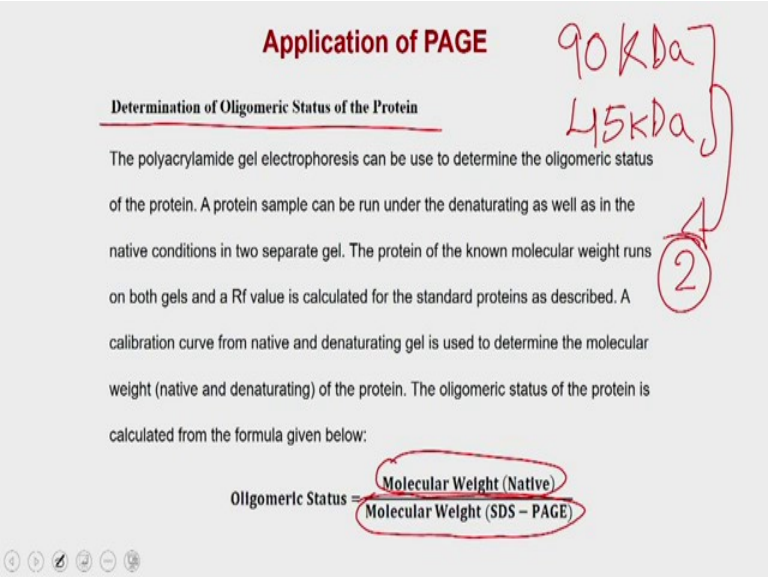

And then you can also, so we have discussed some of the classical examples also, how you can use the SDS page. So if the help of running a protein into a native page versus the SDS page you can be able to even calculate oligomeric status or the protein. So oligomeric status would be (equi) is is going to be equivalent to the molecular weight what you calculate from the native conditions divided by the molecular weight of the what you are going to get the SDS page. For example, if you do 1 native page you are going to get the molecular weight as 90 KDa whereas if you do the SDS page you are going to get the molecular weight as the 45 KDa. That means your oligomeric status is 2. Which means it is a homomeric, homomeric dimer. Which means the similar kind of units are coming together and it will giving you the dimers.

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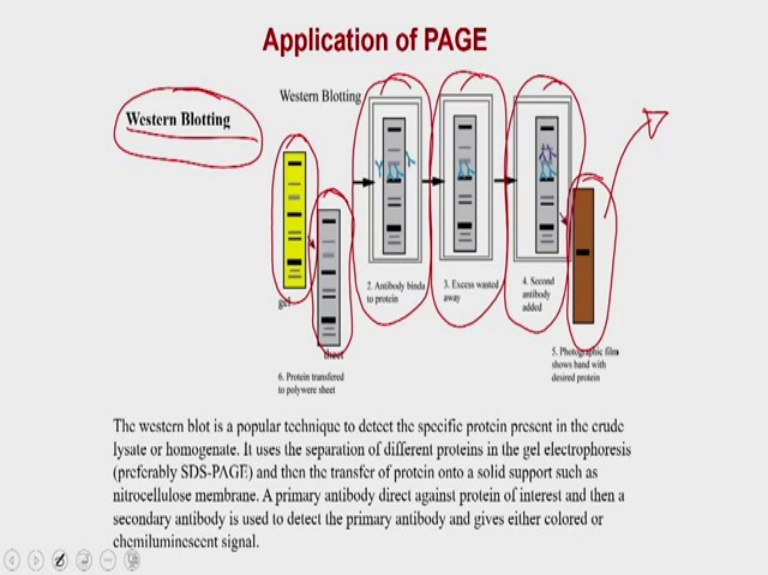

Then we took he examples of Western Blotting, we are also showing you a very in depths demo how to do different steps of western blotting where first you are going to run the SDS page then you are going to transfer the SDS page onto a nitro-cellulose membrane. Then you are going to treat the nitro-cellulose membrane with a primary antibody. Then you are going to do a washing step, then you are going to treat it with the secondary antibodies and then ultimately you are going to use the development. You can do a development with the help of the enzyme substrates such a dab or you can actually use the chemiluminescent substrates. Both are these methods we have discussed while we were showing you the demo.

