pTWIN1

pTWIN1 is an *E. coli* plasmid cloning vector designed for recombinant protein expression, labeling, and cyclization using the IMPACT-TWIN Kit (NEB #E6901) (1). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTWIN1 also contains an M13 origin of replication.

The multiple cloning site (MCS) is positioned to allow translational fusion of an intein tag to the N-terminus, C-terminus, or both, of the cloned target protein. The mini-inteins encoded by pTWIN1, the *Ssp* DnaB intein and the *Mxe* GyrA intein, cleave the peptide bond at their C- and N-termini, respectively (1-4). The chitin binding domain (CBD) from *B. circulans*, fused to each intein, facilitates purification of the intein-target protein precursor.

Transcription of the intein tags is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., NEB #C2566, #C2833 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 (5). Translation of the intein tags utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (ϕ10).

pTWIN2 is identical to pTWIN1 except the *Mth* RIR1 intein is substituted for the *Mxe* GyrA intein (6). The MCS of both vectors are shown on the opposite page; the N-terminal cysteine residues ("Cys_1") of the downstream inteins are shaded.

Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes can be found on the NEB web site (choose Technical Reference > DNA Sequences and Maps). 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.

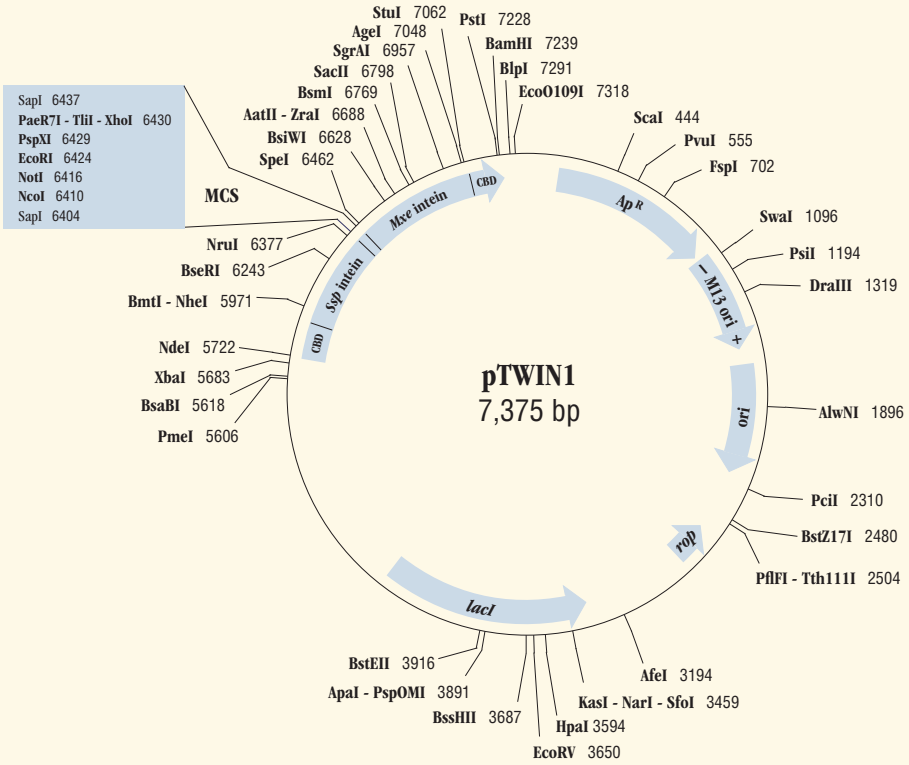
Sequence file available at www.neb.com
See page 164 for ordering information.

Feature	Coordinates	Source
bla (Ap^R)	140-1000	*Tn3*
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
rop	2814-2623	pMB1
lacI	4453-3371	*E. coli*
T7 promoter	5637-5654	T7
expression ORF	5725-7227	–
CBD	5725-5901	*B. circulans*
Ssp DnaB intein	5941-6402	*Synechocystis* sp.
MCS	6403-6444	–
Mxe GyrA intein	6445-7038	*M. xenopi*
CBD	7069-7227	*B. circulans*

ori = origin of replication
Ap = ampicillin

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

(x) = enzyme not available from NEB



References

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(4) Southworth, M.W. et al. (1999) *Biotechniques*, 27, 110–120.
(5) Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol Biol.*, 219, 45–59.
(6) Evans, T.C., Benner, J. and Xu, M.-Q. (1999) *J. Biol. Chem.*, 274, 3923–3926.