

### pET28-SBP-TEV Vector

Source	Constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto
Description	pET28-SBP-tev is an <i>E. coli</i> expression vector derived from vector pET28-SBP (Farrell MacKenzie, SGC) and has a T7 promoter that drives expression of recombinant proteins with the addition of an N-terminal 6xHis-tag and SBP-tag followed by TEV cleavage site. Two stop codons are included in the vector at the C-terminal cloning site.
Antibiotic resistance	Kanamycin
Promoter	T7 - lacO
Cloning Methods	Insertion of a DNA sequence into the cloning/expression region is performed using Clontech's In-fusion enzyme-mediated directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and BseRI linearized vector. Insertion of a 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	MHHHHHHEFMDEKTTGWRGGHVVEGLAGELEQL-RARLEHHPQQQREPSSGRENLVYFQG
5' primer tail for amplification of insert	5' TTGTATTTCCAGGGC --- 3'
3' primer tail for amplification of insert	5' CAAGCTTCGTCATCA --- 3'
5' sequencing primer T7	5' AATTAATACGACTCACTATAGGG 3'
3' sequencing primer T7term	5' ATGCTAGTTATTGCTCAGCGG 3'

**pET28-SBP-TEV sequence (7445bp):**

TGGCGAATGGGACGCGCCCTGTAGCGGGCGCATTAAAGCGCGGGCGGGTGTGGTGGTTACGCGCA  
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTC  
GCCACGTTCCGCCGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCGGATTTA  
GTGCTTTACGGCACCTCGACCCAAAAAATTGATTAGGGTGATGGTTCACGTAGTGGGCCAT  
CGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTT  
GTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTATAAGGGATTTTG  
CCGATTTCCGGCTATTGGTTAAAAAATGAGCTGATTAAACAAAAATTTAACCGGAATTTTAAC  
AAAATATTAACGTTTACAATTTACAGGTGGCACTTTTCGGGGAAAATGTGCGCGGAACCCCTATT  
TGTTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAATTAATTCCTTAGAAAACTCA  
TCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAA  
AGCCGTTTCTGTAATGAAGGAGAAAACCTACCGAGGCAGTTCATAGGATGGCAAGATCCTG  
GTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCTCGTCAAAA  
ATAAGGTTATCAAGTGAGAAAATCACCATGATGACGACTGAATCCGGTGAGAATGGCAAAAG  
TTTATGCATTTCTTCCAGACTTGTTCAACAGGCCATTACGCTCGTCAAAAATCACTC  
GCATCAACCAACCGTTATTCATTTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTG  
TTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATC  
AACAAATTTTTACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATC  
GCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAAATGCTTGATGGTCGGAAGAGG  
CATAAATTCGCTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCT  
TTGCCATGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCA  
CCTGATTGCCCGACATTATCGCGAGCCATTTATACCATATAAATCAGCATCCATGTTGGAA  
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TGTTTTATGTAAGCAGACAGTTTTATTGTTTCATGACCAAAATCCCTAACGTGAGTTTTTCGTTCC  
ACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCG  
TAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCGGTGGTTTTGTTTGCCGGATCAAG  
AGTACCAACTCTTTTTCCGAAGGTAACCTGGCTTACGACAGAGCGCAGATACCAAATACTGTCC  
TTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCCGCTACATACCTCG  
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ACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGGCTGAACGGGGGGTTTCGTGCACA  
CAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGA  
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ACTGAAGATGGCTACCAAGGCGAAGAATCTTTATTTA  
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TAACGATGTAACCTTTACTTACTCACACTTCGCTGTACCTCAAGCGAAAGGAAACAATGTCGT  
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 GCTTCCTGCTGAACATCAAAGGCAAGAAAACATCTGTTGTCAAAGACAGCATCCTTGAACAA  
 GGACAATTAACAGTTAACAAATAAAAACGCAAAAGAAAATGCCGATATCCTATTGGCATTGA  
 CGTCAGGTGGCACTTTTCGAGGAGATCATGCACATGATGACGAAGCTTGCGGGCCGCACTCGAG  
 CACCACCACCACCACCCTGAGATCCGGCTGCTAACAAAGCCCCGAAAGGAAGCTGAGTTGGC  
 TGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAACGGGTCTTGAGGGG  
 TTTTTTGCTGAAAGGAGGAACCTATATCCGGAT

### pET28-SBP-TEV vector map

T7 promoter: 4984-5003  
 N-terminal tag (6x His Tag-SBP-TEV): 5071-5244  
 T7 terminator: 7373-7420  
 f1 origin (reverse complementary): 400-449  
 pBR322 origin: 1504-1544  
*SacB* coding sequence: 5262-7243  
 KanR: 563-1375  
 Lac I coding sequencing: 3515-4597 (1083 bp)

