Vector information sheet

Dated: 8th May 2013

Vector Name	**pNIC-ZB**
Source	Pavel Savitsky
Sequence accession/link	Genbank GU452710

Description	pET expression vector with His$_6$ and Z-basic (ZB) tags followed by a TEV protease cleavage site. The Z-basic tag *(J. Chromatog A, 1161:22-28)* is a 54-aa sequence derived from protein A and modified to have a high positive surface charge, allowing the fusion proteins to bind to S-sepharose at salt concentrations in which most cellular proteins do not bind. The vector 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.	MHHHHHHSSGVDNKFNKERRRARREIRHLPNLNREQRRAFIRSLRDDPSQSANLLAEAKKLNDAQPKGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	8975 Da
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
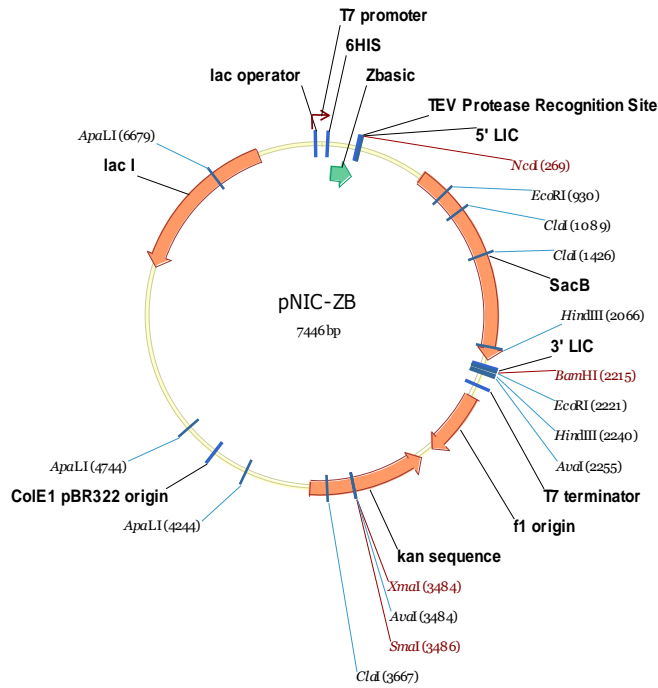
Polylinker region:

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        6HIS
                                              ~~~~~~~~~~~~~~~~~~
                                         NdeI
                                         ~~~~~~~
                                         M   H   H   H   H   H   H  ·
  1 CTAGAAATAA TTTTGTTTAA CTTTAAGAAG GAGATATACA TATGCACCAT CATCATCATC
    GATCTTTATT AAAACAAATT GAAATTCTTC CTCTATATGT ATACGTGGTA GTAGTAGTAG
    6HIS                           Zbasic
    ~~    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     ·  S   S   G   V   D   N   K   F   N   K   E   R   R   A   R   R   E   I   R  ·
 61 ATTCTTCTGG TGTGGATAAC AAGTTCAACA AGGAGCGTCG AAGAGCTCGC CGTGAAATTC
    TAAGAAGACC ACACCTATTG TTCAAGTTGT TCCTCGCAGC TTCTCGAGCG GCACTTTAAG
                              Zbasic
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     ·  H   L   P   N   L   N   R   E   Q   R   R   A   F   I   R   S   L   R   D   D  ·
121 GCCATCTGCC GAACCTGAAC CGCGAACAGC GTCGCGCATT TATTCGCAGC CTGCGCGATG
    CGGTAGACGG CTTGGACTTG GCGCTTGTCG CAGCGCGTAA ATAAGCGTCG GACGCGCTAC
                              Zbasic
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     ·  P   S   Q   S   A   N   L   L   A   E   A   K   K   L   N   D   A   Q   P   K  ·
181 ATCCGAGCCA GAGCGCGAAC CTGCTGGCGG AAGCGAAGAA GCTGAACGAT GCGCAGCCGA
    TAGGCTCGGT CTCGCGCTTG GACGACCGCC TTCGCTTCTT CGACTTGCTA CGCGTCGGCT
                              5' LIC
                         ~~~~~~~~~~~~~~~~~
    Zbasic  TEV Protease Recognition Site
    ~~     ~~~~~~~~~~~~~~~~~~~~~~~~~
                              NcoI
                              ~~~~~~
     ·  G   T   E   N   L   Y   F   Q   S   M
241 AGGGTACCGA GAACCTGTAC TTCCAATCCA TG
    TCCCATGGCT CTTGGACATG AAGGTTAGGT AC
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..(SacB fragment) ....

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

            SalI
                HindIII
            ******~~~~~~~~
   2070   CGTCGACAAG CTTGCGGCCG CACTCGAGCA CCACCACCAC CACCACTGAG ATCCGGCTGC
          GCAGCTGTTC 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 TACTTCCAATCC*ATG* 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-ZB sequence:

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ctagaaataattttgtttaactttaagaaggagatatacatatgcaccatcatcatcatcattcttctg
gtgtggataacaagttcaacaaggagcgtcgaagagctcgccgtgaaattcgccatctgccgaacctga
accgcgaacagcgtcgcgcatttattcgcagcctgcgcgatgatccgagccagagcgcgaacctgctgg
cggaagcgaagaagctgaacgatgcgcagccgaagggtaccgagaacctgtacttccaatccatggaga
ccgacgtccacatatacctgccgttcactattatttagtgaaatgagatattatgatattttctgaatt
gtgattaaaaaggcaactttatgcccatgcaacagaaactataaaaaatacagagaatgaaaagaaaca
gatagattttttagttctttaggcccgtagtctgcaaatcctttttatgattttctatcaaacaaaagag
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```