pET-44 Ek/LIC Vector

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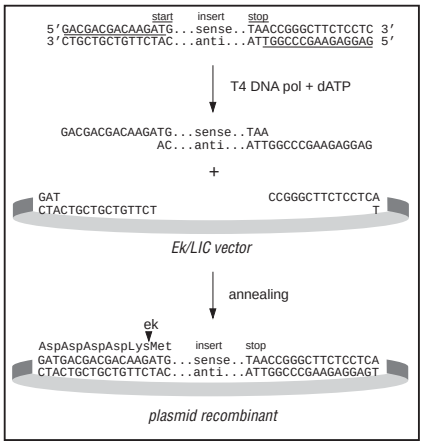
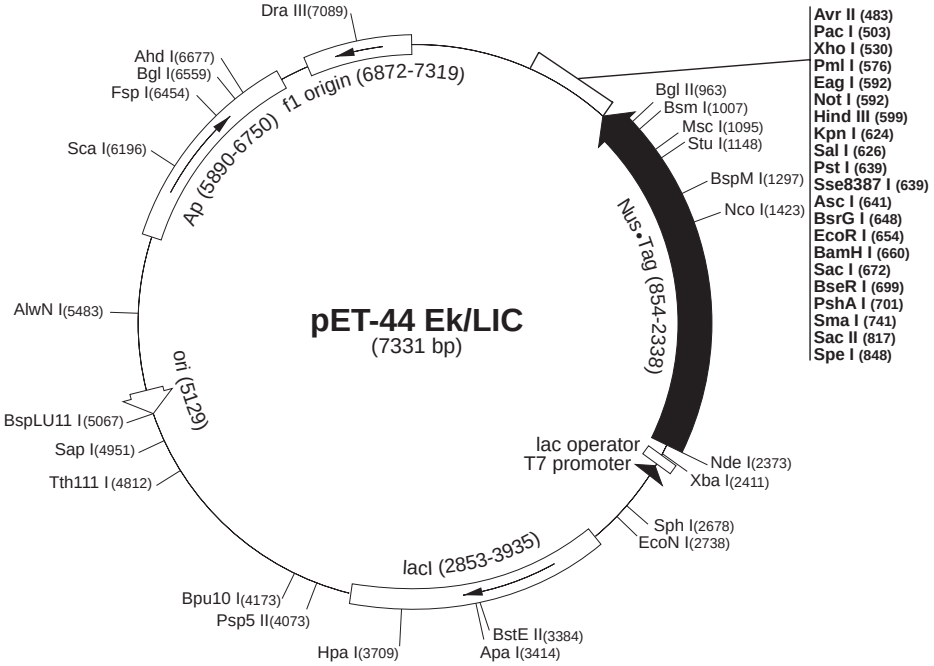
	Cat. No.
pET-44 Ek/LIC DNA	71143-3

