pET28-FLAG Vector

Source	Constructed by Farrell MacKenzie
Company	Structural Genomics Consortium, Toronto

Description	pET28-FLAG is an *E. coli* expression vector derived from vector pET28-MHL (SGC) and has a T7 promoter that drives expression of recombinant proteins with the addition of an N-terminal 6xHis-tag and FLAG-tag. Two stop codons are included in the vector at the C-terminal cloning site.

Antibiotic resistance	Kanamycin
Promoter	T7 - lacO
Cloning Methods	Insertion of a DNA sequence into the cloning/expression region is performed using Clontech's In-fusion enzyme-mediated directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and BseRI linearized vector. Insertion of a target sequence involves replacement of a SacB gene stuffer sequence, which provides for negative selection of the original plasmid on 5% sucrose.
N-terminal fusion sequence	MHHHHHHDYKDDDDK
5' primer tail for amplification of insert	5' GACGATGACGACAAG --- 3'
3' primer tail for amplification of insert	5' CAAGCTTCGTCATCA --- 3'
5' sequencing primer T7	5' AATTAATACGACTCACTATAGGG 3'
3' sequencing primer T7term	5' ATGCTAGTTATTGCTCAGCGG 3'

pET28-FLAG sequence (7,316 bp):

TGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCG
CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCT
TTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTT
CCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTA
GTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAAT
AGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTA
TAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAAC
GCGAATTTTAACAAAATATTAACGTTTACAATTTCAGGTGGCACTTTTCGGGGAAATGTGCGC
GGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAATTAATTCTT
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CAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCC
CCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAG
AATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCA
TCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAA
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