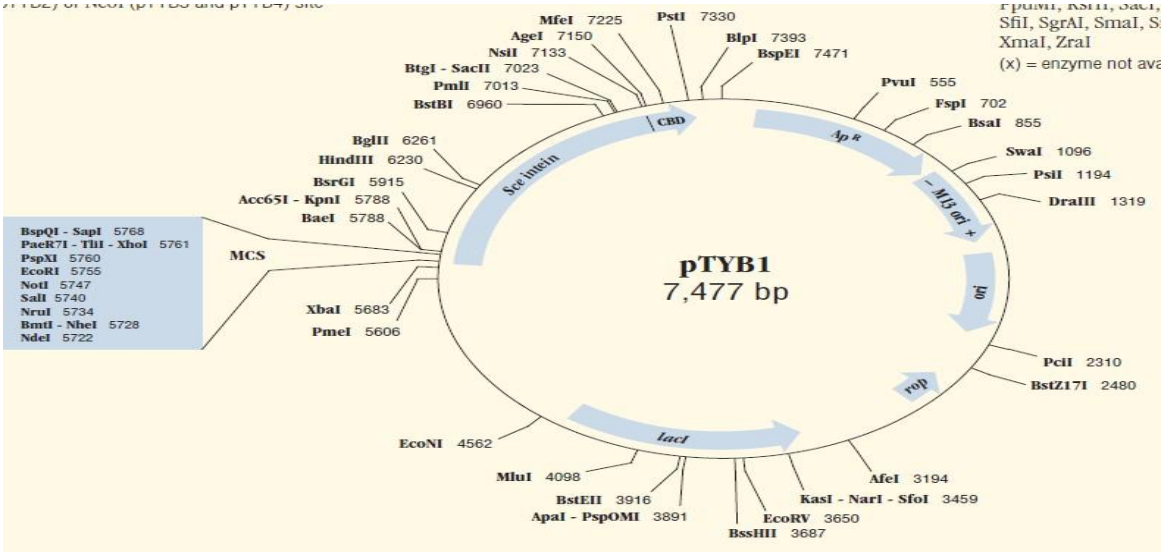
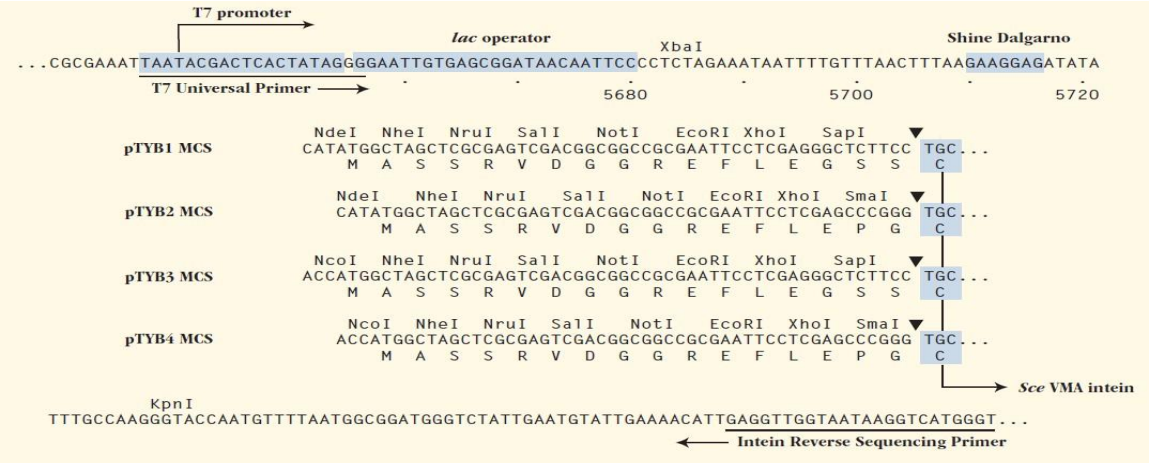
载体基本信息

平台编号	bio-136935
载体名称:	pTYB1
质粒类型:	大肠杆菌表达载体；蛋白纯化
高拷贝/低拷贝:	--
启动子:	T7
克隆方法:	多克隆位点，限制性内切酶
载体大小:	7477 bp
5' 测序引物及序列:	T7: TAATACGACTCACTATAGGG
3' 测序引物及序列:	--
载体标签:	Intein
载体抗性:	Ampicillin
筛选标记:	--
备注:	--
稳定性:	--
组成型:	--
病毒/非病毒:	非病毒

