

# pET-28a-c(+) Vectors

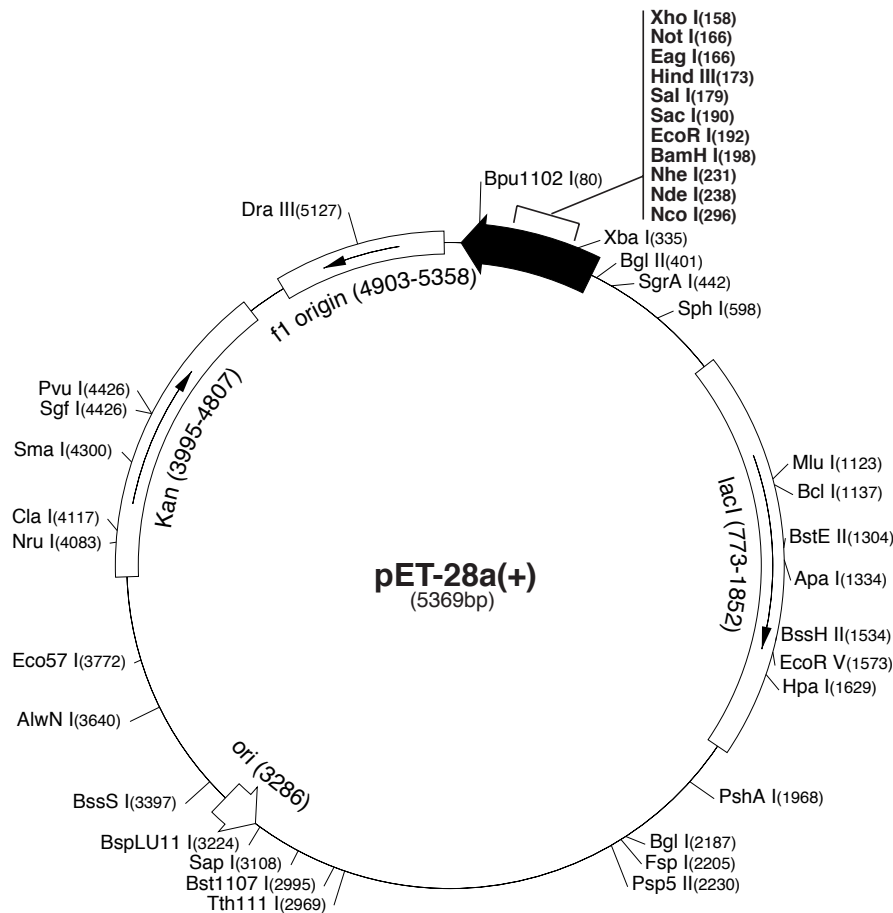
	Cat. No.
pET-28a DNA	69864-3
pET-28b DNA	69865-3
pET-28c DNA	69866-3

The pET-28a-c(+) vectors carry an N-terminal His•Tag®/thrombin/T7•Tag® configuration plus an 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-28a(+) sequence landmarks

T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites ( <i>Bam</i> H I - <i>Xho</i> I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lacI</i> coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.



T7 promoter primer #69348-3

pET upstream primer #69214-3 → *Bgl*II →

T7 promoter      lac operator      *Xba*I      rbs

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AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCGCCCTAGAAATAATTTGTTAACTTTAAGAAGGAGA

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Nco I      His•Tag      Nde I      Nhe I      T7•Tag

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TATACCATGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGC CGCGCGGCCATATGGCTAGCATGACTGGTGACAGCAA
MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerHisMetAlaSerMetThrGlyGlyGlnGln

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thrombin

BamH I   EcoR I   Sac I   Sal I   Hind III   Eag I   Not I   Xho I   His•Tag

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ATGGGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCGCACTCGAGCACCACCACCACCAGCTGAGATCCGGCTGCTAACAAAGCCC pET-28a(+)
MetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProLeuArgSerGlyCysEnd

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...GGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCGCACTCGAGCACCACCACCACCAGCTGAGATCCGGCTGCTAACAAAGCCC pET-28b(+)
...GlyArgAspProAsnSerSerSerValAspLysLeuAlaAlaLeuGluHisHisHisHisHisEnd

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...GGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCGCACTCGAGCACCACCACCACCAGCTGAGATCCGGCTGCTAACAAAGCCC pET-28c(+)
...GlyArgIleArgIleArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrGluIleArgLeuLeuThrLysPro...

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Bpu1102 I      T7 terminator

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GAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGCTTGAGGGGTTTTTTG

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T7 terminator primer #69337-3

