## Vector information sheet

Dated: 8th May 2013

<table>
<thead>
<tr>
<th>Vector Name</th>
<th>pFB-6HZB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Pravin Mahajan</td>
</tr>
<tr>
<td>Sequence accession/link</td>
<td></td>
</tr>
</tbody>
</table>

### Description
Baculovirus transfer vector with His6 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.
- MGHHHHHHSSSVDKFKNKERRRREIRHLPNLREQRRATFIRSLR DDPSQSANLLAEAKLNDAPKGTENLYQ*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: TATTCATACCGTCCCA

### 3’ sequencing primer
- FBAC2: GGGAGGTTTTTTAAAGCAAGTAAA
Polylinker region:

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<table>
<thead>
<tr>
<th>Restriction Enzyme</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>NcoI</td>
<td>TCGGGCGCGG ATCTCGGTCC GAAAACCATG</td>
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<tr>
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<td>GCGCGCCGCC TAGAGCCAGG CTTTTGTAGT</td>
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<td>CGCGGTGTAG TAGTGAAGCA AGAAGACCA</td>
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<td>GTGACATAAC AGTTCAACAA GGAGCGTCGA</td>
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<tr>
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<td>AGAGCTCGCC GTGAAATTCG CCATCTGCCG</td>
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<tr>
<td></td>
<td>CACCTATTGT TCA</td>
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<td></td>
<td>AGTTGTT CCTCGCAGCT TCTCGAGCGG CACTTTAAGC GGTAGACGGC</td>
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<tr>
<td></td>
<td>N  L  Y  F   Q  S</td>
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</tbody>
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Primers for LIC cloning:

Upstream: add TACTTCCAAATCCATG 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:

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actatggagatatttaatgataacccatctgcagaataataatctatatctattatcagtttggatcctgttccgaacacattgctccatctgaacccggtgcctccgggaaacccatttcctgtgtttgttttggagttttggtttaggtatagtatttattctaggttacaattcatttcattccattctctctgttttgattttaatgggttacttttttatatttgatgattacattctttttatattcatatttttagtgtattattagatgtagatattttttaatatttattttatgatttttttcttattttattttttattattattttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt...```