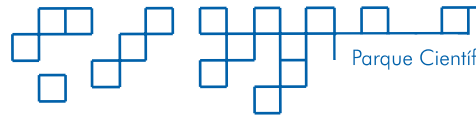




pUC18 *Not* vector



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User's manual
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1. Product Description.

1.1. pUC18Not vector.

pUC18Not is a 2715 basepair plasmid derived from pUC18 vector (GenBank/EMBL sequence accession number L09136), so it can be used as cloning vector in any *E. coli* strain and exhibit the blue/white screening property in conjunction with X-Gal containing plates. pUC18Not carries pUC18 multiple cloning site polylinker (in the same orientation as M13mp18), flanked by Not I cleavage sequence. This vector is an excellent auxiliary plasmid to clone fragments, that will be introduced as Not fragments in other vectors with this restriction site.

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

5. References.

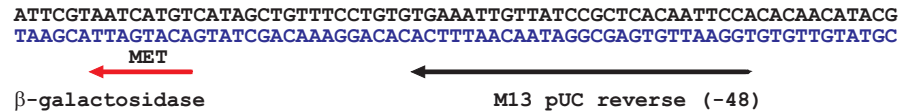
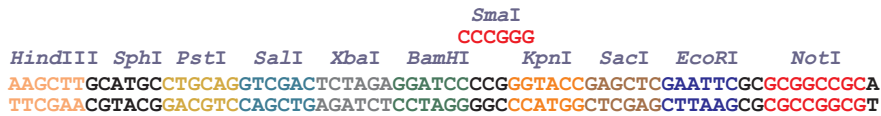
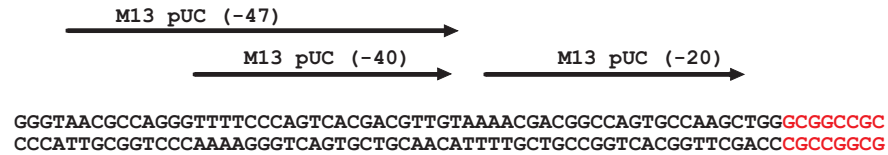
1. Yanisch-Perron C., Vieira J. And Messing J. "Improved M13 phage cloning vectors and host strain: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33, 103-119, 1985.

PRODUCT	QUANTITY	STORAGE	CAT. NO
pUC18Not	8 µg	4°C or -20°C	CV-3282

! 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.

4. Multiple cloning site.



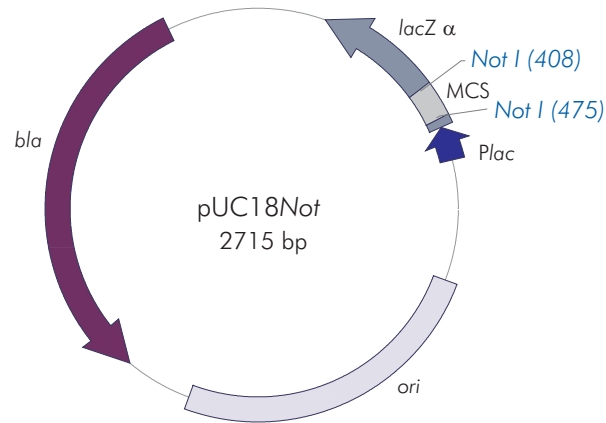
1.1. pUC18-pUC18Not vectors common features.

Because pUC18Not 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.
- NotI. *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: pUC18Not carries a single mutation that eliminates a C and changes the position of the start codon of *lacZ* gene (493-Met instead of 498-Met). This change eliminates the first and second aminoacids of β -galactosidase without changing the structure and function of the *lacZ* alpha product.

2. pUC18Not MAP.



RESTRICTION ANALYSIS

RESTRICTION ENZYMES THAT DO NOT CUT pUC18Not

Aar I	Bfp I	BstE II	Nae I	Psr I
Afe I	BmgB I	BstX I	Nco I	Rsr II
Afl II	Bmt I	BstZ17 I	NgoM I V	Sac II
Age I	Bpl I	Bsu36 I	Nhe I	SanD I
Ale I	Bpu10 I	Btg I	Nru I	SexA I
Alo I	BsaA I	Cla I	Nsi I	Sfi I
Apa I	BsaB I	Dra III	Oli I	Sgf I
Asc I	BseR I	EcoN I	Pac I	SgrA I
AsiS I	Bsg I	EcoR V	PflM I	SnaB I
Avr II	BsiW I	Fal I	Pme I	Spe I
Bae I	Bsm I	Fse I	Pml I	Srf I
Bbs I	BsmF I	FspA I	Ppu10 I	Stu I
BbvC I	BspE I	Hpa I	PpuM I	Sty I
Bcl I	BsrG I	Hfe I	PshA I	Swa I
BfrB I	BssH II	Mlu I	Psi I	Tth111 I
Bgl II	BstB I	Msc I	PspOM I	Xho I

