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

Lecture – 41
Polymerase Chain Reaction (Part-4)

Hello everybody, this is Dr. Vishal Trivedi from, Department of Biosciences and Bioengineering, IIT Guwahati and today we are going to discuss about the application of the PCR. So, far what we have discussed we have discussed about the different aspects related to PCR like how to set up the PCR, what is the basic principle of the PCR and then, in the previous lecture.

We have also discussed about how to design the primers and how you can take the precautions and consideration before you can be able to finalize a primer sequence, which you can use for amplifications. So, with this technical background, now, we would like to go ahead and would like to discuss about how you can be exploit the PCR as a technique to answer some of the questions and as well as how you can be able to utilize the PCR for the understanding many aspects related to science.

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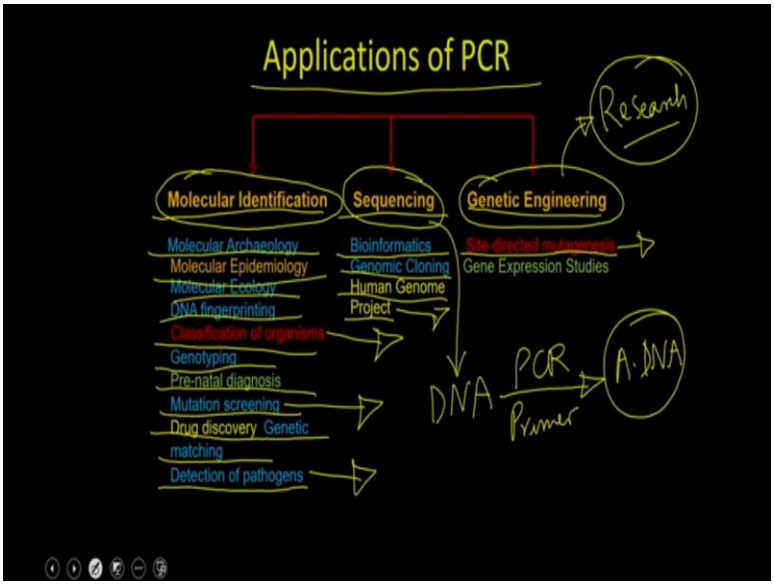

So, mainly the PCR, the application of the PCR lies into the 3 different categories either it could be related to molecular identifications, or it could be used for the sequencing reactions, or it

could be used in the case of genetic engineering which means, it genetic engineer means it can be used even for the research purpose. So, regarding the molecular identifications means; that you are trying to identify a particular organisms or if you are trying to identify and classify.

Suppose, you have isolated a new organisms then you how you can be able to categorize that particular organism. So, see in a traditional world, what people are doing is suppose they have identified a new plant, they will take this plant to experienced taxonomist, and that taxonomist is actually going to based on the experience, and based on the similarity of some of the taxonomical features, it actually going to be able to classify that particular plant as x and y.

But in the recent word, what people are more trying to understand is that what is the pattern of the these DNA and whether can be we can be able to classify the plants and categorize them based on the DNA as well as the amplification products. So in that way, the molecular identification is playing is the place where the PCR is actually playing a crucial role. So molecular identification playing a role in the molecular oncology, molecular epidemiology, ecology, DNA fingerprinting.

And it is being used for the classification of organism because the up you can be able to amplify the DNA and then you can be able to match that DNA with the DNA what is available in the database, and that actually is going to help you in very precisely categorizing a particular plant or animal or even other creatures, then you can use the PCR for the genotyping, prenatal diagnostics, mutation screenings. So the mutation screening is also a place where you can be able to do a PCR.

And it is actually going to tell you whether the particular type of mutation is been persisting into this particular gene or not. And that has a very wide application in terms of the diagnostics because some of the mutations like for example, if there is a mutation, and that mutation is linked to a particular type of disease phenotype, then you can be able to design a PCR and you can be able to design the primers in such a way that it is actually going to detect or it is only going to give you the Amplified product when there will be a mutation.

Other than that, if there will be no mutation then it will not give you the amplified products. So that is how you can be able to identify the mutations in a certain set of genes and that is how you can be able to identify whether a particular any particular cell is having the mutated version of that particular gene or not. Then you can also use the, the PCR for drug discovery as well as the genetic matching and detection of the pathogen.

So, detection of pathogen is also very important area where you can be able to use the PCR because one of the major advantages of the PCR is that it is very sensitive. So, even if you have a very small number of copies available within the particular organisms or within the blood, you can be able to even do amplification, you can increase that number and you can be able to do identification purposes.

Now, the second is the sequencing. So, in the sequencing reactions, you can be able to use the PCR. So, in a typical sequencing reactions, what you are doing is you are simply taking our DNA what you are interested to sequence and then you are doing a PCR with the help of the primers and that is actually going to give you amplified DNA and that amplified DNA is being amplified in such a way that it is actually going to give you the DNA fragments.

For example, if we talk about the Sanger method, the DNA is been amplified with the help of the primers as well as the modified nucleotides, and these modified nucleotides are actually going to give you the small fragments and that small fragments can be used to identify the sequence and that is how you can be able to get reduce the sequence. Once you have got the sequence, you can be able to use that sequence and matches with the database.

And that for you can be able to do that in the bioinformatics similarly the PCR is being used for genomic cloning, as well as in the human genome projects for sequencing the multiple transcripts or multiple DNA fragments, what has been produced from the DNA libraries. Apart from that, in the genetic engineering or the research purposes, the PCR is been widely been used for 2 purposes.

One is that they are being used for generating the site directed mutagenesis, which means you can be able to very precisely generate point mutation into a particular gene. And that is how you can be able to answer the questions like for example, if there is an enzyme which is actually having an aspartate and that aspartate it is very crucial. So then you what you can do is with the help of the PCR, you can be able to do is introduce a mutation and that will be a point mutations.

And that point mutation will replace the aspartate to alanine or glycine or as per your hypothesis and as per the interaction of that aspartate with the neighboring residues. And that is how you can be able to answer the questions that whether that particular aspartate is crucial for a particular activity or not, the other is that the gene expression study, so, with the help of the PCR, you can be able to even study the expression of their particular gene.

So, considering all these 3 different aspects, we have taken , we now going to get into the application part and we are going to take up the application as per the different streams of the science for example, the plant science, animal Science and also so let us start with that.

