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## Supplementary information

# The bacterial toxin colibactin triggers prophage induction

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Supplementary information for

# The bacterial toxin colibactin triggers prophage induction

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**Supplementary Discussion |** Additional discussion pertinent to study findings.

*I. Prophage induction by a native colibactin producer.*

Many studies of colibactin employ strains that heterologously express the *pks* cluster on a BAC.[1] This strategy has proven useful for elucidating colibactin's structure and biosynthesis, but it may fail to capture regulatory features of native colibactin-producing strains. To address this uncertainty, we tested a native colibactin producer, *E. coli* NC101, a murine adherent-invasive strain isolated from mouse models of CRC carcinogenesis.[2,3] As shown in Extended Data Figure 2b and c, WT NC101 activated the $P_R$-*lux* reporter and increased phage titers by approximately 2 orders of magnitude compared to the NC101 Δ*clbP* mutant. While the number of plaques produced was generally lower from the NC101 co-cultures than the heterologously expressed BAC-*pks*, the result demonstrates that a native colibactin-producer induces the SOS response and activates prophages in neighboring cells in a *pks*-dependent manner, and in comparable magnitude compared to the BAC-*pks*-containing laboratory *E. coli*. Moreover, our reporter data showing that $P_R$-*lux* activity is completely abolished when exposed to the NC101 Δ*clbP* mutant, suggests that, under the conditions tested, colibactin production is likely the only mechanism by which NC101 activates the SOS response in neighboring *E. coli* (Extended Data Figure 2b).

*II. The use and specificity of extracellular DNA to interfere with colibactin-dependent prophage induction*

As noted in the main text and displayed in Extended Data Figure 2d-h, we used extracellular DNA (eDNA) as a means of exogenously manipulating the amount of colibactin delivered to target organisms. The rationale for this approach was that, in mammalian cell systems, addition of eDNA

has been shown to attenuate the genotoxicity of *pks*$^{+}$ *E. coli*, presumably by titrating away the reactive colibactin through alkylation of the exogenous DNA (we observed a similar effect in our setup using herring sperm DNA, Extended Data Figure 2e and f).[4] To investigate the importance of the AT-GC richness of the DNA used in this assay, we designed short oligonucleotides with variable AT:GC ratios relative to herring sperm DNA (58% AT). As referenced in the main text (Extended Data Figure 2g and h), AT-rich DNA (75% AT) but not GC-rich DNA (29% AT) attenuated prophage-induction, both in terms of reporter output and plaques produced. Notably, the pattern we observe for AT- versus GC-rich DNA in our assay is consistent with recent reports of colibactin-induced DNA damage and its concomitant mutational signatures occurring predominantly at AT-rich motifs.[5,6]

*III. Bioinformatic prediction of prophages in clbS+ non colibactin producers*

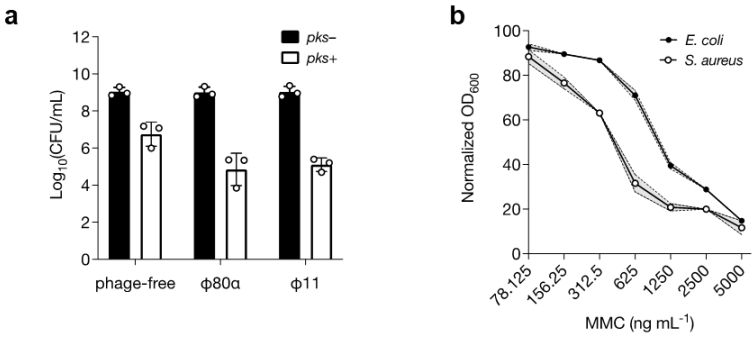
We reasoned that protection against colibactin-mediated DNA damage could provide a mechanism by which these diverse bacteria can avoid induction of native prophages they might carry. Thus, we searched for resident prophages in the available genomic data of the 12 bacteria from which the ClbS panel was assembled (Extended Data Figure 4a). Using a prophage-prediction algorithm (PHASTER),[7] we found a total of 94 prophage regions in the 12 genomes (Extended Data Figure 4b and c), 9 of which were further classified as containing at least one intact prophage (score >90, Extended Data Figure 4c). Like their bacterial hosts, these putative prophages are largely uncharacterized, however, we hypothesize based on domain analysis of the predicted prophage repressors that a subset of these prophages are responsive to DNA damage in a manner similar to phage lambda (Extended Data Figure 4d).

