



Polymerase chain reaction (PCR) V.5

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¹2021 iDEC NEFU_China

Version 5 ▾

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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.

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MATERIALS TEXT

2×High Fidelity Master Mix (MCLAB)/2×Rapid Master Mix (Vazyme)

ddH₂O

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 (MCLAB)/or 2×Rapid Master Mix (Vazyme) 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

Simple PCR for amplifying target DNA fragments

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

A	B
2×High Fidelity Master Mix (MCLAB)	25 μ l
Template	1 μ l
Forward Primer (10 μ M)	1 μ l
Reverse Primer (10 μ M)	1 μ l
ddH ₂ O	22 μ l

- 3 Program the thermocycler as follows:

A	B
Temperature	Time
95/98°C	5 min
95/98°C	30 s
T _m -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.