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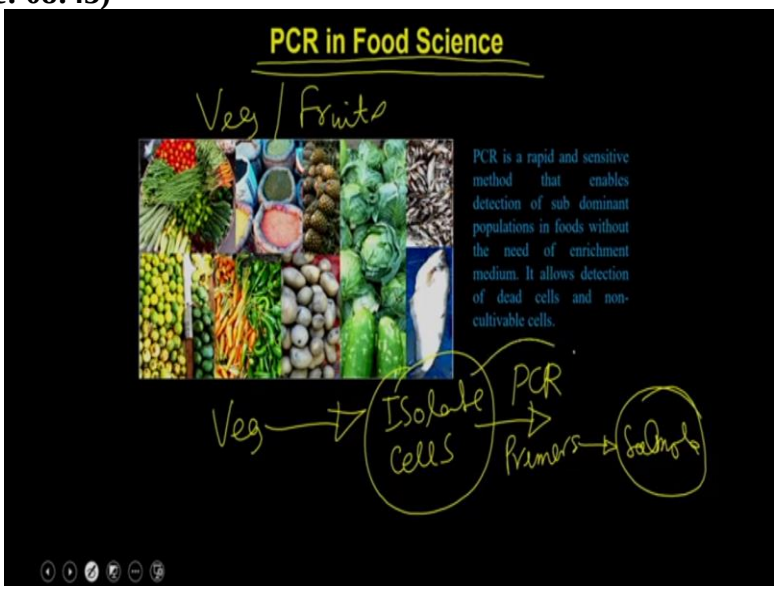

So, the first what we have done is we have taken the example of the PCR how you can be able to use the PCR in the food science. So when you talk about the food science, the food science is all about that you are talking about the vegetables as well as the fruits. So you as you can see in this picture, we have the different types of the fruits and the vegetables. The major problem with

these fruits and vegetables, what you can buy in the market or what has been produced in the farm is that they are infected with the different types of pathogenic organisms.

And if you consume that these pathogenic vegetables or the fruits, the particular bacteria or viruses are actually going to get into the human body and that how these fruits and vegetables are not good for the consumptions. So, how you will know that the particular lot of fruit or the vegetable is good for the human consumptions. So in that case, what you are going to do is you are going to do the PCR and in so suppose I am interested to identify the salmonella is a very dangerous bacteria in it actually causes a lot of disease in humans.

So if you consume a vegetable which is infected with salmonella then it is actually going to cause the toxicity. So, what you are going to do is if you are suspecting a salmonella infections, what you can do is you take the vegetables and then what you can do is you can just isolate the cells. So, you can just crush these vegetables you can just make a homogenate and then from this homogenate you can be able to just do a PCR with the help of the primers.

These primers are nothing but these primers are actually going to be for salmonella. So, in that case what will happen is if there will be even a tiny salmonella contaminations, even if there will be a few salmonella bacteria what is present in this particular plant or in this particular vegetables, it is actually going to give you a amplified DNA.

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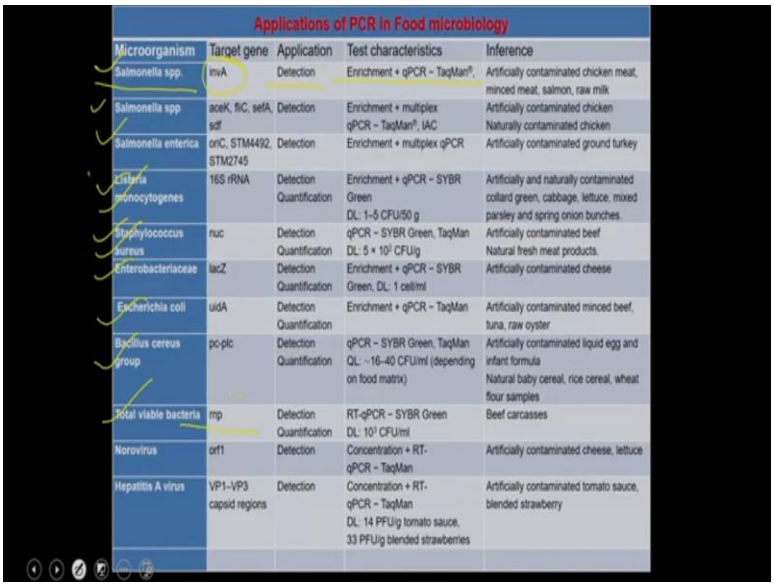

Applications of PCR in Food microbiology

Microorganism	Target gene	Application	Test characteristics	Inference
Salmonella spp.	invA	Detection	Enrichment + qPCR – TaqMan®,	Artificially contaminated chicken meat, minced meat, salmon, raw milk
Salmonella spp	aceK, fliC, sefA, sdf	Detection	Enrichment + multiplex qPCR – TaqMan®, IAC	Artificially contaminated chicken Naturally contaminated chicken
Salmonella enterica	oriC, STM4492, STM2745	Detection	Enrichment + multiplex qPCR	Artificially contaminated ground turkey
Listeria monocytogenes	16S rRNA	Detection Quantification	Enrichment + qPCR – SYBR Green DL: 1–5 CFU/50 g	Artificially and naturally contaminated collard green, cabbage, lettuce, mixed parsley and spring onion bunches.
Staphylococcus aureus	nuc	Detection Quantification	qPCR – SYBR Green, TaqMan DL: 5 × 10^2 CFU/g	Artificially contaminated beef Natural fresh meat products.
Enterobacteriaceae	lacZ	Detection Quantification	Enrichment + qPCR – SYBR Green, DL: 1 cell/ml	Artificially contaminated cheese
Escherichia coli	uidA	Detection Quantification	Enrichment + qPCR – TaqMan	Artificially contaminated minced beef, tuna, raw oyster
Bacillus cereus group	pc-plc	Detection Quantification	qPCR – SYBR Green, TaqMan QL: ~16–40 CFU/ml (depending on food matrix)	Artificially contaminated liquid egg and infant formula Natural baby cereal, rice cereal, wheat flour samples
Total viable bacteria	rrp	Detection Quantification	RT-qPCR – SYBR Green DL: 10^3 CFU/ml	Beef carcasses
Norovirus	orf1	Detection	Concentration + RT-qPCR – TaqMan	Artificially contaminated cheese, lettuce
Hepatitis A virus	VP1–VP3 capsid regions	Detection	Concentration + RT-qPCR – TaqMan DL: 14 PFU/g tomato sauce, 33 PFU/g blended strawberries	Artificially contaminated tomato sauce, blended strawberry

So, let us see besides the salmonella, what are the other things you can be able to detect in which related to the food microbiology. So, one first is the salmonella is being detected with the help of a target gene which is called as inVA, and what you can do is that you can do a qPCR as well as the TaqMan, then you have several species of salmonella. So, the advantage of the PCR is that it allows you to not only detect the particular type of bacteria, but it also allows you a particular species of bacteria.

So, there are so you can actually first identify the salmonella and then you can be able to use another set of primers and that actually will allow you to detect what species of the salmonella species present with the vegetable or the food materials. Apart from that, you are also going to allow you to detect this Listeria or Staphylococcus aureus, Enterobacteriaceae, e coli, bacillus, and that total viable bacteria. So that; is also can be done that you can actually simply go whether there will be a bacterial infection into the vegetable or food or not. And then there are other options as well.

