MB281_IFU_EN_V2401

NZYEasy Cloning kit

Catalogue number	Presentation
MB28101	8 reactions
MB28103	96 reactions

Description

The NZYEasy Cloning kit is an innovative system designed for directional cloning of any PCR-generated fragment into a linearized pHTP0 vector in a single ligase-independent reaction mediated by the NZYEasy enzyme mix. Vector-complementary overhangs containing a specific sequence recognized by the NZYEasy enzyme are incorporated in the PCR product using primers with appropriate 5' extensions. Upon combining the thus-generated insert with the linearized pHTP0 vector, which also contains complementary overhangs, in the presence of the NZYEasy enzyme, the two DNA molecules anneal through base-pair complementation of the single-strand regions. The reaction occurs in a single tube across three temperature-dependent steps. Circular recombinant vector containing the fragment of interest is obtained by transforming the annealed plasmid DNA into competent *Escherichia coli* cells. This system achieves high cloning efficiency (80-100%) without the need for DNA ligases. Moreover, no further treatment (e.g. restriction digestion, phosphorylation, or blunt-end polishing) of the inserts is required.

This kit has been successfully employed in high-throughput (HTP) platforms for the efficient cloning of a large number of genes at a scale compatible with the functional screen of hundreds to thousands of genes.

Shipping & Storage Conditions

This product can be shipped at a range of temperatures from dry ice to blue ice. Upon receipt, store all components at -85 °C to -15 °C in a constant temperature freezer. Immediately after use, swiftly return all components to a temperature between -85 °C and -15 °C. Adhering to these meticulous storage procedures ensures that product delivers consistent and reliable results across its lifespan and usage. Stored as specified, the product will remain stable until the expiry date, ensuring reliable and consistent performance in all applications.

Components

	MB28101 (8 reactions)		MB28103 (96 reactions)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
NZYEasy enzyme mix	1	6 µL	1	50 µL
10x Reaction Buffer for NZYEasy enzyme mix	1	100 µL	1	1 mL
pHTP0, cloning vector	1	10 µL	1	100 µL
Positive control for NZYEasy Cloning kit (*)	1	12 µL	1	12 µL

(*) Provided for 5 experiments. Corresponds to a PCR fragment of 500 bp at 21.0 ng/µL.

Standard Protocol

Recommendations before starting

This document serves as a concise protocol. A more comprehensive protocol, integrated into a compendium of the NZY Easy Cloning System, is available online on the product's webpage under the Product Manuals section. Prior to initiating this protocol, we strongly advise thoroughly reading the Manual for Easy Cloning System provided.

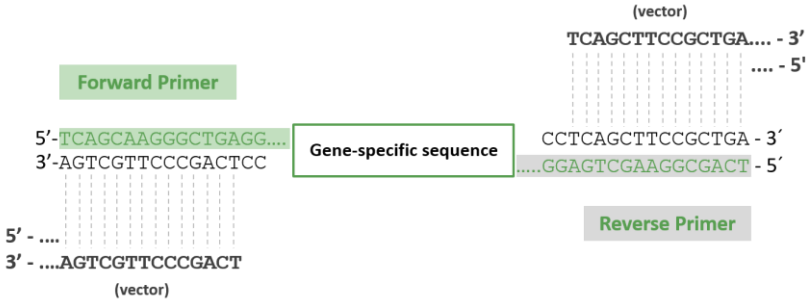
Procedure

Preparing DNA inserts by PCR

1. Design primers according to standard procedures. In addition to the gene-specific sequence, include the following 16 bp overhangs at the 5' ends of both forward and reverse primers to provide the necessary vector-complementary single-strand termini:

   Forward overhang: 5´-**TCAGCAAGGGCTGAGG**…-3´

   Reverse overhang: 5´-**TCAGCGGAAGCTGAGG**…-3´



2. Amplify the gene of interest through PCR, following the guidelines provided in the Technical Note below.
3. Purify the PCR product using spin-column purification, employing the NZYGelpure kit (NZYtech, Cat. No. MB011) or a similar kit. Gel extraction of the desired band should be performed if non-specific amplifications or primer-dimers are observed, thereby enhancing cloning efficiencies.

