

pET-32a-c(+) Vectors

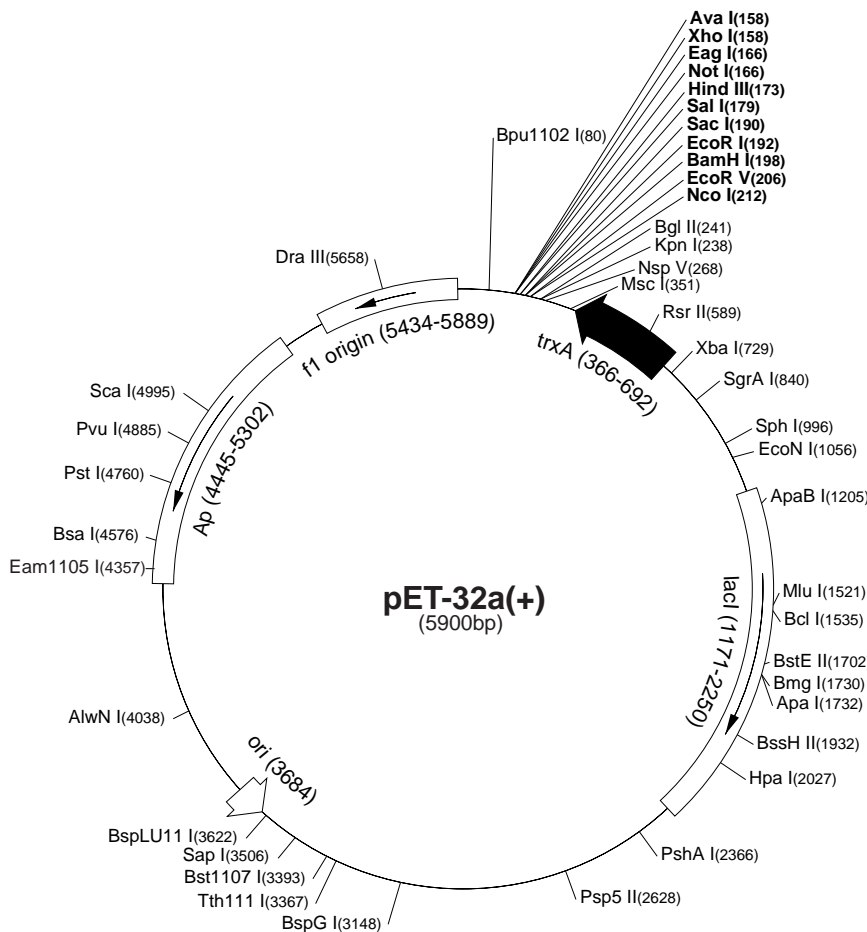
	Cat. No.
pET-32a DNA	69015-3
pET-32b DNA	69016-3
pET-32c DNA	69017-3

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) *Bio/Technology* **11**, 187-193.

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>lac</i> 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*H I at 198. pET-32c(+) is a 5901bp plasmid; add 1bp to each site beyond *Bam*H I at 198 except for *Eco*R V, which cuts at 209.