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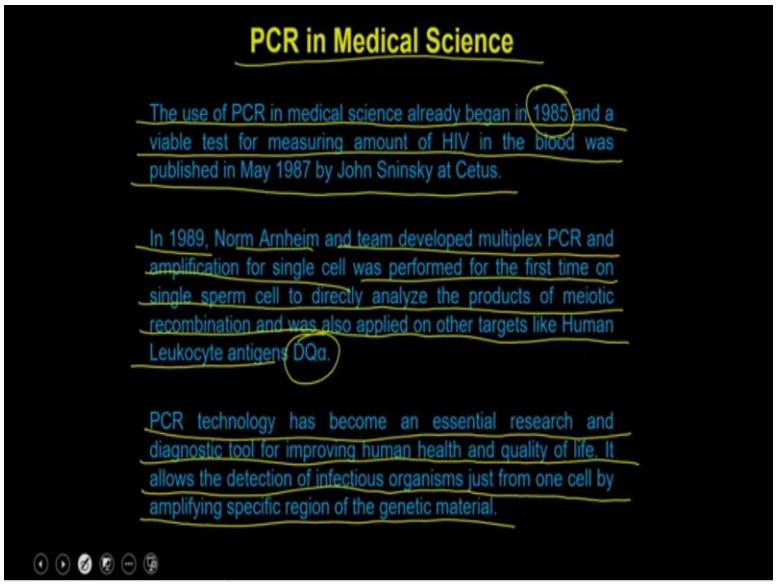

Then you are talking about the PCR in the medical science. So the use of PCR in the case of medical science is very extensive. And, in fact, when the people have designed the PCR technology, they designed PCR technology because of the medical science only. So in the early 80s when the people were trying to amplify the some of the crucial genes, what is been responsible for single cell anaemia and other kinds of diseases, they were having a very difficult task.

So that is why they have decided that, let us have a technique so that it will actually going to allow us to amplifications and that is how the PCR is being used. So, the use of PCR in medical science already started in the year of 1985. And a viable test for measuring the amount of HIV in the blood and that was published in May 1987. So in the 1989, Norm Arnheim and the team developed a multiplex PCR.

And amplification of single cell was performed for the first time on the single sperm cell to directly analyze the product of the meiotic recombination and was also applied on the other target like the human leukocyte antigen at the DQalpha. So PCR technology has become an essential research and the diagnostic tools for improving the human health and the quality of life, it allows the detection of the infectious organism just from 1 cell by the amplification of the specific region of the genetic material.

So one of the major advantage as I said before, also that the sensitivity of this method, so this, because the sensitivity is very high, you can actually be able to detect the infectious organism or the alteration within the host gene itself from a single cell. So the amount of DNA what you are going to receive even from the single cell or amount of genome what you are going to receive even from the single cell that is good enough to give you the enough products.

Which can be analyzed both for sequencing purposes, as well as if you want to clone that you can be able to clone it into our expression vector, you can study a lot of things. So that is why the PCR is been widely been used in the case of medical science as well.

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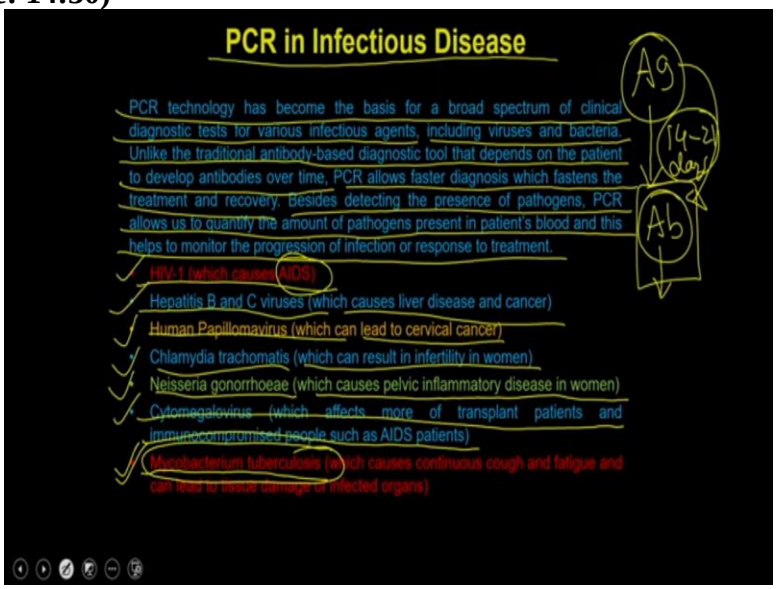

So PCR is being used very extensively for detecting the infectious disease. So PCR technology has become the basis for a broad spectrum of clinical diagnostics tests for various infectious diseases, including viruses and bacteria. Unlike the traditional antibody based diagnostic tool that depends on the patient to develop the antibody over time. PCR allows the faster diagnostics which fastens the treatment and recovery besides detecting the presence of pathogens.

So, PCR allows to; quantify the amount of pathogens present in the patient blood and need help to monitor the progression of infection or response to the treatment, so, what other disease you can be able to detect. So, this is just not the extensive list we this is just a representative list, what you can detect you can detect the HIV which causes the AIDS, then you can detect the hepatitis B and C virus which causes the liver disease and the cancer.

Then you can detect the human papilloma virus, which can lead to the cervical cancer, you can detect the Chlamydia trachomatis, which causes infertility in womens, you can detect Neisseria which causes the pelvic inflammatory disease environments and then you can detect the cytomegalovirus which actually detects the transplant patients and the immunocompromised people such as AIDS patients. And then lastly, you can also detect the mycobacterium tuberculosis which causes the tuberculosis in the patients.

In general now, when you are looking for the detection of these pathogenic organisms, traditionally what people were doing, they simply using the antibody. So, what happened is, when these infectious organisms are entering into the body, they are actually being act as antigen. So, when the antigen is entering into the body, it is actually causing the development of antibody, but in this period, when the antigen is entering into the human body and it is developing the antibodies, it is a very long period.

Which means, it will take at least 14 to 21 days before you could be able to detect some amount of antibody and then you can be able to say this particular infectious organism is present, but that actually requires very large amplification or the large multiplication of the first DNA infectious organisms, then only the body's immune system is going to recognize that and then it is actually going to process that antigen and it is actually going to give you the antibodies.

So, this is a very delayed response and what happens is, we until the PCR was not been developed, the people were using this particular technique, but a major disadvantage of the antibody based test is that it actually going to, before you can be able to detect it will allow the disease to reach to a very serious stage, which means that it will it is actually by the time you will detect the disease in the particular human being or particular patient.