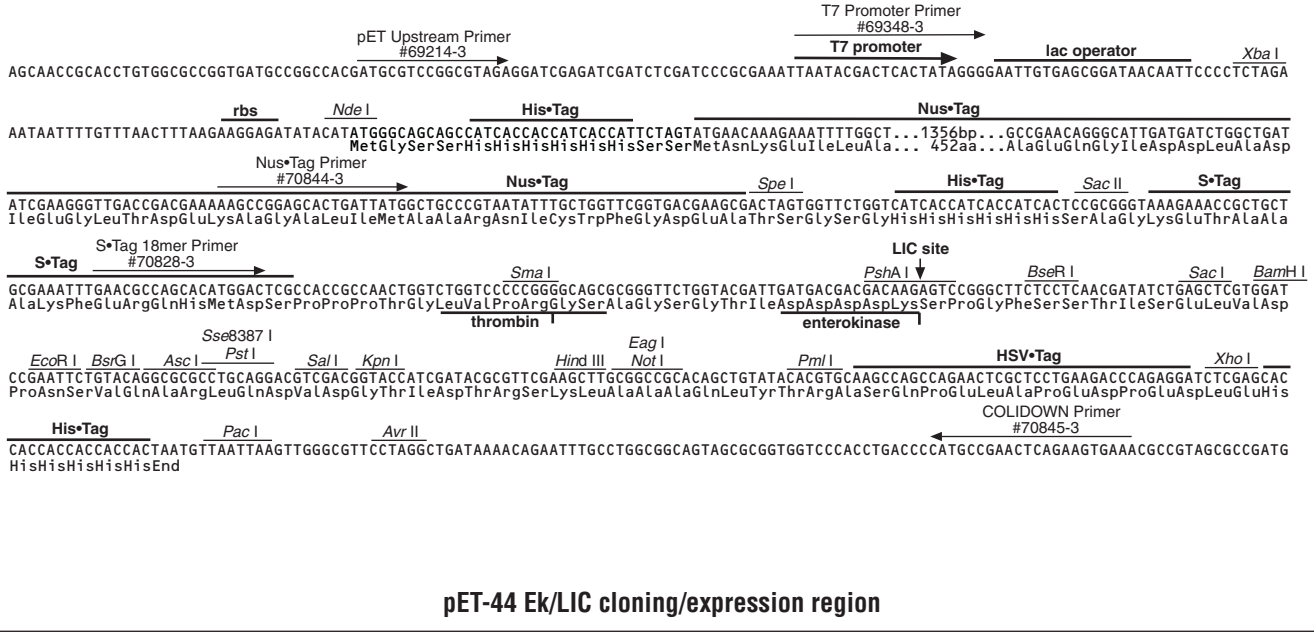
pET-44 EK/LIC sequence landmarks

T7 promoter	2446–2462
T7 transcription start	2445
His•Tag coding sequence	2345–2362
Nus•Tag coding sequence	854–2338
His•Tag coding sequence	821–838
S•Tag coding sequence	767–811
Multiple cloning sites (*Bse*R I–*Xho* I)	699–530
HSV•Tag coding sequence	536–571
His•Tag coding sequence	512–529
T7 terminator	26–73
lacI coding sequence	2853–3932
pBR322 origin	5129
Ap (*bla*) coding sequence	5890–6750
f1 origin	6872–7319



The pET-44 Ek/LIC Vector is prepared for rapid, directional cloning of PCR-amplified DNA for high-level expression of polypeptides fused with N-terminal His•Tag®,Nus•Tag™ and S•Tag™ sequences. Using specifically designed primers for amplification and the pET-44 Ek/LIC Vector Kit (Cat. No. 71144-3), 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 sequences can be completely removed when cloning into the Ek/LIC site (as shown below left) by cleaving the fusion protein with enterokinase.





pET-44 Ek/LIC cloning/expression region

pET-44 Ek/LIC Restriction Sites

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Enzyme	# Sites	Locations				
AatII	2	633	2208			
AccI	4	580	627	1549	4837	
AciI	9					
AflIII	4	575	608	3203	5067	
AhdI	1	6677				
AluI	27					
Alw26I	8	371	2900	3305	3431	3818
		4708	5834	6611		
AlwI	20					
AlwNI	1	5483				
ApaI	1	3414				
ApaLI	4	3183	4881	5381	6005	
ApoI	7	466	654	788	2323	3478
		6891	6902			
AscI	1	641				
AvaI	2	530	739			
AvaII	7	440	745	3755	4073	4352
		6313	6535			
AvrII	1	483				
BamHI	1	660				
BanI	10	620	1470	2525	2546	2660
		3123	3842	3972	6724	7126
BanII	5	672	2587	2601	3414	7164
BbsI	5	538	1228	3349	3688	4185
BbvI	30					
BcgI	3	3529	4644	6139		
BclI	2	1953	3217			
BfaI	11					
BglI	1	6559				
BglII	1	963				
BpmI	4	3041	3530	4594	6608	
Bpu10I	1	4173				
Bpu1102I	2	80	1298			
BsaAI	3	576	4819	7089		
BsaBI	3	2476	2486	4264		
BsaHI	9	200	630	2205	2526	2547
		2661	3160	3843	6137	
BsaI	2	371	6611			
BsaJI	17					
BsaWI	8	2	1596	3522	4025	4256
		5273	5420	6381		
BseRI	1	699				
BsgI	4	1976	3054	3254	4227	
BsiEI	10	595	1135	1273	1809	2190
		3988	4983	5407	6159	6308
BsiHKAI	11					
BsiI	26					
BsmBI	2	3818	4708			
BsmFI	5	453	758	2664	4338	7304
BsmI	1	1007				
Bsp1286I	15					
BspEI	2	2	4256			
BspLU11I	1	5067				
BspMI	1	1297				
BsrBI	6	249	1343	2432	5000	5834
		7233				
BsrDI	6	1093	1474	3250	3616	6443
		6617				
BsrFI	9	1047	1403	1467	1919	2513
		2522	2889	6592	7190	
BsrGI	1	648				
BsrI	29					
BssHII	5	641	1567	1759	2248	3614
BssSI	4	665	1639	5240	6008	
Bst1107I	2	581	4838			
BstEII	1	3384				
BstXI	3	3005	3134	3257		
BstYI	13					
Cac8I	52					
ClaI	5	615	1524	1602	2190	2480
CviJI	114					
DdeI	15					

