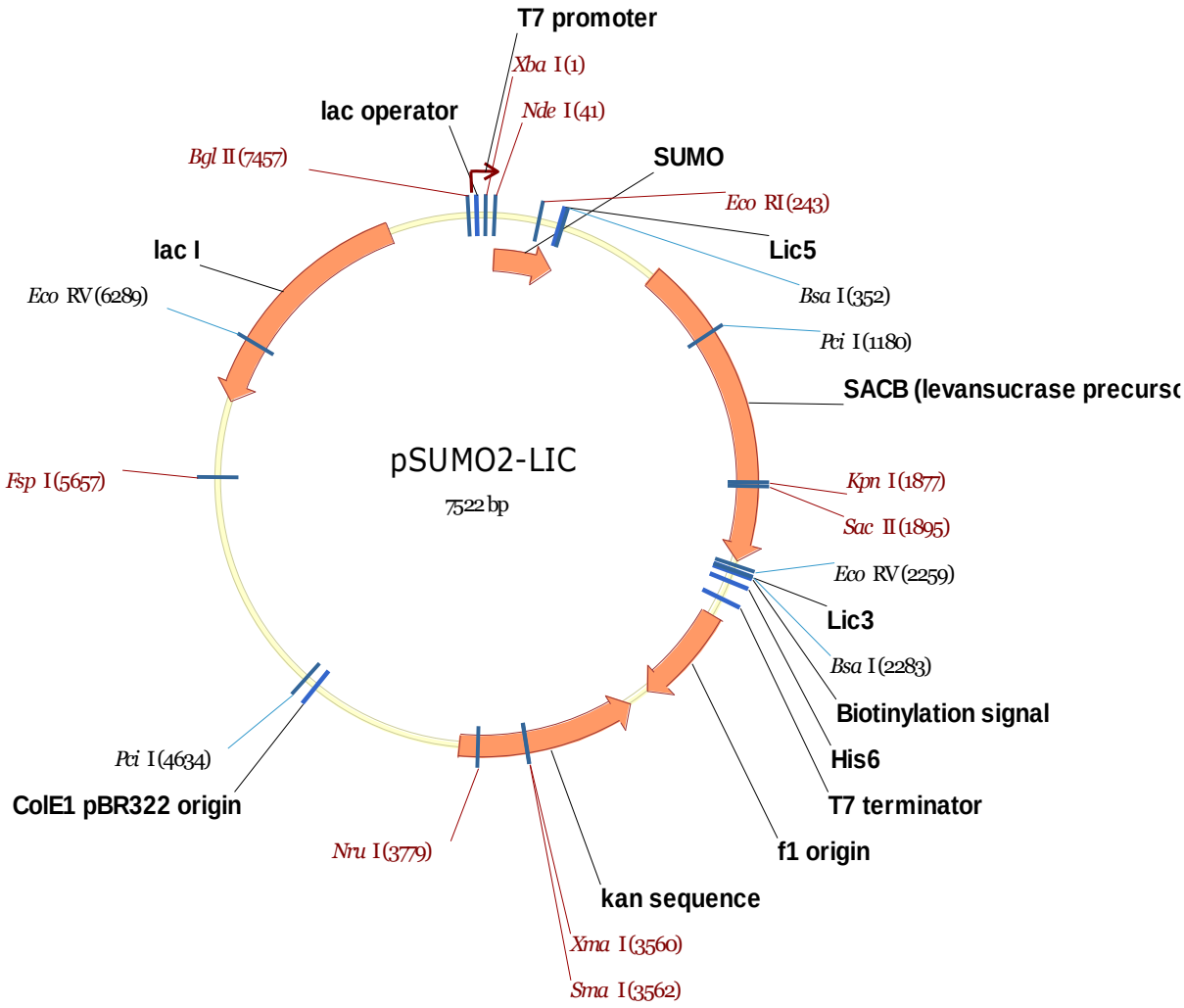
Vector information sheet

Dated: 16th September 2013

Vector Name	**pSUMO2-LIC**
Source	Pavel Savitsky
Sequence accession/link	Genbank XXXXXXX

Description	pET expression vector with N-terminal SUMO (96-aa sequence derived from SUMO1 protein) tags followed by a SUMO protease cleavage site and C-terminal biotinylation andl His_6 tags. SUMO tag has been shown to improve solubility of the fusion partners. 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 dTTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MCSSGSGSGSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEI HFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHT PKELGMEEEDVIEVYQEQTGG* (* - SUMO cleavage site)
N-terminal fusion – MW	11755.1 Da
C-terminal fusion – seq	SGGGLNDIFEAQKIEWHEHHHHHH
C-terminal fusion – MW	2853 Da
Termination codons	No stop codon
Protease cleavage	SUMO
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



