Genetic Engineering: Theory & Applications
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Module VI | Recombinant DNA Technology (Part 2)
Lecture 16 - Protein production in Host: (Part –I)

Hello everybody. This is Dr. Vishal Trivedi from department of biosciences and bioengineering, IIT Guwahati. And in this course what we were discussing so far; we were discussing about the host as well as the transforming agents and you might have seen the scheme which we are following to explain the different steps of generating our genetically modified organisms. So, so far what we have discussed.

We have discussed about the host physiology, host ultra structures and the different types of requirement for the host to grow and how to monitor the growth as well. And in the in the case of transforming agents, we have discussed about the different features of different types of vectors which people are using in the bacterial expression system, eukaryotic systems such as yeast, mammalian system, or the bacteriophage, or as well as the baculovirus based system to over express or to generate the genetically modified organisms.

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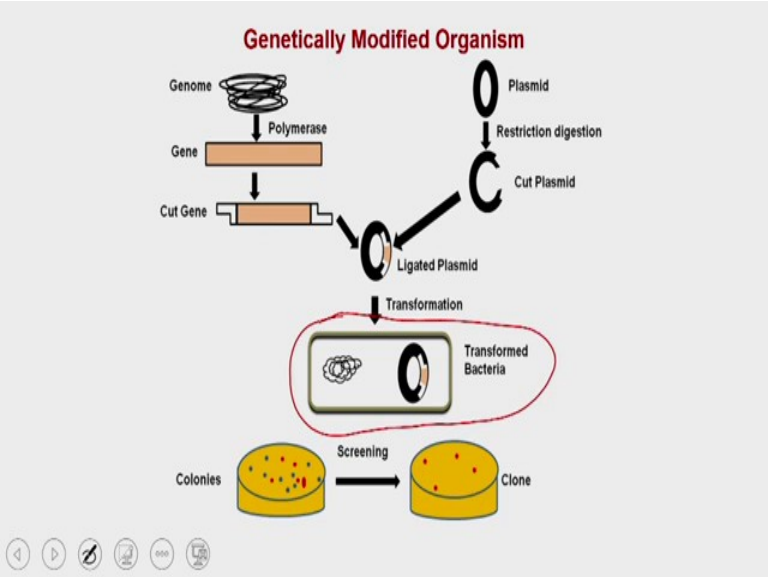

So, at the end, what we have generated is the transformed organism. In this case, we are talking about the transformed bacteria. But at the end what we have, what we are going to generate by

employing these kind of recombinant DNA technology; is we are going to generate the transformed bacteria.

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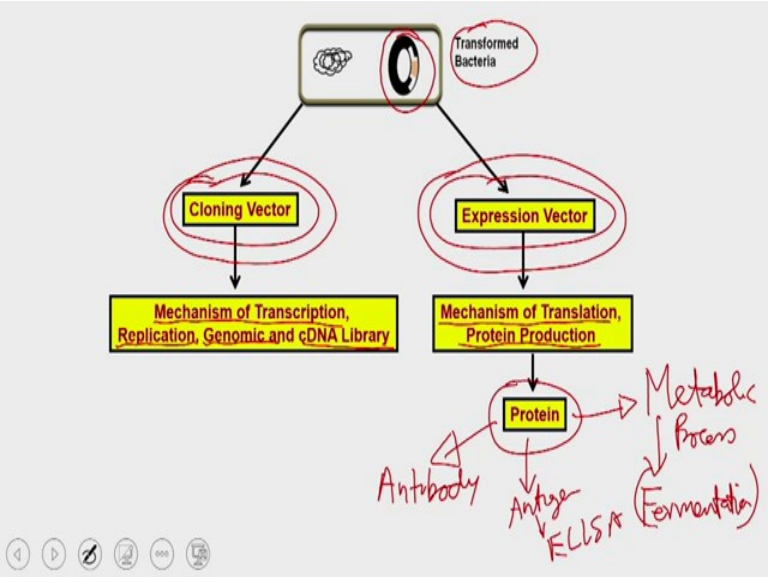

And once you generate the transformed organisms, whether it is bacteria or mammalian cells, you are going to have these transformed organisms containing either the cloning vector or to the expression vector. Because it depends what are the questions or what are the requirement or what are the questions you would like to ask from this transformed host. So, in the case of cloning vector, suppose, you have generated a recombinant clone into a cloning vector then the purpose of these recombinant DNA or recombinant clone would be either to study the mechanism of transcription or to replication as well as, the similar kind of strategies people are using even for generating the genomic as well as the cDNA library.

So, for the, so if you are generating a recombinant clone into the cloning vector, you are actually restricting yourself to the basic research or you are using these constructs only for addressing the different types of questions related to the processes which are happening within the cell or within the, that particular host, Whether it is the transcription or the replication or you can use the similar kind of strategies even to generate the genomic as well as the cDNA libraries. Now, coming back to if you are generating a transformed host containing the expression vector. So, in the case of expression vector, you can use these transformed hosts for addressing the mechanism of translation or you can use these for protein productions.

So, once you use these for protein production. This protein you can use for generating the antibodies or you can use this protein as an antigen to prepare a laser kit or you can generate, you can use these transformed hosts which is over expressing this particular protein for regulating these metabolic processes which means ultimately, what you are going to do is, once you are going to use this particular protein product into the metabolic processes you are actually going to use this for changing the outcomes in terms of the process known as fermentation.

So, once you generate a recombinant transformed host and you are using expression vector D, you are ultimately over expressing the proteins or over expressing the protein over expressing organisms and the utility of this particular thing is much more compared to that you are using this for producing a recombinant clone into the cloning vectors. So, let us today we are going to discuss about how to exploit the transformed host to over express or to express the protein of your interest which you have cloned from a particular gene.

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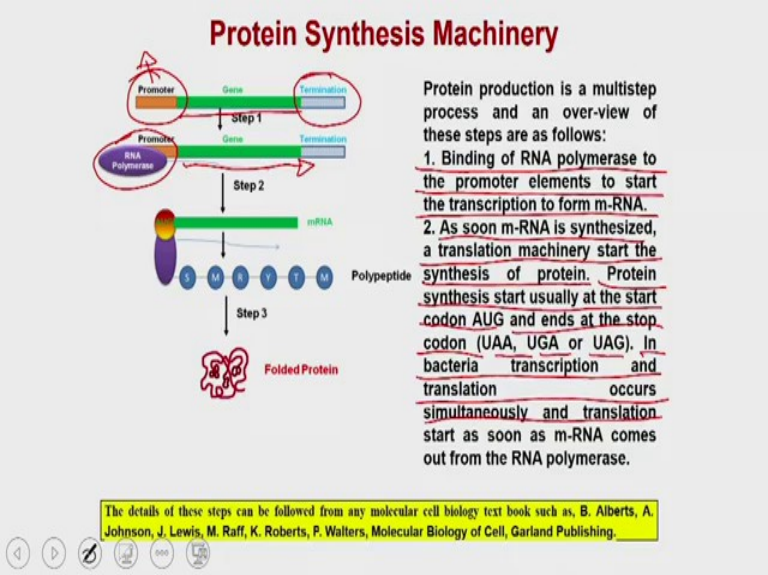

So before getting into the detail of different types of hosts which are important for producing the protein, let us see what are the different steps are involved in a typical protein production machinery. So, in the protein production machinery you have a coding sequence which is called as gene and next to the gene you have a promoter sequence. This promoter sequence is required for providing the docking site for the RNA polymerase as well as different type of transcription

factors and once and onto the 3 prime end you have a termination sequence which is actually going to terminate the transcription to this particular gene.

