

# LITMUS™ U



1-800-632-7799  
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N3640S 002120614061

## N3640S

2 µg (100 reactions)

Lot: 0021206

20 µg/ml

Store at -20°C

Exp: 6/14

**Description:** LITMUS™ U is a linear vector with 8 nucleotide, 3' non-complementary overhangs that can be used for the rapid cloning of PCR fragments without ligation (Figure 1). LITMUS U has opposing T7 promoters which generate dsRNA from a cloned fragment with little additional sequence by *in vitro* transcription with T7 RNA Polymerase.

To clone into LITMUS U, PCR products must be generated using primers that have the following sequences at the 5' end: 5' GGAGACAUNNN... and 5' GGGAAAGUNNN... (U = uracil and NNN... = specific primer sequence). After PCR, the DNA product can be directly cloned into LITMUS U using the

USER Enzyme (NEB #M5505) in a 30 minute reaction without ligation or any purification steps (1) following the USER Friendly Cloning Kit protocol:

1. Amplify your target DNA using *Taq* DNA Polymerase and uracil-containing primers.
2. Assembly Reaction.  
Mix: 10 µl crude PCR sample  
1 µl Linearized LITMUS U (20 ng)  
1 µl USER Enzyme (1 unit)  
12 µl total volume

3. Incubate for 15 minutes at 37°C.
4. Incubate for 15 minutes at room temperature.
5. Transform chemically competent *E. coli* cells with 2–12 µl of the assembly reaction from Step 4.

The cloned insert can be removed by BbvCI cleavage (NEB #R0601).

Supplied in: 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

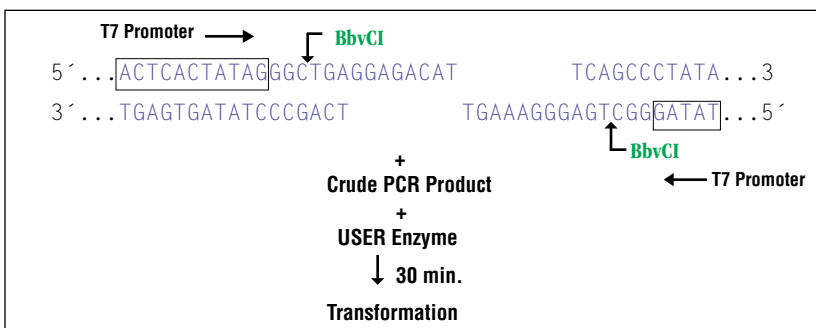


Figure 1: Cloning a PCR Product into LITMUS U

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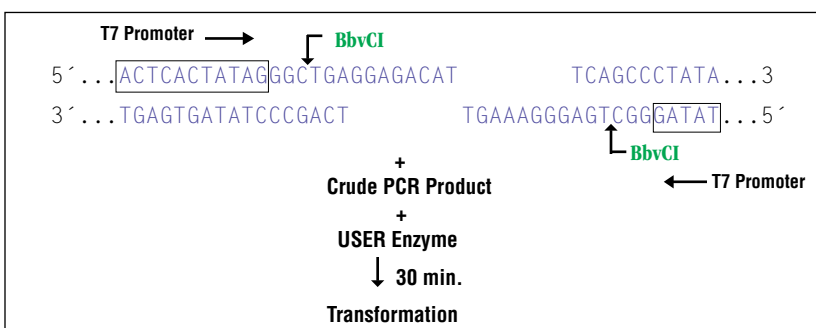


Figure 1: Cloning a PCR Product into LITMUS U

### Quality Control Assays

A standard USER reaction was performed as described in Appendix V of the USER Friendly Cloning Kit Manual (NEB #E5500): [10 µl (100 ng) of a 950 bp control PCR product amplified using *Taq* DNA Polymerase and primers containing uracil, designed as recommended in the USER Friendly Cloning Kit Manual, 20 ng linearized pLITMUS U, and 1 µl USER Enzyme]. After transformation into chemically-competent cells (NEB #ER2267 at 5 x 10<sup>6</sup> c.f.u./µg DNA), 50 µl of the 1 ml outgrowth was spread on Amp plates. A minimum of 200 colonies were obtained and > 95% of these were recombinants.

### Reference:

1. Bitinaite, J. et al. unpublished results.

CERTIFICATE OF ANALYSIS

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