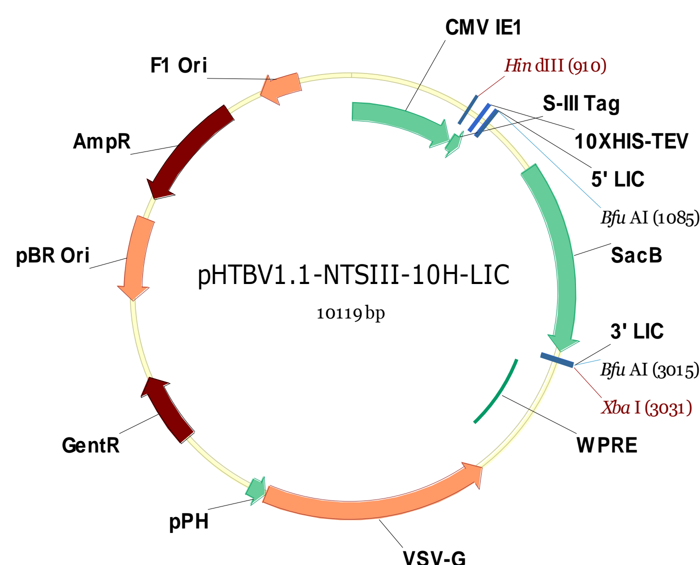
Vector information sheet.

Vector Name	**pHTBV1.1-NTSIII-10H-LIC**
Source	Pravin Mahajan
Sequence accession/link	(SGC)

Description	Baculovirus transfer vector for expression of proteins in mammalian cells, (BacMam) with SIII tag (Twin-Strep) and 10xHis tag in 57-aa N-terminal fusion peptide, with 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 **dGTP**)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKHHHHHHHH HHSSGVDLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	6203.5 Da
Termination codon	Add to reverse PCR primer, if necessary
Protease cleavage	TEV
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. The resulting 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:

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                                  SIII Tag
                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                     M  G    S  A  W    S  H  P  Q    F  E  K    G  G  G
 901                ATGGG CAGCGCTTGG AGCCACCCGC AGTTCGAGAA AGGTGGAGGT
                    TACCC GTCGCGAACC TCGGTGGGCG TCAAGCTCTT TCCACCTCCA
                                                               10XHIS-TEV
                                                               ~~~~~~~~~

                         SIII Tag
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      S  G  G  G    S  G  G    S  A  W    S  H  P  Q    F  E  K    H  H  H
 961 TCCGGAGGTG GATCGGGAGG TTCGGCGTGG AGCCACCCGC AGTTCGAAAA ACACCACCAT
     AGGCCTCCAC CTAGCCCTCC AAGCCGCACC TCGGTGGGCG TCAAGCTTTT TGTGGTGGTA

     10XHIS                                                      TEV
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                                                  5' LIC
                                                                  ~~~~~~
      H  H  H  H    H  H  H    S  S  G    V  D  L  G    T  E  N    L  Y  F
1021 CACCACCATC ATCATCATCA TTCTTCTGGT GTAGATCTGG GTACCGAGAA CCTGTACTTC
     GTGGTGGTAG TAGTAGTAGT AAGAAGACCA CATCTAGACC CATGGCTCTT GGACATGAAG

     10XHIS-TEV
     ~~~~~~
      5' LIC
     ~~~~~~~~~
             BfuAI
         ~~~~~~~~~~~~~~~
      Q  S
1081 CAATCCATGA TCGCAGGT -------------------- SacB fragment --------------------
     GTTAGGTACT AGCGTCCA


                    3' LIC
                ~~~~~~~~~~~~~~~
         BfuAI
     ~~~~~~~~~~~~~~~
3001     ACCTGC AGACAGTAAA GGTGGATA
         TGGACG TCTGTCATTT CCACCTAT
```

Primers for LIC cloning:

Add the following 5’ extensions to the PCR primers:

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.

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