Enzyme	# Sites	Locations				
DpnI	42					
DraI	3	6099	6791	6810		
DraIII	1	7089				
DrdI	3	4760	5175	7044		
DsaI	4	814	984	1423	2640	
EaeI	8	592	991	1093	1380	2511
		2643	3877	6284		
EagI	1	592				
EarI	3	2821	4951	5878		
Ecl136II	1	670				
Eco47III	2	2608	4321			
Eco57I	4	532	1846	5615	6011	
EcoNI	1	2738				
EcoO109I	3	53	2636	4073		
EcoRI	1	654				
EcoRII	13					
EcoRV	3	677	931	1522		
EheI	4	2527	2548	2662	3844	
FauI	20					
Fnu4HI	54					
FokI	14					
FspI	1	6454				
HaeII	15					
HaeIII	31					
HgaI	15					
HhaI	59					
HincII	4	628	920	1315	3709	
HindIII	1	599				
HinfI	16					
HpaI	1	3709				
HphI	31					
KpnI	1	624				
MaeIII	22					
MboII	25					
MluI	2	608	3203			
MnlI	29					
MscI	1	1095				
MseI	33					
MslI	11					
MspA1I	13					
MspI	42					
MwoI	47					
NarI	4	2526	2547	2661	3843	
NciI	20					
NcoI	1	1423				
NdeI	1	2373				
NgoAIV	2	2513	7190			
NlaIII	28					
NlaIV	25					
NotI	1	592				
NruI	3	1230	1655	2279		
NspI	4	2678	4412	4704	5071	
NspV	2	604	2265			
PacI	1	503				
PflMI	2	778	2785			
PleI	12					
PmlI	1	576				
PshAI	1	701				
Psp1406I	6	237	2865	4392	6075	6448
		6874				
Psp5II	1	4073				
PstI	1	639				
PvuI	3	1273	2190	6308		
PvuII	4	586	3803	3896	4658	
RcaI	4	2601	5787	5836	5868	
RsaI	8	622	650	721	976	2198
		3350	4873	6196		
SacI	1	672				
SacII	1	817				
SalI	1	626				
SapI	1	4951				
Sau3AI	42					

Enzyme	# Sites	Locations				
Sau96I	20					
ScaI	1	6196				
ScrFI	33					
SfaNI	23					
SfcI	7	635	2314	2445	5332	5523
		6431	7308			
SgrAI	2	1919	2522			
SmaI	1	741				
SpeI	1	848				
SphI	1	2678				
Sse8387I	1	639				
SspI	2	877	6881			
StuI	1	1148				
StyI	4	57	483	1423	2119	
TaiI	25					
TaqI	28					
TfiI	4	3882	4117	4621	5042	
ThaI	52					
TseI	30					
Tsp45I	10	859	1745	2132	3384	4506
		4719	4814	6204	6415	7262
Tsp509I	23					
TspRI	16					
Tth111I	1	4812				
VspI	4	2460	3888	3947	6502	
XbaI	1	2411				
XcmI	3	3059	3575	3593		
XhoI	1	530				
XmnI	3	2271	4625	6077		

Enzymes that do not cut pET-44 Ek/LIC:

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