

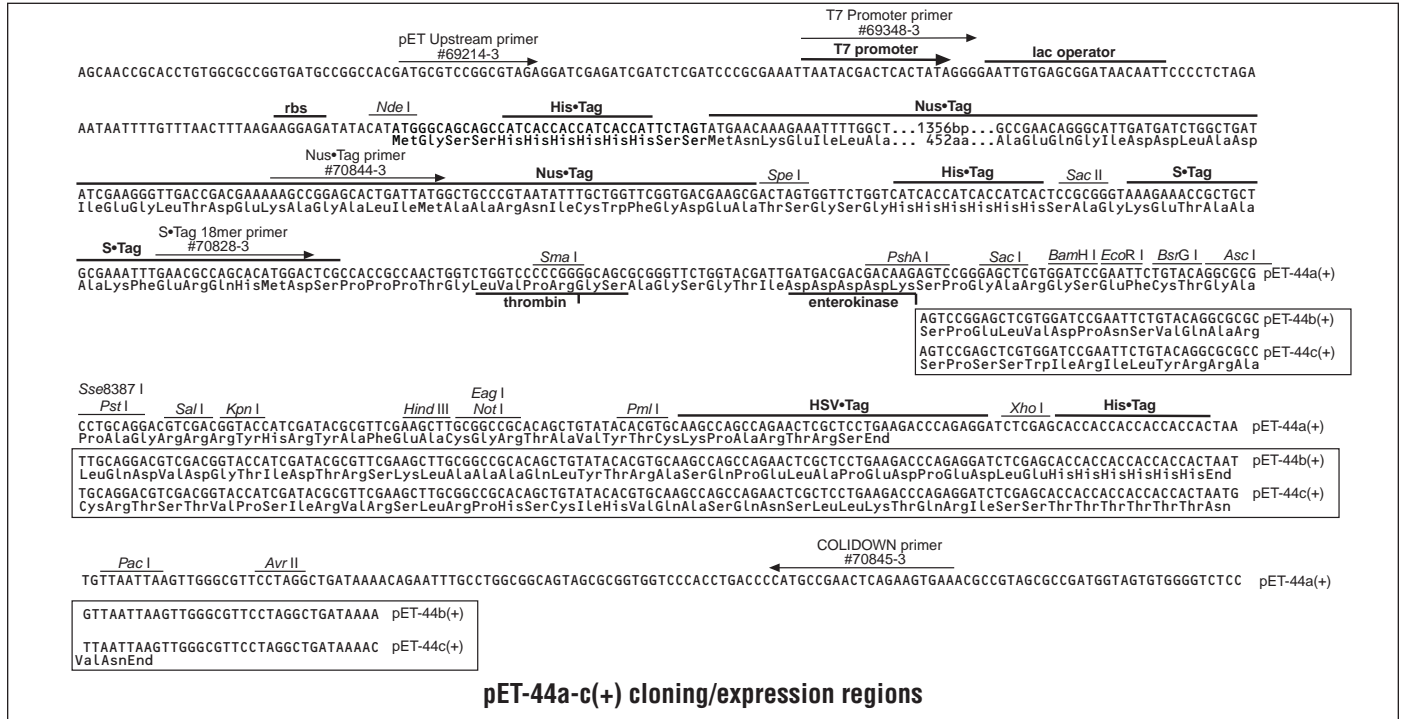
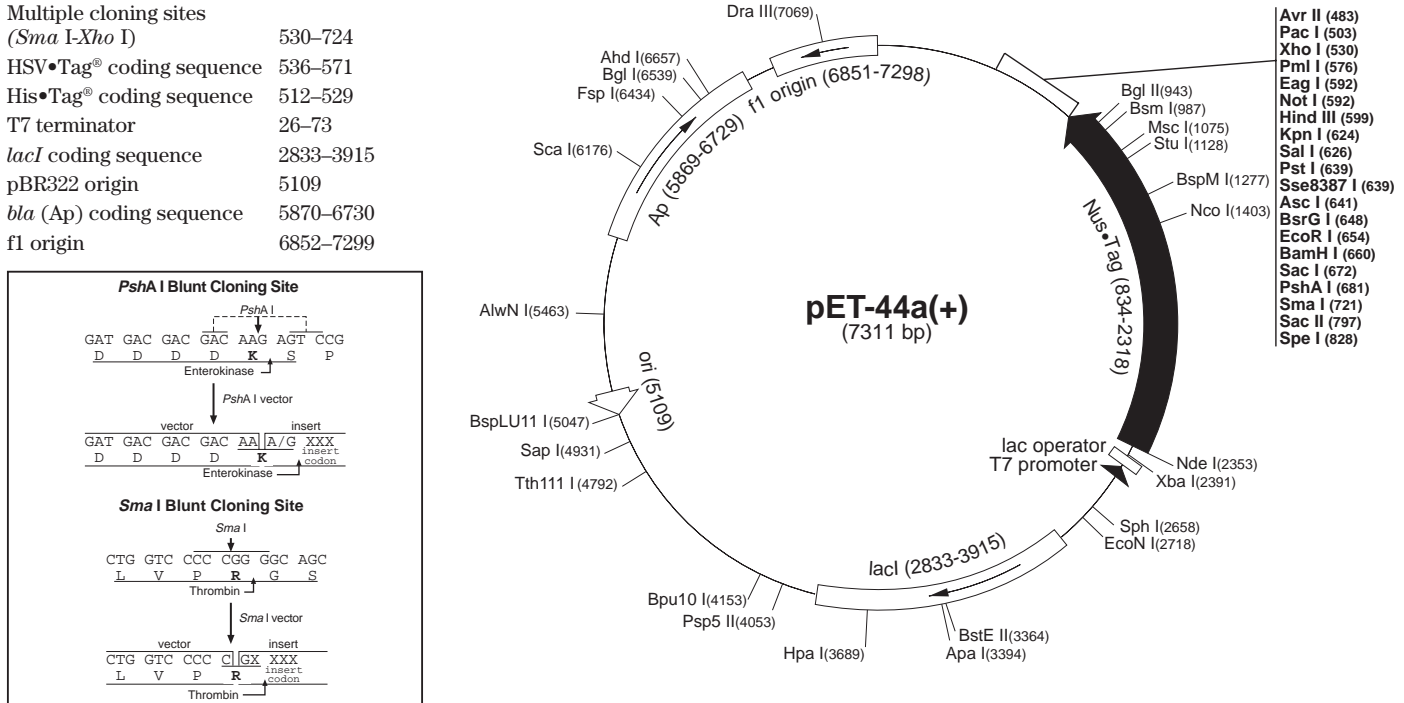
# pET-44a-c(+)<sup>+</sup> Vector

