

## 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 keep 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	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYER DEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSM AIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRY GVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCH KTYLNGDHVTHPDFMLYDALDVVLYMDPMCLD AFPKLVCFFKKRIEAIQIDKYLKSSKYIAWPLQGW QATFGGGDHPKSDGSS <b>EGDATMGHMVHRPFQS</b> <b>AASYPNRPSKTPPRAAESSGRENLYFQG</b>
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
                                .....▶
4968  ctcgatcccg cgaattaat acgactcact ataggggaat tgtgagcggg
      gagctagggc gctttaatta tgctgagtga tatcccctta acactcgct

      ~~~~~ XbaI
5018  taacaattcc cctctagaaa taattttggt taactttaag aaggagatat
      attgttaagg ggagatcttt attaaaaca 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) - -ggtttttag 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
      Aggcttcttc tgcggtggta ccccgatac  cacgtgtccg gaaaggttag

      A A S Y P N R P S K T P P R A A E
5788  cgcggccagt taccccaacc ggcccagcaa gacaccccc  cgagctgcag
      Gcgccgggtca aggggggttg ccgggtcgtt ctgtgggggg gctcgacgtc

                                TEV
                                -----▶
      S S G R E N L Y F Q G BseR I
5838  agagcagcgg cagagaaaac ttgtatttcc agggc/catat gagtt ctctc
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      ----- cassette (2 kb) -----

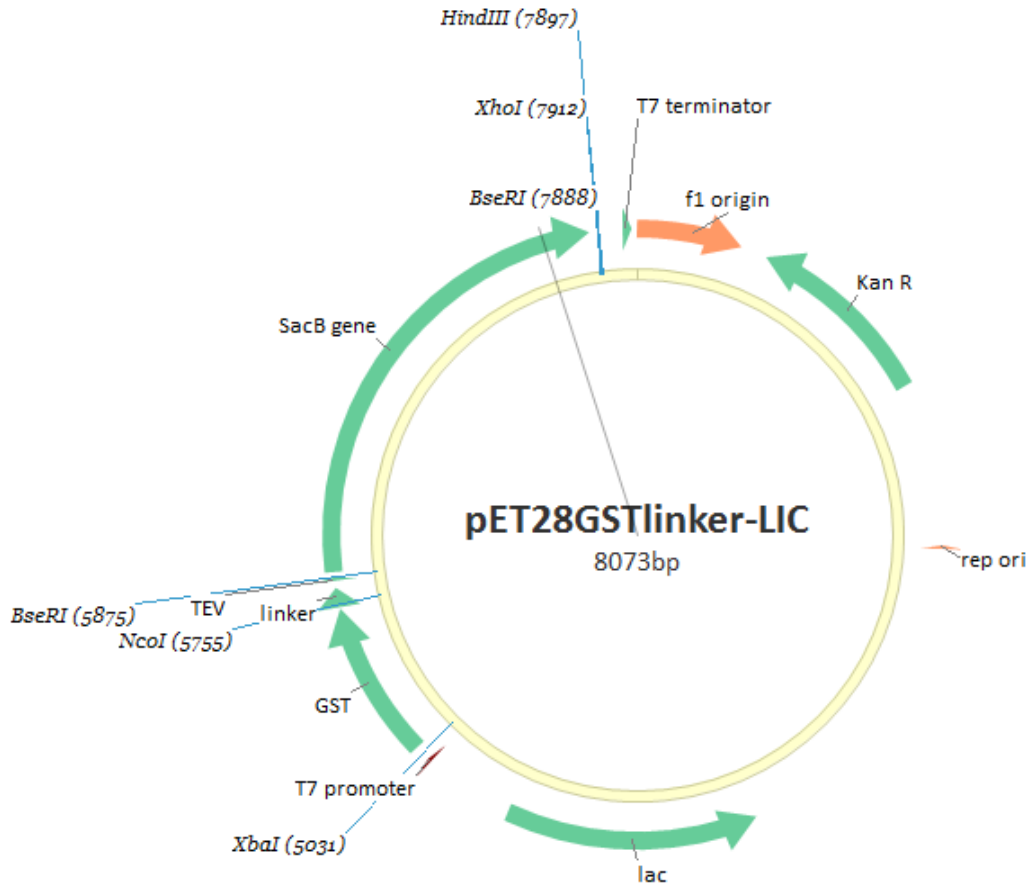
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                                T7 REV
                                ◀-----
7972  agttggctgc tgccaccgct gagcaataac tagcataacc cttgggggcc
      tcaaccgacg acgggtggcga ctcgttattg atcgtattgg ggaaccccg

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# pET28GSTlinker-LIC vector map



## Electronic Sequence of pET28GSTlinker-LIC:

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