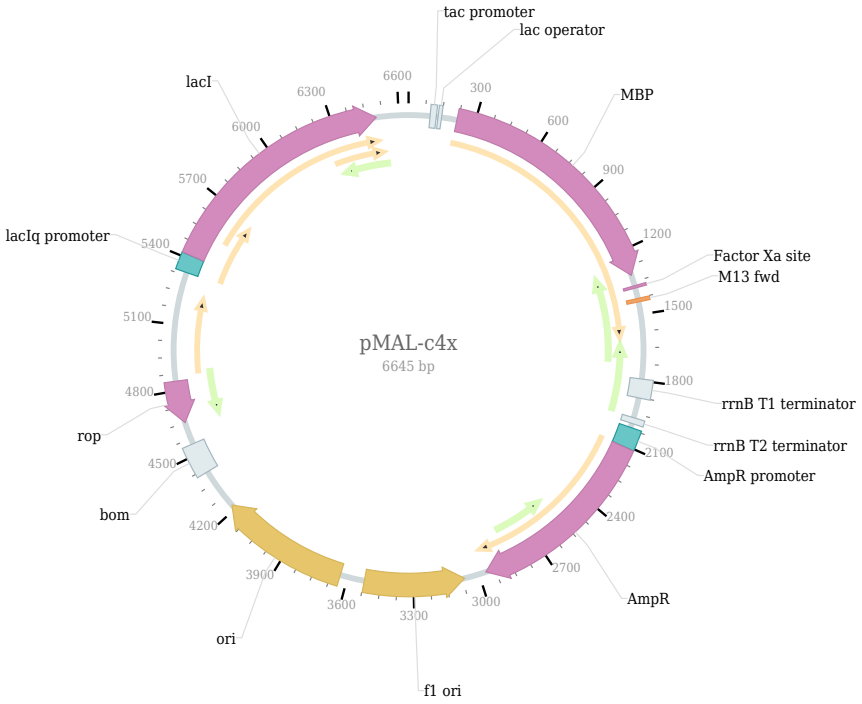
pMAL-c4x

载体编号 Vector ID	V006797
抗性 Antibiotic Resistance	Ampicillin
长度 Length	6645 bp
载体类型 Vector Type	E. coli Expression Vectors

产品信息

复制子 Replication Origin	ori
拷贝数 Copy Number	High copy number
启动子 Promoter	Tac
克隆方法 Cloning Method	Enzyme digestion and ligation
5' 引物 5' Primer	MalE: 5'-GGTCGTCAGACTGTCGATGAAGCC-3'; MBP-F: 5'-gatgaagccctgaaagacgcgcag-3'
3' 引物 3' Primer	pBad-R: 5'-gatttaatctgtatcagg-3'; M13-F: 5'-TGTAAAACGACGGCCAGT-3'
融合标签 Fusion Tag	N-MBP, N-Factor Xa
备注 Comment	The pMAL-4 vectors (Figure 1) provide a method for expressing and purifying a protein produced from a cloned gene or open reading frame. The cloned gene is inserted downstream from the malE gene of E. coli, which encodes maltose-binding protein (MBP), resulting in the expression of an MBP fusion protein (1,2). The MBP in these vectors has been engineered for tighter binding to amylose. The method uses the strong “tac” promoter and the malE translation initiation signals to give high-level expression of the cloned sequences (3,4), and a one-step purification of the fusion protein using MBP’s affinity for maltose (5). The vectors express the malE gene (with or without its signal sequence) fused to the lacZα gene. Restriction sites between malE and lacZα are available for inserting the coding sequence of interest. Insertion inactivates the β-galactosidase α-fragment activity of the malE-lacZα fusion, which results in a blue to white color change on Xgal plates when the construction is transformed into an α-complementing host such as TB1, JM107 or NEB 5-alpha Competent E. coli (6,7). When present, the signal peptide on pre-MBP directs fusion proteins to the periplasm. For fusion proteins that can be successfully exported, this allows folding and disulfide bond formation to take place in the periplasm of E. coli, as well as allowing purification of the protein from the periplasm (8). The vectors carry the lacIq gene, which codes for the Lac repressor. This keeps expression from Ptac low in the absence of IPTG induction. The pMAL-4 vectors also contain the sequence coding for the recognition site of a specific protease, located just 5´ to the polylinker insertion sites. This allows MBP to be cleaved from the protein of interest after purification. The pMAL-c4X and pMAL-p4X vectors that are included in the system encode the site for Factor Xa (9, 10). Factor Xa cleaves after its four amino acid recognition sequence, so that few or no vector-derived residues are attached to the protein of interest, depending on the site used for cloning. pMAL vectors containing sites for alternative proteases are also available (Figure 1). The vectors pMAL-c4G (NEB #N8106) and pMAL-p4G (NEB #N8103) encode the site for GenenaseI (NEB #P8075), which cleaves following the sequence His-Tyr. The vectors pMAL-c4E (NEB #N8105) and pMAL-p4E (NEB #N8102) encode the site for Enterokinase (NEB #P8070), which cleaves following the sequence Asp-Asp-Asp-Asp-Lys.

质粒图谱



复溶方法

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.