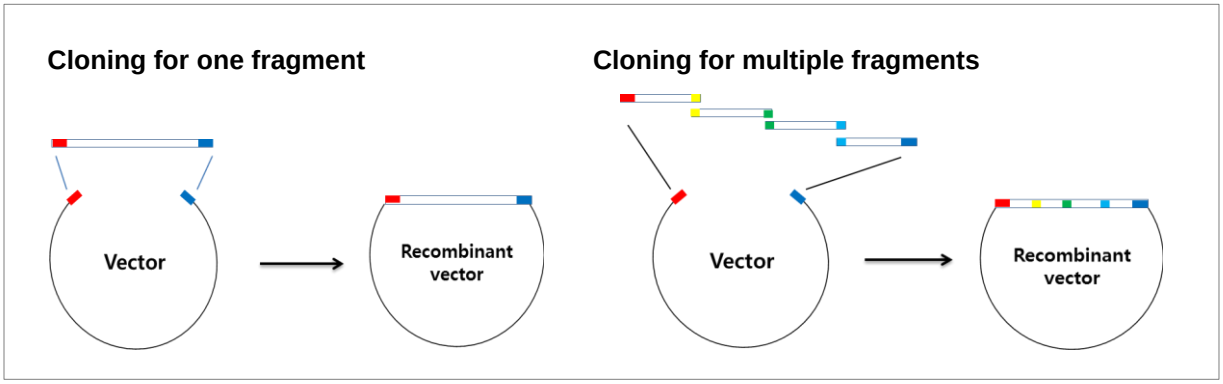
OVERLAP CLONER™ DNA Cloning Kit

Cat No. EBK-1011 (25 reactions)
Cat No. EBK-1012 (100 reactions)

Overview

The Overlap Cloner DNA Cloning Kit provides simple and fast directional cloning of one or more DNA fragments into any vectors. The most advantages of this system are that the insert DNA can be precisely cloned as intended without additional bases and several fragments can be connected with intentional direction at one time reaction. This system requires no site-specific recombination sites or extra DNA sequences and eliminates the needs for restriction enzymatic and DNA ligase treatments. The enzyme mix provided with this system recognizes a more than 15 bp overlap at each ends and precisely connects the DNA fragments sharing this homology. This 15 bp overlap can be generated by PCR amplification with designing primers. This system can be used for up to 4 fragments cloning into vector, totaling up to 10 kb in length.

The Overlap Cloner DNA Cloning Kit offers increased cloning efficiency over classical cloning methods using restriction enzymes and T4 DNA ligase, especially for long fragments and multiple fragments along with no additional bases.



Kit Contents (EBK-1011, EBK-1012)

Components	25 Reactions (EBK-1011)	100 Reactions (EBK-1012)	Storage
Overlap Cloner Enzyme Mix	25 μl	100 μl	-20℃
Overlap Cloner 10x Reaction Buffer	100 μl	500 μl	-20℃
Control Vector (50 ng/μl)	5 μl	25 μl	-20℃
Control Insert (LacZ DNA, 50 ng/μl)	5 μl	25 μl	-20℃
Manual	1 ea	1 ea	-

Storage Condition

The Overlap Cloner DNA Cloning Kit should be stored at -20℃ or below. The kit can be stored for up to 12 months without showing any reduction in performance and quality.

