

Vector information sheet

Dated: 8th May 2013

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.
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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.	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIRYIADKHNMLGGCPKERAISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIA WPLQGQWQATFGGGDHPKSSSENLYFQ*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': CCGGGAGCTGCATGTGTGTCAGAGG

<..... (SacB spacer)
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2941 TCGAGGAGTT TACTAGTAAG TAAAGGTGGA TACTCGAGCG GCCGCATCGT GACTGACTGA
BseRI 3' LIC sequence XhoI NotI

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.

pGTvL1-SGC sequence:

acgttatcgactgcacgggtgcaccaatgcttctggcgtcaggcagccatcggaagctgtgggatggctg
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