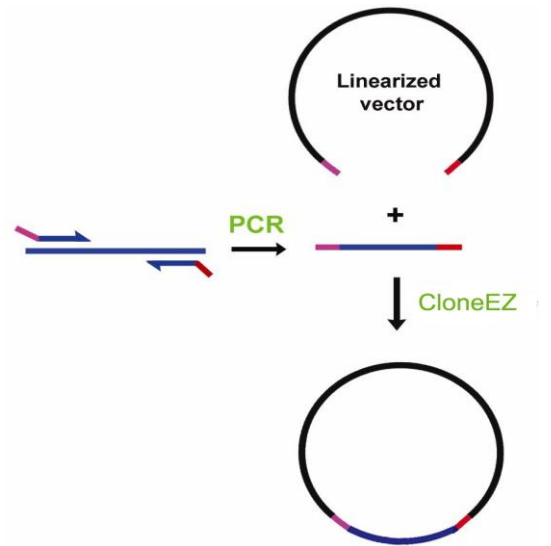


CloneEZ[®] PCR Cloning Kit
Technical Manual No. 0279
Cat. No. L00339
Version: 12232013

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I. DESCRIPTION

GenScript's CloneEZ[®] PCR Cloning Kit is designed for quick and convenient PCR cloning. With our proprietary CloneEZ[®] Enzyme, this kit is especially powerful in high-throughput cloning of PCR products into any destination vector effectively by sidestepping the tedious and limiting tasks such as selecting proper restriction enzymes, phosphatases, or ligases. This kit allows quick and precise directional cloning of PCR DNA into any vector at a very high level of efficiency.



Cloning Procedure

II. APPLICATIONS

GenScript's CloneEZ[®] Kit can be used in a variety of applications:

- PCR cloning up to 12 kb
- Gene transfer from one vector to another
- High-throughput (HTP) PCR cloning
- *In vitro* joining of DNA fragments

III. CONTENTS

Kit Components	L00339
CloneEZ [®] Enzyme (5 U/μl)	50 μl
10X CloneEZ [®] Buffer*	100 μl
pUC57 Linearized with EcoRI/ <i>Hind</i> III, positive control (30 ng/μl)	10 μl
1-kb Control Insert, positive control (100 ng/μl)	10 μl
Manual	1
Note*: The buffer should be always avoid freeze-thaw cycles, and can be stored in aliquots to keep stable.	

IV. KEY FEATURES

- Fast and precise directional cloning procedures completed within 30 minutes.
- PCR DNA and linearized vector immediately used in cloning, no restriction enzymes or ligases are needed.
- Long PCR DNA up to 12 kb can be efficiently cloned into the vectors.

V. STORAGE

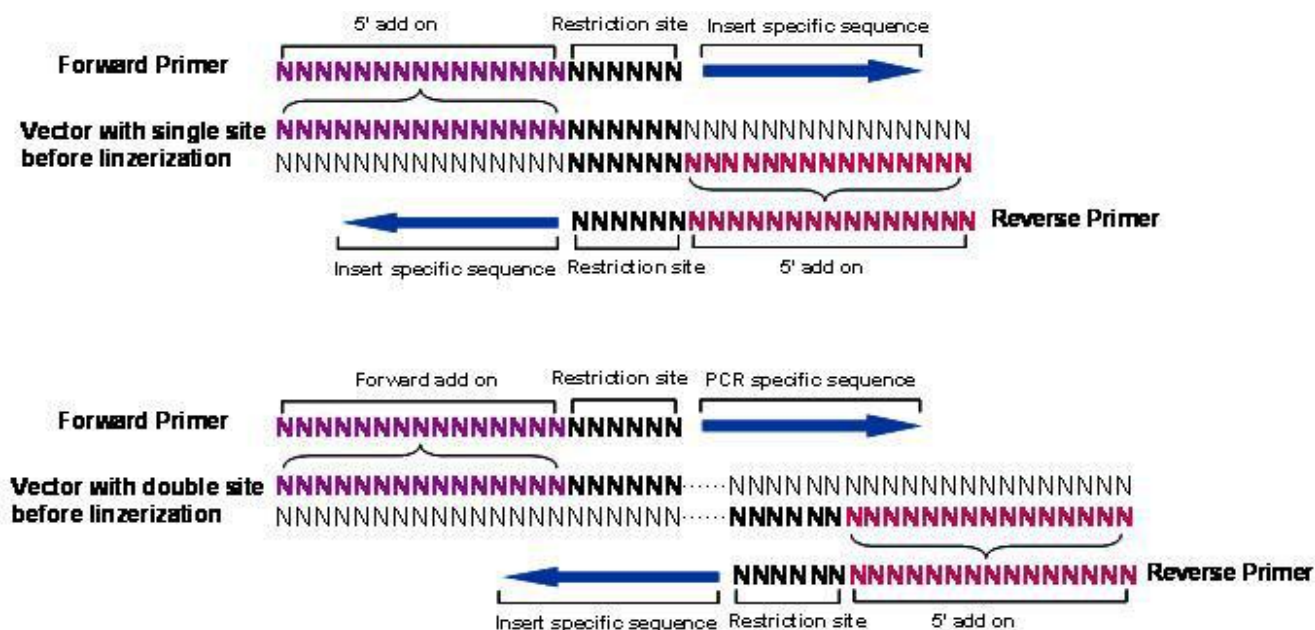
The kit should be stored at -20°C. It will remain stable for at least one year.

