

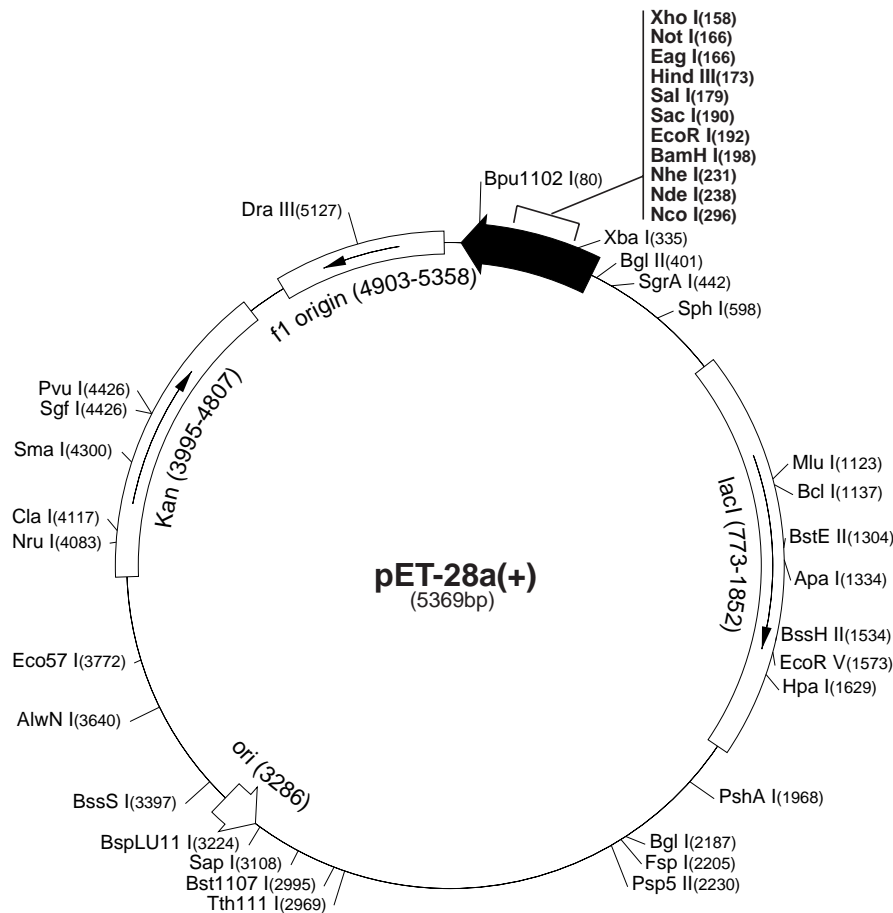
# pET-28a-c(+) Vectors

|             | 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<sup>®</sup>/thrombin/T7•Tag<sup>®</sup> 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<br>( <i>Bam</i> H I - <i>Xho</i> I) | 158-203   |
| His•Tag coding sequence                                    | 140-157   |
| T7 terminator  | 26-72     |
| <i>lac</i> 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

AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCGCCCTAGAAATAATTTGTTAACTTTAAGAAGGAGA

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

TATACCATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGC CGCGCGCCAGCCATATGGCTAGCATGACTGGTGACAGCAA

MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerHisMetAlaSerMetThrGlyGlyGlnGln

Eag I      thrombin      His•Tag

ATGGGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCGCACTCGAGCACCACCACCACCAGCTGAGATCCGGCTGCTAACAAAGCCC pET-28a(+)

MetGlyArgGlySerGlyPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProLeuArgSerGlyCysEnd

BamH I      EcoR I      Sac I      Sal I      Hind III      Eag I      Xho I

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

...GlyArgAspProAsnSerSerSerValAspLysLeuAlaAlaLeuGluHisHisHisHisHisEnd

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

...GlyArgIleArgIleArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrGluIleArgLeuLeuThrLysPro...

