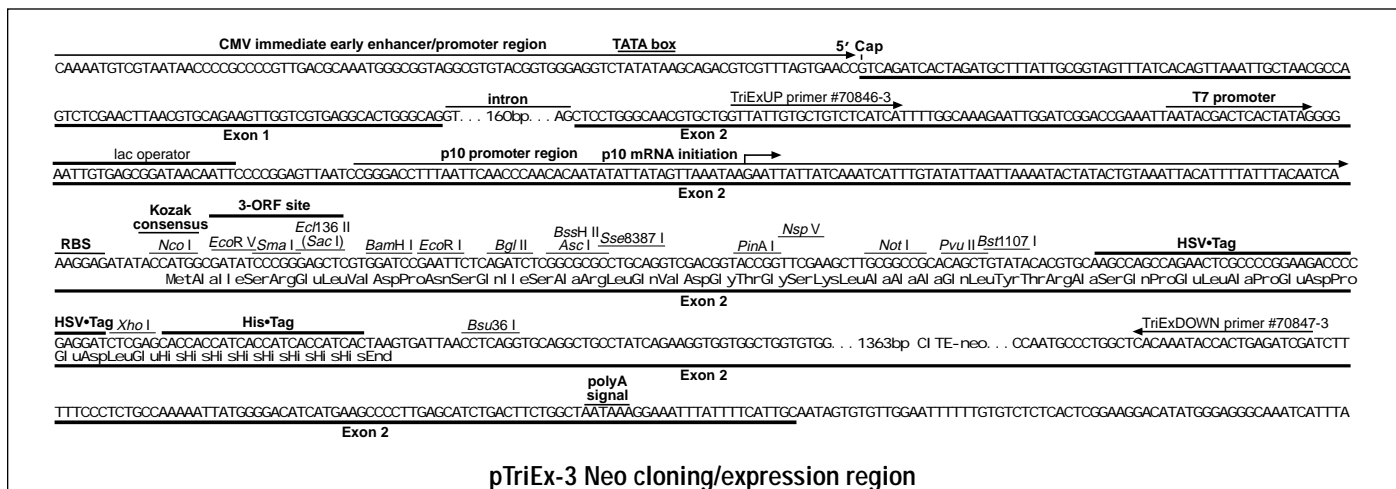
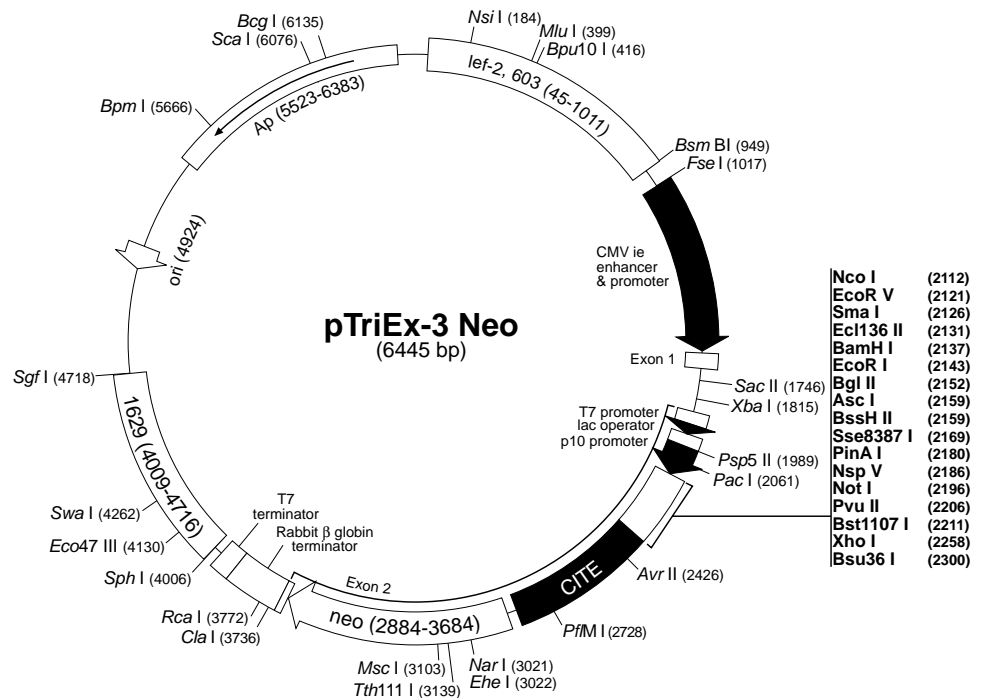


| | |
|--------------------------------------|--------------------------------------|
| Baculovirus Locus | polh |
| Promoters | CMV immediate early p10 T7/lac |
| C-terminal fusion options | HSV•Tag His•Tag |
| Cloning options | polylinker |
| Selectable marker (vertebrate cells) | Neomycin ^R |

