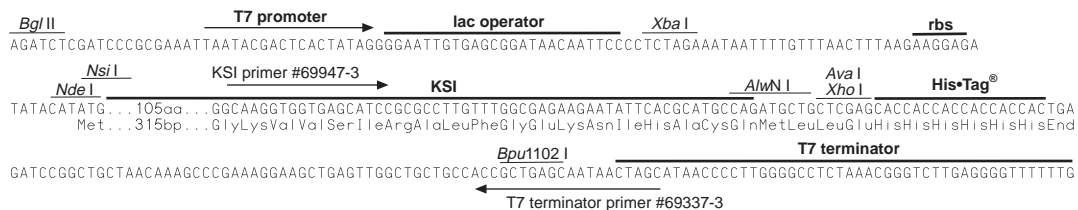
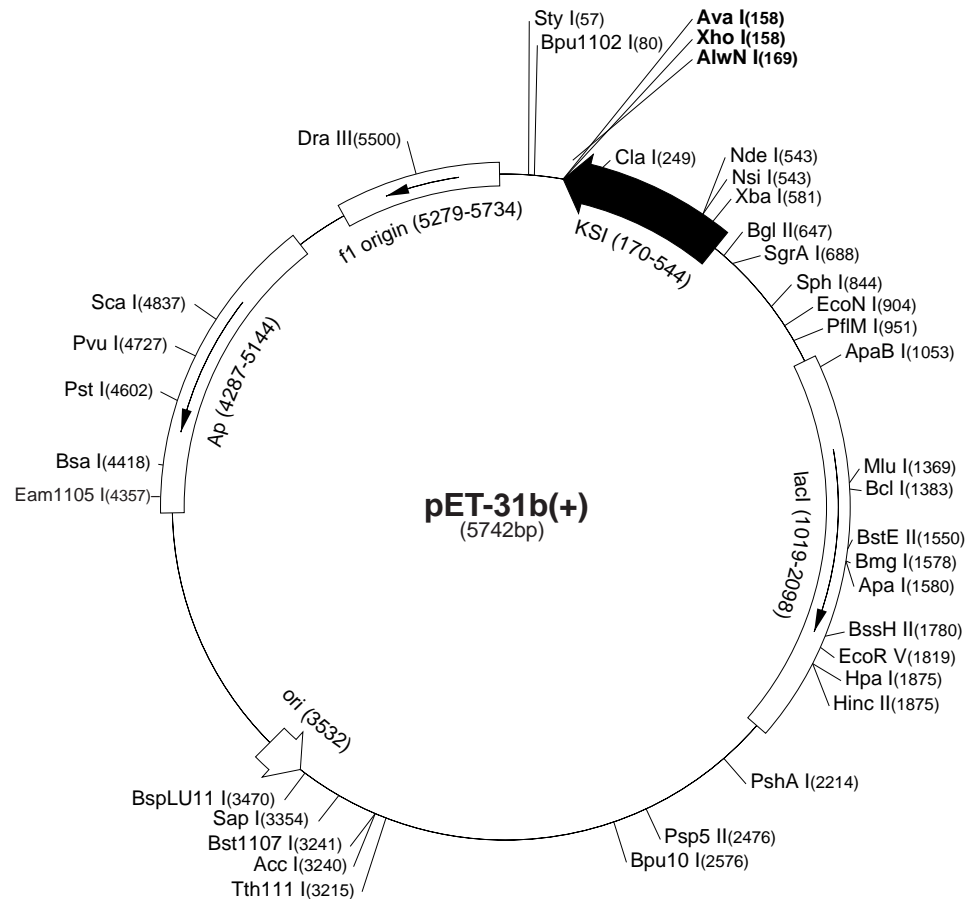


The pET-31b(+) vector (Cat. No. 69952-3) is designed for cloning and high-level expression of peptide sequences fused with the 125aa ketosteroid isomerase protein (1). The unique *AlwN* I cloning site allows the unidirectional insertion of of tandemly repeated peptide coding regions separated by methionine codons. 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).

1. Kuliopulos, A. and Walsh, C.T. (1994) *J. Am. Chem. Soc.* **116**, 4599-4607.

pET-31b(+) sequence landmarks

T7 promoter	616-632
T7 transcription start	615
KSI coding sequence	170-544
<i>AlwN</i> I site	169
His•Tag® coding sequence	140-157
T7 terminator	26-72
<i>lacI</i> coding sequence	1019-2098
pBR322 origin	3532
<i>bla</i> coding sequence	4287-5144
f1 origin	5276-5731



