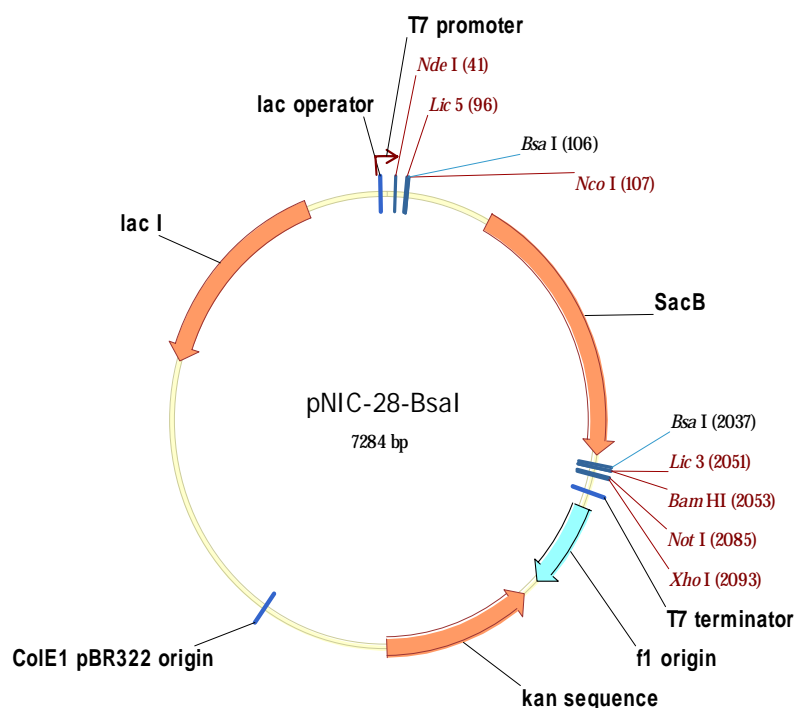


Vector information sheet.

Vector Name	pNIC28-Bsa4
Source	Opher Gileadi
Sequence accession/link	(SGC)

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.	MHHHHHSSGVDLGTENLYFQ*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

```

                T7-forward                pLIC-forward
                ----->                ----->
                lac operator
5101  CTCGATCCCG CGAAATTAAT ACGACTCACT ATAGGGGAAT TGTGAGCGGA TAACAATTCC
      GAGCTAGGGC GCTTTAATTA TGCTGAGTGA TATCCCCTTA ACACTCGCCT ATTGTTAAGG

                NdeI
                ~~~~~
                M H H H H H
5161  CCTCTAGAAA TAATTTTGTT 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
5221  ATCATTCTTC TGGTGTAGAT CTGGGTACCG AGAACCTGTA CTTCCAATCC ATGGAGACCC
      TAGTAAGAAG ACCACATCTA GACCCATGGC TCTTGGACAT GAAGGTTAGG TACCTCTGGC

5281  ACGTCCACAT ..... (SacB fragment) .....
      TGCAGGTGTA

                BsaI                Lower-LIC                BamHI                EcoRI                SacI
                ~~~~~                ~~~~~                ~~~~~                ~~~~~                ~~~~~
7261  GATATCCTAT TGGCATTGAC GGTCTCCAGT AAAGGTGGAT ACGGATCCGA ATTCGAGCTC
      CTATAGGATA ACCGTAACTG CCAGAGGTCA TTCCACCTA TGCCTAGGCT TAAGCTCGAG

      SalI
      HindIII
      *****~
7321  CGTCGACAAG CTTGCGGCGG CACTCGAGCA CCACCACCAC CACCACTGAG ATCCGGCTGC
      GCAGCTGTTC GAACGCCGGC GTGAGCTCGT GGTGGTGGTG GTGGTGACTC TAGCCCGACG
                T7-reverse
      ←-----
7381  TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA
      ATTGTTTCGG GCTTTCCTTC GACTCAACCG ACGACGGTGG CGACTCGTTA TTGATCGTAT
      ←-----
                pLIC-rev

```

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.