

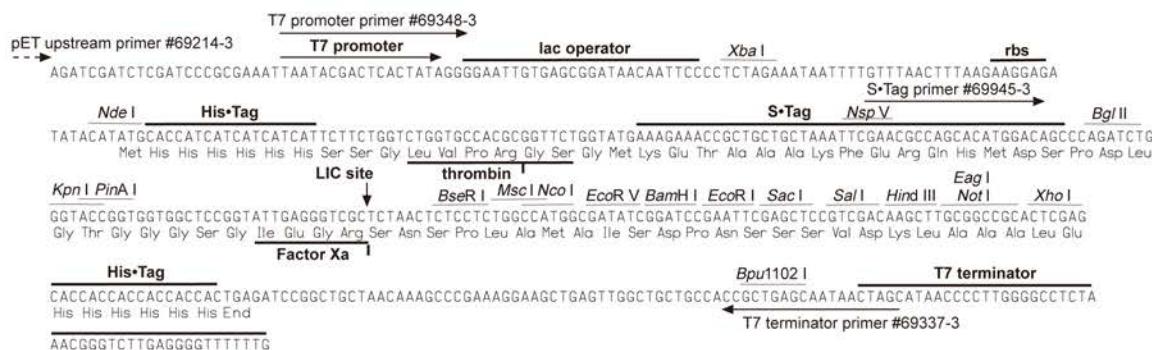
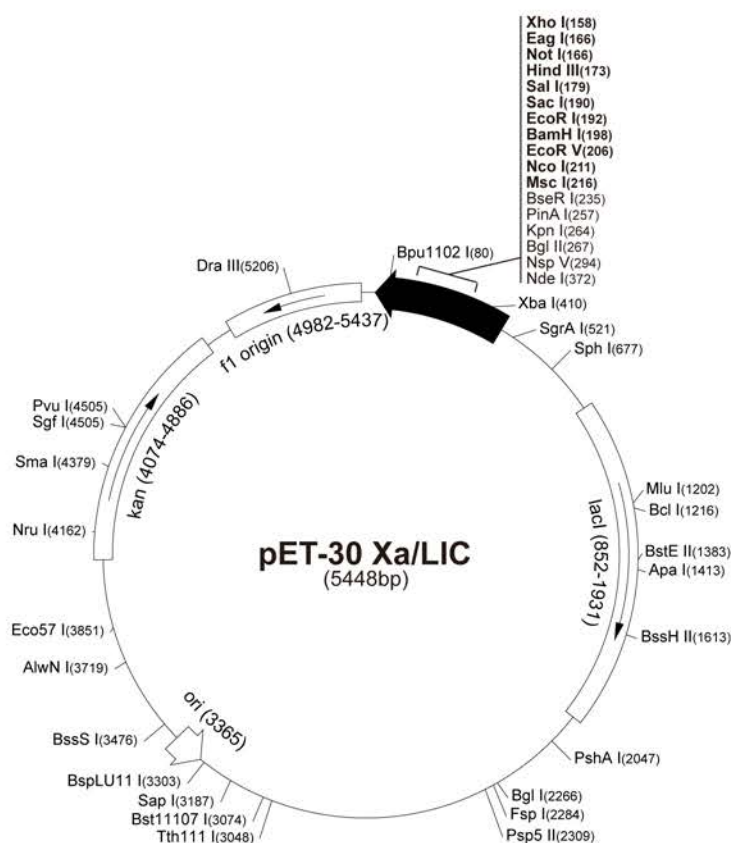
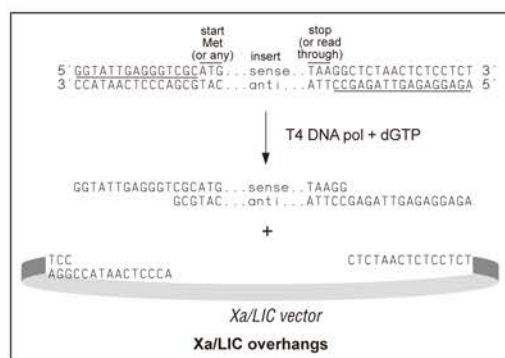
pET-30 Xa/LIC Vector

The pET-30 Xa/LIC vector is designed for rapid, directional cloning of PCR-amplified DNA for high-level expression of polypeptides containing N-terminal His•Tag[®] and S•Tag[™] sequences for detection and purification. Using specifically designed primers for amplification and the pET-30 Xa/LIC Vector Kit (Cat. No. 69073-3), inserts can be efficiently cloned without the need for restriction digestion or ligation, and the resulting fusion proteins can be cleaved with Factor Xa to precisely remove all vector-encoded amino acids. 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 Xa/LIC sequence landmarks

T7 promoter	445-463
T7 transcription start	444
His•Tag coding sequence	353-370
S•Tag coding sequence	275-319
Multiple cloning sites (<i>Bse</i> R I - <i>Xho</i> I)	158-225
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lac</i> I coding sequence	852-1931
pBR322 origin	3365
Kan coding sequence	4074-4886
f1 origin	4982-5437

Primer sequence extensions required for Xa/LIC compatibility are underlined in the diagram below.



