

pTXB1

Sequence file available at www.neb.com.
See page 214 for ordering information.

Feature	Coordinates	Source
<i>bla</i> (Ap ^R)	140-1000	<i>Tn3</i>
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
<i>rop</i>	2814-2623	pMB1
<i>lacI</i>	4453-3371	<i>E. coli</i>
T7 promoter	5637-5654	T7
expression ORF	5725-6558	—
MCS	5722-5775	—
<i>Mxe</i> GyrA intein	5776-6369	<i>M. xenopi</i>
CBD	6400-6558	<i>B. circulans</i>

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), Acc65I, AfIII, AfeI, AscI, AsiSI, AvrII, BaeI, BbvCI, BglII, BmgBI, Bpu10I, BseRI, BspDI, BstBI, Bsu36I, ClaI, CspCI, Eco53KI, FseI, FspAI(x), HindIII, I-CeuI, I-SceI, KpnI, MscI, NcoI, NsiI, P1-PspI, P1-SceI, PacI, PmlI, PpuMI, RsrII, SacI, SanDI(x), SbfI, SexAI, SfiI, SmaI, SnaBI, SrfI(x), TspMI, XmaI

(x) = enzyme not available from NEB

pTXB1 is an *E. coli* plasmid cloning vector designed for recombinant protein expression, purification, and ligation using the IMPACT™ Kit (NEB #E6901) (1,2). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTXB1 also contains an M13 origin of replication.

The multiple cloning site (MCS) is positioned to allow translational fusion of the *Mxe* GyrA intein tag to the C-terminus of the cloned target protein (2,3). The chitin binding domain (CBD) from *B. circulans*, fused to the C-terminus of the intein, facilitates purification of the intein-target protein precursor.

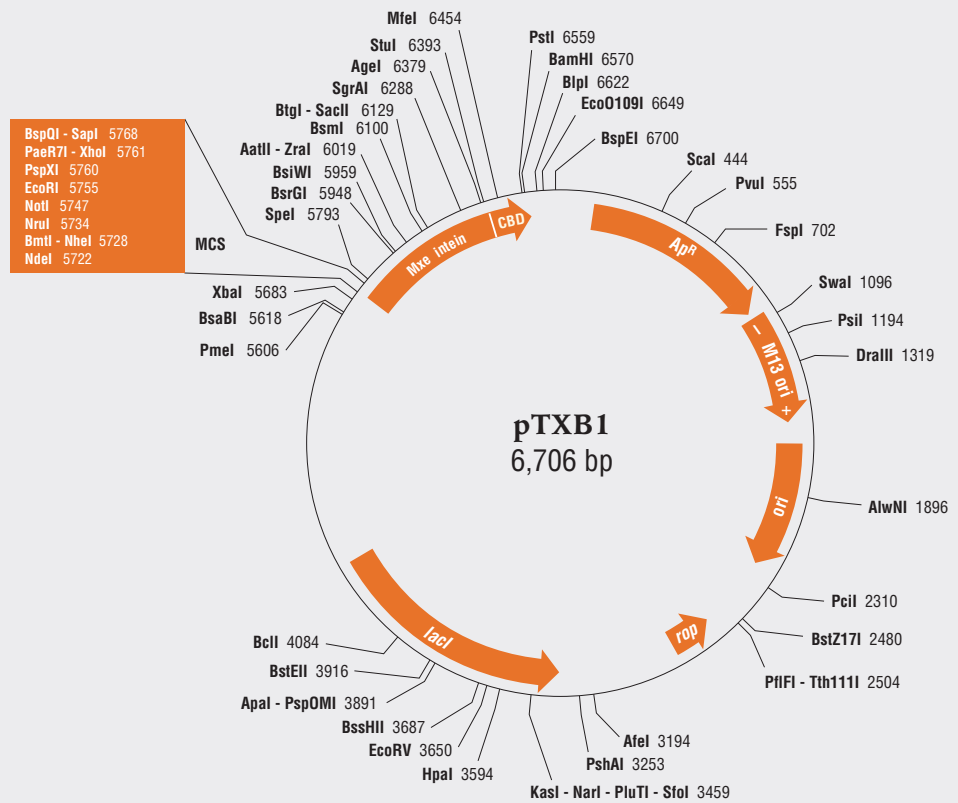
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the *lacI* gene, to the *lac* operator immediately downstream of the T7 promoter (4). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein ($\phi 10$).

pTXB1 and pTXB3 are identical except for the MCS regions: pTXB1 contains an NdeI site, and pTXB3 an NcoI site, overlapping the initiating methionine codon of the intein fusion gene. The N-terminal cysteine residue ("Cys₁") of the intein is shaded.

Enzymes with unique restriction sites are shown in bold type. Location of sites of all NEB restriction enzymes for select plasmids can be found on the NEB website (choose Tools & Resources > DNA Sequences and Maps tool). Restriction site coordinates refer to the position of the 5'-most base on the top strand in each recognition sequence.

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. Component genes or regions of fusion ORFs are indented below the ORF itself.

pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.



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T7 promoter          lac operator          XbaI          Shine Dalgarno
...CGCGAAATTAATACGACTCACTATAGCGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAATTTGTTTAACTTTAA SAAGGAGATATA
                    5680                    5700                    5720
    
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pTXB1 MCS          NdeI  NheI  NruI  SalI  NotI  EcoRI  XhoI  SapI
CATATGGCTAGCTCGCGAGTCGACGGCGGCCGCGAATTCCTCGAGGGCTCTTCC TGC ...
M A S S R V D G G R E F L E G S S C

pTXB3 MCS          NcoI  NheI  NruI  SalI  NotI  EcoRI  XhoI  SapI
ACCATGGCTAGCTCGCGAGTCGACGGCGGCCGCGAATTCCTCGAGGGCTCTTCC TGC ...
M A S S R V D G G R E F L E G S S C
    
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SpeI
ATCACGGGAGATGCACTAGTTGCCCTACCCGAGGGCGAGTCGGTACGCATCGCCGACATCGTGCCG...
    
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References

- Chong, S. et al. (1997) *Gene*, 192, 271–281.
- Evans, T.C., Benner and Xu, M.-Q. (1998) *Protein Sci.*, 7, 2256–2264.
- Southworth, M.W. et al. (1999) *Biotechniques*, 27, 110–120.
- Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.*, 219, 45–59.