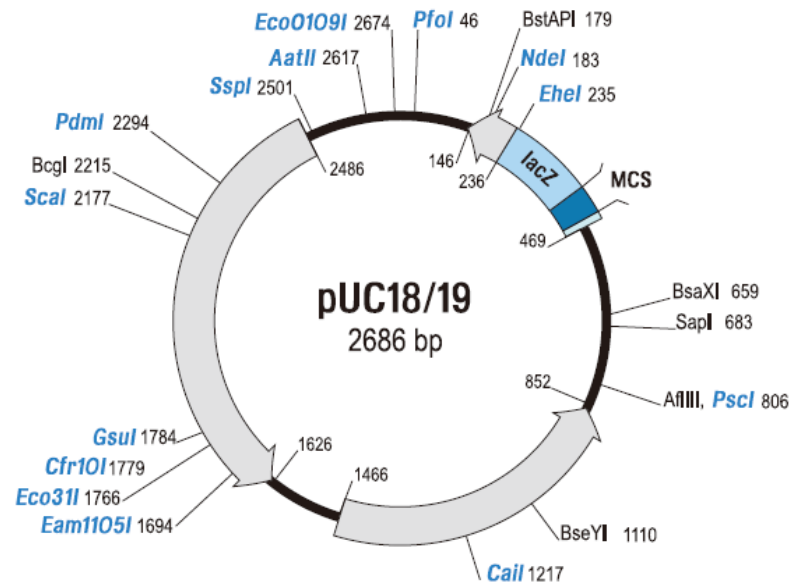


pUC18/19 Vector Map



mp7/pUC7 6163	TCGTATGTTGTGGAAATTGTGAGCGGATACAAATTTACACAGGAACACAGCTATGACCATGATTACGAATTCCTCGGATCCGACCTGCAGGTCGACGGATCCGGGGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCG MetThrMet11eThrAsnSerProAspProSerThrCysArgSerThrAspProGlyAsnSerLeuAlaVal1Val1LeuGlnArgArgAspTrpGluAsnProGly → LacZ
mp8/pUC8 6163	TCGTATGTTGTGGAAATTGTGAGCGGATACAAATTTACACAGGAACACAGCTATGACCATGATTACGAATTCCTCGGATCCGACCTGCAGGTCGACGGATCCCGGGAAATTCACCTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCG MetThrMet11eThrAsnSerArgGlySerVal1AspLeuGlnProSerLeuAlaLeuAlaVal1Val1LeuGlnArgArgAspTrpGluAsnProGly → LacZ
mp9/pUC9 6534	TCGTATGTTGTGGAAATTGTGAGCGGATACAAATTTACACAGGAACACAGCTATGACCATGATTACGCCAAGCTTGCGTGCAGGTCGACGGATCCCGGGGAATTCACCTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCG MetThrMet11eThrProSerLeuAla1a1aGlyArgArg11eProGlyAsnSerLeuAlaVal1Val1LeuGlnArgArgAspTrpGluAsnProGly → LacZ
mp10/pUC12 6164	TCGTATGTTGTGGAAATTGTGAGCGGATACAAATTTACACAGGAACACAGCTATGACCATGATTACGAATTCGAGCTCGCCCGGGGATCCCTCTAGAGTCGACCTGCAGCCCAAGCTTGGCACCTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCG MetThrMet11eThrAsnSerSerSerProGlyAspProLeuGluSerThrCysSerProSerLeuAlaLeuAlaVal1Val1LeuGlnArgArgAspTrpGluAsnProGly → LacZ
mp11/pUC13 6164	TCGTATGTTGTGGAAATTGTGAGCGGATACAAATTTACACAGGAACACAGCTATGACCATGATTACGCCAAGCTTG66CTGCAAGCTCGACTCTAGAGGATCCCGGGCAGGCTCGAATTCACCTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCG MetThrMet11eThrProSerLeuGlyCysArgSerThrLeuGluAspProArgAlaSerSerAsnSerLeuAlaVal1Val1LeuGlnArgArgAspTrpGluAsnProGly → LacZ
mp18/pUC18 6164	TCGTATGTTGTGGAAATTGTGAGCGGATACAAATTTACACAGGAACACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCCTCTAGAGTCGACCTGCAGGGCATGCAAGCTTGGCACCTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCG MetThrMet11eThrAsnSerSerSerVal1ProGlyAspProLeuGluSerThrCysArgHisAlaSerLeuAlaLeuAlaVal1Val1LeuGlnArgArgAspTrpGluAsnProGly → LacZ
mp19/pUC19 6164	TCGTATGTTGTGGAAATTGTGAGCGGATACAAATTTACACAGGAACACAGCTATGACCATGATTACGCCAAGCTTGCAATGCCTGCAGGTCGACTCTAGAGGATCCCGGGTACGAGCTCGAATTCACCTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCCTGGCG MetThrMet11eThrProSerLeuHisAla1aCysArgSerThrLeuGluAspProArgVal1ProSerSerAsnSerLeuAlaVal1Val1LeuGlnArgArgAspTrpGluAsnProGly → LacZ

