

**pTYB21**

pTYB21 is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the IMPACT® Kit (NEB NEB901) (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 VMA intein tag to the N-terminus of the cloned target protein (2). The chitin binding domain (CBD) from *B. circularis* facilitates purification of the intein-target protein precursor.

Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring 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 from the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for transcription of the gene fusion. 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 type.

(Syne Dalgarno sequence) from the strongly expressed T7 gene 10 protein (q10).

pTYB21 contains a Sapl site which allows for cloning of a target gene without any extra amino acids. pTYB21 is identical to pTYB2 except for the MCS regions (see below). pTYB22 contains an Ndel site overlapping the initiating methionine codon of the intein fusion gene. pTYB21 differs from pTYB1 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 website (choose Technical > 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 indicated 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*) gene coordinates include the signal sequence.

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References