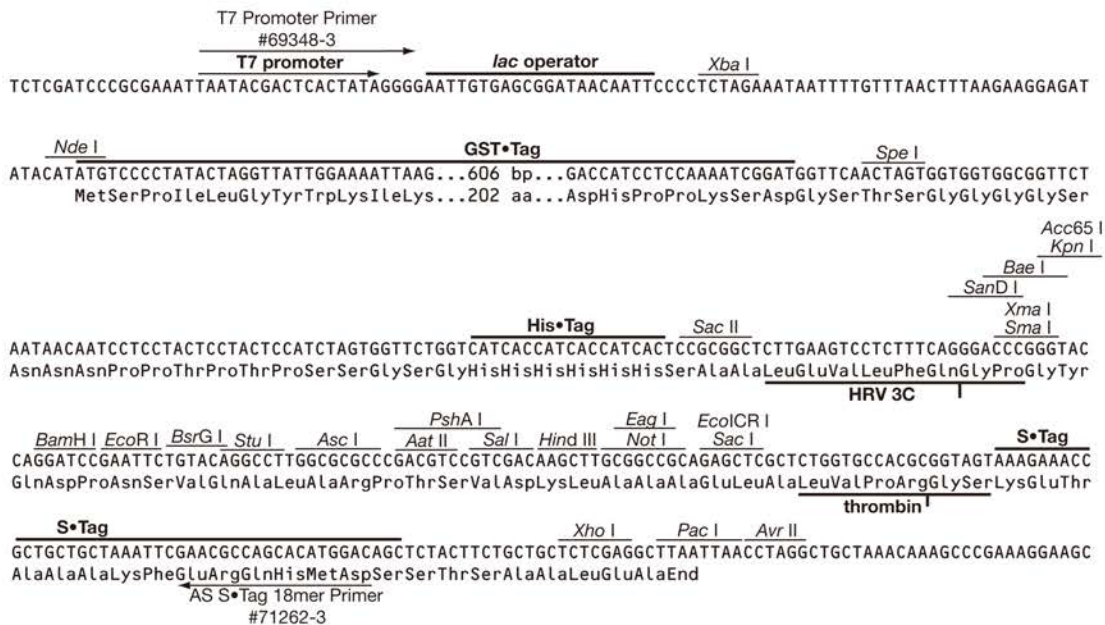
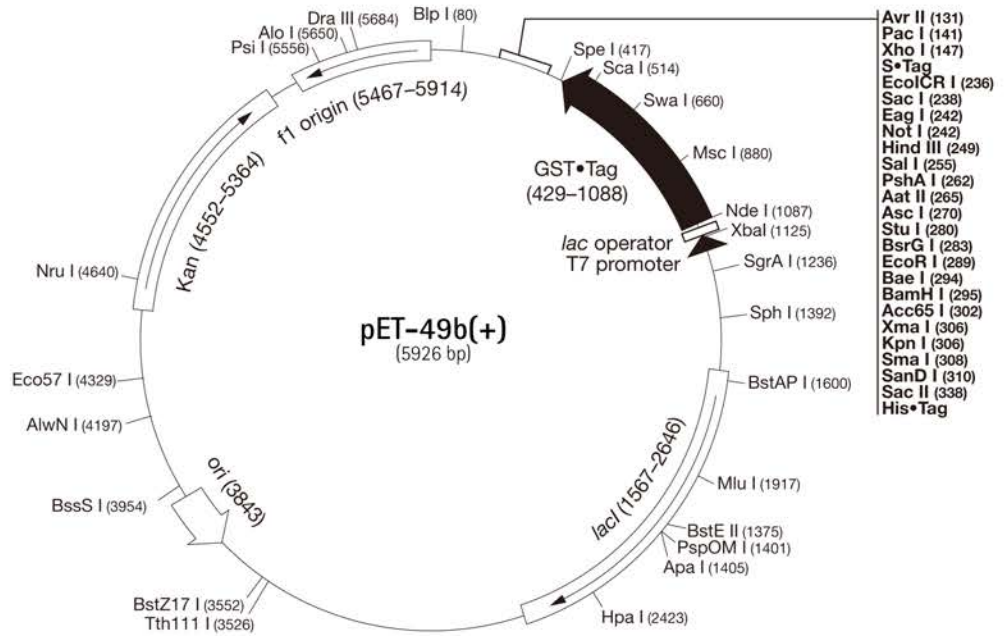


pET-49b(+)⁺ Vector

	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.



