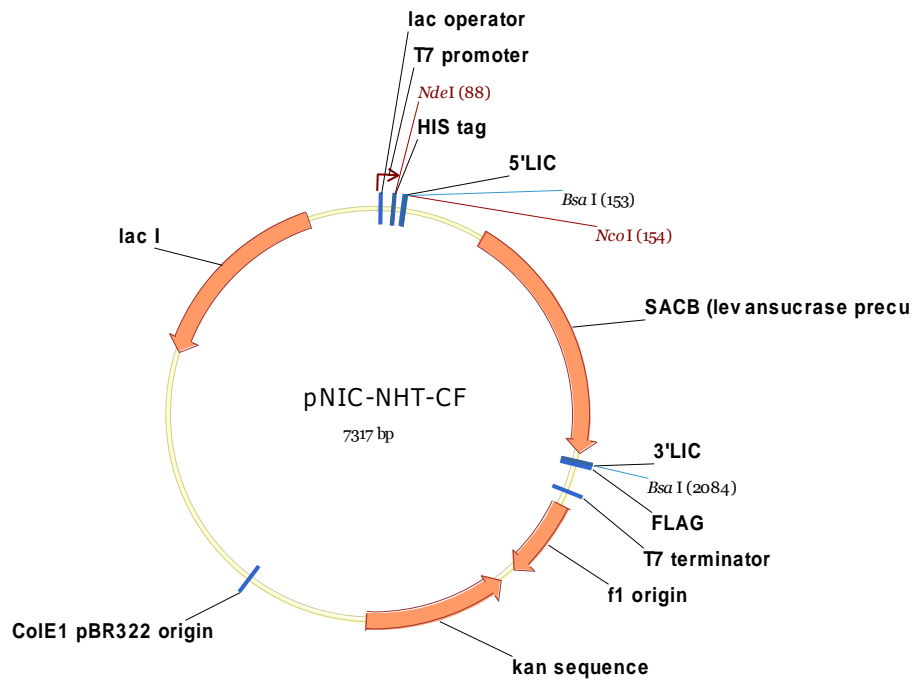


Vector information sheet

Dated: 8th May 2013

Vector Name	pNIC-NHT-CF
Source	Opher Gileadi
Sequence accession/link	Genebank EF199845
Description	pET expression vector with His ₆ tag in 22-aa N-terminal fusion peptide, with TEV protease cleavage site and a C-terminal FLAG peptide. 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.	MHHHHHHSSGVDLG TENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	2684.1 Da including Met (2465.8 Da removed by TEV cleavage)
Termination codon	Downstream of flag tag
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: AGCAGCCAACCTCAGCTTCC



Polylinker region:

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          T7-forward          pLIC-forward
          ----->          ----->
                                lac operator
                                ~~~~~
7222  CTCGATCCCG CGAAATTAAT ACGACTCACT ATAGGGGAAT TGTGAGCGGA TAACAATTCC
      GAGCTAGGGC GCTTTAATTA TGCTGAGTGA TATCCCCTTA ACACTCGCCT ATTGTTAAGG
                                NdeI
                                ~~~~~
                                M H H H H H
7282  CCTCTAGAAA TAATTTTGT TAACTTTAAG AAGGAGATAT ACATATGCAC CATCATCATC
      GGAGATCTTT ATTAAAACAA ATTGAAATTC TTCCTCTATA TGTATACGTG GTAGTAGTAG
                                Upper-LIC      BsaI
                                ~~~~~
      · H S S G V D L G T E N L Y F Q S
58    ATCATTCTTC TGGTGTAGAT CTGGGTACCG AGAACCTGTA CTCCAATCC ATGGAGACCG
      TAGTAAGAAG ACCACATCTA GACCCATGGC TCTTGGACAT GAAGGTTAGG TACCTCTGGC
                                (SacB fragment)
118   ACGTCCACAT ..... (SacB fragment) .....
      TGCAGGTGTA
                                3' LIC
                                ~~~~~
                                BsaI
                                ~~~~~
                                S K G G Y G
2041  CGCAAA AGAAAA TGCCGA TATCCT ATTGGC ATTGAC GGTCTC CAGTAA AGGTGG ATACGG
      GCGTTT TCTTTT ACGGCT ATAGGA TAACCG TAACTG CCAGAG GTCATT TCCACC TATGCC
      FLAG
      ~~~~~
      · S D Y K D D D D K
2101  ATCTGA TTACAA GGATGA CGACGA TAAGTG AAGATC CGAATT CGAGCT CCGTCG ACAAGC
      TAGACT AATGTT CCTACT GCTGCT ATTCAC TTCTAG GCTTAA GCTCGA GGCAGC TGTTCC
2161  TTGCGG CCGCAC TCGAGC ACCACC ACCACC ACCACT GAGATC CGGCTG CTAACA AAGCCC
      AACGCC GCGGTG AGCTCG TGGTGG TGGTGG TGGTGA CTCTAG GCCGAC GATTGT TTCGGG
      T7-reverse
      ~~~~~
2221  GAAAGG AAGCTG AGTTGG CTGCTG CCACCG CTGAGC AATAAC TAGCAT AACCCC TTGGGG

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CTTTCC TTCGAC TCAACC GACGAC GGTGGC GACTCG TTATTG ATCGTA TTGGGG AACCCC

Primers for LIC cloning:

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

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

pNIC-NHT-CF sequence:

taatacgactcactataggggaattgtgagcggataacaattcccctctagaaataatthttgthtaact
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