Bioengineering: An Interface with Biology and Medicine
Prof. Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology- Bombay

Lecture – 10
DNA Tools & Biotechnology-II

So, welcome to this lecture, we are going to continue on DNA tools and biotechnology, in the previous lectures we have been discussing about the hereditary unit or DNA, you have also learnt the theoretical concepts of tools which could help to visualize and amplify DNA by using agarose gel electrophoresis and polymerase chain reaction or the PCR processes. Today, we are going to show you in the laboratory how these techniques could be performed.

So, let us now start the laboratory demonstration for explaining you these techniques and showing you how these experiments could be actually performed in the laboratory.

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Hello everyone, I am Srishty, the TA of this course, so now we will be doing agarose gel electrophoresis to check whether our procedure product has come or not and so for that we will be making our gel.

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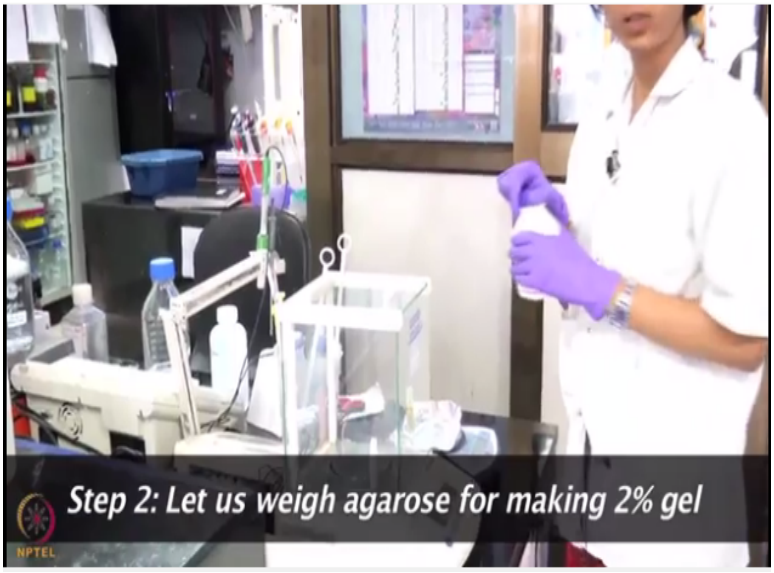

First of all, we will be making 2% gel, we have weigh, 2 grams of agarose powder and we will be adding it to TA buffer.

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So, now we will add 2 gram of agarose to a flask and we will also 100ml of TA buffer, so now they will be dissolving the agarose powder using microwaves.

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So for 2 minutes, it will be putting it inside and in between, we will be stirring it, so now a gel is done and we will be pouring it on a gel casting unit. Now as our gel has cool, we will be adding EtBr here in the ratio of 0.5 microgram per ml.

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Why we are adding EtBr, we are adding EtBr, so that the dye will intercalate with our DNA sample and given fluorescence when observed on the transilluminator, always take care that you discard the tubes of EtBr in biohazard and as it is a potent mutagen.

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Now, we will pour our gel, so as you can see these are the types of casting units we can have, this is the bigger one in which I have already casted a gel, it is a smaller one which we casted right now, when we placing a comb, this is the comb of its size in this before the gel solidifies, these are the different types of combs and casting units available, so this is the electrophoretic unit in which we are replacing our gel after it solidifies for the run.

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And this is the voltage jointer.

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Today, I will be taking you to the PCR process and we will try to learn how the process occurs in real time so basically, what is PCR? PCR is as its name suggests it is polymerase chain reaction.

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In this matter, in this technique what we see is; we have a very small quantity of DNA or any sample then, in this process the sample gets amplified to a million times, so that sometimes when you are working in a clinical field research or there is a crime scene where you have very less quantity of some sample you want to do some research with it or you have to say give some identification then, you cannot do with such small quantity of things, so for that we do PCR.

