Polymerase chain reaction (PCR)

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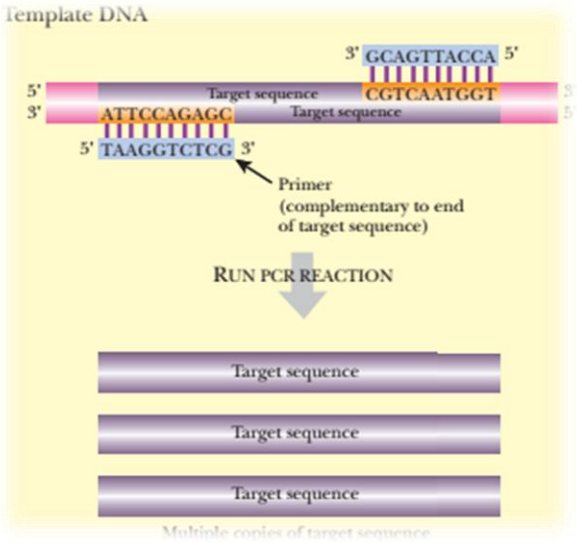

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Polymerase chain reaction (PCR)

➢Polymerase chain reaction (PCR) is a method used widely in molecular biology to make millions to billions of copies of a specific DNA sample rapidly. **Kary Mullis invented PCR in 1987.**

➢If the nucleotide sequences at the ends of a particular DNA region are known, the intervening fragment can be amplified directly by the **PCR**.

➢The PCR allows trace amounts of a DNA sequence to be amplified giving enough DNA for cloning, sequencing or other analyses.

➢PCR is used in clinical diagnosis, genetic analysis, genetic engineering and forensic analysis. In particular, PCR has revolutionized and speeded up the whole area of recombinant DNA technology.

❖The components involved in the polymerase chain reaction are as follows:

1. The original DNA molecule that is to be copied is called the template and the segment of it that will actually be amplified is known as the target sequence. A trace amount of the DNA template is sufficient.

2. Two **PCR primers** are needed to initiate DNA synthesis. These are short pieces of single-stranded DNA that match the sequences at either end of the target DNA segment.

3. The enzyme DNA polymerase is needed to manufacture the DNA copies. The PCR procedure involves several high temperature steps so a heat resistant DNA polymerase is required. This came originally from heat resistant bacteria living in hot springs at temperatures up to 90°C. **Taq polymerase** from ***Thermus aquaticus*** is most widely used.

4. A supply of nucleotides is needed by the polymerase to make the new DNA. These are supplied as the nuceoside triphosphates.

5. Finally we need a **PCR machine** to keep changing the temperature. The PCR process requires cycling through several different temperatures. Because of this, PCR machines are sometimes called **thermocyclers**.

❑Cycling Through the PCR

PCR is a procedure involving multiple cycles of DNA strand separation, binding of primers, and synthesis of new DNA. See the illustration in a diagrammatic manner in the next pages.

1. Denaturation :

The first step of the PCR is to separate the strands of the template DNA by heating the template DNA to 90°C or so for a minute or two.

2. **Annealing of primers:**

Although the primers are present from the beginning, they cannot bind to the template DNA at 90°C. So, the temperature is dropped to around 50°C to 60°C, allowing the primers to anneal to the complementary sequences on the template strands.

3. **Synthesis:**

Next, the temperature is maintained at 70°C for a minute or two to allow the thermostable polymerase to elongate new DNA strands starting from the primers.

- In the second cycle as shown in the Figure, there are now four partly double stranded pieces of DNA. Note again that although they vary in length, they all include double-stranded DNA from the target region.
- As the cycles continue, the single strand overhangs are ignored and are rapidly outnumbered by segments of DNA containing only the target sequence. During the third, the first two pieces of double-stranded DNA that correspond exactly to the target sequence are made. These do not have any dangling single stranded ends. Once past the first two or three cycles, the vast majority of the product is double-stranded target sequence with flush ends. Finally, the DNA generated is run on an agarose gel to assess the size of the PCR fragment.

✓Reverse transcription PCR makes amplified DNA from mRNA

✓In addition to using a variety of types of DNA (plasmids, genomic DNA) as template, PCR can also be performed on cDNA made from mRNA. Isolated mRNA is incubated with reverse transcriptase, then the resulting cDNA is used as a template for PCR. The combination of these two techniques is called *reverse transcription PCR (RT-PCR)*. RT-PCR is useful for three reasons. First, it allows the creation of recombinant DNA molecules containing DNA copies of only the exons of genes. Second, it allows study of the structure and function of gene products. Third, it can be used to determine relative levels of gene expression in cells and tissues.

✓Quantitative RT-PCR can determine levels of an mRNA

Cells often respond to changes in environmental conditions, both internal and external, with changes in gene expression. Analysis of gene expression involves accurate quantification of cellular mRNA levels. One of the fastest and easiest ways to measure relative changes in gene expression is using *reverse transcription quantitative PCR (RT-qPCR)*. This involves isolating mRNA, using reverse transcriptase to convert this to cDNA, then using PCR to amplify specific cDNAs using gene-specific primers. The amount of DNA produced can be measured in real time by the PCR machine. For this reason, quantitative PCR is also known as real-time PCR. Two common techniques are used, both of which depend on the use of fluorescent dyes.

