A Polymerase Chain Reaction (PCR) is a laboratory technique used to amplify a specific segment of DNA, creating millions of copies from a small initial sample.

This protocol outlines the steps for a standard PCR using *Taq* DNA polymerase.

🔬 Requirements

Reagents and Consumables

- **DNA Template**: The DNA sample containing the target sequence you want to amplify.
- **Forward and Reverse Primers**: Short, single-stranded DNA sequences designed to be complementary to the start and end of your target DNA segment.
- **DNA Polymerase**: A heat-stable enzyme that synthesizes new DNA strands. *Taq* polymerase is common.
- **Deoxynucleotide triphosphates (dNTPs)**: The four individual building blocks of DNA (dATP, dCTP, dGTP, dTTP).
- **PCR Buffer**: A solution that provides the optimal chemical environment (pH and salt concentration) for the DNA polymerase. It typically contains magnesium chloride ($MgCl_2$), a necessary cofactor for the enzyme.
- **Nuclease-Free Water**: Ultra-pure water used to bring the reaction to its final volume.
- **PCR Tubes or Plate**: Small, thin-walled tubes designed for efficient heat transfer.

Equipment

- **Thermal Cycler**: A machine that precisely controls the rapid temperature changes required for the PCR cycles.
- **Micropipettes and Filter Tips**: For accurate measurement and transfer of small liquid volumes.
- **Microcentrifuge**: To spin down reagents in the tubes.
- **Vortex Mixer**: For mixing solutions.
- **Ice Bucket**: To keep reagents cool during setup.
- **Gel Electrophoresis System**: For visualizing the PCR product after the reaction is complete.

🧬 Protocol

Work on ice to prevent the polymerase from working prematurely.

1. Prepare the Master Mix

A master mix is a bulk solution containing all the common reagents for every sample. This reduces pipetting errors and ensures consistency. You'll make enough for all your samples plus one extra (N+1 rule) to account for pipetting inaccuracies.

Sample Calculation for a single 25 µL reaction:

Reagent (Stock Concentration)	Volume per Reaction	Final Concentration
10x PCR Buffer	2.5 µL	1x
dNTPs (10 mM)	0.5 µL	200 µM
Forward Primer (10 µM)	1.25 µL	0.5 µM
Reverse Primer (10 µM)	1.25 µL	0.5 µM
Taq Polymerase (5 U/µL)	0.25 µL	1.25 units
Nuclease-Free Water	18.25 µL	-
Total Master Mix Volume	**24.0 µL**	-

*To this, you will add **1.0 µL** of your DNA template in the next step.*

2. Aliquot Master Mix and Add Template

- Gently vortex and briefly centrifuge your master mix.
- Pipette **24 µL** of the master mix into each labeled PCR tube.
- Add **1 µL** of the corresponding DNA template to each tube.
- **Crucially, include a Negative Control**: Prepare one tube where you add **1 µL of nuclease-free water** instead of DNA. This will show if your reagents are contaminated.

3. Load into the Thermal Cycler

- Cap the tubes, gently mix, and spin them down in a microcentrifuge for a few seconds to collect the liquid at the bottom.
- Place the tubes in the thermal cycler and start the program.

4. Set the Thermal Cycler Program

A typical PCR program consists of three main stages repeated in a cycle.

Stage	Temperature	Duration	Purpose
Initial Denaturation	95°C	3-5 minutes	Activates the polymerase and fully separates the initial DNA template strands.
30-35 Cycles			
• **Denaturation**	95°C	30 seconds	Separates the double-stranded DNA into single strands.
• **Annealing**	55-65°C	30 seconds	Allows the forward and reverse primers to bind to their complementary sites on the single-stranded

			DNA. *This temperature is specific to your primers.*
• **Extension**	72°C	1 min per kb	The DNA polymerase synthesizes a new complementary strand, starting from the primers. *The time depends on the length of your target DNA.*
Final Extension	72°C	5-10 minutes	Ensures any remaining single-stranded DNA is fully extended.
Hold	4°C	Indefinitely	Keeps the PCR products cool until you are ready to retrieve them.

5. Analyze the Results

- Once the program is finished, retrieve your samples.
- Analyze the PCR product by running it on an **agarose gel** alongside a **DNA ladder** (a mix of DNA fragments of known sizes).
- A successful reaction will show a bright band of the expected size on the gel. Your negative control should show no band.