Enzyme Science and Technology
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Module - IV
Enzyme Production (Part 2: Over-expression)
Lecture - 20
Over-expression of Enzyme in host (Part-I)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering, IIT, Guwahati. And what we were discussing? We were discussing about the different properties of the enzyme. And in this context so far what we have discuss? We have discuss about the development of this field of the enzymology, we discuss about the nomenclature and classifications in the module 1.

And then subsequent to that, we have also discuss about the structural properties of the enzyme; where we have discussed about the primary structures, secondary structure, tertiary structure and quaternary structures. And in the previous two modules, we are discussing about how you can be able to produce these enzyme in the bulk quantities so, that you can be able to utilize them for studying the property of the property of the enzyme or for other kind of industrial applications.

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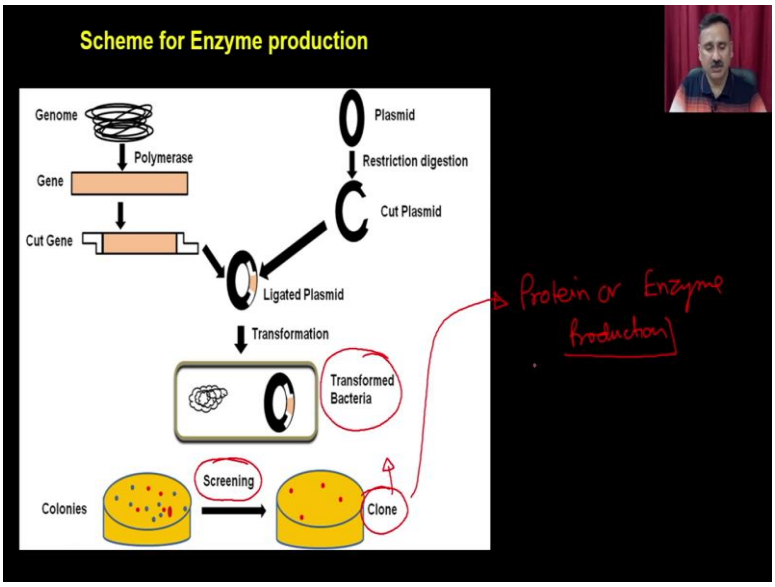

So, in this context so far what we have discuss we have discuss about the cloning, isolating the gene into from the genome either by utilizing the PCR or with the help of the isolating the clone from the genomic library or the cDNA library. Subsequent to that, we have also discuss, how you can be able to clone this fragment into a suitable vector.

And once you got a clone into the vector, you can be able to deliver this DNA into a suitable host. And once you got the transformed colonies, you can be able to utilize them for the screening and as well as subsequent to that for the protein production. So, what we have discuss so far is that, you are going to get the transformed bacteria and or the transformed host that you are going to put it for the screening right.

And once you got the screen, you got the clone containing host cells. Now, this clone can be used for the protein production or the enzyme production, protein or the enzyme production. Now, when we talk about the enzyme production, enzyme production will depends on the type of host what you are going to use for the production.

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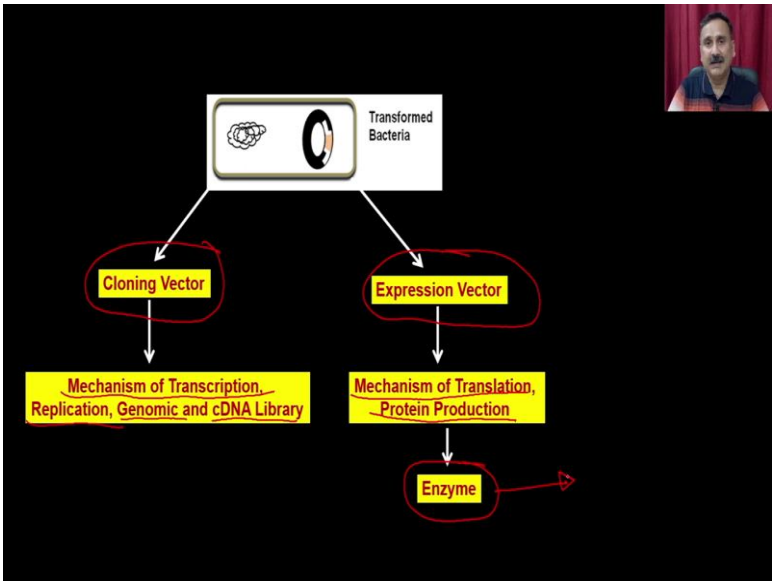

So, as far as the cloning is concerned, the cloning can be done into two different types of vectors; it can be done into the cloning vector or it can be done into the expression vector. In some cases, when you know that the enzyme or the protein what you are expressing is toxic in nature.

So, in those cases, you do not do the cloning reactions, cloning related performance or cloning related operations into a expression vector. Because, then you are going to produce a protein and then eventually it is going to kill the cells; so, that is how you are going to use in the cloning vector.

Apart from the this cloning vector can also be able to use for studying the mechanism of the transcription, replications and the preparation of the genomic and as well as a cDNA Library whereas, the expression vector is exclusively being used for studying the mechanism of the translation or as well as the enzyme production, and ultimately it is going to give you the enzyme of your interest.

Now, when you talk about the enzyme production, the enzyme production is a complicated process and it requires the discrete steps. So, before getting into the different steps with the recombinant DNA, we should first understand how the protein production occurs in a particular cell.

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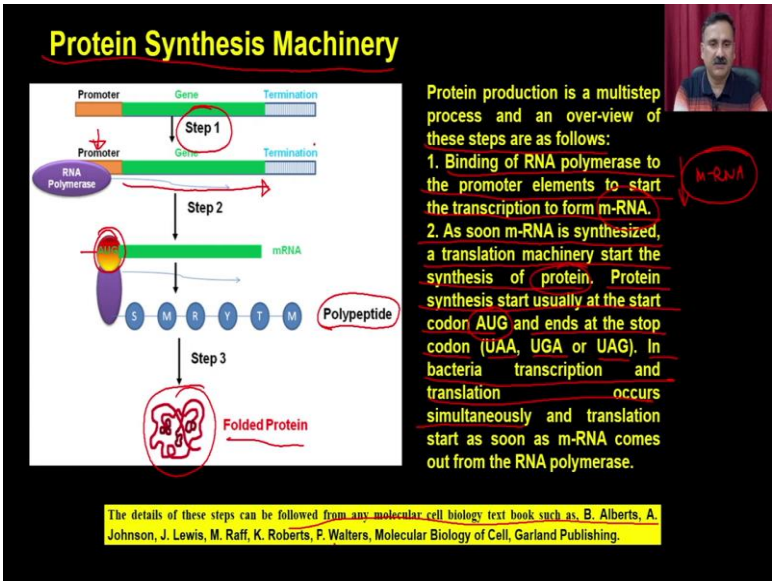

So, protein production is a multi-step process right, in a is a multi-step process and these steps you have to follow following steps. In the step 1, the binding of the RNA polymerase to the promoter element to start the transcription to form the messenger RNA so, in the step 1, you are actually going to produce the messenger RNA and then this messenger RNA is going to be utilized in the step 2.

So, as soon as the messenger RNA is synthesized, a translational machinery start the synthesis of the protein or the enzyme. So, protein synthesis is starts usually at a start codon which is called as AUG and ends at the stop codon which are called as UAA, UGA or UAG.

In bacteria, transcription and translation occurs simultaneously because, there is no nucleus, right; so, transcription and translation occurs simultaneously whereas, in the eukaryotic system, you are going to have the transcription inside the nucleus and then the translation is going to be in the outside the cytosol.

So, that is why in a eukaryotic system, the transcription and translation are not going to be together whereas, in the bacterial system, you are going to have the transcription and translation together. So, in the step 1 from the gene, you are going to produce the messenger RNA. So, RNA polymerase is going to sit on to the promoter region and then it is actually going to synthesize the messenger RNA which is responsible for this.

This messenger RNA is going to be modified you know post translational modification and all that. And then ultimately the first codon, which is the AUG is going to be the place where the ribosome is going to sit and that is how it is actually going to starts forming the synthesis of the proteins; so, and then it is going to synthesize the polypeptide. This polypeptide is going to be get folded and that is how you are going to get the folded functional proteins.

You can actually be able to read this; so, that you can be able to understand the subsequent process what we are going to do inside the host; so, that and it will actually be able to helpful. So, I have given you a reference which you can actually be follow to understand all of these processes. So, you actually require to understand the transcription and translation events, then only you can be able to modulate and you can be able to understand how we are actually over expressing the protein in the host system.

