Basics of Biology
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Lecture 26
Polymerase Chain Reaction

Hello, everyone. This is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering, IIT, Guwahati. And what we were discussing? We were discussing about the living organism. And in that context, in this particular module, we were discussing about the central dogma of life or the central dogma of molecular biology.

So if you recall, in the previous lecture, we were discussing about the DNA dependent DNA synthesis, where we have discussed about the different steps of the replications, we have discussed about the initiation, elongation and terminations. And then we also discussed very briefly about the DNA repair as well as the error detections by the DNA polymerases.

And in this context, as you can see that the replication is actually allowing you to synthesize the DNA dependent DNA synthesis, which means, it can actually be having a very huge replication in terms of developing a technique which is called as the polymerase chain reactions. So the technology this which is based on the development of the application is called as polymerase chain reaction or the PCR.

And this particular technique is allowing the amplification of a particular stretch of DNA. And it has a very wide replications in different types of fields. So let us, in today's lecture, we are going to discuss about the polymerase chain reactions and how you can be able to utilize that technique for detection of the different types of things in the different fields. So let us start discussing about the polymerase chain reactions.

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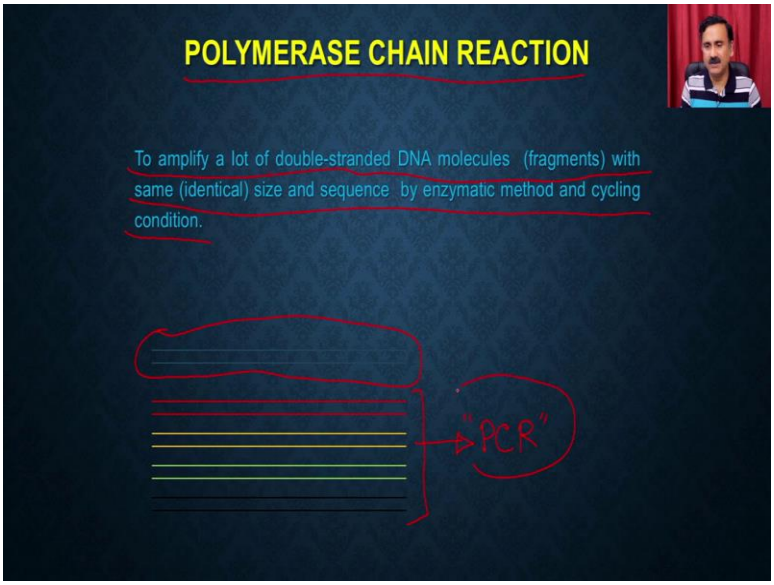

So the polymerase chain reaction as the name suggests, is actually a amplification of the double-stranded DNA. So, to amplify a lot of double stranded molecule or fragment with the same identical sign and sequence by the enzymatic method and under these cyclic conditions. So you can imagine that if this is the template DNA what you have, you can actually be able to synthesize the many copies of this particular DNA with the help of the PCR.

But before discussing about the PCR, we have to discuss about and how this particular technique is being evolved over the course of time, let us discuss first about the piece, the, the replications and then we understand the different components which are actually going to be replaced by the different types of enzymes.

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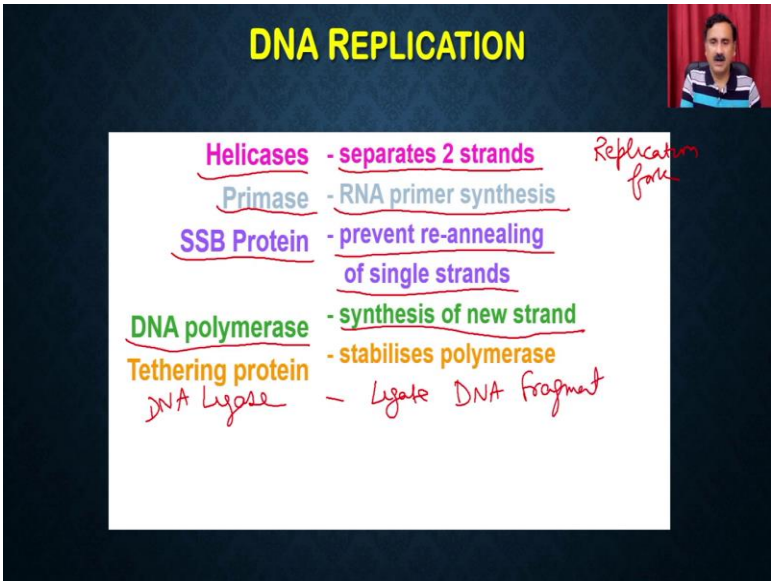

So as we discussed in the previous lecture, we discussed about the role of helicases. So what is the role of helicases, it is actually separates the two strands and that is how it is actually going to make the replication fork. Then you have the primase which is actually going to have the RNA, DNA synthesis of the primers, then you have the single stranded DNA protein, which is actually going to prevent the re-annealing of the single strands.

Then we have the DNA polymerase, and that is actually going to do the synthesis of the new strands and then you also going to have the DNA ligase which is actually going to ligate the DNA fragment. So it is actually going to ligate the DNA fragments. Now, if I want to, So this is what all these molecules are present under the very precise quantities within the cell and that is how these molecules can come and do the DNA synthesis in a very precise manner.

But if I want to do this reaction in an append off or in a test tube, I have to replicate or I have to remove many of these factors.

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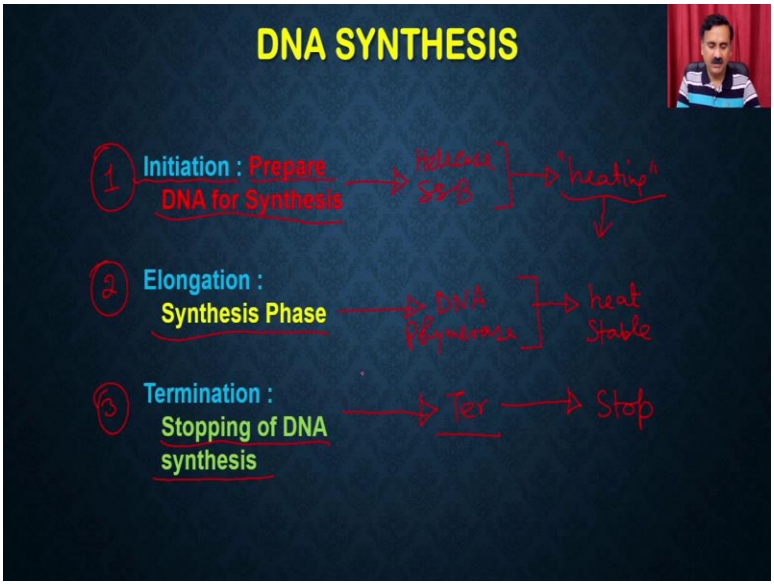

So what we have to do is we have to first do the initiation, where we are going to do the, we are going to do the preparation of the DNA for the synthesis. Under the normal replication this has been done by the helicase, and it has also been done by the single stranded DNA protein, whereas in the case of the primer, the PCR, you actually can do this by the heating.

Remember, when we were discussing about the structure of the DNA, we said that if you heat the DNA, the strands are actually going to get separated from each other. So that is why you actually can do the initiation step simply by heating the DNA for a very high temperature. Then you can also do the elongation.

So elongation, you can have the synthesis phase, which is actually going to be done by the DNA polymerase. DNA polymerase what is present in the, most of the cells are not the thermostable, it is going to be heated labile, whereas in our case, we can actually use the DNA polymerase which is going to be heat since a heat stable. So it actually can withstand this particular high temperature.

And then you can also have the terminations. So the termination is the stopping of synthesis of the DNA. And that is always been done by the Ter sequences in the case of the cell, but that is not required in the case of PCR, because once the DNA machinery

synthesis will reach to the end of the DNA, it is going to be stopped. So, let us see what are the different events happen and through which the PCR is being evolved as a very, very robust technique to do the different types of, to do the DNA synthesis.

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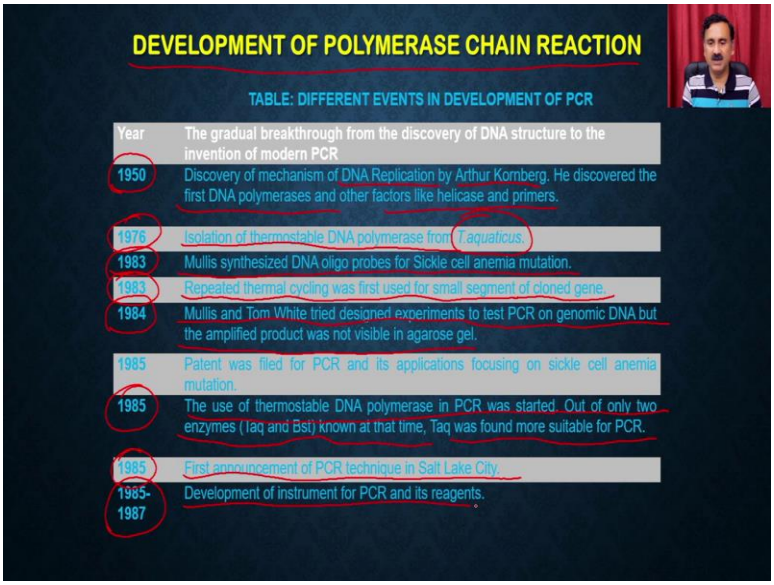

So if you see the deployment of the polymerase chain reaction, so what you will see here is that it started in the year of 1950, where the people have discovered that how the DNA replication is being done by the DNA polymerases, and this has been done by a scientist which is known as the Arthur Kornberg, and he is actually the first scientists who discovered the DNA polymerases and other factors like helicases in primers.

Then the 1976 that we will have isolated the thermostable DNA polymerases from the thermos aquaticus, and that had actually revolutionized the whole field of the PCR, because then you can not be dependent on the rich applying the enzyme again. Then in the era of 1983, the Kary Mullis, actually synthesizes the DNA oligo probes for Sickle cell anemia mutations.

And in the same year, in the 1983, he actually did the, he has done the repeated thermal cycling, and that was used to clone a small fragment of the gene actually. And then in the year of 1984, the Kary Mullis and Tom White tried the design experiment to test the PCR on the genomic DNA and the amplified was not visible in the agarose gel. And then by

the year of the 1985, you can actually have the first announcement of the PCR technique by the salt, in the Salt Lake City actually.

And in the same year, the year 1985, they have actually been able to use of the thermal stable DNA polymerases and PCR was started out of only two enzymes like the Taq and Bst known as that time. So Taq was found more stable for the PCR reaction. And then in the year of 1985 to ’87 people have developed the instrument for the PCR and different types of reagents.

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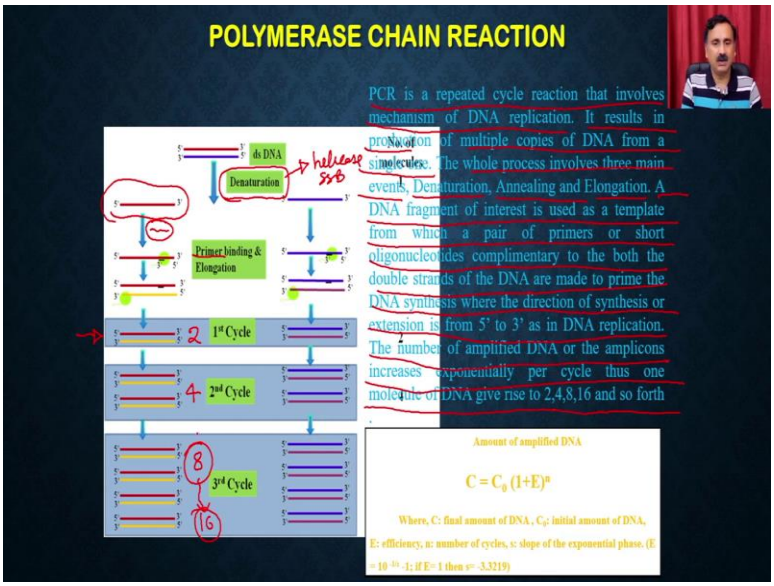

So what is PCR? PCR is polymerase chain reaction. So if you understand, if you want to understand the PCR, it is actually very simple. So PCR is a repeated cycling reaction that involves the mechanism of the DNA replication. It results in the, it results in the production of the multiple copies of DNA from a single chain. So the whole process involves the three main events, the Denaturation, Annealing and Elongations.