VI. GENERAL PROTOCOL of CloneEZ[®] Kit

A. PCR Amplification of Target DNA

To clone any DNA fragment into a linearized vector using this kit, the insert fragment should be obtained by PCR using primers with an add-on of 15 base sequences homologous to either side of the restriction site that is used to linearize the vector. Therefore, a primer should cover a 15-20 base sequence add-on at the 5'-end, an optional restriction site in the middle, and the insert-specific sequence at the 3'-end. How to choose the 15-base sequence add-on is illustrated in the graph below.

(Note: The restriction site in the middle of the primer is optional and does not have to be the same site used to linearize the vector; you may also add any other sequence in the middle for frame adjustment or tag addition.)



PCR amplification can be performed using any thermostable DNA polymerase. However, primers and primer dimers produced in PCR reactions are inhibitory to the CloneEZ[®] PCR cloning reaction. If the PCR produces a single specific band (from an agarose gel), PCR DNA can be purified by simply using a PCR purification kit. GenScript's QuickClean II PCR Purification Kit (Cat. No. L00419) would be a good choice.

B. Preparation of Linearized Vector

To achieve a successful CloneEZ[®] PCR cloning reaction, complete linearization of the vector is critical. Incomplete linearization of the vector will result in high background. The linearized vector should be purified using a gel or PCR purification kit, such as QuickClean II PCR Purification Kit (Cat. No. L00419).

C. CloneEZ[®] Recombination Procedure

1. Set up the following reaction in a 0.5 ml Eppendorf tube by mixing the following reagents gently and then spin down briefly to collect the reagents at the bottom of the tube.

20 µl Reaction Mixture	
Linearized vector (100-200 ng/µl)	6 µl
Purified PCR products (100-200 ng/µl)	n µl
10X CloneEZ [®] Buffer	2 µl
CloneEZ [®] Enzyme	2 µl
Deionized water	up to 20 µl

In general, add more than 10 µl of PCR DNA (n = 10) to the reaction can produce nearly 95% positive clones. In addition, less amount of DNA is appropriate for short PCR DNA fragments. For different sizes of PCR DNA, different amount of DNA is recommended below:

PCR DNA of 1 kb	4 µl
PCR DNA of 2 kb	6 µl
PCR DNA of 3 kb	8 µl
PCR DNA of >3 kb	10 µl

2. Incubate the reactions at 22°C for 30 minutes, and then transfer tubes to ice and incubate on ice for five minutes.
3. Proceed with transformation (Section D). The reaction can also be stored at -20°C for later transformation.

