Molecular Biology

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Module - 12

Applications of Molecular Biology

Lecture-51 Applications of Molecular Biology (Part 3)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT Guwahati and what we were discussing? We were discussing about the different aspects of the molecular biology in this particular course. So, so far what we have discussed? We have discussed about the basic cell biology, we have discussed about the biochemistry of the different biomolecules and then we also discussed about the central dogma of molecular biology followed by we have discussed about the different types of techniques such as the blotting techniques and we have also discussed about the polymerase chain reactions, real time PCR and in the previous module we have also discussed about the genome editing. Now in current module we are discussing about the application of the molecular biology and if you recall in the previous lecture we have discussed about the how the genetic engineering and the transgenic animals are been you know are been used for the different types of applications and how the molecular biology basic principles are been used to perform the genetic engineering and how the genetic engineering in turn will lead to the production of the different types of products. Whether these products are the enzymes or the different types of proteins which is required for correcting the different types of disorders and along with that we have also discussed about that how the genetic engineering lead to the discovery of the different types of transgenic animals and these transgenic animals are mimicking the disease models they are also been used for the protein production they are also been used for various other applications. So, all these we have discussed in the previous lecture.

In today's lecture we are going to discuss about the PCR based applications and we are also going to discuss about the genome editing. So, let us start our discussion about the PCR based applications. So, in the PCR based applications we are going to discuss about the application of the PCR and as well as the real time PCR or RT-PCR. So, let us start our lecture and discuss about the application of the PCR application of the PCR and as well as the real time PCR.

If you recall when we were discussing about the PCR we have discussed in detail about how you can be able to design the primers, how you can be able to perform the PCR and so on. So, it is better if you can actually be able to briefly go through with those lectures. So, that it will be easier for you to understand the application part. So, in today's lecture we are

going to discuss about the PCR based applications. So, we are going to discuss about the PCR based applications and we are also going to discuss about the RT-PCR based applications.

So, let us start about the PCR based applications. So, when we talk about the PCR based application it is highly recommended that you should go through with the previous lectures. So, that it will be easier for you to follow these content because we are not going to discuss about how you can be able to design the primer, how you can be able to perform the PCRs and so on. So, let us start discussing about first the application of the PCR and then we are also going to discuss about the application of the real time PCR. So, when we talk about the PCR application of the PCR, the application of the PCR lies into the three major components or major areas.

First is the molecular identification, the second is the sequencing and the third is the genetic engineering ok. And when we talk about the molecular identifications that is having the in homeliness applications in the different types of fields. For example, it can be in the molecular archaeology, molecular epidemiology, molecular ecology, DNA fingerprinting, it is also being used for the classification of the different types of organisms. So, it is also being used for the genotyping, prenatal diagnosis, mutation screening, their discovery, genetic matching and as well as the detection of the pathogen. Now when we talk about the sequencing it is also being used for the bioinformatics purposes, genetic cloning and as well as the it is been extensively been used in the human genome project.

And as well as the genetic engineering is concerned it is been used for the site directed mutagenesis and as well as for the gene expression studies. So, let us first start with the first aspect and then we will follow the these are the two aspects. So, PCR is extensively been used in the detection of the different types of reagents or agents in the food science. So, PCR is a rapid and sensitive method that enable the detection of the subdominant population of food without the need of the enrichment media. It allows the detection of the dead cells and the non-cultivate cells which means it is actually going to be used for even assessing the quality of the product.

And then the also been used for detection of the different types of microbes for example, the major infectious organism what is present in the food is the salmolar species. So, in the salmolar when you want to detect the salmolar you can actually be able to detect the INVA enzyme and it can be used for the detection and that can be done with the and it is having a application in the terms of the artificially contaminated the chicken meat or the salmon or raw milk and all that. Similarly, you can have other species of the salmolar which are also going to be detected into the food material and that is having a very huge application because it actually helps in getting the salmolar infection. And salmolar is a very very deadly bacteria. So, it actually can cause the food poisoning it also can lead to the death of the human being.

