

pMAL™-p5X

pMAL-p5X is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the pMAL Protein Fusion and Purification System (NEB #E8200) (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) to the N-terminus of the cloned target protein. The pMAL-p5 and -c5 series of vectors differs from the -p4 and -c4 series in that they contain a universal multiple cloning site (MCS) that is compatible with other NEB expression systems and is followed by stop codons in all three reading frames. In addition, *lacZα* and the M13 origin have been removed. In these vectors, 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 Factor Xa protease (3).

Transcription of the gene fusion is controlled by the inducible “lac” promoter (P_{lac}). Basal expression from P_{lac} is minimized by the binding of the Lac repressor, encoded by the *lacI^q* gene, to the *lac* operator immediately downstream of P_{lac}. A portion of the *rrnB* operon containing two terminators, derived from the vector pKK223-2, prevents transcription originating from P_{lac} from interfering with plasmid functions.

pMAL-p5E and pMAL-p5G are identical to pMAL-p5X except they replace the Factor Xa protease cleavage site with Enterokinase and Genenase I™ cleavage sites, respectively.

pMAL-c5-series vectors are identical to the pMAL-p5-series vectors above except for a deletion of the *malE* signal sequence (nt 1531-1605) (1).

Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular 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.

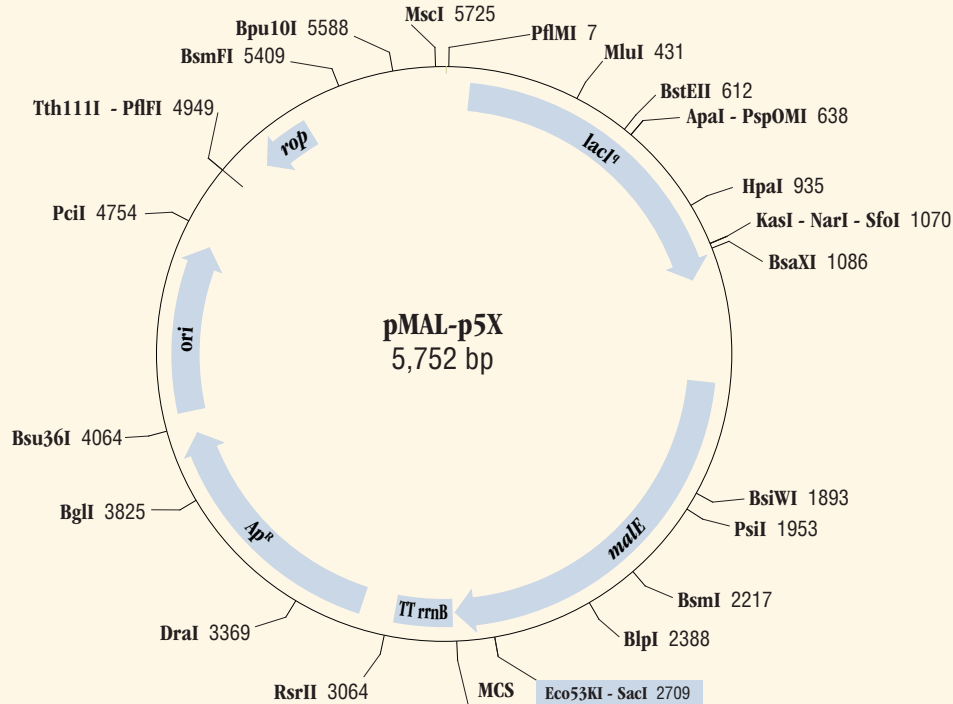
The pMB1 origin of replication includes the region from the -35 promoter sequence of the RNAlI transcript to the RNA/DNA switch point (labeled “ori”) and the *rop* gene, which controls expression of the RNAlI transcript. *bla* (Ap^R) gene coordinates include the signal sequence.

Sequence file available at www.neb.com. See page 202 for ordering information.

Feature	Coordinates	Source
<i>lacI^q</i>	81-1163	<i>E. coli</i>
P _{lac}	1406-1433	–
expression ORF	1528-2832	–
<i>malE</i>	1528-2703	<i>E. coli</i>
MCS	2709-2832	–
<i>bla</i> (Ap ^R)	3162-4022	<i>Tn3</i>
origin	4110-4698	pMB1
<i>rop</i>	5068-5259	pMB1

There are no restriction sites for the following enzymes: AarI(x), AatII, Acc65I, AflIII, AgeI, AleI, AscI, AsiSI, AvrII, BaeI, BbvCI, BmtI, BseRI, BspDI, BsrGI, BstBI, Bsu36I, ClaI, CspCI, EagI, EcoNI, FseI, I-CeuI, I-SceI, KpnI, MfeI, NcoI, NheI, NotI, Nrul, NsiI, P1-PspI, PI-SceI, PacI, PaeR7I, PmeI, PmlI, PshAI, PspXI, RsrII, SanDI(x), SexAI, SfiI, SgrAI, SmaI, SnaBI, SpeI, SphI, SrfI(x), StuI, Styl, TliI, TspMI, XhoI, XmaI, ZraI

(x) = enzyme not available from NEB



Enzyme	Coordinates
Eco53KI - SacI	2709
XmnI	2759
NdeI	2770
NcoI - StyI	2777
NotI	2783
EagI	2784
EcoRV	2791
AccI - SalI	2797
BamHI	2803
EcoRI	2809
SbfI	2815
PstI	2816
HindIII	2832

pMAL-p5X Polylinker:

5' *malE*...CTC GGG ATC GAG GGA AGG ATT TCA CAT ATG TCC ATG GGC GGC CGC GAT ATC
 Aval XmnI NdeI NcoI NotI EcoRV
 Sall BamHI EcoRI SbfI
 GTC GAC GGA TCC GAA TTC CCT GCA GGT AAT TAA ATA A...

pMAL-p5E Polylinker:

5' *malE*...CTC GGG GAT GAC GAT GAC AAG GTA CCG CAT ATG TCC ATG GGC GGC CGC
 EcoRV Sall BamHI EcoRI SbfI
 GAT ATC GTC GAC GGA TCC GAA TTC CCT GCA GGT AAT TAA ATA A...

pMAL-p5G Polylinker:

5' *malE*...CTC GGG CCG GGT GCG GCA CAC TAC GTA CAT ATG TCC ATG GGC GGC CGC
 EcoRV Sall BamHI EcoRI SbfI
 GAT ATC GTC GAC GGA TCC GAA TTC CCT GCA GGT AAT TAA ATA A...

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.