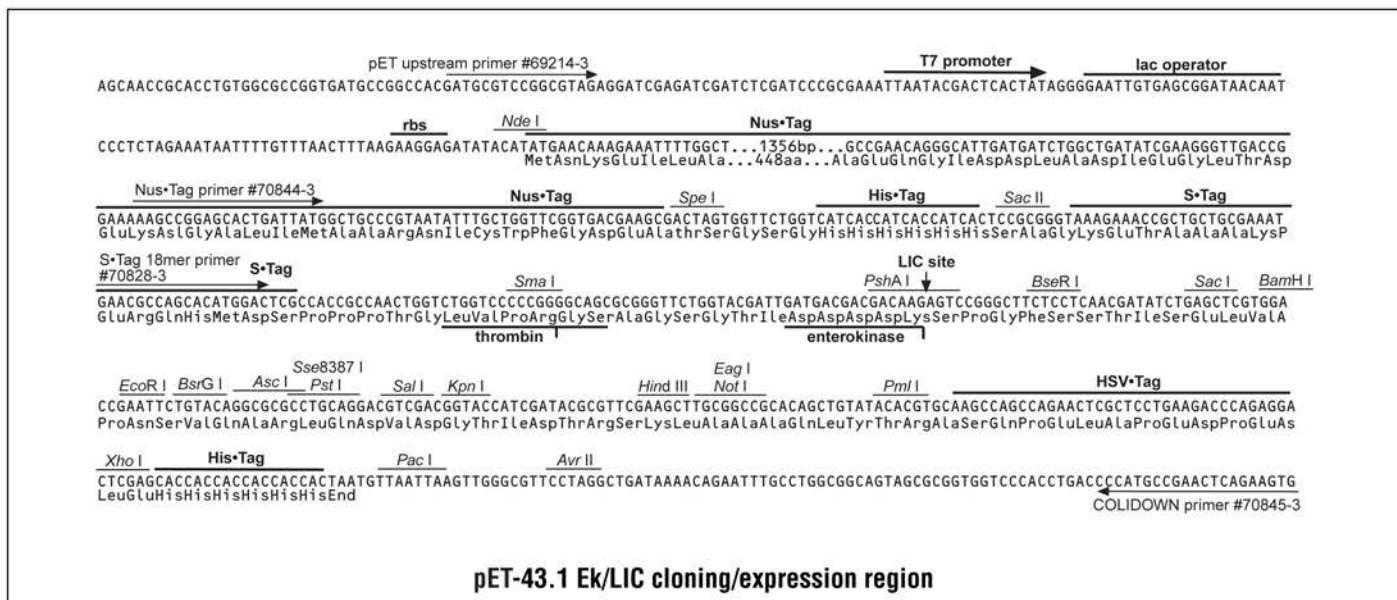
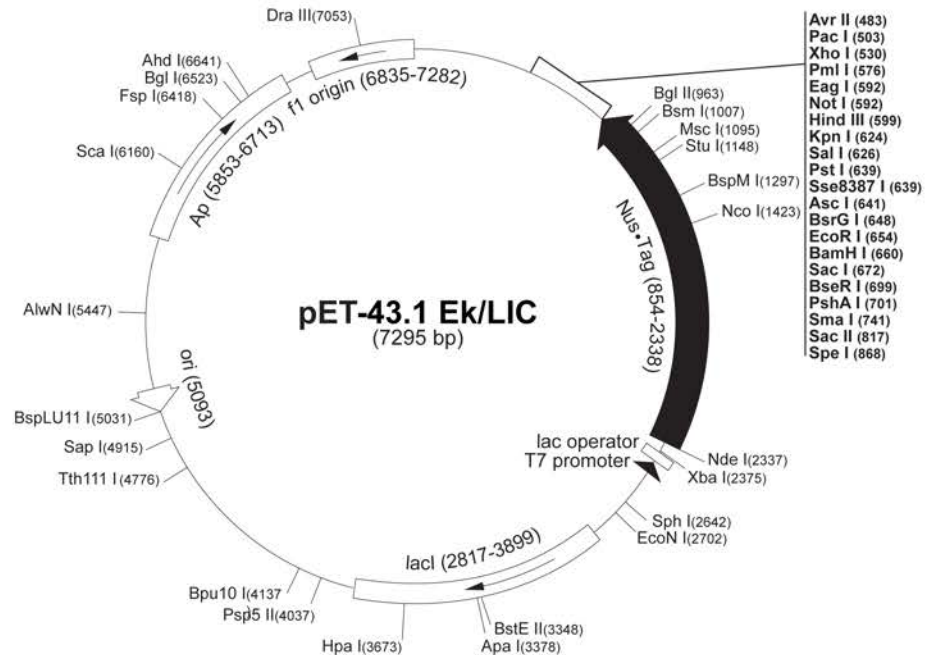
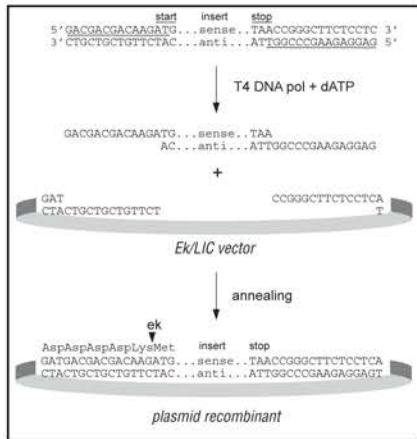