Then we also have the Listeria monocytogenesis Listeria for detecting the Listeria you are going to use the 16 rRNA sequences and it is going to be used for the detection and the quantifications and it is going to be used for detection of this Listeria in the different types of vegetables like cabbage, lettuce and parsley and the onion branches. Then we also have the Stellophagus aureus that is so, for detection of these Stellophagus you are going to use the NUC gene and it is also been used in the majority of these products are being used for detection of the beef or the other meat products. Then we have the enterobacteria we have E. coli we have bacillus celus groups then we have the you know viable bacteria and norovirus. The norovirus is a virus which actually causes the stomach flu in the kids and for detecting the norovirus you can actually be able to use the ORF1 as the gene sequence and you can be able to detect the contamination of the cheese or the lettuce.

Then we also can detect the hepatitis A virus which is actually the leading cause of the hepatitis in the through the food borne hepatitis and you can actually be able to detect using this with the VP1 to VP3 capsid region that gene and it can be used for detecting of the artificially contaminated the tomato sauce or the blended strawberries. So, PCR has a extensive application in the medical sciences. So, the use of PCR in the medical science already began in the year of 1985 and a viable test for measuring the amount of HIV in the blood was published in the May 1987 by the John at CITAS. In 1989 the team has developed the multiplex PCR and amplification of a single cell was performed for the first time on a single sperm cell to directly analyze the product of meiotic recombination and was also applied to another target cells like the human mucocyte antigen DQ alpha. The PCR technology has become an essential research and diagnostic tool for improving the human health and the quality of light.

It allows the detection of the infectious organism just from one cell by amplifying the specific region of the genetic material. The PCR is very extensively been used for detection of the infectious organisms such as it can be used for detecting of the HIV, hepatitis B and C, human papilloma virus, then we also have the Cladiminus tracheodormis, then the ceria and the cytomegalase virus or the CMB virus and it also can be used for detection of the mycobacterium tuberculosis. Majority of these viruses or the bacterial species are known to cause the different types of disease. For example, the hepatitis B and C is causing the liver cirrhosis and the cancer. Similarly, human papilloma virus which can lead to the cervical cancer, then we have this cladomyelitis which can result in the infertility in the woman, then we have an a ceria which cause the pelvic inflammatory disease, then we have the mycobacterium tuberculosis which causes the tuberculosis.

And then how you are going to do the infection or detection by the PCR, what you are going to do is you are going to draw the blood of the patient and then you are going to perform the PCR with the help of the target gene, the primer designed for the target gene and then you are going to analyze it onto a gross gel and then you are going to make it with the marker. So, it is actually going to say whether if the product is being formed, then you are going to say that this particular sample is actually contaminated with this particular

infectious disease, whereas you are also going to run a control sample where you are going to have the healthy individual. And so this based using this particular type of technique, you can be able to do the blood screening and you can be able to detect the different types of diseases like the hepatitis, B and C and HIV and all that. So, and then we also can use the PCR in the plant science. So, there are various fields in plant science which require the use of the PCR technology for its accomplishment.

One of the major area where the PCR in be used for plants species identifications. So, the PCR technique has also been employed in the identification of the plant species using the species and group species primer targeting the chloroplast DNA. These assay allow the identification of the plant based on the size specific amplicon. For example, plant belonging to the same family has closed primer binding site and hence same amplicon sites where the plant belonging to the different species and groups have a different primer binding site and hence will result in the different amplification site. For example, in this case what you see here is that all these species like S1 to S2 and 10, they are very close because their amplicon size is very small or their amplicon size is similar.

Whereas in this case, when the amplicon are widely distributed, they may not be they may not be closer enough right. And that is why when you do the PCR, you are going to see the amplicons coming from the S11 to S20 different and that can be used very nicely to know which one is the close relative and which one is the distant relative. Then we also can study the PCR for detecting the plant microbe interactions. So, in this particular case, you are going to use the PCR and as well as the RT-PCR techniques and you can be able to detect the different types of pathogens which are associated with the particular crop. For example, the clavibacter which is associated with the potato, then we also have the acidovorous which is actually associated with the watermelon and so on.

