pTriEx-4 Neo Vector

TB299 02/01

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

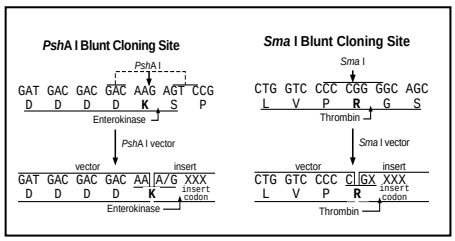
pTriEx-4 Neo sequence landmarks

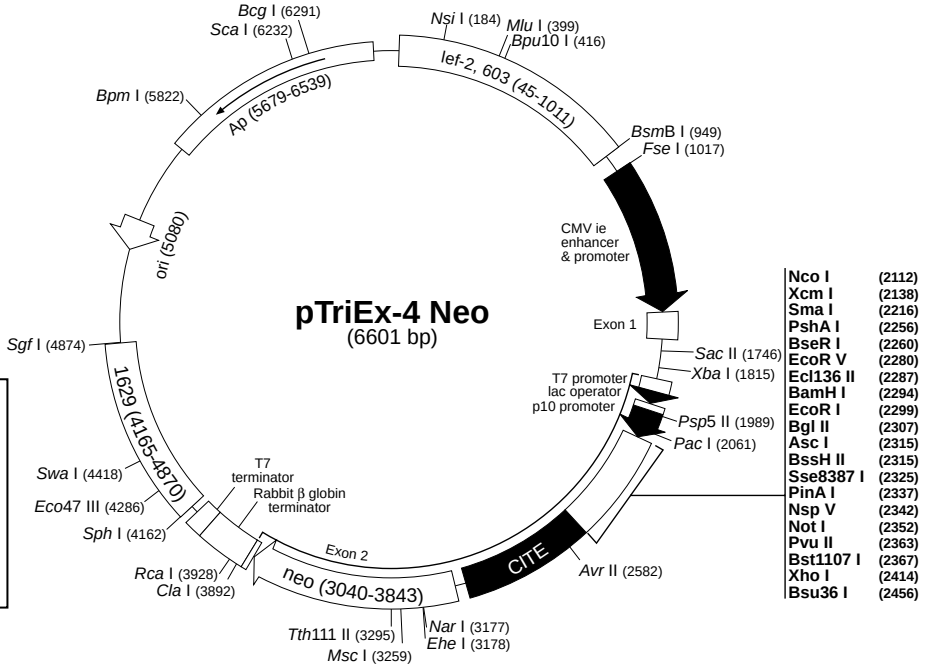
CMV ie enhancer/promoter	1021–1597
Vertebrate transcription start	1598
T7 promoter	1931–1947
T7 transcription start	1948
lac operator	1952–1972
p10 promoter region	1986–2099
p10 transcription start	2030–2031
Multiple cloning sites (*Nco* I–*Bsu*36 I)	2112–2456
His•Tag® coding sequence	2120–2137
S•Tag™ coding sequence	2147–2191
HSV•Tag® coding sequence	2378–2413
His•Tag® coding sequence	2420–2443
CITE sequence	2505–3004
Rabbit globin terminator region	3861–4103
T7 terminator	4104–4151
pUC origin	5080
bla coding sequence	5679–6539

