

	-35	ext.-10	+1	RBS	fMet	
M. bovirh. HAZ141_2_P*	TTGAAA-AAATGTAAAATTTGT	TGATATAAT -AAACTG	<u>A</u> ACGATAAAATAAGAATTTGC	GGAGGT AAAAATATAG	ATG	<i>aadE*</i> →
M. bovirh. HAZ141_2_P* ^{Der}	ATGATACAGTGAATAGATTTTAT	TGATATAAT -GAG-TTATCAACAAATCGGAATTTGT	GGAGGT AAAAATATAG	ATA		<i>aadE*</i> →
	*****	***	*****	*****		
C. coli pCFSAN032805	ATGATACAGTGAATAGATTTTAT	TGATATAAT -GAG-TTATCAACAAATCGGAATTTGT	GGAGGT AAAAATATAG	ATA		<i>aadE*</i> →
C. coli pCFSAN032806	ATGATACAGTGAATAGATTTTAT	TGATATAAT -GAG-TTATCAACAAATCGGAATTTGT	GGAGGT AAAAATATAG	ATA		<i>aadE*</i> →
C. coli pCCDM183	ATGATACAGTGAATAGATTTTAT	TGATATAAT -GAG-TTATCAACAAATCGGAATTTGT	GGAGGT AAAAATATAG	ATA		<i>aadE*</i> →
C. jejuni pGMI16-002	ATGATACAGTGAATAGATTTTAT	TGATATAAT -GAG-TTATCAACAAATCGGAATTTGT	GGAGGT AAAAATATAG	ATA		<i>aadE*</i> →
C. jejuni pCG8245	TTGAAA-AGTGGATAGATTTTAT	TGATACAGT -GAATAGA..+29 bp..GGAATTTGT	GGAGGT AAAAATATAG	ATA		<i>aadE*</i> →
C. jejuni pGB19	GTGTTT-CTTATGCTGTTGTAT	TGGTATgAT -TAGTAAG..+20 bp..AGAATTTGT	GGAGGT AAAAATATAG	ATG		<i>aadE*</i> →
E. faecalis plasmid	TTGTATCGGCAAAAAAGAAGAT	TGTAATAAT -ATAGAAA.....AAATAAAC-	GAGG -AGTATGTAA	ATG		<i>aadE</i> →
S. aureus Tn5405	TTGTATCGGCAAAAAAGAAGAT	TGTAATAAT -ATAGAAA.....AAATAAAC-	GAGG -AGTATGTAA	ATG		<i>aadE</i> →
S. epidermidis plasmid	TTGTATCGGCAAAAAAGAAGAT	TGTAATAAT -ATAGAAA.....AAATAAAC-	GAGG -AGTATGTAA	ATG		<i>aadE</i> →
S. pyogenes NGAS322	TTTTAA-AATAGTAGACTATGT	TGATATAAT -AGAATCG.....GAAGTTGT	GGAGGT AAAAATATAG	ATG		<i>aadE*</i> →
S. suis PP phi-SsUD.1	TTAAAAAATCGTTTATGTT	TGTTCAAAT TATCCTGAA..+35 bp..AAAATAAAC	GAGG -AGTATGTAA	ATG		<i>aadE</i> →

Figure S3: the closest homologous sequences of the putative promoters located upstream of *aadE**- and *aadE*-like genes in genomes of other bacteria. Nucleotide sequence alignment of *Mycoplasma bovirhinis* HAZ141_2 P* and P*^{Der} promoters with their closest homologous identified in genomes of other bacteria. The sequences were aligned using the CLUSTALW [1] public server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=NPSA/npsa_clustalwan.html). Promoter elements, as previously identified for P* [2] are shown with -35 and extended -10 (as ext. -10) above the boldface letters. The 5'RACE experimentally validated transcription start site is shown with +1 above, boldfaced and underlined. Predicted ribosome-binding site is shown with RBS above the boldface italicized letters. Arrow indicates the position and direction of transcription of the *aadE** gene, putative start codons of which (ATG before and ATA after inversion) are shown in bold and underlined. The identical nucleotides identified between *M. bovirhinis* HAZ141_2 P*^{Der} promoter and its homologous sequences in genomes of other bacteria are marked with asterisks.

C. coli pCFSAN032805 - *Campylobacter coli* strain CFSAN032805, plasmid pCFSAN032805 (CP045793.1).

C. coli pCFSAN032806 - *Campylobacter coli* strain CFSAN032806, plasmid pCFSAN032806 (either CP045790.1, or CP023544.1).
C. coli pCCDM183 - *Campylobacter coli* strain BP3183 plasmid pCCDM183 (CP017872.1).
C. jejuni pGMI16-002 - *Campylobacter jejuni* strain CFSAN054107 plasmid pGMI16-002 (CP028186.1).
C. jejuni pCG8245 - *Campylobacter jejuni* strain CG8245, plasmid pCG8245 (AY701528.1).
C. jejuni pGB19 - *Campylobacter jejuni* strain GB19, plasmid pGB19 (CP071593.1).
E. faecalis plasmid - *Enterococcus faecalis* strain OG1RF x UW3114 T-12, plasmid pLG2 (HQ426665.1).
S. aureus Tn5405 – *Staphylococcus aureus* strain, transposon Tn5405 (U73026).
S. epidermidis plasmid - *Staphylococcus epidermidis* strain RP62A, plasmid pSERP (CP000028.1).
S. pyogenes NGAS322 – *Streptococcus pyogenes* strain NGAS322 (CP010449.1).
S. suis PP phi-SsUD.1 - *Streptococcus suis* strain SsUD, prophage phi-SsUD.1 (FN997652.1).

