

- Optimal incubation temperature: for most PCR inserts, the optimal temperature is about 25°C; for some PCR inserts, optimal results can be achieved with higher temperature (up to 37°C).

Transformation

- Add the ligated products to 50 µl of *Trans*1-T1 Phage Resistant Chemically Competent Cell and mix gently (do not mix by pipetting up and down).
- Incubate on ice for 20~30 minutes.
- Heat-shock the cells at 42°C for 30 seconds.
- Immediately place the tube on ice for 2 minutes.
- Add 250 µl of room temperature SOC or LB medium. Shake the tube at 37°C (200 rpm) for 1 hour.
- In the meantime, mix 8 µl of 500 mM IPTG with 40 µl of 20 mg/ml X-gal. Spread them evenly onto a selective LB plate. Place the plate at 37°C for 30 minutes.
- Spread 200 µl or all transformants on the pre-warmed plate. Incubate at 37°C overnight.

Identification of Positive Clones and Sequencing

Analysis of positive clones

- Transfer 5~10 white or light blue colonies into 10 µl ddH₂O and vortex.
- Use 1 µl of the mixture as template for 25 µl PCR using M13 forward and M13 reverse primers.
- PCR reaction conditions

94°C	10 min	} 30 cycles
94°C	30 sec	
55°C	30 sec	
72°C	x min*	
72°C	5-10 min	

* (depends on the insert size and PCR enzymes) the PCR product size from vector self-ligation is 100 bp.

- Analyze positive clones by restriction enzyme digestion and DNA sequencing.
Inoculate positive clones on LB/Amp⁺ or LB/Kan⁺ liquid medium, grow at 37°C for 6 hours at 200 rpm. Isolate plasmid DNA by plasmid MiniPrep Kit. Analyze colonies by restriction enzyme digestion with proper restriction endonuclease.

Sequencing

Analyze the sequence by sequencing with M13 F, M13 R and T7 promoter.

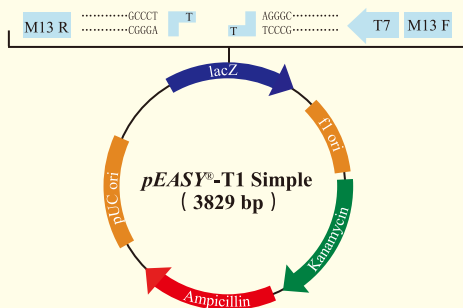
PCR for control insert (700 bp)

Component	Volume	Final Concentration
Control Template (5 ng/µl)	1 µl	0.1 ng/µl
Control Primers (10 µM)	1 µl	0.2 µM
2× <i>EasyTaq</i> [®] PCR SuperMix	25 µl	1×
ddH ₂ O	Variable	-
Total volume	50 µl	-

Thermal cycling conditions for control insert

94°C	2-5 min	} 30 cycles
94°C	30 sec	
55°C	30 sec	
72°C	1 min	
72°C	10 min	

Ligate 1 µl of control PCR insert with 1 µl vector. Hundreds of colonies should be produced with cloning efficiency over 90%.



LacZa fragment: bases 1-445
M13 reverse priming site: bases 205-221
T7 promoter priming site: bases 262-281
M13 forward priming site: bases 288-304
f1 origin: bases 446-883
Kanamycin resistance ORF: bases 1,217-2,011
Ampicillin resistance ORF: bases 2,029-2,889
pUC origin: bases 3,034-3,707

SR Primer

CAG GCT TTA CAC TTT ATG CTT C CG GCT CGT ATG TTG TGT GGA ATT GTG AGC GGA TAA CAA TTT CAC A CA GGA AAC AGC TAT GAC CAT GAT TAC GCC AAG CTG
GTC CGA AAT GTG AAA TAC GAA GGC CGA GCA TAC AAC ACA CCT TAA CAC TCG CCT ATT GTT AAA GTG TG GT CCT TTG TCG ATA CTG GTA CTA ATG CCG TTC GAC

M13 Reverse Primer

T7 Promoter

M13 Forward Primer

CCC TT PCR Product AAA GGG CAG CTT CAA TTC GCC CTA TAG TGA GTC GTA TTA C AA TTC ACT GGC CGT CGT TTT ACA ACG TCG TGA CTG GGA AAA C
GGG AA TT CCC GTC GAA GTT AAG CGG GAT ATC ACT CAG CAT AAT G TT AAG TGA CCG GCA GCA AAA TG T TGC AGC ACT GAC CCT TTT G

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