

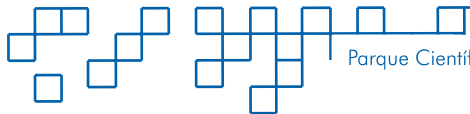


pUC18 *Sfi* vector

User's manual
Biomedal

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pUC18*Sfi* vector

For research use only

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pUC18*Nof* vector

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1. Product Description.

1.1. pUC18Sfi vector.

pUC18Sfi is a 2724 basepair plasmid that carries pUC18 multiple cloning site polylinker (in the same orientation as M13mp18), flanked by *Sfi* I cleavage sequence. This vector is an excellent auxiliary plasmid to clone fragments, that will be introduced like *Sfi* fragments in other vectors with this restriction sites. Note that because of the degeneracy of the *Sfi* I cleavage sequence, the *Sfi* I fragments can only be cloned in vector designed to be compatible with pUC18Sfi. For the same reason, pUC18Sfi - derived fragments can be cloned in only one orientation in other *Sfi*-vectors.

Selection for resistance to	Ampicillin
Replication Origin	pMB1 replicon <i>rep</i> (pBR322)
Antibiotic Resistance	Ampicillin
Host strain	<i>E. coli</i> strains
Copy number	High

2

11

PRODUCT	QUANTITY	STORAGE	CAT. NO
pUC18Sfi	8 µg	4°C or -20°C	CV-3283

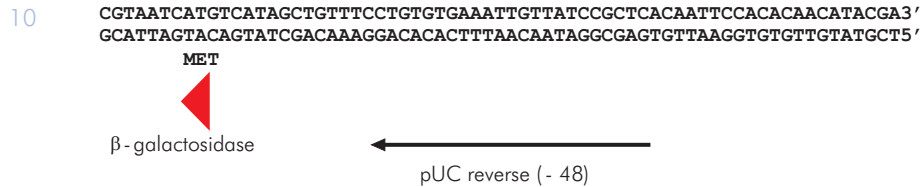
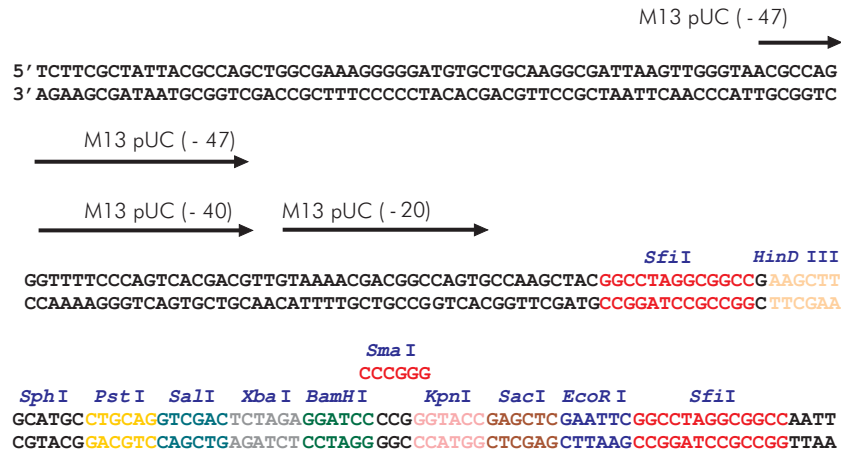
! NOTE:

- The plasmid is shipped at room temperature. Upon arrival, store it according to the direction in the table above.
- The plasmid is supplied dried.

5. References.

1. Yanisch-Perron C., Vieira J. And Messing J. "Improved M13 phage cloning vectors and host strain: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33, 103-119, 1985.

4. Multiple cloning site.



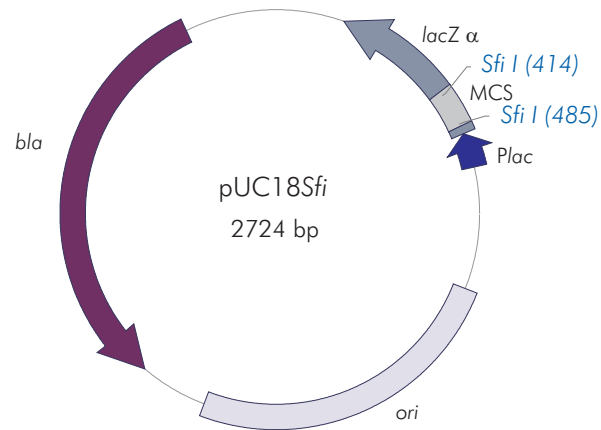
1.1. pUC18-pUC18Sfi vectors common features.

