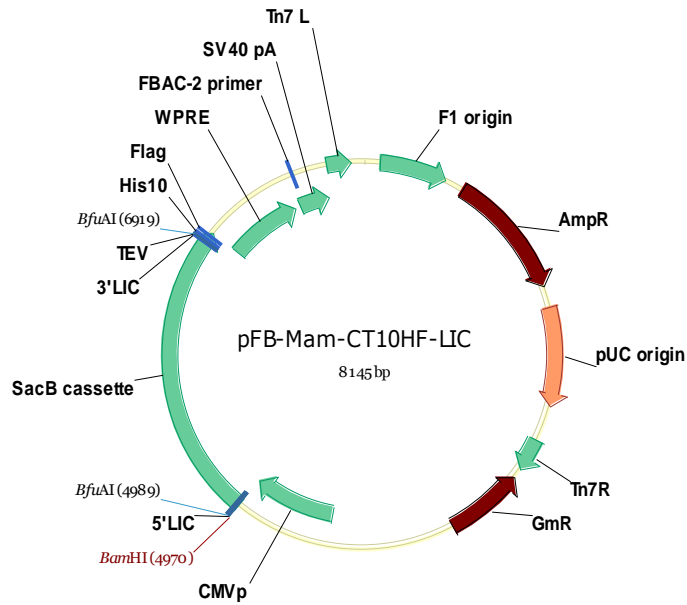


Vector information sheet

Dated: 8th May 2013

Vector Name	pFB-Mam-CT10HF-LIC
Source	Grazyna Kochan
Sequence accession/link	(SGC)
Description	Baculovirus transfer vector, for expression of proteins in mammalian cells, 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 mg/ml
Promoter	CMV
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	2471.47Da
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. Bacmid DNA can be transfected to insect cells to generate recombinant baculovirus. Baculovirus can be used to produce recombinant protein in multiple mammalian cell lines.
5' sequencing primer	pFBM-fwd caaaatgctgtaacaactccgc
	pFBM-rev tagttaagaataccagtcaatctttcac



Polylinker region:

5' end:

```

                                     BfuAI
                                     ~~~~~~
4021  CCATCGGGCG CGGATCTCCT TAAGAAGGAG ATATACTATG CAGGTCGTTT ACTATTATTT
      GGTAGCCCGC GCCTAGAGGA ATTCTTCCTC TATATGATAC GTCCAGCAAG TGATAATAAA

----- SacB fragment -----

                                     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
                                     ~~~~~~

      · H H H H H H H H H D Y K D D D D K
6001  ACCATCATCA CCATCACCAT CACCACCATG ATTACAAGGA TGACGACGAT AAGTGAGGCGGC
      TGGTAGTAGT GGTAGTGGTA GTGGTGGTAC TAATGTTCTT ACTGCTGCTA TTCACTCCGCCG

```

Primers for LIC cloning:

Add the following 5' extensions to the PCR primers:

Upstream: TTAAGAAGGAGATATACTATG (ATG-initiation codon)

