

pET-32 Ek/LIC Vector

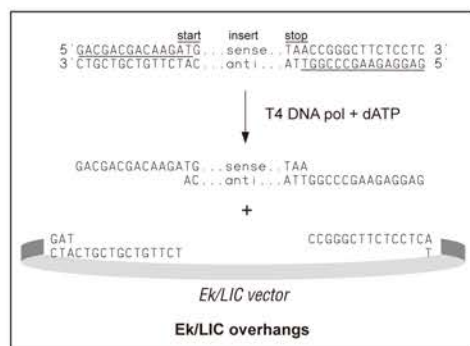
The pET-32 Ek/LIC vector is prepared for rapid, directional cloning of PCR-amplified DNA for high-level expression of polypeptides fused with the 109aa Trx•Tag™ thioredoxin protein (1). Using specifically designed primers for amplification and the pET-32 Ek/LIC Cloning Kit (Cat. No. 69076-3), inserts can be efficiently cloned without the need for restriction digestion or ligation. Fusion proteins also contain 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-32 Ek/LIC sequence landmarks

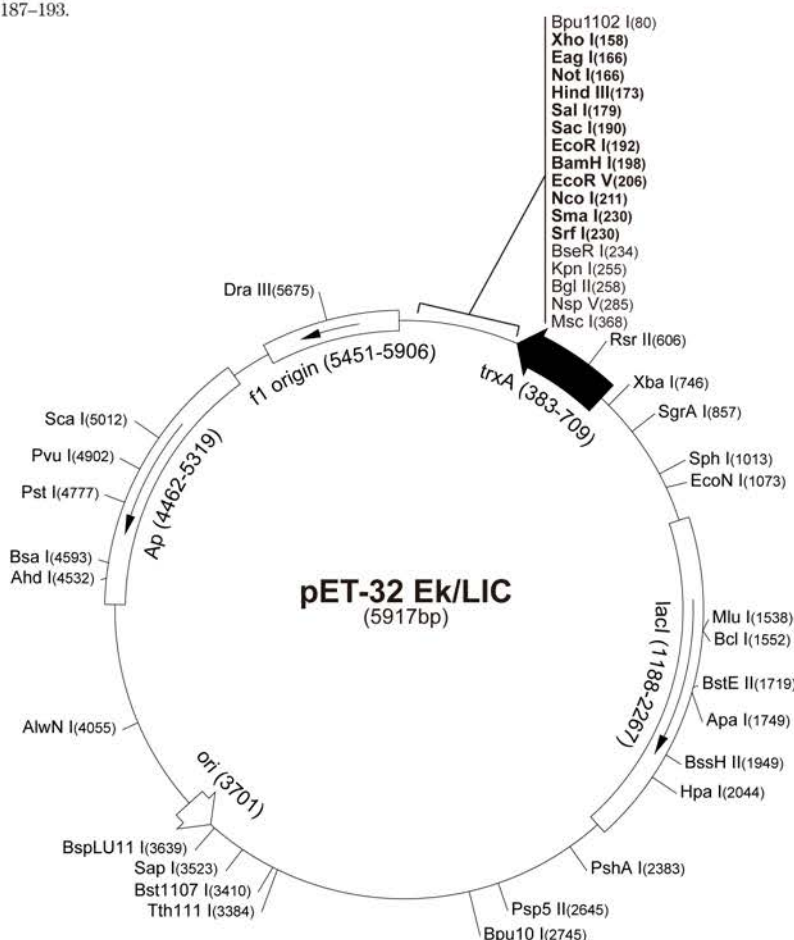
T7 promoter	781-797
T7 transcription start	780
Trx•Tag coding sequence	383-709
His•Tag coding sequence	344-361
S•Tag coding sequence	266-310
Multiple cloning sites	
(BseR I - Xho I)	158-224
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	1188-2267
pBR322 origin	3701
bla coding sequence	4462-5319
f1 origin	5451-5906

Notes: the *Srf*I and *Sma*I sites are destroyed during Ligation Independent Cloning.