So, you can actually be able to take the plant sample and then you actually can do the PCR for these infectious organisms. Then you also can use the PCR in the tissue culture. So, you can actually be able to use the PCR for detecting the different stages of the tissue culture species. So, the use of PCR in tissue culture was already reported in the year of 1992. It was used in the analysis of DNA and a specific gene in the plant cell at the different stages of regeneration during in vitro culture along with the RAPD or the random amplification of the polymeric DNA technology.

The level of polymorphism in the regenerated plant could be revealed by these two technology. PCR could flawlessly amplify the neomesin phosphatase gene and antibiotic which is used for the selective marker into the transgenic animals. And the PCR is also been used in the veterinary parasitology. So, it is a been used for detecting the different types of parasites which are been present on to the animals. For example, you can actually be able to use the Ozuski disease virus of the pigs and this can be detected.

Then we can also use the bovine leukemia virus or BLV. This virus causes the zoonotic

bovine, bovine leukosis and PCR assay for detection was developed in the year of 1991. Then we also can use the virus diarrheal virus or the bovine viral diarrhoea virus. This virus is not only fatal to the cattle, but also causes the contamination in the calf serum used in the cell culture work thus leading to the contamination of the vaccine and the pharmaceutical products. Besides the above example, the PCR has been used in the routine diagnosis of the veterinary viruses such as the porcine, parvovirus, bovine, papilloma virus and avian, polymya virus, chicken anemia, duck hepatitis and so on.

So, PCR is having an extreme potential in detecting the different types of diseases. Then PCR can also be used into the forensic science. So, for example, the criminal investigations. So, each individual has a different DNA profile known as the DNA fingerprinting. A DNA fingerprinting uses the variable number of tandem repeat or VNTR loci as these loci is so variable that the unrelated individuals are unlikely to have the same VNTRs which means the VNTRs pattern of a VNTR is a specific to the particular person not to particular family ok.

So, a sample of the blood, hair root or the tissue left in the crime scene 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 or defending the people originally convicted. So, you can imagine that you have actually you have collected the crimes in DNA and these are the some of the pattern what you got. So, these are the VNTRs what are present and then you can actually be able to do the similar PCR with the different types of suspects. So, you actually know which are actually going to be the prime suspects of or the suspects which were present at the crime site and then what you can do is you can actually be able to do the analysis.

So, what you see here is that the suspect one is actually having a VNTR pattern which is very different. So, for example, in the crime scene DNA what you got you have the VNTR like 1, 2, 3, 4, 5 and 6. Now 1 is correct. So, if you draw the line you will see that the 1 is present in this one 2 is not present in this one 1 is present in this one and 1 is slightly present in this one also. Then 2 is present in this one, but 2 is absent in this one, but 2 is present in this one suspect number 3.

new. then the nu3, nu3 is absent in the suspect number 1 nu3 is present in the suspect number number 2 and nu3 is also present in the suspect number 3 and nu3 is also present in the suspect number 4 which is also absent in suspect number 1. So, suspect number 1 will become the innocent because the majority of those bands are not present and suspect number 2 also does not have. So, it is also going to be innocent whereas, the band number 4 is also present in the suspect number 3, band number 5 is also present in the suspect number 5, suspect number 3 and the band number 6 is also present in the suspect number 3. So, this means the this particular person is actually his DNA profile is matching with the crime scene DNA what you have isolated. This means the suspect number 3 is actually a

person who may be involved in this particular type of criminal activity, he may be the convict and he may be the present at the crime site.

So, that actually can be proved and then you can actually be do the further investigations. Then the you can also be able to use the PCR for the parental testing. So, similar kind of approach. So, PCR technology also being in finding the biological parents of the adopted or the kidnapped child where the DNA of a child is matched with the close relative. The actual biological father of a newborn can also be ruled out.

