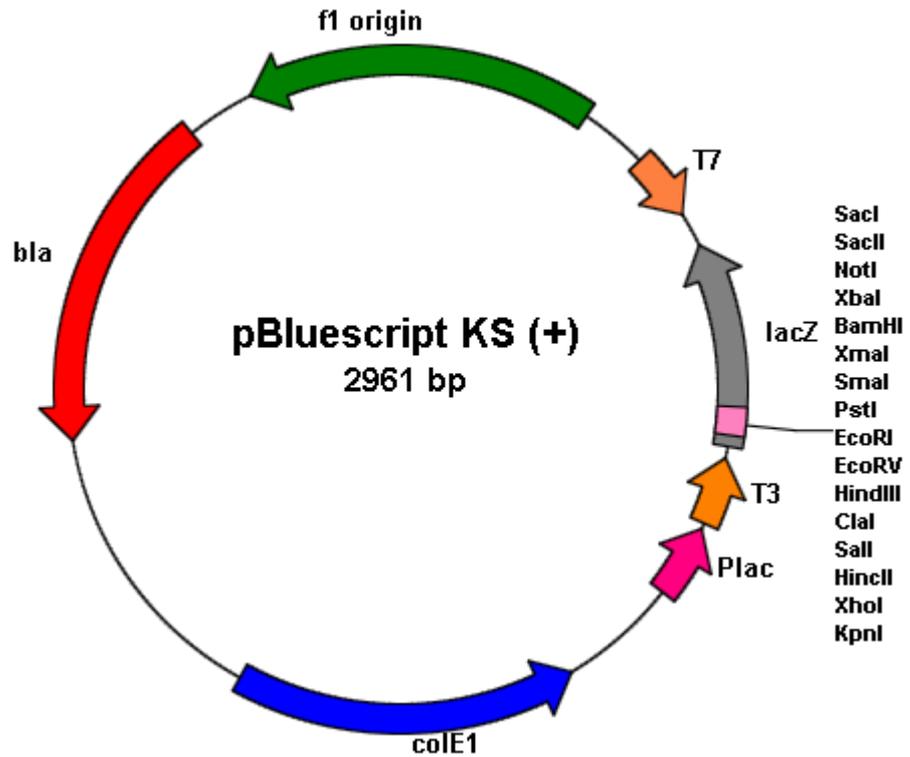


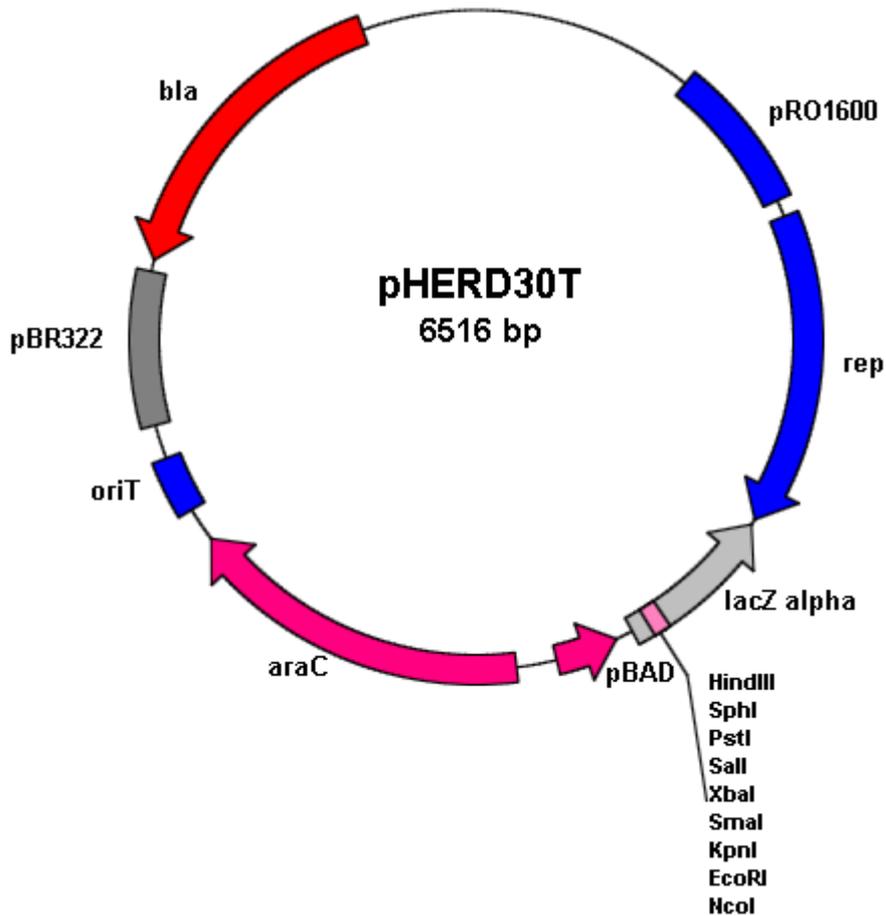
APPENDIX I

Vector map of pBluescriptKS (+)



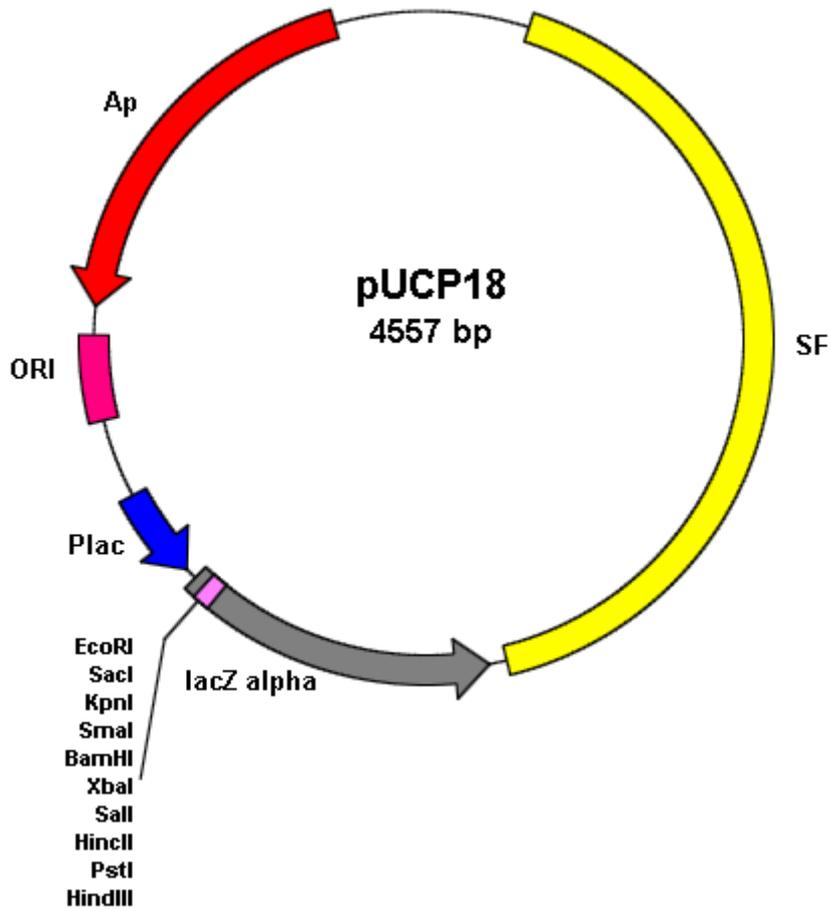
Contains unique restriction sites for cloning in *lacZ* α peptide. T7 and T3 promoters enable *in vitro* transcription of the cloned DNA. β -lactamase renders resistance towards ampicillin and colE1 is the replication origin. f1 is the phage origin of replication.

