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.
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 4 minutes
   - 94°C for 30-60 sec.
   - 45-65°C for 30-60 sec. 20-40 cycles
   - 72°C for 30-60 sec.
   - Final Extension 72°C for 5 minutes

5. **PLACE** tubes on ice. Analyze samples using agarose gel electrophoresis.
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:
   - **Initial denaturation** 94°C for 3-5 min.
   - 94°C for 30-60 sec.
   - 45-65°C for 30-60 sec. 20-40 cycles
   - 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.
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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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**Related Equipment**

See the **EQUIPMENT** section in our Resource Guide for our full range of equipment or visit our website at: [www.edvotek.com](http://www.edvotek.com)

- **Cat. #515**
  - M36 HexaGel™ DNA Electrophoresis Apparatus
  - For 6 Lab Groups

- **Cat. #5010**
  - TetraSource™ 300 Power Supply
  - 30-300 V for 1 to 4 units

- **Cat. #589-#593**
  - EDVOTEK® Variable Micropipets
  - From 0.1 µl to 5000 µl

- **Cat. #534**
  - Piccolo Microcentrifuge
  - Variable speed from 0-6,400 rpm

- **Cat. #510**
  - EdvoCycler™
  - Holds 25 x 0.2 ml tubes

- **Cat. #542**
  - MegaCycler™
  - Holds 49 x 0.2 ml tubes

- **Cat. #544**
  - PCR Bath™
  - 3 individual waterbath chambers allowing multiple temp. settings.
  - Ambient to 99°C

- **Cat. #5067**
  - Classroom PCR LabStation™
  - Supports up to 25 students!