# pET-14b Vector

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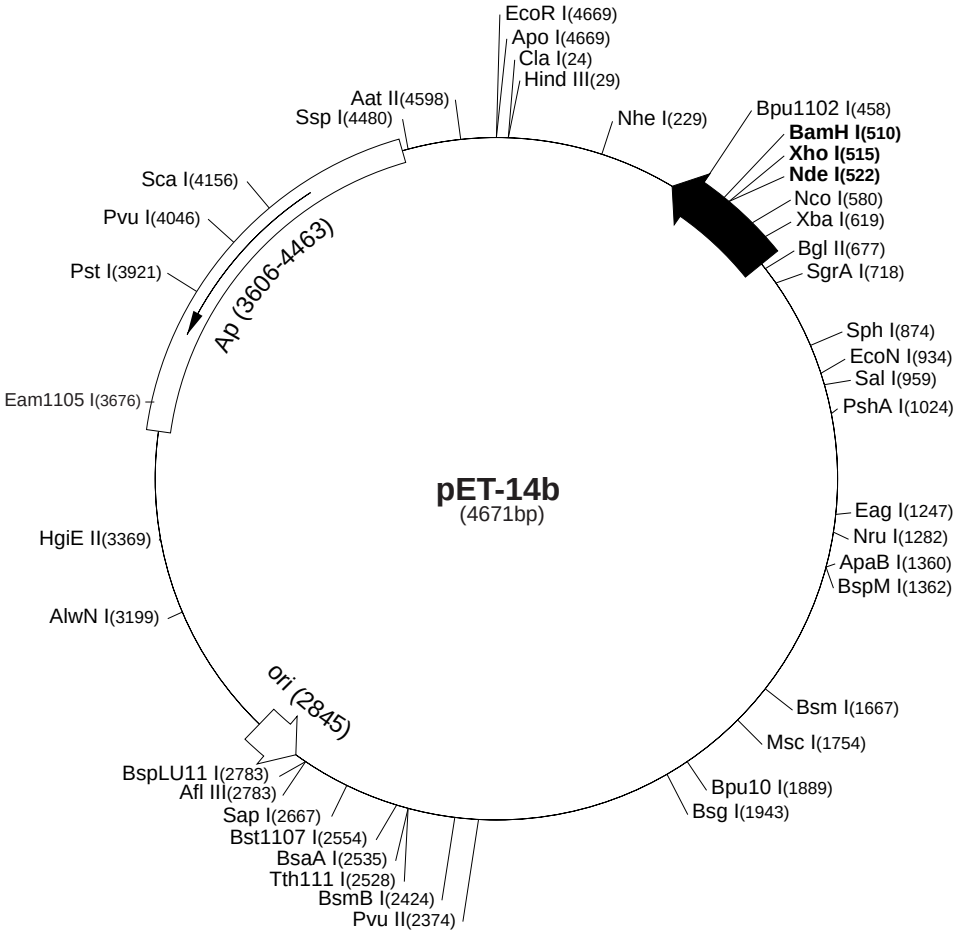
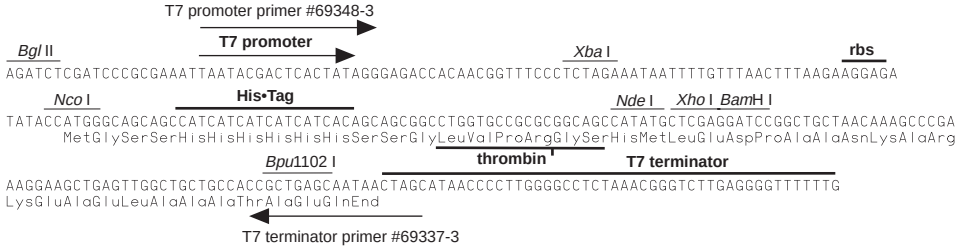
The pET-14b vector (Cat. No. 69660-3) carries an N-terminal His•Tag® sequence followed by a thrombin site and three cloning sites. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below.

