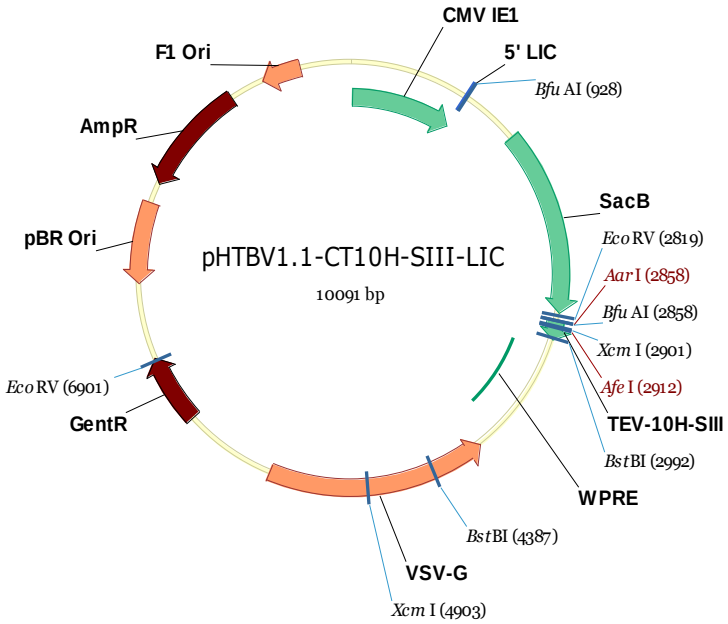
Vector information sheet.

Vector Name	**pHTBV1.1-CT10H-SIII-LIC**
Source	Claire Strain-Damerell
Sequence accession/link	(SGC)

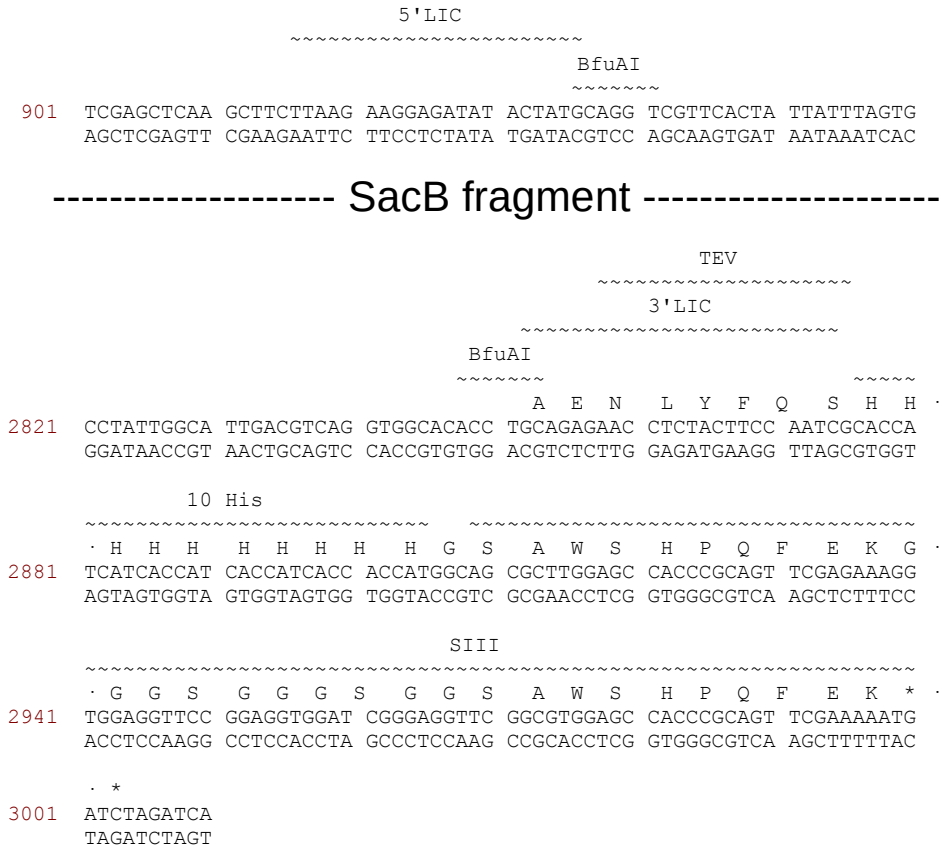
Description	Baculovirus transfer vector for expression of proteins in mammalian cells, with C-terminal His_{10} tag and SIII tag, preceded by a TEV protease cleavage site. Includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection on 5% sucrose. The vector also has full length VSVG for pseudotying of the baculovirus.

Antibiotic resistance	Ampicillin, 100 μg/ml
Promoter	CMV with synthetic intron
Cloning	LIC (vector treated with BfuAI, then with T4 DNA polymerase in presence of dCTP)
Initiation codon	Supplied in PCR primer
C-terminal fusion – seq.	AENLYFQ*SHHHHHHHHHHGSAWSHPQFEKGGGSGGGSG GSAWSHPQFEK (* - TEV cleavage site)
C-terminal fusion – MW	5426.68 Da
Termination codon	Downstream of SIII tag
Protease cleavage	TEV (removes 4560.74 Da)
Additional features	Tn7 sequences for in vivo recombination into bacmid DNA in DH10Bac (using InVitrogen’s Bac-to-bac system).
Preferred host	Initial transformation into any cloning strain, then transform purified plasmid into DH10Bac to generate recombinant bacmid DNA. Bacmid DNA can be transfected to insect cells to generate recombinant baculovirus. Baculovirus can be used to produce recombinant protein in multiple mammalian cell lines.
5’ sequencing primer	pFBM-fwd caaaatgtcgtaacaactccgc
	pFBM-rev tagttaagaataccagtcaatctttcac



Cloning region in the vector:

5' end:



Primers for LIC cloning:

Add the following 5’ extensions to the PCR primers:

Upstream: TTAAGAAGGAGATATACTATG (ATG-initiation codon)

Downstream: GATTGGAAGTAGAGGTTCTCTGC

The purified PCR fragments are treated with T4 DNA polymerase and dGTP, then annealed to the treated vector.