pFBOH-GST Vector

Source	Constructed by Farrell MacKenzie
Company	Structural Genomics Consortium, Toronto

Description	The pFBOH-GST vector is a derivative of the pFBOH-MHL vector (SGC). It is a donor vector for generation of recombinant baculovirus by site-specific transposition in an E. coli host. This vector adds a 231 amino acid N-terminal fusion tag containing a GST tag and TEV cleavage site. Two stop codons are included in the vector at the C-terminal cloning site.

Antibiotic resistance	Ampicillin (plasmid resistance in *E. coli*) Gentamicin (bacmid resistance in DH10Bac *E. coli*)
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	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEG-DKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIAD-KHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDF-ETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDF-MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY-LKSSKYIAWPLQGWQATFGGGDHPPKSDSSGRENLYF-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-GST sequence (7,406 bp):

GACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACC
GCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCAC
GTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTG
CTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCG
CCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTT
GTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTT
GCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAA
CAAAATATTAACGTTTACAATTTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTA
TTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT
GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCC
CTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGA
TGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAG
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