

## pET-12a-c Vectors

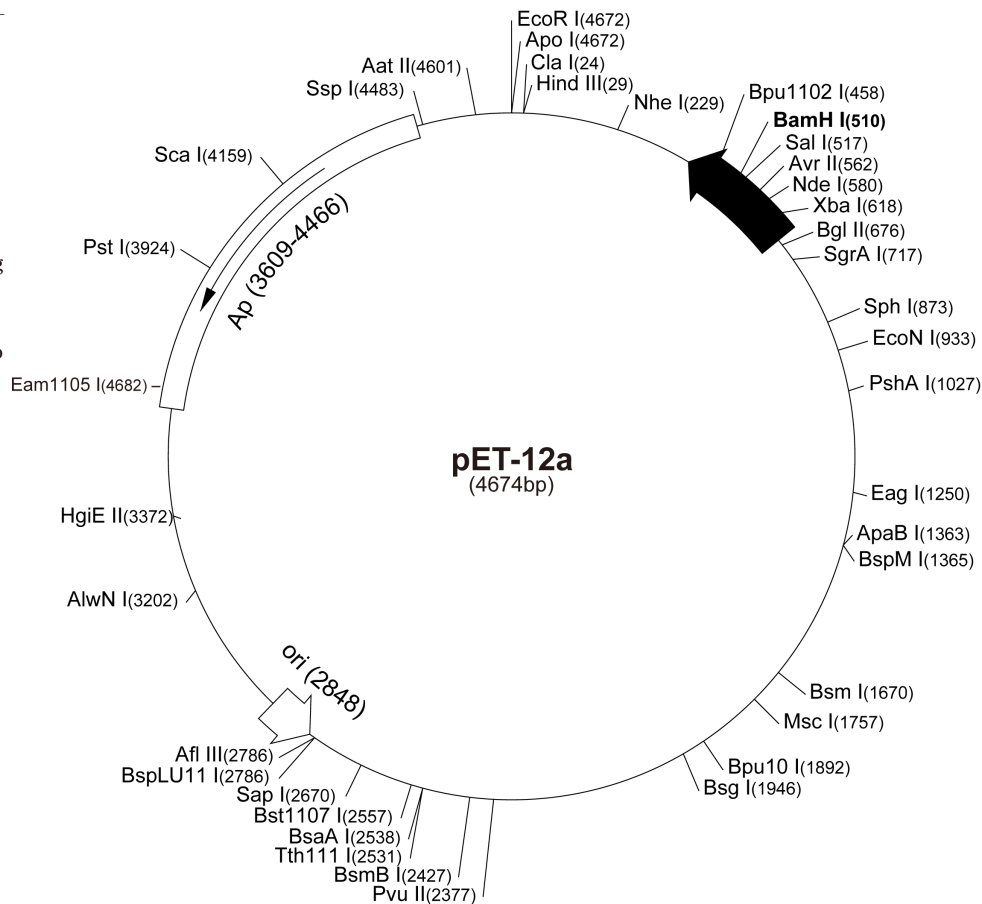
|             | Cat. No. |
|-------------|----------|
| pET-12a DNA | 69440-3  |
| pET-12b DNA | 69441-3  |
| pET-12c DNA | 69442-3  |

The pET-12a-c vectors carry an N-terminal *ompT* sequence for potential periplasmic export of target proteins. 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.

### pET-12a sequence landmarks

|                             |           |
|-----------------------------|-----------|
| T7 promoter                 | 645-661   |
| T7 transcription start      | 644       |
| <i>ompT</i> coding sequence | 516-578   |
| T7 terminator               | 404-450   |
| pBR322 origin               | 2848      |
| <i>bla</i> coding sequence  | 3609-4466 |

The maps for pET-12b and pET-12c are the same as pET-12a (shown) with the following exceptions: pET-12b is a 4673bp plasmid; subtract 1bp from each site beyond *BamH* I at 510. pET-12c is a 4675bp plasmid; add 1bp to each site beyond *BamH* I at 510.