In parental testing the short tandem repeat or STRs are being used as a marker where each person's DNA copy contain the two copy of these markers are often from the father and mother. These markers differ in the length and sometime the sequences. So, DNA marker you can actually be able to have like some of these markers like A, B, C, D, E and the mother and the father will you can test the different types of mothers and you can actually be able to test the different types of probable fathers and mothers and all that. So, in the child the STR what is present in 26 and 30 right and that is the combination of this mother and this father because from the this mother it has taken the 26 and from the father it has taken the 30. So, that is why this is these are the potential father and mother.

Similarly, in the mother we have the 8 and 9 and it has taken the 9 from here right. Similarly, from the here it has taken the 10 actually. So, that is all what you see here is that the child's profile is a combination of mother and father right and that is why this child belongs to this particular mother and father. I am not showing you the more samples or the data from the different samples and so on. So, another sensitive technique that can be used to establish the maternal relationship between the people is called as the mitochondrial DNA analysis which relies on the PCR.

This analysis is better than the fingerprinting for the sample which belongs to too old that are nucleus of the cell get degraded. So, in the some cases you are actually going to analyze the mitochondrial DNA because the mitochondrial DNA remain constant and it runs without getting diluted from the single family. The only difference is that the mitochondrial family mitochondrial DNA analysis will tell you who will be the mother and it is actually going to only follow from the mother side ok. So, it is actually going to tell you who will be the mother, who will be the grandmother, who is going to be the grandmother to mother and so on. So, because of the simple reason that the mitochondrial DNA is always coming from the female side it does not come from the father side.

Then, we also can use the PCR in the research applications. So, PCR can be used for the DNA cloning, PCR can be used for the sequencing, PCR can be used for the sequence tag sites, PCR can be used for the phylogenetic analysis and PCR can be used for the gene expression analysis. So, in the phylogenetic analysis you are going to take the relationship between the different types of organisms. So, this is all about the application of the PCR into the application of the PCR. Now let us move on and we will discuss about the

application of the real time PCR.

So, application of the real time PCR is also very diversified and it is as big as the PCR because it can be used for the gene expression profiling, it can be used in the drug discovery, it can be used for the disease diagnosis and management, the PCR RT-PCR can be used even for the viral quantifications, food testing, GMO food, it can be used for detecting the gene expression as well as the gene copy number and it also can be used for the cancer research. So, let us discuss first about the diagnostic part because that is the major area where the real time PCR is always been used and you might heard about the real time PCR when we were talking about the COVID-19 screening and detect diagnostics. So, let us take an example of the detection of the COVID virus or coronavirus during the COVID-19. So, COVID-19 is an infectious disease caused by the SARS-CoV-2 virus and the common symptom include the high fever, cough and shortness of the birth. So, how to test the SARS-CoV-2? So, what you are going to do is I am sure you might have seen all these steps in television and other places that you are going to collect the sample from the cotton swab or from the otoforangial swab.

So, you are going to collect the sample from the nose or from the mouth. So, that sample will contain the virus and it is going to contain the viral RNA because the COVID is a RNA virus. So, swab is collected in the test tube containing the viral transport media or VTM. Then in the step 2, you are actually going to do you are going to extract the RNA using any of those method what we have discussed you can use actually the RNA extraction method and then you are going to get the purified RNA. Then in the step 3, you are going to connect you are going to you know convert that RNA into the complementary DNA.

So, you are going to make the complementary DNA strands. So, then the CADNA tag with the fluorescence for the performing the real time PCR and then you are going to do a real time PCR with the help of the primers targeting to the COVID virus and then you are going to do the analysis. And what you are going to analyze? You are going to analyze the CT values and it a positive value is actually a CT value beyond a certain copy number is going to tell you that the sample is positive whereas, if the sample is below the baseline then it is going to say the negative. So, this is the step sample is collected from the nasopharyngeal swab and collected in a tube containing the viral transport media. The collected sample are processed to extract the RNA it is the most crucial step in the whole process.

Then obtain the real time PCR kit specifically dedicated for detecting the SARS COVID 2 that include the primer and a probe designed to target the specific region of the viral genome. Then prepare the real time extraction media by adding the extracted RNA primer probe and other necessary component as guided by the manual. Then run the real time PCR this cycle will go to the series of changes in the temperature which lead to the amplification of the viral RNA which it is present in the collection samples. And then the probe which are mixed with the reaction mixture will emit the fluorescence then it binds to the viral RNA and the PCR machine will monitor the fluorescence signal. The real time PCR

machine continuously measure the fluorescence and record the cycle at which the cycle signal crosses the preset threshold and this is called as the threshold cycle or the CT value.