Protocol for Overlap Cloner Reaction

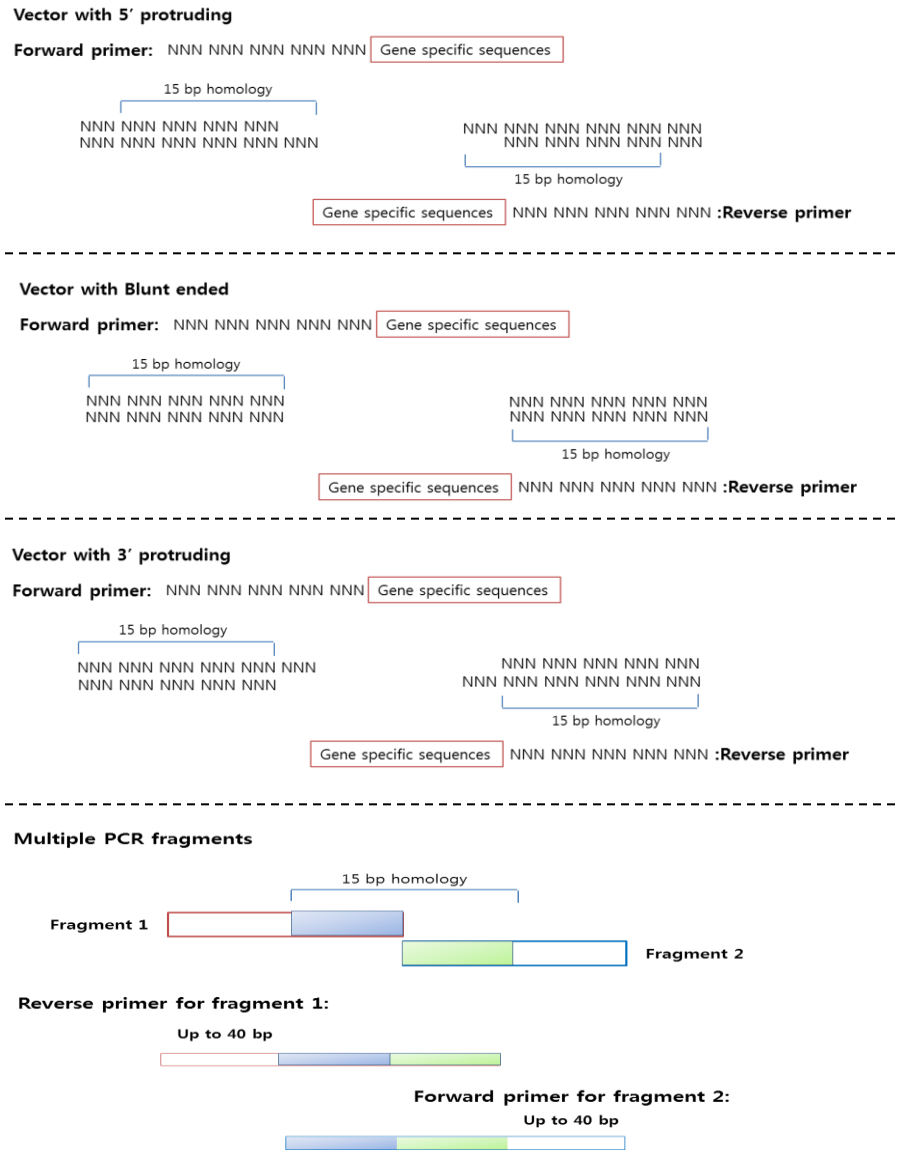
A. Preparing the linearized vector :

- The linearized vector for cloning can be prepared either by restriction enzyme cutting or inverse PCR amplification of whole vector.
- When you use the restriction enzymes, two enzymes are recommended to digest the vector for preventing the background clones.
- The 5'-protruding, 3'-protruding or blunt ended restriction enzymes are acceptable to use in preparing the linearized vector.
- After digestion, we recommend gel purification using the Gel Extraction Kit (EBD-1005) or equivalent.

B. Preparing the inserts by PCR

1. PCR primer design

- The 5'-end of each primer must have at least 15 bases homology with cloning vector or adjacent fragment to be continuously connected (see below for details).
- The 3'-end of each primer must be specific to the insert you want to clone.
- You can design the primers using online site or program to optimize PCR amplification. Generally speaking, including 15 bases homology and 18-25 bases specific to target DNA, up to 40 nucleotides primers are recommended.



2. PCR amplification

- You can perform the conventional PCR reaction using the primers designed such as above. We recommend using *Pfu* Plus Polymerase (EBT-1402 or EBT-1403) for achieving the highest fidelity of your target DNA.
- After PCR reaction, the PCR product should be analyzed by gel electrophoresis and purified by Gel Extraction Kit (EBD-1005).

C. Cloning reaction

1. In a microcentrifuge tube, set up the reaction as follows:

Component	Target reaction	Positive control
Insert (50-200 ng) *	X μl	
Linearized vector (100 ng)	Y μl	
Control insert (LacZ DNA)		2 μl
Control Linearized vector		2 μl
10X reaction buffer	1 μl	1 μl
Enzyme mix	1 μl	1 μl
Deionized water	Up to 10 μl	3 μl

2. Incubate at 37°C for 30 min to I hr.**
3. After reaction, you can go to *E.coli* transformation step.

* In case of multiple DNA fragments, the total amount of inserts would be made up to 200 ng.
** You can also perform the reaction at 30℃ or room temperature, but you may put up with low cloning efficiency. And the reaction time also can be increased until overnight.

