

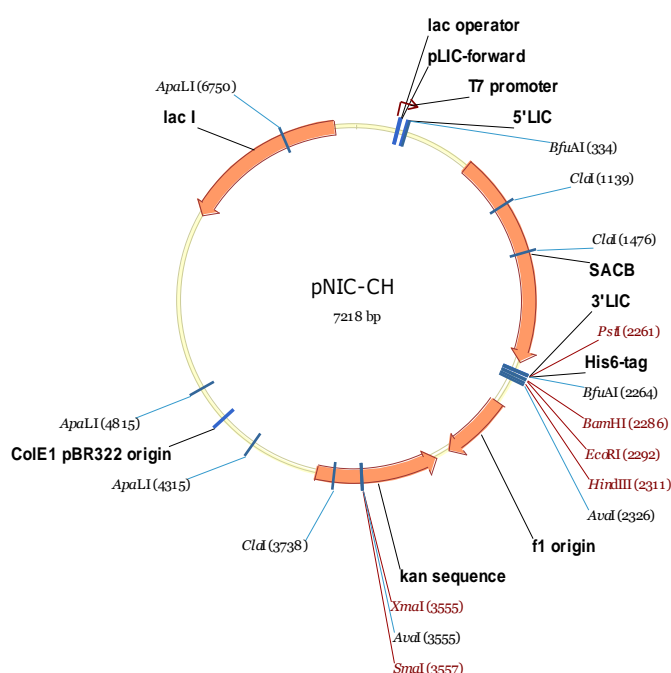
Vector information sheet

Dated: 8th May 2013

Vector Name	pNIC-CH
Source	Opher Gileadi
Sequence accession/link	

Description	pET expression vector with C-terminal His ₆ tag. Includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection on 5% sucrose
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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.	AAHHHHHH
C-terminal fusion – MW	
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: AGCAGCCAACCTCAGCTTCC



Polylinker region:

5' end:

```
                                     BfuAI
                                     ~~~~~~
CTAGAAATAA TTTGTTTAA CCTTAAGAAG GAGA|TATA CT ATGCAGGTCG TTCACTATA
GATCTTTATT AAAACAAATT GGAATTCCTC CTCT ATAT|GA TACGTCCAGC AAGTGATAAT
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----- SacB fragment -----

```
                                     BfuAI
                                     ~~~~~~
                                     PstI
                                     ~~~~~~
                                     A A H H H H H H .
2221 CGATATCCTA TTGGCATTGA CGTCAGGTGG CACACCTGCA GCGCACCATC ATCACCACCA
      GCTATAGGAT AACCGTAACT GCAGTCCACC GTGTGGACGT CGCGTGGTAG TAGTGGTGGT
      BamHI
      ~~~~~~
      . *
2281 TTGAGGATCC
      AACTCCTAGG
```

Primers for LIC cloning:

Add the following 5' extensions to the PCR primers:

Upstream: TTAAGAAGGAGATATACTATG (ATG-initiation codon)

Downstream: ATGGTGGTGATGATGGTGcgc

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

pNIC-CH sequence:

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