pTYB21

Sequence file available at www.neb.com.
See page 214 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	5725-7368	–
MCS	7301-7361	–
Sce VMA intein	5770-7299	*S. cerevisiae*
CBD	6595-6747	*B. circulans*

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), AatII, AflII, AgeI, AscI, AsiSI, AvrII, BbvCI, BmgBI, BseRI, BsiWI, BsmI, BspDI, Bsu36I, ClaI, CspCI, FseI, FspAI(x), I-CeuI, I-SceI, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PpuMI, PspXI, RsrII, SanDI(x), SexAI, SfiI, SgrAI, SmaI, SnaBI, SrfI(x), TspMI, XhoI, XmaI, ZraI

(x) = enzyme not available from NEB

pTYB21 is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification 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, pTYB21 also contains an M13 origin of replication.

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

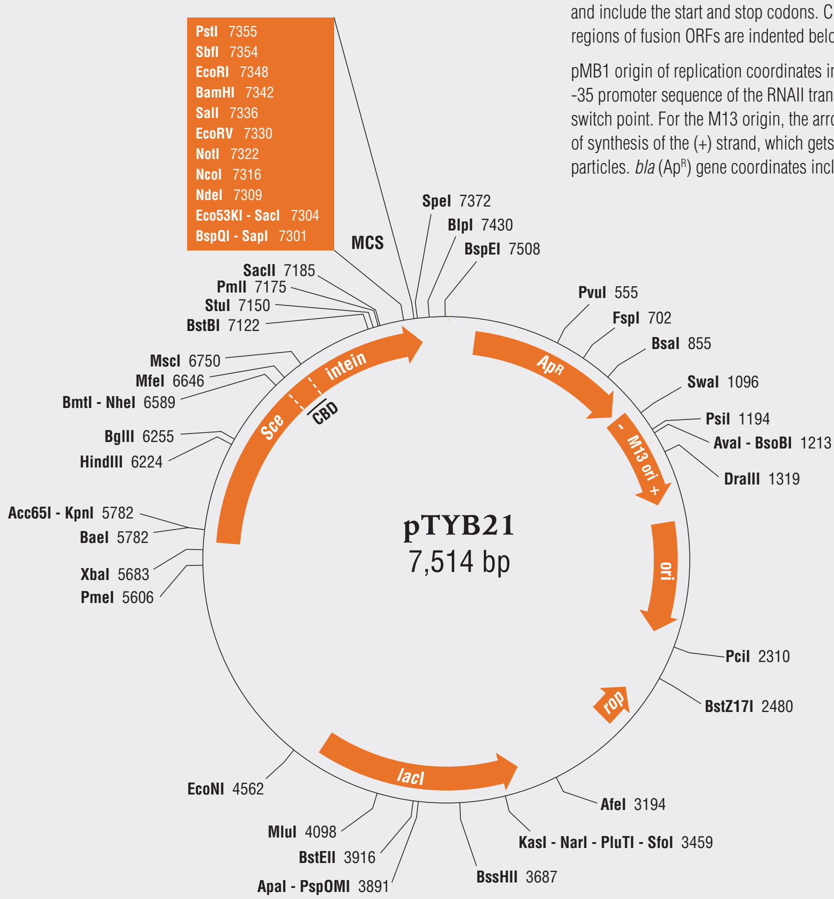
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the *lacI* gene, to the *lac* operator immediately downstream of the T7 promoter (3). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (φ10).

pTYB21 contains a SapI site which allows for cloning of a target gene without any extra amino acids. pTYB22 is identical to pTYB21 except for the MCS regions (see below). pTYB22 contains an NdeI site overlapping the initiating methionine codon of the intein fusion gene. pTYB21 differs from pTYB11 in that it contains a universal MCS that is compatible with all NEB expression systems.

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 (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.

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                                                  Sce VMA Intein      ...    S   D   H  Q   F   L   L   G   S   Q
...TAATACGACTCACTATAGGGGAATTGTG...GAAGACGATTATTATGGGATTACTTTATCTGATGATTCTGATCATCAGTTTTTGCTTGGATCTCAG
      .           .          .          .          .          .          .          .          .
            5650                  7220                  7240                  7260                  7280
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pTYB21 MCS

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      Sce VMA Intein       SacI                                                          SbfI
                        SapI     NdeI     NcoI   NotI              SalI     BamHI  EcoRI     PstI
GTTGTTGTACAGAAC  GGAAGAGCTCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCTGCAGGTAATTAAATAAC...
 V  V  V  Q  N    G  R  A  H  M  S  M  G  G  R  D  I  V  D  G  S  E  F  P  A  G  N  *
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pTYB22 MCS

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      Sce VMA Intein                                                              SbfI
            BsmI         NdeI     NcoI   NotI              SalI     BamHI  EcoRI     PstI
GTTGTTGTACAGAAT  GCTGGTCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCTGCAGGTAATTAAATAAC...
 V  V  V  Q  N    A  G  H  M  S  M  G  G  R  D  I  V  D  G  S  E  F  P  A  G  N  *
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References

(1) Chong et al. (1996) *J. Biol. Chem.*, 271, 22159–22168
(2) Chong et al. (1998) *NAR*, 26, 5109–5115.
(3) Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.*, 219, 45–59.