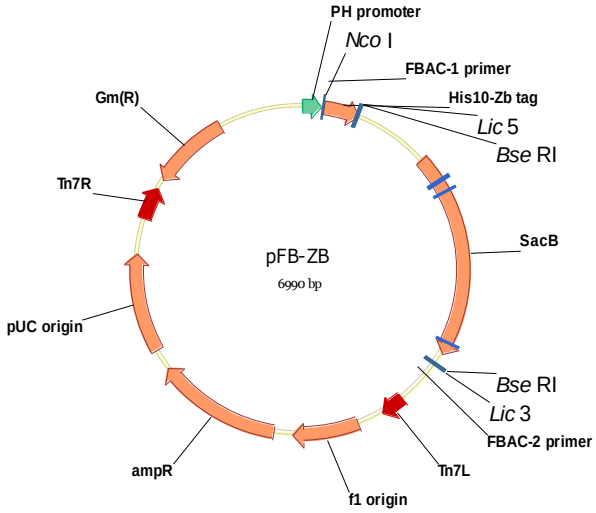
Vector information sheet

Dated: 8th May 2013

Vector Name	**pFB-ZB**
Source	Pavel Savitsky
Sequence accession/link	Genbank JF682518

Description	Baculovirus transfer vector with His_{10} and Z-basic tags, followed by a TEV protease cleavage site. The Z-basic tag *(J. Chromatog A, 1161:22-28)* is a 54-aa sequence derived from protein A and modified to have a high positive surface charge, allowing the fusion proteins to bind to S-sepharose at salt concentrations in which most cellular proteins do not bind. Both the His10 tag and the Zb tag allow purification at stringent conditions. The vector 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.	MGHHHHHHHHHHSSGVDNKFNKERRRARREIRHLPNLNREQRRAFIRSLR DDPSQSANLLAEAKKLNDAQPKGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	9781 Da including Met (9563.15 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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                                  NcoI
                                  ~~~~~~~
                                    M   G  H  H  H   H  H  H   H  H  H
121     TCGGGCGCGG ATCTCGGTCC GAAAACCATG GGCCACCATC ACCATCACCA TCATCATCAT
        AGCCCGCGCC TAGAGCCAGG CTTTTGGTAC CCGGTGGTAG TGGTAGTGGT AGTAGTAGTA

        H  S  S  G   V  D  N   K  F  N   K  E  R  R   R  A  R   R  E  I
181     CATTCTTCTG GTGTGGATAA CAAGTTCAAC AAGGAGCGTC GAAGAGCTCG CCGTGAAATT
        GTAAGAAGAC CACACCTATT GTTCAAGTTG TTCCTCGCAG CTTCTCGAGC GGCACTTTAA

        R  H  L  P   N  L  N   R  E  Q   R  R  A  F   I  R  S   L  R  D
241     CGCCATCTGC CGAACCTGAA CCGCGAACAG CGTCGCGCAT TTATTCGCAG CCTGCGCGAT
        GCGGTAGACG GCTTGGACTT GGCGCTTGTC GCAGCGCGTA AATAAGCGTC GGACGCGCTA

        D  P  S  Q   S  A  N   L  L  A   E  A  K  K   L  N  D   A  Q  P
301     GATCCGAGCC AGAGCGCGAA CCTGCTGGCG GAAGCGAAGA AGCTGAACGA TGCGCAGCCG
        CTAGGCTCGG TCTCGCGCTT GGACGACCGC CTTCGCTTCT TCGACTTGCT ACGCGTCGGC

                             Lic5                          BseRI
                             ~~~~~~~~~~~~~~~~              ~~~~~~
        K  G  T  E   N  L  Y   F  Q  S                    ~~~~~~
361     AAGGGTACAG AGAACCTGTA CTTCCAATCC ATAAGCTAGC TTCTCCTCCT _ _ _ _
        TTCCCATGTC TCTTGGACAT GAAGGTTAGG TATTCGATCG AAGAGGAGGA

                - - - - - - - - SacB - - - - - - - - - - -        CGAGGAGTTT     -2400
                                                                  GCTCCTCAAA
                                                                   ~~~~~~
                                                                   BseRI

                      Lic3
                      ~~~~~~~~~~~~~~~~
2401    ACTAGTAAGT AAAGGTGGAT ACGGATCCGA
        TGATCATTCA TTTCCACCTA TGCCTAGGCT
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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-ZB sequence:

atcatggagataattaaaatgataaccatctcgcaaataaataagtattttactgttttcgtaacagtt
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