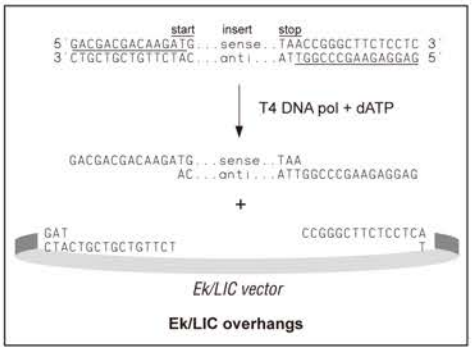
pET-30 Ek/LIC Vector

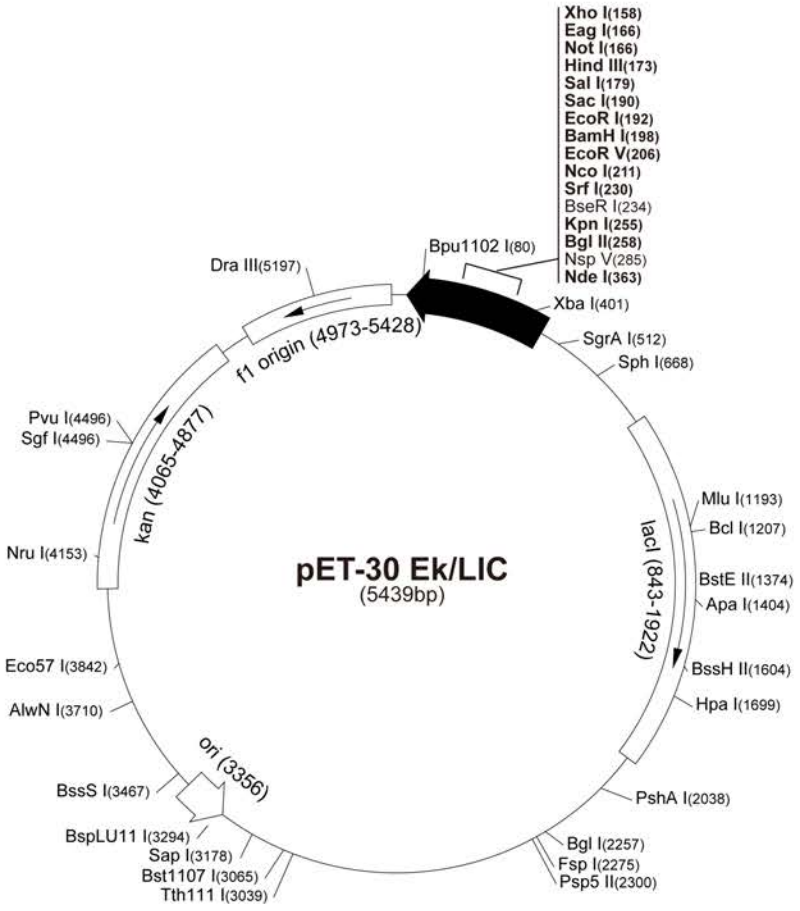
The pET-30 Ek/LIC vector is prepared for rapid, directional cloning of PCR-amplified DNA for high-level expression of polypeptides. Using specifically designed primers for amplification and the pET-30 Ek/LIC Cloning Kit (Cat. No. 69077-3), inserts can be efficiently cloned without the need for restriction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

