Vector information sheet

Dated: 8th May 2013

Vector Name	**pNIC-CTHF**
Source	Opher Gileadi
Sequence accession/link	EF199844

Description	pET expression vector with C-terminal His$_6$ 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	Kanamycin, 50 μg/ml
Promoter	T7 - lacO
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*SHHHHHHDYKDDDDK (* - TEV cleavage site)
C-terminal fusion – MW	2771 (1905 Da removed by TEV cleavage)
Termination codons	supplied in vector
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC
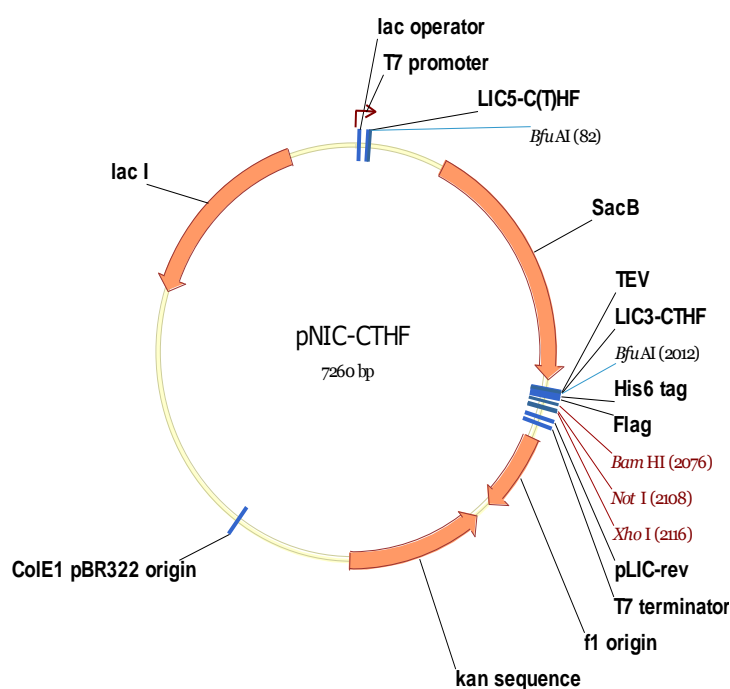
Polylinker region:

5' end:

```
                                                          BfuAI
                                                          ~~~~~~
       CTAGAAATAA TTTTGTTTAA CCTTAAGAAG GAGA|TATA CT ATGCAGGTCG TTCACTATTA
       GATCTTTATT AAAACAAATT GGAATTCTTC CTCT ATAT|GA TACGTCCAGC AAGTGATAAT
```

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

```
                                          BfuAI
                                          ~~~~~~~
                                                  A   E   N   L   Y   F
       CCGATATCCT ATTGGCATTG ACGTCAGGTG GCACACCTGC AGAG|AA CC TCTACTTC
       GGCTATAGGA TAACCGTAAC TGCAGTCCAC CGTGTGGACG TCTC TT GG|AGATGAAG
                                                  -----------------
                                                     (TEV---
         |
       Q  S  H  H   H  H  H   H  D  Y   K  D  D  D   D  K  *   BamHI
  1981 CAATCGCACC ATCATCACCA CCATGATTAC AAGGATGACG ACGATAAGTG AGGATCCGAA
       GTTAGCGTGG TAGTAGTGGT GGTACTAATG TTCCTACTGC TGCTATTCAC TCCTAGGCTT
       ------ ~~~~~~~~~~~~~~~~~  ~~~~~~~~~~~~~~~~~~~~~~~~
        -TEV)          His6 tag              Flag
```

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.