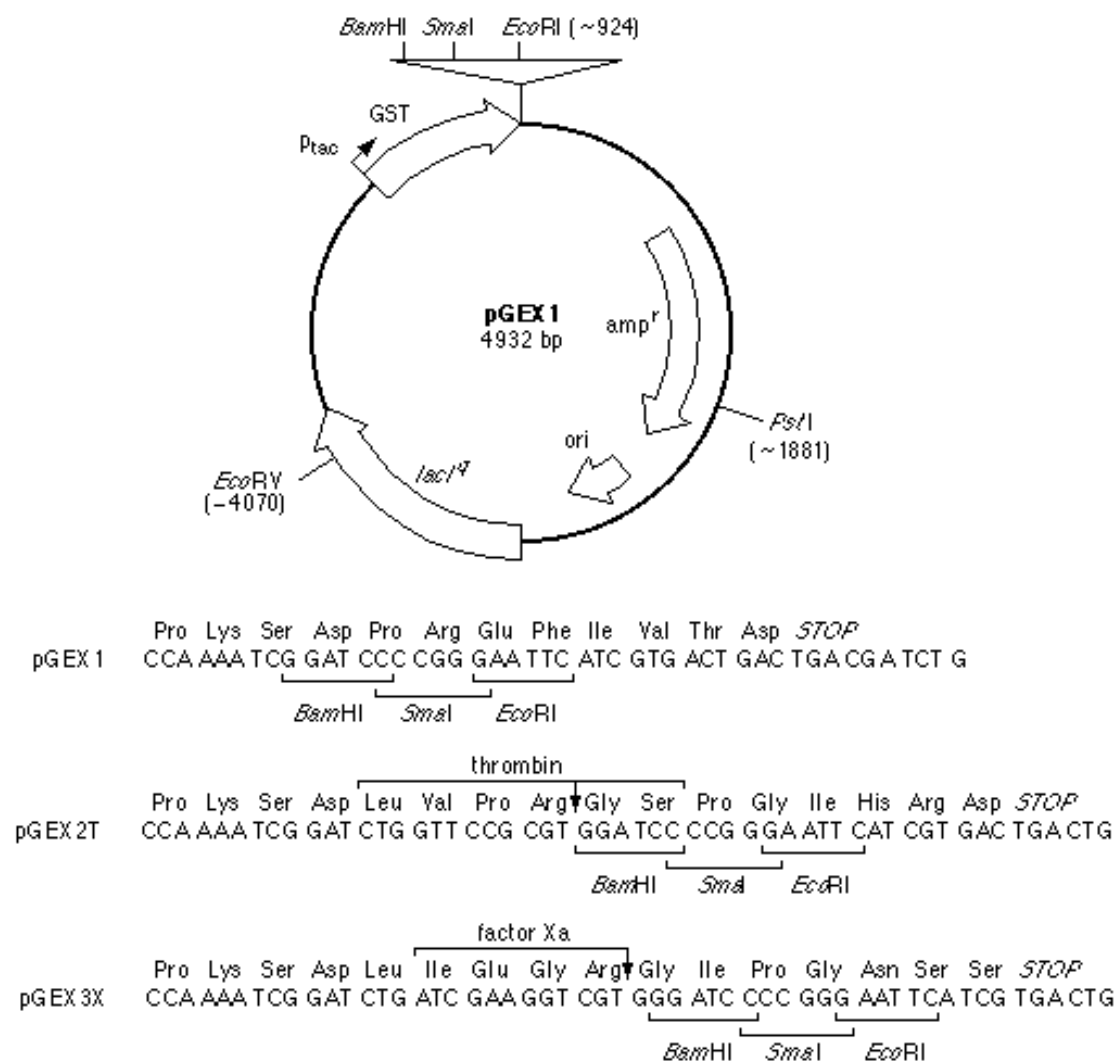
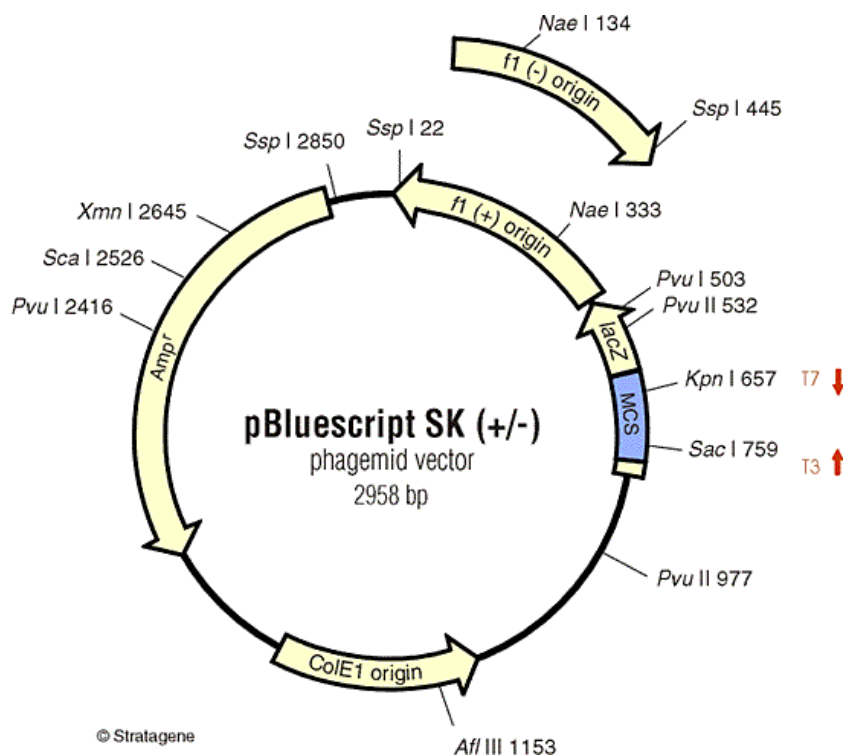


