

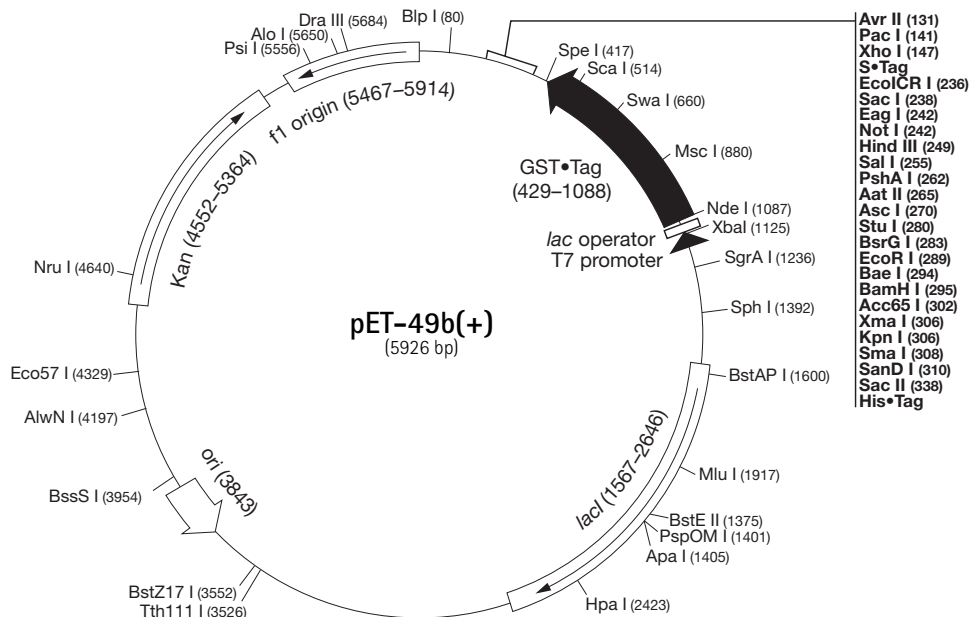
pET-49b(+)⁺ Vector

TB417 0804

	Cat. No.
pET-49b(+) ⁺ DNA	71463-3
pET-49b(+)⁺ sequence landmarks	
T7 promoter	1160-1176
T7 transcription start	1159
GST•Tag coding sequence	429-1088
His•Tag coding sequence	342-359
Multiple cloning sites (<i>SanD</i> I – <i>Avr</i> II)	131-315
S•Tag coding sequence	168-212
T7 terminator	26-73
<i>lacI</i> coding sequence	1567-2646
pBR322 ori	3843
Kan coding sequence	4552-5364
f1 origin	5467-5914

The pET-49b(+)⁺ vector carries N-terminal GST•Tag™ and His•Tag® coding sequences followed by a recognition site for the human rhinovirus (HRV) 3C protease. This protease is highly specific for cleavage of the sequence LEVLFQ↓GP (1), and is active at low temperatures (2). pET-49b(+)⁺ also contains an optional C-terminal thrombin recognition site followed by an S•Tag™ coding sequence. Unique restriction .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 the helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the AS S•Tag 18mer Primer (Cat. No. 71262-3).

1. Cordingley, M.G., Register, R.B., Callahan, P.L., Garsky, V.M., and Colonno, R.J. (1989) *J. Virol.* 63, 5037-5045.
2. Wang, Q.M., Johnson, R.B., Cox, G.A., Villarreal, E.C., and Loncharich, R.J. (1997) *Anal. Biochem.* 252, 238-245.



T7 Promoter Primer
#69348-3

TCTCGATCCGCGAAATTAATACGACTCACTATAGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAATTTGTTAACTTTAAGAAGGAGAT

Nde I
GST•Tag
Spe I

ATACATATGTCCTTACTAGGTTATTGGAAAATTAAG...606 bp...GACCATCTCCAAAATCGGATGGTTCAACTAGTGGTGGTGGCGGTTCT
 MetSerProIleLeuGlyTyrTrpLysIleLys...202 aa...AspHisProProLysSerAspGlySerThrSerGlyGlyGlyGlySer

Acc65 I
Kpn I
Bae I
SanD I
Xma I
Sma I

His•Tag
Sac II

AATAACAATCCTCCTACTCCTACTCCATCTAGTGGTTCTGGTCATCACCATCACCATCACTCCGCGGCTCTTGAAGTCTCTTTAGGGACCCGGGTAC
 AsnAsnAsnProProThrProThrProSerSerGlySerGlyHisHisHisHisHisHisSerAlaAlaLeuGluValLeuPheGlnGlyProGlyTyr

HRV 3C

BamH I
EcoR I
BsrG I
Stu I
Asc I
Aat II
Sal I
Hind III
Not I
Sac I
S•Tag

CAGGATCCGAATTCGTACAGCCCTGGCGCGCCGACGTCGTCGACAAGCTTGGCGCGCAGAGCTCGCTCTGGTGCCACGCGGTAGTAAAGAAACC
 GlnAspProAsnSerValGlnAlaLeuAlaArgProThrSerValAspLysLeuAlaAlaAlaGluLeuAlaLeuValProArgGlySerLysGluThr

thrombin

S•Tag
Xho I
Pac I
Avr II

GCTGCTGCTAAATTCGAACGCCACATGGACAGCTCTACTTCTGCTGCTCGAGGCTTAATTAACCTAGGCTGCTAAACAAAGCCGAAAGGAAGC
 AlaAlaAlaLysPheGluArgGlnHisMetAspSerSerThrSerAlaAlaLeuGluAlaEnd