Vector map of *E. coli* - *Pseudomonas* shuttle plasmid pHERD30T



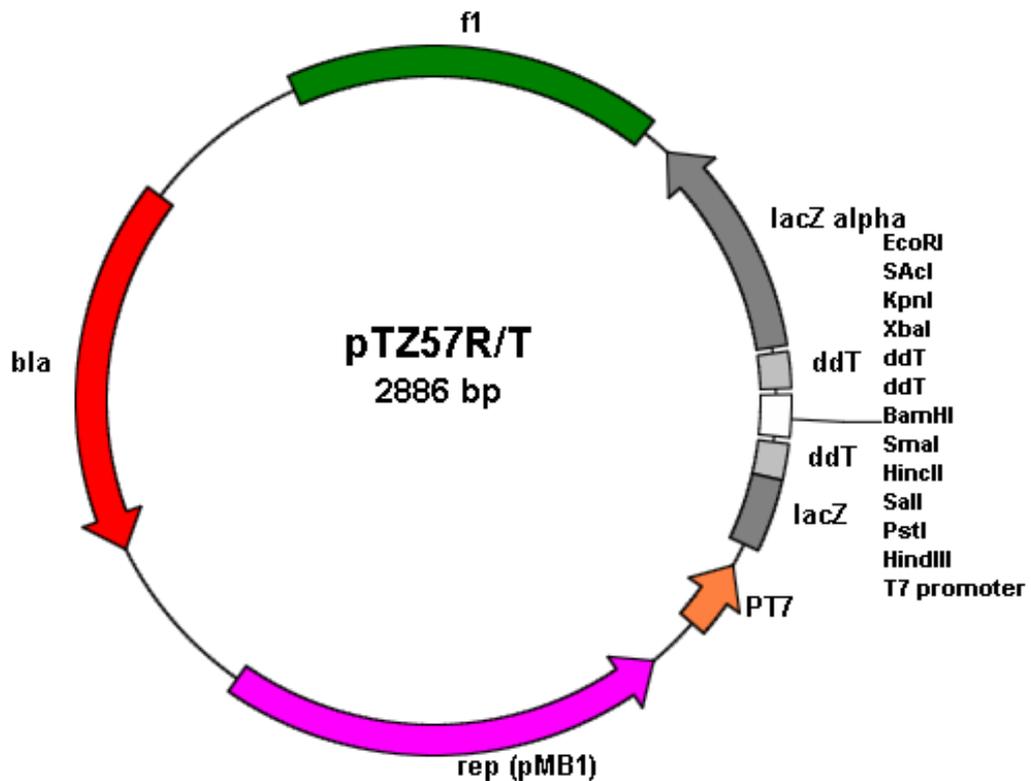
ori_pBR322 and ori_pRO1600 are replication origins for *E. coli* and *Pseudomonas* respectively. pBAD is L-arabinose inducible *ara* promoter. *araC* encodes repressor protein. *oriT* encodes conjugation mediated plasmid transfer. *rep* encodes replication-controlling protein. *lacZ* α contains the multiple cloning site and enables blue-white screening. *aacC1* encoding gentamicin acetyl transferase that confers gentamicin resistance.

Vector map of *E.coli-Pseudomonas* shuttle plasmid pUCP18



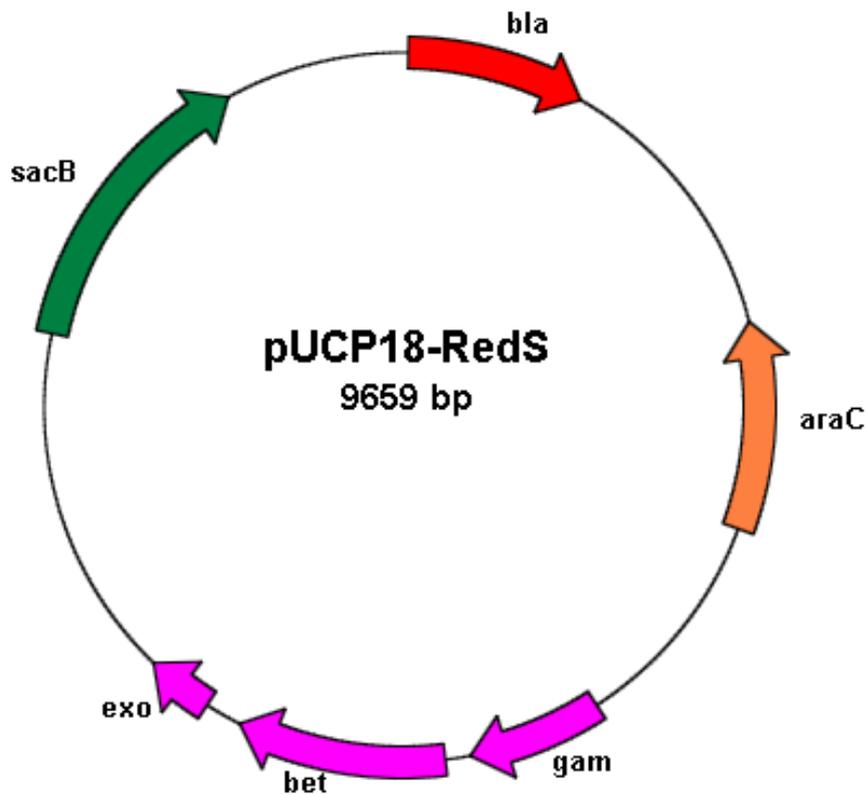
Contains *plac* promoter that constitutively expresses in *Pseudomonas* with the *lacZ* α peptide containing the MCS enabling blue-white screening of recombinant clones. SF- stabilizing fragment with pRO1600 replication origin for *Pseudomonas*. *colE1* origin of replication for *E. coli*. Ap encodes β -lactamase gene conferring ampicillin resistance in *E.coli* and carbenicillin resistance in *Pseudomonas aeruginosa*.