Figure 16.7.1 pGEX1. pGEX1 is a fusion-protein expression vector that expresses a cloned gene as a fusion protein to GST. The *lac* repressor (product of the *lacI* gene) binds to the p_{lac} promoter, repressing the expression of GST fusion protein. Upon induction with IPTG, derepression occurs and GST fusion protein is expressed. The gene of interest can be inserted into the polylinker located at the end of the GST gene. The polylinker sequences are shown below the map of pGEX1, where the restriction endonuclease cloning sites are bracketed. The polylinker of pGEX2T and pGEX3X contains protease cleavage sites so the cloned protein can be released from the GST moiety (or "carrier"). The recognition sequences for thrombin and factor Xa are bracketed above the polylinker sequences, with the actual cleavage site between Arg and Gly. The nucleotide sequence of pGEX1 is available under Genbank accession number M21676.



pBluescript®SK (+/-) phagemid

The pBluescript® SK (+/-) phagemid is a 2958-bp phagemid derived from pUC19. The SK designation indicates the polylinker is oriented such that *lacZ* transcription proceeds from *Sac* I to *Kpn* I.

f1 (+) origin: (6-462 bp) f1 filamentous phage origin of replication allowing recovery of the sense strand of the *lacZ* gene when a host strain containing the pBluescript phagemid is co-infected with helper phage.

f1 (-) origin: (6-462 bp) f1 filamentous phage origin of replication allowing recovery of the antisense strand of the *lacZ* gene when a host strain containing the pBluescript phagemid is co-infected with helper phage.

