

## p28BIOH-LIC Vector (GenBank accession EF442785)

|                                       |  |
|---------------------------------------|--|
| Source                                | Constructed by Peter Loppnau   |
| Company                               | Structural Genomics Consortium, Toronto  |
| Description                           | The p28BIOH-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 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          | MSGLNDIFEAQKIEWHEGSAGGSG   |
| C – terminal fusion sequence          | GGSGHHHHHH**   |
| Termination codons                    | TGATGA included in the vector.   |
| Expression Host                       | BL21 with pCBIRA plasmid   |
| 5' primer for amplification of insert | 5' gctggaggttcaggt --- 3'  |
| 3' primer for amplification of insert | 5' atgaccacttcacc --- 3'<br>Do not add stop codon  |
| 5' sequencing primer T7-Fwd           | 5' AATTAATACGACTCACTATAGGG 3'  |
| 3' sequencing primer T7-Rev           | 5' ATGCTAGTTATTGCTCAGCGG 3'  |

p28BIOH-LIC cloning/expression region

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

~~~~~
5018  taacaattcc  cctctagaaa  taatthttg  taactttaag  aaggagatat
      attgttaagg  ggagatcttt  attaaaacaa  attgaaattc  ttctctata

      AviTag
      M S G L N D I F E A Q K I E W H
5068  accatgagcg  gcctgaacga  tatttttgaa  ggcgagaaaa  ttgaatggca
      tggactcgc  cggacttgct  ataaaaactt  cgcgtctttt  aacttaccgt

      spacer                                BseRI
      E G S A G G S G -
      tgaaggcagc  gctggagggt  caggt/attat  gagttctcct c -----
      acttcgctgc  cgacctcaa  gtcca/taata  ctcaagagga  g

-----SACB cassette (2 kb)----- BseRI - G
      gaggagatca  tgcaca/ggtg
      ctctcttagt  acgtgt/ccac

G S G H H H H H * * HindIII
gaagtgggtca  tcaccacat  catcactgat  gacgaagctt  gcggccgcac
cttcaccagt  agtgggtgta  gtagtgacta  ctgcttcgaa  cgccggcgtg

XhoI
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                                T7 term
                                <.....
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ccttggggcc
ggaaccccg
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>p28BIOH-LIC4 sequence (7373 bp)

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