

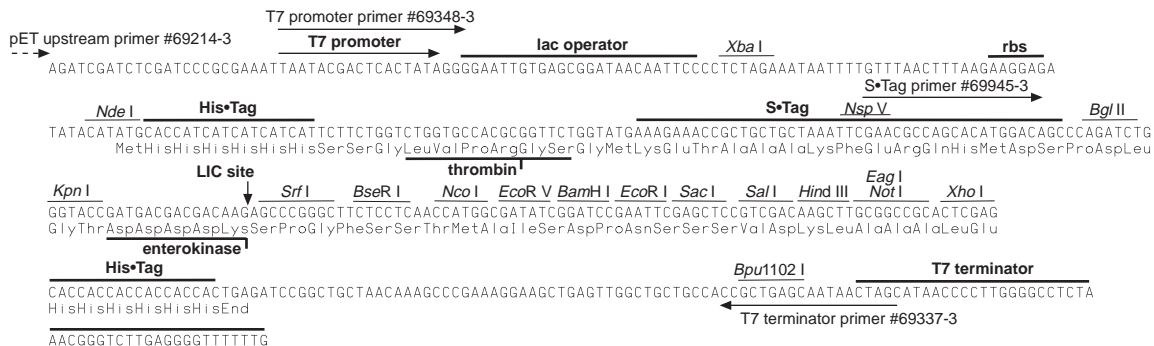
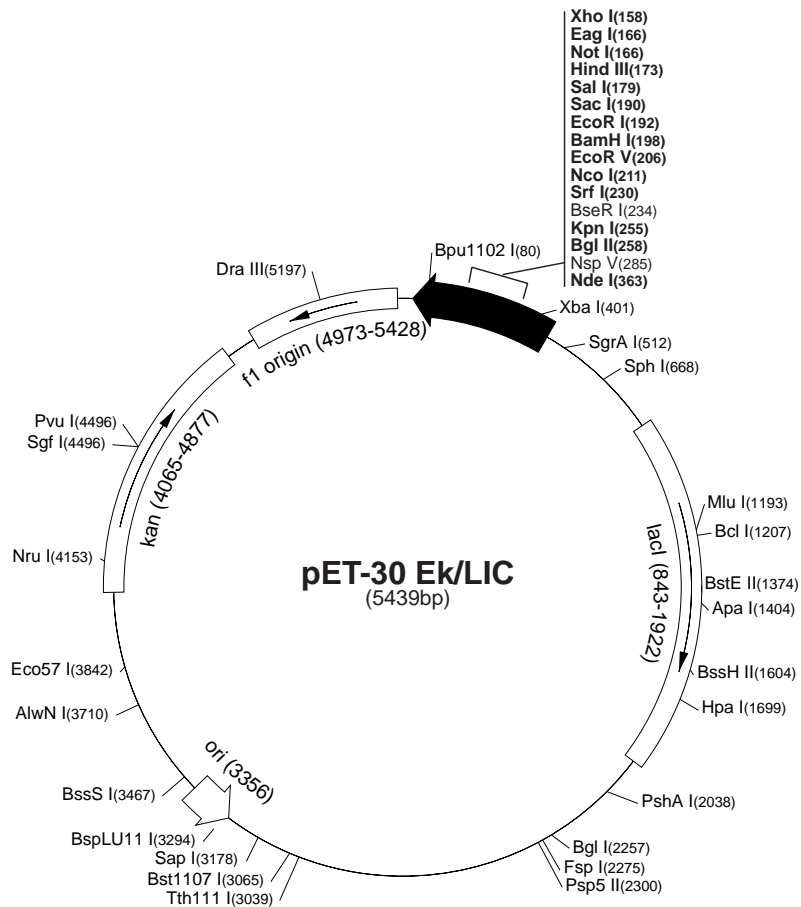
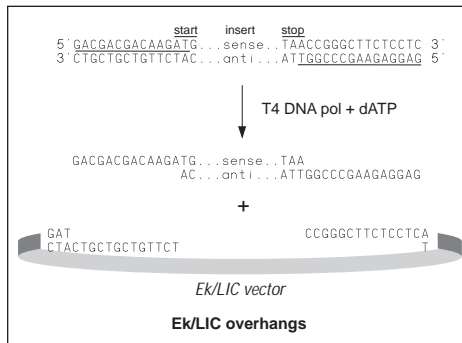
# pET-30 Ek/LIC Vector

