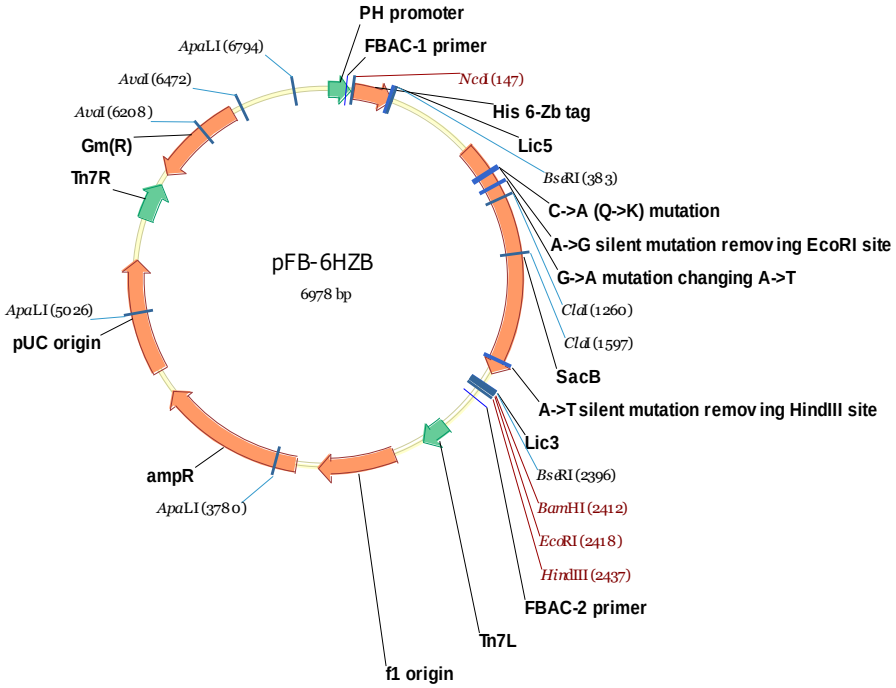
Vector information sheet

Dated: 8th May 2013

Vector Name	**pFB-6HZB**
Source	Pravin Mahajan
Sequence accession/link	

Description	Baculovirus transfer vector with His_6 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 His6 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.	MGHHHHHHSSGVDNKFNKERRRARREIRHLPNLNREQRRAFIRSLR DDPSQSANLLAEAKKLNDAQPKGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	9119 Da including Met
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   S  S  G
121  TCGGGCGCGG ATCTCGGTCC GAAAACCATG GGCCACCATC ATCATCATCA TTCTTCTGGT
     AGCCCGCGCC TAGAGCCAGG CTTTTGGTAC CCGGTGGTAG TAGTAGTAGT AAGAAGACCA
      V  D  N  K   F  N  K   E  R  R   R  A  R  R   E  I  R   H  L  P
181  GTGGATAACA AGTTCAACAA GGAGCGTCGA AGAGCTCGCC GTGAAATTCG CCATCTGCCG
     CACCTATTGT TCAAGTTGTT CCTCGCAGCT TCTCGAGCGG CACTTTAAGC GGTAGACGGC
      N  L  N  R   E  Q  R   R  A  F   I  R  S  L   R  D  D   P  S  Q
241  AACCTGAACC GCGAACAGCG TCGCGCATTT ATTCGCAGCC TGCGCGATGA TCCGAGCCAG
     TTGGACTTGG CGCTTGTCGC AGCGCGTAAA TAAGCGTCGG ACGCGCTACT AGGCTCGGTC
      S  A  N  L   L  A  E   A  K  K   L  N  D  A   Q  P  K   G  T  E
301  AGCGCGAACC TGCTGGCGGA AGCGAAGAAG CTGAACGATG CGCAGCCGAA GGGTACAGAG
     TCGCGCTTGG ACGACCGCCT TCGCTTCTTC GACTTGCTAC GCGTCGGCTT CCCATGTCTC
                                      BseRI
                                      ~~~~~~
      N  L  Y  F   Q  S
361  AACCTGTACT TCCAATCCAT AAGCTAGCTT CTCCTCCTGA AAGATCCATA ACTTCGTATA
     TTGGACATGA AGGTTAGGTA TTCGATCGAA GAGGAGGACT TTCTAGGTAT TGAAGCATAT

          - - - - - - - - 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-6HZB sequence:

atcatggagataattaaaatgataaccatctcgcaaataaataagtattttactgttttcgtaacagtt
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