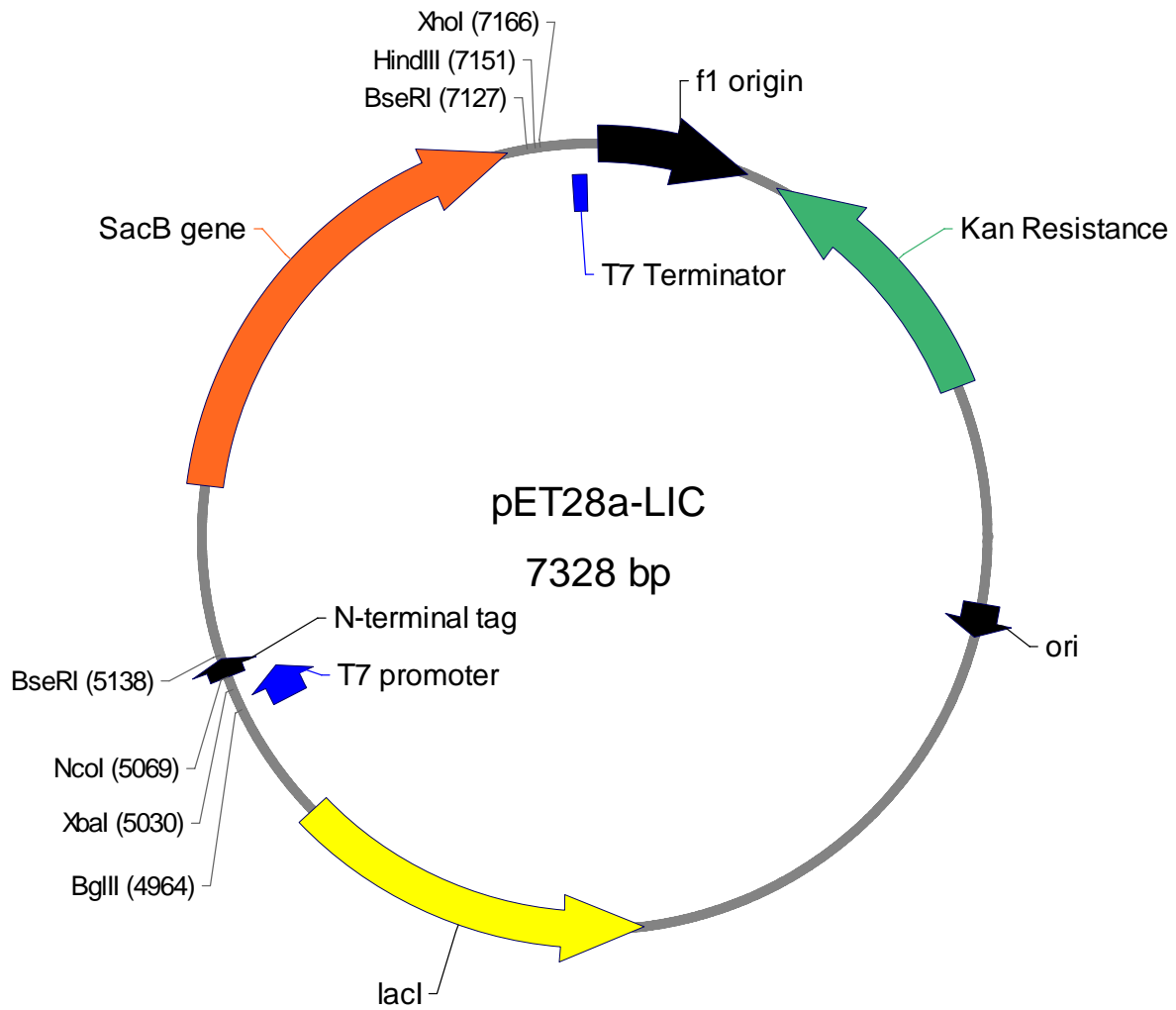


## pET28a-LIC Vector (GenBank accession EF442785)

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	MGSSHHHHHSSGLVPRGS
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 lac operator

.....▶

4968 ctcgatcccc cgaaattaat acgactcact ataggggaat tgtgagcggg  
gagctagggc gctttaatta tgctgagtga tatcccccta acactcgct

~~~~~

5018 taacaattcc cctctagaaa taattttggt taactttaag aaggagatat  
attgttaagg ggagatcttt attaaaacaa attgaaattc ttcctctata

NcoI

M G S S H H H H H S S G L V P

5068 accatgggca gcagccatca tcatcatcat cacagcagcg gcctggttcc  
tgttaccgct cgtcggtagt agtagtagta gtgtcgtcgc cggaccaagg

R G S BseRI

5118 gcgtggtagt/attatgagtt **ctcctc**-----SACB cassette(2 kb)---  
cgcacatca/taatactcaa **gaggag**

BseRI stop HindIII XhoI

7127 **gaggag**atca tgcaca/tgat gacg**agctt** gcggccgcac **tcgag**cacca  
**ctcctc**tagt acgtgt/acta ctgct**tcgaa** cgccggcgtg **agctc**gtgga

7174 ccaccaccac cactgagatc cggctgctaa caaagcccga aaggaagctg  
gggggtgggt gtgactctag gccgacgatt gtttcgggct ttccttcgac

T7 REV

◀.....

7227 agttggctgc tgccaccgct gagcaataac tagcataacc ctttggggcc  
tcaaccgacg acggtggcga ctcgttattg atcgtattgg ggaaccccg