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Anamaria Babosan, Louna Fruchard, Evelyne Krin, André Carvalho, Didier Mazel, Zeynep Baharoglu

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## 1        **Non-essential tRNA and rRNA modifications impact the bacterial response to sub-MIC antibiotic** 2 **stress**

3        Anamaria Babosan<sup>1,§</sup>, Louna Fruchard<sup>1,2,§</sup>, Evelyne Krin<sup>1</sup>, André Carvalho<sup>1,2</sup>, Didier Mazel<sup>1,\*</sup>, Zeynep  
4 Baharoglu<sup>1,\*</sup>

5        § equal contribution

6        \*Corresponding authors: [baharogl@pasteur.fr](mailto:baharogl@pasteur.fr), [mazel@pasteur.fr](mailto:mazel@pasteur.fr)

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9        1. Département Génomes et Génétique, Institut Pasteur, UMR3525, CNRS, Unité Plasticité du  
10        Génome Bactérien, 75015, Paris, France  
11        2. Sorbonne Université, Collège Doctoral, F-75005, Paris, France

### 12 13        **One-sentence summary**

14        Modifications of ribosomal and transfer RNAs impact bacterial fitness in response to antibiotic stress.

### 15 16        **Abstract**

17        Antimicrobial resistance develops as a major problem in infectious diseases treatment. While  
18        antibiotic resistance mechanisms are usually studied using lethal antibiotic doses, lower doses allowing  
19        bacterial growth are now considered as factors influencing the development and selection of  
20        resistance. Starting with a high-density Tn insertion library in *Vibrio cholerae* and following its evolution  
21        by TN-seq in the presence of sub-inhibitory concentrations of antibiotics, we discovered that RNA  
22        modification genes can have opposite fates, being selected or counter-selected. We thus have  
23        undertaken the phenotypic characterization of 23 transfer RNA (tRNA) and ribosomal RNA (rRNA)  
24        modifications deletion mutants, for which growth is globally not affected in the absence of stress. We  
25        uncover a specific involvement of different RNA modification genes in the response to aminoglycosides  
26        (tobramycin, gentamicin), fluoroquinolones (ciprofloxacin),  $\beta$ -lactams (carbenicillin), chloramphenicol  
27        and trimethoprim. Our results identify t/rRNA modification genes, not previously associated to any  
28        antibiotic resistance phenotype, as important factors affecting the bacterial response to low doses of  
29        antibiotics from different families. This suggests differential translation and codon decoding as critical  
30        factors involved in the bacterial response to stress.

### 31 32        **Introduction**

33        Antibiotic overuse and misuse contribute to antimicrobial resistance (AMR), via selective  
34        pressure exerted by treatment during infection, but also in the environment where gradients of  
35        antibiotics are found in soil and water, the natural reservoir of many bacteria among which *Vibrio*.  
36        AMR is increasingly associated with life in the aquatic environment, particularly in aquaculture farms,  
37        where several bacterial species coexist. A World Health Organization report on AMR in enteric  
38        pathogens states that “consideration must be given to the relationship of *Vibrio* with the environment”  
39        to understand AMR development (Sack et al., 2001). Most studies address the bacterial response to  
40        lethal antibiotic concentrations and the effect of gene mutations on antibiotic resistance. Meanwhile,  
41        in their environments, bacteria encounter sub-minimal inhibitory concentrations (sub-MICs) of  
42        antibiotics (Chow et al., 2021), which are stressors, and can lead to transient phenotypic tolerance to  
43        high doses of antibiotics (Andersson and Hughes, 2014). Thus, characterization of the bacterial  
44        responses to such stress and its impact on resistance/tolerance, need to be comprehensively clarified.

45        We have previously demonstrated that several pathways identified for the response to  
46        antibiotic stress in *V. cholerae* are paradigmatic for other bacterial pathogens (Baharoglu et al., 2013;  
47        Baharoglu and Mazel, 2011). Using sub-MIC antibiotics, we aimed at characterizing which bacterial  
48        responses were triggered and allowed the cells to grow and survive, and we asked whether the  
49        identified processes also impact bacterial phenotypes at lethal concentrations of the same antibiotics.

50 Our results point to a central role of transfer RNA (tRNA) and ribosomal RNA (rRNA) modifications in  
51 the response to sub-MIC antibiotic stress, suggesting that RNA modification profiles and translation  
52 may be modified in bacteria by stress.

53 Evolution of resistance requires genetic diversity in populations, yet non-genetic phenotypic  
54 diversity can also contribute. One process generating phenotypic diversity is translation, with an  
55 average error rate of  $10^{-4}$  and up to  $10^{-3}$  substitutions per position under stress conditions (Kramer and  
56 Farabaugh, 2007). Translation errors cause protein misfolding (Liu, 2020; Nedialkova and Leidel, 2015),  
57 aggregation and proteotoxic stress (Drummond and Wilke, 2009). Translation errors can also provide  
58 transient increase in fitness (Samhita et al., 2020), offering cells the necessary time to acquire  
59 beneficial genetic mutations (Whitehead et al., 2008) and to eliminate deleterious ones (Bratulic et al.,  
60 2017), as it was observed upon oxidative stress (Netzer et al., 2009) and proteotoxic stress (Evans et  
61 al., 2019).

62 Codon decoding efficiency can impact translation speed or translation accuracy at specific  
63 mRNAs/codons, and proteome diversity (Kramer and Farabaugh, 2007). Differences in decoding and  
64 reading frame maintenance have already been linked with the presence or absence of certain RNA  
65 modifications (Baudin-Baillieu and Namy, 2021; Valadon and Namy, 2021). In particular, methylation  
66 at specific positions in rRNA stabilizes the binding of initiator tRNA to the ribosome at the start codon  
67 (Burakovsky et al., 2012), and several rRNA methylation factors have been linked to aminoglycoside  
68 (AG) resistance (Doi and Arakawa, 2007; Dunkle et al., 2014). Regarding tRNA modifications, more than  
69 80 have been described in bacteria (de Crecy-Lagard and Jaroch, 2021). They can be involved in tRNA  
70 stability (Motorin and Helm, 2010), abundance (Kramer and Farabaugh, 2007), decay (Hughes et al.,  
71 2020; Kimura and Waldor, 2019) and affinity for the ribosome (Noguchi et al., 1982). While some tRNA  
72 modification genes are essential, (e.g. *trmD*, *tadA*), in many cases their deletion does not confer any  
73 visible phenotype to the unstressed cells (de Crecy-Lagard and Jaroch, 2021) (**Table S1** and references  
74 therein). Few studies address the exact physiological roles of non-essential rRNA (Georgeson and  
75 Schwartz, 2021; Zou et al., 2018) and tRNA modifications in bacterial stress response phenotypes  
76 (Aubee et al., 2016; Chionh et al., 2016; Hou et al., 2017; Thongdee et al., 2019; Toh and Mankin, 2008;  
77 Vecerek et al., 2007), reviewed in (de Crecy-Lagard and Jaroch, 2021).

78 In the present study, we reveal the involvement of various RNA modification genes, in the  
79 response to antibiotics. The RNA modification genes identified here are different from previously  
80 described modifications conferring antibiotic resistance. We show that their inactivation confers, not  
81 resistance, but increased or decreased fitness in presence of antibiotic stress.

82  
83

## 84 **Results**

### 85 **TN-seq identifies rRNA and tRNA modification genes involved in the response to sub-MIC TOB and** 86 **CIP in *V. cholerae***

87 Using TN-seq in *V. cholerae*, we searched for genes that are important for growth in the  
88 presence of sub-MICs of antibiotics targeting the ribosome (TOB: tobramycin belonging to  
89 aminoglycosides (AGs)), or DNA (CIP: ciprofloxacin belonging to fluoroquinolones (FQs)). We  
90 constructed large transposon inactivation libraries in *V. cholerae* as previously performed (Negro et al.,  
91 2019) and we subjected them to growth without or with antibiotics at 50% of the minimal inhibitory  
92 concentration (MIC), during 16 generations. After sequencing and bioinformatics analysis of the  
93 regions flanking the transposon, we identified genes where reads associated to detected transposon  
94 insertions increase or decrease. Loss of detected insertions in a specific gene generally means that the  
95 inactivation of this gene is detrimental in the tested condition, while enrichment means that the  
96 inactivation is beneficial. In some cases, transposon insertion in one gene may also lead to differential  
97 expression of downstream genes. In this study, we searched for genes that are important only during  
98 sub-MIC treatment. We thus compared insertion counts after 16 generations in sub-MIC antibiotics  
99 (TOB or CIP) to those after 16 generations without antibiotics (**Figure 1** and **Tables 1 and S1**). Genes  
100 having a significant impact on fitness (insertions enriched or lost) in the non-treated condition are thus  
101 not included in our analysis. Gene ontology analysis was performed for genes with more than 2-fold

102 changes in the number of insertions and with a significant p-value (**Figure 2** and Table S2). For both  
103 antibiotics, we found common and antibiotic specific RNA modification genes whose number of reads  
104 was impacted, suggesting that their inactivation was either beneficial or detrimental for growth in the  
105 presence of the sub-MIC antibiotic. Results show a 2.43x enrichment ( $p=4.56 \times 10^{-4}$ ) of RNA modification  
106 genes (GO: 0009451) with 23 genes identified out of a total of 80 for TOB and 1.57x enrichment  
107 ( $p=2.3 \times 10^{-2}$ ) for tRNA modification genes (GO: 0006400) with 20 identified out of a total of 48 for CIP.  
108 As expected, other categories identified include expected categories such as proteolysis for TOB or  
109 DNA replication/SOS response for CIP (**Figure 2**).

