

pFBOH-SUMOstar-TEV Vector

Source	Constructed by Farrell MacKenzie
Company	Structural Genomics Consortium, Toronto
Description	The pFBOH-SUMOstar-TEV vector is a derivative of the pFBOH-LIC vector (SGC). It is a donor vector for generation of recombinant baculovirus by site-specific transposition in an <i>E. coli</i> host. This vector adds a 119 amino acid N-terminal fusion tag containing a 6X His, SUMOstar fusion protein and TEV cleavage site. Two stop codons are included in the vector at the C-terminal cloning site.
Antibiotic resistance	Ampicillin (plasmid resistance in <i>E. coli</i>) Gentamicin (bacmid resistance in DH10Bac <i>E. coli</i>)
Promoter	Polyhedrin
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	MGHHHHHGHSLQDSEVNQEAKPEVKPEVKPETHINLKV-SDGSSEIFFKIKKTTPLRRLMEAFKRQKGEMDSLTFLYD-GIEIQADQTPEDLDMEDNDIIEAHREQIGSSGRENLYF-QG
5' primer tail for amplification of insert	5' TTGTATTTCCAGGGC --- 3'
3' primer tail for amplification of insert	5' CAAGCTTCGTCATCA --- 3'
5' sequencing primer pFBOH-fwd	5' CCGGATTATTCATACCGTCCCACCA 3'
3' sequencing primer pFBOH-rev	5' CTGATTATGATCCTCTAGTACTTCT 3'

pFBOH-SUMOstar-TEV sequence (7,070 bp):

GACGCGCCCTGTAGCGGCCGCTTAAGCGCGGGCGGGTGTGGTGGTTACGCGCAGCGTGACC
GCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCTTTCTCGCCAC
GTTCCGCGGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTCCGATTTAGTG
CTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGTATGGTTCACGTAGTGGGCCATCG
CCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTT
GTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTGATTTATAAGGGATTTT
GCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAA
CAAAATATTAACGTTTACAATTTCAAGTGGCACTTTTCGGGAAATGTGCGCGGAACCCCTA
TTTGTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT
GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCCCCTTATTCC
CTTTTTCGCGCATTTCCTTCTGTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGA
TGTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAG
ATCCTTGAGAGTTTTTCGCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTA
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