

## pFBD-LIC1

(SGC 38-C7)

Source	Constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto

Description	pFBD-LIC1 vector is a LIC-adapted derivative of the pFastBac Dual (Invitrogen). It features two promoters for expression of two proteins simultaneously in insect cells when using the Bac-to-Bac® Baculovirus Expression System. N-terminal fusion tag containing a 6 x His tag followed by a thrombin cleavage site is added under Polyhedrin promoter. The P10 promoter is untagged, but the Nco I site under P10 is destroyed which gives more flexibility of removing or replacing His-tag by Nco I and BseR I cut.
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Antibiotic resistance	Ampicillin (plasmid resistance in <i>E. coli</i> ) Gentamicin (bacmid resistance in DH10Bac <i>E. coli</i> )
Promoter	Polyhedrin and P10
Cloning Methods	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 a SacB gene stuffer sequence, which provides for negative selection of the original plasmid on 5% sucrose.
Initiation Codon	ATG start codon within the Nco I restriction site is in frame with N-term His tag.
N – terminal fusion sequence	MGSSHHHHHSSGLVPRGS
Termination codons	Two stop codons TGATGA are included in the C-terminal cloning site.

5' primer tail for amplification of insert	5' GTTCCGCGTGGTAGT --- 3'
3' primer tail for amplification of insert	5' CAAGCTTCGTCATCA --- 3'
5' sequencing primer pFBOH-fwd	5' CCGGATTATTCATACCGTCCCACCA 3'
3' sequencing primer pFBOH-rev	5' CTGATTATGATCCTCTAGTACTTCT 3'

## pFBD-LIC1 cloning/expression region

```
Polyhedrin promoter
~~~~~
4522 gtattttact gttttcgtaa cagttttgta ataaaaaac ctataaatat
cataaaatga caaaagcatt gtcaaaacat tatttttttg gatatttata

      pFBOH-FWD
      ~~~~~>
4572 tccggattat tcataaccgtc ccaccatcgg gcgcggatct cggtcggaaa
aggcctaata agtatggcag ggtggtagcc gcgcgctaga gccaggcttt

NcoI
  M G S   S H H   H H H   H S S G   L V P
4622 accatgggca gcagccatca tcatcatcat cacagcagcg gcctggttcc
tggtagccgt cgtcggtagt agtagtagta gtgtcgtcgc cggaccaagg

      R G S           BseRI
4672 gcgtggtagt/attatgagtt ctctc-----SACB (2 kb) -----
      cgcaccatca/taataactcaa gaggag

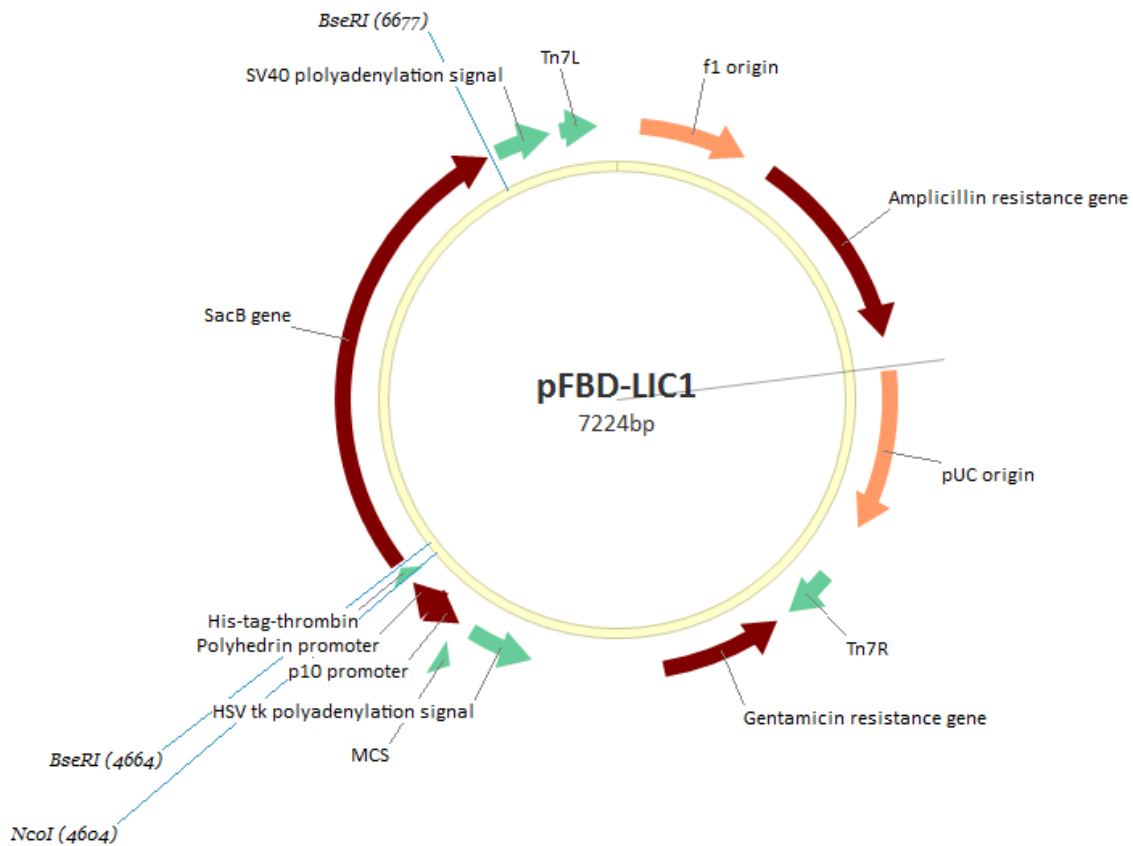
BseRI           stop   HindIII           pFBOH-REV
6681 gaggagatca tgcaca/tgat gacgaagctt tgtcgagaag tactagagga
      ctctccttagt acgtgt/acta ctgcttcgaa acagctcttc atgatctct

      SV40 polyadenylation signal
      ~~~~~
6730 tcataatcag ccataaccaca tttgtagagg ttttacttgc tttaaaaaac
agtagtagtc ggtatgggtg aaacatctcc aaaatgaacg aaattttttg

~~~~~
6780 ctcccacacc tccccctgaa cctgaaacat aaaatgaatg caattgttgt
gaggggtgtg agggggactt ggactttgta ttttacttac gttaacaaca
```

## pFBD-LIC1 Features & Map

f1 origin	102-557
AmpR coding sequence	689-1549
pUC origin	1694-2367
Tn7R	2611-2835
GentR coding sequence	2805-3335 (complimentary)
HSV-tk polydenylation signal	3992-4274 (complimentary)
P10 promoter	4338-4459 (complimentary)
Polyhedrin promoter	4478-4601
N-term His-tag-thrombin	4625-4681
SacB coding sequence	4698-6680
SV40 polyadenylation signal	6728-6968
Tn7L	6997-7162



## Sequence of pFBD-LIC1 Vector: 7224 bp

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