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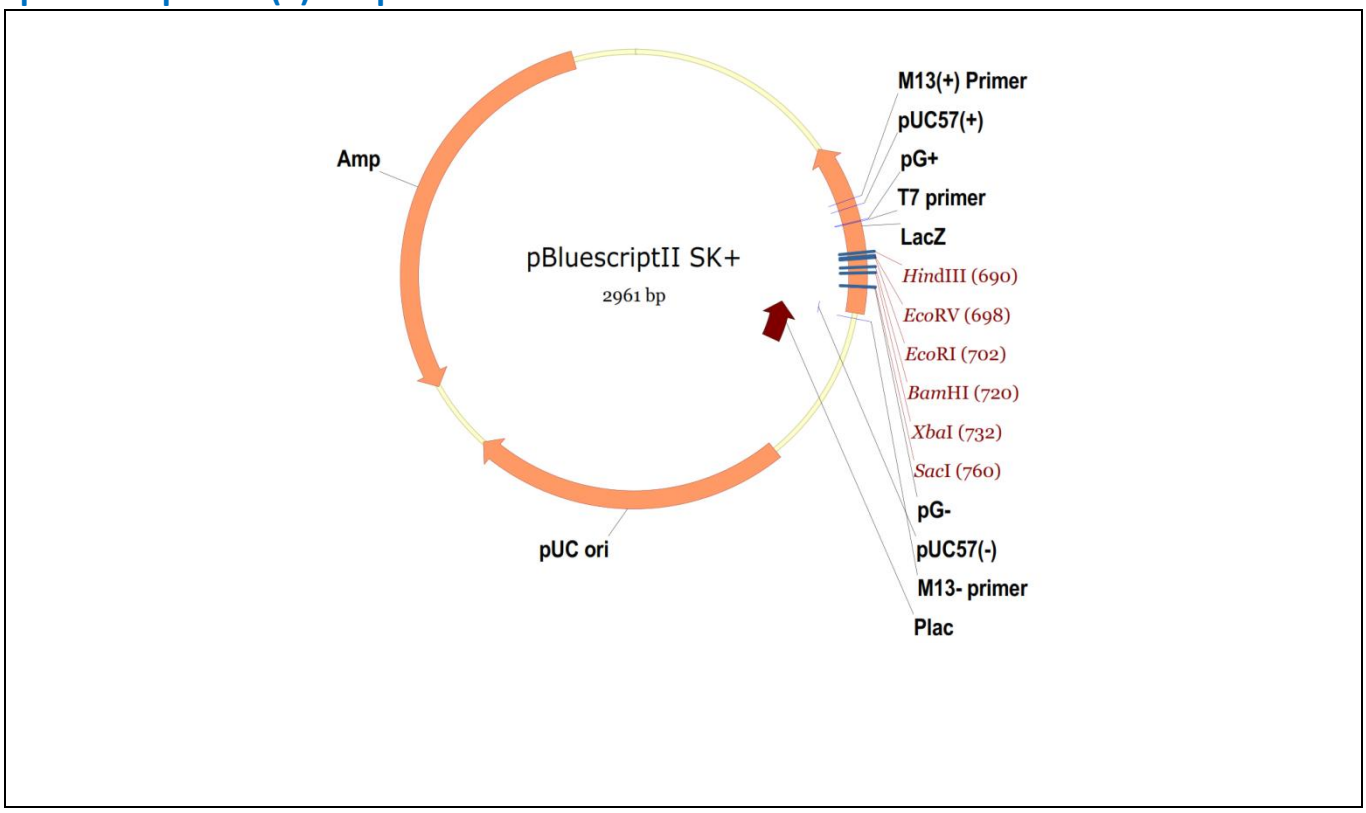
Standard Cloning Vectors

Version 7.1, Revision 2017-09-26

Biomatik has been serving worldwide researchers with quality custom gene synthesis service since 2004. There are several standard vectors can be selected for cloning purpose, free of charge. Please note that the standard cloning vectors are not expression vectors. Biomatik can also subclone your gene into any expression vector of your choice, at additional cost. Important Note: any expression vector supplied by Biomatik for subcloning purposes shall NOT be used for any Commercial Purposes.

