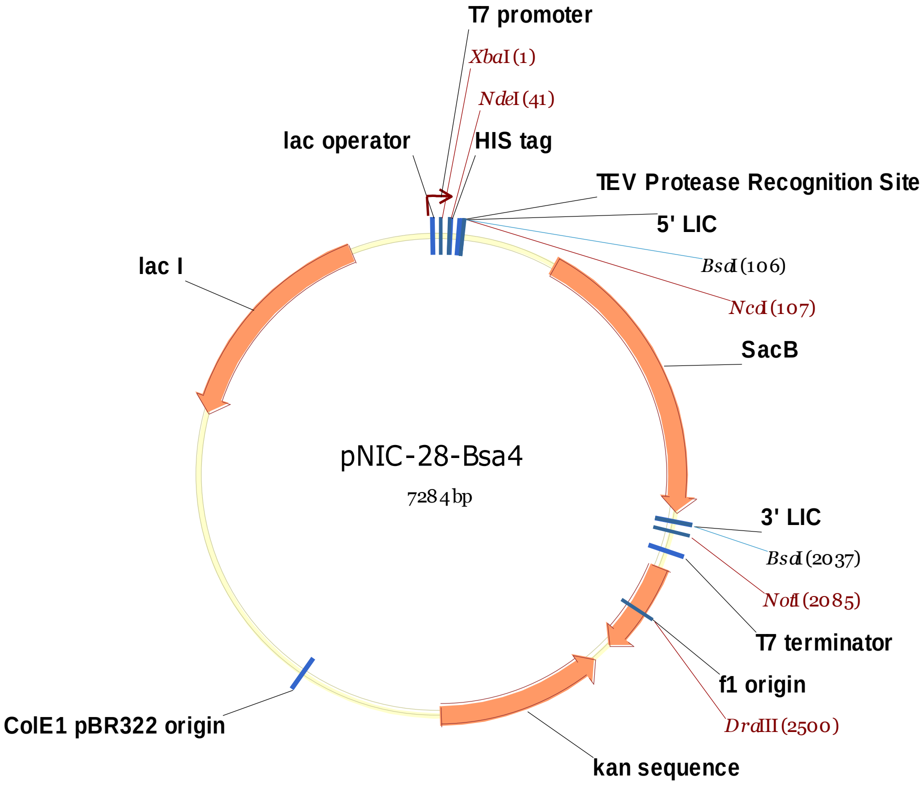
Vector information sheet

Dated: 8th May 2013

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.	MHHHHHHSSGVDLGTENLYFQ*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 ATTGTTAAGG

                                                          NdeI
                                                         ~~~~~~
                                                            M  H   H  H  H  H ·
7282   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
58     ATCATTCTTC TGGTGTAGAT CTGGGTACCG AGAACCTGTA CTTCCAATCC ATGGAGACCG
       TAGTAAGAAG ACCACATCTA GACCCATGGC TCTTGGACAT GAAGGTTAGG TACCTCTGGC

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

                                                                 EcoRI
                                  BsaI     Lower-LIC      BamHI ~~~~~~~ SacI
                                 ______  ~~~~~~~~~~~~~~~~ ~~~~~~       ~~~~~~
2010   GATATCCTAT TGGCATTGAC GGTCTCCAGT AAAGGTGGAT ACGGATCCGA ATTCGAGCTC
       CTATAGGATA ACCGTAACTG CCAGAGGTCA TTTCCACCTA TGCCTAGGCT TAAGCTCGAG

         SalI
              HindIII
        ******~~~~~~~
2070   CGTCGACAAG CTTGCGGCCG CACTCGAGCA CCACCACCAC CACCACTGAG ATCCGGCTGC
       GCAGCTGTTC GAACGCCGGC GTGAGCTCGT GGTGGTGGTG GTGGTGACTC TAGGCCGACG
                                                           T7-reverse
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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.

pNIC28-Bsa4 sequence:

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