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SUMMARY: The polymerase chain reaction (PCR) is a powerful method for the rapid amplification of deoxyribonucleic acid (DNA). The PCR-technique was developed by Dr. K. B. Mullis for which he received the Nobel prize in 1993. The PCR-technique employs the enzyme DNA-polymerase to multiply selectively a single molecule of DNA several million folds in a few hours. It is finding a host of applications not only in fundamental molecular biological research, but also in medicine and clinical medicine particularly in clinical diagnostics.

Key Words: Polymerase chain reaction.

INTRODUCTION

The term polymerase chain reaction (PCR) is in everyday use now in the vocabulary of molecular biologists. The first published account of the PCR-technique came in 1985 through its application to the prenatal diagnosis of sickle-cell anemia (1). Although received with skepticism in 1985, it is now one of the most widespread methods of analyzing deoxyribonucleic acid (DNA). While giving an informative account of the PCR-technique, the discoverer of the technique, Dr. K. B. Mullis, said "Sometimes a good idea comes to you when you are not looking for it. Through an improbable combination of coincidence, naïveté and serendipity mistakes, such a revelation came to me on Friday April 1983, as I gripped the steering wheel of my car and smacked along a moonlit mountain road into northern California's redwood country" (2). The technique, which generates unlimited numbers of copies of specific DNA (either pure or impure) within a short time by a relatively simple enzymatic method, has transformed the study of DNA and ribonucleic acid (RNA).

Excellent reviews and specialist papers on PCR and its applications abound, but the authors of these papers usually assume a familiarity of the reader with molecular biology and its special terminologies and their introduction to the principles of PCR tend to become too technical for less specialized readers (3-5). Therefore, the main objectives of the present review are to a more general audience of biologists, medical scientists and clinicians.

PRINCIPLE AND KEY FEATURES OF THE PCR-TECHNIQUE

The PCR-technique is an in-vitro DNA amplification method by enzymic means at an exponential rate that involves a repeated cycling process composed of the following defined stages (1,5,6):

(a) The first stage is a denaturation step of the double-stranded DNA (ds-DNA) extracted from the material under study (target DNA). This involves increasing the temperature to 95-98°C for usually 60 seconds producing two single strands of DNA (ss-DNA). These ss-DNA serve as patterns or templates for the attachment of synthetic primers;

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(b) The second stage is the annealing step, where the temperature is reduced to approximately 37-50°C for usually 60-120 seconds and primers are added to the reaction mixture in excess (primers are synthetic oligonucleotides, short pieces of single-stranded DNA, consisting of nucleotide bases pairs long). The reduction in temperature allows the primers to bind to their complementary ss-DNA sequences (templates), one to each of the two single strands of template produced in the previous step.

Figure 1: The polymerase chain reaction: (A) the reaction mixture and (B) the sequence of events.

(c) The third and final stage is an extension reaction carried out by an added thermostable DNA-polymerase, an enzyme able to synthesize a complementary copy of the initial single strands by extension of the 3' (but not 5') ends of the annealed primers. This final step, usually carried out at 72°C for 60-120 seconds, is the end of the first cycle of the procedure by which two double strands of DNA have been formed, i.e., a doubling of the original target DNA molecules has been achieved. A second cycle of the procedure is then started with raising of the temperature to separate the strands of DNA, this time yielding four ss-DNA, and repeated cycling through the three heating steps exponentially increases the amount of defined length DNA product generated (target DNA).

The key to the widespread adoption of the PCR was the isolation of an enzyme, Taq polymerase, in 1989 from extreme thermophile bacterium 'Thermus aquaticus' (7) and this enzyme is thermostable up to 95°C. However, amplification requires four main components within the reaction mixture: (i) the enzyme DNA polymerase, (ii) the appropriate buffer containing MgCl₂ to optimize DNA polymerase activity, (iii) equimolar concentrations of the four deoxynucleotide triphosphates (dATP, dCTP, dGTP, dTTP) with which to construct the new DNA strands and (iv) a pair of oligonucleotides that may serve as primers for DNA synthesis. These four components, the polymerase chain reaction, the reaction mixture and the sequence of events are further illustrated in Figure 1. A ds-DNA polymerase product may be visualized by staining with ethidium bromide following agarose gel electrophoresis to confirm the presence and size of a particular target sequence. In other situations several bands may be produced in a multiplex PCR to analyze multiple target regions where multiple products of identical length are produced, it may be necessary to distinguish between them and this can be achieved by southern blotting technique i.e. DNA finger printing (8).