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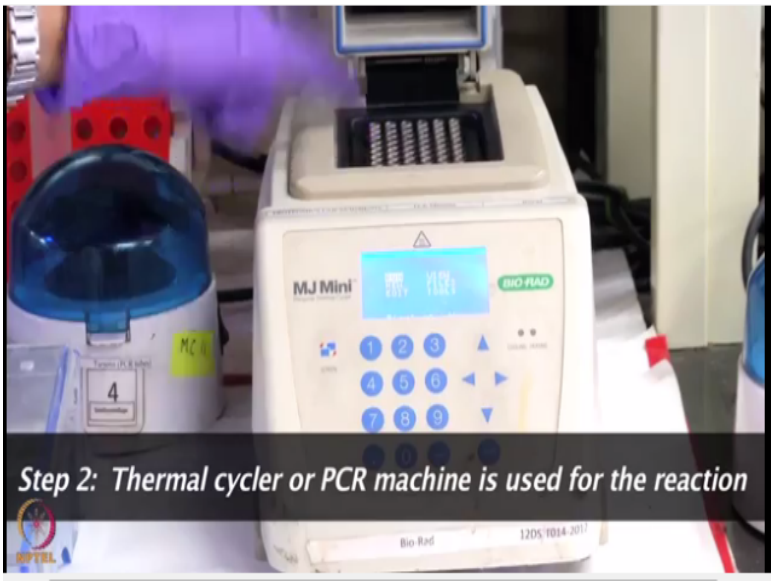

So, this is the machine called thermo cycler which we use for PCR as you can see these are the well plates, where we put a PCR tubes and run a PCR, here we will be putting a PCR product which my colleague has given us and we will checking its result.

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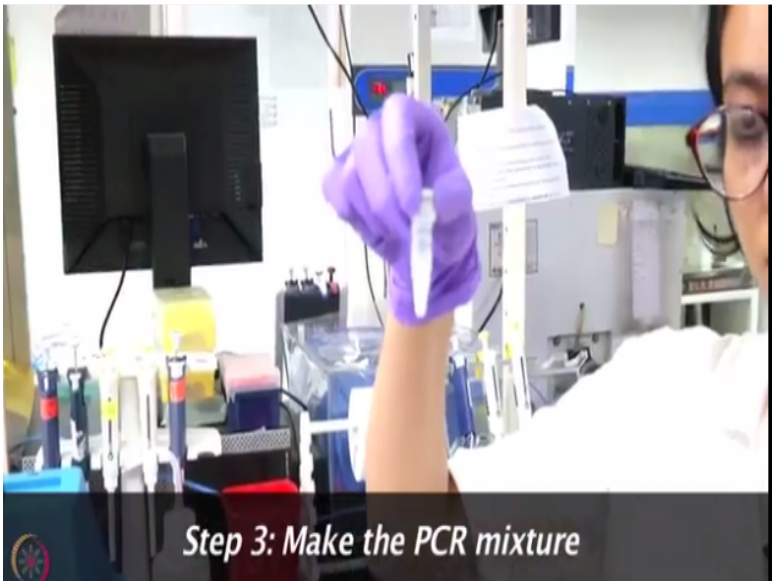

So, before starting the procedure like you know, the basic components of PCR which we need, so first of all this is reaction buffer, this reaction buffer we need.

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It will give proper optimum environment for our enzyme to work, this is our enzyme Taq DNA polymerase.

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These are the 2 primers for our reaction, this is reverse primer, this is forward primer.

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We will be adding all these contents to a master mix tube, this is 2 molar dNTP.

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And you place free water.

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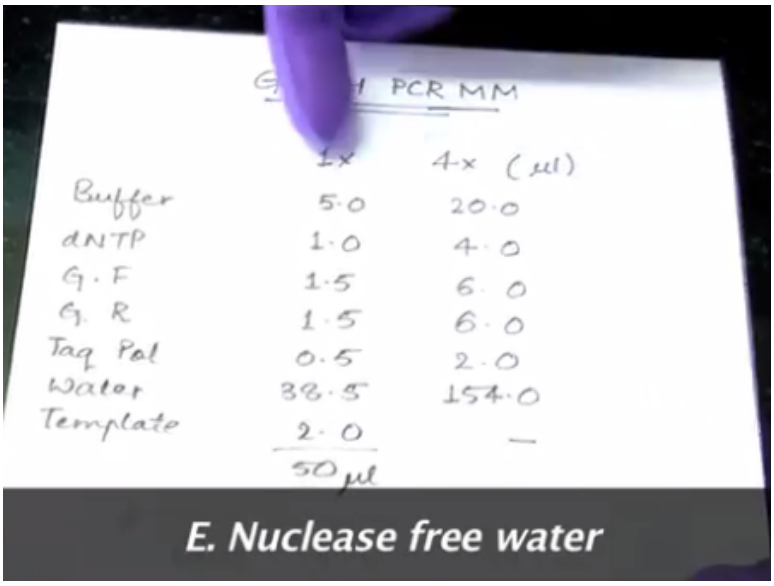

And this is the amount of reagents we add in each reaction, this is for 1x reaction, this is for 4x.