AS S•Tag 18mer Primer
 #71262-3

pET-49b(+)⁺ cloning/expression region

pET-49b(+) Restriction Sites

TB417 0804

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzymes that do not cut pET-49b(+):					
AatII	1	266	BtsI	4	2282 2650 4844 4931	AarI	AfII	AgeI	AhdI	AleI	AsiSI
Acc65I	1	302	ClaI	2	1194 4674	BbvCI	BglI	BglII	BmgBI	BmtI	BpII
AccI	2	256 3551	DraI	2	551 660	BsaI	BseRI	BsiWI	BspMI	Bsu36I	BtrI
AccI	3	1579 3106 5469	DraIII	1	5684	EcoRV	FalI	FseI	FspAI	FspI	MfeI
AfeI	2	1322 3035	DrdI	3	3474 3889 5639	NcoI	NheI	PinAI	PmeI	PmlI	PsrI
AflIII	3	845 1917 3781	EaeI	5	242 878 1225 1357 2591	PstI	PvuI	RsrII	SbfI	SexAI	SfiI
Alol	1	5650	EagI	1	242	SnaBI	SrfI	Sse8387I			
AlwNI	1	4197	EarI	4	1005 1535 3665 4796						
ApaI	1	2128	EarI	3	1708 3843 3989						
ApaLI	3	1897 3595 4095	Eco57I	1	4329						
AscI	1	270	Eco57MI	4	1755 2244 3308 4329						
Asel	5	1174 2602 2661 5182 5371	EcoCRI	1	236						
AvaI	2	147 306	EcoNI	4	318 1076 1452 4895						
AvrII	1	131	EcoO109I	5	53 310 1052 1350 2787						
BaeI	1	294	EcoRI	1	289						
BamHI	1	295	HaeII	13							
BanI	9	223 302 1239 1260 1374	HincII	2	257 2423						
		1837 2556 2686 5721	HindIII	1	249						
BanII	6	238 1301 1315 2128 4638	HpaI	1	2423						
		5759	KasI	4	1239 1260 1374 2556						
BbeI	4	1243 1264 1378 2560	KpnI	1	306						
BbsI	3	2063 2402 2899	MluI	1	1917						
BceAI	6	1436 1776 2403 4283 5302	MscI	1	880						
		5709	MsiI	7	993 1969 2257 2287 2768						
BcgI	4	236 1059 2243 3358			2963 3354						
BciVI	3	2374 3984 5378	NaeI	2	1229 5787						
BclI	2	649 1931	NarI	4	1240 1261 1375 2557						
BfrBI	2	4831 5097	NdeI	1	1087						
BlpI	1	80	NgoMIV	2	1227 5785						
Bme1580I	4	1901 2128 3599 4099	NotI	1	242						
BmrI	5	1446 1843 2080 2720 3520	NruI	1	4640						
BpmI	3	1755 2244 3308	NsiI	2	4833 5099						
Bpu10I	2	2887 5000	NspI	5	849 1392 3126 3418 3785						
BpuEI	5	21 2731 3872 4170 4411	NspV	2	187 688						
BsaAI	2	3533 5684	Pacl	1	141						
BsaBI	3	1190 1200 2978	PciI	2	845 3781						
BsaHI	6	263 1240 1261 1375 1874	PfiMI	4	179 373 1499 5246						
		2557	PfoI	2	1484 3423						
BsaWI	7	2 2236 2739 2970 3987	PpiI	2	4496 5650						
		4134 5118	PpuMI	2	310 2787						
BsaXI	4	331 368 2592 5648	PshAI	1	262						
BseYI	3	2316 2451 4085	PsiI	1	5556						
BsgI	4	803 1768 1968 2941	PspOMI	1	2124						
BsiEI	4	245 2702 3697 4121	PvuII	3	2517 2610 3372						
BsiHKAI	6	238 1417 1901 2775 3599	SacI	1	238						
		4099	SacII	1	338						
BsmAI	6	1614 2019 2145 2532 3422	Sall	1	255						
		4999	SanDI	1	310						
BsmBI	3	2532 3422 4999	SapI	2	1005 3665						
BsmFI	5	296 1096 1378 3052 5899	Scal	1	514						
BsmI	2	4867 4944	Sfcl	4	1159 4046 4237 5903						
Bsp1286I	11		Sfol	4	1241 1262 1376 2558						
BspCNI	9	93 114 2502 2879 3041	SgrAI	1	1236						
		3581 4069 4478 5013	SmaI	1	308						
BspEI	2	2 2970	SmlI	6	36 147 2710 3887 4149						
BspHI	3	1315 4501 5376			4426						
BspLU11I	2	845 3781	SpeI	1	417						
BsrBI	5	796 1146 3714 5382 5828	SphI	1	1392						
BsrDI	2	1964 2330	SspI	2	4908 5476						
BsrFI	5	1227 1236 1603 4937 5785	StuI	1	280						
BsrGI	1	283	StyI	3	57 131 275						
BssHII	2	270 2328	Swal	1	660						
BssSI	1	3954	TaqII	4	2716 3683 5237 5588						
Bst1107I	1	3552	TatI	3	283 512 3585						
BstAPI	1	1600	TspGWI	5	274 2906 3224 4792 4804						
BstBI	2	187 688	Tth111I	1	3526						
BstEII	1	2098	XbaI	1	1125						
BstXI	3	1719 1848 1971	XcmI	3	1773 2289 2307						
BstYI	7	295 1481 2693 2973 4422	XhoI	1	147						
		4433 5232	XmaI	1	306						
BstZ17I	1	3552	XmnI	3	694 3339 5372						
BtgI	2	335 1354	ZraI	1	264						