pFTRYP-EK-N Vector

Source	Constructed by Farrell MacKenzie
Company	Structural Genomics Consortium, Toronto

Description	pFTRYP-EK-N is a donor vector for use in the Bac-to-Bac Baculovirus Expression System for expressing proteins in insect cells, and it is derived from the pFastBac HTa vector (Invitrogen). pFTRYP-EK-N has a polyhedron promoter that drives the expression of proteins targeted for secretion with the addition of an N-terminal *Homo sapiens* trypsin 2 signal peptide followed by an enterokinase cleavage site, 6xHis tag, and TEV cleavage 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 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	MNLLLILTFVAAAVAAPFDDDDKGEHHHHHH-DYDIPENLYFQG
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'

pFTRYP-EK-N sequence (6,826 bp):

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