

pFHMSp LIC C

Source	Constructed by Alma Seitova
Company	Structural Genomics Consortium, Toronto

Description	The pFHMSp-LIC C vector is a derivative of the pFastBac HT A vector (Invitrogen). His tag was replaced by a Honeybee melittin signal sequence and His tag placed into C-term. It is a donor vector for generation of recombinant baculovirus by site-specific transposition into a baculovirus shuttle vector (bacmid) in <i>E. coli</i> host strain, DH10Bac™. For use in Bac-to-Bac Baculovirus Expression System in insect cells for secreted protein expression. This vector adds one or two (A) amino acid after releasing signal peptide by signalase
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Antibiotic resistance	Ampicillin and Gentamicin
Promoter	Polyhedrin Promoter
Cloning Method	Insertion of DNA sequence into the cloning/expression region is performed using BD-Biosciences Infusion enzyme mediated by directional recombination between complementary nucleotide DNA sequences at the ends of the insert (PCR product) and NotI/EcoRI linearized vector. Insertion of target sequence involves replacement of a SacB gene stuffer sequence, which provides for negative selection of the original plasmid on 5% sucrose.
Initiation Codon	ATG codon in Honeybee melittin signal sequence (HBMSS)
N – terminal HBMSS	MKFLVNVALVMVVYISYIYAAA
Termination codons	E F V E <u>H H H H H H H H</u> TAATAG included in vector after 8 His tag

5' primer for amplification of insert	5'TACATTTCTTACATCTATGCGGCCGCT---3'
3' primer for amplification of insert	5' TGG TGG TGC TCG ACG AAT TC ---3'
5' sequencing primer pFHMSp-Frd	5' CCGGATTATTCATACCGTCCCACCA 3'
3' sequencing primer pFHMSp-Rev	5' CTGATTATGATCCTCTAGTACTTCT 3'

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GACGCGCCCTGTAGCGGCGCATTAAAGCGCGGGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTG
CCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTTCGCCGGCTTTCCCG
TCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAA
CTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGG
AGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCGGTCTATTC
TTTTGATTTATAAGGGATTTTGGCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTT
AACCGAATTTTAAACAAAATATTAACGTTTACAATTTTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAA
CCCCTATTTGTTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT
GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTATTCCCTTTTTT
GCGGCATTTTGCCTTCCTGTTTTTGGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGT
TGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCGA
AGAAGTTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCC
GGGCAAGAGCAACTCGGTGCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAG
AAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGTGCCATAACCATGAGTGATAACAC
TGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGG
GATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA
CCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACCTGGCGAACTACTTACTCTAGCTTC
CCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCG
GCTGGCTGGTTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGG
GGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACG
AAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTGAGACCAAGTTTTACTCA
TATATACTTTAGATTGATTTAAAACCTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATA
ATCTCATGACCAAAATCCCTTAACTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA
AGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCA
GCGGTGGTTTTGTTTCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGC
AGATACCAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCC
TACATACTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTTCGTGTCTTACCGGG
TTGGACTCAAGACGATAGTTACC GGATAAGGCGCAGCGGTGCGGGCTGAACGGGGGGTTTCGTGCACACAGC
CCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAAGCGCCACGCT
TCCCGAAGGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTTCGGAACAGGAGAGCGCACGAGGGAG
CTTCCAGGGGAAACGCCTGGTATCTTTATAGTCTGTGCGGGTTTCGCCACCTCTGACTTGAGCGTTCGAT
TTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCTT
GGCCTTTTTGCTGGCCTTTTGTCTACATGTTCTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATT
ACCGCCTTTGAGTGAGCTGATACCGCTCGCCGACCGCAACGACCGAGCGCAGCGAGTCAGTGAGCGAGG
AAGCGGAAGAGCGCCTGATGCGGTATTTTTCTCCTTACGCATCTGTGCGGTATTTACACCCGAGACCAGC
CGCGTAACCTGGCAAAATCGGTTACGGTTGAGTAATAAATGGATGCCCTGCGTAAGCGGGTGTGGGCGGA
CAATAAAGTCTTAACTGAACAAAATAGATCTAACTATGACAATAAAGTCTTAACTAGACAGAATAGT
TGTAAACTGAAATCAGTCCAGTTATGCTGTGAAAAAGCATACTGGACTTTTGTATGGCTAAAGCAAAT
CTTCATTTTTCTGAAGTGCAAATTTGCCCGTTCGTATTAAGAGGGGCGTGGCCAAGGGCATGGTAAAGACTA
TATTCGCGGCGTTGTGACAATTTACCGAACAACCTCCGCGGCCGGAAGCCGATCTCGGCTTGAACGAATT
GTTAGGTGGCGGTACTTGGGTCGATATCAAAGTGCATCACTTCTTCCCGTATGCCCAACTTTGTATAGAG
AGCCACTGCGGGATCGTACCAGTAATCTGCTTGCACGTAGATCACATAAGCACCAAGCGGTTGGCCTCA
TGCTTGAGGAGATTGATGAGCGCGGTGGCAATGCCCTGCCTCCGGTGTCTCGCCGAGACTGCGAGATCAT
AGATATAGATCTCACTACGCGGCTGCTCAAACCTGGGCAGAACGTAAGCCGCGAGAGCGCCAACAACCGC
TTCTTGGTTCGAAGGCAGCAAGCGCGATGAATGTCTTACTACGGAGCAAGTTCCCGAGGTAATCGGAGTCC
GGCTGATGTTGGGAGTAGGTGGCTACGTCTCCGAACCTCACGACCGAAAAGATCAAGAGCAGCCCGCATGG
ATTTGACTTGGTCAGGGCCGAGCCTACATGTGCGAATGATGCCCATACTTGAGCCACCTAACTTTGTTTTT

