CloneEZ® PCR Cloning Kit FAQs

General Question

1 What are the differences between the CloneEZ® kit and other PCR Cloning Kit?

CloneEZ® Seamless cloning technology offers high efficiency, quick and direct cloning, bypassing all the tedious procedures of traditional cloning that often involve restriction, ligation, and sometimes phosphorylation. This technology works with any DNA sequence and any vector. The only requirement is that your PCR primers have to have a sequence containing 15 or more nucleotides homologous to vector sequence.

2 The technical information in patent document is not available in the manual, why is that?

Currently, the patent on CloneEZ® method is invalid, so we can't provide more details about the patent technical information to the general public, and we will not show more detailed explanation in the manual until the patent is valid.

Transformation

3 Why customers are not able to get sufficient colonies when the CloneEZ® reaction is transformed?

This may be caused by four factors.

1) The competent cells have low transformation efficiency.
2) Too much reaction mixture is used.
3) Presence of Inhibitory contaminants from PCR DNA or linearized vector.
4) The molar ratio of vector to insert is off.

Please refer to the manual for TROUBLESHOOTING.

4 Why customers get incorrect colonies when the CloneEZ reaction is transformed?

Two factors may be involved.

1) The cloning vector is not completely linearized.
2) The cloning reaction is contaminated with plasmids having the same antibiotic resistance.

Primer Design

5 What factors need to be considered when designing the primer?

Two sets of primers are used to amplify the gene of interest:

1) 15-bp homology regions to the vector, flanking the cloning site into the insert.
2) Specific gene sequence. The start of the 15 bp homology must begin from the 5' most extension to include the overhang, if the sequence has 5' overhang; if the sequence has a 3' overhang, homology should begin where the DNA becomes double-stranded.

Please refer to the manual for illustration of primer design.

6 To be compatible for CloneEZ® cloning method, what should the purity of my primer?

Desalted oligos (from a qualified supplier) are very suitable for cloning with CloneEZ® PCR cloning Kit.

7 Can multiple fragments be cloned into a single vector using this kit?

Yes, CloneEZ® PCR Cloning Kit can be used for multiple fragments recombination, if there are no many repeated sequences among multiple fragments. But we still recommend to recombinate one by one after several performances of recombining 2 and 3 fragments, the efficient is low as 20%-30%, and it will lead to a direct repeat deletion.

8 Can I use CloneEZ® PCR Cloning Kit with any other vectors?

Yes. GenScript's CloneEZ® PCR Cloning Kit has been tested with any commercial or non-commercial vectors several times, and you will have no problem with it.

9 What factors need to be considered when using CloneEZ® PCR Cloning Kit with customers' vector?

We recommend linearizating your vectors before using CloneEZ® PCR Cloning Kit. Once linearized, the columned and gel-purified vector is ready for CloneEZ reaction.

10 Will the CloneEZ® Cloning reaction work more efficiently if I use primers that contain a longer than 15 base region of homology?

15-20 bp of homology is recommended.

11 How about the stability of the enzyme included in CloneEZ® PCR Cloning Kit?

The liquid enzyme should be stored at -20°C for at least 12 months without activity loss.