## Vector information sheet

Dated: 8th May 2013

<table>
<thead>
<tr>
<th>Vector Name</th>
<th>pNIC-CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Opher Gileadi</td>
</tr>
<tr>
<td>Sequence accession/link</td>
<td></td>
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### 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.
- AAHHHHHHH

### C-terminal fusion – MW
- 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: AGCAGCAAATCTAGCTTCC

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![Diagram of pNIC-CH vector](image)
Polylinker region:

5' end:

```
CTAGAAATTA TTTTGTTTAA CCTTAAGAAG GAGATATA CT
```

--- SacB fragment ---

```
GCTATAGGAT AAGCGTAGCC GTGGCAGGCG CACCACTCAG AGCGCGGGCA
```

Primers for LIC cloning:

Add the following 5’ extensions to the PCR primers:

**Upstream:** TTAAGAAGGAGATACTATGCAG

**Downstream:** ATGGTGAGTGGTGATGCgc

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

**pNIC-CH sequence:**

```
aggtgacgttcgcaagctttgagtctgctgcaaatccctgaacagcaaaaaatgaaaaatataaagttcctgagttcgattcgtccac
```

--- end ---