pEASY®-Uni Seamless Cloning and Assembly Kit

Cat. No. CU101

Storage: *Trans*1-T1 Phage Resistant Chemically Competent Cells at -70°C for six months; others at -20°C for one year.

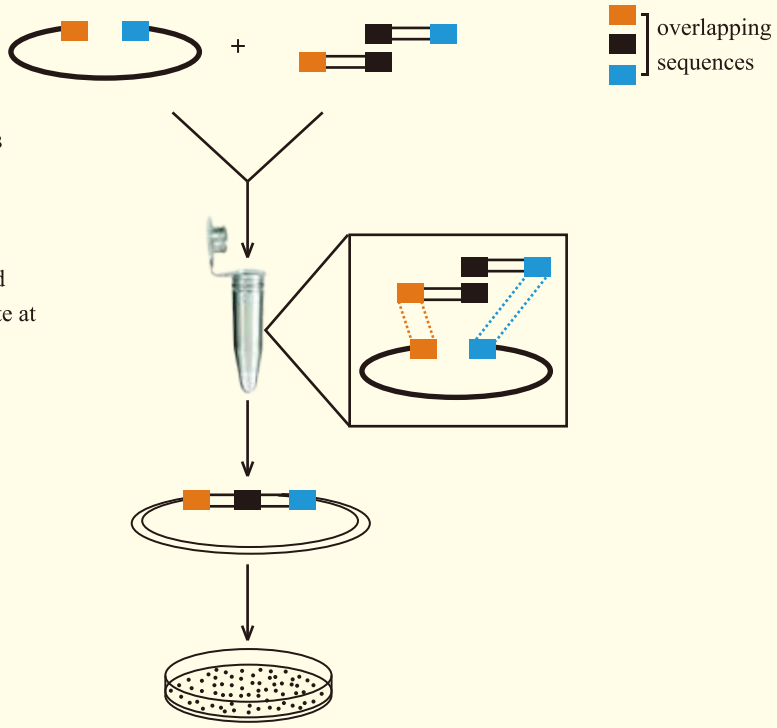
Description

This kit takes advantage of proprietary assembly mix and homologous recombination. This kit can achieve directional cloning of PCR fragments that share 15-25 bp overlapping sequences into any linearized vector.

- Fast: 15 minutes.
- Broad: no restriction enzyme digestions. Can be cloned into any sites.
- High efficiency: up to 95% cloning efficiency.
- Seamless: no extra sequences introduced; up to 5 fragments assembly.

Principle

1. Prepare linearized vector by PCR/Enzyme digestion
2. PCR amplify inserts with 15-25 bp overlapping sequences
3. Mix vector, DNA fragments and Assembly Mix together, incubate at 50°C for 15 minutes
4. Transformation



Applications

Single or multi fragments cloning and assembly.

Kit Contents

Component	CU101-01
2×Assembly Mix	50 µl
*Trans*1-T1 Phage Resistant Chemically Competent Cell	5×100 µl
Linearized pUC19 Control Vector (10 ng/µl)	3 µl
Control Insert (1 kb，20 ng/µl)	3 µl

Cloning

Preparation of Vector and Inserts

A: Preparation of Vector

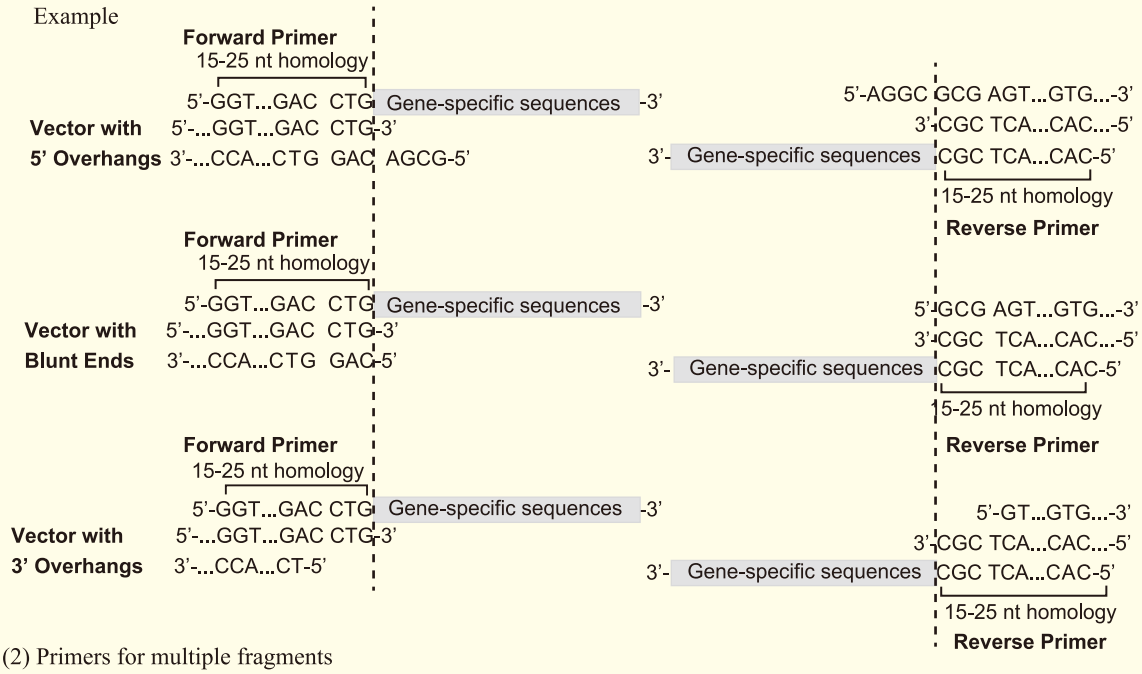
(1) Enzyme digestion: digest plasmid vector with restriction enzyme(s) to generate the linearized vector. Purify the digested vector using Gel Extraction Kit (Cat. No. EG101).

