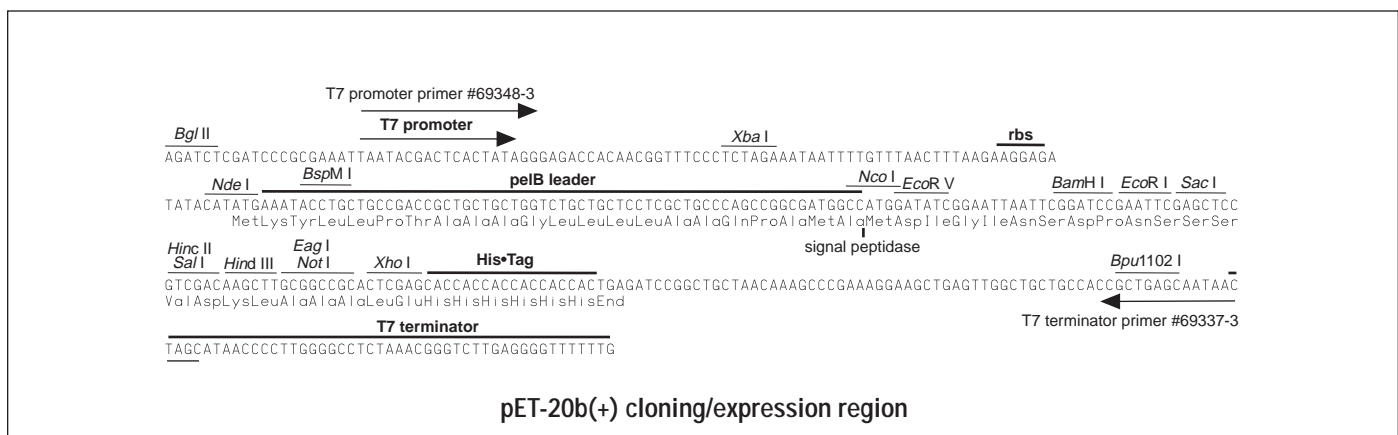
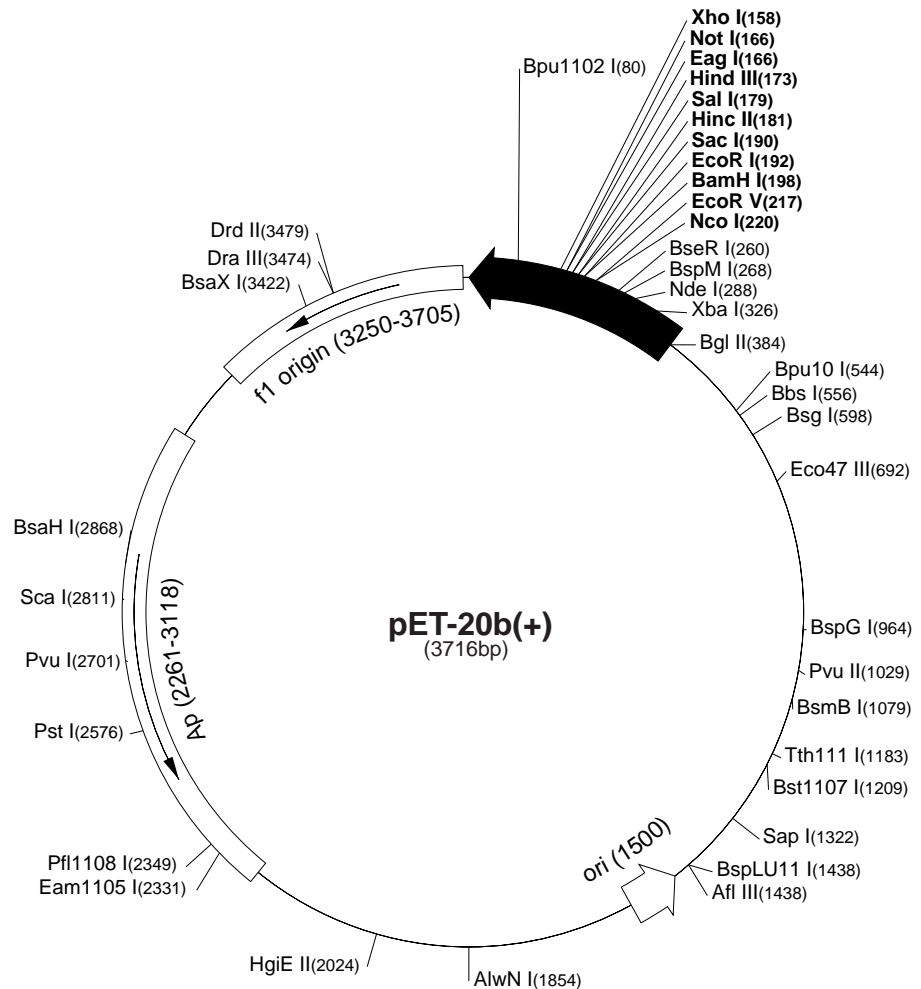