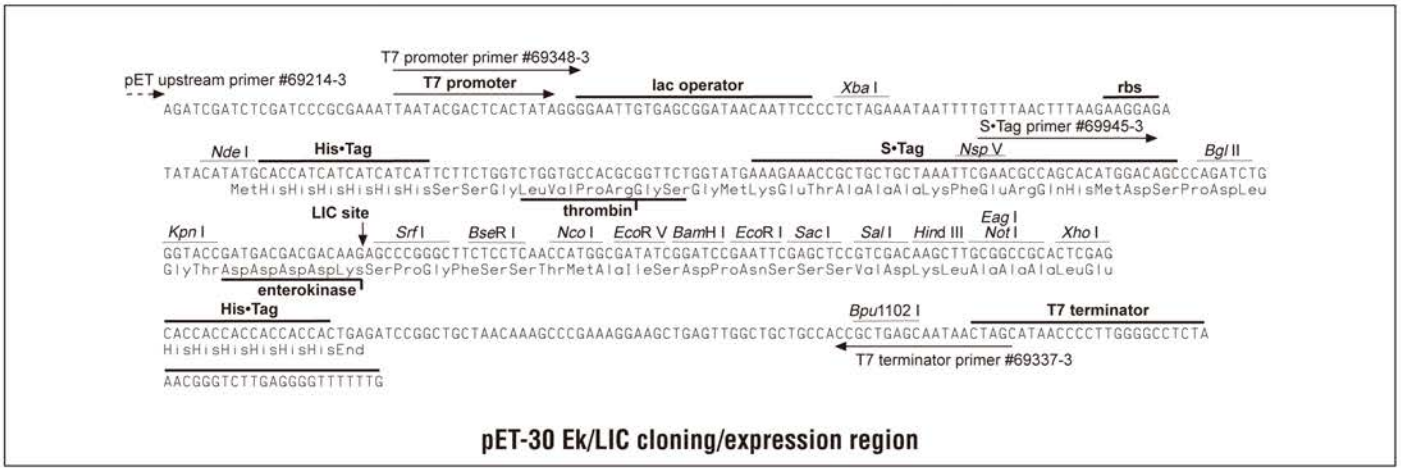
pET-30 Ek/LIC sequence landmarks

T7 promoter	436-452
T7 transcription start	435
His•Tag coding sequence	344-361
S•Tag coding sequence	266-310
Multiple cloning sites	
(*Bse*R I - *Xho* I)	158-224
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI 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				
AccI	2	180	3064			
AceIII	7	960	1688	2019	2803	2944
		3246	5037			
AciI	75					
AflIII	2	1193	3294			
AluI	22					
AlwI	13					
Alw21I	7	159	190	693	1177	2288
		3112	3612			
Alw44I	3	1173	3108	3608		
AlwNI	1	3710				
ApaI	1	1404				
ApaBI	1	877				
ApoI	7	192	287	1468	4109	4293
		4999	5010			
AvaI	3	158	228	4368		
AvaII	5	1745	2121	2209	2300	2579
BamHI	1	198				
BanI	10	251	327	515	536	650
		1113	1832	1962	2088	5234
BanII	7	190	235	577	591	1404
		4151	5272			
BbsI	4	1339	1678	2052	2412	
BbvI	25					
BccI	14					
Bce83I	6	21	2007	2177	3385	3683
		3924				
BcefI	6	712	1053	1680	3796	4815
		5223				
BcgI	8	160	194	1485	1519	2019
		2053	2871	2905		
BclI	1	1207				
BfaI	6	70	402	2308	3789	4096
		5348				
BglI	1	2257				
BglII	1	258				
BmgI	1	1402				
BpmI	4	1031	1520	2154	2821	
Bpu10I	2	2400	4513			
Bpu1102I	1	80				
BsaAI	2	3046	5197			
BsaBI	3	466	476	2491		
BsaHI	5	516	537	651	1150	1833
BsaJI	11					
BsaWI	7	2	1512	2015	2483	3500
		3647	4631			
BsaXI	2	1852	5145			
BsbI	2	3010	5104			
BscGI	11					
BseRI	1	234				
BsgI	3	1044	1244	2454		
BsiI	1	3467				
BsiEI	5	169	1978	3210	3634	4496
BsII	26					
BsmI	2	4380	4457			
BsmAI	6	890	1295	1421	1808	2935
		4512				
BsmBI	3	1808	2935	4512		
BsmFI	4	654	2195	2565	5412	
BsoFI	43					
Bsp24I	10	483	515	1034	1066	1336
		1368	3787	3819	3965	3997
Bsp1286I	13					
BspEI	2	2	2483			
BspGI	1	2820				
BspLU11I	1	3294				
BsrI	21					
BsrBI	4	422	3227	4895	5341	
BsrDI	2	1240	1606			
BsrFI	7	503	512	879	2091	2251
		4450	5298			
BssHII	1	1604				