The pTriEx™-3 Neo vector¹ (Cat. No. 70931-3) is designed to allow rapid characterization of target genes in *E. coli*, insect and vertebrate cells, and to allow rapid selection of stable transfected vertebrate cells expressing high levels of target gene. Expression in vertebrate cells is mediated by the CMV immediate early enhancer and promoter². The drug selection marker is expressed under the control of the EMC virus Cap-Independent Translation Enhancer (CITE) sequence (or IRES), allowing rapid selection of transfected vertebrate cells using the drug G 418 or neomycin sulfate. For expression in insect cells, pTriEx-3 Neo contains flanking baculovirus sequences to permit the generation of recombinant baculoviruses using the BacVector® System. In baculovirus-infected insect cells, expression is driven by the very late p10 promoter. Expression in *E. coli* is regulated by the tightly controlled T7/lac promoter. Expression can be induced in hosts such as NovaBlue by infecting with λCE6, a phage that constitutively expresses T7 RNA polymerase from the λp_L and λp_T promoters. Alternatively, pTriEx recombinant plasmids can be transferred into a (DE3)pLacI host that allows induction with IPTG.

pTriEx-3 Neo sequence landmarks

| | |
|--|-----------|
| CMV ie enhancer/promoter | 1021–1597 |
| Vertebrate transcription start | 1598 |
| T7 promoter | 1931–1947 |
| T7 transcription start | 1948 |
| lac operator | 1952–1972 |
| p10 promoter region | 1986–2099 |
| p10 transcription start | 2030–2031 |
| Multiple cloning sites (Nco I–Bsu36 I) | 2112–2300 |
| HSV•Tag® coding sequence | 2222–2257 |
| His•Tag® coding sequence | 2264–2287 |
| CITE sequence | 2349–2848 |
| neo | 2884–3684 |
| Rabbit globin terminator region | 3705–3947 |
| T7 terminator | 3948–3995 |
| pUC origin | 4924 |
| bla coding sequence | 5523–6383 |