References:

1. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680.
2. Lysnyansky, I; Borovok, I. A GC-rich prophage-like genomic region of *Mycoplasma bovirhinis* HAZ141_2 carries a gene cluster encoding resistance to kanamycin and neomycin. *Antimicrob Agents Chemother.* **2021**, *65*:e01010-20. doi: 10.1128/AAC.01010-20.

SphI pACYC184-R1-SphI →

1 **GCATGCGTAT TAACGAAGCG CTAACC**GTTT TTATCAGGCT CTGGGAGGCA GAATAAATGA TCATATCGTC AATTATTACC TCCACGGGGA
 91 GAGCCTGAGC AAACCTGGCCT CAGGCATTTG AGAAGCACAC GGTACACTG CTTCCGGTAG TCAATAAACC GGTAAACCAG CAATAGACAT
 181 AAGCGGCTAT TTAACGACCC TGCCCTGAAC CGACGACCGG GTCGAATTTG CTTTCGAATT TCTGCCATTC ATCCGCTTAT TATCACTTAT

+A283

271 TCAGGCGTAG **CAACC**AGGCG TTTAAGGGCA CCAATAACTG CCTTAAAAA **ATTAC**GCCCC GCCCTGCCAC TCATCGCAGT ACTGTTGTAA
 220 <<.....**cat**.....<
 * A G G Q W E D C Y Q Q L

397

361 TTCATTAAGC ATTCTGCCGA CATGGAAGCC ATCACAA**ACG** GCATGATGAA CCTGAATCGC CAGCGGCATC AGCACCTTGT CGCCTTGCCT
 <.....**cat**.....<
 207 E N L M R G V H F G D C V A H H V Q I A L P M L V K D G Q T

451 ATAATATTTG CCCATGGTGA AAACGGGGGC GAAGAAGTTG TCCATATTGG CCACGTTTAA ATCAAAACTG GTGAAACTCA CCCAGGGATT
 <.....**cat**.....<
 177 Y Y K G M T F V P A F F N D M N A V N L D F S T F S V W P N

541 GGCTGAGACG AAAAACATAT TCTCAATAAA CCCTTTAGGG AAATAGGCCA GGTTTTACCC GTAACACGCC ACATCTTGCG AATATATGTG
 <.....**cat**.....<
 147 A S V F F M N E I F G K P F Y A L N E G Y C A V D Q S Y I H

631 TAGAAACTGC CGGAAATCGT CGTGGTATTC ACTCCAGAGC GATGAAAACG TTTCAGTTTG CTCATGGAAA ACGGTGTAAC AAGGGTGAAC
 <.....**cat**.....<
 117 L F Q R F D D H Y E S W L S S F T E T Q E H F V T Y C P H V

1680

721 ACTATCCCAT ATCACCAGCT CACCGTCTTT CATTGCCATA CG**A**AATCCG GATGAGCATT CATCAGGCGG GCAAGAATGT GAATAAAGGC
 <.....**cat**.....<
 87 S D W I V L E G D K M A M R F E P H A N M L R A L I H I F A

