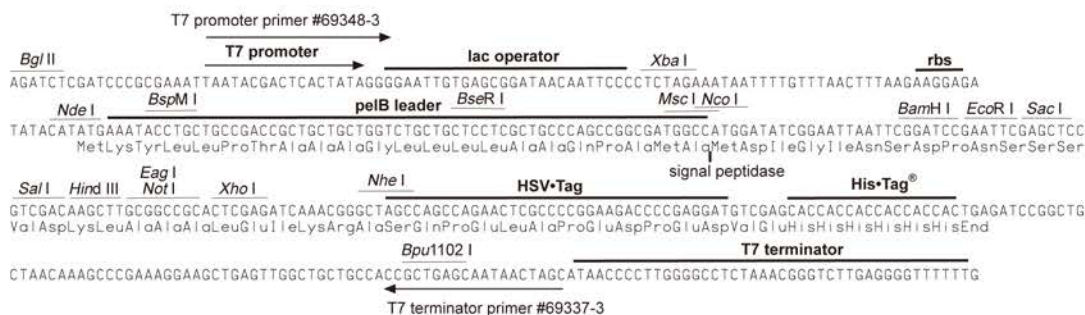
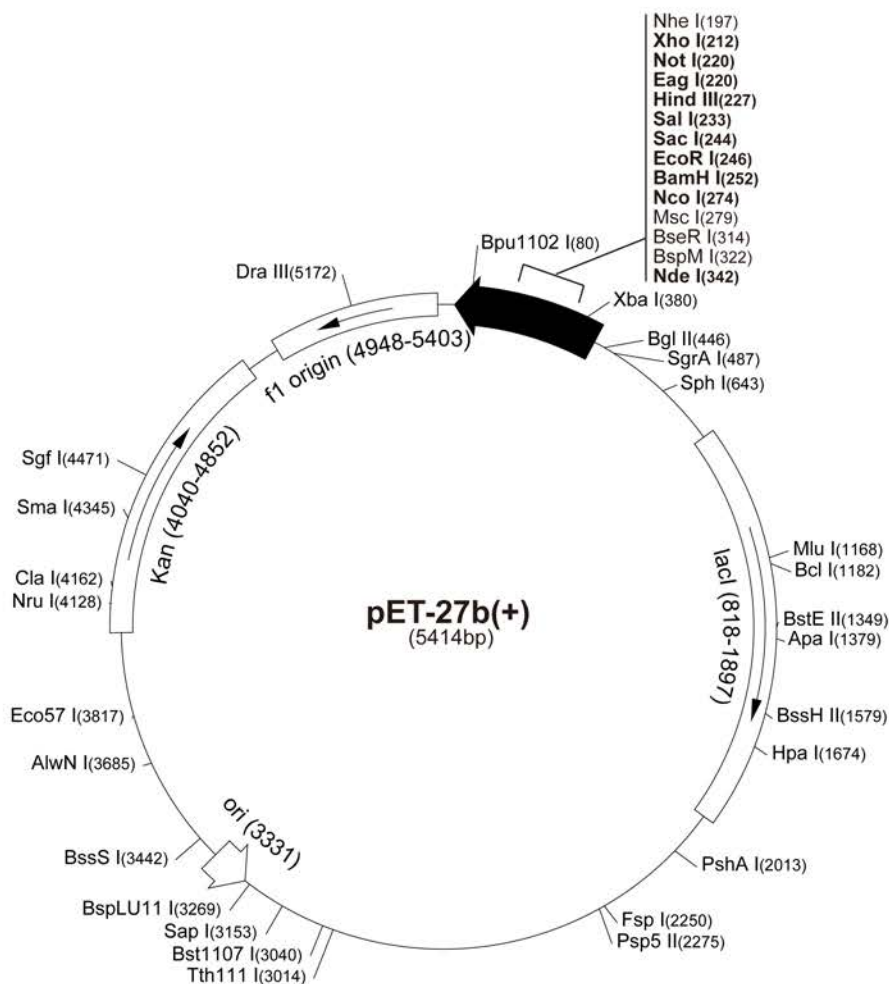


pET-27b(+) Vector

The pET-27b(+) vector (Cat. No. 69863-3) carries an N-terminal *pelB* signal sequence for potential periplasmic localization, plus optional C-terminal HSV•Tag® and His•Tag® sequences. This vector differs from pET-25b(+) only by its selectable marker (kanamycin *vs.* ampicillin resistance). 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 circular 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-27b(+) sequence landmarks

T7 promoter	415-431
T7 transcription start	414
<i>pelB</i> coding sequence	278-343
Multiple cloning sites	
(<i>Nco</i> I - <i>Xho</i> I)	212-279
HSV•Tag coding sequence	164-199
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lacI</i> coding sequence	818-1897
pBR322 origin	3331
Kan coding sequence	4040-4852
f1 origin	4948-5403