*IV. Phage-independent differences in sensitivity to colibactin and mitomycin C by S. aureus and E. coli.*

In our investigations, we observed several notable differences in phage-dependent versus phage-independent responses to colibactin between *S. aureus* and *E. coli.* In particular, the two prophage-

carrying *S. aureus* strains used in this study underwent a four order of magnitude drop in colony forming units (CFU) when cultured with *pks*$^{+}$ versus *pks*$^{-}$ *E. coli*, as compared to a two order of magnitude drop observed for phage-free *S. aureus* (Supplementary Discussion Figure 1a). The reduction of CFUs even in phage-free strains of *S. aureus* contrasts with our earlier finding on the lack of growth inhibition in phage-free *E. coli* (Figure 1b and Extended Data Figure 1a and b), indicating that bacteria can differ in their response to colibactin.[8] While a complete understanding of this difference remains to be resolved, we suspect it is a feature of DNA-damaging agents more generally as phage-free *S. aureus* was significantly more sensitive to MMC than phage-free *E. coli* (Supplementary Discussion Figure 1b).

**Supplemental Discussion Figure 1**




**Supplementary Discussion Figure 1** | *S. aureus* prophages are highly sensitive to colibactin, and *S. aureus* in general is likely more susceptible to other DNA-damaging agents than phage-free *E. coli*.
**a** Colony forming units (CFU) of lysogenic and non-lysogenic (phage-free) *S. aureus* RN450 after being co-cultured with *pks*$^{+}$ and *pks*$^{-}$ *E. coli.* **b** Growth of phage-free strain of *S. aureus* and *E. coli* in the presence of varying doses of MMC. Normalized $OD_{600}$ was calculated as the $OD_{600}$ at a given concentration relative to the $OD_{600}$ of the same strain to which no MMC was added (defined as 100). Data for both panels represented as mean ± SD with n = 3 biological replicates.

*V. Broader implications of colibactin production on prophage induction in complex communities:*

In this study, we investigated the interaction between colibactin-producing and -susceptible bacteria. We show that exposure to colibactin activates lytic development in prophage-carrying bacteria and that this effect occurs via the canonical SOS response. Notably, we observe that colibactin is an effective inducer of prophages across diverse phage-host systems and functions in complex communities. We also find many examples of non-colibactin-producing bacteria that possess colibactin resistance genes, indicating past exposure to this genotoxin. We propose that

the occurrence of colibactin resistance in these non-producing organisms serves as a unique defense mechanism to avoid prophage-mediated cell lysis and to prevent phage outbreaks caused by colibactin producers within a given community. Here, we outline several potentially important roles for colibactin and other bacterial natural products in modulating the activity of phages in microbiomes.

The virulence of certain pathogenic bacteria depends on their lysogenic state, and it is thus interesting to consider how colibactin might operate in these situations either positively, by helping to eliminate the pathogen, or negatively, by activating the expression of prophage-controlled virulence factors the pathogen harbors. For example, in the case of Stx-producing bacteria, the use of quinolone antibiotics is specifically contraindicated because it increases the induction of the SOS-responsive *stx* prophage and concomitant production of the encoded Stx toxin,[9] an effect we also observe with colibactin.

It is also interesting to compare how phage-mediated lysis compares to more direct means of cell-killing and competition (e.g. production of bacteria-encoded toxins). For example, if there were members of the bacterial community susceptible to phages induced by colibactin, production could indirectly stimulate a phage outbreak. By inducing phage outbreaks in microbial communities, colibactin could impact bacteria beyond those which are directly exposed to the metabolite itself. Furthermore, phage infections that lead to lysogeny could have lasting consequences on the infected population as the prophage will be inherited by future generations. We also envision scenarios where prophage induction could be counterproductive for colibactin producers. For example, in a community of closely related species or strains of the same species, phage induction may create phage particles that can go on to infect the colibactin producer, if susceptible. Nevertheless, in most microbial habitats where phages are thought to significantly outnumber the bacterial population,[10,11] it is likely the colibactin producer itself is also a lysogen and hence immune from superinfection. Such a scenario has been examined in a prophage-containing clinical isolate of *Enterococcus faecalis*, where the presence of the resident prophage enhances this isolate's colonization over other closely related phage-susceptible strains both in vitro and in vivo.[12]

**Supplementary Table 1**| tBLASTn result of top 230 *clbS*-like genes encoding proteins matching *E. coli* ClbS, WP_000290498 (tab 1); BLASTp result of top 5,000 ClbS-like proteins using *E. coli* ClbS, WP_000290498 as query (tab 2, in separate excel).

