2025 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.

1.1 Simple 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)	25μl
2 Template	1μl
3 Forward Primer (10μ M)	1μl
4 Reverse Primer (10μ M)	1μl
5 ddH2O	22μl

3. Program the thermocycler as follows:

Temperature	Time
1 98℃	5 min
2 98℃	30 s
3 Tm-3~5℃	30 s
4 72℃	1kb/min
5 72℃	5 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 correct construct was 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 the following reagent to a PCR tube.(10 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.

A	B
1 2×Raqid Master M ix(Vazyme)	5μl
2 Template	0.4μl
3 Forward Primer (1 μM)	0.4μl
4 Reverse Primer (1(μM)	0.4μl
5 ddH2O	3.8μl

4. Program the thermocycler as follows:

Temperature	Time
1 98℃	5 min
2 98℃	30 s
3 Tm-3~5℃	30 s
4 72℃	1kb/min
5 72℃	5 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 construct was present.

1.3Site-directed saturation mutagenesis

1. PCR was performed with pLB 1s-ndmDtB as a template (template plus 20 to 50 ng / 50 μ L) with high-fidelity enzyme and degenerate primers
2. Add the following reagent to a PCR tube.(10 μ l).

A	B
1 2×High Fidelity Master Mix(MCLAB)	25μl
2 Template	1μl
3 Forward Primer (10μ M)	1μl
4 Reverse Primer (10μ M)	1μl
5 ddH2O	22μl

3. Program the thermocycler as follows:

Temperature	Time
1 98℃	5 min
2 98℃	30 s
3 Tm-3~5℃	30 s
4 72℃	1kb/min
5 72℃	5 min
6 25℃	∞

Repeat 25 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 PCR product purification

Preparation of linearized vectors

2. Select an appropriate cloning site on the vector that will be linearized.

3. Vector linearization: the linearized vector can be obtained by digesting the circular vector with restriction enzymes or by reverse PCR.

PCR of the inserts DNA fragments

4. Amplify the insert DNA fragments with homologous sequences (for homologous recombination) of vector-upstream or -downstream by PCR using high fidelity DNA polymerase.

Calculate amount and ratio of linearized vectors and Inserts

5. Detect DNA concentration of linearized vectors and inserts by Nanodrop.
6. Calculation of the amount of vectors:

Molar ratio of vector to insertion is 1:1

Recombination & PCR

7. Setup the following reaction on ice (50μl):

	A	B
1	Forward Primer (10 μM)	1μl
2	Reverse Primer (10 μM)	1μl
3	Fragment1(vector)	X
4	Fragment2(insertion)	Y

5	2×High Fidelity Master Mix (MCLAB)	25μl
6	ddH2O	Add to 50μl

The primer is used to amplify recombinant DNA fragment/circular DNA.

8. Program the thermocycler as follows:

	A	B
1	Temperature	Time
2	95/98℃	5 min
3	95/98℃	30 s
4	Tm-3~5℃	30 s
5	72℃	1kb/min
6	72℃	5~10 min
7	16℃	∞

Repeat 30 times in 3-5 steps

9. Use the palm centrifuge to mix the solution in PCR tube.
10. Put the PCR tube into the thermocycler and Run the program.
11. Using agarose gel electrophoresis to confirm if correct construct was present.