811 CGGATAAAAC TTGTGCTTAT TTTTCTTTAC GGTCTTTAAA AAGGCCGTAA TATCCAGCTG AACGGTCTGG TTATAGGTAC ATTGAGCAAC
 <.....**cat**.....<

```

57   P Y F K H K N K K V T K L F A T I D L Q V T Q N Y T C Q A V

901  T G A C T G A A A T G C C T C A A A T G T T C T T T A C G A T G C C A T T G G A T A T A T C A A C G G T G G T A T A T C C A G T G A T T T T T T C T C CA T T T T A G CTTC
    <.....cat.....<<
27   S Q F A E F H E K R H W Q S I D V T T Y G T I K K E M

                                +1?      ← -10/P5                                ← -35/P5
991  CTT A G C T C C T G A A A T C T C G A T A A C T C A A A A A T A C G C C C G G T A G T A T C T T A T T T C A T T A T G G T G A A A G T T G G A A C C T C T T A C G T G C C G

1081 A T C A A C G T C T C A T T T T C G C C A A A G T T G G C C C A G G G C T T C C C G G T A T C A A C A G G G A C A C C A G G A T T T A T T T A T T C T G C G A A G T G A T C T T C

                                ← IS1_del_Rev      IS1_del_For →
1171 C G T C A C A G G T A T T T A T T C G G C G C A A G T G C G T C G G C G G A G T G T A T A C T G G C T T A C T A T G T T G G C A C T G A T G A G G G T G T C A G T G A A G T G C T

1261 T C A T G T G G C A G G A G A A A A A A G G C T G C A C C G G T G C G T C A G C A G A A T A T G T G A T A C A G G A T A T A T C C G C T T C C T C G C T C A C T G A C T C G C T A

1351 C G C T C G G T C G T T C G A C T G C G G C G A G C G G A A A T G G C T T A C G A A C G G G G C G G A G A T T T C C T G G A A G A T G C C A G G A A G A T A C T T A A C A G G G A A

1441 G T G A G A G G G C C G C G G C A A A G C C G T T TTTCC ATAGGCTCCG CCCCCCTGAC AAGCATCACG AAATCTGACG CTCAAATCAG TGGTGGCGAA
1531 ACCCGACAGG ACTATAAAGA TACCAGGCGT TTCCCCCTGG CGGCTCCCTC GTGCGCTCTC CTGTTCCCTGC CTTTCGGTTT ACCGGTGTCA
1621 TTCCGCTGTT ATGGCCGCGT TTGTCTCATT CCACGCCTGA CACTCAGTTC CGGGTAGGCA GTTTCGCTCCA AGCTGGACTG TATGCACGAA
1711 CCCCCGTTC AGTCCGACCG CTGCGCCTTA TCCGGTAACT ATCGTCTTGA GTCCAACCCG GAAAGACATG CAAAAGCACC ACTGGCAGCA
1801 GCCACTGGTA ATTGATTTAG AGGAGTTAGT CTTGAAGTCA TGCGCCGGTT AAGGCTAAAC TGAAAGGACA AGTTTTGGTG ACTGCGCTCC
1891 TCCAAGCCAG TTACCTCGGT TCAAAGAGTT GGTAGCTCAG AGAACCTTCG AAAAACCGCC CTGCAAGGCG GTTTTTTCGT TTTCAGAGCA
1981 AGAGATTACG CGCAGACCAA AACGATCTCA AGAAGATCAT CTTATTAATC AGATAAAATA TTTCTAGATT TCAGTGCAAT TTATCTCTTC

                                ← NEW_HindIII_pACYC184 HindIII
2071 A A A T G T A G C A C C T G A A G T C A G C C C C A T A C G A T A T A A G T T G T A A T T C T C A T G T A A G C T T