Enzyme	# Sites	Locations				
Bst1107I	1	3065				
BstEII	1	1374				
BstXI	3	995	1124	1247		
BstYI	9	132	198	258	757	1969
		2486	3935	3946	4745	
Cac8I	40					
CjeI	24					
CjePI	18					
ClaI	2	470	4187			
CviJI	85					
CviRI	23					
DdeI	11					
DpnI	23					
DraIII	1	5197				
DrdI	3	2987	3402	5152		
DrdII	2	916	5202			
DsaI	3	211	630	2266		
EaeI	4	166	501	633	1867	
EagI	1	166				
EarI	3	811	3178	4309		
EciI	3	970	3368	3514		
Eco47III	3	598	2099	2548		
Eco57I	1	3842				
EcoNI	2	728	4408			
Eco0109I	3	53	626	2300		
EcoRI	1	192				
EcoRII	9	916	1231	1771	1828	3320
		3441	3454	4384	4741	
EcoRV	1	206				
FauI	17					
FokI	9	1239	1248	2513	2575	2653
		2839	2980	4134	4740	
FspI	1	2275				
GdiII	4	166	501	633	1867	
HaeI	6	921	2242	3309	3320	3772
		4583				
HaeII	14					
HaeIII	23					
HgaI	11					
HgiEII	2	791	3880			
HhaI	46					
Hin4I	4	203	1092	4182	4724	
HincII	2	181	1699			
HindIII	1	173				
HinfI	18					
HpaI	1	1699				
HphI	16					
KpnI	1	255				
MaeII	14					
MaeIII	16					
MboII	13					
MluI	1	1193				
MmeI	7	3509	3693	4138	4332	4694
		4703	5174			
MnlI	26					
MseI	25					
MslI	6	1245	1533	1563	2281	2476
		2867				
MspI	30					
MspA1I	9	84	300	1223	1793	1886
		2885	3004	3636	3881	
MwoI	39					
NarI	4	516	537	651	1833	
NciI	14					
NcoI	1	211				
NdeI	1	363				
NgoAIV	4	503	2091	2251	5298	
NlaIII	26					
NlaIV	23					
NotI	1	166				
NruI	1	4153				
NsiI	2	4346	4612			

Enzyme	# Sites	Locations				
NspI	4	668	2639	2931	3298	
NspV	1	285				
Pfl1108I	1	2080				
PflMI	3	277	775	4759		
PleI	9	450	742	829	1625	3188
		3673	4728	5132	5140	
PshAI	1	2038				
Psp5II	1	2300				
Psp1406I	4	855	2223	2619	4982	
PvuI	1	4496				
PvuII	3	1793	1886	2885		
RcaI	3	591	4014	4889		
RsaI	4	253	1340	3100	4331	
SacI	1	190				
SalI	1	179				
SapI	1	3178				
Sau96I	14					
Sau3AI	23					
ScrFI	23					
SfaNI	23					
SfcI	4	435	3559	3750	5416	
SgfI	1	4496				
SgrAI	1	512				
SmaI	2	230	4370			
SphI	1	668				
SrfI	1	230				
SspI	2	4421	4989			
StyI	2	57	211			
TaqI	17					
TaqII	6	1101	1319	1992	3196	4750
		5101				
TfiI	9	1872	2174	2344	2848	3269
		4407	4463	4635	4726	
ThaI	36					
TseI	25					
Tsp45I	7	1374	2202	2733	2946	3041
		4643	5370			
Tsp509I	21					
Tth111I	1	3039				
Tth111II	8	1032	1725	2755	3884	3891
		3923	4332	4459		
UbaJI	18					
VspI	5	450	1878	1937	4695	4884
XbaI	1	401				
XcmI	3	1049	1565	1583		
XhoI	1	158				
XmnI	2	2852	4885			

Enzymes that do not cut pET-30 Ek/LIC:

AatII	AflII	AgeI	AscI	AvrII
BaeI	BsaI	BspMI	BsrGI	Bsu36I
DraI	Eam1105I	FseI	MscI	MunI
NheI	PacI	PmeI	PmlI	PstI
RleAI	RsrII	SacII	ScaI	SexAI
SfiI	SnaBI	SpeI	Sse8387I	StuI
SunI	SwaI			