

## pET15-MHL Vector (GenBank accession EF456738)

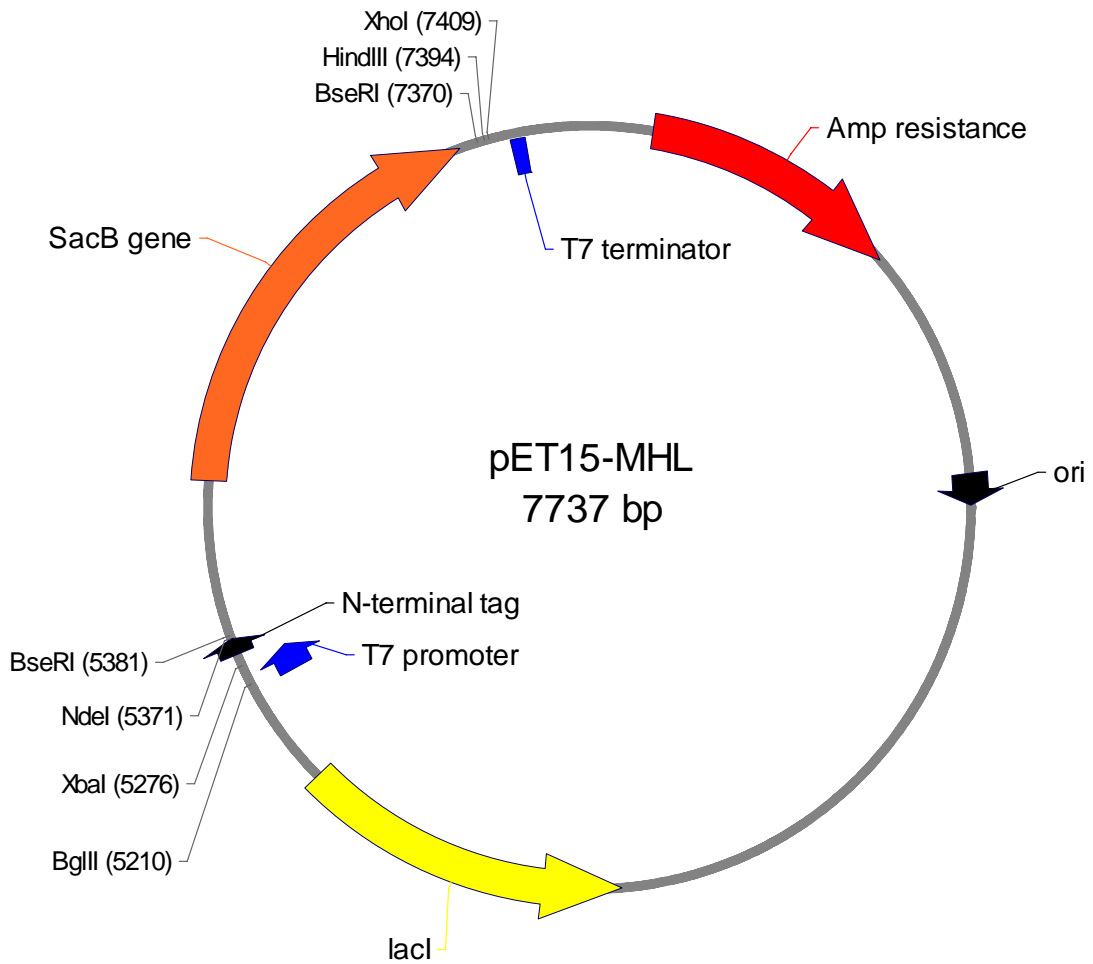
Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto

Description	The pET15-MHL vector is a derivative of pET15b (Novagen) and is for infusion based cloning. 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	Ampicillin 100 ug/ml
Promoter	T7 - lacO
Cloning Method	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 5317, NcoI site was destroyed
N – terminal fusion sequence	MHHHHHHSSGRENLYFQG
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' AAT TAA TAC GAC TCA CTA TAG GG 3'
3' sequencing primer T7-Rev	5' ATG CTA GTT ATT GCT CAG CGG 3'

# pET15-MHL vector map

T7 promoter	5230-5246
N-terminal tag	5317-5370
N-terminal cloning site	5356-5370
C-terminal cloning site	7386-7400
T7 terminator	7479-7525
<i>bla</i> coding region	209-1066
pBR322 origin	1827
<i>lacI</i> coding sequence	3764-4843
<i>sacB</i> coding sequence	5892-7310



pET15-MHL cloning/expression region

T7 FWD

5214 ctcgatcccc cgaataat acgactcact ataggggaat tgtgagcgga  
gagctagggc gctttaatta tgctgagtga tatcccctta acactcgct

XbaI

5264 taacaattcc cctctagaaa taatthtggg taactttaag aaggagatat  
attgttaagg ggagatcttt attaaaacaa attgaaattc ttcctctata

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

5314 accatgcatc atcatcatca tcacagcagc ggcagagaaa acttgtattt  
tggtacgtag tagtagtagt agtgtcgtcg ccgtctcttt tgaacataaa

Q G NdeI BseRI

5364 ccagggc/cat atgagttctc ctc-----SACB(2 kb)-----  
ggtcccg/gta tactcaagag gag

BseRI stop HindIII XhoI

7370 gaggagatca tgcaca/tgat gacgaagctt gcggccgcac tcgaggatcc  
ctcctctagt acgtgt/acta ctgcttcgaa cgccggcgtg agctcctagg

7420 ggctgctaac aaagcccgaag aggaagctga gttggctgct gccaccgctg  
ccgacgattg tttcgggctt tccttcgact caaccgacga cggtgggcagc

T7 REV

7470 agcaataact agcataacc cttggggcct ctaaaccgggt cttgaggggt  
tcgattattga tcgtattggg gaaccccgga gatttgcca gaactcccca