ClonExpress® II

One Step Cloning Kit

Vazyme

www.vazyme.com

Introduction:

The ClonExpress™ II (One Step Cloning) Kit provides a convenience, fast, high-throughput cloning of DNA fragment and generation of precise, directional constructs with any vectors that are ready for any purpose. The Seamless cloning technology allows cloning your gene or sequence of interest into any vector in one day without the need for restriction enzymes, ligase, or blunt-end polishing. The homologous recombination can occur in any site in vector only if the tail of fragment has 15-20 bp homologous to the corresponding fragment or vector.

Component:	**C112-01 (10 rxn)**	**C112-02 (25 rxn)**
5×CE II Buffer	100μL	200μL
Exnase™ II	50μL	100μL
pUC19 control vector, linearized (50ng/μL)	5μL	5μL
500bp control insert (25ng/μL)	5μL	5μL

Storage: store at -20°C, the validity period is half a year.

Product Feature:

I. flexible cloning sites: any sites in any vector.

II. fast and convenient: construct the vector in 30 minutes.

III. accuracy: no extra steps.

IV. efficient: 95% positive clone.

Steps:

I. Linearized Vector Preparation

1. Restriction Enzymes:

Using appropriate enzyme to digest the vector. Double enzymes digestion is strongly recommend
rather than single in order to improve the rate of positive clone. Purify the vector through DNA gel
electrophoresis.

2. PCR:

Design forward and reverse primers in appropriate site. Using high fidelity DNA polymerase to get
better results. Purify the vector through DNA gel electrophoresis to get rid of plasmid template and
improve the rate of positive clone.

II. Fragment Preparation

15-20bp sequences are required in both ends of fragment, which asks a special pair of primers. The sequences should be homologous to the liner vector. As for the primer, each primer should include at least 15bp homologous sequences and 20-25 specific sequences.

Note: Check the reading frame when designing primers which should be purified by HPLC or PAGE. High fidelity DNA polymerase is also needed.

III. Recombination Reaction

1.Mix fragment and vector in appropriate mole ratio in EP tube to reaction on ice. Use pipette mix the solution gently and avoid bubbles.

reaction system

ddH_2O	Up to 20μL
5×CE II Buffer	4μL
linearized cloning vector	xμL （50~200ng）
amplified inserted fragments	xμL (20~200ng）
Exnase™ II	2μL

The most appropriate mass of linearized cloning plasmid = [0.02 × numbers of base pair of plasmid] ng (0.03pmol)
The most appropriate mass of inserted fragment = [0.04 × numbers of base pair of fragment] ng (0.06pmol)

Example: when inserting a 2kb fragment into a 5kb cloning plasmid, the most appropriate masses of linearized cloning plasmid: 0.02 × 5000 = 100ng;
the most appropriate masses of 2kb inserted fragment: 0.04 × 2000 = 80ng

Note:

a) When the length of inserted fragment is bigger than the length of cloning plasmid, the most appropriate mass of cloning plasmid and inserted fragment should be interchanged.
b) The mass of linearized cloning plasmid should be in the scale of 50~200ng. The mass of inserted fragment should be be in the scale of 20~200ng. When the appropriate mass of DNA is out of this scale, please choose the lowest / highest mass.
c) When inserted fragment and linearized cloning plasmid don't undergo purification, the total volume of the added fragments or plasmid shouldn't be over 4μL.
d) The ClonExpress™ MultiS kit has provided pUC19 control vector, linearized (50ng/μL) and 500bp control insert (25ng/μL) 5μL, respectively. When needed you can use them as positive control. Add 1μL per reaction.

2. 37°C water bath the EP tube 30 minutes before putting it on ice. Keep it on ice for 5min.
3. Transform the mixture into competence cell.

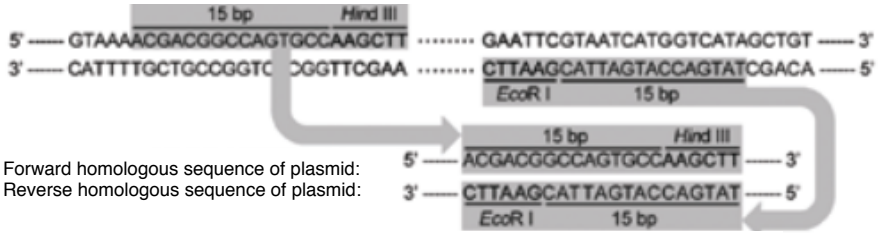
IV. Transformation

1. Add 5μL mixture into 50μL competence cell, keep it on ice for 30min.
2. 42°C water bath 45-90s before put it back on ice at least 2 minutes
3. Add 900μL LB or SOC, 37°C incubation for 10min.
4. 37°C shake culture for 45min.
5. Coat 100μL mixture on LB plate with specific antibiotic, 37°C culture the whole night. Check the clone in next day.