T7 promoter primer #69348-3

*Bgl* II T7 promoter *Xba* I rbs

AGATCTCGATCCCAGAAATTAATACGACTCACTATAGGGAGACCAACAACGGTTTCCCTCTAGAAATAATTTTGTTAACTTTAAGAAGGAGA

*Nde* I *ompT* leader pET-12a *Sal* I *Bam* H I *Bpu*1102 I

TATACATATCGGGCGAAACTCCTAGGAATAGTCTGACAACCCCTATCGGATCAGCTCTTTGCGTGCACGGATCCGGCTGCTAACAAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAG

MetArgAlaLysLeuLeuGlyIleValLeuThrThrProIleAlaIleSerSerPheAlaSerThrGlySerGlyCysEnd

signal peptidase T7 terminator primer #69337-3

|         |   |
|---------|---|
| pET-12b | ...ACGGATCCGGCTGCTAACAAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAG    |
|         | ...ThrAspProAlaAlaAsnLysAlaArgLysGluAlaGluLeuAlaAlaAlaThrAlaGluGlnEnd       |
| pET-12c | ...ACGGGATCCGGCTGCTAACAAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAG   |
|         | ...ThrGlyIleArgLeuLeuThrLysProGluArgLysLeuSerTrpLeuLeuProProLeuSerAsnAsnEnd |