T7 promoter	lac operator	Xba I	rbs
TAATACGACTCACTATAGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAATTTTGTAACTTTAAGAAGGAGA			
	Trx•Tag	Msc I	His•Tag
TATACATATGAGC...315bp...CTGGCCGGTTCTGGTCTGGCCATATGCACCATCATCATCATCTTCTTGGTCTGGTGCACCGGTTCT			
MetSer...105aa...LeuAlaGlySerGlySerGlyHisMethHisHisHisHisHisHisHisHisSerSerGlyLeuValProArgGlySer			
	S•Tag	Nsp V	thrombin I
GGTATGAAGA AACCCCTGCTGCTAAATTCGAACGCCAGCACATGGACAGCCAGATCTGGGTACCGACGACGACGACAAG			
GlyMetLysGluThrAlaIaIaLysPheGluArgGlnHisMetAspSerProAspLeuGlyThrAspAspAspAspLys			
	pET-32a(+)	Eag I	enterokinase
Nco I	EcoR V	BamH I	EcoR I
GCCATGGCTGATATCGGATCCGAATTCGAGCTCCGTCGACAAAGCTTGGCGCCGACTCGAGCACCACCACCACCACCCTGAGATCCGGCTGCTAA			
AlaMetAlaAspIleGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProLeuArgSerGlyCysEnd			
GCCATGGCGATATCGGATCCGAATTCGAGCTCCGTCGACAAAGCTTGGCGCCGACTCGAGCACCACCACCACCACCCTGAGATCCGGCTGCTAA			
AlaMetAlaIleSerAspProAsnSerSerSerValAspLysLeuAlaIaIaLeuGluHisHisHisHisHisHisHisHisEnd			
GCCATGGGATATCTGTGGATCCGAATTCGAGCTCCGTCGACAAAGCTTGGCGCCGACTCGAGCACCACCACCACCACCCTGAGATCCGGCTGCTAA			
AlaMetGlyTyrLeuTrpIleArgIleArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrThrThrGluIleArgLeuLeuThr			
	Bpu1102 I	T7 terminator	
CAAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCCCTGAGCAATAACTAGCATAACCCCTTGGGGCTCTAAACCGGCTTGGAGGGTTTTTGTG			
LysProGluArgLysLeuSerTrpLeuLeuProProLeuSerAsnAsnEnd			
	T7 terminator primer #69337-3		

pET-32a-c(+) cloning/expression region

pET-32a(+) Restriction Sites

Enzyme	# Sites	Locations
AccI	2	180 3392
AccIII	8	1288 2016 2347 3131 3272 3574 4814 5498
Acil	85	
AflIII	2	1521 3622
AluI	25	
AlwI	17	
Alw21I	10	159 190 414 1021 1505 2616 3440 3940 5101 5186
Alw44I	5	410 1501 3436 3936 5182
AlwNI	1	4038
ApaI	1	1732
ApaBI	1	1205
ApoI	5	192 270 1796 5460 5471
AvaI	1	158
Avall	8	589 2073 2449 2537 2628 2907 4653 4875
BamHI	1	198
BanI	11	
BanII	5	190 905 919 1732 5733
BbsI	4	1667 2006 2380 2740
BbvI	29	
BccI	14	
Bce83I	7	21 2335 2505 3713 4011 4252 5120
Bcefl	5	1040 1381 2008 4124 5684
Bcgl	14	
BclI	1	1535
Bfal	7	70 730 2636 4117 4370 4705 5809
BglI	2	2585 4635
BglII	1	241
BmgI	1	1730
BpmI	5	1359 1848 2482 3149 4585
Bpu10I	1	2728
Bpu1102I	1	80
BsaI	1	4576
BsaAI	2	3374 5658
BsaBI	3	794 804 2819
BsaHI	6	844 865 979 1478 2161 5052
BsaJI	9	57 212 469 496 958 964 2156 2594 3782
BsaWI	7	2 1840 2343 2811 3828 3975 4806
BsaXI	2	2180 5606
Bsbl	2	3338 5565
BscGI	14	
Bsgl	3	1372 1572 2782
Bsil	2	3795 5179
BsiEI	6	169 2306 3538 3962 4885 5034
BsII	25	
BsmAI	7	1218 1623 1749 2136 3263 4576 5352
BsmBI	2	2136 3263
BsmFI	4	982 2523 2893 5873
BsoFI	50	
Bsp24I	10	811 843 1362 1394 1664 1696 4115 4147 4293 4325
Bsp1286I	14	
BspEI	2	2 2811
BspGI	1	3148
BspLU11I	1	3622
BsrI	25	
BsrBI	4	750 3555 5356 5802
BsrDI	4	1568 1934 4576 4750
BsrFI	8	363 831 840 1207 2419 2579 4595 5759
BssHII	1	1932
Bst1107I	1	3393
BstEII	1	1702