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Figure S4: sequence of the pACYC_ΔtetΔP2^{vec}ΔP4^{vec} cloning vector used in this study. The 2,128 bp pACYC_ΔtetΔP2^{vec}ΔP4^{vec} cloning vector was constructed from the low-copy-number pACYC184 plasmid (4,245 bp; GenBank X06403.1) by deletion of its P2 and P4 promoters (described

by Stuber & Bujard [1] and designated here as P2^{vec} and P4^{vec}) as well as the *tet* gene as described in Materials and Methods and in Tables S1-2. Names of primers (pACYC184-R1-SphI, IS1_del_For, IS1_del_Rev, and NEW_HindIII_pACYC184) used for assembling PCR shown above the corresponding sequences in bold blue; overlapping sequences of IS1_del_For and IS1_del_Rev are underlined. Both start and stop codons of the *cat* gene (a minus strand) encoding resistance to chloramphenicol shown in bold red and underlined. The *cat*-P5 promoter-related elements including +1 (transcription start site), -10, and -35 elements are bolded and underlined; the predicted ribosome-binding site (RBS) is shown in bold italic. The p15A origin of replication is grey highlighted. One single nucleotide polymorphism (SNP of G to A; a silent mutation) has been identified at position 397 of the pACYC_ΔtetΔP2^{vec}ΔP4^{vec} within the encoding sequence of the *cat* gene results in silent mutation. In addition, an indel (as insertion) of a nucleotide "A" (+A283) was found at position 283 of the pACYC_ΔtetΔP2^{vec}ΔP4^{vec} located downstream of the *cat* gene. The newly designed restriction sites *HindIII* and *SphI*, used for the cloning in this study, are italicized, bolded and underlined. The nucleotide 1680 (in bold and green highlighted) corresponds to the 1st nucleotide of pACYC184 (X06403.1).

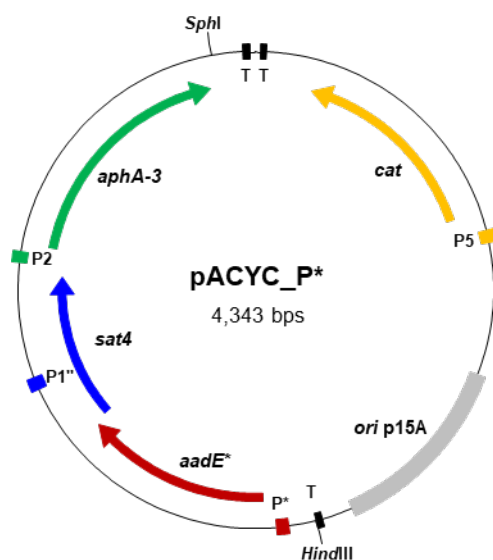
References

1. Stuber, D.; Bujard, H. Organization of transcriptional signals in plasmids pBR322 and pACYC184. *Proc Natl Acad Sci U S A* **1981**, *78*, 167-71.

Figure S5: schematics (as circular maps) of the seven plasmid constructs used in this study and nucleotide sequences of the *aadE**-*sat4*-*aphA*-3 derivatives used for cloning into unique *Hind*III and *Sph*I restriction sites of the pACYC_ΔtetΔP2^{vec}ΔP4^{vec} vector (Fig. S4).

All of schematics contain the following common elements: genes shown as bended arrows colored with yellow for *cat* (resistance to chloramphenicol), red for *aadE**, blue for *sat4*, and green for *aphA*-3; promoters shown as colored rectangles, whose colors correspond to their cognate genes (e.g. P5 of *cat* is yellow, P2 of *aphA*-3 is green etc). The plasmid p15A origin of replication shown as a grey bended rectangle and signed as *ori* p15A. Black rectangles mark positions of predicted transcriptional terminators signed with T capital. Each