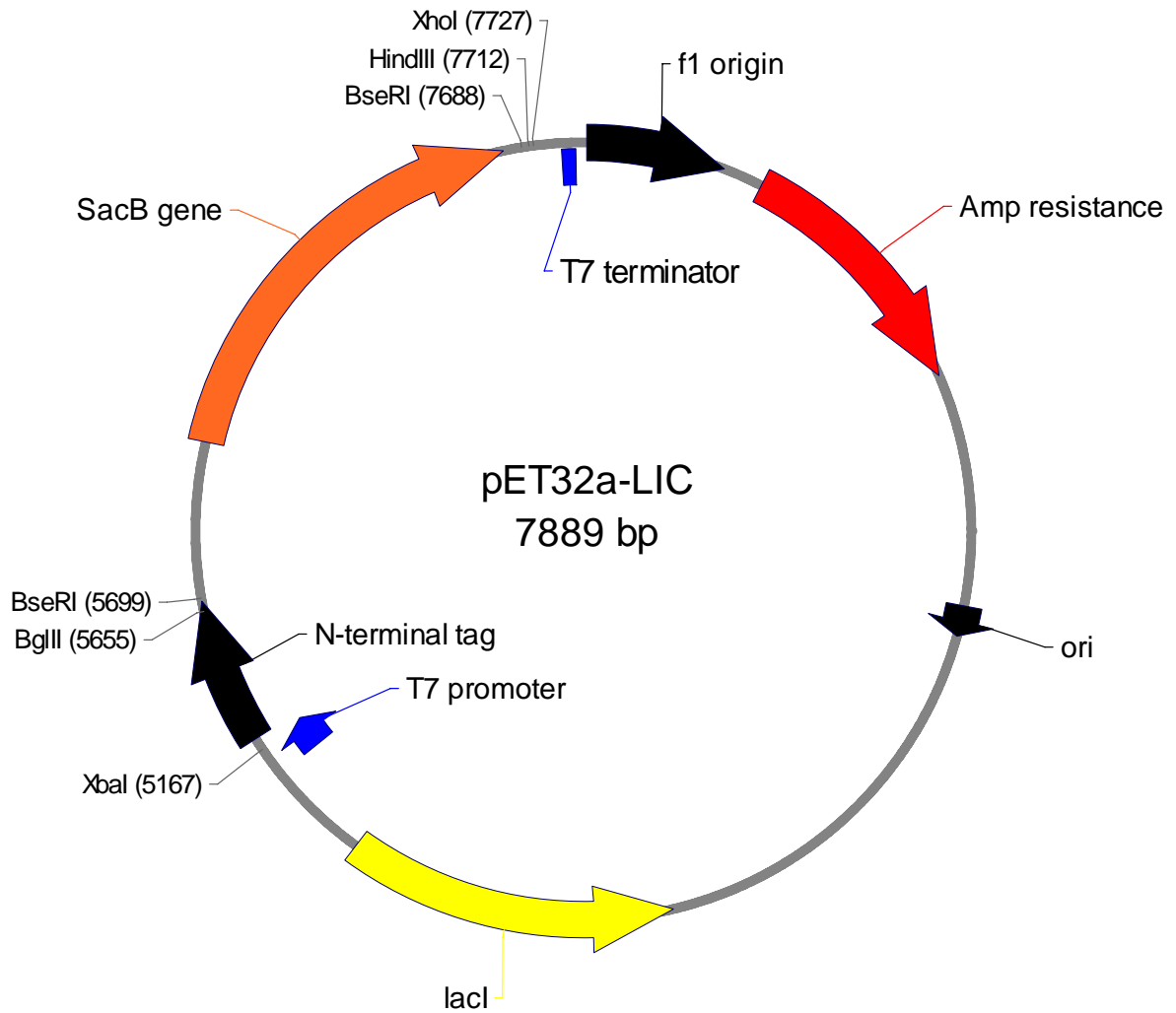


pET32a-LIC Vector (GenBank accession EF442786)

Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto
Description	The pET32a-LIC vector was derived from expression plasmid pET32a (Novagen). It is used for T7 promoter driven expression of recombinant proteins with the addition of a 160 amino acid N-terminal fusion tag containing the Trx-tag followed by a 6X His-tag followed by a thrombin protease cleavage site followed by the S-tag followed by a TEV protease cleavage site. Two stop codons are included in the vector at the C-terminal cloning site.
Antibiotic resistance	Ampicillin, 100 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	NdeI site in vector at 5209 bp
N – terminal fusion sequence	MSDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPC KMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGI RGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLA GSGSGHMH HHHH HSSGLVPRGSGMKETA AAK FERQ HMDSPDLGTENLYFQS
Termination codons	TGATGA included in 3' PCR primer and vector cloning site. No amino acid residues added at cloning junction
Additional features	
Prefered Hosts	
5' primer for amplification of insert	5' TTG TAT TTC CAG AGC --- 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'

pET32a-LIC vector map

T7 promoter	5121-5137
N-terminal tag	5209-5688
Trx-tag coding sequence	5209-5535
His-tag coding sequence	5557-5574
S-tag coding sequence	5608-5652
N-terminal cloning site	5674-5688
C-terminal cloning site	7704-7718
T7 terminator	7818-7864
f1 origin	12-467
<i>bla</i> coding sequence	599-1456
pBR322 origin	2217
<i>lacI</i> coding sequence	3651-4730
<i>sacB</i> coding sequence	6210-7628



pET32a-LIC cloning/expression region

```

                                T7 FWD
                                .....>
5105  ctcgatccccg  cgaaattaat  acgactcact  ataggggaat  tgtgagcgga
      gagctagggc  gctttaatta  tgctgagtga  tatccccctt  acactcgct

                                lac operator
                                ~~~~~

                                XbaI
                                ~~~~~
5155  taacaattcc  cctctagaaa  taatTTTgTT  taactTTtaag  aaggagatat
      attgTTaagg  ggagatcttt  attaaaacaa  attgaaattc  ttcctctata

                                RBS
                                _____

                                NdeI
                                _____
                                M  S
5205  acatattgagc  - - - (105 aa) - - - ctggccgggtt  ctggttctgg
      tgtatactcgc  - - - (315 bp) - - - gaccggccaa  gaccaagacc

                                Trx-tag
                                _____>
                                L  A  G  S  G  S  G

                                His-tag
                                _____>
                                H  M  H  H  H  H  H  S  S  G  L  V  P  R  G  S
5550  ccatatgcac  catcatcatc  atcattcttc  tggTctggTg  ccacgcggTt
      ggtatacgtg  gtagtagtag  tagtaagaag  accagaccac  ggtgcgccaa

                                thrombin
                                _____

                                S-tag
                                _____
                                G  M  K  E  T  A  A  A  K  F  E  R  Q  H  M  D
5600  ctggTatgaa  agaaaccgct  gctgctaaat  tcgaacgccca  gcacatggac
      gaccatactt  tctttggcgt  cgacgattta  agcttgcggT  cgtgtacctg

                                BglII
                                >
                                TEV
                                _____>
                                S  P  D  L  G  T  E  N  L  Y  F  Q  S
5650  agcccagatc  tgggtaccga  aaacttgtat  ttccagagc/c  atatgagttc
      tcgggtctag  acccatggct  tttgaacata  aaggTctcg/g  tataactcaag

                                BseRI
                                _____
5700  tcctc-----SACB cassette(2 kb)---  gaggagatca  tgcaca/tgat
      aggag  ctctcttagt  acgtgt/acta

                                HindIII
                                _____
                                XhoI
                                _____
7708  gacgaagctt  gcgggccgcac  tcgagcacca  ccaccaccac  cactgagatc
      ctgcttcgaa  cgccggcgtg  agctcgtgga  ggtggTggTg  gtgactctag

                                T7 REV
                                .....<
7758  cggctgctaa  caaagcccga  aaggaagctg  agttggctgc  tgccaccgct
      gccgacgatt  gtttcgggct  ttccttcgac  tcaaccgacg  acggTggcga

                                T7 REV
                                .....<
7817  gagcaataac  tagcataacc  cttggggcc  tctaaacggg  tcttgagggg
      ctcgTtattg  atcgTtattg  ggaaccccg  agatttgccc  agaactcccc

```