110 The most important TN-seq hits for TOB include: (i) tRNA modification genes for which inactivation  
111 is detrimental: incorporation of queuosine (Q) by *tgt* (together with the Q synthesis genes *queADEF*),  
112 and i6A37/ms2i6A37 by *miaA/miaB*; or beneficial: dihydrouridine (D) incorporation by *dusB*, *dusA*; and  
113 methylation by *trmH*, *rlmN*; and m<sup>5</sup>U34 incorporation (*gidA*, also called *mnmG*); (ii) rRNA modifications  
114 for which inactivation is detrimental: methylation by *rsml*, *rsmF*, *rsmG*, *rsmH*, *rsmB*, *rsmD*,  
115 pseudouridine ( $\psi$ ) incorporation by *rsuA* ; or beneficial:  $\psi$  by *rluB*. Note that *rsmG* and *rsmF* mutants  
116 have already been associated with increased AG resistance (**Table 1** and references therein), but our  
117 results suggest decreased fitness in AGs for these mutants.

118 For CIP: (i) tRNA modification genes for which inactivation is detrimental were responsible for  $\psi$   
119 incorporation (*truA*) and methylation (*rlmN*); (ii) rRNA methylation genes were also identified in CIP,  
120 some at different positions than those in TOB (detrimental inactivation of *rsmB* and beneficial *rlmE*).  
121 Note that RlmN can modify both tRNA and rRNA.

122 Overall, several non-trivial observations stem from our results: first, the effect of inactivation of  
123 these genes on fitness can either be negative (e.g. *tgt* in TOB), or positive (e.g. *dusB* in TOB). Second,  
124 their impact seems to be an antibiotic specific one. For instance, the inactivation of *dusB/tgt/rluB*  
125 strongly impacts the fitness in TOB, but not in CIP. Our previous transcriptomics results suggest that  
126 sub-MIC TOB could induce the stringent response (Carvalho et al., 2021a). Since RNA modifications  
127 could affect ribosome function, we asked whether the absence of certain modifications could induce  
128 the stringent response and whether there is a correlation between stringent response induction and  
129 changes in fitness upon antibiotic treatment. We constructed a *gfp* fusion under the control of the  
130 rRNA *P1rrn* promoter, which is down-regulated upon stringent response induction (Kolmsee et al.,  
131 2011) and followed fluorescence at various time points throughout growth (**Figure S1**). We found that  
132 the stringent response is significantly induced by sub-MIC TOB (i.e. decrease of fluorescence, **Figure**  
133 **S1, last graph**), but no induction was observed for RNA modification deletion mutants. Unexpectedly,  
134 slightly increased fluorescence was observed during early exponential phase for  $\Delta gidA/\Delta trmE$ , as well  
135 as  $\Delta truA$ ,  $\Delta rsuA$ ,  $\Delta rsmB$  and  $\Delta rluD$ , showing increased transcription from the *P1rrnB* promoter (e.g.  
136 possibly through Fis or another transcriptional activator).

137 Altogether, these observations suggest that the loss of a given modification may affect the bacterial  
138 response in a specific way rather than through a general effect of all modifications on translation.  
139 While AGs, which target the ribosome, could be expected to impact translation related genes, it was  
140 surprising that the response to CIP, which targets DNA, also involves several RNA modification genes,  
141 suggesting that the involvement of RNA modifications may be fundamental upon stress due to  
142 antibiotics from different families.

143

#### 144 **RNA modification gene deletions impact fitness during growth in sub-MIC antibiotics.**

145 We next constructed *V. cholerae* deletion mutants for 23 of the identified RNA modification genes,  
146 selected in TN-seq data for having no (or slight) effect on fitness during growth in the absence of  
147 antibiotics. Many have no known physiological defect, and were not previously associated to antibiotic  
148 related phenotypes (**Table 1**). The following genes were excluded from further study either for known  
149 effects on growth: *miaA*, *rsmA*, *rlmE*; or for known AG related phenotypes: *rsmG*, *rsmA* & *rsmH* (Zou  
150 et al., 2018). We chose *trmK* as a neutral control for TOB and CIP, as it showed no variation in our TN-  
151 seq screens.

152 Since growth curves of monocultures of the mutants were similar to that of the WT in the absence  
153 of treatment (not shown), we decided to perform competition experiments between mutants and the



154 WT strain, to assess effects on fitness in sub-MICs of 6 different antibiotics: the AGs TOB and  
155 gentamicin (GEN), the fluoroquinolone CIP, as used in our TN-seq screen, and additionally the  $\beta$ -lactam  
156 carbenicillin (CRB) targeting the cell envelope, chloramphenicol (CM) targeting translation elongation  
157 and trimethoprim (TRM) which inhibits thymidine synthesis interfering with DNA synthesis. **Figure 3**  
158 shows the competitive index of mutants compared to WT. The results are summarized in **Table 2**.

159 As expected, deletions of the majority of tested genes (with the exception of *trmE*, *rsuA* and *rlmN*)  
160 have no or little effect on competitive index during growth in the absence of antibiotics (**Figure 3**),  
161 emphasizing their specific role during stress, here sub-MIC antibiotics.

162 For the AGs TOB and GEN, among tested genes, deletion of *tgt*, *miaB*, *truA*, *truB*, *rlmI*, *rsmB*, *rsmF*, *rluD*  
163 decreased fitness, while deletion of *gidA*, *dusB*, *trmH*, *rlmN*, *rsmC*, *rluB*, *rluE* conferred a growth  
164 advantage (**Figure 3**). These results were consistent with TN-seq data, with the exception of *truA*, *truB*,  
165 *rluE*, and *rluD* for which the TN-seq data were not statistically significant, and *dusA* and *trmB* for which  
166 the growth advantage observed in TN-seq is not found in competition experiments. For CIP, deletions  
167 of *dusB*, *miaB*, *truA*, *truC*, *rlmI*, *rsmB*, *rluD*, *rluE* were disadvantageous, whereas  $\Delta trmE$  and  $\Delta rsuA$   
168 strains appear to lose the fitness disadvantage they show in absence of CIP compared to WT. Once  
169 again, results were consistent with the statistically significant TN-seq results, except for the *rluD* gene.  
170 For CM, *truA*, *rluD* and *rluE* deletions were detrimental. For TRM, *rluB* and *rluE* deletions were  
171 detrimental, while deletions of *gidA*, *truB*, *trmB*, *rlmN*, *rsmF* conferred a low (up to 10x) but statistically  
172 significant growth advantage. For CRB, detrimental deletions were *gidA*, *trmH*, *rluE*, and advantageous  
173 deletions were *tgt*, *dusB*, *trmE*, *rlmN*, *rsmD*, *rsmF*.

174 In order to test whether these modification genes could be important for the response to another type  
175 of stress, we also performed competitions in the presence of the biocide triclosan (TCL), at 50% of the  
176 MIC. TCL inhibits fatty acid synthesis and can be found in antiseptic consumer products. It has been a  
177 subject of concern for its impact on the aquatic environment (Dhillon et al., 2015) and antibiotic  
178 resistance development (Wesgate et al., 2016). Again, while deletion of many RNA modification genes  
179 decreased fitness in TCL (*tgt*, *gidA*, *dusB*, *miaB*, *truA*, *trmA*, *trmE*, *rlmI*, *rsmB*, *rsmF*, *rluB*, *rluD*), some  
180 were neutral (*dusA*, *trmB*, *trmH*, *rlmN*, *rluE*, *trmK*), and one was beneficial (*rsmD*).

181 These results globally confirm that the effect of a given modification gene is not a general one on  
182 viability but an antibiotic specific one. For instance, regarding tRNA modifications, upon AG treatment  
183 (TOB, GEN), deletion of *tgt* confers a clear 10 to 1000x disadvantage, while it has no major effect in  
184 CIP, TRM, CM and appears to be 10x advantageous in CRB. Deletion of *truA* confers a up to 100x fitness  
185 defect in AGs, CIP and CM but is neutral in TRM and CRB. Deletion of *truB* also appears to affect  
186 specifically growth in AGs. Deletions of *dusB/rlmN*, and *gidA/trmH* are highly (10 to 100x) beneficial in  
187 AGs but respectively deleterious or neutral in CIP. *rlmN* deletion also confers a slight advantage in TRM  
188 and CRB. Deletion of *trmA* shows no major effect in any antibiotics, while *trmB* deletion is only  
189 beneficial in TRM. Regarding rRNA modifications, *rluB* shows a striking phenotype with 10x beneficial  
190 deletion in AGs, highly (1000x) deleterious in TRM, and neutral in presence of the other antibiotics  
191 (Figure 2). Of note, *gidA (mnmG)/ trmE (mnmE)* are known to have pleiotropic phenotypes due to  
192 effects on translation (Bregoon et al., 2001), chromosome replication and cell division (Alam and Clark,  
193 1991; de Crecy-Lagard and Jaroch, 2021; Lies et al., 2015; Ogawa and Okazaki, 1991; Theisen et al.,  
194 1993), in addition to effects on tRNA modification (Elseviers et al., 1984). Regarding TCL, many RNA  
195 modification gene deletions confer a fitness defect. However, the fact that deletion of *rsmD* is  
196 beneficial indicates that the absence or the decrease of the levels of a given RNA modification can also  
197 allow a better fitness upon exposure to toxic chemicals such as antiseptics.

