

pFHMSP LIC N

Source	Constructed by Alma Seitova
Company	Structural Genomics Consortium, Toronto

Description	The pFHMSP-LIC N vector is a derivative of the pFastBac HT A vector (Invitrogen). Honeybee melittin signal sequence was introduced before His tag. 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 a 26 amino acid N-terminal fusion tag containing 6X His followed by a TEV cleavage site.
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Antibiotic resistance	Ampicillin and Gentamicin
Promoter	Polyhedrin Promoter
Cloning Method	Insertion of DNA sequence into the cloning/expression region is preformed using BD-Biosciences Infusion enzyme mediated by directional recombination between complementary nucleotide DNA sequences at the ends of the insert (PCR product) and NcoI/HindIII 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	MKFLVNVALVFMVVYISYIYAAA
Termination codons	TGATGA included in 3' PCR primer
5' primer for amplification of insert	5'CTG TAT TTT CAG GGC GCC ATG GAT ---3'
3' primer for amplification of insert	5'C TCT AGT ACT TCT CGA CAA GCT TCA TCA ---3'
5' sequencing primer pFHMSP-Frd	5' CCGGATTATTCATACCGTCCCACCA 3'
3' sequencing primer pFHMSP-Rev	5' CTGATTATGATCCTCTAGTACTTCT 3'

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GACGCGCCCTGTAGCGGGCGCATTAAAGCGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTG
CCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCTTTCTCGCCACGTTTCGCCGGCTTTCCCGG
TCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAA
CTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTTTCGCCCTTTGACGTTGG
AGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTC
TTTTGATTTATAAGGGATTTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTT
AACGCGAATTTTAAACAAAATATTAACGTTTACAATTTTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAA
CCCCTATTTGTTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT
GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTT
GCGGCATTTTGCCTTCTGTTTTTGGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGT
TGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGA
AGAAGTTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCCTATTGACGCC
GGGCAAGAGCAACTCGGTTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAG
AAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGTGCCATAACCATGAGTGATAACAC
TGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGG
GATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATAACCAAACGACGAGCGTGACA
CCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAATTTAACTGGCGAACTACTTACTCTAGCTTTC
CCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCG
GCTGGCTGGTTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGG
GGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACG
AAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTGAGACCAAGTTTTACTCA
TATATACTTTAGATTGATTTAAAACCTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATA
ATCTCATGACCAAAATCCCTTAACTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA
AGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCA
GCGGTGGTTTTGTTTGC CGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGC
AGATACCAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCC
TACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGG
TTGGACTCAAGACGATAGTTACC GGATAAGGCGCAGCGGTTCGGGCTGAACGGGGGGTTTCGTGCACACAGC
CCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCT
TCCCGAAGGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTTCGGAACAGGAGAGCGCACGAGGGAG
CTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCTGTGGGTTTTCGCCACCTCTGACTTGAGCGTGCAT
TTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCT
GGCCTTTTGTGGCCTTTTGTCTCACATGTTCTTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATT
ACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCACTGAGCGAGG
AAGCGGAAGAGCGCCTGATGCGGTATTTTTCTCTTACGCATCTGTGCGGTATTTACACCCGCAGACCAGC
CGCGTAACCTGGCAAAATCGGTTACGGTTGAGTAATAAATGGATGCCCTGCGTAAGCGGGTGTGGCGGA
CAATAAAGTCTTAAACTGAACAAAATAGATCTAAACTATGACAATAAAGTCTTAAACTAGACGAATAGT
TGTAAACTGAAATCAGTCCAGTTATGCTGTGAAAAAGCATACTGGACTTTTGTTATGGCTAAAGCAAAC
CTTCATTTTTCTGAAGTGCAAATTTGCCCGTCGTATTAAGAGGGGGCGTGGCCAAGGGCATGGTAAAGACTA
TATTCGCGGCGTGTGACAATTTACCGAACAACTCCGCGGCCGGAAGCCGATCTCGGCTTGAACGAATT
GTTAGGTGGCGGTACTTGGGTCGATATCAAAGTGCATCACTTCTTCCCGTATGCCCAACTTTGTATAGAG
AGCCACTGCGGGATCGTCACCGTAATCTGCTTGACGTAGATCACATAAGCACCAAGCGGTTGGCCTCA
TGCTTGAGGAGATTGATGAGCGCGGTGGCAATGCCCTGCCTCCGGTGCTCGCCGGAGACTGCGAGATCAT
AGATATAGATCTCACTACGCGGCTGCTCAAACCTGGGCAGAACGTAAGCCGCGAGAGCGCAACAACCGC
TTCTTGGTGAAGGAGCAAGCGCGATGAATGTCTTACTACGGAGCAAGTTCCCGAGGTAATCGGAGTCC
GGCTGATGTTGGGAGTAGGTGGCTACGTCTCCGAACCTCACGACCGAAAAGATCAAGAGCAGCCCGCATGG
ATTTGACTTGGTCAGGGCCGAGCCTACATGTGCGAATGATGCCATACTTGAGCCACCTAACTTTGTTTT
AGGGCGACTGCCCTGCTGCGTAACATCGTTGCTGCTGCGTAACATCGTTGCTGCTCCATAACATCAAACA
TCGACCCACGGCGTAACGCGCTTGTGCTTGGATGCCCGAGGCATAGACTGTACAAAAAACAGTCATAA
CAAGCCATGAAAACCGCCACTGCGCCGTTACCACCGCTGCGTTCGGTCAAGGTTCTGGACCAGTTGCGTG
AGCGCATACGCTACTTGCATTACAGTTTACGAACCGAACAGGCTTATGTCAACTGGGTTTCGTGCCTTCAT
CCGTTTTCCACGGTGTGCGTCAACCGGCAACCTTGGGCAGCAGCGAAGTCGAGGCATTTCTGTCTGGCTG

