

Vector information sheet

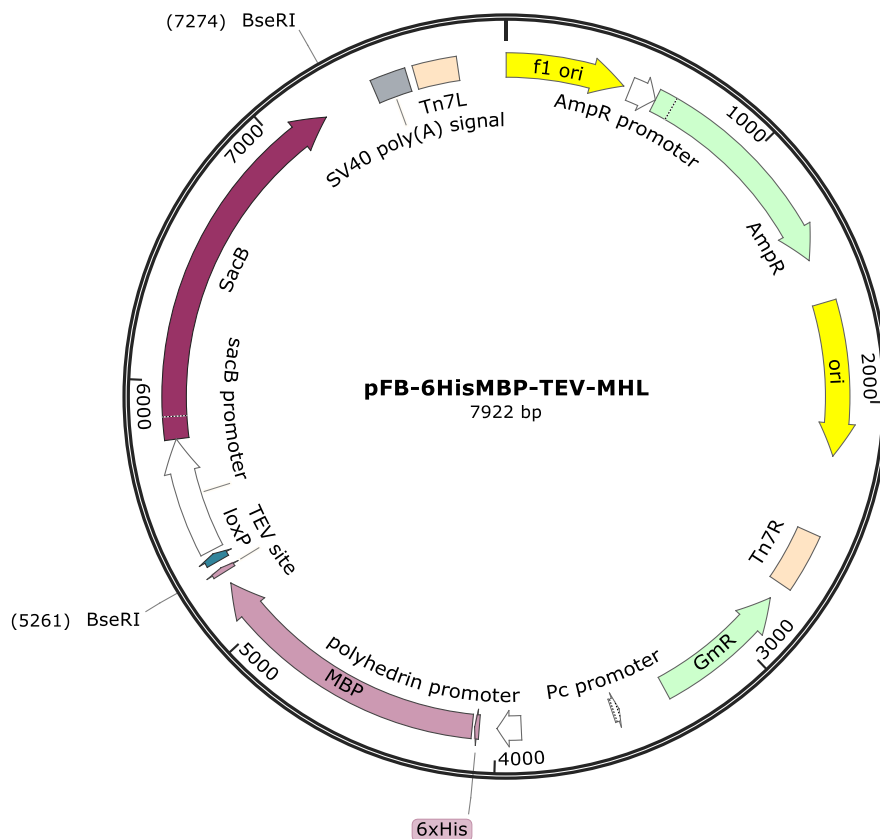
Dated: July 2021

Vector Name	pFB-6HisMBP-TEV-MHL
Source	Designed by Levon Halabelian and constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto
Description	pFB-6HisMBP-TEV-MHL is derived from pFBOH- MHL vector (SGC), a donor vector for generation of recombinant baculovirus by site-specific transposition in an E. coli host. The N-terminal fusion tag was replaced by a PCR product from p6HISMAL-MHL (SGC), containing 6 x His, MBP and TEV cleavage site. Two stop codons are included in the vector at the C-terminal cloning site.
Antibiotic resistance	Ampicillin (plasmid resistance in E. coli) Gentamicin (bacmid resistance in DH10Bac E. coli)
Promoter	Polyhedrin
Cloning Methods	Insertion of DNA sequence into the cloning/expression region is preformed using Infusion enzyme mediated seamless cloning (Clontech cat # 639605). Vector is linearized by Bse RI. Insertion of target sequence involves replacement of a SacB stuffer gene, which provides for negative selection of the original plasmid on 5% sucrose.
N-terminal fusion sequences	MKSSHHHHHHGSSMKIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVE HPDKLEEKFPQVAATGDGPDIIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYP FTWDAVRYNGKLIAYPIAVEALSIIYNKDLLPNPPKTWEEIPALDKELKAGKSA LMFNLQEPYFTWPLIAADGGYAFKYENGYDIKDVGVNAGAKAGLTFVLVLI KNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTF KGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVAL KSYEEELVKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQT VDEALKDAQTNSSNNNNNNNNNNLGENLYFQG
5' primer additions	5'ttgattccagggc--- 3'
3' primer additions	5'caagcttcgcatca--- 3'
Forward primer MBP-nF	5' tggaaaacgccagaaaag---3'
Reverse primer pFB-R	5' taaccattataagctgc---3'

Features of pFB-6HisMBP-TEV-MHL Vector:

Feature Name	Position
F1 Ori	2-245
AmpR	484-1449
Rep Origin	1620-2208
Tn7R	2511-2735
GmR (reverse complementary)	2802-3335
Polyhedrin Promoter	3904-3995
6xHis Tag	4063-4084
MBP Tag	4090-5190
TEV	5239-5259
SacB Gene	5335-5259
SV40 poly(A) Signal	7412-7546
Tn7L	7575-7740

Map of pFB-6HisMBP-TEV-MHL Vector:



pFB-6HisMBP-TEV-MHL Vector cloning/expression region:

polyhedrin promoter

3898 taatagatca tggagataat taaaatgata accatctcgc aaataaataa
attatctagt acctctatta attttactat tggtagagcg tttatttatt

polyhedrin promoter

3948 gtattttact gttttcgtaa cagttttgta ataaaaaac ctataaatat
cataaaatga caaaagcatt gtcaaaacat tatttttttg gatatttata

3998 tccggattat tcataccgtc ccaccatcgg gcgcggatct cggtcgaaa
aggcctaata agtatggcag ggtggtagcc cgcgcctaga gccaggcttt

N-Tag: 6xHis MBP-TEV
M K S S H H H H H H G S S M K

4048 accatgaaat cttctcacca tcaccatcac catggttcctt ctatgaaat
tggactttta gaagagtggg agtggtagtg gtaccaagaa gataactttta

(---1152 bp continued MBP-TEV sequences---)

5250 tttccagggc/catatgagtt **BseRI** **ctcctc** -----**Sac B (1.87 kb)**-----
aaaggtcccg/gtataactcaa **gaggag**

BseRI **2 x stop codons**
7259 **gaggag**atca tgcaca/**tgat gac**gaaagctt gtcgagaagt actagaggat
ctcctctagt acgtgt/acta ctgcttcgaa cagctcttca tgatctccta

Full sequence: 7922 bp

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