Polymerase chain reaction (PCR)

Molecular biology has been revolutionised by PCR, a method that efficiently increases the number of DNA molecules in a logarithmic and controlled fashion. Kary Mullis conceived PCR in 1983 and the first PCR publication appeared in 1985. In 1993, Kary Mullis received the Nobel prize for developing the polymerase chain reaction. Kary Mullis invented PCR while working as a scientist for the Cetus Corporation. Kary Mullis was given a $10,000 bonus by Cetus. Later they sold the technology to Roche for $300,000,000. PCR is used in clinical diagnosis, genetic analysis, genetic engineering and forensic analysis.

PCR components

When performing a PCR reaction, several components are needed:

1. DNA template, which contains the region of the DNA fragment to be amplified. 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.
2. Two primers, which determine the beginning and end of the region to be amplified. These are short pieces of single-stranded DNA that match the sequences at either end of the target DNA segment. PCR primers are made by chemical synthesis of DNA.
3. The DNA polymerase (Taq DNA polymerase) will built a new DNA strand. This came originally from heat resistant bacteria living in hot springs at temperatures up to 90°C.
4. PCR Buffer, which provides a suitable chemical environment for the polymerase to perform the amplification.

5. dNTPs (deoxyiribosenucleotides) which include dATP, dCTP, dGTP and dTTP, they are the monomers that DNA polymerase uses to form DNA
6. Co-factor (magnesium Chloride) to increase the yield of the reaction.
7. Finally we need a PCR machine to keep changing the temperature. PCR machines are sometimes called thermocyclers.

PCR steps

1- Initial Denaturation Step

The complete denaturation of the DNA template at the start of the PCR reaction is of key importance. Incomplete denaturation of DNA results in the inefficient utilization of template in the first amplification cycle and in a poor yield of PCR product. The initial denaturation should be performed over an interval of 1–3 min at 95◦C if the GC content is 50% or less. This interval should be extended up to 10 min for GC-rich templates.

2- Denaturation Step

Usually denaturation for 0.5–2 min at 94–95◦C is sufficient, since the PCR product synthesized in the first amplification cycle is significantly shorter than the template DNA and is completely denatured under these conditions.

3- Primer Annealing Step

After separating the DNA strands, the temperature is lowered so the primers can attach themselves to the single DNA strands. The temperature of this stage depends on the primers and is usually 5◦C below their melting temperature (45–60◦C). A wrong temperature during the annealing step can result in primers not binding to the template DNA at all. Incubation for 0.5–2 min is usually sufficient.

