Appendix

APPENDIX-I

1. VECTOR MAP OF PLASMID pBluescript KS+

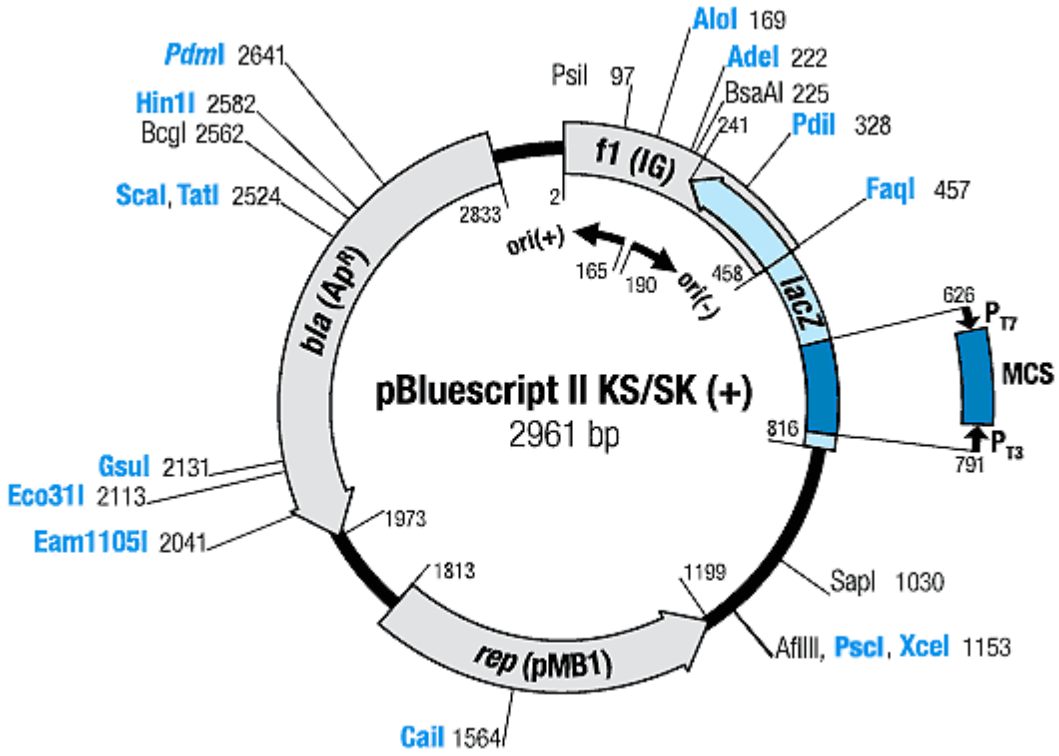


Diagram of pBSKS^{+} plasmid. The multiple cloning site (MCS) is present at the 5' end of the β-galactosidase gene (*lacZ*) and contains the unique restriction enzyme (RE) site forcloning.It includes T7 and T3 promoter for *in vitro* transcription of the gene. It contains ampicillin resistance gene, *bla* (encodes β-lactamase) and the origin of replication, *rep*. Takenfromhttp://www.fermentas.com/techinfo/nucleicacids/mapBluescriptiiskks.htm.

