

## **Reverse transcription polymerase chain reaction: Mechanism and limitations**

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### **Description**

In 1980s, Kary Mullis developed a revolutionary method called Polymerase Chain Reaction. (PCR Reverse transcription polymerase chain response (RT-PCR) is a laboratory method combining opposite transcription of RNA into DNA and amplification of unique DNA goals the usage of polymerase chain response (PCR). It is mainly used to degree the quantity of a particular RNA. This is completed in means of tracking the amplification response the usage of fluorescence, a way known as real-time PCR or quantitative PCR (qPCR). Combined RT-PCR and qPCR are robotically used for evaluation of gene expression and quantification of viral RNA in studies and scientific settings.

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.

### **Mechanism of reverse transcriptase**

DNA polymerase is an enzyme that synthesizes a new strand of DNA complementary to the target sequence. Taq DNA polymerase (from *Thermis Aquaticus*), and PfuDNA 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: 1) they can use DNA templates and primers to generate new DNA strands, and 2) they are heat resistant.

Reverse transcription coupled to the polymerase chain reaction (RT-PCR) is usually used to come across the presence of mRNAs, pre-mRNAs, or different styles of RNA

together with noncoding RNAs. The technique includes the usage of a primer annealed to the RNA. For mRNA, the primer is often a artificial oligo(dT)15-18, a random hexamer mixture (dN)6, or a artificial DNA oligonucleotide this is complementary to a particular transcript (a gene-unique primer). This DNA:RNA hybrid serves as a template all through opposite transcription, where in the enzyme opposite transcriptase (RT) generates a single-stranded cDNA reproduction of a part of the goal RNA molecule cDNA copies of sequences from the complete duration of the mRNAs and pre-mRNAs in a population. On addition of gene-unique primers, a particular DNA fragment similar to a part of the RNA of hobby is generated.

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..

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### **Limitations of PCR and RT-PCR**

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.

### **Conclusion**

This technology includes 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 Analysis (eg; forensic and paternity testing); detection of pathogens in nucleic acid tests to diagnose infectious diseases.