pUC18Not vector

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SEQUENCE REFERENCE POINT

Multiple Cloning Site: 406-480

lacZ: 149-396/486-493 (complementary strand)

lacZ start codon: 493 (complementary strand)

lacZ promoter: 499-572 (complementary strand)

ColE1 origin (origin pUC): 837-1510 (complementary strand)

bla coding region (ampicillin resistance): 1658-2515 (complementary strand)

bla promoter: 2516-2614 (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): 506-529 (complementary strand)

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RESTRICTION ENZYMES THAT CUT ONCE pUC18Not

Aat II	Ava I	BsaX I	Hinc II	Pfo I	Sfo I	Xmn I
Acc I	<u>BamH I</u>	BspM I	<u>HinD III</u>	<u>Pst I</u>	<u>Sma I</u>	Zra I
Acc65 I	Ban II	BsrF I	Kas I	<u>Sac I</u>	<u>Sph I</u>	
Afl III	Bbe I	BstAP I	<u>Kpn I</u>	<u>Sal I</u>	<u>Ssp I</u>	
Ahd I	Bcg I	Ecl136 II	Nar I	<u>Sap I</u>	<u>Xba I</u>	
AlwN I	Bpm I	Eco0109 I	Nde I	Sbf I	<u>Xcm I</u>	
Apo I	Bsa I	<u>EcoR I</u>	Pci I	Sca I	<u>Xma I</u>	

! Underlined: Underlined multicloning restriction sites.

2151 CTGCATAATT CTCTTACTGT TspGWI CATGCCATCC GTAAGATGCT TTTCTGTGAC
 GACGTATTAA GAGAATGACA GTACGGTAGG CATTCTACGA AAAGACACTG
 S C L E R V T M G D T L H K E T V

TatI
 ScaI BcgI
 2201 TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG CGGCGACCGA
 ACCACTCATG AGTTGGTTCA GTAAGACTCT TATCACATAC GCCGCTGGCT

2251 GTTGCTCTTG CCCGGCGTCA ATACGGGATA ATACCGCGCC ACATAGCAGA
 CAACGAGAAC GGGCCGCACT TATGCCCTAT TATGGCGCGG TGTATCGTCT

AclI
 XmnI
 2301 ACTTTAAAAG TGCTCATCAT TGGAAAACGT TCTTCGGGGC GAAAACCTC
 TGAATTTTC ACGAGTAGTA ACCTTTTGCA AGAAGCCCCG CTTTTGAGAG

PpiI TaqII
 2351 AAGGATCTTA CCGCTGTTGA GATCCAGTTC GATGTAACCC ACTCGTGCAC
 TTCCTAGAAT GGGCACAACCT CTAGGTCAAG CTACATTGGG TGAGCACGTG

Eco57I TaqII
 2401 CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC TGGGTGAGCA
 GGTGACTAG AAGTCGTAGA AAATGAAAGT GGTCGCAAAAG ACCCACTCGT

2451 AAAACAGGAA GGCAAAATGC CGCAAAAAG GGAATAAGGG CGACACGGAA
 TTTTGTCTT CCGTTTTACG GCGTTTTTTC CTTTATTTCC GCTGTGCCTT

SspI
 2501 ATGTTGAATA CTCATACTCT TCCTTTTTCA ATATTATTGA AGCATTATC
 TACAACCTTAT GAGTATGAGA AGGAAAAAGT TATAATAACT TCGTAAATAG

BciVI
 2551 AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT
 TCCAATAAC AGAGTACTCG CCTATGTATA AACTTACATA AATCTTTTTA

ZraI
 AatII
 2601 AAACAAATAG GGGTTCGCG CACATTTCCC CGAAAAGTGC CACCTGACGT
 TTTGTTTATC CCCAAGGCGC GTGTAAAGGG GCTTTTCCAG GTGGACTGCA