2. VECTOR MAP OF *E. coli* – STAPHYLOCOCCAL SHUTTLE VECTOR pCN40

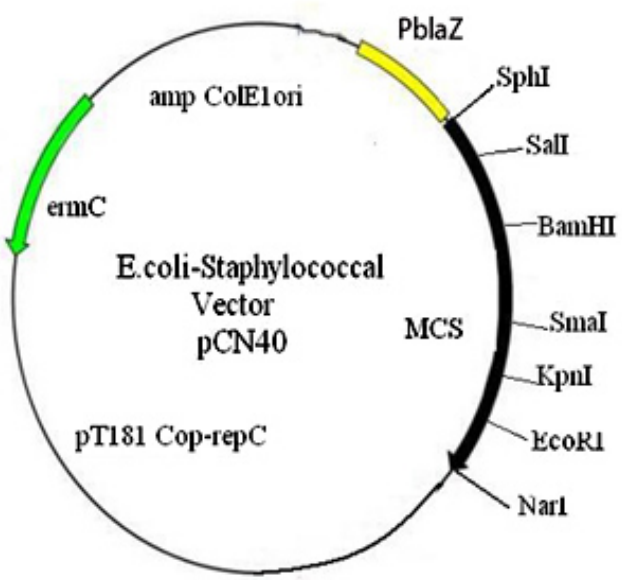
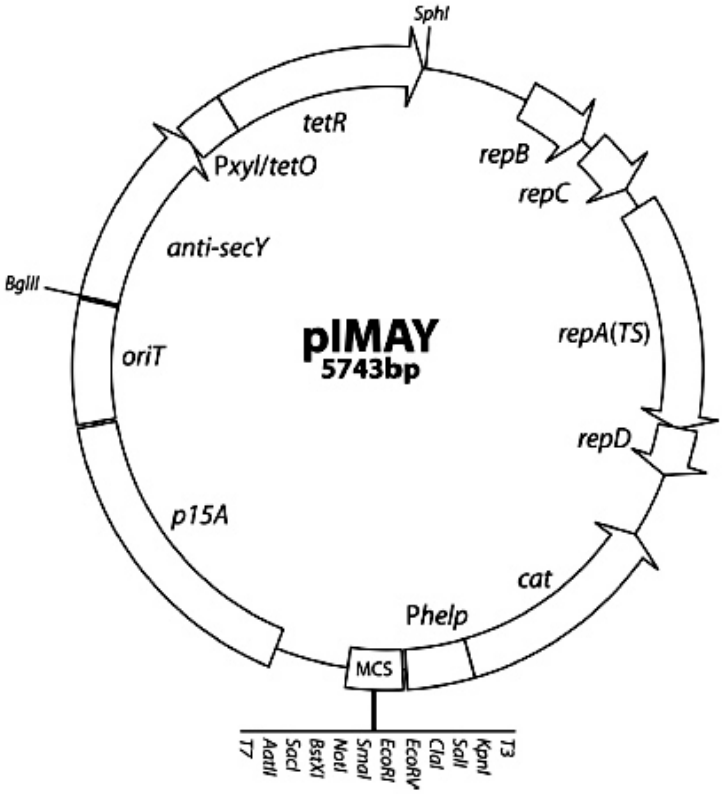


Diagram of pCN40 plasmid. The shuttle plasmid comprises pT181 *cop-wt repC* cassette which contains the origin of replication, the copy control system, and the *repC* gene encoding the replication protein RepC of the pT181 rolling circle replicon for replication in *Staphylococcus* and ColE1 *ori* for replication in *E. coli*. It contains antibiotic resistance gene *ermC* (ribosomal methylase encoding gene of pE194 for erythromycin resistance) and *amp* ColE1 ori (*bla* gene conferring ampicillin resistance). P*blaZ* is the constitutive as well as inducible beta-lactamase promoter.The MCS is derived from pUC19, for cloning purpose (Charpentier *et al.,* 2004).

3. VECTOR MAP OF pIMAY- TEMPERATURE SENSITIVE (TS)*E. coli*-STAPHYLOCOCCAL SHUTTLE PLASMID



Genetic map of pIMAY. The *E. coli*/staphylococcal temperature sensitive plasmid pIMAY comprises the low copy number *E. coli* origin of replication (p15A), an origin of transfer for conjugation (*oriT*), the pBluescript multiple cloning site (MCS) contains unique restriction sites. It containschloramphenicol resistance *cat* gene (P*help-cat*) derived from pIMC. The temperature sensitive replicon for gram positive bacteria (*repBCAD*) and the tetracycline inducible antisense *secY* region (*anti-secY*) is derived from pVE6007 and pKOR1 respectively(Monk *et al*., 2012).

