pET21a-LIC Vector (GenBank accession EF456737)

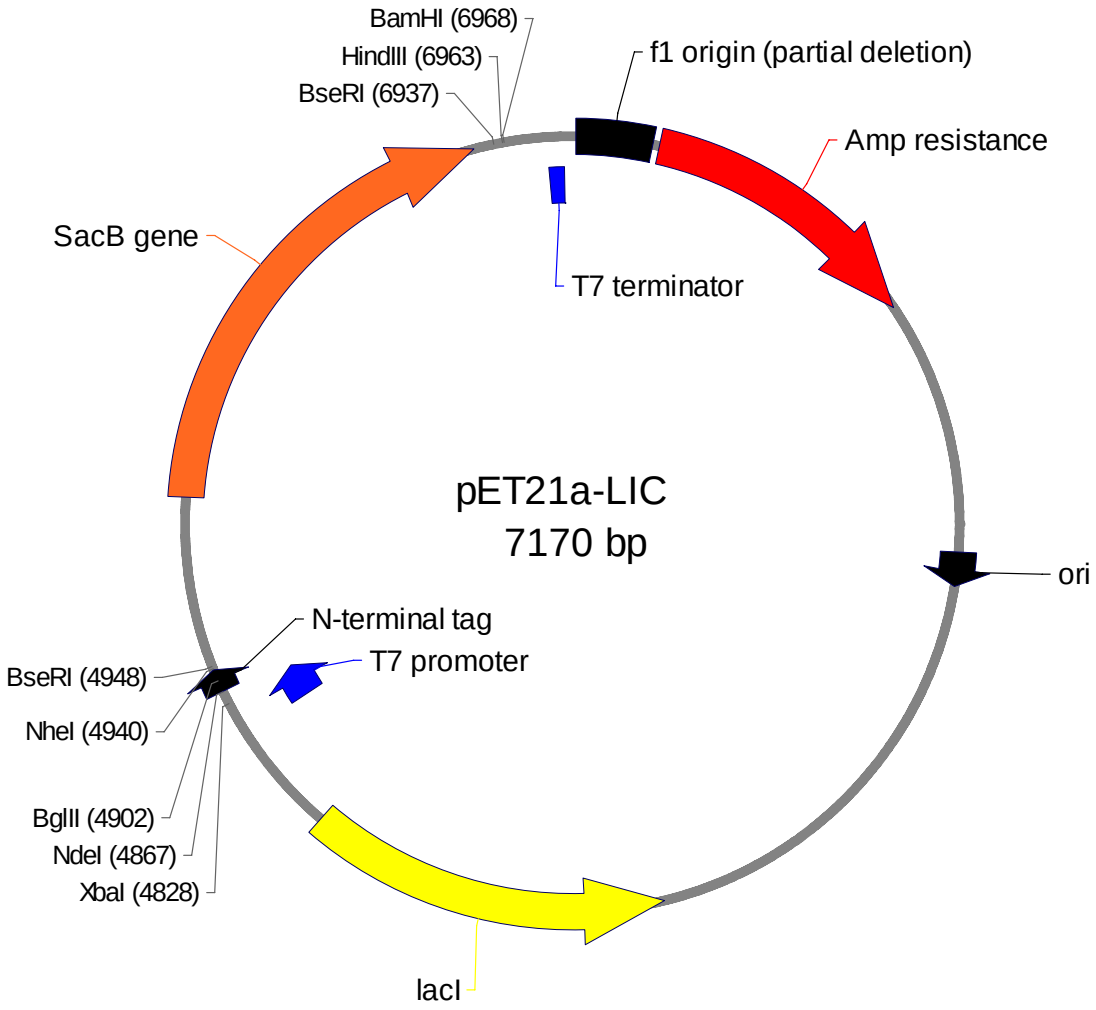
Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto

Description	The pET21a-LIC (aka pLIC-SGC1) vector was derived from expression plasmid pMSCG7 (1). It is used for T7 promoter driven expression of recombinant proteins with the addition of a 23 amino acid N-terminal fusion tag containing a 6X His-tag followed by a TEV protease cleavage site. Two stop codons are included in the primer and vector at the C-terminal cloning site.

