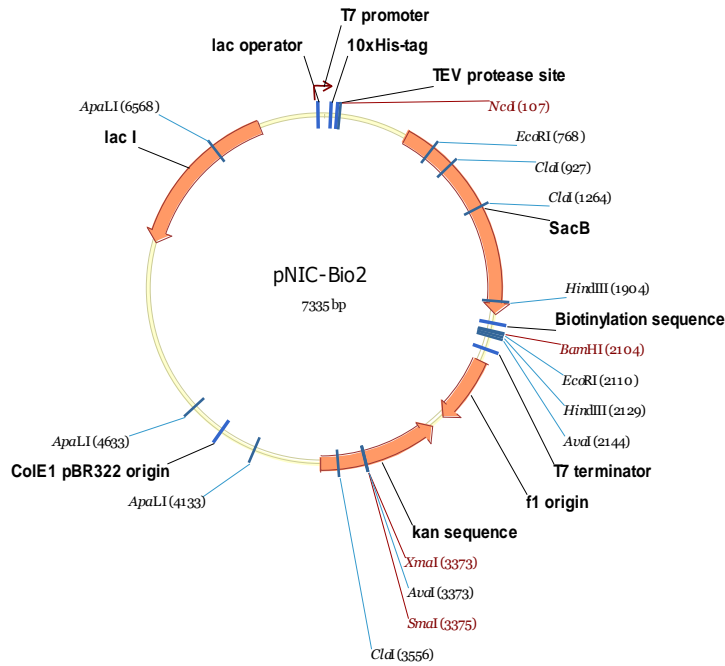


# Vector information sheet

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

Vector Name	<b>pNIC-Bio2</b>
Source	Susanne Gräslund & Opher Gileadi
Sequence accession/link	JF912191
Description	pET expression vector with His <sub>10</sub> tag in 22-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.	MHHHHHHHHHHDLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	2684.1 Da including Met (2465.8 Da removed by TEV cleavage)
C-terminal fusion – seq.	GSKGGYGLNDIFEAQKIEWHE
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 TTTGTTTTAA CTTAAGAAG GAGATATACA TATGCACCAT CATCATCATC
   GATCTTTATT AAAACAAATT GAAATCTTC CTCTATATGT ATACGTGGTA GTAGTAGTAG
                                     LIC5'
                                     ~~~~~~
10xHis-tag                          TEV protease site
~~~~~                               ~~~~~~
                                     BsaI
                                     ~~~~~~
61  · H H H H D L G T E N L Y F Q S M E T D V ·
   ATCACCATCA TCACGATCTG GGTACCGAGA ACCTGFACTT CCAATCCATG GAGACCGACG
   TAGTGGTAGT AGTGCTAGAC CCATGGCTCT TGGACATGAA GGTAGGTAC CTCTGGCTGC
-----SacB-----

                                     LIC3'
                                     ~~~~~~
                                     BsaI
                                     ~~~~~~
1981 TAACAAATAA AAACGCAAAA GAAAATGCCG ATATCCTATT GGCATTGACG GTCTCCAGTA
     ATTGTTTATT TTTGCCTTTT CTTTACGGC TATAGGATAA CCGTAACTGC CAGAGGTCAT
     LIC3'                          Biotinylation sequence
     ~~~~~~
     · G G Y G L N D I F E A Q K I E W H E *
2041 AAGGTGGATA CGGCCTGAAT GATATCTTTG AAGCGCAGAA GATTGAATGG CATGAATGA
     TTCCACCTAT GCCGACTTA CTATAGAAAC TTCGCGTCTT CTAACCTACC GTACTTACT

```

**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-Bio2 sequence:

ctagaataatTTTTGTTAACTTTAAGAAGGAGATATACATATGCACCATCATCATCATCACCATC  
ATCAGGATCTGGGTACCGAGAACCCTGTACTTCCAATCCATGGAGACCGACGTCCACATACCTGCCGT  
TCACTATTATTTAGTGAAATGAGATATTATGATATTTCTGAAATGTGATTA AAAAGGCAACTTTATGC  
CCATGCAACAGAACTATAAAAAATACAGAGAATGAAAAGAAACAGATAGATTTTTTAGTTCTTTAGGC  
CCGTAGTCTGCAAACTCTTTTATGATTTTCTATCAAAACAAAAGAGGAAAAATAGACCAGTTGCAATCCAA  
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CTAAAATGTGTAAGGGCAAAGTGTATACTTTGGCGTCACCCCTTACATATTTAGGTCTTTTTTATT  
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CGGCATTTCCCATATTACACGCCATGATATGCTGCAAACTCCCTGAACAGCAAAAAATGAAAAATCA  
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