NZY-blunt PCR cloning kit

Catalogue number:

MB12101, 24 ligations w/ competent cells
MB12102, 24 ligations

Description

NZY-blunt PCR cloning kit was designed to allow the direct cloning of PCR products with blunt ends, which result from PCR amplifications using proofreading DNA polymerases, into pNZY28. The pNZY28 vector provided was linearized at the EcoR V and dephosphorilated.

Storage temperature

Store competent cells at -80 °C on receipt. Other kit components may be stored at -20 °C or at -80 °C. NZY-blunt PCR cloning kit components are stable for up to one year when stored under the recommended conditions.

Kit components

Component	Concentration	Amount
Phos Buffer	10×	28 µL
Phos Enzyme mix	-	28 µL
Positive control insert	120 ng/µL	8 µL
Linearized pNZY28 vector	20 ng/µL	28 µL
T4 DNA Ligase	2 U/µL	28 µL
NZYStar Competent Cells [a,b]	-	12 × 0.20 mL
Competent Cells Control Plasmid [a,c]	0.1 ng/µL	10 µL

[a]only provided in MB12101 kit.
[b]Genotype of NZYStar competent cells: *end*A1 *hsd*R17(r_k^-, m_k^+) *sup*E44 thi -1 *rec*A1 *gyr*A96 *rel*A1 lac[F´ *pro*A^+B^+ $lacI^qZ\Delta M15$:Tn10(Tc^R)].
[c]Ampicillin resistance.

NZY-blunt cloning protocol

1. Insert preparation

For optimal cloning efficiencies, gel purification of PCR product using NZYTech´s NZYGelpure kit (MB011) is highly recommended. This kit can also be used for a PCR product clean-up which is sufficient in case non-specific amplification or primer-dimer are not apparent.

We recommend using a 1:10 molar ratio of vector:insert and starting with 20 ng of the linearized pNZY28 vector.

To calculate the optimal amount of PCR product required, use the following equation:

$$\frac{\text{ng of vector} \times \text{kb size of insert}}{\text{kb size of vector}} \times \text{molar ratio of } \frac{\text{insert}}{\text{vector}} = \text{ng of insert}$$

Example: If using 50 ng of a vector plasmid with 3 kb, for a 1:10 molar ratio of vector:insert then you will require the following amount of a 500 bp insert:

$$\frac{50 \times 0.5}{3} \times 10 = 83 \text{ ng}$$

2. Phos reaction

2.1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare the following reaction mixture (for a 10 µL reaction):

Component	Volume
Phos buffer	1 µL
PCR fragment*	x µL
Phos Enzyme mix	1 µL
Nuclease-free water	up to 10 µL

*Control reaction: To test the efficiency of the system use 2 µL of the Positive control insert provided.

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

2.3. Incubate the reaction at 37 °C for 20 minutes.

2.4. Heat inactivate the Phos Enzyme mix by incubating at 75 °C for 10 min.

2.5. Cool the reaction on ice for 2 min.

2.6. Centrifuge briefly to collect the reaction components.

3. Ligation reaction

3.1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare the ligation reaction as follows (for a 10 µL reaction):

Component	Volume
Product from step 2.6.	10 µL
Linearized pNZY28 vector (20 ng/µL)	1 µL
T4 DNA Ligase	1 µL

Note: *It is extremely important not to change the ratio of T4 DNA Ligase volume: final volume to prevent a decrease in efficiency of the cloning reactions.*

3.2. Incubate at room temperature (22-23 °C) for 1 hour to overnight. Longer incubation periods lead to higher ligation efficiencies.

4. Transformation

4.1. Thaw the required number of tubes of competent cells on ice. Pipette 100 µL of competent cells into pre-chilled microcentrifuge tubes on ice.