So you can imagine that in the first step the RNA polymerase will bind to the promoter elements to start the transcription to form the messenger RNA. So, the RNA polymerase will sit on to the promoter and will start synthesis of gene and that will produce the messenger RNA. As soon as the messenger RNA will be synthesized, our translational machinery starts the synthesis of protein in some of the eukaryotic cases the RNA which is going to be synthesized, RNA will be synthesized inside the nucleus and then this will be transported outside and that is how the transcription as well as the translation is going to be; will not going to be together. Whereas, in the case of prokaryotic system, once the gene, once the messenger RNA is going to be formed, the ribosome is going to bind to this messenger RNA when we will start the protein synthesis.

Protein synthesis are usually going to start from a start codon which is mostly AUG and then it ends at the stop codon which is called as UAE-UGA or UAG. In bacteria or the prokaryotic system the transcription and the translation occurs simultaneously, Whereas, in the case of eukaryotic system, the transcription occurs inside the nucleus and then, these, this messenger RNA will be transported outside into the cytosol and where the ribosome machinery will going to in bind to this particular messenger RNA and will synthesize the protein.

What I will suggest to all the student is that this is just a brief overview what is the detail of the protein production machinery in any of the hosts but you should study the different steps by going through with any of the molecular biology textbooks; such as you can use the Bruce Alberts or Molecular biology of the cell or you can use the Gene or you can use any of the standard molecular biology textbooks to understand the discrete steps of these protein production machinery to understand as well as to exploit the machinery in such a way that it should give you the better production of your protein.

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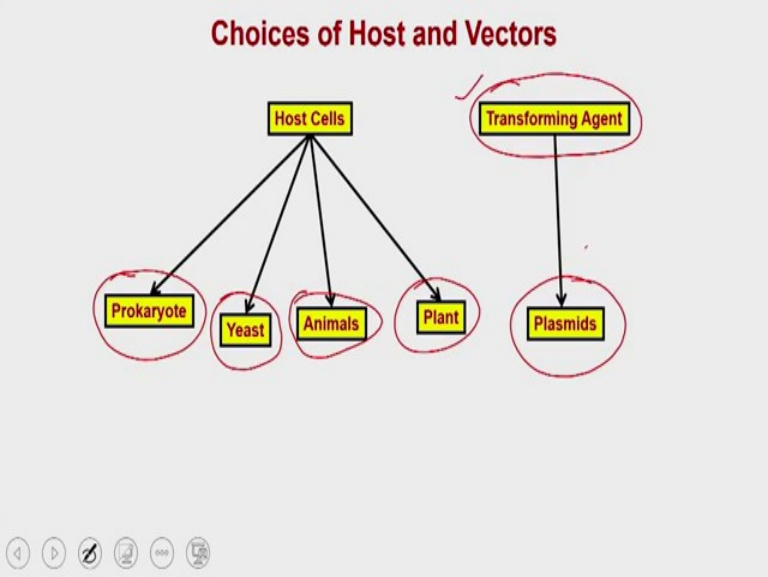

So, now once we have discussed this about the protein synthesis machinery. The protein synthesis is present in different host and what we have discussed so far is that in the case of host cells, you have the prokaryotic host cells, you have eukaryotic host cells such as yeast, animals and plant. We have also have the transforming agents which are complimentary to all of these host cells. So, once you generate a recombinant clone, you have to decide in which particular type of host system you would like to over express or you would like express your protein because accordingly to this particular strategy you have to select the transforming agents as well as the host cells which are going to be complimentary to each other.

So, what will be the basis in which you are going to use the host strain because once you select the host then can automatically will have to select the transforming agent which is the vector which are going to produce the protein in this host cells. We have already discussed about the different types of vector in, which are available for the equalized system, yeast system, mammalian system, as well as the plant systems in our previous lectures. But, the question is what would be the criteria to choose the prokaryotic versus the eukaryotic system from yeast to animals as well as the plant.

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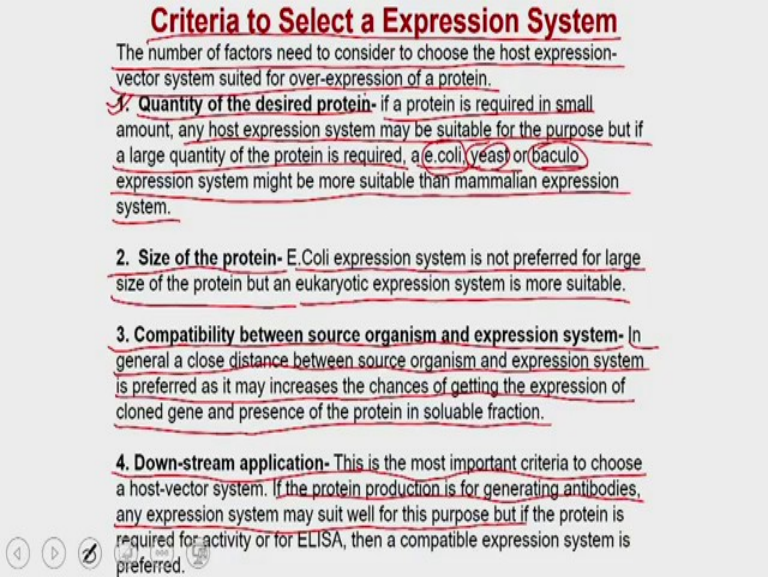

Now, two criteria of selecting expression system. There are number of factors which you need to consider for choosing a host expression vector system which will be suited for the over expression of the protein of your interest. What is the criteria? Number 1 criteria is the quantity of the desired protein; which means whether you need the protein in microgram level or whether you need the protein in milligram or gram level because in some cases, this protein could be the, will be the factor which you are going to use in to the downstream applications for providing the nutrients for or you are going to use these proteins for developing the health drinks. In those cases, the protein would be required in grams or Kgs in concentrations or Kgs amount.

Whereas, if you are simply trying to get this protein for developing the antibodies or developing the antigens or you are trying to use this protein for catalyzing a particular reactions in the bioreactor as well as in the some other kind of metabolic reactions which you would like to catalyze or you would like to use this for transforming one chemical from the toxic chemicals to non toxic chemicals and so on. In those cases, the requirement of these protein products may be low or high. So, the quantity of the protein, desired protein is an important criteria to choose or select the expression system. If the protein what you are expressing is required in a very small quantities but say, such as in the microgram or the milligram region in those cases, you can use any host system which is suitable for the purpose.

But, if the protein is required in a large quantity such as; just now we have discussed, if you suppose, you need a protein to develop a health drink or suppose, you want the proteins in grams level then in those cases you have to use the E coli, yeast or the baculovirus expression system which will be going to give, which will be more suitable compared to the mammalian expression system simply, because of the reason that the E coli, yeast or the baculo expression systems are easy to manipulate. These systems are cheap to grow. For example, if you want to use the E coli as a source of producing your protein then the protein is going; the quantity or the cost what you are going to use to prepare the media or to manipulate these cells is going to be much lower compared to the mammalian expression system.

Same is true for the yeast as well as the baculo expression system because in some cases the protein may be of eukaryotic origin. It may not over express or it may not express very well into the E coli or the yeast system. In those cases, you might have to go to the baculo expression system. And baculo expression system is relatively cheaper compared to the traditional mammalian expression systems. Now, the second is the size of the protein. E coli expression system or the prokaryotic is not been preferred if the size of the protein is very large. For example, if you are interested to over express a protein which is of 300, 500 kilo dalton, which is a multimeric proteins or multimeric protein complexes.

For example, if you interested to suppose, over express, the pyruvate dehydrogenase complex, which is a multimeric, multienzyme complexes. These complexes will not going to over express very or optimally into the E coli expression system. In those cases, you might have to use the eukaryotic system; either the yeast or the mammalian expression system. So, if your protein is very large, the eukaryotic expression system is been optimized or is been trained in such a way that it actually over expresses these kind of protein of large or high molecular weight.