4- Extension Step

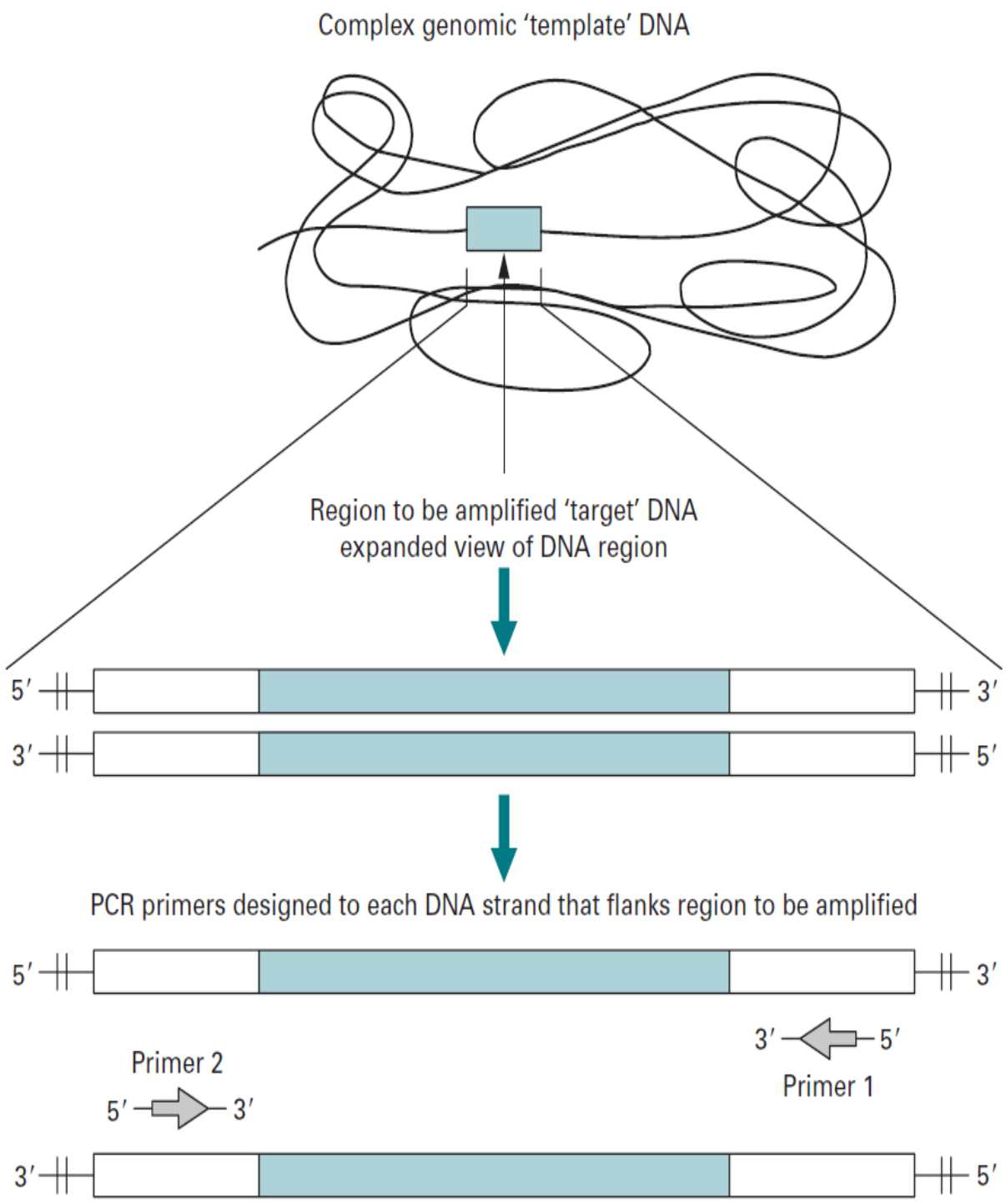
Usually the extension step is performed at 72◦C. The rate of DNA synthesis by *Taq* DNA polymerase is highest at this temperature. Recommended extending time is 1 min for every 1000 bp to be synthesised. When larger DNA fragments are amplified, the extending time is usually increased by 1 min for each 1000 bp. The DNA-Polymerase has to fill in the missing strands. It starts at the annealed primer and works its way along the DNA strand.