T7 terminator

CATAACCCCTTGGGGCTCTAAACGGGTCTTGAGGGGTTTTTTG

**pET-12a-c cloning/expression region**

pET-12a Restriction Sites

| Enzyme   | # Sites | Locations  | Enzyme   | # Sites | Locations  | Enzyme                           | # Sites | Locations                                       |        |          |
|----------|---------|--|----------|---------|--|----------------------------------|---------|---|--------|----------|
| AatII    | 1       | 4601   | Bst1107I | 1       | 2557   | PleI                             | 5       | 659 947 2680 3165 3668                          |        |          |
| AccI     | 2       | 518 2556   | BstYI    | 9       | 510 676 1978 3427 3438<br>3524 3536 4304             | PshAI                            | 1       | 1027  |        |          |
| AccIII   | 6       | 517 1008 2295 2436 2738<br>3978                  | Cac8I    | 32      |  | Psp5II                           | 2       | 1750 1792                                       |        |          |
| Acil     | 70      |  | CjeI     | 16      |  | Psp1406I                         | 4       | 1212 2111 3905 4278                             |        |          |
| AflIII   | 1       | 2786   | CjePI    | 20      |  | PstI                             | 1       | 3924  |        |          |
| AluI     | 19      |  | Clal     | 1       | 24   | PvuI                             | 2       | 963 4049  |        |          |
| AlwI     | 14      |  | CviJI    | 79      |  | PvuII                            | 1       | 2377  |        |          |
| Alw21I   | 8       | 280 898 1489 1780 2604<br>3104 4265 4350         | CviRI    | 21      |  | RcaI                             | 4       | 796 3506 4514 4619                              |        |          |
| Alw44I   | 3       | 2600 3100 4346                                   | DdeI     | 10      | 458 479 1892 2054 2594<br>3061 3470 3636 4176 4602   | RsaI                             | 3       | 165 2592 4159 165                               |        |          |
| AlwNI    | 1       | 3202   | DpnI     | 35      |  | Sall                             | 1       | 517   |        |          |
| ApaBI    | 1       | 1363   | DraI     | 3       | 3545 3564 4256                                       | SapI                             | 1       | 2670  |        |          |
| ApoI     | 1       | 4672   | DrdI     | 2       | 2479 2894  | Sau96I                           | 16      |   |        |          |
| AvaI     | 1736    |  | Dsal     | 2       | 835 1758   | Sau3AI                           | 27      |   |        |          |
| Avall    | 8       | 1110 1198 1447 1750 1792<br>2071 3817 4039       | EaeI     | 6       | 295 706 838 1250 1755<br>4067                        | Scal                             | 1       | 4159  |        |          |
| AvrII    | 1       | 562  | EagI     | 1       | 1250   | ScrFI                            | 16      |   |        |          |
| BamHI    | 1       | 510  | Eam1105I | 1       | 3679   | SfaNI                            | 22      |   |        |          |
| BanI     | 9       | 76 119 720 741 855<br>1077 1516 1600 3627        | EarI     | 2       | 2670 4474  | Sfcl                             | 5       | 138 644 3051 3242 3920                          |        |          |
| BanII    | 2       | 782 796  | Ecil     | 4       | 1706 2860 3006 3834                                  | SgrAI                            | 1       | 717   |        |          |
| BbsI     | 3       | 1041 1904 4657                                   | Eco47III | 4       | 234 803 1088 2040                                    | SphI                             | 1       | 873   |        |          |
| BbvI     | 24      |  | Eco57I   | 2       | 3334 4346  | SspI                             | 1       | 4483  |        |          |
| BccI     | 9       | 767 860 1301 1390 1697<br>1709 3716 3840 4127    | EcoNI    | 1       | 933  | StyI                             | 3       | 435 562 1680                                    |        |          |
| Bce83I   | 7       | 399 996 1166 2877 3175<br>3416 4284              | EcoO109I | 5       | 431 831 1750 1792 4655                               | TaqI                             | 11      |   |        |          |
| Bcefl    | 3       | 917 1478 3288                                    | EcoRI    | 1       | 4672   | TaqII                            | 6       | 981 2688 4027 4212 4365<br>4382                 |        |          |
| BcgI     | 6       | 1008 1042 2363 2397 4184<br>4218                 | EcoRII   | 6       | 129 1369 1752 2812 2933<br>2946                      | TfiI                             | 6       | 1163 1317 1615 1836 2340<br>2761                |        |          |
| Bfal     | 8       | 230 448 563 619 1800<br>3281 3534 3869           | EcoRV    | 2       | 187 378  | Thal                             | 25      |   |        |          |
| BglI     | 3       | 1246 1480 3799                                   | FauI     | 12      |  | Tsel                             | 24      |   |        |          |
| BglIII   | 1       | 676  | FokI     | 12      |  | Tsp45I                           | 9       | 124 212 1191 1458 2225<br>2438 2533 3935 4146   |        |          |
| Bpml     | 4       | 1143 1697 2313 3749                              | Fspl     | 4       | 262 1669 1767 3901                                   | Tsp509I                          | 10      | 58 251 610 660 1630<br>1644 3546 3852 4107 4672 |        |          |
