B. Plasmid Transformation and Cultivating

1	Prepartation	② take the foam box, carrying about 2/3 volume of ice; ② remove 100μL competent cells from the -80 ℃ refrigerator.B
2	Packing	① remove the alcohol lights, tweezers, sterile centrifuge tube and sterile tip; ② ignite the alcohol lights, burning the tweezers 5s on it; ③ remove the 1.5mL sterile centrifuge tube, cover the tube cover and put it on the tube box; ④ open the centrifuge tube with competent cells, remove 50μL competent cells into the sterile centrifuge tube; do these beside the alcohol lights; ⑤ put the above two centrifuge tubes equipped with competent cells in the ice box in the spare.
3	Adhesion	① use a sterile tip to remove 5μL plasmid on the plasmid tray, add it 50μL competent cells; do these beside the alcohol lights, ② open the water bath, and set the temperature of 42 ℃; ③ put the centrifuge tube in the ice box, standing 30min;
4	Heat Shock	① put the ice-bath-after centrifuge tube in 42 ℃ water bath, heat shock 45s; ② Remove the centrifuge tube, placed in the ice box 2min.
5	Recovery	① remove the non-antibiotic liquid medium; ② use sterile tip to remove 500μL liquid medium and add to the centrifuge tube which has been heat shocked; ③ put the centrifuge tube into the 37 ℃ shaker with the tube pad, incubated 1h.
6	Coat Dishes	① remove centrifuge tube from the shaker, centrifuge 7000rpm for 30s or 5000rpm for 1min; ② use sterile tips to abandon 400uL supernatant beside alcohol lights; use a pipette make remaining bacteria liquid well mixed; ③ according to plasmid's resistance, remove the chloramphenicol resistant solid

		medium or ampicillin resistant solid medium; ④ use sterile tips to take 100uL bacteria liquid mentioned in step 3 then add to the surface of solid medium beside alcohol lights; ⑤ apply the bacteria on the solid medium with a coated rake and spread it evenly, cover the culture dish; ⑥ inverted the solid medium, placed in 37 ℃ incubator, cultured 12h.
7	Picking Colony	① remove the non-antibiotic liquid medium, absorb antibiotics, added chloramphenicol 5 ‰ or ampicillin 1 ‰; ② tilt the liquid medium to add antibiotics; ③ open the solid medium, looking for a single colony, then use a tip to row the colony, and put the tip into the liquid medium; ③ place it in the 37 ℃ shaker, culture 12h.