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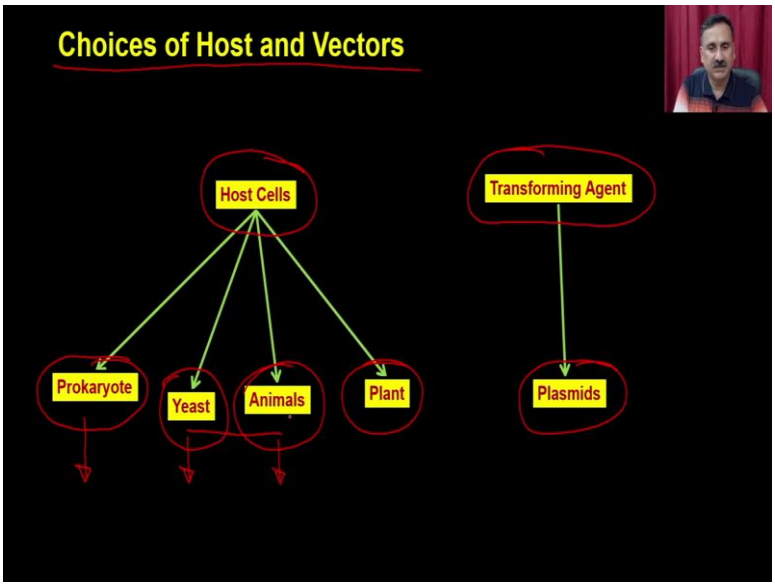

Now, when we talk about the under the in-vitro system, under the in-vitro system you have the two different species. One is you have the host cells which you are going to use as the protein production machinery and then you also going to have the transforming agents, which will actually going to use for providing the instructions, which means, you are going to first use the instructions, and then you put in that into the production machinery and that is how you are going to use that production machinery for the protein production.

So, as far as the host cell is concerned you have the multiple choices. You can use the prokaryotic system either the E COLI or other bacterial cells, you can use the eukaryotic system such as you can use the yeast, animals, and plants. And as far as the transforming agent is concerned, you can use the different types of plasmids, you can use the mammalian vectors, you can use the yeast vectors.

And that all we have discussed when we were discussing about how you can be able to clone a particular gene fragment into a vector of your choice. So, what we are going to discuss is what we are just going to discuss about the prokaryotic expression system, we are going to discuss about the yeast expression system, then we are going to discuss about the animal expression system.

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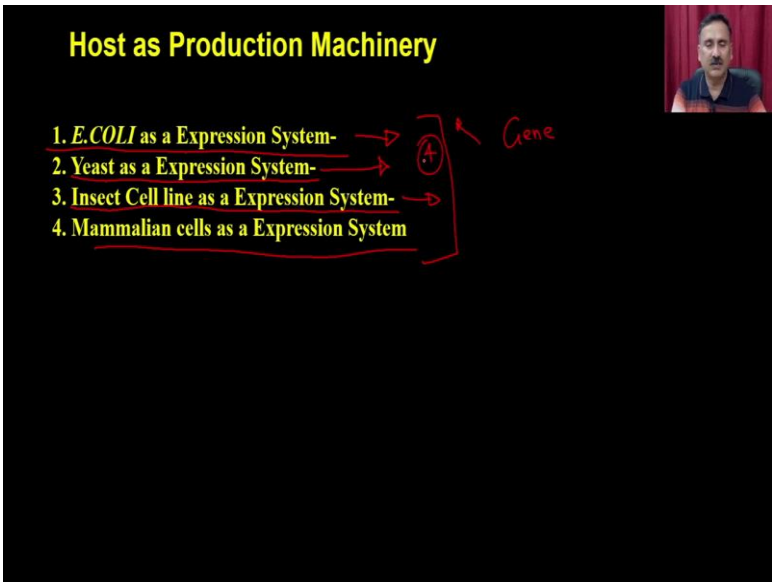

So, these are the things we are going to discuss E.Coli as a expression system which is going to be for the prokaryotic system, yeast as a expression system which is be a part of eukaryotic system. And then we also going to insect cell line as a expression system and the mammalian expression system.

Now, before getting into of these choices; so, you for a particular gene you have the four choices right you have four choices or even more than that ok. The first question comes how you can be able to select and select the particular expression system? Because every expression system has its positive and negatives.

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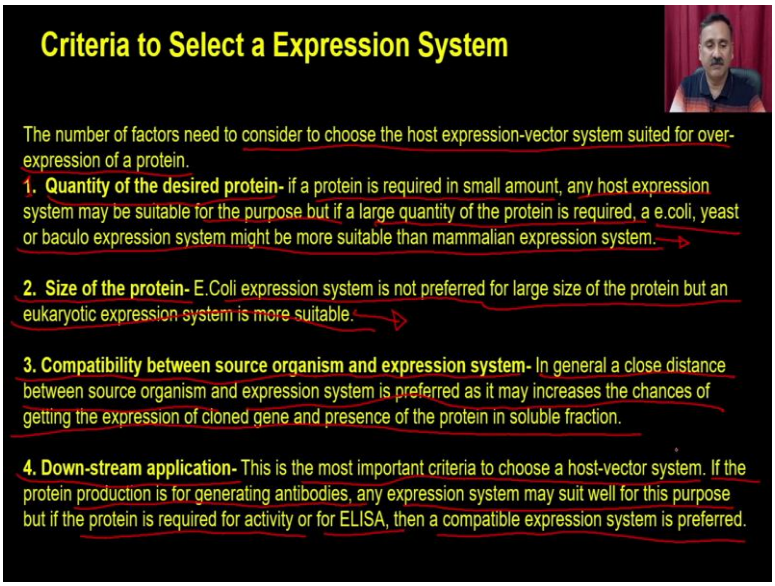

So, the number of factor need to be considered to choose the host expression system suitable for the over expression of a protein. The 1st factor is that the quantity of the desired protein, if the quantity protein required in a small quantity any host expression system can be suitable for the purpose.

If the large quantity of protein is required such a E.coli or yeast or baculo expression system might be more suitable than the mammalian expression system because, mammalian expression system is going to give you a very small amount of proteins. Then it also depends on the size of the protein, the E coli expression system is not preferred for a large protein size of the protein, but an eukaryotic expression system is more suitable for the large size proteins.

Then we have the compatibility, compatibility between the source organism and the expression system. So, in general a close distance between the source organism and the expression system is preferred it may increase the chances of getting the expression of the cloned gene and the presence of protein in the soluble fraction.

Then we also require we have to see the downstream application; so, this is the most important criteria to choose a host vector system. If the protein production is for generating the antibody, any expression system may suit for this purpose, but if the protein is required for activity or for Elisa, then a compatible expression system is preferred.

So, downstream application which means where you are going to use this particular protein is very very important criteria to select any of these four. For example, you cannot use the bacterial expression system in case you are going to use the downstream product for developing the vaccine or utilizing them for using for generation of the antibodies or something.

Because, then there is a chance that you might actually be able to get some bacterial products and these bacterial products are many time causes the allergic reactions into the patients so, if you downstream application is actually going to decide what expression system is going to use.

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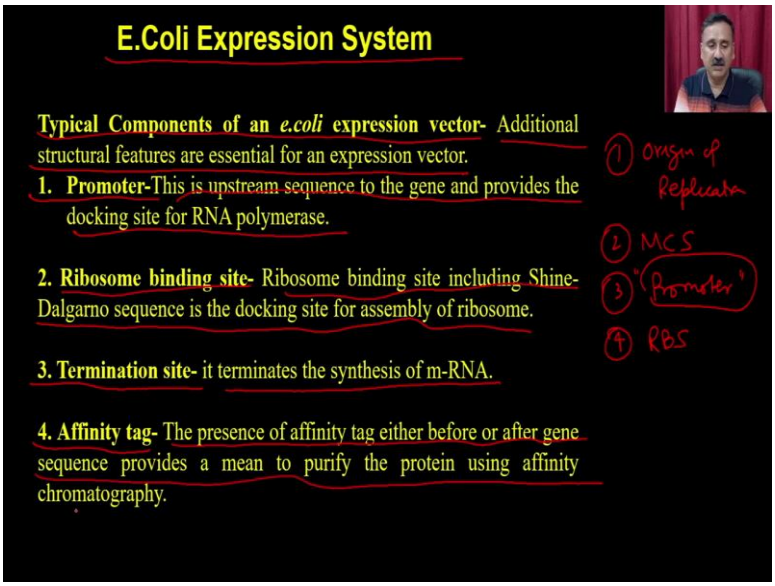

So, in the E.coli expression system; so, in a typical component of an E.coli expression system, additional structural features are essential for an expression vector ok. What we have discussed? When we were discussing about the cloning vector, we said that it should have the origin of replication, it should have a multiple cloning side and so on.