## pET-43.1 Ek/LIC Vector

The pET-43.1 Ek/LIC vector is prepared for rapid, directional cloning of PCR-amplified DNA for high-level expression of polypeptides fused with N-terminal Nus•Tag™, His•Tag® and S•Tag™ sequences. Using specifically designed primers for amplification and the pET-43.1 Ek/LIC Vector Kit (Cat. No. 71072-3), inserts can be efficiently cloned without the need for restriction digestion or ligation. 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 Ek/LIC site (as shown below left) by cleaving the fusion protein with enterokinase.

### pET-43.1 EK/LIC sequence landmarks

T7 promoter	2410-2426
T7 transcription start	2409
Nus•Tag coding sequence	854-2348
His•Tag coding sequence	821-838
S•Tag coding sequence	767-811
Multiple cloning sites ( <i>BseR I-Xho I</i> )	699-530
HSV•Tag coding sequence	536-571
His•Tag coding sequence	512-529
T7 terminator	26-72
<i>lacI</i> coding sequence	2817-3899
pBR322 origin	5093
<i>bla</i> (Ap) coding sequence	5854-6714
f1 origin	6835-7282



### pET-43.1 Ek/LIC cloning/expression region

**pET-43.1 Ek/LIC Restriction Sites**

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations
AatII	2	633 2208	Ddel	15		Sau3AI	42	
AccI	4	580 627 1549 4801	DpnI	42		Sau96I	20	
AcI	99		DraI	3	6063 6755 6774	Scal	1	6160
AflIII	4	575 608 3167 5031	DraIII	1	7053	ScrFI	33	
AhdI	1	6641	DrdI	3	4724 5139 7008	SfaNI	23	
AluI	27		DsaI	4	814 984 1423 2604	Sfcl	7	635 2314 2409 5296 5487
Alw26I	8	371 2864 3269 3395 3782 4672 5798 6575	EaeI	8	592 991 1093 1380 2475 2607 3841 6248	SgrAI	2	6395 7272 1919 2486
AlwI	20		EagI	1	592	SmaI	1	741
AlwNI	1	5447	EarI	3	2785 4915 5842	SpeI	1	848
ApaI	1	3378	Ecl136II	1	670	SphI	1	2642
ApaLI	4	3147 4845 5345 5969	Eco47III	2	2572 4285	Sse8387I	1	639
ApoI	7	466 654 788 2323 3442 6855 6866	Eco57I	4	532 1846 5579 5975	SspI	2	877 6845
AscI	1	641	EcoNI	1	2702	StuI	1	1148
AvaI	2	530 739	EcoO109I	3	53 2600 4037	StyI	4	57 483 1423 2119
Avall	7	440 745 3719 4037 4316 6277 6499	EcoRI	1	654	TalI	25	
AvrII	1	483	EcoRII	13		TaqI	28	
BamHI	1	660	EcoRV	3	677 931 1522	TfiI	4	3846 4081 4585 5006
BanI	10	620 1470 2489 2510 2624 3087 3806 3936 6688 7090	EheI	4	2491 2512 2626 3808	ThaI	52	
