pET-30 Xa/LIC Vector

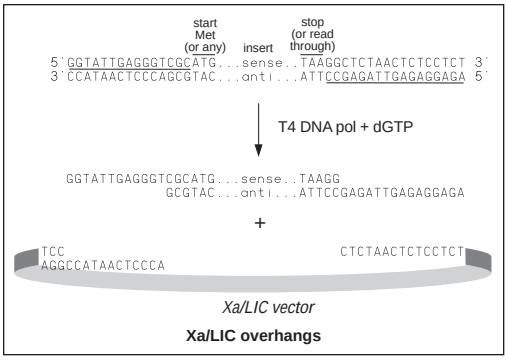
TB185 12/98

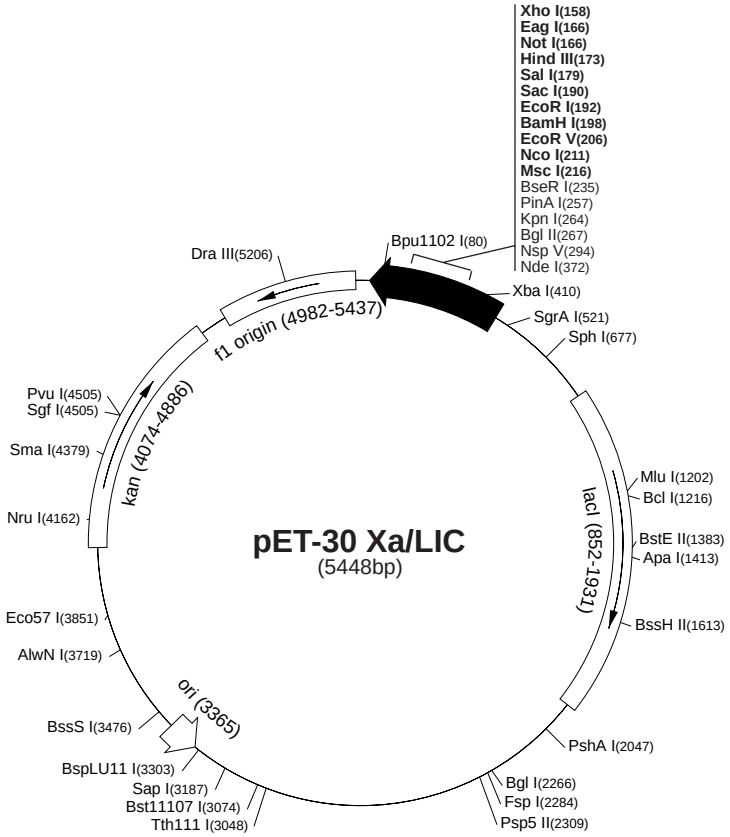
The pET-30 Xa/LIC vector is designed for rapid, directional cloning of PCR-amplified DNA for high-level expression of polypeptides containing N-terminal His•Tag® and S•Tag™ sequences for detection and purification. Using specifically designed primers for amplification and the pET-30 Xa/LIC Vector Kit (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).

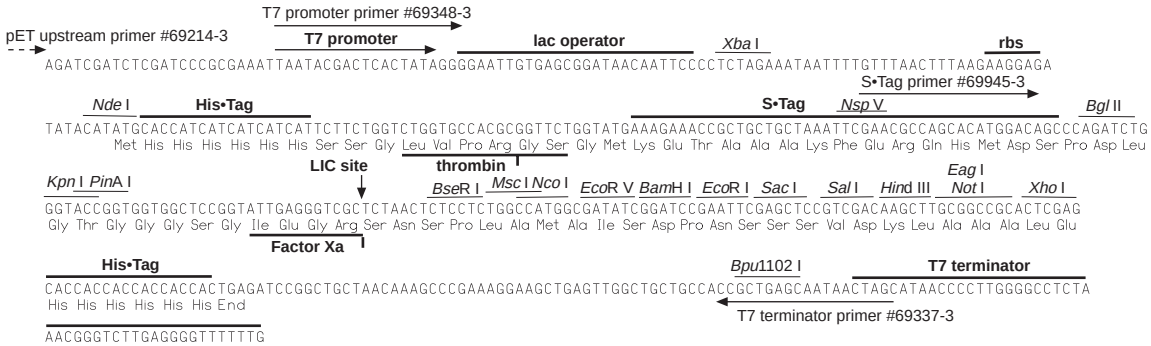
pET-30 Xa/LIC sequence landmarks

T7 promoter	445-463
T7 transcription start	444
His•Tag coding sequence	353-370
S•Tag coding sequence	275-319
Multiple cloning sites	
(*Bse*R I - *Xho* I)	158-225
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI 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					
AflIII	2	1202	3303			
AluI	22					
AlwI	13					
Alw26I	6	899	1304	1430	1817	2944
		4521				
AlwNI	1	3719				
ApaI	1	1413				
ApaLI	3	1182	3117	3617		
ApoI	7	192	296	1477	4118	4302
		5008	5019			
AvaI	2	158	4377			
AvaII	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	
BcgI'	4	160	1528	2028	2880	
BclI	1	1216				
BfaI	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
BsiHKAI	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					
DdeI	11					
DpnI	23					
DraIII	1	5206				
DrdI	3	2996	3411	5161		
DsaI	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					
MboII	13					
MluI	1	1202				
MnlI	27					
MscI	1	216				
MseI	25					
MslI	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					
NotI	1	166				
NruI	1	4162				
NsiI	2	4355	4621			
NspI	4	677	2648	2940	3307	
NspV	1	294				
PflMI	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				
SalI	1	179				
SapI	1	3187				
Sau3AI	23					
Sau96I	14					
ScrFI	21					
SfaNI	23					
SfcI	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	AflII	AhdI	AscI	AvrII
BsaI	BspMI	BsrGI	Bsu36I	DraI
FseI	MunI	NheI	PacI	PmeI
PmlI	PstI	RsrII	SacII	SanDI
ScaI	SexAI	SfiI	SnaBI	SpeI
SrfI	Sse8387I	StuI	SunI	SwaI
UbaEI				