AGGGCGACTGCCCTGCTGCGTAACATCGTTGCTGCTGCGTAACATCGTTGCTGCTCCATAACATCAAACA
TCGACCCACGGCGTAACGCGCTTGGTGGATGCCCGAGGCATAGACTGTACAAAAAACAGTCATAA
CAAGCCATGAAAACCGCCACTGCGCCGTTACCACCGCTGCGTTCGGTCAAGGTTCTGGACCAGTTGCGTG
AGCGCATACGCTACTTGCATTACAGTTTACGAACCGAACAGGCTTATGTCAACTGGGTTTCGTGCCTTCAT
CCGTTTCCACGGTGTGCGTCAACCGGCAACCTTGGGCAGCAGCGAAGTCGAGGCATTTCTGTCCTGGCTG
GCGAACGAGCGCAAGGTTTTCGGTCTCCACGCATCGTCAGGCATTGGCGGCCTTGGTGTTCCTTACGGCA
AGGTGCTGTGCACGGATCTGCCCTGGCTTCAGGAGATCGGAAGACCTCGGCCGTCGCGGGCGCTTGCCGGT
GGTGTGACCCCGGATGAAGTGGTTCGCATCCTCGGTTTTCTGGAAGGCGAGCATCGTTTGTTCGCCCAG
GACTCTAGCTATAGTTCTAGTGGTGGCTACGTATACTCCGGAATATTAATAGATCATGGAGATAATTAA
AATGATAACCATCTCGCAAATAAATAAGTATTTTACTGTTTTTCGTAACAGTTTTGTAATAAAAAAACCTA
3991 TAAATATTCGGGATTATTCATACCGTCCCACCATCGGGCGCGGATCTCGGTCCGAAACC

atgaaattcttagtcaacggttgccttgtttttatggctgatacatttcttacatctat
M K F L V N V A L V F M V V Y I S Y I Y

gcgggcggct --insert---gaattcgctgagcaccaccaccaccaccaccactaataag
A A A E F V E H H H H H H H H Stop

4215 ccaagcttgtcgagaagtactagaggatcataa
P S L S R S T R G S - TCAGCCATACCACATTTGTAGAG
GTTTTACTTGCTTTAAAAAACCTCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTG
TTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAA
AGCATTTTTTTTCACTGCATTCTAGTTGTGGTTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATC
TGATCACTGCTTGAGCCTAGGAGATCCGAACCAGATAAGTGAATCTAGTTCCAAACTATTTTGTCAATTT
TTAATTTTTCGTATTAGCTTACGACGCTACACCCAGTTCCCATCTATTTTGTCACTCTTCCCTAAATAATC
CTTAAAAACTCCATTTCCACCCCTCCCAGTTCCCAACTATTTTGTCCGCCACAGCGGGGCATTTTTCTT
CCTGTTATGTTTTTAAATCAAACATCCTGCCAACTCCATGTGACAAACCGTCATCTTCGGCTACTTTTTCT
CTGTACAGAATGAAAATTTTTCTGTCACTCTTTCGTTATTAATGTTTGTAAATTGACTGAATATCAACGC
TTATTTGCAGCCTGAATGGCGAATGG

Final expressed protein :
AA -----insert----- E F V E H H H H H H H H Stop

Primers for cloning into pFHMSF LIC C

Forward: tacattcttacatctatgcgggcggct

Reverse: tgg tgg tgc tcg acg aat tc