But, if you want to talk about the expression vector, what you require is, you require the promoter. So, for a cloning vector what you require? You require the origin of replications right. Number 1, number 2, you require the multiple cloning site and number 3, you also require a promoter because, the promoter is going to decide the protein production right.

So, promoter this is the upstream sequence to the gene and provides the docking site for the RNA polymerase, then you also require the ribosome binding site. So, ribosome binding site includes a Shine-Dalgarno sequences and it is a docking site for the assembly of ribosomes. So, you also require the RBS right, and RBS is a Ribosome Binding Site and ribosome binding site is very important for the binding of the ribosome; so, that it is actually going to initiate the translation.

Then you also require the termination sites; so, it terminate the synthesis of the messenger RNA. And then some cases you also require the affinity tag; so, affinity tag is not essential; so, These are the component which are essential; the origin of replication, multiple cloning site promoter right.

The presence of affinity tag either before or after the gene sequence provide a mean to purify the protein using the affinity chromatography. So, these are we are going to discuss then only you will understand what is mean by the affinity tag and how it is actually makes the life easy for the researchers to purify the enzyme in a bulk quantities.

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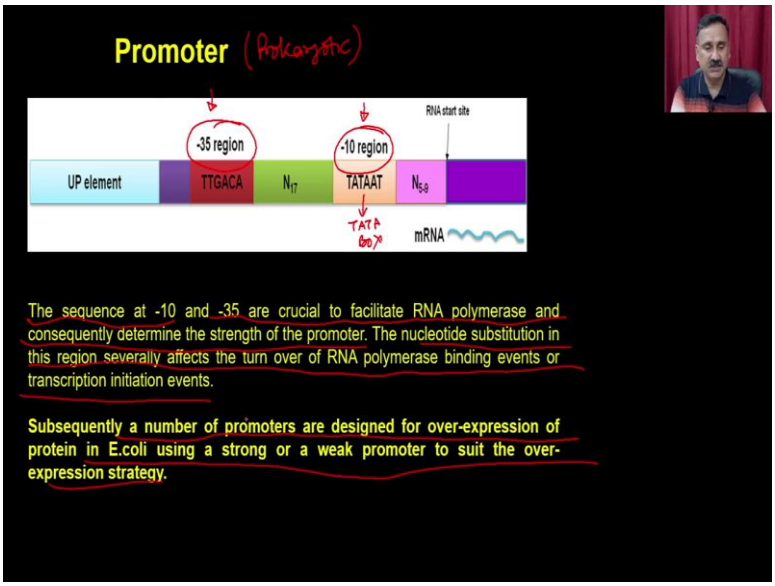

Now, as far as the promoter is concerned in a prokaryotic system, the promoter is containing the some of the classical features such as minus 35 regions and minus 10 region; so, TATAAT box is there. So, you have the TATAAT box and you also have the minus 35 regions. So, sequence at the minus 10 and minus 35 are crucial to facilitate the RNA polymerase and the subsequent determination of the strength of the promoter.

So, as good these sequences are like minus 10 region and minus 35 region it actually going to decide how efficiently the RNA polymerase will go and sit to these region and that is how they are actually going to give you the better transcription. The nucleotide substitution in this region is severely affecting the turnover number of RNA polymerase binding and the transcription initiation side.

Subsequently a number of promoters are designed for the over expression of the protein in E.coli using a strong or weak promoter to suits the over expression strategies.

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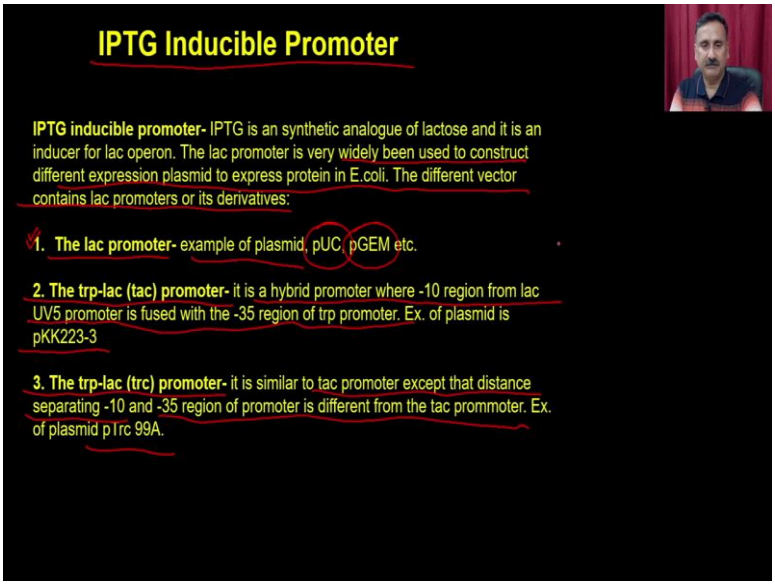

So, we have the IPTG-inducible promoter, it is widely been used for to the construct different expression vector to express the protein in E.coli; the different vector contain the lac promoter or its derivative. So, you have the three different types of promoter; you have the lac promoters, examples of the plasmid is pUC series and pGEM.

Then you also have the tac promoters; so, tac is a hybrid promoter where you have the some region of the tryptophan promoter and a lac promoter. So, it is a hybrid promoter where minus 10 region is a from lac UV5 promoter and it is fused with the minus 35 region of the tryptophan promoter, example is pKK223-3.

Then you also have the trc promoters; so, trc promoter it is similar to the tac promoter except that the distance separating the minus 10 and minus 35 region of the promoter is different from the tac promoter. The example is p trc 99A. So, either of these plasmids

can be used for generating the recombinant DNA and then you can be able to transform that into the suitable host and then you can be able to use this for protein production.

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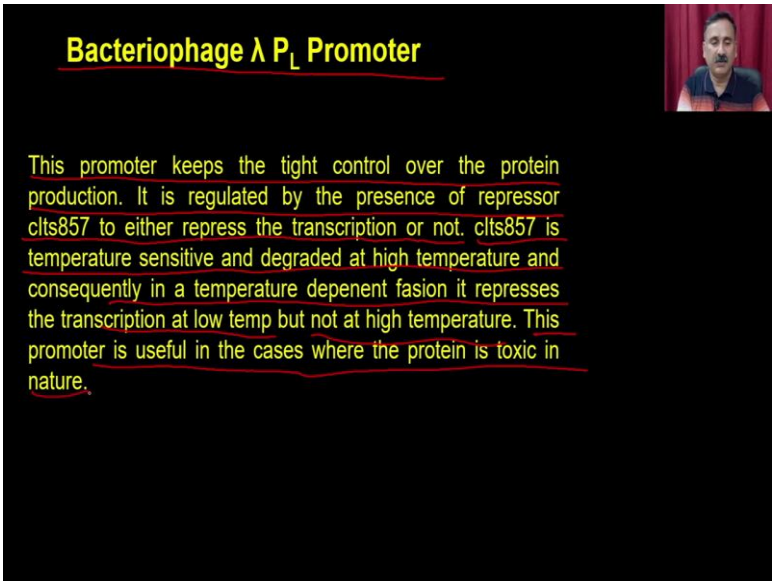

Then we have the Bacteriophage lambda promoters; so, this promoter keeps the tight control over the protein production. It is regulated by the presence of repressors cltps 857 to either repress the transcription or not. cltp5857 is a temperature sensitive and degraded at high temperature and consequently in a temperature dependent fashion. It represses the transcription at low temperature, but not at a high temperature. This promoter is useful in cases where the protein is toxic in nature.