5- Final Extension Step

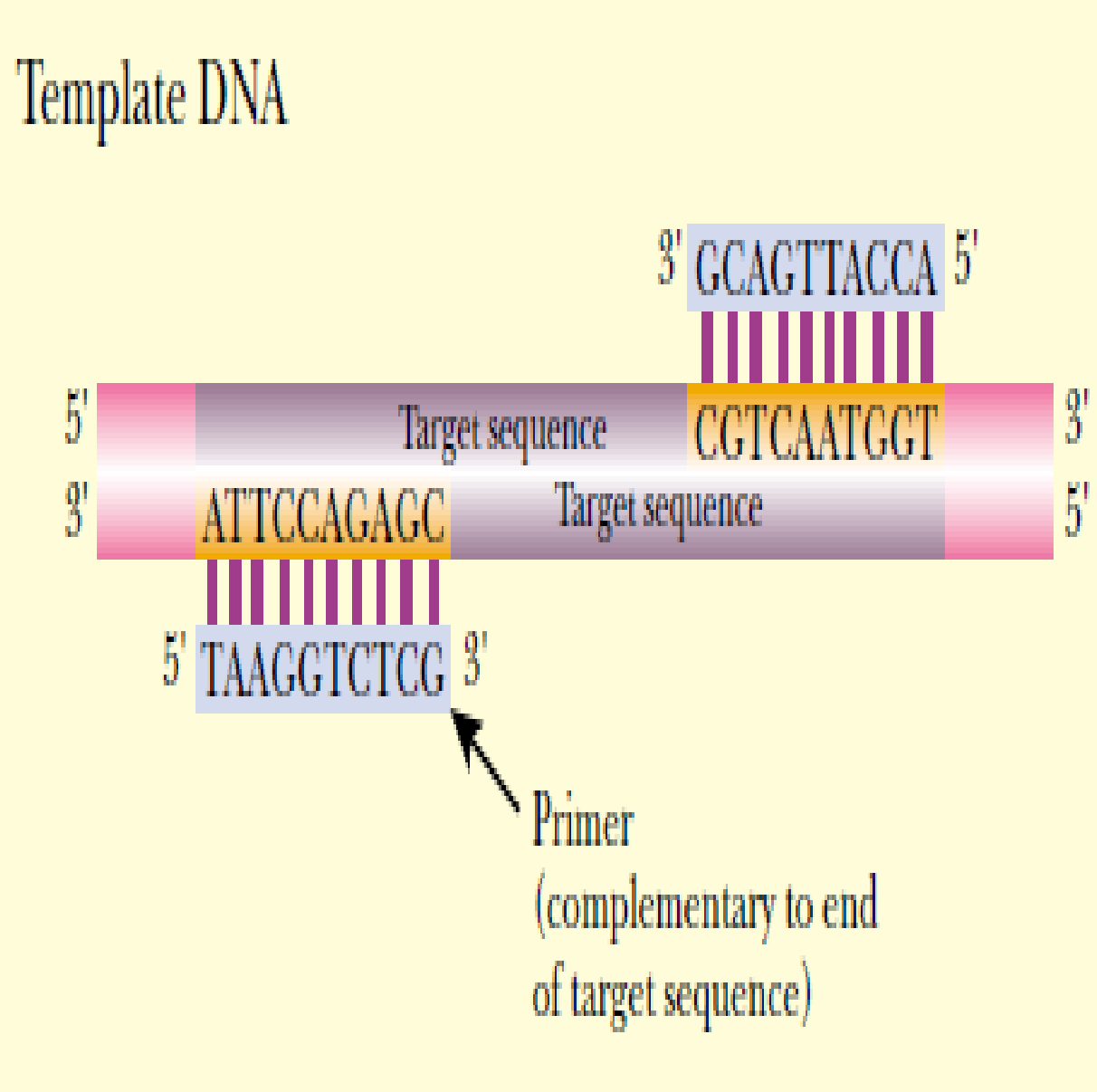
After the last amplification cycle, samples are usually incubated at 72◦C for 5–15 min to fill-in the protruding ends of newly synthesized PCR products. Also, during this step, the terminal transferase activity of the Taq DNA polymerase adds extra A nucleotides to the 3_-ends of PCR products. After the PCR reaction is complete, PCR products can be identified by its size using agarose gel electrophoresis.