The disease is going to be reached to such a level that it is actually non-treatable or it is difficult to treat. So, that is why the people have moved to the PCR based method because in the PCR method, what you have to do is, depending on the site of the infection, for example, if it is a liver

or, sputum or blood, you have to draw that particular type of human tissue. And then you have to just simply, make a homogenate.

And then you have to take a small, few microliter of that particular sample. And that actually is good enough with the help of the PCR, which is going to be specific for these infectious organisms, which means like you are going to have a set of primers, which are actually going to detect the HIV or the you are going to have a set of primer which is actually going to be directed of the some of the classical proteins, what is presenting the mycobacterium tuberculosis.

And that is good enough to detect the mycobacterium tuberculosis because it is at the end you are going to see a band, which is going to be specific for the mycobacterium tuberculosis. And that is how with the help of the PCR people have cut down the detection process. So, that is how the detection is going to be much faster compared to the antibody based method.

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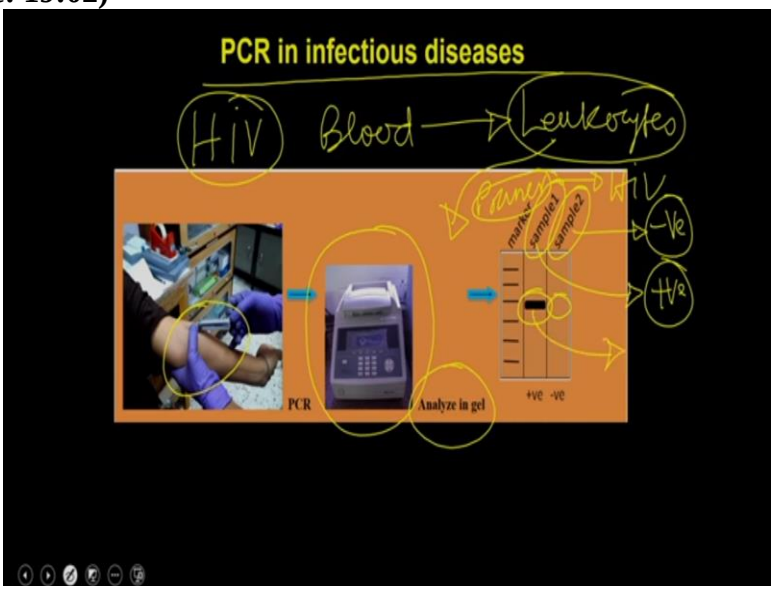

So, in a typical PCR infectious disease method, for example, this is a classic example of HIV. So, what we are showing is that what you have to do is you have to just draw 5 to 10 ml of blood. And then from the blood what you are going to get is what you have to do is you have to first you have to isolate the leukocytes, and from the leukocytes, you can be able to see directly performed the PCR reactions with the help of the primers what is being directed against the HIV.

So, you can use some of the Bessel metabolic pathway in enzymes what is presenting the HIV like either the RNA polymerase or some of the code proteins and that actually is going to allow you to amplifications so when you run it into the PCR machine and then if you analyze that result into the PCR gel, what you are going to see is that we are running 2 samples of sample 1 sample 2, and the sample 1 is going to give you an amplified DNA which is corresponding to the size of the DNA.

What has been reported for at that particular gene in the from the HIV viruses, whereas, the sample 2 does not giving you that particular amplified product, which means, the sample 2 is negative for HIV whereas, the sample 1 is positive for HIV which means, that this sample to the patient of sample 2 is positive for that particular disease. So, this is just an example and the schematic diagram to show that what is the protocol you have to follow for example, in the case of mycobacterium tuberculosis, it is even going to be very simple.

Because then you what you have to do is you have to simply collect the sputum of that particular patient and from the sputum you can be able to just recover the bacteria or you can just simply use the sputum as such, and then you can just perform the PCR with the help of the primers and that is good enough to give you the amplified product if the some amount of bacteria is present. So, even view few cells of the bacteria is actually good enough to give you an amplified product.

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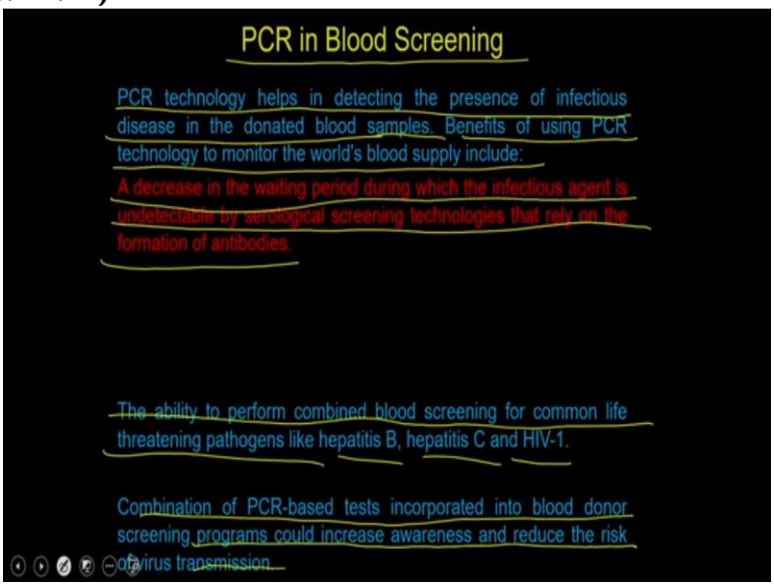

So, PCR is also been used in the blood screening. So, the PCR technology helps in detecting the presence of infectious disease in the donated blood samples. Benefit of using the PCR technology is to monitor include a decrease in the waiting period during which the infectious agent is undetectable by the serological screening technology that relies on the formation of the antibodies. The ability to perform combined blood screening for common life threatening patient and unlike the hepatitis C and HIV

Combination of the PCR based test incorporate into the blood during screen import, increase the awareness and reduce the contamination of the virus transmissions. So, what happens is when you are actually going to give the blood for donations, that blood could have potentially could have the infectious organism. For example, you could, so the donor could be infected with the HIV or donor could be infected with the hepatitis.

So, there are set of infectious organisms, which are already been listed that before you, collect the blood from a donor and before you give this blood to acceptor, you have to perform the detection of the infectious organism. So in that you have to perform the test for the HIV you have to perform the test for the hepatitis and all that, because these are the organism which actually can transmit from one person to another person, if you do not detect that.

So, the PCR is very advantages because you can just simply take a small, few microliter of the blood, and then you can be able to do a PCR and it is actually going to allow you to detect the HIV as well as the hepatitis and all other kinds of infectious organism. The other advantage is that compared to the other technique, like serological techniques, you might have to perform the difference serological reactions like different reactions for detecting the HIV.