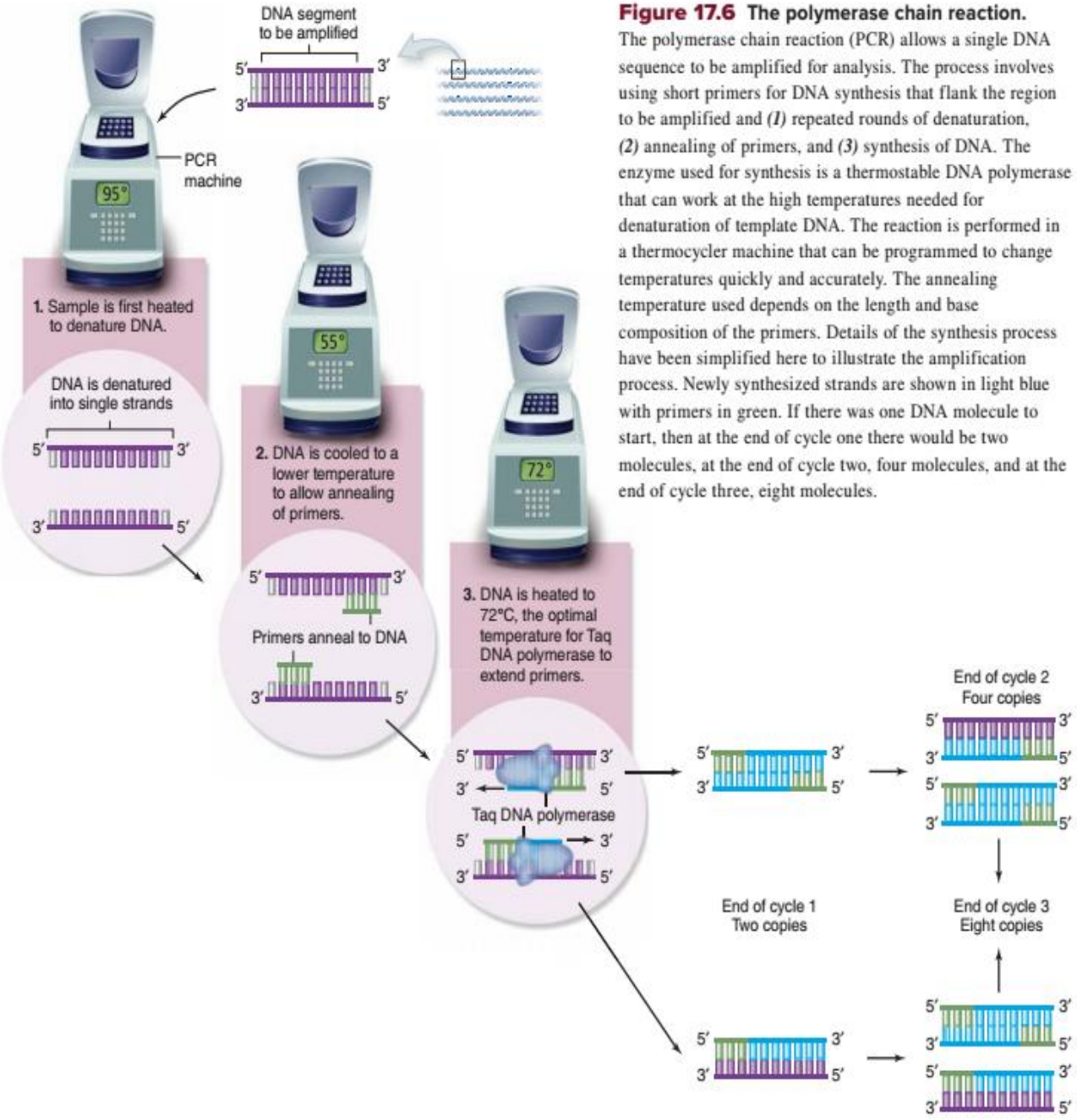

Figure 17.6 The polymerase chain reaction.
The polymerase chain reaction (PCR) allows a single DNA sequence to be amplified for analysis. The process involves using short primers for DNA synthesis that flank the region to be amplified and *(1)* repeated rounds of denaturation, *(2)* annealing of primers, and *(3)* synthesis of DNA. The enzyme used for synthesis is a thermostable DNA polymerase that can work at the high temperatures needed for denaturation of template DNA. The reaction is performed in a thermocycler machine that can be programmed to change temperatures quickly and accurately. The annealing temperature used depends on the length and base composition of the primers. Details of the synthesis process have been simplified here to illustrate the amplification process. Newly synthesized strands are shown in light blue with primers in green. If there was one DNA molecule to start, then at the end of cycle one there would be two molecules, at the end of cycle two, four molecules, and at the end of cycle three, eight molecules.

The Polymerase Chain Reaction (PCR)

Application With PCR, any specific segment (the so-called target sequence) within a DNA sample can be copied many times (amplified), completely *in vitro*.

Technique PCR requires double-stranded DNA containing the target sequence, a heat-resistant DNA polymerase, all four nucleotides, and two 15- to 20-nucleotide DNA strands that serve as primers. One primer is complementary to one end of the target sequence on one strand; the second primer is complementary to the other end of the sequence on the other strand.