载体质粒图谱和多克隆位点信息





Feature	Coordinates	Source
bla (Ap^R)	140-1000	*Tn3*
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
rop	2814-2623	pMB1
lacI	4453-3371	*E. coli*
T7 promoter	5637-5654	T7
expression ORF	5725-7329	—
MCS	5722-5775	—
Sce VMA intein	5776-7137	*S. cerevisiae*
CBD	7171-7329	*B. circulans*

ori = origin of replication
Ap = ampicillin

载体简介

pTYB1 is an E. coli plasmid cloning vector designed for recombinant protein expression and purification using the IMPACT? Kit (NEB #E6901) (1,2). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTYB1 also contains an M13 origin of replication.
The multiple cloning site (MCS) is positioned to allow translational fusion of the Sce VMA intein tag to the C-terminus
of the cloned target protein (1). The chitin binding domain (CBD) from B. circulans, fused to the C-terminus of the intein,

facilitates purification of the intein-target protein precursor.
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring E. coli strains containing integrated
copies of the T7 RNA polymerase gene [e.g., C2566, C2833 or BL21(DE3)] for expression. Basal expression from the T7 promoter
is minimized by the binding of the Lac repressor, encoded by the lacI gene, to the lac operator immediately downstream of the T7 promoter (3). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the
strongly expressed T7 gene 10 protein (φ10).
pTYB1, pTYB2, pTYB3, and pTYB4 are identical except for the MCS regions (opposite page). All four vectors contain either an
NdeI (pTYB1 and pTYB2) or NcoI (pTYB3 and pTYB4) siteoverlapping the initiating methionine codon of the intein fusion gene.
The N-terminal cysteine residue (“Cys1”) of the intein is shaded.
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. Component
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 (ApR) gene coordinates include the signal sequence.

载体序列

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LOCUS       pTYB1  7477 bp  DNA      SYN

DEFINITION  pTYB1

ACCESSION

KEYWORDS

SOURCE

  ORGANISM  other sequences; artificial sequences; vectors.

FEATURES             Location/Qualifiers

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3901 ACAGCGCGAT TTGCTGGTGA CCCAATGCGA CCAGATGCTC CACGCCCAGT CGCGTACCGT

