Vector information sheet

Dated: 8th May 2013
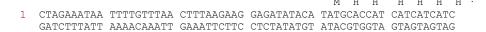
Vector Name	**pNIC-Bio3**
Source	Claire Strain-Damerell (derivative of pNIC-Bio2)
Sequence accession/link	JN792439

Description	pET expression vector with His$_6$ tag in 22-aa N-terminal fusion peptide, with TEV protease cleavage site, and a biotinylation sequence in C-terminal fusion. 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)
C-terminal fusion – seq.	GSKGGYGLNDIFEAQKIEWHE
C-terminal fusion - MW	2396.57 Da
Termination codons	Already included after biotinylation signal at C-terminal
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase. For effective biotinylation, the host should overexpress BirA (e.g. from plasmid pCDF-BIRA, Genbank: JF914075)
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC

Polylinker region:

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                                          M  H  H   H  H  H  H  ·
   1   CTAGAAATAA TTTTGTTTAA CTTTAAGAAG GAGATATACA TATGCACCAT CATCATCATC
       GATCTTTATT AAAACAAATT GAAATTCTTC CTCTATATGT ATACGTGGTA GTAGTAGTAG

                                          Lic5            BsaI
                                          ~~~~~~~~~~~~~~  ~~~~~~
       ·  S  S  G   V  D  L   G  T  E  N   L  Y  F   Q  S  M
  61   ATTCTTCTGG TGTAGATCTG GGTACCGAGA ACCTGTACTT CCAATCCATG GAGACCGACG
       TAAGAAGACC ACATCTAGAC CCATGGCTCT TGGACATGAA GGTTAGGTAC CTCTGGCTGC


---------------------------------------SacB---------------------------------------


                                              BsaI   Lic3
                                              ~~~~~~ ~~~~
                                                            S  K  ·
1981   TAACAAATAA AAACGCAAAA GAAAATGCCG ATATCCTATT GGCATTGACG GTCTCCAGTA
       ATTGTTTATT TTTGCGTTTT CTTTTACGGC TATAGGATAA CCGTAACTGC CAGAGGTCAT

          Lic3
       ~~~~~~~~~~~
       ·  G  G  Y   G  L  N   D  I  F  E   A  Q  K   I  E  W   H  E  *
2041   AAGGTGGATA CGGCCTGAAT GATATCTTTG AAGCGCAGAA GATTGAATGG CATGAATGAT
       TTCCACCTAT GCCGGACTTA CTATAGAAAC TTCGCGTCTT CTAACTTACC GTACTTACTA
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Primers for LIC cloning:

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

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

pNIC-Bio3 sequence:

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ctagaaataattttgtttaactttaagaaggagatatacatatgcaccatcatcatcatcattcttctg
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