DNA fragment of the interest is used as a template from which a pair of primers or the short oligonucleotide complementary to the both of the DNA strands of the DNA are made to prime the DNA synthesis where the direction of the synthesis is 5 prime to 3 prime in the case of DNA replication.

The number of amplified DNA or the amplicons increase exponentially per cycle, that is one molecule of DNA give rise to 2, 4, 8, 6 and 16, so far. So what you happen means you have double stranded DNA, when the first step you are going to do the denaturation, which means you are actually going to mimic the, the reaction, the action what is being done by the helicases as well as the single stranded DNA binding protein, and you are actually going to have the single stranded DNA.

On this single stranded DNA you are actually going to add the small stretch of primers. So in the in the case of primase, so in the case of DNA replication, you are actually adding the primase, whereas in this case you are simply adding a small oligonucleotide. So, that small oligonucleotide is actually going to be called as primer and the primer is actually going to go and bind to the corresponding complementary strand, complementary sequence of that particular DNA.

And then once it binds, then it actually allows the template, allows the DNA polymerase to go and sit onto this DNA. And it is actually going to synthesize the new strand. Same is true for the other strand also. And the same way, so what you see here is that after the first cycle, you are actually going to, if you start from the one copy, you are going to have the two copies.

And after the second cycle, you are going to have the four copies. And after the third cycle, you are actually going to have the eight copies, and so on, so if you are going to have the 16 copies. So that is why the DNA replication is going to be the exponential, so it will going to double after every cycle. So that is why it is going to give you the huge quantity of the DNA.

And the strands, wherever the primer is going to bind, it is actually going to amplify only that particular region of the DNA. And that is how it is actually going to give you the specificity of the reaction's flow.

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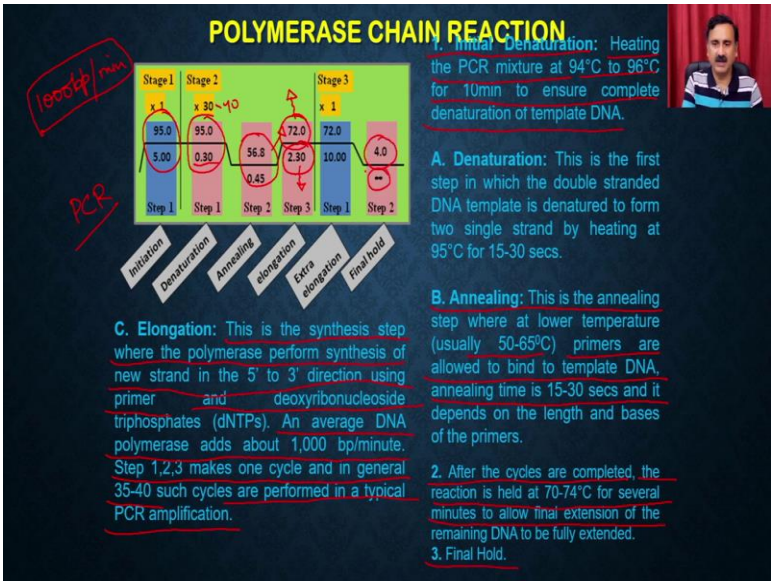

So if you want to perform the polymerase chain reactions, you have to set up the reactions into a PCR machines. So what you have to do is you have to do the first denaturation, so is the first you are going to have the initial denaturation. So where you are going to hit the PCR mixture at 94 to 96 degrees Celsius for 10 minutes to ensure the complete denaturation of the template DNA.

So in the initial 95 degrees Celsius for five minutes, that is going to give you the initial denaturation. And then you are going to enter into the stage to, where you are going to first have denaturation. So you are going to have the denaturation, annealing, and as well as the elongations. So in the denaturation, again, you are going to have the denaturation of the templates, for the 30 seconds.

And then we are going to have the annealing steps. So in the annealing step, you are going to have the, you are going to lower down the temperature. So when you lower down the temperature, the, the primer is actually going to bind to the template. So once you lower down the temperature, like 50 to 65 Celsius, the primers are allowed to bind to the template DNA. And that is all going to anneal for some time.

So once the primer is going to anneal, then they will enter into the elongation step. So you are going to increase the temperature and you will bring it to 72 degrees Celsius so

that you are actually having an enzyme working as an optimal degree. And then you are going to do the elongation for 2 minutes 30 seconds.

That elongations steps depends, the timing for the longest step depends on the, how big, how big your gene is, actually. So if, as I think you remember so, it is actually going to synthesize the 100 base pair per minute. So if you actually do the math, you can actually be able to put the timing for the elongations.

So this is the synthesis step where the polymerase perform the synthesis of the new strand in the 5 prime to 3 prime directions using the primers and the dNTPs. And average DNA polymerase adds approximately 1,000 base pair per minute. So Step 1, 2, 3 can make one cycle. And in general, 35 to 40 such cycles are performed in a typical PCR amplification.

So you are actually going to have the Step 1, 2 and 3, you are going to perform this in a cyclic event. And that is how you are going to do either 30 or 40 times. And that is how you are going to have the cycles. And that is going to give you a very high amplification of that particular DNA.

After the cycles are complete, the reaction is held at 70 to 74 degrees Celsius for a few minutes so that there will be a final extension. And it is going to give you the completion of the, all the DNA fragments. And then you can have the final holds, that final hold could be at 4 degrees for an infinite period of time.

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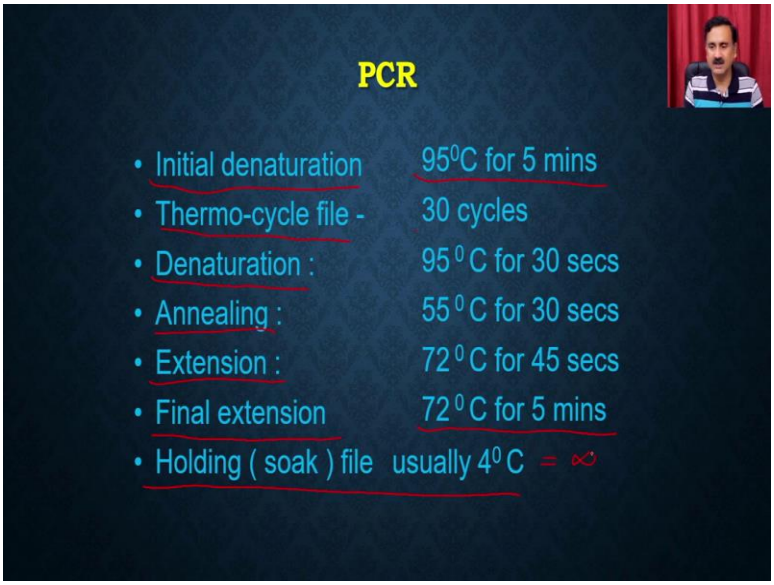

What are the different components are required? So you can have the, these are the PCR reaction. You can have the initial denaturation for 95 degrees Celsius, then you can have the thermal cycler for 30 cycles. So in the 30 cycle, what you are going to have is you are going to have denaturation, annealing and extension, and then you can also have the final extension which is 72 degrees Celsius for 5 minutes. And then you can have the holding of the reaction at 4 degrees for the infinite period of times.

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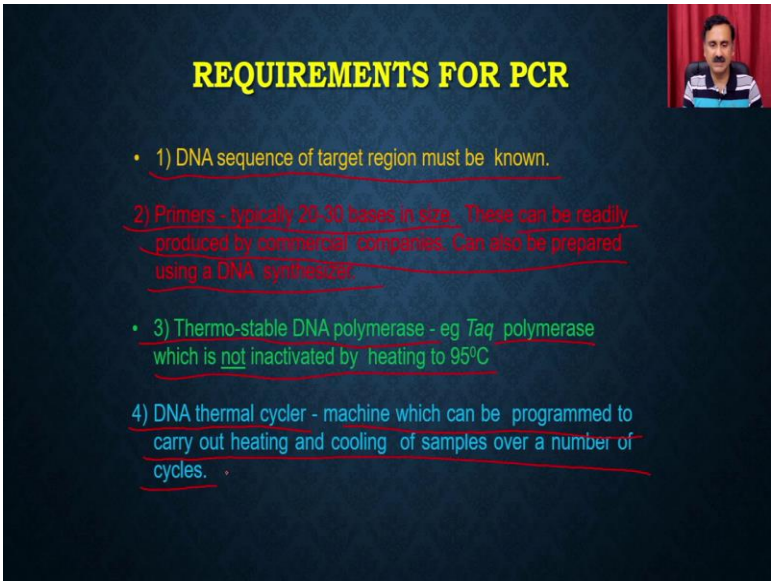

What are different requirement? So requirements for the PCR, you actually require the template DNA or the target DNA, then you require the primers which are actually going to be 20 to 30 base pair in size. And these can be readily produced by the commercial companies and can be prepared using a DNA synthesizer.

Then you also require the thermo-stable DNA polymerase like the Taq DNA polymerase, which is not heat inactivated by the heating at 95 degrees Celsius. And then you also require a thermal cycler, so the machine which can be programmed to carry out the heating and cooling of the sample over number of cycles.

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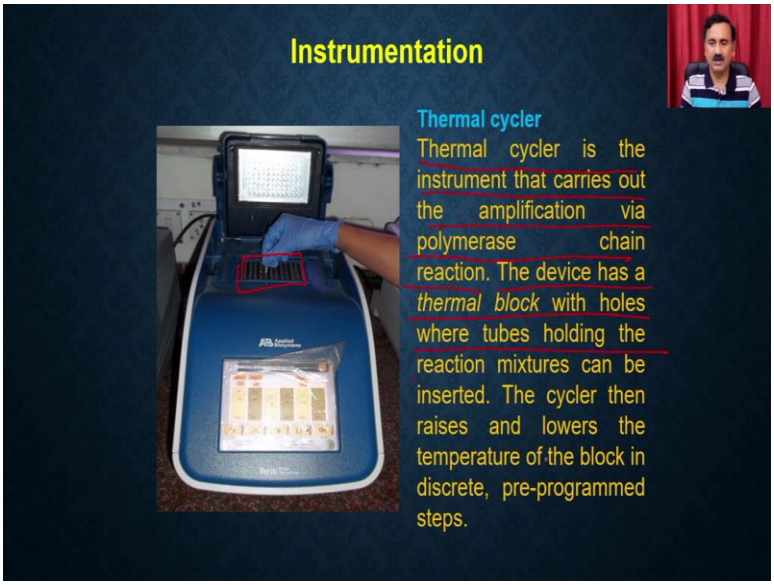

So this is what you are going to see, this is actually a thermal cycler, where you are going to have the, a block where you actually can be able to put the reactions, and a thermal cycler is an instrument that carries out the amplification by the polymerase chain reactions. This device has a thermal block with the hole where tubes holding the reaction mixtures and these cycles can raise and lower down the temperature of the block as per the programming.

