

pFTRYP-EK-C Vector

Source	Constructed by Farrell MacKenzie
Company	Structural Genomics Consortium, Toronto
Description	pFTRYP-EK-C 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-C has a polyhedron promoter that drives the expression of proteins targeted for secretion with the addition of an N-terminal <i>Homo sapiens</i> trypsin 2 signal peptide with enterokinase cleavage site and a C-terminal 8xHis tag.
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	MNLLLILTFVAAAVAAPFDDDDKG
C – terminal fusion sequence	HHHHHHHH
5' primer tail for amplification of insert	5' GATGATGACAAGGGC --- 3'
3' primer tail for amplification of insert	5' ATGATGATGATGGTG --- 3'
5' sequencing primer pFBOH-fwd	5' CCGGATTATTCATACCGTCCCACCA 3'
3' sequencing primer pFBOH-rev	5' CTGATTATGATCCTCTAGTACTTCT 3'

**pFTRYP-EK-C sequence (6,793 bp):**

GACGCGCCCTGTAGCGGCCGATTAAAGCGCGGGCGGGTGTGGTGGTTACGCGCAGCGTGACC  
GCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCTTTCTCGCCAC  
GTTTCGCGGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTCCGATTTAGTG  
CTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGTGGTTACGTTACGTAGTGGGCCATCG  
CCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTT  
GTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTGATTTATAAGGGATTTT  
GCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAA  
CAAAATATTAACGTTTACAATTTCAAGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTA  
TTTGTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT  
GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCCCCTTATTCC  
CTTTTTCGCGCATTTCCTTCTGTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGA  
TCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAAG  
ATCCTTGAGAGTTTTTCGCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTA  
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