pET28GST-LIC Vector (GenBank accession EF456739)

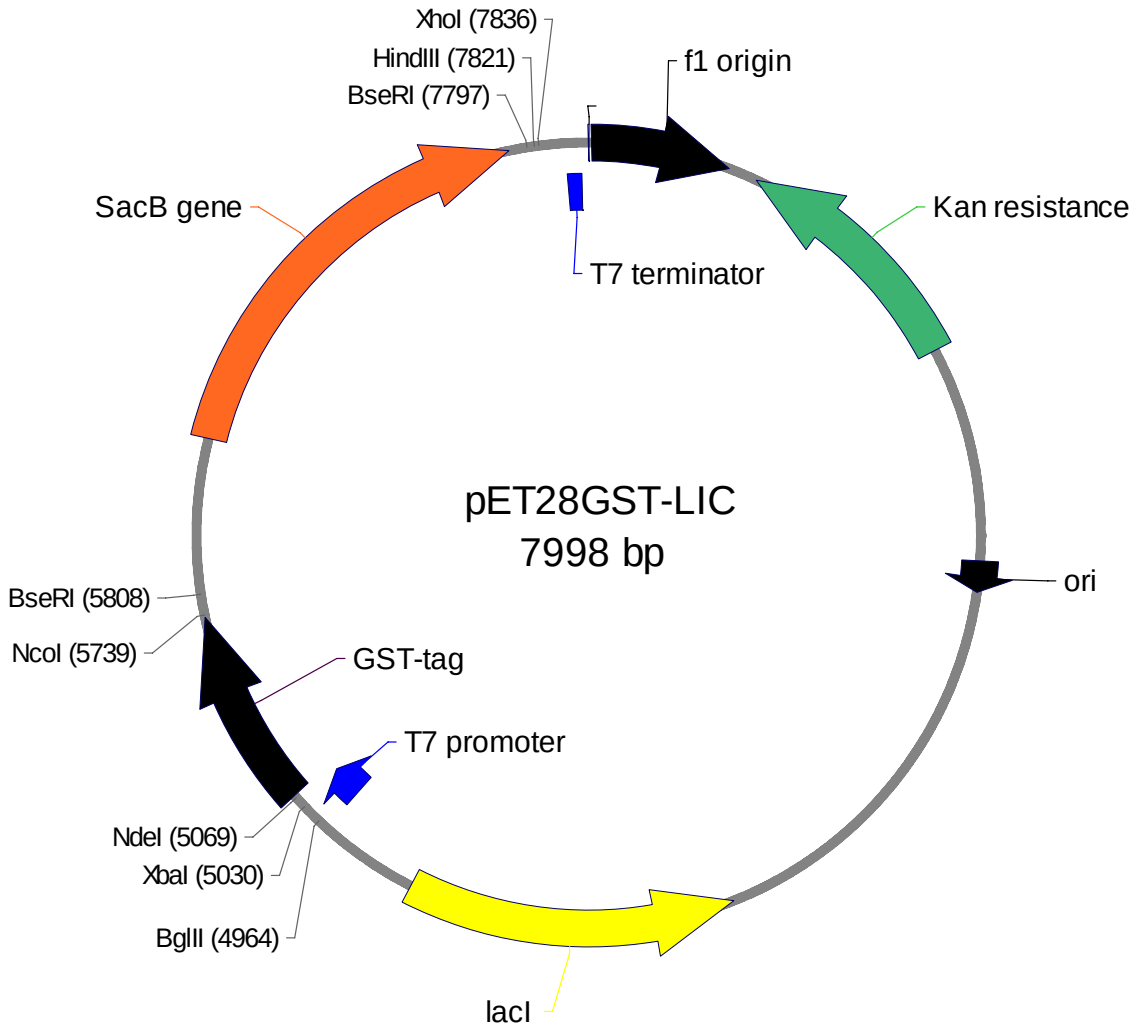
Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto

Description	The pET28GST-LIC vector was derived from expression plasmid pET28a-LIC (SGC) by inserting the GST-tag from pET41a (Novagen) into the XbaI and NcoI sites. It is used for T7 promoter driven expression of recombinant proteins with the addition of a 242 amino acid N-terminal fusion tag containing the 217 amino acid GST-tag protein followed by a 6X His followed by a thrombin cleavage site. 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	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDE GDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRY IADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAY SKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRI EAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD GSSMGSSHHHHHHSSGLVPRGS
Termination codons	TGATGA included in 3’ PCR primer and vector cloning site. No amino acid residues added at cloning junction
Additional features	
5’ primer for amplification of insert	5’ GTT CCG CGT GGT AGT --- 3’
3’ primer for amplification of insert	5’ CAA GCT TCG TCA TCA --- 3’
5’ sequencing primer pETGST-F	5’ ATCGGATGGTTCATCCATGG 3’
3’ sequencing primer T7-Rev	5’ ATGCTAGTTATTGCTCAGCGG 3’

pET28GST-LIC vector map

T7 promoter	4984-5000
N-terminal tag	5072-5797
GST-tag	5081-5731
HIS-tag	5753-5770
Thrombin recognition site	5780-5797
N-terminal cloning site	5783-5797
C-terminal cloning site	7813-7827
T7 terminator	7927-7973
f1 origin	12-467
aph coding sequence	563-1375
pBR322 origin	2084
lacI coding sequence	3518-4597
sacB coding sequence	6319-7737



pET28GST-LIC cloning/expression region