Cloning region in the vector:

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     XbaI                                        NdeI
     ~~~~~                                       ~~~~~~~
                                                    M  C  S   S  G  S  G ·
   1 CTAGAAATAA TTTTGTTTAA CTTTAAGAAG GAGATATACA TATGTGCAGC AGCGGCAGCG

     ·  S  G  S   D  Q  E   A  K  P  S   T  E  D   L  G  D   K  K  E  G ·
  61 GCAGCGGCTC TGACCAGGAG GCAAAACCTT CAACTGAGGA CTTGGGGGAT AAGAAGGAAG

     ·  E  Y  I   K  L  K   V  I  G  Q   D  S  S   E  I  H   F  K  V  K ·
 121 GTGAATATAT TAAACTCAAA GTCATTGGAC AGGATAGCAG TGAGATTCAC TTCAAAGTGA

     ·  M  T  T   H  L  K   K  L  K  E   S  Y  C   Q  R  Q   G  V  P  M ·
 181 AAATGACAAC ACATCTCAAG AAACTCAAAG AATCATACTG TCAAAGACAG GGTGTTCCAA

      EcoRI
      ~~~~~~
     ·  N  S  L   R  F  L   F  E  G  Q   R  I  A   D  N  H   T  P  K  E ·
 241 TGAATTCACT CAGGTTTCTC TTTGAGGGTC AGAGAATTGC TGATAATCAT ACTCCAAAAG

                                                 Lic5          BsaI
                                              ~~~~~~~~~~~~~~~~~~~~ ~~~~
     ·  L  G  M   E  E  E   D  V  I  E   V  Y  Q   E  Q  T   G  G
 301 AACTGGGAAT GGAGGAAGAA GATGTGATTG AAGTTTACCA GGAGCAAACG GGAGGTGAGA

     BsaI
     ~~
 361 CCGACGTCCA CATATACCTG CCGTTCACTA TTATTTAGTG AAATGAGATA TTATGATATT
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SacB

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                                                 EcoRV                BsaI
                                                 ~~~~~~~              ~~~~~
2221  AACAGTTAAC AAATAAAAAC GCAAAAGAAA ATGCCGATAT CCTATTGGCA TTGACGGTCT

      BsaI
      ~
        S  G  G   G  L  N  D   I  F  E   A  Q  K   I  E  W  H   E  H  H ·
2281  CAGCGGTGGC GGTCTGAACG ACATCTTCGA GGCTCAGAAA ATCGAATGGC ACGAACACCA

      · H  H  H   H  *
2341  CCACCACCAC CACTGAGATC CGGCTGCTAA CAAAGCCCGA AAGGAAGCTG AGTTGGCTGC
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Lic cloning scheme:

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Treat vector with dTTP:

V  Y  Q  E  Q  T  G       G    BsaI
GTTTACCAGGAGCAAACGG      GAGGTGAGACCGACGTCCACATatacctgccgttcactattattt
CAAATGGTCCTCGTTTGCCCTCC      ACTCTGGCTGCAGGTGTAtatggacggcaagtgataataaa

(SacB fragment)

                        BsaI      S  G  G  G  L  N  D  I  F  E  A  Q
GATATCCTAT TGGCATTGAC GGTCTCA    GCGGTGGCGGTCTGAACGACATCTTCGAGGCTCAG
CTATAGGATA ACCGTAACTG CCAGAGTCGC    CACCGCCAGACTTGCTGTAGAAGCTCCGAGTC

K  I  E  W  H  E  H   H  H  H   H  H
AAAATCGAATGGCACGAACA CCACCACCAC CACCACTGAGATCCGGCTGC
TTTTAGCTTACCGTGCTTGT GGTGGTGGTG GTGGTGACTCTAGGCCGACG


Treat PCR product with dATP:

ACCAGGAGCAAACGGGAGGT       PCR product       AGCGGTGGCGGTCTG
TGGTCCTCGTTTGCCCTCCA                         TCGCCACCGCCAGAC

Primers LIC extensions:

Forward           ACCAGGAGCAAACGGGAGGT
Reverse           CAGACCGCCACCGCT
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Primers for LIC cloning (treat PCR fragment with T4 DNA polymerase in presence of dATP)

Upstream: add ACCAGGAGCAAACGGGAGGT to the 5’ end of upstream primer

Downstream: add CAGACCGCCACCGCT to 5’ end of downstream primer

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>pSUMO2-LIC
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