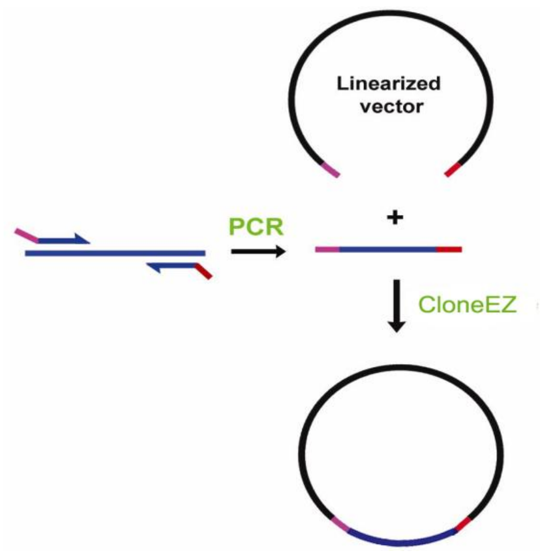
**CloneEZ® Kit** **Cat. No. L00339**

**Technical Manual No. 0279** **Update Date: 01022010**

| | | |
|---|---|---|
| I | Description | 1 |
| II | Applications | 1 |
| III | Contents | 2 |
| IV | Key Features | 2 |
| V | Storage | 2 |
| VI | General Protocol | 2 |
| VII | Examples | 5 |
| VIII | Troubleshooting | 5 |
| IX | Ordering Information | 6 |

## I. DESCRIPTION

The GenScript CloneEZ® PCR Cloning Kit is designed for quick and convenient PCR cloning. With proprietary CloneEZ® Enzyme, this kit is especially powerful in the high-throughput cloning PCR products into any destination vector while bypassing the tedious and limiting tasks of selecting proper restriction enzymes and using phosphatase or ligase. This kit allows quick, precise, directional cloning of PCR DNA into any vector at a very high level of efficiency from any system.




**Cloning Procedure**

## II. APPLICATIONS

The GenScript CloneEZ® Kit can be used in a variety of applications:

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



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## III. CONTENTS

| Kit Components | L00339 |
|---|---|
| CloneEZ® Enzyme (5 U/μl) | 50 μl |
| 10X CloneEZ® Buffer | 100 μl |
| pUC57 Linearized with KpnI/HindIII(100 ng/μl), positive control | 10 μl |
| 1-kb Control Insert (100 ng/μl), positive control | 10 μl |
| Manual | 1 |

## IV. KEY FEATURES

- Fast precise, and directional cloning procedure within 30-min.
- The PCR DNA and linearized vector are immediately usable in cloning without the use of restriction enzymes or ligases.
- Long PCR DNA of up to 12 kb can be efficiently cloned into the vector.

## V. STORAGE

Store the kit at -20°C. It will remain stable for at least one year.

## VI. GENERAL PROTOCOL USING 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 after PCR using primers with an add-on 15 base sequences homologous to either side of the restriction site used to linearize the vector. Therefore, a primer will be composed of an add-on 15-base sequence at the 5'-end, an optional restriction site in the middle, and the insert-specific sequence at the 3'-end. The instruction on how to choose the add-on 15-base sequence is shown 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**.)



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5' add on | Restriction site | Insert specific sequence

Forward Primer

Vector with single site before linzerization

Reverse Primer

Insert specific sequence | Restriction site | 5' add on

Forward add on | Restriction site | PCR specific sequence

Forward Primer

Vector with double site before linzerization

Reverse Primer

Insert specific sequence | Restriction site | 5' add on

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, such as the GenScript QuickClean 5M PCR Purification Kit (Cat. No. L00198). (Note, we have discontinued the gel purification kit, so we should not mention in description)

### 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 5M PCR Purification Kit (Cat. No. L00198).

### 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.

| | |
|---|---|
| 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 |



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In general, add more than 10 μl 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:**

Water bath or heating block (42°C)

SOC liquid medium

DH5α competent cells (>$1\times10^8$ cfu/μg)

1. Thaw one vial of frozen 50 μl competent cells on ice. Tap tube gently to ensure that the cells are suspended.
2. Add 5 to 8 μl of reaction mixture to the competent cells. Tap tube gently and incubate the tube on ice for 30 minutes.
3. Heat shock the cells by placing them 42°C water bath at for 45~90 seconds, and then place the tube on ice for 2-3 minutes.
4. Add 600 μl of SOC medium to the cells and then incubate on a shaker at 250 rpm at 37°C for 60 minutes.
5. Centrifuge the cell down at 4000 rpm for five minutes and remove and discard about 500 μl of medium. Gently suspend the cells.
6. Transfer 10 μl and 100 μl of the suspension to two different plates containing appropriate antibiotics, respectively. Spread the cells evenly on the plates.
7. Incubate the plates overnight at 37 °C.



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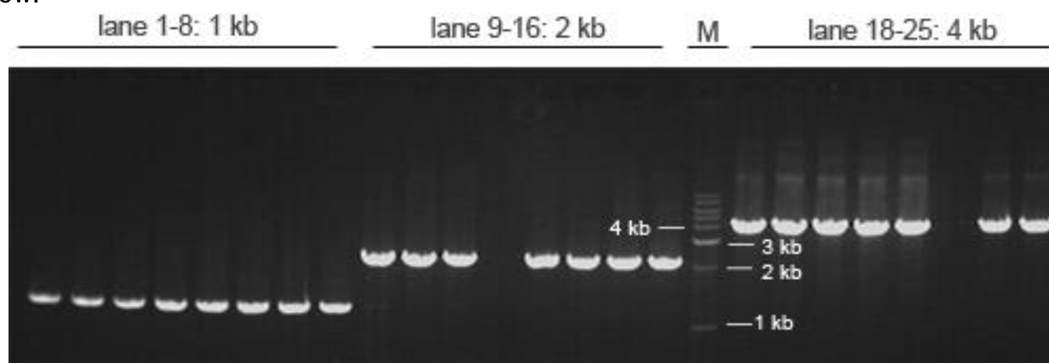
## VII. EXAMPLES

**Cloning of PCR DNA (using lambda DNA as template) into pUC57**

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 summarized 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. Some of 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 $>1\times10^8$ cfu/µg are recommended. |
| | Too much reaction mixture is used. | Do not add more than 10 µl of reaction mixture to 50 µl 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. |
| 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 with the same antibiotic resistance. | Purified PCR DNA may contain the template plasmid, so gel-purify the PCR DNA. |



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## IX. ORDERING INFORMATION

CloneEZ® Kit    Cat. No. L00339.

**For Research Use Only**

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