pET28GSTlinker-LIC Vector

Source	Constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto
Description	The pET28GSTlinker-LIC vector was derived from expression plasmid pET28GST-LIC (SGC). It is used for T7 promoter driven expression of recombinant proteins with N-term GST-tag protein followed by a long linker and TEV cleavage site. The linker is helping to keeping GST tag free in DNA binding screening experiments. 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	NdeI site in vector
N terminal fusion sequence	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYER DEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSM AIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRY GVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCH KTYLNGDHVTHPDFMLYDALDVVLYMDPMCLD AFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGW QATFGGGDHPPKSDGSSEGDATMGHMVHRPFQS AASYPNRPSKTPPRAAESSGRENLYFQG
Termination codons	TGATGA included in 3' PCR primer and vector cloning site. No amino acid residues added at cloning junction
5' primer addition for amplification of insert	5' TTGTATTTCCAGGGC 3'
3' primer addition for amplification of insert	5' CAAGCTTCGTCATCA 3'
5' sequencing primer: pETGSTlinker-F or T7-fw	pETGSTlinker-F: 5' ATAAGTACTTGAAATCCAGC 3' T7-fw: 5' AATTAATACGACTCACTATAGGG 3'
3' sequencing primer: T7-rev	5' ATGCTAGTTATTGCTCAGCGG 3'

pET28GSTlinker-LIC cloning/expression region

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

      ~~~~~~~~~~   XbaI
5018  taacaattcc cctctagaaa taattttgtt taactttaag aaggagatat
      attgttaagg ggagatcttt attaaaacaa attgaaattc ttcctctata


                                GST-tag
       NdeI          ──────────────────────────────────▶
          M  S   P  I  L                  P  K  S   D  G  S
5068  acatatgtcc cctatacta- -(211 aa) - -ccaaaatc ggatggttca
      tgtatacagg ggatatgat- -(633 bp) - -ggttttag cctaccaagt

                  linker
        ──────────────────────────────────────▶

      S E  G   D   A T  M   G  H  M   V  H  R  P   F  Q  S
5738 tccgaaggag acgccaccat ggggcatatg gtgcacaggc ctttccaatc
     Aggcttcctc tgcggtggta ccccgtatac cacgtgtccg gaaaggttag


       A  A S   Y  P  N  R   P  S  K   T  P  P   R   A A  E
5788 cgcggccagt taccccaacc ggcccagcaa gacacccccc cgagctgcag
     Gcgccggtca agggggttgg ccgggtcgtt ctgtgggggg gctcgacgtc

                              TEV
                      ──────────────────▶
       S  S  G   R  E  N   L  Y  F  Q   G                BseR I
5838 agagcagcgg cagagaaaac ttgtatttcc agggc/catat gagtt ctcctc
     Tctcgtcgcc gtctcttttg aacataaagg tcccg/gtata ctcaa gaggag


                  ------ cassette (2 kb)------


      BseR I              stop     HindIII         XhoI
7872  gaggagatca tgcaca/tgat gacgaagctt gcggccgcac tcgagcacca
      ctcctctagt acgtgt/acta ctgcttcgaa cgccggcgtg agctcgtgga


7919  ccaccaccac cactgagatc cggctgctaa caaagcccga aaggaagctg
      ggtggtggtg gtgactctag gccgacgatt gtttcgggct ttccttcgac

                                T7 REV
                        ◀.......................................
7972  agttggctgc tgccaccgct gagcaataac tagcataacc ccttggggcc
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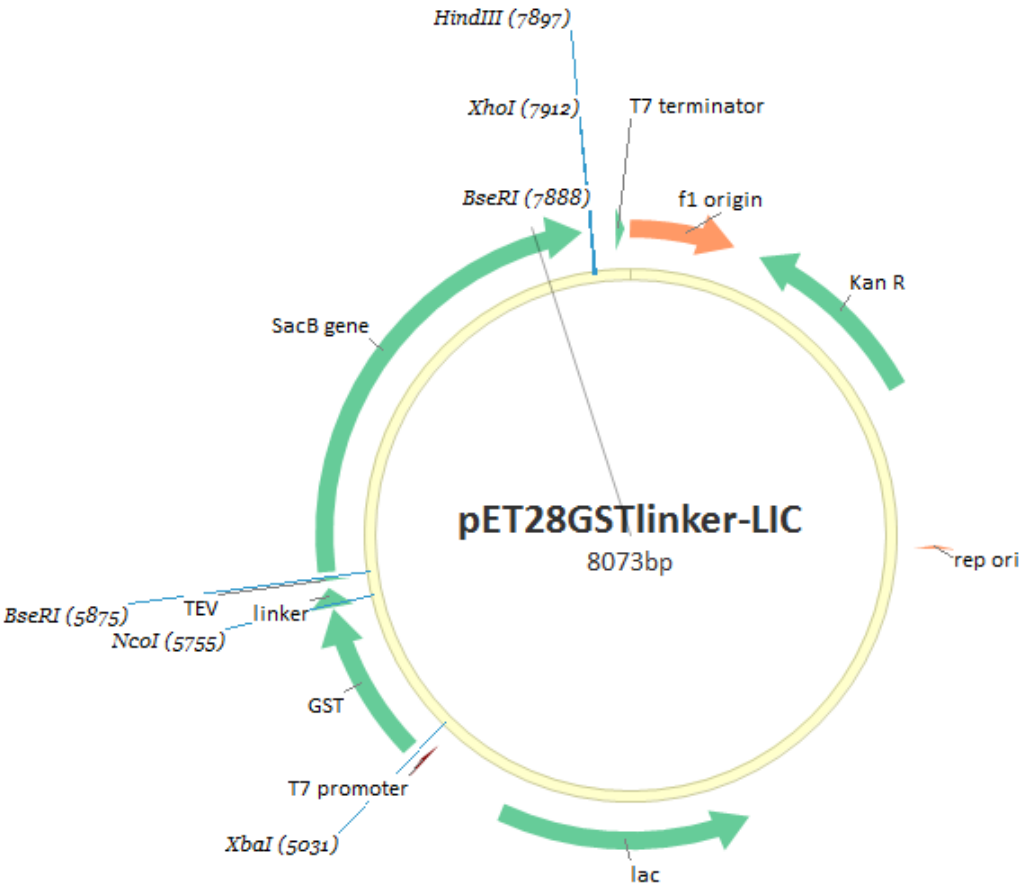
pET28GSTlinker-LIC vector map



Electronic Sequence of pET28GSTlinker-LIC:

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