Different reactions for detecting the hepatitis and different reaction for detecting the mycobacterium tuberculosis whereas in the case of PCR, you can be able to mix all the primers together and you can be able to detect all these infectious organism in a single goal considering that the size of the DNA as well as the similarity of the sequences may not exist. So you can be able to do a single step and detection of all the by infectious organism what is present in the blood of a donor.

And that is how you can be able to very safely, get the blood from the donor person and you can be able to use that blood with a lot of assurance that it will not going to transmit any kind of disease to the acceptors.

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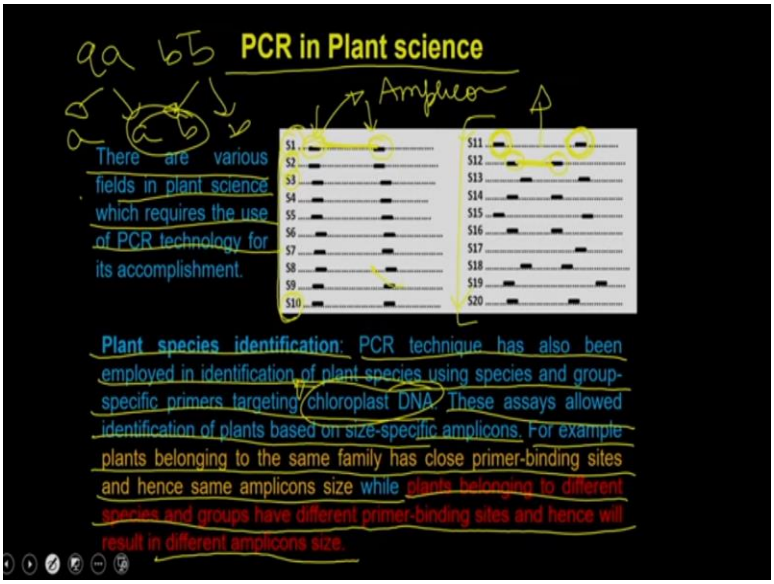

Then the PCR can be used in the plant science so there are various fields or aspects in which of the plant science in which you can use the PCR for PCR technology, one of the major is that you can be able to use the PCR for the plant species identifications so the PCR technique has also been employed in the identification of the plant species using the species and the group specific primers targeting the chloroplast DNA member.

That we are talking about the chloroplast DNA not the genomic DNA because the chloroplast DNA is something what continued from the family to family because as far as the plant is concerned. so genomic DNA is keep altering with every recombination because that if the plant is having a male and female, the male has its own genome the female has its own genome and during the recombination when they are actually going to form the pollen grain as well as the ovaries.

The genome is actually going to go through with the process of meiosis and that is how it is going to be split. So, for example, if you have to say 2 pairs of elisa, then for example, A A and

B B, it is actually going to split. So, it is going to split into A and A like this. So, if you go with the genomic DNA things, you are actually going to see that there will be a contamination where as if you go with the chloroplasts DNA, it is going to be remained constant, because the chloroplast in the plant system also the chloroplast comes from the female side.

So, that actually continued over the multiple generations and it does not get affected if whether the things are coming from the male side or things are coming from the female side. These assays allows the identification of plant based on the size the specific amplicons for example, the plants belonging to the same family has a close primer binding site and hence, they are going to give you the same size of amplicons.

So, this is for example, in all these S 1 to S 10 are actually belonging to the same family and what you see is that the primers are in the genome order in the chloroplast DNA, the primers are binding to a same location and that is how the resulting amplicons are going to be of same size, because it is actually going to give you an amplified DNA of this size, so all the S 1, S 2, S 3 and all that S10 are actually belonging to a same family.

But if you are expecting that, they are not going to belong to the same family, what will happen is that primers are going to bind to the multiple different places. So, as you can see from the S 11 to S 20 all these plants are belonging to different families. And since you are using the primers only for the families, what you see is this primer is binding here and this primer is binding here this means this is the amplified product what you are going to get.

Whereas for the S 12 this primer is binding here and this primer is binding here, which means this is the Amplified product what you are going to get so if the primers will bind to a different location, it is actually going to give you the different size of amplicons. And that is how it is actually going to indicate that these plants species or these plants are belonging to different families, because every family is going to have a signature sequence which you are probing with the help of these primers.

But if they are belonging to a different family, this the locations of these primers are going to be different because that is how the diversity is being present when different families. So, if the plant belonging to a different species and grow groups have been different primer binding site hence will result in a different amplicons size.

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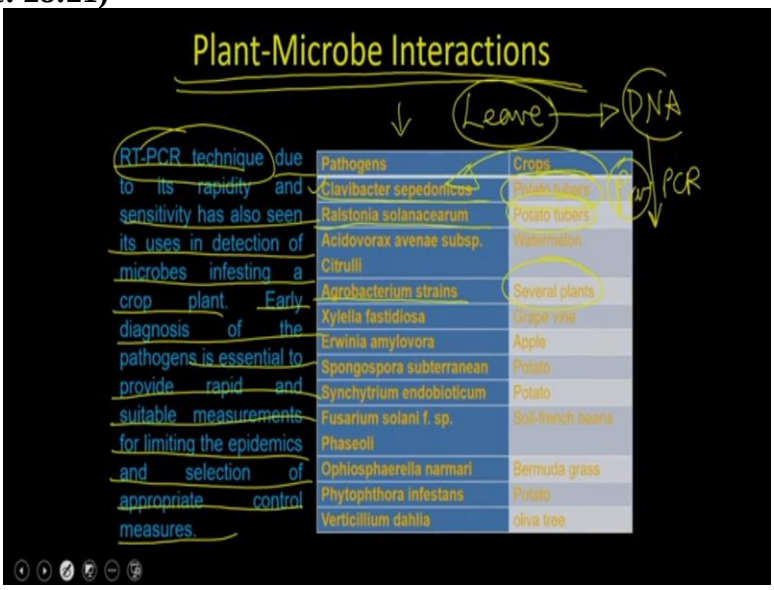

Apart from that, you can be able to also study the plant microbe interactions so the RT PCR technique which is a derived technique from the PCR due to its sensitivity has also been used in the detection of microbes, infesting a crop plant. Early diagnosis of the pathogens is essential to provide rapid and suitable measurements for limiting the epidemics and thus selection of the appropriate control measures. So, these are the few lists which can which can be used.

For example, in the case of the Clavibacter sepedonicus which actually causes the which actually affects the potato tubers, then you have Ralstonia, which actually causes the effect in the potato tubers, then you have the Agrobacterium strains, which actually affects the different types of plants and mechanism remains the same that when you are interested to detect these infectious organisms or microbes.

