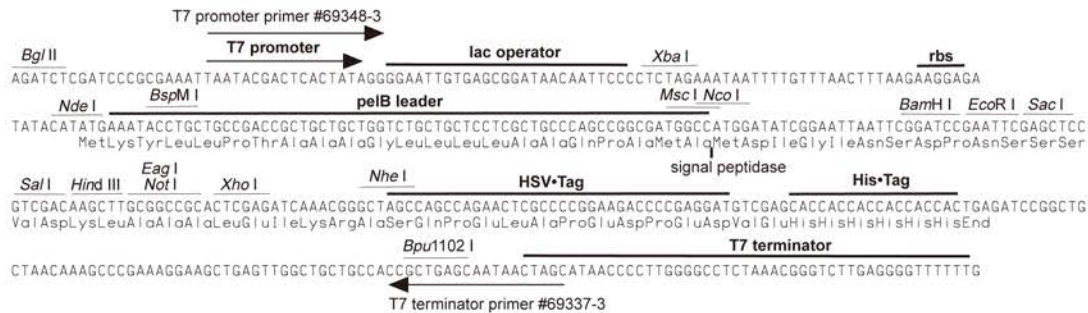
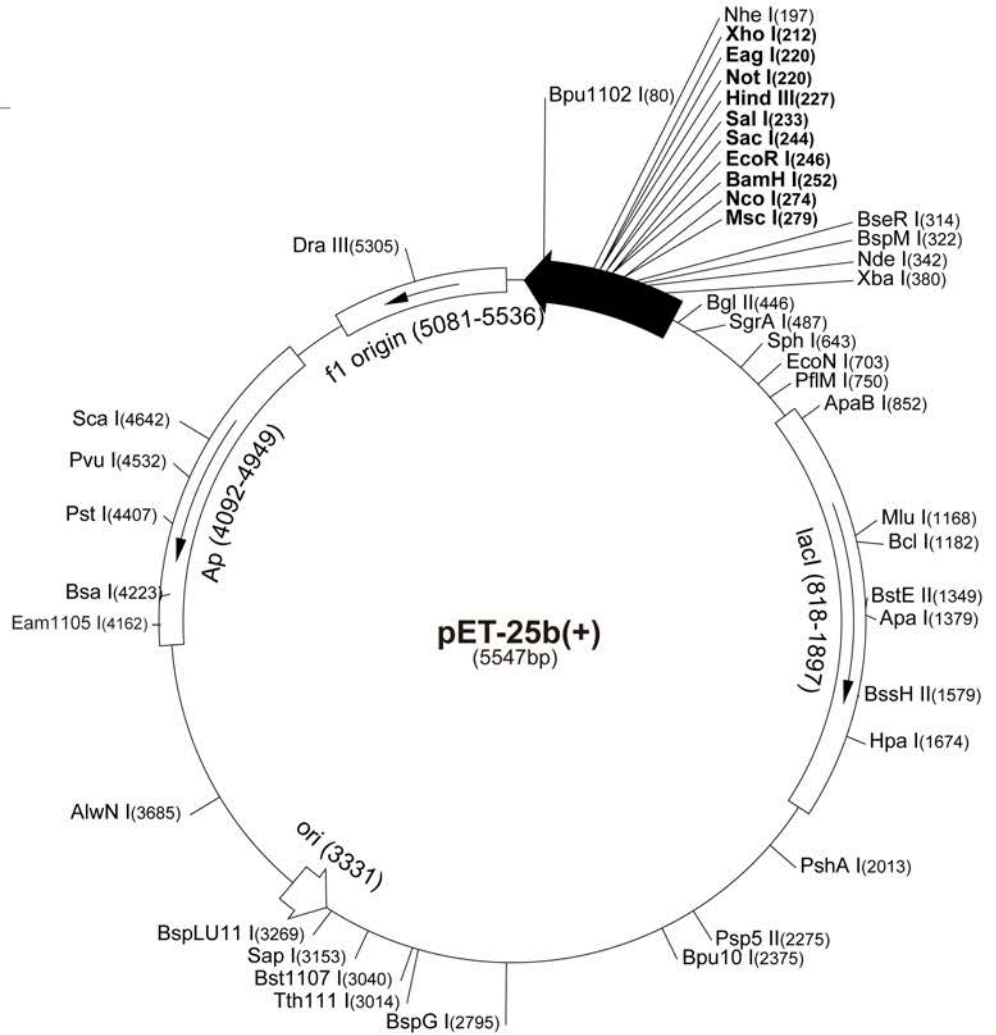


pET-25b(+) Vector

The pET-25b(+) vector (Cat. No. 69753-3) carries an N-terminal *pelB* signal sequence for potential periplasmic localization, plus optional C-terminal HSV•Tag® and His•Tag® 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(+) 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(+) cloning/expression region