Then the third and the most important point is the compatibility between the source organism and the expression system. So, what it is mean is that in general, a close distance between the source organism and the expression system is preferred as it may increase the chances of getting the expression of cloned gene and presence of the protein in soluble fractions. So, this means that if you are trying to over express a protein from the bacteria, then you can use the E coli because

the E coli expression system is going to be very, very close to the bacterial expression system or suppose you want to over express a protein from the streptococcus pneumoniae or mycobacterium tuberculosis then these protein are and mycobacterium tuberculosis is very close to E coli so you can use the genes which are to produce the protein.

So, you can use the gene from the micro bacterium to produce the protein in E coli. But suppose, you want to generate or over express the actin from the rabbit, then you might have to face difficulties in over expressing this kind of protein in the equivalent expression system because the distance between a bacteria and the mammalian cell, mammalian organism is very, very large. So, there are chances that these protein either may not over express or if they over express, the folding system may not exist very proper, very much into the equalized system. So, because of that the E coli may not either provide, either give you the protein or if it gives you the protein, protein may not be properly folded to give you any kind of the activity. So that is why it is always been advisable that you choose the expression system as well as the source of organism which are very close.

For example, in the case of rabbit, we might have to choose the yeast or the baculo expression system because these systems are much closer to the eukaryotic systems or the mammalian system. Now, the third and the fourth and the most important point, is what is the downstream applications of the protein which you are going to over express. So, this is the most important because the ultimate goal of the biotechnology is to over express this protein and utilize them into the downstream applications. So, once you have the idea about downstream applications, then you can choose a certain, you can choose the suitable expression system.

For example, if the protein is produced only for generating the anti bodies or suppose your protein you are simply using for, as an antigen so that you can detect the antibodies from the patient serum which means you are using these proteins simply as an antigen to coat the wells and develop the ELISA detection kits, in those cases it is immaterial whether the protein is active or inactive because in either of these cases the protein epitopes or the antigenic sites may not maybe still be conserved. Because the antigenic sites of a particular protein are present in a very, very small stretch. So, and these stretches may still be intact.

So, even if the protein is being over expressed and it is not present in the soluble fraction or it is not properly folded. Even then the antigenic site may still be remained conserved and you can be able to use despite particular type of proteins for generating the antibodies as well as using these protein as antigen to develop the ELISA kits to diagnose a particular kind of antibodies present in the patient serum. So, for those kind of applications you can easily use any expression system but you can use the E coli because that E coli expression system may give you a large quantity of proteins and as well as the manipulation and the cost to run or cost to operate the E coli expression system would be much cheaper compared to the mammalian or the yeast or the baculo virus expression system.

But suppose you want to produce the protein for the activity. In those cases, or in some cases of some of the proteins may lose its antigenic sites when you are, when they are not properly folded because, you want to use this protein for as an antigen to detect antibodies using the ELISA. In those cases, you have use the host which is very, very compatible to the protein; which means you have to choose the host expression system as well as the protein of the closer association which means, if it is bacteria, you have to use E coli, if it is a eukaryotic system you have the choices of using the yeast, baculo or the mammalian expression system.

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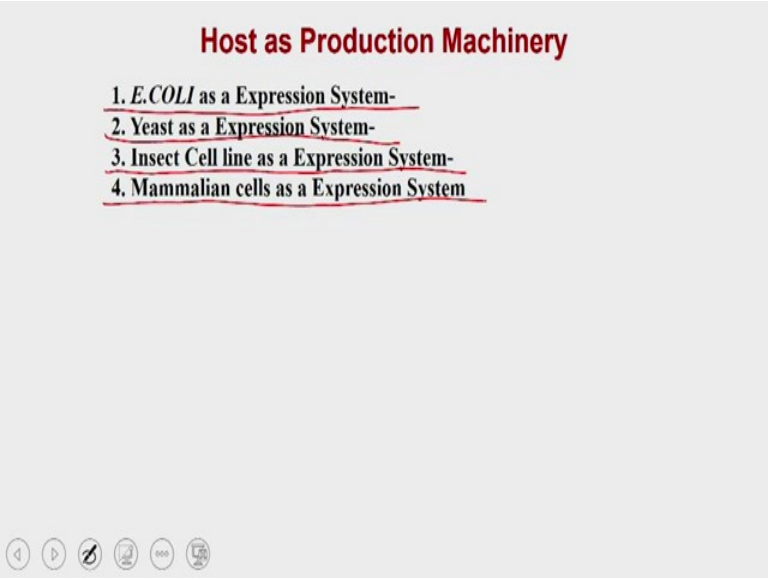

So, in the today's lecture, what we are going to discuss, we are going to discuss 4 different expression system such as E coli expression, as an expression system. We are going to use yeast

as an expression system. We are going to use insect cell line or the baculovirus as an expression system and at the end, we are also going to use the mammalian cell as a expression system. What we are going to discuss in terms of how you can use the, what are the features of these expression system.

How to, what are the different type of vectors which are available or, what are the different features of these expression system and how to produce a protein. Which are the, what are the different schema, protocols which you can use to over express the protein in E coli, yeast or mammalian expression system. In the case of mammalian expression system, we are also going to discuss how to develop, how to make the protein in the transient expression system or how to make protein in the permanent expression system.

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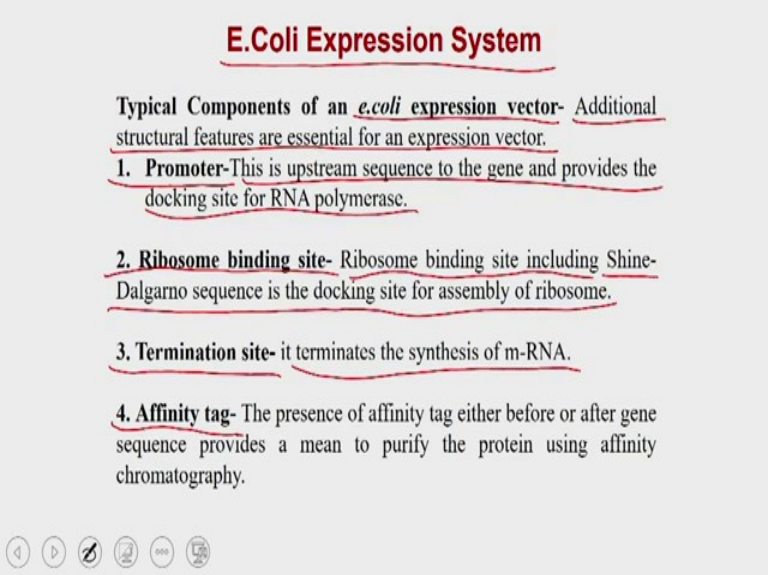

So, let us start with the E coli as an expression system. So, in the case of E coli as an expression system, a typical E coli; the different components which are present in a E coli expression system. Although we are discussing only in the context of E coli expression system, but most of these components are mostly been present in other expression system as well. So, you have the following features which are in addition to the cloning vectors. If you remember or recall, in our previous lectures, we have discussed about what are the features you required for a cloning vector. In the case of cloning vector, what you need?

You need a selection marker, you need the multiple cloning sites you need replication or origin of applications which would be as per the host strain. In most of the prokaryotic system, you have Ori C, whereas in the case of eukaryotic system, you have the origin of replication as per host strain, such as yeast or mammalian systems. And in most of the eukaryotic expression used, most of the eukaryotic cloning vectors, you also have the origin of replications for the prokaryotic system so that you can do the cloning as well as the manipulation within the prokaryotic system and then you can transform this into the respective eukaryotic expression system and over express the protein.