Downstream: GATTGGAAGTAGAGTTCTCTGC

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

pFB-Mam-CT10HF-LIC sequence:

```
ttctctgtcacagaatgaaaatTTTTCTGTCATCTCTTCGTTATTAATGTTTGTAATTGACTGAATATC
aacgcttattttgcagcctgaatggcgaatgggacgcgcctgtagcggcgcattaagcgcggcgggtgt
ggTggTtacgcgcagcgtgaccgctacacttgccagcgccttagcgcggcctcctttcgccttctccc
ttcctttctcgcacggttcgcccgtttccccgctcaagctctaaatcgggggctcccttaggggtccg
atTtagTgcttttacggcacctcgacccccaaaaaacttgattagggTgatggTtcacgtagTgggcatc
gccctgatagacggTTTTTcgcctttgacgTtgagTccacgTtctTtaatagTggactctTgttcca
aactggaacaacactcaaccctatctcggTctattctTTTgatttataagggattTtgccgattTcggc
ctattggTtaaaaaatgagctgatttaacaaaaatttaacgcgaattTtaacaaaaatattaacgtttac
aatttcaggTggcactTTTcggggaaatgtgTcgcggaaccctattTgtttattTTTctaaatacattc
aaatatgtatccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaaggaagagTat
gagTattcaacattTccgTgTcgcccttattccctTTTTTgcgcatTTTgcttctctgTTTTTgctca
cccagaaacgctggTgaaagTaaagatgctgaagatcagTtggTgTcacgagTgggTtacatcgaact
ggatctcaacagcggTaaagatcctTgagagTTTTcgcggcgaagaacgTTTTccaatgatgagcactTt
taaagTctgctatgtggcgcggTattatcccgtattgacgcgggcaagagcaactcggTcgcgcgcat
acactattctcagaatgactTggTtgagTactcaccagTcacagaaaagcatcttacggatggcatgac
agTaaagaaattatgcagTgTgcccataaccatgagTgataaacactgcggccaacttactTctgacaac
gatcggaggaccgaaggagTaaaccgctTTTTTgcacaacatgggggatcatgTaaactcgcctTgatcg
TtgggaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgctTgtagcaatggc
aacaacgTtgcgcaaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactg
gatggaggcggataaaagTtgCaggaccactTctgCgctcgccctTccggctggctggTttattgctga
TaaatctggagccggtgagcgtgggtctcgcggTatcattgcagcactggggccagatggTaaagccctc
ccgTatcgtagTtatctacacgacggggagTcagggcaactatggatgaacgaaatagacagatcgtTga
gatagTgctcactgattaagcattggTaaactgTcagaccaagTtactcatatataactTtagattga
TTTTaaacttcatTTTTtaattTaaaggatctagTgaaagatcctTTTTgataatctcatgaccaaact
ccctTaaacgTgagTTTTcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttctTgaga
TcctTTTTTctgCgctaatctgctgctTgcaacaaaaaaaccaccgctaccagcggTggTttgTtt
gcccgatcaagagctaccaactctTTTTccgaaggTaaactggctTcagcagagcgcagataccaaatac
TgtcctTctagTgtagccgtagTtaggccaaccactTcaagaactctgtagcaccgcctacatacctcgc
TctgctaactcctgTtaccagTggctgctgcccagTggcgataagTcgtgTctTaccgggtTggactcaag
acgatagTtaccggataaggcgcagcggTcgggctgaacgggggTctgTgcacacagcccagctTgga
gcgaacgacctacaccgaactgagatacctacagcgtgagcattgagaaagcgcacgctTcccgaagg
gagaaaggcggacaggtatccggTaaagcggcagggTcggaaacagagagcgcacgagggagctTccagg
gggaaacgcctggTatctTtatagTcctgTcgggtTtcgcccactctgactTgagcgtcgatTTTTgtg
atgctcgtcagggggcgggagcctatggaaaaacgccagcaacgcggcctTTTTacgTtctctggcctt
TtgtTggcctTTTTgctcacatgTtctTctcgtTatcccctgattctgTggataaccgTattaccgc
ctTtgagTgagctgataccgctcgcgcgacccgaacgaccgagcgcagcagTcagTgagcaggaagc
ggaagagcgcctgagTgCGgtattTctcctTaccgatctgTgCGgtattTcacaccgcagaccagcgcg
gTaaactggcaaaatcggTtacggTtgagTaaataaatggatgCctTgCgTaaagcgggtgTgggCGgaca
ataaagTctTaaactgaacaaaatagatctaaactatgacaataaagTctTaaactagacagaatagTt
gTaaactgaaatcagTccagTtatgctgTgaaaaagcataactggaactTtTgttatggctaaagcaact
cttcatTTTTctgaagTgcaaatTgcccgtcgtattTaaagagggcgtggccaaggcatggTaaagact
atattcgcggcgtTgtgacaattTaccgaacaactcccgcggccgggaagccgatctcggctTgaacgaa
TtgtTtaggtggcggTactTgggtcgatatcaaaTgcatcactTctTcccgtatgcccactTtTgtata
gagagccactgcgggatcgtcaccgTaatctgctTgcacgtagatcacataagcaccgaagcgcgtTggc
ctcatgctTgagcagattgatgagcgcggTggcaatgCctTgCctcccgtgctcgcgggagactgCgag
atcatagatatagatctcactacgcggctgctcaaacctgggcagaaCgTaaagccgcgagagcGccaac
aaccgctTctTggTcgaaggcagcaagcgcgatgaatgTctTactacggagcaagTtcccgaggtaatc
ggagTcgggctgatgTtgggagTtagTggctacgTctccgaactcacgaccgaaaagatcaagagcagc
ccgcatggattTgactTggTcagggccgagcctacatgTgCgaaTgatgcccatactTgagccacctaa
ctTtTgtTtagggcgactgCctgctgCgTaaacatcgtTgctgctgCgTaaacatcgtTgctgctccata
acatcaaacatcgacccacggcgtaaacgcgctTgctgctTggatgcccgaggcatagactgtacaaaaa
```