The pET-20b(+) vector (Cat. No. 69739-3) carries an N-terminal *pelB* signal sequence for potential periplasmic localization, plus optional C-terminal His•Tag® sequence. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region 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-20b(+) sequence landmarks

T7 promoter	353-369
T7 transcription start	352
<i>pelB</i> coding sequence	224-289
Multiple cloning sites	
(<i>Nco</i> I - <i>Xho</i> I)	158-225
His•Tag coding sequence	140-157
T7 terminator	26-72
pBR322 origin	1500
<i>bla</i> coding sequence	2261-3118
f1 origin	3250-3705



pET-20b(+) Restriction Sites

Enzyme	# Sites	Locations
AccI	2	180 1208
AccIII	5	947 1088 1390 2630 3314
Acil	49	
AfilIII	1	1438
Alul	18	
AlwI	13	
Alw21I	7	159 190 432 1256 1756
		2917 3002
Alw44I	3	1252 1752 2998
AlwNI	1	1854
ApoI	3	192 3276 3287
AvaI	2	158 388
AvaII	5	402 444 723 2469 2691
BamHI	1	198
BanI	2	2279 3511
BanII	2	190 3549
BbsI	1	556
BbvI	26	
BccI	6	227 2368 2492 2779 3464
		3481
Bce83I	5	21 1529 1827 2068 2936
BceII	2	1940 3500
Bcgl	6	160 194 1015 1049 2836
		2870
Bfal	7	70 327 452 1933 2186
		2521 3625
BglI	2	237 2451
BglII	1	384
Bpml	2	965 2401
Bpu10I	1	544
Bpu1102I	1	80
Bsal	2	351 2392
BsaAI	2	1190 3474
BsaBI	2	383 635
BsaHI	1	2868
BsaJI	4	57 220 410 1598
BsaVI	5	2 627 1644 1791 2622
BsaXI	1	3422
Bsbl	2	1154 3381
BscGI	10	44 414 799 1132 1765
		2111 2332 2356 2878 3568
BseRI	1	260
Bsgl	1	598
Bsil	2	1611 2995
BsiEI	6	169 271 1354 1778 2701
		2850
BsII	12	
BsmAI	4	351 1079 2392 3168
BsmBI	1	1079
BsmFI	2	709 3689
BsoFI	38	
Bsp24I	4	1931 1963 2109 2141
Bsp1286I	8	159 190 432 1256 1756
		2917 3002 3549
BspEI	2	2 627
BspGI	1	964
BspLU11I	1	1438
BspMI	1	268
BsrI	14	
BsrBI	3	1371 3172 3618
BsrDI	2	2392 2566
BsrFI	3	231 2411 3575
Bst1107I	1	1209
BstYI	10	132 198 384 630 2079
		2090 2176 2188 2956 2973
Cac8I	16	
CjeI	10	1451 1484 1925 1958 2003
		2006 2036 2039 2103 2136
CjePI	10	1925 1957 1958 1990 2103
		2136 2223 2256 2695 2728
CviJI	57	
CviRI	13	

