

Vector information sheet.

Vector Name	pBEN1-SGC
Source	Opher Gileadi
Sequence accession/link	(SGC)
Description	pET expression vector with a 94-aa N-terminal fusion derived from pBEn-SBP-SET1a (Stratagene) followed by a TEV protease cleavage site. This tag allows purification on streptavidine columns, and may enhance solubility of fusion proteins. The vector includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection on 5% sucrose.
Antibiotic resistance	Kanamycin, 50 µg/ml
Promoter	T7 - lacO
Cloning	LIC. (vector treated with BsaI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	1 MDPEEASVTS TEETLTPAQE AARTRAANKA 31 RKEAELAAAT AEQTSDEKTT <i>GWRRGGHVVEG</i> 61 <i>LAGELEQLRA</i> <i>RLEHHPQGQR</i> <i>EPSGGCKLGL</i> 91 <i>GLGTENLYFQ</i> SM (SET1 tag: bold; <i>SBP-tag: ital, underline</i>; TEV: bold, underline, cleavage after Q.)
N-terminal fusion – MW	MW of tag (including SM): 10,947.45 Da.
Termination codons	supplied in PCR primer
Protease cleavage	TEV
Additional features	SET1 tag: solubility enhancement tag. SBP -tag: streptavidine binding protein.
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pBEN-for: ACCACCCTCAGGGCCAGCGG
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC

Primers for LIC cloning:

Upstream: add TACTTCCAATCCATG to the 5' end (ATG in-frame with the desired coding sequence).

Downstream: add TATCCACCTTTACTG to 5' end of downstream primer; add termination codon, if necessary.

Tag sequence and cloning junctions:

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                                SET1 tag
                                ~~~~~
                                NdeI
                                ~~~~~
                                M D P E E A S V T S T E E T L T P A .
1  TATACATATG GACCCCGAAG AGGCGAGTGT TACTTCAACA GAAGAAACCT TAACGCCAGC
   ATATGTATAC CTGGGGCTTC TCCGCTCACA ATGAAGTTGT CTTCTTTGGA ATTGCGGTCTG

                                SET1 tag
                                ~~~~~
                                .Q E A A R T R A A N K A R K E A E L A A .
61 ACAGGAGGGCC GCACGCACCC GCGCTGCTAA CAAAGCCCGA AAGGAAGCTG AGTTGGCTGC
   TGTCTCCGG CGTGCCTGGG CGCGACGATT GTTTCGGGCT TTCCTTCGAC TCAACCGACG

                                SET1 tag                                SBP tag
                                ~~~~~                                ~~~~~
                                .A T A E Q T S D E K T T G W R G G H V V .
121 TGCCACCCTG GAACAACTA GTGACGAGAA GACCACCGGC TGGCGGGGCG GCCACGTGGT
   ACGGTGGCGA CTTGTTTGAT CACTGCTCTT CTGGTGGCCG ACCGCCCGCG CGGTGCACCA

                                SBP tag
                                ~~~~~
                                .E G L A G E L E Q L R A R L E H H P Q G .
181 GGAGGGCCTG GCCGGCGAGC TGGAGCAGCT GCGGGCCAGG CTGGAGCACC ACCCTCAGGG
   CCTCCCGGAC CGGCCGCTCG ACCTCGTCGA CGCCCGGTCC GACCTCGTGG TGGGAGTCCC

                                SBP tag                                TEV site
                                ~~~~~                                ~~~~~
                                .Q R E P S G G C K L G L G T E N L Y F Q .
241 CCAGCGGGGAG CCCTCCGGCG GCTGCAAGCT GGGCCTGGGT ACCGAGAACC TGTACTTCCA
   GGTCGCCCTC GGGAGGCCGC CGACGTTTCA CCCGGACCCA TGGCTCTTGG ACATGAAGGT
                                TAG                                ~~~~~
                                ~~~~~                                Lic5

                                NcoI
                                ~~~~~
                                .S M
301 ATCCATGGAG ACCG ----- SacB -----
   TAGGTACCTC TGGC
                                ~~~~~
                                Lic5

                                BsaI                                BamHI                                EcoRI                                SalI                                NotI
                                ~~~~~                                ~~~~~                                ~~~~~                                ~~~~~                                ~~~~~
                                ATTGACGGTC TCCAGTAAAG GTGGATACGG ATCCGAATTC GAGCTCCGTC GACAAGCTTG
2221 TAACTGCCAG AGGTCATTTC CACCTATGCC TAGGCTTAAG CTCGAGGCAG CTGTTTGAAC
                                ~~~~~
                                Lic3

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