

# pTYB21

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 *Scd* 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, 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 (3). Translation of the fusion

utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein ( $\phi 10$ ).

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 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.

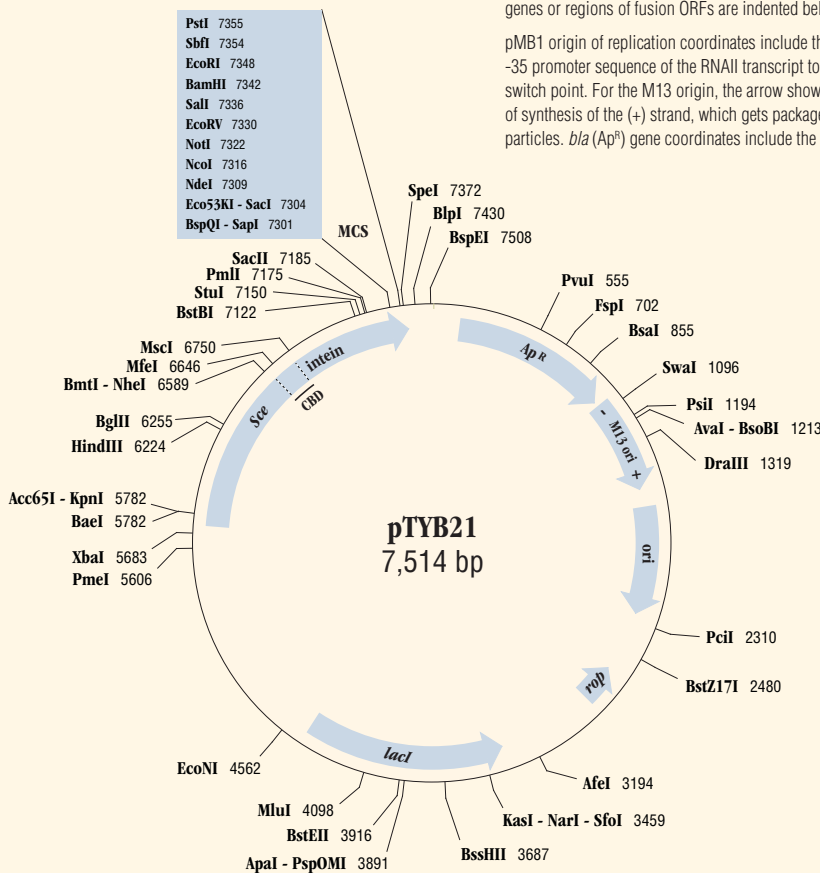
pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (*Ap<sup>r</sup>*) gene coordinates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

Sequence file available at [www.neb.com](http://www.neb.com). See page 205 for ordering information.

Feature	Coordinates	Source
<i>bla</i> ( <i>Ap<sup>r</sup></i> )	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	5725-7368	–
MCS	7301-7361	–
<i>Scd</i> VMA intein	5770-7299	<i>S. cerevisiae</i>
CBD	6595-6747	<i>B. circulans</i>

ori = origin of replication  
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), AatII, AfIII, Agel, AscI, AsiSI, AvrII, BbvCI, BmgBI, BseRI, BsiWI, BsmI, BspDI, Bsu36I, ClaI, CspCI, FseI, FspAI(x), I-CeuI, I-SceI, NruI, NsiI, P1-PspI, P1-Scel, PacI, PaeR7I, PpuMI, PspXI, RsrII, SanDI(x), SexAI, SfiI, SgrAI, SmaI, SnaBI, SrfI(x), TliI, TspMI, XhoI, XmaI, ZraI (x) = enzyme not available from NEB



T7 Universal Primer →  
 ...TAATACGACTCACTATAGGGGAATTGTG...GAAGACGATTATTATGGGATTACTTTATCTGATGATTCTGATCATCAGTTTTTGGTGGATCTCAG  
 5650 7220 7240 7260 7280

Scd VMA Intein → SacI SbfI  
 pTYB21 MCS GTTGTGTACAGAAC GGAAGAGCTCATATGTCATGGCGCGCGGATATCGTCGACGGATCCGAATTCCTGCAGGTAATTAATAAC...  
 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 \*

Scd VMA Intein → BsmI SbfI  
 pTYB22 MCS GTTGTGTACAGAAT GCTGGTCATATGTCATGGCGCGCGGATATCGTCGACGGATCCGAATTCCTGCAGGTAATTAATAAC...  
 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 \*

### References

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