



Comments for pENTR4-FLAG  
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'

\* Sites are not unique

## pENTR4 -FLAG MCS

$\overbrace{\text{GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT}}^{\text{attL1}}$   
 $\text{CCCGGGGTTT ATTACTAAAA TAAAACTGAC TATCACTGGA CAAGCAACGT TGTTTAACTA}$

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*\*                      FLAG epitope                      BamHI\*    *Nco I*\*

**ACC ATG GAT TAC AAG GAC GAT GAC GAC AAA CTC GGA TCC ATG**

**Met Asp Tyr Lys Asp Asp Asp Asp Lys Leu Gly Ser Met**

*Xmn*I                      *Sal*I   *Hin*C II   BamHI\*                      *Kpn*I   *Eco*R I                      *Not*I  
GGA|ACC AAT TCA GTC|GAC TGG ATC CGG TAC|CGA ATT CGC|GGC  
Gly Thr Asn Ser Val Asp Trp Ile Arg Tyr Arg Ile Arg Gly

*Xho*I      *Eco*RV   *Xba*I  
**CGC ACT CGA GAT ATC TAG** ACC AGC TTT CTT GTA CAA A  
 Arg Thr Arg Asp Ile \*

<i>atl2</i>					
GTTGGCATT	TAAGAAAGCA	TTGCTTATCA	ATTTGTTGCA	ACGAACAGGT	CACTATCAGT
CAACCGTAAT	ATTCTTTCGT	AACGAATAGT	TAAACAACGT	TGCTTGTCCA	GTGATAGTCA
<b>reverse primer binding site</b>					
CAAAATAAAA	TCATTATTTG	CCATCCAGCT	GCAGCTCTGG	CCC <sup>GT</sup> GTCTC	AAAATCTCTG
GTTTTATTTT	AGTAATAAAC	GGTAGGTCGA	CGTCGAGACC	GGGCACAGAG	TTTTAGAGAC

ATGTTACATT  
TACAATGTAA

\* Sites not unique