aacagtcataacaagccatgaaaaccgccactgcgccgttaccaccgctgcggttcggtcaaggttctgg
accagttgctgagcgcatacgtacttgcattacagtttacgaaccgaacaggccttatgtcaactggg
ttcgtgccttcatccggttccacgggtgctgctcaccggcaaccttgggcagcagcgaagtcgaggcat
ttctgtcctggctggcgaacgagcgcgaaggttccggtctccacgcatcgtcaggcatggcgccctgc
tgttcttctacggcaaggtgctgtgacggatctgccctggcttcaggagatcggtagacctcggccgt
cgcgccgcttgccgggtgctgaccccggtgaagtgggttcgcatcctcgggttttctggaaggcgagc
atcgtttgttgcgccaggactctagctatagttctagtggttggcctacgtacccttagtggtatggc
agggcttgccgccccgacgttggctgagccctgggcttcacccgaacttgggggttgggggtgggga
aaaggaagaaacgcgggcgatttgggtcccaatgggggtctcgggtggggatcgacagagtgccagccctg
ggaccgaaccccgcttattgaacaaacgacccaacacccgtgctgttttattctgtcttttattgccc
tcatagcggggttccctccgggtattgtctccttccgtgtttcagttagcctccccatctcccggtac
cgcattgctatgcatcagctgctagcaccatgggttttaggatctgtacgggccaagatatacgcgttgaca
ttgattattgactagttattaatagtaatacaattacggggtcattagttcatagcccatataggagtt
ccgcttacataacttacggtaaatggcccgctggctgaccgccaacgaccccgccattgacgctc
aataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggactattt
acggtaaaactgccacttggcagtacatcaagtgtatcatatgccaaagtacgccccctattgacgtcaa
tgacggtaaatggcccgctggcattatgccagtacatgaccttatgggactttcctacttggcagta
catctacgtatttagtcatcgtattaccatgggtgatgcggttttggcagtacatcaatgggctggata
gcggtttgactcacggggatttccaagtctccacccattgacgtcaatgggagtttgttttggacca
aaatcaacgggactttccaaaatgtcgtacaactccgccccattgacgcaaatgggcggtaggcgtgt
acgggtgggaggtctatataagcagagctctctggctaactagagaaccactgcttactggcttatcga
ggatccaattaagaaggagatatactatgcaggtcgttcactattatttagtgaaatgagatattatga
tattttctgaattgtgattaaaaaggcaactttatgcccatgcaacagaaaactataaaaaatacagaga
atgaaaagaaacagatagattttttagttcttttaggcccgtagctcgtcaaatccttttatgattttcta
tcaaacaaaagaggaaaatagaccagttgcaatccaaacgagagtcataatagaatgaggtcgaagagta
aatcgcgcggggtttgttactgataaaagcaggcaagacctaaaatgtgtaaagggcaaagtgtatacttt
ggcgtcacccttacatatttttaggtcttttttattgtgctgtaactaacttggcactcttcaaacagga
gggctggaagaagcagaccgctaacacagtacataaaaaaggagacatgaacgatgaacatcaaaaagt
ttgcaaaaacagcaacagatattaacctttactaccgcaactgctggcaggaggcgcaactcaagcgtttg
cgaaagaaacgaaacaaaagccatataaggaacatacggcatttcccatattacacgctcatgatagc
tgcaaatccctgaacagcaaaaaatgaaaaatataaagttcctgagttcgattcgtccacaattaaaa
atatctcttctgcaaaaggcctggacgtttgggacagctggccattacaaaacactgacggcactgtcg
caactatcacggctaccacatcgtctttgcattagccggagatcctaaaaatgaggatgacacatcga
tttacatgttctatcaaaaagtcggcgaaacttctattgacagctggaaaaacgctggccgctcttta
