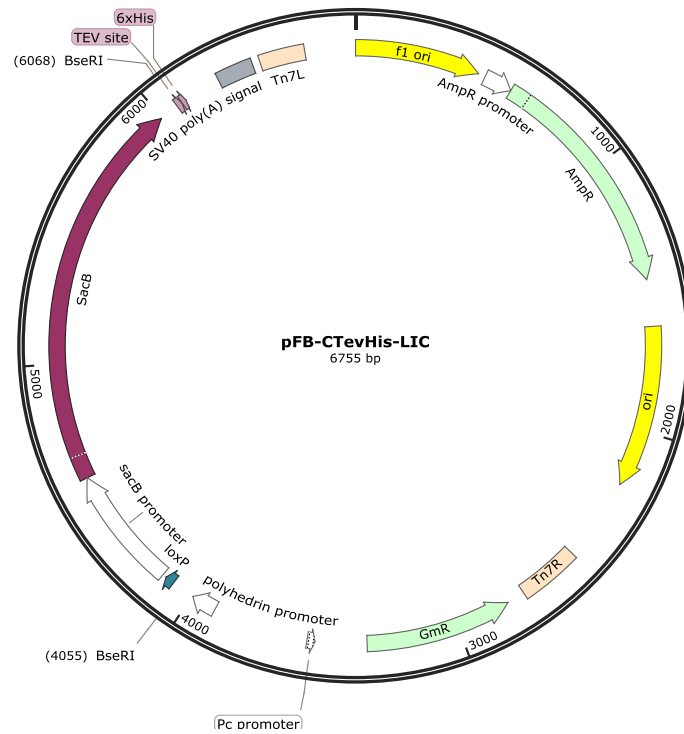


pFB-CTevHis-LIC

Source	Constructed by Yanjun Li, Structural Genomics Consortium, University of Toronto
Description	The pFB-CTevHis-LIC vector is a derivative of the pFBOH-LIC vector (SGC, GenBank accession EF456740). It is a donor vector for generation of recombinant baculovirus by site-specific transposition in an E. coli host. This vector has C-terminal His ₆ tag preceded by TEV protease cleavage site.
Cloning Method	Insertion of a DNA sequence into the cloning/expression region is performed using Clontech's In-fusion enzyme-mediated directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and BseRI linearized vector. Insertion of a target sequence involves replacement of SacB stuffer fragment, which provides for negative selection of the original plasmid on 5% sucrose.
Antibiotic resistance	Ampicillin and Gentamicin
Promoter	Polyhedrin
N-terminal sequence	M
C-terminal fusion sequence	ENLYFQGHHHHHH
5' primer addition for amplification of insert	5' TCGGTCCGAAAACCATG 3'
3' primer addition for amplification of insert	5' GAAATACAAGTTTTC 3'
5' sequencing primer: pFB-F	5' AGTATTTTACTGTTTTCG 3'
3' sequencing primer: pFB-R	5' TAACCATTATAAGCTGC 3'



Locations of Features:

Fi Origin (456 bp): 2-457
AmpR promoter (105 bp): 484-588
AmpR (861 bp): 589-1449
Ori rep_origin (589 bp): 1620-2208
Tn7R (225 bp): 2511-2735
GmR (reverse complementary, 534 bp): 2802-3335
Polyhedrin Promotor (92 bp): 3904-3995
SacB Promotor (446 bp): 4129-4574
SacB Gene (1422 bp): 4575-5996
C Terminal TEV (21 bp) Tag: 6069-6089
C Terminal His (18 bp) Tag: 6090-6107
SV40 poly(A) Signal (135 bp): 6245-6379
Tn7L (166 bp): 6408-6573

pFB-CTevHis-LIC cloning/expression region

Polyhedrin promoter

```
gtattttact gttttcgtaa cagttttgta ataaaaaac ctataaatat
cataaaatga caaaagcatt gtcaaaacat tatttttttg gatatttata

tccggattat tcataccgtc ccaccatcgg gcgcggatct cgggccgaaa
aggcctaata agtatggcag ggtggtagcc cgcgcctaga gccaggcttt
```

```
      M                BseRI                BseRI
accatg/ attatgagtt ctctc---Sac B gene (2 kb)--gaggagatca
Tggtac/ taataactcaa gaggag                ctctcttagt
```

C-TevHis Sequence

```
      E  N  L  Y  F  Q  G  H  H  H  H  H  2x stop
tgcaca/gaaa acttgatatt ccagggccat catcatcatc atcac/tgatga
acgtgt/cttt tgaacataaa ggtcccggta gtagtagtag tagtg/actact
```

pFB-CTevHis-LIC sequences: 6755 bp

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