pET-47b(+) Vector

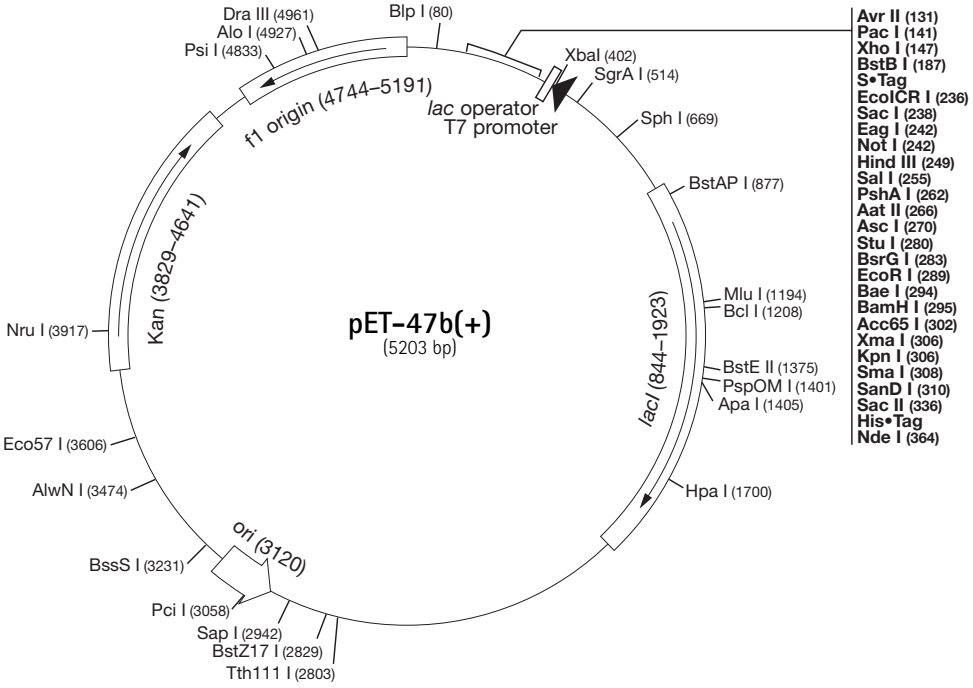
TB415 0804

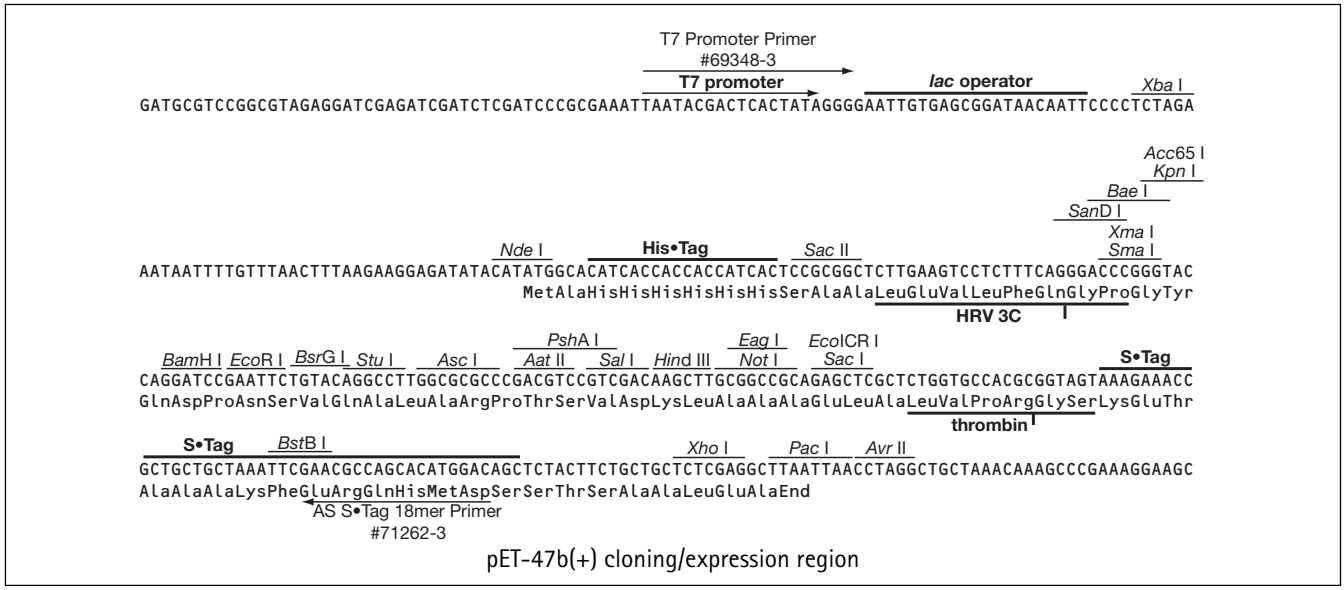
	Cat. No.
pET-47b(+) DNA	71461-3

pET-47b(+) sequence landmarks	
T7 promoter	437–453
T7 transcription start	436
His•Tag coding sequence	341–359
Multiple cloning sites (*San*D I – *Avr* II)	131–315
S•Tag coding sequence	168–212
T7 terminator	26–73
lacI coding sequence	844–1923
pBR322 ori	3120
Kan coding sequence	3829–4641
f1 origin	4744–5191

