pMAL™-p2X

6,721 base pairs
Sequence file available at www.neb.com
See page 138 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	–
*lacZ*α	2810-2991	–
bla (Ap^R)	3493-4353	*Tn3*
M13 origin	4395-4908	M13
origin	5019-5607	pMB1
rop	6228-6037	pMB1

ori = origin of replication
Ap = ampicillin

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., in Ausubel, F.M. et al. (eds), *Current Prot. in Molecular Biol.* (1992) Greene Associates/Wiley Interscience, New York.
4. Zagursky, R.J. et al. (1984) *Gene* 27, 183–191.

pMAL-p2X is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the pMAL Protein Fusion and Purification System (NEB #E8000S) (1-3). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pMAL-p2X 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. MBP's affinity for amylose 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 *lacZ*α, 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.

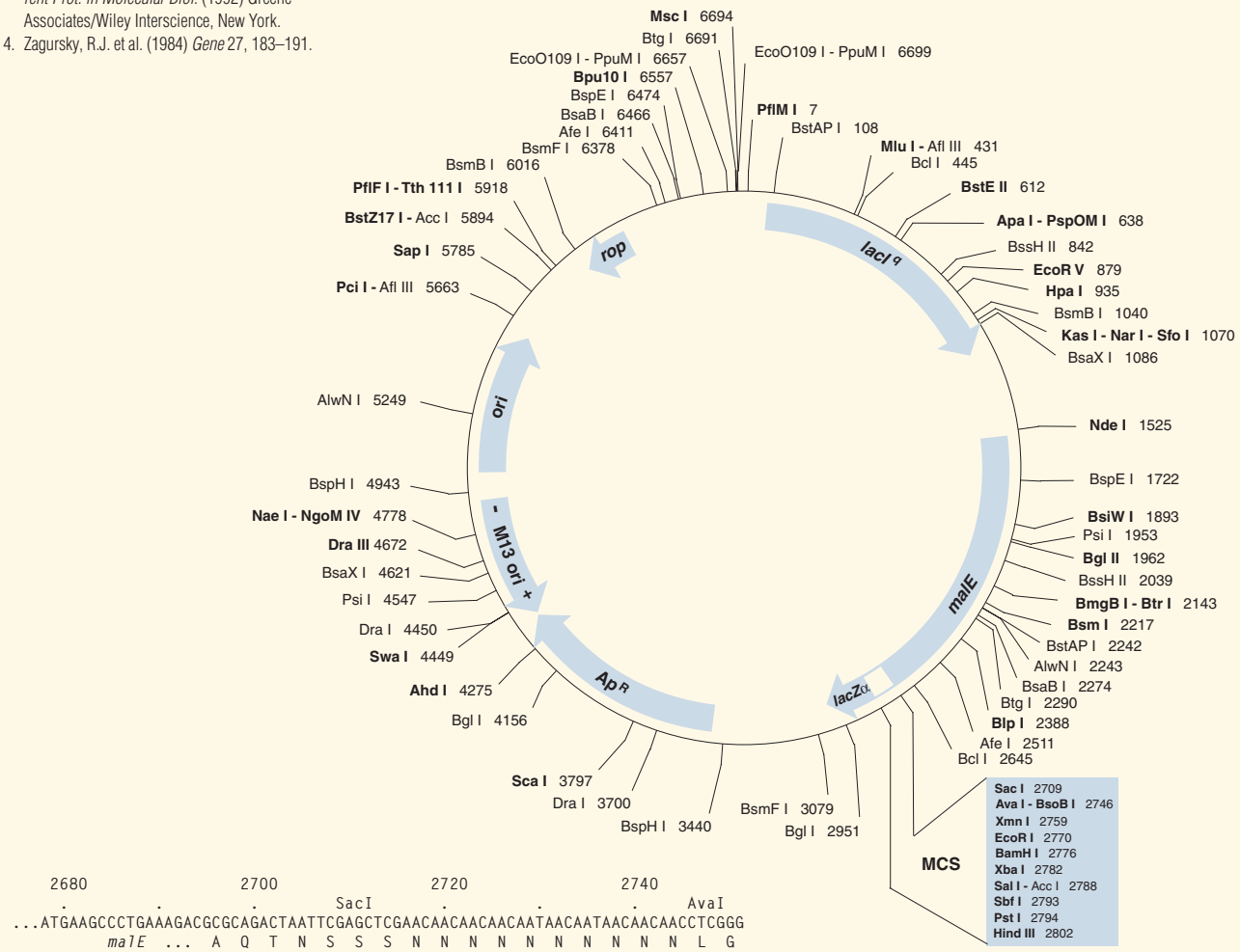
pMAL-p2E and pMAL-p2G are identical to pMAL-p2X except they replace the Factor Xa protease cleavage site with Enterokinase and Genenase I™ cleavage sites, respectively.

pMAL-c2-series vectors are identical to the pMAL-p2-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. The accompanying table shows restriction sites of those enzymes that cut a moderate number of times. 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.




```
2680              2700              2720              2740
 .        .        .       SacI     .        .        .     AvaI
...ATGAAGCCCTGAAAGACGCGCAGACTAATTCGAGCTCGAACAACAACAACAATAACAATAACAACAACCTCGGG
        malE ... A Q T N S S S N N N N N N N N N N N L G
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pMAL-p2X MCS

```
                    XmnI        EcoRI BamHI XbaI  SalI  PstI    HindIII
ATCGAGGGAAGG ATTTCAGAATTCGGATCCTCTAGAGTCGACCTGCAGGCAAGCTTGGCACTGGCCGTCGTT...
 I  E  G  R  I  S  E  F  G  S  S  R  V  D  L  Q  A  S  L  A  ...  lacZα
Factor Xa
```

pMAL-p2E 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
```

pMAL-p2G 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
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