pMAL™-p4X

pMAL-p4X is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the pMAL Protein Fusion and Purification System (NEB #E8000) (1–3). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pMAL-p4X also contains an M13 origin of replication (4).

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. In the pMAL-p4 and -c4 series of vectors, the MBP has been engineered for tighter binding to amylose resin. This allows easy purification of the fusion protein, and the MBP domain can be subsequently removed using Factor Xa protease (3). Cloning of the target gene at the MCS disrupts expression of *lacZα*, allowing for insert screening by α-complementation.

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.

pMAL-p4E and pMAL-p4G are identical to pMAL-p4X except they replace the Factor Xa protease cleavage site with Enterokinase and Genenase I™ cleavage sites, respectively.

pMAL-c4-series vectors are identical to the pMAL-p4-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.
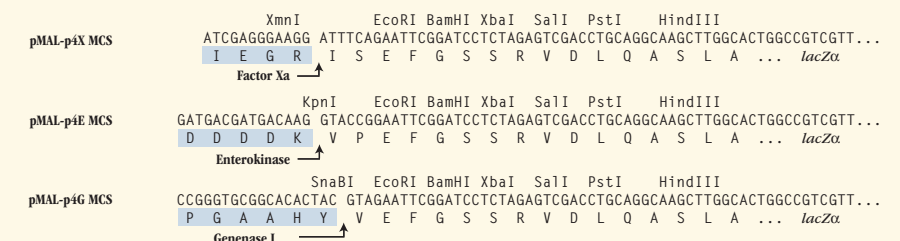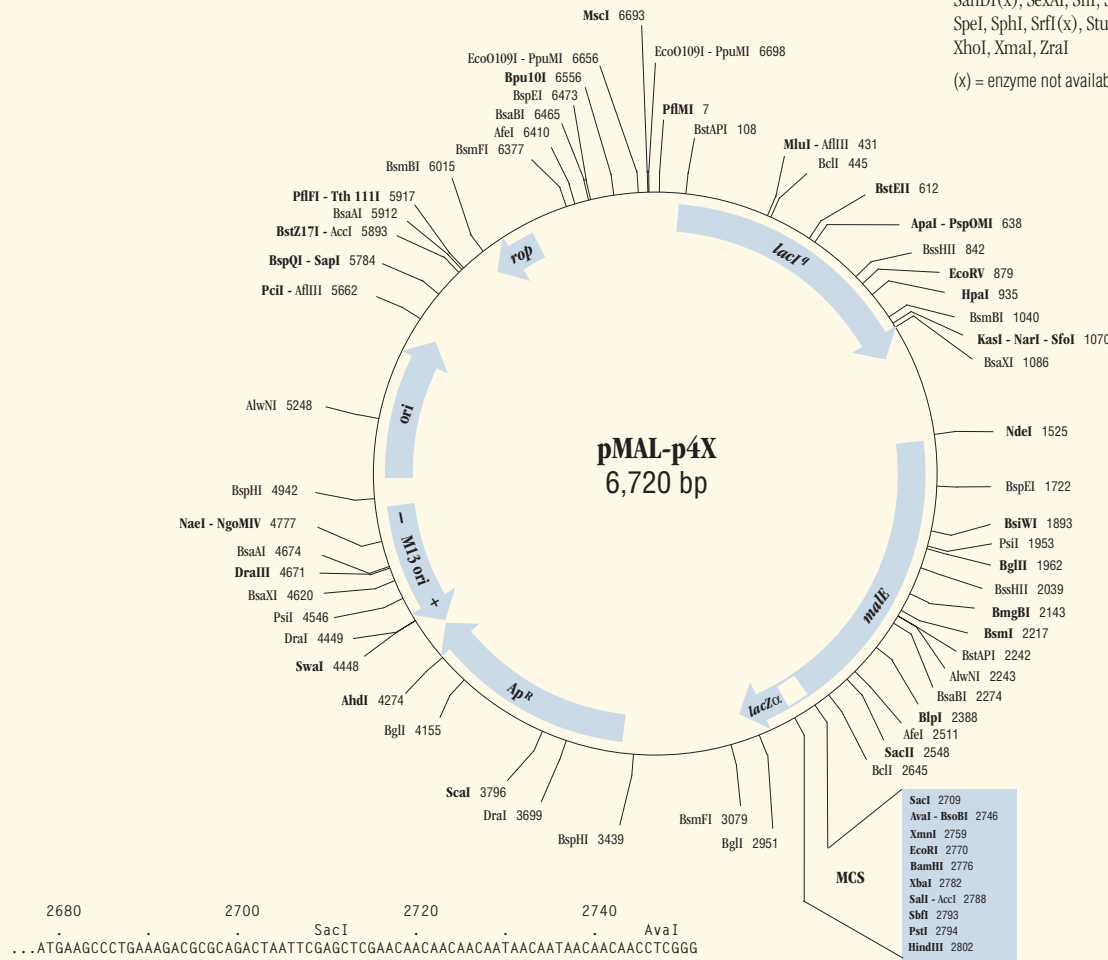
pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.