Standard Cloning Vector	Antibiotic Resistance	Subcloning Cost
pBluescript II SK(+)-Amp	Ampicillin	Free of charge
pBSK(+) Simple-Amp	Ampicillin	Free of charge
pBSK(+) Simple-Kan	Kanamycin	Free of charge
pUC57-Amp	Ampicillin	Free of charge
pUC57-Kan	Kanamycin	Free of charge

pBluescript II SK(+)-Amp



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pBluescript Ⅱ SK(+) MCS

AACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAGCGCGCGTA
M13+
ATACGACTCACTATAGGGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCGACGGTATCG
T7 PstI KpnI XhoI NotI
ATAAGCTTGATATCGAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCC
HindIII EcoRI BamHI XbaI
ACCGCGGTGGAGCTCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTGCGCGCTTGGCGT
SacI
AATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCAC
M13-

>pBluescript II SK(+)

CTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAA
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AAGTGCCAC

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pBSK(+) Simple

pBSK(+) Simple Vector has 2 versions: pBSK(+) Simple-Amp (2907 bp) and pBSK(+) Simple-Kan (3040 bp). Antibiotic resistance is the only difference. pBSK(+) Simple vector is created by removing almost all commonly used restriction sites from the multiple cloning sites (MCS) of the commercial cloning vector pBluescript II SK(+), leaving only one SmaI (CCC*GGG) site and one EcoRV (GAT*ATC) site for blunt end cloning and two HindIII (AAGCTT) sites for gene digestion screening. Similar to pBluescript II SK(+), pBSK(+) Simple vector is designed for DNA cloning, DNA sequencing, *in vitro* mutagenesis and *in vitro* transcription in a single system.

pBSK(+) Simple vector is an excellent choice for subcloning, as it minimizes the unexpected fragments which show up along with the target DNA fragment. Any kind of DNA fragments can be inserted into the vector via blunt end cloning or TA cloning. All the unique restriction sites in the gene will still be unique in the final construct. Features include:

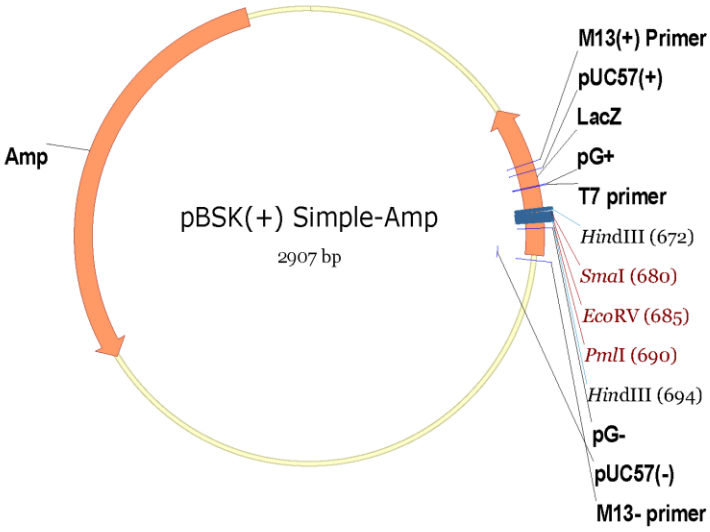
1. *f1* (*IG*) - the intergenic region of phage f1;
2. *rep (*pMB1*)* - the pMB1 replicon responsible for the replication of phagemid. DNA replication initiates at position 1213 (+/- 1) and proceeds in the same direction of pBluescript II SK(+);
3. *bla* (ApR) - gene, coding for beta-lactamase that confers ampicillin resistance;
4. *lacZ* - 5'-terminal part of *lacZ* gene encoding the N-terminal fragment of beta-galactosidase. This fragment allows blue/white screening.

General blunt-end cloning strategy using pBSK(+) Simple vector:

1. Using SmaI (CCC*GGG) or EcoRV (GAT*ATC) site, linearize the pBSK(+) Simple vector;
2. Dephosphorylate the ends of linearized vector (by alkaline phosphatase (AP) treatment) to prevent self re-circularization of the vector during ligation;
3. Perform ligation, transformation and screening according to standard laboratory protocols.

If gene is inserted into the linearized pBSK(+) Simple vector, the SmaI or EcoRV site will be destroyed, and will not be present in the final construct.

pBSK(+) Simple-Amp



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pBSK(+) Simple-Amp MCS

AACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAGCGCGCGTA
M13+ SmaI
ATACGACTCACTATAGGGCGAATTGGGTACGGCCGTCAAGGCCAAGCTTCCCGGGATAT
HindIII EcoRV
CACGTGAAGCTTGCAAGCTCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTGCGCGCTT
HindIII
GGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATT
M13-