198

### 199 **RNA modification gene deletions impact tolerance to high doses of antibiotics without changing the** 200 **resistance.**

201 Next, we addressed whether these genes could be involved in the response to lethal antibiotic  
202 concentrations. We first determined the minimal inhibitory concentrations (MIC) of TOB, CIP, TRM and  
203 CRB for each deletion mutant (**Table 3**). Slight decreases in the MIC of TOB (x 0.9) was observed for  
204  $\Delta rlmI$  and  $\Delta rsmD$ . Slight increases in MIC were observed for  $\Delta gidA$  and  $\Delta rluB$  in TOB (x1.6), for  $\Delta gidA$ ,  
205  $\Delta rluD$ ,  $\Delta trmE$  in CIP (x1.2), (x1.1) for  $\Delta rlmN$  in ampicillin (as a substitute for CRB) and for  $\Delta trmE$  and

206  $\Delta truC$  in TRM (x1.6). Besides these small changes, we found no major differences in MICs, consistent  
207 with the fact that these genes were not previously associated with antibiotic resistance phenotypes.

208 We then tested the survival to lethal concentrations of antibiotic, i.e. the tolerance (**Figure 4**): WT  
209 and mutant bacteria were grown to early exponential phase and then treated for 20 hours with 10xMIC  
210 of TOB, CIP, TRM and CRB as previously performed (Lang et al., 2021).

211 For 10 mutants out of 17 tested (among which 9 tRNA modification mutants), survival profiles were  
212 consistent with fitness profiles shown in **Figure 3**. These were mutants *tgt*, *gidA*, *truB*, *trmE* (except in  
213 CRB) and *rsuA*, for which increased fitness corresponded to increased tolerance and vice-versa; and  
214 *dusA*, *miaB*, *truC*, *trmA*, *trmB* for which the absence of statistically significant effect on tolerance was  
215 also consistent with the absence of differences in fitness. This suggest that a fitness (dis)advantage in  
216 sub-MIC antibiotics in the absence of tRNA (and rRNA) modifications may also impact tolerance to  
217 lethal doses of the same antibiotic, without changing the MIC.

218 For 3 mutants, *dusB*, *trmH*, *rluE*, no significant effect on tolerance was generally observed at 20h of  
219 lethal treatment, while deletion of these genes positively affected fitness in sub-MIC TOB. In order to  
220 address whether differences in tolerance could be detected at earlier times of treatment, we repeated  
221 the experiments and spotted cultures after 30 min, 1 and 2 hours of antibiotic treatment instead of 20  
222 hours (**figure S2**). While  $\Delta dusB$  tolerance was still similar to that of WT,  $\Delta trmH$  and  $\Delta rluE$  strains  
223 displayed increased tolerance to TOB at 30min and 1h of treatment, consistent with their beneficial  
224 effect on fitness in sub-MIC TOB.

225 For the remaining 4 mutants, among which 3 rRNA modification mutants, we observed  
226 contradictory phenotypes between fitness and tolerance at 20h, i.e. decreased TOB tolerance at 20h  
227 in beneficial deletion mutants *rlmN*, *rsmC*, *rluB*; and CRB for *trmE*. However, at earlier time point as  
228 described above, and consistent with fitness profiles, TOB tolerance is clearly increased in *rlmN*, *rsmC*,  
229 *rluB* (**figure S2**), suggesting that mutants with fitness advantage in sub-MIC TOB also survive longer in  
230 the presence of lethal TOB concentrations. However, the final survival after 20h of treatment is not  
231 increased, consistent with unchanged MICs. This phenotype is a characteristic of antibiotic tolerant  
232 populations (Balaban et al., 2019). CRB tolerance of  $\Delta trmE$  remains lower than WT (not shown).

233 To evaluate the levels of correlation between competitive index and survival phenotypes, we  
234 plotted the two sets of data against each other, and for each antibiotic (**Figure 5**). We observe that  
235 there is, or isn't, a correlation between growth in sub-MIC and survival, depending on the gene deletion  
236 and the antibiotics.  $\Delta tgt$ ,  $\Delta gidA$ ,  $\Delta dusB$ ,  $\Delta rsuA$ ,  $\Delta miaB$  for TOB;  $\Delta gidA$ ,  $\Delta trmB$  for TRM; and  $\Delta tgt$ ,  $\Delta gidA$ ,  
237  $\Delta trmE$  for CRB, show similar tendencies for growth in sub-MIC and survival to 20 hours lethal antibiotic  
238 treatment, meaning that in these cases, better growth in sub-MIC and better survival may occur  
239 through the same mechanisms. In other examples, the two phenotypes show opposite directions, as  
240 for  $\Delta rluB$  in TOB or  $\Delta rlmN$  in CRB. Interesting to note, for CIP and TRM, which target DNA and  
241 replication, the two phenotypes seem to be uncorrelated in most cases, while in TOB and CRB both  
242 phenotypes may vary for a given mutant.

243 Overall, tolerance profiles of several mutants correlate with their fitness profiles in sub-MICs of  
244 antibiotics. For those, such as  $\Delta dusB$ , with increase in fitness but not in tolerance, the mechanisms  
245 remain to be determined, and their phenotypes suggest that the effects of RNA modifications during  
246 growth in stressful conditions (sub-MIC antibiotic) do not necessarily affect survival to high antibiotic  
247 doses. rRNA modifications in particular could be expected to have structural effects on ribosomes,  
248 which could lead to pleiotropic effects, and could potentially explain this discrepancy.

249 One such effect is 17S rRNA accumulation, due to a defect of maturation to 16S rRNA (previously  
250 shown for  $\Delta rsmA$  (Smith et al., 2018) and  $\Delta rsmB$ , **Table 1**). We visualized rRNA species purified from  
251 exponentially growing WT and RNA modification deletion mutants (**Figure S3**). We find accumulation  
252 of a pre-16S, consistent with 17S, rRNA species for  $\Delta rsuA$ , for which fitness is most affected also in the  
253 absence of antibiotics. *RsuA* is a 16S rRNA pseudouridine synthase. Apart from  $\Delta rsuA$ , we observed no  
254 differences in rRNA species between the other tested deletion mutants and the WT. This is consistent  
255 with the fact that these strains do not exhibit any major growth defect in the absence of antibiotics.  
256 Further study is needed to clarify the role of identified rRNA modifications in antibiotic specific survival.

257 We also evaluated whether deletion of these RNA modification genes could have any effect on DNA  
258 mutation rates by quantifying the appearance of spontaneous rifampicin resistant mutants (**Figure S4**),  
259 and found no major effect on mutation rates except for  $\Delta gidA$ . This confirms that the fitness  
260 advantage/disadvantage conferred by RNA modification gene deletions are not due to an effect on  
261 mutation rates and/or accumulation of mutations. Finally, we asked whether RNA modification gene  
262 deletions could also have a specific impact on stresses other than antibiotic or chemical treatments.  
263 One such stress is UV irradiation. While 10 mutants did not show any difference with the WT strain,  
264 survival after UV irradiation was increased in 7 mutants (*tgt*, *truA*, *truB*, *trmE*, *trmH*, *rluD* and *rluE*)  
265 (**Figure 6**), suggesting that RNA modifications impact the response to different stress conditions.

### 266 **RNA modification gene deletions also impact *E. coli* growth in sub-MIC antibiotics.**

268 We next sought to determine whether RNA modification genes also play similar roles in bacterial  
269 species other than *V. cholerae*. We constructed deletion mutants in *E. coli* MG1655 of 9 genes selected  
270 for their positive (*gidA*, *dusB*, *rsmC*, *rluB*), neutral (*dusA*, *rsmD*) and negative (*tgt*, *trmE*, *rsuA*) impact  
271 on *V. cholerae* fitness in sub-MIC TOB (**Figure 7** and **Figure S5**). Note that inactivation of *dusA* and *rsmD*  
272 were observed to be respectively beneficial and deleterious in *V. cholerae* TN-seq data, but not in  
273 competitions. Growth curves in 50% MIC TOB display similar and dissimilar phenotypes in *E. coli*  
274 compared to those observed for *V. cholerae*. First, similar to *V. cholerae*, (i) deletions of *dusB*, *rsmC*,  
275 *rluB* and *dusA* and (ii) deletions of *tgt*, *trmE* and *rsmD*, have a positive and a negative impact,  
276 respectively, on growth in sub-MIC TOB in *E. coli*. For  $\Delta tgt$ , we also had some replicates with no  
277 observable effect in sub-MIC TOB (curve in light blue), suggesting heterogeneous response to TOB  
278 stress in this mutant in *E. coli*. On the other hand, unlike in *V. cholerae*,  $\Delta gidA$  decreases while  $\Delta rsuA$   
279 improves growth of *E. coli* MG1655 in TOB. Note that synteny is conserved between the two organisms  
280 for these genes, hence the differences cannot be attributed to an effect of the deletions on  
281 surrounding genes. We also observed the same growth profiles (**Figure S6**) in an *E. coli* BW25113 (Keio)  
282 strain, for all mutants except for the BW25113  $\Delta tgt$  strain which unexpectedly has a positive impact  
283 on growth in sub-MIC TOB in this genetic context. Note that growth curves show differences in growth  
284 but not necessarily in fitness as it is the case for competition experiments where WT and mutant  
285 cultures are mixed. Nonetheless, these results show that the involvement of RNA modification genes  
286 in the response to sub-MIC antibiotic stress is not specific to *V. cholerae* and can be extended to other  
287 bacterial species, although their antibiotic related effects may sometimes be species and even strain-  
288 specific.

