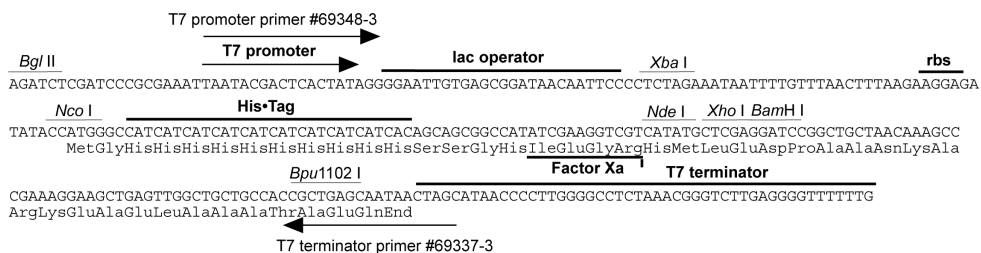
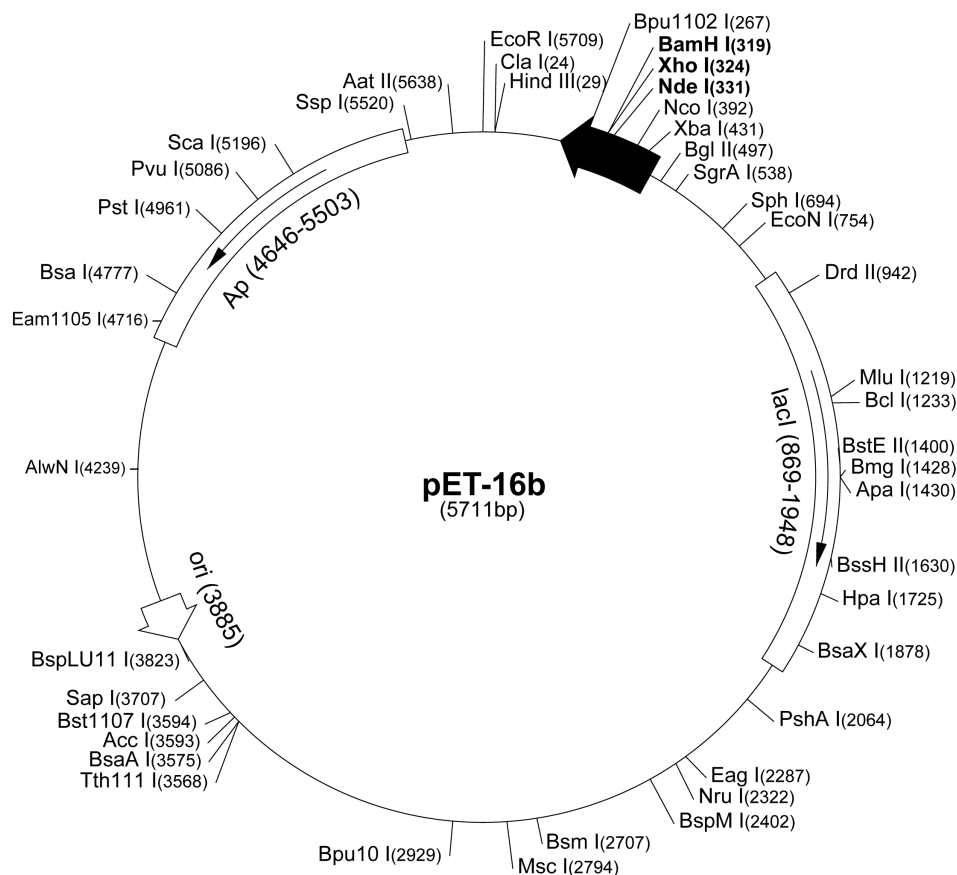


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The pET-16b vector (Cat. No. 69662-3) carries an N-terminal His•Tag® sequence followed by a Factor Xa site and three cloning sites. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below.