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So now, as you know the components of the PCR and we have added into the master mix will be adding it to a PCR tubes. This tubes I have already added 23 microliter of master mix and 2 microliters of our; so now as we know, what does a master mix contain; we have already added it in proper amount, so now we will be adding it to a PCR tubes, which I have already added, we have already made a master mix.

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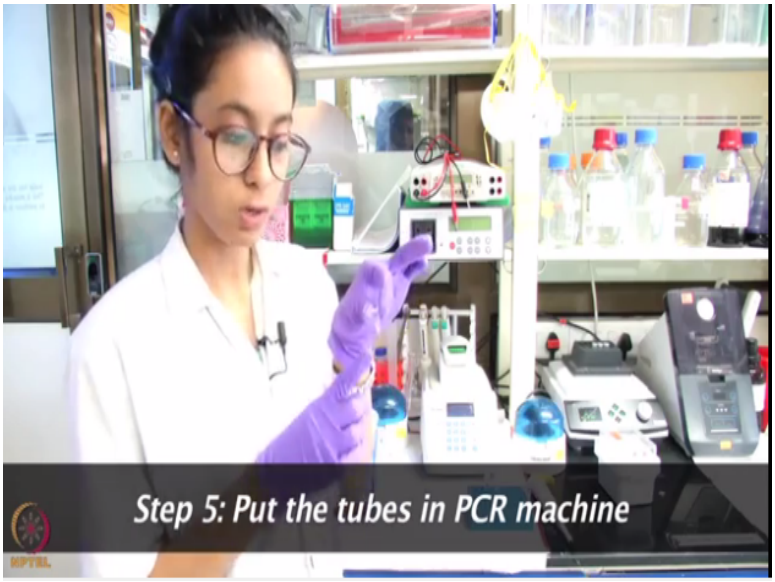

So, from this master mix, we will be adding 23 micro litre of a master mix to a PCR tubes, in this tubes we already have added 2 micro litre of our DNA which we want to; loop is here, now we will be putting a tubes into the PCR machine, before putting the tubes in PCR machine make sure you give it a spin, so we will be spinning our tube, so that to ensure that our contents are properly mixed.

So, now once our tubes are ready, so now as you can see a machine is on, this is a thermo cycler, so in this thermo cycler, we will be adding a tubes and in this way and we will be closing it, make sure you tighten the cap now, as you can see here, we can see many options; run, new, edit, view, files and tools, I have already set the program which is gap DH because we are going to check here for Gap DH gene.

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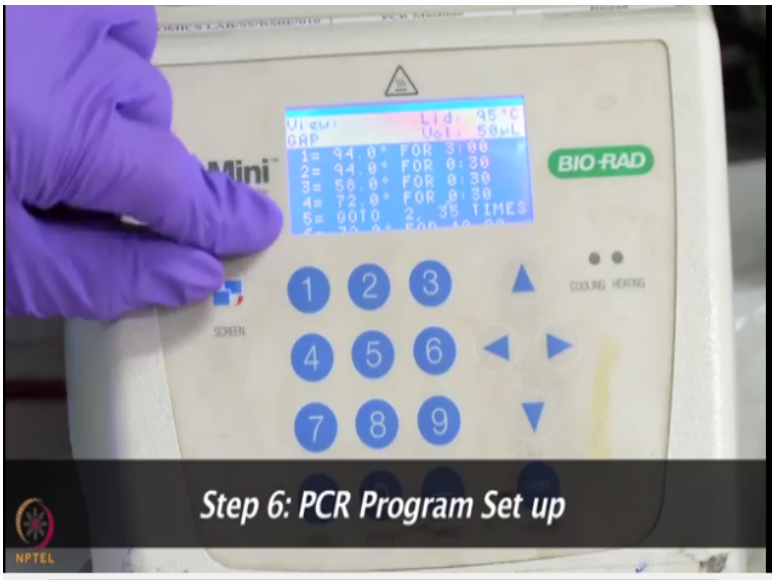

So, here sample volume is 25, enter, view the program, this is the program we will be using as you can see you are able to read multiple temperatures here and number of steps, so the first step is initialization. In initialization, the enzyme will be activated for further denaturation, second step is 94 degree for 30 seconds, in this step denaturation of a DNA will occur, in denaturation both the strength will separate out.