(2) PCR amplification: prepare the linearized vector by high-fidelity DNA polymerase. If a single expected band is generated, use PCR Purification Kit (Cat. No. EP101) to purify the product. Otherwise, use Gel Extraction Kit to recover the product.

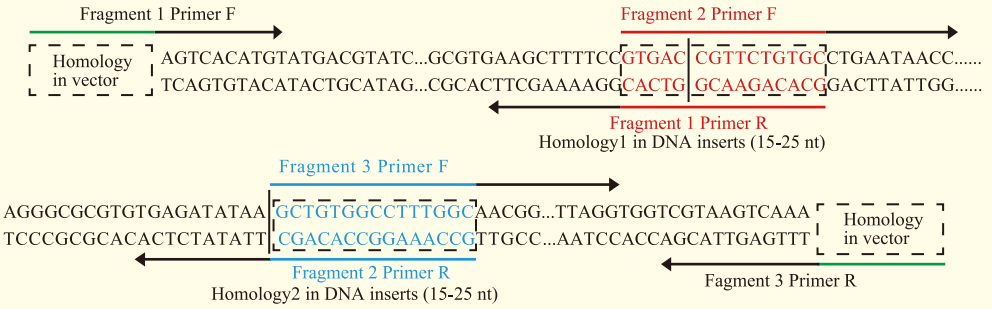
In order to increase the positive cloning efficiency, we suggest using DMT enzyme to digest plasmid template before PCR purification or gel extraction. Add DMT enzyme (Cat. No. GD111) after PCR amplification (1 μl of DMT enzyme for a 50 μl PCR system), and incubate at 37°C for 30 minutes.

B: Preparation of Inserts

(1) Forward primer (5'-3'): 15-25 nt homology of linearized vector + 20-25 nt target specific sequence.
Reverse primer (5'-3'): 15-25 nt homology of linearized vector + 20-25 nt target specific sequence.



(2) Primers for multiple fragments



(3) We suggest to use high-fidelity DNA polymerases to generate both the linear vector and fragments.

(4) Reaction conditions

- Use 0.2-0.4 μM (final concentration) primers for PCR.
- Use 60-68°C as annealing temperature.

(5) Purification of target DNA fragments

- To increase the cloning efficiency, if the recombinant vector has the same selection marker as the parental plasmid for PCR fragments, pretreat the PCR fragments with DMT enzyme before purification.
- If product is single band, we recommend using PCR Purification Kit (Cat. No. EP101) to purify your fragments.
- If products are multibands, we recommend using Gel Extraction Kit (Cat. No. EG101) to recover your fragments.

Setting up the cloning reaction

2×Assembly Mix	5 μl
Linearized vector (5-100 ng)	x μl*
Inserts	y μl*
ddH_2O	to 10 μl

* In a 10 μl system, we recommend using 0.01-0.025 pmols of vector and insert respectively, for optimal cloning efficiency, use 1:2 (vector: insert) molar ratio.

pmols= (weight in ng)/(base pairs×0.65 kDa)

For example

100 ng of 2000 bp insert is equal to 100/(2000×0.65) which is about 0.08 pmols.

100 ng of 5000 bp insert is equal to 100/(5000×0.65) which is about 0.03 pmols.

Gently mix and incubate at 50°C for 15 minutes. Place it on ice for a few seconds. The reaction mixture can be directly used for transformation or stored at -20°C.

Transformation

(1) Thaw a vial of *Trans*1-T1 Phage Resistant Chemically Competent Cell on ice.

(2) Transfer 2 μl of reaction mixture into 50 μl of *Trans*1-T1 Phage Resistant Chemically Competent Cell and mix gently by flicking the tube (do not vortex). Incubate on ice for 30 minutes.

(3) Heat-shock at 42°C for 30 seconds, and immediately place on ice for 2 minutes.

(4) Add 450 μl of room temperature SOC/LB medium. Incubate at 37°C for 1 hour at 250 rpm.

(5) Pre-warm LB plate containing the appropriate selection antibiotic at 37°C.

(6) Spread 100 μl of cells on the selection plate and incubate overnight at 37°C.

Analysis of Positive Clones

- **Analyzing positive clones by PCR**

(1) Pick single colony into 10 μl of sterile water. Mix by vortexing or pipetting up and down.

(2) Add 1 μl of mixture into 25 μl of PCR system. Identify the positive clones by appropriate forward and reverse primer.

- **Analyzing positive clones by restriction enzyme digestion**

  Pick single colonies and culture them overnight in LB medium containing the appropriate selection antibiotic. Isolate plasmid DNA by *EasyPure*® Plasmid MiniPrep Kit (Cat. No.EM101). Analyze the plasmids by restriction enzyme digestion.

- **Sequencing**

  Perform sequence analysis using vector universal primers

Cloning reaction for control insert

2×Assembly Mix	5 μl
Linearized pUC19 Control Vector	1 μl
Control Insert	1 μl
ddH_2O	3 μl

Reaction conditions, transformation and analysis of positive clones are the same as above.

Notes

- Cloning efficiency decreases with the size (vector + insert) increases. For higher efficiency, we recommend to use *Trans*2-Blue Chemically Competent Cell (Cat. No. CD411) for transformation.
- For multi fragments cloning, increasing the length of the overlapping sequences can increase the cloning effiency.

Website www.transbionovo.com
E-mail trade@transbionovo.com
Customer Service +86-10-57815027
Phone +86-10-57815087