Sequence file available at www.neb.com
See page 163 for ordering information.

Feature	Coordinates	Source
lacI^q	81-1163	*E. coli*
P_{tac}	1406-1433	–
expression ORF	1528-2991	–
malE	1528-2703	*E. coli*
MCS	2704-2809	–
lacZa	2810-2991	–
bla (Ap^R)	3492-4352	*Tn3*
M13 origin	4394-4907	M13
origin	5018-5606	pMB1
rop	6227-6036	pMB1

There are no restriction sites for the following enzymes: AarI(x), AatII, Acc65I, AflII, 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, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PmeI, PmlI, PshAI, PspXI, RsrII, SanDI(x), SexAI, SfiI, SgrAI, SmaI, SnaBI, SpeI, SphI, SrfI(x), StuI, StyI, TliI, TspMI, XhoI, XmaI, ZraI

(x) = enzyme not available from NEB

pMAL-p4X
6,720 bp

MscI 6693
EcoO109I - PpuMI 6656
EcoO109I - PpuMI 6698
Bpu10I 6556
BspEI 6473
BsaBI 6465
AfeI 6410
BsmFI 6377
BsmBI 6015
PflMI 7
BstAPI 108
MluI - AflIII 431
BclI 445
BstEII 612
ApaI - PspOMI 638
BssHII 842
EcoRV 879
HpaI 935
BsmBI 1040
KasI - NarI - SfoI 1070
BsaXI 1086
PflFI - Tth 111I 5917
BsaAI 5912
BstZ17I - AccI 5893
BspQI - SapI 5784
PciI - AflIII 5662
NdeI 1525
BspEI 1722
AlwNI 5248
BsiWI 1893
PsiI 1953
BglII 1962
BssHII 2039
BmgBI 2143
BsmI 2217
BstAPI 2242
AlwNI 2243
BsaBI 2274
BspHI 4942
NaeI - NgoMIV 4777
BsaAI 4674
DraIII 4671
BsaXI 4620
PsiI 4546
DraI 4449
SwaI 4448
BlpI 2388
AfeI 2511
SacII 2548
BclI 2645
AhdI 4274
BglII 4155
ScaI 3796
DraI 3699
BspHI 3439
BsmFI 3079
BglII 2951

MCS
SacI	2709
AvaI - BsoBI	2746
XmnI	2759
EcoRI	2770
BamHI	2776
XbaI	2782
SalI - AccI	2788
SbfI	2793
PstI	2794
HindIII	2802

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      2680              2700              2720              2740
       .     .     .     .     .  SacI .     .     .     .   AvaI
...ATGAAGCCCTGAAAGACGCGCAGACTAATTCGAGCTGCGAACAACAACAATAACAATAACAACAACCTCGGG
      malE  ...  A  Q  T  N  S  S  S  N  N  N  N  N  N  N  N  N  L  G
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pMAL-p4X MCS       XmnI      EcoRI BamHI XbaI  SalI  PstI   HindIII
               ATCGAGGGAAGG AATTCAGAATTCGGATCCTCTAGAGTCGACCTGCAGGCAAGCTTGGCACTGGCCGTCGTT...
                I  E  G  R   I  S  E  F  G  S  S  R  V  D  L  Q  A  S  L  A ...  lacZα
               Factor Xa ──▶
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```
pMAL-p4E MCS       KpnI      EcoRI BamHI XbaI  SalI  PstI   HindIII
               GATGACGATGACAAG GTACCGGAATTCGGATCCTCTAGAGTCGACCTGCAGGCAAGCTTGGCACTGGCCGTCGTT...
                D  D  D  D  K   V  P  E  F  G  S  S  R  V  D  L  Q  A  S  L  A ...  lacZα
               Enterokinase ──▶
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```
pMAL-p4G MCS       SnaBI     EcoRI BamHI XbaI  SalI  PstI   HindIII
               CCGGGTGCGGCACACTAC GTAGAATTCGGATCCTCTAGAGTCGACCTGCAGGCAAGCTTGGCACTGGCCGTCGTT...
                P  G  A  A  H  Y   V  E  F  G  S  S  R  V  D  L  Q  A  S  L  A ...  lacZα
               Genenase I ──▶
```

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..
(4) Zagursky, R.J. et al. (1984) *Gene*, 27, 183–191.