BanII	5	672 2551 2565 3378 7128	FauI	20		TseI	28	
BbsI	5	538 1228 3313 3652 4149	Fnu4HI	52		Tsp45I	10	859 1745 2132 3348 4470 4683 4778 6168 6379 7226
BbvI	28		FokI	14		Tsp509I	23	
BcgI	3	3493 4608 6103	FspI	1	6418	TspRI	16	
BclI	2	1953 3181	HaeII	15		Tth111I	1	4776
Bfal	10	70 484 849 2376 4010 4045 5526 6448 6783 7204	HaeIII	31		VspI	4	2424 3852 3911 6466
BglI	1	6523	HgaI	15		XbaI	1	2375
BglIII	1	963	HhaI	59		XcmI	3	3023 3539 3557
BpmI	4	3005 3494 4558 6572	HincII	4	628 920 1315 3673	XhoI	1	530
Bpu10I	1	4137	HindIII	1	599	XmnI	3	2271 4589 6041
Bpu1102I	2	80 1298	Hinfl	16				
BsaAI	3	576 4783 7053	HpaI	1	3673			
BsaBI	3	2440 2450 4228	HphI	29				
BsaHI	9	200 630 2205 2490 2511 2625 3124 3807 6101	KpnI	1	624			
BsaI	2	371 6575	MaeIII	22				
BsaJI	17		MbolI	25				
BsaWI	8	2 1596 3486 3989 4220 5237 5384 6345	MluI	2	608 3167			
BseRI	1	699	MnlI	29				
BsgI	4	1976 3018 3218 4191	MscI	1	1095			
BsiEI	10	595 1135 1273 1809 2190 3952 4947 5371 6123 6272	MseI	33				
BsiHKAI	11		MslI	11				
BsII	26		MspA1I	13				
BsmBI	2	3782 4672	MspI	42				
BsmFI	5	453 758 2628 4302 7268	MwoI	47				
BsmI	1	1007	NarI	4	2490 2511 2625 3807			
Bsp1286I	15		NciI	20				
BspEI	2	2 4220	NcoI	1	1423			
BspLU11I	1	5031	NdeI	1	2337			
BspMI	1	1297	NgoAIV	2	2477 7154			
BsrBI	6	249 1343 2396 4964 5798 7197	NlaIII	28				
BsrDI	6	1093 1474 3214 3580 6407 6581	NlaIV	25				
BsrFI	9	1047 1403 1467 1919 2477 2486 2853 6556 7154	NotI	1	592			
BsrGI	1	648	Nrul	3	1230 1655 2279			
BsrI	29		NspI	4	2642 4376 4668 5035			
BssHII	5	641 1567 1759 2248 3578	NspV	2	604 2265			
BssSI	4	665 1639 5204 5972	Pacl	1	503			
Bst1107I	2	581 4802	PfIMI	2	778 2749			
BstEII	1	3348	PleI	12				
BstXI	3	2969 3098 3221	PmlI	1	576			
BstYI	13		PshAI	1	701			
CacBI	52		Psp1406I	6	237 2829 4356 6039 6412 6838			
ClaI	5	615 1524 1602 2190 2444	Psp5II	1	4037			
CviJI	113		PstI	1	639			
			PvuI	3	1273 2190 6272			
			PvuII	4	586 3767 3860 4622			
			RcaI	4	2565 5751 5800 5832			
			RsaI	8	622 650 721 976 2198 3314 4837 6160			
			SacI	1	672			
			SacII	1	817			
			Sall	1	626			
			SapI	1	4915			

Enzymes that do not cut pET-43.1 Ek/LIC:  
AflIII Bsu36I FseI MunI NheI NsiI PinAI  
PmeI RsrII SanDI SexAI Sfil Sgfl SnaBI  
SrfI SunI SwaI