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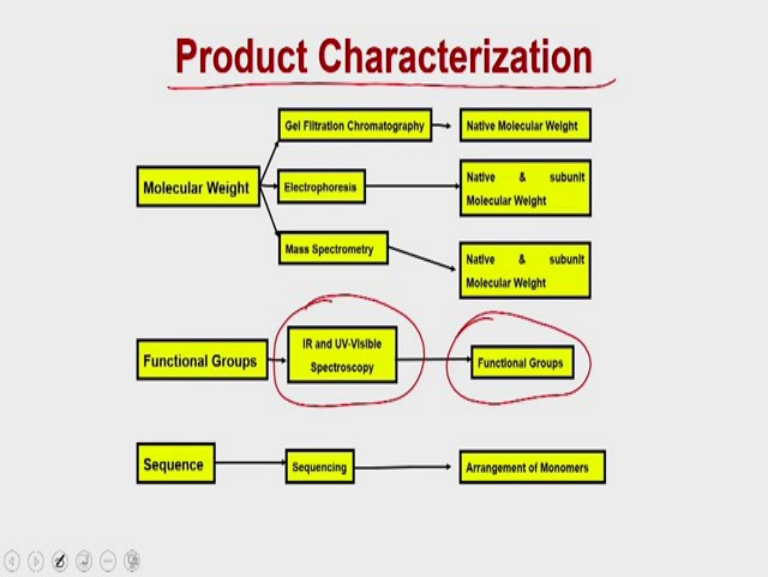

And then with this we have we were done with the (prod) product characterization. We have also discussed about the IR and UV visible spectroscopy. How that can be helpful in terms of predicting that this could be type of functional group is present in your protein or DNA or small molecules.

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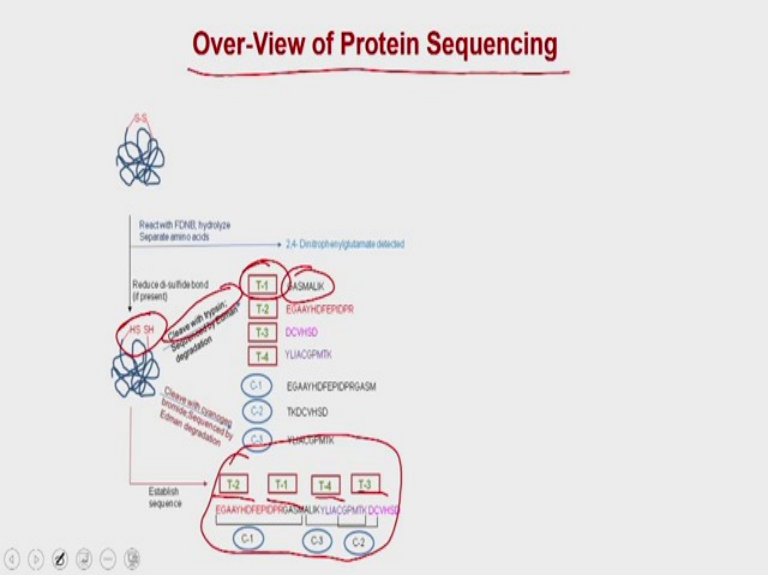

After this we discuss about the sequencing techniques. So in a typical sequencing technique what you are doing is, you are actually cleaving the proteins with the help of trypsin. First you are, first you are removing the disulphide bonds then you are cleaving the protein with the trypsin

and you know where the trypsin is actually cutting. So you are going to get different types of fragments and these fragments you can actually be able to sequence and then once you sequence the individual fragments, you can be able to put them together and that is how you are going to get the full length sequence. So you can put like T-2, T-1, T-4 and T-3 as per its overlapping sequences, taking the overlapping sequences into the consideration and that is how it is going to give you the sequence of the full length protein.

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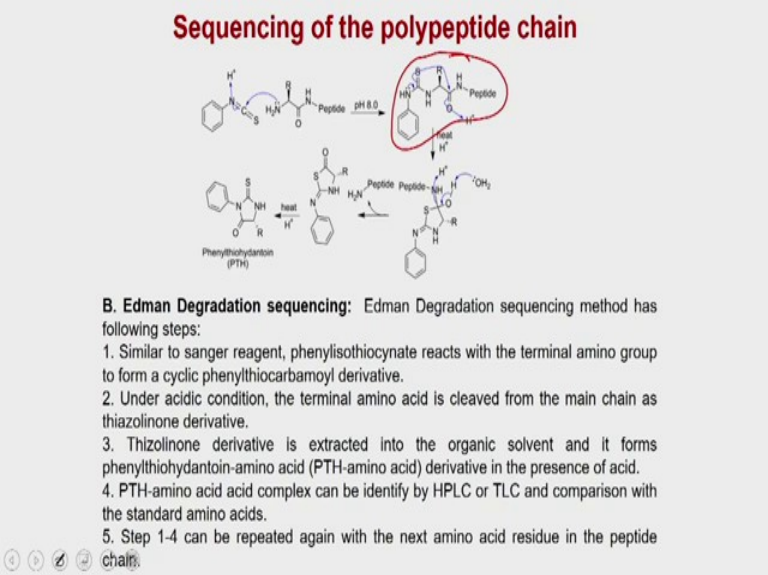