PCR amplification cycle number

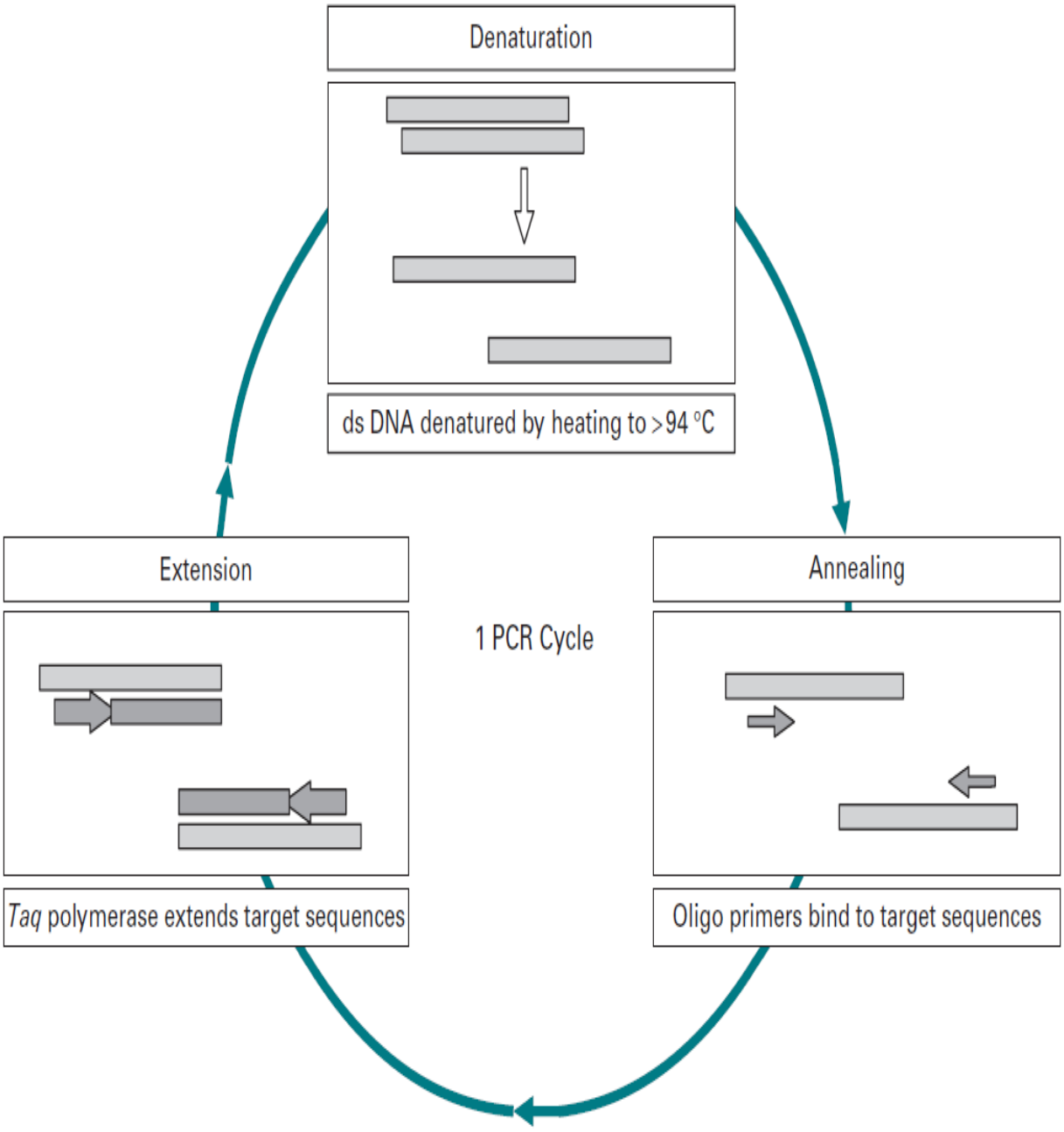
The number of cycles necessary to obtain a sufficient amount of PCR product depends strongly on the concentration of the DNA template. Usually the PCR process consists of a series of 30–40 cycles. Each cycle consists of three steps (step 2, step 3 and step 4).



