**Source:** pNEB206A is isolated from *E. coli* by a standard purification procedure, digested to completion with Xba I and nicked with N.BbvC IB. The DNA is phenol extracted and resuspended in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.

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

**Quality Control Assays**

A standard USER reaction was performed as described in Appendix V of the USER Friendly Cloning Kit (NEB #E5500) manual [20 ng linearized pNEB206A, 1 µl USER Enzyme and 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]. After transformation into chemically-competent cells (NEB #ER2267 at $5 \times 10^6$ c.f.u./µg pNEB206A), 50 µl of the 1 ml outgrowth was spread on Amp + Xgal + IPTG plates. A minimum of 200 colonies were obtained and > 90% of these were white (i.e., contained recombinant molecules).

U.S. Publication No. US-2009-0042258