

pFHMSPN-avi-TEV-LIC Vector
(SGC 37-C3)

Source	Constructed by Yanjun Li
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Description	The pFHMSPN-avi-TEV-LIC is a derivative of the pFBOH-LIC vector (SGC). It is a donor vector for generation of recombinant baculovirus by site-specific transposition in an <i>E. coli</i> host. This vector has N-terminal TEV cleavable fusion tags of a honeybee melittin signal, a 6X His tag, an avi tag for <i>in vivo</i> biotinylation. Two stop codons are included in the vector at the C-terminal cloning site.
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 BseRI 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	MKFLVNVALVFMVVYISYIYAAPEMHHHHHHEFMSGLNDI FEAQKIEWHEGSAGGSGENLYFQG
5' primer tail for amplification of insert	5' TTGTATTTCCAGGGC --- 3'
3' primer tail for amplification of insert	5' CAAGCTTCGTCATCA --- 3'
5' sequencing primer pFBOH-fwd	5' CCGGATTATTCATACCGTCCCACCA 3'
3' sequencing primer pFBOH-rev	5' CTGATTATGATCCTCTAGTACTTCT 3'

pFHMSPN-avi-TEV-LIC cloning/expression region

Polyhedrin promoter

gtattttact gttttcgtaa cagttttgta ataaaaaac ctataaatat
cataaaatga caaaagcatt gtcaaaacat tatttttttg gatatttata

pFBOH-FWD

----->
tccggattat tcataccgtc ccaccatcgg gcgcggatct cgggccgaaaacc
aggcctaata agtatggcag ggtggtagcc cgcgcctaga gccaggcttttg

M K F L V N V A L V F M V V Y I S Y I Y A
atgaaattct tagtcaacgt tgccttgtt tttatggcgc tatacttctt acatctatgc
tactttaaga atcagttgca acgggaacaa aaataccagc atatgtaaagaa ttagatagc

A A P E M H H H H H H E F M S G L
ggccgctccg gaaatgcatca tcaccatcac catgaattca tgagcggcct
ccggcgaggc ctttacgtagt agtggttagtg gtacttaagt actcgccgga

N D I F E A Q K I E W H E G S A G
Gaacgatatt tttgaagcgc agaaaattga atggcatgaa ggcagcgcgtg
Cttgctataa aaacttcgcg tcttttaact taccgtaact ccgctcgcgac

G S G E N L Y F Q G BseRI

gaggttcaggt gaaaacttg tatttccagggc /attatgagtt **ctcctc**
Ctccaagtcca cttttgaac ataaaggtccc /taataactcaa **gaggag**

-----SACB(2 kb)-----

BseRI stop HindIII pFBOH-REV
gaggagatca tgcaca/tgat gacg**agc**tt gtcgagaag tactagagga
ctcctctagt acgtgt/acta ctgct**tcgaa** cagctcttc atgatctcct

SV40 polyadenylation signal
tcataatcag ccataccaca tttgtagagg ttttacttgc tttaaaaaac
agtatttagtc ggtatggtgt aaacatctcc aaaatgaacg aaattttttg

ctccacacc tcccctgaa cctgaaacat aaaatgaatg caattgtttg
gaggggtgtg agggggactt ggactttgta ttttacttac gttaacaaca

Electronic sequence of pFHMSPN-avi-TEV-LIC: 6908 bp

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