



**Comments for pENTR4 ccdB⁻
nucleotides**

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'

pENTR4 -ccDB MCS

at1.1

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 ***Xmn I*** ***Sal I*** ***HinC II*** ***BamHI*** ***KpnI*** ***EcoR I*** ***NotI***
ACC ATG GGA ACC AAT TCA GTC GAC TGG ATC CGG TAC CGA ATT CGC GGC CGC
TGG TAC CCT TGG TTA AGT CAG CTG ACC TAG GCC ATG GCT TAA GCG CCG GCG

XhoI ***EcoRV*** ***Xba I***
ACT CGA GAT ATC TAG ACC AGC TTT CTT GTA CAA A
TGA GCT CTA TAG ATC TGG TCG AAA GAA CAT GTT T

at1.2

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

reverse primer binding site
CAAAATAAAA TCATTATTTG CCATCCAGCT GCAGCTCTGG CCCGTGTCTC AAAATCTCTG
GTTTTATTTT AGTAATAAAC GGTAGGTCGA CGTCGAGACC GGGCACAGAG TTTTAGAGAC

ATGTTACATT
TACAATGTAA