The pET-30 Ek/LIC vector is prepared for rapid, directional cloning of PCR-amplified DNA for high-level expression of polypeptides. Using specifically designed primers for amplification and the pET-30 Ek/LIC Cloning Kit (Cat. No. 69077-3), inserts can be efficiently cloned without the need for restriction digestion or ligation. Fusion proteins contain N-terminal cleavable His•Tag<sup>®</sup> and S•Tag<sup>™</sup> 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).

## pET-30 Ek/LIC sequence landmarks

T7 promoter	436-452
T7 transcription start	435
His•Tag coding sequence	344-361
S•Tag coding sequence	266-310
Multiple cloning sites ( <i>BseR</i> I - <i>Xho</i> I)	158-224
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lacI</i> coding sequence	843-1922
pBR322 origin	3356
Kan coding sequence	4065-4877
f1 origin	4973-5428

Note: the *Srf*I site is destroyed during Ligation Independent Cloning. Primer sequence extensions required for LIC compatibility are underlined in the diagram below.



## pET-30 Ek/LIC cloning/expression region

# pET-30 Ek/LIC Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations		
AccI	2	180 3064	Bst1107I	1	3065	NspI	4	668 2639 2931 3298		
AccIII	7	960 1688 2019 2803 2944	BstEII	1	1374	NspV	1	285		
		3246 5037	BstXI	3	995 1124 1247	Pfi1108I	1	2080		
AcI	75		BstYI	9	132 198 258 757 1969	PfiMI	3	277 775 4759		
AflIII	2	1193 3294			2486 3935 3946 4745	PleI	9	450 742 829 1625 3188		
AluI	22		Cac8I	40				3673 4728 5132 5140		
AlwI	13		CjeI	24		PshAI	1	2038		
Alw21I	7	159 190 693 1177 2288	CjePI	18		Psp5II	1	2300		
		3112 3612	Clal	2	470 4187	Psp1406I	4	855 2223 2619 4982		
Alw44I	3	1173 3108 3608	CviJI	85		PvuI	1	4496		
AlwNI	1	3710	CviRI	23		PvuII	3	1793 1886 2885		
Apal	1	1404	Ddel	11		Rcal	3	591 4014 4889		
ApaBI	1	877	Dpnl	23		Rsal	4	253 1340 3100 4331		
ApoI	7	192 287 1468 4109 4293	DrallI	1	5197	SacI	1	190		
		4999 5010	DrdI	3	2987 3402 5152	Sall	1	179		
AvaI	3	158 228 4368	DrdII	2	916 5202	SapI	1	3178		
AvaII	5	1745 2121 2209 2300 2579	Dsal	3	211 630 2266	Sau96I	14			
BamHI	1	198	EaeI	4	166 501 633 1867	Sau3AI	23			
BanI	10	251 327 515 536 650	EagI	1	166	ScrFI	23			
		1113 1832 1962 2088 5234	EarI	3	811 3178 4309	SfaNI	23			
BanII	7	190 235 577 591 1404	Ecil	3	970 3368 3514	Sfcl	4	435 3559 3750 5416		
		4151 5272	Eco47III	3	598 2099 2548	Sgfl	1	4496		
BbsI	4	1339 1678 2052 2412	Eco57I	1	3842	SgrAI	1	512		