**Supplementary Table 2**| Strains used in this study.

| Strain | Genotype | Reference |
|---|---|---|
| *E. coli* DH10β | *F– mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara-leu)7697 galU galK λ– rpsL(Str$^R$) endA1 nupG* | NEB |
| *E. coli* BW25113 | *laclq, rrnBT14, ΔlacZWJ16, hsdR514,ΔaraBADAH33, ΔrhaBADLD78* | Baba et al. 2006 [13] |
| *E. coli* BW25113 λ | BW25113 lysogenic for λ | This study |
| *E. coli* BW25113 *lamB::kan* | *laclq, rrnBT14, ΔlacZWJ16, hsdR514,ΔaraBADAH33, ΔrhaBADLD7 ΔlamB732::kan* | Baba et al. 2006 [13] (JW3996-1) |
| *E. coli* MG1655 KIlac | MG1655 with Lac Operon removed from native locus and re-integrated at the Tn7 locus | Eames et al. 2012 [14] (Addgene: #52696) |
| *E. coli* MG1655 (delta-Z) | KIlac with *lacZ* deletion | Eames et al. 2012 [14] (Addgene: #52706) |
| *E. coli* NC101 | Murine isolate of *E. coli* (causes colitis in gnotobiotic IL-10−/− mice) | Tomkovich et al. 2017 [15] |
| *E. coli* NC101 Δ*clbP* | Δ*clbP* mutant of *E. coli* NC101 | Tomkovich et al. 2017 [15] |
| *S.* Typhimurium D23580 ΔΦ (JH3949) | D23580, cured of 5 native prophages | Owen et al. 2017 [16] |
| *S.* Typhimurium D23580 ΔΦ [P22] (SSO128) | D23580 ΔΦ lysogenic for P22 | Owen et al. 2021 [17] |
| *S.* Typhimurium polylysogen (SVO2) | D23580 BTP1, Gifsy-1 polylysogen | Owen et al. 2017 [16] |
| *S.* Typhimurium ΔBTP1 (BL1) | D23580 ΔBTP1 | Owen et al. 2017 [16] |
| *S.* Typhimurium ΔGifsy-1 (BL2) | D23580 ΔΦ Δ*waaG*::*aph* | This study |
| *C. rodentium* (Φ*stx*$_{2dact}$) | DBS770 | Mallick et al. 2012 [18] |

| | | |
|---|---|---|
| *S. aureus* RN450 | NCTC8325 cured of Φ11, Φ12, Φ13 | Novick 1967 [19] |
| *S. aureus* RN10359 | RN450 lysogenic for Φ80a | Úbeda et al. 2007 [20] |
| *S. aureus* RN451 | RN450 lysogenic for Φ11 | Novick 1967 [19] |
| *Enterococcus faecium* E1007 | Isolated from feces of a healthy individual | Lebreton et al. 2017 [21] |
| *Escherichia albertii* 07-3866 | *Escherichia albertii* isolated from human in the U.S. | Lindsey et al. 2019 [22] |
| | | |

**Supplementary Table 3** | Plasmids used in this study.

| Plasmid | Relevant fragment | Resistance | Reference |
|---|---|---|---|
| JSS-2347 | empty BAC (pBeloBAC11) | Cm | NEB |
| JSS-2348 | BAC-*pks* | Cm | Gift of Bonnet Lab |
| JSS-2367 | BAC-*pks* Δ*clbP* | Kan Cm | Gift of Bonnet Lab |
| JSS-2435 | empty BAC2 (cos sites removed) | Cm | This study |
| JSS-2403 | $P_R$-*lux* | Kan | This study |
| JSS-2587 | pBR322-empty (*ampR*) | Amp | This study |
| JSS-2436 | pEVS143-empty (*kanR*) | Kan | This study |
| JSS-2442 | pEVS143-empty (*kanR-cmR*) | Kan Cm | This study |
| JSS-2470 | pTrc-*clbS* | Amp | This study |
| JSS-2696 | pTrc-*clbS* | Kan | This study |
| JSS-2482 | pTrc-Δ*clbS* (pTrc-*clbS* with *clbS* deleted) | Amp | This study |
| JSS-2522 | pTrc-$clbS_{Dickeya}$ (*Dickeya dadantii*) | Amp | This study |
| JSS-2523 | pTrc-$clbS_{Mixta}$ (*Mixta theicola*) | Amp | This study |
| JSS-2528 | pTrc-$clbS_{Ecoli69}$ (*Escherichia coli 69,* plasmid) | Amp | This study |

