Polymerase chain reaction (PCR) V.3

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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/2×Rapid Master Mix
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/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

step case

Simple PCR for amplifying target DNA fragments

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
ddH2O	22 µl

3 Program the thermocycler as follows:

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

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