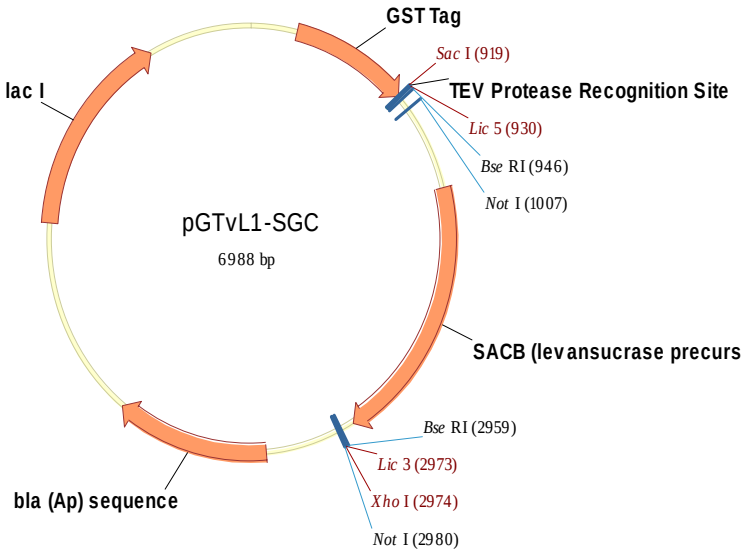
Vector information sheet.

Vector Name	**pGTvL1-SGC**
Source	Jonathan Elkins (SGC, Oxford)
Sequence accession/link	(SGC)

Description	pGEX expression vector with N-terminal GST tag and TEV protease cleavage site. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection on 5% sucrose.

Antibiotic resistance	ampicillin
Promoter	Tac promoter (lac/IPTG inducible)
Cloning	LIC. (vector treated with BseRI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	(readthrough from GST gene).
N-terminal fusion – seq.	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSSSENLYFQ*S(M) (* - TEV cleavage site)
N-terminal fusion – MW	26752.6 including Methionine (26534.3 removed by TEV cleavage)
Termination codons	supplied in PCR primer
Protease cleavage	TEV protease
Additional features	
Preferred host	Many E. coli strains (not dependent on T7 RNA polymerase)
5' sequencing primer	pGEX-5': GGGCTGGCAAGCCACGTTTGGTG
3' sequencing primer	pGEX-3': CCGGGAGCTGCATGTGTCAGAGG



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Polylinker region:

GST protein·····>

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                          M   S  P  I  L   G  Y  W   K  I  K   G  L  V  Q ·
241   CACAGGAAAC AGTATTCATG TCCCCTATAC TAGGTTATTG GAAAATTAAG GGCCTTGTGC
     · P  T  R   L  L  L   E  Y  L  E   E  K  Y   E  E  H   L  Y  E  R ·
301   AACCCACTCG ACTTCTTTTG GAATATCTTG AAGAAAAATA TGAAGAGCAT TTGTATGAGC
     · D  E  G   D  K  W   R  N  K  K   F  E  L   G  L  E   F  P  N  L ·
361   GCGATGAAGG TGATAAATGG CGAAACAAAA AGTTTGAATT GGGTTTGGAG TTTCCCAATC
     · P  Y  Y   I  D  G   D  V  K  L   T  Q  S   M  A  I   I  R  Y  I ·
421   TTCCTTATTA TATTGATGGT GATGTTAAAT TAACACAGTC TATGGCCATC ATACGTTATA
     · A  D  K   H  N  M   L  G  G  C   P  K  E   R  A  E   I  S  M  L ·
481   TAGCTGACAA GCACAACATG TTGGGTGGTT GTCCAAAAGA GCGTGCAGAG ATTTCAATGC
     · E  G  A   V  L  D   I  R  Y  G   V  S  R   I  A  Y   S  K  D  F ·
541   TTGAAGGAGC GGTTTTGGAT ATTAGATACG GTGTTTCGAG AATTGCATAT AGTAAAGACT
    ·  E  T  L   K  V  D   F  L  S  K   L  P  E   M  L  K   M  F  E  D ·
601   TTGAAACTCT CAAAGTTGAT TTTCTTAGCA AGCTACCTGA AATGCTGAAA ATGTTCGAAG
     · R  L  C   H  K  T   Y  L  N  G   D  H  V   T  H  P   D  F  M  L ·
661   ATCGTTTATG TCATAAAACA TATTTAAATG GTGATCATGT AACCCATCCT GACTTCATGT
     · Y  D  A   L  D  V   V  L  Y  M   D  P  M   C  L  D   A  F  P  K ·
721   TGTATGACGC TCTTGATGTT GTTTTATACA TGGACCCAAT GTGCCTGGAT GCGTTCCCAA
     · L  V  C   F  K  K   R  I  E  A   I  P  Q   I  D  K   Y  L  K  S ·
781   AATTAGTTTG TTTTAAAAAA CGTATTGAAG CTATCCCACA AATTGATAAG TACTTGAAAT
     · S  K  Y   I  A  W   P  L  Q  G   W  Q  A   T  F  G   G  G  D  H ·
841   CCAGCAAGTA TATAGCATGG CCTTTGCAGG GCTGGCAAGC CACGTTTGGT GGTGGCGACC
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TEV protease cleavage ↓

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     P  P  K   S  S  S   E  N  L  Y   F  Q  S
901   CCTCCAAA ATCGAGCTCA GAGAACCTGT ACTTCCAATC CATAAGCTAG CTTCTCCTCC
                                  5′ LIC sequence              BseRI
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<····· (SacB spacer) ·····>

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2941  TCGAGGAGTT TACTAGTAAG TAAAGGTGGA TACTCGAGCG GCCGCATCGT GACTGACTGA
        BseRI          3′ LIC sequence      XhoI   NotI
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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.

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