V. Example of Primer Design:

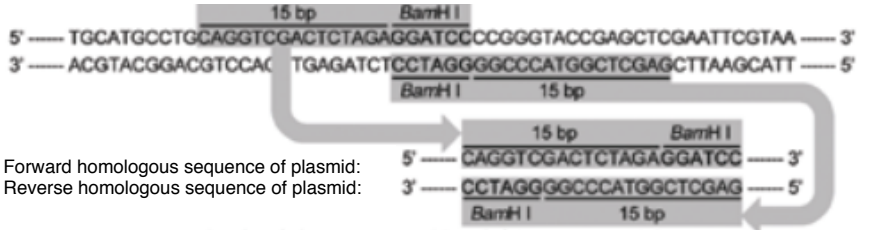
1. Liner Fragment Primer

A. If cloning plasmid are digested by double enzymes (*Hind III* + *EcoR I*):



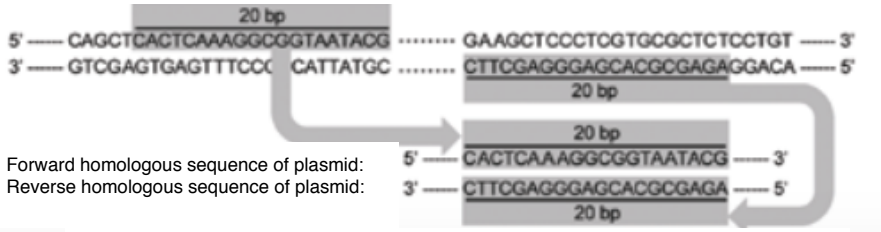
Forward homologous sequence of plasmid:
Reverse homologous sequence of plasmid:

B. If cloning plasmid are digested by single enzyme (*BamH I*):



Forward homologous sequence of plasmid:
Reverse homologous sequence of plasmid:

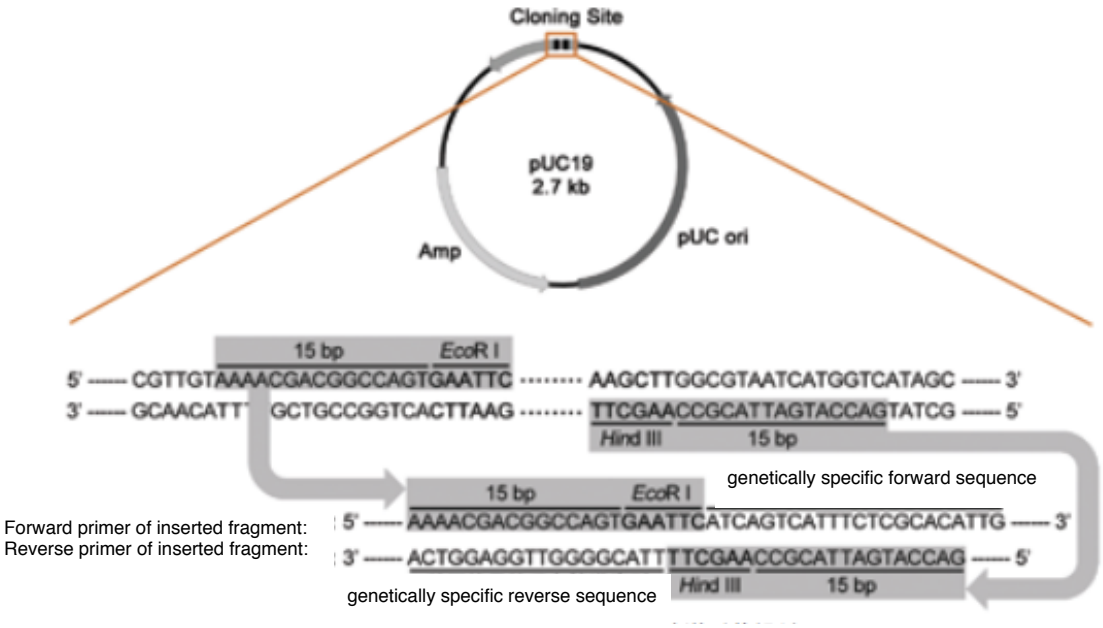
C. If cloning plasmid are amplified by PCR:



Forward homologous sequence of plasmid:
Reverse homologous sequence of plasmid:

(Note: the inserted fragment will be ligated with plasmid scarlessly when cloning completed)

2. Primer for Ligation of Nearby Fragment



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