3961 CTTCATGGGA GAAAATAATA CTGTTGATGG GTGTCTGGTC AGAGACATCA AGAAATAACG

4021 CCGGAACATT AGTGCAGGCA GCTTCCACAG CAATGGCATC CTGGTCATCC AGCGGATAGT

4081 TAATGATCAG CCCACTGACG CGTTGCGCGA GAAGATTGTG CACCGCCGCT TTACAGGCTT

4141 CGACGCCGCT TCGTTCTACC ATCGACACCA CCACGCTGGC ACCCAGTTGA TCGGCGCGAG

4201 ATTTAATCGC CGCGACAATT TGCGACGGCG CGTGCAGGGC CAGACTGGAG GTGGCAACGC

4261 CAATCAGCAA CGACTGTTTG CCCGCCAGTT GTTGTGCCAC GCGGTTGGGA ATGTAATTCA

4321 GCTCCGCCAT CGCCGCTTCC ACTTTTTCCC GCGTTTTCGC AGAAACGTGG CTGGCCTGGT

4381 TCACCACGCG GGAAACGGTC TGATAAGAGA CACCGGCATA CTCTGCGACA TCGTATAACG

4441 TTACTGGTTT CACATTCACC ACCCTGAATT GACTCTCTTC CGGGCGCTAT CATGCCATAC

4501 CGCGAAAGGT TTTGCGCCAT TCGATGGTGT CCGGGATCTC GACGCTCTCC CTTATGCGAC

4561 TCCTGCATTA GGAAGCAGCC CAGTAGTAGG TTGAGGCCGT TGAGCACCGC CGCCGCAAGG

4621 AATGGTGCAT GCCGGCATGC CGCCCTTTCG TCTTCAAGAA TTAATTCCCA ATTCCCCAGG

4681 CATCAAATAA AACGAAAGGC TCAGTCGAAA GACTGGGCCT TTCGTTTTAT CTGTTGTTTG

4741 TCGGTGAACG CTCTCCTGAG TAGGACAAAT CCGCCGGGAG CGGATTTGAA CGTTGCGAAG

4801 CAACGGCCCG GAGGGTGGCG GGCAGGACGC CCGCCATAAA CTGCCAGGAA TTAATTCCCC

4861 AGGCATCAAA TAAAACGAAA GGCTCAGTCG AAAGACTGGG CCTTTCGTTT TATCTGTTGT

4921 TTGTCGGTGA ACGCTCTCCT GAGTAGGACA AATCCGCCGG GAGCGGATTT GAACGTTGCG

4981 AAGCAACGGC CCGGAGGGTG GCGGGCAGGA CGCCCGCCAT AAACTGCCAG GAATTAATTC

5041 CCCAGGCATC AAATAAAACG AAAGGCTCAG TCGAAAGACT GGGCCTTTCG TTTTATCTGT

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5161 GCGAAGCAAC GGCCCGGAGG GTGGCGGGCA GGACGCCCGC CATAAACTGC CAGGAATTAA

5221 TTCCCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT TCGTTTTATC

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5341 GTTGCGAAGC AACGGCCCGG AGGGTGGCGG GCAGGACGCC CGCCATAAAC TGCCAGGAAT

5401 TAATTCCCCA GGCATCAAAT AAAACGAAAG GCTCAGTCGA AAGACTGGGC CTTTCGTTTT

5461 ATCTGTTGTT TGTCGGTGAA CGCTCTCCTG AGTAGGACAA ATCCGCCGGG AGCGGATTTG

5521 AACGTTGCGA AGCAACGGCC CGGAGGGTGG CGGGCAGGAC GCCCGCCATA AACTGCCAGG

5581 AATTGGGGAT CGGAATTAAT TCCCGGTTTA AACCGGGGAT CTCGATCCCG CGAAATTAAT

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5701 TAACTTTAAG AAGGAGATAT ACATATGGCT AGCTCGCGAG TCGACGGCGG CCGCGAATTC

5761 CTCGAGGGCT CTTCCTGCTT TGCCAAGGGT ACCAATGTTT TAATGGCGGA TGGGTCTATT

5821 GAATGTATTG AAAACATTGA GGTTGGTAAT AAGGTCATGG GTAAAGATGG CAGACCTCGT

5881 GAGGTAATTA AATTGCCCAG AGGAAGAGAA ACTATGTACA GCGTCGTGCA GAAAAGTCAG

5941 CACAGAGCCC ACAAAAGTGA CTCAAGTCGT GAAGTGCCAG AATTACTCAA GTTTACGTGT

6001 AATGCGACCC ATGAGTTGGT TGTTAGAACA CCTCGTAGTG TCCGCCGTTT GTCTCGTACC

6061 ATTAAGGGTG TCGAATATTT TGAAGTTATT ACTTTTGAGA TGGGCCAAAA GAAAGCCCCC

6121 GACGGTAGAA TTGTTGAGCT TGTCAAGGAA GTTTCAAAGA GCTACCCAAT ATCTGAGGGG

6181 CCTGAGAGAG CCAACGAATT AGTAGAATCC TATAGAAAGG CTTCAAATAA AGCTTATTTT

6241 GAGTGGACTA TTGAGGCCAG AGATCTTTCT CTGTTGGGTT CCCATGTTCG TAAAGCTACC

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 6421 ATTGGTGATG GATTGTCTGA CAGGGCAACT TTTTCGGTTG ATTCCAGAGA TACTTCTTTG

 6481 ATGGAACGTG TTACTGAATA TGCTGAAAAG TTGAATTTGT GCGCCGAGTA TAAGGACAGA

 6541 AAAGAACCAC AAGTTGCCAA AACTGTTAAT TTGTACTCTA AAGTTGTCAG AGGTAATGGT

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 6721 GAAACATTTC TTGCTGGTCT AATTGATTCT GATGGCTATG TTACTGATGA GCATGGTATT

 6781 AAAGCAACAA TAAAGACAAT TCATACTTCT GTCAGAGATG GTTTGGTTTC CCTTGCTCGT

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 7321 CTTCAATGAC TGCAGGAAGG GGATCCGGCT GCTAACAAAG CCCGAAAGGA AGCTGAGTTG

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