pET-27b(+) cloning/expression region

pET-27b(+) Restriction Sites

Enzyme	# Sites	Locations					Enzyme	# Sites	Locations					Enzyme	# Sites	Locations				
AccI	2	234	3039				BsrFI	8	285	478	487	854	2066	NlaIV	21					
AccIII	7	935	1663	1994	2778	2919			2226	4425	5273		NotI	1	220					
		3221	5012				BssHII	1	1579				NruI	1	4128					
Acil	74						Bst1107I	1	3040				NsiI	2	4321	4587				
AlfIII	2	1168	3269				BstEII	1	1349				NspI	4	643	2614	2906	3273		
AluI	22						BstXI	3	970	1099	1222		PII1108I	1	2055					
AlwI	13						BstYI	9	132	252	446	732	1944	PIIMI	2	750	4734			
Alw21I	7	159	244	668	1152	2263			2461	3910	3921	4720	PleI	9	429	717	804	1600	3163	
		3087	3587				Cac8I	42							3648	4703	5107	5115		
Alw44I	3	1148	3083	3583			CjeI	24					PshAI	1	2013					
AlwNI	1	3685					CjePI	18					Psp5II	1	2275					
Apal	1	1379					Clal	1	4162				Psp1406I	4	830	2198	2594	4957		
ApaBI	1	852					CviJI	87					PvuI	1	4471					
ApoI	6	246	1443	4084	4268	4974	CviRI	22					PvuII	3	1768	1861	2860			
		4985					Ddel	11					RcaI	3	566	3989	4864			
AvaI	3	167	212	4343			DpnI	22					RsaI	3	1315	3075	4306			
AvaII	5	1720	2096	2184	2275	2554	DraIII	1	5172				SacI	1	244					
BamHI	1	252					DrdI	3	2962	3377	5127		Sall	1	233					
BanI	8	490	511	625	1088	1807	DrdII	2	891	5177			SapI	1	3153					
		1937	2063	5209			Dsal	3	274	605	2241		Sau96I	14						
BanII	6	244	552	566	1379	4126	EaeI	5	220	277	476	608	1842	Sau3AI	22					
		5247					EagI	1	220					ScrFI	22					
BbsI	5	166	1314	1653	2027	2387	EarI	3	786	3153	4284			SfaNI	23					
BbvI	28						Ecil	3	945	3343	3489			Sfcl	4	414	3534	3725	5391	
BccI	14						Eco47III	3	573	2074	2523			Sgfl	1	4471				
Bce83I	6	21	1982	2152	3360	3658	Eco57I	1	3817					SgrAI	1	487				
		3899					EcoNI	2	703	4383				SmaI	1	4345				
BceII	6	687	1028	1655	3771	4790	EcoO109I	3	53	601	2275			SphI	1	643				
		5198					EcoRI	1	246					Sspl	2	4396	4964			
Bcgl	8	214	248	1460	1494	1994	EcoRII	9	891	1206	1746	1803	3295	StyI	2	57	274			
		2028	2846	2880					3416	3429	4359	4716		TaqI	16					
BclI	1	1182					EcoRV	2	271	1618				TaqII	6	1076	1294	1967	3171	4725
BfaI	7	70	198	381	2283	3764	FauI	17							5076					
		4071	5323				FokI	10	149	1214	1223	2488	2550	TfiI	9	1847	2149	2319	2823	3244
BgII	2	291	2232						2628	2814	2955	4109	4715		4382	4438	4610	4701		
BglII	1	446					FspI	1	2250					ThaI	35					
BmgI	1	1377					GdIII	4	220	476	608	1842		TseI	28					
BpmI	4	1006	1495	2129	2796		HaeI	7	279	896	2217	3284	3295	Tsp45I	7	1349	2177	2708	2921	3016
Bpu10I	2	2375	4488						3747	4558					4618	5345				
Bpu1102I	1	80					HaeII	14						Tsp509I	22					
BsaAI	2	3021	5172				HaeIII	24						Tth111I	1	3014				
BsaBI	3	445	451	2466			HgaI	11						Tth111II	8	1007	1700	2730	3859	3866
BsaHI	5	491	512	626	1125	1808	HgiEII	2	766	3855					3898	4307	4434			
BsaJI	12						HhaI	46						UbaJI	18					
BsaWI	7	2	1487	1990	2458	3475	Hin4I	3	1067	4157	4699			VspI	6	261	429	1853	1912	4670
		3622	4606				HincII	2	235	1674					4859					
BsaXI	2	1827	5120				HindIII	1	227					XbaI	1	380				
Bsbl	2	2985	5079				HinfI	18						XcmI	3	1024	1540	1558		
BscGI	12						Hpal	1	1674					XhoI	1	212				
BseRI	1	314					HphI	16						XmnI	3	262	2827	4860		
BsgI	3	1019	1219	2429			Maell	14						Enzymes that do not cut pET-27b(+):						
Bsil	1	3442					MaellI	16						AatII	AfIII	AgeI	AscI	AvrII		
BsIEI	6	223	325	1953	3185	3609	MbolI	13						Bael	Bsal	BsrGI	Bsu36I	DraI		
		4471					MluI	1	1168					Eam1105I	FseI	KpnI	MunI	NspV		
BslI	23						MmeI	7	3484	3668	4113	4307	4669	Pacl	PmeI	PmlI	PstI	RleAI		
BsmI	2	4355	4432						4678	5149				RsrII	SacII	Scal	SexAI	Sfil		
BsmAI	6	865	1270	1396	1783	2910	MnII	27						SnaBI	SpeI	SrfI	Sse8387I	StuI		
		4487					MscI	1	279					SunI	Swal					
BsmBI	3	1783	2910	4487			Msel	26												
BsmFI	4	629	2170	2540	5387		MslI	6	1220	1508	1538	2256	2451							
BsoFI	46								2842											
Bsp24I	10	458	490	1009	1041	1311	MspI	31												
		1343	3762	3794	3940	3972	MspA1I	9	84	321	1198	1768	1861							
Bsp1286I	12								2860	2979	3611	3856								
BspEI	2	2	2458				Mwol	40												
BspGI	1	2795					NarI	4	491	512	626	1808								
BspLU11I	1	3269					NciI	13												
BspMI	1	322					NcoI	1	274											
Bsrl	21						NdeI	1	342											
BsrBI	4	401	3202	4870	5316		NgoAIV	5	285	478	2066	2226	5273							
BsrDI	2	1215	1581				NheI	1	197											
							NlaIII	25												