ColE1 origin: (1032-1912 bp) Plasmid origin of replication used in the absence of helper phage.

***lacZ* gene:** (*lac* promoter: 816-938 bp) This portion of the *lacZ* gene provides α -complementation for blue/white color selection of recombinant phagemids. An inducible *lac* promoter upstream from the *lacZ* gene permits fusion protein expression with the β -galactosidase gene product.

MCS: (657-759 bp) Multiple cloning site flanked by T3 and T7 RNA promoters (please see the polylinker sequence below).

Ampicillin: (1975-2832 bp) Ampicillin-resistance gene (*Amp^r*) for antibiotic selection of the phagemid vector.

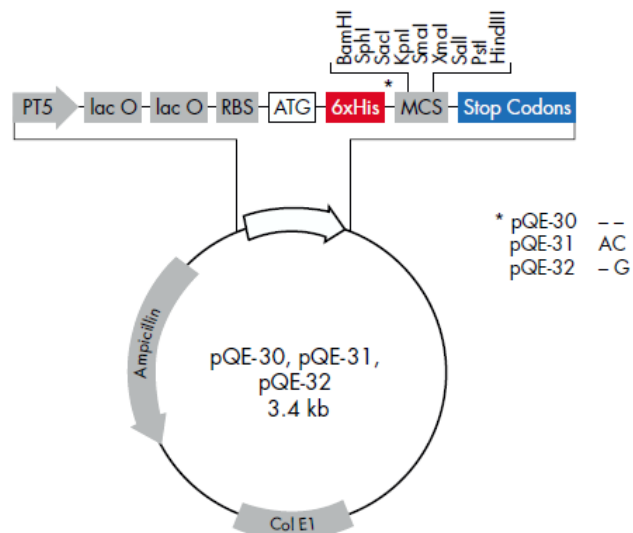
Please Note: The upper strand is designated the (+) strand and the lower strand is designated the (-) strand.

GenBank® # 52325 [SK(+)] & #52324 [SK(-)]

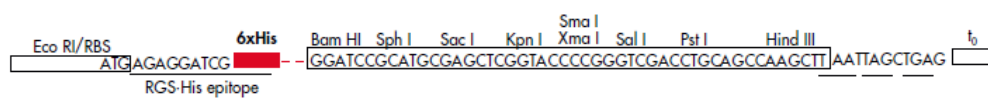
MET
 5' GGAAACAGCTATGACCATTAGCCGAAGCTCGAAATTAACCTCTCACTAMAGGGAACAAGCTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACCTAGTGGATCCCGGGGTGCAGG
 3' CCTTTGTCTGATACTGGTACTAATGCGGTTCGAGCTTAAATGGGAGTGAATTCGCTGTTTTCGAGCTCGAGGTGGCCCACTCCCGGCGAGATCTTGATCACCCTAGGGGGCCGACGCTCTTAA
 816
 β-Galactosidase →
 T3 promoter +1 →
 BstXI SacI NotI EcoRI
 SacII XbaI SpeI BamHI PstI
 EcoRV HindIII
 AATTCGATATCAAGCTTTATCGATACCGTTCGACTTGGAGGGGGGGCCGGTACCCCAATTGCGCCTCTAGTGGCTGTATTCAGTAATCACTGGCCGCTGTTTACAA 3' (+)
 GCTATAGTTCGAATAGCTATGGCAGCTGGAGCTCCCGCGGCCATGGGTAAAGGGGATATCACTTCAGCATATCTTAAGTGAACGGCAGCAAAATGTT 5' (-)
 759 A/A
 Eco109I
 Hinc II
 Cla I
 Acc I
 Sal I
 Xba I
 Kpn I
 +1 T7 promoter
 657