So, apart from those features like selection marker, MCS, origin of replications, you also need the additional feature for the vector to express the protein. What are these features? The first and the most important feature is a promoter. The promoter is a upstream sequence to the gene and it provides the docking site for the RNA polymerase. Just now when we were discussing about the protein production machinery, we discussed about the promoter where, the RNA polymerase goes and bind then it start the transcription of the messenger RNA.

Then you need the ribosome binding site. The ribosome binding site is essential because it will allow the ribosomes to go and bind and that includes the crucial Shine-Dalgarno sequences which is actually going to provide the docking site for the assembly of the ribosome subunits, so that the ribosome is going to assemble onto the messenger RNA and then it will start the translation process. The third and is also the termination sites because once you start the synthesis or transcription, you have to terminate so that you are going to get the fully matured messenger RNA.

And then, at the end, because you want to also facilitate the purification of that particular proteinaceous factor, you also could put the affinity tags. These affinity tags are important because you can use the compatible affinity beats and that actually will allow you to purify this protein without going through the conventional process of different types of chromatography columns. For example, if you do not have affinity tags, you might have to use the conventional chromatography techniques such as anion exchange chromatography, cationic exchange chromatography or hydrophobic interaction chromatography, gel filtration chromatography.

But and even after using these combinations you may end up in losing the proteins in different types of chromatography techniques, you may also going to lose the time because by running these chromatography techniques you are going to waste lot of time and then at the end, you also going to waste a lot of chemicals. So by putting affinity tag whether it is the 'His tag' or 'GST tag' or any other affinity tag.

You are actually going to use a particular breed which has only affinity for this tag. So, tag is just like you are putting a stretch of antigenic region and that actually has only affinity for that particular breeds. So once you pass through the host bacterial lysate or the host lysate, your protein will go and bind to the breeds. All other proteins will come out. So because of that you could be able to achieve the better purification in a simpler way as well as in a single step.

In most of the cases, you will get the more than 90% purified protein in a single step and that actually has an advantage because it lose, it basically makes the efficiency of the production more. Number 2, it also reduces the cost of its production because it is reuse, it reduces the wastage in terms of running the different chromatography columns. And the third is the level of purity what you are going to get just after the single column is going to be way more compared to using the traditional chromatography columns or traditional chromatography techniques.

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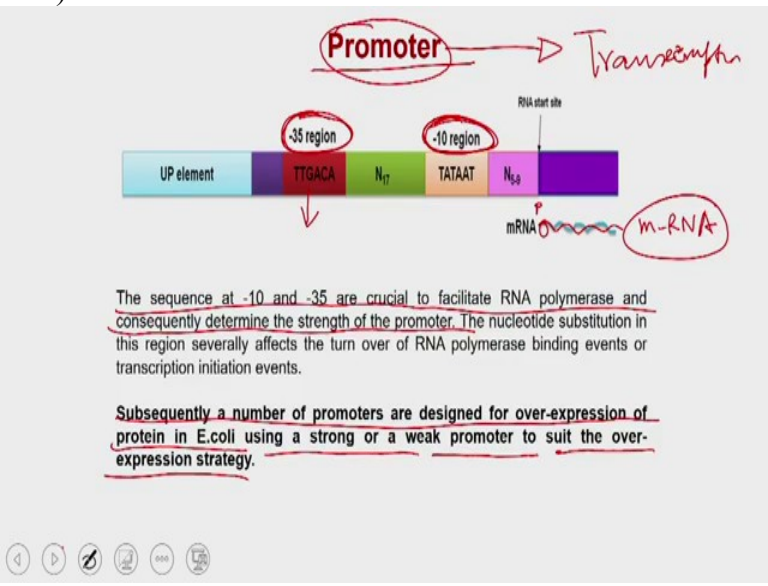

So let us start with the E coli. So the promoter, as I said in a promoter is the region which actually facilitate the binding of RNA polymerase and that is how it actually initiates the transcription. And in the case of E coli as we said, the transcription and translation are linked to each other. So as soon as you have, you started the synthesis of messenger RNA, you are going to also initiate the binding of ribosomes to the RNA, to the messenger RNA and that is how you are going to initiate. So that process is completely been controlled by the promoter because the promoter is going to control the transcription and so, in a typical promoter you have 2 regions. 1 is called minus 35 region, the other one is called as the minus 10 region. And this is your site 0, where from where the messenger RNA is going to synthesized so all these promoters are upstream to the RNA, to the initial, to the start site.

And the minus 35 region as well as the minus 10 region is containing the different types of nucleotide sequences. And the presence of different types of nucleotide sequences are the assets actually decides the strength of these promoter because if the minus 35 region as well as the minus 10 region is very strong in terms of facilitating the binding of transcription factor as well as the binding of RNA polymerase then this promoter is going to be very, very strong. If these regions are going to be weak, which means they do not facilitate the binding of the transcription factor as well as the binding of RNA polymerase then, the promoter is not going to be strong, which means it does not allow the more rounds of RNA polymerase binding; which means ultimately it is going to reduce the level of messenger RNA into the particular transcription.

After the transcription, the level of messenger RNA, which you are going to be produced from this particular promoter is going to be low and that is why, the promoter may fall into 2 category. The promoter which are of the strong promoter where, the minus 35 region as well as the minus 10 region facilitate the rapid docking of the RNA polymerase. So, since it is allowing the rapid docking, it will actually going to increase the messenger RNA level within the cell and the other category of promoter is where you are going to have the promoter of the weak promoters. So, in the weak promoters in the minus 35 region as well as the minus 10 region are going to be, will not going to facilitate the RNA polymerase binding that strongly. And because of that, the number of cycles through which the RNA polymerase can go and bind to the promoter is going to be low.

And as a result, the level of messenger RNA which was going to be produced from this promoter is going to be less compared to the strong promoter. Hence consequently the amount of protein which you are going to produce from the low, the weak promoter is going to be less compared to the strong promoters. So, sequence at the minus 10 and minus 35 are crucial for facilitating the RNA polymerase binding. Consequently, it determine the strength of the promoter. It could be a strong promoter or the weak promoter and if you play with the composition of the minus 35 region and the minus 10 region by simply by switching the nucleotides or you are making the mutations into the promoter, you could be able to generate the strong promoter versus the weak promoter.

Because of this, a number of promoters are being designed for over expression of a protein in E coli using a strong or the big promoter to suits the over expression strategies. For example, in some of the cases, it is not almost important or essential that you use the strong promoter. In some of the cases, you also prefer to use the weak promoter because the advantage of weak promoter is that it actually lower down the protein production. So, once it is lowering down the protein production it actually giving the more time for the protein to get folded. So for example, suppose you are trying to over express a eukaryotic protein into the E coli as an expression system then in those cases, this mammalian system or mammalian protein, if you produce very rapidly the equalized system may not be able to fold this particular protein into the proper conformation.

But if you reduce the kinetics of the protein production, you may give the chance to the folding machinery and that may give you the chance that it may get folded properly and you may get the active protein. So in those kind of context sometime people prefer to use the weak promoter versus the strong promoter. So based on these weak or strong promoter, people have developed different types of vectors and that is how they have developed the different types of E coli expression systems.