### 289 **Discussion**

291 Using antibiotics at sub-MICs, we identify here the importance of rRNA and tRNA modification  
292 genes, not previously associated to any antibiotic resistance/tolerance phenotypes (**Table S1** and  
293 references therein). Among these are rRNA methylation factors RsmB/C/D/H/I and RlmI, rRNA  
294 pseudouridine synthases RsuA and RluD; and Tgt, DusB, TruA/B/C, TrmA/B/E/H and RlmN for tRNA  
295 modifications.

296 Most t/rRNA modifications influence translation rate, fidelity and precision of codon decoding  
297 (Chan et al., 2018). Errors in decoding can lead to transient tolerance to stress (Samhita et al., 2020),  
298 increasing the cell's chances to acquire genetic mutations allowing adaptation (Bratulic et al., 2017).  
299 For instance, increased survival after 20h antibiotic treatment as described above for several mutants,  
300 may be due to tolerance or persistence, a transient state of phenotypic (non-heritable) resistance to  
301 lethal antibiotic concentrations. The idea that RNA modifications can act on such phenotypic  
302 adaptation is interesting, and worth pursuing.

303 Since the genetic code is degenerate, and one tRNA can decode several codons, decoding efficiency  
304 can logically be impacted by tRNA modification (Quax et al., 2015). Thus, the link between tRNA  
305 modification-dependent differences in translation, proteome diversity, and the bacterial response to  
306 antibiotics and more generally to changing environments, is an attractive area to explore further. It is  
307 known that codon usage has an impact on translation (Gingold and Pilpel, 2011; Krafczyk et al., 2021;  
308 Nieuwkoop et al., 2020). Highly translated mRNAs, such as those of ribosomal proteins, display a codon

309 usage profile different than the general codon usage in the genome (Plotkin and Kudla, 2011). It was  
310 proposed that codon usage of highly expressed genes is determined by the abundance of tRNAs, so as  
311 to prevent titration of tRNAs, hence allowing efficient translation of the rest of the proteome (Frumkin  
312 et al., 2018). We can speculate that codon usage of these genes can also be a function of decoding  
313 efficiency displayed by modified vs. unmodified tRNAs. Various transcriptional regulators also show  
314 codon usage biases, and RNA modifications may impact their translation and thus lead to differential  
315 transcription of the regulon that they control (Aubee et al., 2016; Chionh et al., 2016; Thongdee et al.,  
316 2019; Vecerek et al., 2007).

317 Stress regulated RNA modifications would facilitate homeostasis by reprogramming the translation  
318 of stress response genes (Pollo-Oliveira and de Crecy-Lagard, 2019). Although RNA modifications were  
319 initially thought to be static, studies reveal the existence of dynamic modifications depending on  
320 growth phase and rate (Moukadiri et al., 2014), environmental changes (reviewed in (Barraud and  
321 Tisne, 2019; Georgeson and Schwartz, 2021)) or stress (Galvanin et al., 2020), leading to differential  
322 translation of stress response transcripts and translational reprogramming (Pollo-Oliveira and de  
323 Crecy-Lagard, 2019). In this process, RNA modifications and modification levels have an impact on the  
324 translation of regulators, which were thus defined as modification tunable transcripts, or  
325 MoTTs (Endres et al., 2015).

326 Such processes were described in *E. coli* for the general stress sigma factor *rpoS* carrying leucine  
327 codons necessitating MiaA-modified tRNAs (Aubee et al., 2016); for the iron sensing *fur* regulator,  
328 carrying serine codons decoded by MiaB-modified tRNA, in response to low iron (Vecerek et al., 2007);  
329 for the response to magnesium levels through TrmD modification dependent decoding of proline  
330 codons in *mgtA* (Hou et al., 2017). In *Pseudomonas aeruginosa*, TrmB modification increases  
331 translation efficiency of phenylalanine and aspartate enriched catalase mRNAs during oxidative stress  
332 (Thongdee et al., 2019), suggesting tRNA methylation mediated translational response to H<sub>2</sub>O<sub>2</sub>. During  
333 the mycobacterial response to hypoxic stress (Chionh et al., 2016), differential translation of specific  
334 stress response genes was linked, first *in silico*, then experimentally, to their codon usage bias. Our  
335 results highlight tRNA dependent translational reprogramming as a promising subject to be addressed  
336 in bacteria in regard to antibiotic stress.

337 This study reveals the existence of an epigenetic control of the response to sub-MIC antibiotics at  
338 the RNA level, adding upon our previous report of an epigenetic tolerance to aminoglycosides at DNA  
339 level (Carvalho et al., 2021b). Such a response may also involve gene sequences which co-evolve with  
340 the specific bacterial species so that translational regulation of the response to antibiotics becomes  
341 associated with other stress response genes bearing differentially decoded sequences, i.e.  
342 modification tunable transcripts. It can also not be excluded that certain of these RNA modification  
343 enzymes also exert their effect through mRNA modification (Eyler et al., 2019; Hurt et al., 2007).  
344 Molecular study of codon decoding particularities of each RNA modification mutant, coupled to  
345 proteomics, ribosome profiling and *in silico* analysis of genes with differential codon usage, could allow  
346 for the identification of specific pathways post-transcriptionally regulated by a given RNA modification.  
347 A sequence specific action was recently shown for antibiotics targeting the ribosome (Syroegin et al.,  
348 2022; Tsai et al., 2022), where the identity of the amino-acid at the penultimate position determines  
349 whether the ribosome will stop or continue translation in the presence of the antibiotic. In this case, a  
350 specific 23S rRNA methylation (by Cfr) leads to structural changes, which hinders antibiotic access.  
351 Interestingly, two very similar antibiotics, which have the same sequence specificities (here linezolid  
352 and radezolid), can be affected differently by rRNA modifications, inhibiting one and not the other.  
353 Such results, together with our observation that multiple rRNA modifications can impact antibiotic  
354 related phenotypes, may initiate future studies raising the possibility of the development of antibiotics  
355 inhibiting translation of specific proteins.

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365

## 366 **Tables**

367 **Table 1.** Phenotypes associated to RNA modification genes identified by TN-seq. In grey: not selected  
368 for further study.

369 **Table 2.** Summary of the competitive index of the *V. cholerae* deletion mutants compared to WT during  
370 growth in sub-MIC antibiotics

371 **Table 3.** Minimal inhibitory concentrations determined using *Etests*.

## 372 **Supplementary Tables**

373 **Table S1.** TN-seq analysis for the whole genome. Excel file. First tab is TOB, genes were annotated  
374 using current Genbank format (VC\_RSxxxx) and genes names when annotated. Second tab is CIP,  
375 genes were annotated using the old locus tag (VCxxxx) and gene names when annotated. Due to  
376 nomenclature changes between the two TN-seq experiments (TOB and CIP), gene locus tab numbers  
377 are different. Third tab shows correspondence between the old locus tag (e.g. VC0002), current locus  
378 tag (e.g. VC\_RS00005) and gene name (e.g. *mioC*).

379 **Table S2.** Gene ontology enrichment analysis for gene with at least 2-fold change in TN-seq data at  
380 16 generations compared to non-treated condition, and with an adjusted (Bonferroni correction) p-  
381 value <0,05.

382 **Table S3.** Strains and plasmids.

