

pET28-MKH8SUMO Vector (GenBank accession N/A)

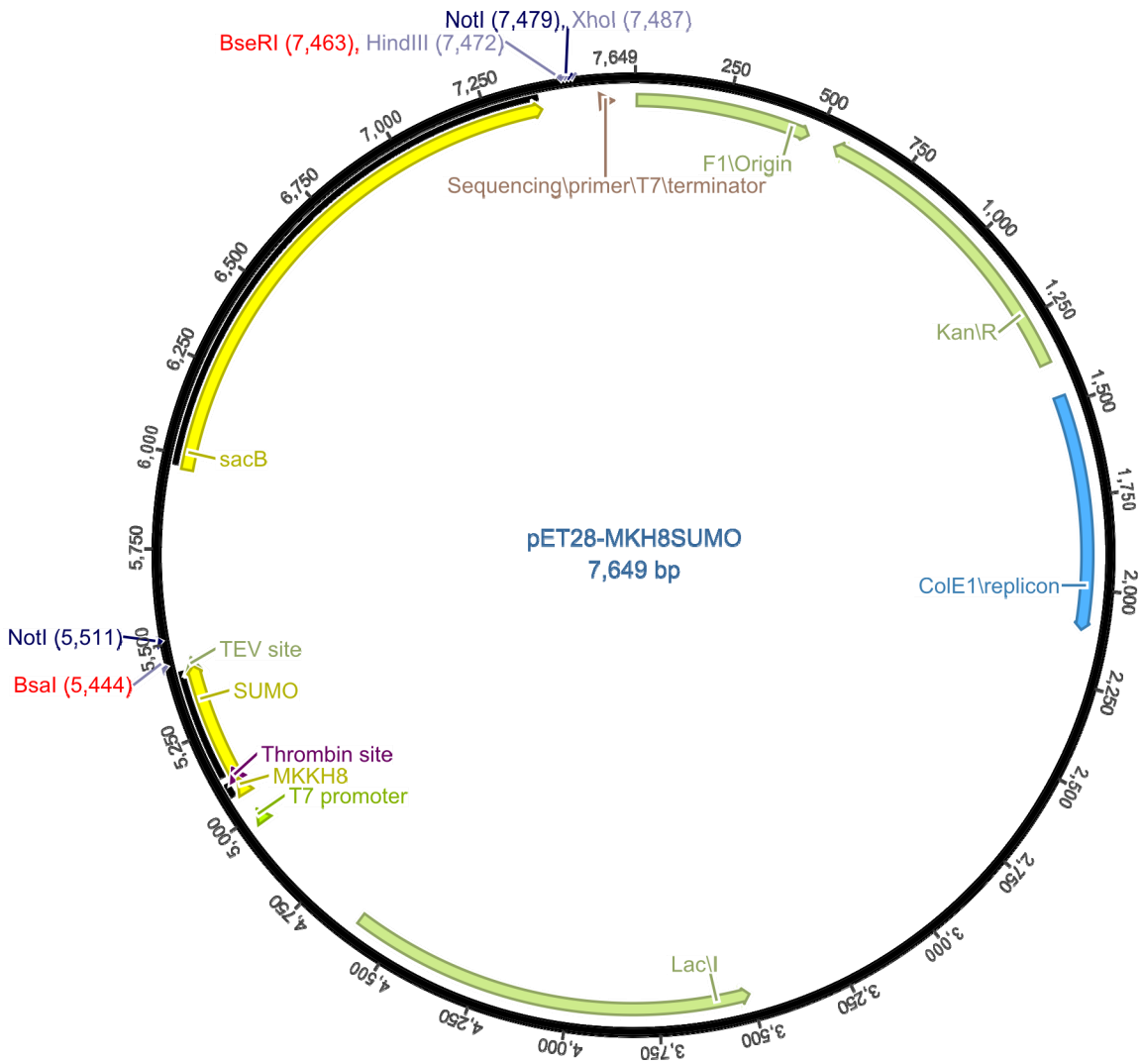
Source	Constructed by Yufeng Tong
Company	Structural Genomics Consortium, Toronto