| Bpu10I   | 1       | 1892   | GdIII    | 5       | 295 706 838 1250 4067                                | Tth111I                          | 1       | 2531  |        |          |
| Bpu1102I | 1       | 458  | HaeI     | 7       | 1231 1303 1360 1757 2801<br>2812 3264                | Tth111III                        | 5       | 2247 3376 3383 3415 4671                        |        |          |
| Bsal     | 2       | 643 3740   | Haell    | 11      |  | UbaII                            | 21      |   |        |          |
| BsaAI    | 1       | 2538   | HaellI   | 23      |  | Vspl                             | 2       | 659 3851  |        |          |
| BsaBI    | 3       | 675 681 1983                                     | Hgal     | 12      |  | XbaI                             | 1       | 618   |        |          |
| BsaHI    | 6       | 721 742 856 1517 4216<br>4598                    | HgiEI    | 1       | 3372   | XmnI                             | 2       | 2344 4278                                       |        |          |
| BsaJI    | 10      | 115 129 435 562 835<br>841 1478 1680 1758 2946   | Hhal     | 33      |  | Enzymes that do not cut pET-12a: |         |   |        |          |
| BsaWI    | 6       | 380 1004 1975 2992 3139<br>3970                  | Hin4I    | 6       | 16 334 958 1452 3678<br>3752                         | AflIII                           | Agel    | Apal  | AscI   | BaeI     |
| Bsbl     | 2       | 2502 4222  | HincII   | 2       | 519 4220   | BclI                             | BmgI    | BsaXI   | BseRI  | BsrGI    |
| BscGI    | 14      |  | HindIII  | 1       | 29   | BssHII                           | BstEII  | BstXI   | Bsu36I | DraIII   |
| BsgI     | 1       | 1946   | Hinfl    | 11      |  | DrdII                            | FseI    | HpaI  | KpnI   | MluI     |
| Bsil     | 3       | 2959 4343 4650                                   | HphI     | 12      |  | MunI                             | NcoI    | NotI  | NsiI   | NspV     |
| BsiEI    | 8       | 289 963 967 1253 2702<br>3126 4049 4198          | MaeII    | 10      | 1212 1268 1857 1881 2111<br>2537 3489 3905 4278 4598 | Pacl                             | PmeI    | PmlI  | RleAI  | RsrII    |
| BsII     | 21      |  | MaeIII   | 17      |  | Sacl                             | SacII   | SexAI   | Sfil   | SgfI     |
| BsmI     | 1       | 1670   | MbolI    | 11      |  | Smal                             | SnaBI   | SpeI  | SrfI   | Sse8387I |
| BsmAI    | 4       | 643 2427 3740 4516                               | Mmel     | 4       | 222 309 3001 3185                                    | StuI                             | SunI    | Swal  | XcmI   | XhoI     |
| BsmBI    | 1       | 2427   | MnlI     | 30      |  |                                  |         |   |        |          |
| BsmFI    | 4       | 859 1184 1409 2057                               | MscI     | 1       | 1757   |                                  |         |   |        |          |
| BsoFI    | 45      |  | MseI     | 18      |  |                                  |         |   |        |          |
| Bsp24I   | 8       | 688 720 3279 3311 3457<br>3489 4583 4615         | MslI     | 7       | 1342 1773 1968 2359 3931<br>4090 4449                |                                  |         |   |        |          |
| Bsp1286I | 10      | 280 782 796 898 1489<br>1780 2604 3104 4265 4350 | MspI     | 28      |  |                                  |         |   |        |          |
| BspEI    | 2       | 380 1975   | MspA1I   | 7       | 462 1452 2377 2496 3128<br>3373 4314                 |                                  |         |   |        |          |
| BspGI    | 3       | 1370 1447 2312                                   | Mwol     | 36      |  |                                  |         |   |        |          |
| BspLU11I | 1       | 2786   | NarI     | 4       | 721 742 856 1517                                     |                                  |         |   |        |          |
| BspMI    | 1       | 1365   | NciI     | 10      | 171 842 1570 1796 2124<br>2430 2465 3166 3862 4213   |                                  |         |   |        |          |
| BsrI     | 19      |  | NdeI     | 1       | 580  |                                  |         |   |        |          |
| BsrBI    | 2       | 2719 4520  | NgoAIV   | 4       | 708 1080 1240 1594                                   |                                  |         |   |        |          |
| BsrDI    | 2       | 3740 3914  | NheI     | 1       | 229  |                                  |         |   |        |          |
| BsrFI    | 7       | 160 708 717 1080 1240<br>1594 3759               | NlaIII   | 26      |  |                                  |         |   |        |          |
|          |         |  | NlaIV    | 25      |  |                                  |         |   |        |          |
|          |         |  | NruI     | 2       | 538 1285   |                                  |         |   |        |          |
|          |         |  | NspI     | 4       | 873 2131 2423 2790                                   |                                  |         |   |        |          |
|          |         |  | Pfl1108I | 2       | 1069 3697  |                                  |         |   |        |          |
|          |         |  | PfIMI    | 2       | 1632 1681  |                                  |         |   |        |          |