In the third step, it is 58 degree for 30 seconds, now this is the underlying step, where the primers are going to anneal, when primers will anneal, the process will go on further, next is 72 degree for 30 seconds that is the extension step, so in extension once the synthesis, so in this extension step it is for 30 seconds.

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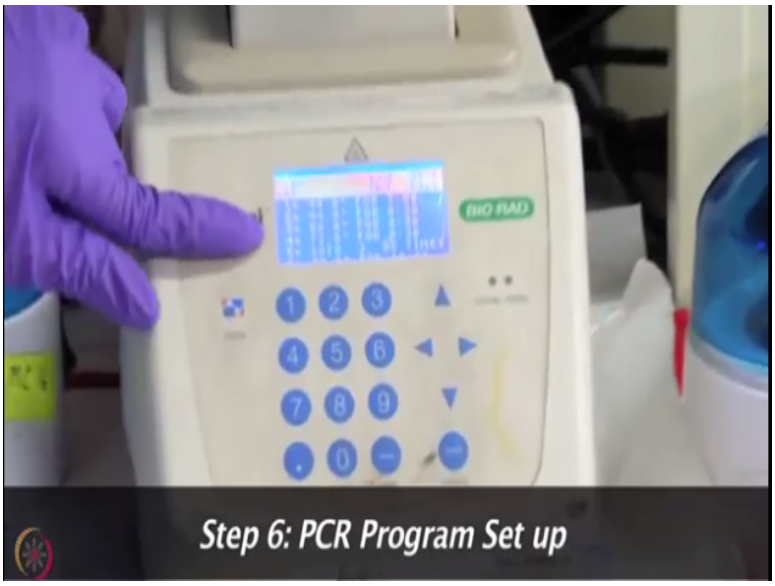

In extension step, what will occur that synthesis will go on again and again and once a product is formed another product will be taken as template and new product will be formed and then we will be repeating this cycle for 35 times as you can see here, 35 times go to 2, so these 3 steps will be repeated, we will be getting multiple copies. Next is again 72 degrees for 10 minutes, so for final extension if any product is left for extension, it will be occurring again.

And last is hold; hold at 4 degree, why will be holding to bring down the temperature to normal to for cooling up the chamber, so now as we know in the program, we will be running a PCR program. So, as you can see now it has started and here we can see how the steps are going, so it will take this much of time to complete a program, so now we will run the program; run, now we can see a PCR has started and it will take this much of time to complete.

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So, now as you can see a gel has solidified and we will be putting it on a running unit, as you can see it is properly solidified, so now we will be taking out a combs and putting a gel in the unit, this is 1x TAE and we have to add it in such a way that our whole gel is immersed in it, so, (FL) so now we will be adding a DNA to the loading dye which have spotted here.

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This is 1x loading dye, to it we will be adding a PCR product which is, which will be taking as 8 micro litre and after adding it by pipetting, they will be mixing it properly.

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And will be loading it on a gel, so now to check whether the products; PCR products are of a desired one or not we will check it by loading DNA ladder into it, the size of Gap DH here is 595 base pair, so we have loaded 100 base pair ladder and the loading dye we used is this one, so the ladder we have used here is 100 base pair DNA ladder because the product size is 595 base pair.

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Now, we will be running a gel make sure that you make the connections properly and now, so now we will be setting up our voltage is; we will keeping 100 volts, as you can see for 1 hour and press run, so as you can see here bubbles will start coming out so, the gel has started running.

