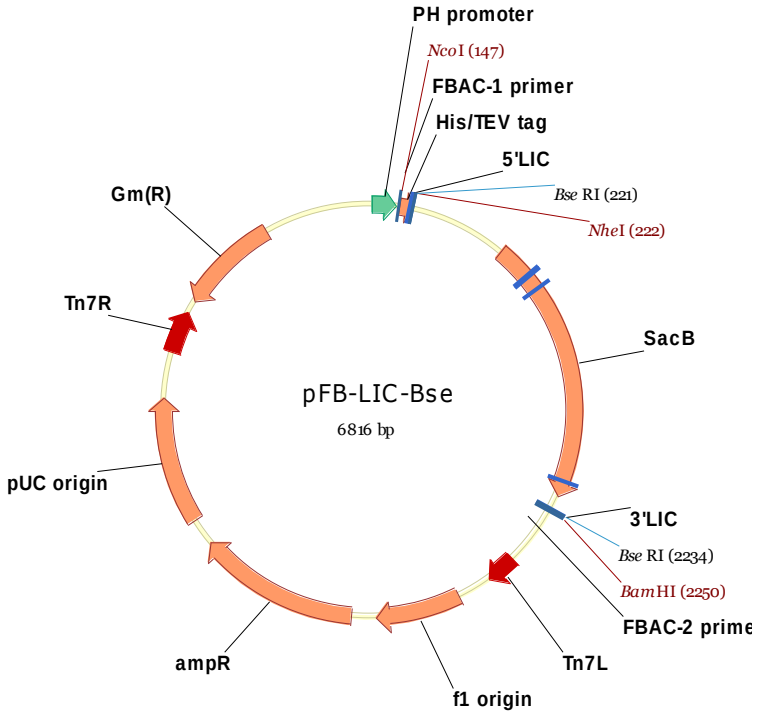
Vector information sheet

Dated: 8th May 2013

Vector Name	**pFB-LIC-Bse**
Source	Opher Gileadi
Sequence accession/link	EF199842

Description	Baculovirus transfer vector with His_6 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



Polylinker region:

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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
       AGCCCGCGCC TAGAGCCAGG CTTTTGGTAC 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 CATTCATTTC 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 TATCCACCTTTACTG to 5' end of downstream primer; add termination codon, if necessary.

pFB-LIC-Bse sequence:

atcatggagataattaaaatgataaccatctcgcaaataaataagtattttactgttttcgtaacagtt
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