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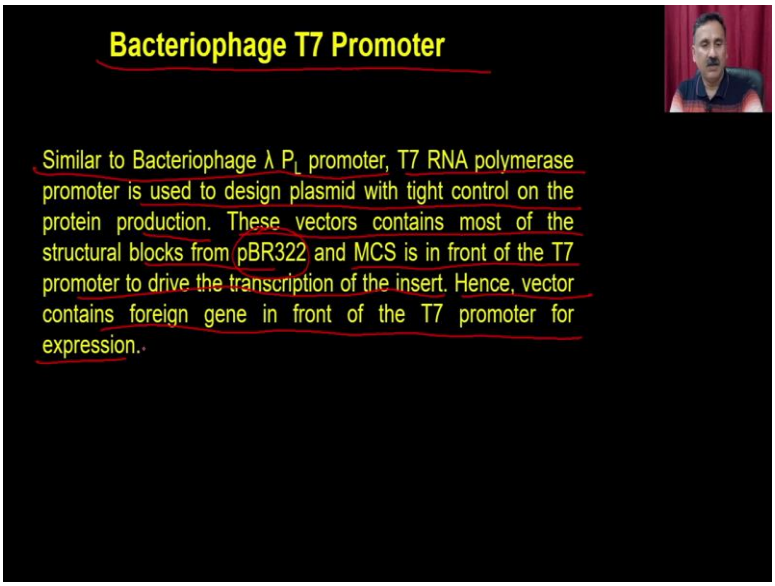

So, then we also have the bacteriophage T7 promoters; so, similar to the bacteriophage P L promoter, the T7 promoters is used to design the plasmid with tight control on the protein production. These vector contain most of the structural blocks from the pBR322 and the MCS in front of the T7 promoter to drive the transcription of the insert. Hence, vector containing the foreign gene in front of the T7 promoter for the expression.

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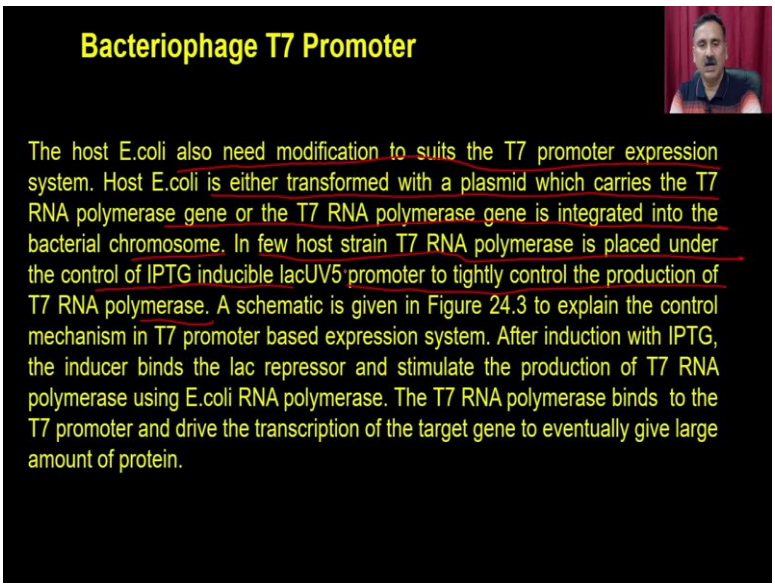

So, T7 the host E.coli also needs the modification to suits the T7 promoter. And host E.coli is being transformed with a plasmid which carries the T7 RNA polymerase gene

or the T7 RNA polymerase, gene is integrated into the bacterial chromosome. In few host strain, T7 RNA polymerase is placed under the tight control of IPTG, inducible lac UV-5 promoter to tightly control the production of the T7 polymerase.

So, either of these promoters first step is that you are going to transform the recombinant DNA into the host and then you are going to do the protein production.

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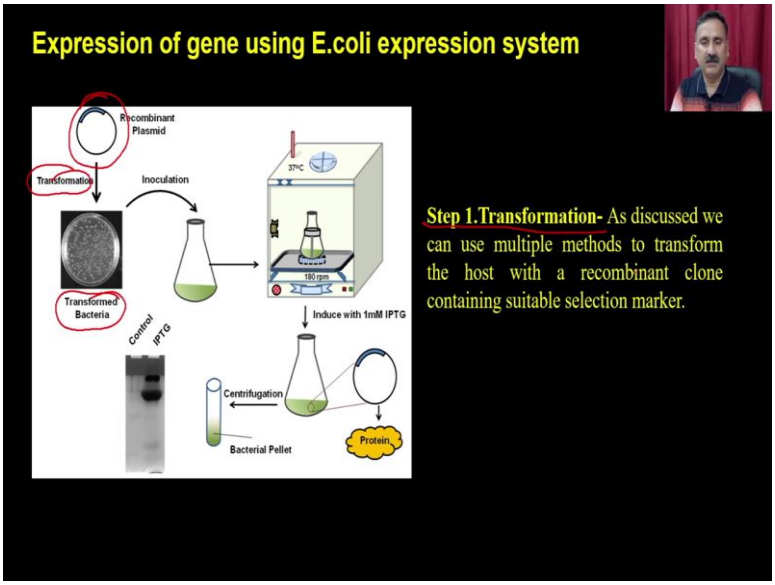

How you are going to do the protein production? In the step 1, you are going to do the transformations. So, what you are going to do is you are going to take the recombinant plasmid and you are going to do the transformation into the suitable bacterial species or bacterial strain and that is why you are going to get the transformed bacteria. And you can use the multiple method of transformation, you can use a calcium chloride method or you can use the electroporation.

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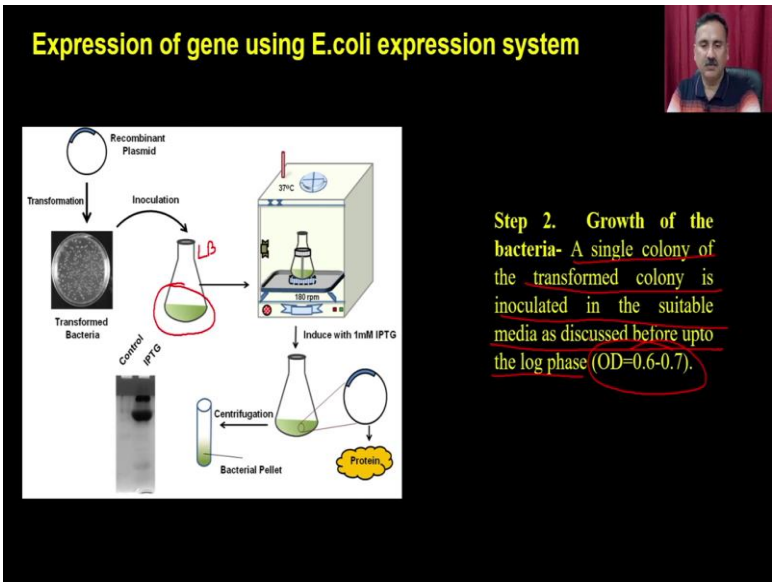

Then there is the step 2, you are going to inoculate the single colony into a suitable bacterial media such as LB media right. And you can allow them to grow into a 37 incubator. So, a single colony of the transformed colony is inoculated into a suitable media and it can grow up to a log phase such as the OD is 0.6 to 0.7.

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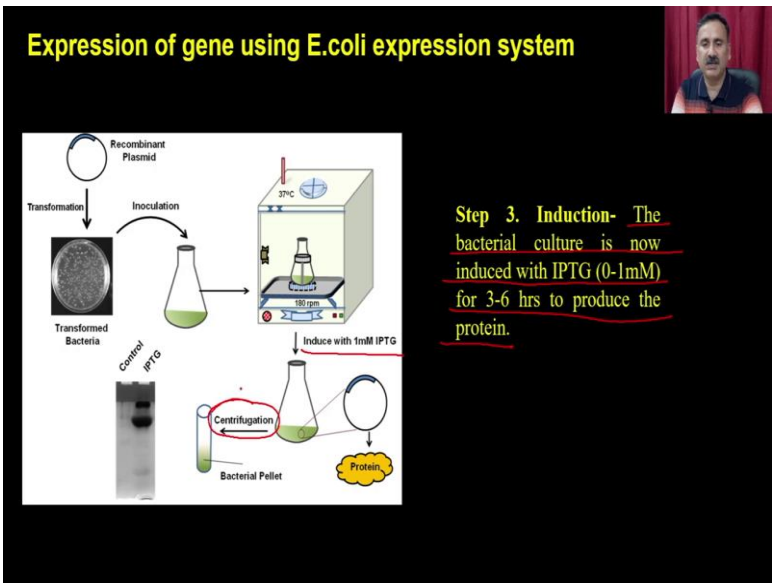

And then what you are going to do is, you are going to induce; so, you are going to in the 3rd step you are going to induce the bacterial species with the induces such as IPTG for 3

to 6 hours to produce the proteins. So, you can do like that and then you are going to do a centrifugation or the collection of the bacterial cells.

