2023 NEFU_China

Polymerase chain reaction (PCR)

ABSTRACT

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

BEFORE STARTING

- Setup 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.

1.1Simple PCR for amplifying target DNA fragments

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

	A	B
1	2×High Fidelity Master Mix (MCLAB)	25 μl
2	Template	1 μl
3	Forward Primer (10 μM)	1 μl
4	Reverse Primer (10 μM)	1 μl
5	ddH_2O	22 μl

3.Program the thermocycler as follows:

	Temperature	Time
1	95/98℃	5 min
2	95/98℃	30 s
3	Tm-3~5℃	30 s
4	72℃	1kb/min
5	72℃	5~10 min
6	16℃	∞

Repeat 30 times in 3-5 steps

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 the correct construction is present.

1.2.Colony PCR

2.Pick colonies as the template for colony PCR. Mix the colonies with 2.5μl LB and pick 1μl as PCR template and 1.5μl for culture.

3.Add following reagents to a PCR tube.(10 μl).

	A	B
1	2×Raqid Master Mix (Vazyme)	5 μl
2	Template	0.4 μl
3	Forward Primer (10 μM)	0.4 μl
4	Reverse Primer (10 μM)	0.4 μl
5	ddH_2O	3.8 μl

There is no need to add Gold View as colouring agent for agarose gel eletrophoresis when using 2×Rapid Master Mix (Vazyme) as PCR enzyme.

4.Program the thermocycler as follows:

	Temperature	Time
1	95/98℃	5 min
2	95/98℃	30 s
3	Tm-3~5℃	30 s
4	72℃	1kb/min
5	72℃	5~10 min
6	16℃	∞

Repeat 30 times in 3-5 steps

5.Use the palm centrifuge to mix the solution in PCR tube.

6.Put the PCR tube into the thermocycler and run the program.

7.Using agarose gel electrophoresis to confirm if correct construction is present.

1.3 Error-prone PCR

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

	A	B
1	2×Raqid Master Mix (Vazyme)	25 μl
2	Template	2 ng
3	Forward Primer (10 μM)	2 μl
4	Reverse Primer (10 μM)	2 μl
5	Mn^{2+}	1 μl (final concentration 0.02 mM)
6	Mg^{2+}	1 μl (final concentration 1.25 mM)
7	dNTP	1 μl (final concentration 2 mM)
8	ddH_2O	15.5 μl

3.Program the thermocycler as follows:

	Temperature	Time
1	95/98℃	5 min
2	95/98℃	30 s
3	Tm-3~5℃	30 s
4	72℃	1kb/min
5	72℃	5~10 min
6	16℃	∞

Repeat 30 times in 3-5 steps

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 the correct construction is present.