

## p28BIOHTEV-LIC Vector

Source	Constructed by Elena Dobrovetsky
Company	Structural Genomics Consortium, Toronto
Description	The p28BIOHTEV-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 TEV cleavable N-terminal AviTag for <i>in vivo</i> biotinylation and a C-terminal 6X HIS tag. Two stop codons are included in the vector at the C-terminal.
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	5071 bp
N – terminal fusion sequence	MSG LNDIFE AQKIEWHEGSAGGSGENLYFQG
C – terminal fusion sequence	GGSGHHHHHH**
Termination codons	TGATGA included in the vector.
Expression Host	BL21 DE3 with Biotin ligase expression vector
5' primer for amplification of insert	5' ttgtattccagggc --- 3'
3' primer for amplification of insert	5' atgaccactccacc --- 3' Do not add stop codon
5' sequencing primer T7-Fwd	5' AATTAATACGACTCACTATAGGG 3'
3' sequencing primer T7-Rev	5' ATGCTAGTTATTGCTCAGCGG 3'

p28BIOHTEV-LIC cloning/expression region

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                                T7 FWD                               lac operator
                                .....>.....
4968  ctcgatccccg  cgaaattaat  acgactcact  ataggggaat  tgtgagcgga
      gagctagggc  gctttaatta  tgctgagtga  tatcccctta  aactcgcct

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5018  taacaattcc  cctctagaaa  taatdddgtt  taactttaag  aaggagatat
      attgtdaagg  ggagatcttt  attaaaacaa  attgaaattc  ttctctata

      AviTag
      M S G L N D I F E A Q K I E W H
5068  accatgagcg  gcctgaacga  tattdddgaa  gcgcagaaaa  ttgaatggca
      tggtdactcgc  cggacttgct  ataaaaactt  cgcgtctddd  aacttaccgt

      Spacer                                TEV
      E G S A G G S G E N L Y F Q ^G
      tgaaggcagc  gctggagggt  caggtgaaaa  ctdgtatddd  cagggc/atta
      acttdcgtcg  cgacctcaa  gtccactddd  gaacataaag  gtcccg/taat

      BseRI                                BseRI      - G
      tgagtdctcc tc ---SACB(2 kb)---- gagggatca tgcaca/ggtg
      actcaagagg ag ---          ---- ctctctagt acgtgt/ccac

      Spacer      Hexa-His
      G S G H H H H H * * HindIII
      gaagtdgtca  tcaccaccat  catcactgat  gacgaagctt  gcggccgcac
      ctdcaccagt  agtdgtdgta  gtdagtdacta  ctgcttcgaa  cgccggtcgt

      XhoI
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      ctdtdggggcc
      ggaaccccgg

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>p28BIOHTEV-LIC sequence (7394 bp)

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