D. Transformation

Materials needed but not provided along with the kit:

Water bath or heating block (42°C)

SOC liquid medium

DH5α competent cells (>1×10⁸ cfu/µg)

1. Thaw one vial of frozen 100 µl competent cells on ice. Tap the tube gently to ensure that the cells are suspended.
2. Add 20 µl of reaction mixture to the competent cells. Tap the tube gently and incubate the tube on ice for 30 minutes.
3. Heat shock the cells by placing them in 42°C water bath for 90 seconds and then place the tube on ice for 5 minutes.
4. Add 1 ml of SOC medium to the cells and then incubate the cells on a shaker set at 200 rpm at 37°C for 60 minutes.
5. Centrifuge the cell down at 5000 rpm for five minutes and then remove and discard about 100 µl of medium. Gently suspend the cells by tapping the tube.
6. Incubate the plates overnight at 37 °C.

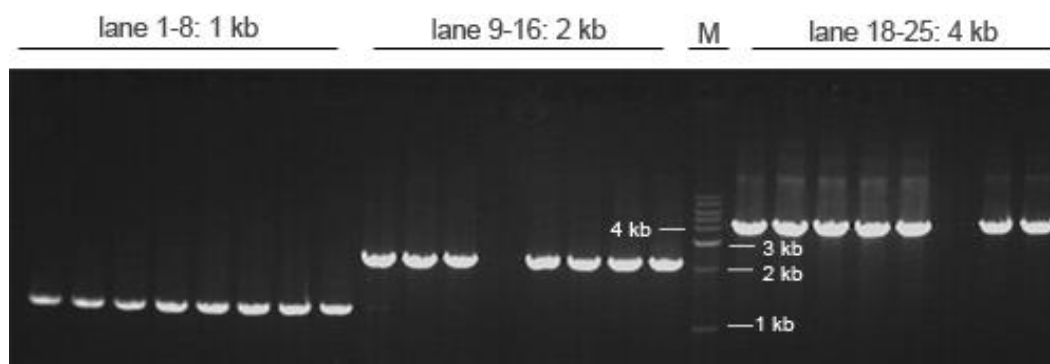
VII. EXAMPLES

Cloning of PCR DNA (using lambda DNA as template) into pUC57 (Cat. No. SD1176, GenScript)

Three PCR DNA fragments of 1 kb, 2 kb, and 4 kb, respectively, were cloned into pUC57 using the kit. The total transformants and successful recombinant rates are displayed in the table below.

	1 kb	2 kb	4 kb
Transformants	≈ 1200	≈ 800	221
Recombinant rate	100%	95%	93%

The inserts were detected by PCR **colony** screening on single colonies. The results are displayed in the figure below.



VIII. TROUBLESHOOTING

Problem	Probable Cause	Solution
Few or no colonies are obtained from the transformation.	The competent cells have low transformation efficiency.	Check the transformation efficiency. Competent cells with $>1 \times 10^8$ cfu/ μ g are recommended.
	Too much reaction mixture is used.	Do not add more than 20 μ l of reaction mixture to 50 μ l of competent cells. Too much reaction mixture inhibits the transformation.
	There are inhibitory contaminants from PCR DNA or from linearized vector.	Both of the PCR DNA and the linearized vector should be purified.
	The molar ratio of vector to insert is off.	Usually an insert/vector molar ratio of 2:1 is optimal. If the insert is as large as the linearized vector, a molar ratio of 1:1 can also be used.
	Too long or short recombination time	It is proper to keep the recombination procedure within 30 min

	The cloning vector or primer linearized is completely large	Connect the product to the target step recombinant vector
Most of the colonies contain no insert.	The cloning vector is not completely linearized.	Gel-purify the linearized vector.
	The cloning reaction is contaminated with plasmids having the same antibiotic resistance.	Purified PCR DNA may contain the template plasmid, so gel-purify the PCR DNA.

IX. ORDERING INFORMATION

CloneEZ[®] PCR Cloning Kit

Cat. No. L00339.

GenScript USA Inc.
 860 Centennial Ave., Piscataway, NJ 08854
 Tel: 732-885-9188
 Fax: 732-210-0262, 732-885-5878
 Email: product@genscript.com
 Web: www.genscript.com
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