>pBSK(+) Simple-Amp

```
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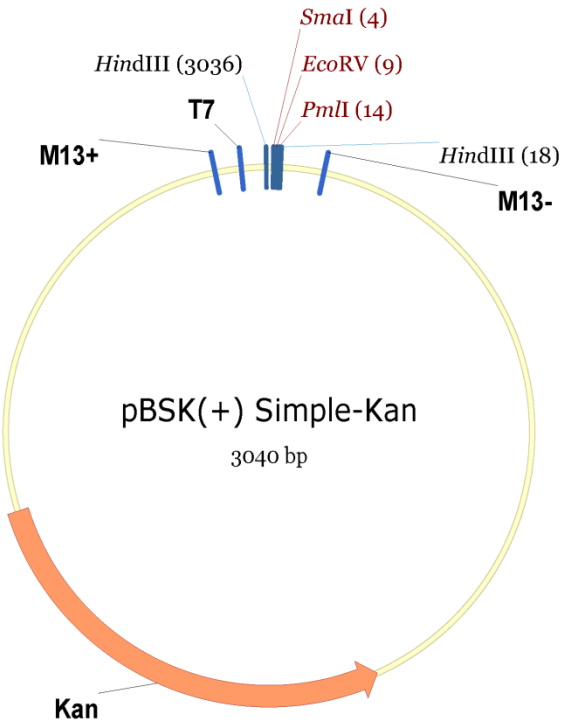
pBSK(+) Simple-Kan



pBSK(+) Simple-Kan MCS

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M13+
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HindIII
EcoRV
CACGTGAAGCTTGCAAGCTCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTGCGCGCTT
HindIII
GGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATT
M13-

>pBSK(+) Simple-Kan

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TCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCT
GGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCA
GCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCG
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AAGCTT

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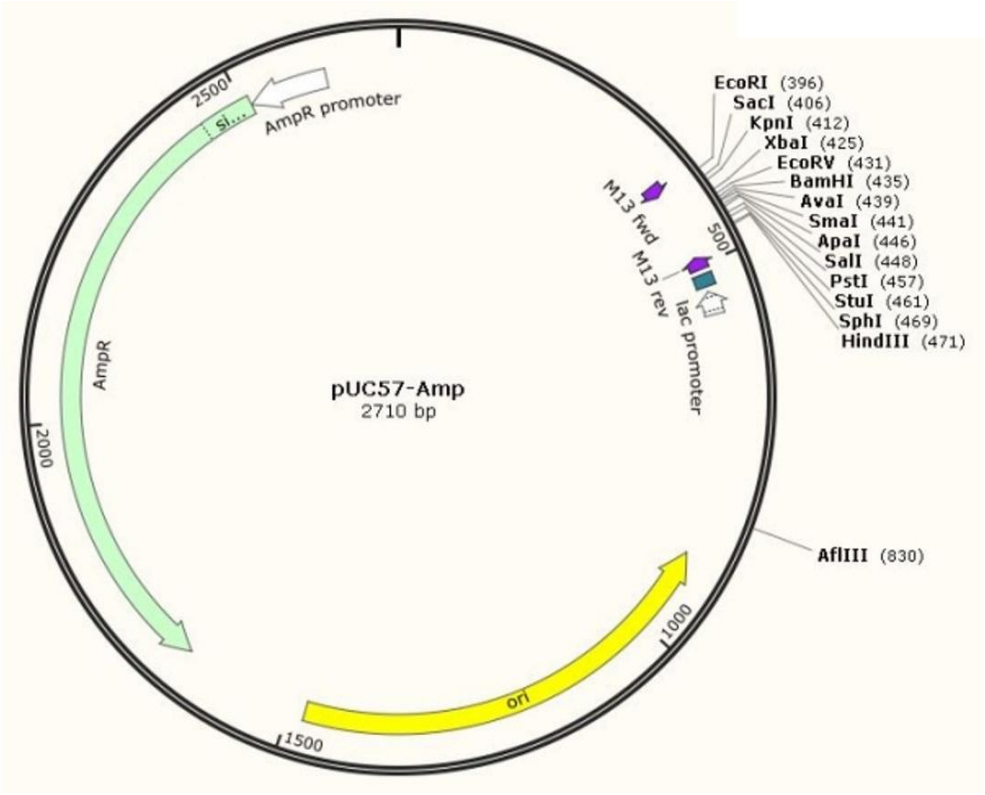
pUC57-Amp

pUC57-Amp is a derivative of pUC19 plasmid. pUC57 MCS contains 6 restriction sites with 3' sticky ends, which are resistant to *E.coli* exonuclease III. The exact position of genetic elements is shown on the map (termination codons included). DNA replication initiates at position 890 (+/-1) and proceeds in indicated direction. The *bla* gene coding for beta-lactamase confers ampicillin resistance.