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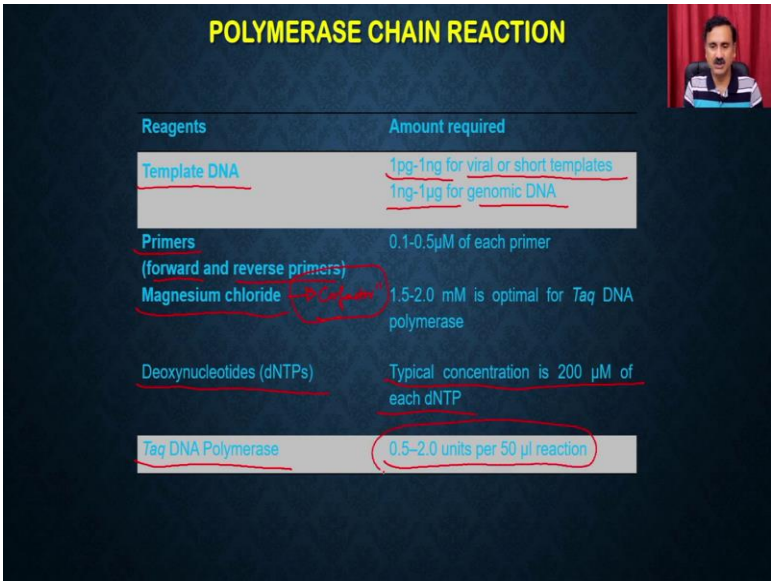

Then we can have the, different types of reagents what are required. So you can have the template DNA, so you can have the template DNA in the range of 1 Pico gram to 1 nano gram, if you are talking about the short stretch of DNA, or it could be 1 nano gram to 1 microgram, if you are talking about the genomic DNA. Then you require the forward primers. You can require the two primers; you require a forward primer as well as reverse primers.

Then you require the magnesium chloride. So magnesium chloride is actually a cofactor, which is responsible or which is required for the activity of the Taq DNA polymerase. And then you also require the deoxynucleotides or the dNTPs, which is required for the synthesis of the DNA. So, and the typical concentration of the dNTP is 200 micro molars, and then you also require the Taq DNA polymerase, which is from the 0.5 to 2 units per 50 micro liters reactions.

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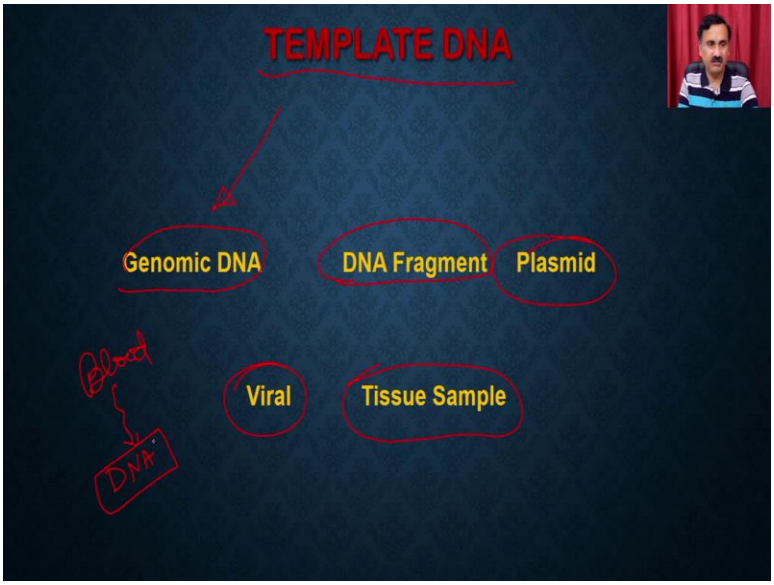

As far as the template DNA is fragment, the template DNA could be of different types. You can have the genomic DNA, you can have the DNA fragments, you can have the plasmids, you can have the viral DNA, you can have the tissue samples, and you can also have the template DNA isolated from the crime sites.

So for example, like you can have the blood from the crime site, and then in that, from the blood, you can actually be able to isolate the DNA and that can be used for the different types of activities to identify the criminals.

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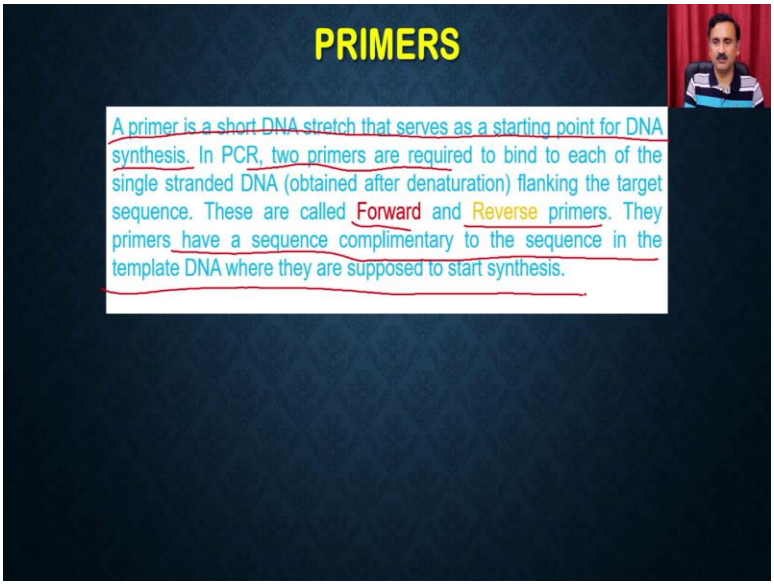

Then you can also have the primers. So primer is a short stretch of DNA that serves as a starting point for the DNA synthesis. In PCR, you can have the two different types of primers, you can have the forward primer and you can have the reverse primers. And these primers have a sequence complementary to the sequence in the template DNA where they are supposed to start the synthesis.

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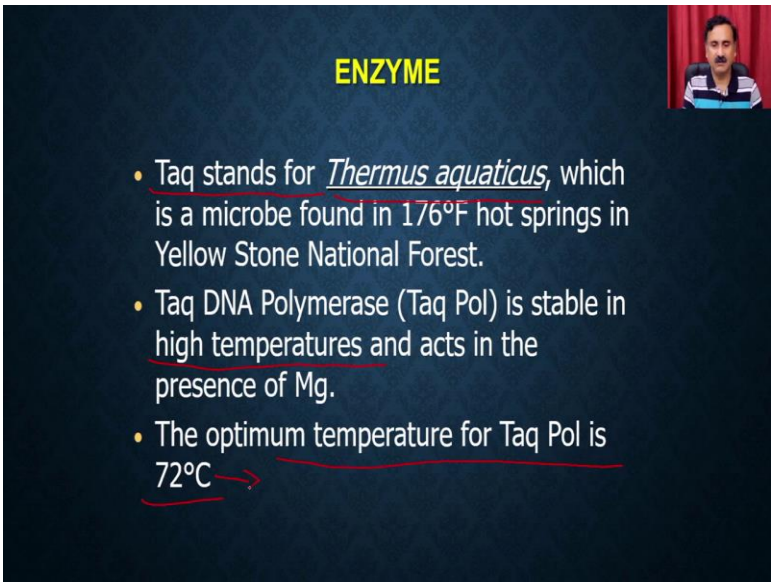

Then as far as the enzyme is concerned, the enzyme, you can have the Taq DNA polymerases. So Taq actually is a poly, is a, is an enzyme which has been isolated from the Thermus aquaticus, and which is, which is actually thermostable enzyme. So it is actually not going to destroy when you are going to have the denaturation steps and all that. And the optimal temperature for the Taq DNA polymerase is 72 degrees Celsius.

And that is why we are actually going to do the extension at 72 degrees Celsius. But Taq DNA polymerase has its own disadvantages. So it has an advantage that it is a thermostable enzyme, but there are some disadvantages.

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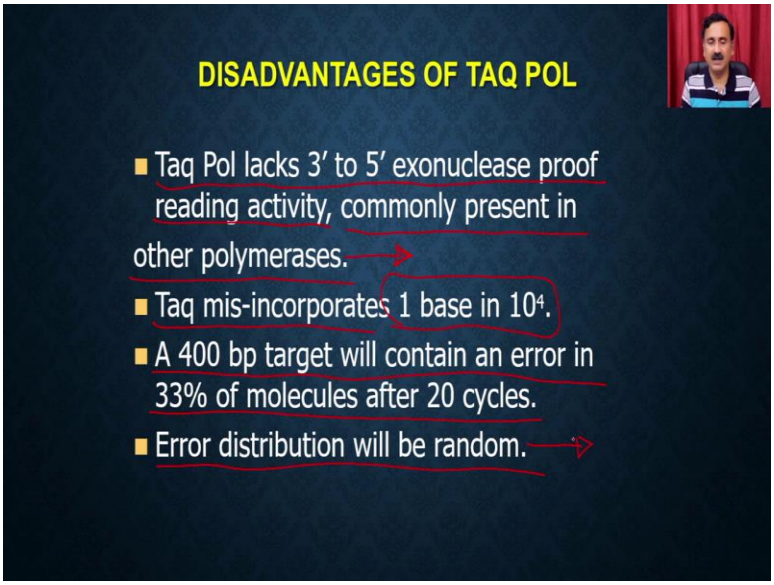

What are the different disadvantages that Tac DNA polymerase actually lacks the 3 prime to 5 prime exonuclease proofreading activities, which means it can actually do the synthesis of the DNA, but it cannot check whether the DNA synthesis what has been done is correct or not, so which is very problematic, because if it cannot check that errors, it actually can incorporate the different types of errors?

Then the Taq DNA polymerase mis-incorporate at least 1 base in the 10 to power 4 bases, which is actually going to generate the mutations into the amplified DNA. A 400 base pair target will contain an error in the 33 percent of the molecule after the cycle. So if you actually going to amplify a 400 base pair target, it is actually going to have the 33 percent

of those molecules after the 20 cycle, which are going to have the error one or other place.

And this error distribution is going to be random, which means it is actually going to give you a DNA which is actually going to be mutations, it is going to have the different types of mutations.

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So what is the advantage, what is the alternative? So alternative is that you can actually go with the Pfu DNA polymerase, which is from the Pyrococcus furiosus, and it actually processes the 3 prime to 5 prime exonuclease proofreading activity. The error rate is only 3.5 percent After 20 cycle and more amount of primer is needed to avoid the primer dimering. For unexplored genes, the primer used in closely related species are used.

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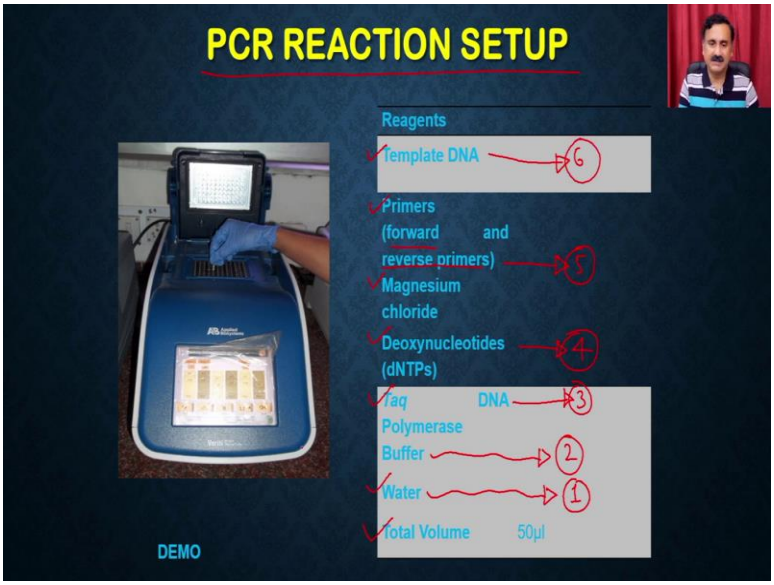

Now how you can be able to set up the PCR reactions. So you can actually require the different reagents, you require the template DNA, you require the primers like forward primers and reverse primers, you require the magnesium, you require the dNTPs, you require the Taq DNA polymerase, water and total molecules.

So what you are going to do is you are going to first take the water, so you are going to first add the water into the reactions. So as per the calculation, the water is going to be remaining, then you are going to add the buffer. So this is your buffer what you are going to add, then you are actually going to add the Tac DNA polymerase, you are going to add the dNTPs and then you are going to add the forward as well as the reverse primer and then at the end, you are actually going to add the templates.

So I, we have prepared a small demo clips which actually going to explain you how to set up the PCR reactions and how to actually set up the different cycles into the reactions.

