

Vector information sheet

Dated: 8th May 2013

Vector Name	pBEN1-SGC
Source	Opher Gileadi
Sequence accession/link	GenBank GU725055

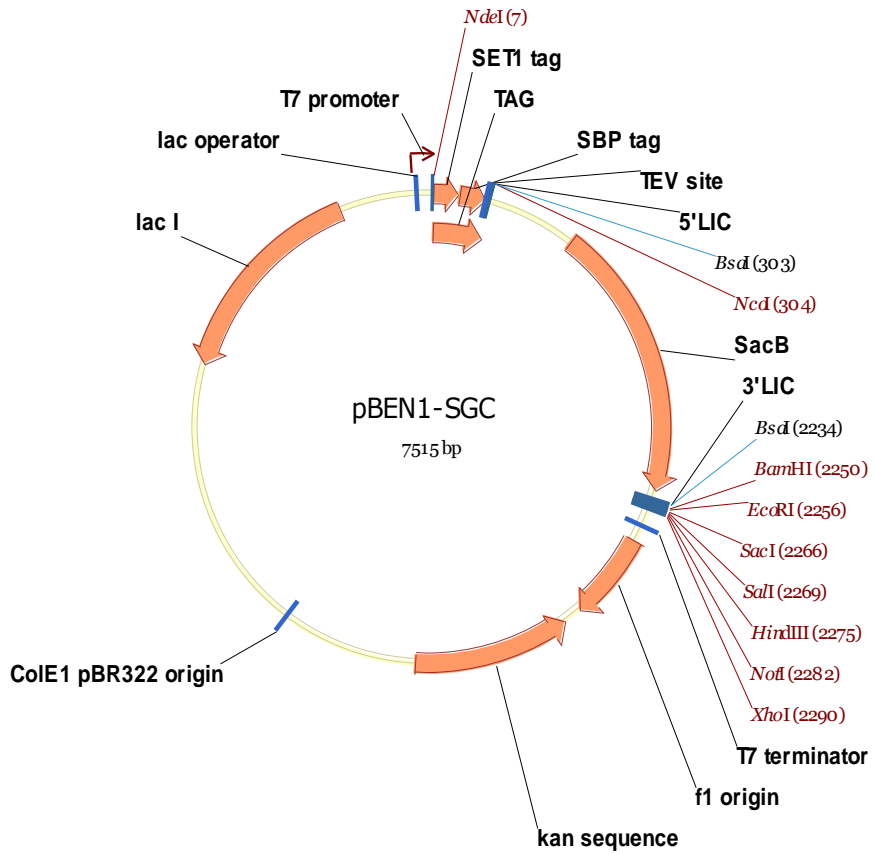
Description	<p>pET expression vector with a 94-aa N-terminal fusion derived from pBEN-SBP-SET1a (Stratagene) followed by a TEV protease cleavage site. This tag allows purification on streptavidine columns, and may enhance solubility of fusion proteins.</p> <p>The vector includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection on 5% sucrose.</p>
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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.	<p>1 MDPEEASVTS TEETLTPAQE AARTRAANKA</p> <p>31 RKEAELAAAT AEQTSDEKTT <i>GWRGGHVVEG</i></p> <p>61 <i>LAGELEQLRA RLEHHPQGQR EPSGGCKLGL</i></p> <p>91 GLGTENLYFQ SM</p> <p>(SET1 tag: bold; <i>SBP-tag: ital, underline</i>; TEV: bold, underline, cleavage after Q.)</p>
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: ACCACCCTCAGGCCAGCGG
3' sequencing primer	pLIC-rev: AGCAGCCAACCTCAGCTTCC

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.



Polylinker region:

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

                                SET1 tag
                                ~~~~~
61 ·Q E A A R T R A A N K A R K E A E L A A
ACAGGAGGCC GCACGCACCC GCGCTGCTAA CAAAGCCCGA AAGGAAGCTG AGTTGGCTGC
TGTCCTCCGG CGTGCGTGGG CGCGACGATT GTTTCGGGCT TTCCTTCGAC TCAACCGACG

                                SET1 tag                                SBP tag
                                ~~~~~                                ~~~~~
121 ·A T A E Q T S D E K T T G W R G G H V V
TGCCACCGCT GAACAAACTA GTGACGAGAA GACCACCGGC TGGCGGGGCG GCCACGTGGT
ACGGTGGCGA CTTGTTTGAT CACTGCTCTT CTGGTGGCCG ACCGCCCCGC CGGTGCACCA

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

                                SBP tag                                TEV site
                                ~~~~~                                ~~~~~
241 ·Q R E P S G G C K L G L G T E N L Y F Q
CCAGCGGGAG CCCTCCGGCG GCTGCAAGCT GGCCTGGGT ACCGAGAACC TGTACTTCCA
GGTCGCCCTC GGGAGGCCGC CGACGTTCGA CCCGGACCA TGGCTCTGG ACATGAAGGT
TAG
~~~~~
                                Lic5

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

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

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pBEN-SGC-1 sequence:

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