D. Transformation

- You can follow the general transformation protocol.
- The 1-5 μl reaction mixture can be used to 50 μl competent cells.
- After recovery step, you can plate the diluted transformed cells (1:10) depend on the expected colony number.
- In control reaction supplied in this kit, transformants are plated onto LB/ampicillin and check the blue color of colonies in the presence of x-gal.

Quality Control

The performance of Overlap Cloner DNA Cloning Kit is monitored routinely on a lot number. The quality of kit is tested with control vector and insert DNA by the criteria of enzyme performance, transformation efficiency, molecular analysis of resulted plasmid DNAs from several clones.

Notes for Overlap Cloner DNA Cloning Kit

- The key condition of Overlap Cloner is the homologous recombination between both ends of two DNA fragments. Therefore, the inserts and vector DNA should be linear.
- Although, we have had success with cloning up to 6 fragments using Overlap Cloner, we recommend using this kit for cloning up to 4 fragments. Since the cloning efficiency would be lower up on increasing number of DNA fragments.
- Please keep all components at -20℃ or below. Repeated freezing and thawing can decrease enzymatic performance. It is the best choice to aliquot enzyme mix immediately after product reception.

Related Products

Pfu Plus DNA Polymerase (500 unit)	EBT-1402
Pfu Plus 5x PCR Master Mix (1ml, 250 rxn)	EBT-1403
Dpn I (500 unit)	EBR-1045
Dokdo-Prep™ Gel Extraction Kit (spin-type)	EBD-1005

Troubleshooting Guide

Problem 1. No or few transformation colonies

<Check 1> Transformation efficiency is too low.
Test the transformation efficiency of your competent cell with supercoiled plasmid DNA. Use competent cell showing efficiency at least more than 1x 10^7 / μg DNA.

<Check 2> Transformed bacteria should be inoculated in medium containing the appropriate antibiotics.
Antibiotic selection should be applied at all stages of growth. Use the appropriate antibiotics for clonal selection. If you are using the linear pUC19 cloning vector, use 50 μg/mL ampicillin containing LB plate or liquid media.

<Check 3> Too much reaction volume may be toxic to competent cells .
You can obtain better results if you dilute the reaction. It may be better to dilute the reaction with TE buffer 5-10 times prior to transformation and use as transformation protocol.

<Check 4> Vector or insert DNA concentration is too low.
Appropriate DNA concentration is required in Overlap Cloner reaction. We recommend using 50-200 ng of vector or insert DNA in reaction.

<Check 5> Vector or insert DNA fragments do not have required 15 bp homology.
You have to check the primer sequences to ensure that they have more than 15 bp homology at each ends of the DNA fragments. Sometimes the PCR fragments have been damaged at the ends during handling by UV light. Be careful to minimize the potential damage.

<Check 6> Enzyme mix of Overlap Cloner is handled incorrectly.
Repeated freezing and thawing of enzyme mix may be the reason of reduced activity of the enzyme. Also, do not leave the enzyme mix at room temperature for a long time. For a long term storage, keep the enzyme mix at -70°C .

<Check 7> Reaction temperature or time is not appropriated.
We recommend that the reaction temperature is 37°C. At room temperature or 30°C incubation may cause the low number of transformants. Too short reaction time may also be the reason of low transformation efficiency.

Problem 2. Most transformants contain no insert

<Check 1> Vector DNA may not be completely linearized.
It is important to use completely linearized vector in Overlap Cloner reaction. Uncut vector should be removed prior to use in reaction. If necessary, recut the vector DNA and purify again. The formation of any colonies in control reaction with vector DNA only means that your vector DNA is not completely digested.

<Check 2> Contaminant DNA may be included in the reaction.
The template DNA for PCR amplification may contaminated in the reaction. Circular DNA remained in PCR reaction critically hamper the transformation step. Before purification, use linearized DNA for PCR reaction or treat the PCR product with Dpn I restriction enzyme to remove methylated template DNA.

<Check 3> Insert DNA contains non-specific products.
Suboptimal PCR condition may produce the non-specific product as different size. Before adding insert DNA to the reaction, make sure to remove non-specific products and obtain the correct insert by using gel purification.

Customer & Technical Services

For technical assistance and more information please call one of the Elpis-Biotech., Inc.

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