So this is the Edman Degradation Method. So Edman degradation method also we have discussed and the Edman degradation method similar to the sanger reagents. The reagent is very different but the overall working principle and other things are very very similar kind of thing. Where you actually are making adduct with the terminal (phospha) terminal amino acid and this adduct is what you are characterizing in the Edman degradation method also.

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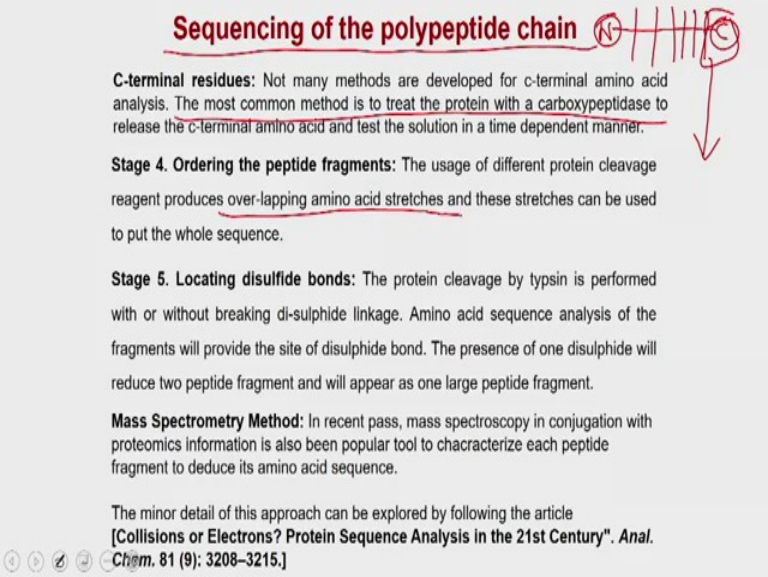

Apart from the end terminal sequencing, you can also do the c-terminal sequencing. So for the c-terminal (meth) sequencing, the the most appropriate method is that you treat the protein with a carboxypeptidase. So carboxypeptidase is a very very unique peptidase and it only choose the protein from the carboxyl side. So what will happen is, suppose you have protein like this and this is the end terminus and this is the c- terminus and suppose if you treat it with the carboxypeptidase, it is going to start cleaving the protein from the backside.

So if you get this particular fragment and then you if you run the sanger (reag) sanger sequencing method or if you use the Edman degradation method, it is actually going to give you the adduct. And that adduct when you identify it will tell you that this is the protein this is the particular amino acid present at the c-terminal of this particular protein. With the help of the mass spec and all those kind of high-end techniques and with the overlapping amino acid stretches, you can be able to put these fragments into the into a proper order and that is how you are going to get the full length sequence.

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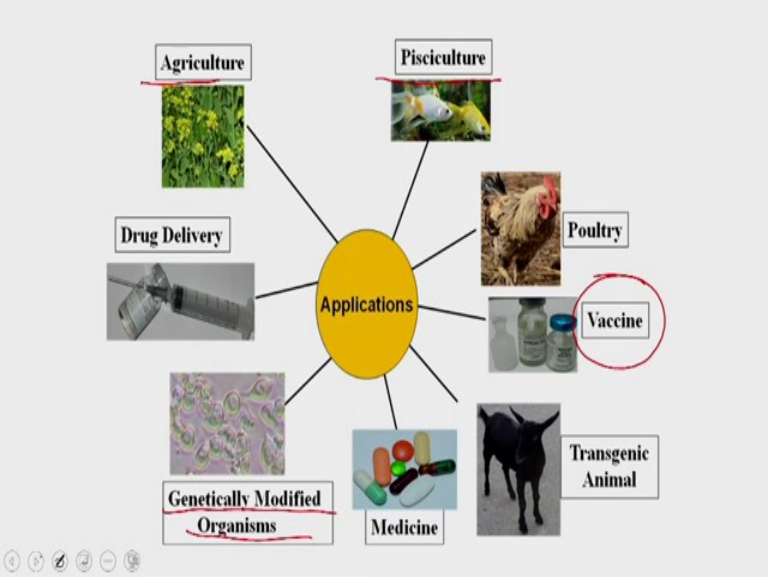

At the end in the (la) in the module eleventh we have also discuss about the different types of biotechnology applications. So we have discuss the application of biotechnology in agriculture, we have discuss the application is the pisciculture, we have discussed about the application of biotechnology in the case of medical field and then we have also discuss about the applications of the biotechnology in the generating the genetically modified organisms and so on.

