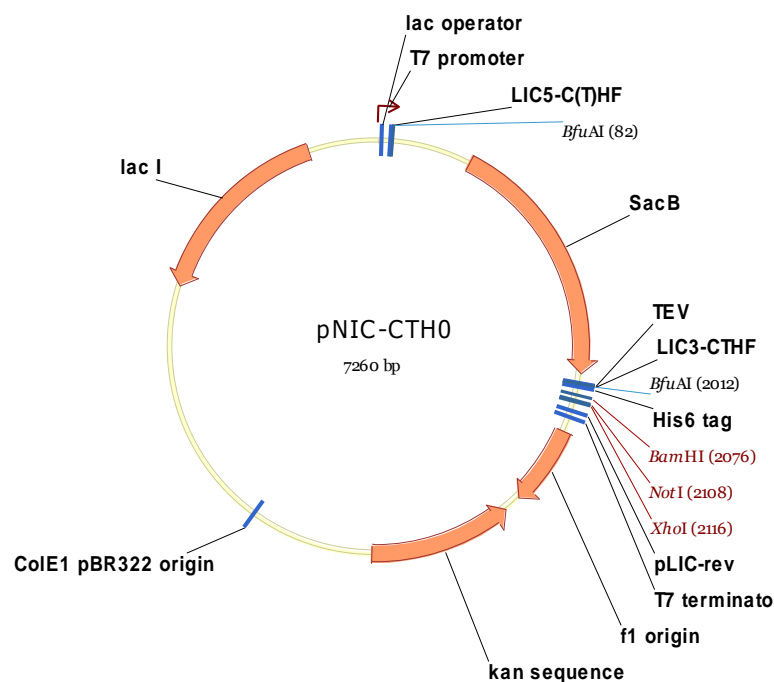


# Vector information sheet

Dated: 8<sup>th</sup> May 2013

Vector Name	<b>pNIC-CTH0</b>
Source	Pavel Savitsky
Sequence accession/link	
Description	pET expression vector with C-terminal His <sub>6</sub> 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: AGCAGCCAACCTCAGCTTCC



Cloning region in the vector:

5' end:

```

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

----- 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 GTGGACGCTC CTTGGAGATG AAGGTTAGCG TGGTAGTAGT
    His6 tag
    ~~~~~~
                                     BamHI
                                     ~~~~~~
    . H H
2041 CCACCATTGA TACAAGGATG ACGACGATAA GTGAGGATCC GAATTCGAGC TCCGTCGACA
    GGTGGTAACT ATGTTCTTAC 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
    GGGCTTTTCT TCGACTCAAC CGACGACGGT GCGGACTCGT TATTGATCGT ATTGGGGAAC
    ~~~~~~
    pLIC-rev
2221 GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGCTGAAAGG AGGAACTATA TCCGGATTGG
    CCCGGAGATT TGCCCAGAAC TCCCCAAAAA ACGACTTTC TCCTTGATAT AGGCCTAACC

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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.

pNIC-CTHO sequence:

```

taatacgactcactataggggaattgtgagcggataacaattcccctctagaaataatTTTgtttaacc
ttaagaaggagatatactatgcagggtcgttcactattatTTtagtgaaatgagatattatgatatTTTct
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