EDVOTEK® Quick Guide: Polymerase Chain Reaction



What is the Polymerase Chain Reaction (PCR)?

PCR is a technique that allows researchers to quickly create many copies of a specific region of DNA in vitro.

What do I need to perform PCR?

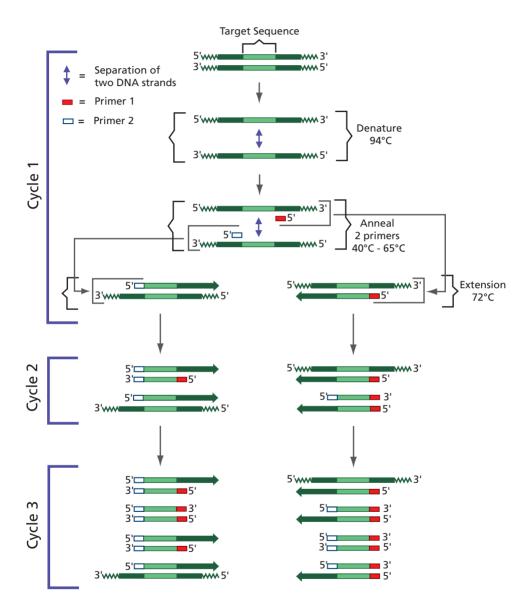
Template – the purified, double-stranded piece of DNA we want to copy Primers – short synthetic DNA molecules that target a specific DNA sequence for amplification *Taq* DNA Polymerase – thermostable enzyme used to copy DNA Free nucleotides – the building blocks of DNA Thermal Cycler (a.k.a. PCR machine) – a specialized machine that rapidly heats and cools the samples.

How does PCR Work?

To perform PCR, the template is mixed with primers, *Taq* polymerase and nucleotides. The mixture is heated to 94°C to denature the DNA duplex (i.e., unzip it into single strands). Next, the sample is then cooled to 45°C-60°C, allowing the primers to base pair with the target DNA sequence (called "annealing"). Lastly, the temperature is raised to 72°C, the optimal temperature at which *Taq* polymerase will extend the primer to synthesize a new strand of DNA. Each "PCR cycle" (denaturation, annealing, extension) doubles the amount of the target sequence in less than five minutes. In order to produce enough DNA for analysis, twenty to forty cycles may be required.

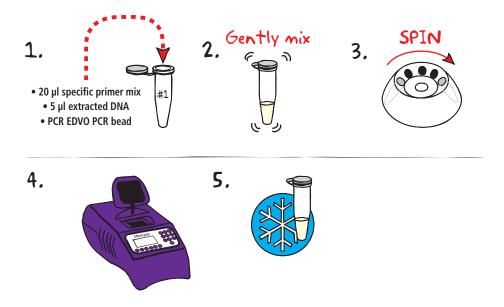


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- 1. ADD 20 µL specific primer mix, 5 µl extracted DNA and the EDVO PCR bead to a labeled 0.2 mL tube.
- 2. MIX the PCR sample. Make sure the EDVO PCR bead is completely dissolved.
- 3. **CENTRIFUGE** to collect the sample at the bottom of the tube.
- 4. **AMPLIFY** DNA using PCR:

PCR cycling conditions:

Initial denaturation 94°C for 3-5 min. 94° C for 30-60 sec. 45-65° C for 30-60 sec. 72° C for 30-60 sec. Final Extension 72° C for 5-10 min.

5. **PLACE** tubes on ice. Analyze samples using agarose gel electrophoresis.

Related Equipment

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