Enzyme	# Sites	Locations
DdeI	10	80 101 136 544 706
		1246 1713 2122 2288 2828
DpnI	19	
DraI	3	2197 2216 2908
DrallI	1	3474
DrdI	3	1131 1546 3429
DrdII	1	3479
Dsal	2	220 410
EaeI	4	166 223 407 2719
EagI	1	166
Eam1105I	1	2331
EarI	2	1322 3126
EciI	3	1512 1658 2486
Eco47III	1	692
Eco57I	2	1986 2998
EcoO109I	3	53 402 444
EcoRI	1	192
EcoRII	4	404 1464 1585 1598
EcoRV	1	217
FauI	8	368 453 734 920 1141
		1151 3611 3680
FokI	8	657 719 797 983 1124
		2297 2478 2765
FspI	2	419 2553
GdIII	2	166 2719
HaeI	5	225 409 1453 1464 1916
HaeII	6	611 694 1316 1686 3625
		3633
HaeIII	14	
Hgal	6	974 1131 1549 2127 2857
		3691
HgiEI	1	2024
HhaI	23	
Hin4I	2	2330 2404
HincII	1	181
HindIII	1	173
Hinfl	9	359 488 992 1338 1413
		1809 2326 3401 3423
HphI	9	483 1058 1067 2174 2401
		2817 3023 3058 3475
Maell	12	
MaellI	15	
MbolI	8	556 1309 2100 2171 2926
		3004 3113 3614
MmeI	3	1653 1837 3451
MnlI	20	
MscI	2	225 409
MseI	23	
Msil	6	425 620 1011 2583 2742
		3101
MspI	18	
MspA1I	7	84 267 1029 1148 1780
		2025 2966
MwoI	19	
NciI	7	448 776 1082 1117 1818
		2514 2865
NcoI	1	220
NdeI	1	288
NgoAIV	2	231 3575
NlaIII	15	
NlaIV	15	
NotI	1	166
NspI	3	783 1075 1442
Pfi1108I	1	2349
PleI	6	367 1332 1817 2320 3409
		3417
Psp5II	2	402 444
Psp1406I	4	763 2557 2930 3259
PstI	1	2576
PvuI	1	2701
PvuII	1	1029
RcaI	2	2158 3166

Enzyme	# Sites	Locations
RsaI	2	1244 2811
SacI	1	190
Sall	1	179
SapI	1	1322
Sau96I	10	53 402 444 723 910
		2373 2452 2469 2691 3465
Sau3AI	19	
Scal	1	2811
ScrFI	11	
SfaNI	13	
SfcI	5	352 1703 1894 2572 3693
SspI	2	3135 3266
StyI	7	159 180 190 381 1538
		2982 3507
TaqII	6	1340 2679 2864 3017 3034
		3378
TfiI	3	488 992 1413
ThaI	16	
TseI	26	
Tsp45I	6	877 1090 1185 2587 2798
		3647
Tsp509I	11	
Tth111I	1	1183
Tth111II	4	899 2028 2035 2067
UbaII	12	
VspI	3	207 367 2503
XbaI	1	326
XhoI	1	158
XmnI	3	208 996 2930

Enzymes that do not cut pET-20b(+):

AatII	AfilI	AgeI	ApaI	ApaBI
AscI	AvrII	BaeI	BclI	BmgI
BsmI	BsrGI	BssHII	BstEII	BstXI
Bsu36I	Clal	EcoNI	FseI	HpaI
KpnI	MluI	MunI	NarI	NheI
NruI	NsiI	NspV	PacI	PfiMI
PmeI	PmlI	PshAI	RleAI	RsrII
SacII	SexAI	SfiI	SgfI	SgrAI
Smal	SnaBI	SpeI	SphI	SrfI
Sse8387I	StuI	SunI	Swal	XcmI