

pET-32 Xa-LIC Vector

Cat. No. 70072-3

pET-32 Xa-LIC sequence landmarks

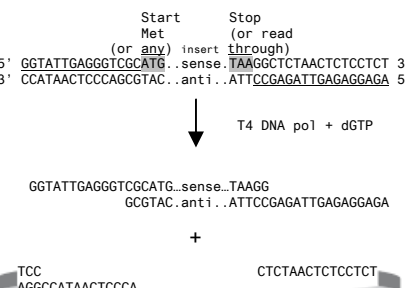
T7 promoter	790-806
T7 transcription start	789
Trx•Tag coding sequence	392-718
His•Tag coding sequence	353-370
S•Tag coding sequence	275-319
Multiple cloning sites	
(BseR I - Xho I)	158-225
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	1197-2276
pBR322 origin	3710
bla coding sequence	4471-5328
f1 origin	5460-5915

Notes

All corresponding maps and tables are generated using VectorNTI® software. Restriction sites are numbered in accordance with the conventions of the respective software.

The *SrfI* and *SmaI* sites are destroyed during Ligation Independent Cloning.

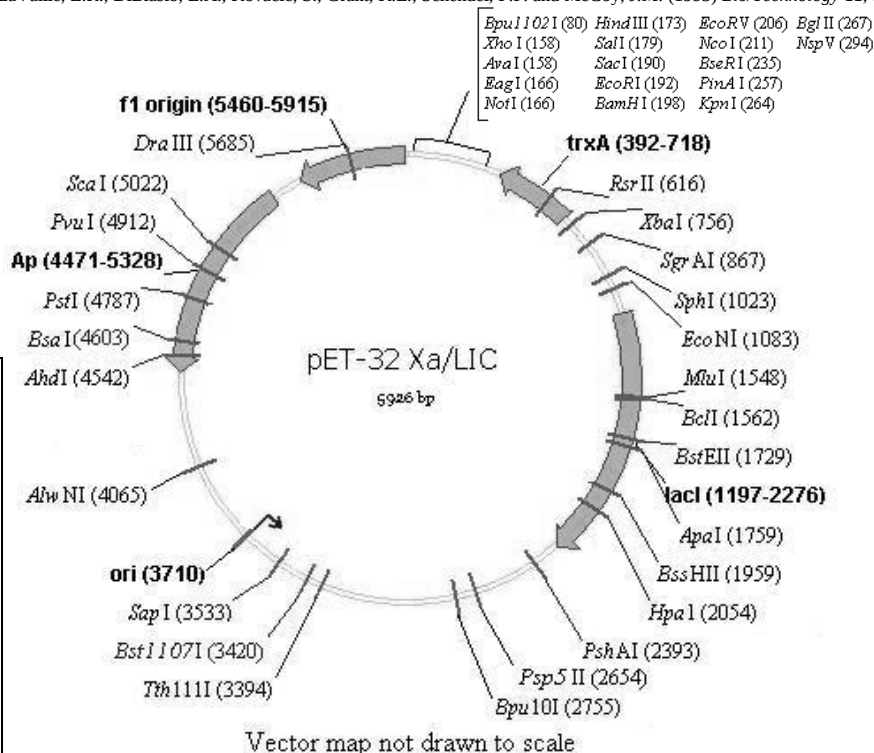
Primer sequence extensions required for Xa/LIC compatibility are underlined in the overhangs diagram below.



The pET-32 Xa/LIC vector is designed for rapid, directional cloning of PCR-amplified DNA for high-level expression of polypeptides fused with the 109aa Trx•Tag™ thioredoxin protein (1), His•Tag® and S•Tag™ sequences. With the use of specifically designed primers for amplification and the pET-32 Xa/LIC Vector Kit, 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).

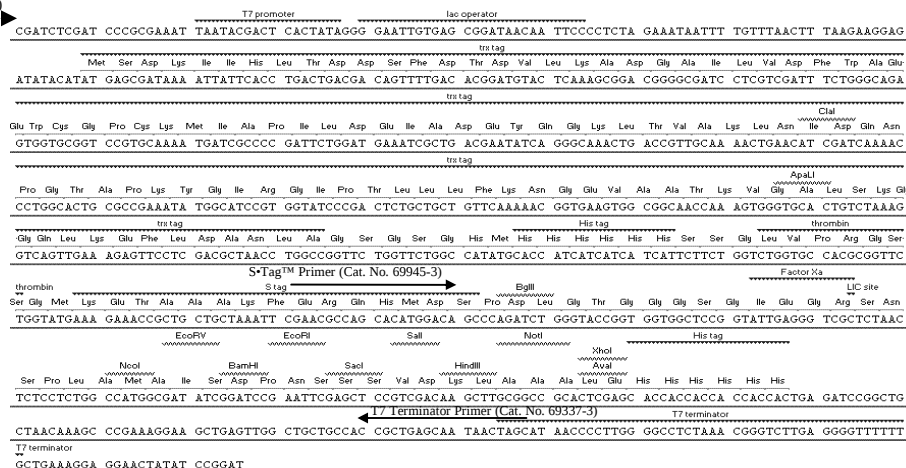
References

1. LaVallie, E.R., DiBlasio, E.A., Kovacic, S., Grant, K.L., Schendel, P.F. and McCoy, J.M. (1993) *Bio/Technology* **11**, 187-193