The advent of the use of Taq polymerase has transformed the PCR by facilitating the development of automated PCR machines which are programmed to control the repetitive stages of heating and cooling and each full cycle of the PCR takes only about 5 minutes. In practice, some 20-30 cycles are used resulting in the production of more than 10⁶ copies of the original target DNA molecule within 1.5-2.5 hours.

SOME APPLICATIONS OF THE PCR-TECHNIQUE: CURRENT PERSPECTIVE

The significance of the above general description of the PCR technique is best demonstrated by reference to specific examples of some of its applications as noted below:

a) Evolutionary Biology: The PCR analysis of spec-
imens from archeological material and museum collections is proving to be of great assistance in assessing the past and present genetic make up of extinct and endangered animal populations. An example is the quagga, a relative of the zebra, which became extinct 100 years ago and left zoologists wondering whether or not it was a distinct species. Samples from the skin of stuffed specimens were subjected to the PCR and subsequent analysis of the amplified DNA revealed the ‘quagga’ to be a variant of the zebra and not a distinct species. More dramatic examples are studies on a 7500-years old mummified brain and a 40,000-years old woolly mammoth frozen in a glacier. The assessment of the genetic make-up and relations among endangered populations by PCR may help to prevent this loss of genetic variation by employing appropriate management procedures (6).

d) Environmental Applications: Of particular interest of the application of PCR related to environment is the possibility of detecting bacteria that cannot be cultured and the monitoring of the distribution of genetically engineered microorganisms that have been released to the environment. It may also be applied in the further enhancement of existing rapid procedures for monitoring water quality by the detection of coliform bacteria and Escherichia coli, the specific indicator of human faecal contamination (6).

e) Routine Diagnostic Laboratory: From the foregoing examples of applications, it should be apparent that the PCR technique is more suited to use in research than in the busy routine diagnostic laboratory. However, once an exotic tool of the research scientists, PCR could realize its full potential only if made accessible to the clinical laboratory. Indeed, problems of cross-contamination, specimen preparation and containment, and the need for areas of the laboratory to be specified to the PCR work, together with other factors such as costs and training needs, will dictate when PCR will be routinely used in the diagnostic laboratory. In fact, some of the commercial companies such as Roche Diagnostics Systems, Switzerland, took this challenge seriously and a massive international collaborative effort have made PCR accessible for clinical diagnostic use. For example, Roche Diagnostics Systems, Switzerland, has simplified the sophisticated technology of PCR by incorporating familiar laboratory techniques into the PCR procedure and addressing the needs and concerns of the clinician to provide an optimized, integrated system with laboratory ready kits for specimen preparation, amplification and detection. To control the serious problem of potential carryover contamination, Roche has developed a unique and ingenious integral sterilization system ‘AmpErase’. Pre-amplification inactivation by ampErase ensures selective distribution of the previously amplified DNA.
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while leaving target DNA intact and thus provides effective carryover control with no additional steps required. For clinical use, PCR dramatically improves accuracy and speed in the diagnosis of infectious diseases, tissue matching and diagnosis of inherited disorders. It appears, therefore, that this is only the beginning of a new era in the diagnostic certainty.

THE FUTURE

Furthermore, necessary modifications and refinements of the PCR continue at a rapid pace. For example, there are now two new polymerases in use that bring advantages in specificity over Taq polymerase; they are from Thermococcus litoralis (VENT polymerase) Pyrococcus furiosus, also both extreme thermophiles. It should also be born in mind that although PCR is the most important technique currently in use for the amplification of nucleic acid sequences in vitro, other techniques have been, are being, and will continue to be developed e.g. ligase chain reaction (LCR) technique (6).

CONCLUSIONS

The PCR is continually transforming the way we approach fundamental problems in many areas of biology as well as medicine and laboratory medicine. As advances continue in protocols, reagents, kits and instrumentation, PCR will be more widely applied to routine laboratory diagnosis and to a variety of problems in an ever increasing range of areas including environmental pollution and monitoring of compliance with the International Biological Weapons Convention. As per medicine and laboratory medicine are concerned PCR has dramatically improved accuracy and speed in the diagnosis of many infectious diseases, methods of tissue matching allowing greater success in organ and tissue transplantation, and the ability to diagnose inherited diseases. The cost effectiveness should ensure that the basic principles of PCR will become one component of future clinical laboratory diagnostic investigation within almost all fields. This is only the beginning of a new era in diagnostic certainty.

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REFERENCES


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