Because pUC18Sfi is a derivative vector from pUC18, both of vectors have common features. These vectors contain:

- pMB1 replicon *rep*, as origin of replication.
- *bla* gene, which confers resistance to ampicillin.
- *E. coli* operon *lac* region, that contains CAP protein binding site, promoter P_{lac} , *lac* repressor binding site and 5'-terminal region of the *lacZ* gene encoding the N-terminal fragment of β-galactosidase. This fragment is capable of alpha-complementation with a defective form of β-galactosidase encoding by host. The synthesis of β-galactosidase N-terminal fragment is induced by IPTG. In presence of IPTG, both fragments of the enzyme are synthesised and form blue colonies on media with X-Gal. Insertion of DNA into the MCS abolishes the alpha complementation. Bacteria carrying recombinant plasmids will give rise to white colonies.

! NOTE: pUC18Sfi carries a single mutation that eliminates a C and changes the position of the start codon of *lacZ* gene (502-ATG instead of 507-ATG). This change eliminates the first and second aminoacids of β-galactosidase without changing the structure and function of the *lacZ* alpha product.

2. pUC18*Sfi* MAP.



RESTRICTION ENZYMES ANALYSIS

RESTRICTION ENZYMES THAT DO NOT CUT pUC18*Sfi*

Aar I	Bcl I	BsiW I	Bsu36 I	Mfe I	Oli I	Psr I	Stu I
Afe I	BfrB I	Bsm I	Btg I	Mlu I	Pac I	Rsr II	Swa I
Afl II	Bgl II	BsmF I	Btr I	Msc I	PflM I	Sac II	Tth111 I
Age I	Blp I	BspE I	Cla I	Nae I	Pme I	SanD I	Xcm I
Alo I	Bpl I	BsrG I	Dra III	Nco I	Pml I	SexA I	Xho I
Apa I	Bpu10 I	BssH II	EcoN I	NgoM I V	Ppu10 I	Sgf I	
Asc I	BsaA I	BstB I	EcoR V	Nhe I	PpuM I	SgrA I	
Bae I	BsaB I	BstE II	Fse I	Not I	PshA I	SnaB I	
Bbs I	BseR I	BstX I	FspA I	Nru I	Psi I	Spe I	
BbvC I	Bsg I	BstZ17 I	Hpa I	Nsi I	PspOM I	Srf I	

pUC18Not vector

4 SEQUENCE REFERENCE POINT

Multiple Cloning Site: 406-490
lacZ: 148-405/491-507 (complementary strand)
lacZ start codon: 502 (complementary strand)
lacZ promoter: 508-581 (complementary strand)
 ColE1 origin (origin pUC): 846-1519 (complementary strand)
bla coding region (ampicillin resistance): 1667-2525 (complementary strand)
bla promoter: 2525-2623 (complementary strand)
 Binding site of pUC/M13 Forward Sequencing Primer (-20): 379-395
 Binding site of pUC/M13 Forward Sequencing Primer (-40): 359-375
 Binding site of pUC/M13 Forward Sequencing Primer (-47): 352-375
 Binding site of pUC/M13 Reverse Sequencing Primer (-48): 515-538 (complementary strand)

RESTRICTION ENZYMES THAT CUT ONCE pUC18*Sfi*

Aat II	Apo I	Bcg I	BstAPI	<u>HinD III</u>	<u>Pst I</u>	Sfo I	Xmn I
Acc I	Ava I	Bpm I	Eag I	Kas I	<u>Sac I</u>	<u>Sma I</u>	
Acc65 I	Avr I	Bsa I	<u>Ecl136 II</u>	<u>Kpn I</u>	<u>Sal I</u>	<u>Sph I</u>	
Afl III	<u>BamHI</u>	BsaXI	<u>EcoO109 I</u>	Nar I	Sap I	Ssp I	
Ahd I	Ban II	BspMI	<u>EcoR I</u>	Nde I	Sbf I	<u>Xba I</u>	
AlwN I	Bbe I	BsrFI	<u>Hinc II</u>	Pci I	Sca I	Xma I	

! Underlined: Underlined multicloning restriction sites.

2051 AGTTACATGA TCCCCATGT TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC
TCAATGTACT AGGGGGTACA ACACGTTTTT TCGCCAATCG AGGAAGCCAG

PvuI
2101 CTCGATCGT TGTCAGAAGT AAGTTGGCCG CAGTGTATC ACTCATGGTT
GAGGCTAGCA ACAGTCTTCA TTCAACCGGC GTCACAATAG TGAGTACCAA

TspGI
2151 ATGGCAGCAC TGCATAATC TCTTACTGTC ATGCCATCCG TAAGATGCTT
TACCGTCGTG ACGTATTAAG AGAATGACAG TACGGTAGGC ATTCTACGAA

TatI
ScaI
2201 TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC
AAGACACTGA CCACTCATGA GTTGGTTCAG TAAGACTCTT ATCACATACG

