



# Polymerase chain reaction (PCR) V.2

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Version 2 ▾

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1 Works for me

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## ABSTRACT

This protocol is used to amplify target DNA fragment for plasmid construction or other use.

## DOI

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## PROTOCOL CITATION

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## PROTOCOL INTEGER ID

53758

## MATERIALS TEXT

2×High Fidelity Master Mix/2×Rapid Master Mix  
ddH<sub>2</sub>O  
Template  
F/R Primer  
Thermo cycler.

## SAFETY WARNINGS

Please wear gloves for the experiment, don't try to touch the lid after PCR program initiation.

## BEFORE STARTING

Set up a small box with ice, put DNA and 2×high Fidelity Master Mix/or 2×Rapid Master Mix into it before going into the thermocycler.

- 1 Choose one case from the cases below.  
Step 1 includes a Step case.

### Simple PCR for amplifying target DNA fragments

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- 2 Add the following reagent to a PCR tube.(50 µl).

A	B
2×High Fidelity Master Mix (Enzyme)	25 µl
Template	1 µl
Forward Primer (10 µM)	1 µl
Reverse Primer (10 µM)	1 µl
ddH <sub>2</sub> O	22 µl

- 3 Program the thermocycler as follows:

A	B
Temperature	Time
95/98°C	5 min
95/98°C	30 s
T <sub>m</sub> -3~5°C	30 s
72°C	1kbp/min
72°C	5~10 min
16°C	∞

- 4 Use the palm centrifuge to mix the solution in PCR tube.
- 5 Put the PCR tube into the thermocycler and Run the program.
- 6 Using agarose gel electrophoresis to confirm if correct construct was present.