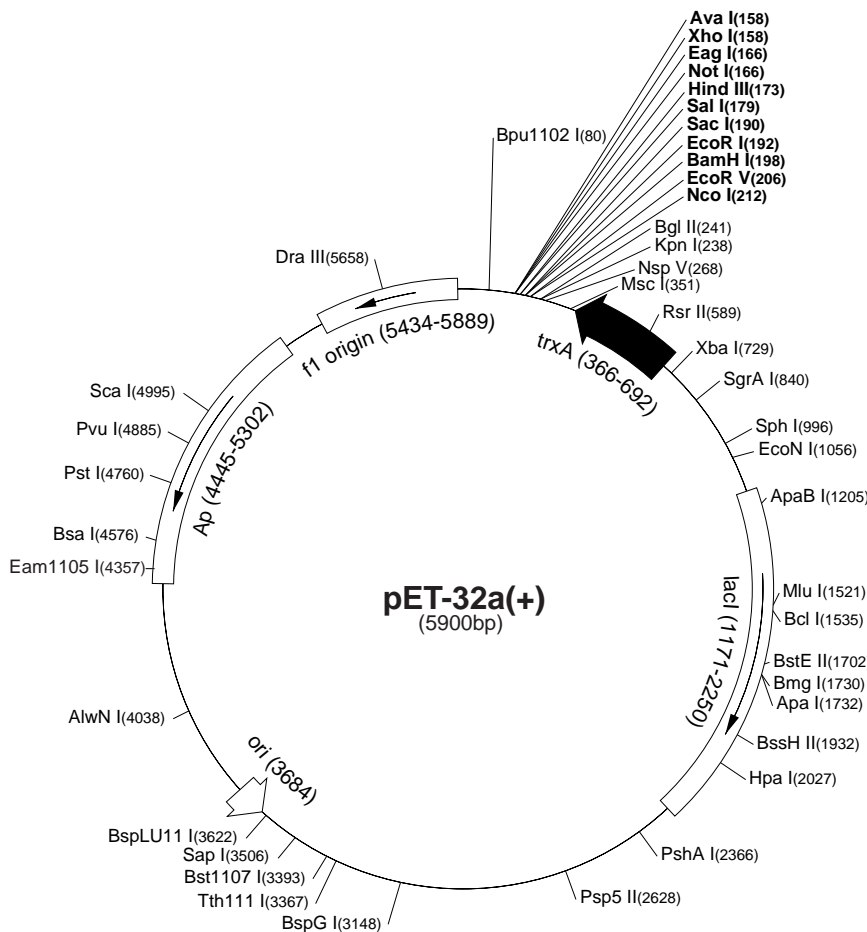# pET-32a-c(+) Vectors

	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 (*Nco* I - *Xho* I)	158-217
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	1171-2250
pBR322 origin	3684
bla 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.

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

pET-32a(+) Restriction Sites

Enzyme	# Sites	Locations				
AccI	2	180	3392			
AceIII	8	1288	2016	2347	3131	3272
		3574	4814	5498		
AciI	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				
AvaII	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			
BcefI	5	1040	1381	2008	4124	5684
BcgI	14					
BclI	1	1535				
BfaI	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			
BsbI	2	3338	5565			
BscGI	14					
BsgI	3	1372	1572	2782		
BsiI	2	3795	5179			
BsiEI	6	169	2306	3538	3962	4885
		5034				
BslI	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					
Cac8I	39					
CjeI	26					
CjePI	18					
ClaI	2	508	798			
CviJI	90					
CviRI	28					
DdeI	11					
DpnI	32					
DraI	3	4381	4400	5092		
DraIII	1	5658				
DrdI	3	3315	3730	5613		
DrdII	3	357	1244	5663		
DsaI	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		
EciI	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					
HgiEII	3	419	1119	4208		
HhaI	46					
Hin4I	4	203	1420	4514	4588	
HincII	2	181	2027			
HindIII	1	173				
HinfI	16					
HpaI	1	2027				
HphI	18					
KpnI	1	238				
MaeII	15					
MaeIII	18					
MboII	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					
NotI	1	166				
NspI	4	996	2967	3259	3626	
NspV	1	268				

Enzyme	# Sites	Locations				
Pfl1108I	2	2408	4533			
PflMI	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				
SalI	1	179				
SapI	1	3506				
Sau96I	19					
Sau3AI	32					
ScaI	1	4995				
ScrFI	21					
SfaNI	21					
SfcI	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				
ThaI	35					
TseI	29					
Tsp45I	8	1702	2530	3061	3274	3369
		4771	4982	5831		
Tsp509I	18					
Tth111I	1	3367				
Tth111II	6	1360	2053	3083	4212	4219
		4251				
UbaJI	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	MunI	NheI	NruI
NsiI	PacI	PmeI	PmlI	RleAI
SacII	SexAI	SfiI	SgfI	SmaI
SnaBI	SpeI	SrfI	Sse8387I	StuI
SunI	SwaI			