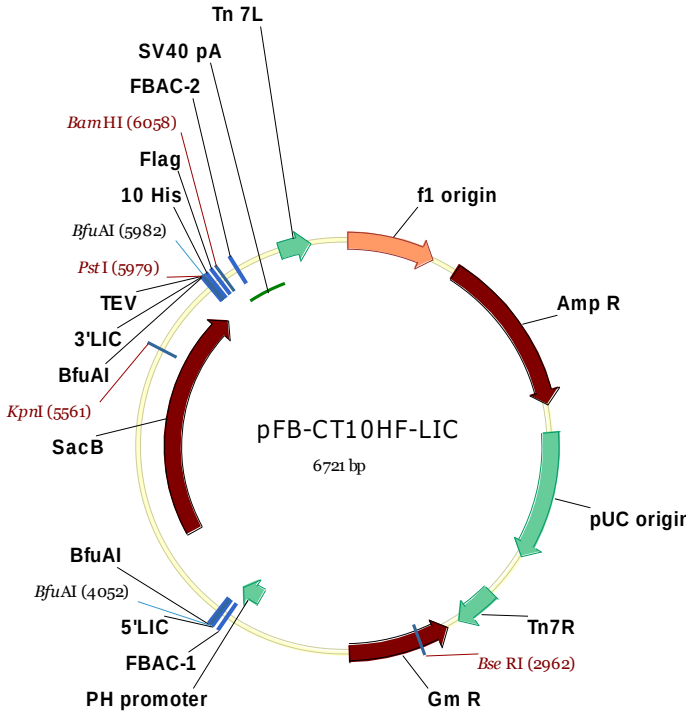
Vector information sheet

Dated: 8th May 2013

Vector Name	**pFB-CT10HF-LIC**
Source	Grazyna Kochan
Sequence accession/link	(SGC)

Description	Baculovirus transfer vector with with C-terminal His_{10} tag and FLAG tag, preceded by a TEV protease cleavage site. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection on 5% sucrose

Antibiotic resistance	Ampicillin, 100 μg/ml
Promoter	Polyhedrin
Cloning	LIC. (vector treated with BfuAI, then with T4 DNA polymerase in presence of dCTP)
Initiation codon	Supplied in PCR primer
C-terminal fusion – seq.	AENLYFQ*SHHHHHHHHHHDYKDDDDK (* - TEV cleavage site)
C-terminal fusion – MW	
Termination codon	Downstream of flag tag
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 or FBAC3: TTAAAATGATAACCATCTCG
3' sequencing primer	FBAC2: GGGAGGTTTTTTAAAGCAAGTAAA



Polylinker region:

5' end:

```
                                                  BfuAI
                                                 ~~~~~~~
4021  CCATCGGGCG CGGATCTCCT TAAGAAGGAG ATATACTATG CAGGTCGTTC ACTATTATTT
      GGTAGCCCGC GCCTAGAGGA ATTCTTCCTC TATATGATAC GTCCAGCAAG TGATAATAAA
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---------- SacB fragment ----------

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                                                        TEV
                                                 ~~~~~~~~~~~~~~~~~~~~
                                                      3'LIC
                                              ~~~~~~~~~~~~~~~~~~~~~~~~~~
                                          BfuAI                          10 His
                                          ~~~~~~                         ~
                                          BfuAI
                                          ~~~~~~
                                             PstI
                                            ~~~~~~
                                               A  E   N  L  Y   F  Q  S  H ·
5941  ATATCCTATT GGCATTGACG TCAGGTGGCA CACCTGCAGA GAACCTCTAC TTCCAATCGC
      TATAGGATAA CCGTAACTGC AGTCCACCGT GTGGACGTCT CTTGGAGATG AAGGTTAGCG
                10 His
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                  Flag
                                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                                 BamHI
                                                                 ~~~~
      · H  H  H   H  H  H   H  H  H  D   Y  K  D   D  D  D   K
6001  ACCATCATCA CCATCACCAT CACCACCATG ATTACAAGGA TGACGACGAT AAGTGAGGAT
      TGGTAGTAGT GGTAGTGGTA GTGGTGGTAC TAATGTTCCT ACTGCTGCTA TTCACTCCTA
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Primers for LIC cloning:

Add the following 5’ extensions to the PCR primers:

Upstream: TTAAGAAGGAGATATACTATG (ATG-initiation codon)

Downstream: GATTGGAAGTAGAGGTTCTCTGC

The purified PCR fragments are treated with T4 DNA polymerase and dGTP, then annealed to the treated vector.

pFB-CT10HF-LIC sequence:

gacgcgccctgtagcggcgcattaagcgcggcgggtgtggtggttacgcgcagcgtgaccgctacactt
gccagcgccctagcgcccgctcctttcgctttcttcccttcctttctcgccacgttcgccggctttccc
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