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In this video, we will be demonstrating how to set up a PCR reaction and analyze the results using agarose gel electrophoresis. PCR or polymerase chain reaction is a widely used molecular biology technique to amplify a particular segment of DNA. It is also implied in biomedical research and forensic medicine. The main application of this Polymerase Chain Reaction is cloning.

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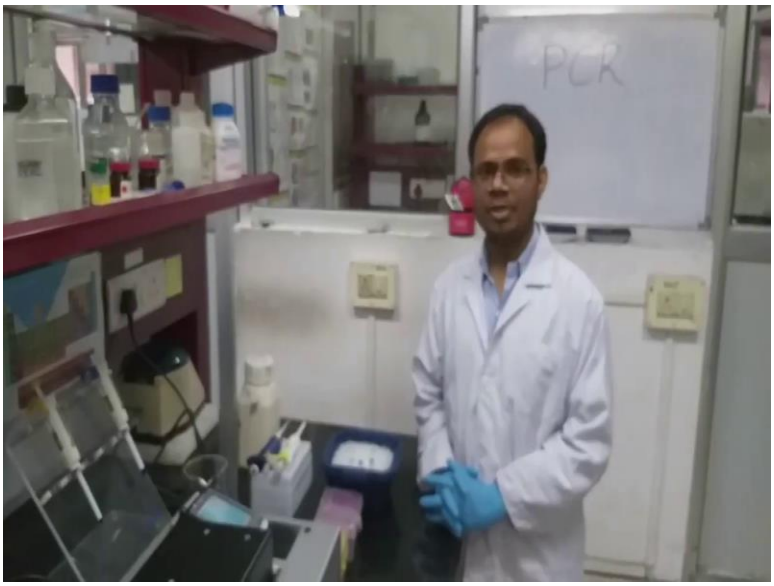

To set up a PCR reaction, we need template DNA, site specific primers, dNTP mix, nucleus free water and Taq polymerase. For a 50 micro liters reaction in a typical concentrations of 10 to 100 nano grams of template DNA use and 5 Pico mols of each primer will be used.

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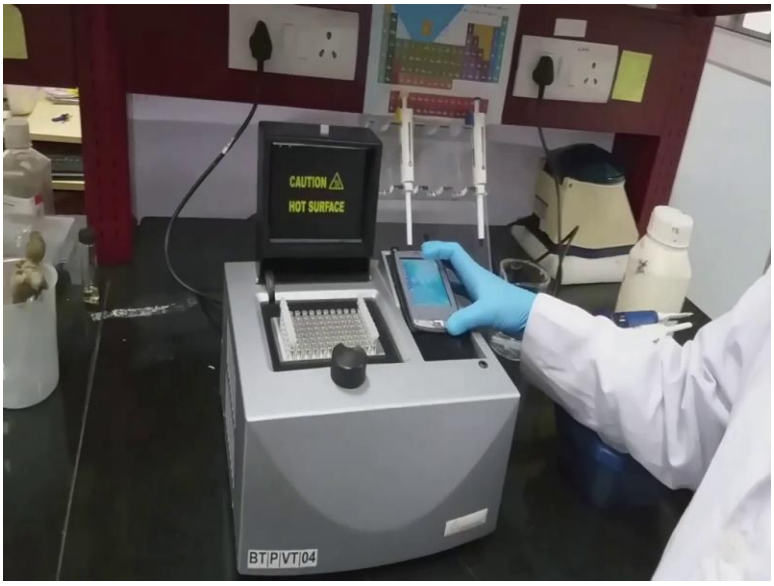


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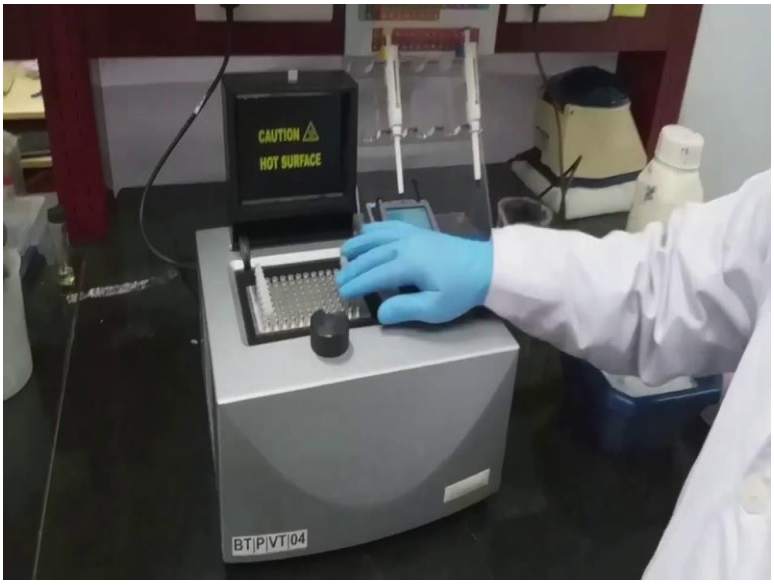
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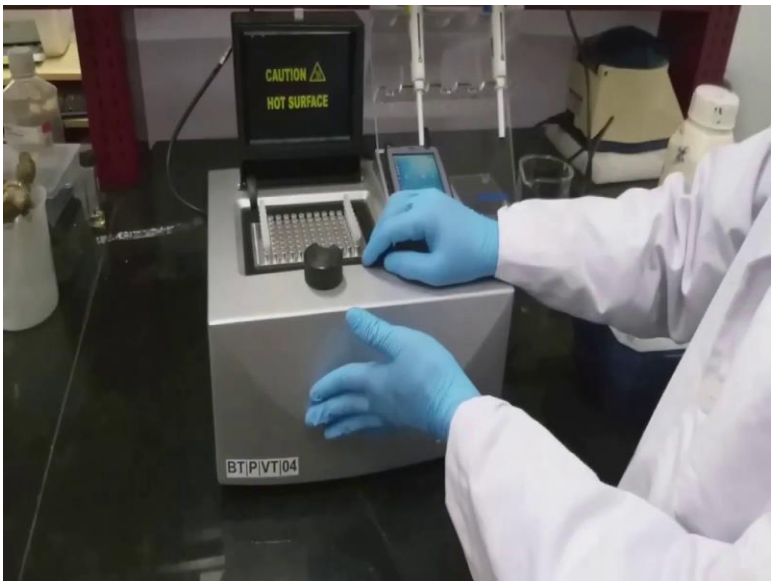

This is an earlier version of thermal cycler which contains display unit where we can observe the parameters and change the parameters. This is the hot shield, this is sample holder, and inside there is a Peltier system which can maintain the temperature fluctuations.

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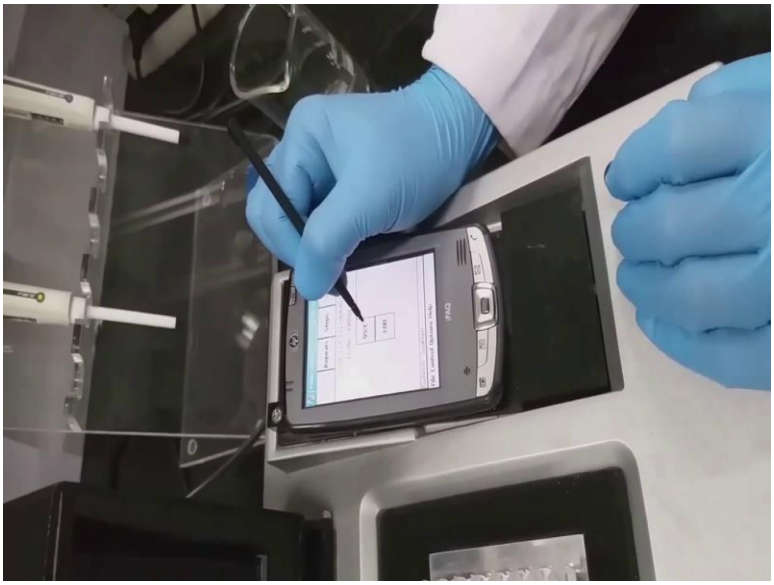

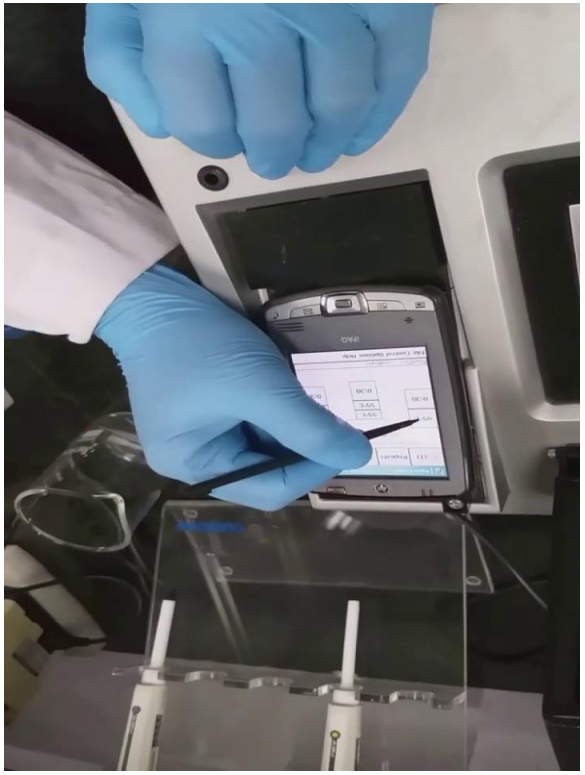


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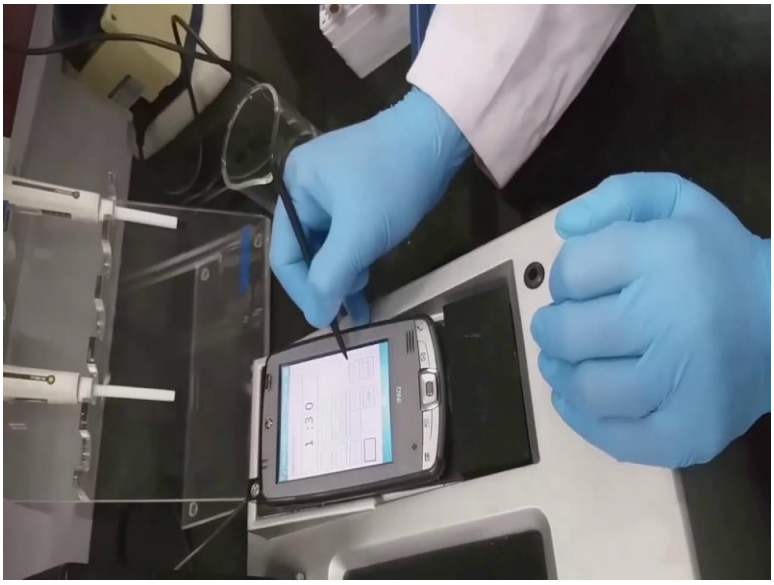
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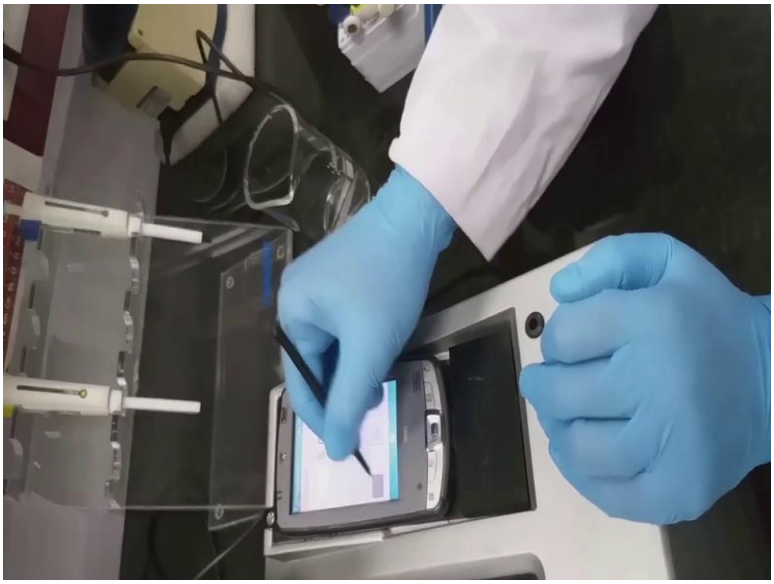

