Epidemiology: Open Access

Mini Review Open Access

A Brief Note on Retroviral Therapy

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Abstract

Reverse transcription is the process of creating complementary DNA (cDNA) from an RNA template using an enzyme known as a reverse transcriptase (RT). Eukaryotic cells use reverse transcriptases to extend the telomeres at the ends of their linear chromosomes, retrotransposon mobile genetic elements proliferate within the host genome, and viruses like HIV and hepatitis B use reverse transcriptases to replicate their genomes. Because transfers of information from RNA to DNA are explicitly held possible, the process does not violate the flows of genetic information as described by the classical central dogma. Retroviral RT has three sequential biochemical activities: ribonuclease H (RNase H), DNA-dependent DNA polymerase activity, and RNA-dependent DNA polymerase activity The enzyme is able to transform single-stranded RNA into double-stranded cDNA as a result of these activities taken together.

Keywords: Reverse transcription, Eukaryotic cells

Introduction

This cDNA can then integrate into the host genome in retroviruses and retrotransposons, allowing for the production of new RNA copies through host-cell transcription. In the laboratory, the same sequence of reactions is frequently used to convert RNA to DNA for use in molecular cloning, RNA sequencing, PCR, or genome analysis. There are three genome-sequenced and identified exogenous retroviruses in the Epsilonretrovirus genus, which includes two known types (WEHV-1 and WEHV-2) associated with walleye epidermal hyperplasia disease. The walleye epidermal hyperplasia viruses are two species of retroviruses that fall under the family Retroviridae. The neoplastic condition in the freshwater fish species North American walleye (Sander vitreus) has been confirmed to be caused by both viral types. The presence of retrovirus-like particles (observed with electron microscopy) and reverse transcriptase activity (measured with reverse transcriptase polymerase chain reaction methods) from neoplastic tissue support the particular association that retroviral infection has with proliferative lesions in fish. Even though both types of viruses have been found in fish lesions, only one virus lives in each cell of the infected tissue. The polymerase chain reaction (PCR) is a method that is widely used to rapidly make millions to billions of copies (complete or partial) of a specific DNA sample. This allows scientists to take a very small sample of DNA and amplify it (or a part of it) to a large enough amount to study in detail. Transmission studies have also shown that WEHV-2 has been the more proliferative agent of the condition than WEHV-1. American biochemist Kary Mullis created PCR at Cetus Corporation in 1983; In 1993, Mullis and biochemist Michael Smith were jointly awarded the Nobel Prize in Chemistry for their contributions to the development of additional important methods for manipulating DNA [1-5].

Many of the methods used in genetic testing and research, such as analyzing ancient DNA samples and identifying infectious agents, rely on PCR. In a series of temperature changes, copies of very small amounts of DNA sequences are exponentially amplified using PCR. The majority of PCR methods rely on thermal cycling, which is now a common and frequently necessary method in medical laboratory research for a wide range of applications, including biomedical research and criminal forensics. Reactants are subjected to repeated heating and cooling cycles during thermal cycling to enable various temperature-dependent reactions, particularly DNA melting and enzyme-driven DNA replication. Primers (short single-strand DNA fragments known as oligonucleotides that are a complementary sequence to the target DNA region) and a DNA polymerase are the two main reagents used in PCR.

Discussion

Nucleic acid denaturation is the physical separation of the two strands of the DNA double helix at a high temperature in the first step of PCR. The temperature is decreased in the second step, and the primers bind to the DNA's complementary sequences. After that, the two DNA strands serve as templates for DNA polymerase, which uses the enzyme to enzymatically assemble a new DNA strand from free nucleotides, which are the components that make up DNA. The generated DNA is used as a template for replication as PCR progresses, initiating an exponential amplifying chain reaction of the initial DNA template.

A heat-stable DNA polymerase like Taq polymerase, an enzyme originally isolated from the thermophilic bacterium Thermus aquaticus, is used in almost all PCR applications. The denaturation step's high temperatures would cause the polymerase used to denature if it was heat-susceptible. DNA cloning for sequencing, gene cloning and manipulation, and gene mutagenesis are all examples of the technique's applications. Prior to the use of Taq polymerase, DNA polymerase had to be manually added to each cycle, which was time-consuming and costly. the creation of DNA-based phylogenies or the study of genes' functions; monitoring and diagnosis of genetic diseases; amplification of ancient DNA; genetic fingerprint analysis for DNA profiling (for forensic science and parentage testing, for instance); and pathogen detection in nucleic acid tests for infectious disease diagnosis. By selectively amplifying a particular region of DNA, PCR permits the isolation of DNA fragments from genomic DNA. The productions of hybridization probes for northern or southern hybridization and DNA cloning, both of which require greater quantities of DNA to represent a

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Received: 04-Jan-2023, Manuscript No: ECR-23-86723; **Editor assigned:** 06-Jan-2023, Pre-QC No: ECR-23-86723 (PQ); **Reviewed:** 20-Jan-2023, QC No: ECR-23-86723; **Revised:** 23-Jan-2023, Manuscript No: ECR-23-86723 (R); **Published:** 30-Jan-2023, DOI: 10.4172/2161-1165.1000478

Citation: Marco V (2023) A Brief Note on Retroviral Therapy. Epidemiol Sci, 13: 478.

specific DNA region, are two examples of how this use of PCR enhances other methods. These methods can analyze DNA samples even from very small amounts of starting material because PCR provides these techniques with large amounts of pure DNA.

Isolation of a DNA sequence to expedite recombinant DNA technologies involving the insertion of a DNA sequence into a plasmid, phage, or cosmid (depending on size) or the genetic material of another organism, as well as DNA sequencing to determine unknown PCR-amplified sequences in which one of the amplification primers may be used in Sanger sequencing are two additional applications of PCR.

Throughout the course of a retrovirus's life cycle, there are three distinct replication systems. The reverse transcriptase synthesis of viral DNA from viral RNA is the first step, which results in the formation of new complementary DNA strands. When the integrated viral DNA is replicated by the host cell's DNA polymerase, the second replication process takes place. The proviral DNA is transcribed into RNA by RNA polymerase II, which is then packed into virions. Reverse transcriptase has a high error rate when transcribing RNA into DNA because, unlike the majority of other DNA polymerases, it lacks the ability to proofread. Mutations can occur during any one or all of these replication steps [6-10].

Conclusion

Mutations can accumulate more quickly than proofread forms of replication due to this high error rate. It has been hypothesized that the template switching activity of reverse transcriptase, which can be fully demonstrated in vivo, may have been one of the reasons for the discovery of several thousand unannotated transcripts in the genomes of model organisms. Promega's commercially available reverse transcriptases have error rates in the range of 1 in 17,000 bases for AMV and 1 in 30,000 bases for M-MLV, according to their manuals. In addition to producing single-nucle

In paternity testing, some PCR fingerprint methods have high discriminative power and can be used to identify genetic relationships between people, such as parent-child or siblings. When particular molecular clocks (such as microorganisms' recA and 16S rRNA genes) are utilized, this method can also be used to identify evolutionary relationships between organisms.

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