

pFBOH-avi-TEV-LIC Vector
(SGC 37-C2)

Source	Constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto

Description	The pFBOH-avi-TEV vector 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 6 X His-tag and an avi-tag for <i>in vivo</i> biotinylation. Two stop codons are included in the vector at the C-terminal cloning site.
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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	MHHHHHHEFMSGLNDIFEAQKIEWHEGSAGGSGENLYFQG
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'

pFBOH-avi-TEV-LIC cloning/expression region

Polyhedrin promoter
gtatatttact gttttcgtaa cagttttgta ataaaaaaaaac ctataaatat
cataaaatga caaaagcatt gtcaaaacat tatttttttg gatatttata

pFBOH-FWD

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tccggattat tcataaccgtc ccaccatcgg gcgcggatct cggtcgaaa
aggcctaata agtatggcag ggtggtagcc cgcgcctaga gccaggcttt

M H H H H H H E F M S G L N D I
ccatgcatca tcaccatcac catgaattca tgagcggcct gaacgatatt
ggtacgtagt agtggtagtg gtacttaagt actcgccgga cttgctataa

F E A Q K I E W H E G S A G G S G
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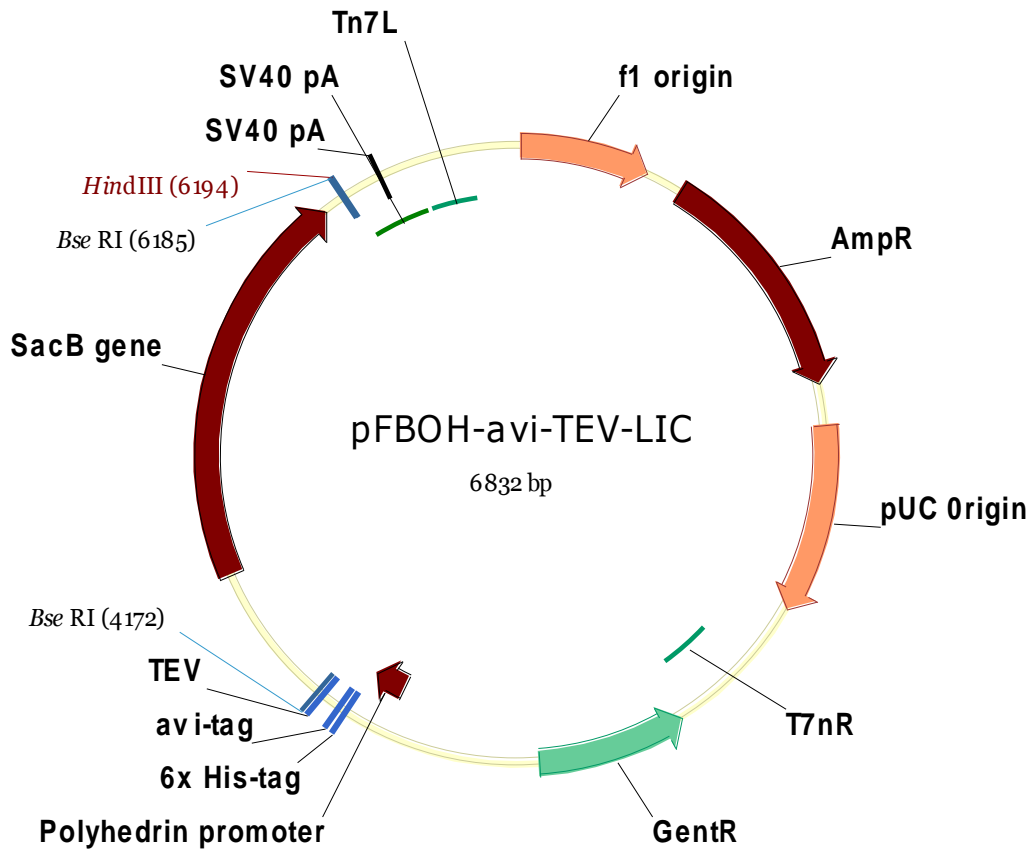
E N L Y F Q G BseRI
tgaaaacttg tatttccagggc /attatgagtt **ctcctc**
acttttgaac ataaagggtccc /taatactcaa **gaggag**

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

BseRI stop HindIII pFBOH-REV
gaggagatca tgcaca/tgat gacga**agctt** gtcgagaag tactagagga
ctcctctagt acgtgt/acta ctgct**tcgaa** cagctcttc atgatctct

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

ctccacacc tccccctgaa cctgaaacat aaaatgaatg caattgttgt
gaggggtgtg agggggactt ggactttgta ttttacttac gttaacaaca



Electronic sequence of pFBOH-avi-TEV-LIC (6832bp)

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