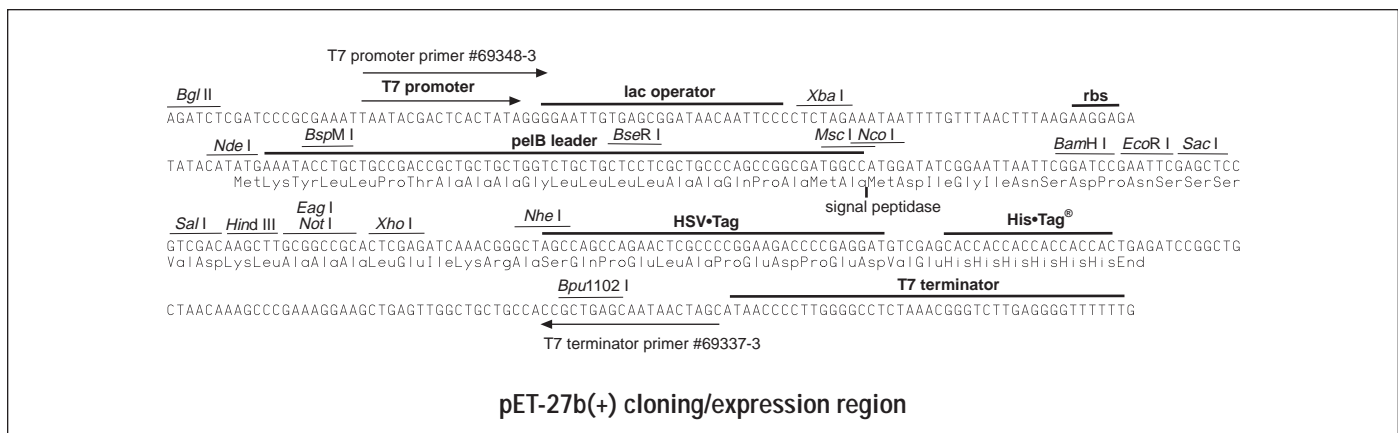
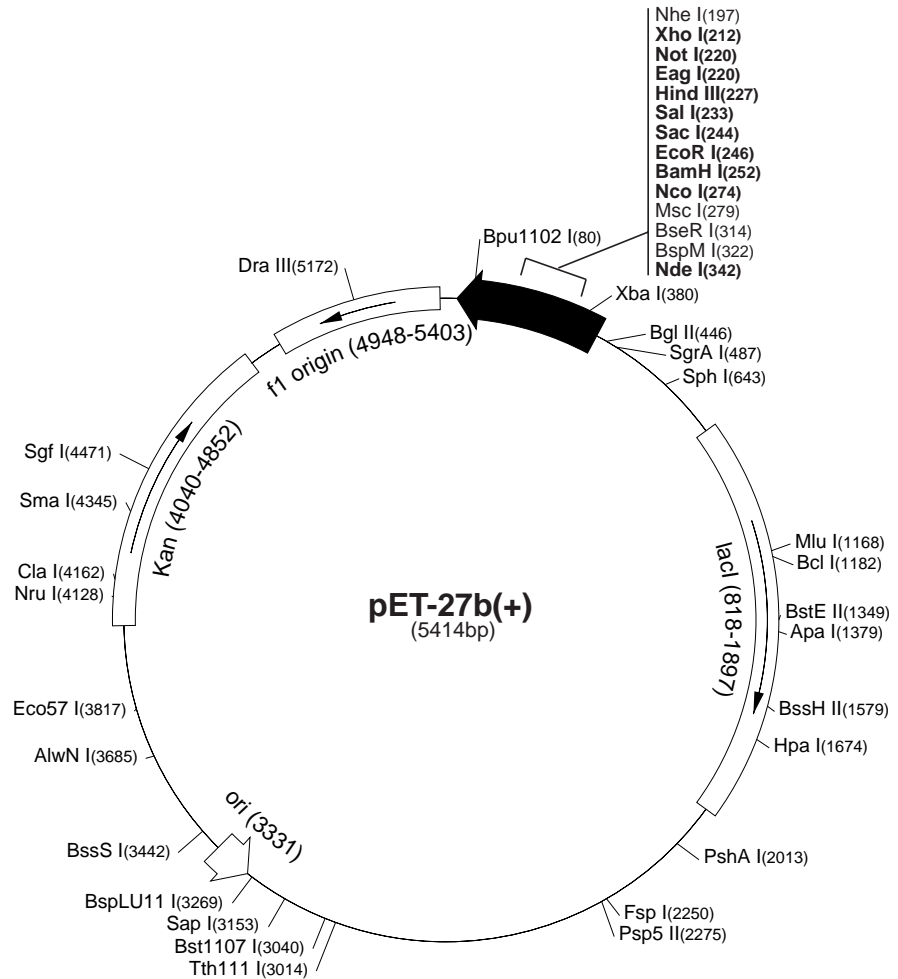


pET-27b(+) Vector

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>lac</i> I coding sequence	818-1897
pBR322 origin	3331
Kan coding sequence	4040-4852
f1 origin	4948-5403



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	BssHIII	1	1579	NruI	1	4128		
Acil	74		Bst1107I	1	3040	Nsil	2	4321 4587		
AfIII	2	1168 3269	BstEII	1	1349	NspI	4	643 2614 2906 3273		
AluI	22		BstXI	3	970 1099 1222	Pfi1108I	1	2055		
AlwI	13		BstYI	9	132 252 446 732 1944	PfiMI	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	DdeI	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	SgfI	1	4471		
Bce83I	6	21 1982 2152 3360 3658	Eco57I	1	3817	SgrAI	1	487		
		3899	EcoNI	2	703 4383	Smal	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		
BcgI	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		
Bfal	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		
BglI	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	Hgal	11		Tth111II	8	1007 1700 2730 3859 3866		
BsaHI	5	491 512 626 1125 1808	HgiEII	2	766 3855			3898 4307 4434		
BsaJI	12		Hhal	46		UbaII	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	Hinfl	18		XcmI	3	1024 1540 1558		
BscGI	12		HpaI	1	1674	XhoI	1	212		
BseRI	1	314	HphI	16		XmnI	3	262 2827 4860		
BsgI	3	1019 1219 2429	Maell	14						
Bsil	1	3442	MaellI	16		Enzymes that do not cut pET-27b(+):				
BsiEI	6	223 325 1953 3185 3609	MbolI	13		AatII	AflII	AgeI	AscI	AvrII
		4471	MluI	1	1168	BaeI	BsaI	BsrGI	Bsu36I	DraI
BsII	23		MmeI	7	3484 3668 4113 4307 4669	Eam1105I	FseI	KpnI	MunI	NspV
BsmI	2	4355 4432			4678 5149	PacI	PmeI	PmlI	PstI	RleAI
BsmAI	6	865 1270 1396 1783 2910	MnII	27		RsrII	SacII	Scal	SexAI	SfiI
		4487	MscI	1	279	SnaBI	SpeI	SrfI	Sse8387I	StuI
BsmBI	3	1783 2910 4487	Msel	26		SunI	Swal			
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					
BsrI	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						