

B. The Transformation of Plasmid pKD46

1	The Preparation of Competent Cells(CaCl ₂)	<ul style="list-style-type: none"> a. Shake the E.coli BW25113 overnight at 30°C, then transfer it into a new liquid no resistant LB medium. b. Detect OD600 each hour. c. When OD600 is between 0.3 and 0.4, take 1.5ml bacterium solution into a 1.5ml sterile centrifuge tube, putting it into ice for 15-30min. d. Centrifuge the bacterium solution in the conditions of 4000rpm, 2min, 4°C, abandoning the supernatant. e. Add 800μl pre-cooling 0.1mol/L sterilized CaCl₂ solution to suspend the sediment. f. 30min ice bath. g. Centrifuge the bacterium solution in the conditions of 4000rpm, 2min, 4°C, abandoning the supernatant. h. Add 200μl pre-cooling 0.1mol/L sterilized CaCl₂ solution to suspend the sediment, take 50μl to transform.
2	The Transformation of Plasmid pKD46	<ul style="list-style-type: none"> a. Add 50μl competent cells and 5μl plasmid pKD46 to 1.5ml sterile centrifuge tube, taking 30min ice bath. b. After a 45s heating at 42°C water, put the tube into ice rapidly for 2min, then adding 500μl liquid no resistant LB medium. c. Shake the tube for 1.5h at 30°C. d. Centrifuge the bacterium solution in the conditions of 5000rpm for 1min, abandoning the supernatant. e. Coat the ampicillin resistant solid LB medium with the bacterium solution, than cultivated overnight at 30°C. f. Picking a single bacterial colony into the ampicillin resistant liquid LB medium, cultivated overnight at 30°C.
3	The Verification	<ul style="list-style-type: none"> a. Bacteria keeping: add 700μl bacterium solution and 700μl 50% sterile glycerin into a 1.5ml sterile centrifuge tube, storing at -20°C. b. Extract plasmids: TIANprep Mini Plasmid Kit. c. AGE