

## pET28-MHL Vector (GenBank accession EF456735)

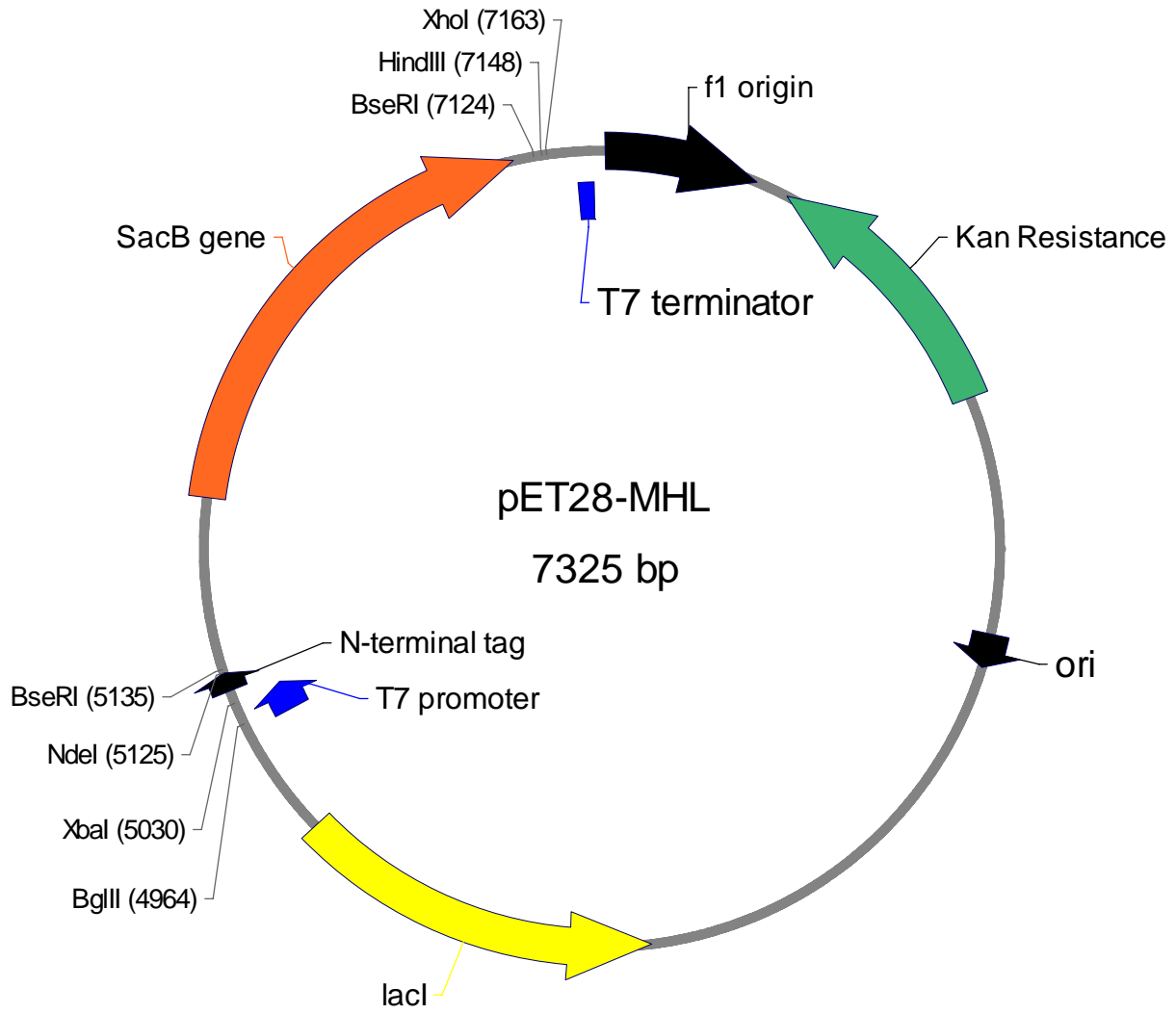
Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto

Description	The pET28-MHL vector was derived from expression plasmid pET28a-LIC (SGC). It is used for T7 promoter driven expression of recombinant proteins with the addition of an 18 amino acid N-terminal fusion tag containing 6X His followed by a TEV cleavage site. The GSS residues after the Met start site were removed to reduce N-terminal gluconoylation via preventing N-terminal Met excision. Two stop codons are included in the vector at the C-terminal cloning site.
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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	ATG site in vector at bp 5071, NcoI site was destroyed
N – terminal fusion sequence	MHHHHHSSGRENLYFQG
Termination codons	TGATGA included in 3' PCR primer and vector cloning site. No amino acid residues added at cloning junction
Additional features	
Prefered Hosts	
5' primer for amplification of insert	5' TTG TAT TTC CAG GGC --- 3'
3' primer for amplification of insert	5' CAA GCT TCG TCA TCA --- 3'
5' sequencing primer T7-Fwd	5' AATTAATACGACTCACTATAGGG 3'
3' sequencing primer T7-Rev	5' ATGCTAGTTATTGCTCAGCGG 3'

pET28-MHL vector map

T7 promoter	4984-5000
N-terminal tag	5071-5124
N-terminal cloning site	5110-5124
C-terminal cloning site	7140-7154
T7 terminator	7254-7300
f1 origin	12-467
<i>aph</i> coding sequence	563-1375
pBR322 origin	2084
<i>lacI</i> coding sequence	3518-4597
<i>sacB</i> coding sequence	5646-7064



pET28-MHL cloning/expression region

T7 FWD lac operator

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4968 ctcgatcccg cgaaattaat acgactcact ataggggaat tgtgagcggg  
gagctagggc gctttaatta tgctgagtga tatcccctta acactcgct

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5018 taacaattcc cctctagaaa taattttggt taactttaag aaggagatat  
attgttaagg ggagatcttt attaaaacaa attgaaattc ttcctctata

M H H H H H H S S G R E N L Y F

5068 accatgcatc atcatcatca tcacagcagc ggcagagaaa acttgatatt  
Tggtacgtag tagtagtagt agtgctgctg ccgtctcttt tgaacataaa

Q G NdeI BseRI

5118 ccagggc/**cat atgagtt ctctctc**-----SACB cassette(2 kb)---  
Ggtcccg/**gta tactcaa gaggag**

BseRI stop HindIII XhoI

7124 **gaggagatca** tgcaca/tgat gacga**aagctt** gcggccgcac **tcgag**cacca  
**ctctctctag**t acgtgt/acta ctgct**tcgaa** cgccggcgtg **agctc**gtgga

7171 ccaccaccac cactgagatc cggctgctaa caaagcccga aaggaagctg  
ggtgggtggtg gtgactctag gccgacgatt gtttcgggct ttccttcgac

T7 REV

◀.....

7224 agttggctgc tgccaccgct gagcaataac tagcataacc ctttggggcc  
tcaaccgacg acggtggcga ctctgttattg atcgtattgg ggaaccccg