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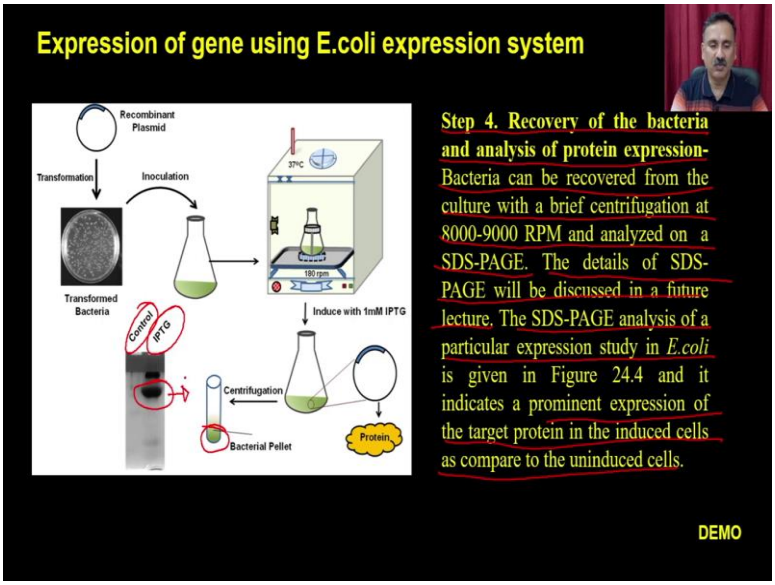

So, in the step 4, you are going to recover the bacterial bacteria and analyze the protein expression. So, bacteria can be recovered from the culture with a brief centrifugation at 8000 to 9000 RPM and analyzed onto the SDS page. The detail of the SDS page will be discussed in a future experiment ok. The SDS page analysis of a particular expression study in is given right and it indicates a prominent expression of the target protein in the induced cell as compared to the un-induced cells.

So, once induction is over, you can actually be able to do the centrifugation and that will actually going to give you the bacterial pellet. This bacterial pellet can be analyzed for the protein production. So, what you can see here is this is the un-induced cells and these are the IPTG induced cells and what you see here is a very prominent band of the protein of your interest. So, we have prepared a small demo clips to explain you how you can be able to perform or express a particular gene into the E coli expression system.

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Hi everyone, myself, Sooram Banesh, Research Scholar at Department of Biosciences Bioengineering, IIT Guwahati. In this video, we will show you how to induce protein expression in bacterial cells and how to analyze the induction. Before that, the gene of interest which we want to express in bacterial expression system, we have to transform that construct into BL21DE3 strain.

So, BL21 cells specifically used for expression of a particular protein. Once transformed into BL21, we have to pick the single colony and inoculate in a small volume of culture. That culture we will use in scale up. So, I will show you how to inoculate, how to take single colony and inoculate one colony in 5ml of LB media and that we will use for further experiments.

So, this inoculation should be done in laminar air flow. So, we will use laminar flow to inoculate this colony. And also we have to note that the expression if you having any resistant marker; like, ampicillin resistance, a kanamycin resistant, you have to include that antibiotic also in your culture media. So, that it will specifically grows our strain or our strain which expresses protein rather than non-specific bacteria, although it is highly impossible, but we still it is good to be cautious.

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We have inoculated single colony to LB media with suitable antibiotic. Now, what we have to do is keep in an incubator till we get growth of 0.4 or 0.5 OD before inoculating into large culture.

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So, I will keep these first in incubator shaker, this is the incubator shaker. So, we can actually rotate the base; so, that uniformly the culture spready throughout the area. After we get growth, then we will inoculate into another culture that we will use for the

induction analysis. As we can see the bacteria the (Refer Time: 23:50) is around the 0.37 to 0.4; so, this is the right time for induction.

We will use isopropyl beta D thiogalactoside galactoside as inducing agent, which we call it as short in short form we call it as IPTG. So, we will induce with the IPTG and also at the same time we have to add antibiotic equivalent to this media. So, that will prevent any contamination which may be accidentally comes into the flask by doing induction.

So, this process should be carry out in receptive conditions that is in, that is why we will use laminar air flow, for this purpose. So, let us induce the samples then we will keep it keep again back to the incubator shaker. This is 1 milli, 1 molar IPTG, the amount of IPTG which we have to add for induction is depends on how much expression your protein, how much expression you getting.

So, you have to optimize using different concentrations of IPTG these 0.1 millimole 0.51 millimole respectively; so, I am going to induce it. After induction we have to keep for 4 hours; now, the time is our 4 hours is over. So, we have to centrifuge and get the pellet that we will use for the sonication and protein purification.

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As we can see that it is almost over; so, we can take out the gel then we will do stain and destain it. Generally, what we will do is we will, there are two ways of staining and

destaining process; one is we can do quick staining like we have to create it with the staining solution which contains coomassie brilliant blue and along with methanol and water.

So, then we will try to destain with the water by heating, but in another way the simplest way is we will just stain the gel for 2 hours, then we will destain overnight. So, I am going to show the simplest way, first we will stain in coomassie brilliant blue staining solution. Then we will de-staining methanol water containing salt; so, I am going to start the trailer, then I will remove it, I will show you how to remove the gel.

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Here we have to be very careful while taking out gel. Otherwise the short plates may broke; on a corner we have to take and lift the gel like this. Then keep the, but it can sustain the, I will keep it for a rotation for an a shaker for at least 2 hours, then we will de-stain over it.

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So, once the time is over after 2 hours we will de-stain this machine. We kept 2 hours in staining solution, we as we can see the staining is over, like we can see the gel completely turned into blue.

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So, we remove the solution; then, I am going to add de-staining solution and I will keep this on a rocker for 2 hours for de-staining. So, the composition contains per 100 ml of de-staining solution 40 ml of water double distilled water and 40 ml of methanol and 10 ml of glacial acetic acid; so, I am going to take this on a rocker. We have run the gel and

stained right stained and de-stained; now, we will capture the gel image. So, you can see manually also, but for required purpose we have to capture it through gel doc.

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So, this is the gel doc imaging system from biorad; so, I will show you how to take the capture the images; so, let.

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So, here we will use white tray, there is another one grey or UV tray is also there. So, there you can see any fluorescent one or stained with the ethidium bromide or blast,

chemiluminescence blast you can use that. But for normal protein imaging, we can use this white tray; so, I am going to keep the gel doc on this one.

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So, we have to open properly, this is very important step, you have to align the tray in a proper way. So, otherwise the it will show error; so, once it is over, you just push it back.

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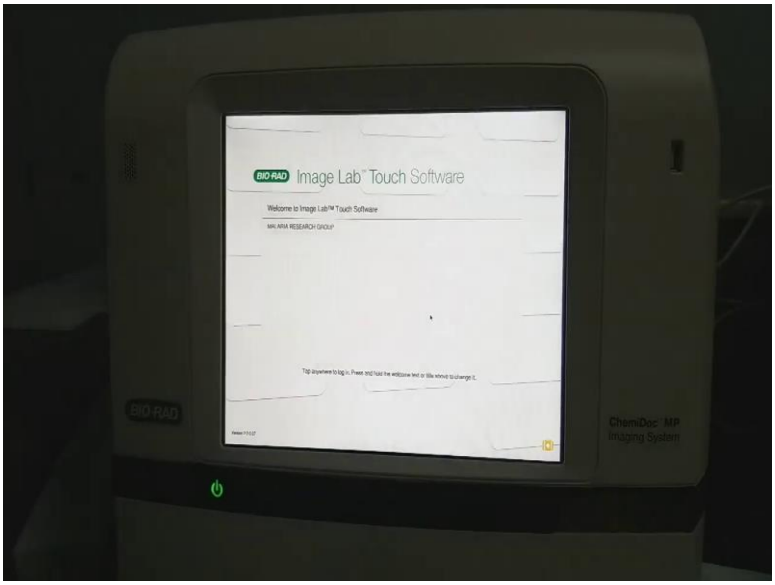

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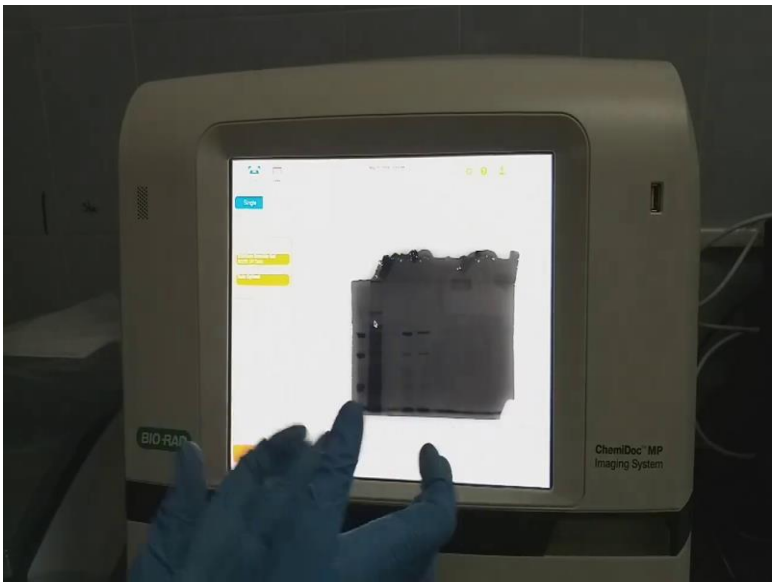

So we have to log on to account; so, this is a SDS page gel, you can select the application whatever you want.