pET-31b(+) cloning/expression region

pET-31b(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations		
AccI	1	3240	BstYI	11		RcaI	3	767 4184 5192		
AcII	84		Cac8I	40		RsaI	6	332 403 512 1516 3276		
AflIII	2	1369 3470	CjeI	28				4837		
AluI	26		CjePI	20		SapI	1	3354		
AlwI	14		Clal	1	249	Sau96I	20			
Alw21I	8	159 869 1353 2464 3288	CviJI	89		Sau3AI	28			
		3788 4943 5028	CviRI	25		Scal	1	4837		
AlwNI	1	169	Ddel	11		ScrFI	20			
ApaI	1	1580	Dpnl	28		SfaNI	23			
ApaBI	1	1053	DraI	3	4223 4242 4934	Sfcl	5	615 3735 3920 4598 5719		
ApaLI	4	1349 3284 3784 5024	DraIII	1	5500	SgrAI	1	688		
ApoI	3	1644 5302 5313	DrdI	4	470 3163 3578 5455	SphI	2	177 844		
Avai	1	158	DrdII	2	1092 5505	Sspl	3	184 5161 5292		
Avall	8	407 1921 2297 2385 2476	Dsal	5	424 439 514 806 2442	StyI	1	57		
		2755 4495 4717	EaeI	4	677 809 2043 4745	TaqI	13			
BanI	10	227 691 712 826 1289	Eam1105I	1	4357	TaqII	9	1277 1495 2168 3372 4705		
		2008 2138 2264 4305 5537	EarI	3	987 3354 5152			4890 5043 5060 5404		
BanII	5	417 753 767 1580 5575	Ecil	4	1146 3544 3690 4512	TfiI	6	391 2048 2350 2520 3024		
BbsI	5	424 1515 1854 2228 2588	Eco47III	4	507 774 2275 2724			3445		
BbvI	28		Eco57I	2	4012 5024	Thal	38			
BccI	14		EcoNI	1	904	TseI	28			
Bce83I	7	21 2183 2353 3561 3859	EcoO109I	3	53 802 2476	Tsp45I	8	1550 2378 2909 3122 3217		
		4094 4962	EcoRII	8	409 1092 1407 1947 2004			4613 4824 5673		
Bcefl	8	385 456 532 888 1229			3496 3617 3630	Tsp509I	15			
		1856 3966 5526	EcoRV	1	1819	Tth111I	1	3215		
Bcgl	8	1661 1695 2195 2229 3047	FauI	17		Tth111III	8	212 470 1208 1901 2931		
		3081 4862 4896	FokI	11				4054 4061 4093		
BclI	1	1383	Fspl	2	2451 4579	UbaII	20			
Bfal	7	70 582 2484 3959 4212	GdIII	4	677 809 2043 4745	VspI	4	630 2054 2113 4529		
		4547 5651	HaeI	5	1097 2418 3485 3496 3942	XbaI	1	58		
BglI	2	2433 4477	HaeII	16		XcmI	3	1225 1741 1759		
BglII	1	647	HaeIII	25		XhoI	1	158		
BmgI	1	1578	HgaI	14		XmnI	2	3028 4956		
Bpml	5	1207 1696 2330 2997 4427	HgiEI	2	967 4050					
Bpu10I	1	2576	HhaI	52		Enzymes that do not cut pET-31b(+):				
Bpu1102I	1	80	Hin4I	4	412 1268 4356 4430	AatII	AflII	AgeI	AscI	AvrII
BsaI	1	4418	HincII	1	1875	BaeI	BamHI	BseRI	BsmI	BspMI
BsaAI	2	3222 5500	Hinfl	15		BsrGI	Bsu36I	EagI	EcoRI	FseI
BsaBI	3	646 652 2667	HpaI	1	1875	HindIII	KpnI	MunI	NcoI	NheI
BsaHI	8	228 444 692 713 827	HphI	19		NotI	NruI	NspV	Pacl	PmeI
		1326 2009 4894	MaeII	15		PmlI	RleAI	RsrII	Sacl	SacII
BsaJI	10	57 410 424 439 514	MaeIII	18		Sall	SexAI	Sfil	Sgfl	SmaI
		806 812 2004 2442 3630	MbolI	16		SnaBI	SpeI	SrfI	Sse8387I	StuI
BsaWI	8	2 404 1688 2191 2659	Mlul	1	1369	SunI	Swal			
		3676 3823 4648	MmeI	3	3685 3869 5477					
BsaXI	2	2028 5448	MnlI	27						
Bsbl	2	3186 5407	MseI	28						
BscGI	14		MslI	10	519 1421 1709 1739 2457					
Bsil	2	3643 5021			2652 3043 4609 4768 5127					
BsiEI	6	325 2154 3386 3810 4727	MspI	34						
		4876	MspA1I	10						
BsII	22		Mwol	46						
BsmAI	7	1066 1471 1597 1984 3111	NarI	5	228 692 713 827 2009					
		4418 5194	NciI	12						
BsmBI	2	1984 3111	NdeI	1	543					
BsmFI	4	830 2371 2741 5715	NgoAIV	6	224 482 679 2267 2427					
BsoFI	48				5601					
Bsp24I	10	659 691 1210 1242 1512	NlaIII	25						
		1544 3957 3989 4135 4167	NlaIV	26						
Bsp1286I	13		Nsil	1	543					
BspEI	2	2 2659	NspI	5	177 844 2815 3107 3474					
BspGI	2	475 2996	Pfi1108I	1	951					
BspLU11I	1	3470	PfiMI	2	395 569					
BsrI	24		PleI	9	630 918 1005 1801 3364					
BsrBI	4	602 3403 5198 5644			3849 4346 5435 5443					
BsrDI	4	1416 1782 4418 4592	PshAI	1	2214					
BsrFI	9	224 482 679 688 1055	Psp5II	1	2476					
		2267 2427 4437 5601	Psp1406I	6	1031 2399 2795 4583 4956					
BssHII	1	1780			5285					
Bst1107I	1	3241	PstI	1	4602					
BstEII	1	1550	PvuI	1	4727					
BstXI	3	1171 1300 1423	PvuII	4	1969 2062 3061 3880					