Enzyme	# Sites	Locations
BstXI	3	1323 1452 1575
BstYI	12	
CacBI	39	
CjeI	26	
CjePI	18	
Clal	2	508 798
CviJI	90	
CvIRI	28	
DdeI	11	
DpnI	32	
DraI	3	4381 4400 5092
DrallI	1	5658
DrdI	3	3315 3730 5613
DrdII	3	357 1244 5663
Dsal	4	212 469 958 2594
EaeI	7	166 349 365 829 961 2195 4903
EagI	1	166
Eam1105I	1	4515
EarI	3	1139 3506 5310
Ecil	4	1298 3696 3842 4670
Eco47III	3	926 2427 2876
Eco57I	2	4170 5182
EcoNI	1	1056
EcoO109I	3	53 954 2628
EcoRI	1	192
EcoRII	9	367 495 1244 1559 2099 2156 3648 3769 3782
EcoRV	1	206
FauI	17	
FokI	13	
FspI	2	2603 4737
GdIII	6	166 365 829 961 2195 4903
HaeI	7	217 351 1249 2570 3637 3648 4100
HaeII	14	
HaeIII	27	
HgaI	13	
HgiEI	3	419 1119 4208
HhaI	46	
Hin4I	4	203 1420 4514 4588
HincII	2	181 2027
HindIII	1	173
HinfI	16	
Hpal	1	2027
HphI	18	
KpnI	1	238
MaeI	15	
MaeII	18	
MbolI	15	
MluI	1	1521
MmeI	3	3837 4021 5635
MnlI	28	
MscI	1	351
MseI	28	
MslI	9	1573 1861 1891 2609 2804 3195 4767 4926 5285
MspI	32	
MspA1I	10	84 283 1551 2121 2214 3213 3332 3964 4209 5150
MwoI	41	
NarI	4	844 865 979 2161
NciI	12	
NcoI	1	212
NdeI	2	346 691
NgoAIV	4	831 2419 2579 5759
NlaIII	26	
NlaIV	27	
NottI	1	166
NspI	4	996 2967 3259 3626
NspV	1	268

Enzyme	# Sites	Locations
Pfi1108I	2	2408 4533
PfiMI	2	260 1103
PleI	10	466 778 1070 1157 1953 3516 4001 4504 5593 5601
PshAI	1	2366
Psp5II	1	2628
Psp1406I	6	1183 2551 2947 4741 5114 5443
PstI	1	4760
PvuI	1	4885
PvuII	3	2121 2214 3213
RcaI	3	919 4342 5350
RsaI	5	236 642 1668 3428 4995
RsrII	1	589
SacI	1	190
Sall	1	179
SapI	1	3506
Sau96I	19	
Sau3AI	32	
Scal	1	4995
ScrFI	21	
SfaNI	21	
Sfcl	5	763 3887 4078 4756 5877
SgrAI	1	840
SphI	1	996
SspI	2	5319 5450
StyI	2	57 212
TaqI	18	
TaqII	10	429 1429 1647 2320 3524 4863 5048 5201 5218 5562
TfiI	6	566 2200 2502 2672 3176 3597
Thal	35	
Tsel	29	
Tsp45I	8	1702 2530 3061 3274 3369 4771 4982 5831
Tsp509I	18	
Tth111I	1	3367
Tth111II	6	1360 2053 3083 4212 4219 4251
UbaII	21	
VspI	4	778 2206 2265 4687
XbaI	1	729
XcmI	3	1377 1893 1911
XhoI	1	158
XmnI	3	388 3180 5114

Enzymes that do not cut pET-32a(+):

AatII	AflII	AgeI	AscI	AvrII
BaeI	BseRI	BsmI	BspMI	BsrGI
Bsu36I	FseI	MluI	NheI	NruI
NsiI	PacI	PmeI	PmlI	RleAI
SacII	SexAI	SfiI	SgfI	SmaI
SnaBI	SpeI	SrfI	Sse8387I	StuI
SunI	Swal			