كلية المستقبل الجامعة / قسم تقنيات المختبرات الطبية
المرحلة الثالثة
المحاضرة الثالثة/ مادة التقنيات المختبرية
(الجزء العملي)

POLYMERASE CHAIN REACTION

Polymerase chain reaction (**PCR**) is a common laboratory technique used to make many copies (millions or billions!) of a particular region of DNA. This DNA region can be anything the experimenter is interested in. For example, it might be a gene whose function a researcher wants to understand, or a genetic marker used by forensic scientists to match crime scene DNA with suspects.

Typically, the goal of PCR is to make enough of the target DNA region that it can be analyzed or used in some other way. For instance, DNA amplified by PCR may be sent for sequencing, visualized by gel electrophoresis, or cloned into a plasmid for further experiments.

PCR is used in many areas of biology and medicine, including molecular biology research, medical diagnostics, and even some branches of ecology.

Taq polymerase

Like DNA replication in an organism, PCR requires a DNA polymerase enzyme that makes new strands of DNA, using existing strands as templates. The DNA polymerase typically used in PCR is called ***Taq* polymerase**, after the heat-tolerant bacterium from which it was isolated (*Thermus* ***aquaticus***).

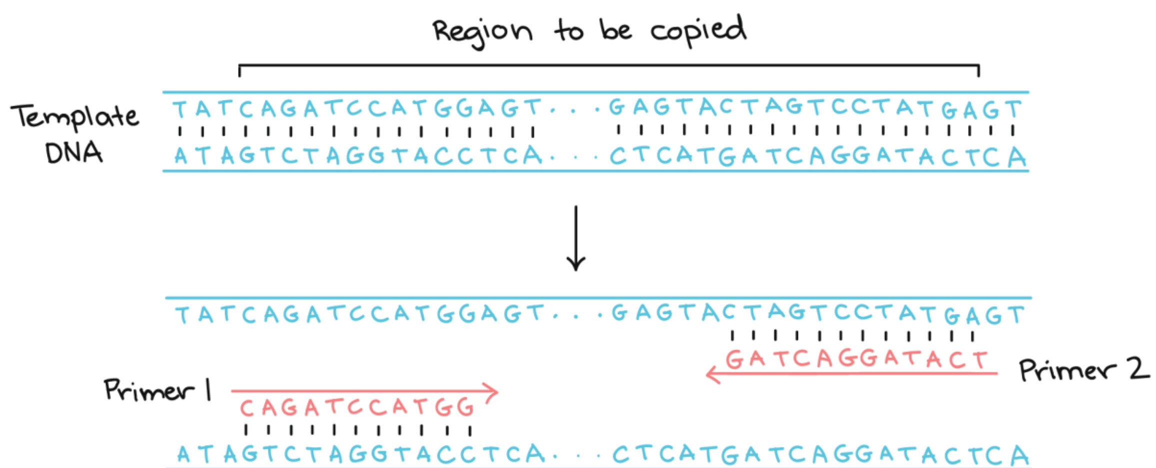
T. aquaticus lives in hot springs and hydrothermal vents. Its DNA polymerase is very heat-stable and is most active around 70 °\text C70°C70, °, start text, C, end text (a temperature at which a human or *E. coli* DNA polymerase would be nonfunctional). This heat-stability makes

Taq polymerase ideal for PCR. As we'll see, high temperature is used repeatedly in PCR to **denature** the template DNA, or separate its strands.

PCR primers

Like other DNA polymerases, *Taq* polymerase can only make DNA if it's given a **primer**, a short sequence of nucleotides that provides a starting point for DNA synthesis. In a PCR reaction, the experimenter determines the region of DNA that will be copied, or amplified, by the primers she or he chooses.

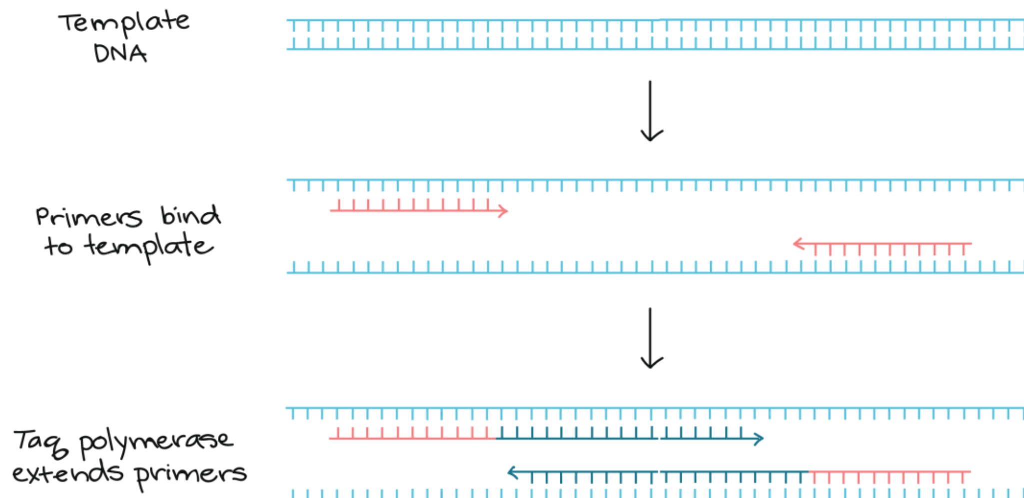
PCR primers are short pieces of single-stranded DNA, usually around 202020 nucleotides in length. Two primers are used in each PCR reaction, and they are designed so that they flank the target region (region that should be copied). That is, they are given sequences that will make them bind to opposite strands of the template DNA, just at the edges of the region to be copied. The primers bind to the template by complementary base pairing.



