Genetic Engineering: Theory & Applications
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Module XII
Summary and Conclusions
Lecture 38
Summary and Conclusions (Part –I)

Hello everybody, this is Dr. Vishal Trivedi from Department of biosciences and bioengineering, IIT Guwahati. And in this course, so far we have discuss many aspects related to biotechnology as well as related to genetic engineering. So now today, we are going to resummarize or summarize the, whatever we have learnt so far in this course. So if you see this course has (11) 12 modules and the content of this course is being divided into all these 12 modules.

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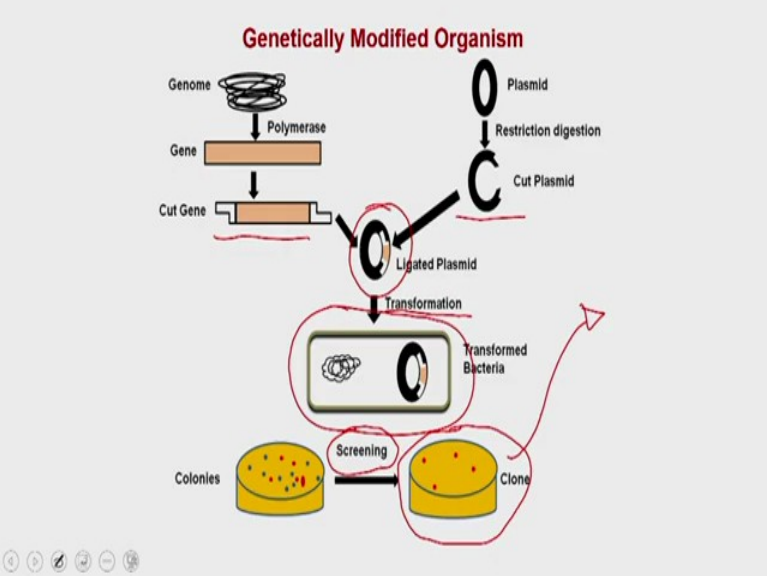

By now you might be very much familiar with this figure which actually will be the core figure through which we were discussing the different aspects related to genetic engineering. So we started with a very basic thing about understanding or about discussing the host. And then we discuss about the how you can isolate the gene from the host and then you are going to cut these genes with the restriction enzymes. And then similarly you are, we have also discuss about different types of plasmids and then we discuss about how you can do a restriction digestion and that will actually going to generate the cohesive ends.

So you are going to have the cohesive ends on to the gene, you are going to have the cohesive ends onto the plasmids and then you are going to put these two components for a ligation

reaction that is how you are going to get the recombinant DNA and then you are going to employ different types of DNA delivery methods to transform the bacteria. And that is how you are going to have the genetically modified organisms.

And once you have this you are actually going do a screening process so that you will be able to screen the transform bacteria from the non-transform bacteria and ultimately once you got a, your desirable clone. This desirable clone can be used for protein productions and subsequently to this we have also discuss about how to purify the protein, how to break open the host cell, what are the different methods are available for break open the host cell so that can be able to release the content from the different storage compartment available within the host.

And at the end, we have also discuss once you got the product you can how you can characterize that product utilizing the electrophoresis techniques, you are utilizing the chromatography techniques and UV visibles as well as the IR spectroscopy. And at the end we have also discuss about the, what is application of genetic engineering or the biotechnology in different fields. For example, the animal sciences, plant sciences, the we have discuss about what is application of the medical sciences and we have also this about the application of genetic engineering in all different types of aspects related to the society.

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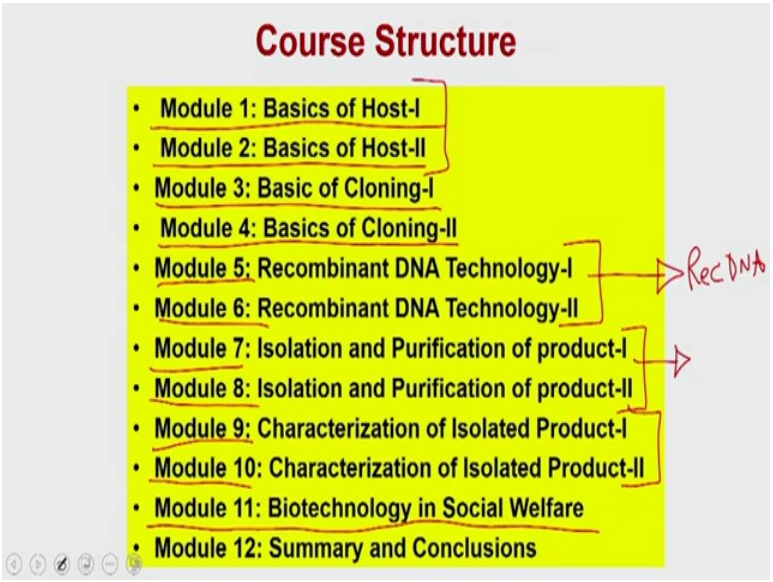

So the course of, the content of this course is being divided into 12 different modules. The module 1 is going to deal with, module 1 and 2 are dealing with the basics of the host. The module 3 and 4 are actually covering more about the basics of cloning 1 and 2. Module 5 and

6 are mostly being developing or dealing with the recombinant DNA technology, so that you can by visualizing all these you can be able to generate the recombinant DNA.

Once you generated the recombinant DNA then next step is that you actually going to over express, so in the module 5 and 6we also discuss about different types of over expression strategies. What you are going to use in different types of host strains whether it is a bacterial strain or the yeast or bacteriophage or insects cell lines or mammalian cells. Then in the module 7 and 8, we have mainly focused on to the isolation and purification of the product, what you are going to over express in these host cells and then module 9 and 10 is more on towards the characterization of the isolated product. So in the module 9 and 10, we have discuss about the different types of the techniques what are available to categorize the product.

For example, you can run, you can determine the molecular weight utilizing the gel filtration chromatography, electrophoresis, maldi mass or you can be able to determine the characteristic absorption or emission spectra of molecule using the UV visible spectroscopy or you can be able to run the IR spectroscopy to know what are the different functional groups are present in the your compound.

And then in the module 11 we have discuss about the different applications of genetic engineering as well as the biotechnology which is for the social welfare. So let us start from the module 1 and 2 and understand what we have discuss so far. This summary and the conclusion is a very very important and very important because in due course you are going to face the exam of this course, so we are just going to review the everything what we have discuss so far in a very very fast forward mode so that you will be able to refresh your memories.

And if anything which is not going to be covered or which is actually not going to be understand and then you actually can go back to the particular module. You can again listen those video lectures and at that actually be the way you can be able to prepare yourself for the exam. The assignment what you are going to get in this particular week is actually probably a kind of a mock exam. If you do that you are actually going to prepare yourself for the real example which is going to happen very soon.

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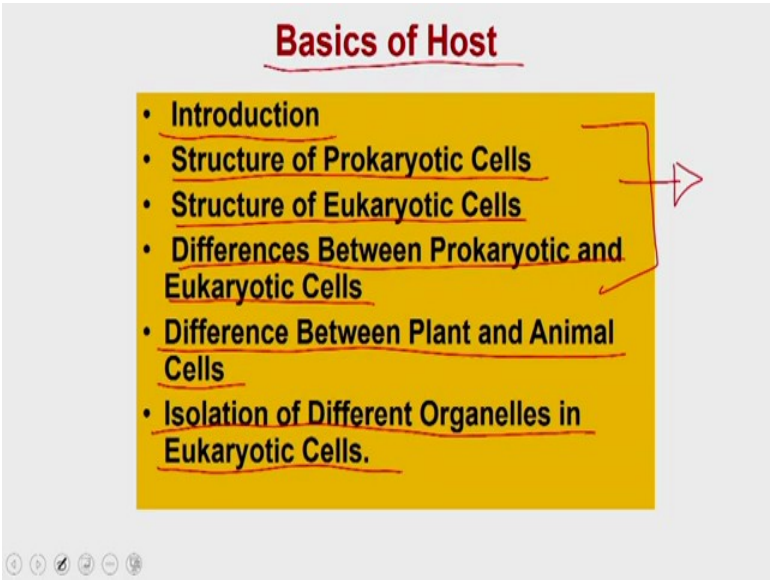

So in the module 1 and 2, we have discuss about a basics of host. And what we have discuss? We have initially discuss about the introduction of biotechnology. How the biotechnology is helping the human society and then we have discuss about the structure of Prokaryotic Cells, structure of Eukaryotic cells. Then we have discuss about the differences between prokaryotic and the eukaryotic cells. This is important because once you know the cellular structures of prokaryotic as well as the eukaryotic cells.

As well as the differences between them you can able to utilize or exploit the host strain and you can be able to choose the perfect host strain what is required for over expressing your protein, or over expressing your factors. Then we have also discuss about the difference between the plant and the animal cell. And then at the end in the lecture 3 we have also discuss about the isolation of different organelles or the fractionation strategies from the eukaryotic cell as well as we have also discuss about the isolation of pre plasmid fraction from the prokaryotic cells.

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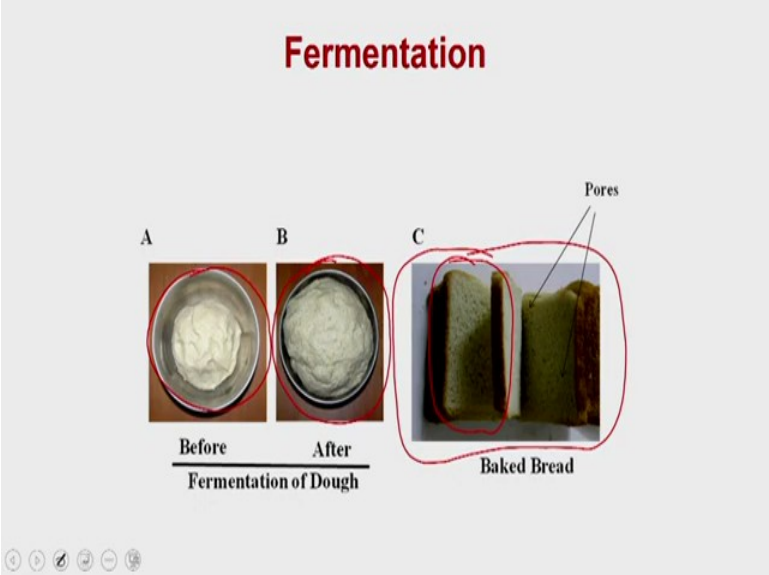

So we started very basic that with the very simple example of formation of bread and that is a very very classical example through which we have started with the course saying that this is happening because people were doing the people were making a dough and then they were adding the very small quantity of yeast and what will happen is that when the yeast is utilizing the glucose which is present in this dough, it is actually producing the carbon dioxide and that carbon dioxide when it comes out from the dough it actually makes a dough very fluffy and that is how you are going to a spongy texture of the bread. And that is what, one of the classical example of the utilization of biotechnology for the social welfare.

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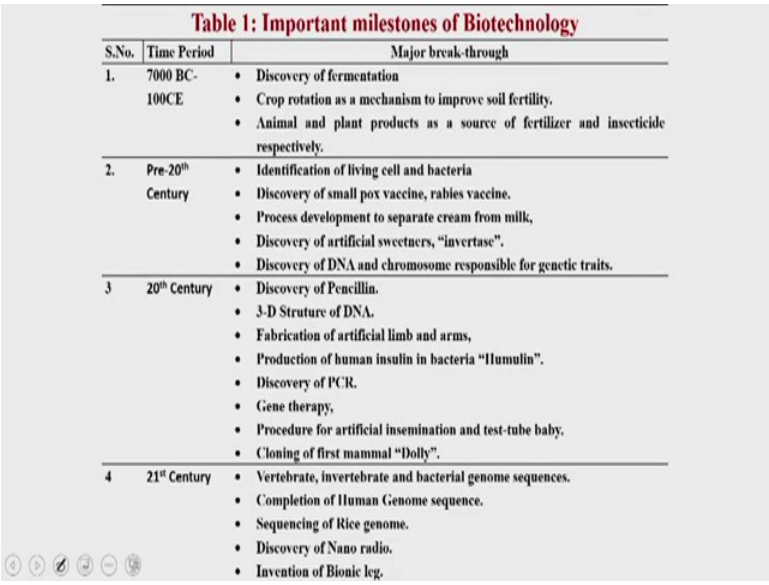

Table 1: Important milestones of Biotechnology

S.No.	Time Period	Major break-through
1.	7000 BC-100CE	• Discovery of fermentation • Crop rotation as a mechanism to improve soil fertility. • Animal and plant products as a source of fertilizer and insecticide respectively.
2.	Pre-20th Century	• Identification of living cell and bacteria • Discovery of small pox vaccine, rabies vaccine. • Process development to separate cream from milk, • Discovery of artificial sweeteners, "invertase". • Discovery of DNA and chromosome responsible for genetic traits.
3	20th Century	• Discovery of Pencillin. • 3-D Struture of DNA. • Fabrication of artificial limb and arms, • Production of human insulin in bacteria "Humulin". • Discovery of PCR. • Gene therapy, • Procedure for artificial insemination and test-tube baby. • Cloning of first mammal "Dolly".
4	21st Century	• Vertebrate, invertebrate and bacterial genome sequences. • Completion of Human Genome sequence. • Sequencing of Rice genome. • Discovery of Nano radio. • Invention of Bionic leg.

Then we moved on and talked about many of the important discoveries of the biotechnology in terms of the different eras whether it is the pre-genomic era or the post-genomic era. Most of these discoveries were done simply because they were be a requirement of that particular type of product and that is how the people started doing the research as well as the different types of experiments and that is how they have discovered many of these techniques or many of these products they have evolved. So I am not going to discuss all these because this all we have discuss in the lecture 1.

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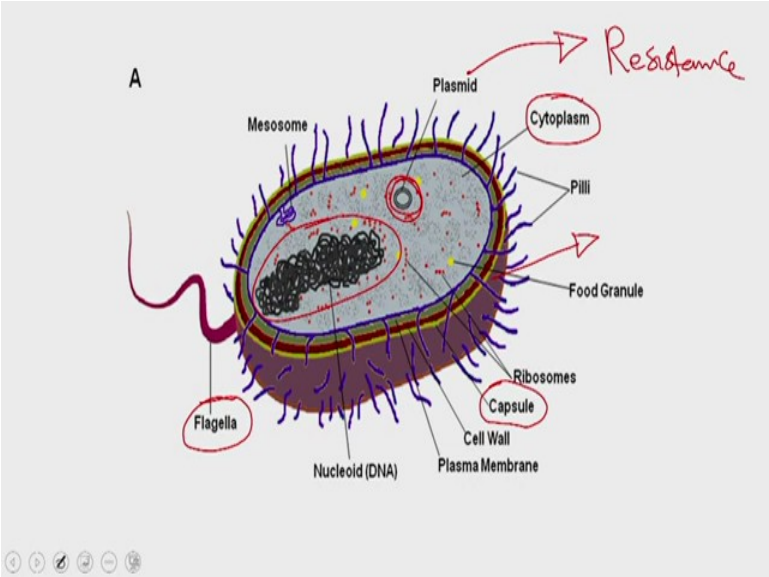

Then we have discuss about the structure of the bacterial cell where we have said that the bacterial cell has the plasma membrane, bacterial cell has a circular plasmids or circular genome. And then it has the extra chromosomal DNA that is called as the plasmid and that plasmid is important for providing the resistance or different types of phenotype which actually are happening in a dynamic fashion so that the bacteria is continuously exchanging their plasmids between the different bacteria present in the within the colony.

And then you have the flagella, the flagella is actually a important cellular structures of the bacterial cell because it actually allows the bacterial cell to reach to the food particles. And it allows different the bacteria to swim into the water or other kind of liquid media and then we have the cytoplasm. So the bacterial cell does not contain a proper nucleus or double walled nucleus and infect there is no cellular organelle available which is double walled cell organelle present in the prokaryotic cell such as bacteria. And bacteria has a very thick cell wall and outer to this safe cell wall there is a capsule. This capsule is made up of

polysaccharides and this capsule is actually helping the bacteria to withstand that is the very high-quality of the dehydration and all other kind of environmental stresses.

