DNA Amplification by Polymerase Chain Reaction (PCR)

The introduction of cell-free methods for multiplying DNA fragments of defined origin (DNA amplification) in 1985 ushered in a new era in molecular genetics (the principle of PCR is contained in earlier publications). This fundamental technology has spread dramatically with the development of automated equipment used in basic and applied research.

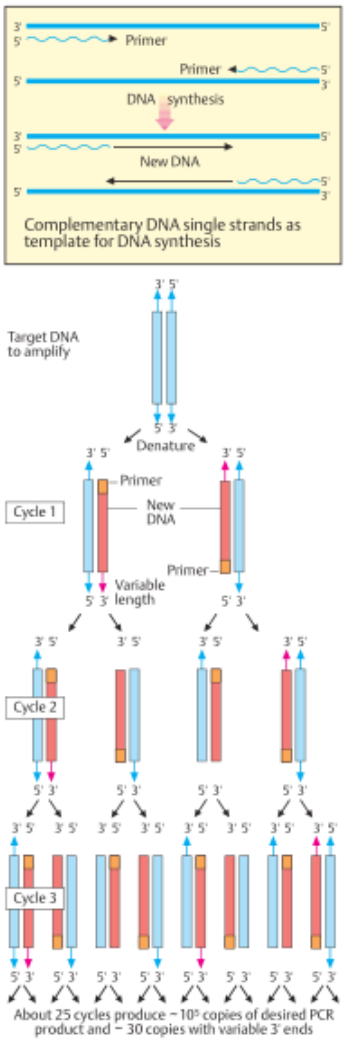
Polymerase chain reaction (PCR)

PCR is a cell-free, rapid, and sensitive method for cloning DNA fragments. A standard reaction and a wide variety of PCR-based methods have been developed to assay for polymorphisms and mutations. Standard PCR is an in vitro procedure for amplifying defined target DNA sequences, even from very small amounts of material or material of ancient origin. Selective amplification requires some prior information about DNA sequences flanking the target DNA. Based on this information, two oligonucleotide primers of about 15–25 base pairs length are designed. The primers are complementary to sequences outside the 3′ ends of the target site and bind specifically to these. PCR is a chain reaction because newly synthesized DNA strands act as templates for further DNA synthesis for about 25–35 subsequent cycles. Theoretically each cycle doubles the amount of DNA amplified. At the end, at least 10^5 copies of the specific target sequence are present. This can be visualized as a distinct band of a specific size after gel electrophoresis. Each cycle, involving three precisely time-controlled and temperature-controlled reactions in automated thermal cyclers, takes about 1–5min. The three steps in each cycle are (1) denaturation of double-stranded DNA, at about

93–95°C for human DNA, (2) primer annealing at about 50–70°C depending on the expected melting temperature of the duplex DNA, and (3) DNA synthesis using heat-stable DNA polymerase (from microorganisms living in hot springs, such as Thermophilus aquaticus, Taq polymerase), typically at about 70–75°C. At each subsequent cycle the template (shown in blue) and the DNA newly synthesized during the preceding cycle (shown in red) act as templates for another round of synthesis. The first cycle results in newly synthesized DNA of varied lengths (shown with an arrow) at the 3′ ends because synthesis is continued beyond the target sequences. The same happens during subsequent cycles, but the variable strands are rapidly outnumbered by new DNA of fixed length at both ends because synthesis cannot proceed past the terminus of the primer at the opposite template DNA.

cDNA amplification and RT-PCR

A partially known amino acid sequence of a polypeptide can be used to obtain the sequence information required for PCR. From its mRNA one can derive cDNA, and determine the sequence of the sense and the antisense strand to prepare appropriate oligonucleotide primers (1). When different RNAs are available in small amounts, rapid PCR based methods are employed to amplify cDNA from different exons of a gene. cDNA is obtained by reverse transcriptase from mRNA, which is then removed by alkaline hydrolysis (2). After a complementary new DNA strand has been synthesized, the DNA can be amplified by PCR (3). Reverse transcriptase PCR (RT-PCR) can be used when the known exon sequences are widely separated within a gene. With rapid amplification of cDNA ends (RACE-PCR), the 5? and 3? end sequences can be isolated from cDNA.



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