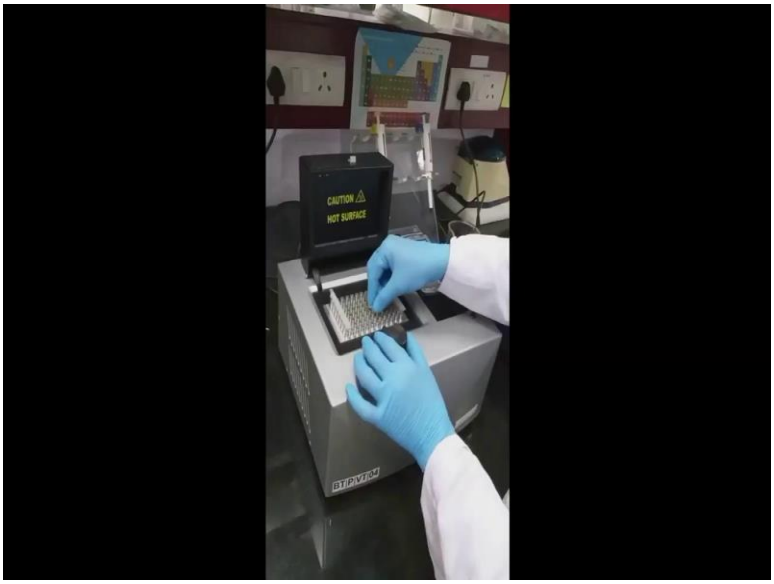
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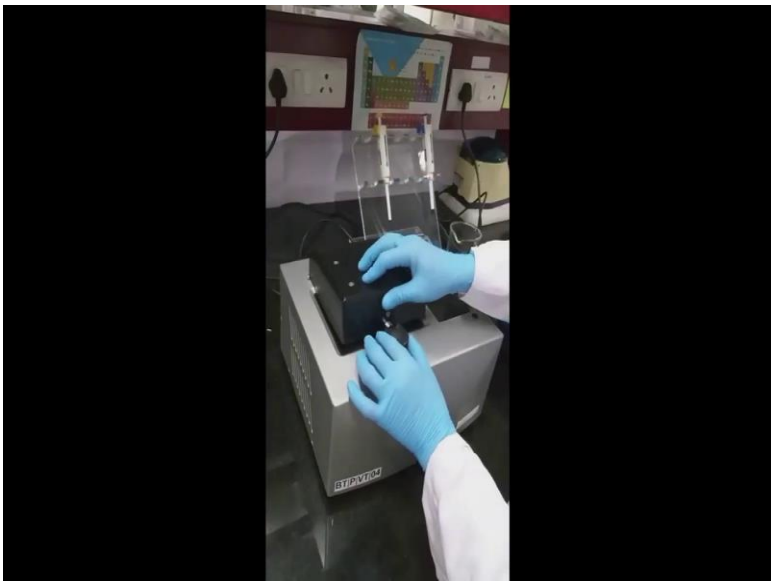

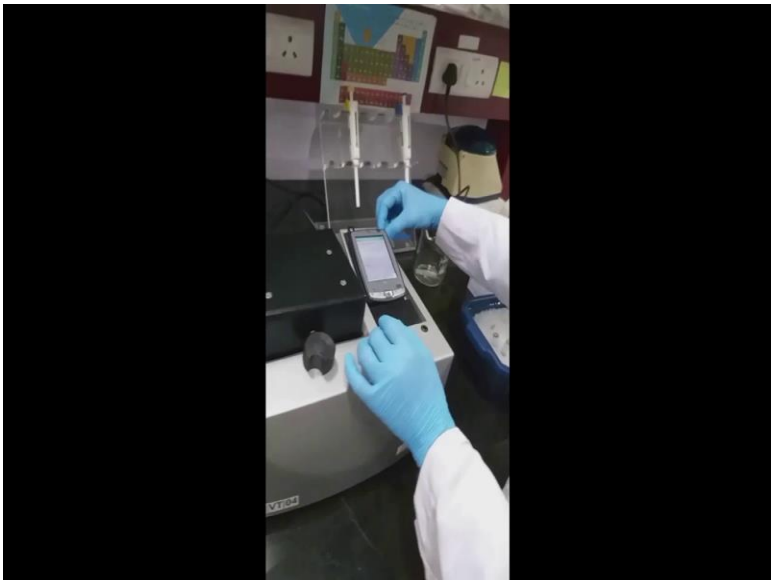

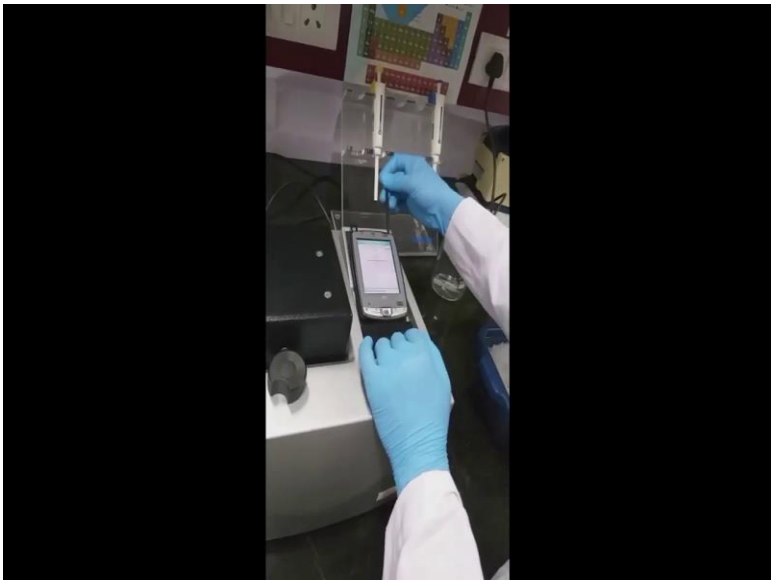

For setting up a PCR reaction, initial denaturation at 95 degrees Celsius, 3 minutes, and this steps will use 30 repeats where initial denaturation will be 30 seconds and annealing at extension, extension time should be given. 1 minute per kb. And here final extension should be given 10 minutes and hold it 4 degrees Celsius, 10 minutes.

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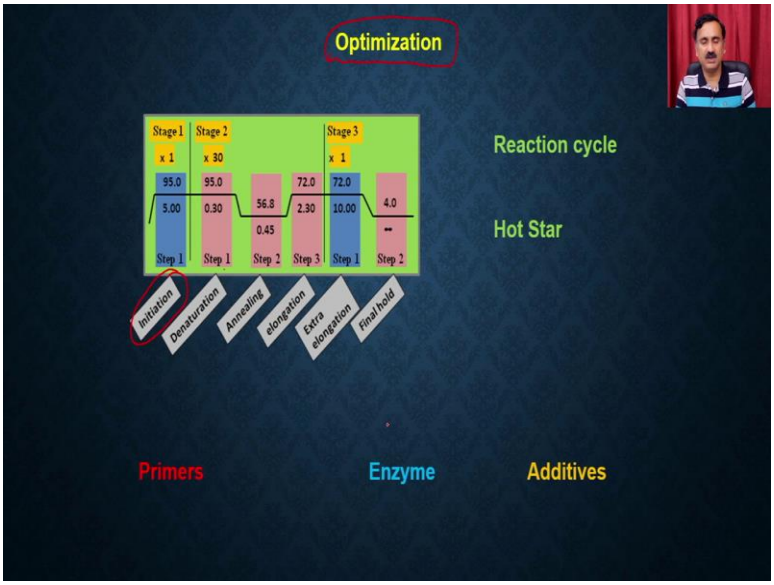

So in this demo clip, students have discussed many aspects, how you can be able to set up the different types of reactions, whether it is the initial denaturation, or the denaturation into the cycles, like the first denaturation, annealing and elongations. And then you have the extended elongations. And then you can have the final hold.

So you can also have the different types of modification into the PCR reaction. And that is how you can be able to do the optimizations to achieve your target DNA.

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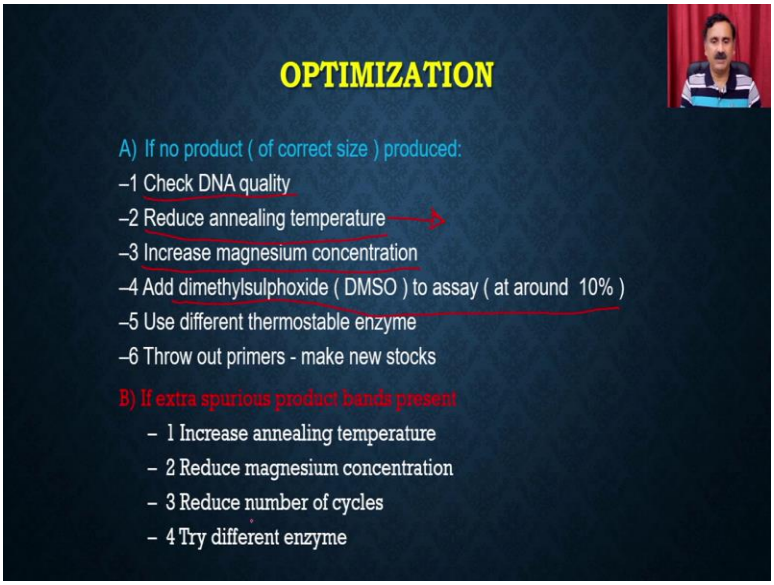

These optimization can be done at different level, like for example, you can actually check the quality of the DNA what you are using as a template, or you can actually reduce the annealing temperature so that the primer is actually going to bind adequately into the templates, you can actually increase the concentration of the magnesium and that also is actually going to the increase the productivity.

You can also add the DMSO. And that is also going to have the, reduce the, it is also going to help you in optimization of the DSA. So there are many aspects or many things that you can do for optimizations. And once you are done the optimization, and you are going to perform the PCRs, you are actually going to see the amplify DNA. So now let us see how the amplified DNA will look like.

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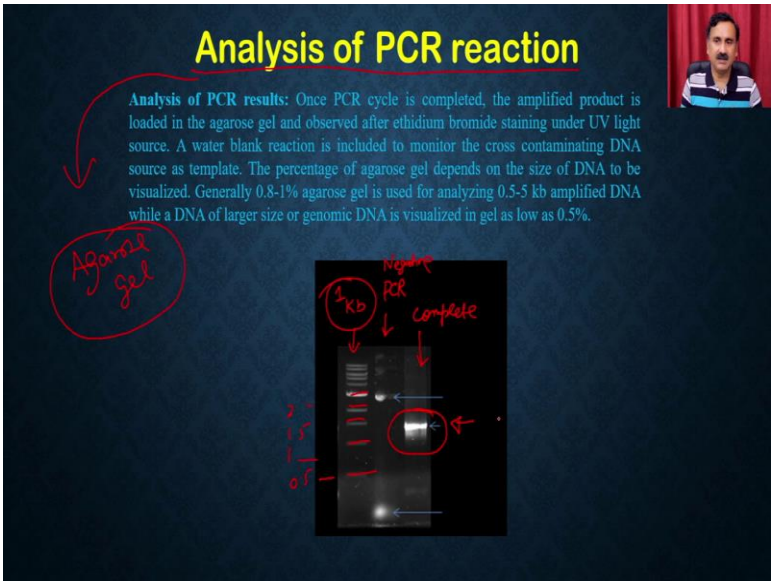

So in the amplified DNA, what you are going to do is once the PCR is over, then what you can do is you can just analyze the PCR product into agarose gel, and that agarose gel is actually going to give you the pattern of the DNA. So what you are going to see here is this is the 1 Kb ladder. So this is going to be a 1 Kb ladder, which is actually going to give you the fragments, like the 0.5 Kb, 1 Kb, 1.5, Kb two Kb like that.

