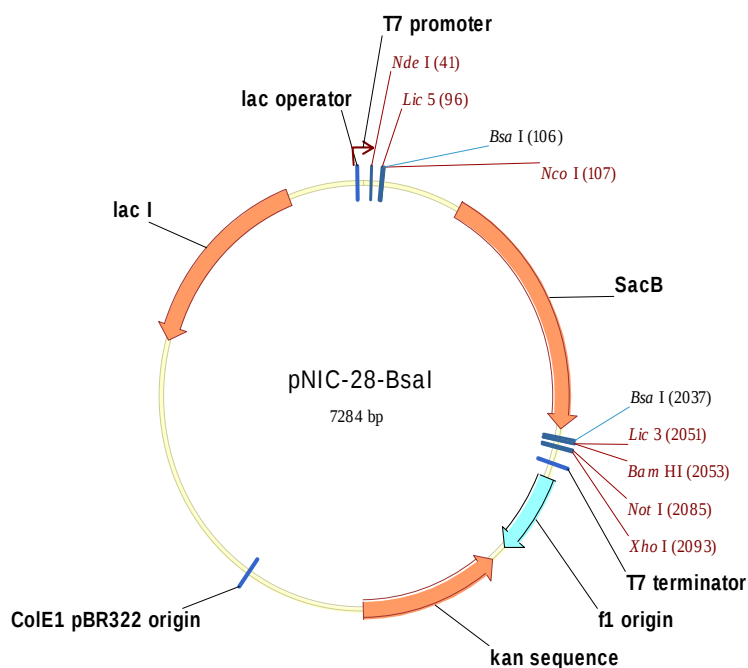


Vector information sheet.

Vector Name	pNIC28-Bsa4
Source	Opher Gileadi
Sequence accession/link	Genebank EF198106
Description	pET expression vector with His ₆ tag in 22-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
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 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-forward      pLIC-forward
                                ----->          ----->
                                -----
                                lac operator
                                -----
7222 CTCGATCCCG CGAAATTAAT ACGACTCACT ATAGGGGAAT TGTGAGCGGA TAACAATTCC
     GAGCTAGGGC GCTTTAATTA TGCTGAGTGA TATCCCCTTA ACACTCGCCT ATTGTAAAGG

                                NdeI
                                -----
                                M H H H H H .
7282 CCTCTAGAAA TAATTTTGT TAACTTTAAG AAGGAGATAT ACATATGCAC CATCATCATC
     GGAGATCTTT ATTAACAACA 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 CTTCCAATCC ATGGAGACCG
     TAGTAAGAAG ACCACATCTA GACCATGGC TCTTGGACAT GAAGGTTAGG TACCTCTGGC

118  ACGTCCACAT ..... (SacB fragment) .....
     TGCAGGTGTA

                                BsaI      Lower-LIC      BamHI      EcoRI
                                -----
2010 GATATCCTAT TGGCATTGAC GGTCTCCAGT AAAGGTGGAT ACGGATCCGA ATTCTGAGCTC
     CTATAGGATA ACCGTAAC TGAGAGTCA TTTCCACCTA TGCTAGGCT TAAGCTCGAG

                                Sali
                                -----
                                HindIII
                                *****
2070 CGTCGACAAG CTTGCGGCCG CACTCGAGCA CCACCACCAC CACCACTGAG ATCCGGCTGC
     GCAGCTGTTT GAACGCCGGC GTGAGCTCGT GGTGGTGGTG GTGGTGACTC TAGGCCGACG
                                T7-reverse
                                -----
2130 TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA
     ATTGTTTCGG GCTTTCCTTC GACTCAACCG ACGACGGTGG CGACTCGTTA TTGATCGTAT
                                -----
                                pLIC-rev

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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.