pMAL-c6T Map

Sequence file available at www.neb.com.
For ordering information, see Protein Expression & Purification.

There are no restriction sites for the following:

AatII, AbsI(x), Acc65I, AflII, AgeI, AjuI(x), AleI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BarI(x), BbvCI, BmtI, BpiI(x), BsaAI, BseRI, BsmFI, BspDI, BsrGI, BstBI, BstZ17I, ClaI, CspCI, DraIII, EcoNI, FalI(x), FseI, FspAI(x), KfiI(x), KpnI, MauBI(x), MreI(x), MscI, MteI(x), NaeI, NcoI, NdeI, NgoMIV, NheI, NruI, NsiI, PacI, PaeR7I, PaqCI, PasI(x), PmeI, PmlI, PshAI, PspXI, PsrI(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

Feature	Coordinates	Source
lacI^q	80-1162	*E. coli*
Ptac	1405-1432	–
expression ORF	1527-2761	–
malE	1527-2721	*E. coli*
MCS	2722-2761	–
bla (ApR)	3101-3961	*Tn3*
origin	4049-4637	pMB1
rop	5007-5198	pMB1

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.

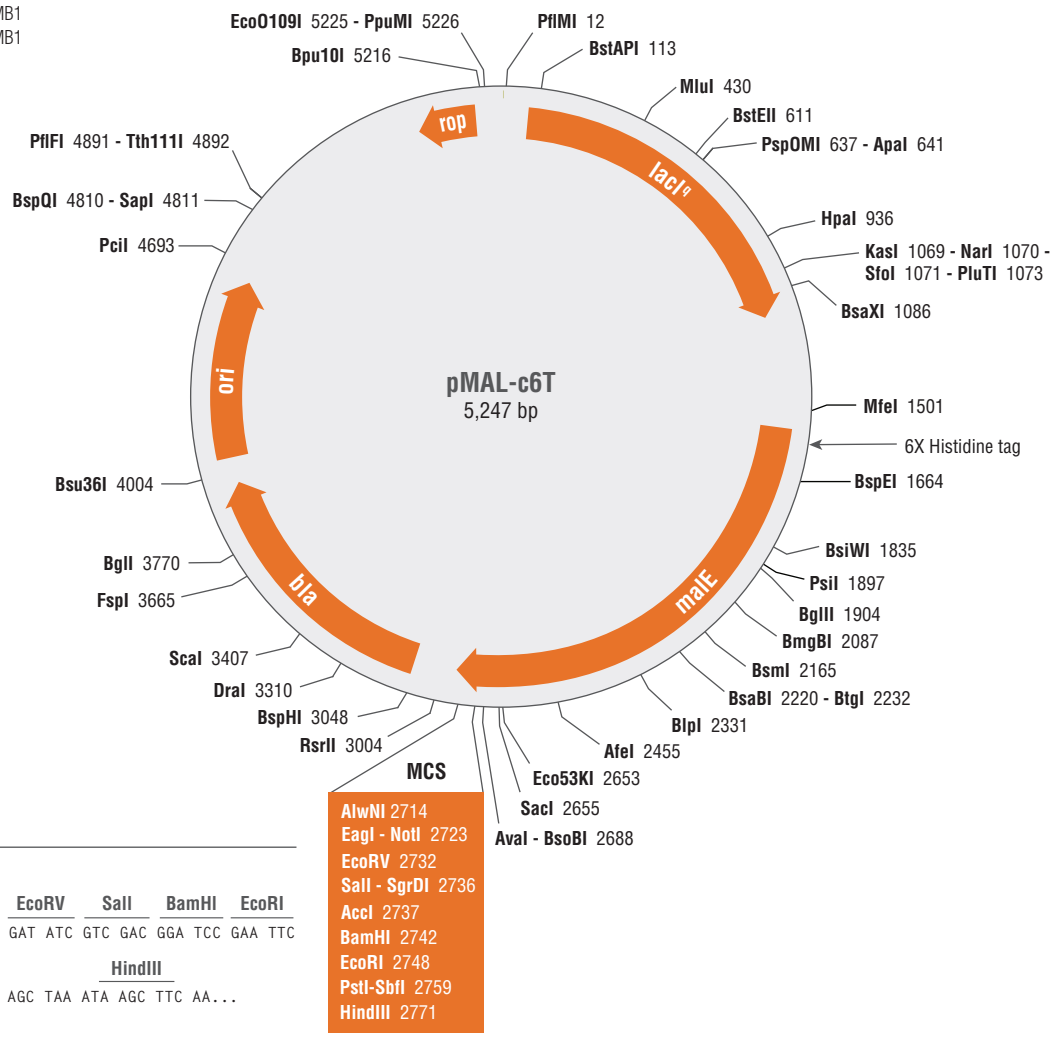
The multiple cloning site (MCS) is positioned to allow translational fusion of the *E. coli* maltose binding protein (MBP, encoded by the malE gene lacking its secretory signal sequence) to the *N*-terminus of the cloned target protein, thus resulting in an MBP-fusion protein localized in the cytoplasm. 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 "tac" promoter (P_{tac}). Basal expression from P_{tac} is minimized by the binding of the Lac repressor, encoded by the *lacI*^{q} gene, to the *lac* operator immediately downstream of P_{tac}. A portion of the *rrnB* operon containing two terminators, derived from the vector pKK233-2, prevents transcription originating from P_{tac} from interfering with plasmid functions.

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

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 RNAII transcript to the RNA/DNA switch point (labeled "ori") and the rop gene, which controls expression of the RNAII transcript. *bla* (Ap^R) gene coordinates include the signal sequence.



pMAL-c6T Polylinker:

	AlwNI	NotI/EagI	EcoRV	SalI	BamHI	EcoRI
5´ *malE* ...	CAG ATG CTG GGC GGC CGC GAT ATC GTC GAC GGA TCC GAA TTC					

	PstI/SbfI	HindIII
	CCT GCA GGT AATTAA ATA AGC TAA ATA AGC TTC AA...	

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.