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Biochemistry, Polymerase Chain Reaction (PCR)

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Introduction

Polymerase chain reaction (PCR) refers to a technique employed widely in the basic and biomedical sciences. PCR is a laboratory technique utilized to amplify specific segments of DNA for a wide range of laboratory and/or clinical applications. Building on the work of Panet and Khorana's successful amplification of DNA in-vitro, Mullis and coworkers developed PCR in the early 1980s, having been met with a Nobel prize only a decade later. Allowing for more than the billion-fold amplification of specific target regions, it has become instrumental in many applications including the cloning of genes, the diagnosis of infectious diseases, and the screening of prenatal infants for deleterious genetic abnormalities.

Fundamentals

The main components of PCR are a template, primers, free nucleotide bases, and the DNA polymerase enzyme. The DNA template contains the specific region that you wish to amplify, such as the DNA extracted from a piece of hair for example. Primers, or oligonucleotides, are short strands of DNA complementary to the 3' end of each target region. Both a forward and a reverse primer are required, one for each complementary strand of DNA. DNA polymerase is the enzyme that carries out DNA replication. Thermostable analogues of DNA polymerase I, such as Taq polymerase, which was originally found in a bacterium that grows in hot springs, is a common choice due to its resistance to the heating and cooling cycles necessary for PCR.[1]

PCR takes advantage of the complementary base pairing, double-stranded nature, and melting temperature of DNA molecules. This process involves cycling through 3 sequential rounds of temperature dependent reactions: DNA melting (denaturation), annealing and enzyme-driven DNA replication (elongation). Denaturation begins by heating the reaction to about 95 C, disrupting the hydrogen bonds that hold the two strands of template DNA together. Next, the reaction is reduced to around 50 to 65 C, depending on the physicochemical variables of the primers, enabling annealing of complementary base pairs.[2] The primers, which are added to the solution in excess, bind to the beginning of the 3' end of each template strand and prevent re-hybridization of the template strand with itself. Lastly, enzyme-driven DNA replication begins by setting the reaction temperature to the amount which optimizes the activity of DNA polymerase, which is around 75 to 80 C. At this point, DNA polymerase, which needs double-stranded DNA to begin replication, synthesizes a new DNA strand by assembling free-nucleotides in solution in the 3' to 5' direction to produce 2 full sets of complementary strands. The newly synthesized DNA is now identical to the template strand and will be used as such in the progressive PCR cycles.

Given that previously synthesized DNA strands serve as templates, the amplification of DNA using PCR increases at an exponential rate, where the copies of DNA double at the end of each replication step. The exponential replication of the target DNA eventually plateaus around 30 to 40 cycles mainly due to reagent limitation, but can also be due to inhibitors of the polymerase reaction found in the sample, self-annealing of the accumulating product, and accumulation of pyrophosphate molecules.[3]

Real-Time PCR

At its advent, PCR technology was limited to qualitative and or semi-quantitative analysis due to limitations on the ability to quantitate nucleic acids. At that time, to verify if the target gene was amplified successfully, the DNA product was separated by size via agarose gel electrophoresis. Ethidium bromide, a molecule that fluoresces when bound to dsDNA, could give a rough estimate of DNA amount by roughly comparing the brightness of separated bands, but was not sensitive enough for rigorous quantitative analysis.

Improvements in fluorophore development and instrumentation led to thermocyclers that no longer required measurement of only end-product DNA. This process, known as real-time PCR, or quantitative PCR (qPCR), has allowed for the detection of dsDNA during amplification. qPCR thermocyclers are equipped with the ability to excite fluorophores at specific wavelengths, detect their emission with a photodetector, and record the values. The sensitive collection of numerical values during amplification has strongly enhanced quantitative analytical power.

There are two main types of fluorophores used in qPCR: those that bind specifically to a given target sequence and those that do not. The sensitivity of fluorophores has been an important aspect of qPCR development. One of the most effective and widely used non-specific markers, SYBR Green, after binding to the minor groove of dsDNA, exhibits a 1000-fold increase in fluorescence compared to being free in solution. However, if even more specificity is desired, a sequence-specific oligonucleotide, or hybridization probe, can be added, which binds to the target gene at some point in front of the primer (after the 3' end). These hybridization probes contain a reporter molecule at the 5' end and a quencher molecule at the 3' end. The quencher molecule effectively inhibits the reporter from fluorescing while the probe is intact. However, upon contact with DNA polymerase I, the hybridization probe is cleaved, allowing for the fluorescence of the dye.

