pNEB206A

Sequence file available at www.neb.com.

There are no restriction sites for the following enzymes: AbsI(x), Acc65I, AccI, AfeI, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AsiSI, AvaI, AvrII, BaeI, BarI(x), BbsI, BclI, BfuAI, BglII, BlpI, BmgBI, BmtI, BplI(x), BsaAI, BsaBI, BsgI, BsiWI, BsmFI, BsmI, BsoBI, BspDI, BspEI, BspMI, BsrGI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FalI(x), FseI, FspAI(x), HincII, HpaI, KflI(x), KpnI, MauBI(x), MfeI, MluI, MreI(x), MscI, MteI(x), NaeI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PaeR7I, PaqCI, PasI(x), PflFI, PflMI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, PsrI(x), RsrII, SacII, SalI, SexAI, SfiI, SgrAI, SgrDI(x), SmaI, SnaBI, SpeI, SphI, SrfI, StuI, StyI, SwaI, TspMI, Tth111I, XcmI, XhoI, XmaI

(x) = enzyme not available from NEB

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

pNEB206A is an *E. coli* plasmid vector designed for fast and efficient cloning of PCR products to be used in conjunction with USER Enzyme (NEB #M5505; 1). It is derived from pNEB193 containing the high-copy pUC19 origin of replication and *lacZ*α gene for screening of insertions at the cloning site using α-complementation (2).

The plasmid is supplied in a linearized form 2,706 bp in length (with bp 438-453 excised from the circular form), flanked by two noncomplementary 8-base 3´ overhangs at the intended cloning site. Amplification with deoxyuridine-containing primers and subsequent treatment (as defined in the protocol "Cloning with USER Enzyme" found on our website), results in PCR products with 5´ overhangs complementary to those in pNEB206A. These products can be directionally cloned into pNEB206A at high efficiency without the use of restriction enzymes or DNA ligase, forming recombinant circular molecules.

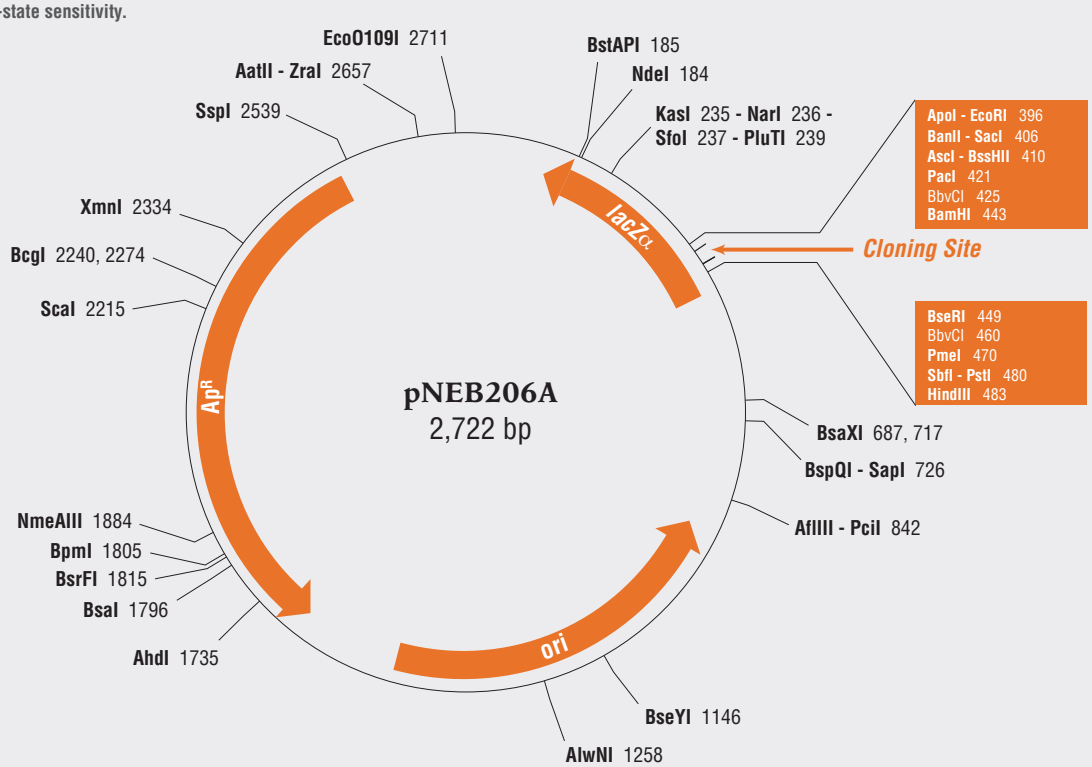
Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence. Cloning site coordinates include those bases in the circular form that are single-stranded in or missing from the supplied linear form.

Feature	Coordinates	Source
*lacZ*α	505-146	–
cloning site	430-461	–
origin	1491-903	pUC19
bla (Ap^R)	2522-1662	*Tn3*

ori = origin of replication
Ap = ampicillin



pNEB206A (linearized form) cloning site:

```
          Eco53KI
            SacI        BssHII
    EcoRI           AscI        PacI        BbvCI
agtgAATTCGAGCTCAGGCGCGCCTTAATTAAGCTGAGGGAAAGT
tcacTTAAGCTCGAGTCCGCGCGGAATTAATTCGACT
      .           .           .           .
      400         410         420         430

...S  N  S  S  L  R  A  K  I  L  S  L  S  L
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                                  SbfI
        BbvCI    PmeI        PstI     HindIII
          TCAGCGTTTAAACCCTGCAGGAAGCTTGGcgtaatcatggtcat
TACAGAGGAGTCGCAAATTTGGGACGTCCTTCGAACCgcattagtaccagta
      .           .           .           .           .
      460         470         480         490         500

  T  E  E  A  N  L  G  Q  L  F  S  P  T  I  M  T  M
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*lacZ*α translational start

References
(1) Bitinaite, J. and Vaiskunaite, R. (2003) unpublished observations.
(2) Yanisch-Perron, C. et al. (1985) *Gene,* 33, 103–119.