| | | | |
|---|---|---|---|
| JSS-2529 | pTrc-*clbS*$_{Snod}$ *(Snodgrassella alvi wkB2)* | Amp | This study |
| JSS-2530 | pTrc-*clbS*$_{Gibsiella}$ (*Gibbsiella quercinecans* DSM 25889) | Amp | This study |
| JSS-2531 | pTrc-*clbS*$_{Samsonia}$ (*Samsonia erythrinae* DSM 16730) | Amp | This study |
| JSS-2532 | pTrc-*clbS*$_{Frischella}$ (*Frischella perrara*) | Amp | This study |
| JSS-2555 | pTrc-*clbS*$_{Bifido}$ (*Bifidobacterium longum subsp. infantis*) | Amp | This study |
| JSS-2556 | pTrc-*clbS*$_{Ealbertii}$ (*Escherichia albertii* CB9786) | Amp | This study |
| JSS-2557 | pTrc-*clbS*$_{Metako}$ (*Metakosakonia sp.* MRY16-398) | Amp | This study |
| JSS-2576 | P$_{Metako}$-*lux* | Kan | This study |
| | | | |

**Supplementary Table 4** | Gene blocks and oligonucleotides used in this study.

| ID | Sequence (5-3') |
|---|---|
| JSgblock-0133 (*Dickeya dadantii*) | ATATTAATGTATCGATTAAATAAGGAGGAATAAACCATGGGCGTGCCACAAACAAAATCCGAACTACT TTTCGCTATTGATAAAAATTTTACGAAATTAATCGGTTACCTCAATGCGATTCCGCCAGAGCTCACTG CGGATAAATCGCTGGATGGCCATGCAAAAGGCACGGAGATGAGTGTTTGTGAGCTTGTTTCGTATTT GCTGGGATGGAACACTCTGGTTGTAAAATGGATTAGCGGTGACGCAACAGGGCAACCTGTTGATTT CCCGGAAACCGGTTACAAGTGGAATCAGCTCGGGCTTCTGGCGCAGAAATTTTACTTTGATTACAAA GAATTAAATTACCAGACGTTAATCAATAACCTTCAGTCGGTAAAAGACGAAATAGTGAGGCTGATTGA TGAGCGCACTGATGAAGATCTGTATGGCAAGCCATGGTATGGCAAATGGACAATGGGACGTATGAT CTCATTGAACACGTCATCACCCTATGCCAATGCTAATGGAAGATTAAGGCAGTGGGCAAAGAACAAT CACCTGCGTTTAAAATGAGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGATAC |
| JSgblock-0134 (*Frischella perrara*) | ATATTAATGTATCGATTAAATAAGGAGGAATAAACCATGGCAGTACCTGAATCTAAAACTGAGTTAAT AGAAGCGATTAATAAAAATTATGCTTCGTTAGTCAAAAAGCTATCAGCTGTACCAGCAGAAAAGGCTT TCCAACCATTAATGGAGGGGCATGCCAAAGGCACGACTATGAGTGCAGCACAACTTGTTTCTTATTT AATAGGTTGGGGCGAATTAGTCTTATCTTGGCATGCACAAGAAGCACATGGGCATAGCATCGTTTTT CCTGAAGTGGGCTTTAAATGGAATGAATTAGGAAAACTAGCACAGAAATTTTATCAAGACTATCAAAA CATCACTGATTACAAGGAGCTATTACAACGGTTAGAAAAAAATAAAAATGATCTTATTAAATTGATTGA AGGTTTTACGAATAATGATTTATATGGTTCGAGTTGGTGTGAAAAATACACTCGAGGACGAATGATTC AGCTAAATACGTCATCACCGTATAGAAATGCAAGTGGTCGCATCGCGACATTCCTCAAAACTATCGG CAATTAAGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGATAC |
| JSgblock-0135 (*Mixta theicola*) | ATATTAATGTATCGATTAAATAAGGAGGAATAAACCATGGCGGTACCGGGATCAAAAGCTGAATTAAT TAAAGCGATCAATAGTAATTTCGCGTTGCTGAATAAAAAGCTGGAGGCTATTGCGCCAGCGCTTGCC TTTGAGCCGCTTATGGAAGGGCATGCCAAAGGGAGCACGATGAGCGTGGCGCAGCTGGTCTCCTG GCTGATTGGCTGGGGCGAATTGGTGTTGCACTGGCATAACCAAGAGGCGAAAGGGGAAACCATCGT GTTTCCTGCAGAGGGCTATAAATGGAATGAGCTGGGACAGCTGGCGCAGAAATTCTACCGTGACTA TGAAGACATCAACAATTATGAAACCTTGTTGGCGCGGTTAAGGGAGAACAAACAGCGGCTCTTGATG CTGACTGAGCAATTTAGCAACGATGAACTTTACGGTACGCCCTGGTATGGCAAATGGACGCGAGGA CGAATGATTCAATTTAATACCGCCTCGCCCTATAGAAATGCCGCAGGACGATTAAACAAATTGAAAAA ACAAACTGCTGCGTAAGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGATAC |
| JSgblock-0136 (*Escherichia coli* 69, plasmid) | ATATTAATGTATCGATTAAATAAGGAGGAATAAACCATGAGTGTGCCGCAAACAAAAGCTGAACTGCT TTTAGCTATTGATAAAAATTTTAGTAAATTAATTAGTTACCTCAACACAATCCCACCAGAAATTACTTCA GATAAATCAATGGACGGACACGCCAAAGGAACGGAGATGAGTGTTCGTGATCTCGTTTCGTATCTGC TTGGATGGAATGCTCTTGTTGTAAAGTGGATCGCTTCTGATGCTAAAGGTCTGCCTGTCGATTTTCC GGAAACTGGCTATAAATGGAATCAGCTTGGCCTTCTTGCTCAAAAATTTTACTCAGATTACAGTGAGT TAAGTTATGAGTTGTTAGTAGCTGAACTTCAGACTGTAAAAAATGAGATTGTGAACCTTATTAATGATC GTACCGATGATATTTTGTATGGAAGACCATGGTACACAAAATGGACGATGGGGAGAATGATCTCATT TAACACATCTTCGCCTTACGCCAACGCTAATGGAAGATTAAGAAAGTGGGCAAAAAATAATAATATCA GTTTAAAGTAAGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGATAC |
| JSgblock-0137 (*Snodgrassella alvi* wkB2) | ATATTAATGTATCGATTAAATAAGGAGGAATAAACCATGGCAATACCAGAATCAAAACAGGAACTAAT TGAAGCAATTAATAAAAACTATACACTACTGACCAAAAAGCTGGCTGCTGTACCTGAACAGAAAGCCT ATCTGCCTCTGATGGAAGGCCATGCTAAAGGTACAATGATGAGTGCTGCACAGCTTGTGTCTTATTT AATTGGCTGGGGAGAACTGGTGCTGTCATGGCATAAACAGGAACAGTCAGGGCAGAAAATTGCCTT TCCGGAAGCAGGATATAAATGGAATCAACTGGGATTACTGGCACAGAAATTTTATCGTGATTATCAG GACATTACCGATTTCAAACAGTTACTCTCGCTTCTGGAGACCAACAAAAAGAATCTGATTTCACTTAT TGATAGCTTTAGTAATGAAGAGCTGTATGGCTCACCCTGGCATGAAAAATACACACGCGGACGCATG ATTCAGTTTAATACCTCCTCCCCTTATAAAAATGCCACAGGACGATTGAATAAATTGTTGAAACAGAT CAAAGAATAAGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGATAC |
| JSgblock-0138 (*Gibbsiella quercinecans* DSM 25889) | ATATTAATGTATCGATTAAATAAGGAGGAATAAACCATGGCCGTGCCGGAATCAAAAGGCGCATTGA TCAAAGCGGTGAACAGCAATTTCGCGCTGCTGATGAAAAAACTGGACGACATACCGGCAGAAAAGG CGTTTGAGCCGCTGATGCCGGGGCATGCCAAGGGCAGCGTAATGAGCGTTGCCCAACTGGTGGCC TATCTGATTGGCTGGGGGGAACTGGTTTTGTCCTGGCATGCGAAAGAAAGACAGGGCCTTGAAATC GCCTTCCCCGAAGACGGTTTTAAGTGGAACCAATTGGGGTTGCTGGCGCAAAAGTTTTACCGTGATT ATGCGCAGATTACGGACTACGGCGCCCTGCTGATGCGGCTTGCCGATAATAAACGGCAGATTATCG CGTTGATTGACGGCTTTAGCGATGAGGCGCTCTACGGCCAGCCGTGGTACGGCAAATGGACGCGT GGCCGGATGATTCAGTTCAACACGGCGTCACCGTATAAGAACGCCTGCGGCAGGCTAAACGCATTG CGAAAAGTAGGCTGTGTCCCGTAAGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGATAC |
| JSgblock-0139 (*Samsonia erythrinae* DSM 16730) | ATATTAATGTATCGATTAAATAAGGAGGAATAAACCATGGCGGTGCCAGAATCAAAAGATGAACTCAT TAAAGCGATCAATAGTCACTTTGCGTTATTGCAGAAAAAGCTGGGGGCAGTCCTGCCTGAGCAGTCC TTTGAACCGACAATGGAAGGGCATGCAAAAGGGAGCGTGATGAGCGTGGCCCAATTGGTGTCTTAT CTTATTGGGTGGGGAGAATTGGTCTTGCACTGGCACGAACAAGAAGCGAAAGGGGAAACGATAAAC TTTCCTGAAGAGGGCTATAAATGGAATGAATTGGGGCGCCTGGCGCAGAAGTTTTATCGCGACTATG AAGACATAAAGGATTATGAAATCTTATTGTTGCGATTAAAGGAAAACAAACAGCAGCTTTTGACGCTG ATTGAGCGCTTTAGTAACGAGGAACTTTACGGTAGTCCTTGGTATGGCAAATGGACGCGGGGGCGA ATGATTCAATTTAATACCTCCTCGCCTTATAAAAATGCTTCAGGGCGATTAAATAAATTGAAAAAACAG GCTCCGATATGAGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGATAC |
| JSgblock-0140 (*Bifidobacterium longum subsp. infantis*) | ATATTAATGTATCGATTAAATAAGGAGGAATAAACC.ATGGCAGCCGGGAGGTGCATAGTGAGAACAT ACGAGAACGCGCAGGAGCTCAAAAAGGAGATCGGCGCGGCGTTTCGAAAATACATTGCGGAGTTCG ACGACATTCCGGAAGCCCTGAAAGACAAGCGCATCGACGAGGTCGAGAGGACCCCGGCGGAAAAC CTCGCCTATCAGGTTGGTTGGACCACCCTGCTGCTGCAATGGGAGGATCGCGAGCGGAAGGGCCT TCCGGTGCGAACGCCGTCGGACGAGTTCAAGTGGAACCAGCTCGGAAAGCTGTACCAGTGGTTCAC CGACACCTACGCCCACCTTTCGCTGCGGGAGCTGAAGGGCATGTTGACCGACAACGTCGACGCCAT ATACGCCATGATCGATGCGATGAGCGAGGACGAGCTGTTCAAGCCGCATATGAGGAAGTGGGCCG ACGACGCCACCAAAACGGCGGTATGGGAAGTGTACAGGTTCATTCATGTGAACACCGTCGCCCCGT TCGGATCGTTCAGGACGAAGATCCGCAAGTGGAAGCGGATGGCCCTATAG.GTTTTGGCGGATGAG AGAAGATTTTCAGCCTGATAC |