So it is going to give you are fragments in the, so it is going to give you a different bands. And all these bands are actually corresponding to the, their sites. And this is the what is actually a complete reaction. So this is the PCR reactions, and this is the negative reaction. So this is the negative reaction where the template is missing.

So what you see here is that the, there is no amplification, what you see here is a nonspecific amplification, whereas, this is the complete PCR reactions, where you have the template and as well as the primer. So what you see here is that the, you have a amplified DNA products of a desirable length. So this is what you are actually can do to analyze the PCR reactions.

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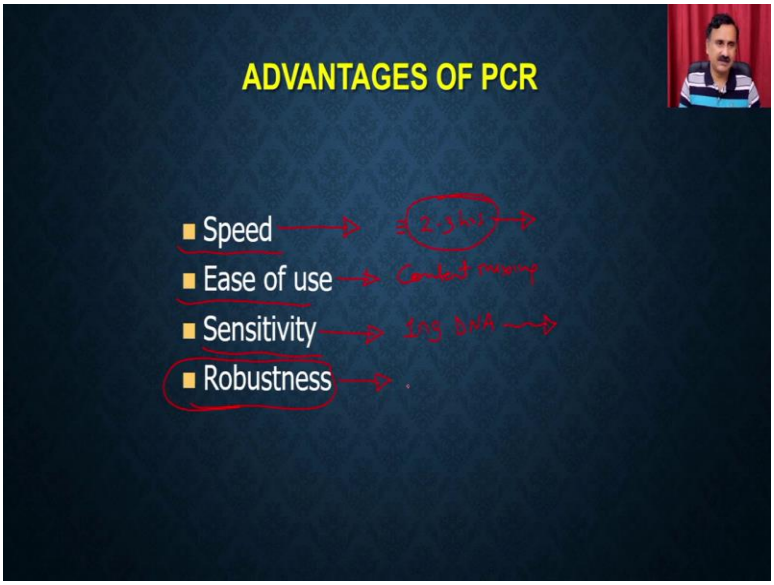

Now, what is the advantage of the PCR? Advantage of the PCR is that it is actually going to be very high speed. So it actually can give you the amplified DNA at a very, very small, at a very high speed, which means approximately two to three hours of the PCR actually can give you the very high quantity of amplified DNA. It is easy to add, it is actually a content mixing assays. So you can actually do the content mixing and then you just put it into the thermal cycler, it is actually going to give you.

It is very sensitive. So even the very small quantity of DNA is actually going to give you So example in the case of 1 nano gram, even though you have a 1 nano gram of DNA, it is actually going to give you the amplified products Then it is also robust. So it is reproducible actually. So even if you do 100 times, it is actually going to give you the reproducible results.

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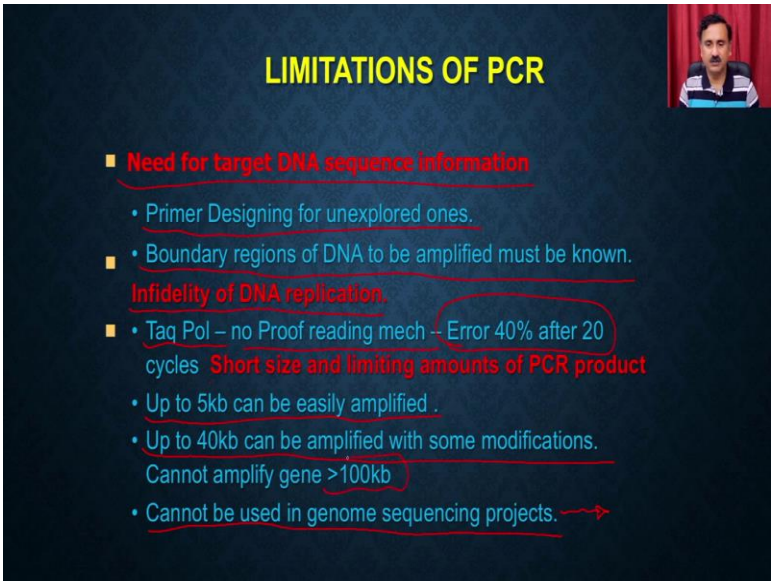

Now, apart from the advantages, what are the limitations of the PCR? So the limitation is that you require a target DNA. If you want to do a PCR you always require a target DNA for doing the, the, for doing the PCR. Why you require a target DNA sequence because you have to use the target DNA sequence to design the primers.

And you also require the prime boundary region of the DNA to be amplified because these two information is required to design the forward as well as the reverse primers. Then the fidelity of the DNA replication. So fidelity of DNA replication is very compromised. For example, in the Taq DNA polymerase, there is no proofreading activity. So there will be approximately a 40 percent error in the Amplified products.

And the, you cannot use the PCR to amplify a very, very large DNA fragments. For example, you can actually amplify up to 5 Kb, but if you want to amplify 40 Kb DNA, then that is very, very difficult to do. And that is why the PCR cannot be used in the genome sequencing project. Remember then we are, when we were discussing about the DNA sequencing, when we were talking about DNA as a biomolecule.

We said that you are going to perform the PCR and that is how you are going to incorporate the modified nucleotide. But that cannot be done even if you are doing the genome sequencing because in the genome sequencing, you might not, you will not be

able to use the PCR to sequence the whole genome, because you cannot be able to perform the amplification of a DNA fragment which is bigger to this.

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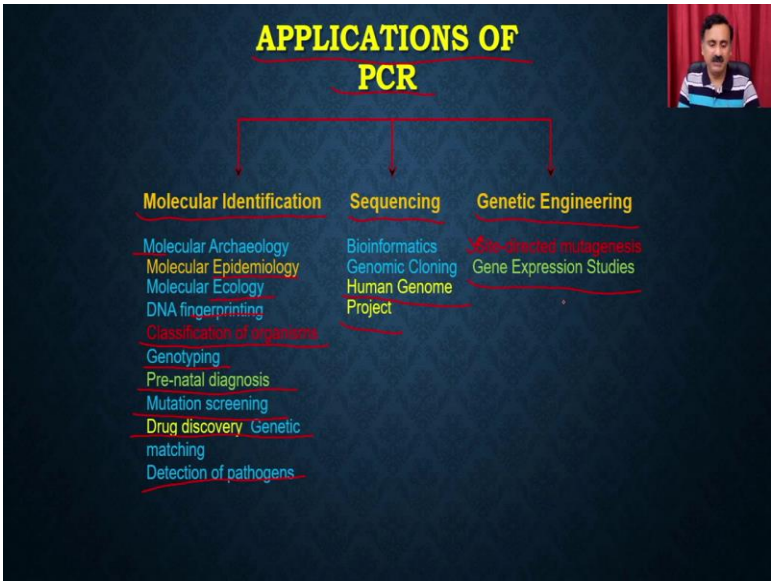

Now, what is the application of the PCR? So application of the PCR lies into the three areas. One is, you can have the molecular identifications, you can use the PCR for sequencing, and then you can also use the PCR for the genetic engineering. In the case of the molecular identification you have a huge applications.

Like you can have the application in the Archaeology, you can have the application in the Epidemiology, you can have the replication in the Ecology, DNA fingerprinting, you can have the, PCR be used to classify the organisms, Genotyping, Pre-natal diagnosis, mutational screening, drug discovery and the detection of the pathogens.

Whereas in the sequencing you can use for the human genome sequence and other kinds of sequencing projects. So you cannot do the sequencing of the whole genome by the PCR but you can actually sequence the small fragments. And in the case of genetic engineering, you can use the PCR to generate the site-directed mutagens.

Or you can actually be able to use the PCR to clone a particular gene fragment and that is how you can be able to use for applications.

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So PCR in the food science, so in the food science, the PCR is a rapid and sensitive method that enable the detection of the sub dominant population in food without the need of the enrichment media, it allows the detection of the dead cells and the non-cultivable cells.

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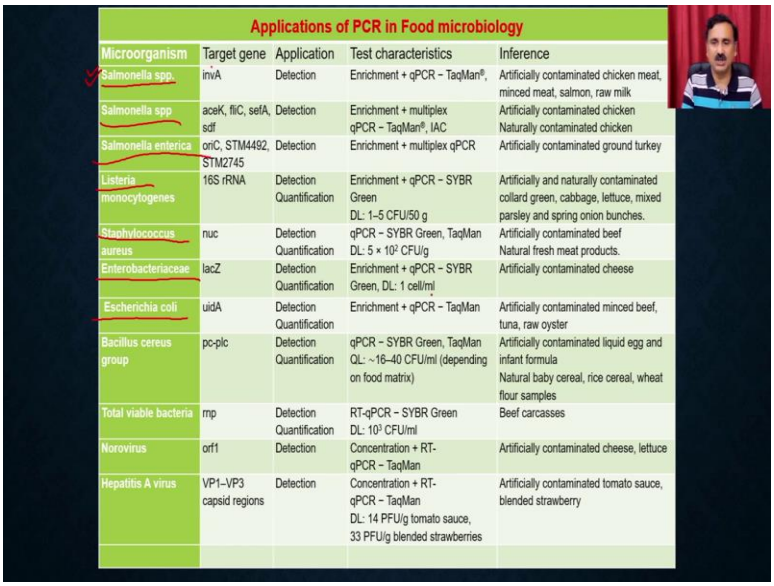

Microorganism	Target gene	Application	Test characteristics	Inference
Salmonella spp.	invA	Detection	Enrichment + qPCR – TaqMan®,	Artificially contaminated chicken meat, minced meat, salmon, raw milk
Salmonella spp	aceK, fliC, sefA, sdf	Detection	Enrichment + multiplex qPCR – TaqMan®, IAC	Artificially 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	rnp	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

For example, you can have the list of the applications or list of the places where you actually can be able to detect these, these contaminating or these micro organisms into

the food products. For example, you can detect the salmonella species, the difference, even the salmonella different species into the different types of foods and that is how you can be able to, screen out these products.

Then you can have the Listeria, you can have the Staphylococcus, Enterobacteriaceae, E.Coli all these bacterias can be detected by using their target gene, you can use the detection as well as the quantification into the different food products.

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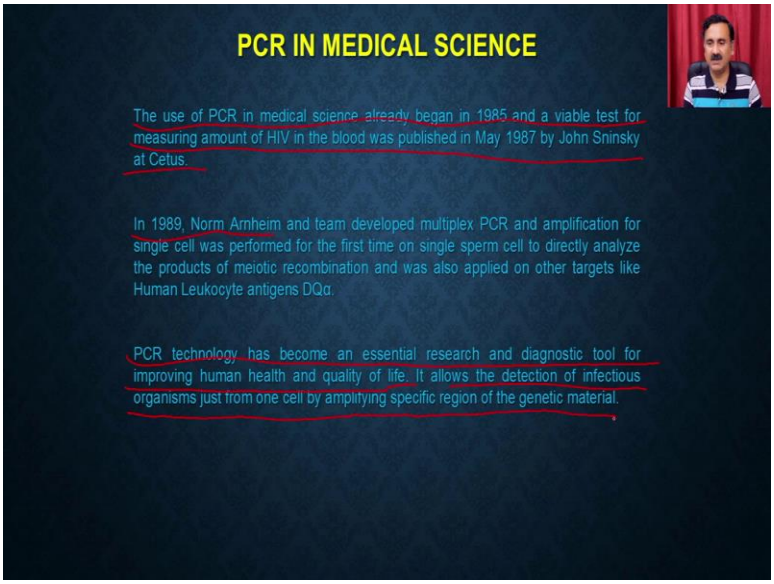

Then the PCR can be used in the medical science. So the use of PCR in medical science has already began in the 1985 and a viable test for measuring amount of HIV in blood was published in the 1987 by John, and in 1985 and all that, So PCR technology has become an essential research and diagnostic tool for improving the human health and quality of life. It allows the detection of the infectious organism from one cell by amplifying the specific region of the genetic material.