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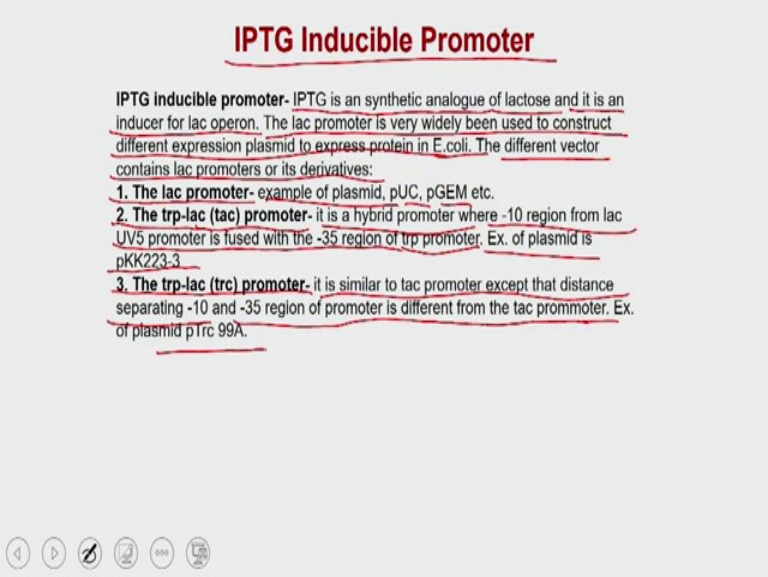

So, first of the promoter is the IPTG inducible promoter. So, IPTG is an synthetic analogue of lactose. So mostly the IPTG inducible promoter are actually the lactose promoter or lac operons and they are being used to induce the lac operon. The lac operon is very widely being used to construct different expression plasmid to express protein in E coli. The different vector contains lac promoters or its derivatives.

One of the example is the lac promoter. Example is like the plasmid, pUC, pGEM, etc. Then you have the tac promoter which is called trp-lacose promoter. It is a hybrid promoter where the minus 10 region from the lactose UV5 promoter is fused with the minus 35 region of the trptophan promoter. Example in this case is pKKK223-3 which means in this case, you are actually taking a minus 10 region from the lactose, lac promoter and you are taking the minus 35 promoter from the tryptophan promoter.

Then the third is the trc promoter. The trc promoter is the tryptophan lactose promoter. So, it is exactly the reverse of this. So, it is similar to the tac promoter, except that the distance separating minus 10 and minus 35 region of promoter is different from the tac promoter. Example in this case is pTrc99A. So these are the 3 different types of promoters which are being derived to work as a IPTG inducible promoter and IPTG is a chemical analogue of lactose.

What is the difference between IPTG and lactose is that IPTG is non degradable compared to the lactose which is going to be degrade by the system into the constituent sugars. So if you use the lactose instead of IPTG. Lactose will give you the high induction in the beginning but as the protein will start producing, the lactose will start degrading by the bacterial machinery and as result you are not going to get the continuous same level of induction throughout your induction period. Whereas, in the case of IPTG, since IPTG is non hydrolyzable lactose analogue, it will going to induce the lac operon but that induction will remain constant from the starting of the experiment to the end of the experiment.

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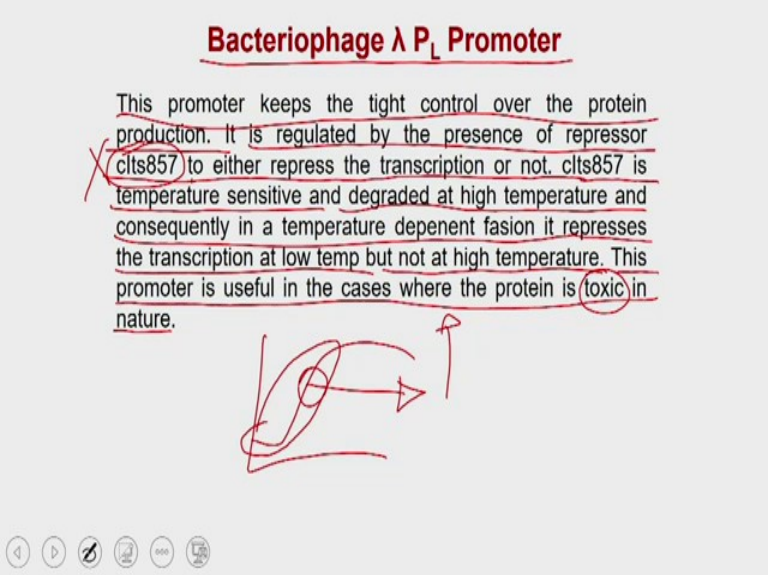

Now the second promoter what the people use in the E coli expression system is called the bacteriophage Pl promoter. This promoter keeps the tight control over the protein production. It is regulated by the presence of a repressor called clts857 to either repress the transcription or not. clts857 is a temperature sensitive and it degrades at the high temperature and consequently in a temperature dependent fashion it represses the transcription at low temperature but not at high temperature, which means this is actually a temperature sensitive factor. So if you increase the temperature, the factor will get degraded and as a result it is not going to repress. So at low temperature, you are, it is going to function, so it will going to repress the transcription. But once you change the temperature which means like if you change the temperature from 37 to 42 that actually will going to degrade this particular repressor.

And once it is getting degraded, there will be no repression or there will be no repression on to the promoter and as a result it is going to give you the protein. This promoter is useful in the cases where the protein is toxic in nature. So what the way this particular type of unique promoter is being used is that, if the protein is very toxic what will happen is that, if you will use the IPTG based inducible promoter, it is actually going to start producing the protein because the bacteria normally contains a small amount of lactose which it will take up from the media.

So, as a result you are going to get the protein production from the beginning itself and because the protein is toxic, it is going to kill the bacteria. So, as a result what will happen is, suppose you inoculated the bacteria into the media, it will not let for the bacteria to grow and reach to a certain growth stage. Which means it will not let you to grow the bacteria to grow from the lag phase to log phase and then stationary phase. It will not let the bacteria go to the log phase because before it will get, because the bacteria will be in a lag phase and in that lag phase itself the protein will be produced which is toxic and it is going to kill the bacteria.

So to avoid that you put the gene under the control of this particular type of promoter, what will happen is, you grow the bacteria at low temperature. So that actually will going to suppress or repress the production of this particular protein. So what will happen is that this is your typical bacterial growth kinetics. So what you do is, you keep the temperature low until it reaches to the log phase and once it reaches the log phase then you what you do is you increase the temperature.

And even at this stage, the bacteria is going to die. It hardly matters because the number of bacteria which you are going to have in the log phase is way more than the number of bacteria what you have in the lag phase. And as a result, the bacteria will die but it is going to produce this particular toxic protein which you can collect later on and use it for the downstream applications. So that is how you can utilize or exploit the bacteriophage lambda PL based promoters.

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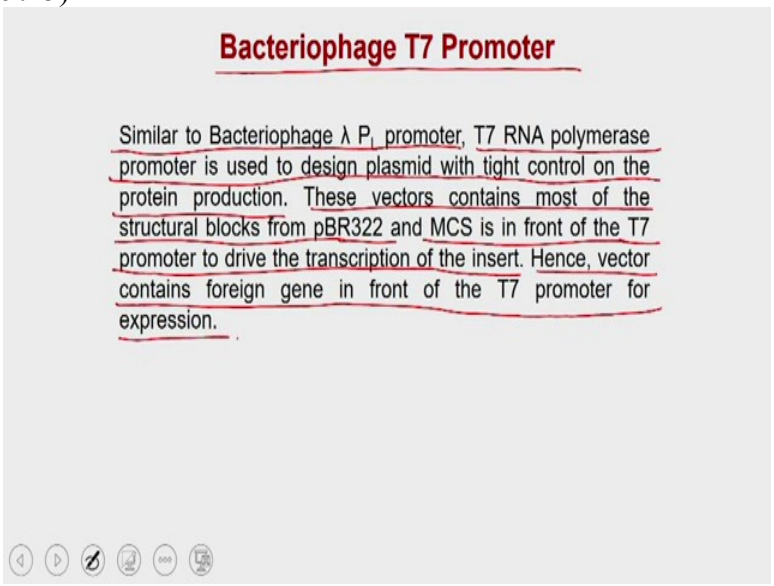

Now you have the bacteriophage T7 promoter, so bacteriophage T7 promoter is very, very popular in the case of E coli as an expression vector. Since it is similar to the bacteriophage promoter lambda promoter. But the T7 promoter, T7 RNA polymerase is used to design plasmid with tight control on the protein production. These vectors contains most of the structural blocks such as the selection markers and the multiple cloning sites and everything from the PBR 322 and the in front of the MCS you have the T7 promoter to drive the transcription of the your gene which you are going to use as an insert. Hence the vector contains foreign gene in front of the T7 promoter for expression.

