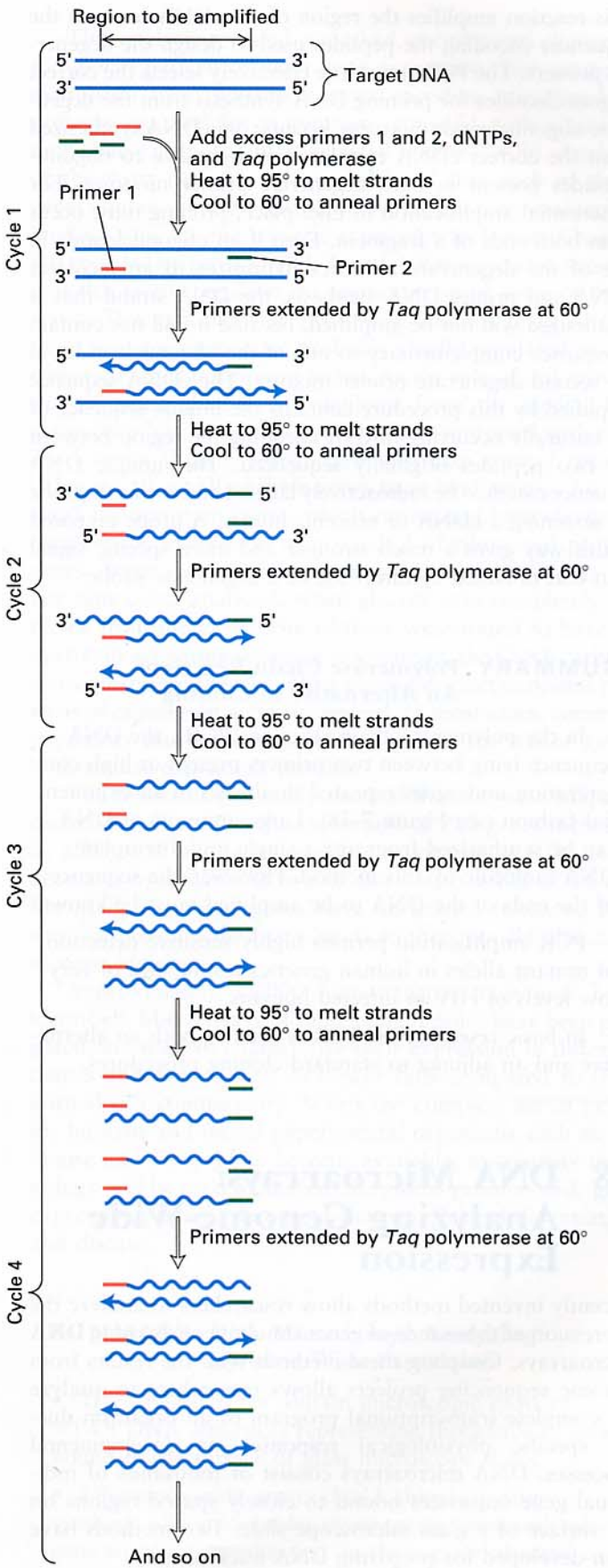

FIGURE 7-38 The polymerase chain reaction. The starting material is a double-stranded DNA. Large numbers of primers are added, each with the sequence found in one strand at the end of the region to be amplified. The thermostable *Taq* polymerase and dNTPs are also added. In the first cycle, heating to 95 °C melts the double-stranded DNA and subsequent cooling to 60 °C then allows the excess primers to hybridize (anneal) to their complementary sequences in the target DNA. The *Taq* polymerase then extends each primer from its 3′ end by polymerization of dNTPs, generating newly synthesized strands (wavy lines) that extend in the 3′ direction to the 5′ end of the template restriction fragment. In the second cycle, the original and newly made DNA strands are separated at 95 °C and primers annealed to their complementary sequences at 60 °C. (For simplicity, subsequent events involving only newly made strands are shown; these soon greatly outnumber the original strands.) Each annealed primer again is extended by *Taq* polymerase to the end of the other primer sequence at the 5′ end of the template strand. Thus the strands (amplimers) synthesized in this cycle exactly equal the length of region to be amplified. In the third cycle, two double-stranded DNA molecules are generated equal to the sequence of the region to be amplified. These two are doubled in the fourth cycle and are doubled again with each successive cycle. [Adapted from J. D. Watson et al., 1992, *Recombinant DNA*, 2d ed., Scientific American Books.]

Another medical application of the PCR is early detection of infection with HIV, the virus that causes acquired immunodeficiency syndrome (AIDS). The PCR is so sensitive that it can detect HIV at very early stages in the disease (before symptoms appear) when only a few thousand blood cells in a patient are infected with the virus.

DNA Sequences Can Be Amplified for Use in Cloning and as Probes

In basic research, the PCR also has numerous applications. For example, this procedure allows the recovery and rapid amplification of the entire DNA region between any two ends whose sequences are known; the amplified DNA fragment then can be ligated into standard cloning vectors. Fragments of ≈2 kb or less can be amplified readily, and recent refinements of the technique allow amplification of regions of >30 kb.

The PCR also provides an alternative approach for preparing probes to screen genomic or cDNA libraries for clones encoding a protein of interest. The amino acid sequence of two peptides isolated from the purified protein are used to design two degenerate oligonucleotide mixtures containing all possible DNA sequences encoding the two peptides (see Figure 7-19). Rather than using these oligonucleotides as probes for direct screening of a cDNA library, as described previously, they are used as primers in a PCR. First, cDNA is synthesized from total cellular mRNA using reverse transcriptase. The cDNA is then used as the template for a PCR performed with the two degenerate oligonucleotide primers.