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A Short Note on Reverse Transcription Polymerase Chain Reaction and its Applications

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Description

In 1980s, Kary Mullis developed a revolutionary method called Polymerase Chain Reaction (PCR). PCR relies on the ability to use DNA polymerase to synthesize new DNA strands that are complementary to the provided template strand. Since DNA polymerase can only add nucleotides to the pre-existing 3'OH groups, primers are needed to add the first nucleotide. This requirement makes it possible to map specific regions of the template sequence that researchers want to amplify. The specific sequence will accumulate billions of copies (amplicons), at the end of the PCR reaction.

DNA sample that contains the target sequence is called DNA template. In order to separate the strands from each other at the beginning of the reaction, a high temperature is applied to the original double-stranded DNA molecule.

DNA polymerase is an enzyme that synthesizes a new strand of DNA complementary to the target sequence. Taq DNA polymerase (from *Thermis Aquaticus*), and Pfu DNA polymerase (from *Pyrococcus furiosus*) are the first and the most commonly used enzymes due to their higher fidelity in copying DNA. There are two functions that make them suitable for PCR even though these enzymes are slightly different. The reasons include: they can use DNA templates and primers to generate new DNA strands and they are heat resistant.

Primer is a small single-stranded DNA fragment that is complementary to the target sequence. The polymerase synthesizes new DNA from the ends of the primers.

Nucleotides (DNTP or Deoxy Nucleotide Tri-Phosphate) are a single unit of A, T, G and C bases. They are basically the "building blocks" of the new DNA chain.

RT-PCR (Reverse Transcription PCR) is a PCR that first uses reverse transcriptase to convert a sample of RNA into cDNA. Most PCR methods are based on thermal cycling. In especially DNA fusion and enzyme-driven DNA replication, the thermal cycling expose reagents to repeated cycles of heating and cooling to allow for different temperature-dependent reactions. PCR uses two main reagents: primers (they are short pieces of single-stranded DNA, called oligonucleotides, which are sequences complementary to the target DNA region) and DNA polymerase. In the first step of PCR, the two strands of the DNA double helix are physically separated at high temperature in a process called nucleic acid denaturation. The second step is to lower the temperature and combine the primers with complementary DNA sequences. The two strands of DNA are then converted into DNA polymerase templates and a new strand of DNA is assembled from free nucleotides (a component of DNA) enzymatically. In order to start a chain reaction, the generated DNA itself is used as a replication template to start the chain reaction as PCR progresses, in which the original DNA template is exponentially amplified.

The PCR reaction begins to generate copies of the target sequence exponentially. Only in the exponential phase of the PCR reaction is it possible to extrapolate to determine the initial amount of target sequence contained in the sample. Due to the inhibitors of the polymerase reaction found in the sample, the limitations of the reagents, the accumulation of pyrophosphate molecules, and the self-cyclization of the accumulated products, the PCR reaction eventually stopped amplifying the target sequence to an exponential speed, forming a "platform" effect. Make PCR products an unreliable quantitative endpoint. This is the characteristic of PCR, which makes quantitative real-time RT-PCR necessary.

Applications

Applications of this technology include DNA cloning, cloning and manipulation, genetic sequencing of genetic mutations; DNA-based phylogenetic construction or genetic function analysis; diagnosis and monitoring of genetic diseases; ancient DNA amplification; fingerprints for DNA analysis (eg; forensic and paternity testing); detection of pathogens in nucleic acid tests to diagnose infectious diseases.

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