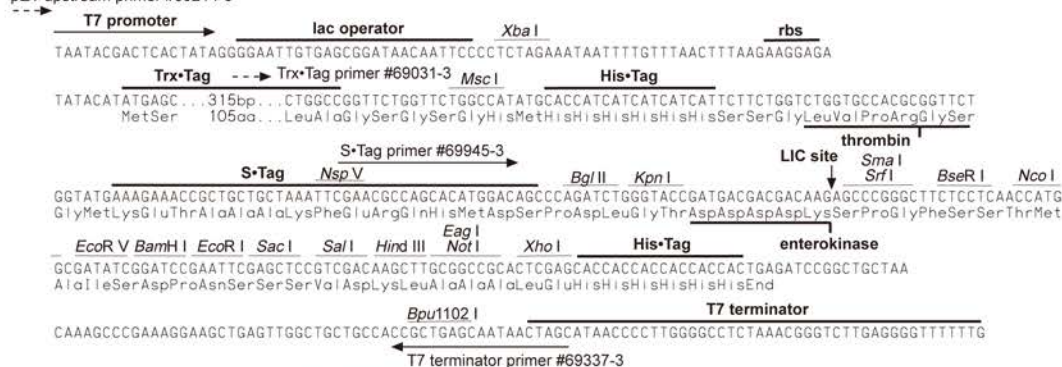
Primer sequence extensions required for LIC compatibility are underlined in the diagram below.



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.