BcgI
2251 GGCACCCGAG TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGCGCCA
CCGCTGGGCTC AACGAGAACG GGCCGCAGTT ATGCCCTATT ATGGCGCGGT

AclI
XmnI
2301 CATAGCAGAA CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTCGGGGCG
GTATCGTCTT GAAATTTTCA CGAGTAGTAA CCTTTTGCAA GAAGCCCCGG

PpiI
2351 AAAACTCTCA AGGATCTTAC CGCTGTTGAG ATCCAGTTCG ATGTAACCCA
TTTTGAGAGT TCCTAGAATG GCGACAACCT TAGGTCAAGC TACATTGGGT

TaqII Eco57I TaqII
2401 CTCGTGCACC CAACTGATCT TCAGCATCTT TACTTTTAC CAGCGTTTTCT
GAGCACGTGG GTTGACTAGA AGTCGTAGAA AATGAAAGTG GTCGCAAAGA

2451 GGGTGAGCAA AAACAGGAAG GCAAAAATGCC GCAAAAAGG GAATAAGGGC
CCCCTCGTT TTTGTCCCTC CGTTTTACGG CGTTTTTTCC CTTATTCGGC

TspGI SspI
2501 GACACGGAAA TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA
CTGTGCCTTT ACAACTTATG AGTATGAGAA GGAAAAGTT ATAATAACTT

BciVI
2551 GCATTATCA GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT
CGTAAATAGT CCAATAACA GAGTACTCGC CTATGTATAA ACTTACATAA

2601 TAGAAAAATA AACAAATAGG GGTCCGCGC ACATTTCCCC GAAAAGTGCC
ATCTTTTTAT TTGTTTATCC CCAAGGCGCG TGTAAGGGG CTTTTACGGC

ZraI
AatII
2651 ACCTGACGTC TAAGAAACCA TTATTATCAT GACATTAACC TATAAAAATA
TGGACTGCAG ATTCTTTGGT AATAATAGTA CTGTAATTGG ATATTTTTAT

EcoO109I BsmBI
2701 GGCGTATCAC GAGGCCCTTT CGTC
CCGCATAGTG CTCCGGGAAA GCAG

3. Sequence and restriction analysis.

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

PfoI
BsmBI DrdI
51 GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
CTCTGCCAGT GTCGAACAGA CATTTCGCTA CGGCCCTCGT CTGTTCCGGC

101 TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
AGTCCCAGCG AGTCGCCAC AACC GCCAC AGCCCCGACC GAATTGATAC

NdeI
151 CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
GCCGTAGTCT CGTCTAACAT GACTCTCAG TGGTATACGC CACACTTTAT

SfoI
NarI
KasI
BbeI
201 CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC ATTCGCCATT
GGCGTGCTA CGCATTCCTC TTTTATGGCG TAGTCCGCGG TAAGCGGTAA

FspI PvuI
251 CAGGCTGCGC AACTGTGGG AAGGCGGATC GGTGCGGGCC TCTTCGCTAT
GTCCGAGCGG TTGACAACCC TTCCCGCTAG CCACGCCCCG AGAAGCGATA

PvuII
301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA
ATGCGGTGCA CCGCTTTCCC CCTACACGAC GTTCCGCTAA TTCAACCCAT

BmrI
351 ACGCCAGGGT TTTCCAGCTC ACGACGTTGT AAAACGACGG CCAGTGCCAA
TGCGGTCCCA AAAGGGTCAG TGCTGCAACA TTTTGCTGCC GGTACGGT

StyI BspMI
AvrII SphI PstI HincII
401 GCTACGGCCT AGGCGGCCGA AGCTTGCATG CCTGCAGGTC GACTCTAGAG
CGATGCCGGA TCCGCCGGCT TCGAACGTAC GGACGTCCAG CTGAGATCTC

KpnI
Acc65I
XmaI SacI EcoRI StyI
SmaI EcoICRI AvrII
AvaI BanII ApoI SfiI
451 GATCCCCGGG TACCGAGCTC GAATTCGGCC TAGGGCGCCA ATTCGTAATC
CTAGGGGCC ATGGCTCGAG CTTAAGCCGG ATCCGCCGGT TAAGCATAG

501 ATGTCATAGC TGTTTCTGT GTGAAATTGT TATCCGCTCA CAATTCCACA
TACAGTATCG ACAAAGGACA CACTTAAACA ATAGGCGAGT GTAAAGTGT

551 CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG
GTTGTATGCT CGGCCTTCGT ATTTACATT TCGGACCCCA CGGATTACTC

601 TGAGCTAACT CACATTAATT GCGTTGCGCT CACTGCCCCG TTTCCAGTCG
ACTCGATTGA GTGTAATTAA CGCAACGCGA GTGACGGGCG AAAGGTCAGC

651 GGAACCTGT CGTGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG
CCTTTGGACA GCACGGTCTGA CGTAATTACT TAGCCGGTTG CGCGCCCCTC

