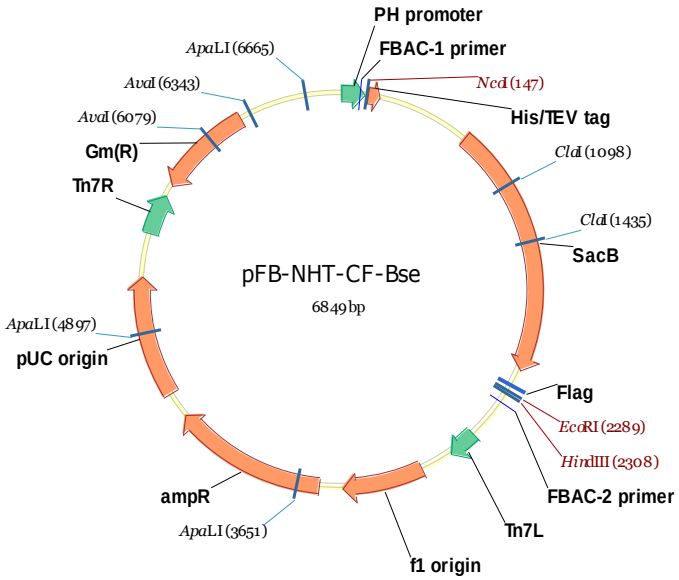
Vector information sheet

Dated: 8th May 2013

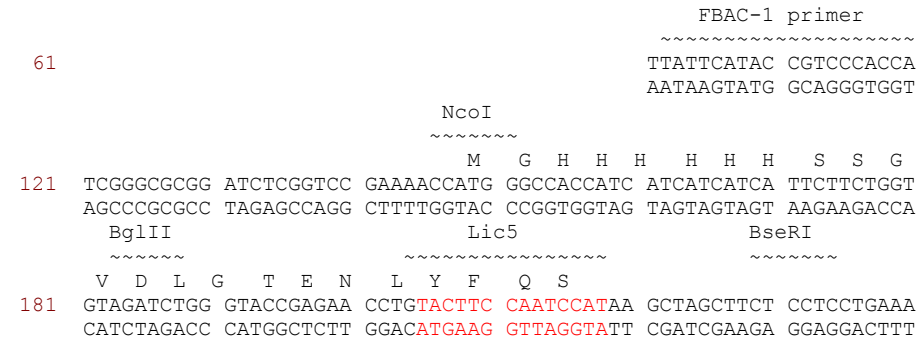
Vector Name	**pFB-NHT-CF-Bse**
Source	Opher Gileadi
Sequence accession/link	

Description	Baculovirus transfer vector with His_6 tag in 22-aa N-terminal fusion peptide, with TEV protease cleavage site and a C-terminal Flag tag. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection of transformed bacteria on 5% sucrose

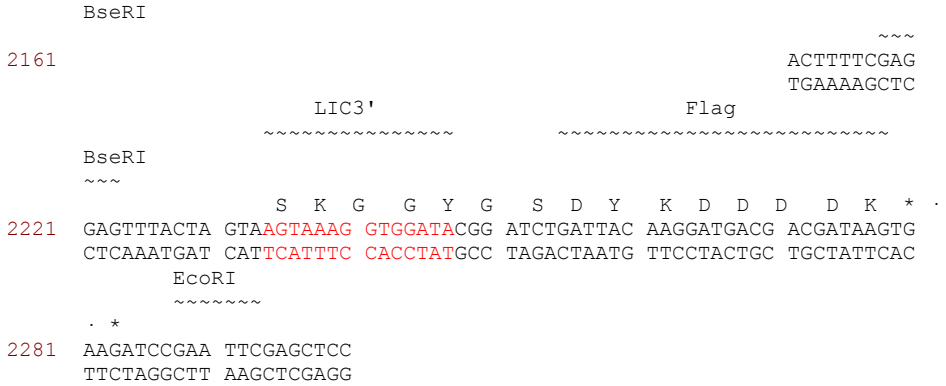
Antibiotic resistance	Ampicillin, 100 μg/ml
Promoter	Polyhedrin
Cloning	LIC. (vector treated with BseRI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MGHHHHHHSSGVDLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	2630 Da including Met (2411.8 Da removed by TEV cleavage)
C-terminal fusion - MW	DYKDDDDK
Termination codons	None
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:



--SacB linker--



Primers for LIC cloning:

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

Downstream: add TATCCACCTTTACTGCC to 5’ end of downstream primer. Do not add a stop codon.
NB. Additional 2 bases (CC) added onto reverse primer.

pFB-NHT-CF-Bse sequence:

atcatggagataattaaaatgataaccatctcgcaaataaataagtattttactgttttcgtaacagtt
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