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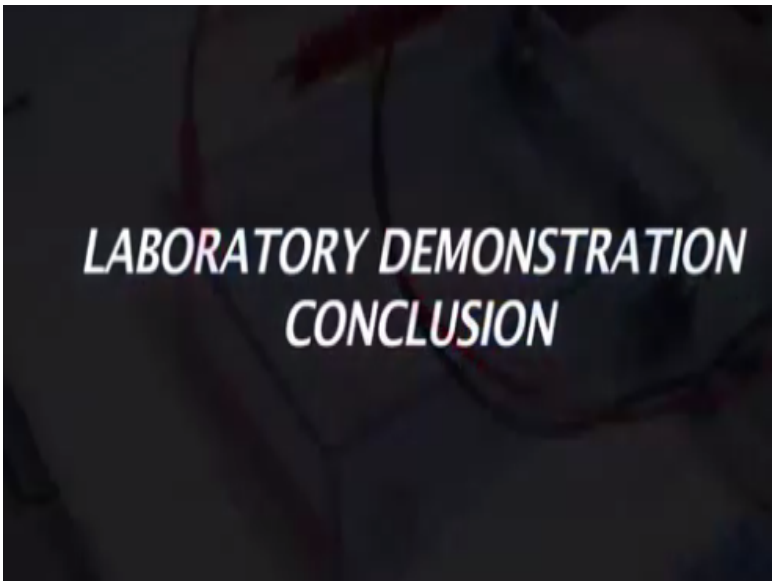

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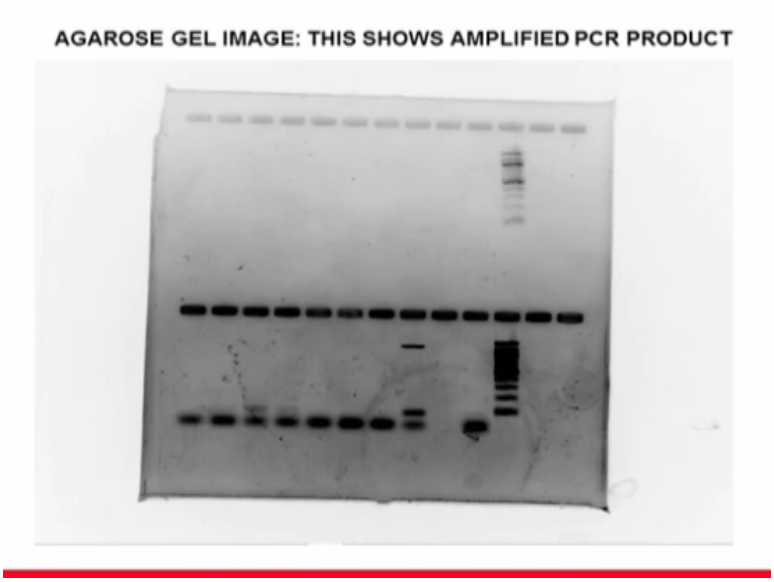

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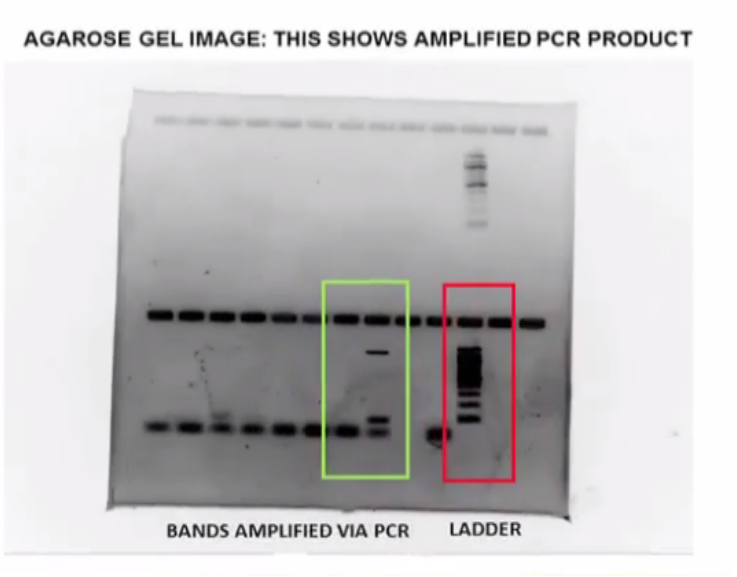