APPENDIX II

Genomic sequence of SprX1 in *S. aureus* Newman (156bp)

ACACATGCATCAACTATTTACATCTATCCTTGTTCACCCAAGCATGTCACTGGGTGTTTTTTCTTATGATA
GAGAGCATAGTTTTCATACTACTCCCTCGTAGTATATATGACTTTA**GCATTCCCGTATAATAGTTTACGGG**
GTGCTTTTTATGTT

Region of SprX1 amplified and cloned (279 bp)

<ATAATCTTTCTAGACGTATTCAAAGGACGTCTTTTTAGATTGTATGTTATAGC**TAGCCT**TCCGGTTAATT
TTT**TGTTATGAT**GTGTT**<**ACACATGCATCAACTATTTACATCTATCCTTGTTCACCCAAGCATGTCACTGG
GTGTTTTTTCTTATGATAGAGAGCATAGTTTTCATACTACTCCCTCGTAGTATATATGACTTTAGCATTCC
CGTATAATAGTTTACGGGGTGCTTTTTATGTT**>**ATAATTAATTGTATATAGTAGGAGTGAACTATATAGCC
TG**>**

SprX in pCN40 (pMNSprX)

CAGCTTACTATGCCATTATTAATAACTTAGCCATTTCAACACCTTCTTTCAAATATTTATAATAAACTATT
GACACCGATATTACAATTGTAATATTATTGATTTATAAAAATTACAACTGTAATATCGGAGGGTTTATTCT
GCAGGTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAGCCC**<**ATAATCTTTCTAGACGTATTCAAA
GGACGTCTTTTTAGATTGTATGTTATAGC**TAGCCT**TCGGGCTAGTTTTT**TGTTATGAT**GTGTT**<**ACACATG
CATCAACTATTTACATCTATCCTTGTTCACCCAAGCATGTCACTGGGTGTTTTTTCTTATGATAGAGAGCA
TAGTTTTCATACTACTCCCTCGTAGTATATATGACTTTA**GCATTCCCGTATAATAGTTTACGGGGTGCTTT**
TTATGTT>ATAATTAACTGTATATAGTAGGAGTGAACTATATAGCCTG**>**GGGGGGATCCCCGGGTACC

$SprX_{AS}$ in pCN40 (pMN$SprX_{AS}$)

CAGCTTACTATGCCATTATTAATAACTTAGCCATTTCAACACCTTCTTTCAAATATTTATAATAAACTATT
GACACCGATATTACAATTGTAATATTATTGATTTATAAAAATTACAACTGTAATATCGGAGGGTTTATTCT
GCAGGTCGACTCTAGAGGATCCC**<**CAGGCTATATAGTTCACTCCTACTATATACAGTTAATTATAACATAA
AAAGCACCCCGTAAACT**ATTATA**CGGGAATGCTAAAGTC**ATATATACT**ACGAGGGAGTAGTATGAAAACTA
TGCTCTCTATCATAAGAAAAAACACCCAGTGACATGCTTGGGTGAACAAGGATAGATGTAAATAGTTGATG
CATGTGTAACACATCATAACAAAAAACTAGCCCGAAAGCTAGCTATAACATACAATCTAAAAAGACGTCCC
TTGAATACGTCTAGAAAGATTAT**>**GGGCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGA
GGGGGGGCCCGGTACC

Underlined region is the SprX1 and $SprX1_{AS}$ transcripts; Bold letters and underlined indicate TT- transcription terminator; Light shaded box GTCGAC- SalI; GGATCC- BamHI; GGTACC- KpnI indicate the restriction enzymes; Thick shaded box (Bold and underlined) indicate the putative inherent promoter of the SprX1 and Thick shaded box (but not bold and underlined) indicate the vector borne promoter PblaZ.

