

## pMAL-c6T

Sequence file available at [www.neb.com](http://www.neb.com).  
See page 229 for ordering information.

Feature	Coordinates	Source
<i>lacI<sup>q</sup></i>	80-1162	<i>E. coli</i>
P <sub>lac</sub>	1405-1432	—
expression ORF	1527-2761	—
<i>malE</i>	1527-2721	<i>E. coli</i>
MCS	2722-2761	—
<i>bla</i> (Ap <sup>R</sup> )	3101-3961	<i>Tn3</i>
origin	4049-4637	pMB1
<i>rop</i>	5007-5198	pMB1

There are no restriction sites for the following enzymes: AatII, AbsI(x), Acc65I, AfIII, AgeI, AjuI(x), AelI, AroI(x), AscI, AsiSI, AvrII, BaeI, BarI(x), BbvCI, BmtI, BpII(x), BsaAI, BseRI, BsmFI, BspDI, BsrGI, BstBI, BstZ17I, ClaI, CspCI, DraIII, EcoNI, Fall(x), FseI, FspAI(x), KfiI(x), KpnI, MauBI(x), MreI(x), MscI, MteI(x), NaeI, NcoI, NdeI, NgoMIV, NheI, NruI, NsiI, PacI, PaeR7I, PaqCI, PaeI(x), PmeI, PmlI, PshAI, PspXI, PstI(x), SacII, SexAI, SfiI, SgrAI, SmaI, SnaBI, SpeI, SphI, SrfI, StuI, StyI, SwaI, TspMI, XbaI, XhoI, XmaI, XmnI, ZraI

(x) = enzyme not available from NEB



We recommend NEBcutter at [NEBcutter.com](http://NEBcutter.com) to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

pMAL-c6T is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the NEBExpress MBP Fusion and Purification System (NEB #E8201) (1–3). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322.

The multiple cloning site (MCS) is positioned to allow translational fusion of the *E. coli* maltose binding protein (MBP, encoded by the *malE* gene lacking its secretory signal sequence) to the N-terminus of the cloned target protein, thus resulting in an MBP-fusion protein localized in the cytoplasm. The pMAL-c6T vector contains a multiple cloning site (MCS) that is compatible with other NEB expression systems and is followed by stop codons in all three reading frames. In this vector, MBP has been engineered for tighter binding to amylose. This allows easy purification of the fusion protein, and the MBP domain can be subsequently removed using TEV Protease (NEB #P8112).

Transcription of the gene fusion is controlled by the inducible "lac" promoter (P<sub>lac</sub>). Basal expression from P<sub>lac</sub> is minimized by the binding of the Lac repressor, encoded by the *lacI<sup>q</sup>* gene, to the *lac* operator immediately downstream of P<sub>lac</sub>. A portion

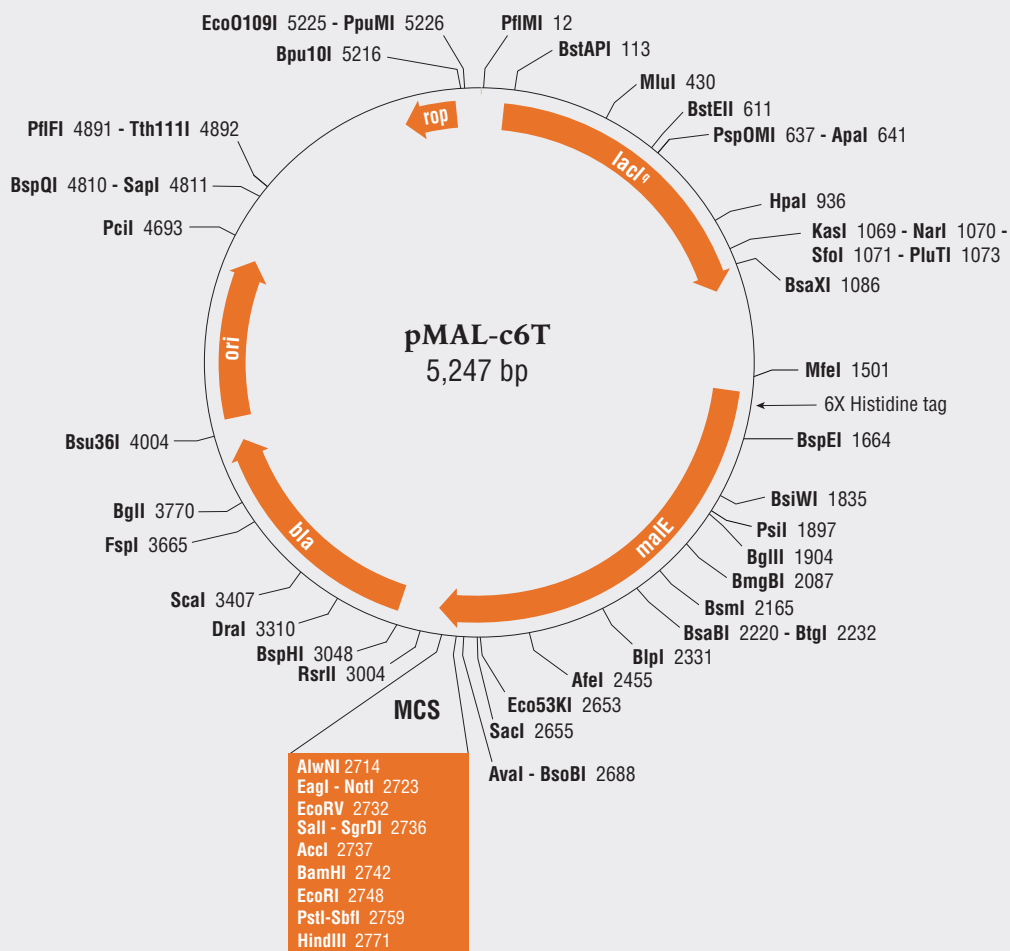
of the *rnnB* operon containing two terminators, derived from the vector pKK233-2, prevents transcription originating from P<sub>lac</sub> from interfering with plasmid functions.

Enzymes with unique restriction sites are shown in bold type.

**Coordinates indicate position of cutsite on the top strand. In previous catalogs, coordinates referred to the position of the 5' most base on the top strand, please make note of new numbering system.**

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.

The pMB1 origin of replication includes the region from the -35 promoter sequence of the RNAlI transcript to the RNA/DNA switch point (labeled "ori") and the *rop* gene, which controls expression of the RNAlI transcript. *bla* (Ap<sup>R</sup>) gene coordinates include the signal sequence.



## pMAL-c6T Polylinker:

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AlwNI   NotI/EagI   EcoRV   Sall   BamHI   EcoRI   PstI/SbfI
5' maIE...ATG CTG ATG GGC GGC CGC GAT ATC GTC GAC GGA TCC GAA TTC CCT GCA GGT AAT
          HindIII
TAA ATA AGC TAA ATA AGC TTC AA...

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## References

- (1) Guan, C. et al. (1987) *Gene*, 67, 21–30.
- (2) Maina, C.V. et al. (1988) *Gene*, 74, 365–373.
- (3) Riggs, P.D. (1992). In F.M. Ausubel, et al. (Eds.), *Current Prot. in Molecular Biol.* New York: John Wiley & Sons, Inc.