

Vector information sheet

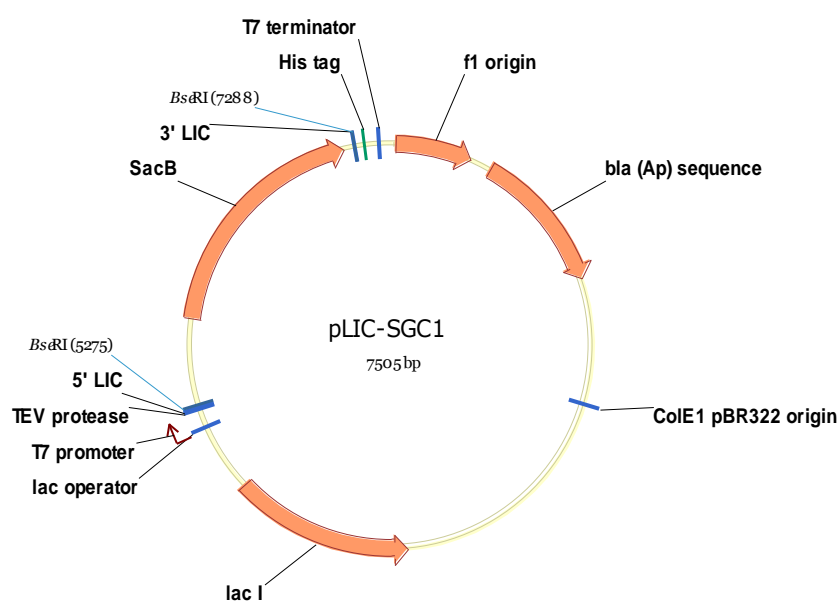
Dated: 8th May 2013

Vector information sheet.

Vector Name	pLIC-SGC1
Source	Sujata Sharma, Toronto/ Argonne
Sequence accession/link	(SGC)

Description	pET expression vector with His ₆ 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
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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: AGCAGCCAACCTCAGCTTCC



Polylinker region:

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

                                NdeI
                                ~~~~~
                                M H H H H H
7462  CCTCTAGAAA TAATTTTGT TAACTTTAAG AAGGAGATAT ACATATGCAC CATCATCATC
      GGAGATCTTT ATTAACAACAA 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 TCTTGACAT GAAGGTTAGG TATTCGATCG

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

                                BseRI
                                ~~~~~
                                Lower-LIC
                                ~~~~~
                                BamHI ~~~~~
                                EcoRI ~~~~~
                                SacI ~~~~~
2057  TGGCACTTTT CGAGGAGTTT ACTAGTAAGT AAAGGTGGAT ACGGATCCGA ATTCGAGCTC
      ACCGTGAAAA GCTCCTCAA TGCATATCA TTCCACCTA TGCCTAGGCT TAAGCTCGAG

      SalI
      HindIII
      *****
2117  CGTCGACAAG CTGCGGCCG CACTCGAGCA CCACCACCAC CACCACTGAG ATCCGGCTGC
      GCAGCTGTTC GAACGCCGCG GTGAGCTCGT GGTGGTGGTG GTGGTGACTC TAGGCCGACG

                                T7-reverse
                                <-----
2177  TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA
      ATTGTTTCGG 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.

pLIC-SGC1 sequence:

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