

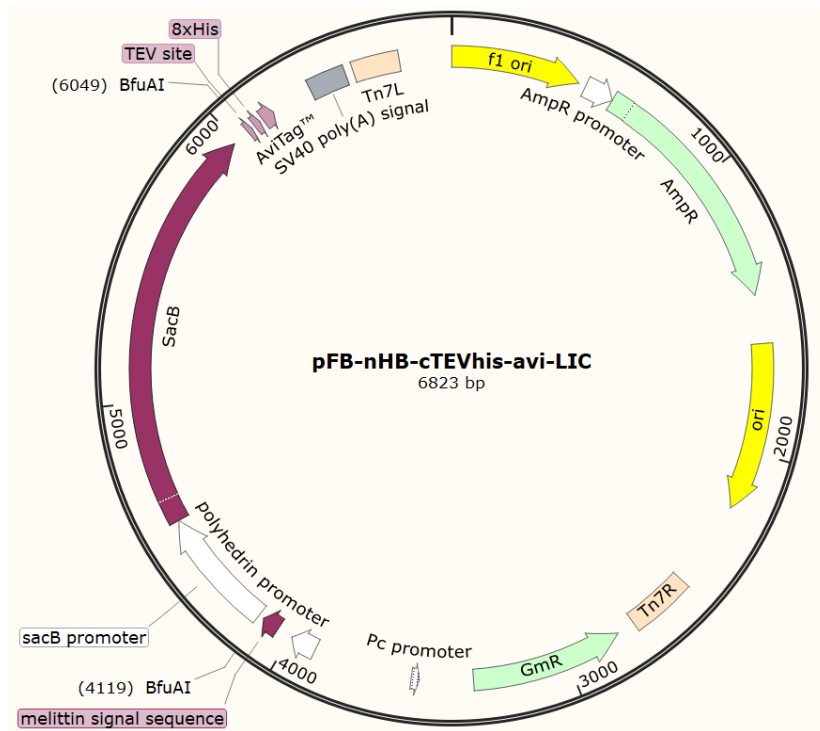
pFB-nHB-cTEVhisAvi-LIC Vector

Source	Designed by Bryan Fraser, constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto
Description	The pFB-nHB-cTEVhisAvi-LIC is a derivative of the pFHMSPN-avi-TEV-LIC (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 honeybee melittin signal peptide and C-terminal fusion tags of TEV cleavage site followed by 8His and an avi tag for <i>in vivo</i> biotinylating. 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 by LIC method, enzyme-mediated directional recombination using TaKaTa's In-fusion HD EcoDry (cat#639691). Insertion of target PCR product and BfuAI linearized vector involves the replacement of SacB gene stuffer sequence by negative selection on 5% sucrose.
N-Term Tag Sequence	MKFLVNVALVFMVVYISYIYAAA
C-Term Fusion Tag Sequence	ENLYFQGGGAGHHHHHHHEFMSGLNDIFEAQKIEWHEGSAG
5' primer addition	5' atctatgcgccgct 3'
3' primer addition	5' tgctccctggaaataaaggtttc 3'
5'sequencing Oligo: Fast-F	5' agtattttactgttttcg 3'
3'sequencing Oligo: Fast-R	5' taaccattataagctgc 3'

Electronic DNA Sequence and Map of pFB-nHB-cTEVhisAvi-LIC:

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cloning/expression region of pFB-nHB-cTEVhisAvi-LIC:

Polyhedrin promoter

atcatggaga taattaaaat gataaccatc tcgcaaataa ataagtattt
tagtacctct attaatttta ctattggtag agcgtttatt tattcataaa

tactgttttc gtaacagttt tgtaataaaa aaacctataa atattccgga
atgacaaatg cattgtcaaa acattatfff tttgatatt tataaggcct

honeybee melittin signal M

ttattcatac cgtcccacca tcggggcgcg atctcgggcc gaaaaccatg
aataagtatg gcagggtggt agcccgcgcc tagagccagg cttttggtac

K F L V N V A L V F M V V Y I S Y
aaattccttag tcaacgttgc cttgttttt atggtcgtat acatttctta
ttaaagaatc agttgcaacg ggaacaaaaa taccagcata tgtaaagaat

I Y A A A Bfu AI
catctatgcg gccgct/**tata ctatgcaggt**
cgagatacgc cggcga/**atat gatacgtcca**

-----SACB(1.8 kb)-----

Bfu AI C-fusion tags
E N L Y F Q G G S A G H H H
acctgcagag/ gaaaaccttt atttccaggg aggcagtgct ggccaccacc
tgagctctc/ cttttggaaa taaagggtccc tccgtcacga ccggtggtgg

H H H H H E F M S G L N D I F E
atcatcacca tcaccatgaa ttcattgagcg gcctgaacga tatttttgaa
tagtagtggt agtgggtactt aagtactcgc cggacttgct ataaaaactt

A Q K I E W H E G S A G 2x stops
gcgcaaaaa ttgaatggca tgaaggcagc gctggatgatga
cgcgtctttt aacttaccgt acttccgtcg cgacctactact