Vector map of pTZ57R/T



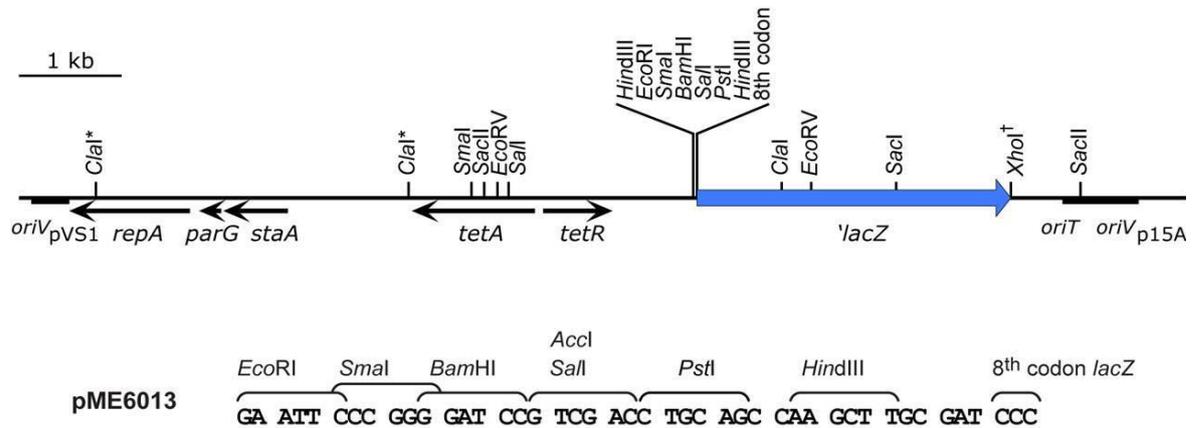
Linearized ddT tailed cloning vector for cloning of PCR products generated with thermostable non-proof reading enzymes that add extra A overhangs at the 3' end of the PCR product. *rep* from the pMB1 plasmid for replication, *bla* conferring resistance to ampicillin, *lacZ* α peptide for blue white screening, phage f1 origin for synthesis of single stranded DNA, T7 promoter for *in vitro* transcription of insert DNA with T7 RNA polymerase.

Vector map of pUCP18-RedS



Recombinant proficient vector expressing *Red* operon (*gam*, *bet*, *exo*) enabling homologous recombination. *araC* fragment- repressor of *ara* operon, *sacB* encodes levansucrase, *bla*- β -lactamase confers resistance to ampicillin.

Vector map of pME6013



Plasmid for translational fusion with *lacZ* truncated at the 8th codon. p15A-pVS1 replicons for maintenance in *E. coli* and *Pseudomonas* respectively. *tetA* conferring resistance to tetracycline.

APPENDIX II

>Sequence of PhrD (73 bp)

ATGCCAAGACTAGGAGCAGCTCCAGTCACAAATGGCCTCTACAGTCGTCTAGTCTCCTGTTTACGTTTGTGTT

>Sequence of PhrD cloned in pHERD30T (152 bp)

GCTCTAGAATGCCAAGACTAGGAGCAGCTCCAGTCACAAATGGCCTCTACAGTCGTCTAGTCTCCTGTTTACGTT
TGTGTTGCCCTTAGGCTCGGCTCGGGCTCGATGCCAAAGGGCTTCACAAATAGTAAATCCCCGCTCTGCAGAC
GT

*Xba*I and *Pst*I sites used for cloning are underlined.