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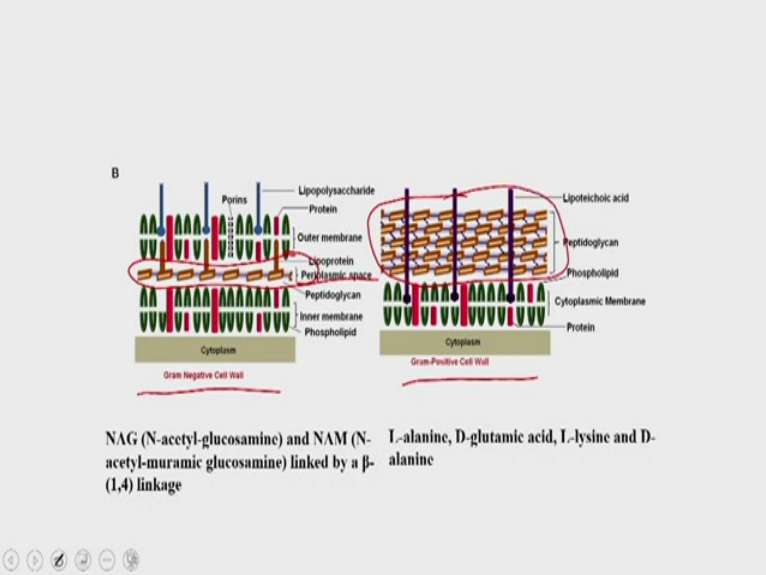

Then we have also discuss about the composition of the cell wall, the composition of cell wall in the gram negative bacteria or the gram positive bacteria. The major difference is the presence of the peptidoglycan layer, so what you see is a thin layer of peptidoglycan which is present in the gram negative bacteria whereas there is a thick layer of peptidoglycan in the (positive) gram positive bacteria and that actually makes a things very different between the gram negative versus gram positive. And because there is a difference in the thickness of the peptidoglycan layer the people have discovered the strains which actually can be used to distinguish between the gram positive as well as the gram negative bacteria.

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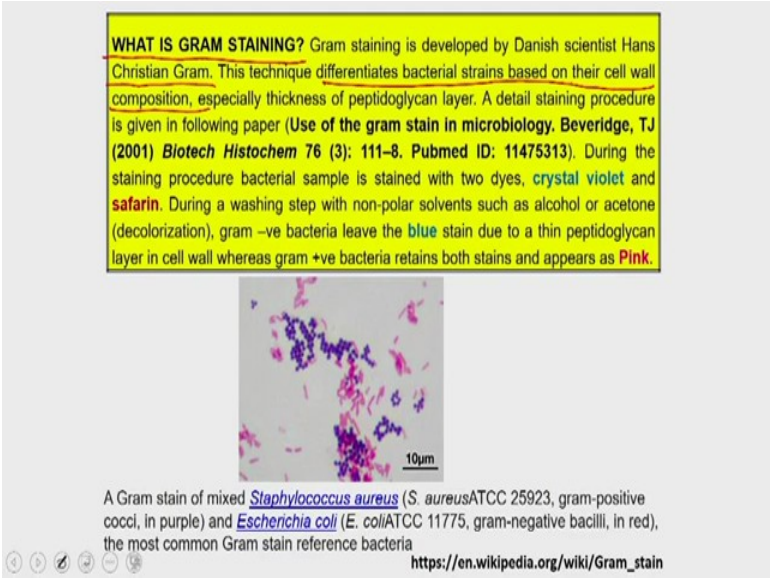

So what you see is there is a stain which is called as the gram stain and the gram stain is being discovered by a scientist called as the Hans Christian Gram and this technique is useful for differentiating the bacterial strain based on their cell wall composition, specially the composition of the peptidoglycan layer and a detail procedure anyway we have discussed when we were discussing about the bacterial cell wall as well as the utilization of the gram negative, gram stains in differentiating the gram positive bacteria versus the gram negative bacteria.

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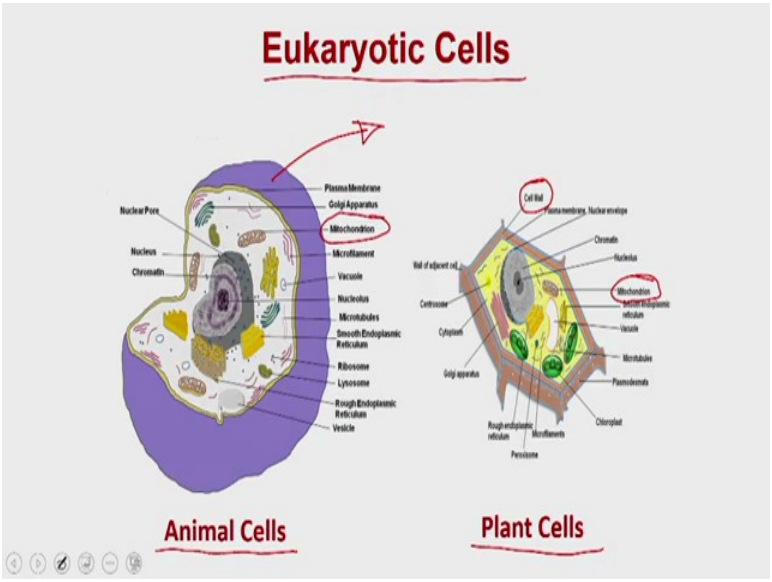

Then we moved on and we discussed about a eukaryotic cell so in the eukaryotic cell we have taken up the examples of animal cell as well as the plant cells. So majority of the organelles

are common between the animals cell as well as the plant cell except that the there are few differences. For example, in the plant cell it has a very thick cell wall which is completely absent in the animal cell, compared to that the animal cell has large quantity of mitochondria because the animal cell is energetically very active compared to the plant cell, so the plant cell contains very lower quantity of mitochondria. Similarly, there is a difference of the chloroplast, the plant cell contains the chloroplast whereas the animal cell does not contain the chloroplast and there are some more differences between the animal as well as the plant cell that also we have discussed.

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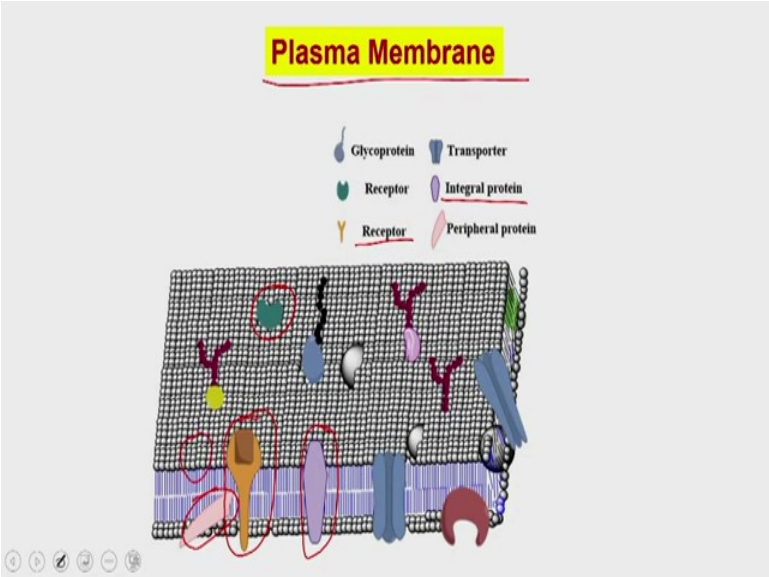

And then we have discuss about the plasma membrane, the composition of the plasma membrane. The plasma membrane is made up of 2 components, one is lipid bilayer and the protein which are present within the plasma membranes. And plasma membrane is a bilayer structures where the proteins are integrated. There are multiple types of proteins, the protein which are actually running all over the plasma membranes or the protein which are either present onto the top layer or the bottom layer.

So plasma membrane contains different types of receptors, this receptor could be for the food particles, this receptor for the capturing the signals or they have a many of the integral protein as well as the peripheral protein. So what you see is the protein which is going through all over the plasma membrane which means it is covering the both layer of the lipid bilayer then that is called as a integral protein whereas the protein which is present either on onto the inner side or to the upper side that is called as the peripheral proteins.

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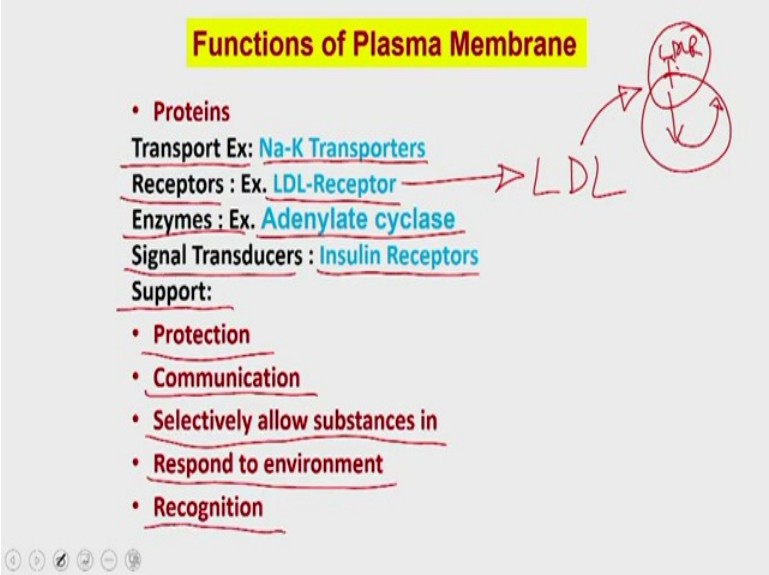

The function of the plasma membrane is that the protein which are present on to the plasma membrane are working as a transporters. The classical example is the sodium potassium transporters. Then it also have receptors, the receptor as I said this receptor could be for receiving the food particle. The classical example is the LDL receptor which actually take of the LDL from the blood and it actually does the recycling of the LDL receptor from the surface, inside the cell and so on.

So what happen is when the LDL present, LDL goes and bind to the LDL receptor and this LDL receptor containing, containing LDL is goes inside and that is how it delivers the the LDL. And after the delivery it returns back to the plasma membrane and that is how the receptor cycling is keep continuing into the cells. Then it has the enzymes for example, the Adenylate cyclase to catalyze the reactions then it has the receptors which are not participating into uptake of the food particles but it is actually participating into the signal transductions.

Classical example is insulin receptors, if you remember we have talked about that insulin which is flowing into the blood when it goes and binds to the cells onto the which is when it binds to the insulin receptor present onto the cell it actually drives a phosphorylation reaction downstream and these downstream phosphorylation reactions actually changes the cellular metabolism in such a way that the glucose get converted into that the start the cell starts taking up the glucose from the external environment and that is how that glucose start converting into the glycogen and it gets stored in to those cells. When you require the energy

then the glycogen is going to be broken down and glucose is available for the cell to utilize into the metabolic reactions.

Apart from that it also provides as a support system. That support system it actually required for providing the protections for communication to the other neighboring cells. And then it also allow the selective entry or the exit of substances within the or outside the cell. Then it responds to the environment and it also works as a recognition particle which means a plasma membrane contains some of the receptors or some of the effectors which actually being recognized by the cell. One of the classical example is the MSC class two or MSC class one which actually being recognized by the system of the immune system to say whether particular cell is of the same person which means whether the cell if of the self origin or the external origins.

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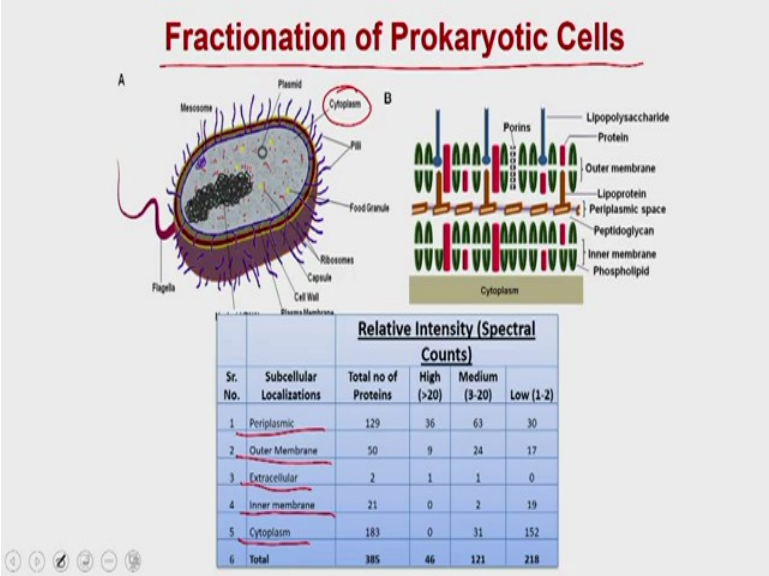

Sr. No.	Subcellular Localizations	Relative Intensity (Spectral Counts) Total no of Proteins	High (>20)	Medium (3-20)	Low (1-2)
1	Periplasmic	129	36	63	30
2	Outer Membrane	50	9	24	17
3	Extracellular	2	1	1	0
4	Inner membrane	21	0	2	19
5	Cytoplasm	183	0	31	152
6	Total	385	46	121	218

Then we also discuss about the fractionation of the prokaryotic as well as the eukaryotic cells. Within the prokaryotic cell we have discuss about how the we can actually get the protein from the different compartments. So suppose you are over expressing the proteins it can actually be present into the cytoplasm or it could be present into the periplasmic fractions or it could be present onto the outer membrane or the inner membrane. So these are the different locations you can have the protein on the periplasmic fractions, outer membrane, it could be inner membrane, some protein might be secretary.

So it may go into the extra solar media or the protein will be present into the cytoplasm. So how you can actually fractionate the bacteria in such a way so that if your protein of interest

is present into the periplasmic fractions you could just isolate the periplasmic fraction and that actually is going to help you in terms of the higher recovery of your protein as well as it actually going to give you the better purification as well as the better yield at the end.

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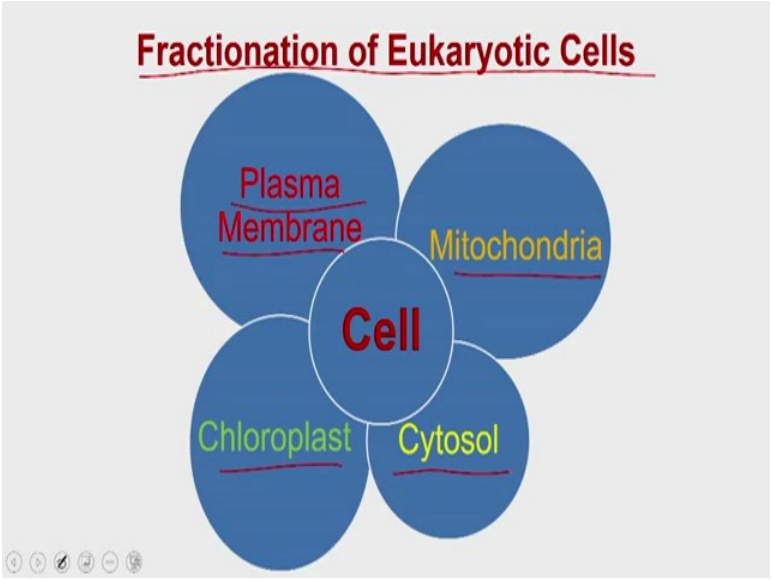

And then we have also discuss about the fractionation of the eukaryotic cells. In that we have discuss how you can actually prepare the plasma membranes, mitochondria, chloroplast as well as the cytosol. Then all this fractionation during this discussion about a fractionation of eukaryotic cell we have discuss about how you can use the density gradient centrifugations or the differential centrifugation techniques to achieve the targets which means you can use the differential centrifugations to get the plasma membrane, mitochondrias, cytosol or the chloroplast. Whereas you can do the same thing by the differential centrifugation which means you can actually centrifuged the particles at a different speeds and that is how you are actually going to get the different particles at a different sedimentation coefficients.

