



rrnB T1 transcription termination sequence: bases 106-149

rrnB T2 transcription termination sequence: bases 281-308

attL1: bases 358-457 (complementary strand)

multiple cloning site (MCS): bases

attL2: bases

Kanamycin resistance gene: bases

pUC origin: bases

Recommended *E.coli* strain for propagation: TOP10F'

* Sites are not unique

pENTR4 -V5 MCS

attL1

GGGCCCAAA TAATGATTTC ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAAATTGAT
CCCAGGGTTT ATTACTAAAA TAAAAGTAC TATCACTGGA CAAGCAACGT TGTTAACTA

AAG CAA TGC TTT TTT ATA ATG CCA ACT TTG TAC AAA AAA GCA GGC TCC
TTC GTT ACG AAA AAA TAT TAC GGT TGA AAC ATG TTT CGT CCG AGG

Nco I* V5 epitope

ACC ATG GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG
Met Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr

BamHI* Nco I* XmnI Sall HinC II BamHI* KpnI EcoR I NotI

GGA TCC ATG GGA ACC AAT TCA GTC GAC TGG ATC CGG TAC CGA ATT CGC GGC
Gly Ser Met Gly Thr Asn Ser Val Asp Trp Ile Arg Tyr Arg Ile Arg Gly

XhoI Eco RV XbaI

CGC ACT CGA GAT ATC TAG ACC AGC TTT CTT GTA CAA A
Arg Thr Arg Asp Ile *

attL2

GTTGGCATTA TAAGAAAGCA TTGCTTATCA ATTTGTTGCA ACGAACAGGT CACTATCAGT
CAACCGTAAT ATTCTTTCGT AACGAATAGT TAAACAAACGT TGCTTGTCCA GTGATAGTCA
CAAATAAAAA TCATTATTTG CCATCCAGCT GCAGCTCTGG CCCGTGTCTC AAAATCTCTG
GTTTTATTTT AGTAATAAAC GGTAGGTCGA CGTCGAGACC GGGCACAGAG TTTTAGAGAC

reverse primer binding site

ATGTTACATT
TACAATGTAA

* Sites not unique