**pET-14b sequence landmarks**

| | |
|---|---|
| T7 promoter | 646-662 |
| T7 transcription start | 645 |
| His•Tag coding sequence | 554-571 |
| Multiple cloning sites | |
| (*Nde* I -*Bam*H I) | 510-526 |
| T7 terminator | 404-450 |
| pBR322 origin | 2845 |
| *bla* coding sequence | 3606-4463 |




**pET-14b cloning/expression region**

# pET-14b Restriction Sites

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| Enzyme | # Sites | Locations |
|---|---|---|
| AatII | 1 | 4598 |
| AccI | 2 | 960 2553 |
| AceIII | 5 | 1005 2292 2433 2735 3975 |
| AciI | 72 | |
| AflIII | 1 | 2783 |
| AluI | 18 | |
| AlwI | 14 | |
| Alw21I | 8 | 280 899 1486 1777 2601 3101 4262 4347 |
| Alw44I | 3 | 2597 3097 4343 |
| AlwNI | 1 | 3199 |
| ApaBI | 1 | 1360 |
| ApoI | 1 | 4669 |
| AvaI | 2 | 515 1733 |
| AvaII | 8 | 1107 1195 1444 1747 1789 2068 3814 4036 |
| BamHI | 1 | 510 |
| BanI | 10 | 76 119 537 721 742 856 1074 1513 1597 3624 |
| BanII | 2 | 783 797 |
| BbsI | 3 | 1038 1901 4654 |
| BbvI | 28 | |
| BccI | 10 | 570 768 861 1298 1387 1694 1706 3713 3837 4124 |
| Bce83I | 7 | 399 993 1163 2874 3172 3413 4281 |
| BcefI | 3 | 918 1475 3285 |
| BcgI | 6 | 1005 1039 2360 2394 4181 4215 |
| BfaI | 7 | 230 448 620 1797 3278 3531 3866 |
| BglI | 3 | 1243 1477 3796 |
| BglII | 1 | 677 |
| BpmI | 4 | 1140 1694 2310 3746 |
| Bpu10I | 1 | 1889 |
| Bpu1102I | 1 | 458 |
| BsaI | 2 | 644 3737 |
| BsaAI | 1 | 2535 |
| BsaBI | 3 | 676 682 1980 |
| BsaHI | 6 | 722 743 857 1514 4213 4595 |
| BsaJI | 10 | 115 129 435 580 836 842 1475 1677 1755 2943 |
| BsaWI | 6 | 380 1001 1972 2989 3136 3967 |
| BsbI | 2 | 2499 4219 |
| BscGI | 13 | |
| BsgI | 1 | 1943 |
| BsiI | 3 | 2956 4340 4647 |
| BsiEI | 7 | 289 964 1250 2699 3123 4046 4195 |
| BsII | 21 | |
| BsmI | 1 | 1667 |
| BsmAI | 4 | 644 2424 3737 4513 |
| BsmBI | 1 | 2424 |
| BsmFI | 4 | 860 1181 1406 2054 |
| BsoFI | 52 | |
| Bsp24I | 8 | 689 721 3276 3308 3454 3486 4580 4612 |
| Bsp1286I | 10 | 280 783 797 899 1486 1777 2601 3101 4262 4347 |
| BspEI | 2 | 380 1972 |
| BspGI | 3 | 1367 1444 2309 |
| BspLU11I | 1 | 2783 |
| BspMI | 1 | 1362 |
| BsrI | 19 | |
| BsrBI | 2 | 2716 4517 |
| BsrDI | 2 | 3737 3911 |
| BsrFI | 7 | 160 709 718 1077 1237 1591 3756 |
| Bst1107I | 1 | 2554 |
| BstYI | 9 | 510 677 1975 3424 3435 3521 3533 4301 4318 |
| Cac8I | 31 | |
| CjeI | 16 | |
| CjePI | 22 | |
| ClaI | 1 | 24 |
| CviJI | 81 | |
| CviRI | 21 | |
| DdeI | 10 | 458 479 1889 2051 2591 3058 3467 3633 4173 4599 |
| DpnI | 25 | |
| DraI | 3 | 3542 3561 4253 |
| DrdI | 2 | 2476 2891 |
| DsaI | 3 | 580 836 1755 |
| EaeI | 6 | 295 707 839 1247 1752 4064 |
| EagI | 1 | 1247 |
| Eam1105I | 1 | 3676 |
| EarI | 2 | 2667 4471 |