>Sequence of PhrD gene disruption cassette

GATCCGGGAGCGAACCCCGAAGCGCAACTGGACGAGGAGCCCCAGGAGGTCAGCGAAGCGCCGGCCTATCGCGTG
GATGACCTCACCTTCTGTACCTCACCCATGAGGTCTATCTGGAACCCACGTTTCCGCGTCCGAAAGTCCCTG
GGCGAACGTTCTGACGTCGCTGTGGAACGAAACCTATTCGCGCCGCTCCCCGCTGAATTTCTGGGAATACGAC
GGGGGCGACTTCCGCGCTCAACGACCCGGCAGTGTGTTGGCCGAGGGAAAGTTCGCCAGCGAGCAGCGCATC
GGCGAAGCGCCCTGCGGCTGCCCCGATATCGATCTGGACCAGTTGGAGCGGGCCTACTCGCTGATGATGTAA
AGGAGAGGAACCTTCCGGCTGCGTCTCCGCCGAAATCCATGGGGCGCTTCCGATCTTCCCGGGCTTTACGCGGT
AAAGCCTGAAAAAGCAAGCCCTGGCACTGGTGTGTTGTTGTCAGGGGCTTTTGCATTGGGATCTGGAGCGGGCGAAG
GGAATCGAACCCCTTTAAAAATAGGGTTTTGAAGCGTTCCATACATTTCTAATGCCACTGCCTTCGATTTTTAGGCG
TCTTACTCCGTAGCTCTTCGTGTCATTGATTTTCGACACATTTTCGACAGGTCTGCATGAGGATTCATTTTTAT
CCTGGTTGCTTTATGATCACCTT**ATGCCAAGACTAGGAGCAGCTCCAGTCACAAATGGC**<GACGCACACCGTGGAA
AACGGATGAAGGCACGAACCCAGTTGACATAAGCCTGTTCCGTTTCGTAACCTGTAATGCAAGTAGCGTATCGCGT
CACGCAACTGGTCCAGAACCTTGACCGAACGCAGCGGTGGTAACGGCCAGTGGCGGTTTTTCATGCTTGTATG
ACTGTTTTTTGTACAGTCTATGCCCTCGGGCATCCAAGCAGCAAGCGGTTACGCCGTGGGTCGATGTTTGTATGT
TATGGAGCAGCAACG**ATGTTACGCAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAAAACAAAGTTAGGT**
GGCTCAAGTATGGGCATCATTTCGCACATGTAGGCTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCCTGAT
CTTTTTCGGTTCGTGAGTTTCGGAGACGTAGCCACCTACTCCCAACATCAGCCGGACTCCGATTACCTCGGGAACCTG
CTCCGTAGTAAGACATTCATCGCGCTTGCTGCCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGCTTACGTT
CTGCCAGGTTTGAGCAGCCGCTAGTGAGATCTATATCTATGATCTCGCAGTCTCCGGCAGCACCAGGAGGCAG
GGCATTGCCACCGCTCATCAATCTCCTCAAGCATGAGGCCAACGCGCTTGGTGCCTTATGTGATCTACGTGCAA
GCAGATTACGGTACGATCCCGCAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAAGTATGCACTTTGAT
ATCGACCCAAGTACCGCCACCTAACAATTCGTTCAAGCCGAGATCGGCTTCCCGGCCGCGGAGTTGTTCCGGTAAA
TTGTCACAACGCCG>**CTCTACAGTCGTCTAGTCTCCTGTTTACGTTTGTGTT**GCCCTTTAGGCTCGGCTCGGGCT
CGATGCCCTAAAGGGCTTCACAAATAGTAAATCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCC
CCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCT
CCCCGCTCCCTCGATCTTTTACTCATCCCCCTGCTCTGGGTCTACCGTTTTTCTCTACTGGGACTATCACGCCT
TTGCGTCTTCTCCCTTCCATTGTACCTTCTATGCGCTGTGCTACGAGCTTTATGGTGAGTCTTGTTCGCACCAA
TGTACATTTATTGCTGTTGGTATCGACTGGGTTCCAATATACGATGGAACCATAGATTTCTCGGCAGATGAGATA
TGAAGAAGAGACCTTTAGAAGATCTTTTTTTTTGCGATGTACAGAGGGAAGCAGAGTTTTCGAACACTTTGCCAATT
GCTCTGTCGAAGGTTTATATGAGCCGGTGTATGGTGAATGGACGACTCGTATATAAAACCGATAAGGACTCTGCGTG
CGTATCATCGGTTTCTAAATAAATCTTGTGTTGAAAGGCTTCCGTTGTTGATGATGTTGTGTTTTCTTATCGTA
AAGGTGTTAACGCCGTAGATGCTGTCGAAAAGCATGCAGGTAGTAAGTTTTTATTTTCAAACCTGATTTAAGGGATT
TCTTCAGTAGTCTAACTAAGTCTGATGTTAGAAGAACTCTGGAATGGCGAAAGATGTTTTG