	Cat. No.
pET-44a(+) <sup>+</sup> DNA	71122-3
pET-44b(+) <sup>+</sup> DNA	71123-3
pET-44c(+) <sup>+</sup> DNA	71124-3

## pET-44a(+)<sup>+</sup> sequence landmarks

T7 promoter	2426-2442
T7 transcription start	2425
His•Tag <sup>®</sup> coding sequence	2325-2342
Nus•Tag <sup>™</sup> coding sequence	834-2318
His•Tag <sup>®</sup> coding sequence	801-818
S•Tag <sup>™</sup> coding sequence	747-791
Multiple cloning sites ( <i>Sma</i> I- <i>Xho</i> I)	530-724
HSV•Tag <sup>®</sup> coding sequence	536-571
His•Tag <sup>®</sup> coding sequence	512-529
T7 terminator	26-73
<i>lacI</i> coding sequence	2833-3915
pBR322 origin	5109
<i>bla</i> (Ap) coding sequence	5870-6730
f1 origin	6852-7299

The pET-44 vectors are designed for cloning and high-level expression of peptide sequences fused with the 495 aa Nus•Tag<sup>™</sup> protein. Compared to the pET-43.1 series, the pET-44 vectors encode an additional N-terminal His•Tag. 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 COLIDOWN primer (Cat. No. 70845-3). Vector encoded sequence can be completely removed when cloning into the *PshA* I or *Sma* I sites (as shown below) by cleaving the Nus•Tag fusion protein with enterokinase or thrombin, respectively.