pET upstream primer #69214-3



pET-32 Ek/LIC cloning/expression region

pET-32 Ek/LIC Restriction Sites

Enzyme	# Sites	Locations					Enzyme	# Sites	Locations					Enzyme	# Sites	Locations				
AccI	2	180	3409				Bst1107I	1	3410				NspI	4	1013	2984	3276	3643		
AccII	8	1305	2033	2364	3148	3289	BstEII	1	1719				NspV	1	285					
		3591	4831	5515			BstXI	3	1340	1469	1592		Pfl1108I	2	2425	4550				
Acil	85						BstYI	12					PfIIMI	2	277	1120				
AtfIII	2	1538	3639				Cac8I	39					PleI	10	483	795	1087	1174		
AluI	25						CjeI	26							3533	4018	4521	5610		
AlwI	17						CjePI	18					PshAI	1	2383			5618		
Alw21I	10	159	190	431	1038	1522	Clal	2	525	815			Psp5II	1	2645					
		2633	3457	3957	5118	5203	CviJI	90					Psp1406I	6	1200	2568	2964	4758		
Alw44I	5	427	1518	3453	3953	5199	CviRI	28							5460			5131		
AlwNI	1	4055					Ddel	11					PstI	1	4777					
Apal	1	1749					DpnI	32					PvuI	1	4902					
ApaBI	1	1222					DraI	3	4398	4417	5109		PvuII	3	2138	2231	3230			
ApoI	5	192	287	1813	5477	5488	DraIII	1	5675				RcaI	3	936	4359	5367			
AvaI	2	158	228				DrdI	3	3332	3747	5630		RsaI	5	253	659	1685	3445		
AvaII	8	606	2090	2466	2554	2645	DrdII	3	374	1261	5680		RsrII	1	606			5012		
		2924	4670	4892			DsaI	4	211	486	975	2611	SacI	1	190					
BamHI	1	198					EaeI	7	166	366	382	846	Sall	1	179					
BanI	11								2212	4920			SapI	1	3523					
BanII	6	190	235	922	936	1749	EagI	1	166				Sau96I	19						
		5750					Eam1105I	1	4532				Sau3AI	32						
BbsI	4	1684	2023	2397	2757		EarI	3	1156	3523	5327		Scal	1	5012					
BbvI	29						Ecil	4	1315	3713	3859	4687	ScrFI	23						
BccI	14						Eco47III	3	943	2444	2893		SfaNI	21						
Bce83I	7	21	2352	2522	3730	4028	Eco57I	2	4187	5199			Sfcl	5	780	3904	4095	4773		
		4269	5137				EcoNI	1	1073				SgrAI	1	857			5894		
BceII	5	1057	1398	2025	4141	5701	EcoO109I	3	53	971	2645		SmaI	1	230					
Bcgl	14						EcoRI	1	192				SphI	1	1013					
BclI	1	1552					EcoRII	9	384	512	1261	1576	SrfI	1	230					
Bfal	7	70	747	2653	4134	4387			2173	3665	3786	3799	Sspl	2	5336	5467				
		4722	5826				EcoRV	1	206				StyI	2	57	211				
BglI	2	2602	4652				FauI	17					TaqI	18						
BglII	1	258					FokI	13					TaqII	10	446	1446	1664	2337		
BmgI	1	1747					FspI	2	2620	4754					4880	5065	5218	2337		
BpmI	5	1376	1865	2499	3166	4602	GdiII	6	166	382	846	978			583	2217	2519	2689		
Bpu10I	1	2745							4920						3614			3193		
Bpu1102I	1	80					HaeI	6	368	1266	2587	3654	3665							
Bsal	1	4593							4117					Thal	35					
BsaAI	2	3391	5675				HaeII	14						TseI	29					
BsaBI	3	811	821	2836			HaeIII	26						Tsp45I	8	1719	2547	3078		
BsaHI	6	861	882	996	1495	2178	HgaI	13							4788	4999	5848	3291		
		5069					HgiEI	3	436	1136	4225			Tsp509I	18			3386		
BsaJI	10	57	211	228	486	513	HhaI	46						Tth111I	1	3384				
		975	981	2173	2611	3799	Hin4I	4	203	1437	4531	4605		Tth111II	6	1377	2070	3100		
BsaWI	7	2	1857	2360	2828	3845	HincII	2	181	2044					4268			4229		
		3992	4823				HindIII	1	173					UbaJI	21					
BsaXI	2	2197	5623				HinfI	16						VspI	4	795	2223	2282		
Bsbl	2	3355	5582				HpaI	1	2044					XbaI	1	746		4704		
BscGI	14						HphI	18						XcmI	3	1394	1910	1928		
BseRI	1	234					KpnI	1	255					XhoI	1	158				
BsgI	3	1389	1589	2799			MaeI	15						Xmnl	3	405	3197	5131		
Bsil	2	3812	5196				MaeIII	18					Enzymes that do not cut pET-32 Ek/LIC:							
BsiEI	6	169	2323	3555	3979	4902	MbolI	15					AatII	AfIII	AgeI	AscI	AvrII			
		5051					MluI	1	1538				BaeI	BsmI	BspMI	BsrGI	Bsu36I			
BsII	25						MmeI	3	3854	4038	5652		FseI	MunI	NheI	NruI	NsiI			
BsmAI	7	1235	1640	1766	2153	3280	MnlI	29					PacI	PmeI	PmlI	RleAI	SacII			
		4593	5369				MscI	1	368				SexAI	SfiI	Sgfi	SnaBI	SpeI			
BsmBI	2	2153	3280				MseI	28					Sse8387I	StuI	SunI	Swal				
BsmFI	4	999	2540	2910	5890		MslI	9	1590	1878	1908	2626								
BsoFI	50								3212	4784	4943	5302								
Bsp24I	10	828	860	1379	1411	1681	MspI	33												
		1713	4132	4164	4310	4342	MspA1I	10	84	300	1568	2138	2231							
Bsp1286I	15								3230	3349	3981	4226	5167							
BspEI	2	2	2828				Mwol	41												
BspGI	1	3165					NarI	4	861	882	996	2178								
BspLU11I	1	3639					NciI	14												
Bsrl	25						NcoI	1	211											
BsrBI	4	767	3572	5373	5819		NdeI	2	363	708										
BsrDI	4	1585	1951	4593	4767		NgoAIV	4	848	2436	2596	5776								
BsrFI	8	380	848	857	1224	2436	NlaIII	26												
		2596	4612	5776			NlaIV	27												
BssHII	1	1949					NotI	1	166											