PhrD is in green bold, gentamicin with its promoter and terminator is enclosed within brackets and coding sequence of gentamicin is highlighted.

>Sequence of P18 (134 bp)

GGCGGCGTCTACCTCAGCCCCTGTTTCGTCTATGCCCTGCTGGCGCTGGCGGCCACCGCCGCGCTGCGGGTGTG
ATCCAGAAAAACGCCGTTGCGTCGCTGGGTCTGGCACGAGGCGCTGTTCTGACTGCGCCCT

>Sequence of P18 cloned in pHERD30T (184 bp)

GCTCTAGAGGCGGCGTCTACCTCAGCCCCTGTTTCGTCTATGCCCTGCTGGCGCTGGCGGCCACCGCCGCGCTGC
GGGTGCTGATCCAGAAAAACGCCGTTGCGTCGCTGGGTCTGGCACGAGGCGCTGTTCTGACTGCGCCCTGTTTCATT
TGATGTTGAGTGGCATTACCTGGGCTGCAGTGCA

*Xba*I and *Pst*I sites used for cloning are underlined.

>Sequence of P18 gene disruption cassette (2014 bp)

CGCAACCAGATGGACTACAACCTCGCGACCCTGCTGAATTCGCCCCAGGGCGTGTGATCGGAGTCGGCTTCGCG
GTGGTGATCTCCGCTTGTGACCTTGCCTCCGGGCTGGCTGACCCGACGCTGCTGGACGCCACCTGCGTCGAT
CTCGGCCGCCCTCACCCGGCGCCCGCTGGCGCAGGCGGAGAACTGGTTCGGCGGGCGCATGGCCGACCGCCTGTTG
CGCTGGCGCGGCACGCCAACCTGTTGCCGGAGCAGGAGCGGCGGCGTGGGACGATGGCCTCCTGGCGCTCGAC
CTGGGTAACGAACTGATCCACCTGCGGGCCTGCCGGAAGGCGCCCGTGGCGTCCGCGCAAGGCGCGCACCGC
TTCTCGGGGAACTGGGCGAACTGCTTGAGGCCGGTCCCGCGGGTCCGCGCAGTGACCGCCTGGAGCGGATCAGC
CTGCCGTTGCGGAAGCCCTGGAGCGTGACGCTCGCCACGACGACGAAGCCAGCCGCTGGCGCGCGCGGCCCTG
GCGCAACTGCTGTTACCTGGCAGCAGTGGTGCCATCAGGAGGAAAGCCATGAGTCTGCATGAATCGGCCTGG
GGCGGCTCTACCTCAGCCCCTGTTTCGTCTATGCCCTC<GACGCACACCGTGGAAACGGATGAAGGCACGAACCCA
GTTGACATAAGCCTGTTTCGGTTCGTAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAACCTT
GACCGAACGCAGCGGTGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGTATGACTGTTTTTTTTGTACAGTCTAT
GCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGTTCGATGTTTATGATGTTATGGAGCAGCAACG**ATGTTAC**
GCAGCAGCAACGATGTTACGCAGCAGGGCAGTTCGCCCTAAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATT
GCACATGTAGGCTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATCTTTTCGGTTCGTGAGTTCGGAG
ACGTAGCCACCTACTCCCAACATCAGCCGGACTCCGATTACCTCGGGAACCTGCTCCGTAGTAAGACATTCATCG
CGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGCTTACGTTCTGCCAGGTTTGAGCAGCCGC
GTAGTGAGATCTATATCTATGATCTCGCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCA
ATCTCCTCAAGCATGAGGCCAACGCGCTTGGTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGATCCCG
CAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACTTTGATATCGACCCAAGTACCGCCACCT
AACAATTCGTTCAAGCCGAGATCGGCTTCCCGCCGCGGAGTTGTTTCGGTAAATTGTCACAACGCCG>**GACTGGC**
CCCTGTTTCATTTTGATGTTGAGTGGCATTACCTGGGCGGCTACGACCTGAATCGTCGCCCGAGAAGGAGATTG
CATGCGTGCATCGCTTCGCGTCGTCACCCCTGCTGGTGGTTCGCTGCCGCTGTCCCTCGCCGGCATCTGGCTCTG
GCGCTACTACATGCTCTCGCCCTGGACCCGCGATGCCCGGGTGCGGGCGACGTGGTGGTGGTGGCGCCGGACGT
GTCCGGCTGGGTGACCGACCTGGAGGTCAAGGACAACCAGGTGGTCAAGGTCGGCGACGTGCTGATGCGCATCGA
CCAGGAACGCTACCAGGCCAACCTGGAGCAGGCCCGCGCCGTCGCCGAGACCCGTCACCAGCAATACCTGTTGCG
CCAGAACGAGGCGGCCCGGCGCAGCCGCTGGGCATCGGCGGATCAGCGCCGAGGACAAGGAAAACGCCAGAT
CAATGCCGCCATCGCTCGCAGCGAGTACCAGGAGGCGTGGCCAGGTGAAGATCGCCGAATC

