NZYEasy Cloning kit

Catalogue number:

MB28101, 8 reactions
MB28103, 96 reactions

Description

NZYEasy Cloning kit was designed to allow 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 by using primers with appropriate 5' extensions. When you combine the insert thus generated with the linearized pHTP0 vector, also containing complementary overhangs, in the presence of NZYEasy enzyme, the two DNA molecules will anneal through base-pair complementation of the single-strand regions. The reaction occurs in a single-tube along 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. The system allows achieving high cloning efficiency (80-100%) and does not require the use of DNA ligases. In addition, no further treatment (e.g. restriction digestion, phosphorylation, or blunt-end polishing) of the inserts is required**.**

This kit has been successfully used 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.

Storage temperature

Kit components may be stored at -20 °C or at -80 °C.

Kit components

Component	8 reactions	24 reactions	96 reactions
10x Reaction Buffer	8 µL	24 µL	96 µL
NZYEasy enzyme mix	4 µL	12 µL	48 µL
pHTP0 vector	8 µL	24 µL	96 µL
Positive control [1]	10 µL	10 µL	10 µL

[1] Positive Control: PCR fragment provided for 5 experiments.

NZYEasy cloning protocol

Before you start using this protocol, please read carefully the NZYEasy Cloning Expression System User Guide available at the product resources tab of the product.

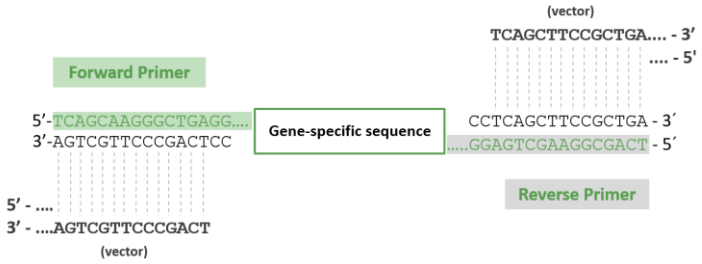
1. Preparing DNA inserts by PCR

1.1 Guidelines for Primers Design:

Besides gene-specific sequences, the following 16 bp overhangs must be included on 5' ends of both forward and reverse primers, in order to provide the required vector-complementary single-strand terminals:

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

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



1.2 Guidelines for PCR amplification:

- We strongly recommend using a high-fidelity enzyme to reduce error rate.
- When genes are isolated 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.
- For optimal cloning efficiencies, spin-column purification of the PCR product using NZYGelpure kit (NZYTech, cat. No. MB011) or other similar kit is highly recommended. Gel-extraction of the desired band should be ever performed in case non-specific amplifications or primer-dimers are formed, thus enhancing cloning efficiencies.

2. Ligase-independent cloning reaction

Please use the table below to determine the optimal amount of the insert DNA, in nanograms, to be used in a cloning reaction:

Fragment length (bp)[1]	Optimal DNA quantity for Cloning reaction (ng)
100	8.3
300	25.0
500	41.5
1000	83.0
2000	166.0
3000	249.0
4000	332.0

[1] ng of insert required = DNA fragment length (bp) × 0.083
e.g. 1348 bp gene = 1348 x 0.083 = 114.9 ng of DNA

2.1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare the following reaction mixture:

Component	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

[1] Use a maximum of 7.5 µL of purified PCR insert when it is not possible to use the recommended optimal amount.
[2] Positive Control: PCR fragment of 500 bp is provided at 21.0 ng/µL (enough for 5 experiments). Please use 2 µL per reaction.
[3] pHTP vectors are provided in a ready-to-use form.

2.2. Mix the reactions by pipetting and spin to collect contents at the bottom of the tubes.

2.3. Perform the cloning reaction in a thermal cycler programmed with the following protocol:

Temperature (°C)	Time (min)
37	60
80	10
30	10
4	∞

2.4. Centrifuge briefly to collect the reaction components.

3. Transformation

3.1. Add 10 µL of ligation product directly into 100 µL NZY5α cells (NZYTech cat No. MB004) competent cells.

3.2. Place the mixture on ice for 30 min. Heat shock cells at 42 °C for 40 seconds. Place tube on ice for 2 minutes.

3.3. Add 900 µL of pre-warmed SOC media and incubate at 200 rpm at 37 °C for 1 hour.

3.4. Centrifuge at 5000 rpm for 1 min. Remove 900 µL of supernatant.

3.5. Re-suspend cells by gentle pipetting. Spread 100 µL of the cells onto the selection LB agar plates containing 200 µg/mL ampicillin.

3.6. Incubate inverted plates overnight at 37 °C.

Note: *Significantly lower cloning efficiencies can result from using other E. coli strains than DH5α.*

4. Screening for recombinant clones

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

pHTP0 forward primer:
5´- GAGCGGATAACAATTTCACACAGG -3´

pHTP0 reverse primer:
5´- GTTTTCCCAGTCACGACGTTG -3'

Note: *After running on an agarose gel, the expected size of the insert amplified using the pHTP0 vector-specific primers will be incremented by extra 268 bp.*

pHTP0 vector

Nucleotide sequence and properties of pHTP0 cloning vector is available for download at the product resources tab of the product.

Multiple fragment cloning protocol

NZYEasy Cloning & Expression System offers the possibility to clone multiple inserts simultaneously into one vector in a single reaction. Please read the Manual for Multiple Fragment Cloning using pHTP vectors available for download on the product page on our website.

NZYEasy Cloning & Expression System

For more details, please read the NZYEasy Cloning & Expression System Manual available for download on the product page on our website.

Quality control assays

Purity
NZYEasy enzyme mix is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by Bluesafe (MB152) staining.

Nucleases assay
All components of the kit are tested for nucleases activities, using 0.2-0.3 µg of pNZY28 plasmid DNA. Following incubation at 37 °C for 14-16 hours, the DNA is visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the DNA.

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.

V2001

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases assay	Pass
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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