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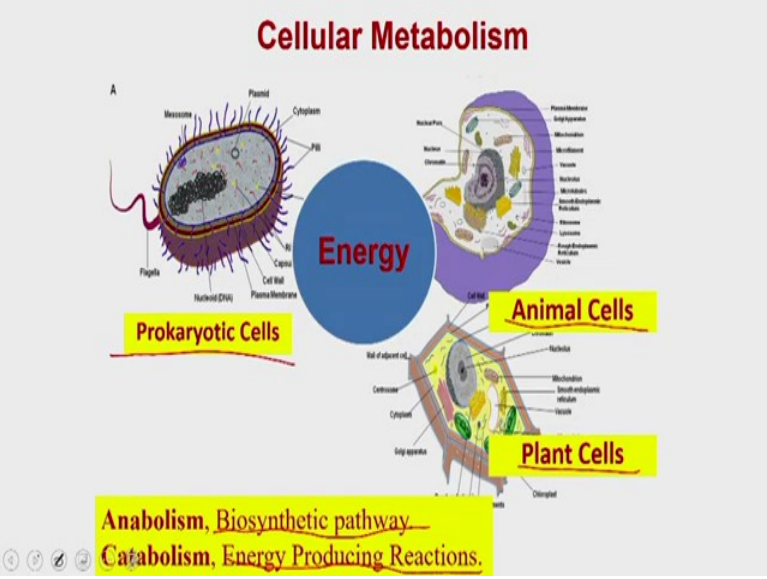

So apart from that once we were done with the in the module 1 with the basics of the host then we moved on and understand the metabolism of the host. And in that context we have discuss about metabolism which is operating within the prokaryotic cells. The metabolism which is operating within the animal cell and the metabolism which is operating within the any plant cells. So we have discuss about the anabolic pathways which means the biosynthetic pathway or the catabolic pathway which means the energy producing reactions.

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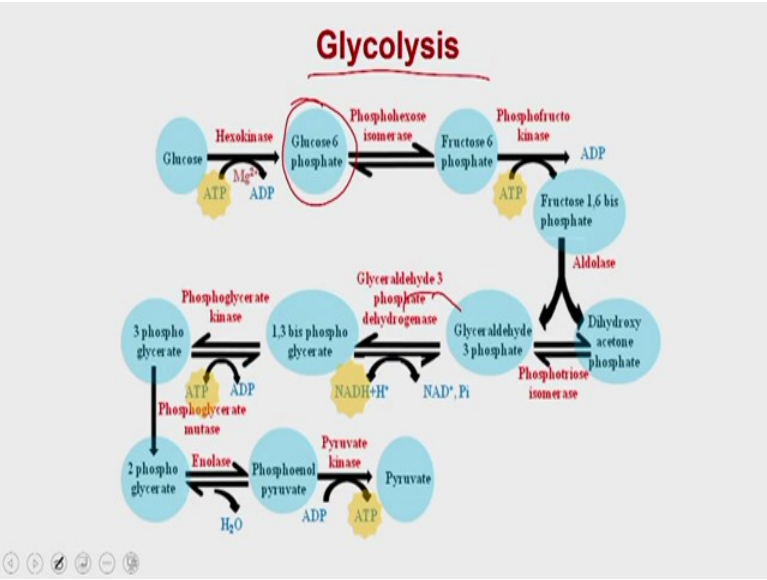

In this context we have discuss about the glycolysis, so glycolysis is the central pathway which actually started as soon as the glucose enter into the cytosol and then it starts the glucose get channelized into that glycolysis with the help of an enzyme known as

Hexokinase. And once the glucose phosphorylated and produce the glucose-6-phosphate then this glucose is going to be committed for the glucose metabolism that is the glycolysis. Once this glucose-6-phosphate is being generated it actually has no other option but to enter into the glycolysis and then eventually it is going to produce the pyruvic acid during this whole cycle it is going to generate the NADH as well as the ATP molecules.

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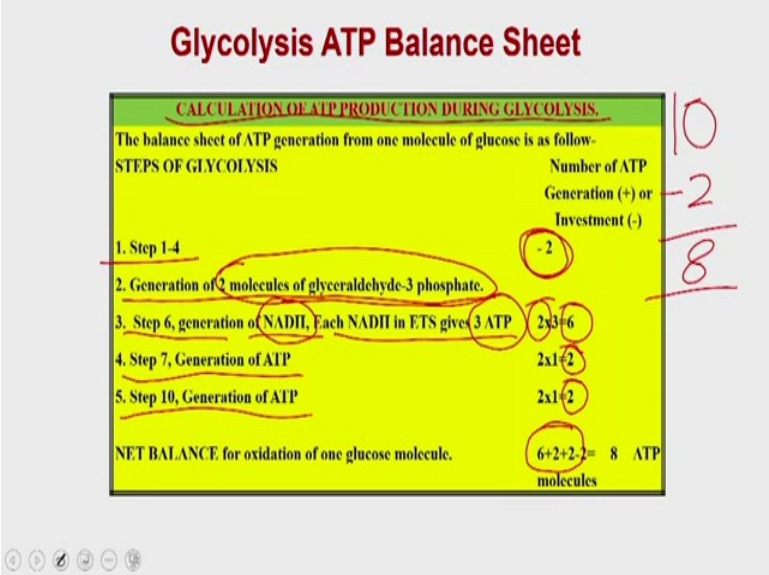

So we have also discuss about what will be the balance sheet of the ATP productions if you consider that the glucose is being utilized and local is going to be oxidized completely in the electron transport chains. So what will have done in the 1, to, step 1 to 4, the two ATP molecule are being used, they are being used when the, you are producing the glucose-6-phosphate and as well as the fructose 1, 6, bisphosphate. So that time you have invested the 2 ATP molecule.

After that there is a generation of 2 molecule of glyceraldehyde-3 phosphates, below this whatever you see is going to be a split, so glyceraldehyde-3 phosphate then will be converted and in the step 6 there will be a generation of NADH. So each NADH in electron transport chain is going to generate the 3 ATP. So there is a generation of one NADH but because the 2 molecule of glyceraldehyde-3 phosphates are being generated, you are going to multiply this number by 2.

So this means a molecules of NADH are going to be generated and since 1 each NADH in ETS is going to give you the 3 ATP molecules. Ultimately it is going to give you 6 molecules. Then in the step 7 you are going to generate 1 molecule which means you are

going to generate 2 ATP molecules. And in the last step when the pyruvate is going to be generated when you are going to generate the other 2 ATP molecules.

Ultimately what will be the intake or what will be the total production of glucose ATP? The total production of ATP is going to be 2, you have invested the 2 ATP molecules. So the 2 molecules are going to be subtracted which means the eight is the net ATP which is going to be generated from the glycolysis, if the NADH, if NADH is going to go to the electron transport chain and it is going to be oxidized completely.

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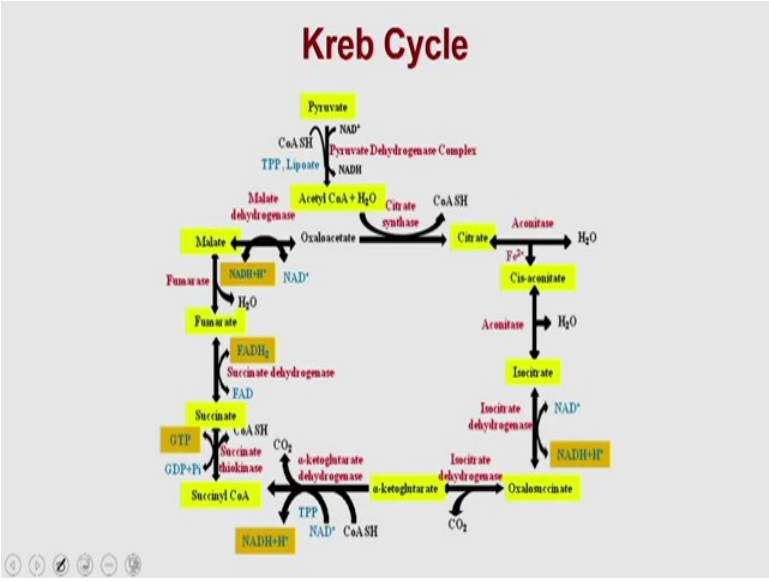

Once the pyruvate is generated it enters into the another cycle which is called as the Kreb Cycles. So the pyruvate is going to be converted into the acetyl CoA. And in this step you are going to generate 1 molecule of NADH then and remember that you are going to generate 2 molecules of a pyruvate because you have generated the 2 molecules of glyceraldehyde-3 phosphate. And then acetyl CoA will gets channelized into the Kreb cycle to go with these intermediates and as result is also going to generate large quantity of NADH, FADH 2, ATP and GTP.

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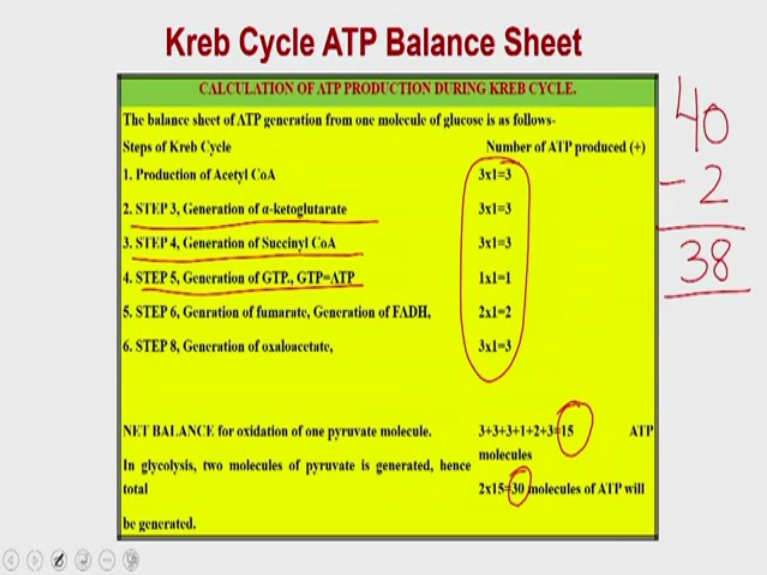

So if you see the balance sheet what you are going to do? There is no investment of ATP as it was done in the glycolysis, so glycolysis we are investing 2 ATP so that the molecules are getting channelized into carbohydrate metabolism. Whereas in the Kreb Cycle you are just simply using the, you are generating different intermediates. So that is how you are actually producing the different types of ATP molecules at different steps.

In step 3, you are generating NADH molecule then you generating NADH molecule and so on. And ultimately what is happening? You are actually making the 15 ATP molecule from the 1 pyruvate which you are getting from the glycolysis. But since you from glycolysis you are getting 2 ATP molecules, you are going to have the 30 molecules of ATP total ATP generated after the kreb cycle. So if I ask you how many ATP are going to be produced during the carbohydrate metabolism if you channelized 1 glucose molecule into the glycolysis followed by the carbohydrate, it is going to be 40. Whereas the 2 molecule you are going to utilize. So 38 is the net ATP gain after the 1 molecule of glucose oxidation considering that the glucose is going to be oxidized completely.

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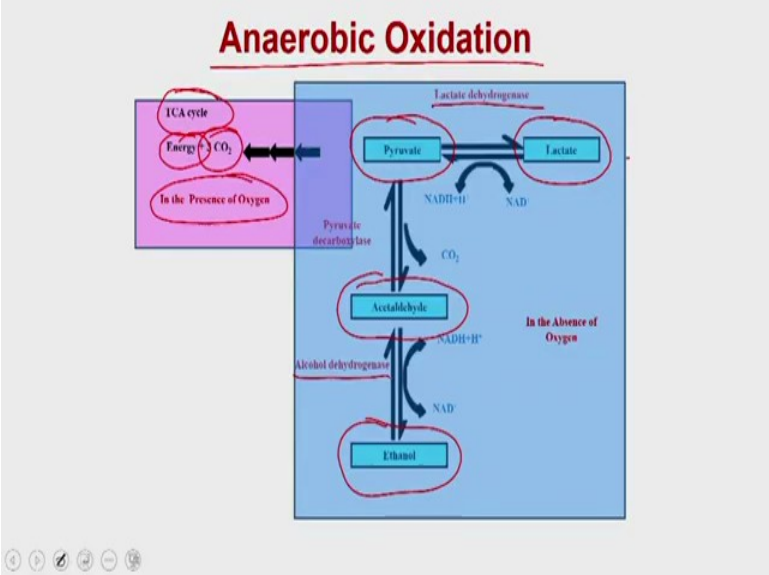

So after understanding this we have moved on and discuss about the anaerobic oxidations. So you understand that the glycolysis or the Kreb cycles are operatings, because there is a plenty of oxygen available in the system. And that oxygen actually allows the electron transport chain to run. But in the absence of electron transport chain you are going to have the anaerobic oxidations. So if you have the oxygen present then you are going to use the kreb cycle or the TCA cycle and that actually is going to generate the energy as well as the 3 carbon dioxide molecule. But if the if the oxygen is not present then the pyruvate which is being generated after the glycolysis is going to be channelized and produce the lactic acid or the pyruvate is going to be channelized to produce the acetaldehyde followed by the ethanol.

And in the so there are 2 enzymes which are actually working in this anaerobic oxidation. The enzyme one is called as the Lactate Dehydrogenase and the enzyme two is called as a Alcohol Dehydrogenase. And those these are the 2 enzyme or in under these conditions the because of the anaerobic oxidations you are actually, what you are doing is, you are converting the pyruvate to lactate or you are converting the pyruvate to ethanol. So that has a long significance in terms of for the organism as well as it also has significance for the industrial applications. We have discuss all these we have discuss about the reaction mechanism of both the reactions in detail in the, in that particular lectures. So if you would like to know the mechanism you can actually follow those you can follow that particular lecture again and that will actually give you the details.

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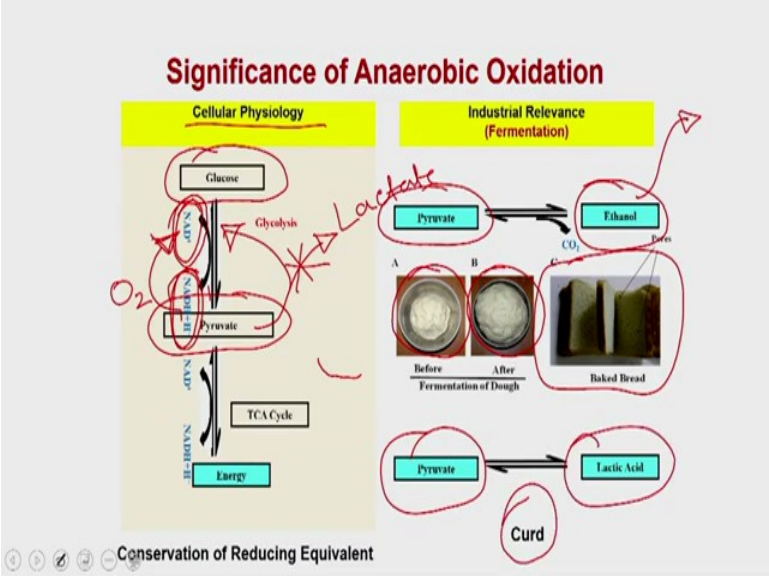

So what is the significance of this? For cellular physiology, the anaerobic oxidation is that when the glucose is being utilized it is actually producing the pyruvate. And in this process, it is actually converting a 1 molecule of NADH to NADH. This NADH will be get converted into NAD plus only if the oxygen is present. But since the oxygen is not present, what will happen is, this NADH is there will be no NAD plus present in the cell if the all the NAD plus get converted into NADH. All the only the NADH is going to be present but there will be no NAD plus present.