383 **Table S4.** Primers

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387

**Table 1. Phenotypes associated to RNA modification genes identified by TN-seq. “N/A” means: not selected for further study. NS : non-significant p value.**

<i>gene</i>	Function	Known physiological phenotypes In literature	Known effects on translation In literature	TN-seq Fold Change (T16 antibiotic/T16MH) - This study
<b>tRNA modification</b>				
<i>tgt</i>	tRNA-guanine transglycosylase GUN anticodon tRNAs	No significant biological difference but defect in stationary phase viability (Noguchi et al., 1982)	UAG readthrough (Frey et al., 1989) reading frame maintenance defect (Urbonavicius et al., 2001)	<b>TOB: -15.8 (p=0.002)</b> <b>CIP: -2 (p=0.04)</b>
<i>gidA</i> <i>mnmG</i>	5-carboxymethylaminomethyluridine-tRNA synthase. (m <sup>5</sup> U34)	Pleiotropic phenotypes on DNA replication, cell division, tmRNA tagging defect, reviewed in (Nedialkova and Leidel, 2015)	decoding NNG codons (Kurata et al., 2008) reading frame maintenance defect (Urbonavicius et al., 2001)	TOB: +1.9 (NS) <b>CIP: -2.3 (p=0.05)</b>
<i>dusA</i>	tRNA-dihydrouridine synthase A	N/A (Bou-Nader et al., 2018)		<b>TOB : +3.8 (p=0.0002)</b> CIP : +1.4 (NS)
<i>dusB</i>	tRNA-dihydrouridine synthase B	N/A (Bou-Nader et al., 2018)		<b>TOB : +12.2 (p=10<sup>-4</sup>)</b> CIP : +1.3 (NS)
<i>miaA</i> N/A	tRNA dimethylallyltransferase (i6A37)	Mutator phenotype (Zhao et al., 2001). Reduced tetracycline resistance (Taylor et al., 1998). Evolvability of resistance to ciprofloxacin (Mehi et al., 2013). Stress specific phenotype: RpoS/IraP translation (Aubee et al., 2016)	reading frame maintenance defect (Urbonavicius et al., 2001)	<b>TOB: -18 (p=10<sup>-4</sup>)</b>
<i>miaB</i>	isopentenyl-adenosine A37 tRNA methylthiolase (ms2i6A37) U- starting codons	Stress specific phenotype: Fur translation and iron levels (Vecerek et al., 2007)	(Taylor et al., 1998)	<b>TOB : -5.9 (p=0,0007)</b> CIP: +1.1 (NS)
<i>truA</i> <i>hisT</i>	tRNA pseudouridine3 8-40 synthase	growth defect in minimal medium (Tsui et al., 1991)	reading frame maintenance defect (Urbonavicius et al., 2001) mistranslation (Bruni et al., 1977; Parker, 1982)	TOB: +2.4 (NS) CIP: -2.1 (NS)
<i>truB</i>	tRNA pseudouridine5 5 synthase	sensitivity to heatshock (Kingham et al., 2002)		TOB: -1.4 (NS) CIP: -1.9 (NS)
<i>truC</i>	tRNA pseudouridine6 5 synthase tRNA <sup>Ala1</sup> and tRNA <sup>Asp</sup>	N/A (Del Campo et al., 2001)		TOB: 1 CIP: 1
<i>trmA</i>	tRNA m <sup>5</sup> U54 methyltransferase and Y341 of tmRNA	N/A (Bjork and Neidhardt, 1975) Sensitivity to heatshock of <i>truB</i> <i>trmA</i> (Kingham et al., 2002)		<b>TOB: -2.3 (p=0.003)</b> CIP: -1.2 (NS)

<i>trmB</i>	tRNA m <sup>7</sup> G46 methyltransferase	N/A (De Bie et al., 2003)		<b>TOB: +2.7 (p=0.008)</b> CIP: +3.4 (p=0.06 NS)
<i>trmE</i> <i>mnmE</i> <i>E</i>	5-carboxymethylaminomethyluridine-tRNA synthase m <sup>5</sup> U modification of U34 in tRNA	Pleiotropic, acts with <i>gidA</i> , see <i>gidA</i> above	reading frame maintenance defect (Urbonavicius et al., 2001) UAG readthrough (Elseviers et al., 1984) Mistranslation (Hagervall et al., 1998)	<b>TOB: +3.9 (p=0.01)</b> CIP: +2.4 (NS)
<i>trmH</i>	tRNA (Gm18) 2'-O-methyltransferase	N/A (Persson et al., 1997)		<b>TOB : +5.4 (p=0,0002)</b> CIP : -1.3 (NS)
<i>trmK</i>	tRNA (m <sup>1</sup> A22)methyltransferase	(Kimura et al., 2020)		TOB : 1 CIP : -1.3 (NS)
<b>rRNA modification (positions described in <i>E. coli</i>)</b>				
<i>rlmB</i>	23S rRNA 2'-O-ribose G2251 methyltransferase	No obvious growth defect (Lovgren and Wikstrom, 2001)		<b>TOB: -2.6 (p=0,001)</b> <b>CIP: +2 (p=0.05)</b>
<i>rlmI</i>	23S rRNA m <sup>5</sup> C1962 methyltransferase	Decrease in biofilm formation (Herzberg et al., 2006) Slight growth defect (Purta et al., 2008b)		TOB: -1.4 (NS) CIP: +1.3 (NS)
<i>rlmH</i> N/A	23S rRNA m <sup>3</sup> Ψ1915 methyltransferase	Slight growth defect (Purta et al., 2008a)		<b>TOB: -3 (p=0.003)</b> CIP: -1.3 (NS)
<i>rlmE/rrmJ</i> N/A	23S rRNA 2'-O-ribose U2552 methyltransferase	Decreased growth rate (Caldas et al., 2000; Pletnev et al., 2020; Toh and Mankin, 2008) Sparsomycin and tiamulin sensitive (Toh and Mankin, 2008) Lincomycin sensitive (Caldas et al., 2000)	Frameshift and stop codon readthrough (Widerak et al., 2005) Accumulation of ribosomal subunit intermediates (Pletnev et al., 2020)	TOB: +1.2 (NS) <b>CIP: +825 (p=0.003)</b>
<i>rsmB</i>	16S rRNA m <sup>5</sup> C967 methyltransferase	No obvious growth defect (Gu et al., 1999; Pletnev et al., 2020)	Accumulation of 17S rRNA (Pletnev et al., 2020) Translation initiation (Arora et al., 2013; Burakovsky et al., 2012)	<b>TOB: -6.7 (p=0.007)</b> CIP: -2.9 NS
<i>rsmC</i>	16S rRNA m <sup>2</sup> G1207 methyltransferase	No obvious growth defect (Pletnev et al., 2020)	Correct folding of 16S rRNA (Gc et al., 2020)	<b>TOB: +2.3 (p=0.05)</b> CIP: -1.2 (NS)
<i>rsmD</i>	16S rRNA m <sup>2</sup> G966 methyltransferase	No obvious growth defect (Lesnyak et al., 2007; Pletnev et al., 2020)	Translation initiation (Arora et al., 2013; Burakovsky et al., 2012)	<b>TOB: -5.7 (p=0.006)</b> CIP: 1.1 (NS)
<i>rsmF/yebU</i>	16S rRNA m <sup>5</sup> C1407 methyltransferase	No obvious (Pletnev et al., 2020) or slight (Andersen and Douthwaite, 2006) growth defect	Role in translation initiation (Das et al., 2008)	<b>TOB: -2.5 (p=0.002)</b> CIP: -1.8 (NS)

		Increased resistance to some aminoglycosides reported (Gutierrez et al., 2012)		
<i>rsmG</i> N/A	16S rRNA m7G527 methyltransferase	Mutations found in streptomycin resistant MTB clinical isolates (Okamoto et al., 2007)		<b>TOB: -7.7 (p=0.0009)</b>
<i>rsmH</i> N/A	16S rRNA m <sup>4</sup> C1402 methyltransferase	No obvious growth defect (Dassain et al., 1999) <i>ΔrsmH ΔrsmI</i> has a growth defect (Kimura and Suzuki, 2010)	Decoding fidelity (Kimura and Suzuki, 2010)	<b>TOB: -8.4 (p=0.0009)</b>
<i>rsmI</i> N/A	16S rRNA 2'-O-ribose C1402 methyltransferase	No obvious growth defect <i>ΔrsmH ΔrsmI</i> has a growth defect (Kimura and Suzuki, 2010)	Decoding fidelity (Kimura and Suzuki, 2010)	<b>TOB: -8.4 (p=10<sup>-4</sup>)</b>
<i>rsuA</i>	16S rRNA pseudouridine5 16 synthase	No obvious growth defect (Conrad et al., 1999) Overexpression leads to resistance to HOCl (Chen et al., 2021)	Accumulation of 17S rRNA (the present study)	<b>TOB: -2.3 (p=0.01)</b> CIP: 1
<i>rluB</i>	23S rRNA pseudouridine2 605 synthase	No obvious growth defect (Del Campo et al., 2001; Toh and Mankin, 2008) Increased CM and linezolid sensitivity (Toh and Mankin, 2008)	50S subunit maturation (Jiang et al., 2007)	<b>TOB: +9.4 (p=10<sup>-4</sup>)</b> CIP: +1.2 (NS)
<i>rluC</i> N/A	23S rRNA pseudouridine9 55/2504/2580 synthase	No obvious growth defect (Conrad et al., 1998) Cold sensitivity (Jiang et al., 2007) clindamycin, linezolid, and tiamulin sensitivity (Toh and Mankin, 2008)		<b>TOB: +1.8 (p=10<sup>-4</sup>)</b> CIP: +1.3 (NS)
<i>rluD</i>	23S rRNA pseudouridine1 911/1915/1917 synthase	Reported to cause a large growth defect in <i>E. coli</i> but independently of pseudouridines (Gutgsell et al., 2001)	ribosome assembly (Gutgsell et al., 2005)	<b>TOB: +1.4 (p=0.03)</b> <b>CIP: +6.6 (p=0.002)</b>
<i>rluE</i>	23S rRNA pseudouridine2 457 synthase	No obvious growth defect (Del Campo et al., 2001)		<b>TOB: -1.5 (p=0.002)</b> CIP: -2.4 (NS)
<b>Modification of both tRNA and rRNA</b>				
<i>rluF</i>	23S rRNA pseudouridine2 604 / tRNA <sup>Tyr</sup> pseudouridine3 5 synthase	No obvious growth defect (Del Campo et al., 2001; Pletnev et al., 2020; Toh and Mankin, 2008) Decreased linezolid resistance (Toh and Mankin, 2008)	Effect on translation of Tyr codons (Addepalli and Limbach, 2016)	TOB: -1.2 (NS) CIP: -1.2 (NS)
<i>rlmN</i>	tRNA m2A37 methyltransferase / 23S rRNA m2A2503 methyltransferase	No obvious growth defect (Benitez-Paez et al., 2012) Slightly increased susceptibility to certain peptidyl transferase-targeting antibiotics (Toh and Mankin, 2008)	UAG readthrough (Benitez-Paez et al., 2012)	<b>TOB: +2.3 (p=0.005)</b> <b>CIP: -2.2 (p=0.0003)</b>



