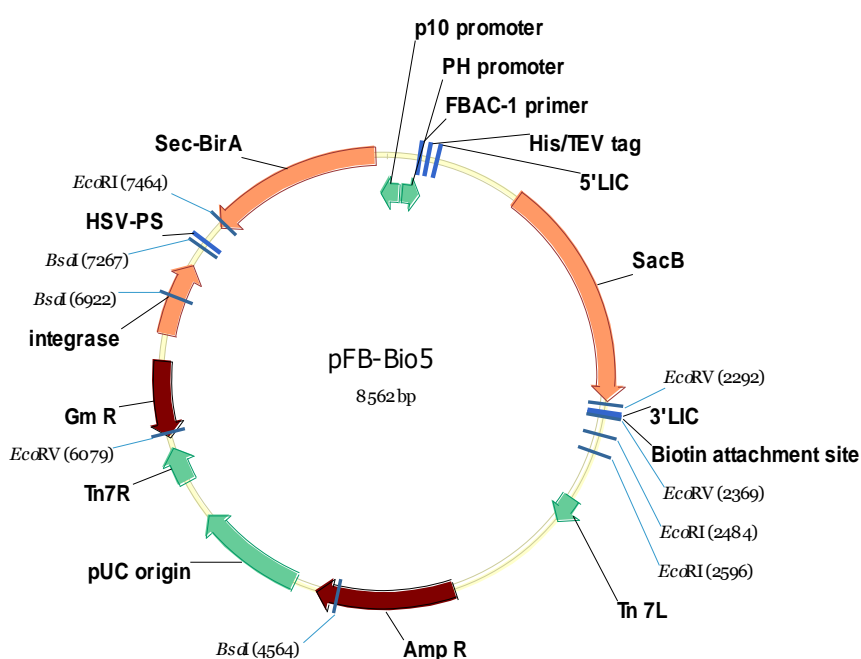


Vector information sheet.

Vector Name	<b>pFB-Bio5</b>
Source	Opher Gileadi and Claire Strain-Damerell
Sequence accession/link	
Description	Baculovirus transfer vector with His <sub>6</sub> tag in 22-aa N-terminal fusion peptide, with TEV protease cleavage site and C-terminal biotin attachment site. The vector also includes a secreted version of <i>birA</i> under the control of the p10 promoter for <i>in vivo</i> biotinylation. Includes sites for LIC cloning, and a “stuffer” fragment that includes the <i>SacB</i> 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.	MGHHHHHHSSGVDLG TENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	2630 Da including Met (2411.8 Da removed by TEV cleavage)
C-terminal fusion – seq.	SSKGGYGLNDIFEAQKIEWHE
C-terminal fusion – MW	2408.6 Da
Termination codons	supplied in PCR primer
Protease cleavage	TEV (cleaves His-tag only)
Additional features	Tn7 sequences for <i>in vivo</i> 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



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## Cloning region in the vector:

```

                                Fbacl
                                ----->
181  AATAAAAAAA CCTATAAATA TTCCGGATTA TTCATACCGT CCCACCATCG GGCGCGCTTA
    TTATTTTTTT GGATATTAT  AAGGCCTAAT AAGTATGGCA GGGTGGTAGC CCGCGCGAAT

                                M G  H H H  H H H S  S G V  D L G
241  AGAAGGAGAT ATACTATGGG CCACCATCAT CATCATCATT CTTCTGGTGT AGATCTGGGT
    TCTTCCTCTA TATGATACCC GGTGGTAGTA GTAGTAGTAA GAAGACCACA TCTAGACCCA
                                BseRI
                                ~~~~~~
    T E N L  Y F Q
301  ACCGAGAACC TGTACTTCCA ATCCATAAGC TAGCTTCTCC TCCTGAAAGA TCCATAACTT
    TGGCTCTTGG ACATGAAGGT TAGGTAATTCG ATCGAAGAGG AGGACTTTCT AGGTATTGAA
                                LIC5'

```

## --SacB linker--

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                                BseRI
                                ~~~~~~
2281 AAAATGCCGA TATCCTATTG GCATTGACGT CAGGTGGCAC TTTTCGAGGA GTTTACTAGT
    TTTTACGGCT ATAGGATAAC CGTAACCTGA GTCCACCGTG AAAAGCTCCT CAAATGATCA

    S K G  G Y G L  N D I  F E A  Q K I E  W H E
2341 AAGTAAAGGT GGATACGGCC TGAATGATAT CTTTGAAGCG CAGAAGATTG AATGGCATGA
    TTCAATTTCCA CCTATGCCGG ACTTACTATA GAAACTTCGC GTCTTCTAAC TTACCGTACT
    LIC3'

    . * *
2401 ATGATAAGCA GAGAACCTCT ACTTCCAATC GGGTCTGAAC GACATCTTCG AAGCTCAGAA
    TACTATTCTG CTCTTGAGAG TGAAGGTTAG CCCAGACTTG CTGTAGAAGC TTCGAGTCTT

2461 AATTGAATGG CACTGAGGAT CCGAATTCTGA GCTCCGTCGA CAATTGGGCA GAGAACCTCT
    TTAACCTTACC GTGACTCCTA GGCTTAAGCT CGAGGCAGCT GTTAACCCGT CTCTTGAGAG

2521 ACTTCCAATC GCACCATCAT CACCATCACC ATCACCACCA TGATTACAAG GATGACGACG
    TGAAGGTTAG CGTGGTAGTA GTGGTAGTGG TAGTGGTGGT ACTAATGTTT CTACTGCTGC

2581 ATAAGTGAGG ATCCGAATTC GAGCTCCGTC GACAAGCTTG TCGAGAAGTA CTAGAGGATC
    TATTCACCTC TAGGCTTAAG CTCGAGGCAG CTGTTTCGAAC AGCTCTTCAT GATCTCCTAG
                                FBac2
                                <-----
2641 ATAATCAGCC ATACCACATT TGTAGAGGTT TTAATTGCTT TAAAAACCT CCCACACCTC
    TATAGTTCGG TATGGTGTAA ACATCTCCAA AATGAACGAA ATTTTTTGGA GGGTGTGGAG

```

## Primers for LIC cloning:

Upstream: add TACTTCCAATCCATG to the 5' end (ATG in-frame with the desired coding sequence).

Downstream: add TATCCACCTTTACTGCT to 5' end of downstream primer. Do not add termination codon as this will remove the biotin attachment site.