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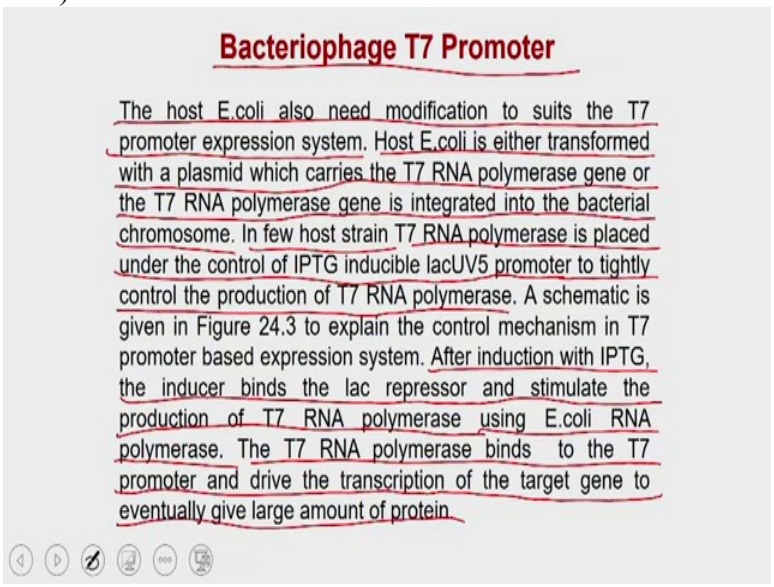

Now in some of the cases when you use the bacteriophage T7 promoters, you can also modify the host strain such as the E coli strain. You can over express some of the protein on to the chromosome of these E coli and that is how you can modify the host to make even metal tight control over the protein production.

So host cells also need to modify to suit to the T7 promoter expression system. Host E coli is either 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 control of IPTG inducible lacUV5 promoter to tightly control the production of T7 RNA polymerase, okay.

After the induction of with IPTG, the inducer binds the lac repressor and stimulate the production of T7 RNA polymerase using E coli RNA polymerase. The T7 RNA polymerase binds to the T7 promoter and drive the transcription of the target gene to eventually give large amount of protein which means...

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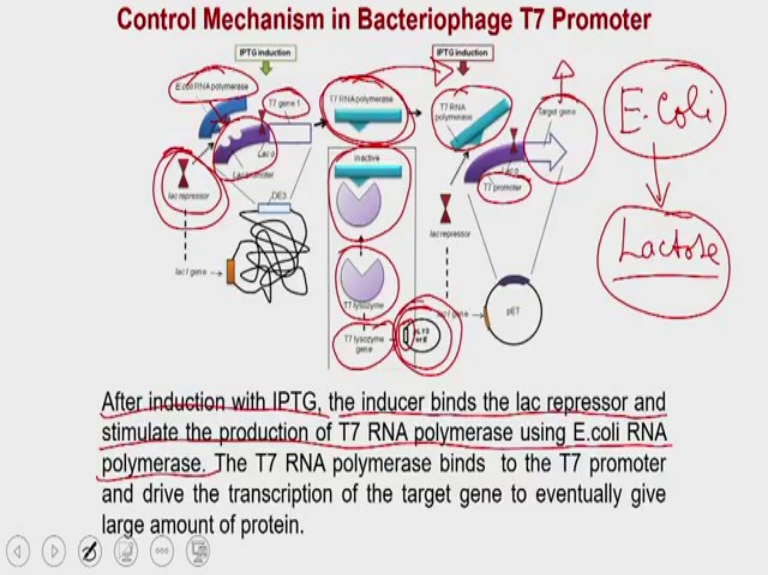

What we are trying to say is once you do the induction with IPTG, the inducer is going to bind to the lac repressor and stimulate the production of T7 RNA polymerase using the E coli RNA polymerase. Once you going to put the inducer, it will not going to, it will allow the E coli RNA

polymerase to go and bind to the lac promoter and that is how, onto the lac promoter, under the influence of lac promoter you have the T7 gene which is going to produce the T7 RNA polymerase. And once the T7 RNA is been produced, it will go and bind to the T7 promoter and that actually will going to drive the transcription of the target gene.

But in some of the cases, once you want to make a very tight control. So what will happen is that you are using the E coli as an expression system and E coli is going to have very low amount of lactose. So even you are not adding the IPTG which means you are not adding the inducer the lactose which is present inside the cell is going to produce or going to bind to the repressor and as a result it will going to induce the RNA polymerase, the E coli RNA polymerase to go and bind to the lac promoter and that is how it is going to induce the, first it will going to induce the production of T7 polymerase and that eventually going to start synthesizing the target gene in the absence of the inducer as well.

Which means if even, if you do not add the IPTG, the lactose which is present inside the cell will going to function at a very, very low level to produce some amount of T7 RNA polymerase and that T7 RNA polymerase will drive the production of your target gene. So in some of the cases, as just we discussed in the bacteriophage PL lambda promoter, some of these target proteins which you are over expressing may be toxic or may not be good for the E coli growth. In those cases, you will not going to see the E coli cells number is increasing while you are inoculating. So to make it more tight control what people have designed is they have made 2 more plasmids. And in those plasmids what they have done is; they have over expressed the T7 lysozyme gene.

So once the T7 lysozyme gene is going to produce the T7 lysozyme and this T7 lysozyme is going to destroy or inactivate the low level of T7 RNA polymerase which is being produced under the influence of lactose which is present inside the E coli. So that actually to destroy or going to kill the background protein production in the absence of IPTG but once you add the IPTG, the IPTG is going to be very, very strong inducible into inducers compared to the lactose. The low level of lactose which is present inside E coli.

So as a result what will happen is a large quantity of T7 RNA polymerase is going to be produced and then going to see a induction once you add the IPTG. So in the presence of these 2, these construct which contains the T7 lysozyme gene, you are going to kill the background expression and that is how actually you can over express the protein which are toxic in nature in the this RNA T7 promoter system as well.

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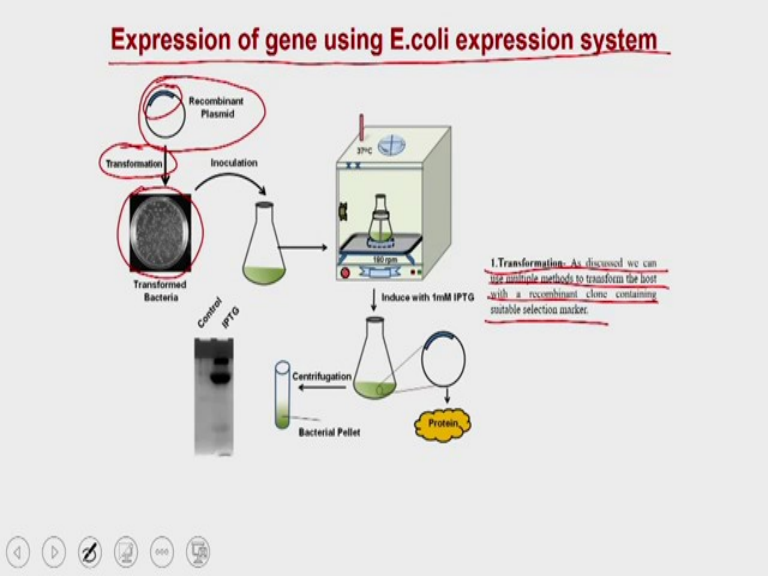

Now let us discuss about how to express the gene using the E coli expression system. So once what are the different steps? In the step 1, you are going to produce the recombinant plasmid which is containing your foreign gene. The first step you are going to do the transformation which means you are going to deliver this particular recombinant plasmid into the bacterial system and then you are going to screen them for your transformed bacteria. So you can use the different types of selection markers depending on the what kind of selection marker is present is present on that particular recombinant plant.