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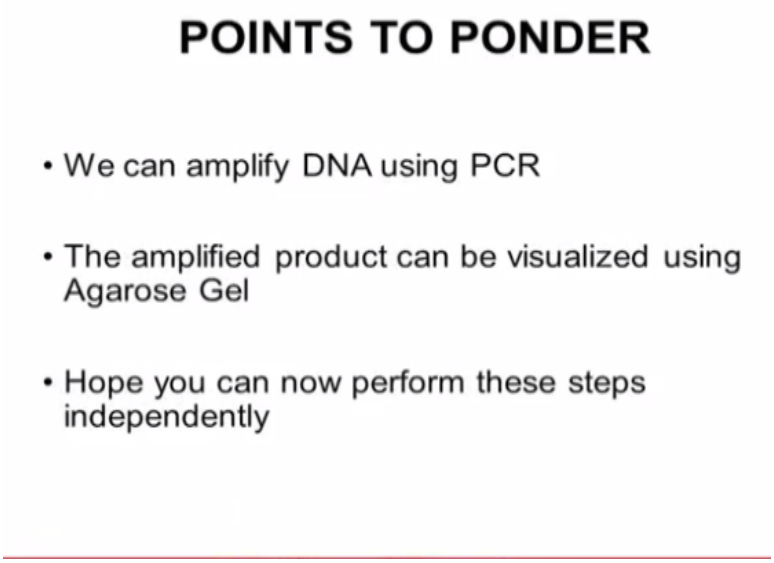

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Let us watch the following animation to understand this concept better.

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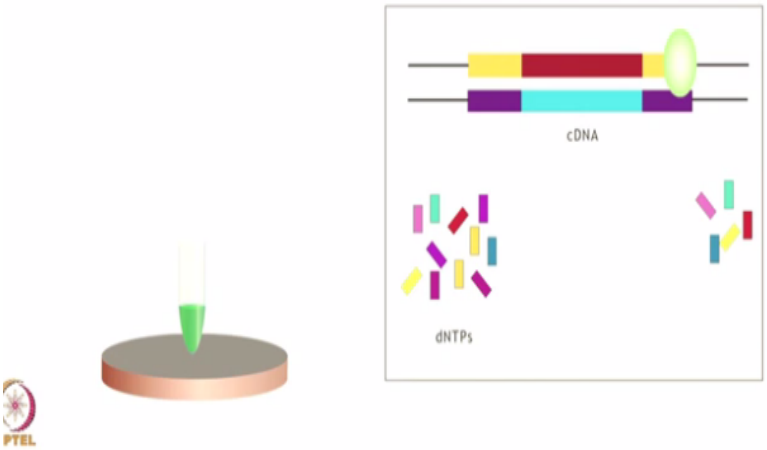

Reverse transcription PCR is used to generate multiple copies of DNA with RNA as a starting material. The template RNA molecule is first reverse transcribed into the corresponding cDNA by means of the enzyme reverse transcriptase, this enzyme which is commonly found in viruses is capable of synthesizing DNA from an RNA template.

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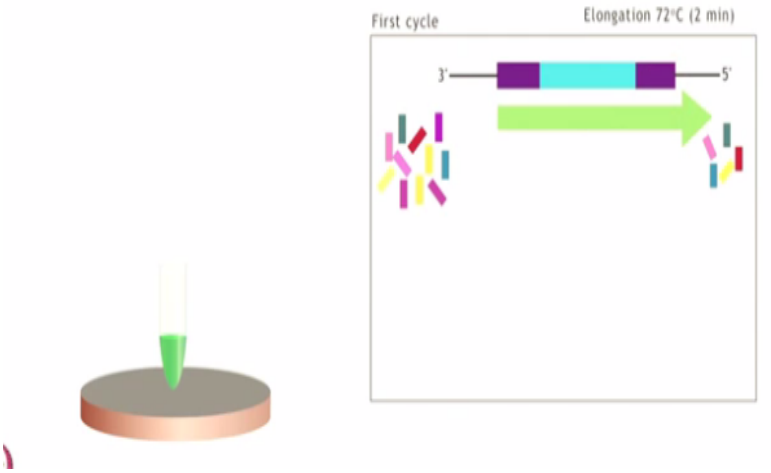

Template strand at 72.