BbvI	25		EcoNI	2	728 4408	SmaI	2	230 4370		
BccI	14		EcoO109I	3	53 626 2300	SphI	1	668		
Bce83I	6	21 2007 2177 3385 3683	EcoRI	1	192	SrfI	1	230		
		3924	EcoRII	9	916 1231 1771 1828 3320	Sspl	2	4421 4989		
Bcefl	6	712 1053 1680 3796 4815			3441 3454 4384 4741	StyI	2	57 211		
		5223	EcoRV	1	206	TaqI	17			
BcgI	8	160 194 1485 1519 2019	FauI	17		TaqII	6	1101 1319 1992 3196 4750		
		2053 2871 2905	FokI	9	1239 1248 2513 2575 2653			5101		
BclI	1	1207			2839 2980 4134 4740	TfiI	9	1872 2174 2344 2848 3269		
Bfal	6	70 402 2308 3789 4096	Fspl	1	2275			4407 4463 4635 4726		
		5348	GdIII	4	166 501 633 1867	Thal	36			
BglI	1	2257	HaeI	6	921 2242 3309 3320 3772	TseI	25			
BglII	1	258			4583	Tsp45I	7	1374 2202 2733 2946 3041		
BmgI	1	1402	HaeII	14				4643 5370		
BpmI	4	1031 1520 2154 2821	HaeIII	23		Tsp509I	21			
Bpu10I	2	2400 4513	HgaI	11		Tth111I	1	3039		
Bpu1102I	1	80	HgiEII	2	791 3880	Tth111II	8	1032 1725 2755 3884 3891		
BsaAI	2	3046 5197	HhaI	46				3923 4332 4459		
BsaBI	3	466 476 2491	Hin4I	4	203 1092 4182 4724	UbaII	18			
BsaHI	5	516 537 651 1150 1833	HincII	2	181 1699	VspI	5	450 1878 1937 4695 4884		
BsaJI	11		HindIII	1	173	XbaI	1	401		
BsaWI	7	2 1512 2015 2483 3500	Hinfl	18		XcmI	3	1049 1565 1583		
		3647 4631	HpaI	1	1699	XhoI	1	158		
BsaXI	2	1852 5145	HphI	16		XmnI	2	2852 4885		
Bsbl	2	3010 5104	KpnI	1	255					
BscGI	11		MaeI	14		Enzymes that do not cut pET-30 Ek/LIC:				
BseRI	1	234	MaeII	16		AatII	AfII	AgeI	AscI	AvrII
BsgI	3	1044 1244 2454	MbolI	13		BaeI	BsaI	BspMI	BsrGI	Bsu36I
Bsil	1	3467	MluI	1	1193	DraI	Eam1105I	FseI	MscI	MunI
BsiEI	5	169 1978 3210 3634 4496	MmeI	7	3509 3693 4138 4332 4694	NheI	Pacl	PmeI	PmlI	PstI
BsII	26				4703 5174	RleAI	RsrII	SacII	Scal	SexAI
BsmI	2	4380 4457	MnlI	26		SfiI	SnaBI	SpeI	Sse8387I	StuI
BsmAI	6	890 1295 1421 1808 2935	MseI	25		SunI	Swal			
		4512	Msil	6	1245 1533 1563 2281 2476					
BsmBI	3	1808 2935 4512	MspI	30						
BsmFI	4	654 2195 2565 5412	MspAII	9	84 300 1223 1793 1886					
BsoFI	43				2885 3004 3636 3881					
Bsp24I	10	483 515 1034 1066 1336	MwoI	39						
		1368 3787 3819 3965 3997	NarI	4	516 537 651 1833					
Bsp1286I	13		NciI	14						
BspEI	2	2 2483	NcoI	1	211					
BspGI	1	2820	NdeI	1	363					
BspLU11I	1	3294	NgoAIV	4	503 2091 2251 5298					
BsrI	21		NlaIII	26						
BsrBI	4	422 3227 4895 5341	NlaIV	23						
BsrDI	2	1240 1606								
BsrFI	7	503 512 879 2091 2251	NotI	1	166					
		4450 5298	NruI	1	4153					
BssHII	1	1604	Nsil	2	4346 4612					