**pET-28a-c(+) cloning/expression region**

# pET-28a(+) Restriction Sites

Enzyme	# Sites	Locations
AccI	2	180 2994
AccIII	7	890 1618 1949 2733 2874 3176 4967
Acil	77	
AflIII	2	1123 3224
AluI	22	
AlwI	13	
Alw21I	7	159 190 623 1107 2218 3042 3542
Alw44I	3	1103 3038 3538
AlwNI	1	3640
ApaI	1	1334
ApaBI	1	807
ApoI	6	192 1398 4039 4223 4929 4940
AvaI	2	158 4298
AvaII	5	1675 2051 2139 2230 2509
BamHI	1	198
BanI	9	253 445 466 580 1043 1762 1892 2018 5164
BanII	6	190 507 521 1334 4081 5202
BbsI	4	1269 1608 1982 2342
BbvI	27	
BccI	14	
Bce83I	6	21 1937 2107 3315 3613 3854
BceII	6	642 983 1610 3726 4745 5153
BcgI	9	160 194 228 1415 1449 1949 1983 2801 2835
BclI	1	1137
Bfal	7	70 232 336 2238 3719 4026 5278
BglI	1	2187
BglII	1	401
BmgI	1	1332
BpmI	4	961 1450 2084 2751
Bpu10I	2	2330 4443
Bpu1102I	1	80
BsaAI	2	2976 5127
BsaBI	3	400 406 2421
BsaHI	5	446 467 581 1080 1763
BsaJI	10	57 296 560 566 1758 2196 3384 4297 4298 4699
BsaWI	7	2 1442 1945 2413 3430 3577 4561
BsaXI	2	1782 5075
Bsbl	2	2940 5034
BscGI	11	
BsgI	3	974 1174 2384
Bsil	1	3397
BsiEI	5	169 1908 3140 3564 4426
BsII	23	
BsmI	2	4310 4387
BsmAI	6	820 1225 1351 1738 2865 4442
BsmBI	3	1738 2865 4442
BsmFI	4	584 2125 2495 5342
BsoFI	48	
Bsp24I	12	
Bsp1286I	12	
BspEI	2	2 2413
BspGI	1	2750
BspLU11I	1	3224
BsrI	22	
BsrBI	4	356 3157 4825 5271
BsrDI	2	1170 1536
BsrFI	7	433 442 809 2021 2181 4380 5228
BssHII	1	1534
Bst1107I	1	2995

Enzyme	# Sites	Locations
BstEII	1	1304
BstXI	3	925 1054 1177
BstYI	9	132 198 401 687 1899 2416 3865 3876 4675
Cac8I	40	
CjeI	26	
CjePI	30	
Clal	1	4117
CviJI	86	
CviRI	22	
DdeI	11	
DpnI	21	
DraIII	1	5127
DrdI	3	2917 3332 5082
DrdII	2	846 5132
Dsal	3	296 560 2196
EaeI	4	166 431 563 1797
EagI	1	166
EarI	3	741 3108 4239
Ecil	3	900 3298 3444
Eco47III	3	528 2029 2478
Eco57I	1	3772
EcoNI	2	658 4338
EcoO109I	3	53 556 2230
EcoRI	1	192
EcoRII	10	256 846 1161 1701 1758 3250 3371 3384 4314 4671
EcoRV	1	1573
FauI	17	
FokI	9	1169 1178 2443 2505 2583 2769 2910 4064 4670
FspI	1	2205
GdIII	4	166 431 563 1797
HaeI	6	851 2172 3239 3250 3702 4513
HaeII	14	
HaeIII	24	
Hgal	11	
HgiEI	2	721 3810
HhaI	47	
Hin4I	3	1022 4112 4654
HincII	2	181 1629
HindIII	1	173
Hinfl	18	
HpaI	1	1629
HphI	16	
MaeII	14	
MaeIII	16	
MbolI	12	
MluI	1	1123
MmeI	7	3439 3623 4068 4262 4624 4633 5104
MnlI	25	
MseI	25	
MslI	6	1175 1463 1493 2211 2406 2797
MspI	29	
MspAII	9	84 264 1153 1723 1816 2815 2934 3566 3811
MwoI	39	
NarI	4	446 467 581 1763
NciI	12	
NcoI	1	296
NdeI	1	238
NgoAIV	4	433 2021 2181 5228
NheI	1	231
NlaIII	26	
NlaIV	22	
NottI	1	166
NruI	1	4083
Nsil	2	4276 4542
NspI	4	598 2569 2861 3228

Enzyme	# Sites	Locations
Pfl1108I	1	2010
PflMI	2	705 4689
PleI	9	384 672 759 1555 3118 3603 4658 5062 5070
PshAI	1	1968
Psp5II	1	2230
Psp1406I	4	785 2153 2549 4912
PvuI	1	4426
PvuII	3	1723 1816 2815
RcaI	3	521 3944 4819
RsaI	3	1270 3030 4261
SacI	1	190
SalI	1	179
SapI	1	3108
Sau96I	14	
Sau3AI	21	
ScrFI	22	
SfaNI	23	
SfcI	4	369 3489 3680 5346
SgfI	1	4426
SgrAI	1	442
SmaI	1	4300
SphI	1	598
SspI	2	4351 4919
StyI	2	57 296
TaqI	15	
TaqII	6	1031 1249 1922 3126 4680 5031
TfiI	9	1802 2104 2274 2778 3199 4337 4393 4565 4656
ThaI	38	
TseI	27	
Tsp45I	7	1304 2132 2663 2876 2971 4573 5300
Tsp509I	20	
Tth111I	1	2969
Tth111II	8	962 1655 2685 3814 3821 3853 4262 4389
UbaJI	21	
VspI	5	384 1808 1867 4625 4814
XbaI	1	335
XcmI	3	979 1495 1513
XhoI	1	158
XmnI	2	2782 4815

Enzymes that do not cut pET28a(+):

AatII	AflII	AgeI	AscI	AvrII
BaeI	BsaI	BseRI	BspMI	BsrGI
Bsu36I	DraI	Eam1105I	FseI	KpnI
MscI	MunI	NspV	PaeI	PmeI
PmlI	PstI	RleAI	RspII	SacII
Scal	SexAI	SfiI	SnaBI	SpeI
SrfI	Sse8387I	StuI	SunI	Swal