Bpu1102 I      T7 terminator

GAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGCTTGAGGGGTTTTTTG

T7 terminator primer #69337-3

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

# pET-28a(+) Restriction Sites

| Enzyme   | # Sites | Locations                | Enzyme   | # Sites | Locations                | Enzyme                             | # Sites  | Locations                |       |       |
|----------|---------|--------------------------|----------|---------|--------------------------|------------------------------------|----------|--------------------------|-------|-------|
| AccI     | 2       | 180 2994                 | BstEII   | 1       | 1304                     | PfiI108I                           | 1        | 2010                     |       |       |
| AccIII   | 7       | 890 1618 1949 2733 2874  | BstXI    | 3       | 925 1054 1177            | PfiMI                              | 2        | 705 4689                 |       |       |
|          |         | 3176 4967                | BstYI    | 9       | 132 198 401 687 1899     | PleI                               | 9        | 384 672 759 1555 3118    |       |       |
| AcI      | 77      |                          |          |         | 2416 3865 3876 4675      |                                    |          | 3603 4658 5062 5070      |       |       |
| AflIII   | 2       | 1123 3224                | Cac8I    | 40      |                          | PshAI                              | 1        | 1968                     |       |       |
| AluI     | 22      |                          | CjeI     | 26      |                          | Psp5II                             | 1        | 2230                     |       |       |
| AlwI     | 13      |                          | CjePI    | 30      |                          | Psp1406I                           | 4        | 785 2153 2549 4912       |       |       |
| Alw21I   | 7       | 159 190 623 1107 2218    | Clal     | 1       | 4117                     | PvuI                               | 1        | 4426                     |       |       |
|          |         | 3042 3542                | CviJI    | 86      |                          | PvuII                              | 3        | 1723 1816 2815           |       |       |
| Alw44I   | 3       | 1103 3038 3538           | CviRI    | 22      |                          | RcaI                               | 3        | 521 3944 4819            |       |       |
| AlwNI    | 1       | 3640                     | DdeI     | 11      |                          | RsaI                               | 3        | 1270 3030 4261           |       |       |
| ApaI     | 1       | 1334                     | DpnI     | 21      |                          | SacI                               | 1        | 190                      |       |       |
| ApaBI    | 1       | 807                      | DraIII   | 1       | 5127                     | Sall                               | 1        | 179                      |       |       |
| ApoI     | 6       | 192 1398 4039 4223 4929  | DrdI     | 3       | 2917 3332 5082           | SapI                               | 1        | 3108                     |       |       |
|          |         | 4940                     | DrdII    | 2       | 846 5132                 | Sau96I                             | 14       |                          |       |       |
| AvaI     | 2       | 158 4298                 | Dsal     | 3       | 296 560 2196             | Sau3AI                             | 21       |                          |       |       |
| AvaII    | 5       | 1675 2051 2139 2230 2509 | EaeI     | 4       | 166 431 563 1797         | ScrFI                              | 22       |                          |       |       |
| BamHI    | 1       | 198                      | EagI     | 1       | 166                      | SfaNI                              | 23       |                          |       |       |
| BanI     | 9       | 253 445 466 580 1043     | EarI     | 3       | 741 3108 4239            | SfiCI                              | 4        | 369 3489 3680 5346       |       |       |
|          |         | 1762 1892 2018 5164      | Ecil     | 3       | 900 3298 3444            | Sgfl                               | 1        | 4426                     |       |       |
| BanII    | 6       | 190 507 521 1334 4081    | Eco47III | 3       | 528 2029 2478            | SgrAI                              | 1        | 442                      |       |       |
|          |         | 5202                     | Eco57I   | 1       | 3772                     | Smal                               | 1        | 4300                     |       |       |
| BbsI     | 4       | 1269 1608 1982 2342      | EcoNI    | 2       | 658 4338                 | SphI                               | 1        | 598                      |       |       |
| BbvI     | 27      |                          | EcoO109I | 3       | 53 556 2230              | Sspl                               | 2        | 4351 4919                |       |       |
| BccI     | 14      |                          | EcoRI    | 1       | 192                      | StyI                               | 2        | 57 296                   |       |       |
| Bce83I   | 6       | 21 1937 2107 3315 3613   | EcoRII   | 10      | 256 846 1161 1701 1758   | TaqI                               | 15       |                          |       |       |
|          |         | 3854                     |          |         | 3250 3371 3384 4314 4671 | TaqII                              | 6        | 1031 1249 1922 3126 4680 |       |       |
| BceII    | 6       | 642 983 1610 3726 4745   | EcoRV    | 1       | 1573                     |                                    |          | 5031                     |       |       |
|          |         | 5153                     | FauI     | 17      |                          | TfiI                               | 9        | 1802 2104 2274 2778 3199 |       |       |
| BcgI     | 9       | 160 194 228 1415 1449    | FokI     | 9       | 1169 1178 2443 2505 2583 |                                    |          | 4337 4393 4565 4656      |       |       |
|          |         | 1949 1983 2801 2835      |          |         | 2769 2910 4064 4670      | Thal                               | 38       |                          |       |       |
| BclI     | 1       | 1137                     | FspI     | 1       | 2205                     | Tsel                               | 27       |                          |       |       |
| Bfal     | 7       | 70 232 336 2238 3719     | GdiII    | 4       | 166 431 563 1797         | Tsp45I                             | 7        | 1304 2132 2663 2876 2971 |       |       |
|          |         | 4026 5278                | HaeI     | 6       | 851 2172 3239 3250 3702  |                                    |          | 4573 5300                |       |       |