pET-30 Xa/LIC cloning/expression region

pET-30 Xa/LIC Restriction Sites

Enzyme	# Sites	Locations					
AccI	2	180	3073				
AcII	75						
AluIII	2	1202	3303				
AluI	22						
AlwI	13						
Alw26I	6	899	1304	1430	1817	2944	
		4521					
AlwNI	1	3719					
Apal	1	1413					
ApaLI	3	1182	3117	3617			
ApoI	7	192	296	1477	4118	4302	
		5008	5019				
AvaI	2	158	4377				
Avall	5	1754	2130	2218	2309	2588	
BamHI	1	198					
BanI	10	260	336	524	545	659	
		1122	1841	1971	2097	5243	
BanII	6	190	586	600	1413	4160	
		5281					
BbsI	4	1348	1687	2061	2421		
BbvI	25						
BcgI	4	194	1494	2062	2914		
Bcgl'	4	160	1528	2028	2880		
BclI	1	1216					
Bfal	6	70	411	2317	3798	4105	
		5357					
BglI	1	2266					
BglII	1	267					
BpmI	4	1040	1529	2163	2830		
Bpu10I	2	2409	4522				
Bpu1102I	1	80					
BsaAI	2	3055	5206				
BsaBI	3	475	485	2500			
BsaHI	5	525	546	660	1159	1842	
BsaJI	10	57	211	639	645	1837	
		2275	3463	4376	4377	4778	
BsaWI	9	2	245	257	1521	2024	
		2492	3509	3656	4640		
BseRI	1	235					
BsgI	3	1053	1253	2463			
BsiEI	5	169	1987	3219	3643	4505	
BsiHKAII	7	159	190	702	1186	2297	
		3121	3621				
BsII	26						
BsmI	2	4389	4466				
BsmBI	3	1817	2944	4521			
BsmFI	4	663	2204	2574	5421		
Bsp1286I	12						
BspEI	2	2	2492				
BspLU11I	1	3303					
BsrI	21						
BsrBI	4	431	3236	4904	5350		
BsrDI	2	1249	1615				
BsrFI	8	257	512	521	888	2100	
		2260	4459	5307			
BssHII	1	1613					
BssSI	1	3476					
Bst1107I	1	3074					
BstEII	1	1383					
BstXI	3	1004	1133	1256			
BstYI	9	132	198	267	766	1978	
		2495	3944	3955	4754		
Cac8I	40						
ClaI	2	479	4196				
CviJI	85						
Ddel	11						
DpnI	23						
DrallI	1	5206					
DrdI	3	2996	3411	5161			
Dsal	3	211	639	2275			
EaeI	5	166	214	510	642	1876	
EagI	1	166					

Enzyme	# Sites	Locations					
EarI	3	820	3187	4318			
Eco47III	3	607	2108	2557			
Eco57I	1	3851					
EcoNI	2	737	4417				
EcoO109I	3	53	635	2309			
EcoRI	1	192					
EcoRII	9	925	1240	1780	1837	3329	
		3450	3463	4393	4750		
EcoRV	1	206					
FauI	17						
Fnu4HI	43						
FokI	9	1248	1257	2522	2584	2662	
		2848	2989	4143	4749		
FspI	1	2284					
HaeII	14						
HaeIII	24						
HgaI	11						
HhaI	46						
HincII	2	181	1708				
HindIII	1	173					
HinfI	18						
HpaI	1	1708					
HphI	16						
KpnI	1	264					
MaeIII	16						
MbolI	13						
MluI	1	1202					
MnII	27						
MscI	1	216					
MseI	25						
MsiI	6	1254	1542	1572	2290	2485	
		2876					
MspI	31						
MspA1I	9	84	309	1232	1802	1895	
		2894	3013	3645	3890		
MwoI	39						
NarI	4	525	546	660	1842		
NciI	12						
NcoI	1	211					
NdeI	1	372					
NgoAIV	4	512	2100	2260	5307		
NlaIII	26						
NlaIV	24						
NottI	1	166					
NruI	1	4162					
NsiI	2	4355	4621				
NspI	4	677	2648	2940	3307		
NspV	1	294					
PIIMI	3	286	784	4768			
PinAI	1	257					
PleI	9	459	751	838	1634	3197	
		3682	4737	5141	5149		
PshAI	1	2047					
Psp1406I	4	864	2232	2628	4991		
Psp5II	1	2309					
PvuI	1	4505					
PvuII	3	1802	1895	2894			
RcaI	3	600	4023	4898			
RsaI	4	262	1349	3109	4340		
SacI	1	190					
Sall	1	179					
SapI	1	3187					
Sau3AI	23						
Sau96I	14						
ScrFI	21						
StaNII	23						
StcI	4	444	3568	3759	5425		
SgfI	1	4505					
SgrAI	1	521					
SmaI	1	4379					
SphI	1	677					
SspI	2	4430	4998				

Enzyme	# Sites	Locations					
StyI	2	57	211				
TaiI	14						
TaqI	17						
TfiI	9	1881	2183	2353	2857	3278	
		4416	4472	4644	4735		
ThaI	36						
TseI	25						
Tsp45I	7	1383	2211	2742	2955	3050	
		4652	5379				
Tsp509I	21						
TspRI	13						
Tth111I	1	3048					
VspI	5	459	1887	1946	4704	4893	
XbaI	1	410					
XcmI	3	1058	1574	1592			
XhoI	1	158					
XmnI	2	2861	4894				

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

AatII	AflIII	AhdI	AscI	AvrII
BsaI	BspMI	BsrGI	Bsu36I	DraI
FseI	MunI	NheI	PacI	PmeI
PmlI	PstI	RsrII	SacII	SanDI
Scal	SexAI	SfiI	SnaBI	SpeI
SrfI	Sse8387I	StuI	SunI	Swal
UbaEI				