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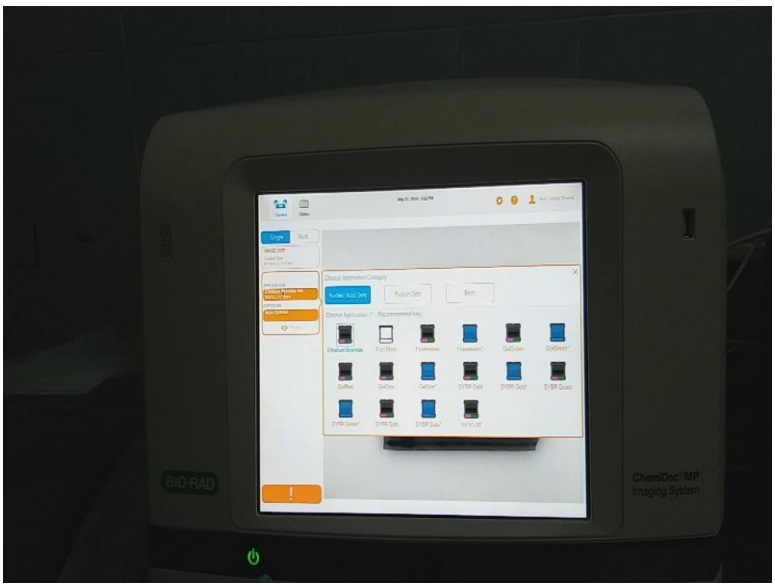

So, here nucleic acids, protein gels, blast, three different categories are there. So, we are observing here protein gels; protein gels stained with the commassie blue or you can use white tray, we are using white tray; so, this is the right tray. You can use commassie blue stained one gray tray also, but we are using as we are using white tray. So, we will use commassie blue.

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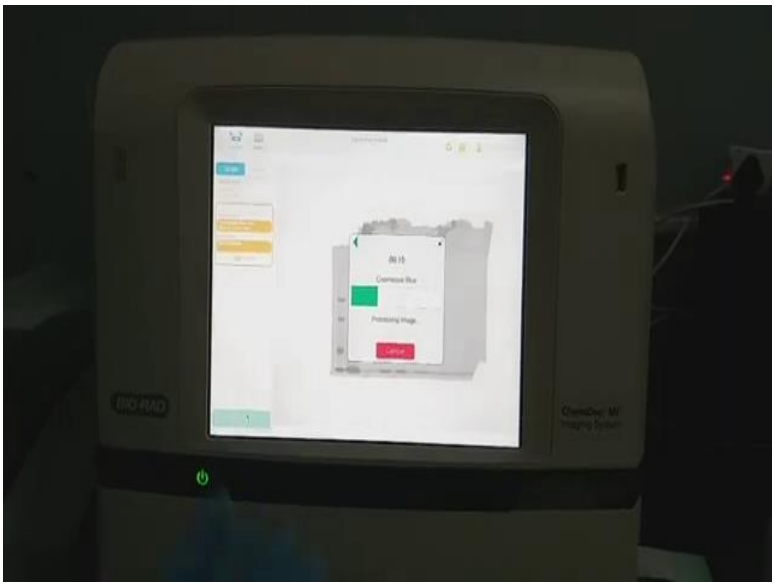

So, auto-optimal then I will ask for capture; so, it will take 1 to 3 minutes based on the signal intensity. So, as we can see it is optimizing the signal intensity, you can minimize this one also; so, that you can see the gel image, so, now it is over.

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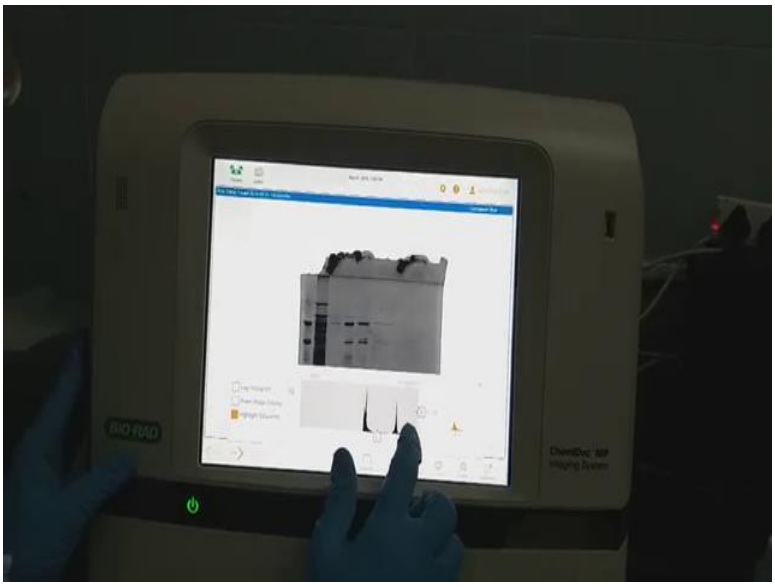

If you want to do any modifications to images, for suppose you want to decrease or increase the signal intensity; so, this kind of changes you can do; so, if you want to send this gel.

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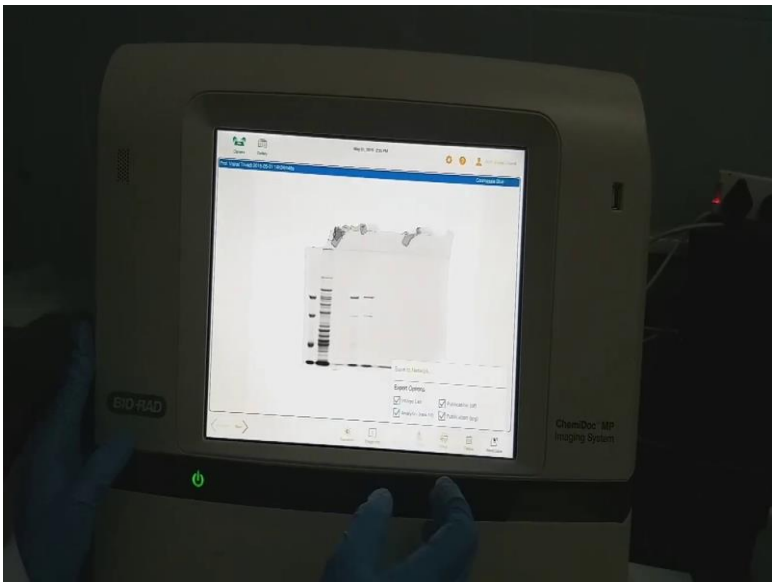

You can have send and save; if you have any drive connected to this one, you can send directly to that is not that the. So, for image analysis part, we will show in the upcoming video how to analyze the what this band of interest correspond to which molecular weight. So, we already loaded the molecular weight one; so, we can easily find out using Magellan software.

In this video we have learned that how to prepare a SDS page gel and how to run it, what are the precautions need to be taken while preparing the gel and how to observe, how to record the gel using gel documentation system. So, I hope this will give you this will give you a (Refer Time: 34:07) of how to prepare and run a SDS page get and analyze the protein sample; thank you for watching.

So, in this demo clip, we have discussed different steps what you have to perform and then how you can be able to check the expression into a SDS page. Now, there are many factors which are actually going to decide the protein production into the E.coli expression system.

