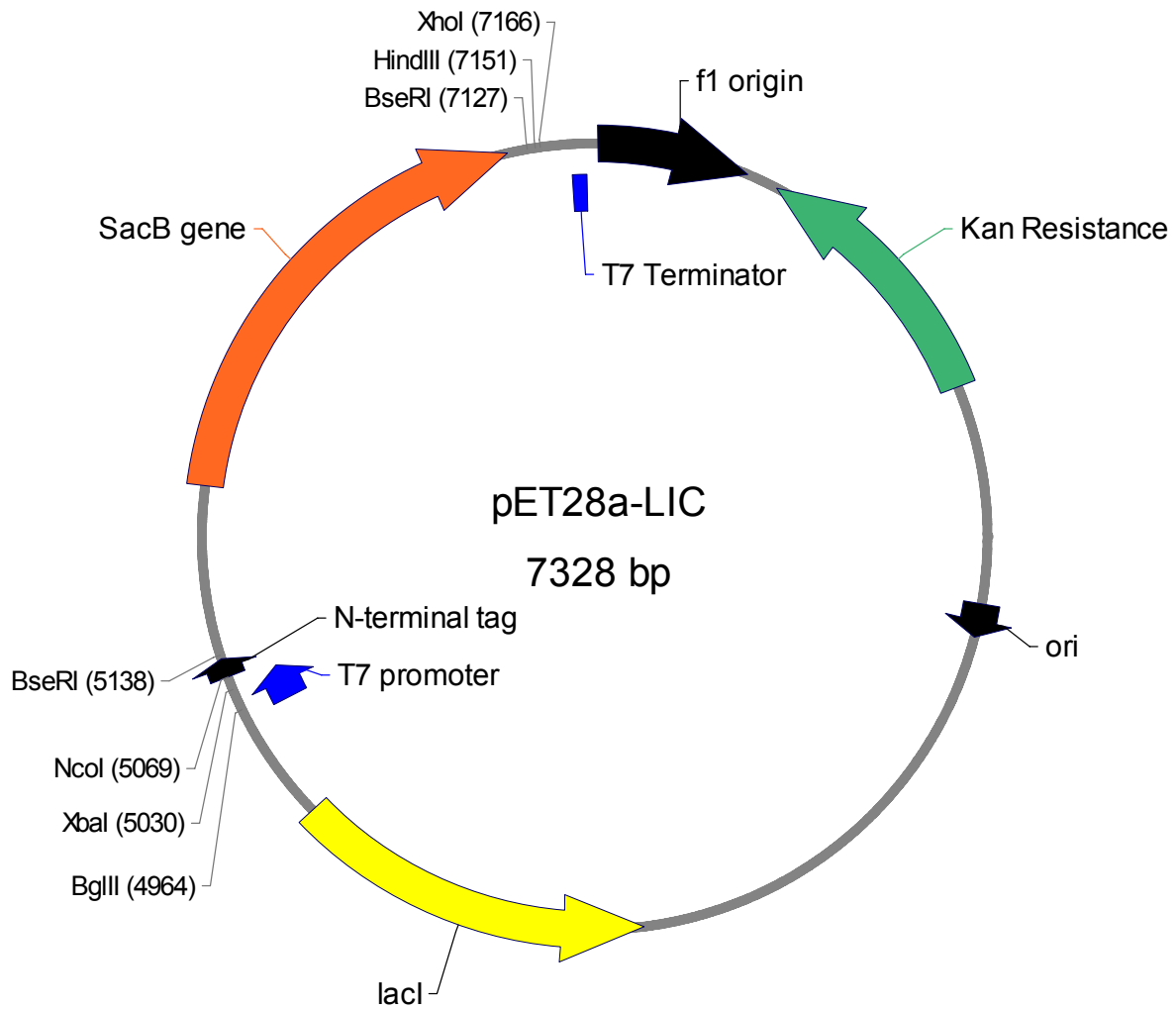


pET28a-LIC Vector (GenBank accession EF442785)

Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto
Description	The pET28a-LIC vector was derived from expression plasmid pET28a (Novagen). It is used for T7 promoter driven expression of recombinant proteins with the addition of a 19 amino acid N-terminal fusion tag containing a 6X His-tag followed by a thrombin protease cleavage site. Two stop codons are included in the vector at the C-terminal cloning site.
Antibiotic resistance	Kanamycin, 50 ug/ml
Promoter	T7 - lacO
Cloning Methods	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. Insertion of target sequence involves replacement of a SacB gene stuffer sequence, which provides for negative selection of the original plasmid on 5% sucrose.
Initiation Codon	NcoI site in vector at 5071 bp
N – terminal fusion sequence	MGSSHHHHHHSSGLVPRGS
Termination codons	TGATGA included in 3' PCR primer and vector cloning site. No amino acid residues added at cloning junction
Additional features	
5' primer for amplification of insert	5' GTT CCG CGT GGT AGT --- 3'
5' primer for amplification of insert without tag for NcoI/BseRI cloning	5' AAG AAG GAG ATA TAC CAT G --- 3'
3' primer for amplification of insert	5' CAA GCT TCG TCA TCA --- 3'
5' sequencing primer T7-Fwd	5' AATTAATACGACTCACTATAGGG 3'
3' sequencing primer T7-Rev	5' ATGCTAGTTATTGCTCAGCGG 3'

pET28a-LIC vector map

T7 promoter	4984-5000
N-terminal tag coding sequence	5071-5127
N-terminal cloning site	5113-5127
C-terminal cloning site	7143-7157
T7 terminator	7257-7303
f1 origin	12-467
<i>aph</i> coding sequence	563-1375
pBR322 origin	2084
<i>lacI</i> coding sequence	3518-4597
<i>sacB</i> coding sequence	5649-7067



pET28a-LIC cloning/expression region

```

                                T7 FWD
                                .....▶
4968  ctcgatccccg  cgaaattaat  acgactcact  ataggggaat  tgtgagcgga
      gagctagggc  gctttaatta  tgctgagtga  tatccccctta  acactcgct

~~~~~

5018  taacaattcc  cctctagaaa  taattttggt  taactttaag  aaggagatat
      attgttaagg  ggagatcttt  attaaaacaa  attgaaattc  ttcctctata

      NcoI
      M G S   S H H   H H H   H S S G   L V P
5068  accatgggca  gcagccatca  tcatcatcat  cacagcagcg  gcctggttcc
      tggtagccgt  cgtcggtagt  agtagtagta  gtgtcgtcgc  cggaccaagg

      R G S           BseRI
5118  gcgtggtagt/attatgagtt  ctcctc-----SACB cassette (2 kb)---
      cgcacatca/taatactcaa  gaggag

      BseRI           stop           HindIII           XhoI
7127  gaggagatca  tgcaca/tgat  gacgaagctt  gcggcgcgac  tcgagcacca
      ctcctctagt  acgtgt/acta  ctgcttcgaa  cgccggcgtg  agctcgtgga

7174  ccaccaccac  cactgagatc  cggctgctaa  caaagcccga  aaggaagctg
      ggtggtggtg  gtgactctag  gccgacgatt  gtttcgggct  ttccttcgac

                                T7 REV
                                ◀.....
7227  agttggctgc  tgccaccgct  gagcaataac  tagcataacc  ctttggggcc
      tcaaccgacg  acggtggcga  ctcgttattg  atcgtattgg  ggaaccccg

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