

ClonExpress[®] MultiS

One Step Cloning Kit



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Introduction :

The ClonExpress™ MultiS (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:	C113-01 (10 rxn)	C113-02 (25 rxn)
5×CE MultiS Buffer	40μL	100μL
Exnase™ MultiS	20μL	50μL
pUC19 control vector, linearized (50ng/μL)	5μL	5μL
Control insert Mix (0.5kb, 10ng/μL; 1kb, 20ng/μL; 2kb, 40ng/ 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 ₂ O	Up to 20μL
5×CE MultiS Buffer	4μL
linearized cloning vector	xμL (50~200ng)
amplified inserted fragments	xμL (>10ng)
Exnase™ MultiS	2μL

The most appropriate mass of each fragment = [0.02 × numbers of base pair of fragment] ng (0.03pmol)

Example: when inserting three 0.5kb, 1kb, 2kb fragments respectively into a 5kb cloning plasmid, the most appropriate masses of each fragment are:

the most appropriate masses of linearized cloning plasmid: $0.02 \times 5000 = 100\text{ng}$

the most appropriate masses of 0.5kb inserted fragment: $0.02 \times 500 = 10\text{ng}$

the most appropriate masses of 1kb inserted fragment: $0.02 \times 1000 = 20\text{ng}$

the most appropriate masses of 2kb inserted fragment: $0.02 \times 2000 = 40\text{ng}$

Note:

- a) The mass of linearized cloning plasmid should be in the scale of 50~200ng. When the appropriate mass of DNA is out of this scale, please choose the lowest / highest mass.
- b) The mass of inserted fragment should be more than 10ng. When the appropriate mass of DNA is less than 10ng, please choose 10ng as the mass of inserted fragment.
- 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 4 μ L.
- d) The ClonExpress™ MultiS kit has provided pUC19 control vector, linearized (50ng/ μ L) and Control insert Mix (0.5kb, 10ng/ μ L; 1kb, 20ng/ μ L; 2kb, 40ng/ μ L) 5 μ L, respectively. When needed you can use them as positive control. Add 1 μ L 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

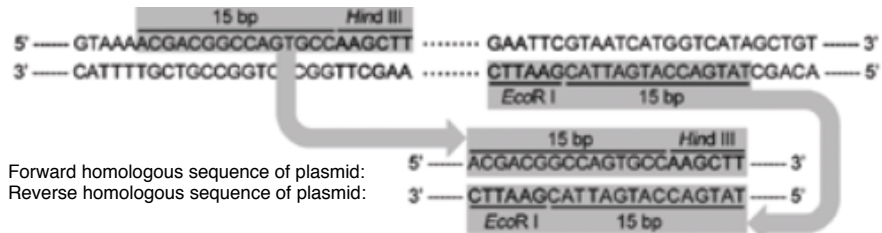
1. Add 5 μ L mixture into 50 μ L competence cell, keep it on ice for 30min.
2. 42°C water bath 45-90s before put it back on ice at least 2 minutes
3. Add 900 μ L LB or SOC, 37°C incubation for 10min.
4. 37°C shake culture for 45min.
5. Coat 100 μ L mixture on LB plate with specific antibiotic, 37°C culture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

V. Example of Primer Design :

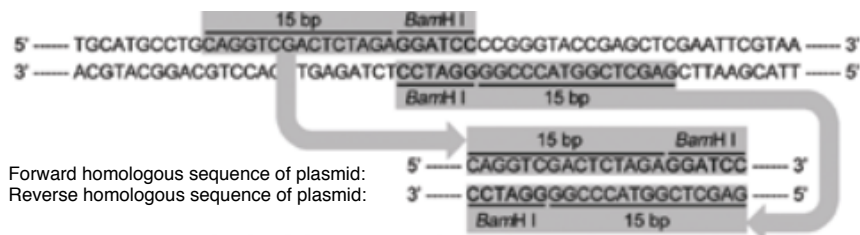
1. Liner Fragment Primer



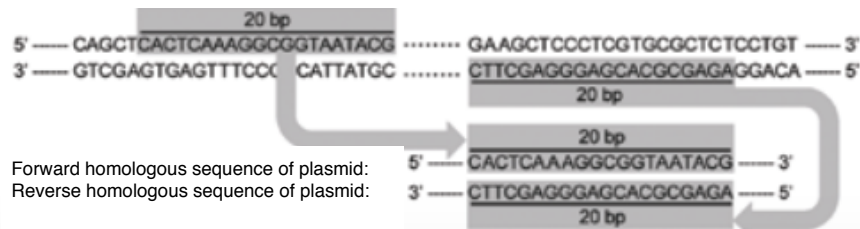
A. If cloning plasmid are digested by double enzymes (*Hind III* + *EcoR I*):



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



C. If cloning plasmid are amplified by PCR:



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

2. Primer for Ligation of Nearby Fragment