of schematics is followed with a nucleotide sequence of the corresponding cloned insert. Open reading frames (ORFs) of *aadE**, *sat4* and *aphA*-3 are shown in bold and colored as described above – red for *aadE**, blue for *sat4*, and green for *aphA*-3 except a four-nucleotide overlapping between the stop codon of *aadE** (TGA) and the start of *sat4* (GTG), which colorless and underlined. An ORF remnant (if there is) shown with same color as that of an intact ORF. Start and stop codons of ORFs underlined. Promoter sequences shown in bold and highlighted using the following colors – grey for P* and P*^{Der}, yellow for P1'', and green for P2.

pACYC_P* (4,343 bp)



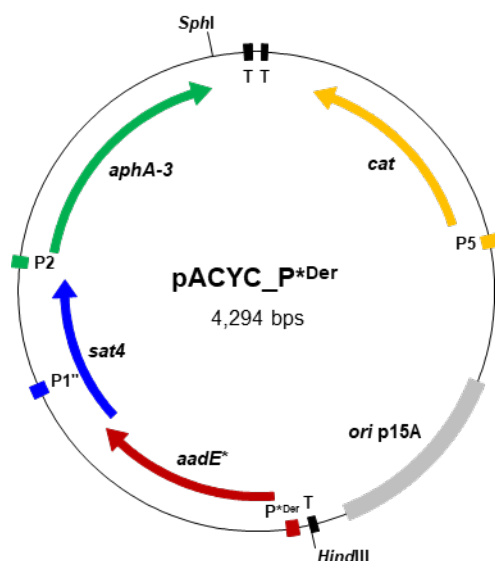
pACYC_P* (an insert size is 2,215 bp; without flanking *HindIII* and *SphI* sites)

HindIII

AAGCTTGCACATTCGGTAACGGAAGCAGTTACAATCTCCTGCAAAGTCGGGAGATTTTTTCGTTATACGGTAGGTGAATG
 TAT**TTGAAAAAATGTAAAAATTTGTGATATAAT**AAACTGAACGATAAATAAGAATTTGCGGAGGTAAAATATAG**ATGAATCA**
AGGTAGAATTATTGTAATCACAGGTGCGCCGGGGACAGGAAAACTACAACGGCATCTGCTGTTGCAAAAGAATCAGATT
TGGAAAAGTCTGTGCATATGCACACAGATGACTTTTATCATTATCATGTAAGAAAGCCAAGCGCAAGGGAGTATGATGAT
TGCTGCAATGAATTTTGAATGTAACAGCTTATGTTATTAAAGGATTGTGCCGTAAGGAAATTTTATTTGCTATTGATCA
TTTTAATCAGATTGTTTCGCCATGAGCTGCTGAGAATGATATCATGGAAGGTCGGCATCGAAACAGGCTTTAAATTAAGTG
TAGGCAAGAACTATAAGTTTATTGAAAGGTATGTATCCGAGGATTTGTGGGAGAACTTTTGTCCACCTACCGGATGGAT
TCCTATGAAAACATATGGGAAGCATTATTTCTATGCCATCAATTGTTTCAGGGCGGTATCCGGTGAGGTGGCGGAATGGCT
TCATTATGCCTATCCGGAGTATGATAGGAATATAACAAAATATACCAGGGACATGTATAAAAAATACACTGGTAAAACCG
GCTGCCTGGATAGCACATATGCCGCTGATATAGAAGAGAGGCGGGAACA**GTGA****TTACAGAAATGAAAGCAGGGCACCTGA**
AAGATATCGATAAACCAGCGAACCATTTGAGGTGATAGGTAAGATTATACCGAGGTATGAAAACGAGAATTGGACCTTT
ACAGAATTACTCTATGAAGCGCCATATTTAAAAAGCTACCAAGACGAAGAGGATGAAGAGGATGAGGAGGCAGATTGCCT
TGAATATA**TTGACAATACTGATAAGATAATATATCTTT**TACTACCAAGACGATAAATGCGTCGGAAAAGTTAAACTGCGAA
 AAAATTGGAACCGGTACGCTTATATAGAAGATATCGCCGTATGTAAGGATTTTCAGGGGGCAAGGCATAGGCAGCGCGCTT
 ATCAATATATCTATAGAATGGGCAAAGCATAAAAACTTGCATGGACTAATGCTTGAAACCCAGGACAATAACCTTATAGC
 TTGTAAATCTATCATAATTGTGGTTTCAAATCGGCTCCGTCGATACTATGTTATACGCCAACTTTGAAAACAACCTTG
AAAAAGCTGTTTTCTGGTATTTAAGGTTTTAGAATGCAAGGAACAGTGAATTGGAGTTCGTC**TTGTTATAATTAGCTTCT**
TGGGGTATCTTTAAATACTGTAGAAAAGAGGAAGGAATAATAA**ATGGCTAAATGAGAATATCACCGGAATTGAAAAAA**
CTGATCGAAAAATACCGCTGCGTAAAAGATACGGAAGGAATGTCTCCTGCTAAGGTATATAAGCTGGTGGGAGAAAATGA
AAACCTATATTTAAAAATGACGGACAGCCGGTATAAAGGGACCACCTATGATGTGGAACGGGAAAAGGACATGATGCTAT
GGCTGGAAGGAAAGCTGCCTGTTCCAAAGGTCCTGCACTTTGAACGGCATGATGGCTGGAGCAATCTGCTCATGAGTGAG
GCCGATGGCGTCCTTTGCTCGGAAGAGTATGAAGATGAACAAAGCCCTGAAAAGATTATCGAGCTGTATGCGGAGTGCAT