The CT value provide the information about the concentration of the viral RNA present in the collected sample. Lower the CT value high will be the viral concentration higher will be the CT value the lower will be the viral concentrations. A positive result is typically been determined by comparing the CT value with the predetermined the threshold value. If the CT value falls below the threshold value of the sample then it is considered as the SARS COVID 2 or it is going to be called as the positive for the COVID 19. Quality control include the positive and negative control to check for the contamination and to the validate the results.

Apart from that you can also do the gene expression analysis. So, I am sure when we were discussing about the real time PCR we have shown you a complete demo how you can be able to do the gene expression profiling. So, this we are not going to discuss in detail like where in this one the real time PCR is a powerful technique to analyze the gene expression and in a biological sample. So, what you are going to do is you are going to do a cDNA synthesis, you are going to do the PCR amplification, you are going to analyze the sample to know what is the threshold value then you are going to do a quantification and ultimately in the step 5 you are going to do the data analysis. And this all we have discussed in detail when we were discussing about the real time PCR both theoretically and as well as the experimentally where the students have shown you how to perform the analysis part, how to perform the cDNA synthesis and so on.

Then you can also be able to do the gene copy number. So, utilizing the similar kind of approach you can also be able to do the gene copy number and you can be able to detect the gene copy number utilizing the real time PCR. Then we can also be able to use the real time PCR for detecting the GMOs what is present in the particular or GMOs present in the particular sample and there are multiple ways and in which you can be able to do that. So, in this one what you are going to do is you are going to collect the sample right which is including any GMO sequences and then you design the gene primer which is a specific to the selected gene sequence that are unique to the GMO of the interest. Then you are going to perform the real time PCR reactions and then you are going to do the amplification and detection. Ultimately you are going to know the CT values and if there will be a CT value beyond the threshold then it is actually going to say that the particular genetically modified organism sequences are present in your DNA.

Then you can also use the real time PCR for the cancer cell detections. So, quantifying the cancer cell using the real time PCR involves detection and measurement of a specific gene marker associated with the cancer and this is been published in one of the such article which is called as BMC cancer where they have used the gene expression profiling of circulating tumor cell in the breast cancer by the RT-PCR. So, here also you are going to take the sample you are going to collect the samples then you are going to do the gene marker

selection. So, you are going to select the gene which is specific only for the cancer cells then you are going to design the primers and probes and all that and then you are going to perform the real time reactions then you are going to analyze the data and you are going to say whether the CT value is above the threshold or not and then based on this data you can be able to say whether the particular sample has a cancer cell or not. So and then real time PCR is also been used for the drug discovery.

So, it can actually be able to use for the target identification, pharmacogenomics, biomarker analysis, gene expression profiling, toxicity assessment, pharmacokinetics and pharmacodynamics and assay development and as well as the high-to-fruit screening. So, this is very extensively been you know exploited the real time PCR and as well as the PCR for detect for doing the many types of you know applications. And what we have discussed we have discussed only the application which are actually very very popular right for example, the detection of the COVID and all those kind of things, but that is not the limit of these techniques. These techniques are having the enormous potentials to be used and they can be exploited in a multiple ways in which to know the particular type of. So, apart from the their contribution in the clinical practices or diagnostics purposes they are also having the enormous applications in the basic sciences and so on.

So, all those we have not discussed in detail because then it becomes a very very extensive lecture and it may not be possible to discuss all those in the in a single lecture or in this particular course. So, with this I would like to conclude my lecture here in our subsequent lecture we are going to discuss about the application of the genome editing and how the genome editing tools what we have discussed in the previous module and how they can be used and exploited for the human welfare. So, with this I would like to conclude my lecture here in a subsequent lecture we are going to discuss some more applications of the molecular biology. Thank you. .