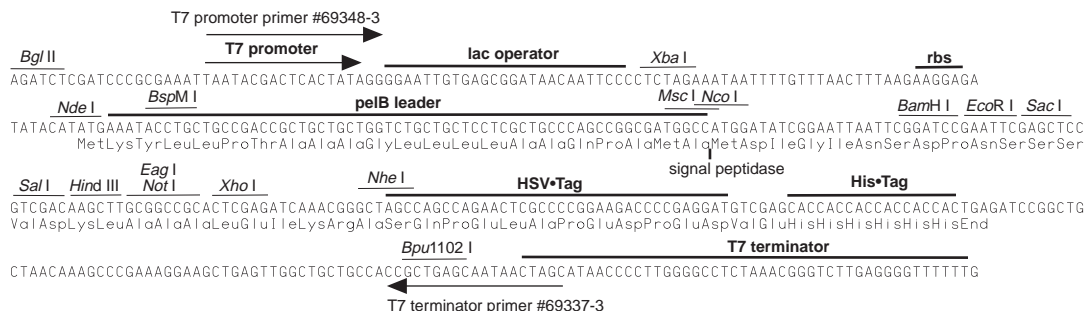
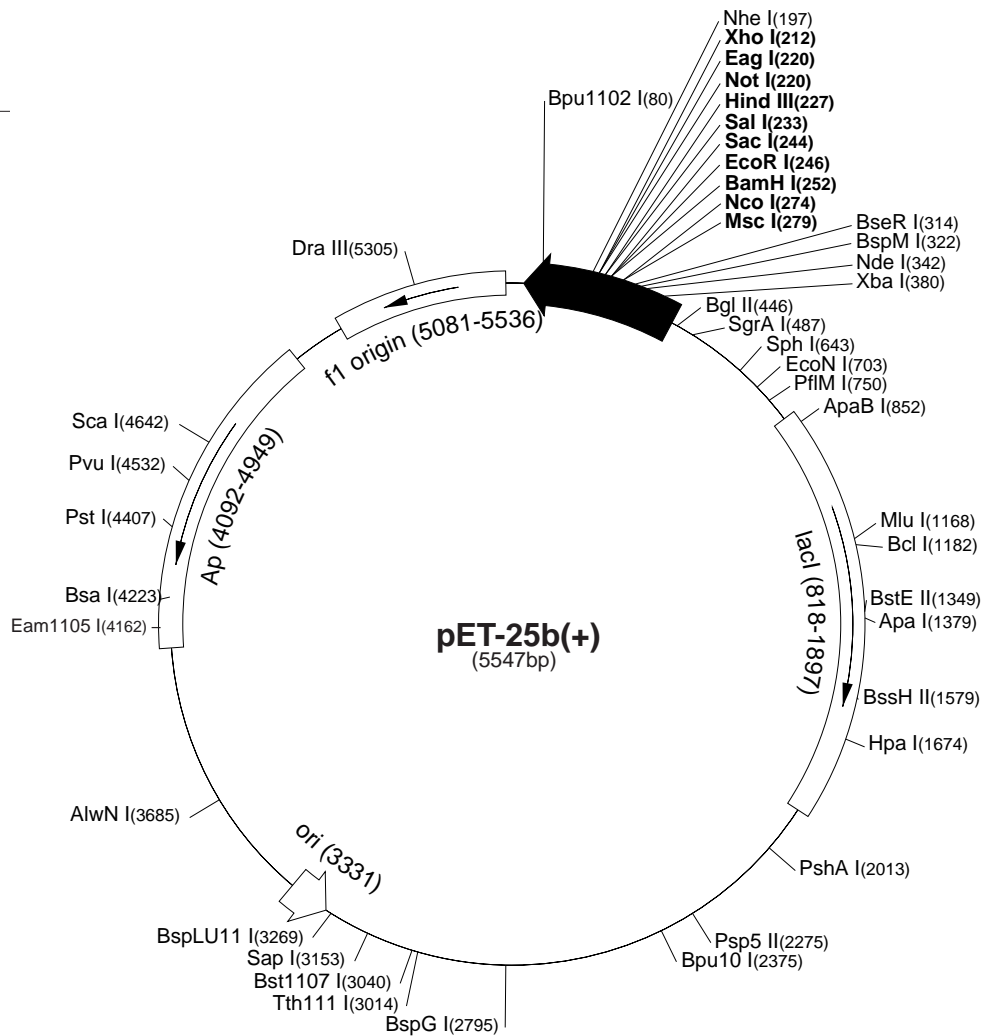


# pET-25b(+)<sup>+</sup> Vector

The pET-25b(+)<sup>+</sup> vector (Cat. No. 69753-3) carries an N-terminal *pelB* signal sequence for potential periplasmic localization, plus optional C-terminal HSV•Tag<sup>®</sup> and His•Tag<sup>®</sup> sequence. 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-25b(+)<sup>+</sup> 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
<i>bla</i> coding sequence	4092-4949
f1 origin	5081-5536



