pTWIN1 is an E. coli plasmid cloning vector designed for recombinant protein expression, labeling, and cyclization using the IMPACT-TWIN Kit (NEB #E69801) (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 initiating tag to the N-terminus, C-terminus, or both, of the cloned target protein. The mini-inteins encoded by pTWIN1, the SapI and the MreGyR 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 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, requiring transcription of the intein tags is controlled by the inducible T7 promoter, requiring synthesis of the (+) strand, which gets packaged into phage particles. bla (Ap+) gene coordinates include the signal sequence.

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.

There are no restriction sites for the following enzymes: AarI(x), Acc65I, AfII, AlwI, Ascl, Aval, AvrII, BaeI, BbvCI, BglII, BmgBI, BsaBI, BglII, BstBI, BstBI, BsmBI, CaiI, CclI, CfoI, FstI, HpaI, Hpy18I, KlaiI, KpnI, MluI, MscI, NciI, PciI, PstI, SacI, SbfI, SfiI, SspI, StuI, TspMI, XmaI, XmnI (x) = enzyme not available from NEB

References