For example, if you if you have the ampicillin resistant gene, you can use the transform. You can plate the cells on to a plate that contains the Ampicillin and in that case the resistant cells are going to grow onto the plate, whereas the non-transformed colonies will going to die, so in the first step, you will do the transformation and we have discussed many methods, how to transform a host with a recombinant clone containing suitable selection markers. So you will do the transformation as well as the selection of the transformed bacteria.

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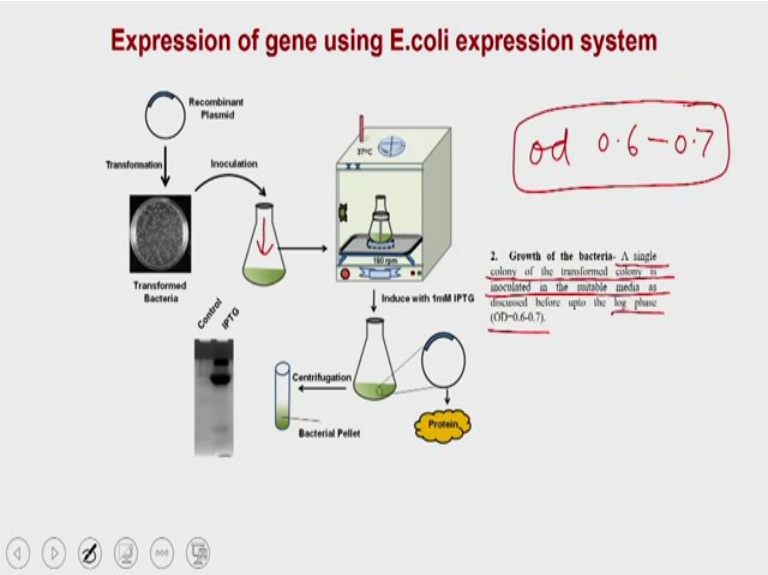

Then in the second step, you are going to grow. So, what you are going to do is, you will take out few colonies from this particular transformed plate. You will inoculate into the growth media. We have also discussed types of growth media which you can use for growing the E coli cells. Then a single colony or few colonies of transformed colony you inoculate into the suitable media. And let the bacteria to go grow into the log phase which is in the OD of 0.6 to 0.7. If you remember, we have also discussed how to monitor the growth of these bacteria, Okay and you can put them into the 37 incubators with a shaking of 180 RPM.

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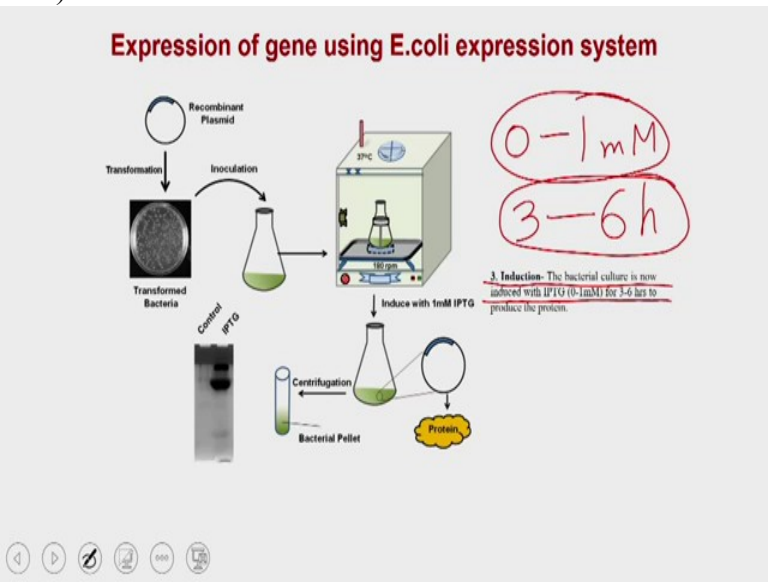

Now in the third step, you will do the induction. So what will happen is, you will take out the bacterial colony culture. You add the IPTG. Mostly people use 0 to 1millimolar IPTG and you can use the and then you put it back into the incubator at 37 with 180 RPM. And you can optimize the time through which you can do induction. So you can optimize the IPTG concentration as well as the time of induction. Mostly people use 3 to 6 hours induction but this induction time can be optimized even for longer period or the higher period.

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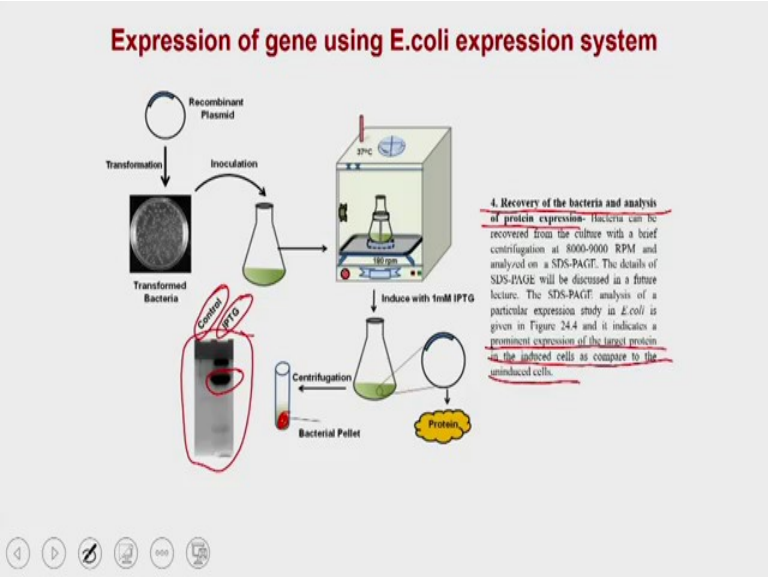

Once you do the induction, the protein will start producing into these bacteria. Then you will do is, you will recover the bacterial cells. So, you can recover the bacterial cells simply by centrifugation. So that actually will give you the bacterial plates with bacterial palette. And that bacterial palette you can lies in the, with the help of SDS as well as other kind of detergents and then you can release them onto the SDS page. As you can see here, this is the control bacteria which means untransformed bacteria or un-induced bacteria and this is the IPTG induced bacteria and what you will see is that we have the enhanced production of that particular protein compared to all other proteins. So you will see that there is a prominent expression of the target protein in the inducible cell as compared to the un-induced cells.

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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 BL21 DE3 strain, so BL 21 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 scalar.

So I will show you how to inoculate, how to take a single colony and inoculate 1 colony in 5 ml of LB media and that we will use for further experiments. So, this inoculation should be done in lambda L pro. So we will use lambda 2 to inoculate this colony and also we have to note that the expression if you having any resistant marker like ampicillin resistance kanamycin resistant, you have to include that antibiotic also in your culture media. So that it will specifically gross 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 medium with suitable antibiotic. Now what we have to do is keep in a incubator till we get growth of point 4 or point 5 OD before inoculating into large culture.