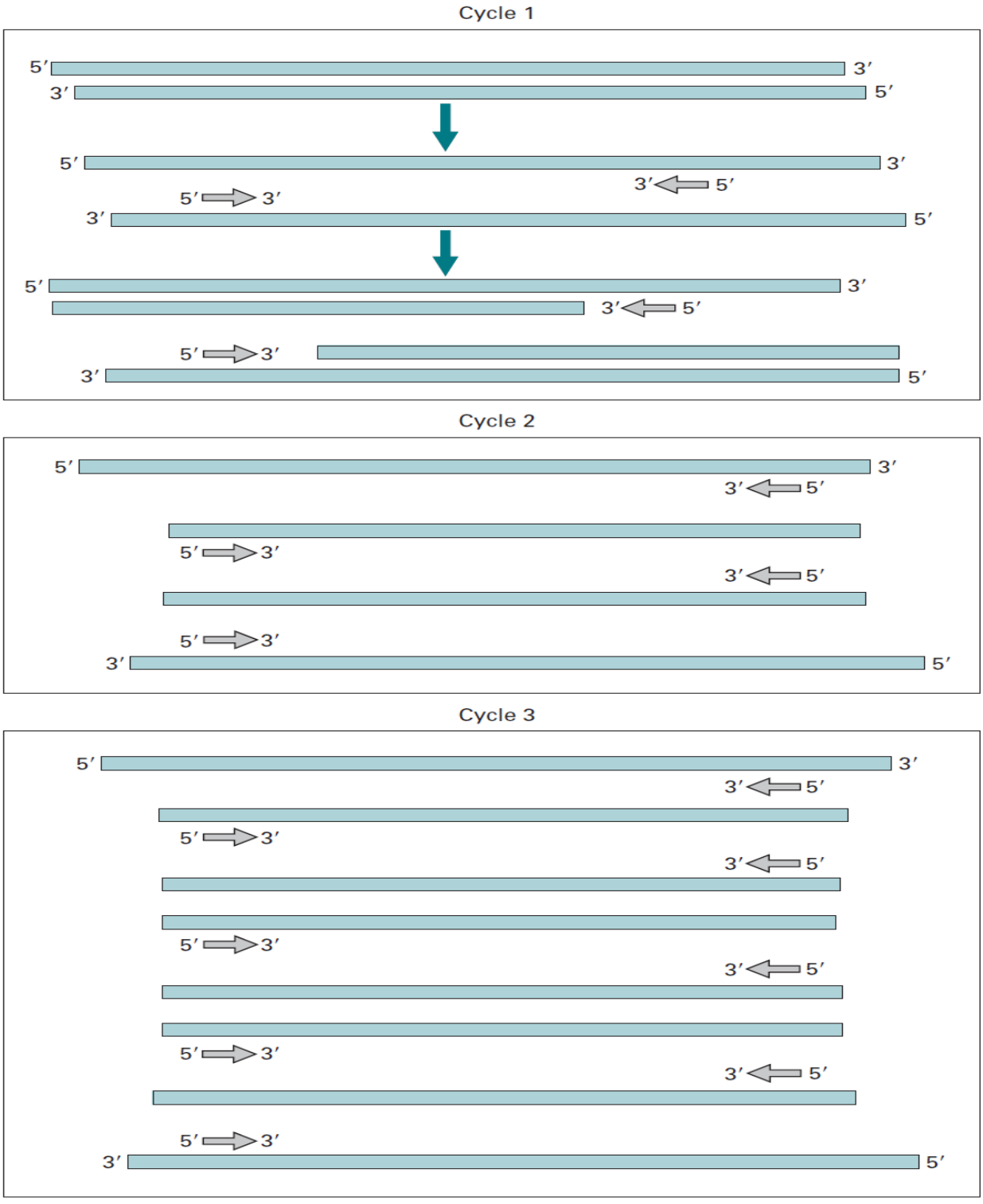
The region of DNA (e.g. a gene) to be amplified from a complex starting material of genomic template DNA