## pET-44a-c(+)<sup>+</sup> cloning/expression regions

## pET-44a(+) Restriction Sites

TB330 Rev. A 0502

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations
AatII	2	633 2188	DraI	3	6079 6771 6790	Scal	1	6176
AccI	4	580 627 1529 4817	DraIII	1	7069	ScrFI	33	
Acil	99		DrdI	3	4740 5155 7024	SfaNI	23	
AflIII	4	575 608 3183 5047	Dsal	4	794 964 1403 2620	Sfcl	7	635 2294 2425 5312 5503
AhdI	1	6657	EaeI	8	592 971 1073 1360 2491			6411 7288
AluI	27				2623 3857 6264	SgrAI	2	1899 2502
Alw26I	8	371 2880 3285 3411 3798	EagI	1	592	Smal	1	721
		4688 5814 6591	EarI	3	2801 4931 5858	SpeI	1	828
AlwI	20		Ecl136II	1	670	SphI	1	2658
AlwNI	1	5463	Eco47III	2	2588 4301	Sse8387I	1	639
Apal	1	3394	Eco57I	4	532 1826 5595 5991	Sspl	2	857 6861
ApaLI	4	3163 4861 5361 5985	EcoNI	1	2718	StuI	1	1128
ApoI	7	466 654 768 2303 3458	EcoO109I	3	53 2616 4053	StyI	4	57 483 1403 2099
		6871 6882	EcoRI	1	654	Tal	25	
AscI	1	641	EcoRII	13		TaqI	28	
AvaI	2	530 719	EcoRV	2	911 1502	TfiI	4	3862 4097 4601 5022
Avall	7	440 725 3735 4053 4332	EheI	4	2507 2528 2642 3824	Thal	52	
		6293 6515	FauI	20		TseI	30	
AvrII	1	483	Fnu4HI	54		Tsp45I	10	
BamHI	1	660	FokI	14		Tsp509I	23	
BanI	10	620 1450 2505 2526 2640	FspI	1	6434	TspRI	16	
		3103 3822 3952 6704 7106	HaeII	15		Tth111I	1	4792
BanII	5	672 2567 2581 3394 7144	HaeIII	31		VspI	4	2440 3868 3927 6482
BbsI	5	538 1208 3329 3668 4165	HgaI	15		XbaI	1	2391
BbvI	30		HhaI	59		XcmI	3	3039 3555 3573
BcgI	3	3509 4624 6119	HincII	4	628 900 1295 3689	XhoI	1	530
BclI	2	1933 3197	HindIII	1	599	XmnI	3	2251 4605 6057
BfaI	11		HinfI	16				
BglI	1	6539	HpaI	1	3689			
BglIII	1	943	HphI	31				
Bpml	4	3021 3510 4574 6588	KpnI	1	624			
Bpu10I	1	4153	MaeIII	22				
Bpu1102I	2	80 1278	MbolI	25				
BsaAI	3	576 4799 7069	MluI	2	608 3183			
BsaBI	3	2456 2466 4244	MnlI	28				
BsaHI	9	200 630 2185 2506 2527	MscI	1	1075			
		2641 3140 3823 6117	MseI	33				
BsaI	2	371 6591	MslI	11				
BsaJI	17		MspA1I	13				
BsaWI	8	2 1576 3502 4005 4236	MspI	42				
		5253 5400 6361	MwoI	47				
BsgI	4	1956 3034 3234 4207	NarI	4	2506 2527 2641 3823			
BsiEI	10	595 1115 1253 1789 2170	NciI	20				
		3968 4963 5387 6139 6288	NcoI	1	1403			
BsiHKAI	11		NdeI	1	2353			
BsII	26		NgoAIV	2	2493 7170			
BsmBI	2	3798 4688	NlaIII	28				
BsmFI	5	453 738 2644 4318 7284	NlaIV	25				
BsmI	1	987	NotI	1	592			
Bsp1286I	15		NruI	3	1210 1635 2259			
BspEI	2	2 4236	NspI	4	2658 4392 4684 5051			
BspLU11I	1	5047	NspV	2	604 2245			
BspMI	1	1277	PacI	1	503			
BsrBI	6	249 1323 2412 4980 5814	PfiMI	2	758 2765			
		7213	PleI	12				
BsrDI	6	1073 1454 3230 3596 6423	PmlI	1	576			
		6597	PshAI	1	681			
BsrFI	9	1027 1383 1447 1899 2493	Psp1406I	6	237 2845 4372 6055 6428			
		2502 2869 6572 7170			6854			
BsrGI	1	648	Psp5II	1	4053			
BsrI	29		PstI	1	639			
BssHII	5	641 1547 1739 2228 3594	PvuI	3	1253 2170 6288			
BssSI	4	665 1619 5220 5988	PvuII	4	586 3783 3876 4638			
Bst1107I	2	581 4818	RcaI	4	2581 5767 5816 5848			
BstEII	1	3364	RsaI	8	622 650 701 956 2178			
BstXI	3	2985 3114 3237			3330 4853 6176			
BstYI	13		SacI	1	672			
CacBI	52		SacII	1	797			
Clal	5	615 1504 1582 2170 2460	Sall	1	626			
CviJI	113		SapI	1	4931			
Ddel	14		Sau3AI	42				
DpnI	42		Sau96I	20				

Enzymes that do not cut pET-44a(+):

 AflIII, BseRI, Bsu36I, FseI, MunI, NheI,  
 NsiI, PinAI, PmeI, RsrII, SanDI, SexAI,  
 SfiI, SgfI, SnaBI, SrfI, SunI, SwaI