Protocol: Amplification of fragments by PCR

Last updated: 2026-07-21

Reviewed by: Patrik

The PCR protocol was originally provided by Jena Bioscience. Additional details, clarifications, and tips for practical use in the lab were provided by last year's iGEM Team.

Note:

- Always work on ice.
- Wear gloves.
- Use nuclease-free water and sterile tubes.
- Completely thaw and thoroughly mix all reagents before use.
- Avoid repeated freeze-thaw cycles of reagents.
- **Important:** The first thing you do should be reading through the protocol and thawing all reactants on ice. This will take some time so the earlier the better.

Materials and reactants:

- Jena Bioscience Red Load Master
    - Red Load Taq Master Mix (5x) - red cap
    - Nuclease free water (PCR-grade) - white cap
- Forward Primer
- Reverse Primer
- Template DNA (or colonies)
- Sterile 0.2 ml PCR tubes
- 10 µL sterile pipette tips
- Micropipette 10 µL
- Thermal cycler

Red Load Taq Master (Jena Bioscience)

Part A. Preparation of Reaction Mix

1. Thaw Red Load Master Mix, primer solutions and template DNA. It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salt.
2. Below is a scheme of how to make a PCR reaction mix for a recommended final volume of 20 µL. In every PCR tube you should have a total volume of 20 µL.

    - Note that the different genes use different primers; make sure that you pick the correct primers for the gene.
    - Make sure that you dilute the primer solutions to the correct concentration (0.5 µM).

Table 1: PCR reaction mix. *The Red Load Taq Master contains thermostable DNA polymerase, dATP, dCTP, dGTP, dTTP, KCl, MgCl2, red dye, gel loading buffer and stabilizers.*

.

Component	20 µL Reaction	Final Concentration
5x Taq Master Mix (Red Cap)	10 µL	1×
Forward Primer (10 µM)	1 µL	0.5 µM
Reverse Primer (10 µM)	1 µL	0.5 µM
Template DNA	X µL	2 - 50 ng
Milli-Q H_2O	X µL	Fill up to reach a final volume of 20 µl
Total volume	20 µl	-

3. Mix the reaction mix thoroughly and dispense 20 µl into each PCR tube. Mix gently by pipetting up and down.

4. Program the thermal cycler as shown in Table 2. Adjust annealing temperature based on primer melting temperature (typically 3–5°C below Tm). Program the thermal cycler according to Table 2.

Table 2: Thermocycling protocol, based on Red Load Taq Master protocol.

Step	Temperature	Duration of cycles	Cycles
Initial Denaturation	94 °C	2 min	1x
Denaturation	94°C	30 s	30x
Annealing*	X°C	30 s	
Elongation**	72°C	X min*	
Final Extension	72°C	5 min	1x
Hold	4 °C	∞	

* *The annealing temperature depends on the melting temperature of the primers used (as a rule of thumb, melting temperature of the primer - 3/5).*

** The elongation time depends on the length of the fragments to be amplified. A time of **1 min/kb** is recommended.

Note: For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary for each new template DNA and/or primer pair.

6. Place tubes in the thermal cycler and start the PCR.
7. Run gel electrophoresis (*see separate protocol*). Loading dye is not needed, as this Master Mix contains it.
8. Confirm the correct size and yield of amplicons.

Cleanup

- Dispose all tubes that are not needed after the experiments.
- Wipe the gel imaging station with a wet paper towel.
- Close all programs and clean up your bench.