Ligase-independent cloning reaction

1. Gently mix all reaction components and pulse briefly.
2. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture by combining the following components:
   Note: *If setting up more than one reaction, prepare a reaction mixture volume 10% greater than the total required for the number of reactions to be performed.*

COMPONENT	1 REACTION VOLUME
Purified DNA fragment	x µL(1,2)
pHTP0 vector (3)	1 µL
10x Reaction Buffer	1 µL
NZYEasy enzyme mix	0.5 µL
Nuclease-free water	up to 10 µL
FINAL VOLUME =	10 µL

(1) To determine the optimal quantity of the insert, please refer to the Technical Note below. Use a maximum of 7.5 µL of purified PCR insert when it is not possible to use the recommended optimal amount.
(2) For the positive control reaction: use 2 µL of the Positive control for NZYEasy Cloning kit provided.
(3) pHTP vectors are provided in a ready-to-use form.

3. Mix the reactions by pipetting and centrifuge briefly to collect the contents at the bottom of the tube.
4. Perform the cloning reactions in a thermal cycler programmed with the following protocol:

CYCLES	TEMP.	TIME
1	37 °C	60 min
1	80 °C	10 min
1	30 °C	10 min
1	4 °C	∞

5. Centrifuge briefly to collect the reaction components.
6. Store the cloning product at -85 °C to -15 °C or proceed to transformation following instructions below.

Transformation

1. Add 10 µL of ligation product directly into 100 µL of NZY5α competent cells (NZYtech Cat No. MB004) or other *DH5α-derivatives*.
   Note: *Significantly lower cloning efficiencies can result from using other E. coli strains than DH5α.*
2. Place the mixture on ice for 30 min. Heat shock cells at 42 °C for 40 seconds. Place tube on ice for 2 minutes.
3. Add 900 µL of pre-warmed SOC media and incubate at 200 rpm at 37 °C for 1 hour.
4. Centrifuge at 5000 rpm for 1 min. Remove 900 µL of supernatant.

5. Re-suspend cells by gentle pipetting. Spread 100 µL of the cells onto the selection LB agar plates containing 200 µg/mL ampicillin.
6. Incubate inverted plates overnight at 37 °C.

Screening for recombinant clones

Screening for recombinants can be easily achieved through colony-PCR, restriction analysis, and/or sequencing. For colony PCR or sequencing, utilize the following pHTP0 vector-specific primers:

pHTP0 forward primer: 5´- **GAGCGGATAACAATTTCACACAGG** -3´

pHTP0 reverse primer: 5´- **GTTTTCCCAGTCACGACGTTG** -3'

Note: *After electrophoresis on an agarose gel, the anticipated size of the insert amplified using the pHTP0 vector-specific primers will be increased by an additional 268 base pairs.*

Technical Notes

Insert / PCR product

To amplify the gene of interest, we strongly recommend using a high-fidelity DNA polymerase to reduce the error rate. When isolating genes from plasmids with ampicillin resistance (same as pHTP0), use 0.1-0.5 ng of plasmid template per 50 µL PCR reaction. Digestion with DpnI (NZYtech, cat. No. MB078) is recommended when high amounts of template are used. Prior to PCR, purification of the amplified DNA is highly recommended for optimal cloning efficiencies.

To determine the optimal amount of the PCR product to be used in the cloning reaction, please refer to the following table for guidance:

FRAGMENT LENGTH (*)	OPTIMAL DNA QUANTITY FOR CLONING REACTION
100 bp	8.3 ng
300 bp	25.0 ng
500 bp	41.5 ng
1000 bp	83.0 ng
2000 bp	166.0 ng
3000 bp	249.0 ng
4000 bp	332.0 ng

(*) The number of nanograms of insert required = DNA fragment length (bp) × 0.083. For example: a 1348 bp gene would require 1348 x 0.083 = 114.9 ng of DNA.

pHTP0 vector

The nucleotide sequence and properties of the pHTP0 cloning vector are available for download on the product's webpage under the Product Manuals section: User Guide - pHTP0 map.

Multiple fragment cloning protocol

The NZYEasy Cloning & Expression System offers the possibility to clone multiple inserts simultaneously into one vector in a single reaction. Please refer to the Manual for Easy Cloning System provided on the product's webpage under the Product Manuals section.

Quality control

Purity

The NZYEasy enzyme mix is >90% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 DNA are incubated with the kit component in test for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the kit component in test for 1 h at 37°C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids. Similar tests are performed with reaction buffer.

Functional assay

All components of the kit are functionally tested in a ligase-independent cloning reaction, followed by a transformation assay. >90% of the recombinant plasmids must contain the appropriate insert

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