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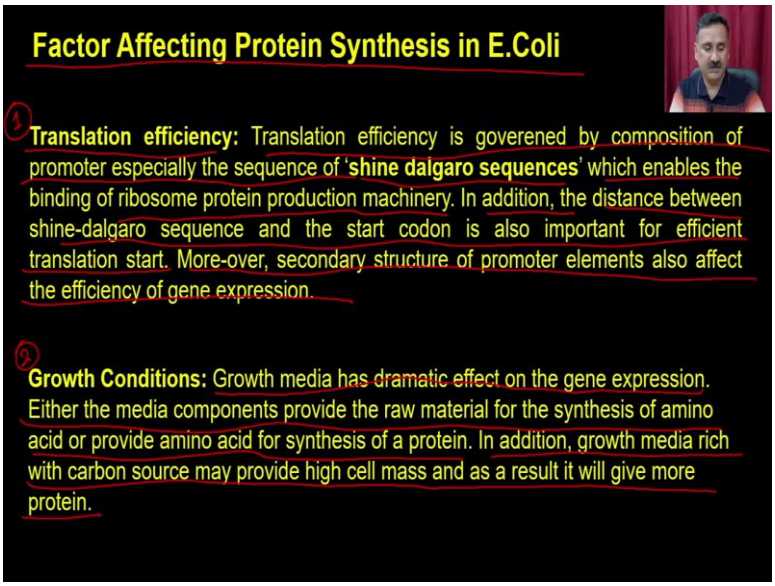

So, factor affecting the protein synthesis in E.coli ok. So, 1st factor is the translational efficiency ok; so, translational efficiency is governed by the composition of the promoter, especially the sequence of the shine dalgaro sequences, which enables the binding of the ribosome protein production machinery. In addition, the distance between the shine dalgaro sequence and the start codon is also important for the efficient translation.

Moreover, secondary structure of the promoter elements also affect the efficiency of the gene expression. Then the step 2 is the growth conditions, growth media has a drastic effect on the protein production. Either the media component provide the raw material for the synthesis of the amino acid or provide the amino acid for the synthesis of a protein. In addition, the growth media rich with carbon source may provide high cell mass and as a result it will give you the more amount of proteins.

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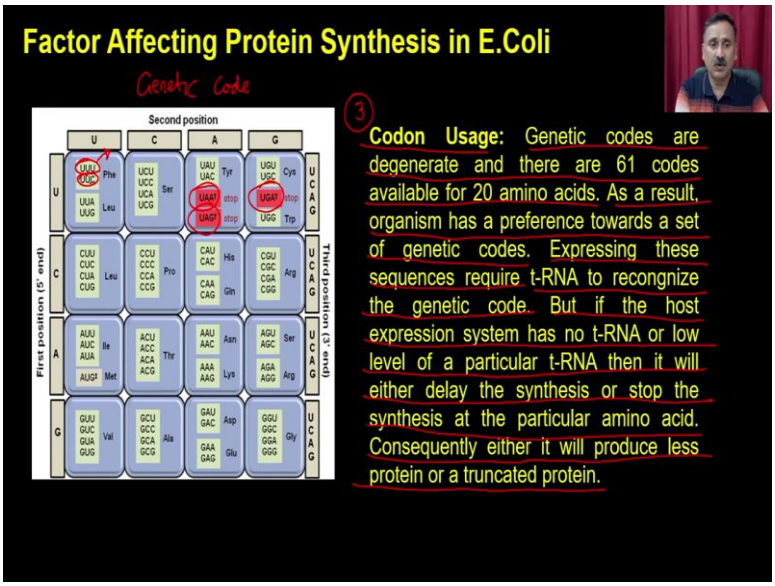

Then the 3rd is the codon usage ok; so, third is the codon usage. Genetic codes are degenerate and there are 61 codes which are available for the 20 amino acids; so, this is the genetic code what you see right. And there are 61 codes which codes for the proteins, whereas, 3 codons which are for the stock codons. So, these are the stock codons what you see and which is does not code for any amino acid. Except these, you are going to have the codon, which is coding for one or other amino acids.

As a result, the organism has a preference towards a set of genetic code. Expressing these sequence requires the t-RNA to recognize the genetic code. But if the host expression system has no t-RNA or low level of particular t-RNA, then it will either delay the synthesis or stop the synthesis of a particular amino acid. Consequently, either it will produce less protein or the truncated protein.

So, what it mean is that every organism has a preference over using some of the codons; for example, in the case of the phenylalanine, you have the two codons, UUU, UUC right. It is possible that in E.coli probably the UUU, is more preferred codon. So, in that case, it will actually going to have the t-RNAs only for the UUU.

But if you are taking a protein which is you know which does not have this codon, if you have the other codon, then either it will take the time for synthesis of these t-RNA molecules or it will actually going to truncate the protein synthesis at that stage. So, that

is a very, very important criteria to select the host as per the codon what is present in your gene.

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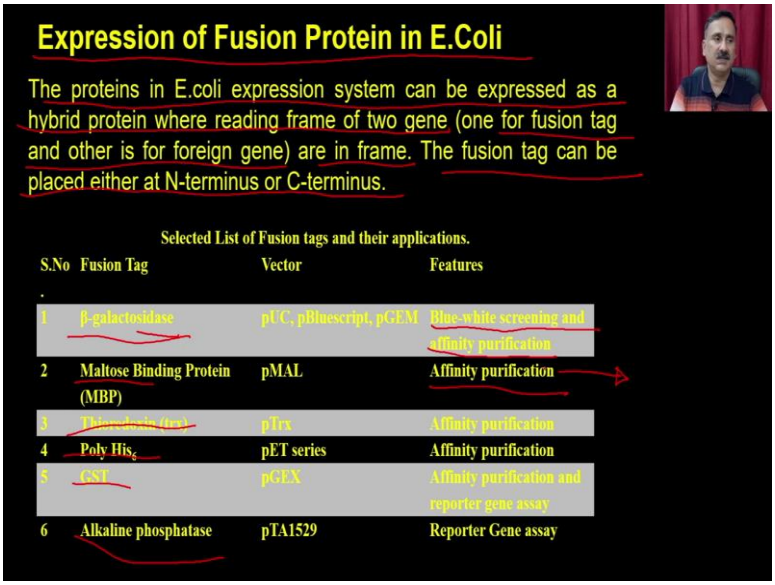

Then we have the expression of the fusion protein in E.coli. The protein in the E.coli expression system can be expressed as a hybrid protein where the reading frame of the two gene, one for the fusing tag and the other one is for the foreign gene are in a frame. The fusion tag can be placed either at the N-terminus or the C-terminus; so, these are the some of the fusion tag, beta-galactosidase, MBP, thioredoxin, polyhistidine, GST and alkaline phosphatase.

And you can actually be able to use these are the vectors, what you can use for tag. And what is the advantage? For example, if you have beta-galactosidase, you can use the you can use that for blue blue-white screening and as well as for affinity purification . Mostly these fusion tags are being used for affinity purification.

So, that you can be you know avoid the contaminating protein, because the fusion tag will not be present in other protein, but only present in your protein. So, if you pass through this to a affinity column, only this protein is actually going to bind and the rest protein will not bind and that is how you will get the purification in single step.

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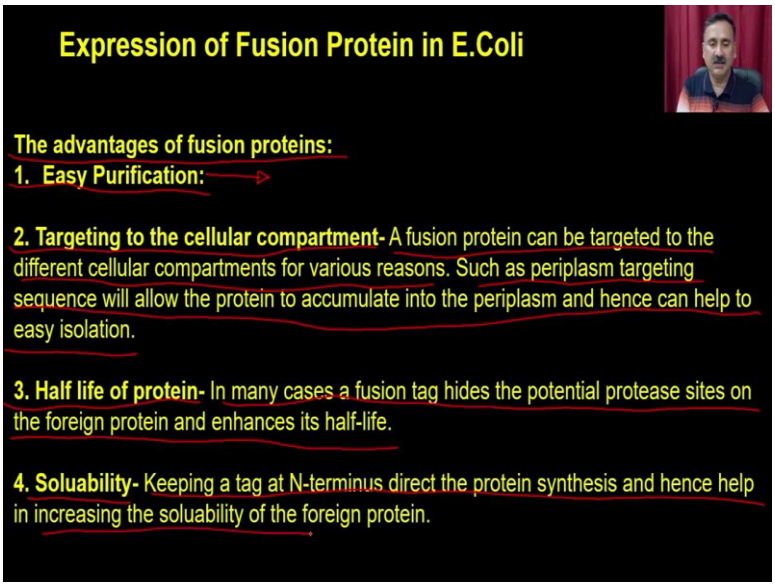

The advantage of the fusion proteins, it is going to make the easy purification. You might have seen that the with the help of the affinity purification, it can be a single step purification. Sometime the tags are being put; so, that you can be able to target a protein into a particular compartment. So, fusion protein can be targeted to the different cellular compartment for various reasons, such as the periplasm targeting sequences will allow the protein to accumulate into the periplasm and hence can help to the easy isolation.