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|---|---|
| JSgblock-0142 (*Escherichia albertii* CB9786) | ATATTAATGTATCGATTAAATAAGGAGGAATAAACC.ATGTCTGTACCAGAAAGCAAAGCTGCACTGTT<br>GCTGGAAATGGAAAAATCATGGAATGGACTTACAAAAAAGTTATCTCGCATTCCAGAAGAAAAAGCTT<br>TTCTCATCACAATGGAAGGACATGCCAGCGGCACTGTGATGAGTGCGGCTAACCTGGTAAGCTATTT<br>AATAGGTTGGGGAGAGCAGGTTCTTACCTGGCACAGACAAGAAGAAGCAGGAATCCCCATTGATTTT<br>CCGGCTAAAGACCATAAGTGGAATGAACTTGGGAAACTAGCGCAGACATTTTACGCTAATTATTCCC<br>ATGTCACATCATGGTCCCAATTGTGCGAAATGTTAGAAACCAATCATCAGCAACTCAAATCGCTAGTG<br>GAACGCTACAGCGATAACGAGTTATACCATCATCCCTGGTATGGCAAATGGACGCGTGGTCGAATG<br>ATCCAATTTAATACCGTATCACCCTATAGAAATGCAAGCACACGTCTGAACGCCCTACTTAAACAATT<br>CTGAGTTTTGGCGGATGA.GAGAAGATTTTCAGCCTGATAC |
| JSgblock-0143 (*Metakosakonia* sp. MRY16-398 / *Kluyvera intestini* strain GT-16) | ATATTAATGTATCGATTAAATAAGGAGGAATAAACCATGGCCGTGCCAGAAAGCAAAACTGCCTTACT<br>GGAAGCAATGGAAAAGTCGGCAAGCGCCCTGCGCAAAAAACTCATCCGCATTCCGGCGGACATCGC<br>TTATCAGCAATGCCTGGAAGGGCACGTGGCTGGTAGCCGCATGAGTGTTGCTAATCTTGTCAGCTAT<br>CTGATTGGCTGGGGAGAGCAAGTTCTCTTCTGGCACCAACAGGAAGCCGCAGGCGAAGAAATAGAC<br>TTCCCGGCAAAAGGCTTTAAATGGAATGAACTGGGGAAGCTTGCGCAAAAATACTACGCTGATTACC<br>AGCATATTACGTCATGGCCAACGCTGCTTGCCATGCTGGAAGAGAATCAACAGCAACTGATATCACT<br>GGTGAATGGCTTTAGTGACGATGAGTTGTACCACCAGCTCTGGTACGGAAAATGGACGCGAGGACG<br>TATGATTCAATTCAACAGCGCCTCACCGTATAAGAATGCCAGCGCCAGGCTGAACAGTCTGTTAAAA<br>ACGCTCGCCGAAGCCTGACGCTCGCCGAAGCCTGAGTTTTGGCGGATGAGAGAAGATTTTCAGCCT<br>GATAC |
| JSgblock-0151 (*Metakosakonia* sp. MRY16-398_2 phage) | CGGGTACCGCTCGAGTTAATTAAGGCATAAAAAAACCAGCCGAAGCTGGTTTTTTATATGTATGAGT<br>CTCACCCCAAACGGTTGTATGACATTTGCCATCTACCGACAACTAAGCCCTGAATGTGAAACTCGCT<br>TTGATCAGTTTCAGATACAAACCATTTTTCGTAAGTTGAATTATCGCTGATGACTACCAGCTTGTCCTT<br>AAGCAGCTGTAAGCGTTTGATATGAAAGTTGTCACCATAAACGAACGCATAAATCCCATCACCTATCA<br>ACCGGTTTACCGTAACGTCTATAACGACAAGATCCCCAGGGAAAATAGTCCCGAGCATGCTATCGCC<br>GACAACCGTGCAGATTTTCAGGGAGGTAGGGCTACGCCCACCAAACATCCTTTTAGCTTCCTCTGGA<br>TCAAGTTCAATCGATTTAACTATTTCAGGATAGTCCATGTTCATCCTGCCACTTCCGCAACTGAACTC<br>AGTGTCCAGCACCTCAATTCGGTATTGTTGCTGATTTTTTGTCAGTGGATTTATGAAAAAGTTAGGCT<br>TGGTACCAGATGCATCAACATCTGCTCTCTTTGAATCCATCCAGCCATGGGGCAGGCCCATACTTGA<br>CTCAATTCTCCTAGCTATTATGTCACCAATGTTACGAGAGGCGTTAGAGCCAGATAACTGACTCAGTT<br>GAGAAGGGGGTATACCAATTTTTTCAGCAATTTGTGCCTTGGTATAACCTTCCACGATGTACTCACCA<br>ATCAACCTGTTTAGGTTCTGACGTCGAATGTTTTTTATGTCCATGACCAAATACTCCCACTTTTTAGCA<br>ATATGATAAATACCCAATTTGATAAATTTATCTTGCCTCATAGTTATCGTAAAGATAAACTTTACCAAAA<br>TGATAAATTGAGGTGTGGGTATGACTAAAAAAATTTCATTCATTATTAACGGCCAG |
| JSO-1758: pTrc backbone | GTTTTGGCGGATGAGAGAAG |
| JSO-1759: pTrc backbone | GGTTTATTCCTCCTTATTTAATCGATACATTAA |
| JSO-1756: *clbS* (into pTrc) | ATGGCTGTTCCATCATCAAAAGAAGAG |
| JSO-1757: *clbS* (into pTrc) | CTATTCTGCAAGACATTTCTGCAGTTTATTTAACC |
| JSO-1766: *clbS* deletion from pTrc-*clbS* (construction of pTrc-Δ*clbS*) | TTCTTTTGATGATGGAACAGCCAT |
| JSO-1767: *clbS* deletion from pTrc-*clbS* (construction of pTrc-Δ*clbS*) | CTGCAGAAATGTCTTGCAGAATAG |
| JSO-1727: $P_R$-*lux* insert | TGAATGAAATTTTTTTAGTCATACAACCTCCTTAGTACATGCA |
| JSO-1730: $P_R$-*lux* insert | GGTACCGCTCGAGTTAATTAAGATCAGCCAAACGTCTCTTC |
| JSO-1691: $P_R$-*lux*, $P_{metako}$-*lux* and pEVS143-empty (kanR) | TTAATTAACTCGAGCGGTACCCG |
| JSO-1692: $P_R$-*lux* and $P_{metako}$-*lux* | ATGACTAAAAAAATTTCATTCATTATTAACGGCCAG |
| JSO-1731: pBR322-empty (*ampR*) | TTAATTAACTCGAGCGGTACCTCTTAC |
| JSO-1743: pBR322-empty (*ampR*) and pEVS143-empty (*kanR*, with JSO-1691) | GGATCCGGTGATTGATTGAGC |
| JSO-1741: empty BAC2 (cos sites removed) | GTATTTTGTCCACATAACCGTGCG |
| JSO-1742: empty BAC2 (cos sites removed) and cmR (with JSO-1745) for pEVS143-empty (*kanR-cmR*, ligated with primer product used for pEVS143-empty *kanR*) | GAGTGAGCTAACTCACATTAATTGCG |
| JSO-1998: pTrc-clbS *ampR* to *kanR* | GCCCTCTCACTTCCCTGTTAAG |
| JSO-1999: pTrc-clbS *ampR* to *kanR* | TCAAGGTGTACTGCCTTCCAG |
| JSO-2000: pTrc-clbS *ampR* to *kanR* | GCATTGGTAACTGTCAGACCAAGT |
| JSO-2001: pTrc-clbS *ampR* to *kanR* | GGGTTATTGTCTCATGAGCGGATAC |
| JSO-1973: lambda red *clbS* recombineering from *E. albertii* | GGACCATGGCTAATTCCCATCAAATATATTGTCAGAATTGTTTAAGTAGGGCGTT |
| JSO-1972: lambda red *clbS* recombineering from *E. albertii* | TCACTATGCGCTGTAAAACGC |

