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.

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 10^6 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:

Figure 1: Cloning a PCR Product into LITMUS U