|                                                          |           |
|----------------------------------------------------------|-----------|
| T7 promoter                                              | 466-482   |
| T7 transcription start                                   | 465       |
| His•Tag coding sequence                                  | 360-389   |
| Multiple cloning sites<br>( <i>Nde</i> I- <i>Bam</i> HI) | 319-335   |
| T7 terminator                                            | 213-259   |
| <i>lacI</i> coding sequence                              | 869-1948  |
| pBR322 origin                                            | 3885      |
| <i>bla</i> coding sequence                               | 4646-5503 |



**pET-16b cloning/expression region**

# pET-16b Restriction Sites

| Enzyme   | # Sites | Locations |      |      |      |      | Enzyme   | # Sites | Locations |      |      |      |        | Enzyme                           | # Sites  | Locations |        |       |      |      |      |
|----------|---------|-----------|------|------|------|------|----------|---------|-----------|------|------|------|--------|----------------------------------|----------|-----------|--------|-------|------|------|------|
| AatII    | 1       | 5638      |      |      |      |      | BssHII   | 1       | 1630      |      |      |      |        | PfiIMI                           | 3        | 801       | 2669   | 2718  |      |      |      |
| AccI     | 1       | 3593      |      |      |      |      | Bst1107I | 1       | 3594      |      |      |      |        | PleI                             | 7        | 480       | 768    | 855   | 1651 | 3717 |      |
| AccIII   | 7       | 986       | 1714 | 2045 | 3332 | 3473 | BstEII   | 1       | 1400      |      |      |      |        |                                  |          | 4202      | 4705   |       |      |      |      |
|          |         | 3775      | 5015 |      |      |      | BstXI    | 3       | 1021      | 1150 | 1273 |      |        |                                  |          |           |        |       |      |      |      |
| Acil     | 89      |           |      |      |      |      | BstYI    | 11      |           |      |      |      |        | PshAI                            | 1        | 2064      |        |       |      |      |      |
| AfIII    | 2       | 1219      | 3823 |      |      |      | Cac8I    | 41      |           |      |      |      |        | Psp5II                           | 2        | 2787 2829 |        |       |      |      |      |
| AluI     | 24      |           |      |      |      |      | Cjel     | 26      |           |      |      |      |        | Psp1406I                         | 5        | 881       | 2249   | 3148  | 4942 | 5315 |      |
| AlwI     | 16      |           |      |      |      |      | CjePI    | 28      |           |      |      |      |        | PstI                             | 1        | 4961      |        |       |      |      |      |
| Alw21I   | 8       | 719       | 1203 | 2526 | 2817 | 3641 | Clal     | 1       | 24        |      |      |      |        | PvuI                             | 1        | 5086      |        |       |      |      |      |
|          |         | 4141      | 5302 | 5387 |      |      | CviI     | 96      |           |      |      |      |        | PvuII                            | 3        | 1819      | 1912   | 3414  |      |      |      |
| Alw44I   | 4       | 1199      | 3637 | 4137 | 5383 |      | CviRI    | 26      |           |      |      |      |        | RcaI                             | 4        | 617       | 4543   | 5551  | 5656 |      |      |
| AlwNI    | 1       | 4239      |      |      |      |      | Ddel     | 11      |           |      |      |      |        | Rsal                             | 4        | 165       | 1366   | 3629  | 5196 |      |      |
| Apal     | 1       | 1430      |      |      |      |      | Dpnl     | 29      |           |      |      |      |        | SapI                             | 1        | 3707      |        |       |      |      |      |
| ApaBI    | 2       | 903       | 2400 |      |      |      | DraI     | 3       | 4582      | 4601 | 5293 |      |        | Sau96I                           | 22       |           |        |       |      |      |      |
| ApoI     | 2       | 1494      | 5709 |      |      |      | DrdI     | 2       | 3516      | 3931 |      |      | Sau3AI | 37                               |          |           |        |       |      |      |      |
| AvaI     | 2       | 324       | 2773 |      |      |      | DrdII    | 1       | 942       |      |      |      |        | Scal                             | 1        | 5196      |        |       |      |      |      |
| Avall    | 9       | 1771      | 2147 | 2235 | 2484 | 2787 | Dsal     | 3       | 392       | 656  | 2795 |      | ScrFI  | 24                               |          |           |        |       |      |      |      |
|          |         | 2829      | 3108 | 4854 | 5076 |      | EaeI     | 7       | 349       | 527  | 659  | 1893 | 2287   | SfaNI                            | 24       |           |        |       |      |      |      |
|          |         |           |      |      |      |      |          |         | 2792      | 5104 |      |      |        | Sfcl                             | 5        | 138       | 465    | 4088  | 4279 | 4957 |      |