Gene cassette (*sprX1::kan*) of *sprX1* disrupted with 1.4 kb of kanamycin gene (2.9 kb)

GGATCCCCCGGTACCGCCATGCTCAAAAGAGGTTTATTATTTTTAACTGTTTTATTGTTATTATTCTCATT
TTCTTCAATTACTAATGAGGTAAGTGCATCAAGTTCATTCGACAAAGGAAAATATAAAAAAGGCGATGACG
CGAGTTATTTTGAACCAACAGGCCCGTATTTGATGGTAAATGTGACTGGAGTTGATGGTAAAGGAAATGAA
TTGCTATCCCCTCATTATGTCGAGTTTCCTATTAAACCTGGGACTACACTTACAAAAGAAAAAATTGAATA
CTATGTCGAATGGGCATTAGATGCGACAGCATATAAAGAGTTTAGAGTAGTTGAATTAGATCCAAGCGCAA
AGATCGAAGTCACTTATTATGATAAGAATAAGAAAAAAGAAGAAACGAAGTCTTTCCCTATAACAGAAAAA
GGTTTTGTTGTCCCAGATTTATCAGAGCATATTAAAAACCCTGGATTCAACTTAATTACAAAGGTTATTAT
AGAAAAGAAATAAAACAAAATAGTTGTTTATTATAGAAAGCAATGTCTTGATTGAATATGTGTAGTGAAAA
TTATCTTTCATCAAATTCTCATTCATGCACGAATGGTTCTTCCCCACCTAATCAGATATTAGGTGACTTAT
GGGGAGAAATCAGTTAGGATGAAAAAGTGGATAATCCTTTTTTAGGCAGGTACTTCGGTACTTGCCTATTT
TTTTATGTTATAATCTTTCTAGACGTATTCAAGGGACGTCTTTTTAGATTGTATGTTATAGCTAGCTTTCG
GGCTAGTTTTTTGTTATGATGTGTTACACATGCAT<GCAACATGTGAGAGGCGGTTTGCGTATTGGGCGCA
TGCATAAAAACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAAACGGCATGATGAACC
TGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATATTTGCCCATGGGGGTGGGCGAAGAACT
CCAGCATGAGATCCCCGCGCTGGAGGATCATCCAGCCGGCGTCCCGGAAAACGATTCCGAAGCCCAACCTT
TCATAGAAGGCGGCGGTGGAATCGAAATCTCGTGATGGCAGGTTGGGCGTCGCTTGGTCGGTCATTTCGAA
CCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGC
GATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCA
ACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTT
TCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGCCGTCGGGCATGCGCGC
CTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTTCGTCCAGATCATCCTGATCGACAA
GACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCC
GGATCAAGCGTATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGA
TGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGA
GCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTCGTCCTGCAGTTCATTC
AGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGCGGC
ATCAGAGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAAC
CTGCGTGCAATCCATCTTGTTCAATCATGCGAAACGATCCTCATCCTGTCTCTTGATCAGATCTTGATCCC
CTGCGCCATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTTGCAGGGCTTCCCAACCTTACCAGA
GGGCGCCCCAGCTGGCAATTCCGGTTCGCTTGCTGTCCATAAAACCGCCCAGTCTAGCTATCGCCATGTAA
GCCCACTGCAAGCTACCTGCTTTCTCTTTGCGCTTGCGTTTTCCCTTGTCCAGATAGCCCAGTAGCTGACA
TTCATCCCAGGTGGCACTTTTCGGGGCAACATGTATGCAT>CAACTATTTACATCTATCCTTGTTCACCCA
AGCATGTCACTGGGTGTTTTTTCTTATGATAGAGAGCATAGTTTTCATACTACTCCCTCGTAGTATATATG
ACTTTAGCATTCCCGTATAATAGTTTACGGGGTGCTTTTTATGTTATAATTAACTGTATATAGTAGGAGTG
AACTATATAGCCTGTTAAGTGGCCTAGTAACCTAACACTTATCCTGCAATTGATATCCTTTTTGCCCTTCA
CTCGATACATATATCTCAACAACATAGAAATATTACAGTCGCTACACCGCATCTTAAATGGTGTGGTTATT
TTTATTGGAAGTGTGTATCAGGTATCAGTAATGTTAAAACACCAGCTAAAAATGAAAAGAATTCACCAGTG
CCAGCAGGTTATACACTCGATAAAAACAATGTACCGTATAAAAAAGAGACTGGTTATTACACAGTTGCCAA
TGTTAAAGGTAATAACGTGAGGGATGGCTATTCAACTAATTCAAGAATTACAGGTGTATTACCCAATAACG
CAACTATCAAATATGACGGCGCATATTGCATTAATGGCTATAGATGGATTACTTATATTGCTAATAGTGGA
CAACGTCGTTATATAGCGACAGGAGAGGTAGACAAGGCAGGTAATAGAATAAGCAGTTTTGGTAAGTTTAG
TGCAGTTTGATAATTAGATATATAAAGGTTTGGCAAGTTATGAAATGTCTGCCAAACCGGATCCGGGCTGC
AG