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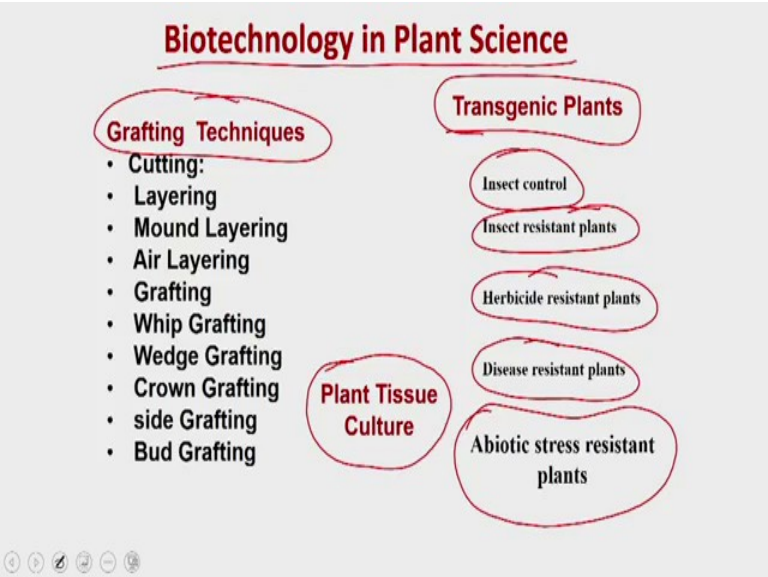

So in general what we have tried is that we have tried to give you a glimpse of the applications of biotechnology in separate fields. That does not mean that these are the only limited, only

applications of biotechnology. Biotechnology is a very very vast field, it has a lot of scope. So the what we are showing you are the classical examples of the applications of biotechnology. But there is a lot of applications which are people are discovering and people are using the biotechnology for many purposes.

So in the case of biotechnology in plant sciences, we discuss about how to generate the transgenic plants or we have discuss about many techniques which were popular, (bethor) before the genomic era, where people were using different types of grafting techniques to make the new species of the plant or at least to improve the crop yield and other kind of applications. But once the genomic era started, then people were started developing the insect control or insect resistant plant.

Then we they started developing the herbicide resistant plants and then they started developing the disease resistant plants and then ultimately they started developing the abiotic or the biotic stress plants. So that it will actually going to improve the overall yield of that particular plant, so that it will going to give you the more food grains and that actually will going to help the human society. And at the in this, in this context also we have discussed about the plant tissue culture as well.

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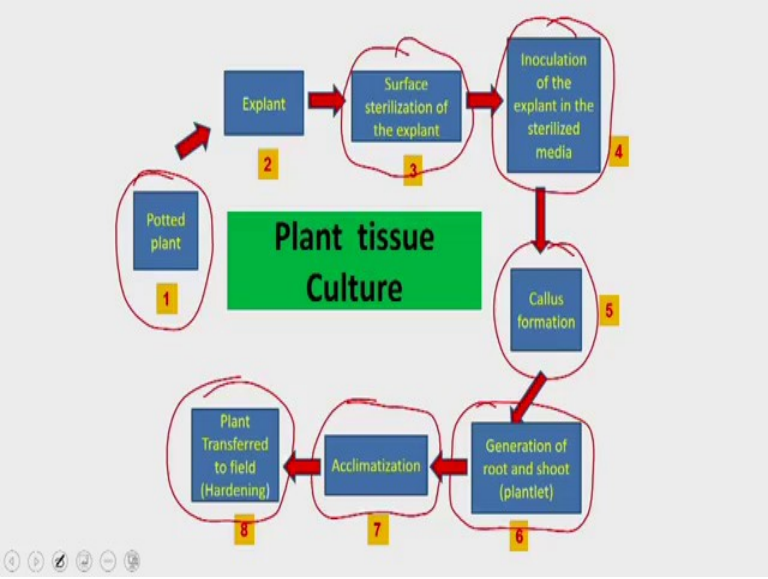

So while we were discussing about the plant tissue culture we took you to the, our laboratory. We started with the, a plotted plant, how to select the explant, how to do the surface sterilization

of the explant, how you inoculate the explant, and then once the callus is been formed. We have shown you how to you can change the media components and that actually will allow the root and shoot formations and then ultimately we have shown you how to do the acclimatization as well as the hardening step, so that you will be able to develop these plantlets into a fully grown tree. And or the plantlets which are actually going to have the applications in the fields.