389 **Figure legends**

390 **Figure 1. TN-seq identifies rRNA and tRNA modification genes affecting fitness of *V. cholerae* in the**  
391 **presence of sub-MIC TOB and CIP.** tRNA modification genes are indicated in red. rRNA modification  
392 genes are indicated in blue. *rlmN* modifies both tRNAs and rRNA. Volcano plots show genes for which  
393 the number of transposon inactivation is increased (beneficial) or decreased (detrimental) after 16  
394 generations of growth, compared to growth without antibiotics. A: TOB 50% of the MIC, B: CIP 50% of  
395 the MIC. X axis represents log<sub>2</sub> fold change of the number of transposon reads associated with gene  
396 inactivations, detected after 16 generations in the indicated antibiotic versus non-treated condition.  
397 The Y-axis represents the negative log<sub>10</sub> *p* value. Gene inactivations which show the strongest antibiotic  
398 specific effects are highlighted in yellow. Dotted lines in the Y-axis indicate *p*-values of 0.05 (lower line)  
399 and 0.01 (upper line). Dotted lines in the X-axis represent 2-fold decrease (left) and 2-fold increase  
400 (right).

401 **Figure 2: Gene ontology analysis for TN-seq data.** The analyzed lists were for each antibiotic (TOB/CIP),  
402 genes (plotted on Fig. 1) with at least 2-fold change in TN-seq data at 16 generations compared to non-  
403 treated condition, and with an adjusted (Bonferroni correction) *p*-value <0.05. Red arrows indicate  
404 RNA modification processes. The total number of uploaded genes list to be analyzed were 449 genes  
405 for TOB and 1004 genes for CIP. The reference gene list was *Vibrio cholerae* (all genes in database),  
406 3782 genes. Annotation Version: PANTHER Overrepresentation Test (Released 20220712). GO  
407 Ontology database DOI: 10.5281/zenodo.6399963 Released 2022-03-22. Only the results with the Fold  
408 Enrichment of the most specific categories are shown, the detailed results are shown in **Table S2**.

409 **Figure 3. Impact of RNA modification gene deletions on fitness during growth in sub-MIC antibiotics.**  
410 In vitro competition experiments of *V. cholerae* WT and mutant strains in the absence or presence of  
411 different antibiotics at sub-MICs (50% of the MIC). TCL: triclosan 0.01 mM, TOB: tobramycin 0.6 µg/ml;  
412 GEN: gentamicin 0.5 µg/ml; CIP: ciprofloxacin 0.002 µg/ml, CM: chloramphenicol 0.4 µg/ml, TRM:  
413 trimethoprim 0.4 µg/ml, CRB: carbenicillin 2.5 µg/ml. The Y-axis represents log<sub>10</sub> of competitive index  
414 value calculated as described in the methods. A competitive index of 1 indicates equal growth of both  
415 strains. NT: no antibiotic treatment. For multiple comparisons, we used one-way ANOVA on  
416 GraphPad Prism. \*\*\*\* means *p*<0.0001, \*\*\* means *p*<0.001, \*\* means *p*<0.01, \* means *p*<0.05. Only  
417 significant *p* values are represented. Number of replicates for each experiment: 3<*n*<8.

418 **Figure 4: Survival to lethal antibiotic treatment.** *V. cholerae* WT and deletion mutant cultures were  
419 grown without antibiotics up to early exponential phase. Total number of bacteria was determined by  
420 plating on MH plates before addition of the indicated antibiotic at >MIC, at time T<sub>0</sub>. After 20 hours of  
421 incubation with the antibiotic, the number of surviving bacteria was determined by plating on MH  
422 plates (T<sub>20</sub>). Survival was calculated for each strain by dividing the number of surviving bacteria at T<sub>20</sub>  
423 by the initial number of bacteria. The Y-axis represents the log<sub>10</sub> survival ratio of a given mutant over  
424 the survival of the WT strain. Antibiotic concentrations: tobramycin (TOB) 10 µg/ml, ciprofloxacin (CIP)  
425 0.04 µg/ml, trimethoprim (TRM) 50 µg/ml, carbenicillin (CRB) 50 µg/ml. Means and geometric means  
426 for logarithmic values were calculated using GraphPad Prism. For multiple comparisons, we used one-  
427 way ANOVA on GraphPad Prism. \*\*\*\* means *p*<0.0001, \*\*\* means *p*<0.001, \*\* means *p*<0.01, \*  
428 means *p*<0.05. Only significant *p* values are represented. Number of replicates for each experiment:  
429 3<*n*<8.

430 **Figure 5. Fitness versus survival.** For each antibiotic, survival values from figure 3 (Y-axis, log<sub>10</sub> values)  
431 were plotted against competitive index values from figure 2 (X-axis, log<sub>10</sub> values). TOB: tobramycin,

432 CIP: ciprofloxacin, TRM: trimethoprim, CRB: carbenicillin. Names of genes with significant changes are  
433 indicated.

434 **Figure 6. Survival of *V. cholerae* WT and RNA modification deletion mutants after UV irradiation.**  
435 Survival of indicated  $\Delta$ -mutant relative to WT after UV irradiation at 60J. For multiple comparisons, we  
436 used one-way ANOVA on GraphPad Prism. \*\*\*\* means  $p < 0.0001$ , \*\*\* means  $p < 0.001$ , \*\* means  
437  $p < 0.01$ .

438 **Figure 7. Growth of *E. coli* MG1655 WT and derivatives deleted for selected RNA modification genes  
439 in sub-MIC TOB.** Overnight cultures were diluted 100x in fresh MH medium, on 96 well plates. Plates  
440 were incubated with shaking in TECAN plate reader device at 37°C, OD 620 nm was measured every  
441 15 minutes. Grey: no treatment. Blue: sub-MIC TOB, at 0.2  $\mu\text{g/ml}$  (50% of the MIC for MG1655 in MH  
442 liquid culture). The Y-axis represents the OD of the mutant divided by the OD of the WT strain in the  
443 same growth conditions, and thus reflects slower (below 1) or faster (above 1) growth. Standard  
444 deviation is shown.

445

446 **Supplementary Figure legends**

447 **Figure S1: Stringent response induction measured with *P1rrn-gfp* reporter.** *P1rrn-gfp* reporter  
448 fluorescence decreases upon induction of stringent response. Graphs shows fluorescence (Y-axis) as a  
449 function of growth (X-axis: OD 620nm). The last graph shows the effect of sub-MIC TOB in the WT  
450 strain. All experiments were performed in triplicates, the means and standard deviations are shown.

451 **Figure S2: Survival to short lethal antibiotic treatment.** *V. cholerae* WT and deletion mutant cultures  
452 were grown without antibiotics up to early exponential phase, and serial dilutions were spotted on MH  
453 medium without antibiotics. Exponential phase cultures were then treated with antibiotics at lethal  
454 concentrations for 30min, 1h and 2 hours. At each time point, dilutions were spotted on MH. TOB:  
455 tobramycin 5 or 10 µg/ml.

456 **Figure S3. Effect of rRNA modification gene deletions on detected rRNA species during growth in the  
457 absence of antibiotics.** A and B. Bioanalyzer results. C: 23S/16S ratio. For *ΔrsuA* which displayed an  
458 extra-peak, we represent two independent biological replicates.

459 **Figure S4. Frequency of appearance of spontaneous mutants in *V. cholerae* WT and RNA modification  
460 deletion mutants.** Stationary phase cultures were plated in parallel on MH and MH plate  
461 supplemented with RIF: rifampicin 1 µg/ml. The mutation frequency was calculated as CFU MH +  
462 RIF/total CFU on MH. The Y-axis represents the log<sub>10</sub> resistant mutant frequency. Data were first log  
463 transformed in order to achieve normal distribution, and statistical tests were performed on these log-  
464 transformed data. Means and geometric means for logarithmic values were calculated using GraphPad  
465 Prism. For multiple comparisons, we used one-way ANOVA on GraphPad Prism. \*\*\* means p<0.001.  
466 Only significant p values are represented. Number of replicates for each experiment: 3<n<8.