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So I will keep these ones in incubator shaker. This is the incubator shaker. So we can actually rotate the base so that uniformly in the culture is spread throughout the media. So after we get growth, we will inoculate into another culture that we will use for the induction analysis.

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As we can see the bacteria the (())(59:02) is around 0.37 to 0.4. So, this is the right tray for induction. We will use isopropyl beta-d-1-thiogalactopyranoside as inducing agent which we will call it as. In short form we will call it as IPTG.

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So we will induce with the IPTG and also at the same time we have to add antibiotic deeper into this media so that that will prevent any contamination which may be accidentally comes into the flask while doing induction. So this process should be carried out in a septic conditions, that is why we will use lambda l pro for this purpose. So let us induce these sample and we will keep again back to the incubation.

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This is 1 millimolar IPTG. The amount of IPTG which we have to add for induction, it depends on how much expression you have to, how much expression you get. So you have to optimize using different concentrations of IPTG, viz 0.1 milimole or 0.51 milimole respectively. So, I am going to induce.

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After induction, we have to keep for 4 hours. The time is, 4 hours is over. So we have to centrifuge and get the palette that we will use for the sonication and protein purification.

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As we can see the, it is almost over. So you can take out the gene, then we will stain and de-stain it. Generally what we will do is, we will, there are two ways of staining and anti staining. One is we can do quick staining like we have to keep it with the staining solution which contains coomassie brilliant blue and along with methanol water. So then we will try to de-stain with water by heat. But in another way, the simplest way is, we will just stain the gene for 2 hours, then we will we will de-stain over night. So I am going to show the simplest way. First we will

stain in coomassie brilliant blue staining solution. Then we will de-stain it with methanol water containing solution.

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So I am going to start the stainer, then I will remove it and show you how to remove the gel.

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Take out the glass blades.

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Here we have to be very careful while taking out gel, otherwise the sharp blades may broke.

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On a corner we have to take and lift the gel like this.

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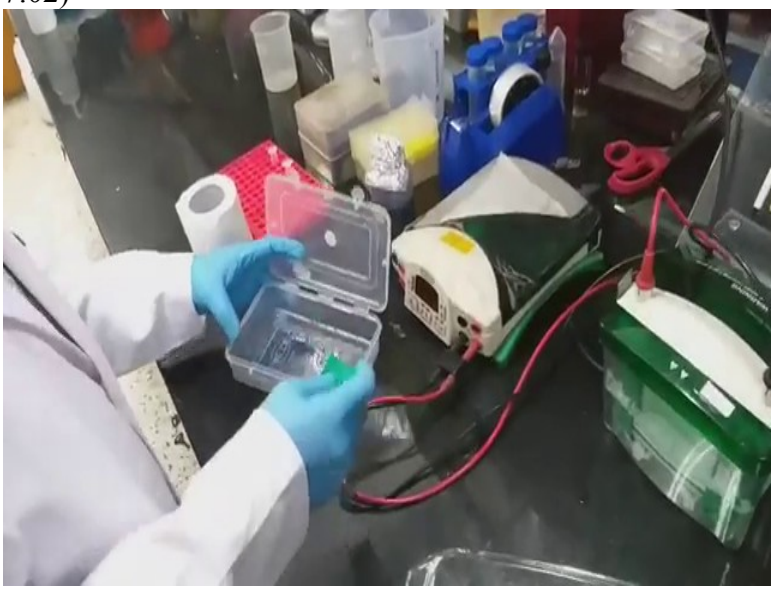

Keep the gel in a staining box which is more or less a plastic one but it can sustain it.

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So then I am going to add the staining solution.

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I will keep it on a rotation for, on a shaker for at least 2 hours then we will de-stain, over. So, once the time is over, after 2 hours, we will de-stain this.

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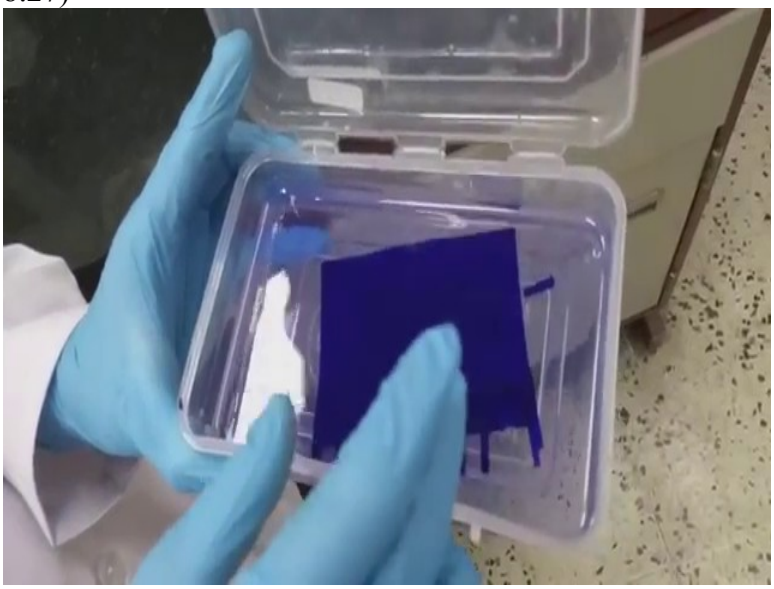

We kept 2 hours in staining solution, we will. As we can see, the staining is over. Like you can see, the gel completely turned into blue.

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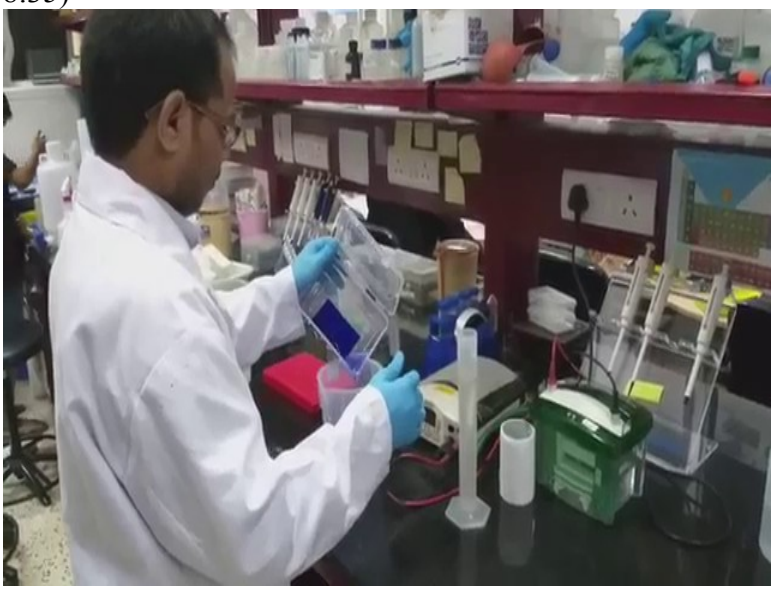

So we remove the solution.

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Then I am going to add de-staining solution.

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And I will keep this on a rocker for 2 hours for de-staining. So the composition contains for 100 ml of destining 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 keep this on a rocker.

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Now it is time to document the gel. So we have to identify whether we got any single (()) (1:09:32) or not. So this is the gel. I kept on white tray. Now just close it.

To summarise what we have discussed so far in terms of over expressing a particular foreign gene into the E coli expression system. What you are supposed to do is, you have to first transformed the bacteria with a recombinant clone and then you inoculate the single colonies into

the media and then let the bacteria to grow in the log phase which is from 0.3 to 0.6 OD. And then after you recover the bacteria, lies them and then analyze the production, induction on to the SDS page. So with this we would like to conclude our lecture here and in our subsequent lecture we are going to discuss about the yeast as well as the mammalian expression system. Thank you.