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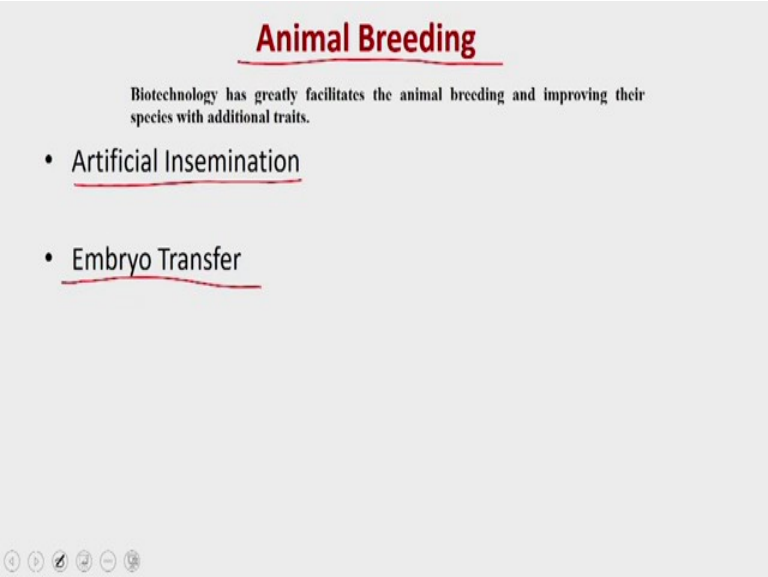

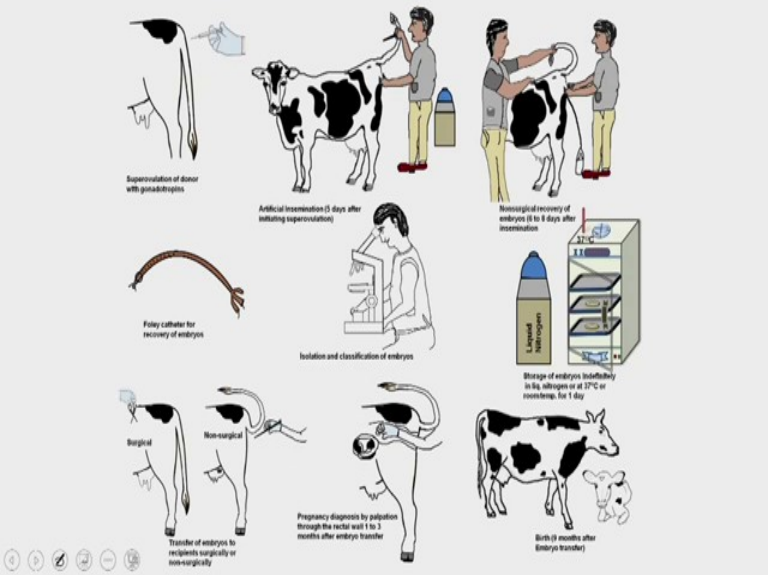

In the animal sciences, we discuss about the artificial insemination as well as the embryo transfer and what we have discussed in the embryo transfer is that you many times what will happen is that when you have a very good breed cow or buffalo or the kettles, you cannot afford to have

the, those species but what you can afford is having the embryos. So what will happen is that the embryos are been transferred from the good cow to or the high yield cow to a low yield cow so that you will have more numbers of these cows. And then you will actually be able to have the more production of milk and other kind of things. So we discuss all these details in, discuss all these in detail.