| EciI | 4 | 1703 2857 3003 3831 |
| Eco47III | 4 | 234 804 1085 2037 |
| Eco57I | 2 | 3331 4343 |
| EcoNI | 1 | 934 |
| EcoO109I | 5 | 431 832 1747 1789 4652 |
| EcoRI | 1 | 4669 |
| EcoRII | 7 | 129 540 1366 1749 2809 2930 2943 |
| EcoRV | 2 | 187 378 |
| FauI | 11 | |
| FokI | 12 | |
| FspI | 4 | 262 1666 1764 3898 |
| GdiII | 5 | 295 707 839 1247 4064 |
| HaeI | 7 | 1228 1300 1357 1754 2798 2809 3261\ |
| HaeII | 11 | |
| HaeIII | 24 | |
| HgaI | 11 | |
| HgiEII | 1 | 3369 |
| HhaI | 32 | |
| Hin4I | 5 | 16 334 1449 3675 3749 |
| HincII | 2 | 961 4217 |
| HindIII | 1 | 29 |
| HinfI | 11 | |
| HphI | 12 | |
| MaeII | 10 | 1209 1265 1854 1878 2108 2534 3486 3902 4275 4595 |
| MaeIII | 17 | |
| MboII | 11 | |
| MmeI | 4 | 222 309 2998 3182 |
| MnlI | 31 | |
| MscI | 1 | 1754 |
| MseI | 18 | |
| MslI | 7 | 1339 1770 1965 2356 3928 4087 4446 |
| MspI | 28 | |
| MspA1I | 8 | 462 548 1449 2374 2493 3125 3370 4311 |
| MwoI | 36 | |
| NarI | 4 | 722 743 857 1514 |
| NciI | 10 | 171 843 1567 1793 2121 2427 2462 3163 3859 4210 |
| NcoI | 1 | 580 |
| NdeI | 1 | 522 |
| NgoAIV | 4 | 709 1077 1237 1591 |
| NheI | 1 | 229 |
| NlaIII | 27 | |
| NlaIV | 26 | |
| NruI | 1 | 1282 |
| NspI | 4 | 874 2128 2420 2787 |
| Pfl1108I | 2 | 1066 3694 |
| PflMI | 2 | 1629 1678 |
| PleI | 5 | 660 948 2677 3162 3665 |
| PshAI | 1 | 1024 |
| Psp5II | 2 | 1747 1789 |
| Psp1406I | 4 | 1209 2108 3902 4275 |
| PstI | 1 | 3921 |
| PvuI | 1 | 4046 |
| PvuII | 1 | 2374 |
| RcaI | 4 | 797 3503 4511 4616 |
| RsaI | 3 | 165 2589 4156 |
| SalI | 1 | 959 |
| SapI | 1 | 2667 |
| Sau96I | 16 | |
| Sau3AI | 25 | |
| ScaI | 1 | 4156 |
| ScrFI | 17 | |
| SfaNI | 22 | |
| SfcI | 5 | 138 645 3048 3239 3917 |
| SgrAI | 1 | 718 |
| SphI | 1 | 874 |
| SspI | 1 | 4480 |
| StyI | 3 | 435 580 1677 |
| TaqI | 10 | 24 339 516 674 682 960 1435 1576 2883 4327 |
| TaqII | 6 | 978 2685 4024 4209 4362 4379 |
| TfiI | 6 | 1160 1314 1612 1833 2337 2758 |
| ThaI | 26 | |
| TseI | 28 | |
| Tsp45I | 9 | 124 212 1188 1455 2222 2435 2530 3932 4143 |
| Tsp509I | 10 | 58 251 611 661 1627 1641 3543 3849 4104 4669 |
| Tth111I | 1 | 2528 |
| Tth111II | 5 | 2244 3373 3380 3412 4668 |
| UbaJI | 24 | |
| VspI | 2 | 660 3848 |
| XbaI | 1 | 619 |
| XhoI | 1 | 515 |
| XmnI | 2 | 2341 4275 |

Enzymes that do not cut pET-14b:

| | | | | |
|---|---|---|---|---|
| AflII | AgeI | ApaI | AscI | AvrII |
| BaeI | BclI | BmgI | BsaXI | BseRI |
| BsrGI | BssHII | BstEII | BstXI | Bsu36I |
| DraIII | DrdII | FseI | HpaI | KpnI |
| MluI | MunI | NotI | NsiI | NspV |
| PacI | PmeI | PmlI | RleAI | RsrII |
| SacI | SacII | SexAI | SfiI | SgfI |
| SmaI | SnaBI | SpeI | SrfI | Sse8387I |
| StuI | SunI | SwaI | XcmI | |