CAGGCTCTTTCACTCCATCGACATATCGGATTGTCCCTATACGAATAGCTTAGACAGCCGCTTAGCCGAATTGGATTACT
TACTGAATAACGATCTGGCCGATGTGGATTGCGAAAACCTGGGAAGAAGACACTCCATTTAAAGATCCGCGCGAGCTGTAT
GATTTTTTTAAAGACGGAAAAGCCCAGAGGAACTTGTCTTTTCCACGGCGACCTGGGAGACAGCAACATCTTTGTGAA
AGATGGCAAAGTAAGTGGCTTTATTGATCTTGGGAGAAGCGGCAGGGCGGACAAGTGGTATGACATTGCCTTCTGCGTCC

GGTCGATCAGGGAGGATATCGGGGAAGAACAGTATGTCGAGCTATTTTTTGA^{CTTACTGGGGATCAAGCCTGATTGGGAG}
 AAAATAAAATATTATATTTTACTGGATGAATTGTTTTAGTACCTAGATTTAGATATCTAAAGCATGC
SphI

pACYC_P*Der (4,294 bp)



pACYC_P*Der (an insert size is 2,166 bp; a putative alternative promoter is underlined)

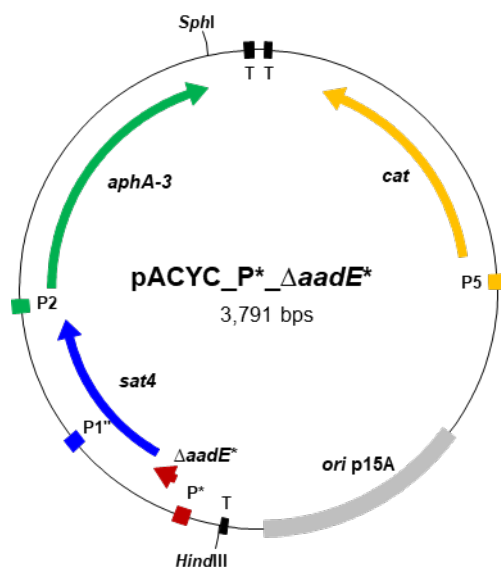
HindIII

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 GAATTTGTGGAGGTAAAATATAGATAAATCAAGGTAGAATTATTGTAATCACAGGTGCGCCGGGGACAGGAAAACTACA
 ACGGCATCTGCTGTTGCAAAAGAATCAGATTTGGAAAAGTCTGTGCATATGCACACAGATGACTTTTATCATTATCATGT
 AAGAAAGCCAAGCGCAAGGGAGTATGATGATTGCTGCAATGAATTTTGGAAATGTAAACAGCTTATGTTATTAAAGGATTGT
 GCCGTAAGGAAATTTTATTTGCTATTGATCATTTTAATCAGATTGTTTCGCCATGAGCTGCTGAGAATGATATCATGGAAG

GTCGGCATCGAAACAGGCTTTAAATTAAGTGTAGGCAAGAACTATAAGTTTATTGAAAGGTATGTATCCGAGGATTTGTG
 GGAGAACTTTTTGTCCACCTACCGGATGGATTCCCTATGAAAACATATGGGAAGCATTATTTCTATGCCATCAATTGTTCA
 GGGCGGTATCCGGTGAGGTGGCGGAATGGCTTCATTATGCCTATCCGGAGTATGATAGGAATATAACAAAATATACCAGG
 GACATGTATAAAAAATACACTGGTAAAACCGGCTGCCTGGATAGCACATATGCCGCTGATATAGAAGAGAGGCGGGAACA
GTGAATTACAGAAATGAAAGCAGGGCACCTGAAAGATATCGATAAACCCAGCGAACCATTGAGGTGATAGGTAAGATTAT
 ACCGAGGTATGAAAACGAGAATTGGACCTTTACAGAATTACTCTATGAAGCGCCATATTTAAAAAGCTACCAAGACGAAG
 AGGATGAAGAGGATGAGGAGGCAGATTGCCTTGAATATATTGACAATACTGATAAGATAATATATCTTTACTACCAAGAC
 GATAAATGCGTCGGAAAAGTTAACTGCGAAAAAATTGGAACCGGTACGCTTATATAGAAGATATCGCCGTATGTAAGGA
 TTTCAGGGGGCAAGGCATAGGCAGCGCGCTTATCAATATATCTATAGAATGGGCAAAGCATAAAAACTTGCATGGACTAA
 TGCTTGAAACCCAGGACAATAACCTTATAGCTTGTAATTCCTATCATAATTGTGGTTTCAAATCGGCTCCGTCGATACT
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 TAAAATGAGAATATCACCGGAATTGAAAAAATGATCGAAAAATACCGCTGCGTAAAAGATACGGAAGGAATGTCTCCTG
 CTAAGGTATATAAGCTGGTGGGAGAAAATGAAAACCTATATTTAAAAATGACGGACAGCCGGTATAAAGGGACCACCTAT
 GATGTGGAACGGGAAAAGGACATGATGCTATGGCTGGAAGGAAAGCTGCCTGTTCCAAAGGTCTGCACTTTGAACGGCA
 TGATGGCTGGAGCAATCTGCTCATGAGTGAGGCCGATGGCGTCCTTTGCTCGGAAGAGTATGAAGATGAACAAAGCCCTG
 AAAAGATTATCGAGCTGTATGCGGAGTGCATCAGGCTCTTTCACTCCATCGACATATCGGATTGTCCCTATACGAATAGC
 TTAGACAGCCGCTTAGCCGAATTGGATTACTTACTGAATAACGATCTGGCCGATGTGGATTGCGAAAACGGGAAGAAGA
 CACTCCATTTAAAGATCCGCGCGAGCTGTATGATTTTTTAAAGACGGAAAAGCCGAAGAGGAACTTGTCTTTTCCCACG
 GCGACCTGGGAGACAGCAACATCTTTGTGAAAGATGGCAAAGTAAGTGGCTTTATTGATCTTGGGAGAAGCGGCAGGGCG
 GACAAGTGGTATGACATTGCCTTCTGCGTCCGGTCGATCAGGGAGGATATCGGGGAAGAACAGTATGTCGAGCTATTTTT