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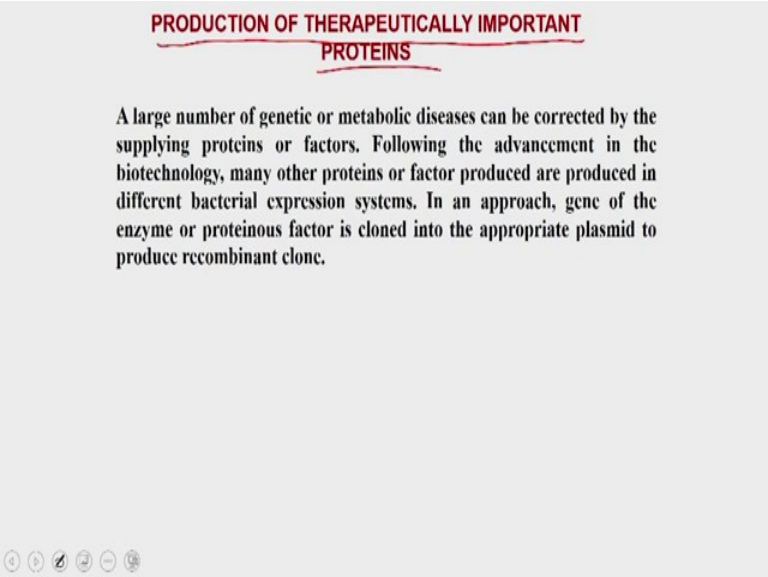

Then we discuss about the application of the biotechnology in the medical field and we discuss about that how the (biotchno) biotechnology has allowed to to, to produce the therapeutically important proteins.

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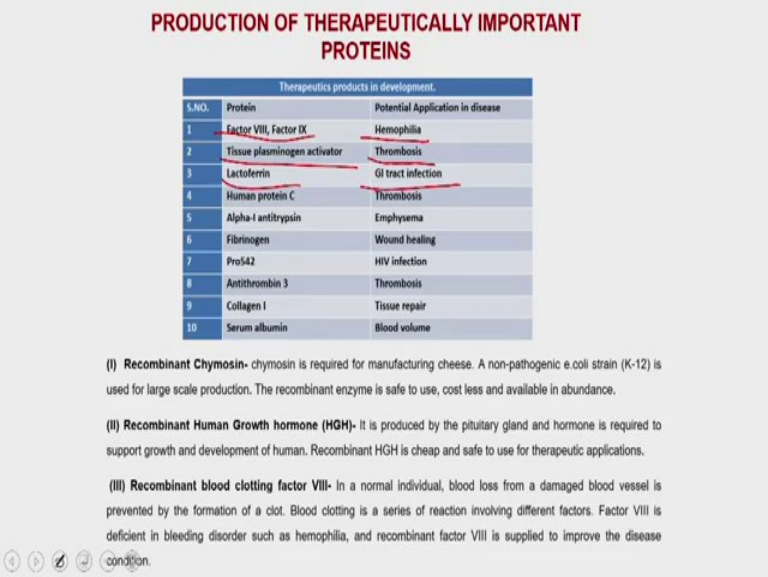

And we took a few examples like factor VIII, factor IX for hemophilia, tissue plasminogen activator for thrombosis, lactoferrin for Gi tract infection and so on. And there are many more proteins which people are more and more exploring and producing into the recombinant expression systems and they are trying it into the clinical settings to see whether that particular recombinant protein is doing the same function what it was suppose to do under the (invi) invivo conditions because when you produce a protein under the recombinant conditions, first of all you actually make the protein in a very very high quantity. Second you can have the full control over its quality as well as the quantity. The third, the you can be able to bring down the cost in a very very big way so that it will be actually going to help the human society at the end.

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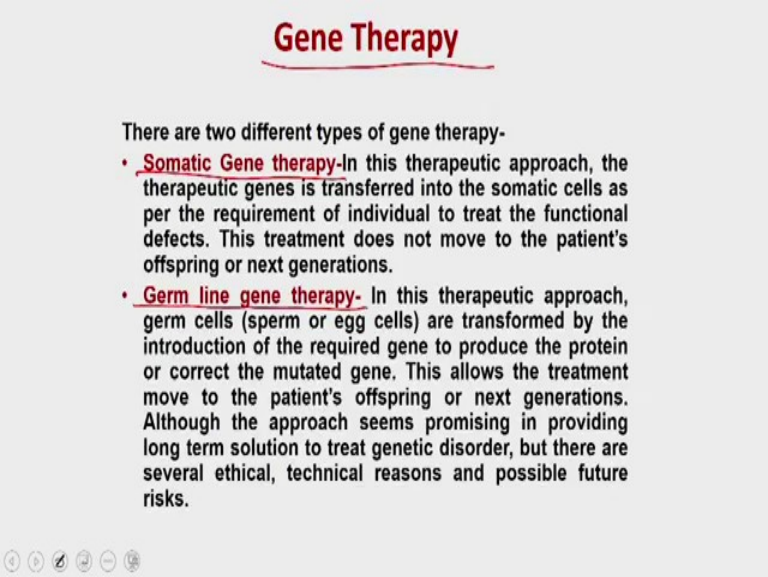

Then we also discuss about the gene therapy. We discuss about the somatic gene therapy approaches and the germ line gene therapy approaches.