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| JSO-1971: lambda red *clbS* recombineering from *E. albertii* | GTGATGAAAGGTCTATTATCATCGGC |
| JSO-1970: lambda red *clbS* recombineering from *E. albertii* | GAAGCAGCTCCAGCCTACACCTATTTATTCGCTGATTTTTCTGCTCGT |
| JSO-1969: lambda red *clbS* recombineering from *E. albertii* | GTTGATGGAAATTGTTATGCCTCAGA |
| JSO-1968: lambda red *clbS* recombineering from *E. albertii* | AGCATGAACCCGATGTTACC |
| JSO-1967: pKD3 *cmR* amplification | GAAGCAGCTCCAGCCTACACCATGGGAATTAGCCATGGTCC |
| JSO-1966: pKD3 *cmR* amplification | GCAGAAAAATCAGCGAATAAATAGGTGTAGGCTGGAGCTGCTTC |
| JWO-1116: *Enterococcus faecium* E1007 phage phi1 | GATTTGGCTGGTACGGAGTTGC |
| JWO-1117: *Enterococcus faecium* E1007 phage phi1 | AGTTGCAGAGGAAAATGGGGG |
| JWO-1120: *Enterococcus faecium* E1007 | GACCAGTTGCAAGGCGAAGG |
| JWO-1121: *Enterococcus faecium* E1007 | TCACCGTTTGTCGAATCGCC |
| JSO-1745: *cmR* for pEVS143-empty (*kanR*-*cmR*); ligated with primer product used for pEVS143-empty (*kanR*) | GGCATTTATTCTCAGGATAATTGTTTCAGC |
| JWO-1046: GC-rich probe | GCCGCATGCCGCATGCCGCATGCCGCATGCCGCATGCCGCATGCCGCATGCCGCATGCCGCAT |
| JWO-1047: GC-rich complement | ATGCGGCATGCGGCATGCGGCATGCGGCATGCGGCATGCGGCATGCGGCATGCGGCATGCGGC |
| JWO-1044: AT-rich probe | CACACATTTGCAAATTTCGCAAATTTGCACAGATCTTGCAAATTTGCAAATTTGCAAATTTCA |
| JWO-1045: AT-rich complement | TGAAATTTGCAAATTTGCAAATTTGCAAGATCTGTGCAAATTTGCGAAATTTGCAAATGTGTG |

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