2651 CTAAGAAACC ATTATTATCA TGACATTAAC CTATAAAAAT AGGCGTATCA
 GATTCTTTGG TAATAATAGT ACTGTAATTG GATATTTTTA TCCGCATAGT

EcoO109I BsmBI
 2701 CGAGGCCCTT TCGTC
 GCTCCGGGAA AGCAG

3. Sequence and restriction analysis.

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT PfoI GCAGCTCCCC
 AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

BsmBI DrdI
 51 GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCC
 CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTGCGGC

101 TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
 AGTCCCAGC AGTCGCCAC AACCGCCAC AGCCCCGACC GAATTGATAC

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

SfoI
 NarI
 KasI
 201 CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC BbeI BglI
 GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCCGCGG TAAGCGGTAA

FspI PvuI
 251 CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT
 GTCCGACGCG TTGACAACCC TTCCCCTAG CCACGCCCGG AGAAGCGATA

PvuII
 301 TACGCCAGCT GCGCAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA
 ATGCGGTCTA CCGCTTTCCC CCTACACGAC GTTCCGCTAA TTCAACCCAT

BmrI XcmI
 BceAI
 351 ACGCCAGGGT TTTCCAGTGC ACGACGTGT AAAACGACGG CCAGTGCCAA
 TGCGGTCCCA AAAGGGTCAG TGCTGCAACA TTTTGCTGCC GGTACGGTT

SalI XmaI
 SmaI
 AvaI
 BspMI
 NotI SphI PstI HincII
 BseI EagI HindIII SbfI AccI XbaI BamHI
 401 GCTGGGCGGC CGCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGGATCCC
 CGACCCGCGC GCGTTTGAAC GTACGGACGT CCAGCTGAGA TCTCTAGGG

SacI
 EcoICRI
 KpnI BanII EcoRI EagI
 Acc65I ApoI NotI
 451 CGGGTACCGA GCTCGAATTC GCGCGGCCG AATTCGTAAT CATGTCATAG
 GCCCATGGCT CGAGCTTAAG CGCGCCGGCG TTAAGCATTG GTACAGTATC

501 CTGTTTCTG TGTGAAATTG TTATCCGCTC ACAATTCCAC ACAACATACG
 GACAAAGGAC AACTTTAAC AATAGCGGAG TGTTAAGGTG TGTGTATGC

551 AGCCGGAAGC ATAAAGTGTA AAGCCTGGGG TGCCTAATGA GTGAGCTAAC
TCGGCCTTCG TATTTTCACAT TTCGGACCCC ACGGATTACT CACTCGATTG

601 TCACATTAAT TGC GTTGC GC TCACTGCCCC CTTTCCAGTC GGGAAACCTG
AGTGTAATTA ACGCAACGCG AGTGACGGGC GAAAGGTCAG CCCTTTGGAC

651 PvuII TCGTGCCAGC TGCATTAATG TfiI AATCGGCCAA CGCGCGGGGA BsaXI GAGGCGGTTT
AGCACGGTCG ACGTAATTAC TTAGCCGGTT GCGCGCCCCT CTCCGCCAAA

701 SapI GCGTATTGGG CGCTCTTCGG CTTCTCGCT CACTGACTCG CTGCGCTCGG
CGCATAACCC GCGAGAAGGC GAAGGAGCGA GTGACTGAGC GACGCGAGCC

751 TCGTTCGGCT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAAATACGG
AGCAAGCCGA CGCCGCTCGC CATAGTCGAG TGAGTTTCCG CCATTATGCC

801 TfiI TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACATGT AflIII GAGCAAAAGG
AATAGGTGTC TTAGTCCCTT ATTGCGTCTT TTCTTGTAACA CTCGTTTTCC

851 CCAGCAAAAG GCCAGGAACC GTAAAAAGGC CGCGTTGCTG GCGTTTTTCC
GGTCGTTTTTC CGGTCTCTGG CATTTTTTCCG GCGCAACGAC CGCAAAAAGG

901 DrdI ATAGGCTCCG CCCCCTGAC GAGCATCACA AAAATCGAGC CTCAAGTCAG
TATCCGAGGC GGGGGGACTG CTCGTAGTGT TTTTAGCTGC GAGTTCAGTC

951 AAGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAGGCGT TTCCCCTGG
TCCACCGCTT TGGGCTGTCC TGATATTTCT ATGGTCCGCA AAGGGGGACC

1001 MmeI AAGCTCCCTC GTGCGCTCTC CTGTTCGGAC CCTGCGCTT BciVI ACCGGATACC
TTCGAGGGAG CACGCGAGAG GACAAGGCTG GGACGGCGAA TGGCCTATGG