pQE-30, pQE-31, and pQE-32 Vectors

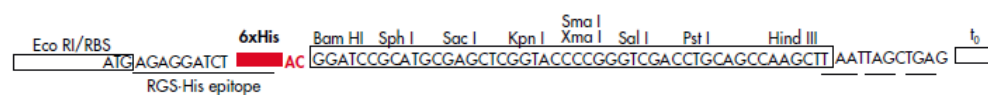
Positions of elements in bases	pQE-30	pQE-31	pQE-32
Vector size (bp)	3461	3463	3462
Start of numbering at <i>Xho</i> I (CTCGAG)	1–6	1–6	1–6
T5 promoter/lac operator element	7–87	7–87	7–87
T5 transcription start	61	61	61
6xHis-tag coding sequence	127–144	127–144	127–144
Multiple cloning site	145–192	147–194	146–193
Lambda <i>t</i> _o transcriptional termination region	208–302	210–304	209–303
<i>rrnB</i> T1 transcriptional termination region	1064–1162	1066–1164	1065–1163
ColE1 origin of replication	1638	1640	1639
β-lactamase coding sequence	3256–2396	3258–2398	3257–2397



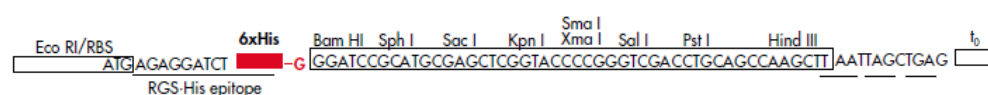
pQE-30



pQE-31



pQE-32



pET-28a-c(+) Vectors

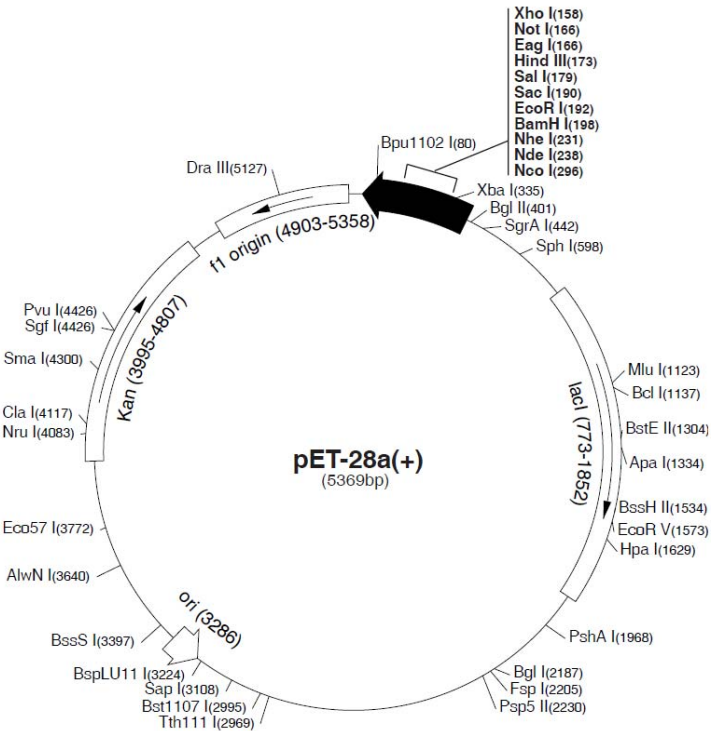
TB074 12/98

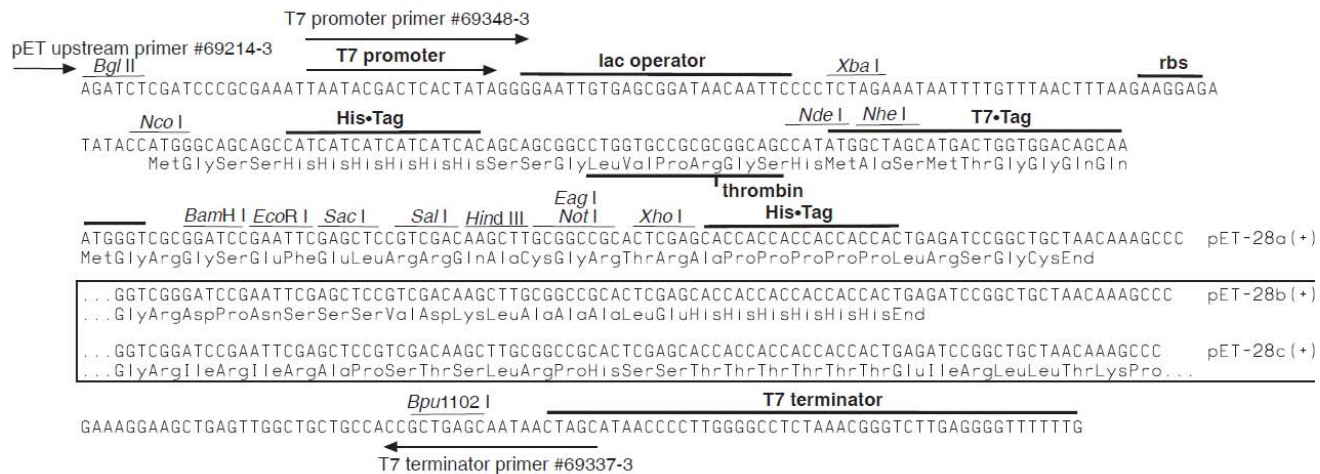
	Cat. No.
pET-28a DNA	69864-3
pET-28b DNA	69865-3
pET-28c DNA	69866-3

The pET-28a-c(+) vectors carry an N-terminal His•Tag®/thrombin/T7•Tag® configuration plus an optional C-terminal His•Tag sequence. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer (Cat. No. 69337-3).

pET-28a(+) sequence landmarks	
T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites (<i>Bam</i> HI - <i>Xho</i> I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lac</i> I coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*HI at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*HI at 198.





