One-Step Cloning

Materials

2×Basic Assembly Mix(TransGen catalog no. CU201-02)
Linearized vector
Inserts
Nucleasw-free Water
LB agar plates
LB medium
chemo-competent E.coli trans1-T1 cells
LB agar plates(with selection marker)
LB medium

Procedure

Recombine:

1. Calculate the amount of carrier and fragment to be added according to the following proportion.In the 10 μL reaction system, the recommended dosage of vectors and each insertion fragment was 0.01-0.25 pmols. The optimal molar ratio of the vector to each inserted fragment is1:2.

Pmols = quality ng /(fragment length bpX0.65kda)

For example: 100 ng of 2000 bp fragment is equal to 100/(20000.65) about 0.08 pmols.

100 ng of 5000 bp fragment is equal to 100/5000*0.65) about 0.03 pmols.

2×Basic Assembly Mix	5 uL
Linearized vector(5-100ng)	x uL
Inserts	y uL
Nucleasw-free Water	To 10 uL

2. Mix gently and react at 50°C for 15 minutes. After the reaction, place the centrifuge tube on ice. Cool for a few seconds. The recombinant product can then be stored at 20C or used directly in the transformation.

Transformation:

1. Melt trans1-T1 receptor cells on ice.
2. Add 5 μL of recombinant product into 25 μL cells, gently shake the centrifugal tube wall to mix (vortex is forbidden), and place on ice for 30 minutes.
3. 42°C water bath in heat shock for 90 seconds, after the horse. Transfer to ice and cool for 2 minutes.

4. Add 970 μL LB medium at room temperature, then culture in 37℃ shaker at 250 rpm for 1 hour.
5. The LB plate suitable for resistance was preheated in 37℃.
6. Evenly spread 100 μL cells on the plate. And then incubated overnight in incubator 37℃.