The pET-47b(+) vector carries an N-terminal His•Tag® coding sequence 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-47b(+) 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-47b(+) cloning/expression region

pET-47b(+) Restriction Sites

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Enzyme	# Sites	Locations
AatII	1	266
Acc65I	1	302
AccI	2	256 2828
AclI	3	856 2383 4746
AfeI	2	599 2312
AflIII	2	1194 3058
AloI	1	4927
AlwNI	1	3474
ApaI	1	1405
ApaLI	3	1174 2872 3372
AscI	1	270
AseI	5	451 1879 1938 4459 4648
AvaI	2	147 306
AvrII	1	131
BaeI	1	294
BamHI	1	295
BanI	9	223 302 516 537 651 1114 1833 1963 4998
BanII	6	238 578 592 1405 3915 5036
BbeI	4	520 541 655 1837
BbsI	3	1340 1679 2176
BceAI	6	713 1053 1680 3560 4579 4986
BcgI	3	236 1520 2635
BciVI	3	1651 3261 4655
BclI	1	1208
BfrBI	2	4108 4374
BlpI	1	80
Bme1580I	4	1178 1405 2876 3376
BmrI	5	723 1120 1357 1997 2797
BpmI	3	1032 1521 2585
Bpu10I	2	2164 4277
BpuEI	5	21 2008 3149 3447 3688
BsaAI	2	2810 4961
BsaBI	3	467 477 2255
BsaHI	6	263 517 538 652 1151 1834
BsaWI	7	2 1513 2016 2247 3264 3411 4395
BsaXI	3	331 1869 4925
BseYI	3	1593 1728 3362
BsgI	3	1045 1245 2218
BsiEI	4	245 1979 2974 3398
BsiHKAI	6	238 694 1178 2052 2876 3376
BsmAI	6	891 1296 1422 1809 2699 4276
BsmBI	3	1809 2699 4276
BsmFI	4	296 655 2329 5176
BsmI	2	4144 4221
Bsp1286I	11	
BspCNI	9	93 114 1779 2156 2318 2858 3346 3755 4290
BspEI	2	2 2247
BspHI	3	592 3778 4653
BspLU11I	1	3058
BsrBI	4	423 2991 4659 5105
BsrDI	2	1241 1607
BsrFI	5	504 513 880 4214 5062
BsrGI	1	283
BssHII	2	270 1605
BssSI	1	3231
Bst1107I	1	2829
BstAPI	1	877
BstBI	1	187
BstEII	1	1375
BstXI	3	996 1125 1248
BstYI	7	295 758 1970 2250 3699 3710 4509
BstZ17I	1	2829
BtgI	2	335 631
BtsI	4	1559 1927 4121 4208
ClaI	2	471 3951
DraIII	1	4961
DrdI	3	2751 3166 4916
EaeI	4	242 502 634 1868
EagI	1	242
EarI	3	812 2942 4073
EciI	3	985 3120 3266
Eco57I	1	3606
Eco57MI	4	1032 1521 2585 3606
EcoICRI	1	236
EcoNI	3	318 729 4172
EcoO109I	4	53 310 627 2064
EcoRI	1	289
HaeII	13	
HincII	2	257 1700
HindIII	1	249
HpaI	1	1700
KasI	4	516 537 651 1833
KpnI	1	306
MluI	1	1194
MslI	6	1246 1534 1564 2045 2240 2631
NaeI	2	506 5064
NarI	4	517 538 652 1834
NdeI	1	364
NgoMIV	2	504 5062
NotI	1	242
NruI	1	3917
NsiI	2	4110 4376
NspI	4	669 2403 2695 3062
NspV	1	187
PacI	1	141
PciI	1	3058
PflMI	3	179 776 4523
PfoI	2	761 2700
PpiI	2	3773 4927
PpuMI	2	310 2064
PshAI	1	262
PsiI	1	4833
PspOMI	1	1401
PvuII	3	1794 1887 2649
SacI	1	238
SacII	1	338
SalI	1	255
SanDI	1	310
SapI	1	2942
SfcI	4	436 3323 3514 5180
SfoI	4	518 539 653 1835
SgrAI	1	513
SmaI	1	308
SmlI	6	36 147 1987 3164 3426 3703
SphI	1	669
SspI	2	4185 4753
StuI	1	280
StyI	3	57 131 275
TaqII	4	1993 2960 4514 4865
TatI	2	283 2862
TspGWI	5	274 2183 2501 4069 4081
Tth111I	1	2803
XbaI	1	402
XcmI	3	1050 1566 1584
XhoI	1	147
XmaI	1	306
XmnI	2	2616 4649
ZraI	1	264

Enzymes that do not cut pET-47b(+):

AarI	AflII	AgeI	AhdI	AleI	AsiSI
BbvCI	BglI	BglII	BmgBI	BmtI	BplI
BsaI	BseRI	BsiWI	BspMI	Bsu36I	BtrI
DraI	EcoRV	FalI	FseI	FspAI	FspI
MfeI	MscI	NcoI	NheI	PinAI	PmeI
PmlI	PsrI	PstI	PvuI	RsrII	SbfI
ScaI	SexAI	SfiI	SnaBI	SpeI	SrfI
Sse8387I	SwaI				