Transformation

1. Take competent cells out from -80℃ storage, incubate the competent cells on the ice for 10 min so that it thaws.

2. Add ligation product gently.

System component	volume	**Note**:competent cells are Trans-T1 Phage Resistant Chemically ones or Trans5 α Chemically ones from TransGen Biotech. Solution III is Transformation Enhancer from TaKaRa DNA Ligation Kit Ver.2.1.
Ligation product	5ul	
Competent Cell	30~50ul	
Solution III	1/10 of total	

3. Incubate the mixture on ice for 30 min.

4. Heat shock in 42℃ water bath for 50 sec.

5. Put the mixture on the ice immediately for 2 min.

6. Add 500ul LB non-antibiotic medium, incubate in shaker at 200rpm, 37℃ for 1 h.

7. Take the culture out from shaker, centrifuge for 1 min at 3000~4000rpm, discard 300~400ul supernatants.

8. Blend the cells and the remaining supernatants, plate the mixture on LB plate containing corresponding antibiotics.

9. Incubate in 37℃ overnight.