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 \(\mu\)g/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

**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-forward: TGTGAGCGGATAACAATTCC

**3’ sequencing primer**: pLIC-reverse: AGCAGCCAACTCAGCTTCC

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**Diagram**:

- **pNIC-CTHO**: 7260 bp
- **lac I**
- **lac operator**
- **T7 promoter**
- **LIC5-(T)HF**
- **BfuAI (82)**
- **SacB**
- **TEV**
- **LIC3-CTHF**
- **BfuAI (2012)**
- **His6 tag**
- **Kan HI (2007)**
- **NotI (2008)**
- **XhoI (2016)**
- **pLIC-reverse**
- **T7 terminato**
- **f1 origin**
- **ColE1 pBR322 origin**
- **kan sequence**
Polylinker region:

5’ end:

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SacB fragment ---

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Primers for LIC cloning:

Add the following 5’ extensions to the PCR primers:

Upstream: TTAAGAAGGAGATATACTATG (ATG-initiation codon)

Downstream: GATTGGAAGTACAGGTCTCTCTG

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

pNIC-CTHO sequence: