Universal USER Cassette

1-800-632-7799
info@neb.com
www.neb.com

N5504S 001120714071

N5504S

50 pmol **Lot: 0011207** **Exp: 7/14**

2.5 pmol/ µl **Store at –20°C**

Description: The Universal USER Cassette enables the construction of USER compatible vectors by ligation of an unphosphorylated double-stranded oligonucleotide cassette into any blunt-ended phosphorylated vector. Once the cassette is ligated into the vector of choice, this vector is digested with nicking enzyme Nt.BbvCI and the restriction enzyme XbaI which creates DNA overhangs compatible with the USER processed PCR product described in the USER Friendly PCR Cloning Kit (NEB #E5500).

Nt.BbvCI XbaI XbaI Nt.BbvCI
5'-GCTGAGGGAAAGTCTAGAGGATCCTCTAGATGTCTCCTCAGC-3'
3'-CGACTCCCTTTCAGATCTCCTAGGAGATCTACAGAGGAGTCG-5'

Reagents Supplied: Preannealed, unphosphorylated oligonucleotides (50 pmol, 2.5 pmol/µl) in 20 µl of 10 mM Tris-HCl, 1 mM EDTA (pH 8.0).

Quality Controls

The DNA sequence of the cassette was confirmed by DNA sequencing after cloning the Universal USER Cassette into LITMUS 38 digested with StuI. Using either the High Concentration T4 DNA Ligase Reaction protocol or Quick Ligation Reaction protocol outlined below, 20-40% of transformants had the cassette inserted into the vector.

Cassette Cloning Protocol

1. Either high concentration T4 DNA Ligase (NEB #M0202T/M) or the Quick Ligation Kit (NEB #M2200) may be used to ligate the Universal USER Cassette into the vector of choice. Follow Step 1a or 1b accordingly:

1a. High Concentration T4 DNA Ligase Reaction

Phosphorylated, blunt-ended Vector 0. 1 pmol (see protocol notes for calculation)

Universal USER Cassette (2.5 pmol/µl)	2 µl
10X T4 DNA Ligase Reaction Buffer	2 µl
Concentrated T4 DNA Ligase	1 µl
H_2O	to 20 µl
Total volume	20 µl

Incubate 2 hours at room temperature. Proceed to transformation step 2 or store ligation reaction at –20° C.

-OR-

1b. Quick Ligation Reaction

Phosphorylated, blunt-ended Vector 0. 1 pmol (see protocol notes for calculation)

Universal USER Cassette (2.5 pmol/µl)	2 µl
2X Quick Ligation Reaction Buffer	10 µl
Quick T4 DNA Ligase	1 µl
H_2O	to 20 µl
Total volume	20 µl

Incubate 10 min at room temperature. Longer incubation times may reduce ligation efficiency. Proceed to transformation step 2 or store ligation reaction at –20° C.

2. Transformation of ligation reaction: It is unnecessary to purify the DNA from either the high concentration ligase reaction or the Quick Ligase reaction if *E. coli* chemically competent cells ($CaCl_2$ or $RbCl_2$ treated cells) are used to recover recombinants. Use 5 µl of ligation reaction for 50 µl of competent cells. If using electrocompetent cells, it is necessary to purify the DNA after either ligation protocol prior to electroporation. We recommend spin column purification.

Notes:

Vector Constraints: The starting vector of choice cannot have any pre-existing XbaI sites. XbaI is used to generate compatible ends for the USER PCR.

Ideally, your vector will have no other Nt.BbvCI sites. If any additional Nt.BbvCI sites are present, but greater than 100 bp from each other, or from the new Nt.BbvCI sites in the Universal USER Cassette, these sites will be nicked but no intervening DNA will be removed. However, long-term storage of such a vector, and repeated freeze/thaws, could result in some decay of the

(See other side)

CERTIFICATE OF ANALYSIS

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H_2O	to 20 µl
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Incubate 10 min at room temperature. Longer incubation times may reduce ligation efficiency. Proceed to transformation step 2 or store ligation reaction at –20° C.