Reverse-Transcription PCR

Since its advent, PCR technology has been creatively expanded upon, and reverse-transcription PCR (RT-PCR) is one of the most important advances. Real-time PCR is frequently confused with reverse-transcription PCR, but they are separate techniques. In RT-PCR, the DNA amplified is derived from mRNA by using reverse-transcriptase enzymes, DNA polymerases expressed by RNA-containing retroviruses, to generate a complementary DNA (cDNA) library. Using primers sequences for genes of interest, traditional PCR methods can be used with the cDNA to study the expression of genes qualitatively. Currently, reverse-transcription PCR is commonly used with real-time PCR, which allows one to quantitatively measure the relative change in gene expression across different samples.

Issues of Concern

One disadvantage of PCR technology is that it is extremely sensitive. Trace amounts of RNA or DNA contamination in the sample can produce extremely misleading results. Another disadvantage is that the primers designed for PCR requires sequence data, and therefore can only be used to identify the presence or absence of a known pathogen or gene. Another limitation is that sometimes the primers used for PCR can anneal non-specifically to sequences that are similar, but not identical, to the target gene.[4]

Another potential issue of using PCR is the possibility of primer dimer (PD) formation. PD is a potential by-product and consists of primer molecules that have hybridized to each other due to the strings of complementary bases in the primers. The DNA polymerase amplifies the PD, leading to competition for PCR reagents that could be used to amplify the target sequences.[5]

Clinical Significance

PCR amplification is an indispensable tool with various applications within medicine. Often, it is used to test for the presence of specific alleles, such as in the case of prospective parents screening for genetic carriers, but it can also be used to diagnose the presence of disease directly and for mutations in the developing embryo. For example, the first time PCR was used in this way was for the diagnosis of sickle cell anemia through the detection of a single gene mutation.[6]

Additionally, PCR has greatly revolutionized the diagnostic potential for infectious diseases, as it can be used to rapidly determine the identity of microbes that were traditionally unable to be cultured, or that required weeks for growth.[7] Pathogens routinely detected using PCR include Mycobacterium tuberculosis, human immunodeficiency virus, herpes simplex virus, syphilis, and countless other pathogens. Moreover, qPCR is not only used for testing the qualitative presence of microbes but also to quantify the bacterial, fungal, and viral loads.[8]

The sensitivity of diagnostic tools for mutations to oncogenes and tumor suppression genes has been improved at least 10,000 fold due to PCR, allowing for earlier diagnosis of cancers like leukemia. PCR has also enabled more nuanced and individualized therapies for cancer patients. Additionally, PCR can be used for the tissue typing done that is vital to organ implantation and has even been proposed as a replacement for antibody-based tests for blood type. PCR also has clinical applications in the field of prenatal testing for various genetic diseases and/or clinical pathologies. Samples are obtained either via amniocentesis or chorionic villus sampling.

In forensic medicine, short pieces of repeating, highly polymorphic DNA, coined short tandem repeats (STRs), are amplified and used to compare specific variation within genes to differentiate individuals.[9] Primers specific for the loci of these STRs are used and amplified using PCR. Various loci contain STRs in the human genome, and the statistical power of this technique is enhanced by checking multiple sites.