1051 TGTCGGCCTT TCFCCTTCG GGAAGCGTGG CGCTTTCTCA TAGCTCACGC
ACAGGCGGAA AGAGGGAAGC CCTTCGCACC GCGAAAGAGT ATCGAGTGCG

1101 BseYI TGTAGGTATC TCAGTTCGGT GTAGGTGCTT CGCTCCAAGC TGGGCTGTGT
ACATCCATAG AGTCAAGCCA CATCCAGCAA GCGAGGTTCC ACCCGACACA

1151 GCACGAACCC CCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACATC
CGTGCTTGGG GGGCAAGTCG GGCTGGCGAC GCGGAATAGG CCATTGATAG

1201 MmeI GTCTTGTAGT CAACCCGGTA AGACACGACT TATCGCCACT AlwNI GGCAGCAGCC
CAGAACTCAG GTTGGGCCAT TCTGTGCTGA ATAGCGGTGA CCGTCGTCGG

1251 ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT
TGACCATTGT CCTAATCGTC TCGCTCCATA CATCCGCCAC GATGTCTCAA

1301 BceAI CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA GTATTTGGTA
GAACTTACC ACCGGATTGA TGCCGATGTG ATCTTCTGT CATAAACCAT

1351 Eco57I TCTGCGCTCT GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT
AGACGCGAGA CGACTTCGGT CAATGGAAGC CTTTTTCTCA ACCATCGAGA

1401 TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTTGCAA
ACTAGGCCGT TTGTTTGGTG GCGACCATCG CCACCAAAAA AACAAACGTT

1451 GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT CCTTTGATCT
CGTCGTCTAA TGCGCGTCTT TTTTCTCTAG AGTTCTTCTA GGAAACTAGA

1501 PpiI TTTCTACGGG GTCTGACGCT CAGTGGAAAG AAAACTCAGC TTAAGGGATT
AAAGATGCC CAGACTGCGA GTCACCTTGC TTTTGAGTGC AATTCCCTAA

1551 TTGGTCATGA GATTATCAA AAGGATCTTC ACCTAGATCC TTTTAAATTA
AACCAGTACT CTAATAGTTT TTCCTAGAAG TGGATCTAGG AAAATTTAAT

1601 AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG
TTTFACTTCA AAATTTAGTT AGATTTTCTA TATACTCATT TGAACCAGAC

1651 ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA
TGCAATGGT TACGAATTAG TCACTCCGTG GATAGAGTCG CTAGACAGAT

1701 AhdI TTTCTGTCAT CCATAGTTGC CTGACTCCCC GTCGTGTAGA TAACTACGAT
AAAGCAAGTA GGTATCAACG GACTGAGGGG CAGCACATCT ATTGATGCTA

1751 BmrI BsrDI BsaI ACGGGAGGGC TTACCATCTG GCCCCAGTGC TGCAATGATA CCGCGAGACC
TGCCCTCCCG AATGGTAGAC CGGGGTCACG ACGTTACTAT GGCGCTCTGG

1801 BsrFI BpmI BglI CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC AGCCGGAAGG
GTGCGAGTGG CCGAGGTCTA AATAGTCGTT ATTTGGTCGG TCGGCCTTCC

1851 AvaII GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA TCCAGTCTAT
CGGCTCGCGT CTTACCAGG ACGTTGAAAT AGGCGGAGGT AGGTCAGATA

1901 FspI TAATTGTGTG CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTTGC
ATTAACAACG GCCCTTCGAT CTCATTCATC AAGCGGTCAA TTATCAAACG

1951 AclI BsrDI GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTACG CTCGTCGTTT
CGTTGCAACA ACGGTAACGA TGTCCTGAGC ACCACAGTGC GAGCAGCAAA

2001 GGTATGGCTT CATTACGTC CGGTTCCCAA CGATCAAGGC GAGTTACATG
CCATACCAGAA GTAAGTCGAG GCCAAGGGTT GCTAGTTCCG CTCAATGTAC

2051 AvaII PvuI ATCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT CCTCCGATCG
TAGGGGGTAC AACACGTTTT TTCGCCAATC GAGGAAGCCA GGAGGCTAGC

2101 TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT CACTCATGGT TATGGCAGCA
AACAGTCTT ATTCAACCGG CGTCAACAATA GTGAGTACCA ATACCGTCTG