It can also modulate the half-life of the protein, in many cases a fusion tag hides the potential protease site which are present on the foreign protein and enhances its half-life. Then it also increases the solubility, keeping the tag at N-terminus direct the protein synthesis and hence help in increasing the solubility of the foreign protein.

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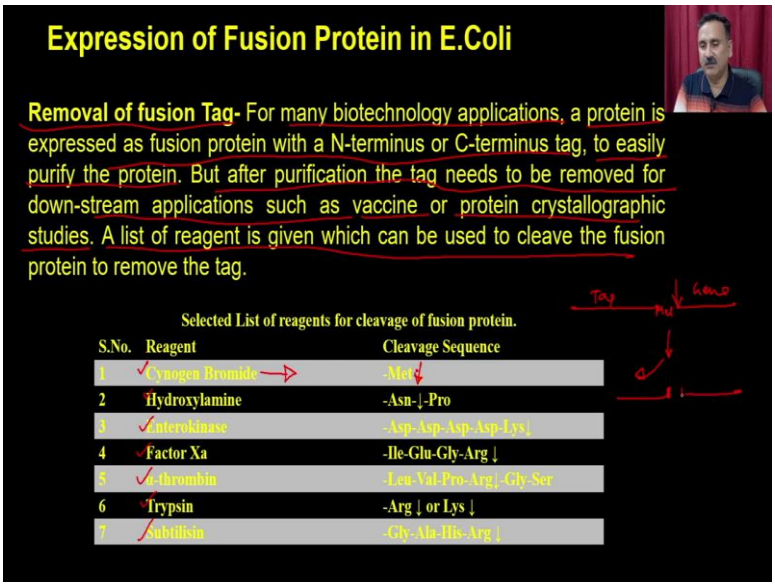

Then we have the how we can be able to remove the fusion tag so for many biotechnology application of protein is expressed as a fusion tag with N-terminus or C-terminus tag to easily purify the protein. But after the purification, the tag need to be removed for the downstream application, such as vaccine or the protein crystallographic studies.

A list of reagent is given right; so, these are the reagent what you can used. You can use the cynogen bromide, you can use hydroxylamine, enterokinase, factor 10A, alpha-thrombin, trypsin and subtilisin. And they are all mostly the proteases except the cynogen bromide which is a chemical and that is going to cleave just after the methionine.

So, if you have a tag and if you have a methionine here right; so, this is the tag actually and this is your gene of interest right; so, it is have having a methionine in between. So, what will happen is if you treat that with the cynogen bromide, it is actually going to cleave and that is how you are going to get two fragments, your tag and the gene of your interest ok.

So, in many cases it is very very essential, especially the places where you are going to use this protein as a drug for example, like for example, if you are going to use that like insulin for example. So, if you are going to put as a affinity tag on the insulin, it may

actually cause the allergic reactions to the to the patient, That is why these affinity tag has to be removed.

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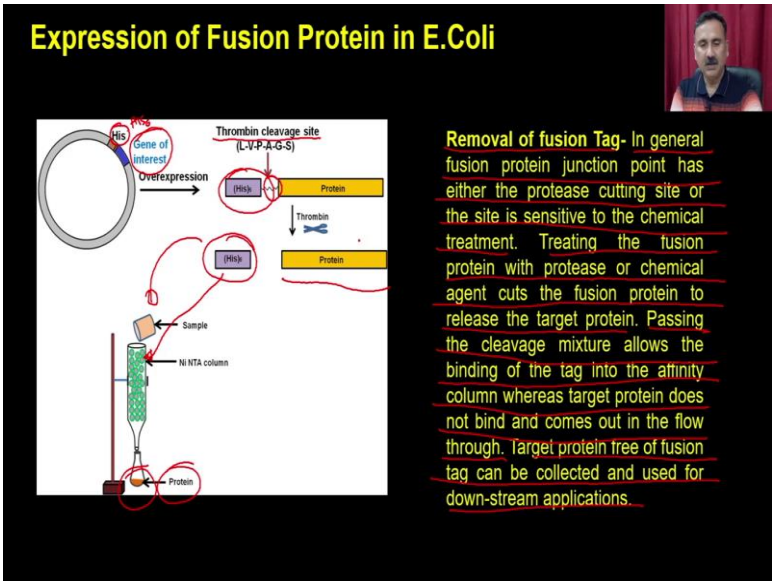

How you are going to remove the fusion tags? So, what you are going to do is in general, the fusion tag junction point has either the protease cutting site or the site is sensitive for the chemical treatment. Treating the fusion protein with a protease or the chemical agent, cut the fusion tag to release the target protein passing the cleavage mixture allows the binding of the tag into the affinity column; whereas, the target protein does not bind and comes out in the flow through.

Target proteins, free of fusion tag can be collected and used for the downstream application. So, this is what you have in this particular we have taking an example of the his tag; so, you have made a chimeric protein. So, that is having the his tag on one side and the protein of your interest on the other side. And then what you can do is, you can actually be able to treat this with the thrombin because it has the thrombin cleavage size in between.

So, what thrombin is going to do is, it is going to remove the his tag and the protein. And now what you do is, you load this onto a affinity column and as a result what will happen is that his tag will go and bind to the beads whereas, the protein of your protein is going to come out into the flow through. And that is how you can be able to separate the tag from the protein of your interest.

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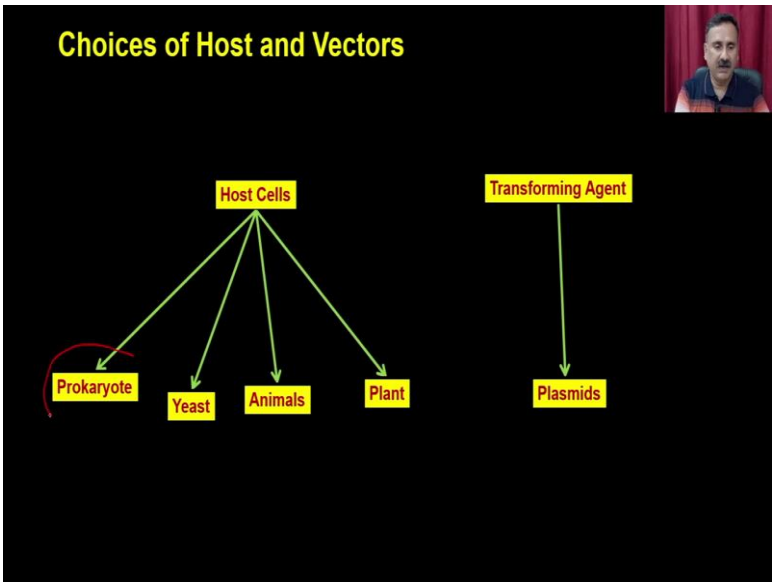

So, this is all about the bacteria as the host system for protein production. And what we have discussed? We have discuss about the different steps what you have to follow, which means in the step 1 you are going to transform the bacterial species bacteria with a with your recombinant DNA. And we have discussed many method, what you can use for transformations.

Number 2, you are going to do the screening or the selection of the transform clones. Number 3, you are going to grow a single colony, you are going to inoculate into the media and that is why you are going to induce. And the step 4, you are going to induce that bacterial culture with the help of the inducers; so, in this particular example, we have taken an inducer as the IPTG.

And then, once the induction is over for 3 to 4 hours, then you are going to collect these cells by centrifugations. And then you are going to utilize these cells for analyzing the protein production in the SDS page. And once you are sure that the protein is being produced, then you can be able to lyse the cells and you can purify the protein for downstream applications.

So, this is all about the different aspects of the protein production in E.coli as an expression system. In our subsequent lectures, we are going to discuss more about the yeast expression system and as well as the mammalian expression system; so, with this, I would like to conclude my lecture here.

Thank you.