pNIC-CTHF sequence:

```
taatacgactcactatagggggaattgtgagcggataacaattcccctctagaaataattttgtttaacc
ttaagaaggagatatactatgcaggtcgttcactattatttagtgaaatgagatattatgatattttct
gaattgtgattaaaaaggcaactttatgcccatgcaacagaaactataaaaaatacagagaatgaaaag
aaacagatagattttttagttctttaggcccgtagtctgcaaatccttttatgattttctatcaaacaa
aagaggaaaatagaccagttgcaatccaaacgagagtctaatagaatgaggtcgaaaagtaaatcgcgc
gggtttgttactgataaagcaggcaagacctaaaatgtgtaaagggcaaagtgtatactttggcgtcac
cccttacatattttaggtctttttttattgtgcgtaactaacttgccatcttcaaacaggagggctgga
agaagcagaccgctaacacagtacataaaaaaggagacatgaacgatgaacatcaaaaagtttgcaaaa
caagcaacagtattaacctttactaccgcactgctggcaggaggcgcaactcaagcgtttgcgaaagaa
acgaaccaaaagccatataaggaaacatacggcatttcccatattacacgccatgatatgctgcaaatc
cctgaacagcaaaaaaatgaaaaatataaagttcctgagttcgattcgtccacaattaaaaatatctct
tctgcaaaaggcctggacgtttgggacagctggccattacaaaacactgacggcactgtcgcaaactat
cacggctaccacatcgtctttgcattagccggagatcctaaaaatgcggatgacacatcgatttacatg
ttctatcaaaaagtcggcgaaacttctattgacagctggaaaaacgctggccgcgtctttaaagacagc
gacaaattcgatgcaaatgattctatcctaaaagaccaaacacaagaatggtcaggttcagccacattt
acatctgacggaaaaatccgtttattctacactgatttctccggtaaacattacggcaaacaaacactg
acaactgcacaagttaacgtatcagcatcagacagctctttgaacatcaacggtgtagaggattataaa
tcaatctttgacggtgacggaaaaacgtatcaaaatgtacagcagttcatcgatgaaggcaactacagc
```

```
tcaggcgacaaccatacgctgagagatcctcactacgtagaagataaaggccacaaatacttagtattt
gaagcaaacactggaactgaagatggctaccaaggcgaagaatctttatttaacaaagcatactatggc
aaaagcacatcattcttccgtcaagaaagtcaaaaacttctgcaaagcgataaaaaacgcacggctgag
ttagcaaacggcgctctcggtatgattgagctaaacgatgattacacactgaaaaaagtgatgaaaccg
ctgattgcatctaacacagtaacagatgaaattgaacgcgcgaacgtctttaaaatgaacggcaaatgg
tacctgttcactgactcccgcggatcaaaaatgacgattgacggcattacgtctaacgatatttacatg
cttggttatgtttctaattctttaactggcccatacaagccgctgaacaaaactggccttgtgttaaaa
atggatcttgatcctaacgatgtaacctttacttactcacacttcgctgtacctcaagcgaaaggaaac
aatgtcgtgattacaagctatatgacaaacagaggattctacgcagacaaacaatcaacgtttgcgcct
agcttcctgctgaacatcaaaggcaagaaaacatctgttgtcaaagacagcatccttgaacaaggacaa
ttaacagttaacaaataaaaacgcaaaagaaaatgccgatatcctattggcattgacgtcaggtggcac
acctgcagagaacctctacttccaatcgcaccatcatcaccaccatgattacaaggatgacgacgataa
gtgaggatccgaattcgagctccgtcgacaagcttgcggccgcactcgagcaccaccaccaccaccact
gagatccggctgctaacaaagcccgaaaggaagctgagttggctgctgccaccgctgagcaataactag
cataacccttggggcctctaaacgggtcttgaggggttttttgctgaaaggaggaactatatccggat
tggcgaatgggacgcgccctgtagcggcgcattaagcgcggcgggtgtggtggttacgcgcagcgtgac
cgctacacttgccagcgccctagcgcccgctcctttcgctttcttcccttcctttctcgccacgttcgc
cggctttccccgtcaagctctaaatcgggggctcccttagggttccgatttagtgctttacggcacct
cgaccccaaaaaacttgattagggtgatggttcacgtagtgggccatcgccctgatagacggttttttcg
ccctttgacgttggagtccacgttctttaatagtggactcttgttccaaactggaacaacactcaaccc
tatctcggtctattcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagct
gatttaacaaaaatttaacgcgaattttaacaaaatattaacgtttacaatttcaggtggcactttcg
gggaaatgtgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgaa
ttaattcttagaaaaactcatcgagcatcaaatgaaactgcaatttattcatatcaggattatcaatac
catattttgaaaaagccgtttctgtaatgaaggagaaaactcaccgaggcagttccataggatggcaa
gatcctggtatcggtctgcgattccgactcgtccaacatcaatacaacctattaatttcccctcgtcaa
aaataaggttatcaagtgagaaatcaccatgagtgacgactgaatccggtgagaatggcaaaagtttat
gcatttctttccagacttgttcaacaggccagccattacgctcgtcatcaaaatcactcgcatcaacca
aaccgttattcattcgtgattgcgcctgagcgagacgaaatacgcgatcgctgttaaaaggacaattac
aaacaggaatcgaatgcaaccggcgcaggaacactgccagcgcatcaacaatattttcacctgaatcag
gatattcttctaatacctggaatgctgttttcccggggatcgcagtggtgagtaaccatgcatcatcag
gagtacggataaaatgcttgatggtcggaagaggcataaattccgtcagccagtttagtctgaccatct
catctgtaacatcattggcaacgctacctttgccatgtttcagaaacaactctggcgcatcgggcttcc
catacaatcgatagattgtcgcacctgattgcccgacattatcgcgagcccatttatacccatataaat
