

Protocol of FastCloning

1-5™ 2x High-Fidelity Master Mix (TSINGKE™)

Step 1: PCR amplification of vector and insert

Vector: PET-28a Plasmid

System: 50µl

H ₂ O	variable
DNA polymerase: 1-5™ 2x High-Fidelity Master Mix	25µl
Primer: PET-28a-Forward	0.5µl
Primer: PET-28a-Reverse	0.5µl
Template: PET-28a Plasmid	0.5µl
Total	50µl

Insert: eGFP plasmid

System: 50µl

H ₂ O	variable
DNA polymerase: 1-5™ 2x High-Fidelity Master Mix	25µl
Primer: eGFP-28a -Forward	0.5µl
Primer: eGFP -28a-Reverse	0.5µl
Template: eGFP Plasmid	0.5µl
Total	50µl

The PCR cycling parameters:

Step	Temperature,	Time	Number of cycles
Initial denaturation	98°C	2 min	1
Denaturation	98°C	15 sec	18
Annealing	T _m -5	15 sec	
Extension	72°C	15 sec/kb	
Final extension	72°C	5 min	1
Store	4°C	infinite	1

Gel electrophoresis confirmation of PCR products

Samples: 5µl PCR products

1% agarose gel electrophoresis with ethidium bromide staining run at 110 V for 20 min.

Visualize the PCR products under a UV transilluminator.

Step 2: DpnI digestion of DNA templates

System: 50µl

QuickCut Buffer	5µl
Vector: PCR product of PET-28a	22.5µl
Insert: PCR product of eGFP-28a fragment	22.5µl
DpnI	1.2µl
Total	50µl

Digested in 30°C for 1h

Step 3: Transformation into competent cells

1. Thaw a tube (100 μ l) of Competent DH5 α E.coli cells on ice until the last crystals disappear (about 10 min).
2. Add 10 μ l of the digested vector-insert mixture to the cell mixture. Carefully flick the tube 4–5 times to mix cells and DNA. Do not vortex.
3. Incubate the mixture on ice for 30 min. Do not mix.
4. Heat shock at exactly 42°C for 1 min. Do not mix.
5. Place the mixture on ice for 10 min. Do not mix.
6. Pipette 650 μ l of room temperature (25°C) LB medium into the mixture.
7. Place at 37°C for 1h. Shake vigorously (180 rpm) or rotate.
8. Warm K+C+ selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting. Centrifuge at 12000 rpm for 1 min, then discard the supernatant to keep 300ul in the tube.
10. Spread 300 μ l onto a K+C+ selection plate and incubate overnight at 37°C.