701 AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC
TCCGCCAAAC GCATAACCCG CGAGAAGGCG AAGGAGCGAG TGACTGAGCG

751 TGCCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG
ACGGGAGCCA GCAAGCCGAC GCCGCTCGCC ATAGTCGAGT GAGTTTCCGC

801 GTAATACGGT TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG
CATTATGCCA ATAGGTGTCT TAGTCCCTTA TTGCGTCCTT TCTTGTACAC

851 AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG
TCGTTTTCCG GTCGTTTTCC GGTCCCTTGGC ATTTTTCCGG CGCAACGACC

901 CGTTTTTCCA TAGGCTCCGC CCCCTGACG AGCATCACAA AAATCGACGC
GCAAAAAGGT ATCCGAGGCG GGGGACTGC TCGTAGTGTT TTTAGTGGC

951 TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT
AGTTCAGTCT CCACCGCTT GGGCTGTCTT GATATTTCTA TGGTCCGCAA

1001 TCCCCTGGA AGCTCCCTCG TGCCTCTCC TGTTCGACC CTGCCGCTTA
AGGGGACCT TCGAGGGAGC ACGCGAGAGG ACAAGGCTGG GACGGCGAAT

1051 CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTCTCAT
GGCCTATGGA CAGGCGGAAA GAGGGAAGCC CTTTCGCACC CGAAAGAGTA

1101 AGCTCACGCT GTAGGTATCT CAGTTCGGTG TAGGTCGTTT GCTCCAAGCT
TCGAGTGCGA CATCCATAGA GTCAAGCCAC ATCCAGCAAG CGAGGTTCTGA

1151 GGGCTGTGTG CACGAACCCC CCGTTCAGCC CGACCCTGC GCCTTATCCG
CCCACACAC GTGCTTGGGG GGCAAGTCGG GCTGGCGACG CGGAATAGGC

1201 GTAACATCG TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG
CATTGATAGC AGAACTCAGG TTGGGCCATT CTGTGCTGAA TAGCGGTGAC

1251 GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC

1301 TACAGAGTTC TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG
ATGTCTCAAG AACTTCACCA CCGGATTGAT GCCGATGTGA TCTTCTGTCT

1351 TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT
ATAAACATA GACGCGAGAC GACTTCGGTC AATGGAAGCC TTTTCTCAA

1401 GGTAGCTCTT GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT
CCATCGAGAA CTAGGCCGTT TGTTTGGTGG CGACCATCGC CACCAAAAAA

1451 TGTTTGCAAG CAGCAGATTA CGCGCAGAAA AAAAGGATCT CAAGAAGATC
ACAAACGTTT GTCGTCTAAT GCGCGTCTTT TTTTCTTAGA GTTCTTCTAG

1501 CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AACTCACGT
GAAACTAGAA AAGATGCCCC AGACTGCGAG TCACCTTGCT TTTGAGTGCA

1551 TAAGGGATT TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT
ATTCCCTAAA ACCAGTACTC TAATAGTTTT TCCTAGAAGT GGATCTAGGA

1601 TTTAAATTA AAATGAAGTT TTAATCAAT CTAAAGTATA TATGAGTAAA
AAATTTAATT TTTACTTCAA AATTTAGTTA GATTTCTAT ATACTCATTT

1651 CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG
GAACCAGACT GTCAATGGTT ACGAATTAGT CACTCCGTGG ATAGAGTCGC

1701 ATCTGTCTAT TTCGTTTCATC CATAGTTGCC TGACTCCCCG TCGTGTAGAT
TAGACAGATA AAGCAAGTAG GTATCAACGG ACTGAGGGGC AGCACATCTA

1751 AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGTCT GCAATGATAC
TTGATGTAT GCCCTCCCGA ATGGTAGACC GGGGTCACGA CGTTACTATG

1801 CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA
GCGCTCTGGG TGCAGTGGC CGAGGTCTAA ATAGTCTGTA TTTGGTCCGT

1851 GCCGGAAGGG CCGAGCGCAG AAGTGGTCTT GCAACTTTAT CCGCCTCCAT
CGGCCTTCCC GGCTCGCGTC TTCACCAGGA CGTTGAAATA GGCGGAGGTA

1901 CCAGTCTATT AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA
GGTCAGATAA TTAACAACGG CCCTTCGATC TCATTCATCA AGCGGTCAAT

1951 ATAGTTTTCG CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC
TATCAAACGC GTTGCAACAA CCGTAACGAT GTCCTGAGCA CCACAGTGGC

2001 TCGTCGTTTG GTATGGCTTC ATTCAGCTCC GGTCCCAAC GATCAAGGCG
AGCAGCAAAC CATACCGAAG TAAGTCGAGG CCAAGGGTTG CTAGTTCCGC

AvaII