 TGACTTACTGGGGATCAAGCCTGATTGGGAGAAAATAAAATATTATTTTACTGGATGAATTGTTTTAGTACCTAGATT
 TAGATATCTAAAGCATGC

SphI

pACYC_P*_ Δ aadE* (3,791 bp)



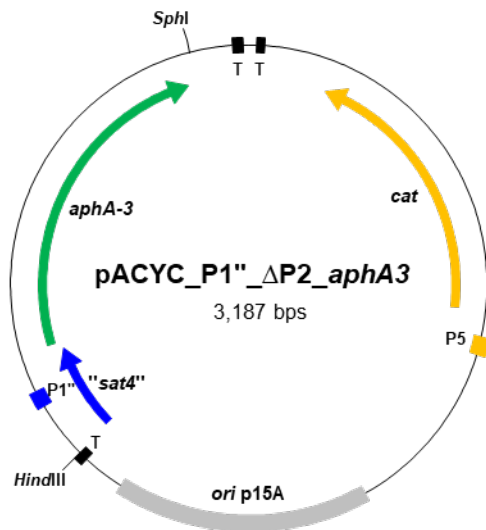
pACYC_P*_ Δ aadE* (an insert size is 1,663 bp)

*Hind*III

AAGCTTGCACATTCGGTAACGGAAGCAGTTACAATCTCCTGCAAAGTCGGGAGATTTTTTCGTTATACGGTAGGTGAATG
 TAT**TTGAAAAAATGTAAAATTTGTGATATAAT**AAACTGAACGATAAATAAGAATTTGCGGAGGTAAAATATAG**ATGAATCA**
AGGTAGAATTATTGTAATCACAGGTGCGCCGGGGACAATAGAAGAGAGGCGGGAACA**GTGATTACAGAAATGAAAGCAGG**
GCACCTGAAAGATATCGATAAACCCAGCGAACCATTTGAGGTGATAGGTAAGATTATACCGAGGTATGAAAACGAGAATT
GGACCTTTACAGAATTACTCTATGAAGCGCCATATTTAAAAAGCTACCAAGACGAAGAGGATGAAGAGGATGAGGAGGCA
GATTGCCTTGAATATATTGACAATACTGATAAGATAATATATCTTTACTACCAAGACGATAAATGCGTCGGAAAAGTTAA
ACTGCGAAAAAATTGGAACCGGTACGCTTATATAGAAGATATCGCCGTATGTAAGGATTTCAGGGGGCAAGGCATAGGCA
GCGCGCTTATCAATATATCTATAGAATGGGCAAAGCATAAAAACTTGCATGGACTAATGCTTGAAACCCAGGACAATAAC

CTTATAGCTTGTAATTCTATCATAATTGTGGTTTCAAAATCGGCTCCGTCGATACTATGTTATACGCCAACTTTGAAAA
 CAACCTTTGAAAAAGCTGTTTTCTGGTATTTAAGGTTTTAGAAATGCAAGGAACAGTGAATTGGAGTTTCGTC TTGTTATAAT
 TAGCTTCTTGGGGTATCTT TAAATACTGTAGAAAAGAGGAAGGAAATAATAA ATGGCTAAAATGAGAATATCACCGGAAT
 TGAAAAAACTGATCGAAAAATACCGCTGCGTAAAAGATACGGAAGGAATGTCTCCTGCTAAGGTATATAAGCTGGTGGGA
 GAAAATGAAAACCTATATTTAAAAATGACGGACAGCCGGTATAAAGGGACCACCTATGATGTGGAACGGGAAAAGGACAT
 GATGCTATGGCTGGAAGGAAAGCTGCCTGTTCCAAAGGTCCTGCACCTTTGAACGGCATGATGGCTGGAGCAATCTGCTCA
 TGAGTGAGGCCGATGGCGTCCTTTGCTCGGAAGAGTATGAAGATGAACAAAGCCCTGAAAAGATTATCGAGCTGTATGCG
 GAGTGCATCAGGCTCTTTCACCTCCATCGACATATCGGATTGTCCCTATACGAATAGCTTAGACAGCCGCTTAGCCGAATT
 GGATTACTTACTGAATAACGATCTGGCCGATGTGGATTGCGAAAACCTGGGAAGAAGACACTCCATTTAAAGATCCGCGCG
 AGCTGTATGATTTTTTAAAGACGGAAGCCGAAGAGGAACCTGTCTTTTCCCACGGCGACCTGGGAGACAGCAACATC
 TTTGTGAAAGATGGCAAAGTAAGTGGCTTTATTGATCTTGGGAGAAGCGGCAGGGCGGACAAGTGGTATGACATTGCCTT
 CTGCGTCCGGTCGATCAGGGAGGATATCGGGGAAGAACAGTATGTCGAGCTATTTTTTTGACTTACTGGGGATCAAGCCTG
 ATTGGGAGAAAATAAAATATTATATTTTACTGGATGAATTGTTTTAGTACCTAGATTTAGATATCTAAA GCATGC
SphI

pACYC_P1''_ΔP2_aphA3 (3,187 bp)



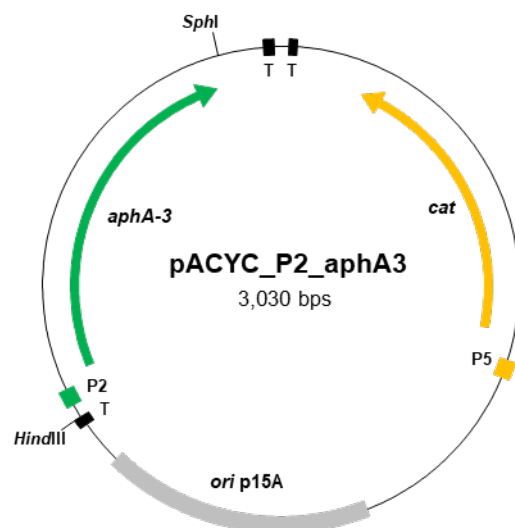
pACYC_P1" _ΔP2_aphA3 (an insert size 1,059 bp)

*Hind*III

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*Sph*I

pACYC_P2_aphA3 (3,030 bp)



pACYC_P2_aphA3 (an insert size 902 bp)

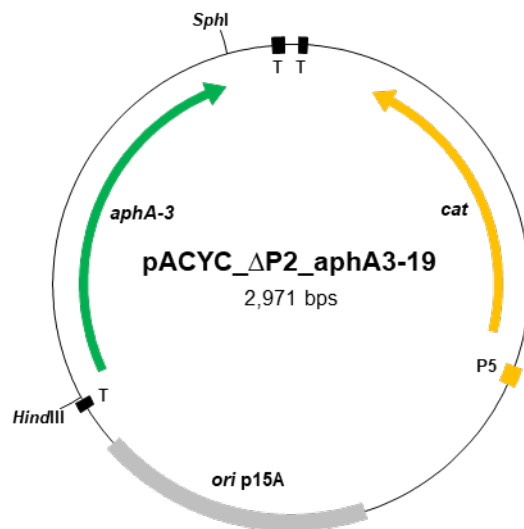
HindIII

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SphI

pACYC_ΔP2_aphA3-19 (2,971 bp)



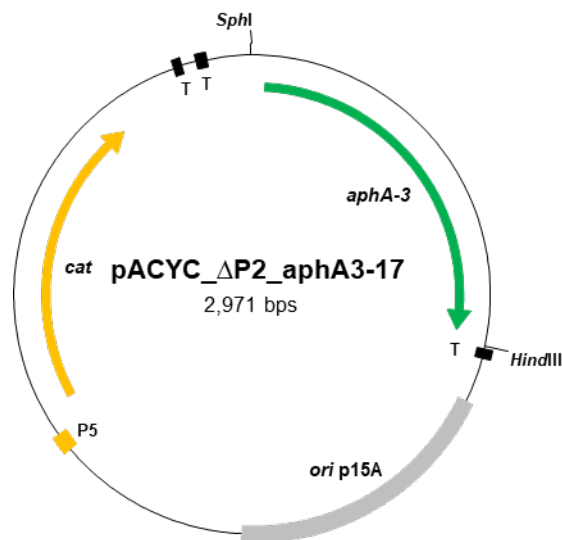
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HindIII

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SphI

pACYC_ΔP2_aphA3-17 (2,971 bp)



