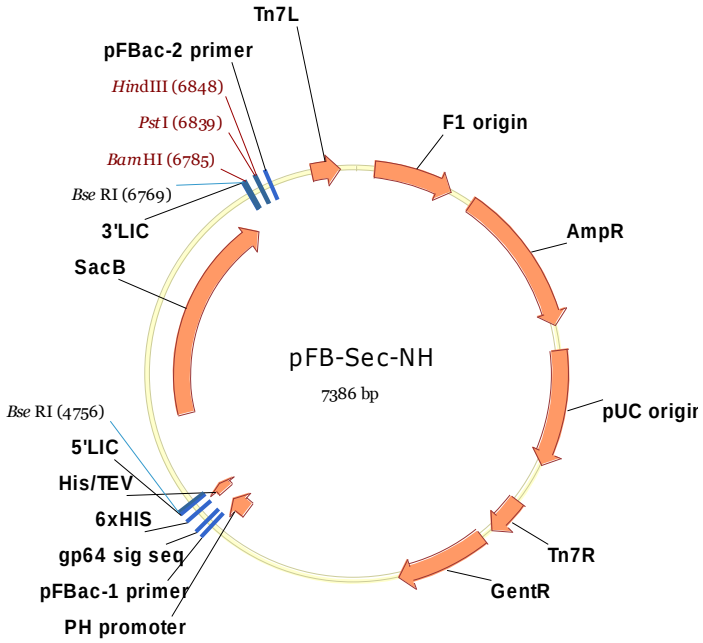
Vector information sheet

Dated: 8th May 2013

Vector Name	**pFB-Sec-NH**
Source	Grazyna Kochan
Sequence accession/link	(SGC)

Description	Baculovirus transfer vector with gp64 signal sequence and His_6 tag in 44-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.	MVSAIVLYVLLAAAAHSAFAAAMGHHHHHHSSGVDLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	5012 Da including Met (4682.9 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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                    PH promoter
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          pFBac-1 primer                          gp64 sig seq
                        ~~~~~~~~~~~~~~~~~~~~~                     ~~~~
                                                                   M  V ·
4561  CCTATAAATA TTCCGGATTA TTCATACCGT CCCACCATCG GGCGCGGATC TAAAACATGG
      GGATATTTAT AAGGCCTAAT AAGTATGGCA GGGTGGTAGC CCGCGCCTAG ATTTTGTACC
                           gp64 sig seq
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      · S  A  I   V  L  Y   V  L  L  A   A  A  A   H  S  A   F  A  A  A ·
4621  TAAGCGCTAT TGTTTTATAT GTGCTTTTGG CGGCGGCGGC GCATTCTGCC TTTGCGGCCG
      ATTCGCGATA ACAAAATATA CACGAAAACC GCCGCCGCCG CGTAAGACGG AAACGCCGGC
                                 His/TEV
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                    6xHIS                                                5'LIC
               ~~~~~~~~~~~~~~~~~~~~~                                     ~
      · M  G  H   H  H  H   H  H  S  S   G  V  D   L  G  T   E  N  L  Y ·
4681  CCATGGGCCA CCATCATCAT CATCATTCTT CTGGTGTAGA TCTGGGTACC GAGAACCTGT
      GGTACCCGGT GGTAGTAGTA GTAGTAAGAA GACCACATCT AGACCCATGG CTCTTGGACA
      His/TEV
      ~~~~~~~~
          5'LIC
      ~~~~~~~~~~~~~~~
                                BseRI
                                ~~~~~~
      ·  F  Q
4741  ACTTCCAATC CATAAGCTAG CTTCTCCTCC
      TGAAGGTTAG GTATTCGATC GAAGAGGAGG
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--SacB linker--

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                                                                3'LIC
                                                             ~~~~~~~~~~~~~~
                                          BseRI
                                          ~~~~~~
6721  CCTATTGGCA TTGACGTCAG GTGGCACTTT TCGAGGAGTT TACTAGTAAG TAAAGGTGGA
      GGATAACCGT AACTGCAGTC CACCGTGAAA AGCTCCTCAA ATGATCATTC ATTTCCACCT
      3'LIC
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          BamHI                                                  PstI
          ~~~~~~                                                 ~~~~~~
6781  TACGGATCCG AATTCGAGAA TCGAATTCCC GCGGCCGCTT TCGAATCTAG AGCCTGCAGT
      ATGCCTAGGC TTAAGCTCTT AGCTTAAGGG CGCCGGCGAA AGCTTAGATC TCGGACGTCA
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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-Sec-NH sequence:

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