

pFGP64-N Vector

Source	Constructed by Farrell MacKenzie
Company	Structural Genomics Consortium, Toronto
Description	pFGP64-N is a donor vector for use in the Bac-to-Bac Baculovirus Expression System for expressing proteins in insect cells. The vector is a derivative of the pFastBac HTa vector (Invitrogen). pFGP64-N has a polyhedron promoter that drives the expression of proteins targeted for secretion with the addition of an N-terminal <i>Autographa californica nuclear polyhedrosis virus</i> (AcNPV) gp64 signal peptide followed by a 6xHis tag and TEV cleavage 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 BfuAI 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	MVSAIVLYVLLAAAAHSAFAGEHHHHHHHDYDIPENLYFQG
5' primer tail for amplification of insert	5' CTGTATTTTCAGGGC --- 3'
3' primer tail for amplification of insert	5' CGACAAGCTTCATCA --- 3'
5' sequencing primer pFBOH-fwd	5' CCGGATTATTCATACCGTCCCACCA 3'
3' sequencing primer pFBOH-rev	5' CTGATTATGATCCTCTAGTACTTCT 3'

**pFGP64-N sequence (6,817 bp):**

GACGCGCCCTGTAGCGGCATTAAAGCGCGGGTGTGGTGGTTACGCGCAGCGTGACC  
GCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCTTTCTCGCCAC  
GTTTCGCGGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTG  
CTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGTGGTTACGTTACGTTAGTGGGCCATCG  
CCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTT  
GTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTT  
GCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAA  
CAAAATATTAACGTTTACAATTTCAAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTA  
TTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT  
GCTTCAATAATATTGAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTATTCC  
CTTTTTTTCGGCATTTCCTTCCCTGTTTTGCTCACCCAGAAACGCTGGTGAAGTAAAAGA  
TGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAG  
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