P18 is in bold, gentamicin with its promoter and terminator is enclosed within brackets and coding sequence of gentamicin is highlighted.

APPENDIX III

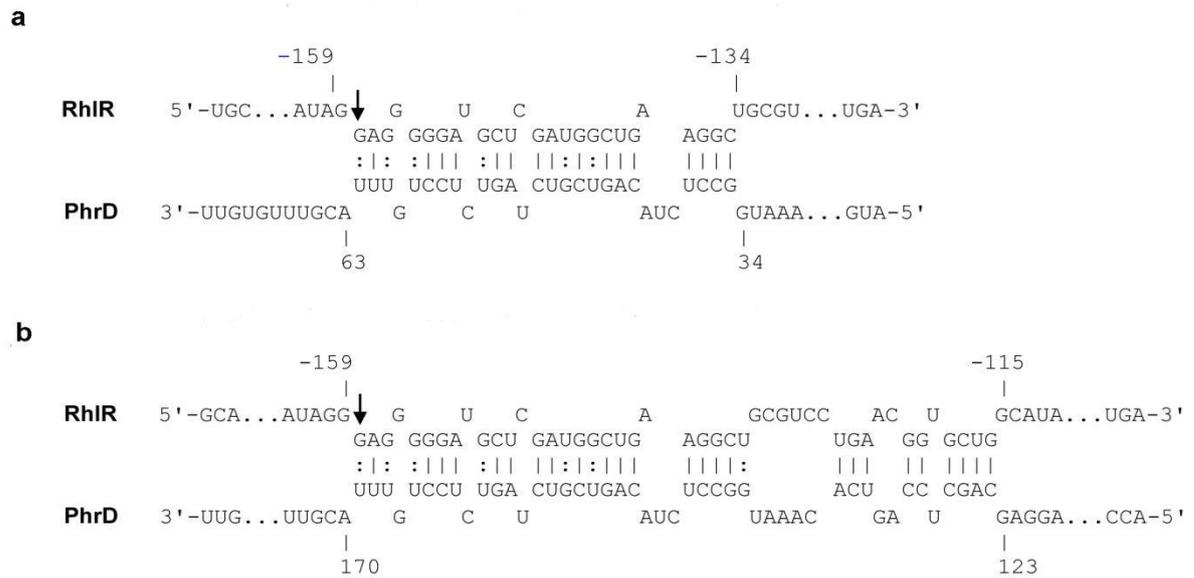


Figure: Comparison of RNA-RNA interaction of *RhlR* with PhrD expressed from the chromosome (a) and plasmid (b). The former interaction is completely in the later as indicated by IntaRNA program. The numbers on PhrD indicate nucleotide from the transcription start site