pTriEx-3 Neo cloning/expression region

pTriEx-3 Neo Restriction Sites

| Enzyme | # Sites | Locations | Enzyme | # Sites | Locations | Enzyme | # Sites | Locations | | |
|----------|---------|--|----------|---------|--|---------------------------------------|---------|---|-------|-------|
| AatII | 6 | 1140 1193 1276 1462 1583 4453 | DraI | 4 | 429 3892 4262 6173 | RsrII | 2 | 1921 3537 | | |
| AccI | 3 | 244 2172 2210 | DraIII | 2 | 2290 2638 | SacI | 1 | 2133 | | |
| AcI | 59 | | DrdI | 2 | 3048 4969 | SacII | 1 | 1746 | | |
| AflIII | 7 | 399 2213 2590 2765 4523 4673 4862 | DsaI | 3 | 1743 2112 2809 | SalI | 2 | 243 2171 | | |
| AhdI | 2 | 499 5596 | EaeI | 9 | 1011 2196 2578 2891 2927 3101 3492 3519 5984 | SapI | 2 | 3365 3575 | | |
| AluI | 21 | | EagI | 2 | 2196 2927 | Sau3AI | 29 | | | |
| Alw26I | 8 | 285 949 1449 1659 1897 3858 5657 6433 | EarI | 5 | 51 547 3365 3575 6391 | Sau96I | 18 | | | |
| AlwI | 16 | | Ecl136II | 1 | 2131 | Scal | 1 | 6076 | | |
| AlwNI | 2 | 1865 5277 | Eco47III | 1 | 4130 | ScrFI | 22 | | | |
| Apal | 2 | 1712 2392 | Eco57I | 5 | 2720 3167 3599 5409 6263 | SfaNI | 16 | | | |
| ApaLI | 3 | 2752 5175 6263 | EcoO109I | 6 | 1709 1989 2388 2744 2798 3964 | Sfcl | 9 | 1857 1943 2165 2523 2873 3070 5126 5317 5837 | | |
| ApoI | 11 | | EcoRI | 1 | 2143 | Sgfl | 1 | 4718 | | |
| AscI | 1 | 2159 | EcoRII | 11 | | SmaI | 1 | 2126 | | |
| AvaI | 4 | 1732 2124 2249 2258 | EcoRV | 1 | 2121 | SnaBI | 2 | 1355 4172 | | |
| Avall | 6 | 1921 1989 3537 4081 5734 5956 | EheI | 1 | 3022 | SphI | 1 | 4006 | | |
| AvrII | 1 | 2426 | FauI | 9 | 1108 1134 1301 1529 1712 1738 1775 2868 3144 | Sse8387I | 1 | 2169 | | |
| BamHI | 1 | 2137 | Fnu4HI | 42 | | SspI | 4 | 425 4266 4463 6400 | | |
| BanI | 7 | 1480 2177 2568 2716 3020 3055 5544 | FokI | 6 | 2717 3345 3370 5562 5743 6030 | StyI | 3 | 2112 2426 3959 | | |
| BanII | 4 | 1712 2133 2392 3386 | FseI | 1 | 1017 | Swal | 1 | 4262 | | |
| BbsI | 5 | 498 2250 2401 2515 4631 | FspI | 3 | 659 3123 5818 | Tal | 25 | | | |
| BbvI | 22 | | HaeII | 3 | 3024 4132 5109 | TaqI | 18 | | | |
| Bcgl | 1 | 6135 | HaeIII | 25 | | TfiI | 5 | 446 3506 3640 4135 4837 | | |
| Bfal | 12 | | Hgal | 9 | 146 503 966 1541 4072 4232 4658 4972 6122 | Thal | 16 | | | |
| BglI | 5 | 1105 1227 1298 2577 5716 | Hhal | 23 | | TseI | 22 | | | |
| BglIII | 1 | 2151 | HincII | 3 | 245 1531 2173 | Tsp45I | 5 | 1802 3141 3447 5852 6063 | | |
| Bpml | 1 | 5666 | HindIII | 3 | 2189 2499 2847 | Tsp509I | 41 | | | |
| Bpu10I | 1 | 416 | Hinfl | 13 | | TspRI | 14 | | | |
| BsaAI | 5 | 1355 2216 2591 3325 4172 | HphI | 10 | 183 879 2264 2270 2665 3199 5666 6082 6288 6323 | Tth111I | 1 | 3139 | | |
| BsaHI | 10 | 495 1137 1190 1273 1459 1580 3021 4450 4650 6133 | KpnI | 2 | 2181 2720 | VspI | 5 | 1022 1930 2057 4657 5768 | | |
| Bsal | 2 | 285 5657 | MaeIII | 15 | | XbaI | 1 | 1815 | | |
| BsaJI | 15 | | MboII | 20 | | XhoI | 1 | 2258 | | |
| BsaWI | 7 | 833 2180 2346 3052 5067 5214 5887 | MluI | 1 | 399 | XmnI | 2 | 2487 6195 | | |
| BsgI | 2 | 1689 2326 | MnlI | 34 | | Enzymes that do not cut pTriEx-3 Neo: | | | | |
| BsiEI | 7 | 2199 2930 4718 4778 5201 5966 6115 | MscI | 1 | 3103 | AflIII | BclI | Bpu1102I | BsaBI | BseRI |
| BsiHKAI | 8 | 2133 2265 2756 3134 3324 5179 6182 6267 | MseI | 38 | | BspEI | BstEII | EcoNI | HpaI | NheI |
| BsII | 17 | | MslI | 8 | 950 1380 1837 3458 3901 5848 6007 6366 | NruI | PmeI | PshAI | SanDI | SexAI |
| BsmBI | 1 | 949 | MspA1I | 7 | 655 1745 2206 2545 5203 5448 6231 | SfiI | SgrAI | SpeI | SrfI | StuI |
| BsmFI | 10 | 1190 1341 1509 1764 1783 2002 2828 3172 3780 4578 | MspI | 30 | | SunI | XcmII | | | |
| Bsml | 2 | 2421 2460 | MunI | 2 | 4161 4506 | | | | | |
| Bsp1286I | 13 | | Mwol | 29 | | | | | | |
| BspLU11I | 2 | 2765 4862 | NarI | 1 | 3021 | | | | | |
| BspMI | 5 | 1686 2158 2614 2908 3289 | NciI | 11 | | | | | | |
| BsrBI | 4 | 1728 1960 3634 4795 | NcoI | 1 | 2112 | | | | | |
| BsrDI | 6 | 79 2386 3254 3823 5657 5831 | NdeI | 4 | 1249 3872 3932 3940 | | | | | |
| BsrFI | 8 | 42 781 1013 1806 2180 3340 3521 5676 | NgoAIV | 3 | 781 1013 3521 | | | | | |
| BsrGI | 3 | 49 768 4502 | NlaIII | 19 | | | | | | |
| Bsrl | 16 | | NlaIV | 20 | | | | | | |
| BssHII | 1 | 2159 | NotI | 1 | 2196 | | | | | |
| BssSI | 4 | 2132 3613 5034 6260 | Nsil | 1 | 184 | | | | | |
| Bst1107I | 1 | 2211 | NspI | 4 | 2760 2769 4006 4866 | | | | | |
| BstXI | 2 | 167 3712 | NspV | 1 | 2186 | | | | | |
| BstYI | 9 | 2137 2151 2254 2732 3192 5502 5513 6221 6238 | Pacl | 1 | 2061 | | | | | |
| Bsu36I | 1 | 2300 | PfIMI | 1 | 2728 | | | | | |
| CacBI | 28 | | PinAI | 1 | 2180 | | | | | |
| Clal | 1 | 3736 | PleI | 8 | 150 1419 1931 2669 3444 4505 5240 5585 | | | | | |
| CviJI | 100 | | PmII | 2 | 2216 2591 | | | | | |
| Ddel | 9 | 416 2148 2287 2300 3684 3730 5136 5553 6093 | Psp1406I | 2 | 5822 6195 | | | | | |
| Dpnl | 29 | | Psp5II | 1 | 1989 | | | | | |
| | | | PstI | 3 | 2169 2877 3074 | | | | | |
| | | | PvuI | 2 | 4718 5966 | | | | | |
| | | | PvuII | 1 | 2206 | | | | | |
| | | | Rcal | 1 | 3772 | | | | | |
| | | | Rsal | 17 | | | | | | |