KIT PARA CLONACIÓN DE FRAGMENTOS

B632219

BIO BASIC
Quality-Affordable Research

Product information

Seamless Cloning Master Mix

Catalog #: B632219-0020 | B632219-0040
Size: 20 rxns | 40 rxns
Storage: Transport at 4°C. Store at -20°C. Avoid repeated freeze-thaw cycles.

Components

Components	B632219-0020, 20 rxns	B632219-0040, 40 rxns
Seamless cloning Master Mix	170 ul	170 ul x 2
Sterilized ddH2O	1 ml	1 ml x 2
Protocol	1	1

Product Description

The Seamless Cloning Master Mix is a ready-to-use reac?on mix for seamless cloning reac?ons. It allows for successful assembly of mul?ple DNA fragments, regardless of fragment length or end compa?bility. It has been rapidly adopted by the synthe?c biology community due to its ease-of-use, flexibility and suitability for large DNA constructs.

The Seamless Cloning Master Mix efficiently joins mul?ple overlapping DNA fragments in a single-tube isothermal reac?on. The system allows the cloning of the DNA fragments into virtually any linearized Escherichia. coli vector, requires no pre-exis?ng recombina?on sites or extra DNA sequences, and eliminates the need for extensive enzyma?c treatments of the DNA such as restric?on and liga?on. The enzyme mix provided with the Seamless Cloning Master Mix recognizes and precisely assembles the DNA fragments sharing a 15-20 base pair (bp) end homology that can be created by PCR-amplifica?on.

The end result is a double-stranded fully sealed DNA molecule that can serve as template for PCR, RCA or a variety of other molecular biology applica?ons, including direct transforma?on. Using the Seamless Cloning Master Mix, you can clone mul?ple DNA fragments into a single vector without subcloning, create modular expression vectors with interchangeable parts, construct seamless fusion proteins, delete and replace DNA segments, make internal protein fusions, swap tags on a gene, add UTRs to a cDNA, insert restric?on sites, and more.

Protocol

Genera?ng a Linearized E. coli Cloning Vector:

1. Prepare the linearized E. coli cloning vector using restric?on enzymes (single or double digest) or using PCR amplifica?on. When genera?ng the linearized vector by restric?on digest, we recommend that you digest the vector with two restric?on enzymes rather than a single enzyme to reduce the amount of background.

2. Use of restric?on enzymes that leave 3' protruding, 5' protruding, or blunt ends to linearize your cloning vector are all compa?ble with this kit. However, for best results we recommend use of a restric?on enzyme that produces blunt or 3' protruding ends when possible for achieving maximum cloning efficiency.

3. It is very important to have a complete digest (i.e. very low background of uncut vector). Therefore, we recommend increasing the enzyme diges?on ?me (2–3 hours to overnight) and the reac?on volume. Double diges?on with two restric?on enzymes is the most efficient way of linearizing your cloning vector. A double digest followed by PCR amplifica?on of your linear vector virtually eliminates any background.

4. Analyze your restric?on diges?on products using agarose gel electrophoresis to verify that the digest is complete and then purify the digested vector.

Preparing DNA Inserts by PCR:

1. Seamless Cloning reac?on requires that each DNA fragment share a 15-20bp (base pair) end-terminal homology with the adjacent fragment (including the cloning vector). Therefore, PCR primers used for genera?ng inserts must have 15-nucleo?de overhangs on their 5' ends to provide this homology with the adjacent fragments; however, this homology may be split between the primers used for adjacent PCR-amplified DNA fragments.

2. Restric?on enzyme-treated vectors can have 5´-overhangs, 3´-overhangs or blunt ends. When vector is linearized by restric?on diges?on, the en?re overlap sequence must originate from the vector sequence and must be added to primers that will be used to amplify the insert. The overlap region of the forward primer for the gene of interest should line up with the 3´end of the overhang on the vector's le? arm and extend back un?l the Tm ? 48°C. This primer also includes gene-specific sequence at the 3´-end. PCR primers should be up to 40 nucleo?des in length (15 nucleo?des to provide the requisite homology at the 5' end and 18–25 nucleo?des specific to your DNA element).

3. It should be noted that the restric?on site, which was used to digest the vector, will be lost in the assembled product. However, addi?onal nucleo?des may be added between the overlap region and gene-specific sequence region to restore the pre-exis?ng restric?on site, or to introduce a new, unique restric?on site. A similar principle is applied to the design of the reverse primer for the gene of interest.

Performing the Seamless Cloning Reaction

1. Set up the following reac?on on ice:

Component	Volume(ul)
Seamless cloning Master Mix	8.5
Fragments	X
Linearized vector	X
Sterilized ddH2O	Up to 20 ul

Note:

- Op?mized cloning efficiency is 50 ng of vectors with 3 fold of excess inserts. Use 100 ng of vectors with 3 fold of excess inserts if vector size is more than 8000 bps.
- For assembly of mul?ple fragments into a vector, we recommend using equimolar ra?o of fragments.

2. Incubate at 50°C for 30 minutes to 1 hour, depending on number of fragments being assembled.

3. Transform 4 µl of the reac?on mixture into competent E. coli, or use the mixture directly in other applica?ons.