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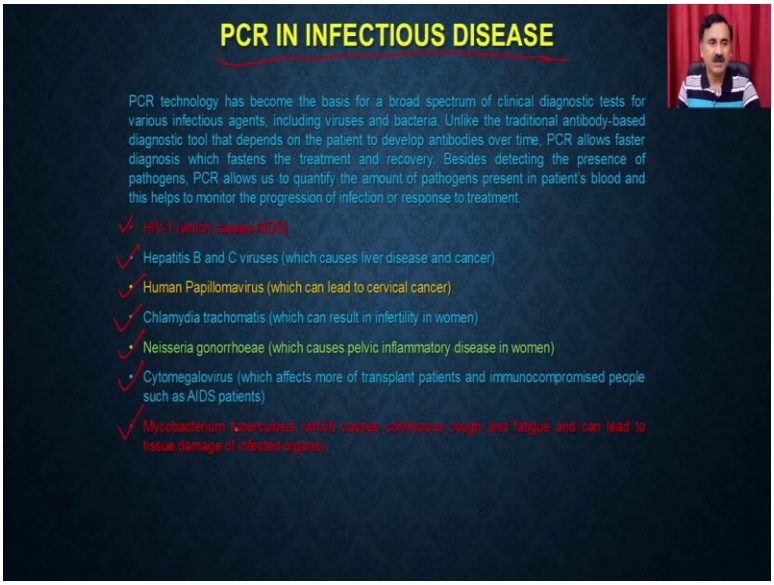

So how you can be able to use the detection of the amplified, infectious diseases? So it can actually be use a particular gene which is only present in infectious organism and then you can be able to do the amplifications. And as I said, the PCR is very sensitive. So you can just require a very, 1 nano gram DNA, and that actually is going to give you the DNA.

So for example, you can use that for detection of the HIV, hepatitis, Human Papillomavirus, Chlamydia virus, Neisseria, Cytomegalovirus or the Mycobacterium tuberculosis. Now, let us see how you can do that.

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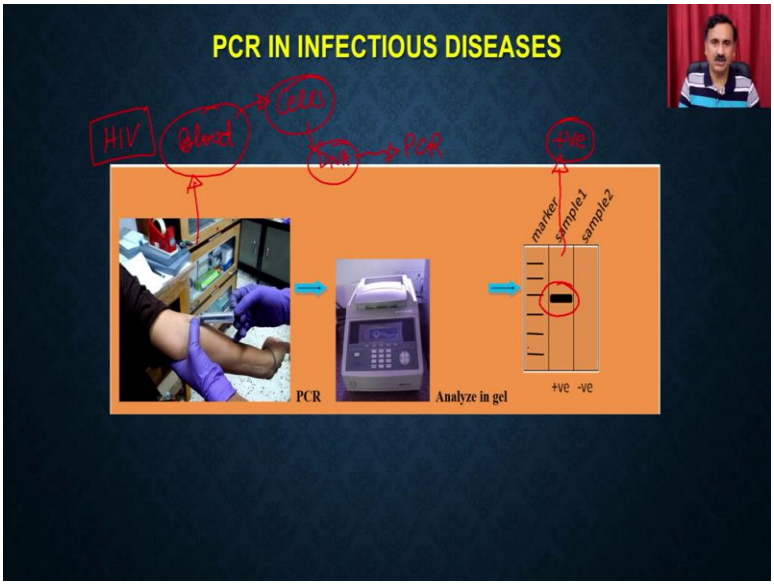

In a particular diagnostics what you are going to do is suppose, this is the experiment where we are showing is that how you can be able to do the detection of the HIV. So what you have to do is first you have to withdraw the blood and the, from the blood, what you have to do is you have to isolate the cells and from the cells, you can actually be able to isolate the DNA, and from the DNA, you can actually be able to do the PCR in the help of the PCR thermal cycler.

And then you analyze that onto a gel. So what you are going to see is that there will be an amplified DNA. So that amplified DNA is actually going to say that the, it is actually the that particular organism is present in this particular sample, and that you can actually be, and you can also run a negative control and that actually going to tell you that the, that can actually be able to use to authenticate the reaction mixtures.

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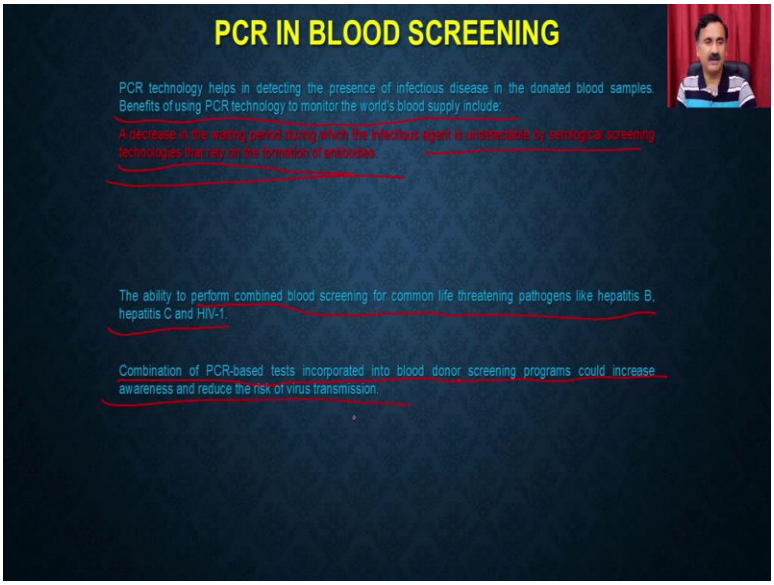

Then PCR is also can be used in blood screening. So PCR technology in detecting the presence of infectious disease in the donated blood sample. Benefits of using PCR technology monitors the blood supply includes, that includes the decrease in the waiting period, like because you can actually do the infectious disease screening very fast with the help of the PCR.

Then ability to perform the combined screening for different types of pathogen at the same time. You can actually do the screening for the hepatitis, you can do the screening for HIV you can do the screening for TB and all that. And that combination of the PCR based test incorporate into the blood donor screening scheme could increase the awareness and reduce the risk of virus transmissions.

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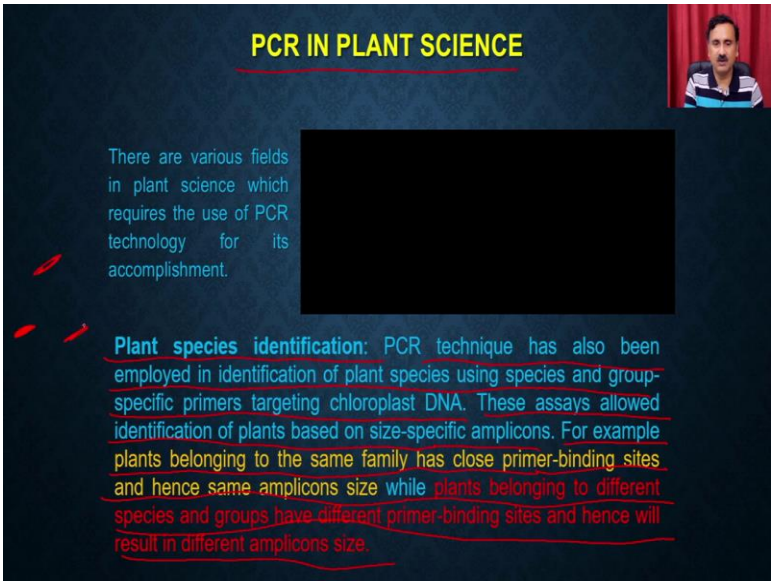

Then we have the PCR in the various fields, like PCR in the plant science. So in the PCR in the plant sciences, that you can actually use the plant species identification. So PCR technique has been employed in identification of the plant species using the species and the group specific primer targeting the chloroplast DNA. These assays allowed the identification of the PCR plants based on the size-specific amplicons.

For example, plants belonging to the same family has a close primer-binding site and hence same amplicons size, while the plant belonging to the different species and group have the different primer binding site and hence will result in the different amplicon size, which means, if you, if the two plants are of closely related size, they are actually going to give you the size DNA of the same size. But if they are of the different species, then they are actually going to give you the DNA of the different size.

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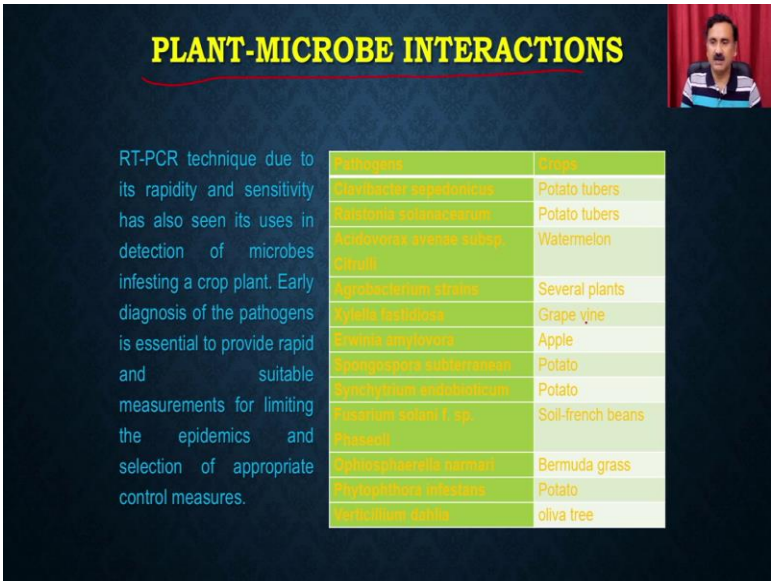

This is what you are going to see here. You can actually be able to use the, even the PCR or the RT-PCR techniques to detect the plant microbes interaction. So you can actually be able to detect the different types of microbes associated with the different types of plants.

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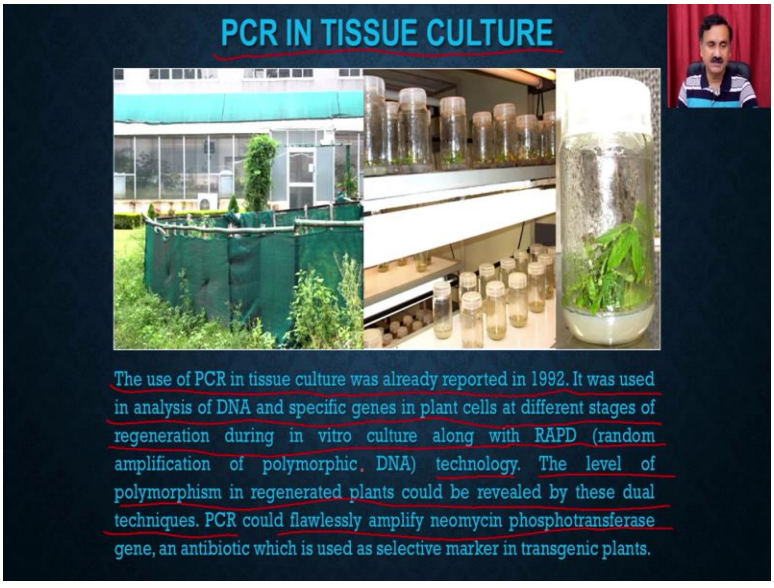

Then PCR can also be used to study that, in tissue culture. So you know that in tissue culture, you have the different stages of the particular plants, you can have the callus, you can have the differential stages of the in vitro culture. So you are actually able to use the

PCR and, so the use of PCR in tissue culture was already reported in 1992. It was used in analysis of DNA and specific genes in plant cell had the different stages of the regeneration during in vitro cultivation, along with the RAPD technology.

The level of polymorphism in generated plant could be revealed by these dual character. So PCR could flawlessly amplify the neomycin phosphotransferase gene, an antibiotic which is used in a selection marker in the transgenic plants. So you can actually be able to even cream the transgenic plant from the non transgenic plants.