What do you have to do is you have to just take the plant that part for example, you can simply take a plant leaf and you can just, crush this leaf and you can be able to isolate the DNA. And then what you can do is you can just do a PCR with the help of the primers which are belonging to this particular infectious organism. And that is how it is actually going to give you if it is

going to give you an amplified product. Which means that particular infectious organism is associated with the leaves.

So, depending on that site of infestations and depending of the site of infections, you can be able to isolate that particular plant part for example, in some cases, it may not be leaf it could be stem or the root. So, that particular organ of the plant has to be isolated and then from that organ you can just simply isolate the DNA and then you can just perform the PCR or the RT PCR.

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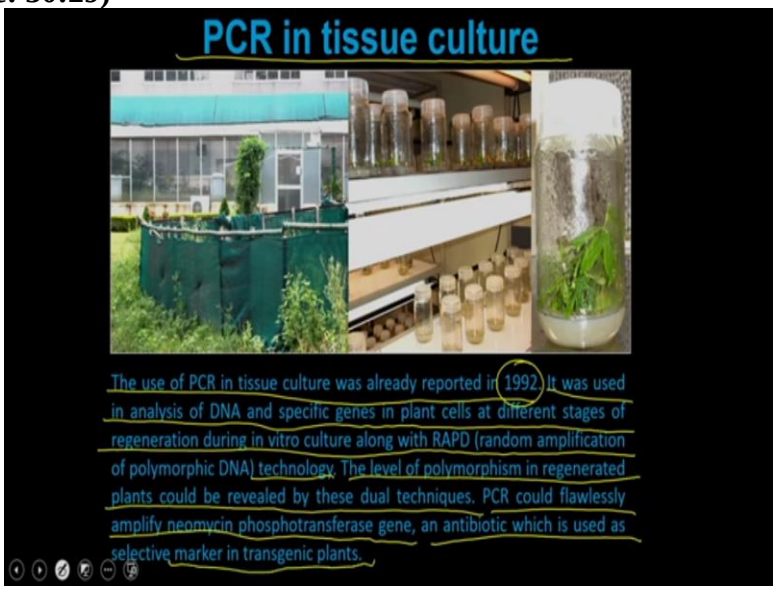

PCR can be used even in the tissue culture because the in the tissue culture when you are starting with a with a single cell or with a small plant part and then eventually you are generating a differentiated mass of cells, which is called as the callus and then from the callus you are actually putting the differentiating hormones, so, you are actually generating the callus to shoot and the root. So, all these events are associated with the up regulation and the down regulation of the different types of genes.

And that expression as well as the pattern can be also studied with the help of the PCR and that how you can be able to study with the help of the PCR at what stage your plant is present because how you will know that the plant has formed the callus or the plant has formed the shoots and roots. So, all these events can be even categorized or characterized with the help of the PCR as well.

So, the use of PCR in tissue culture was already reported in the case of 1992 it was used in the analysis of DNA and the specific genes in the plant cell at a different stage of the regeneration during in vitro culture along with the RAPD technology. The level of the polymorphism in the regenerated plant could be revealed by these dual techniques, PCR code flawlessly amplified neomycin phosphotransferase gene and antibiotics which is used as a selection marker in the transgenic plant.

So, apart from the looking at the different stages, you can also be able to see, whether the plant has taken up the foreign gene and it is been integrated into the genome or not whether the plant has been converted into a transgenic species or not, and whether the transgenic species is stable or unstable, because once you remove the selection pressure, whether the genes remains within the plant or not or whether it gets excluded from the plant.

So, that all that kind of questions can be asked simply by using the PCR because in most of the transgenic species, you are going to use some of the marker enzymes as a selection pressure. So, those genes for those selection pressures can be used to detect whether that particular gene is associated with the plants or not.

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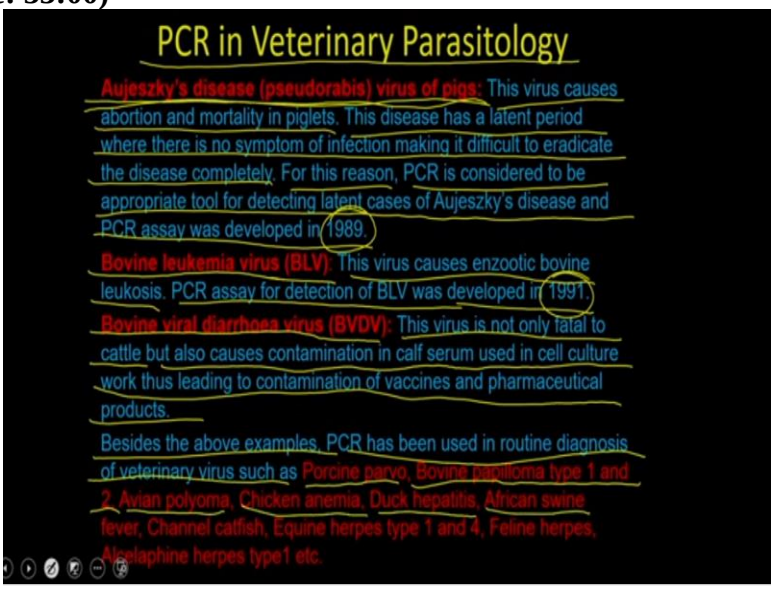

And, apart from that, the PCR is also been used in the veterinary parasitology. So, when the veterinary pathology you can be able to detect the for example, this particular disease virus in the pig so, this virus causes the abortion and the mortality in piglets. Disease has a latent period

where there is no symptom of infection making it difficult to eradicate the disease completely. For this reason, the PCR is considered to be the appropriate tool for detecting the latent case of this disease.

And the PCR assay was developed in the case of 1989. Then you have the Bovine leukemia virus. This virus causes the enzootic Bovine leukosis. The PCR assay for detection of the BLV was developed in the case in the year of 1991. And then you have the bovine viral diarrhoea virus. So this virus is not only fatal to the cattle, but also causes contamination in calf serum used in the cell culture work, thus leading to the contamination of vaccines and the pharmaceutical products.

So besides the above example, the PCR has been used routinely in the diagnosis of the veterinary viruses such as the porcine parvo, Bovine papilloma type 1 and type 2, Avian Polyoma, Chicken anemia, Duck hepatitis and so on, like the swine flu viruses and all that kind of thing. So, PCR can be used even to detect some of the infectious organism what has been affecting the veterinary or animals and the dogs and all that kind of thing.