### pET-25b(+)<sup>+</sup> cloning/expression region

# pET-25b(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations		
AccI	2	234 3039	BsrFI	8	285 478 487 854 2066	NotI	1	220		
AccIII	8	935 1663 1994 2778 2919				NspI	4	643 2614 2906 3273		
		3221 4461 5145	BssHII	1	1579	Pfi1108I	2	2055 4180		
Acil	81		Bst1107I	1	3040	PfiMI	1	750		
AfIII	2	1168 3269	BstEII	1	1349	PleI	9	429 717 804 1600 3163		
AluI	25		BstXI	3	970 1099 1222			3648 4151 5240 5248		
AlwI	16		BstYI	12		PshAI	1	2013		
Alw21I	9	159 244 668 1152 2263	Cac8I	41		Psp5II	1	2275		
		3087 3587 4748 4833	CjeI	24		Psp1406I	6	830 2198 2594 4388 4761		
Alw44I	4	1148 3083 3583 4829	CjePI	18				5090		
AlwNI	1	3685	CviJI	89		PstI	1	4407		
Apal	1	1379	CviRI	24		PvuI	1	4532		
ApaBI	1	852	DdeI	11		PvuII	3	1768 1861 2860		
ApoI	4	246 1443 5107 5118	DpnI	28		RcaI	3	566 3989 4997		
AvaI	2	167 212	DraI	3	4028 4047 4739	RsaI	3	1315 3075 4642		
Avall	7	1720 2096 2184 2275 2554	DrallI	1	5305	SacI	1	244		
		4300 4522	DrdI	3	2962 3377 5260	Sall	1	233		
BamHI	1	252	DrdII	2	891 5310	SapI	1	3153		
BanI	9	490 511 625 1088 1807	Dsal	3	274 605 2241	Sau96I	18			
		1937 2063 4110 5342	EaeI	6	220 277 476 608 1842	Sau3AI	28			
BanII	4	244 552 566 1379			4550	Scal	1	4642		
BbsI	5	166 1314 1653 2027 2387	EagI	1	220	ScrFI	20			
BbvI	31		Eam1105I	1	4162	SfaNI	21			
BccI	14		EarI	3	786 3153 4957	Sfcl	5	414 3534 3725 4403 5524		
Bce83I	7	21 1982 2152 3360 3658	Ecil	4	945 3343 3489 4317	SgrAI	1	487		
		3899 4767	Eco47III	3	573 2074 2523	SphI	1	643		
Bcefl	5	687 1028 1655 3771 5331	Eco57I	2	3817 4829	Sspl	2	4966 5097		
Bcgl	10	214 248 1460 1494 1994	EcoNI	1	703	StyI	2	57 274		
		2028 2846 2880 4667 4701	EcoO109I	3	53 601 2275	TaqI	14			
BclI	1	1182	EcoRI	1	246	TaqII	9	1076 1294 1967 3171 4510		
Bfal	8	70 198 381 2283 3764	EcoRII	7	891 1206 1746 1803 3295			4695 4848 4865 5209		
		4017 4352 5456			3416 3429	TfiI	5	1847 2149 2319 2823 3244		
BglI	3	291 2232 4282	EcoRV	2	271 1618	Thal	34			
BglII	1	446	FauI	17		Tsel	31			
BmgI	1	1377	FokI	12		Tsp45I	8	1349 2177 2708 2921 3016		
Bpml	5	1006 1495 2129 2796 4232	FspI	2	2250 4384			4418 4629 5478		
Bpu10I	1	2375	GdIII	5	220 476 608 1842 4550	Tsp509I	18			
Bpu1102I	1	80	HaeI	6	279 896 2217 3284 3295	Tth111I	1	3014		
Bsal	1	4223			3747	Tth111II	6	1007 1700 2730 3859 3866		
BsaAI	2	3021 5305	HaeII	14				3898		
BsaBI	3	445 451 2466	HaeIII	25		UbaII	20			
BsaHI	6	491 512 626 1125 1808	Hgal	12		VspI	5	261 429 1853 1912 4334		
		4699	HgiEII	2	766 3855	XbaI	1	380		
BsaJI	9	57 166 178 274 605	Hhal	45		XcmI	3	1024 1540 1558		
		611 1803 2241 3429	Hin4I	3	1067 4161 4235	XhoI	1	212		
BsaWI	7	2 1487 1990 2458 3475	HincII	2	235 1674	XmnI	3	262 2827 4761		
		3622 4453	HindIII	1	227					
BsaXI	2	1827 5253	Hinfl	14		Enzymes that do not cut pET-25b(+):				
Bsbl	2	2985 5212	HpaI	1	1674	AatII	AfilI	AgeI	AscI	AvrII
BscGI	14		HphI	16		BaeI	BsmI	BsrGI	Bsu36I	Clal
BseRI	1	314	MaeII	15		FseI	KpnI	MunI	NruI	Nsil
BsgI	3	1019 1219 2429	MaeIII	18		NspV	Pacl	PmeI	PmlI	RleAI
Bsil	2	3442 4826	MbolI	15		RsrII	SacII	SexAI	Sfil	SgfI
BsiEI	7	223 325 1953 3185 3609	Mlul	1	1168	Smal	SnaBI	SpeI	SrfI	Sse8387I
		4532 4681	Mmel	3	3484 3668 5282	Stul	SunI	Swal		
BsII	20		MnII	28						
BsmAI	7	865 1270 1396 1783 2910	MscI	1	279					
		4223 4999	Msel	29						
BsmBI	2	1783 2910	Msil	9	1220 1508 1538 2256 2451					
BsmFI	4	629 2170 2540 5520			2842 4414 4573 4932					
BsoFI	51		MspI	33						
Bsp24I	10	458 490 1009 1041 1311	MspAII	10	84 321 1198 1768 1861					
		1343 3762 3794 3940 3972			2860 2979 3611 3856 4797					
Bsp1286I	12		MwoI	39						
BspEI	2	2 2458	NarI	4	491 512 626 1808					
BspGI	1	2795	NciI	13						
BspLU11I	1	3269	NcoI	1	274					
BspMI	1	322	NdeI	1	342					
BsrI	25		NgoAIV	5	285 478 2066 2226 5406					
BsrBI	4	401 3202 5003 5449	NheI	1	197					
BsrDI	4	1215 1581 4223 4397	NlaIII	25						
			NlaIV	24						