pMAL™-pIII

Sequence file available at www.neb.com.

Feature	Coordinates	Source
lacI^q	81-1163	*E. coli*
P_{tac}	1406-1433	–
expression ORF	1528-2976	–
pIII leader seq.	1528-1581	M13KE
malE	1591-2688	*E. coli*
MCS	2689-2794	–
lacZα	2795-2976	–
bla (Ap^R)	3478-4338	Tn3
M13 origin	4380-4893	M13
origin	5004-5592	pMB1
rop	6213-6022	pMB1

There are no restriction sites for the following enzymes: AarI(x), AatII, AflII, AgeI, AleI, AscI, AsiSI, AvrII, BaeI, BbvCI, BglII, BmtI, BseRI, BspDI, BsrGI, BstBI, Bsu36I, ClaI, CspCI, EcoNI, FseI, I-CeuI, I-SceI, MfeI, NcoI, NheI, NotI, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PciI, PmeI, PmlI, PshAI, PspXI, RsrII, SacII, 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pMAL-pIII is a derivative of pMAL-p2X in which the *malE* leader signal sequence has been replaced by the gene pIII leader sequence from M13KE. It is designed for cloning selected peptide sequences isolated using any of the Ph.D. Phage Display libraries (NEB #E8100, #E8110 or #E8120) as N-terminal fusions to maltose binding protein (MBP, encoded by the *malE* gene).

pIII-displayed peptide sequences can be subcloned from M13KE to pMAL-pIII using the Acc65I/KpnI and EagI cloning sequences common to both vectors. In pMAL-pIII, these sequences are expressed as translational fusions to the N-terminus of MBP. The pIII leader sequence upstream of the cloning sites directs the fusion protein to the periplasm and is cleaved off during secretion. Expression as MBP fusion allows examination of peptide properties in a monovalent context and in the absence of downstream phage-encoded amino acids from pIII itself (1).

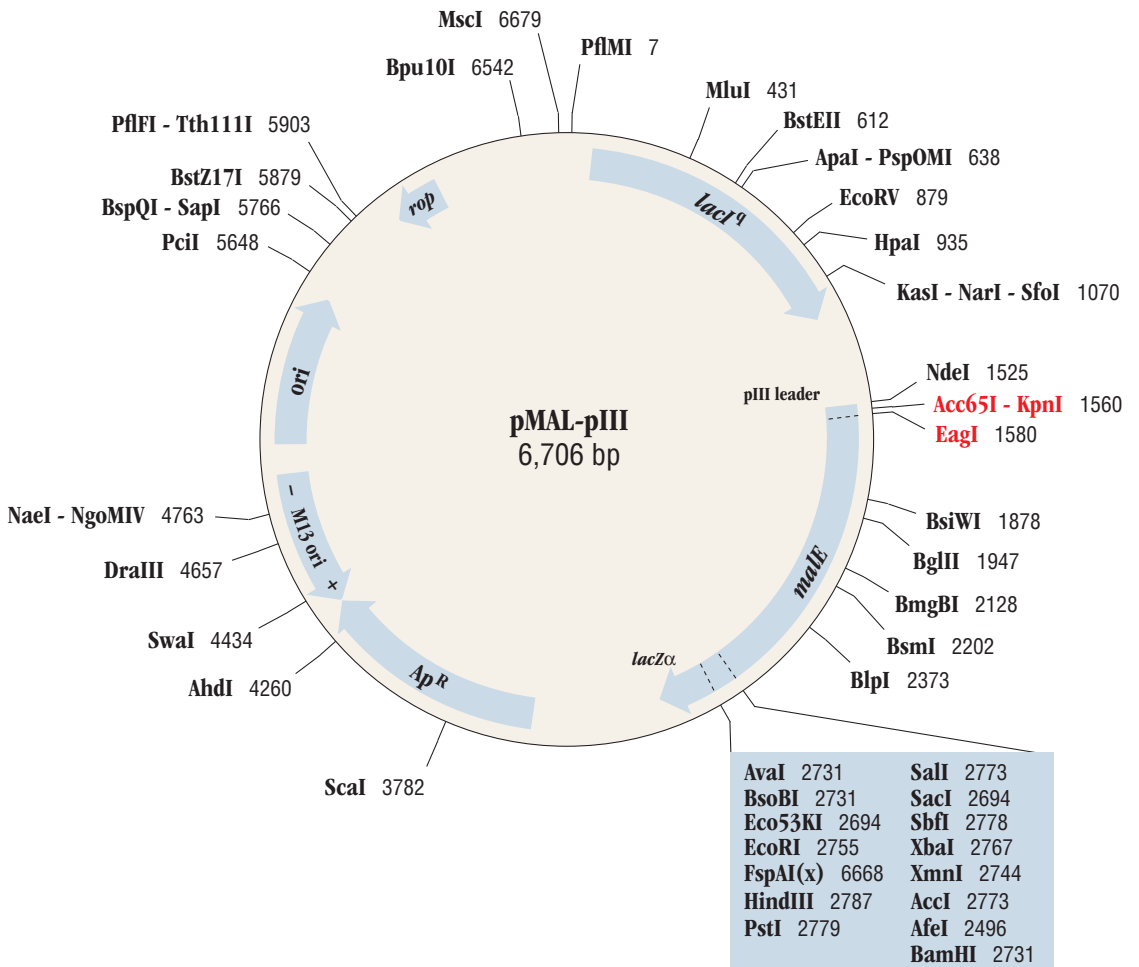
Transcription of the peptide:MBP fusion is controlled by the inducible "tac" promoter (P_{tac}). Basal expression from P_{tac} is minimized by the binding of the Lac repressor, encoded by the *lacI*^{q} gene, to the lac operator immediately downstream of P_{tac}. A portion of the *rrnB* operon containing two terminators, derived from the vector pKK233-2, prevents transcription originating from P_{tac} from interfering with plasmid functions.

pMAL-pIII contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, it contains an M13 origin of replication (2). The multiple cloning site (MCS) from pMAL-p2X is present in pMAL-pIII but is not used.

Enzymes with unique restriction sites are shown in **bold** 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. Components genes or regions of fusion ORFs are indented below the ORF itself.

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.



References

(1) Zwick, M.B. et al. (1998) *Anal. Biochem.,* 264, 87–97.

(2) Zagursky, R.J. et al. (1984) *Gene,* 27, 183–91.