cagcatccatgttggaatttaatcgcggcctagagcaagacgtttcccgttgaatatggctcataacac
cccttgtattactgtttatgtaagcagacagttttattgttcatgaccaaaatcccttaacgtgagttt
tcgttccactgagcgtcagacccccgtagaaaagatcaaaggatcttcttgagatcctttttttctgcgc
gtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagagcta
ccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtag
ccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgtta
ccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggat
aaggcgcagcggtcgggctgaacggggggttcgtgcacacagcccagcttggagcgaacgacctacacc
gaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacagg
tatccggtaagcggcagggtcggaacaggagagcgcacgagggagcttccaggggaaacgcctggtat
ctttatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcagggggg
cggagcctatggaaaaacgccagcaacgcggcctttttacggttcctggccttttgctggccttttgct
cacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgat
accgctcgccgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcctgatg
cggtattttctccttacgcatctgtgcggtatttcacaccgcatatatggtgcactctcagtacaatct
gctctgatgccgcatagttaagccagtatacactccgctatcgctacgtgactgggtcatggctgcgcc
ccgacacccgccaacacccgctgacgcgccctgacgggcttgtctgctcccggcatccgcttacagaca
agctgtgaccgtctccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgaggca
gctgcggtaaagctcatcagcgtggtcgtgaagcgattcacagatgtctgcctgttcatccgcgtccag
ctcgttgagtttctccagaagcgttaatgtctggcttctgataaagcgggccatgttaagggcggtttt
ttcctgtttggtcactgatgcctccgtgtaagggggatttctgttcatgggggtaatgataccgatgaa
acgagagaggatgctcacgatacgggttactgatgatgaacatgcccggttactggaacgttgtgaggg
taaacaactggcggtatggatgcggcgggaccagagaaaaatcactcagggtcaatgccagcgcttcgt
taatacagatgtaggtgttccacagggtagccagcagcatcctgcgatgcagatccggaacataatggt
gcagggcgctgacttccgcgtttccagactttacgaaacacggaaaccgaagaccattcatgttgttgc
tcaggtcgcagacgttttgcagcagcagtcgcttcacgttcgctcgcgtatcggtgattcattctgcta
accagtaaggcaacccgccagcctagccgggtcctcaacgacaggagcacgatcatgcgcacccgtgg
```

```
ggccgccatgccggcgataatggcctgcttctcgccgaaacgtttggtggcgggaccagtgacgaaggc
ttgagcgagggcgtgcaagattccgaataccgcaagcgacaggccgatcatcgtcgcgctccagcgaaa
gcggtcctcgccgaaaatgacccagagcgctgccggcacctgtcctacgagttgcatgataaagaagac
agtcataagtgcggcgacgatagtcatgccccgcgcccaccggaaggagctgactgggttgaaggctct
caagggcatcggtcgagatcccggtgcctaatgagtgagctaacttacattaattgcgttgcgctcact
gcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaatcggccaacgcgcggggagagg
cggtttgcgtattgggcgccaggtggttttttcttttcaccagtgagacgggcaacagctgattgccct
tcaccgcctggccctgagagagttgcagcaagcggtccacgctggtttgccccagcaggcgaaaatcct
gtttgatggtggttaacggcgggatataacatgagctgtcttcggtatcgtcgtatcccactaccgaga
tatccgcaccaacgcgcagcccggactcggtaatggcgcgcattgcgcccagcgccatctgatcgttgg
caaccagcatcgcagtgggaacgatgccctcattcagcatttgcatggtttgttgaaaaccggacatgg
cactccagtcgccttcccgttccgctatcggctgaatttgattgcgagtgagatatttatgccagccag
ccagacgcagacgcgccgagacagaacttaatgggcccgctaacagcgcgatttgctggtgacccaatg
cgaccagatgctccacgcccagtcgcgtaccgtcttcatgggagaaaataatactgttgatgggtgtct
ggtcagagacatcaagaaataacgccggaacattagtgcaggcagcttccacagcaatggcatcctggt
catccagcggatagttaatgatcagcccactgacgcgttgcgcgagaagattgtgcaccgccgctttac
aggcttcgacgccgcttcgttctaccatcgacaccaccacgctggcacccagttgatcggcgcgagatt
taatcgccgcgacaatttgcgacggcgcgtgcagggccagactggaggtggcaacgccaatcagcaacg
actgtttgcccgccagttgttgtgccacgcggttgggaatgtaattcagctccgccatcgccgcttcca
cttttttcccgcgttttcgcagaaacgtggctggcctggttcaccacgcgggaaacggtctgataagaga
caccggcatactctgcgacatcgtataacgttactggtttcacattcaccaccctgaattgactctctt
ccgggcgctatcatgccataccgcgaaaggttttgcgccattcgatggtgtccgggatctcgacgctct
cccttatgcgactcctgcattaggaagcagcccagtagtaggttgaggccgttgagcaccgccgccgca
aggaatggtgcatgcaaggagatggcgcccaacagtcccccggccacggggcctgccaccatacccacg
ccgaaacaagcgctcatgagcccgaagtggcgagcccgatcttccccatcggtgatgtcggcgatatag
gcgccagcaaccgcacctgtggcgccggtgatgccggccacgatgcgtccggcgtagaggatcgagatc
tcgatcccgcgaaat
```