| BamHI    | 1       | 319       |      |      |      |      | EagI     | 1       | 2287      |      |      |      |        | SgrAI                            | 1        | 538       |        |       |      |      |      |
| BanI     | 12      |           |      |      |      |      | Eam1105I | 1       | 4716      |      |      |      |        | SphI                             | 1        | 694       |        |       |      |      |      |
| BanII    | 3       | 603       | 617  | 1430 |      |      | EarI     | 3       | 837       | 3707 | 5511 |      | Sspl   | 1                                | 5520     |           |        |       |      |      |      |
| BbsI     | 5       | 1365      | 1704 | 2078 | 2941 | 5694 | Ecil     | 5       | 996       | 2743 | 3897 | 4043 | 4871   | StyI                             | 3        | 244       | 392    | 2717  |      |      |      |
| BbvI     | 28      |           |      |      |      |      | Eco47III | 3       | 624       | 2125 | 3077 |      |        | TaqI                             | 14       |           |        |       |      |      |      |
| BccI     | 16      |           |      |      |      |      | Eco57I   | 2       | 4371      | 5383 |      |      |        |                                  | TaqII    | 8         | 1127   | 1345  | 2018 | 3725 | 5064 |
| Bce83I   | 7       | 208       | 2033 | 2203 | 3914 | 4212 | EcoNI    | 1       | 754       |      |      |      |        |                                  |          | 5249      | 5402   | 5419  |      |      |      |
|          |         | 4453      | 5321 |      |      |      | EcoO109I | 5       | 240       | 652  | 2787 | 2829 | 5692   | TfiI                             | 7        | 1898      | 2200   | 2354  | 2652 | 2873 |      |
| BceII    | 5       | 738       | 1079 | 1706 | 2515 | 4325 | EcoRI    | 1       | 5709      |      |      |      |        |                                  |          | 3377      | 3798   |       |      |      |      |
| Bcgl     | 8       | 1511      | 1545 | 2045 | 2079 | 3400 | EcoRII   | 10      | 129       | 942  | 1257 | 1797 | 1854   |                                  |          |           |        |       |      |      |      |
|          |         | 3434      | 5221 | 5255 |      |      |          |         | 2406      | 2789 | 3849 | 3970 | 3983   |                                  |          |           |        |       |      |      |      |
| BclI     | 1       | 1233      |      |      |      |      | EcoRV    | 2       | 187       | 1669 |      |      |        |                                  | Thal     | 39        |        |       |      |      |      |
| Bfal     | 6       | 257       | 432  | 2837 | 4318 | 4571 | FauI     | 18      |           |      |      |      |        | TseI                             | 28       |           |        |       |      |      |      |
|          |         | 4906      |      |      |      |      | FokI     | 14      |           |      |      |      |        | Tsp45I                           | 9        | 124       | 1400   | 2228  | 2495 | 3262 |      |
| BglI     | 3       | 2283      | 2517 | 4836 |      |      | FspI     | 3       | 2706      | 2804 | 4938 |      |        |                                  |          | 3475      | 3570   | 4972  | 5183 |      |      |
| BglII    | 1       | 497       |      |      |      |      | GdIII    | 6       | 349       | 527  | 659  | 1893 | 2287   | Tsp509I                          | 16       |           |        |       |      |      |      |
| BmgI     | 1       | 1428      |      |      |      |      |          |         | 5104      |      |      |      |        | Tth111I                          | 1        | 3568      |        |       |      |      |      |
| BpmI     | 6       | 1057      | 1546 | 2180 | 2734 | 3350 | HaeI     | 8       | 947       | 2268 | 2340 | 2397 | 2794   | Tth111II                         | 7        | 1058      | 1751   | 3284  | 4413 | 4420 |      |
|          |         | 4786      |      |      |      |      |          |         | 3838      | 3849 | 4301 |      |        |                                  |          | 4452      | 5708   |       |      |      |      |
| Bpu10I   | 1       | 2929      |      |      |      |      | HaeII    | 13      |           |      |      |      |        | UbaII                            | 24       |           |        |       |      |      |      |
| Bpu1102I | 1       | 267       |      |      |      |      | HaeIII   | 29      |           |      |      |      |        | VspI                             | 4        | 480       | 1904   | 1963  | 4888 |      |      |
| BsaI     | 1       | 4777      |      |      |      |      | Hgal     | 15      |           |      |      |      |        | XbaI                             | 1        | 431       |        |       |      |      |      |
| BsaAI    | 1       | 3575      |      |      |      |      | HgiEII   | 2       | 817       | 4409 |      |      |        | XcmI                             | 3        | 1075      | 1591   | 1609  |      |      |      |
| BsaBI    | 3       | 496       | 502  | 3020 |      |      | HhaI     | 44      |           |      |      |      |        | XhoI                             | 1        | 324       |        |       |      |      |      |
| BsaHI    | 8       | 542       | 563  | 677  | 1176 | 1859 | Hin4I    | 5       | 16        | 1118 | 2489 | 4715 | 4789   | XmnI                             | 2        | 3381      | 5315   |       |      |      |      |