Vector map not drawn to scale

T7 Promoter Primer (Cat. No. 69214-3)



pET-32 Xa-LIC cloning expression region

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Enzyme	#	Locations						Enzyme	#	Locations						Enzyme	#	Locations					
AccI	2	181	3419					BssHII	1	1959					NlaIII	26							
AcII	85							BssSI	2	3822	5206				NlaIV	28							
AflIII	2	1548	3649					Bst1107I	1	3420					NotI	1	167						
AhdI	1	4542						BstEII	1	1729					NspI	4	1023	2994	3286	3653			
AluI	25							BstXI	3	1350	1479	1602			NspV	1	295						
AlwI	17							BstYI	12						PfIMI	2	287	1130					
Alw26I	7	1245	1650	1776	2163	3290		Cac8I	39						PinAI	1	258						
		4603	5379					ClaI	2	535	825				PleI	10	493	805	1097	1184	1980		
AlwNI	1	4065						CviJI	90								3543	4028	4531	5620	5628		
Apal	1	1759						DdeI	11						PshAI	1	2393						
ApaLI	5	437	1528	3463	3963	5209		DpnI	32						Psp1406I	6	1210	2578	2974	4768	5141		
ApoI	5	193	297	1823	5487	5498		DraI	3	4408	4427	5119					5470						
AvaI	1	159						DraIII	1	5685					Psp5II	1	2655						
Avall	8	616	2100	2476	2564	2655		DrdI	3	3342	3757	5640			PstI	1	4787						
		2934	4680	4902				Dsal	4	212	496	985	2621		PvuI	1	4912						
BamHI	1	199						EaeI	8	167	215	376	392	856	PvuII	3	2148	2241	3240				
BanI	11									988	2222	4930			RcaI	3	946	4369	5377				
BanII	5	191	932	946	1759	5760		EagI	1	167					RsaI	5	263	669	1695	3455	5022		
BbsI	4	1694	2033	2407	2767			EarI	3	1166	3533	5337			RsrII	1	616						
BbvI	29							Eco47III	3	953	2454	2903			SacI	1	191						
Bcgl	7	195	502	591	1840	2408		Eco57I	2	4197	5209				Sall	1	180						
		3260	5047					EcoNI	1	1083					SapI	1	3533						
Bcgl'	7	161	468	625	1874	2374		EcoO109I	3	54	981	2655			Sau3AI	32							
		3226	5081					EcoRI	1	193					Sau96I	19							
BclI	1	1562						EcoRII	9	394	522	1271	1586	2126	Scal	1	5022						
Bfal	7	71	757	2663	4144	4397				2183	3675	3796	3809	ScrFI	21								
		4732	5836					EcoRV	1	207					SfaNI	21							
BglI	2	2612	4662					FauI	17						Sfcl	5	790	3914	4105	4782	5904		
BglII	1	268						Fnu4HI	50						SgrA1	1	867						
BpmI	5	1386	1875	2509	3176	4612		FokI	13						SphI	1	1023						
Bpu10I	1	2755						FspI	2	2630	4764				SspI	2	5346	5477					
Bpu1102I	1	81						Haell	14						StyI	2	58	212					
BsaI	1	4603						HaellI	27						TaiI	15							
BsaAI	2	3401	5685					Hgal	13						TaqI	18							
BsaBI	3	821	831	2846				HhaI	46						TfiI	6	593	2227	2529	2699	3203		
BsaHI	6	871	892	1006	1505	2188		HincII	2	182	2054						3624						
		5079						HindIII	1	174					TseI	29							
BsaJI	9	58	212	496	523	985		HinfI	16						Tsp45I	8	1729	2557	3088	3301	3396		
		991	2183	2621	3809			HpaI	1	2054							4798	5009	5858				
BsaWI	9	3	246	258	1867	2370		HphI	18						Tsp509I	18							
		2838	3855	4002	4833			KpnI	1	265					TspRI	17							
BseRI	1	236						MaeIII	18						Tth111I	1	3394						
BsgI	3	1399	1599	2809				MbolI	15						XbaI	1	756						
BsiEI	6	170	2333	3565	3989	4912		MluI	1	1548					XcmI	3	1404	1920	1938				
		5061						MnlI	30						XhoI	1	159						
BsiHKAI	10	160	191	441	1048	1532		MscI	2	217	378				Xmnl	3	415	3207	5141				
		2643	3467	3967	5128	5213		MseI	28														
BslI	25							MslI	9	1600	1888	1918	2636	2831									
BsmBI	2	2163	3290							3222	4794	4953	5312										
BsmFI	4	1009	2550	2920	5900			MspI	34														
Bsp1286I	14							MspA1I	10	85	310	1578	2148	2241									
BspEI	2	3	2838							3240	3359	3991	4236	5177									
BspLU11I	1	3649						MwoI	41														
BsrI	25							NarI	4	871	892	1006	2188										
BsrBI	4	777	3582	5383	5829			NciI	12														
BsrDI	4	1595	1961	4603	4777			NcoI	1	212													
BsrFI	9	258	390	858	867	1234		NdeI	2	373	718												
		2446	2606	4622	5786			NgoAIV	4	858	2446	2606	5786										

Enzymes that do not cut pET-32 Xa/LIC:

AatII	AflII	AscI	AvrII	BsmI
BspMI	BsrGI	Bsu36I	FseI	MunI
NheI	NruI	NsiI	Pacl	PmeI
PmlI	SacII	SanDI	SexAI	SfiI
Sgfi	SmaI	SnaBI	SpeI	SrfI
Sse8387I	StuI	SunI	SwaI	UbaEI

Enzymes that do not cut pET-32 Xa/LIC:					
AatII	AflII	AscI	AvrII	BsmI	
BspMI	BsrGI	Bsu36I	FseI	MunI	
NheI	NruI	NsiI	PacI	PmeI	
PmlI	SacII	SanDI	SexAI	SfiI	
SgfI	SmaI	SnaBI	SpeI	SrfI	
Sse8387I	StuI	SunI	SwaI	UbaEI	

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