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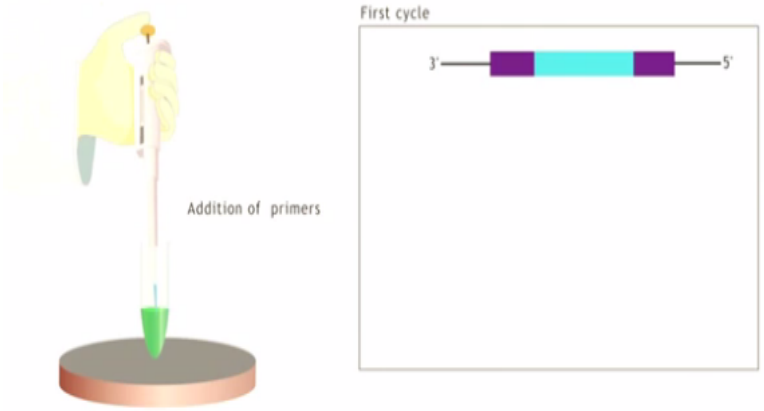

Traditional PCR is then performed on the cDNA obtained by addition of primers which are allowed to anneal at 54 degrees centigrade.

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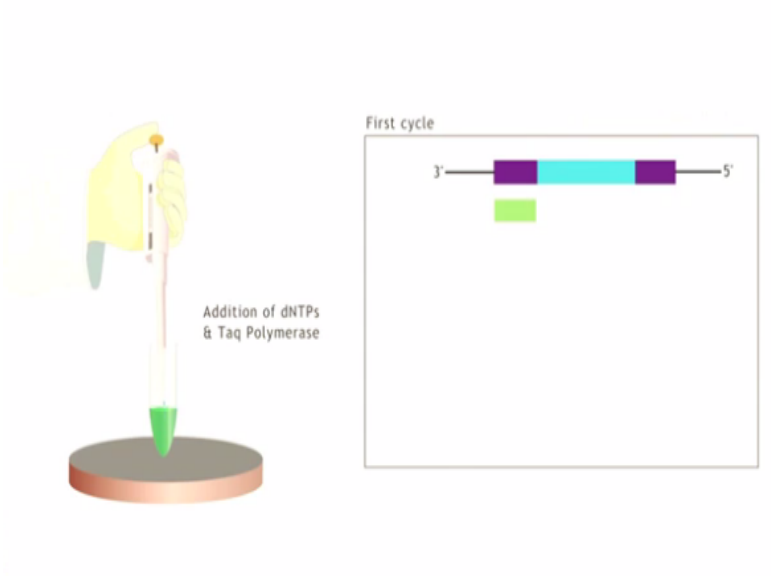

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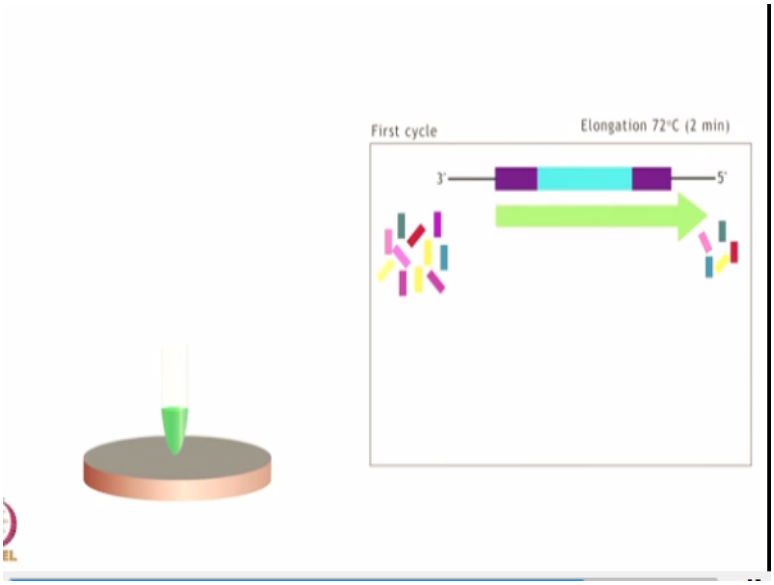

This is followed by addition of nucleotides and taq polymerase which performs elongation of the template strand at 72 degrees centigrade.