467 **Figure S5. Growth of *E. coli* MG1655 WT and derivatives deleted for selected RNA modification  
468 genes.** Overnight cultures were diluted 100x in fresh MH medium, on 96 well plates. Plates were  
469 incubated with shaking in TECAN plate reader device at 37°C, OD 620 nm was measured every 15  
470 minutes. Antibiotics were used at sub-MIC for MG1655 in MH liquid culture: TOB 0.2 µg/ml and 0.4  
471 µg/ml, CRB 3 µg/ml, TRM 0.15 µg/ml, GEN 0.3 µg/ml and 0.4 µg/ml, CIP 0.006 µg/ml. The Y-axis  
472 represents the OD of the mutant grown in a given antibiotic divided by the OD of the WT strain in the  
473 same antibiotic, and thus reflects slower (below 1) or faster (above 1) growth.

474 **Figure S6. Growth of *E. coli* BW25113 WT and derivatives deleted for selected RNA modification  
475 genes in sub-MIC TOB.** Overnight cultures were diluted 100x in fresh MH medium, on 96 well plates.  
476 Plates were incubated with shaking in TECAN plate reader device at 37°C, OD 620 nm was measured  
477 every 15 minutes. Grey: no treatment. Blue: sub-MIC TOB, at 0.2 µg/ml (50% of the MIC for MG1655  
478 in MH liquid culture). The Y-axis represents the OD of the mutant grown in a given antibiotic divided  
479 by the OD of the WT strain in the same growth conditions, and thus reflects slower (below 1) or faster  
480 (above 1) growth.

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## 486 **Mat & Met**

### 487 **Bacterial Strains and Plasmids**

488 All *V. cholerae* strains used in this study are derivative of *V. cholerae* N16961 *hapR+*, and were  
489 constructed by allelic exchange. *V. cholerae* mutants were constructed in the  $\Delta lacZ$  strain (K329). All *E.*  
490 *coli* strains used in this work are derivatives of *E. coli* MG1655, and were constructed by transduction  
491 using *E. coli* Keio knockouts strains. Strains, plasmids and primers are listed in **Tables S3 and S4** for  
492 more details.

### 493 **Media and Growth Conditions**

494 Colonies on plates grew at 37°C, in Mueller-Hinton medium (MH) media. Liquid cultures grew at 37°C  
495 in appropriate media in aerobic conditions, with 180 rotations per minute

### 496 **Transposon insertion sequencing**

497 Libraries were prepared as previously described (Baharoglu et al., 2014; Chiang and Rubin, 2002). to  
498 achieve a library size of 600.000 clones, and subjected to passaging in MH and MH+TOB 0.5 or MH+CIP  
499 0,001 for 16 generations (Negro et al., 2019). A saturated mariner mutant library was generated by  
500 conjugation of plasmid pSC1819 from *E. coli* to *V. cholerae* WT. Briefly, pSC189 (Baharoglu et al., 2014;  
501 Chiang and Rubin, 2002) was delivered from *E. coli* strain 7257 ( $\beta$ 2163 pSC189::spec, laboratory  
502 collection) into the *V. cholerae* WT strain. Conjugation was performed for 2 h on 0.45  $\mu$ M filters. The  
503 filter was resuspended in 2 ml of MH broth. Petri dishes containing 100  $\mu$ g/ml spectinomycin were  
504 then spread. The colonies were scraped and resuspended in 2 ml of MH. When sufficient single  
505 mutants were obtained (>600 000 for 6X coverage of non-essential regions), a portion of the library  
506 was used for gDNA extraction using Qiagen DNeasy® Blood & Tissue Kit as per manufacturer's  
507 instructions. This was used for library validation through insert amplification by nested PCR using a  
508 degenerate primer (ARB6), which contains 20 defined nucleotides followed by a randomized sequence.  
509 This was combined with a primer anchored in the edge of the transposon sequence (MV288)  
510 (Baharoglu et al., 2014; Negro et al., 2019). After this, primer ARB3, which contains the first 20  
511 nucleotides of ARB6 was used for nested amplification in combination with MV288. After validation,  
512 the libraries were passaged in MH media for 16 generations with or without 50%MIC of TOB or CIP, in  
513 triplicate. gDNA from time point 0 and both conditions after 16 generation passage in triplicate was  
514 extracted. Sequencing libraries were prepared using Agilent's SureSelect XT2 Kit with custom RNA baits  
515 designed to hybridize the edges of the Mariner transposon. The 100 ng protocol was followed as per  
516 manufacturer's instructions. A total of 12 cycles were used for library amplification. Agilent's 2100  
517 Bioanalyzer was used to verify the size of the pooled libraries and their concentration. HiSeq Paired-  
518 end Illumina sequencing technology was used producing 2x125bp long reads. Reads were then filtered  
519 through transposon mapping to ensure the presence of an informative transposon/genome junction  
520 using a previously described mapping algorithm (Pierle et al., 2014). Informative reads were extracted  
521 and mapped. Reads were counted when the junction was reported as mapped inside the CDS of a gene  
522 plus an additional 50 bp upstream and downstream. Expansion or decrease of fitness of mutants was  
523 calculated in fold changes with normalized insertion numbers. Normalization calculations were applied  
524 according to van Opijnen et al (van Opijnen et al., 2009). Expansion or decrease of fitness of mutants  
525 was calculated in fold changes with normalized insertion numbers. Baggerly's test on proportions  
526 (Baggerly et al., 2003) was used to determine statistical significance as well as a Bonferroni correction  
527 for multiple hypotheses testing.

528

### 529 **Gene Ontology (GO) enrichment analysis.**

530 GO enrichment analyses were performed on <http://geneontology.org/> as follows: Binomial test was  
531 used to determine whether a group of gene in the tested list was more or less enriched than expected  
532 in a reference group. The annotation dataset used for the analysis was GO biological process complete.  
533 The analyzed lists were for each antibiotic (TOB/CIP), genes (plotted on Fig. 1) with at least 2-fold  
534 change in TN-seq data at 16 generations compared to non-treated condition, and with an adjusted  
535 (Bonferroni correction) p-value <0,05. The total number of uploaded genes list to be analyzed were  
536 449 genes for TOB and 1004 genes for CIP.

537 The reference gene list was *Vibrio cholerae* (all genes in database), 3782 genes. Annotation Version:  
538 PANTHER Overrepresentation Test (Released 20220712). GO Ontology database DOI:  
539 10.5281/zenodo.6399963 Released 2022-03-22

### 540 **Competition experiments**

541 Overnight cultures from single colonies of mutant lacZ<sup>+</sup> and WT lacZ<sup>-</sup> strains were washed in PBS  
542 (Phosphate Buffer Saline) and mixed 1:1 (500µl + 500µl). At this point 100µl of the mix were serial  
543 diluted and plated on MH agar supplemented with X-gal (5-bromo-4-chloro-3-indolyl-β-D-  
544 galactopyranoside) at 40 µg/mL to assess T0 initial 1:1 ratio. At the same time, 10 µl from the mix were  
545 added to 2 mL (approximately 5x10<sup>5</sup> cells/mL) of MH or MH supplemented with sub-MIC antibiotics  
546 (TCL: triclosan 0.01 µM, TOB: tobramycin 0.6 µg/ml; GEN: gentamicin 0.5 µg/ml; CIP: ciprofloxacin  
547 0.002 µg/ml, CM: chloramphenicol 0.4 µg/ml, TRM: trimethoprim 0.4 µg/ml, CRB: carbenicillin 2.5  
548 µg/ml) and incubated with agitation at 37°C for 20 hours, which corresponds to 9 generations. Cultures  
549 were then diluted and plated on MH agar plates supplemented with X-gal. Plates were incubated  
550 overnight at 37°C and the number of blue and white CFUs was assessed. Competitive index was  
551 calculated by dividing the number of blue CFUs (lacZ<sup>+</sup> strain) by the number of white CFUs (lacZ<sup>-</sup> strain)  
552 and normalizing this ratio to the T0 initial ratio.

### 553 **MIC determination**

554 Stationary phase cultures grown in MH were diluted 20 times in PBS, and 300 µL were plated on MH  
555 plates and dried for 10 minutes. Etests (Biomérieux) were placed on the plates and incubated overnight  
556 at 37°C. The Etest for ampicillin (AMP) was used for the evaluation of the MIC of carbenicillin.

557 **Survival/tolerance tests** were performed on early exponential phase cultures. In order to clear the  
558 culture from previously non-growing cells that could potentially be present from the stationary phase  
559 inoculum, we performed a two-step dilution protocol, before antibiotic treatment. Overnight *V.*  
560 *cholerae* cultures were first diluted 1000x in 4 ml fresh Mueller-Hinton (MH) medium, and incubated  
561 at 37°C with shaking. When the OD 620 nm reached ~0.2, cultures were diluted 1000x a second time,  
562 in order to clear them from non-growing cells, in Erlenmeyers containing 25 ml fresh MH medium, and  
563 were allowed to grow at 37°C. When cultures reached an OD 620 nm between 0.25 and 0.3 (early  
564 exponential phase), appropriate dilutions were plated on MH plates to determine the total number of  
565 CFUs in time zero untreated cultures. Note that for *V. cholerae*, it was important to treat cultures at  
566 the precise OD 620 nm 0.25-0.3, as persistence levels seem to be particularly sensitive to growth phase  
567 in this species, where they decline in stationary phase. 5 ml of cultures were collected into 50 ml Falcon  
568 tubes and treated with lethal doses of desired antibiotics (10 times the MIC: tobramycin 10 µg/ml,  
569 carbenicillin 50 µg/ml, ciprofloxacin 0.025 µg/ml, trimethoprim 5 µg/ml) for 20 hours at 37°C with

570 shaking in order to guarantee oxygenation. Appropriate dilutions were then plated on MH agar without  
571 antibiotics and proportion of growing CFUs were calculated by doing a ratio with total CFUs at time  
572 zero. Experiments were performed 3 to 8 times.

