

PCR (Polymerase chain reaction)

Materials

2×Taq PCR Master Mix was obtained from Biomed (Beijing, China). Forward primer and reverse primer were obtained from Genscript (Jiangsu, China).

Components

Component	Volume
2×Taq PCR Master Mix	12.5 µL
ddH ₂ O	9.5 µL
<i>mecA</i> Plasmid	2 µL (1ng)
Forward Primer	0.5 µL
Reverse Primer	0.5 µL
Total volume	25 µL

Thermocycling conditions for PCR

Step	Temperature	Duration	Cycles
Initial Denaturation	94 °C	5 min	1
Denaturation	94 °C	30s	
Annealing	57 °C	30s	30
Extension	72 °C	2 min 30s	
Final extension	72 °C	5 min	1

PCR product purification

Materials

Binding Buffer (BB), Wash Buffer (WB), Elution Buffer (EB), Spin Column EC with Collection Tubes were all supplied by quick Midi Purification Kit from Biomed (Beijing, China).

Procedures

1. Add 100mL anhydrous ethanol to the wash buffer.
2. Transfer the PCR product from the PCR tube to the centrifuge tube. Add ddH₂O to the centrifuge tube to adjust the volume to 100 µL. Add 500 µL binding buffer to the centrifuge tube.
3. Put the spin column EC into the collection tube.
4. Transfer the solution from the previous step to the spin column EC. Let the spin column EC stand at room temperature for 1 minute. Centrifuge the spin column EC at 12,000 rpm for 1 minute and discard the waste liquid in the collection tube. Put the spin column EC back into the collection tube.
5. Add 500 µL wash buffer to the spin column EC. Centrifuge the spin column

EC at 12,000 rpm for 30 seconds and discard the waste liquid in the collection tube. Put the spin column EC back into the collection tube.

6. Repeat step 5.
7. Centrifuge the spin column EC at 12,000 rpm for 2 minutes and discard the waste liquid in the collection tube.
8. Put the spin column EC into a new collection tube.
9. Let the spin column EC stand for 3 minutes with the cap opened.
10. Add 30 μ L elution buffer to the center of the spin column EC (the elution buffer is preheated to 70 degrees Celsius). Let the spin column EC stand at room temperature for 2 minutes.
11. Centrifuge the spin column EC at 12,000rpm for 1 minute.
12. Pipette the elution buffer in the collection tube back to the center of the spin column EC. Let the spin column EC stand at room temperature for 2 minutes. Centrifuge the spin column EC at 12,000rpm for 1 minute.
13. The purified DNA is preserved at -20 degrees Celsius.

sgRNA/Cas9 Incubation

Materials

Cas9 Nuclease (10mg/mL) and 10 \times Cas9 Nuclease Reaction Buffer were obtained from KeyGen BioTECH (Jiangsu, China).

sgRNA was obtained from Genscript (Jiangsu, China).

Groups

sgRNA- Δ mecA + Cas9 + purified <i>mecA</i> gene	15.64 μ L ddH ₂ O
	2 μ L 10 \times Cas9 Nuclease Reaction Buffer
	1 μ L sgRNA- Δ mecA
	0.16 μ L Cas9 Nuclease
	1.2 μ L <i>mecA</i> gene (100ng)
sgRNA- Δ EGFP + Cas9 + purified <i>mecA</i> gene	15.64 μ L ddH ₂ O
	2 μ L 10 \times Cas9 Nuclease Reaction Buffer
	1 μ L sgRNA- Δ EGFP
	0.16 μ L Cas9 Nuclease
	1.2 μ L <i>mecA</i> gene (100ng)
sgRNA- Δ mecA + purified <i>mecA</i> gene	15.8 μ L ddH ₂ O
	2 μ L 10 \times Cas9 Nuclease Reaction Buffer
	1 μ L sgRNA- Δ mecA
	1.2 μ L <i>mecA</i> gene (100ng)

Cas9 + purified <i>mecA</i> gene	16.64 µL ddH ₂ O 2 µL 10 × Cas9 Nuclease Reaction Buffer 0.16 µL Cas9 Nuclease 1.2 µL <i>mecA</i> gene (100ng)
ddH ₂ O + purified <i>mecA</i> gene	16.8 µL ddH ₂ O 2 µL 10 × Cas9 Nuclease Reaction Buffer 1.2 µL <i>mecA</i> gene (100ng)

Procedures

1. Add all the components except DNA to the PCR tubes.
2. Put the PCR tubes into the thermocycler and incubate the PCR tubes at 25 degrees Celsius for 10 minutes.
3. Add 1.2 µL purified DNA (100ng) to each PCR tube.
4. Put the PCR tubes into the thermocycler and incubate the PCR tubes at 37 degrees Celsius for 1 hour, followed by incubation at 85 degrees Celsius for 5 minutes.

Agarose Gel Electrophoresis

Materials

Trans5K DNA marker and Gelstain were obtained from TransGen Biotech (Beijing, China).

6×DNA loading buffer was obtained from Toroivd (Shanghai, China).

Procedures

Preparation of agarose gel:

1. Add 0.6g agarose and 60mL 1×TAE buffer in the flask.
2. Mix the agarose solution and heat the solution in a microwave oven until it boils 3 times.
3. Cool down the agarose solution in the ice box and add 6 µL Gelstain to the solution before the solution gelates.
4. Insert combs into the gel casting tray and pour the agarose solution into the gel casting tray.
5. Wait 30 minutes for the gel to gelate.

Electrophoresis:

1. Add 6×loading buffer into the DNA sample of each group.
2. Place the gel into the electrophoresis chamber.
3. Pour 1×TAE buffer into the chamber until it covers the gel.

4. Load the DNA marker and DNA sample of each group into the loading wells of the gel.
5. Start the electrophoresis with certain voltage, amperage and duration.
6. After electrophoresis, put the gel under UV light to observe the DNA band of each group.