GCGAACGAGCGCAAGGTTTTCGGTCTCCACGCATCGTCAGGCATTGGCGGCCTTGCTGTTCTTCTACGGCA
AGGTGCTGTGCACGGATCTGCCCTGGCTTCAGGAGATCGGAAGACCTCGGCCGTCGCGGCGCTTGCCGGT
GGTGTGACCCCGGATGAAGTGGTTCGCATCCTCGGTTTTCTGGAAGCGGAGCATCGTTTGTTCGCCCAG
GACTCTAGCTATAGTTCTAGTGGTTGGCTACGTATACTCCGGAATATTAATAGATCATGGAGATAATTAA
AATGATAACCATCTCGCAAATAAATAAGTATTTTACTGTTTTTCGTAACAGTTTTGTAAATAAAAAAACCTA
3991 TAAATATTCCGGATTATTCATACCGTCCCACCATCGGGCGCGGATCTCGGTCCGAAACC

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

cgggccgctccggaacatcaccatcaccatcacgattacgatatcccaacgaccgaaaac
A A A P E H H H H H H D Y D I P T T E N

ctgtatTTTTcagggcgccatggat
L Y F Q G A M D --- insert (SacB) -TGATGA---

4218 agcttgtcgagaagtactagaggatcataa
S L S R S T R G S - TCAGCCATACCACATTTGTAGAG
GTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTG
TTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAA
AGCATTTTTTTTCACTGCATTCTAGTTGTGGTTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATC
TGATCACTGCTTGAGCCTAGGAGATCCGAACCAGATAAGTGAAATCTAGTTCCAAACTATTTTTGTCATTT
TTAATTTTTCGTATTAGCTTACGACGCTACACCCAGTTCCCATCTATTTTTGTCACTCTTCCCTAAATAATC
CTTAAAAACTCCATTTCCACCCCTCCCAGTTCCCAACTATTTTTGTCCGCCACAGCGGGGCATTTTTTCTT
CCTGTTATGTTTTTAAATCAAACATCCTGCCAACTCCATGTGACAAACCGTCATCTTCGGCTACTTTTTCT
CTGTACAGAATGAAAATTTTTCTGTCTATCTCTCGTTATTAATGTTTGTAAATTGACTGAATATCAACGC
TTATTTGCAGCCTGAATGGCGAATGG

one more time highlighting changed area in original vector

3991 TAAATATTCCGGATTATTCATACCGTCCCACCATCGGGCGCGGATCTCGGTCCGAAACC

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

cgggccgctccggaacatcaccatcaccatcacgattacgatatcccaacgaccgaaaac
A A A P E H H H H H H D Y D I P T T E N
↑ signalase

ctgtatTTTTcagggcgccatggat
L Y F Q G A M D --- insert-TGATGA---

4218 agcttgtcgagaagtactagaggatcataa
S L S R S T R G S - TCAGCCATACCACATTTGTAGAG

Final expressed protein from this vector: (one or two alanine AA), depending where signalase cuts

A A P E H H H H H H D Y D I P T T E N L Y F Q G A
M D ----- insert-TGATGA-----

Red: Honeybee melittin signal peptide
Blue: TEV recognition site: cuts between Q/G

Vector linearizes by Nco1 and Hind III

Primers for pFHMS LIC N vector: overhands

Forward: ctgtatttcagggcgccatggat

Final reverse: ctctagtacttctcgacaagcttcatca