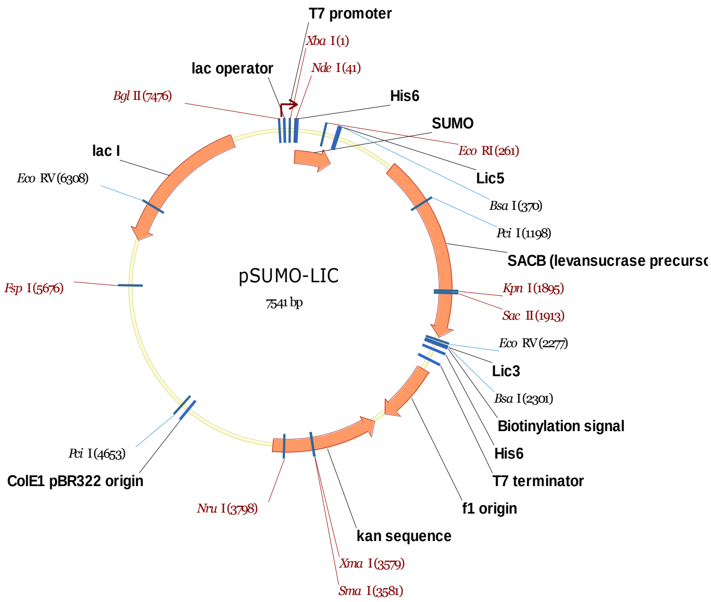
Vector information sheet

Dated: 16th September 2013

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

Description	pET expression vector with His_6 and SUMO (96-aa sequence derived from SUMO1 protein) tags followed by a SUMO protease cleavage site. 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. If no stop codon is included in reverse primer, protein of interest will be fused to biotinylation tag followed by additional His_6 tag.

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.	MCSSHHHHHHGSGSGSDQEAKPSTEDLGDKKEGEYIKLKVIG QDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRI ADNHTPKELGMEEEDVIEVYQEQTGG* (* - SUMO cleavage site)
N-terminal fusion – MW	12577.9 Da
Termination codons	supplied in PCR primer
Protease cleavage	SUMO
Additional features	Optional non-cleavable biotinylation and His_6 tags if reverse primer does not encode stop codon.
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   H   H   H
   1  CTAGAAATAA TTTTGTTTAA CTTTAAGAAG GAGATATACA TATGTGCAGC AGCCATCATC
      GATCTTTATT AAAACAAATT GAAATTCTTC CTCTATATGT ATACACGTCG TCGGTAGTAG
                                                    SUMO
                                     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        H   H   H    G   S   G    S   G   S   D    Q   E   A    K   P   S    T   E   D   L
  61  ATCATCATCA CGGCAGCGGC AGCGGCTCTG ACCAGGAGGC AAAACCTTCA ACTGAGGACT
      TAGTAGTAGT GCCGTCGCCG TCGCCGAGAC TGGTCCTCCG TTTTGGAAGT TGACTCCTGA
                                   SUMO
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
        G   D   K    K   E   G    E   Y   I   K    L   K   V    I   G   Q    D   S   S   E
 121  TGGGGGATAA GAAGGAAGGT GAATATATTA AACTCAAAGT CATTGGACAG GATAGCAGTG
      ACCCCCTATT CTTCCTTCCA CTTATATAAT TTGAGTTTCA GTAACCTGTC CTATCGTCAC
                                   SUMO
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      · I   H   F    K   V   K    M   T   T   H    L   K   K    L   K   E    S   Y   C   Q
 181  AGATTCACTT CAAAGTGAAA ATGACAACAC ATCTCAAGAA ACTCAAAGAA TCATACTGTC
      TCTAAGTGAA GTTTCACTTT TACTGTTGTG TAGAGTTCTT TGAGTTTCTT AGTATGACAG
                                   SUMO
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      · R   Q   G    V   P   M    N   S   L   R    F   L   F    E   G   Q    R   I   A   D
 241  AAAGACAGGG TGTTCCAATG AATTCACTCA GGTTTCTCTT TGAGGGTCAG AGAATTGCTG
      TTTCTGTCCC ACAAGGTTAC TTAAGTGAGT CCAAAGAGAA ACTCCCAGTC TCTTAACGAC
                                   SUMO
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      · N   H   T    P   K   E    L   G   M   E    E   E   D    V   I   E    V   Y   Q   E
 301  ATAATCATAC TCCAAAAGAA CTGGGAATGG AGGAAGAAGA TGTGATTGAA GTTTACCAGG
      TATTAGTATG AGGTTTTCTT GACCCTTACC TCCTTCTTCT ACACTAACTT CAAATGGTCC
           SUMO
      ~~~~~~~~~~~~~~~
      · Q   T   G    G
 361  AGCAAACGGG AGGT
      TCGTTTGCCC TCCA
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.... **(SacB fragment)** ....

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                     BsaI
                    ~~~~~~
                                    G   L   N   D    I   F   E    A   Q   K   I ·
2281  TATTGGCATT GACGGTCTCC AGCGGTGGCG GTCTGAACGA CATCTTCGAG GCTCAGAAAA
      ATAACCGTAA CTGCCAGAGG TCGCCACCGC CAGACTTGCT GTAGAAGCTC CGAGTCTTTT
      · E   W   H    E   H   H    H   H   H   H    *
2341  TCGAATGGCA CGAACACCAC CACCACCACC ACTGAGATCC GGCTGCTAAC AAAGCCCGAA
      AGCTTACCGT GCTTGTGGTG GTGGTGGTGG TGACTCTAGG CCGACGATTG TTTCGGGCTT
             pLIC-rev               T7-reverse
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          <------------------  <-----------------
2401  AGGAAGCTGA GTTGGCTGCT GCCACCGCTG AGCAATAACT AGCATAACCC CTTGGGGCCT
      TCCTTCGACT CAACCGACGA CGGTGGCGAC TCGTTATTGA TCGTATTGGG GAACCCCGGA
```

Lic cloning scheme:

```
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 GGTCTCC     AGCGGTGGCGGTCTGAACGACATCTTCGAGGCTCAG
CTATAGGATA ACCGTAACTG CCAGAGGTCGC     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       TGAAGCGGTGGCGGTCTG
TGGTCCTCGTTTGCCCTCCA                         AGTTCGCCACCGCCAGAC

Primers LIC extensions:

Forward          ACCAGGAGCAAACGGGAGGT
Reverse1         CAGACCGCCACCGCT
Reverse2         CAGACCGCCACCGCTTGA
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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; add termination codon, if necessary.

pSUMO-LIC sequence:

```
ctagaaataattttgtttaactttaagaaggagatatacatatgtgcagcagccatcatcatcatcatc
acggcagcggcagcggctctgaccaggaggcaaaaccttcaactgaggacttgggggataagaaggaag
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