pET-25b(+) Restriction Sites

Enzyme	# Sites	Locations
AccI	2	234 3039
AccII	8	935 1663 1994 2778 2919 3221 4461 5145
Acil	81	
AfilI	2	1168 3269
AluI	25	
AlwI	16	
Alw21I	9	159 244 668 1152 2263 3087 3587 4748 4833
Alw44I	4	1148 3083 3583 4829
AlwNI	1	3685
Apal	1	1379
ApaBI	1	852
ApoI	4	246 1443 5107 5118
AvaI	2	167 212
Avall	7	1720 2096 2184 2275 2554 4300 4522
BamHI	1	252
BanI	9	490 511 625 1088 1807 1937 2063 4110 5342
BanII	4	244 552 566 1379
BbsI	5	166 1314 1653 2027 2387
BbvI	31	
BccI	14	
Bce83I	7	21 1982 2152 3360 3658 3899 4767
BceII	5	687 1028 1655 3771 5331
Bcgl	10	214 248 1460 1494 1994 2028 2846 2880 4667 4701
BclI	1	1182
Bfal	8	70 198 381 2283 3764 4017 4352 5456
BglI	3	291 2232 4282
BgIII	1	446
BmgI	1	1377
BpmI	5	1006 1495 2129 2796 4232
Bpu10I	1	2375
Bpu1102I	1	80
BsaI	1	4223
BsaAI	2	3021 5305
BsaBI	3	445 451 2466
BsaHI	6	491 512 626 1125 1808 4699
BsaJI	9	57 166 178 274 605 611 1803 2241 3429
BsaWI	7	2 1487 1990 2458 3475 3622 4453
BsaXI	2	1827 5253
Bsbl	2	2985 5212
BscGI	14	
BseRI	1	314
BsgI	3	1019 1219 2429
Bsil	2	3442 4826
BsiEI	7	223 325 1953 3185 3609 4532 4681
BsII	20	
BsmAI	7	865 1270 1396 1783 2910 4223 4999
BsmBI	2	1783 2910
BsmFI	4	629 2170 2540 5520
BsoFI	51	
Bsp24I	10	458 490 1009 1041 1311 1343 3762 3794 3940 3972
Bsp1286I	12	
BspEI	2	2 2458
BspGI	1	2795
BspLU11I	1	3269
BspMI	1	322
BsrI	25	
BsrBI	4	401 3202 5003 5449
BsrDI	4	1215 1581 4223 4397

Enzyme	# Sites	Locations
BsrFI	8	285 478 487 854 2066 2226 4242 5406
BssHII	1	1579
Bst1107I	1	3040
BstEI	1	1349
BstXI	3	970 1099 1222
BstYI	12	
Cac8I	41	
CjeI	24	
CjePI	18	
CviJI	89	
CviRI	24	
Ddel	11	
Dpnl	28	
DraI	3	4028 4047 4739
DraIII	1	5305
DrdI	3	2962 3377 5260
DrdII	2	891 5310
Dsal	3	274 605 2241
EaeI	6	220 277 476 608 1842 4550
EagI	1	220
Eam1105I	1	4162
EarI	3	786 3153 4957
Ecil	4	945 3343 3489 4317
Eco47III	3	573 2074 2523
Eco57I	2	3817 4829
EcoNI	1	703
EcoO109I	3	53 601 2275
EcoRI	1	246
EcoRII	7	891 1206 1746 1803 3295 3416 3429
EcoRV	2	271 1618
FauI	17	
FokI	12	
FspI	2	2250 4384
GdIII	5	220 476 608 1842 4550
HaeI	6	279 896 2217 3284 3295 3747
HaeII	14	
HaeIII	25	
HgaI	12	
HgiEI	2	766 3855
Hhal	45	
Hin4I	3	1067 4161 4235
HincII	2	235 1674
HindIII	1	227
Hinfl	14	
HpaI	1	1674
HphI	16	
MaeII	15	
MaeIII	18	
MbolI	15	
MluI	1	1168
MmeI	3	3484 3668 5282
MnlI	28	
MscI	1	279
MseI	29	
MsiI	9	1220 1508 1538 2256 2451 2842 4414 4573 4932
MspI	33	
MspA1I	10	84 321 1198 1768 1861 2860 2979 3611 3856 4797
MwoI	39	
NarI	4	491 512 626 1808
NciI	13	
NcoI	1	274
NdeI	1	342
NgoAIV	5	285 478 2066 2226 5406
NheI	1	197
NlaIII	25	
NlaIV	24	

Enzyme	# Sites	Locations
NotI	1	220
NspI	4	643 2614 2906 3273
PflI1108I	2	2055 4180
PfIMI	1	750
PleI	9	429 717 804 1600 3163 3648 4151 5240 5248
PshAI	1	2013
Psp5II	1	2275
Psp1406I	6	830 2198 2594 4388 4761 5090
PstI	1	4407
PvuI	1	4532
PvuII	3	1768 1861 2860
RcaI	3	566 3989 4997
RsaI	3	1315 3075 4642
SacI	1	244
Sall	1	233
SapI	1	3153
Sau96I	18	
Sau3AI	28	
Scal	1	4642
ScrFI	20	
SfaNI	21	
Sfcl	5	414 3534 3725 4403 5524
SgrAI	1	487
SphI	1	643
SspI	2	4966 5097
StyI	2	57 274
TaqI	14	
TaqII	9	1076 1294 1967 3171 4510 4695 4848 4865 5209
TfiI	5	1847 2149 2319 2823 3244
ThaI	34	
TseI	31	
Tsp45I	8	1349 2177 2708 2921 3016 4418 4629 5478
Tsp509I	18	
Tth111I	1	3014
Tth111II	6	1007 1700 2730 3859 3866 3898
UbaII	20	
VspI	5	261 429 1853 1912 4334
XbaI	1	380
XcmI	3	1024 1540 1558
XhoI	1	212
XmnI	3	262 2827 4761

Enzymes that do not cut pET-25b(+):

AatII	AflIII	AgeI	AscI	AvrII
BaeI	BsmI	BsrGI	Bsu36I	Clal
FseI	KpnI	MunI	NruI	NsiI
NspV	Pacl	PmeI	PmlI	RleAI
RsrII	SacII	SexAI	SfiI	SgII
SmaI	SnaBI	SpeI	SrfI	Sse8387I
StuI	SunI	Swal		