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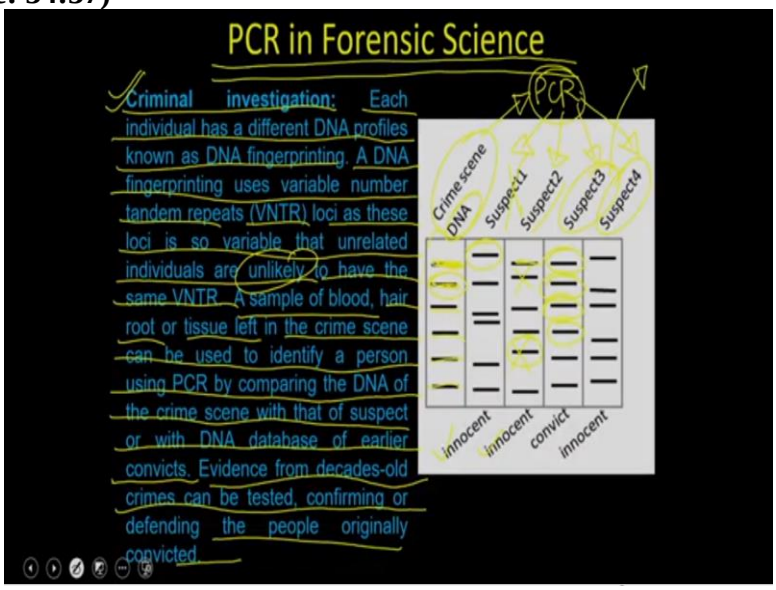

PCR can be used in the forensic science. So in the forensic science, the PCR has been used in 2 aspects one is the criminal investigations. So in the criminal investigations, what you are going to do is the each individual has a different set of DNA profile, known as the DNA fingerprinting.

a DNA fingerprinting uses variable number of tandem repeat which is called as the VNTR loci as these loci is so vulnerable that the unrelated individuals are unlikely to have the same VNTR.

A sample of blood, hair, root or tissue left in the crime scene can be used to identify a person using PCR by comparing the DNA of the crime scene with that of the suspect or with the DNA database or the earlier convicts, evidence from the decade old crime can be tested confirming or depending on the people originally convicted. So, in the PCR, PCR can be used even in the forensic science in 2 aspects.

One aspect is it can be used for criminal investigations. So that every human being has a particular set of DNA repeats, and these repeats are peculiar to that particular person, and it cannot be matched with the next person. So that can be used and can be explored with the help of the PCR. So if you use the primers to detect or to amplify these marker sequences, it is actually going to give you the whether that particular suspect is a criminal or not.

Because what you are going to do is you are going to isolate the tissue, you can be able to isolate the blood, or you can be able to isolate the hair and all that kind of biological samples from the crime scene, and then you can be able to detect the DNA, and then you can just simply do a PCR. And once you do the PCR, it is actually going to give you the particular pattern of the DNA, and then you can do a simple PCR for the suspected person like 1 and 2 and all that.

And then you do the exactly the same that you are going to run all these on agarose gel, and what you can see in this case is that all these suspects, you are what you have to do is you have to do image analysis, and then you have to do a side by side comparison. So, this band is present here, but it is absent here. So this suspect 1 is innocent actually. So then suspect 2 is having this band, so this band is present, but it is not having the band number 2, so the band number 2 is absent.

Whereas the band number, some of the other bands are also these bands also not present. So this guy is also innocent, this guy first guy is also innocent. And the third guy is actually having the similar pattern of the DNA, what it is been present from the DNA, what is been isolated from the

crime site. So that is why the suspect number 3 is actually a convicted person, it may be having a role in that particular type of crime.

And you can suspect or you can actually do some more background studies, and you can be able to do some more additional evidences, and you can collect some more evidences to confirm whether this person is involved in that particular crime or not. But what surely this analysis is going to say that this person was present at the size of the crime. Similarly, for the suspect number 4 also some of the padding DNA patterns are not matching.

So this guy is also an innocent guy, he was not present at the site of the action. Apart from that, if you also want to do what people also do, or what the police is also doing is it is actually having this kind of pattern of all these people like the criminal people, what they are doing is they are generating a database of these criminal peoples, they are generating a DNA library. And that is how once they got a DNA, and once they got a pattern, what they can do is simply take this pattern and blast into their database.

And then it will say, it is matching with these 4 people. And that is how it is actually going to say that these are the potential criminals possibly involved in this particular type of action. And that is how they can be able to pinpoint, cash those guys and do the photo investigations.

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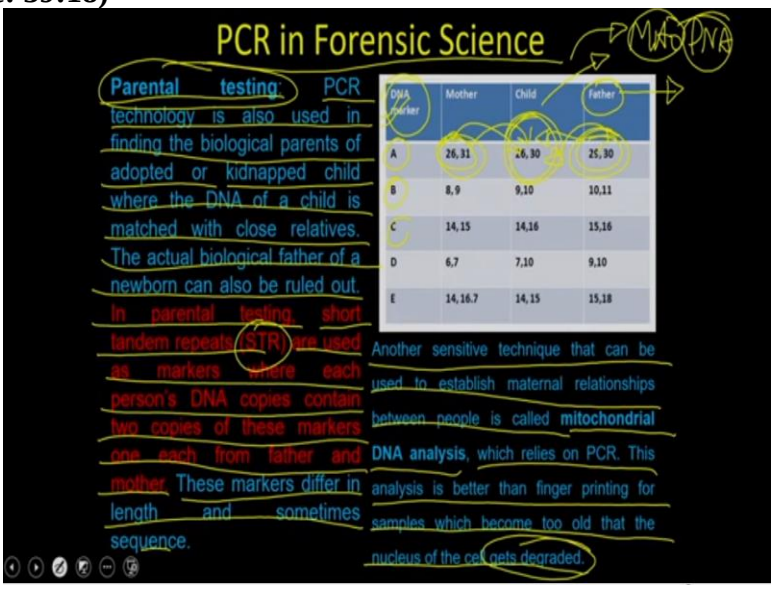

The second aspect where the PCR is being used in the forensic science is the parental testing. So in the parental testing, what happened is, so sometime when the kids are being kidnapped or killed, intervene, there will be a mismatch of the kids, even in the hospital as well. Then there will be always a question of who are going to be the parent of this particular kid. So in those kinds of parental disputes, the people are also going with the DNA based assays.

So mostly what they are doing is they are simply going with the PCR and then matching the PCR results with the parents like the mother and father as well as the other persons. And so, the PCR technology is also being used in the finding the biological parents have adopted or the kidnapped child where the DNA of a child is matched with the close relatives. The actual biological father of a new bond can also be ruled out.

In parental testing, the short tandem repeats or the STRs are used as a marker where each persons DNA copies contain 2 copies of these markers, and one each from the father as well as the mother. These markers differ in length and sometime in the sequence as well. So, what you can see is that you have the different types of DNA markers like A, B, C, D, and E, and all these markers are present in the mother as well as in the father,

But a combination is going to be present into the child for example; in this case, what you see is for the DNA marker A the 26 is present from the mother and 30 is present from the father, which means the child is going to be a hybrid of the father and mother. So, that is why it will although mother is having 26 and 31, the father is having 29 and 30, the children is going to have the 26 and 30. So, same is this kind of analysis is going to allow identifying the father.