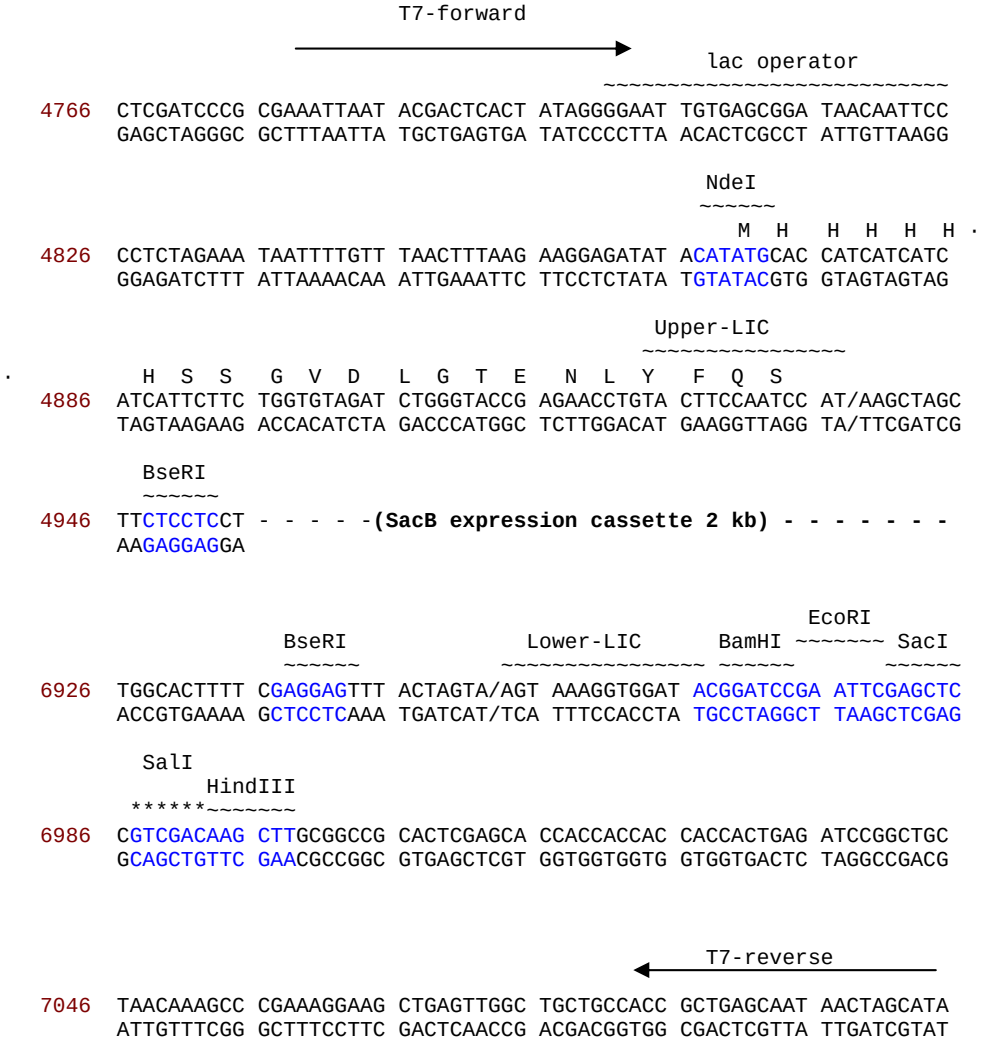
Antibiotic resistance	Ampicillin, 100 ug/ml
Promoter	T7 - lacO
Cloning Methods	Designed for ligation independent cloning. Vector treated with BseRI, then with T4 DNA polymerase in presence of dGTP). PCR insert treated with T4 DNA polymerase in presence of dCTP. Negative selection of the original plasmid on 5% sucrose. Alternative is infusion cloning. Insertion of DNA sequence into the cloning/expression region is preformed using BD-Biosciences Infusion enzyme mediated directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and BseRI linearized vector.
Initiation Codon	NdeI site in vector at 4870 bp
N – terminal fusion sequence	MHHHHHHSSGVDLGTENLYFQ*SM--- (* - TEV cleavage site)
Termination codons	*TAA*CAG*TAA* included in 3' PCR primer and vector. No amino acid residues added at cloning junction
Additional features	
Prefered Hosts	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' primer for amplification of insert	5'-TACTTCCAATCCAT*G* ---3' The 'ATG' is the first codon of the inserted gene
3' primer for amplification of insert	5'-TATCCACCTTTACTG*TTA*---3' *CTA*---3' *TCA*---3' Any one of the three stop codons can be used
5' sequencing primer T7-Fwd	5' AATTAATACGACTCACTATAGGG 3'
3' sequencing primer T7-Rev	5' ATGCTAGTTATTGCTCAGCGG 3'

pET21a-LIC vector map

T7 promoter	4782-4798
N-terminal tag coding sequence	4870-4937
N-terminal cloning site	4924-4937
C-terminal cloning site	6953-6966
T7 terminator	7099-7145
f1 origin (partially deleted)	12-239
bla coding sequence	260-1117
pBR322 origin	1878
lacI coding sequence	3312-4391
sacB coding sequence	5459-6877



pET21a-LIC cloning/expression region

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4766  CTCGATCCCG CGAAATTAAT ACGACTCACT ATAGGGGAAT TGTGAGCGGA TAACAATTCC
      GAGCTAGGGC GCTTTAATTA TGCTGAGTGA TATCCCCTTA ACACTCGCCT ATTGTTAAGG

4826  CCTCTAGAAA TAATTTTGTT TAACTTTAAG AAGGAGATAT ACATATGCAC CATCATCATC
      GGAGATCTTT ATTAAAACAA ATTGAAATTC TTCCTCTATA TGTATACGTG GTAGTAGTAG

4886  ATCATTCTTC TGGTGTAGAT CTGGGTACCG AGAACCTGTA CTTCCAATCC AT/AAGCTAGC
      TAGTAAGAAG ACCACATCTA GACCCATGGC TCTTGGACAT GAAGGTTAGG TA/TTCGATCG

4946  TTCTCCTCCT - - - - -(SacB expression cassette 2 kb) - - - - - - -
      AAGAGGAGGA

6926  TGGCACTTTT CGAGGAGTTT ACTAGTA/AGT AAAGGTGGAT ACGGATCCGA ATTCGAGCTC
      ACCGTGAAAA GCTCCTCAAA TGATCAT/TCA TTTCCACCTA TGCCTAGGCT TAAGCTCGAG

6986  CGTCGACAAG CTTGCGGCCG CACTCGAGCA CCACCACCAC CACCACTGAG ATCCGGCTGC
      GCAGCTGTTC GAACGCCGGC GTGAGCTCGT GGTGGTGGTG GTGGTGACTC TAGGCCGACG

7046  TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA
      ATTGTTTCGG GCTTTCCTTC GACTCAACCG ACGACGGTGG CGACTCGTTA TTGATCGTAT
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Reference
1. Stols L., Minyi G., Dieckman L., Raffen R., Collart F.R., and Donnelly M. I. 2002. A new vector for high-throughput, ligation-independent cloning encoding a tobacco etch virus protease cleavage site. Protein Expression and Purification 2, 8-15.