So if that happens there are so many reactions where the NAD plus required and that actually is going to stop the cellular metabolism. So because of that, what will happen is that the pyruvate is going to be get converted into the lactate and in that process the NADH is going to be utilized to produce the NAD plus. So that is actually being done simply by running the anaerobic oxidation which means it is actually a futile cycle where the glucose is getting converted into pyruvate and the pyruvate is getting converted into lactate and in this process what will happen is the NADH is getting generated.

so this means it is actually making the pool of NAD plus available for the cells to run its cellular metabolism which means to other reactions not the glycolysis or the kreb cycle but all other reactions. So that the cell will be sustaining for longer period of time and in that process if the oxygen will be available on a later date, then the automatically this pathway is going to be cut down and then the main pathway will be running and then starts producing the energy. What is the industrial relevance? Industrial relevance is that under these

conditions when the pyruvate is getting converted into ethanol, the pyruvate we are actually using this for producing the alcohol and the one of the classical example is that you can use yeast as a molecule to run the alcohol productions.

Or the other example is the fermentation where you are actually adding the yeast molecules and because the dough has very limited quantity of oxygen it is actually going to go through with the anaerobic oxidation. And under the anaerobic oxidations it is actually going to generate and that is how the, it is going to give you the baking of the bread as well. Because the pyruvate is getting converted into lactate, so that actually is the example of where the milk is getting converted into the curd. Because the lactic acid is going to reduce the pH of that particular and in that process is the milk is going to be get converted will converted into the curd.

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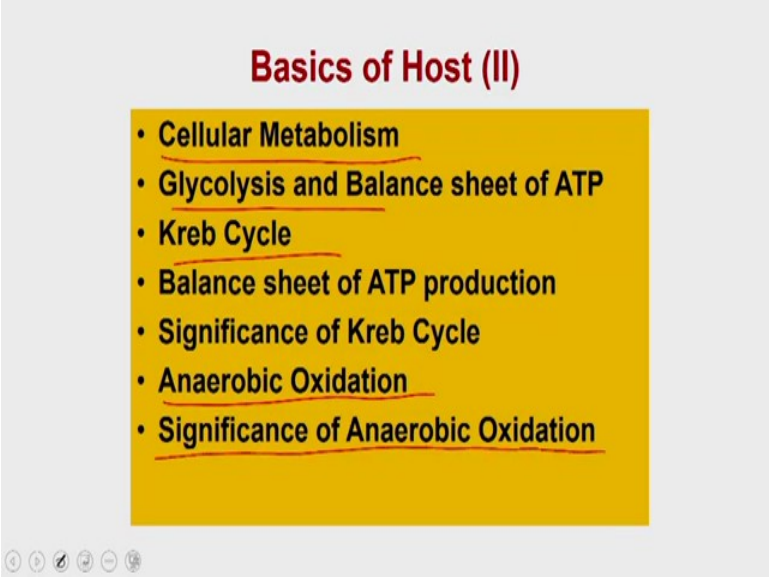

So this is a, what we discuss so far in the in terms of basics of hosts. We have discuss about a cellular metabolism, we have discuss glycolysis, kreb cycle then we have discuss about the anaerobic oxidations and at the end we have discuss about the significance of anaerobic oxidations. So in the module 1 and 2, we have discuss more about the hosts whether in terms of initially in terms of the cellular structures and then subsequently in terms of the metabolism.

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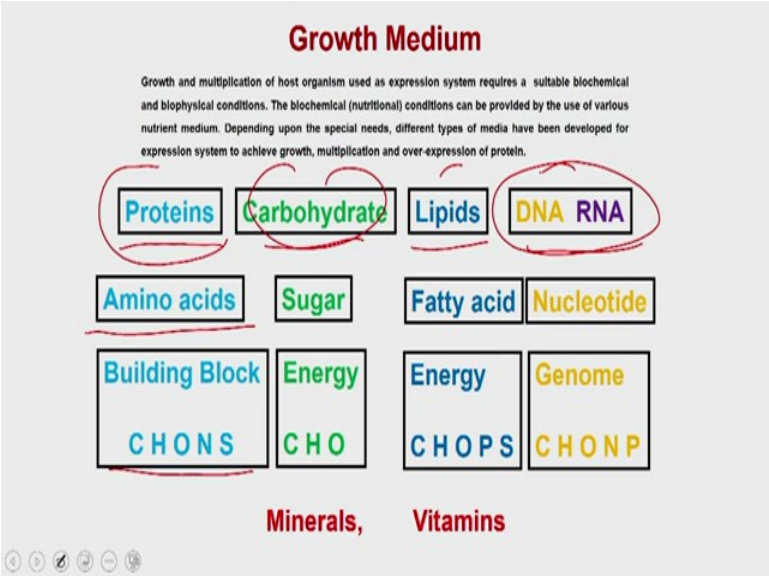

But in the next module, we have more discuss about the growth media so because you once you understand the metabolism you also should understand how to grow the bacteria or how to grow your host. So for that then we have discuss about the different types of growth media and the purpose of your growth media is to provide the protein, carbohydrate, lipids and the DNA, why it is so? Because if you provide the amino acid or the building blocks you can allow the organism to take up these molecules and generate the protein, carbohydrate, lipids as well as the DNA and RNA.

And together all these components are going to facilitate the growth of that particular organisms. So we have discuss different types of growth media for prokaryotic cells, for yeast, for mammalians cells, for insect cell lines and so on. In this particular type of module we have also given you a demos to how to prepare the bacterial culture media or the cell culture media and what are the different precautions you should take while you are preparing these medias.

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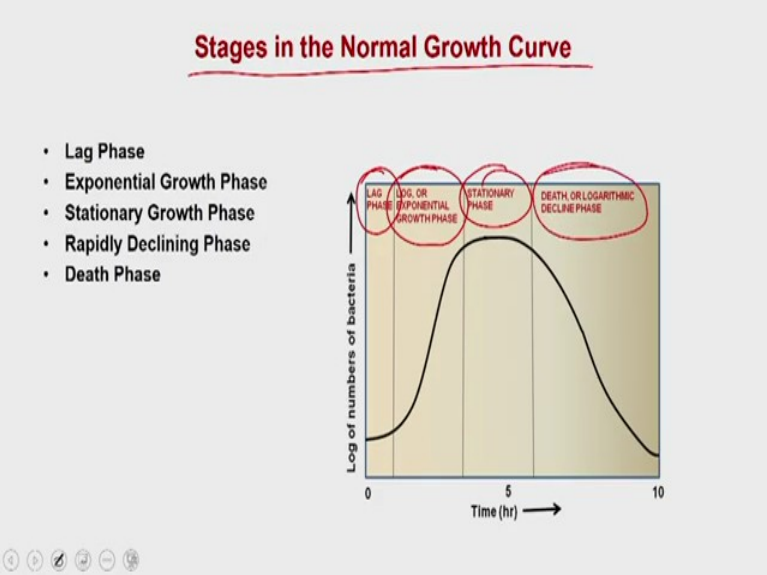

When, then we also discuss about the growth kinetics of a typical bacterial growth kinetics that which will we have lag phase. We discuss about the significance of log phase, stationary phase and as well as death phase.

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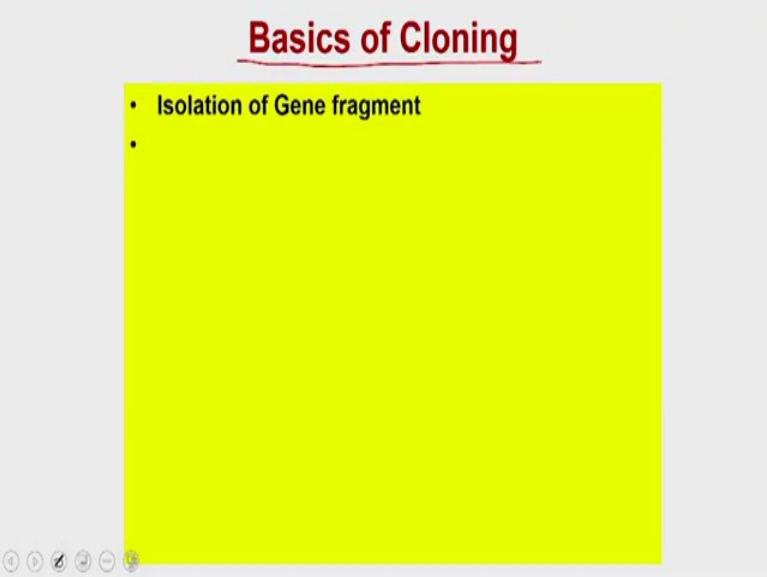

So this is all about the basics of the host where we have discuss about the initially we have discussed about the cellular structures followed by the metabolism and followed by the different types of growth media as well as the growth kinetics. And that actually will allow you to understand the potential of each host, how to grow that, how to manipulate and so on. And then once you know about the host we moved on and try to give you the idea about the different types of the techniques how you can be able to isolate a gene fragment under the

next module that is called as the basics of cloning. Because if you want to generate a recombinant DNA you have to get a recombinant clone gene you have to get your gene of interest from the different sources.

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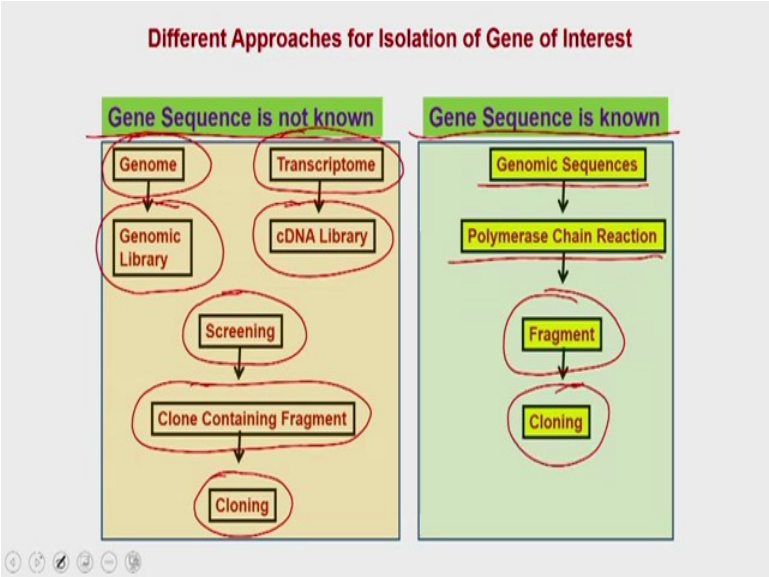

So we discuss about the 3 sources, the sequence, the 3 conditions with a condition when the gene sequence is not known or a sequence or the condition when the gene sequence is known. When the gene sequence is not known you either have the (()) (34:29) of getting these gene sequence from the genome by the help of generating a gnomic library or you can have the if the protein is getting expressed you can have the transcriptome then you can actually generate the cDNA library.

Ultimately you are going to screen either of these libraries and then you will going to get the clone containing your fragments and that you can use for cloning reactions. Similarly, if the gene sequence is known that you have the gene sequence then you (generate) you prepare the primers. You get the polymerase chain reactions that is going to give you the fragment and that fragment you can be able to clone into the vector of your interest.

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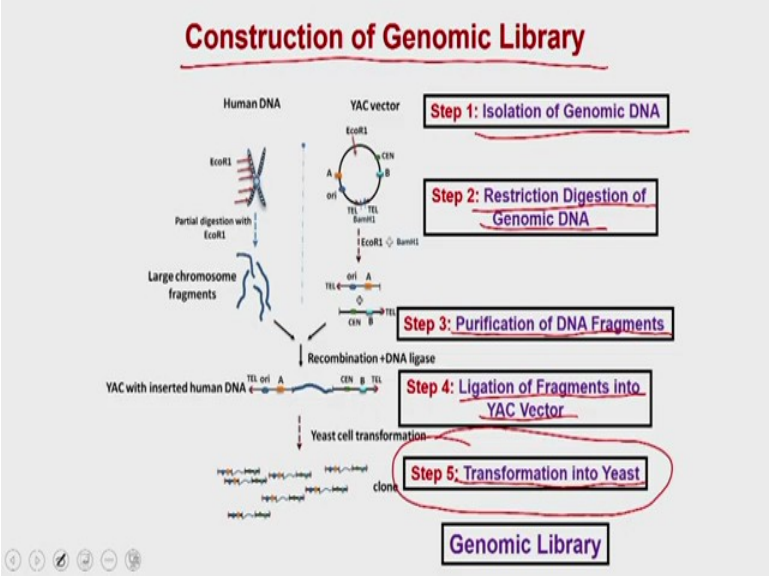

So the different steps what you have to go through with the construction of genomic library. So in the step 1, you are going to do a isolation of genomic DNA and a step 2 you are going to do a restriction digestion of genomic DNA. Step 3, you are going to do a purification of DNA fragments. Step 4, you are going to do a ligation of fragments into the YAC vectors and then step 5, you are going to do the transformation into the yeast and the that actually is going to give you the genomic library into the yeast.

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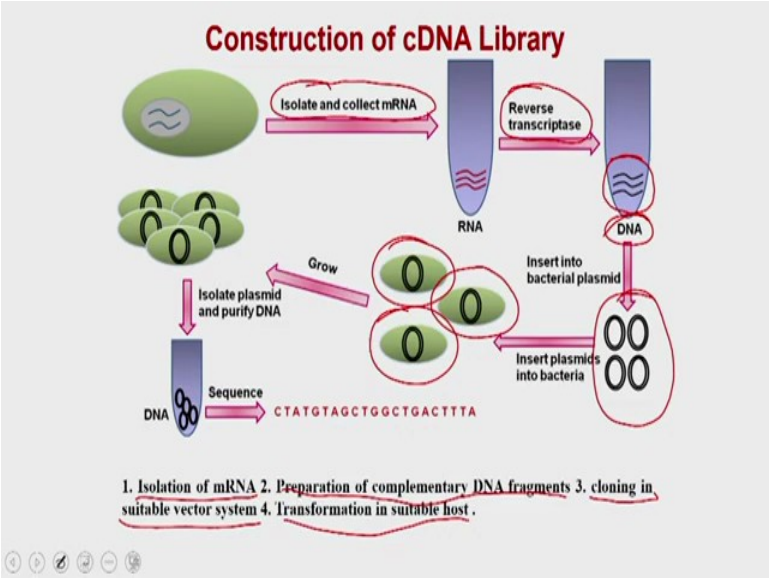

Apart from that for the cDNA library, first step is that you isolate and collect the messenger RNA then with the help of the enzyme reverse transcriptase you produce the DNA, or the cDNA, then you clone this insert, clone this cDNA into the plasmid of plasmid or the bacteria

and then you to do a transformation so that it will be taken up by the bacterial cell and that is how you are going to get the cDNA library. So these are the steps isolation of messenger RNA, preparation of complementary DNA fragments cloning into the suitable vector and then transformation into the suitable host.