pET-28a-c(+) cloning/expression region

pET-32a-c(+) Vectors

	Cat. No.
pET-32a DNA	69015-3
pET-32b DNA	69016-3
pET-32c DNA	69017-3

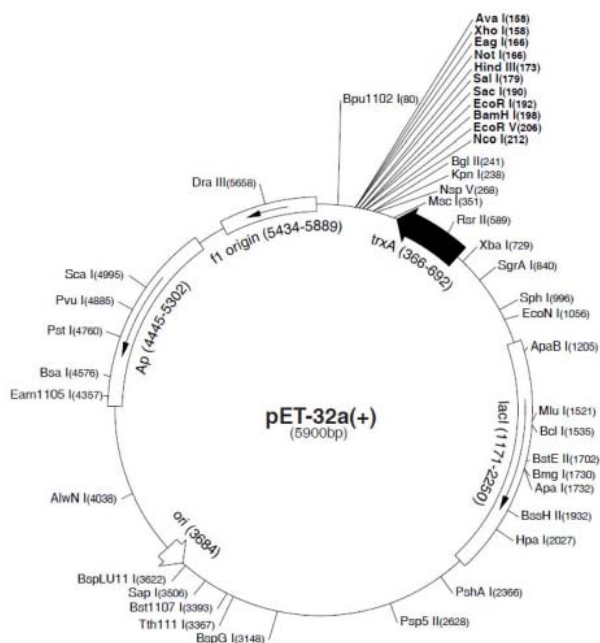
pET-32a(+) sequence landmarks

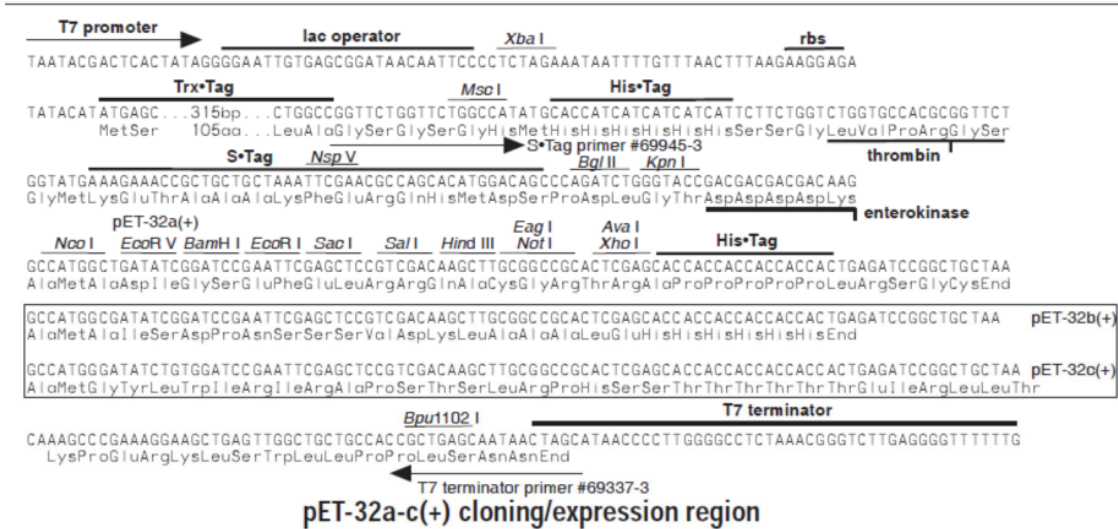
T7 promoter	764-780
T7 transcription start	763
Trx•Tag coding sequence	366-692
His•Tag coding sequence	327-344
S•Tag coding sequence	249-293
Multiple cloning sites (<i>Nco</i> I - <i>Xho</i> I)	158-217
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lacI</i> coding sequence	1171-2250
pBR322 origin	3684
<i>bla</i> coding sequence	4445-5302
f1 origin	5434-5889

The maps for pET-32b(+) and pET-32c(+) are the same as pET-32a(+) (shown) with the following exceptions: pET-32b(+) is a 5899bp plasmid; subtract 1bp from each site beyond *Bam*HI at 198. pET-32c(+) is a 5901bp plasmid; add 1bp to each site beyond *Bam*HI at 198 except for *Eco*R V, which cuts at 209.

The pET-32 series is designed for cloning and high-level expression of peptide sequences fused with the 109aa Trx•Tag™ thioredoxin protein (1). Cloning sites are available for producing fusion proteins also containing cleavable His•Tag™ and S•Tag™ sequences for detection and purification. