C. Electroporation

1	The Preparation of Competent Cells(glycerin)	a. Add 100μl pKD46-transformed E.coli BW25113 to 7ml liquid LB medium, along with 7μl ampicillin solution, shaking overnight at 30℃. b. Take 5% of bacterium solution into 30ml no resistant liquid LB medium within 30μl ampicillin solution, cultivating at 30℃ shaker. c. Detect OD600 each hour until it is between 0.3 and 0.4. d. Add L-Arabinose until its concentration is 30mmol/L. Then shake it at 30℃ for 1h. e. Take all of the bacterium solution into 1.5ml centrifuge tubes, ice bath for 10min. f. Centrifuge the bacterium solution in the conditions of 5000rpm and 4℃ for 10min, abandoning the supernatant. g. Suspend the sediment with pre-cooling 50% sterile glycerin till 1ml, centrifuging in the conditions of 5000rpm and 4℃ for 2min, repeat for 3 times. h. Suspend the sediment with 300μl glycerin.
2	Electroporation	a. Take 100μl bacterium solution and 10μl kan targeting vector into 1.5ml sterile centrifuge tube, mix, then adding into a pre-cooling electroporation cup with no bubbles. b. The size of electroporation cup:1mm or 2mm. c. Electroporation parameters: 2000kV, 25μF, controller 200Ω
3	Follow- up	a. Add 1 ml no resistant liquid LB medium immediately after electroporation, mix. b. Suck all the liquid out of the electroporation cup into a new sterile 1.5ml centrifuge tube, shaking it at 30℃, 180rpm for 1-1.5h. c. Coat the ampicillin resistant solid LB medium with the bacterium solution, cultivated overnight at 37℃.