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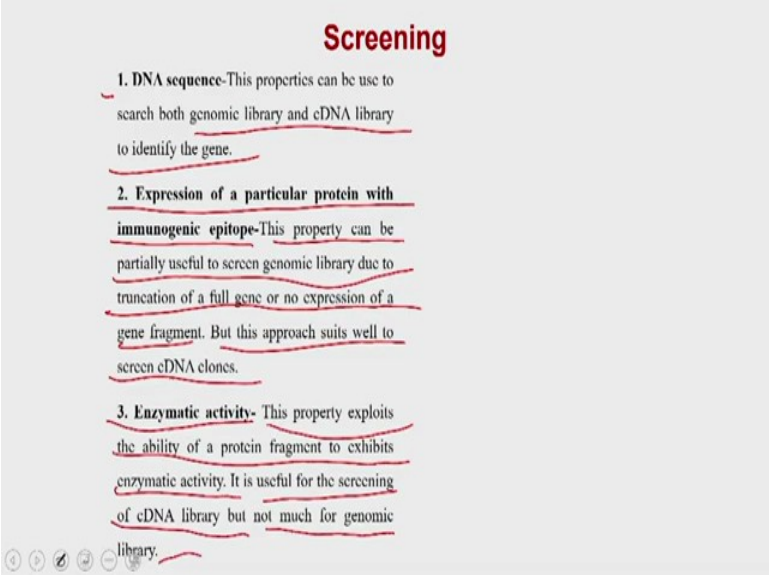

Then we have also discuss about the different types of screening methods what are available. So screening method can be worked on to the 3 criteria, one is called DNA sequence. So DNA sequence property can be use to screen both the genomic library as well as the cDNA library. Then if the protein is getting expressed then you can use the immunogenic epitopes with the help of the antibodies.

So this property can be partially useful to screen the genomics library due to the truncation of a full gene or no expression of a gene fragment but this approach suits well to the screen cDNA clones. Then the third is enzymatic activity, this property exploits the ability of a protein fragment to exhibit enzymatic activity. It is useful for the screening of cDNA library but not much for the genomic library because again the same thing because the protein may not express completely because the small protein fragment might be present into the clone or you that particular fragment may not be giving you the protein or the enzyme.

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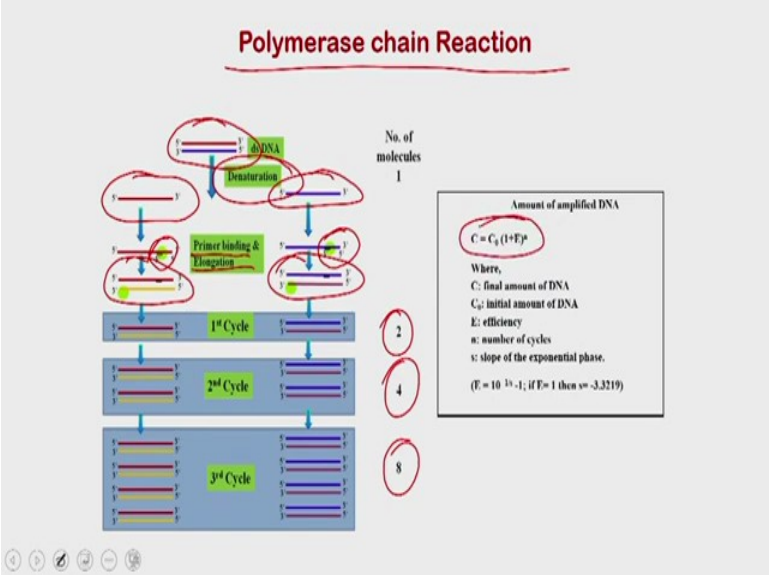

Apart from that we have also discuss in detail about the PCR, we have discuss about how the PCR works, where the single template is being utilized by the help of the forward primer and reverse primer and then you have the different steps where the annealing, denaturations and extensions and that is how you are going to get the amplifications. So you are going to have 3 steps, one is the denaturations. In the denaturation step, the template is going to be broken down into the individual fragments then you have the annealing step.

Annealing step, your primer is going to bind, so the forward primer will bind into this, the reverse primer will bind into this. And then you have elongation step where the primer if with the help of a primer and DNA polymerase. The next strand is going to be synthesized and that is how when you if you started with the single molecule in the beginning, after the first cycle you are going to have the 2 molecules, second cycle you are going to have 4 molecules and the third cycle you are going to have the 8 molecule and so on. And if you would like to use or utilize or want to quantitate what is the amount of DNA what you are going to get used this formula to calculate.

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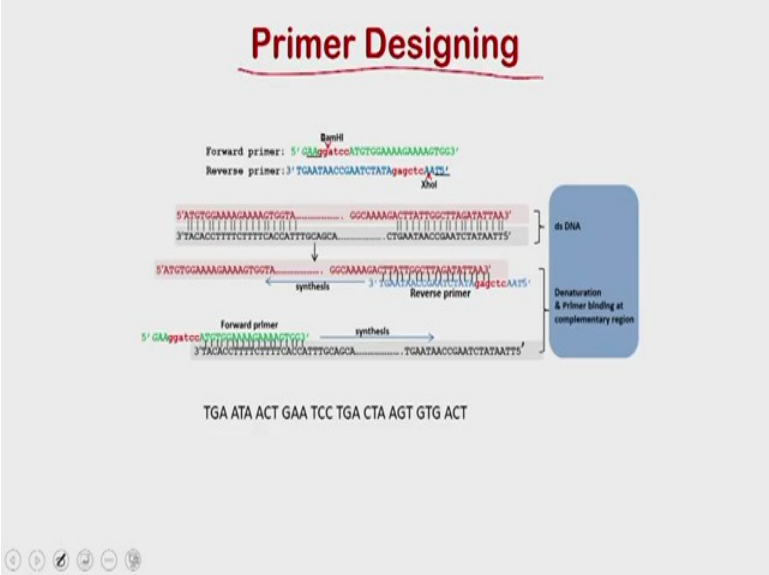

We have also discuss about how to design the primers whether it is the forward primer or the reverse primer. We also given you a demo of the different types of problems what you are going to encounter or how to actually validate the primer sequences so that you will be able to understand when you have designed a better primer or not. For example, you can be able to see whether the loop formation is there not or different types of secondary structures are forming or not or whether the primer dimer is forming or not and so on. And how you can be able to overcome these problems either by utilizing some of the reagents or chemicals or you can be able to change the sequences in such a way so that you will it will still be able to amplify that particular region. But you will be able to overcome the formation of loops or the self-ligations.

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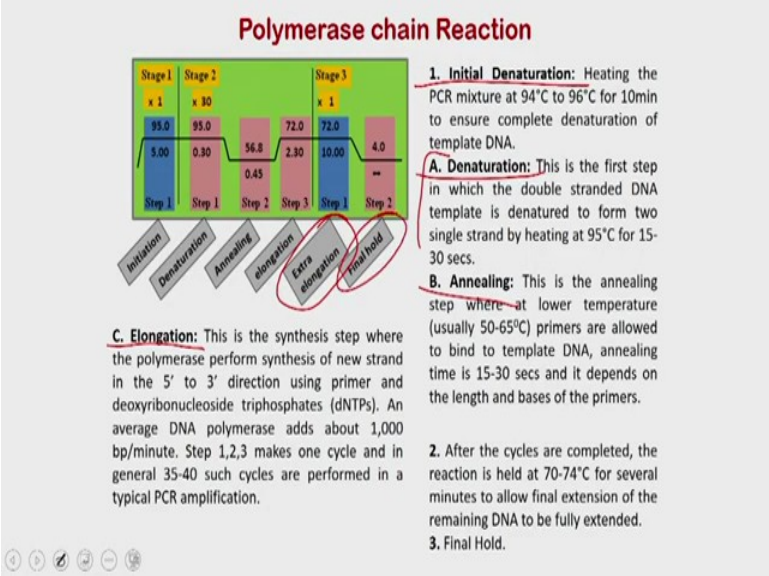

Then polymerase chain reaction has multiple steps as I said initial denaturations. Then you have the multiple cycles of denaturations, annealing and then elongations. And that actually will continue for at least 30 cycles and then you have the extra elongations. And then you have the final hold at 4 degree and that is how you are going to get the PCR done. And we have also discuss about how to analyze the PCR results and how to know that whether the amplification is being done or not and what are different troubleshooting you have (don) to do.

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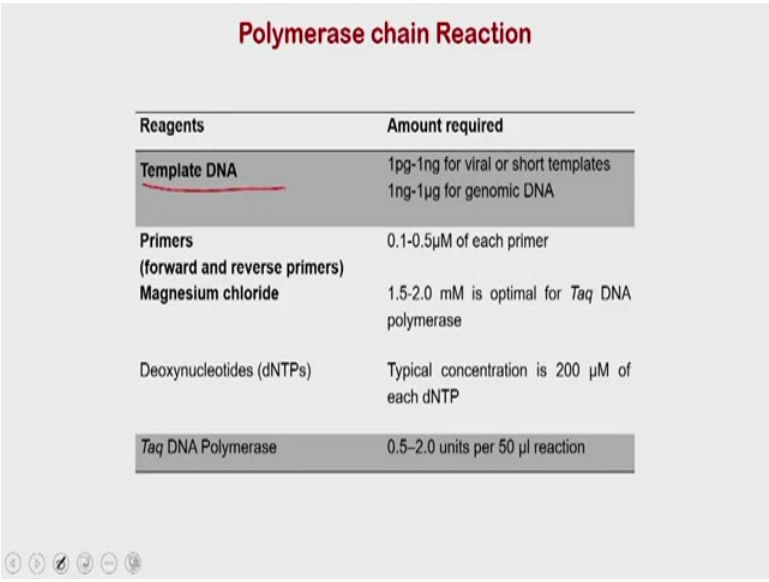

We are also discuss about how to setup the PCR reactions and so on.

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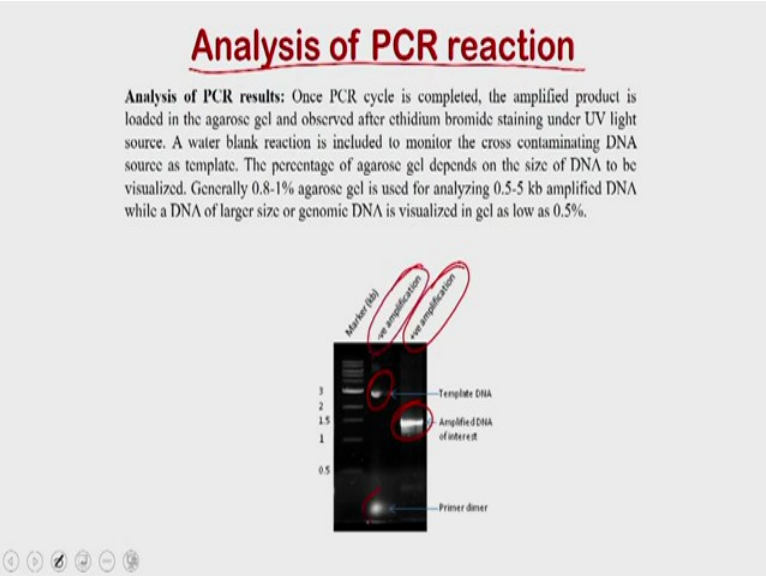

Then we have also discuss about the analyses of the PCR reactions. So you can see that in a negative amplification you are only going to see a template DNA that you if the amplification is done then you are going to see the template DNA as well. So if it is a negative amplifications you are going to see a primer dimers. Because the primer are not going to be utilized into the reactions but if it is a positive reactions you are going to see a amplifier product. And the primer are mostly being utilized into the formation of the particular amplify DNA.

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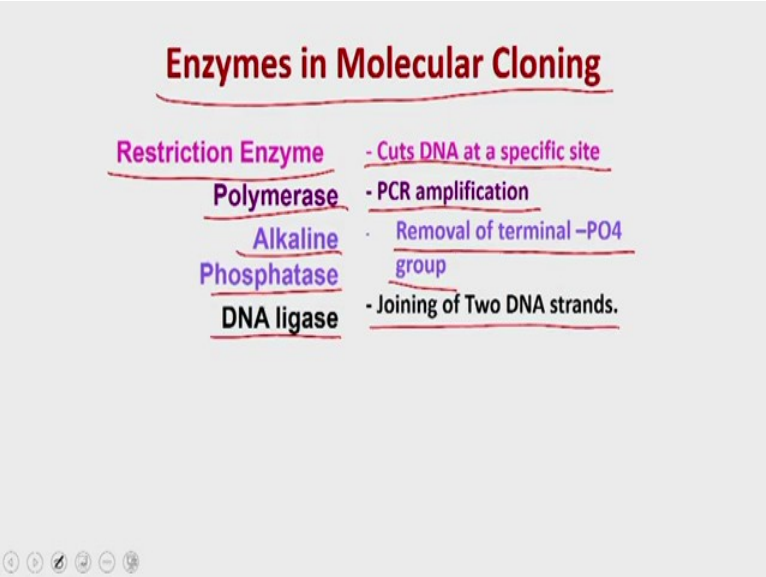

Then we further moved on and we discuss about the role of different types of enzyme in the molecular cloning. We have discuss about the restriction enzyme, we have discuss about the polymerases, we discuss about the alkaline phosphatases and then we also discuss about the DNA ligase. Restriction enzyme is required for cutting the DNA at a specific site. Polymerase is required for PCR amplifications. Alkaline phosphotase is required for removing the terminal phosphate groups. And the ligase is required for joining of two DNA so that you are going to get the recombinant DNA.

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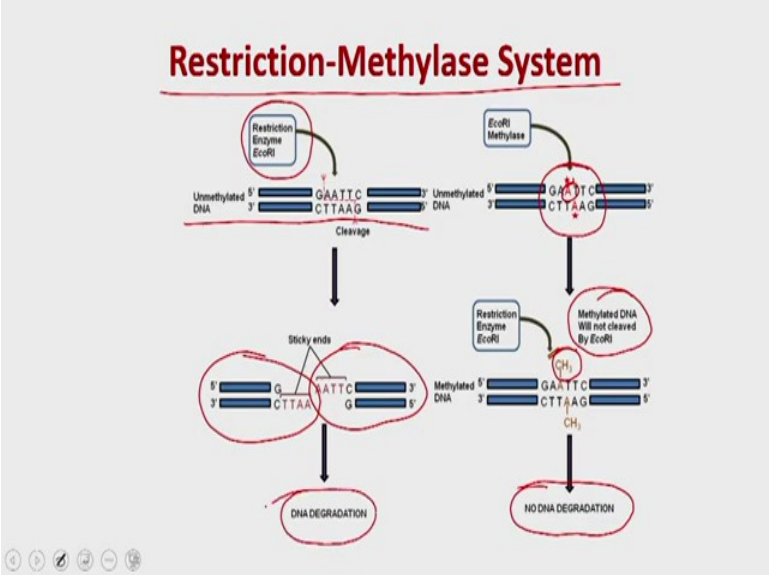

So restriction methylase system, restriction methylase system is like a immune system. It is actually helping the bacteria or the prokaryotic cells to understand whether the particular DNA belongings to them or to the other organisms. So if it is belonging to the other organisms it is not going to be methylated whereas if it is belonging to them it is going to be methylated and that is how they have the restriction methylase system. So in the restriction methylase system, the enzyme for example in this case the Eco R 1 is going to go to the DNA and find and then it will look for whether the adenine which is bound is methylated or non-methylated.

So if the adenine is non-methylated for example in this case, both the adenine molecules are un-methylated. So in that case what will happen is, that the enzyme will bind to this DNA and it is going to cut this DNA into the 2 fragments. Whereas if the adenine is methylated for example, in this particular DNA, the restriction enzyme will not be able to make the closed complex because the methylation is on the present is present on the adenine group. And because of that the methylated DNA will not going to be cleaved by the Eco R 1 and that is

how there will be no DNA degradations and there will be DNA degradation if there will be no methylation. So it is a kind of protection mechanism exist within the prokaryotic cells.