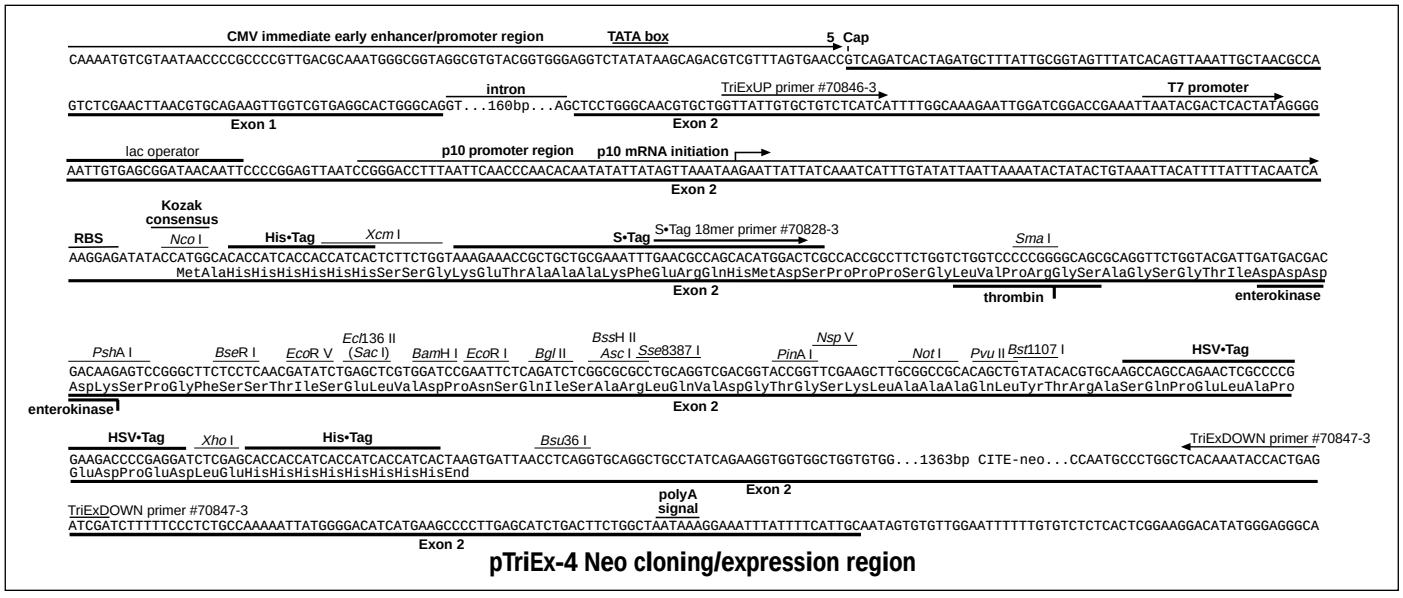
The pTriEx™-4 Neo vector[1] (Cat. No. 70933-3) is designed to allow rapid characterization of target genes in *E. coli*, insect and vertebrate cells, and to allow rapid selection of stable transfected vertebrate cells expressing high levels of target gene. Expression in vertebrate cells is mediated by the CMV immediate early enhancer and promoter[2]. 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-4 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_I promoters. Alternatively, pTriEx recombinant plasmids can be transferred into a (DE3)pLacI host that allows induction with IPTG. Native protein can be expressed by cloning into the *Nco* I site, or generated by cloning into the *Psh*A I or *Sma* I sites and cleaving the fusion protein with enterokinase or thrombin, respectively.

[1] patent pending

[2] The CMV promoter is covered by U.S. Patent nos. 5,168,062 and 5,385,839 issued to the University of Iowa Research Foundation and is licensed for research use only.