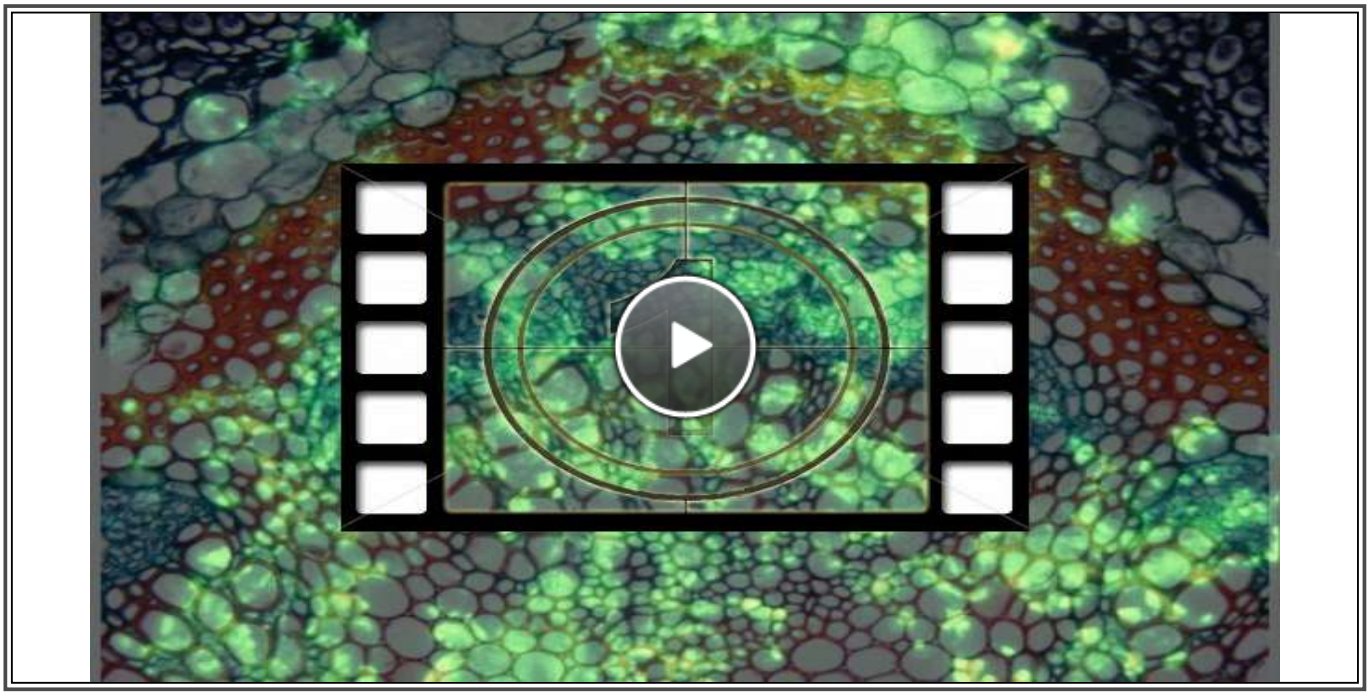
Questions

To access free multiple choice questions on this topic, click here.

References

1. Mullis KB. The unusual origin of the polymerase chain reaction. Sci. Am. 1990 Apr;262(4):56-61, 64-5. [PubMed: 2315679]
2. Wittwer CT, Herrmann MG, Moss AA, Rasmussen RP. Continuous fluorescence monitoring of rapid cycle DNA amplification. 1997. BioTechniques. 2013 Jun;54(6):314-20. [PubMed: 23905170]
3. Ishmael FT, Stellato C. Principles and applications of polymerase chain reaction: basic science for the practicing physician. Ann. Allergy Asthma Immunol. 2008 Oct;101(4):437-43. [PubMed: 18939735]
4. Smith CJ, Osborn AM. Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. FEMS Microbiol. Ecol. 2009 Jan;67(1):6-20. [PubMed: 19120456]
5. Brownie J, Shawcross S, Theaker J, Whitcombe D, Ferrie R, Newton C, Little S. The elimination of primer-dimer accumulation in PCR. Nucleic Acids Res. 1997 Aug 15;25(16):3235-41. [PMC free article: PMC146890] [PubMed: 9241236]
6. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. 1985. Biotechnology. 1992;24:476-80. [PubMed: 2999980]
7. Mackay IM. Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. 2004 Mar;10(3):190-212. [PubMed: 15008940]
8. Muldrew KL. Molecular diagnostics of infectious diseases. Curr. Opin. Pediatr. 2009 Feb;21(1):102-11. [PubMed: 19242246]
9. Tamaki K, Jeffreys AJ. Human tandem repeat sequences in forensic DNA typing. Leg Med (Tokyo). 2005 Jul;7(4):244-50. [PubMed: 15939656]

Figures



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1. Steps of traditional PCR process 2. Non-specific fluorophore detection in qPCR 3. Specific hybridization probe detection in qPCR. Contributed by Mousa Ghannam

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