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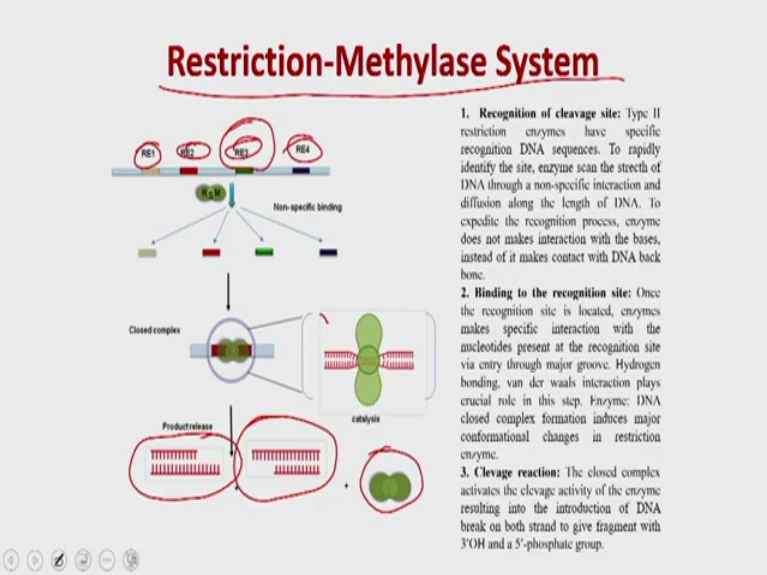

We have also discuss about the mechanism of the restriction methylase systems so in that particular thing what we have discuss is that suppose you have the different types of restriction sites RE1, RE2, RE3, RE4 and suppose you have added the restriction enzymes, so what will happen is, the restriction enzyme will go and bind all these restriction sites but it will only going to make the closed complex when it will go and bind to its respective restriction site. For example in this case, the RE3 is the restriction site for which the enzyme is being added. And so once it form the closed complex it is actually going to cleave the DNA and going to generate the fragments. Once it cleaves the DNA then the enzyme is going to be released.

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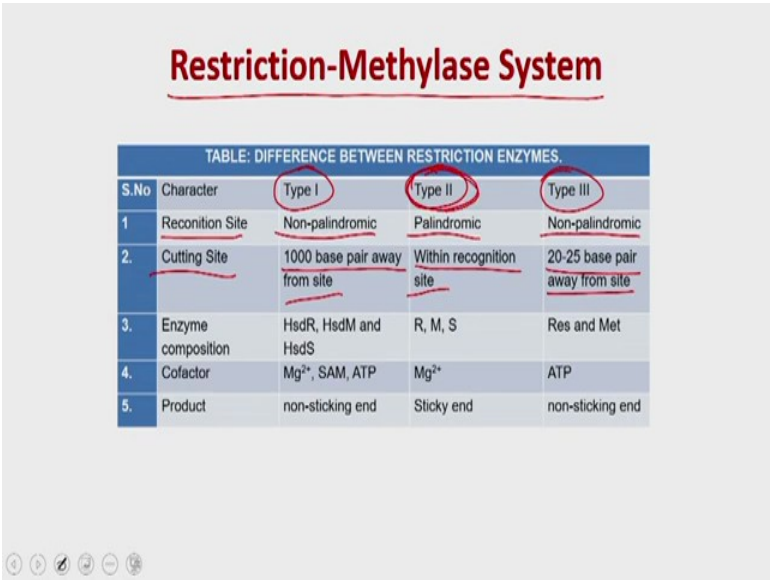

Then we have also compared the different types of restriction enzymes present in the system so you have the type I enzymes, you have type II enzymes and you have the type III enzymes. They are very different in terms of their properties, their cutting properties, their cleavage site as well as their recognition sites. So for example, in the case of recognition sites that type I it is non-palindromic, type II it is palindromic and in the type III also it is non-palindromic. Whereas the cutting site, the cutting site is 1000 base pair away from the recognition sites.

Type II it is within the recognition sites and the type III it is 20 to 25 base pair away from the recognition sites which means type II enzymes are actually going to first of all they are going to have palindromic sites and on the other hand they are also going to cleave the DNA within the restriction site. So actually you know precisely where that enzyme is going to cut whereas in the case of type I or type III they are going to cut away from where they are actually going to sit and bind. So that is why the type II restriction enzymes are preferred for the molecular cloning applications compared to the all type I or type III.

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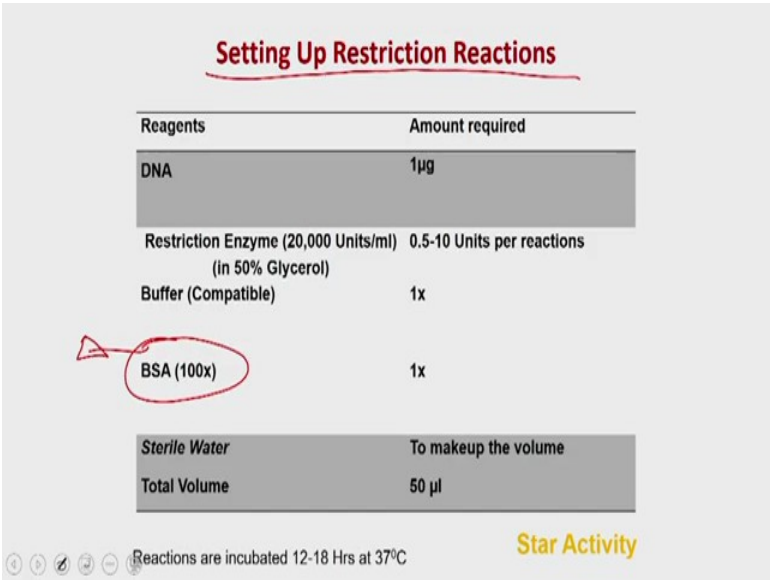

Then we have also discuss about how to setup the restriction reactions. So you are going to take restriction enzymes, you are going to add the BSA and the we have also discuss about that you are going to add a BSA only to reduce the star activity of the enzymes.

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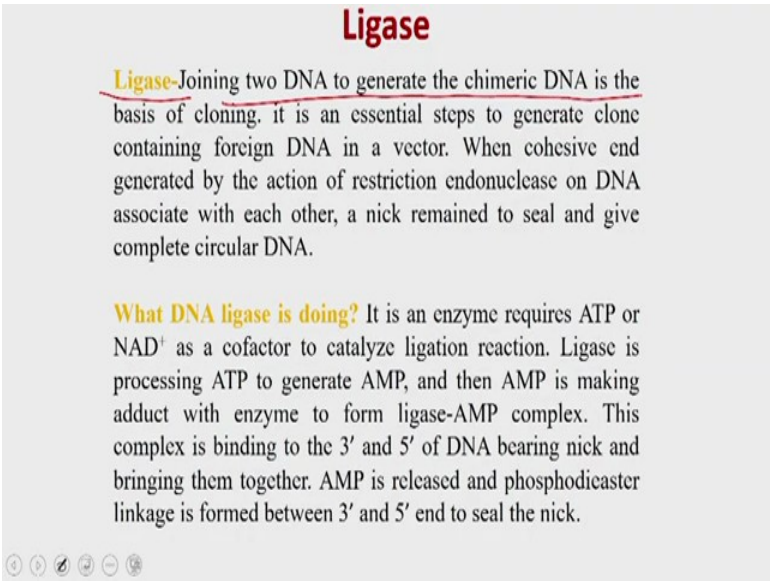

Then we discuss about the ligase, ligase is an enzyme which is actually joined the two DNA to generate chimeric DNA and that is actually the basis of the cloning. So what you are going to do is, you are going to cut the gene fragment which you have isolated from the genome. You are going to cut the vectors and then you are going to cut them with the same set of restriction enzymes so that they are going to have the similar set of cohesive ends. And that is how you are going to put them into a ligation reactions.

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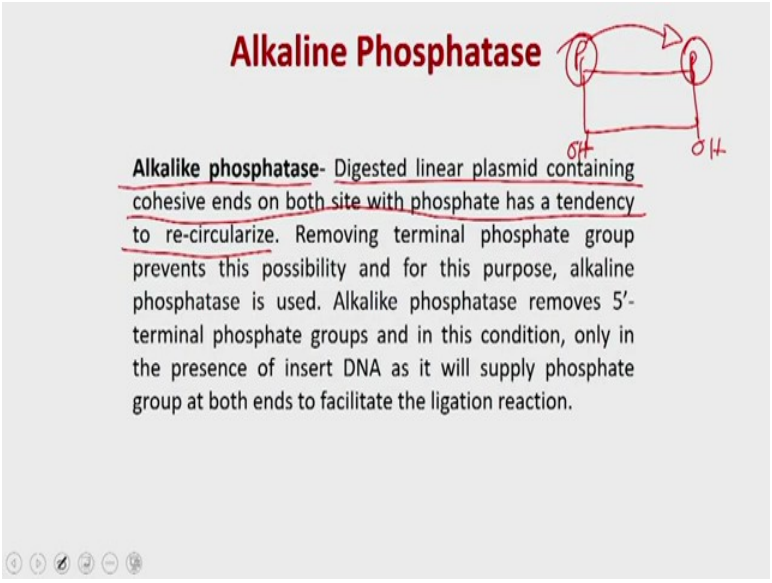

And that ligation reaction also we have discussed and how to setup the ligation reactions, at what temperature you should incubate the ligation reactions and what are the different precautions you should take while you are doing the ligation reactions. And then we have also discuss about the alkaline phosphatase, so digested linear plasmid containing cohesive ends, ends up in, ends up in both sides with phosphate has a tendency to recircularize, which means if you have a fragment and you have the phosphate on both the ends that actually has a tendency that these 2 actually will going to bind to the which which are present onto the other side. And because of that if you remove this phosphate groups with the help of the alkaline phosphatase, this fragment is not going to recircularize until it is going to get the other DNA.

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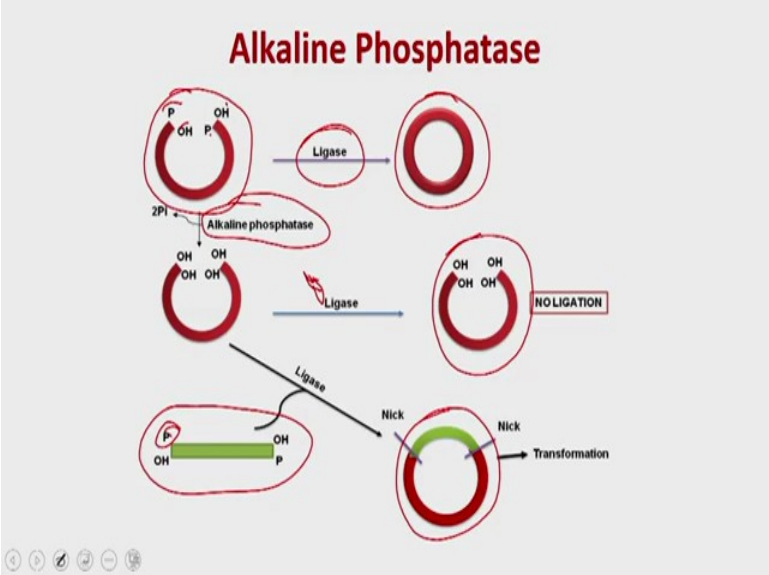

So that is what is shown here, if you have a circular plasmids which is actually going to be cut by an enzyme so you have a phosphate, you have a OH, you have a OH, you have a phosphate. So if you put it for the ligation reaction this plasmid is actually going to bind to each other to give you a circular plasmid instead of having a insert within that. So if you treat this within alkaline phosphatase it is actually going to remove the phosphate which is present onto the, this particular plasmid. And what will happen is, it is now going to have the OH, OH. So in that case what will happen is, if you put the inserts, the insert phosphate group is there, so it actually going to utilize the insert phosphate group. And as a result it is going to give you the recombinant DNA whereas the OH, OH is not going to be get recircularized and it will not going to give you the colonies.

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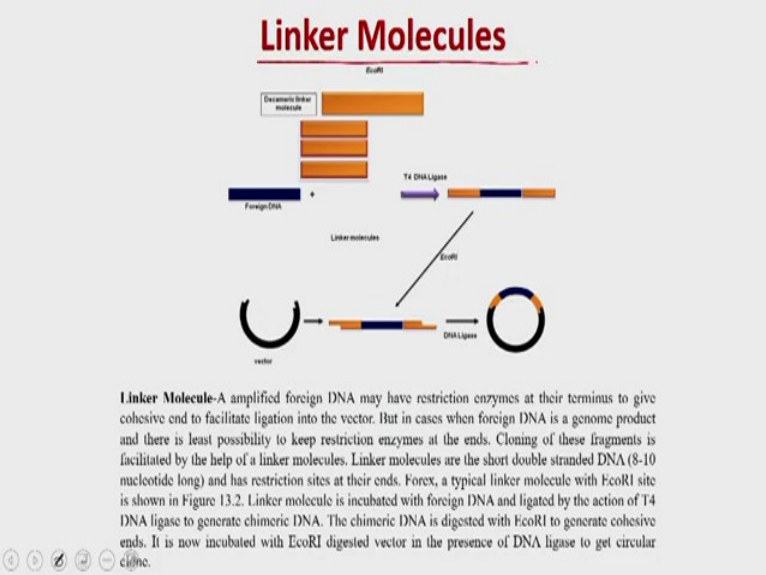

Subsequently we discuss about the linker molecules, the significance of the linker molecules, the utilization of linker molecules and so on. Then we discuss about the adapter molecule as well, so in what conditions you use the linker molecules, in what condition you use the adapter molecules.

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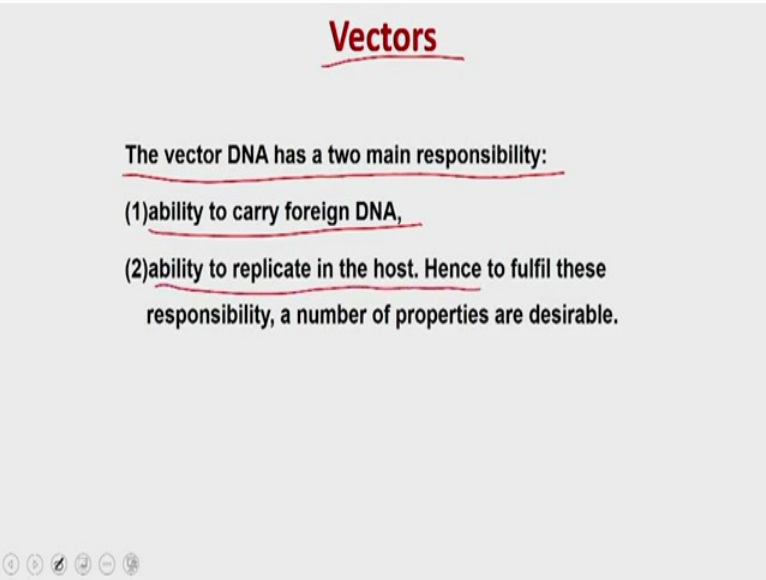

Then in the molecular cloning we have also discuss about the vectors. So the vector is the DNA which has a 2 responsibility, ability to carry the foreign DNA or and the ability to replicate in host of your interest which means it should have the ability or it should have the features so that you could be able to integrate your foreign DNA. And then it should also have the feature to go into the host. This means you need a system for some machinery so

that it should have the ability to replicate within the host of your choice and then it should be having the regions so which you can use to integrate tour foreign DNA.

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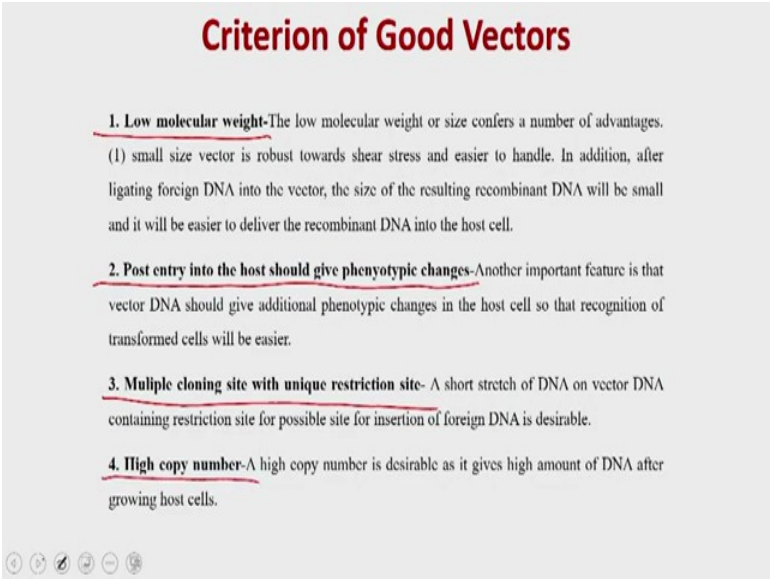

So what are the different criteria? The vector should be low molecular weight. Vector should be, give you the phenyotypic changes when it enters into the host which means it either should give you the antibiotic resistance, it should give some color or something like that. Then it should have a multiple cloning sites, so that you can be able to integrate the foreign DNA and then it should be of high copy number, so that your desirable things should be more.