pUC57 MCS

EcoRI SacI

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M13+ EcoRV PstI

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KpnI XbaI BamHI SalI

ATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCA

HindIII M13-

CAAT

>pUC57-Amp

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CCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTT
CGTC

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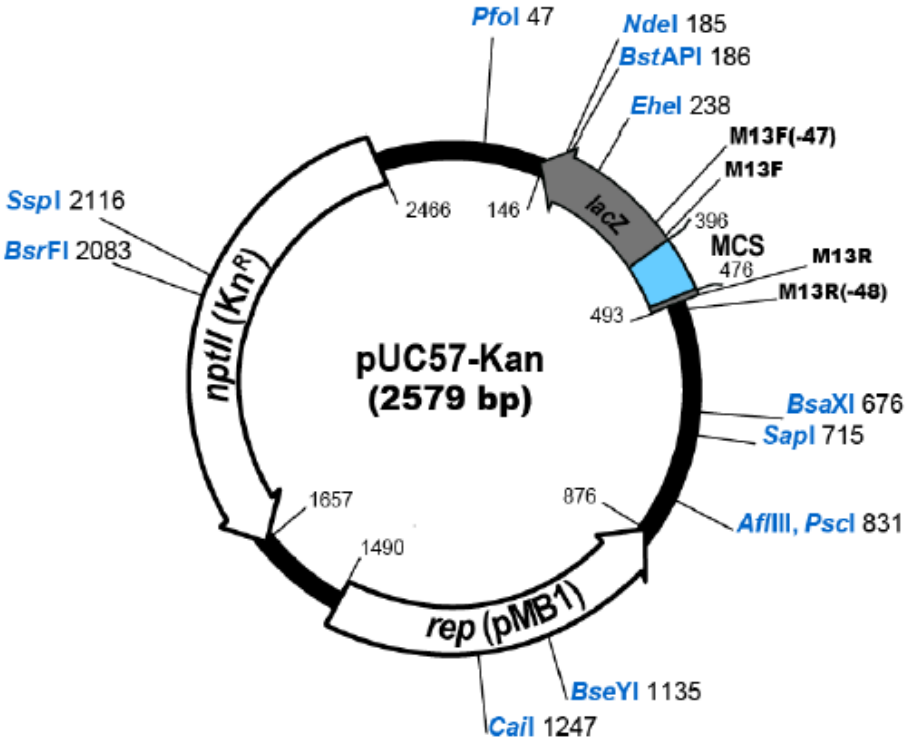
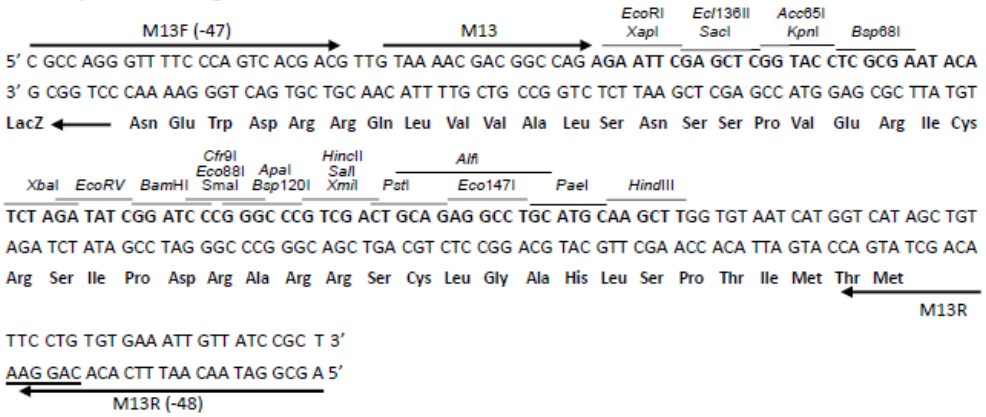


Multiple Cloning Sites:



M13F (-47):5′-d (CGC CAG GGT TTT CCC AGT CAC GAC)-3′
M13F: 5′-d (GTA AAA CGA CGG CCA G)-3′
M13R: 5′-d (CAG GAA ACA GCT ATG AC)-3′
M13R (-48): 5′-d (AGC GGA TAA CAA TTT CAC ACA GGA)-3′

>pUC57-Kan

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGC
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TGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCGAGCTCGGT
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