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CERTIFICATE OF ANALYSIS

vector due to strand separation, reducing the yield of colonies. Removing the Nt.BbvCI sites from the vector backbone will solve this problem. If the final vector designed has Nt.BbvCI site(s) closer that 100 bp, we strongly recommend removing extraneous Nt.BbvCI site(s) prior to use. It is absolutely necessary to remove these site(s) because the DNA strands are likely to separate.

Using the recommended 1:50 molar ratio of vector DNA to Universal USER Cassette ensures that a reasonable percentage (20-40%) of the transformants will contain an insertion. Using more or less vector DNA than recommended will result in lower numbers of transformants and fewer transformants with a Universal USER Cassette.

The digested vector of choice must have blunt phosphorylated ends since the 5' ends of the Universal USER cassette are unphosphorylated. If the researcher prefers to use a dephosphorylated vector, the Universal USER Cassette will need to be phosphorylated prior to ligation. T4 Polynucleotide Kinase (NEB #M0201) and T4 DNA Ligase Buffer (NEB #B0202) can be used for the phosphorylation reaction of the Universal USER Cassette. See the New England Biolabs catalog or website for the phosphorylation protocol as detailed in the T4 Polynucleotide Kinase product information. After the kinase reaction and inactivation, the amount of Universal USER Cassette used in the ligation reaction should be reduced from 50 fold molar excess to three fold molar excess over vector to reduce the frequency of vectors containing multiple USER cassettes.

Use 0.1 pmol of cut vector in the ligation reaction. See below for calculation of how many ng of vector is required for 0.1 pmol.

Converting ng of Vector DNA into pmol:

To calculate how much vector (in ng) is necessary for 0.1 pmol, the following equation can be used:

(# of base pairs of vector) x (650 g/mol base pair) x (10^9 ng/g) x 10^{-13} mol (i.e. 0.1 pmol)= x ng.

Example: How many ng of vector pBR322 (4361 bp) is required for 0.1 pmol?

(4361 base pairs) x (650 g/mol base pair) x (10^9 ng/g) x 10^{-13} mol (i.e. 0.1 pmol)= 283 ng

Recommended Controls:

We also encourage performing several controls:

1) An uncut vector control gives a measure of transformation efficiency.
2) A cut vector control to which no ligase is added, measures the background of undigested vector.
3) A cut vector to which no universal USER Cassette was added in the ligation reaction confirms that the ends of the vector were blunt and ligatable.

USER-Friendly Vector Preparation Protocol:

Any standard plasmid DNA purification can be used as long as a phenol/chloroform extraction and subsequent ethanol precipitation step are employed. We have found that Qiagen DNA column purifications may not be completely digested unless the DNA is phenol/chloroform extracted and ethanol precipitated. This protocol is for 10 µg DNA but can, of course, be scaled up. All parts of the protocol should be followed exactly.

1. Digest plasmid with Xba I restriction endonuclease (NEB #R0145):

Plasmid DNA (at least 640µg/ml)	10 µg
NEBuffer 4	10 µl
BSA (10 mg/ml)	1 µl
XbaI(40 units)	2 µl
H_2O	to 100 µl

Incubate overnight at 37˚C

2. Nick the XbaI-linearized vector with Nt.BbvCI (NEB #R0632) by adding 2 µl (20 units) of Nt.BbvCI (10,000 units/ml) to the above reaction and incubating for 1 hour at 37˚C

3. Purify linearized, nicked vector by phenol-chloroform extraction. Resuspend in 100 µl of TE buffer .

4. Determine vector concentration. Dilute to 20 µg/ml final concentration as an alternative to the pNEB206A cut vector in the USER Friendly Cloning Kit Protocol.

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vector due to strand separation, reducing the yield of colonies. Removing the Nt.BbvCI sites from the vector backbone will solve this problem. If the final vector designed has Nt.BbvCI site(s) closer that 100 bp, we strongly recommend removing extraneous Nt.BbvCI site(s) prior to use. It is absolutely necessary to remove these site(s) because the DNA strands are likely to separate.

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