Description	The pET28-MKH8SUMO 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 N-terminal fusion tag containing 8X His followed by a thrombin cleavage site, a SUMO, and a TEV cleavage site. Two lysines were encoded after the Met start site. Two stop codons are included in the vector at the downstream cloning site.
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Antibiotic resistance	Kanamycin, 50 ug/ml
Promoter	T7 - lacI
Cloning Methods	Insertion of DNA sequence into the cloning/expression region is preformed using ClonTech In-Fusion™ enzyme mediated directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and BseRI/BsaI 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 5070, NcoI site was destroyed
N – terminal fusion sequence	<i>mkkhhhhhhhhssglvprgmsdsevnqeakpevkpevkpethinlkvsdg sseiffkikkttplrrlmeafakrqgkemdslrflydgiriqadqtpedldmedn diieahreqiggtlenlyfqg</i>
Termination codons	TGATGA included in 3' PCR primer and vector cloning site. No amino acid residues added at cloning junction
Additional features	The 15-bp primer extensions are compatible with those for pET28-MHL, such that the PCR inserts prepared for pET28-MHL can be directly used for cloning into pET28-MKH8SUMO.
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'

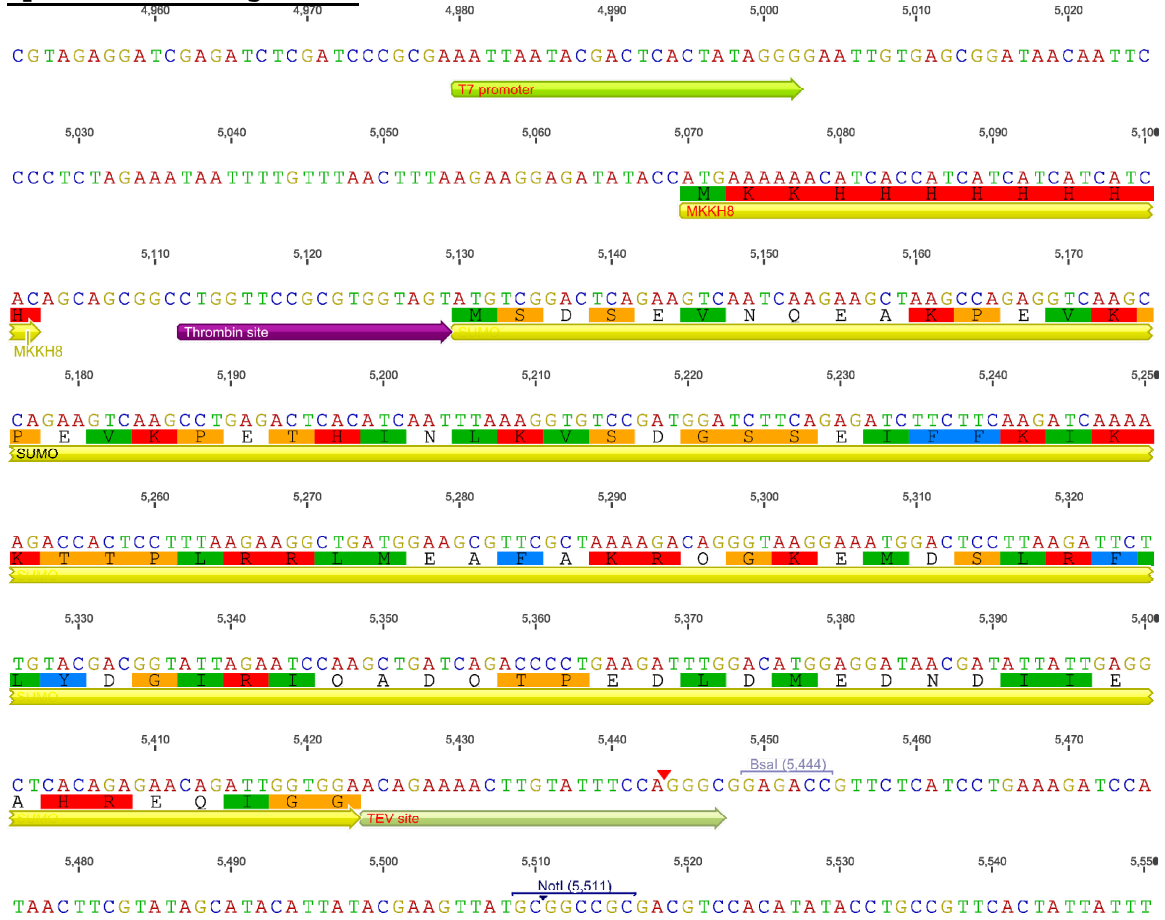
pET28-MKH8SUMO vector map

T7 promoter	4980-5002
N-terminal tag	5070-5447
Upstream BsaI site	5454-5449
Downstream BseRI site	7140-7154
T7 terminator	7254-7300
f1 origin	12-467
<i>KmR</i> coding sequence	562-1374
<i>ColE1</i> replicon	1485-2099
<i>lacI</i> coding sequence	3517-4596
<i>sacB</i> coding sequence	5969-7387



pET28-MKH8SUMO cloning/expression region

Upstream cloning site:



Downstream cloning site:

