

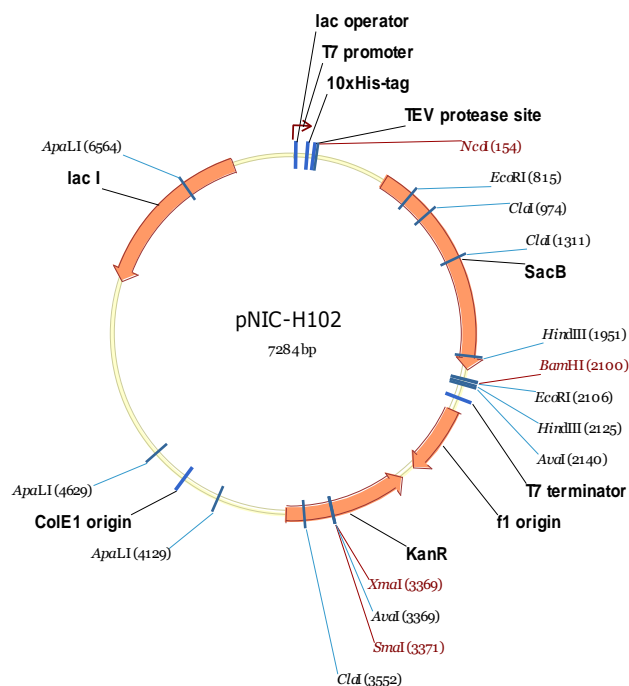
Vector information sheet

Dated: 8th May 2013

Vector Name	pNIC-H102
Source	Opher Gileadi
Sequence accession/link	Genebank JF912192

Description	pET expression vector with His ₁₀ tag in 26-aa N-terminal fusion peptide, with TEV protease cleavage site. 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 BsaI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MHHHHHHHHHHSSGV [*] DLGTENLYFQ [*] SM (* - TEV cleavage site)
N-terminal fusion – MW	2788 Da including Met (2701.89 Da removed by TEV cleavage)
Termination codons	supplied in PCR primer
Protease cleavage	TEV
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



Polylinker region:

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          T7 promoter                lac operator
          ~~~~~                      ~~~~~
1  TAATACGACT CACTATAGGG GAATTGTGAG CGGATAACAA TTCCCCTCTA GAAATAATTT
   ATTATGCTGA GTGATATCCC CTTAACACTC GCCTATTGTT AAGGGGAGAT CTTTATTA
                                     10xHis-tag
                                     ~~~~~
61 TGTTTAACTT TAAGAAGGAG ATATACATAT GCACCATCAT CATCATCATC ACCATCATCA
   ACAAATTGAA ATTCTTCCTC TATATGTATA CGTGGTAGTA GTAGTAGTAG TGGTAGTAGT
   10xHis-tag          TEV protease site
   ~~~~~
                                     NcoI
                                     ~~~~~
   · D L G   T E N L   Y F Q   S M E   T D V H   I Y
121 CGATCTGGGT ACCGAGAACC TGTACTTCCA ATCCATGGAG ACCGACGTCC ACATATACCT
   GCTAGACCCA TGGCTCTTGG ACATGAAGGT TAGGTACCTC TGGCTGCAGG TGTATATGGA
181 GCCGTTCACT ATTATTTAGT GAAATGAGAT ATTATGATAT TTTCTGAATT GTGATTA
   CCGCAAGTGA TAATAAATCA CTTTACTCTA TAATACTATA AAAGACTTAA CACTAATTTT
241 AGGCAACTTT ATGCCCATGC AACAGAAACT ATAAAAAATA CAGAGAATGA AAAGAAACAG
   TCCGTTGAAA TACGGGTACG TTGTCTTGA TATTTTTTAT GTCTCTFACT TTTCTTTGTC
301 ATAGATTTTT TAGTCTTTA GGCCCGTAGT CTGCAAATCC TTTTATGATT TTCTATCAAA
   TATCTAAAAA ATCAAGAAAT CCGGGCATCA GACGTTTAGG AAAATACTAA AAGATAGTTT
361 CAAAAGAGGA AAATAGACCA GTTGCAATCC AAACGAGAGT CTAATAGAAT GAGGTCGAAA
   GTTTTCTCCT TTTATCTGGT CAACGTTAGG TTTGCTCTCA GATTATCTTA CTCCAGCTTT
421 AGTAAATCGC GCGGGTTTGT TACTGATAAA GCAGGCAAGA CCTAAAATGT GTAAAGGGCA
   TCATTTAGCG CGCCCAAACA ATGACTATTT CGTCCGTTCT GGATTTTACA CATTTCCTCGT
481 AAGTGTATAC TTTGGCGTCA CCCCTTACAT ATTTTAGGTC TTTTTTTATT GTGCGTAACT
   TTCACATATG AAACCGCAGT GGGGAATGTA TAAATCCAG AAAAAAATAA CACGCATTGA
541 AACTTGCCAT CTTCAAACAG GAGGGCTGGA AGAAGCAGAC CGCTAACACA GTACATAAAA
   TTGAACGGTA GAAGTTTGTG CTCCCGACCT TCTTCGTCTG GCGATTGTGT CATGTATTTT
601 AAGGAGACAT GAACG
   TTCCTCTGTA CTTGC -----SacB gene-----

1981                                     TAAAAA
                                       ATTTTT
                                       BamHI
                                       ~~

2041 CGCAAAAGAA AATGCCGATA TCCTATTGGC ATTGACGGTC TCCAGTAAAG GTGGATACGG
   GCGTTTTCTT TTACGGCTAT AGGATAACCG TAACTGCCAG AGGTCATTTT CACCTATGCC
   EcoRI
   ~~~~~

BamHI                                HindIII                                AvaI
~~~~~                                ~~~~~                                ~~~~~

2101 ATCCGAATTC GAGCTCCGTC GACAAGCTTG CGGCCGCACT CGAGCACCAC CACCACCACC
   TAGGCTTAAG CTCGAGGCAG CTGTTCGAAC GCCGGCGTGA GCTCGTGGTG GTGGTGGTGG
2161 ACTGAGATCC GGCTGCTAAC AAAGCCCGAA AGGAAGCTGA GTTGGCTGCT GCCACCCTG
   TGACTCTAGG CCGACGATTG TTTCGGGCTT TCCTTCGACT CAACCGACGA CGGTGGCGAC
                                     T7 terminator
                                     ~~~~~

2221 AGCAATAACT AGCATAACCC CTGGGGCCCT CTAAACGGGT CTGAGGGGT TTTTGTCTGA
   TCGTTATTGA TCGTATTGGG GAACCCCGGA GATTTGCCCA GAACTCCCA AAAAACGACT

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Primers for LIC cloning:

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

Downstream: add TATCCACCTTTACTG to 5' end of downstream primer; add termination codon, if necessary.

pNIC-H102 sequence:

taatacgactcactataggggaattgtgagcggataacaattcccctctagaaataatgtttaaact
ttaaagaaggagatatacatatgcaccatcatcatcatcaccatcatcacgatctgggtaccgagaa
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