**pFHMSP LIC C**

<table>
<thead>
<tr>
<th>Source</th>
<th>Constructed by Alma Seitova</th>
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<tr>
<td>Company</td>
<td>Structural Genomics Consortium, Toronto</td>
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**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 *E. coli* 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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<tr>
<th>Antibiotic resistance</th>
<th>Ampicillin and Gentamicin</th>
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<td>Promoter</td>
<td>Polyhedrin Promoter</td>
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**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**
MKFLVNVAMVVFVYISYIYAAA

**Termination codons**
E F V E H H H H H H H H H H H TAATAG included in vector after 8 His tag

**5’ primer for amplification of insert**
5’TACATTCTTCTTACATCTATGCACGCCCT---3’

**3’ primer for amplification of insert**
5’ TGG TGG TGC TCG ACG AAT TC ---3’

**5’ sequencing primer pFHMSF-Frd**
5’ CCGGATTATTTCATACCGTCCCCACCA 3’

**3’ sequencing primer pFHMSF-Rev**
5’ CTGATTATGACTCCTCCTAGTACTTCT 3’
AGGGCGACTGCCCTGCTGTAACATCGTTGCTGCTGCGTAACATCGTTGCTGCTCCATAACATCAAACA
TCGACCCACGCGCGTAACGCGCTTGCTGCTTGGATGCCCGAGGCATAGACTGTACAAAAAAACAGTCATA
CAAGCCATGAAAACCGCCACTGCGCCGTTACCACCGCTGCGTTCGGTCAAGGTTCTGGACCAGTTGCCG
AGCGCATACGCTACTTGCATTACAGTTTACCTTGTTGACTGCCGCCCTCGCTCCAT
CGTTCACAGGCATCGCTACGTGTTGCTGCTTGACCTTTTGCTTGGGATTGCTCGACG
AGGCGGATGCGGAGAGGCACGAGCGCAAGGTTTCGGTCTCCACGCGCATCGCAGGCGGCTTGGCTTGC
GTCGACCCCGGATGAAGTGGTTCGCATCCTCGGTTTTCTGGAAGGCGAGCATCGTTTGTTCGCCCAG
GACTCTAGCTATAGTTCTAGTGGCTGACATGATTACCTCGGAAATATAGATCATGGAGATAATTAA
AATGATAAACCATCTCGCAAAATAATAGATTTATTCTGTACACTTTTGTGTAATAAATTTTTTTCT
3991 TAAATATTCCCGGATATTTCATACCGTCCCACCATCGGGCG
atgaaattcttagctaacggtgccccttgtttttatggtcgtatacatttcttacatctat
M  K  F  L  V  N  V  A  L  V  F  M  V  V  Y  I  S  Y  I  Y
A  A  A  A  E  F  V  E  H  H  H  H  H  H  H  H  H  Stop
4215 ccagcttgctgagcaccaccaaccacaccacaccaactaatag
PSL S R S R T R G S - TCAAGCCATACGAGATTTTAGGG
GTTTTACTTGCTTTAAAAACCTCACCACCTCCCTGGACTGAAACATATAATGAATGGAAATTGTGG
TGTTGAACTGTTTATGCTACGCATATATGTGTTACAAATATAAGGAATAGCATCAGAATAATTTCTGGAAT
AGCATTATTTCATCGCATCTCAGGTGTTGTTGTTGGCACCACACATCTCATATCTGATCAGATG
TGATCGATGCTGAGCCATGAGACCCGAAAGCAGATAAGGAAATCTAGTTCCAAACTATTTTTTGCATTT
TTAAATTCTGATTAGTACGCAGCTACCCACCTTCCCTCATTATTGTGCACTCTCCCTAAATATCTCC
CTTAAAACACTCCATTTCACCACCCCTCCAGTTCCAACATTATTGTCGCCACAGGCGGACATTTTTCT
CCAGATGTTTTTTAACTCAAACATCCCTGCAACCTGCAACAGATGCAAAACGTCATCTCGCTGACTTTTCT
CTGTCAGAAATGAAAATTTTTTTCTGCTACTCTCTGTATATAGTTGGTAATTGAATATCAGAATATACACG
TTATTTGACGCTGAAATGGGAAAT
Final expressed protein:
AA -----insert-------- E F V E H H H H H H H H H H Stop

Primers for cloning into pFHMSP LIC C
Forward: tacatttctcatcatgtagccggccgt
Reverse: tgg tgg tgc tgc acg aat tc