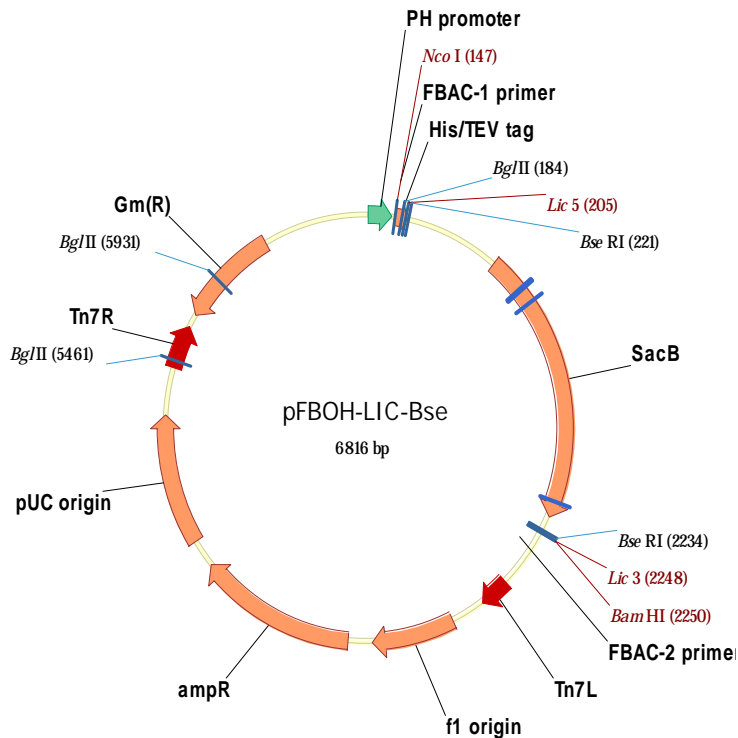


Vector information sheet.

Vector Name	<b>pFB-LIC-Bse</b>
Source	Opher Gileadi
Sequence accession/link	(SGC)
Description	Baculovirus transfer vector with His <sub>6</sub> tag in 22-aa N-terminal fusion peptide, with TEV protease cleavage site. Includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection of transformed bacteria on 5% sucrose
Antibiotic resistance	Ampicillin, 100 µg/ml
Promoter	Polyhedrin
Cloning	LIC. (vector treated with BseRI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MGHHHHHHSSGVDLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	2630 Da including Met (2411.8 Da removed by TEV cleavage)
Termination codons	supplied in PCR primer
Protease cleavage	TEV
Additional features	Tn7 sequences for in vivo recombination into bacmid DNA in DH10Bac (using InVitrogen’s Bac-to-bac system).
Preferred host	Initial transformation into any cloning strain, then transform purified plasmid into DH10Bac to generate recombinant bacmid DNA
5’ sequencing primer	FBAC1: TATTCATACCGTCCCACCA
3’ sequencing primer	FBAC2: GGGAGGTTTTTTAAAGCAAGTAAA



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                                                FBAC-1 primer
                                                ~~~~~
61      TTATTCATAC CGTCCCACCA
        AATAAGTATG GCAGGGTGGT

                NcoI
                ~~~~~
                M G H H H H H H S S G
121     TCGGGCGCGG ATCTCGGTCC GAAAACCATG GGCCACCATC ATCATCATCA TTCTTCTGGT
        AGCCC GCGCC TAGAGCCAGG CTTTGGTAC CCGGTGGTAG TAGTAGTAGT AAGAAGACCA
        BglII                Lic5                BseRI
        ~~~~~                ~~~~~                ~~~~~
        V D L G T E N L Y F Q S
181     GTAGATCTGG GTACCGAGAA CCTGTACTTC CAATCCATAA GCTAGCTTCT CCTCCTGAAA
        CATCTAGACC CATGGCTCTT GGACATGAAG GTTAGGTATT CGATCGAAGA GGAGGACTTT

                                                BseRI
--SacB linker--
2161                                         ACTTTTCGAG
                                                TGAAAAGCTC

                BamHI
                ~~~~~
        BseRI                Lic3'
        ~~~~~                ~~~~~
2221     GAGTTTACTA GTAAGTAAAG GTGGATACGG ATCCGAATTC GAGCTCCGTC GACAAGCTTG
        CTCAAATGAT CATTCATTC CACCTATGCC TAGGCTTAAG CTCGAGGCAG CTGTTCGAAC

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Primers for LIC cloning:

Upstream: add TACTTCCAATCCATG to the 5' end (ATG in-frame with the desired coding sequence).

Downstream: add TATCCACCTTACTG to 5' end of downstream primer; add termination codon, if necessary.