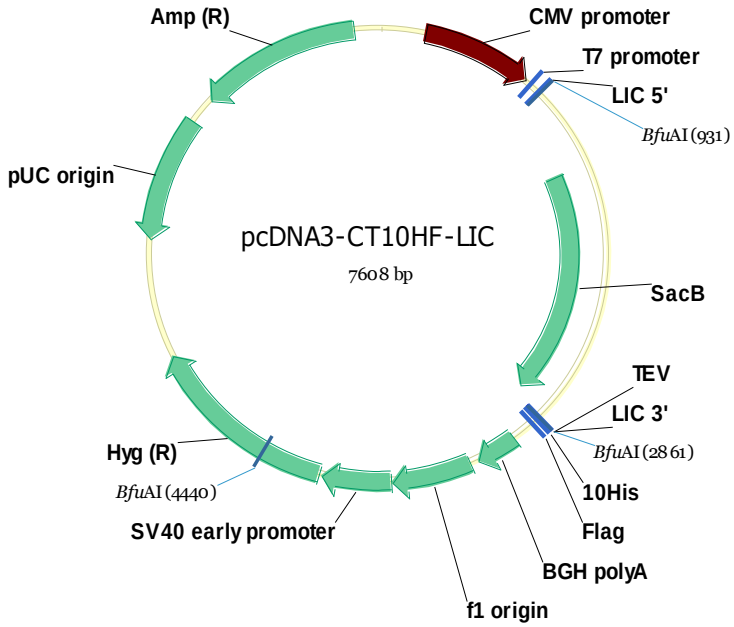
Vector information sheet

Dated: 8th May 2013

Vector Name	**pcDNA3-CT10HF-LIC**
Source	Grazyna Kochan
Sequence accession/link	(SGC)

Description	Mammalian expression 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	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	2471.47 Da
Termination codon	Downstream of flag tag
Protease cleavage	TEV
Additional features	
Preferred host	Mammalian cell lines (HEK HeLa, BHK BSC 1etc.
5' sequencing primer	pcDNA3-fwd (50), and T7fwd. TCCAAAATGTCGTAACAACTCC
3' sequencing primer	pcDNA3-rev (48): TTTTATTAGGAAAGGACAGTGG



Polylinker region:

5' end:

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                                                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.

pcDNA3-CT10HF-LIC sequence:

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gacggatcgggagatctcccgatcccctatggtcgactctcagtacaatctgctctgatgccgcatagt
taagccagtatctgctccctgcttgtgtgttggaggtcgctgagtagtgcgcgagcaaaatttaagcta
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