Template DNA:

5' TATCAGATCCATGGAGT...GAGTACTAGTCCTATGAGT 3' 3' ATAGTCTAGGTACCTCA...CTCATGATCAGGATACTCA 5'

When the primers are bound to the template, they can be extended by the polymerase, and the region that lies between them will get copied.



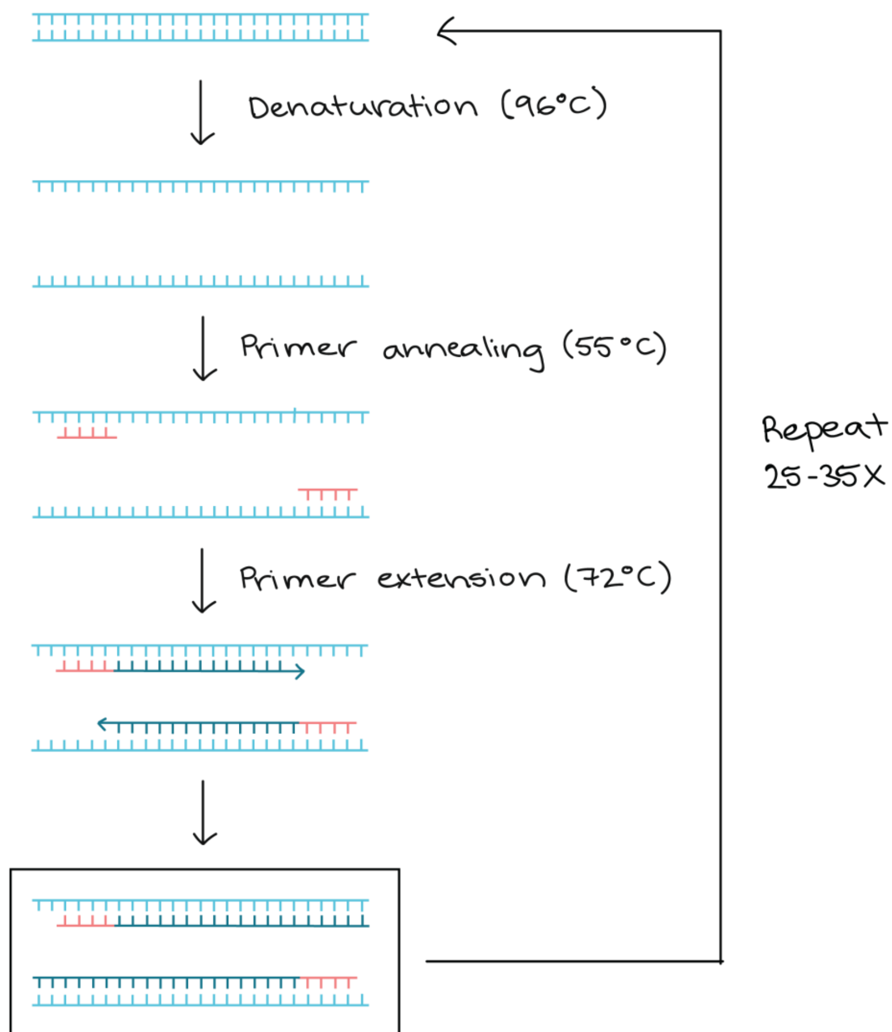
The steps of PCR

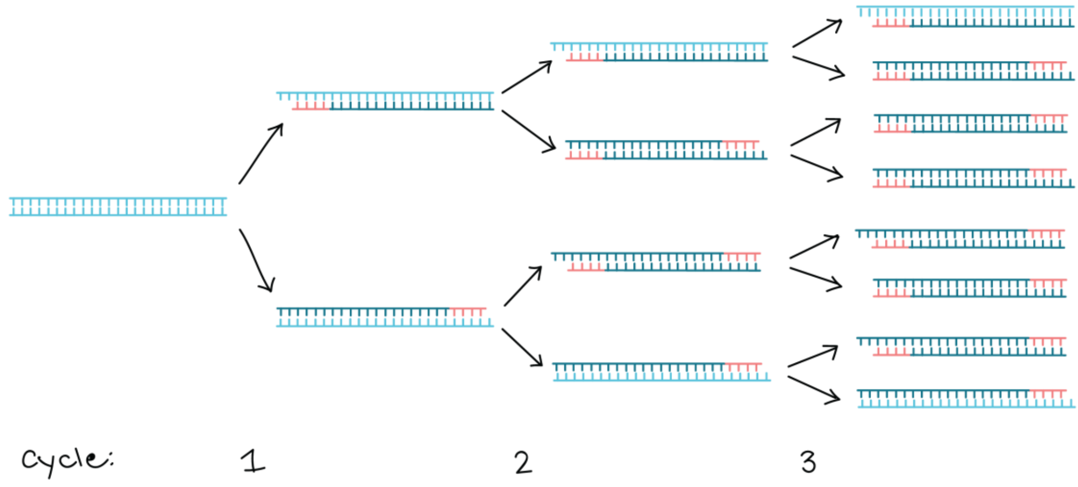
The key ingredients of a PCR reaction are *Taq* polymerase, primers, template DNA, and nucleotides (DNA building blocks). The ingredients are assembled in a tube, along with cofactors needed by the enzyme, and are put through repeated cycles of heating and cooling that allow DNA to be synthesized.

The basic steps are:

1. **Denaturation** (96 °\text C96°C96, °, start text, C, end text): Heat the reaction strongly to separate, or denature, the DNA strands. This provides single-stranded template for the next step.

2. **Annealing** (555555 - 656565°\text C°C°, start text, C, end text): Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.
3. **Extension** (72 °\text C72°C72, °, start text, C, end text): Raise the reaction temperatures so *Taq* polymerase extends the primers, synthesizing new strands of DNA.

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