

pFBOH-LIC Vector (GenBank accession EF456740)

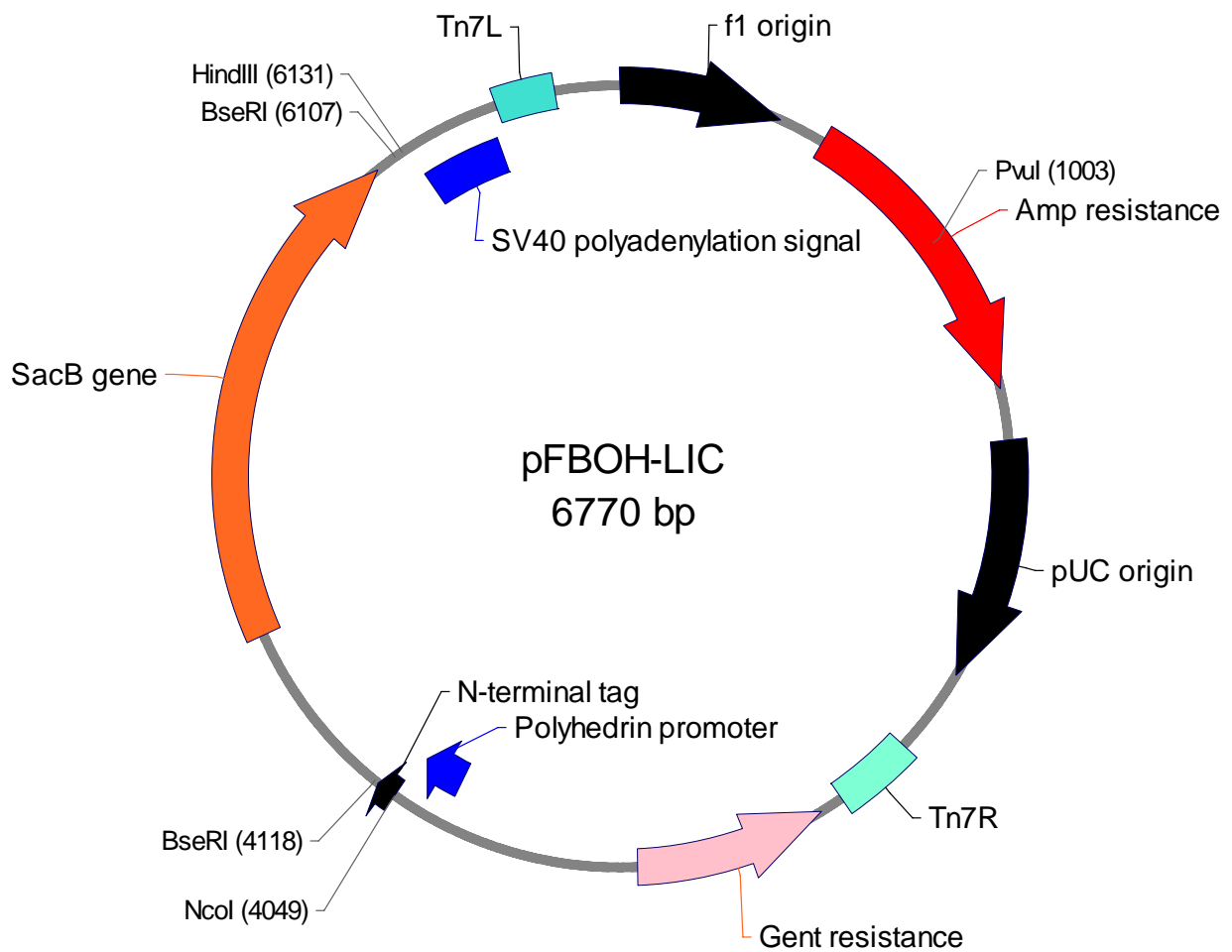
Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto

Description	The pFBOH-LIC vector is a derivative of the pFastBac vector (Invitrogen). A false start site was removed. It is a donor vector for generation of recombinant baculovirus by site-specific transposition in a <i>E. coli</i> host. For use in Baculovirus expression system in insect cells. This vector adds a 19 amino acid N-terminal fusion tag containing 6X His followed by a thrombin cleavage site. Two stop codons are included in the vector at the C-terminal cloning 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 directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and BseRI 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	NcoI site in vector
N – terminal fusion sequence	MGSSHHHHHSSGLVPRGS
Termination codons	TGATGA included in 3' PCR primer and vector cloning site. No amino acid residues added at cloning junction
Additional features	
Prefered Hosts	
5' primer for amplification of insert	5' GTT CCG CGT GGT AGT --- 3'
3' primer for amplification of insert	5' CAA GCT TCG TCA TCA --- 3'
5' sequencing primer pFBOH-FWD	5' CCGGATTATTCATACCGTCCCACCA 3'
3' sequencing primer pFBOH-REV	5' CTGATTATGATCCTCTAGTACTTCT 3'

pFBOH-LIC vector map

Polyhedrin promoter	3904-4032
N-terminal tag	4051-4107
N-terminal cloning site	4093-4107
C-terminal cloning site	6123-6137
SV40 polyadenylation signal	6154-6394
Tn7L	6423-6588
Tn7R	2511-2735
f1 origin	2-457
<i>bla</i> coding sequence	589-1446
<i>aacC1</i> coding sequence	2805-3335
pUC origin	1594-2267
<i>sacB</i> coding sequence	4629-6047



pFBOH-LIC cloning/expression region

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Polyhedrin promoter
~~~~~
3948  gtattttact gttttcgtaa cagttttgta ataaaaaac ctataaatat
      cataaaatga caaaagcatt gtcaaaacat tatttttttg gatatttata

      pFBOH-FWD
      ~~~~~
3998  tccggattat tcataccgtc ccaccatcgg gcgcggatct cgggccgaaa
      aggcctaata agtatggcag ggtggtagcc cgcgcctaga gccaggcttt

      NcoI
      M G S S H H H H H S S G L V P
4048  accatgggca gcagccatca tcatcatcat cacagcagcg gcctggttcc
      tggtagccgt cgtcggtagt agtagtagta gtgtcgtcgc cggaccaagg

      R G S BseRI
4098  gcgtggtagt/attatgagtt ctctc-----SACB(2 kb)-----
      cgcaccatca/taatactcaa gaggag

      BseRI stop HindIII pFBOH-REV
      ~~~~~
6107  gaggagatca tgcaca/tgat gacgaagctt tgctcgagaag tactagagga
      ctctcttagt acgtgt/acta ctgcttcgaa acagctcttc atgatctcct

      SV40 polyadenylation signal
      ~~~~~
6156  tcataatcag ccataaccaca tttgtagagg ttttacttgc tttaaaaaac
      agtattagtc ggtatggtgt aaacatctcc aaaatgaacg aaattttttg

      ~~~~~
6206  ctcccacacc tccccctgaa cctgaaacat aaaatgaatg caattgttgt
      gagggtgtgg agggggactt ggactttgta ttttacttac gttaacaaca

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