



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'

* Site is not unique

pENTR4 -V5_2 MCS

atf1

GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
CCCGGGGTTT ATTACTAAAA TAAAACTGAC TATCACTGGA CAAGCAACGT TGTTTAACTA

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 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

EcoRV Xba I

GGA TCC ATG GGA ACC AAT TCA GTC GAG ATA TCT AGA CCA GCT TTC TTG TAC AAA
Gly Ser Met Gly Thr Asn Ser Val Glu Ile Ser Arg

atf2

GTTGGCATT TAAGAAAGCA TTGCTTATCA ATTTGTTGCA ACGAACAGGT CACTATCAGT
CAACCGTAAT ATTCTTTTCGT AACGAATAGT TAAACAACGT TGCTTGTCCA GTGATAGTCA

reverse primer binding site

CAAAATAAAA TCATTATTTG CCATCCAGCT GCAGCTCTGG CCCGTGTCTC AAAATCTCTG
GTTTTATTTT AGTAATAAAC GGTAGGTCGA CGTCGAGACC GGGCACAGAG TTTTAGAGAC

ATGTTACATT
TACAATGTAA

* Site not unique