| BglI     | 1       | 2187                     |          |         | 4513                     | Tsp509I                            | 20       |                          |       |       |
| BglII    | 1       | 401                      | HaeII    | 14      |                          | Tth111I                            | 1        | 2969                     |       |       |
| BmgI     | 1       | 1332                     | HaeIII   | 24      |                          | Tth111II                           | 8        | 962 1655 2685 3814 3821  |       |       |
| BpmI     | 4       | 961 1450 2084 2751       | Hgal     | 11      |                          |                                    |          | 3853 4262 4389           |       |       |
| Bpu10I   | 2       | 2330 4443                | HgiEII   | 2       | 721 3810                 | UbaII                              | 21       |                          |       |       |
| Bpu1102I | 1       | 80                       | Hhal     | 47      |                          | VspI                               | 5        | 384 1808 1867 4625 4814  |       |       |
| BsaAI    | 2       | 2976 5127                | Hin4I    | 3       | 1022 4112 4654           | XbaI                               | 1        | 335                      |       |       |
| BsaBI    | 3       | 400 406 2421             | HincII   | 2       | 181 1629                 | XcmI                               | 3        | 979 1495 1513            |       |       |
| BsaHI    | 5       | 446 467 581 1080 1763    | HindIII  | 1       | 173                      | XhoI                               | 1        | 158                      |       |       |
| BsaJI    | 10      | 57 296 560 566 1758      | Hinfl    | 18      |                          | XmnI                               | 2        | 2782 4815                |       |       |
|          |         | 2196 3384 4297 4298 4699 | HpaI     | 1       | 1629                     |                                    |          |                          |       |       |
| BsaWI    | 7       | 2 1442 1945 2413 3430    | HphI     | 16      |                          | Enzymes that do not cut pET28a(+): |          |                          |       |       |
|          |         | 3577 4561                | MaeII    | 14      |                          | AatII                              | AflII    | AgeI                     | AscI  | AvrII |
| BsaXI    | 2       | 1782 5075                | MaeIII   | 16      |                          | BaeI                               | BsaI     | BseRI                    | BspMI | BsrGI |
| Bsbl     | 2       | 2940 5034                | MbolI    | 12      |                          | Bsu36I                             | DraI     | Eam1105I                 | FseI  | KpnI  |
| BscGI    | 11      |                          | MluI     | 1       | 1123                     | MscI                               | MunI     | NspV                     | Pacl  | PmeI  |
| BsGI     | 3       | 974 1174 2384            | MmeI     | 7       | 3439 3623 4068 4262 4624 | PmlI                               | PstI     | RleAI                    | RsrII | SacII |
| Bsil     | 1       | 3397                     |          |         | 4633 5104                | Scal                               | SexAI    | Sfil                     | SnaBI | SpeI  |
| BsiEI    | 5       | 169 1908 3140 3564 4426  | MnlI     | 25      |                          | SrfI                               | Sse8387I | StuI                     | SunI  | Swal  |
| BsII     | 23      |                          | MseI     | 25      |                          |                                    |          |                          |       |       |
| BsmI     | 2       | 4310 4387                | MslI     | 6       | 1175 1463 1493 2211 2406 |                                    |          |                          |       |       |
| BsmAI    | 6       | 820 1225 1351 1738 2865  |          |         | 2797                     |                                    |          |                          |       |       |
|          |         | 4442                     | MspI     | 29      |                          |                                    |          |                          |       |       |
| BsmBI    | 3       | 1738 2865 4442           | MspAII   | 9       | 84 264 1153 1723 1816    |                                    |          |                          |       |       |
| BsmFI    | 4       | 584 2125 2495 5342       |          |         | 2815 2934 3566 3811      |                                    |          |                          |       |       |
| BsoFI    | 48      |                          | MwoI     | 39      |                          |                                    |          |                          |       |       |
| Bsp24I   | 12      |                          | NarI     | 4       | 446 467 581 1763         |                                    |          |                          |       |       |
| Bsp1286I | 12      |                          | NciI     | 12      |                          |                                    |          |                          |       |       |
| BspEI    | 2       | 2 2413                   | NcoI     | 1       | 296                      |                                    |          |                          |       |       |
| BspGI    | 1       | 2750                     | NdeI     | 1       | 238                      |                                    |          |                          |       |       |
| BspLU11I | 1       | 3224                     | NgoAIV   | 4       | 433 2021 2181 5228       |                                    |          |                          |       |       |
| BsrI     | 22      |                          | NheI     | 1       | 231                      |                                    |          |                          |       |       |
| BsrBI    | 4       | 356 3157 4825 5271       | NlaIII   | 26      |                          |                                    |          |                          |       |       |
| BsrDI    | 2       | 1170 1536                | NlaIV    | 22      |                          |                                    |          |                          |       |       |
| BsrFI    | 7       | 433 442 809 2021 2181    | NottI    | 1       | 166                      |                                    |          |                          |       |       |
|          |         | 4380 5228                | NruI     | 1       | 4083                     |                                    |          |                          |       |       |
| BssHII   | 1       | 1534                     | Nsil     | 2       | 4276 4542                |                                    |          |                          |       |       |
| Bst1107I | 1       | 2995                     | Nspl     | 4       | 598 2569 2861 3228       |                                    |          |                          |       |       |