So, imagine that you have the multiple candidates who are claiming that they could be a father of this particular child. So then what you can do is you can simply do analysis of the multiple candidates with the help of the different types of DNA markers, and then you can check whether that is matching or with the child or not. So, another sensitive technique that can be used to establish the maternal relationship between people is called as the mitochondrial DNA analysis.

Which relies on the PCR this analysis is better than the fingerprinting for sample which becomes too old, that the nucleus of the cells get degraded. So apart from these people are also using the mitochondrial DNA because as I said in the past, also the mitochondrial DNA is something which remained conserved throughout the family. So by because the mitochondrial DNA is what people are having, whether it is a male or the female is always coming from the mothers side rather than a father side.

So if you analyze the mitochondrial DNA, which is not going to be diluted because all the genomic DNA is going to be diluted, in every off springs, like after every generation, you are going to have the half from your father and half from your mother. And but that mitochondrial DNA is going to be remain intact. So if you follow the mitochondrial DNA very precisely, you can be able to collect the pedigree of that particular family and you can be able to even identify the father as well as the mothers.

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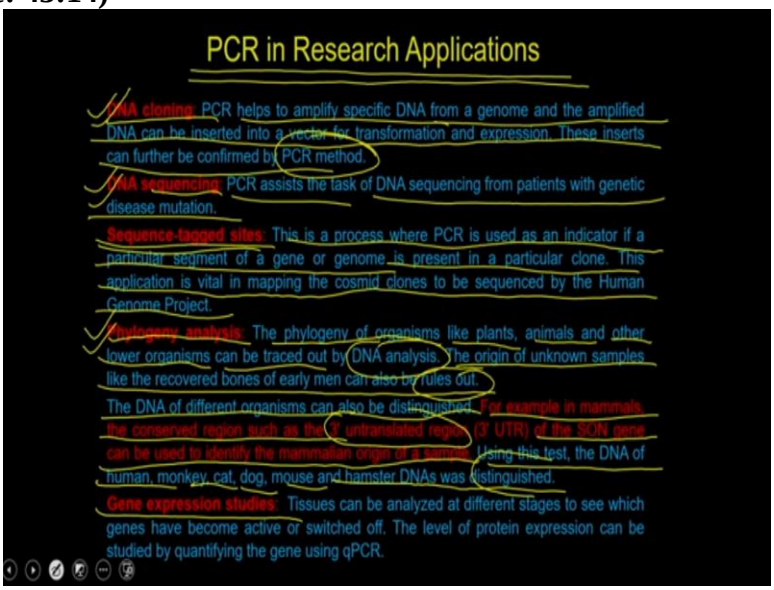

Then the PCR is also been used very successfully in the case of the research applications. So PCR is being used in the case of DNA cloning. So, PCR is being used in the DNA sequencing, and then you are also being used in the sequence based sites. It is also being used in the phylogenetic analysis, and it is also been used in the gene expression studies. So in the case of DNA cloning, the PCR helps to amplify the specific DNA from a genome.

And the amplified DNA can be inserted into a vector for transformation and the expression this insert can further be confirmed by the PCR methods, and then DNA sequencing the PCR assess the task for DNA sequencing from the patient with the genetic disease mutations, then the sequence tax sites. This is a process where the PCR is used and an indicator if a particular segment of a gene or genome is present in a particular clone.

This application is vital in mapping the cosmic clones to be sequenced by the human genome projects. Then it also been used in the phylogeny analysis. So the phylogeny of organisms like the plant, animals and other lower organisms can be traced out by the DNA analysis. The origin of unknown sample like the recovered bone of early man can also be ruled out. The DNA of different organism can also be distinguished.

For example, in the case of mammals, the conserved region such as the 3 prime UTR of the Son gene can be used to identify the mammalian origin of a sample using this test the DNA of the human, monkey cat, goat mouse and the hamster DNA was distinguished. In the Gene Expression studies also the PCR can be used to monitor the expression of that particular gene.

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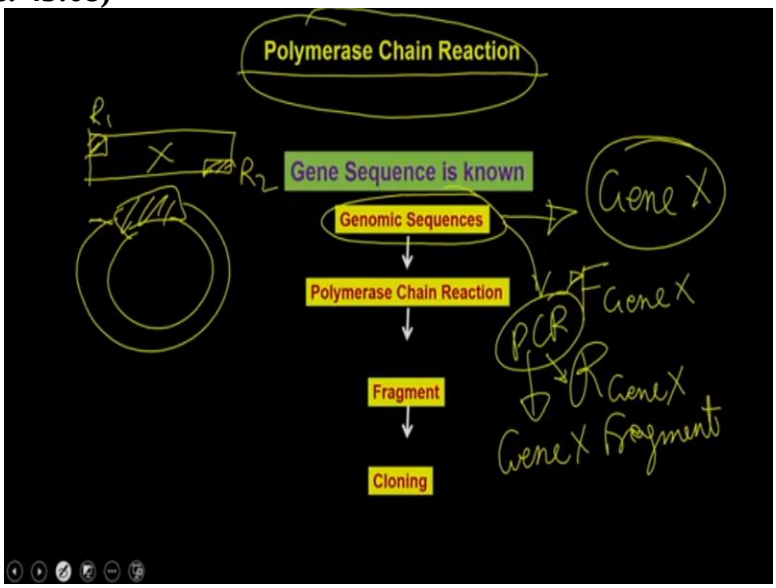

So, in a simple process, the PCR can be used for cloning reactions. So, what you are going to do is you are just going to take the genome sequence based on the genome sequence, suppose you are interested to isolate the gene x, what you are going to use is you are going to generate a

forward primer, which is for the gene x, and then you are actually going to have the reverse primer which is for the gene x.

And then you are going to isolate the genome and from the genome you are going to set up a PCR with the help of the primer as well as the reverse primer and ultimately what you are going to get, you are going to get the gene x fragment and this gene x fragment is going to have these restriction sites on the both sides which means, this gene fragment of the gene x is going to have the restriction sites on both the ends and these restriction sites can be used to integrate these gene x into a vector of your choice.

So, you can digest the vector with the help of these set frames that are enzyme like for example, R 1 and R 2, and you can be able to use that to integrate that into the your plasmids and then you can use this plasmid for downstream applications like you can use it for expression analyses or you can use it for the some other questions like whether you are interested to monitor the replications and transcriptions and translations.

And so, you can do some basic research as well and you can do some application oriented work as well. So, this is all about the application of the PCR in different fields of the science. So, we started with the plant science and then we end up with a forensic science as well as the research applications. So with this, I would like to conclude my lecture here. Thank you.