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circle map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer (Cat. No. 69337-3).

1. LaVallie, E.R., DiBlasio, E.A., Kovacic, S., Grant, K.L., Schendel, P.F. and McCoy, J.M. (1993) *BioTechnology* 11, 187-193.





LISTA DE SITIOS PARA ENDONUCLEASAS DE RESTRICCIÓN de uso frecuente

Secuencia reconocida	Enzima	Secuencia reconocida	Enzima
GGGCC/C	Apal	CTGCA/G	PstI
C/YCGRG	Aval	CGAT/CG	PvuI
G/GWCC	Avall	CAG/CTG	PvuII
G/GATCC	BamHI	GAGCT/C	SacI
A/GATCT	BglII	CCGC/GG	SacII
AT/CGAT	ClaI	G/TCGAC	Sall
G/ATC	DpnI	/GATC	Sau3AI
/GATC	DpnII	CCC/GGG	SmaI
G/AATTC	EcoRI	A/CTAGT	SpeI
GAT/ATC	EcoRV	GCATG/C	SphI
RGCGC/Y	HaeII	AAT/ATT	SspI
GTY/RAC	HincII	T/CTAGA	XbaI
A/AGCTT	HindIII	C/TCGAG	XhoI
GTT/AAC	HpaI	C/CCGGG	XmaI
GGTAC/C	KpnI		
/GATC	MboI		
C/CATGG	NcoI		
CA/TATG	NdeI		
GC/GGCCGC	NotI		

• Código	• Ambigüedad
• N	• A,C,G,T
• M	• A,C
• R	• A,G
• W	• A,T
• S	• C,G
• Y	• C,T
• K	• G,T
• B	• C,G,T
• D	• A,G,T
• H	• A,C,T
• V	• A,C,G