1. Extract the plasmid

(1) Centrifuge 2 ml of thallus at 12000 RPM for 1 min, pour out the supernatant, add 2 ml of bacterial solution if the plaque is small and repeat the operation.
(2) Add 250μl solutionI, Blow and mix repeatedly to make sure there are no lumps.
(3) Add 250 μl solutionII, Slowly invert, turn the test tube several times to gently mix, stand for two minutes, get a transparent cleavage product.
(4) Add 350 μl solutionIII, Immediately invert and mix well.
(5) Centrifuge at 12000 RPM for 15 min.
(6) Carefully absorb the supernatant into the purification column, rather less adsorption supernatant does not adsorption precipitation.
(7) Add 500 μl HBC washing Buffer, 12000 rpm 1min, Abandon the filtrate.
(8) Add 700 μl DNA washing Buffer, 12000 rpm 1 min, Abandon the filtrate.
(9) Repeat (8).
(10) Empty column centrifugation, 12000 rpm 2min.
(11) Oven drying until without ethanol.
(12) Placing the purification column into a clean 1.5ml EP tube and adding 30-100 μl of elution buffer preheated at 60 ° C to the column, and leaving it for 2 min, Centrifuge at 12000 RPM for 1 min.
(13) Repeat (12) but except leaving it for 2 min.

2. Whole plasmid PCR

Reaction system

2 × Phanta Max Master Mix	50 μl
Upstream primer	2 μl
Downstream primer	2 μl
DNA	2 μl
dd H_20	44 μl

PCR temperature

95 ℃	30 sec	
95 ℃	15 sec	35 cycles
58 ℃	15 sec	
72 ℃	3.5 min	
72 ℃	2 min	
12 ℃	∞	

3. Electrophoresis

DNA electrohoresis is performed in agarose gel which is 1% and TAE buffer, stained with etidium bromide and imaged under UV light.

4. Gel purification

(1) Cut the glue as thin as possible, add the same volume of Buffer GDP, bath in 58℃ water.
(2) The liquid was transferred into the purification column and allowed to stand for 5 min.
(3) 12000rpm 1 min, return the suction column, 12000rpm 1 min.
(4) Discard the solution and add 300 μl Buffer GDP and centrifuge at 12000rpm for 1 min.
(5) Add 700 μl SPW wash Buffer, centrifuge at 12000rpm for 1 min.
(6) Repeat (5).
(7) Drop the solution, centrifuge at 12000rpm for 1 min.
(8) Open the cover and drying at 37℃ incubator for 15-20 min.
(9) Place the purification column and 20 μl of ddH2O preheated at 60 ° c was added to the center of the membrane and left for 5 min.
(10) 12000rmp 1min.
(11) Add the solution back to the purification column and centrifuge at 12000rmp for 1 min.

5. Homologous recombination

The carrier	50 μl
Fragment 1	2 μl
Fragment 2	2 μl
……	2 μl
2xSoSoo Mix	5 μl
dd H_20	Up to 50 μl

The carrier is 10-100ng, The moore of the carrier : the Moore of the fragment is 1:2-1:10, the Moore of each fragment is 1.

6. Plasmid transformation

TOP10:

(1) Put 20 μl of competent E. coli melted on ice into a precooled 1.5 ml EP tube
(2) Add 2 μl plasmid into the E. coli.
(3) Put the E. coli into the 42℃ water for 90 sec.
(4) Put the E. coli on the ice for 2-3 min.
(5) Add 500μl LB without ampicillin and put it on 37℃ Shaking table for 1h.
(6) 5000rmp 2min, pour out some of the supernatant and use the rest for blending and Spread E. coli evenly on the plate.

BL21:

(1) Put 20 μl of competent E. coli melted on ice into a precooled 1.5 ml EP tube.
(2) Add the product into the E. coli and put it on the ice for 25-30min.
(3) Put the E. coli into the 42℃ water for 90 sec.
(4) Put the E. coli on the ice for 2-3 min.
(5) Spread E. coli evenly on the plate.

7. Colony PCR

Reaction system

2 × Taq	12.5 μl
Upstream primer	0.5 μl
Downstream primer	0.5 μl
dd H_20	11.5 μl

PCR temperature

95 ℃	30 sec	
95 ℃	15 sec	35 cycles
58 ℃	15 sec	
72 ℃	30 sec	
72 ℃	2 min	
12 ℃	∞	

8. Gene express

(1) Extract 50μl of bacterial solution from the retaining tube.
(2) Add into 5ml LB medium to shake the bacteria for 2h.
(3) 2ml of the mixture of bacterial solution and medium is extracted into 80ml LB medium.
(4) When the OD value is between 0.6 and 0.8, add 40ul IPTG to induce E. coli to produce protein, and wait for 12h.

9. Protein purification

(1) Column balance: Put the filler into the column and add 10mL of 0mM imidazole to the column.
(2) Loading sample: put the filler into the tube, add 10mL crushing liquid and shake for 10min. The mixture of packing and flow through the liquid is added to the column, and the flow through the liquid passes through the column again.
(3) Washing: 6ml10mM imidazole is added to the column.
(4) Elution: put the filler into the tube, combine with 6mL100mM imidazole and shake for 10 min. Add to the column and wash it again.

10. SDS-PAGE

The protein glue is assembled with the instrument, 40ul of sample liquid and 10ul of corresponding buffer are extracted and heated at 100℃ for 10min, and 30ul of sample is dotted int o the glue.

11. Enzyme activity measurement

phosphate buffer	40 µl
Na_2S (100 µM)	40 µl
Decyl ubiquinone (50 µM)	20µl
dd H_20	100 µl

Use 275nm light and test the OD number each 2 min. Use the OD number to draw the line.