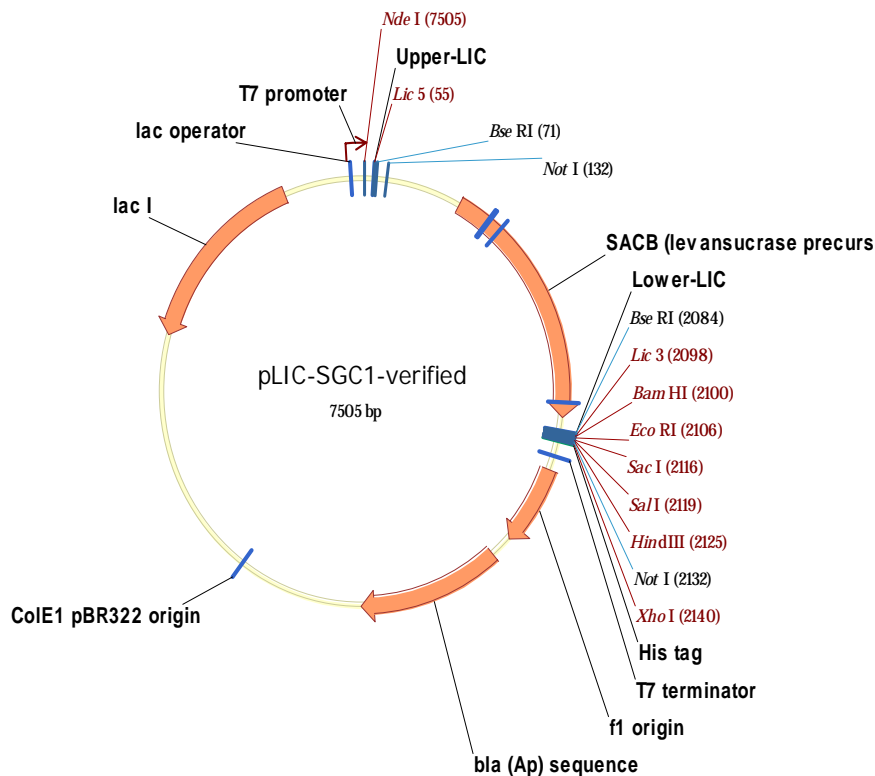


Vector information sheet.

Vector Name	<b>pLIC-SGC1</b>
Source	Sujata Sharma, Toronto/ Argonne
Sequence accession/link	(SGC)
Description	pET expression vector with His <sub>6</sub> tag in 23-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	ampicillin
Promoter	T7 - lacO
Cloning	LIC. (vector treated with BseRI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MHHHHHHSSGVDLGTENLYFQ*S (* - TEV cleavage site)
N-terminal fusion – MW	2684.1 Da including Met (2465.48 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

```

                                T7-forward
                                ----->
                                lac operator
                                ~~~~~
7402  CTCGATCCCG  CGAAATTAAT  ACGACTCACT  ATAGGGGAAT  TGTGAGCGGA  TAACAATTCC
      GAGCTAGGGC  GCTTTAATTA  TGCTGAGTGA  TATCCCCTTA  ACACTCGCCT  ATTGTTAAGG

                                NdeI
                                ~~~~~
                                M H H H H H
7462  CCTCTAGAAA  TAATTTTGT  TAACTTTAAG  AAGGAGATAT  ACATATGCAC  CATCATCATC
      GGAGATCTTT  ATTAATAACA  ATTGAAATTC  TTCCTCTATA  TGTATACGTG  GTAGTAGTAG

                                Upper-LIC
                                ~~~~~
      · H S S G V D L G T E N L Y F Q S
17    ATCATTCTTC  TGGTGTAGAT  CTGGGTACCG  AGAACCTGTA  CTTCCAATCC  ATAAGCTAGC
      TAGTAAGAAG  ACCACATCTA  GACCCATGGC  TCTTGGACAT  GAAGGTAGG  TATTCGATCG

      BseRI
      ~~~~~
77    TTCTCCTCCT ..... (SacB fragment) .....
      AAGAGGAGGA

                                BseRI          Lower-LIC          BamHI  EcoRI  SacI
                                ~~~~~          ~~~~~          ~~~~~  ~~~~~  ~~~~~
2057  TGGCACTTTT  CGAGGAGTTT  ACTAGTAAGT  AAAGGTGGAT  ACGGATCCGA  ATTCGAGCTC
      ACCGTGAAAA  GCTCCTCAA  TGATCATTCA  TTTCCACCTA  TGCTTAGGCT  TAAGCTCGAG

      Sali
      HindIII
      *****~
2117  CGTCGACAAG  CTTGCGGCCG  CACTCGAGCA  CCACCACCAC  CACCACTGAG  ATCCGGCTGC
      GCAGCTGTTC  GAACGCCGGC  GTGAGCTCGT  GGTGGTGGTG  GTGGTGACTC  TAGGCCGACG

                                T7-reverse
                                -----<
2177  TAACAAAGCC  CGAAAGGAAG  CTGAGTTGGC  TGCTGCCACC  GCTGAGCAAT  AACTAGCATA
      ATGTTTCGG  GCTTTCCTTC  GACTCAACCG  ACGACGGTGG  CGACTCGTTA  TTGATCGTAT

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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.