4.2. Add 5 µL of ligation mix directly into the cells. Stir gently to mix.

4.3. Incubate the transformation reaction for 30 min on ice.

4.4. Heat shock cells at 42 °C for exactly 40 seconds (**do not shake**).

4.5. Place on ice for 2 minutes.

4.6. Add 900 µL of pre-warmed SOC media (not provided).

4.7. Shake the tubes at 200 rpm at 37 °C for 1 hour.

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

4.9. Re-suspend cells by gentle pipetting. Plate 100 µL of cells onto LB agar plates containing 100 µg/mL ampicillin, 15 µg/mL tetracycline♦, 100 µg/mL X-gal and 0.5 mM IPTG.

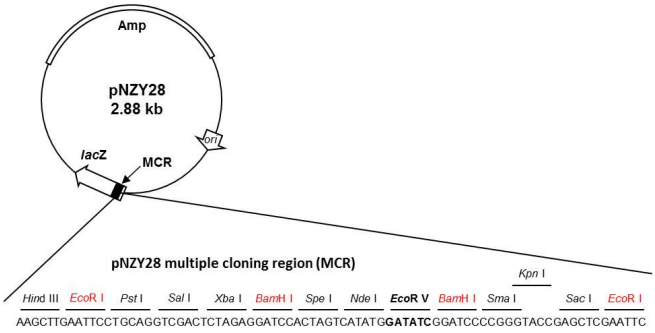
♦ For other cells than NZYStar Competent Cells, please check first if strain is resistant to tetracycline. Remove tetracycline from plates if using an *E. coli* strain without this resistance.

4.10. Incubate inverted plates overnight at 37 °C.

4.11. Screening for recombinants can easily be achieved by cutting with EcoR I or BamH I to excise the cloned insert from pNZY28 (the pNZY28 multiple cloning region is illustrated below), colony-PCR or sequencing.

pNZY28 vector

The provided vector was prepared by cutting pNZY28 with EcoR V and dephosphorilated. The nucleotide sequence and properties of pNZY28 are available at www.nzytech.com.



Sequencing pNZY28 recombinant derivatives

pNZY28 recombinant derivatives can be used for double stranded dideoxy sequencing using the T7 promoter, M13 reverse and U-19mer primers.

Troubleshooting

No colonies
• Competent cells are damaged
Check the transformation efficiency of *E. coli* competent cells with competent cells control plasmid.
• A specific component is missing in the ligation reaction
Repeat ligation reaction and transformation.
Low number or no white colonies
• PCR product without blunt ends
Check if your PCR insert was amplified with a proofreading DNA polymerase that creates a blunt ends.
• Incorrect insert/vector ratio
Optimise the ligation using other insert to vector ratios.
• Ligation is not optimal
Increase the time of ligation reaction (1 hour to overnight).
• Salts or ethanol present in the PCR insert
Repeat PCR and gel-purify the PCR product for a new ligation and transformation.
• PCR product is damaged
Verify quality of insert by gel electrophoresis. Limit DNA exposure to UV light to a few seconds or use safe dyes, like GreenSafe Premium (MB132), to detect DNA in a less aggressive environment.
• Low amount of PCR product
Re-quantify the PCR product by reading Abs 260 nm. If required increase amount of insert in ligation reaction.
White colonies without insert of interest or with incorrect inserts
• PCR product is used un-purified in the ligation reaction
Gel-purify the PCR band of interest in order to remove non-specific PCR products or primer-dimers that were generated

Quality control assays

Purity

Recombinant T4 DNA Ligase and enzymes from Phos Enzyme mix are >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe (MB152) staining.

Nucleases assay

All components of the NZY-blunt PCR cloning kit, excluding the pNZY28-A vector, 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 NZY-blunt PCR cloning kit are tested in a control experiment with the Positive control insert following the NZY-blunt cloning protocol described above. A 5 µL of the ligation mix was used to transform 100 µL of NZYStar competent cells. >90% of the recombinant plasmids must contain the appropriate insert.

V1901

Certificate of Analysis

Test	Result
Enzyme Purity	Pass
Nucleases assay	Pass
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

For research use only.

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