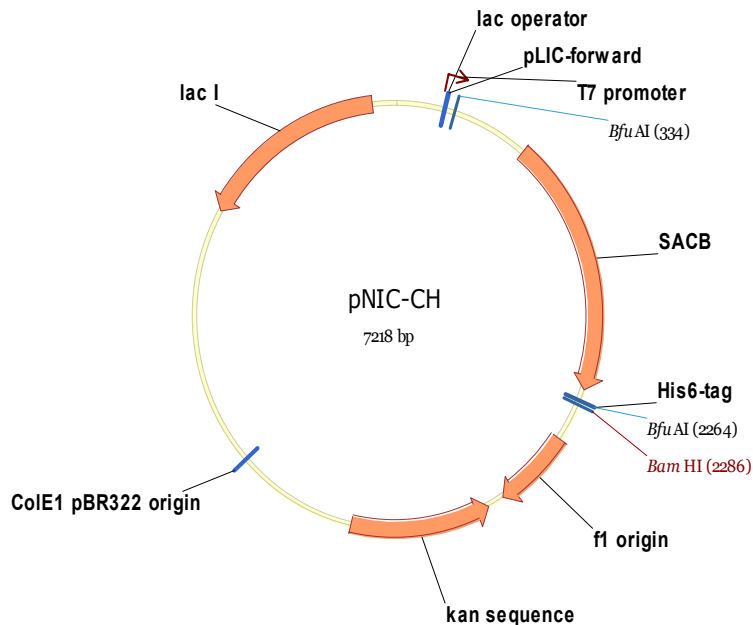


Vector information sheet.

Vector Name	<b>pNIC-CH</b>
Source	Opher Gileadi
Sequence accession/link	EF199843
Description	pET expression vector with C-terminal His <sub>6</sub> tag. 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.	AHHHHHH
C-terminal fusion – MW	894 Da
Termination codons	supplied in vector
Protease cleavage	none
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



## Cloning region in the vector:

5' end:

```

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

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

                                     His6-tag
                                     ~~~~~~

                                     BfuAI
                                     ~~~~~
2231 TTGGCATTGA CGTCAGGTGG CACACCTGCA GCG|CACC ATC ATCACCACCA TTGAGGATCC
AACCGTAACT GCAGTCCACC GTGTGGACGT CGC GTGG|TAG TAGTGGTGGT AACTCCTAGG
His6-tag
~
      EcoRI          SalI          NotI
      ~~~~~          ~~~~~          ~~~~~
      BamHI          SacI          HindIII          XhoI
      ~~~~~          ~~~~~          ~~~~~          ~~~~~
. *
2281 GAATTCGAGC TCCGTCGACA AGCTTGCGGC CGCACTCGAG CACCACCACC
CTTAAGCTCG AGGCAGCTGT TCGAACGCCG GCGTGAGCTC GTGGTGGTGG
```

## Primers for LIC cloning:

Add the following 5' extensions to the PCR primers:

Upstream: **TTAAGAAGGAGATATACTATG** (ATG-initiation codon)

Downstream: **AATGGTGGTGATGATGGTGCGC**

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