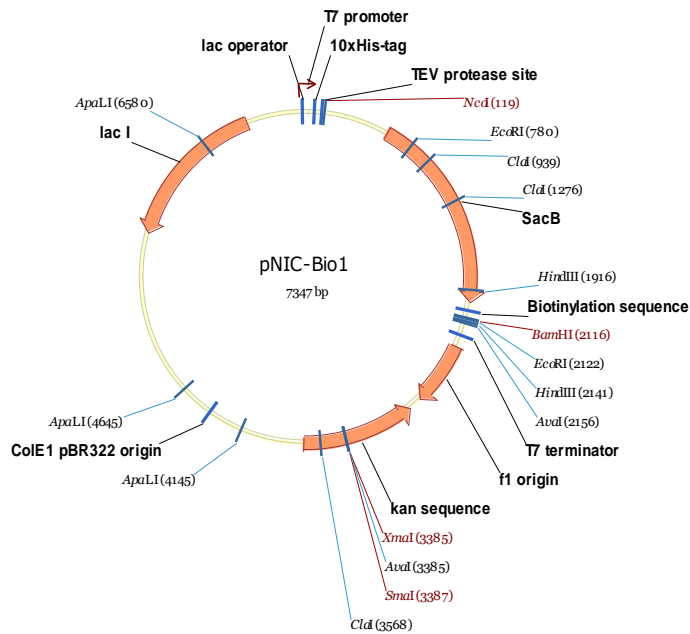


# Vector information sheet

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

Vector Name	<b>pNIC-Bio1</b>
Source	Susanne Gröslund & Opher Gileadi
Sequence accession/link	
Description	pET expression vector with His <sub>10</sub> tag in 25-aa N-terminal fusion peptide, with TEV protease cleavage site, and a biotinylation sequence in C-terminal fusion. 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 BsaI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MHHHHHHHHHHSSGV <sup>*</sup> DLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	3119.3 Da including Met (3032.2 Da removed by TEV cleavage)
C-terminal fusion – seq.	SSKGGYGLNDIFEAQKIEWHE
C-terminal fusion - MW	2396.57 Da
Termination codons	Already included after biotinylation signal at C-terminal
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase. For effective biotinylation, the host should overexpress BirA (e.g. from plasmid pCDF-BIRA, Genbank: JF914075)
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACCTCAGCTTCC



**Polylinker region:**

```

10xHis-tag
                                     M H H H H H H H
1  CTAGAAATAA TTTGTTTAA CTTAAGAAG GAGATATACA TATGCACCAT CATCATCATC
   GATCTTTATT AAAACAAATT GAAATCTTC CTCTATATGT ATACGGTGGTA GTAGTAGTAG
                                     LIC5'
                                     ~~~~~
10xHis-tag                               TEV protease site
~~~~~                                     ~~~~~
                                     NcoI
                                     ~~~
· H H H H S S G V D L G T E N L Y F Q S M ·
61 ATCACCATCA TCACTCTTCT GGTGTAGATC TGGGTACCGA GAACCTGTAC TTCCAATCCA
   TAGTGGTAGT AGTGAGAAGA CCACATCTAG ACCCATGGCT CTTGGACATG AAGGTTAGGT
   LIC5'
   ~~~
   TEV protease site
   ~~~
       BsaI
       ~~~~~
NcoI
~~~~~
· E T D V H I
121 TGGAGACCGA CGTCCACATA
    ACCTCTGGCT GCAGGTGTAT
-----SacB-----

                                     LIC3'           Biotinylation sequence
                                     ~~~~~
       BsaI
       ~~~~~
· V S S K G G Y G L N D I F E A Q K I E W ·
2041 CGGTCTCCAG TAAAGGTGGA TACGGCCTGA ATGATATCTT TGAAGCGCAG AAGATGGAAT
     GCCAGAGGTC ATTTCCACCT ATGCCGGACT TACTATAGAA ACTTCGCGTC TTCTAACTTA
     Biotinylation sequence
     ~~~~~
· H E *
2101 GGCATGAATG A
     CCGTACTTAC T

```

Primers for LIC cloning:

Upstream: add TACTTCCAATCCATG to the 5' end (ATG in-frame with the desired coding sequence).

Downstream: add TATCCACCTTTACTGCT to 5' end of downstream primer.

pNIC-Bio1 sequence:

```
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