pACYC_ΔP2_aphA3-17 (an insert size 843 bp)

SphI
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 TATATTTTACTGGATGAATTGTTTTAGTACCTAGATTTAGATATCTAAA**AAGCTT**

HindIII

References:

1. Caillaud, F.; Trieu-Cuot, P.; Carlier, C.; Courvalin, P. Nucleotide sequence of the kanamycin resistance determinant of the pneumococcal transposon Tn1545: evolutionary relationships and transcriptional analysis of *aphA-3* genes. *Mol Gen Genet.* **1987**, *207*, 509-513.
2. Trieu-Cuot, P.; Gerbaud, G.; Lambert, T.; Courvalin, P. *In vivo* transfer of genetic information between gram-positive and gram-negative bacteria. *EMBO J* **1985**, *4*, 3583-3587.
3. Trieu-Cuot, P.; Klier, A.; Courvalin, P. DNA sequences specifying the transcription of the streptococcal kanamycin resistance gene in *Escherichia coli* and *Bacillus subtilis*. *Mol Gen Genet.* **1985**, *198*, 348-352.

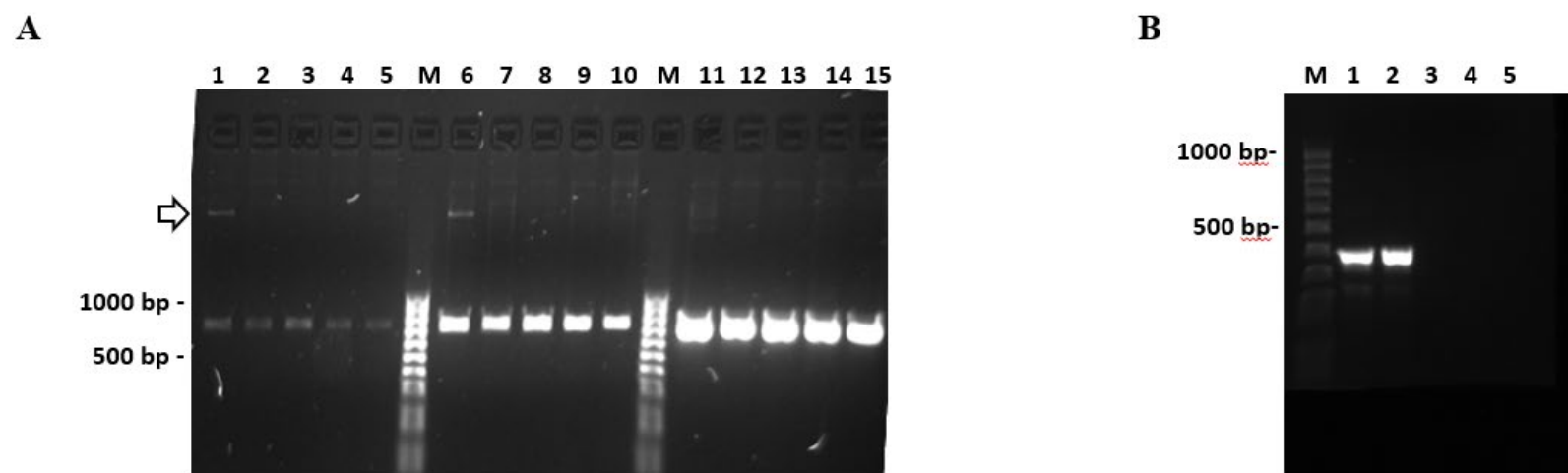


Figure S6: analyses of a potential loss of the *aadE**-*sat4*-*aphA*-3 gene cluster as well as prophage during multiple passages of *M. bovirhinis* HAZ141_2 under nonselective conditions. **(A).** Semi-quantitative PCR was performed to detect a potential increase in amount of the PCR product (727 bp in length) available upon excision of the *aadE**-*sat4*-*aphA*-3 gene cluster during nonselective serial passages (in Kn-free broth). Genomic DNA (50 ng) of *M. bovirhinis* HAZ141_2 parent strain (p.3) as well as DNAs of clonal passages were subjected to PCR amplification with 0922_down_F1 and up-aadE_F2 primers (Table S1) using different number of amplification cycles. *M. bovirhinis* HAZ141_2 p.3 (lanes 1, 6, and 11), p.30 (lanes 2, 7 and 12) and p.50 (lanes 3, 8 and 13), p. 80 (lanes 4, 9 and 14) and p.100 (lanes 5, 10 and 15). The numbers of PCR cycles were as the following: 22 – lanes 1-5; 26 – lanes 6-10; and 30 – lanes 11-15. A weaker band near 6.6 kb appeared in lanes 1 and 6 represents an intact genomic *aadE**-*sat4*-*aphA*-3 containing region present in the wild type configuration. **(B).** Potential excision of *M. bovirhinis* HAZ141_2 prophage was tested using pseudo_R and tRNA-Leu_F primers (Table S1). The PCR allows identification of the predicted restored junction upon excision of the prophage as well as a "prophage-empty place" in prophagesless *M. bovirhinis* strains. *M. bovirhinis* type strain PG43 (lane 1), Israeli *M. bovirhinis* strain 316981 (lane 2) and *M. bovirhinis* HAZ141_2 p.3, p.50 and p.100 clones (lanes 3-5, respectively). PCR product of 378 bp was obtained only in prophagesless strains (lanes 1-2). The 100-bp ladder (BioRad, California, USA) is shown as M.