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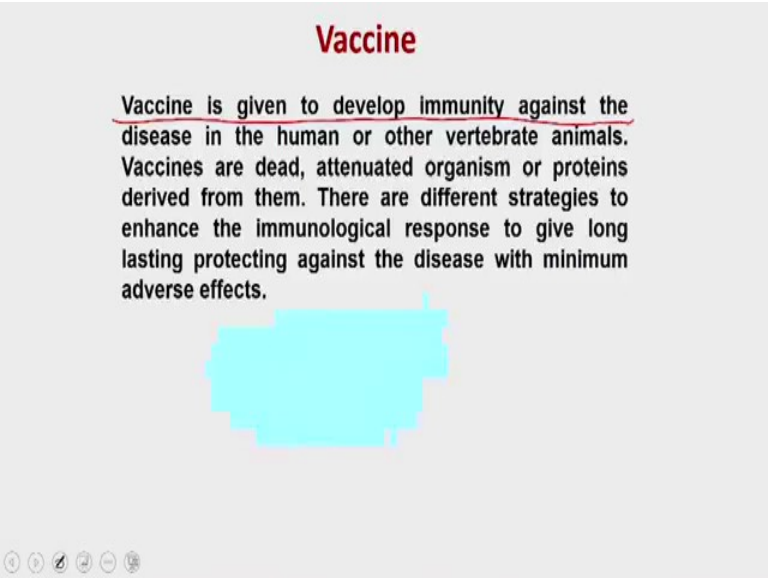

And we discuss about the different types of vaccines which are being developed to produce the immunity into the, into the kids as well as into the adults.

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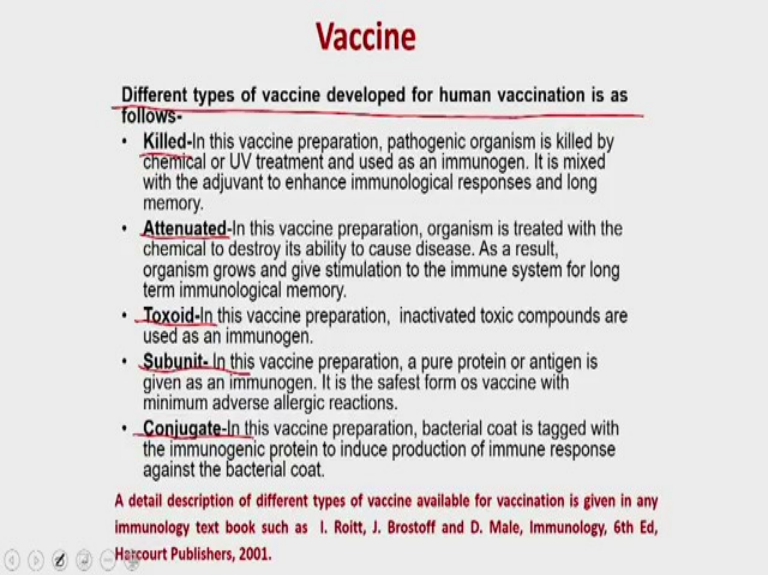

And we discuss about the different types of vaccine which are been developed whether it is the killed vaccines or attenuated vaccines or toxoids or subunit vaccines or the conjugated vaccines. So we have discussed many types of the vaccines and there their utility and their drawbacks and all those kind of things while we were discussing about the application of biotechnology in the medical field.

