**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.

Transcription of the gene fusion is controlled by the inducible "lac" promoter (Plac). Basal expression from Plac is minimized by the binding of the Lac repressor, encoded by the lacI gene, to the lac operator immediately downstream of Plac. A portion of the nMB operon containing two terminators, derived from the vector pKK233-2, prevents transcription originating from PnMB 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 Genemase 1 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 References > 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 (ciswaise) strand, regardless of the direction of transcription and include the start and stop codons.

Plasmid 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*) gene coordinates include the signal sequence.

Protein Fusion and Purification System (NEB #E8000) (1–3).

**References**