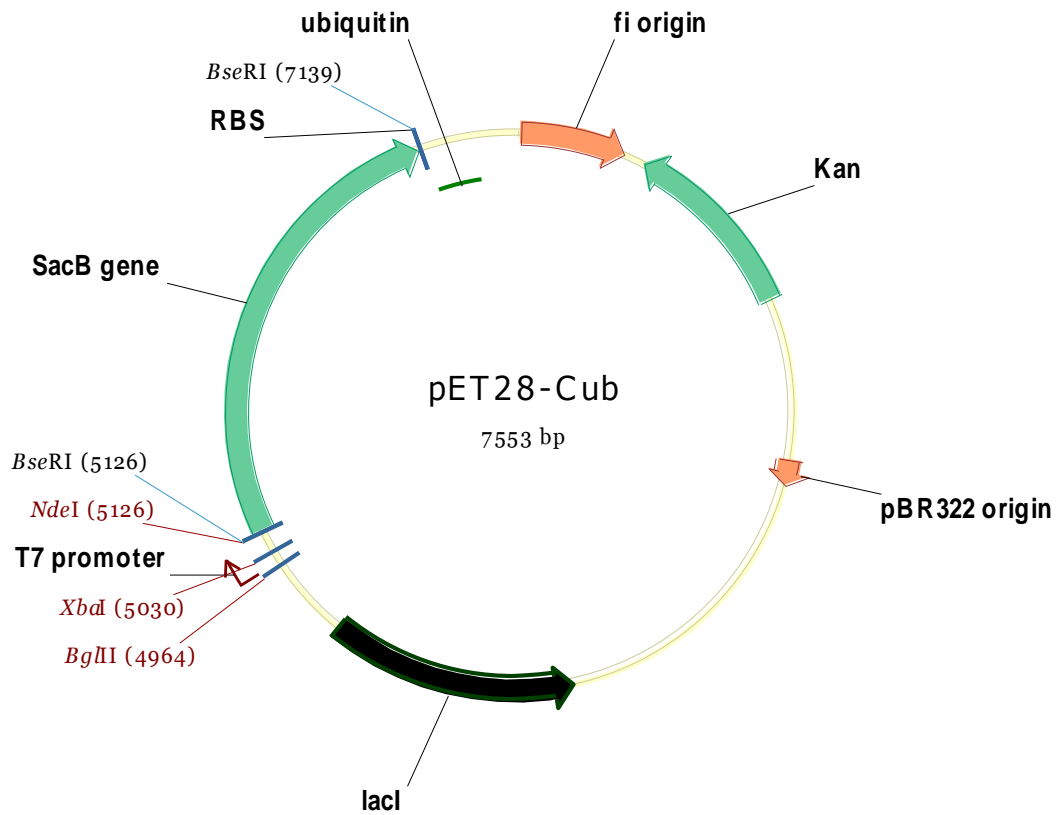


pET28-Cub Vector

Source	Constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto
Description	The pET28-Cub vector was derived from expression plasmid pET28-MHL (made by Peter Loppnau, SGC). It is designed for T7 promoter driven co-expression of recombinant proteins with ubiquitin located at c-terminal. The C-terminal ubiquitin is preceded by the ribosomal binding site (RBS) and followed by two stop codons. The pET28-Cub keeps the features of pET28-MHL as addition of 18 amino acid N-terminal fusion tag containing 6X His followed by a TEV cleavage site as well as removing of the GSS residues after the Met start site to reduce N-terminal gluconoylation via preventing N-terminal Met excision.
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.
N – terminal fusion sequence	MHHHHHSSGRENLYFQG
Termination codons	TGATGA is right downstream of ubiquitin located at C terminal of this vector.
5' primer for amplification of insert	5' TTGTATTTCCAGGGC 3'
3' primer for amplification of insert	5'TATCTCCTTCTTTTA 3'
5' sequencing primer T7-Fwd	5' AATTAATACGACTCACTATAGGG 3'
3' sequencing primer T7-Rev	5' ATGCTAGTTATTGCTCAGCGG 3'
Primer mix for screening	T7- forward and reverse mix

pET28-Cub vector map:

T7 promoter	4985-4980
N-terminal tag	5071-5124
N-terminal cloning site	5109-5123
C-terminal cloning site	7139-7153
T7 terminator	7481- 7528
f1 origin	12-466
KanR coding sequence	563-1374
pBR322 origin	2087
lacI coding sequence	3517-4596
SacB coding sequence	5140-7122



pET28-Cub cloning/expression region:

T7 FWD → lac operator
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4967 ctcgatcccg cgaaattaat acgactcact ataggggaat tgtgagcgga  
gagctagggc gctttaatta tgctgagtga tatcccctta acactcgct  
~~~~~

5017 taacaattcc cctctagaaa taatTTTgTT taactTTaag aaggagatat
attgTTaagg ggagatctTT attaaaacaa attgaaattc tTcctctata

M H H H H H H S S G R E N L Y F
5' addition

5067 accatgcatc atcatcatca tcacagcagc ggcagagaaa actTgtattT
Tggtacgtag tagtagtagt agtgtcgtcg ccgtctctTT tgaacataaa

Q G NdeI BseRI

5117 ccagggc/cat atgagtt ctctc-----SACB cassette(1983bp)---
Ggtcccg/gta tactcaa gaggag

BseRI RBS 2xSTOP

7123 gaggagatca tgcaca/TAAAAGAAGGAGATATACC/ubiquitin(228bp)/TGATGA
ctcctctagt acgtgt/ATTTTCTTCCTCTATATGG/ubiquitin(228bp)/ACTACT
3' addition

7392 tcgagcacca ccaccaccac cactgagatc cggctgctaa caaagcccga

T7 REV ←

7442 aaggaagctg agttggctgc tgccaccgt gagcaataac tagcataacc

Whole Sequence of pET28-Cub: 7553 bp

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