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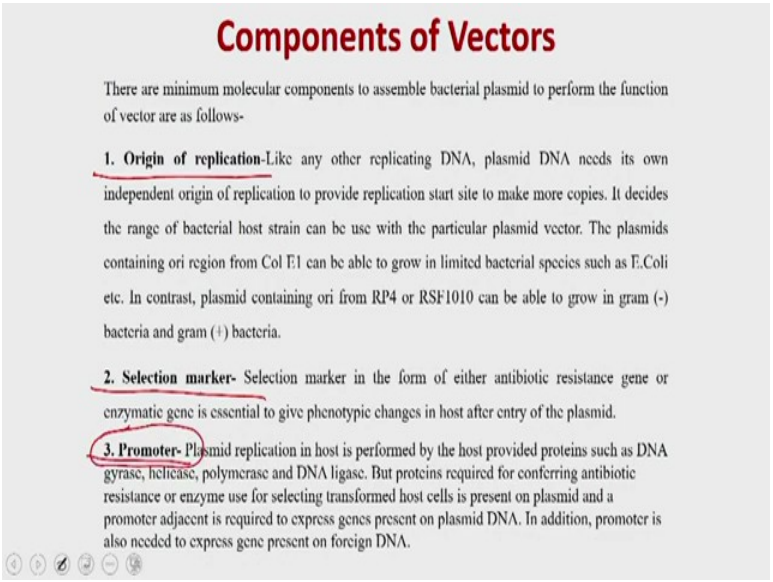

So what are the minimum component required to make a vector? You need a origin of replication, so origin of replication is required so that the vector is going to replicate within the host. Origin of replication is going to be specific for that particular post if it is if you are preparing vector for the bacterial system. Then it should be for bacteria, if it is for mammalian system then it should have the mammalian system. If it is for the yeast then it should be yeast. Similarly it should have a selection marker so that it should give the phenotypic changes it should and those phenotypic changes can be selected afterwards. And then it should have a promoter, so the promoter is the requirement for those plasmids where you would like to have the over expressions or the protein productions

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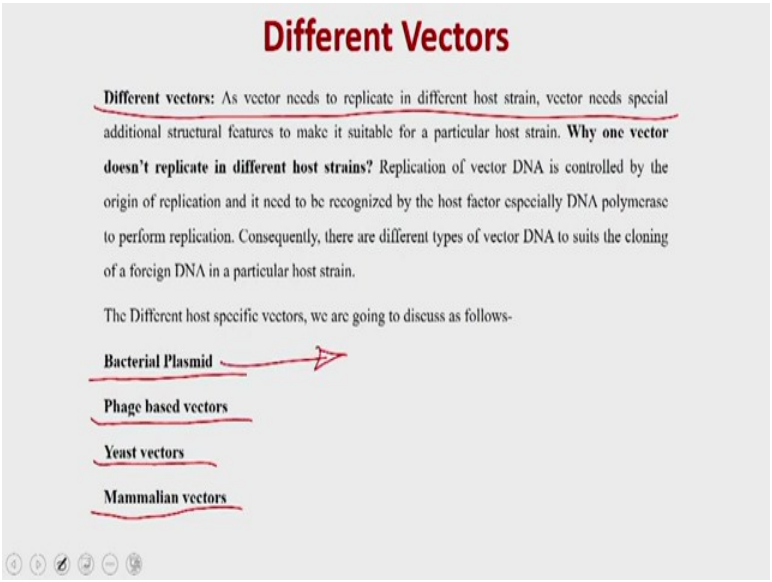

Then we have discussed about the different types of vectors, so we need we have discuss about the bacterial plasmids, we have discuss about the phage based reactors, we have discuss about the yeast vectors and we have also discuss about the mammalian vectors. In all these classes we have discuss about the inducible system and non-inducible system which means we have discuss about the inducible system and we have discuss about the constitutive expression vectors. We have discuss about the yeast vectors which are actually in having the inducible promoters, so that you can use the inducers. Similarly we have discuss about the inducible as well as constitutive vectors in the case of mammalian cells as well.

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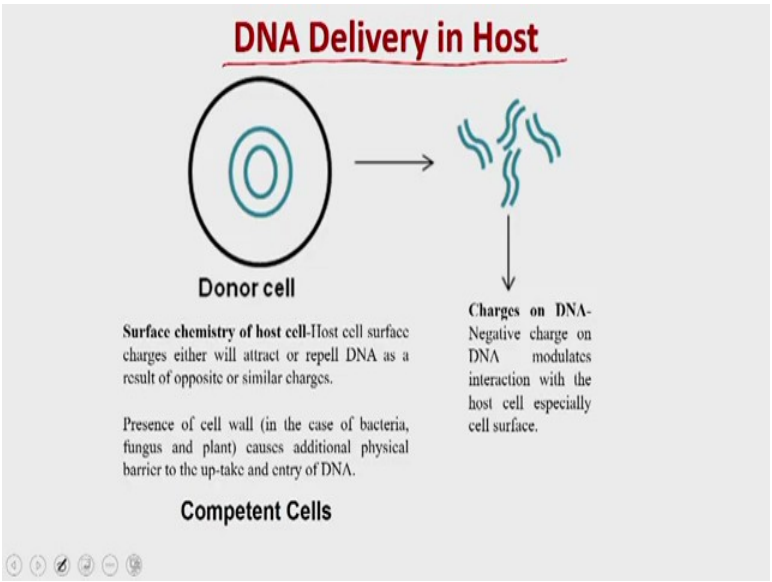

And then we have also discuss about the DNA delivery in host, so we have the first initially we discuss about what is the philosophy of the uptake of the foreign DNA by the host. And later on we have discuss about the different types of methods or strategies available in different types of host systems.

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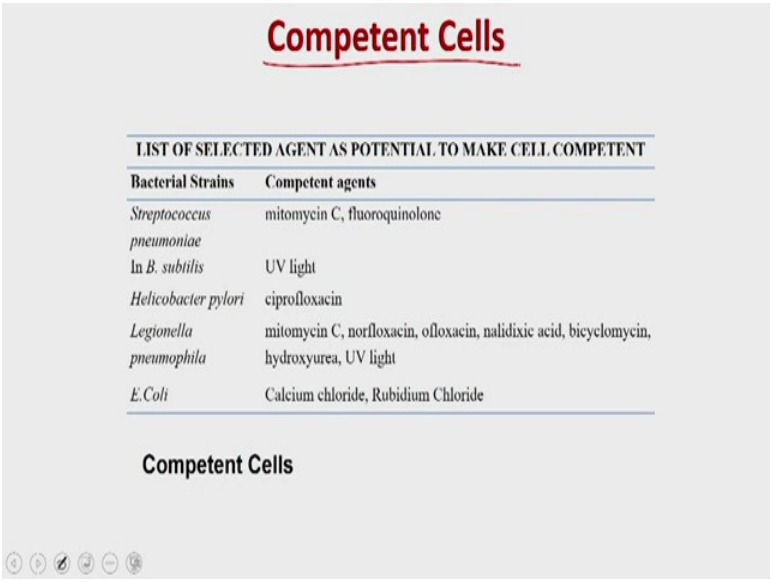

We discuss about the (transformation) preparation of competent cells to understand the transformations for different bacteria the competenting reagent is very different. And this competent cells are going to take the foreign DNA and they will get transforms.

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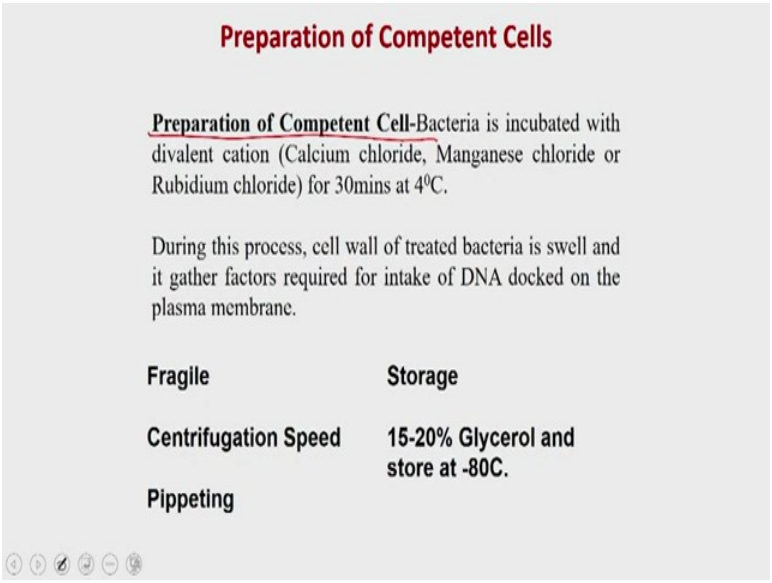

And then we have discuss about how to prepare the competent cells. We have also given you a very small demo, so that you can be able to understand the different steps and different precautions, what you should take while you are preparing the competent cells.

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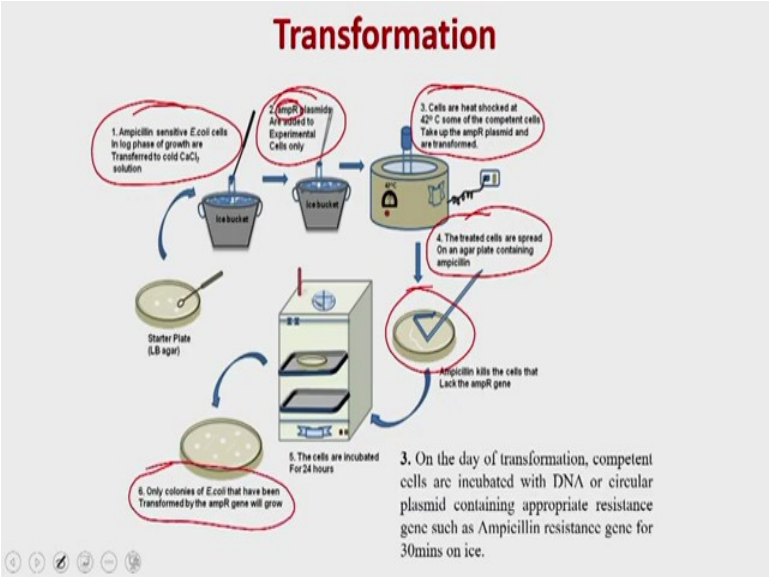

And in a typical transformations what you are going to do is, you are going to first grow the bacterial cells. You are going to add the, your plasmid of your choice. Suppose example in this case, we are taking a plasmid which is ampicillin resistance incubated onto the ice. Then you give them a heat shock at 42 degrees Celsius for 90 minutes. Then you put them for recovery phase and after that you do them plating. After the plating you leave the cell you plate into the incubator for 24 hours or 16 hours. And then that actually will going to give you the different types of E. coli colonies.

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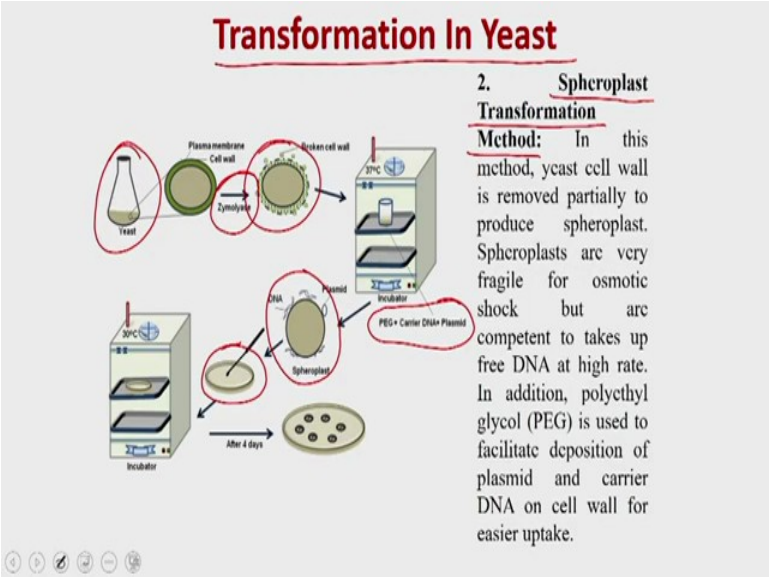

Similarly we have also discuss about the transformation in yeast, so we have discuss about different methods of transformation available in yeast, one of the method is spheroplast method. So in the spheroplast method, you first grow yeast cells then you are actually going to treat a yeast cell with the zymolyase, the zymolyase is going to remove the partially remove the cell walls and that actually is going to generate the spheroplast. And then you add the DNA plasmid as well as the PEG to the spheroplast and that is how the spheroplast will take up the DNA. Then you do the plating and incubate in into the 30 degree for 4 days and that actually is going to give you the transform yeast cells.

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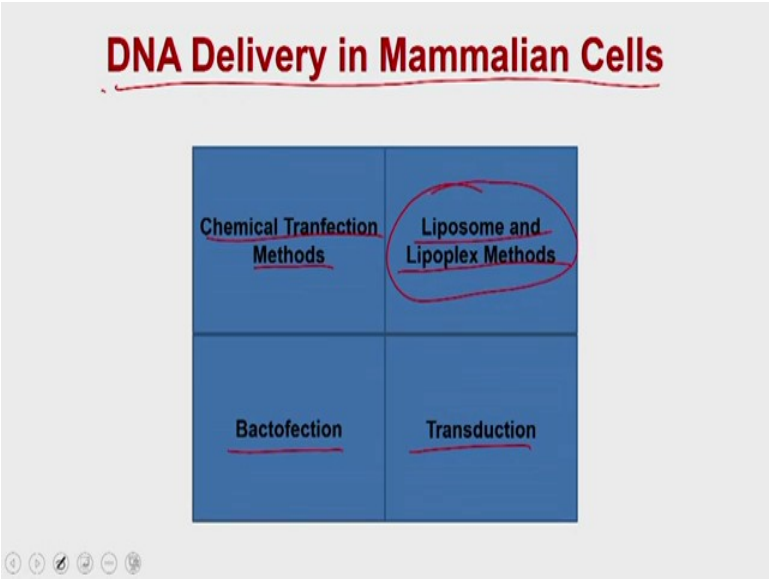

Subsequently we have also discuss about a different delivery methods available in the mammalian cells. We have discuss about the chemical transfection method, we have discuss about the liposome or the lipoplex methods. We have also discuss about the bactofecting and we also discussed about the transduction. Individually also we have taken up many of these methods we have given you the demo how to do the transfections into the bacterial cell into the mammalian cells. What are the different steps you should follow? So we have taken an example of liposome or lipoplex methods and that is how we have actually shown you a demo to do, to tell you, what are the different precautions you should take while you are doing a transfections into the mammalian cells.

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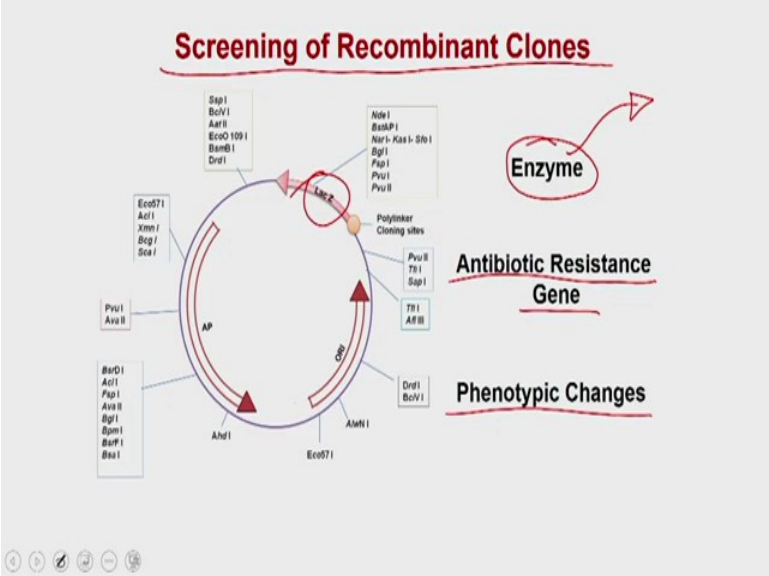

Then we have also once you done the transformation you also have to screen the recombinant DNA. For screening of the recombinant DNA you have the 3 choices either you go with the enzymatic activity which is going to be present. For example in this case, you can use a Lac Z as an enzyme, so in the case of enzyme you can have the insertional inactivation which means if you clone within this enzyme it is going to inactivated. So your untransformed one is going to give you the reactions whereas the transformed ones are not going to give reactions. Similarly you have the antibiotic resistance genes as a screening method or you can have the phenotypic changes for such as the blue white screening.

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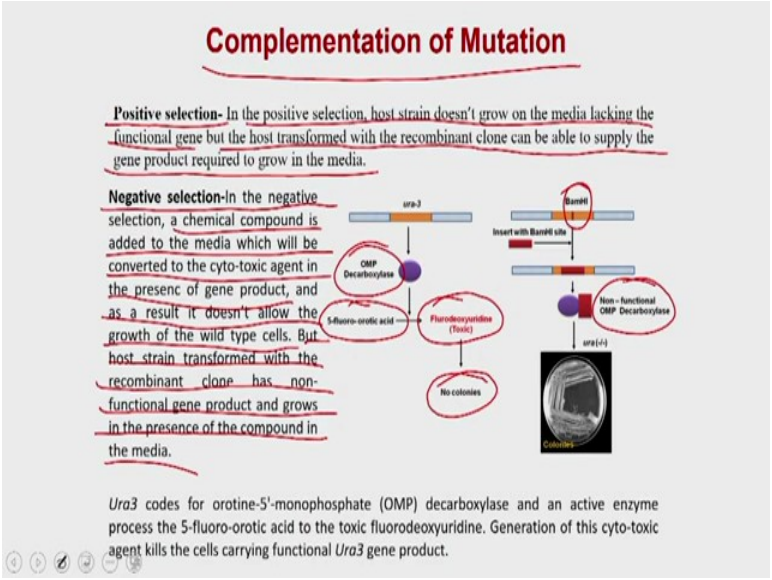

So in the, and then you have the complementation of the mutation that is a typical technique or typical way of screening the things into the yeast. In the complementations it could be a positive complementations. In the positive complementations, the host does not grow on the media lacking the functional gene. But the host transform with the recombinant clone can be able to supply the gene product and required to grow in the media. So complementation media always being done onto the, those strain which are actually growing onto a media which are deficient of that particulars thing.

So once the deficiency is being provided by the transformation it actually grows and that is the positive selection. Whereas in the case of negative selections, a chemical compound is added to the media which will be converted to the cytotoxic agent in the presence of gene product. And as a result it does not allow the grow of the wild type cell. But the host strain transforms with the recombinant clone has non-functional gene product and grows in the presence of compound in the media.

Classical example is OMP decarboxylase which actually is going to process the 5 fluoroorotic acid to a fluorodeoxyuridine and the fluorodeoxyuridine is the toxic compound, so it is going to give you, it is going to give the, it is going to kill a yeast cells. And that is how going to, not going to form any colonies. But if you do a cloning within that gene, it is going to produce the non-functional OMP decarboxylase and that is how it is not going to catalyze this reactions and it is going to give you the colonies.

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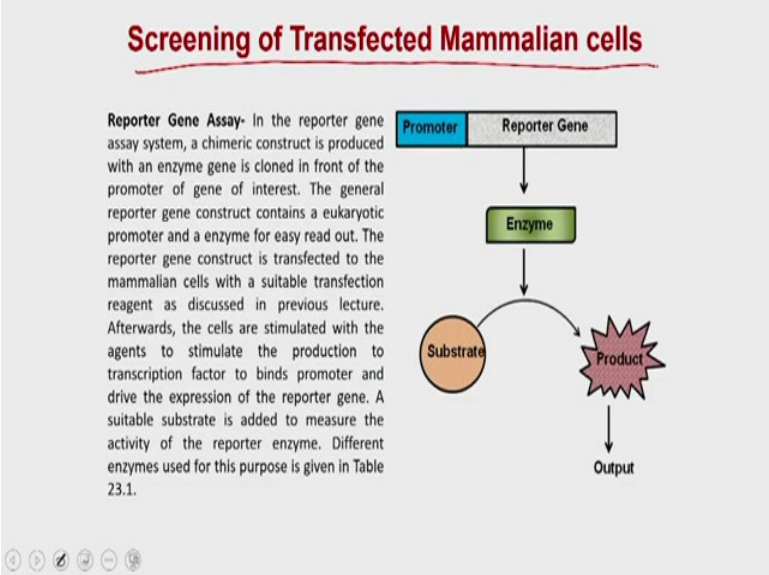

Then we have also discuss about the screening of transfected mammalian cells. We have discuss about the reporter gene assays which you can use to see whether the particular mammalian cell is being transfected or not.

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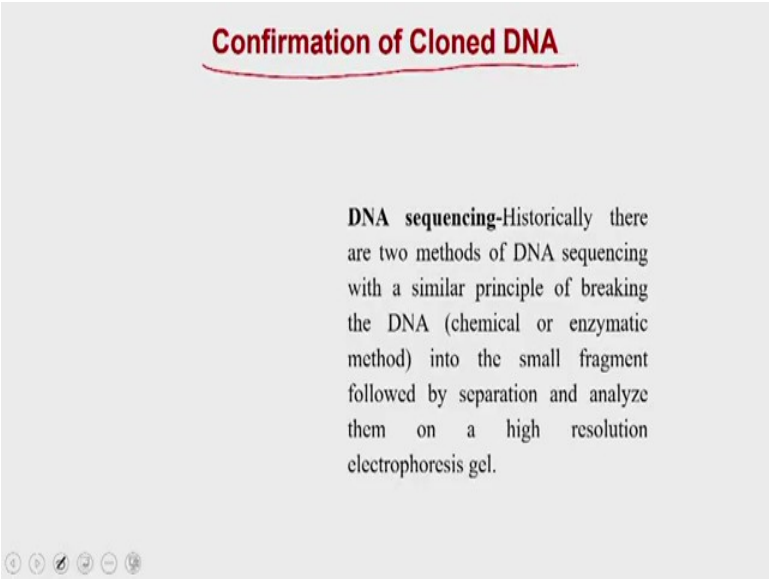

And ultimately we have also discuss about the confirmation of the cloned DNA. So confirmation of the cloned DNA is that the cloning of that particular recombinant clone what you got after the cloning is actually having the right sequence or the right a nucleotide sequence. You can done, you can do that by simply sequencing the DNA. So you have the 2 methods, dideoxy method or the Sanger method. And that those 2 method you can use to

sequence the DNA and that actually will give you the confirmation that the DNA that you are, the clone what you have prepared is correct

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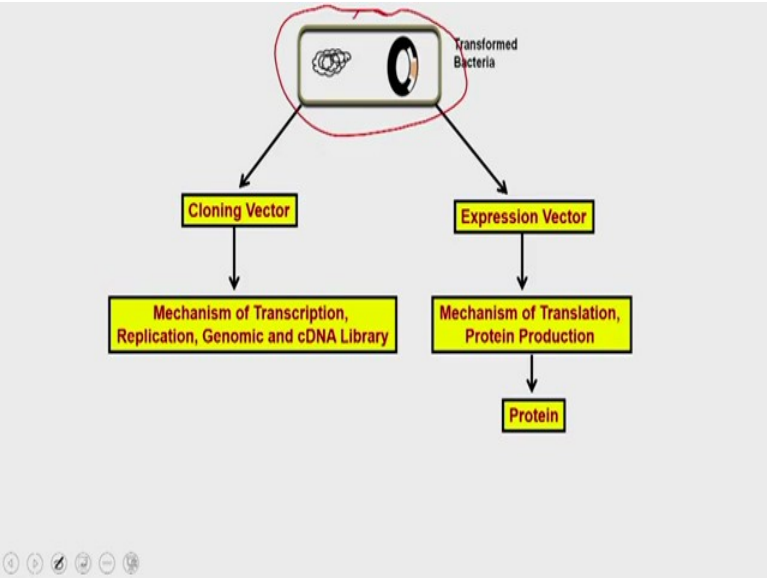

So after that we have also discuss about how to do the over expression of a particular protein into the host. So once you generated the transform bacteria you screen them you are actually having the 2 possibilities, you have done the cloning into the cloning vector or into the expression vector. Cloning vector is always being used to answer the question which are related to the mechanism of transcription, replications or it can be used for preparation of genomic or the cDNA library. Whereas the expression vectors you can use the mechanism of translation or the protein productions. So ultimately if you clone it into the expression vector you could be able to over express the protein into that particular host cells

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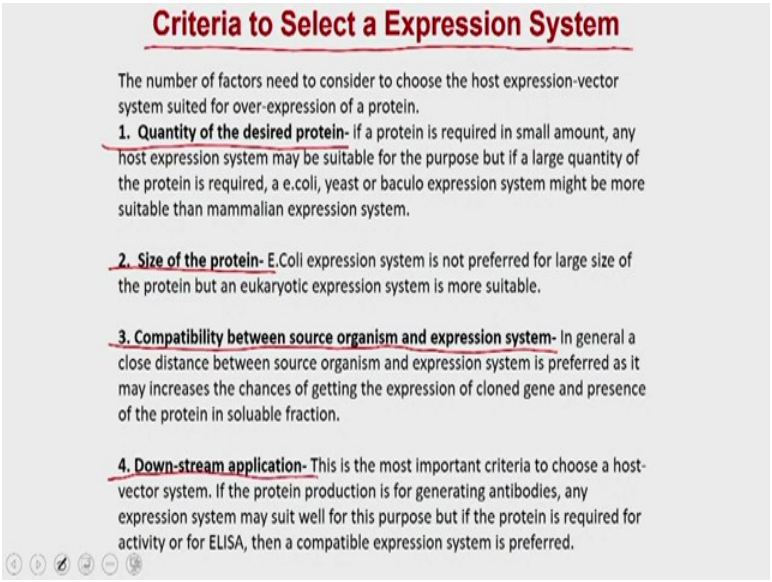

We have discuss about what are the criteria to select a particular expression system. It depends on the quantity of the desired protein, it depends on the size of the particular protein, it depends on the compatibility between the source organism on the expression system. And then it also depends on the downstream applications. So considering these criteria you can be able to choose the different types of host whether it is bacterial host, yeast host, insects or the mammalian cells.

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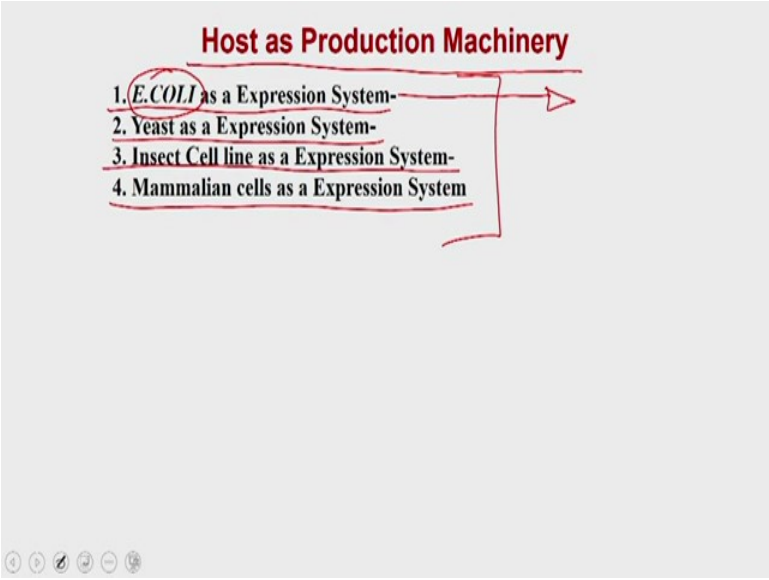

So in the host as the production machinery we discuss different types of expression system, we discuss E.COLI as an expression system. We discuss yeast as an expression system. We discuss the insect cell line as an expression system and we discuss about the mammalian

expression system. In the within the, within of in all these expression system we have also discuss about how you can, because you know all this expression system have different types of promoters. So they can have the different types of inducers which you can actually use to induce the protein production and that is how they are very different. So when you are this when you taking care of the E.COLI or yeast, the manipulation methods are going to be very different from the when you are talking about the insect cell line or the mammalian cell lines.

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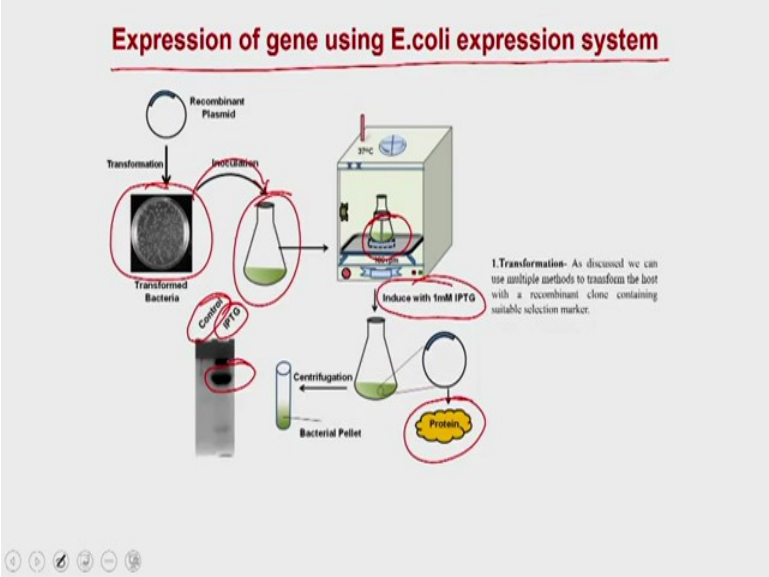

So expression of a gene using the E.coli expression system is very simple first you do the transformations, you get the transform colonies. You inoculate few colonies into your media, let the media to grow and then when she admits the, it achieves the OD 2.6 then you the induction, you induce it with the one minimizer IPTG. It will actually going to start producing the protein after that you centrifuge and check. So you can see or if you run it on the SDF page, what you see is, in the control you do not have the bands for that particular over express protein whereas in the case of IPTG induce samples you have a protein of your interest produced by the bacteria.

So this is all about so far what we have discuss until the module 6. And in the subsequent lecture we are going to summarize what we discuss from the module 6 to module 11 and in that particular process we are going to discuss about how to purify the protein, how to isolate, how to break open the host cells and different strategies what we discuss so far in this course. How to purify, what are the different chromatography techniques what we have discuss and ultimately we are also going to discuss few of the classical biotechnology applications.

And that actually is going to refresh your memory that is actually going to let you to understands few of the missed point but I might not have discuss while I was taking those lectures. So I hope this kind of revision is going to helpful for you to understand and prepare for the, your exams, which you are going to face in due course. With this I would like to conclude our lecture here. Thank you.