|          |         | 2554      | 5253 | 5635 |      |      | HincII   | 2       | 1725      | 5257 |      |      |        | Enzymes that do not cut pET-16b: |          |           |        |       |      |      |      |
| BsaJI    | 11      |           |      |      |      |      | HindIII  | 1       | 29        |      |      |      |        | AfIII                            | Agel     | Ascl      | AvrII  | Bael  |      |      |      |
| BsaWI    | 7       | 189       | 1538 | 2041 | 3012 | 4029 | Hinfl    | 14      |           |      |      |      |        | BseRI                            | BsrGI    | Bsu36I    | DraIII | FseI  |      |      |      |
|          |         | 4176      | 5007 |      |      |      | HpaI     | 1       | 1725      |      |      |      |        | KpnI                             | MunI     | NheI      | NotI   | NsiI  |      |      |      |
| BsaXI    | 1       | 1878      |      |      |      |      | HphI     | 17      |           |      |      |      |        | NspV                             | PacI     | PmeI      | PmlI   | RleAI |      |      |      |
| Bsbl     | 2       | 3539      | 5259 |      |      |      | Maell    | 12      |           |      |      |      |        | RsrII                            | SacI     | SacII     | Sall   | SexAI |      |      |      |
| BscGI    | 13      |           |      |      |      |      | MaeII    | 18      |           |      |      |      |        | Sfil                             | Sgfl     | Smal      | SnaBI  | SpeI  |      |      |      |
| BsgI     | 3       | 1070      | 1270 | 2983 |      |      | MbolI    | 15      |           |      |      |      |        | Srfl                             | Sse8387I | Stul      | SunI   | Swal  |      |      |      |
| Bsil     | 3       | 3996      | 5380 | 5687 |      |      | MluI     | 1       | 1219      |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BsiEI    | 6       | 2004      | 2290 | 3739 | 4163 | 5086 | MmeI     | 2       | 4038      | 4222 |      |      |        |                                  |          |           |        |       |      |      |      |
|          |         | 5235      |      |      |      |      | MnII     | 34      |           |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BsII     | 22      |           |      |      |      |      | MscI     | 1       | 2794      |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BsmI     | 1       | 2707      |      |      |      |      | MseI     | 24      |           |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BsmAI    | 7       | 916       | 1321 | 1447 | 1834 | 3464 | MslI     | 10      | 1271      | 1559 | 1589 | 2379 | 2810   |                                  |          |           |        |       |      |      |      |
|          |         | 4777      | 5553 |      |      |      |          |         | 3005      | 3396 | 4968 | 5127 | 5486   |                                  |          |           |        |       |      |      |      |
| BsmBI    | 2       | 1834      | 3464 |      |      |      | MspI     | 35      |           |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BsmFI    | 4       | 680       | 2221 | 2446 | 3094 |      | MspA1I   | 11      |           |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BsoFI    | 52      |           |      |      |      |      | Mwol     | 44      |           |      |      |      |        |                                  |          |           |        |       |      |      |      |
| Bsp24I   | 12      |           |      |      |      |      | NarI     | 5       | 542       | 563  | 677  | 1859 | 2554   |                                  |          |           |        |       |      |      |      |
| Bsp1286I | 11      |           |      |      |      |      | NciI     | 14      |           |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BspEI    | 2       | 189       | 3012 |      |      |      | NcoI     | 1       | 392       |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BspGI    | 3       | 2407      | 2484 | 3349 |      |      | NdeI     | 1       | 331       |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BspLU11I | 1       | 3823      |      |      |      |      | NgoAIV   | 4       | 529       | 2117 | 2277 | 2631 |        |                                  |          |           |        |       |      |      |      |
| BspMI    | 1       | 2402      |      |      |      |      | NlaIII   | 31      |           |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BsrI     | 25      |           |      |      |      |      | NlaIV    | 28      |           |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BsrBI    | 3       | 452       | 3756 | 5557 |      |      | NruI     | 1       | 2322      |      |      |      |        |                                  |          |           |        |       |      |      |      |
| BsrDI    | 4       | 1266      | 1632 | 4777 | 4951 |      | NspI     | 4       | 694       | 3168 | 3460 | 3827 |        |                                  |          |           |        |       |      |      |      |
| BsrFI    | 8       | 160       | 529  | 538  | 905  | 2117 | Pfi1108I | 2       | 2106      | 4734 |      |      |        |                                  |          |           |        |       |      |      |      |
|          |         | 2277      | 2631 | 4796 |      |      |          |         |           |      |      |      |        |                                  |          |           |        |       |      |      |      |