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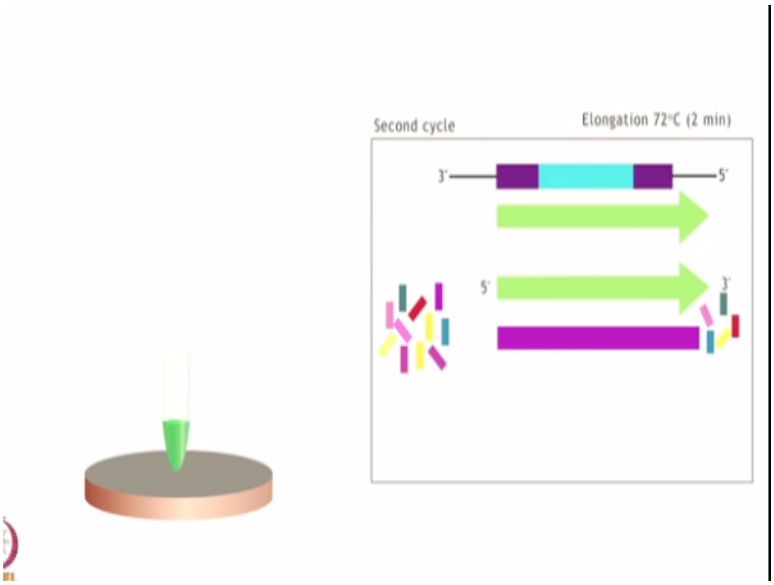

Second and subsequent rounds of PCR result in further amplification of the cDNA of interest strand separation is performed at 95 degrees centigrade followed by primer annealing and elongation respectively in this way, the new mRNA transcript originally used is amplified in the form of its corresponding cDNA, which can then be studied further.

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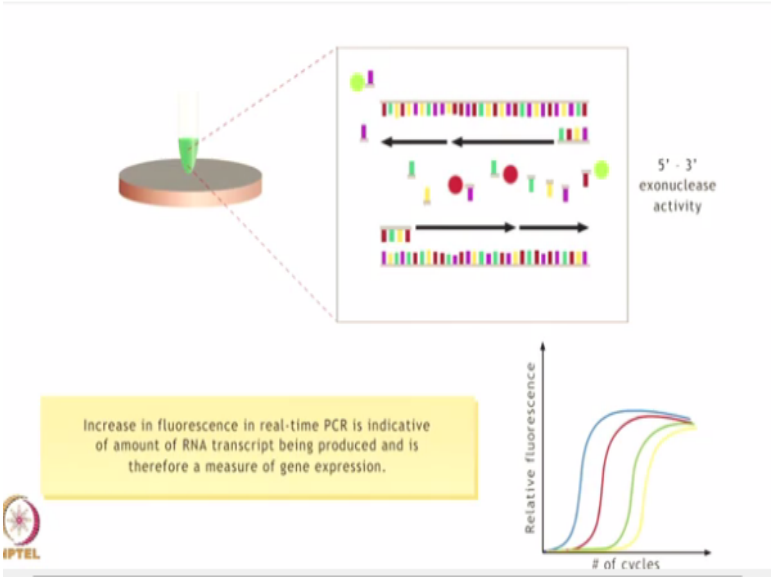

The double stranded DNA that needs to be amplified is heated to 95 degrees centigrade to bring about strand separation, once the strands are separated primers are added along with the probe DNA molecules which have the quencher and reporter molecule bound to its ends. Once these have annealed to the template DNA strands at 54 degrees centigrade, taq polymerase and

nucleotides are added and the temperature is again increased to 72 degrees centigrade to carry out strand elongation.

The taq polymerase continues to elongate the DNA strand based on the corresponding template DNA, when it reaches the bound probe molecule, the 5 prime to 3 prime exonuclease activity of taq polymerase degrades the probe into its nucleotide fragments and continues to elongate the DNA strand. The released reporter dye thus gets separated from the quencher molecule during this process and the fluorescence emitted can then be detected using a suitable detector.

The increase in fluorescence in real time PCR is directly indicative of the amount of nucleotide being synthesized and is therefore, a useful tool for measuring gene expression. I hope know your concepts for the tools which are used for the amplification and visualization of DNA is clear now. In today's session, we have just got a glimpse of how to perform agarose gel electrophoresis and polymerase chain reaction.

And actually, whole experiment takes a long time but to give you a demo we have done the things in a very short time but you can definitely, think about that you know in which way now you can use these particularly you know the simple tools to design your experiments and how you can do the molecular biology to understand some specific biological concepts when it comes to the research areas, you can now start planning those experiments.

And these tools are actually much simpler which you can easily implement in the lab settings, I hope you have enjoyed the lab session, thank you.