

pET28-CFP-YFP Vector

Source	Constructed by Farrell MacKenzie
Company	Structural Genomics Consortium, Toronto

Description	pET28-CFP-YFP is an <i>E. coli</i> expression vector derived from vector pET28-MHL (SGC) and Plasmid 22866: K9 HMR in pcDNA3 (Addgene). The vector has a T7 promoter that drives expression of recombinant proteins with the addition of an N-terminal 6xHis-tag, TEV protease cleavage site and a cyan fluorescent protein (CFP), as well as a C-terminal yellow fluorescent protein (YFP). Two stop codons are included in the vector at the C-terminal cloning site. The vector is designed to generate reporter of histone methylation constructs when a methyllysine binding domain and a histone peptide are inserted in between the CFP and YFP.
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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 Bsal 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	MHHHHHHSSGRENLYFQGMVSKGEELFTGVVPILVELDGDVNGHRFVSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTTLTWGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYISHNVYITADKQKNGIKAHFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHMLLEFVTAA
C-terminal fusion sequence	MVSKGEELFTGVVPILVELDGDVNGHKFVSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYSYQSALS KDPNEKRDHMLLEFVTAAGITLGMDELYK
5' primer tail for amplification of insert	5' TTCGTGACCGCCGCC --- 3'
3' primer tail for amplification of insert	5' GCCCTTGCTCACCAT --- 3'
5' sequencing primer CFP-fwd	5' CTACCTGAGCACCCAGTCC 3'
3' sequencing primer T7term	5' ATGCTAGTTATTGCTCAGCGG 3'

pET28-CFP-YFP sequence (8,362 bp):

TGGCGAATGGGACGCGCCCTGTAGCGGCCGATTAAGCGCGGGCGGGTGTGGTGGTTACGCG
CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTCCT
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CCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTA
GTGGGCCATCGCCCTGATAGACGGTTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAAT
AGTGGACTCTTGTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTA
TAAGGGATTTTGCCGATTTCCGGCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAAC
GCGAATTTTAAACAAAATATTAACGTTTACAATTTTCAAGGTGGCACTTTTTCGGGGAAATGTGCGC
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