aagacagcgacaaaattcgatgcaaatgattctatcctaaaagacaaaacacaagaatggtcagggttcag
ccacatttacatctgacggaaaaatccggttattctacactgatttctccggtaaacattacggcaaac
aaacactgacaactgcacaagttaacgtatcagcatcagacagctctttgaacatcaacgggtgtagagg
attataaatcaatctttgacgggtgacggaaaaacgtatcaaaaatgtacagcagttcatcgatgaaggca
actacagctcaggcgacaaccatacgtgagagatcctcactacgtagaagataaaggccacaaactact
tagtatttgaagcaaacactggaactgaagatggctaccaaggcgaagaatctttatttaacaaagcat
actatggcaaaagcacatcattcttccgtcaagaaagtcaaaaacttctgcaaaagcgataaaaaacgca
cggctgagtttagcaaacggcgctctcgggtatgattgagctaaaacgatgattacacactgaaaaaagtga
tgaaaccgctgattgcatctaacacagtaacagatgaaattgaacgcgcgaacgtctttaaatagaacg
gcaaatgggtacctgttactgactcccgcggatcaaaaatgacgattgacggcattacgtctaacgata
tttacatgcttgggttatgtttctaattctttaactggccatacaagccgctgaacaaaactggccttg
tgtaaaaaatggatcttgatcctaacgatgtaacctttacttactcacacttcgctgtacctcaagcga
aaggaaacaatgtcgtgattacaagctatatgacaaaacagaggattctacgcagacaaaacaatcaacgt
ttgcgcttagcttctcgtgctgaacatcaaaaggcaagaaaacatctgttgtcaaaagacagcatccttgaac
aaggacaattaacagttaacaaaataaaaacgcaaaagaaaatgccgatatcctattggcattgacgtca
gggtggcacacctgcagagaacctctacttccaatcgcaccatcatcaccatcaccatcaccacatgat
tacaaggatgacgacgataagtgaggcggcccaagcttgatatcaagcttatcgataatcaacctct
ggattacaaaatttgtgaaagattgactgggtattcttaactatgttgctccttttacgctatgtggata
cgctgctttaaagcctttgtatcatgctattgcttcccgatggctttcattttctcctccttgataa
atcctgggtgctgtctctttatgaggagttgtggcccggtgtcaggcaacgtggcgtgggtgtgactgt
ggttgctgacgcaacccccactgggtggggcattgccaccctgtcagctcctttccgggactttcgc
ttccccctccctattgccacggcggaactcatcgccgctgcttgcggctgctggacaggggctcg
gctgttgggcaactgacaattccgtgggtgtgtcggggaaatcatcgtcctttccttggctgctcgccctg
tgttgccacctggattctgcgcgggacgtccttctgctacgtcccttcggccctcaatccagcggacct
tcttccccggggcgccccctcctcacggcgagcgaagcttgtcgagaagtactagaggatctctaga
gctcgcagctcgcacaagcttgtcgagaagtactagaggatcataatcagccataccacattttagag
gttttacttgcctttaaanaaacctcccacacctccccctgaacctgaaacataaaaatgaatgcaattggt

gttgttaacttgtttattgcagcttataatggttacaataaagcaatagcatcaciaatttcaciaat
aaagcatttttttactgcattctagttgtggtttgtccaaactcatcaatgtatcttatcatgtctgg
atctgatcactgcttgagcctaggagatccgaaccagataagtgaaatctagttccaaactatttgtc
attttaattttcgtattagcttacgacgctacaccagttcccatctatttgtcactcttcctaaa
taatccttaaaaactccatttccaccctcccagttcccaactatttgtccgcccacagcggggcatt
tttcttctgttatgtttttaatcaaacatcctgccaaactccatgtgacaaaccgtcatcttcggctac
ttt