ATGCAT- Thick shaded box indicates NsiI enzyme; Underlined region is the disrupted *sprX1* region; **<>** - closed bracket enclosed the region of kanamycin gene. **GGATCC**-Bold and underlined region is BamHI enzyme.

Genomic sequence of SprB in *S. aureus* Newman (~110bp)

GACGACATGCGCGAACATGTCGTCTAAGCAAGCCAAACGCTGGCTTCTAAAATATTTTTTTAACCATTCCA
ACTTGCCAAAGTTAATGCGGAATGGTTTTTTTATTT

Region of SprB amplified and cloned (140 bp)

AAATGTAAAAATAA**<**GACGACATGCGCGAACATGTCGTCTAAGCAAGCCAAACGCTGGCTTCTAAAATATT
TTTTTAACCATTCCAACTTGCCAAAGTTAATGCGGAATGGTTTTTTTATTT**>**TCCGCTAATTGAAATAAAA

SprB in pCN40 (pMNSprB)

CAGCTTACTATGCCATTATTAATAACTTAGCCATTTCAACACCTTCTTTCAAATATTTATAATAAACTATT
GACACCGATATTACAATTGTAATATTATTGATTTATAAAAATTACAACTGTAATATCGGAGGGTTTATTCT
GCAGGTCGACGGTATCGATAAGCTTGATA**TCGAAT**TCCTGCAGCCC**<**AAA**TGTAAAAAT**AAGACGACATGC
GCGAACATGTCGTCTAAGCAAGCCAAACGCTGGCTTCTAAAATATTTTTTTAACCATTCCAACTTG**CCAAA**
GTTAATGCGGAATGGTTTTTTTATTTTCCGCTAATTGAAATAAAA**>**TCTAGAGGGGGATCCCCGGGTACCG
AGCTC

SprB$_{AS}$ in pCN40 (pMNSprB$_{AS}$)

CAGCTTACTATGCCATTATTAATAACTTAGCCATTTCAACACCTTCTTTCAAATATTTATAATAAACTATT
GACACCGATATTACAATTGTAATATTATTGATTTATAAAAATTACAACTGTAATATCGGAGGGTTTATTCT
GCAG**GTCGAC**TCTAGA**GGATCC<**TTTTATTTCAATTAGCGGAAAATAAAAAAACCATTCCGCATTAACTTT
GGCAAGTTGGAATGGTTAAAAAAATATTTTAGAAGCCAGCGTTTGGCTTGCTTAGACGATATGTTCGCGCA
TGTCGTCTTATTTTTACATTT**>**GGGCTGCAGGAATTCGATATC**AAGCTT**ATCGATACC**GTCGAC**CTCGAGG
GGGGGCCC**GGTACC**GAGCTC

Underlined region is the SprB and SprB$_{AS}$ transcripts; Bold letters and underlined indicate TT- transcription terminator; Light shaded box GTCGAC- SalI; GGATCC- BamHI; GGTACC- KpnI indicates the restriction enzymes; Thick shaded box (Bold and underlined) indicate the putative inherent promoter of SprB; Thick shaded box (but not Bold and underlined) indicate the vector promoter PblaZ.