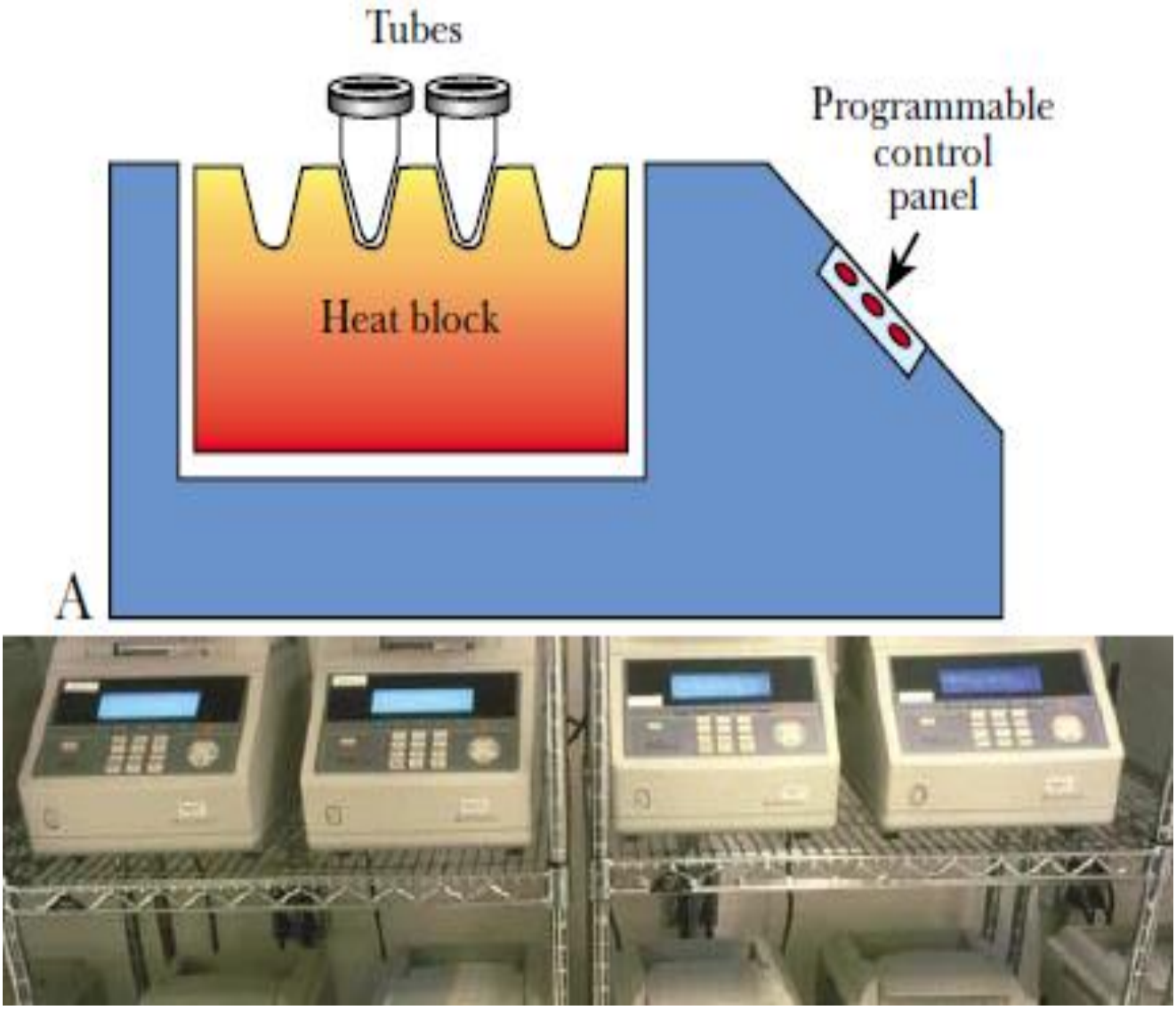
The primers anneal to complementary sequences at either end of a target sequence on a piece of denatured template DNA



A simplified scheme of one PCR cycle that involves denaturation, annealing and extension. ds, double-stranded



Three cycles in the PCR



The thermocycler or PCR machine can be programmed to change temperature rapidly