pTriEx-4 Neo cloning/expression region

pTriEx-4 Neo Restriction Sites

TB299 02/01

Enzyme	# Sites	Locations
AatII	6	1140 1193 1276 1462 1583 4609
AccI	3	244 2328 2366
AciI	61	
AflIII	7	399 2369 2746 2921 4679 4829 5018
AhdI	2	499 5752
AluI	21	
Alw26I	8	285 949 1449 1659 1897 4014 5813 6589
AlwI	16	
AlwNI	3	1865 2231 5433
ApaI	2	1712 2548
ApaLI	3	2908 5331 6419
ApoI	12	
AscI	1	2315
AvaI	4	1732 2214 2405 2414
AvaII	7	1921 1989 2209 3693 4237 5890 6112
AvrII	1	2582
BamHI	1	2293
BanI	7	1480 2333 2724 2872 3176 3211 5700
BanII	4	1712 2289 2548 3542
BbsI	5	498 2406 2557 2671 4787
BbvI	25	
BcgI	1	6291
BfaI	12	
BglI	5	1105 1227 1298 2733 5872
BglII	1	2307
BpmI	1	5822
Bpu10I	1	416
BsaAI	5	1355 2372 2747 3481 4328
BsaHI	10	495 1137 1190 1273 1459 1580 3177 4606 4806 6289
BsaI	2	285 5813
BsaJI	17	
BsaWI	7	833 2336 2502 3208 5223 5370 6043
BseRI	1	2260
BsgI	2	1689 2482
BsiEI	7	2355 3086 4874 4934 5357 6122 6271
BsiHKAI	8	2289 2421 2912 3290 3480 5335 6338 6423
BslI	19	
BsmBI	1	949
BsmFI	11	
BsmI	2	2577 2616
Bsp1286I	13	
BspLU11I	2	2921 5018
BspMI	6	1686 2216 2314 2770 3064 3445
BsrBI	4	1728 1960 3790 4951
BsrDI	6	79 2542 3410 3979 5813 5987
BsrFI	8	42 781 1013 1806 2336 3496 3677 5832
BsrGI	3	49 768 4658
BsrI	16	
BssHII	1	2315
BssSI	4	2288 3769 5190 6416
Bst1107I	1	2367
BstXI	2	167 3868
BstYI	9	2293 2307 2410 2888 3348 5658 5669 6377 6394
Bsu36I	1	2456
Cac8I	29	
ClaI	1	3892
CviJI	101	
DdeI	10	416 2283 2304 2443 2456 3840 3886 5292 5709 6249
DpnI	29	
DraI	4	429 4048 4418 6329
DraIII	2	2446 2794
DrdI	2	3204 5125
DsaI	3	1743 2112 2965
EaeI	9	1011 2352 2734 3047 3083 3257 3648 3675 6140
EagI	2	2352 3083
EarI	6	51 547 2143 3521 3731 6547
Ecl136II	1	2287
Eco47III	1	4286
Eco57I	5	2876 3323 3755 5565 6419
EcoO109I	6	1709 1989 2544 2900 2954 4120
EcoRI	1	2299
EcoRII	11	
EcoRV	1	2280
EheI	1	3178
FauI	9	1108 1134 1301 1529 1712 1738 1775 3024 3300
Fnu4HI	45	
FokI	6	2873 3501 3526 5718 5899 6186
FseI	1	1017
FspI	3	659 3279 5974
HaeII	3	3180 4288 5265
HaeIII	25	
HgaI	9	146 503 966 1541 4228 4388 4814 5128 6278
HhaI	24	
HincII	3	245 1531 2329
HindIII	3	2345 2655 3003
HinfI	15	
HphI	11	183 879 2117 2420 2426 2821 3355 5822 6238 6444
KpnI	2	2337 2876
MaeIII	15	
MboII	21	
MluI	1	399
MnlI	35	
MscI	1	3259
MseI	38	
MslI	8	950 1380 1837 3614 4057 6004 6163 6522
MspA1I	8	655 1745 2156 2362 2701 5359 5604 6387
MspI	31	
MunI	2	4317 4662
MwoI	29	
NarI	1	3177
NciI	12	
NcoI	1	2112
NdeI	4	1249 4028 4088 4096
NgoAIV	3	781 1013 3677
NlaIII	20	
NlaIV	21	
NotI	1	2352
NsiI	1	184
NspI	4	2916 2925 4162 5022
NspV	1	2342
PacI	1	2061
PflMI	2	2182 2884
PinAI	1	2336
PleI	10	150 1419 1931 2180 2265 2825 3600 4661 5396 5741
PmlI	2	2372 2747
PshAI	1	2256
Psp1406I	2	5978 6351
Psp5II	1	1989
PstI	3	2325 3033 3230
PvuI	2	4874 6122
PvuII	1	2362
RcaI	1	3928
RsaI	18	
RsrII	2	1921 3693
SacI	1	2289
SacII	1	1746
SalI	2	243 2327
SapI	2	3521 3731
Sau3AI	29	
Sau96I	19	
ScaI	1	6232
ScrFI	23	
SfaNI	16	
SfcI	9	1857 1943 2321 2679 3029 3226 5282 5473 5993
SgfI	1	4874
SmaI	1	2216
SnaBI	2	1355 4328
SphI	1	4162
Sse8387I	1	2325
SspI	4	425 4422 4619 6556
StyI	3	2112 2582 4115
SwaI	1	4418
TaiI	25	
TaqI	18	
TfiI	5	446 3662 3796 4291 4993
ThaI	16	
TseI	25	
Tsp45I	5	1802 3297 3603 6008 6219
Tsp509I	42	
TspRI	14	
Tth111I	1	3295
VspI	5	1022 1930 2057 4813 5924
XbaI	1	1815
XcmI	1	2138
XhoI	1	2414
XmnI	2	2643 6351

Enzymes that do not cut pTriEx-4 Neo:

AflII	BclI	Bpu1102I	BsaBI	BspEI
BstEII	EcoNI	HpaI	NheI	NruI
PmeI	SanDI	SexAI	SfiI	SgrAI
SpeI	SrfI	StuI	SunI	