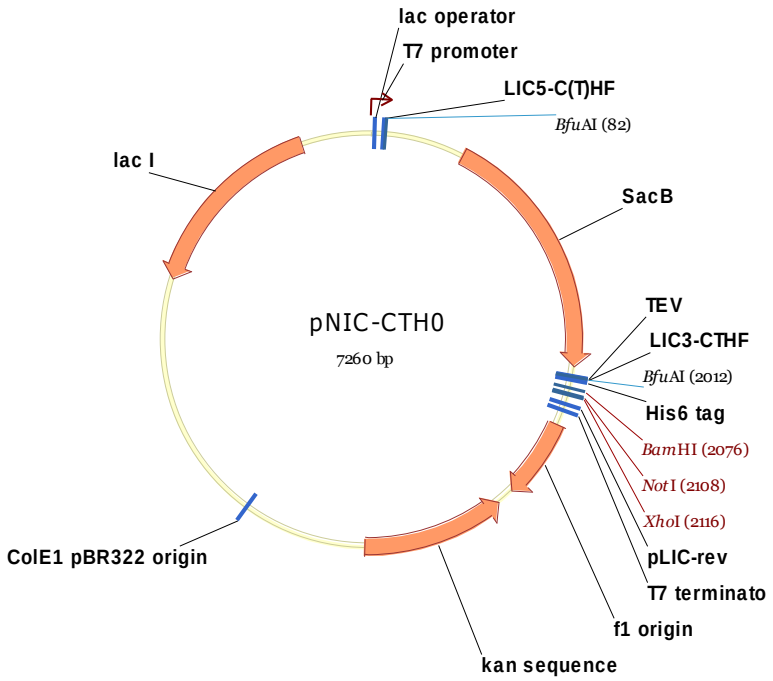
Vector information sheet

Dated: 8th May 2013

Vector Name	**pNIC-CTHO**
Source	Pavel Savitsky
Sequence accession/link	

Description	pET expression vector with C-terminal His_6 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*SHHHHHH (* - TEV cleavage site)
C-terminal fusion – MW	1793 (1077 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
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---------- SacB fragment ----------

```
                                        LIC3-CTHF
                                      ~~~~~~~~~~~~~~~~~~
                                        TEV              His6 tag
                                    ~~~~~~~~~~~~~~~~~~~~   ~~~~~~~~~~~~
                            BfuAI
                            ~~~~~~
                          T   P  A  E   N  L  Y   F  Q  S  H   H  H  H ·
1981  GGCATTGACG TCAGGTGGCA CACCTGCAGA GAACCTCTAC TTCCAATCGC ACCATCATCA
      CCGTAACTGC AGTCCACCGT GTGGACGTCT CTTGGAGATG AAGGTTAGCG TGGTAGTAGT
      His6 tag
      ~~~~~~~
                                        BamHI
                                        ~~~~~~~
      · H  H
2041  CCACCATTGA TACAAGGATG ACGACGATAA GTGAGGATCC GAATTCGAGC TCCGTCGACA
      GGTGGTAACT ATGTTCCTAC TGCTGCTATT CACTCCTAGG CTTAAGCTCG AGGCAGCTGT
            NotI     XhoI
          ~~~~~~~~~ ~~~~~~~
2101  AGCTTGCGGC CGCACTCGAG CACCACCACC ACCACCACTG AGATCCGGCT GCTAACAAAG
      TCGAACGCCG GCGTGAGCTC GTGGTGGTGG TGGTGGTGAC TCTAGGCCGA CGATTGTTTC
2161  CCCGAAAGGA AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA ATAACTAGCA TAACCCCTTG
      GGGCTTTCCT TCGACTCAAC CGACGACGGT GGCGACTCGT TATTGATCGT ATTGGGGAAC
            ~~~~~~~~~~~~~~~~~~~~~
                  pLIC-rev
2221  GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGCTGAAAGG AGGAACTATA TCCGGATTGG
      CCCGGAGATT TGCCCAGAAC TCCCCAAAAA ACGACTTTCC TCCTTGATAT AGGCCTAACC
```

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-CTHO sequence:

```
taatacgactcactataggggaattgtgagcggataacaattcccctctagaaataattttgtttaacc
ttaagaaggagatatactatgcaggtcgttcactattatttagtgaaatgagatattatgatattttct
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