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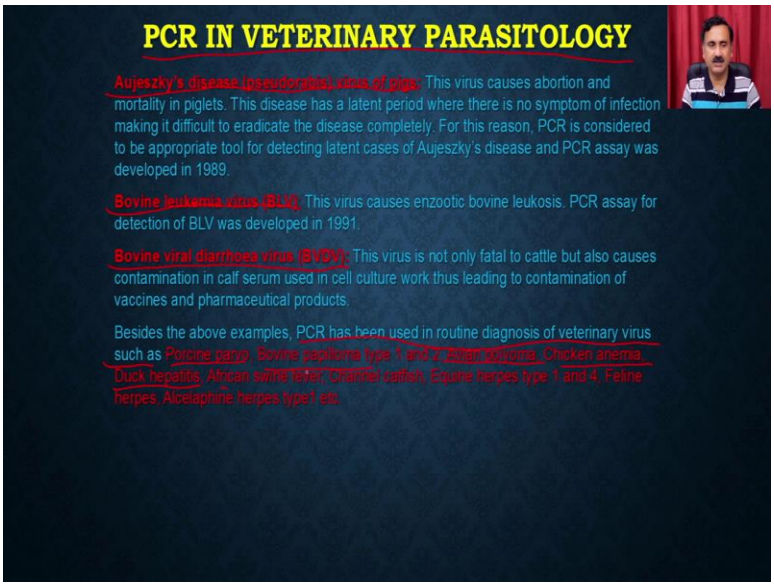

Then you can also use the PCR in the case of veterinary technologies or parasitologies where you can actually be able to use the PCR to detect the different types of the pathogens what are being found into a different type of animals. For example, you can use the, you can detect the Aujeszky's disease, virus of the pigs, you can use the Bovine leukaemia virus, you can detect the Bovine viral diarrhoea.

And besides the above example, the PCR has been used in routine diagnosis of the veterinary viruses such as Porcine parvo, Bovine papilloma virus, Avian polyoma virus, Chicken anemia, Duck hepatitis and all that.

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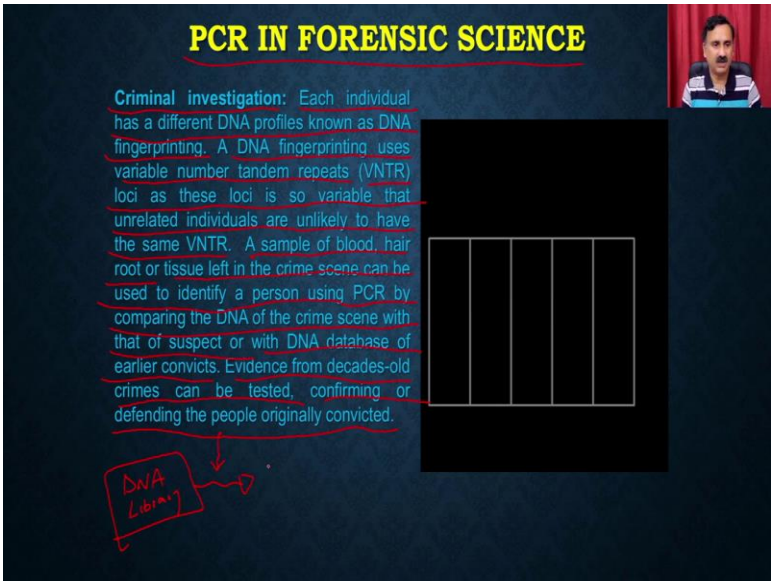

Apart from that the PCR can be used even in the case of the forensic science. So, for example, the criminal investigation, so each individual has a different DNA profile such as the DNA fingerprinting. DNA fingerprinting uses the variable number tandem repeat or VNTRs loci, these loci is so variable that the unrelated individuals are unlikely to have the same VNTR.

What you can do is you can take a sample of the blood, hair root, or the tissue left in the crime site and can be used to identify a person using the PCR by comparing the DNA of the crime scene with that of the suspect, or with the DNA database of the earlier convicts. Evidences from the decade old crime can be tested, confirming order depending on the people originally convict.

So in this particular type of criminal investigations, what you require is you are actually required a DNA library. So this DNA library is actually going to have the, these VNTR, the information about the VNTRs for the different criminals. And then what you can do is you can actually be able to identify, calculate or determine the VNTRs into the, this particular blood which you have collected from the crime site, and then if you match this, it is actually going to give you the identity of that particular convict.

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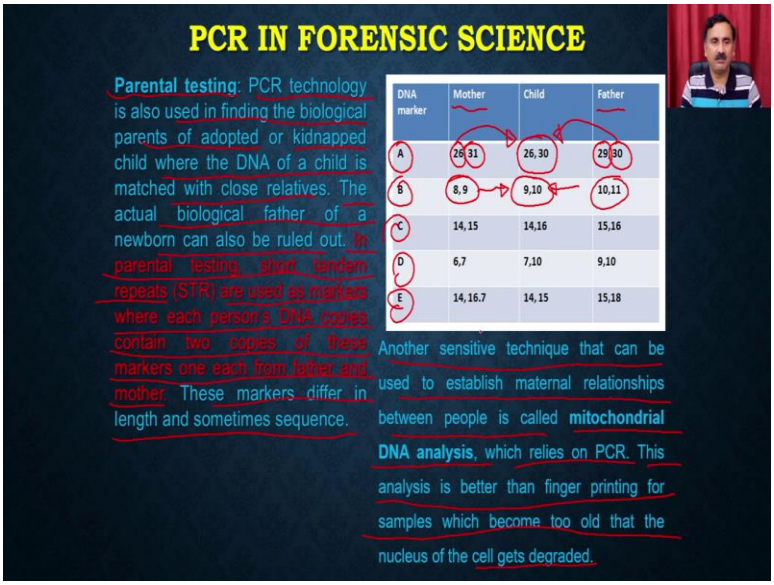

Then the PCR can be used even in the parental testing. So you know that there are many times when the kids are actually get kidnapped or they get, so in those cases you can actually be able to match the DNA of the kids with the mothers and the Father. And that is how you can be able to resolve the any kind of parental disputes.

So the PCR technology is also being used in finding the biological parents of the adopted or the kidnapped children, where the DNA of a child is matched with the close relative. The actual biological father of a newborn can also be ruled out. So in the parental testing, the short tandem repeats, or the STRs are used as a marker which, each parent's DNA copies contain two copies of these markers, one each from the father and the mother.

These markers differ in the length and sometimes the sequence. So what you can see here is that you have the different types of DNA marker like A, B, C, D, and E. And in the case of Mother, you have the STRs like the 26 and 31. And in the case of Father, you have the 29 and 31, 30. So, whereas in the case of child, you are going to have the combination of these two, that is why you can have the 26 and 30.

So 26 come from the mother, and the 30 comes from the father. So that is why if your child is having the 26 and 30, it is actually going to say that these are the mother and these are the father combinations. Similarly, you see the DNA marker B, you have 8 and

9 for the mother, you have the 10 and 11 for the father, and you have the 9 and 10 for the child. It means this also has the similar kind of pattern.

So by calculate, by looking at the DNA, DNA markers, what is present in the child and what is present in the mother and suspected fathers, you can be able to detect the, and you can be able to identify the parents. Another sensitive technique that can be used to establish the parental relationship between the 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 this cell get damaged.

So you can also do the mitochondrial DNA analysis. You know that when we were discussing about the different types of cells and we were talking about the mitochondria, we said that the mitochondria can be used for detecting the family history, so, or family tree. So because the mitochondria remains consistent, so it goes from the mother to mother and that is how it actually can be well preserved in a particular family. So you can actually also do the mitochondrial DNA analysis with the help of the PCR.

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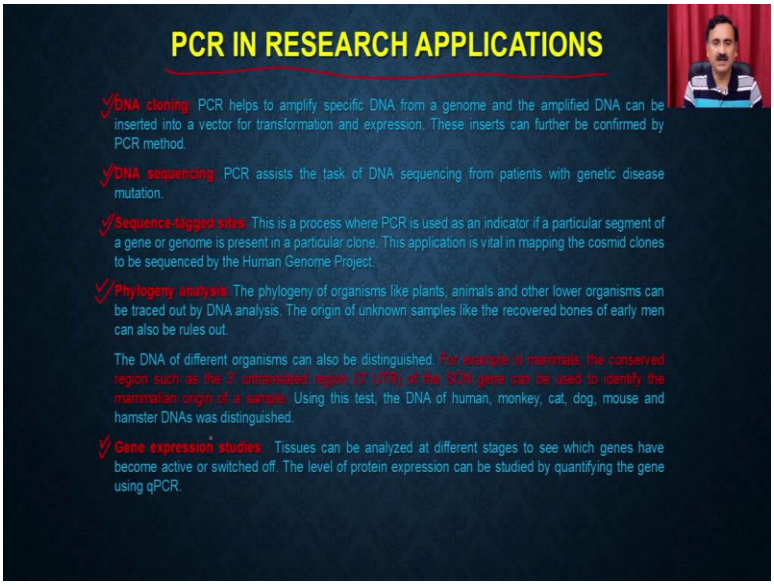

Apart from that, the PCR is very extensively been used in the research replications. PCR can be used for DNA cloning, PCR can be used for DNA sequencing, PCR can be used

for the sequence tagged sites, PCR can be used for the phylogenetic analysis and PCR can also used for the gene expression studies.

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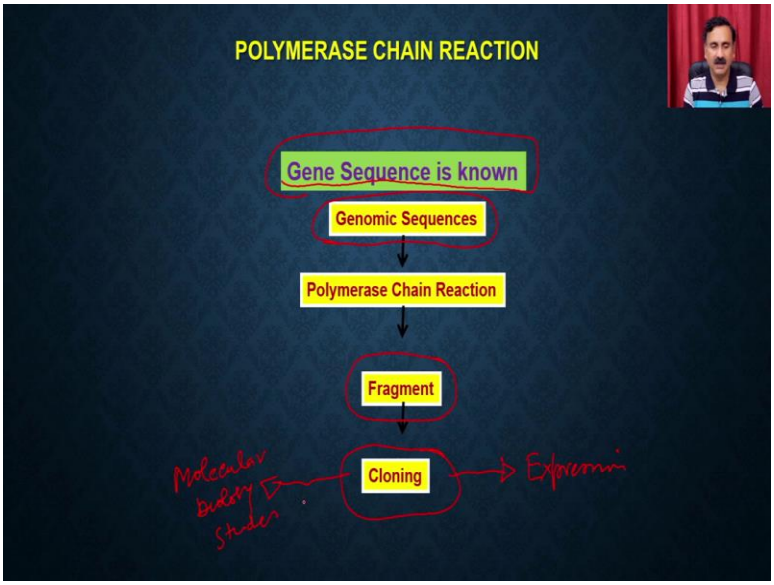

How the PCR can be used for the DNA cloning? What you can do is you can actually be able to just know the sequence, sequence of that particular gene which you are interested to clone, then what you can do is you can just isolate the genome, and then you can just design the site-specific primers with the help of this particular genome sequence or gene sequence, then you are actually going to do the PCR.

Once you do the PCR, it is actually going to give you a fragment which is identical to that particular gene sequence. And then you can take this fragment and you can clone it into the particular vector. And that is how you can actually be able to use this clone for, either for the expression of that particular protein or you can actually be able to use that for the other kinds of molecular biology studies.

So you can actually use for the molecular biology studies. So this is all about the PCR and we have discussed about the different aspects related to PCR. And the PCR is a technique which is completely depend on the, the DNA of replications, and it has a very huge role, and what we require is we require the template DNA, we require the site

specific primers and then we require the DNA polymerase, magnesium chloride and water. And once, and you also require a thermal cycler.

So once you set up these reactions, you put it into the thermal cycler and then you go for the, these PCR cycles where you are going to have the initial denaturation and then you are going to have the elongation, and then you are going to have the all other steps and that is how you are actually going to get the huge amplified product of the that particular region which you have selected by designing a forward as well as the reverse primers.

So and lastly, we have also discussed about the application of the PCR in the different fields. We discussed the application of the PCR in the medical, field agricultural field, plant microbe interactions and the other kinds of criminal investigations or the parental testing. And so with this, I would like to conclude my lecture here.

In our subsequent lecture, we are going to discuss more aspects related to the central dogma of life or to the central dogma of molecular biology. So with this, I would like to conclude my lecture here. Thank you.