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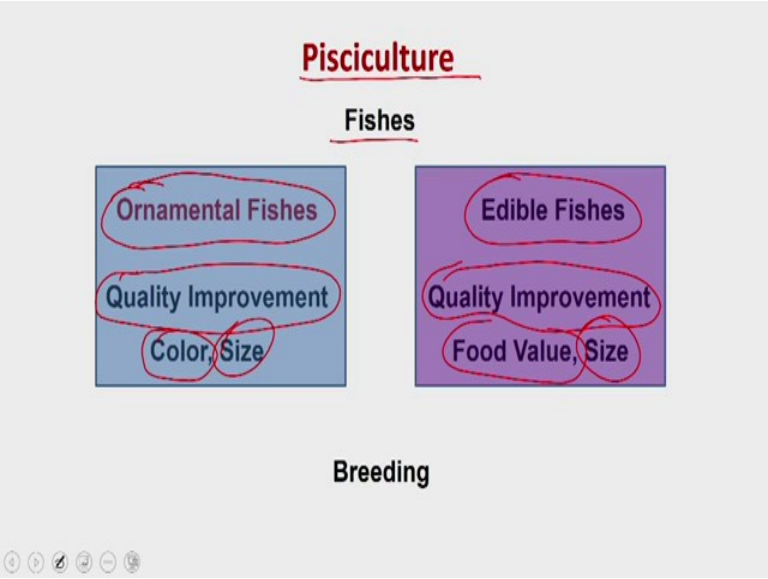

And then we moved on to the pisciculture. In the pisciculture we took the example of how the biotechnology is helping in getting the improvement in the ornamental fishes versus the edible fishes. So in the case of ornamental fishes they are actually helping in terms of quality

improvement such as color and size. Whereas in the case of edible fishes it is actually generating the quality improvement, food values as well as the size and it with the help of the biotechnology you can also be overcome with the breeding problems.

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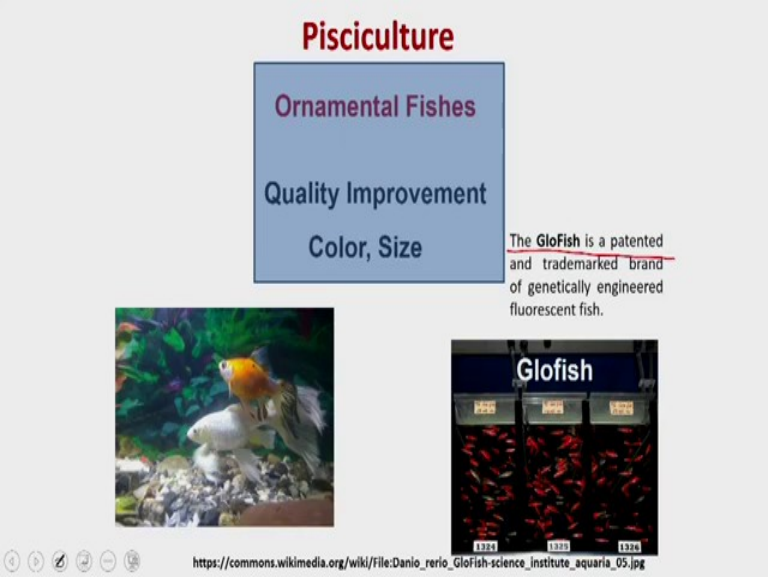

So in the case of ornamental fishes, we discuss about how the people have discovered the Glo fishes which actually the fluorescently flourescent (proti) florescent fishes where the scientist have over expressed the red color, green color or yellow color proteins and that is how these proteins, these fishes are actually (growing) glowing in the aquarium. And these fishes are very very popular in the western countries because they have a very high, they because they are very attractive, because even if you do not turn on the lights these fishes are glowing on their own. And that is why the name has been given as the glo fishes.

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So this is all about the biotechnology applications in the, for the human society. We have not covered as I said you know biotechnology has the application in many fields, for example, in the case of environmental field, biotechnology is producing lot of bacteria which is actually overcome, which is actually being used in taking, in taking care of different types of pollutants which people are, which which are been either generated by the human made or by natural pollutants and and so on. So this is very difficult to overcome or this is very difficult to cover the all the aspects of biotechnology or all the applications of biotechnology. So so far we have summarized the all the aspect what we have discussed in this course.

The purpose of summarizing and revising these aspect is simply to refresh your memories. You may be missing some points and also in case you want to read or you want to study or you want to discuss those points again, you can be able to have an opportunity at this moment that you can discuss these points with me and we can actually sort out your your confusions or any kind of things. Because ultimately at after this particular module you are actually going to face the exam and the assignment what you are going to get in this particular week is also going to have the real flavor how the end sem exam is going to be (ove), end sem exam is going to be the or the pattern of the particular type of end sem exam.

So at the end I hope you might have enjoyed this course and you might have learned many things and I have tried my level best to explain you different aspects of genetic engineering. I have also

discuss about many of the things which people are following after cloning the product as well and the ultimate goal of this course is that it should help you in advancing your research, it should help you to explore different aspects related to biotechnology and so on. So with this I would like to conclude our lecture here. Thank you.