pET-49b(+)⁺ cloning/expression region

pET-49b(+) Restriction Sites

Enzyme	# Sites	Locations
AatII	1	266
Acc65I	1	302
AccI	2	256 3551
AccII	3	1579 3106 5469
AfeI	2	1322 3035
AfIII	3	845 1917 3781
Alol	1	5650
AlwNI	1	4197
Apal	1	2128
ApalI	3	1897 3595 4095
AscI	1	270
AseI	5	1174 2602 2661 5182 5371
AvaI	2	147 306
AvrII	1	131
BaeI	1	294
BamHI	1	295
BanI	9	223 302 1239 1260 1374 1837 2556 2686 5721
BanII	6	238 1301 1315 2128 4638 5759
BbeI	4	1243 1264 1378 2560
BbsI	3	2063 2402 2899
BceAI	6	1436 1776 2403 4283 5302 5709
BcgI	4	236 1059 2243 3358
BciVI	3	2374 3984 5378
BclI	2	649 1931
BfrBI	2	4831 5097
BlpI	1	80
Bme1580I	4	1901 2128 3599 4099
BmrI	5	1446 1843 2080 2720 3520
BpmI	3	1755 2244 3308
Bpu10I	2	2887 5000
BpuEI	5	21 2731 3872 4170 4411
BsaAI	2	3533 5684
BsaBI	3	1190 1200 2978
BsaHI	6	263 1240 1261 1375 1874 2557
BsaWI	7	2 2236 2739 2970 3987 4134 5118
BsaXI	4	331 368 2592 5648
BseYI	3	2316 2451 4085
BsgI	4	803 1768 1968 2941
BsiEI	4	245 2702 3697 4121
BsiHKA1	6	238 1417 1901 2775 3599 4099
BsmAI	6	1614 2019 2145 2532 3422 4999
BsmBI	3	2532 3422 4999
BsmFI	5	296 1096 1378 3052 5899
BsmI	2	4867 4944
Bsp1286I	11	
BspCNI	9	93 114 2502 2879 3041 3581 4069 4478 5013
BspEI	2	2 2970
BspHI	3	1315 4501 5376
BspLU111	2	845 3781
BsrBI	5	796 1146 3714 5382 5828
BsrDI	2	1964 2330
BsrFI	5	1227 1236 1603 4937 5785
BsrGI	1	283
BssHII	2	270 2328
BssSI	1	3954
Bst1107I	1	3552
BstAPI	1	1600
BstBI	2	187 688
BstEII	1	2098
BstXI	3	1719 1848 1971
BstYI	7	295 1481 2693 2973 4422 4433 5232
BstZ17I	1	3552
BtgI	2	335 1354

Enzyme	# Sites	Locations
BtsI	4	2282 2650 4844 4931
Clal	2	1194 4674
Dral	2	551 660
DraIII	1	5684
DrdI	3	3474 3889 5639
EaeI	5	242 878 1225 1357 2591
EagI	1	242
EarI	4	1005 1535 3665 4796
Ecil	3	1708 3843 3989
Eco57I	1	4329
Eco57MI	4	1755 2244 3308 4329
EcoCRI	1	236
EcoNI	4	318 1076 1452 4895
EcoO109I	5	53 310 1052 1350 2787
EcoRI	1	289
HaeII	13	
HincII	2	257 2423
HindIII	1	249
HpaI	1	2423
KasI	4	1239 1260 1374 2556
KpnI	1	306
MluI	1	1917
MscI	1	880
MslI	7	993 1969 2257 2287 2768 2963 3354
NaeI	2	1229 5787
NarI	4	1240 1261 1375 2557
NdeI	1	1087
NgoMIV	2	1227 5785
NotI	1	242
NruI	1	4640
NsiI	2	4833 5099
NspI	5	849 1392 3126 3418 3785
NspV	2	187 688
Pacl	1	141
PciI	2	845 3781
PfiMI	4	179 373 1499 5246
PfoI	2	1484 3423
Ppil	2	4496 5650
PpuMI	2	310 2787
PshAI	1	262
Psil	1	5556
PspOMI	1	2124
PvuII	3	2517 2610 3372
SacI	1	238
SacII	1	338
Sall	1	255
SanDI	1	310
SapI	2	1005 3665
Scal	1	514
SfcI	4	1159 4046 4237 5903
SfoI	4	1241 1262 1376 2558
SgrAI	1	1236
Smal	1	308
SmlI	6	36 147 2710 3887 4149 4426
SpeI	1	417
SphI	1	1392
Sspl	2	4908 5476
StuI	1	280
StyI	3	57 131 275
Swal	1	660
TaqII	4	2716 3683 5237 5588
TatI	3	283 512 3585
TspGWI	5	274 2906 3224 4792 4804
Tth111I	1	3526
XbaI	1	1125
XcmI	3	1773 2289 2307
XhoI	1	147
XmaI	1	306
XmnI	3	694 3339 5372
ZraI	1	264

Enzymes that do not cut pET-49b(+):

AarI	Afill	AgeI	AhdI	AleI	AsiSI
BbvCI	BglI	BglII	BmgBI	BmtI	BpII
Bsal	BseRI	BsiWI	BspMI	Bsu36I	BtrI
EcoRV	FalI	FseI	FspAI	Fspl	MfeI
NcoI	NheI	PinAI	PmeI	PmlI	PsiI
PstI	PvuI	RsrII	SbfI	SexAI	SfiI
SnaBI	SrfI	Sse8387I			