### 573 **RNA purification and analysis of rRNA species**

574 For RNA extraction, overnight cultures were diluted 1:1000 in MH medium and grown with agitation  
575 at 37°C until an OD600 of 0.3 (exponential phase). 0.5 mL of these cultures were centrifuged and  
576 supernatant removed. Pellets were homogenized by resuspension with 1.5 mL of cold TRIzol Reagent.  
577 Next, 300 µL chloroform were added to the samples following mix by vortexing. Samples were then  
578 centrifuged at 4°C for 10 minutes. Upper (aqueous) phase was transferred to a new 2mL tube and  
579 mixed with 1 volume of 70% ethanol. From this point, the homogenate was loaded into a RNeasy Mini  
580 kit (Qiagen) column and RNA purification proceeded according to the manufacturer's instructions.  
581 Samples were then subjected to DNase treatment using TURBO DNA-free Kit (Ambion) according to  
582 the manufacturer's instructions. Total RNA samples were then analyzed on an Agilent 2100 Bioanalyzer  
583 (Agilent Technologies) using the Agilent RNA 6000 nano kit according to the instructions of the  
584 manufacturer.

### 585 **Rifampicin spontaneous mutation tests.**

586 Stationary phase cultures were plated in parallel on MH and MH plate supplemented with RIF:  
587 rifampicin 1 µg/ml. The mutation frequency was calculated as CFU MH + RIF/total CFU on MH.

### 588 **Growth of *E. coli* on microtiter plate reader**

589 Overnight cultures were diluted 100x in fresh MH medium, on 96 well plates. Each well contained 200  
590 µL. Plates were incubated with shaking on TECAN plate reader device at 37°C, OD 620 nm was  
591 measured every 15 minutes. Antibiotics were used at sub-MIC for MG1655 in MH liquid culture: TOB  
592 0.2 and 0.4 µg/ml, CRB 3 µg/ml, TRM 0.15 µg/ml, GEN 0.3 and 0.4 µg/ml, CIP 0.006 µg/ml.

### 593 **Stringent response measurements.**

594 *P1rrnB-gfp* fusion was constructed using *gfp* ASV (Andersen et al., 1998), and cloned into plasmid  
595 pSC101. *P1rrnB-GFP<sub>ASV</sub>* transcriptional fusion was amplified from strain R438 (*E. coli* MG1655  
596 *attB::P1rrnB gfp-ASV::kan* provided by Ivan Matic) using primers AFC060 and AFC055 thus including 42  
597 bp upstream of *rrnB* transcription initiation site. PCR product was then cloned in pTOPOblunt vector  
598 and subcloned to pSC101 by EcoRI digestion and ligation. The final construct was confirmed by sanger  
599 sequencing. The plasmid was then introduced by electroporation into the tested strains. Overnight  
600 cultures were performed in MH + carbenicillin 100 µg/mL and diluted 500x in 10 mL fresh MH, in an  
601 Erlenmeyer. At time points 0 min, and every 30 during 3 hours, the OD 620 nm was measured and  
602 fluorescence was quantified in flow cytometry. For each experiment, 50,000 to 100,000 events were  
603 counted on the Miltenyi MACSquant device.

### 604 605 **UV sensitivity measurements.**

606 Overnight cultures were diluted 1:100 in MH medium and grown with agitation at 37°C until an OD  
607 600 nm of 0.5-0.7. Appropriate dilutions were then plated on MH agar. The proportion of growing CFUs  
608 after irradiation at 60 Joules over total population before irradiation was calculated, doing a ratio with  
609 total CFUs. Experiments were performed 3 to 8 times

610

611 **Quantification and statistical analysis.**

612 First an F-test was performed in order to determine whether variances are equal or different between  
613 comparisons. For comparisons with equal variance, Student's t-test was used. For comparisons with  
614 significantly different variances, we used Welch's t-test. For multiple comparisons, we used one-way  
615 ANOVA. We used GraphPad Prism to determine the statistical differences (p value) between groups.  
616 \*\*\*\* means  $p < 0.0001$ , \*\*\* means  $p < 0.001$ , \*\* means  $p < 0.01$ , \* means  $p < 0.05$ . For survival tests, data  
617 were first log transformed in order to achieve normal distribution, and statistical tests were performed  
618 on these log-transformed data. Number of replicates for each experiment was  $3 < n < 6$ . Means and  
619 geometric means for logarithmic values were also calculated using GraphPad Prism. For persistence  
620 tests, data were first log transformed in order to achieve normal distribution, and statistical tests were  
621 performed on these log-transformed data.

622 **Data availability**

623 TN-seq data is available through the GEO accession number: GSE198341 for TOB and accession  
624 numbers SRR8351961, SRR8351962, SRR8351957, SRR8351958, SRR8351959, SRR8351960,  
625 SRR8351965, SRR8351966, SRR8351963, SRR8351964, SRR8351967, and SRR8351968 for CIP.

626

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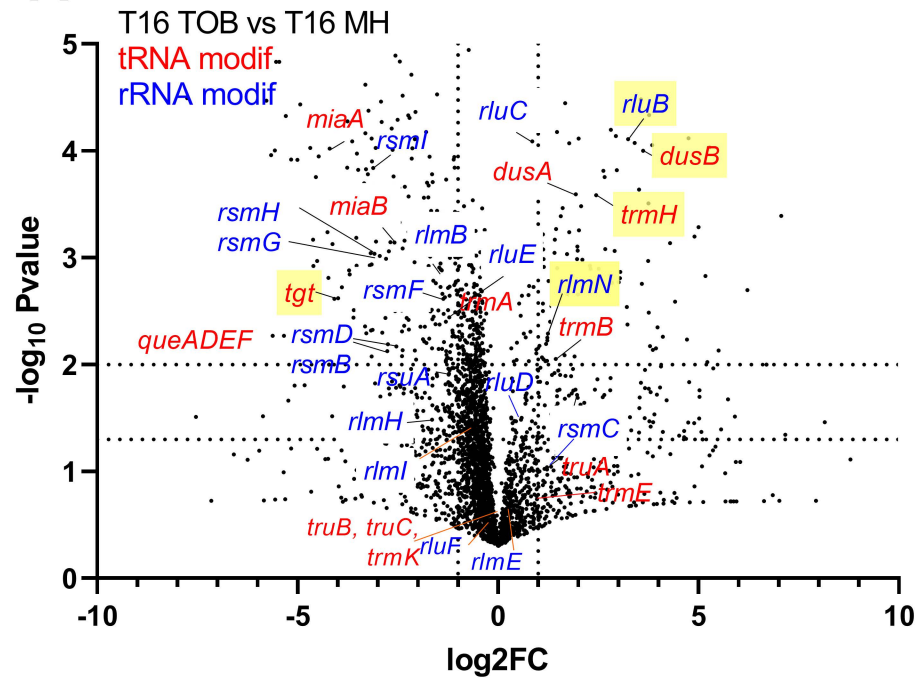
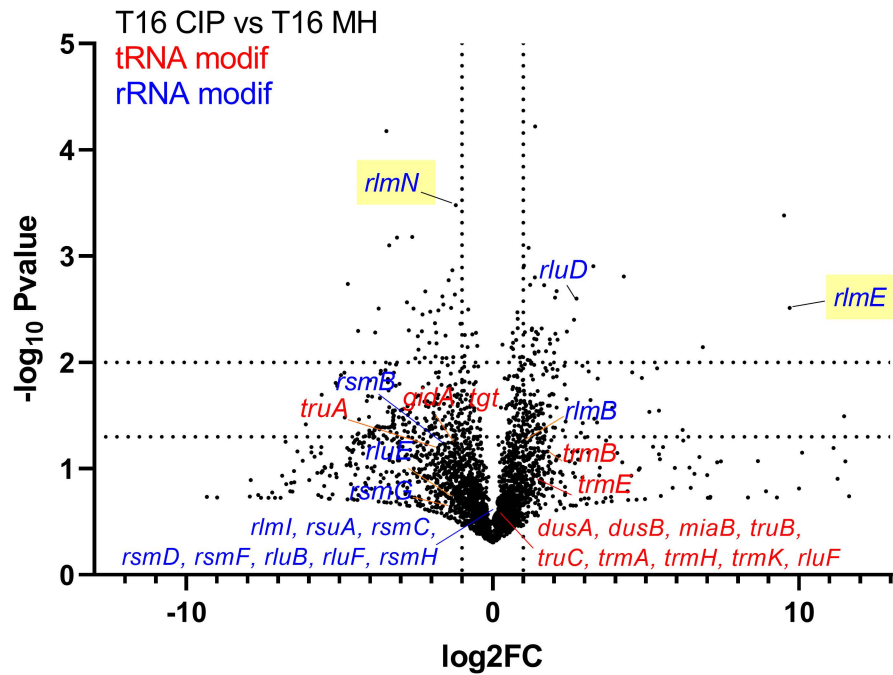
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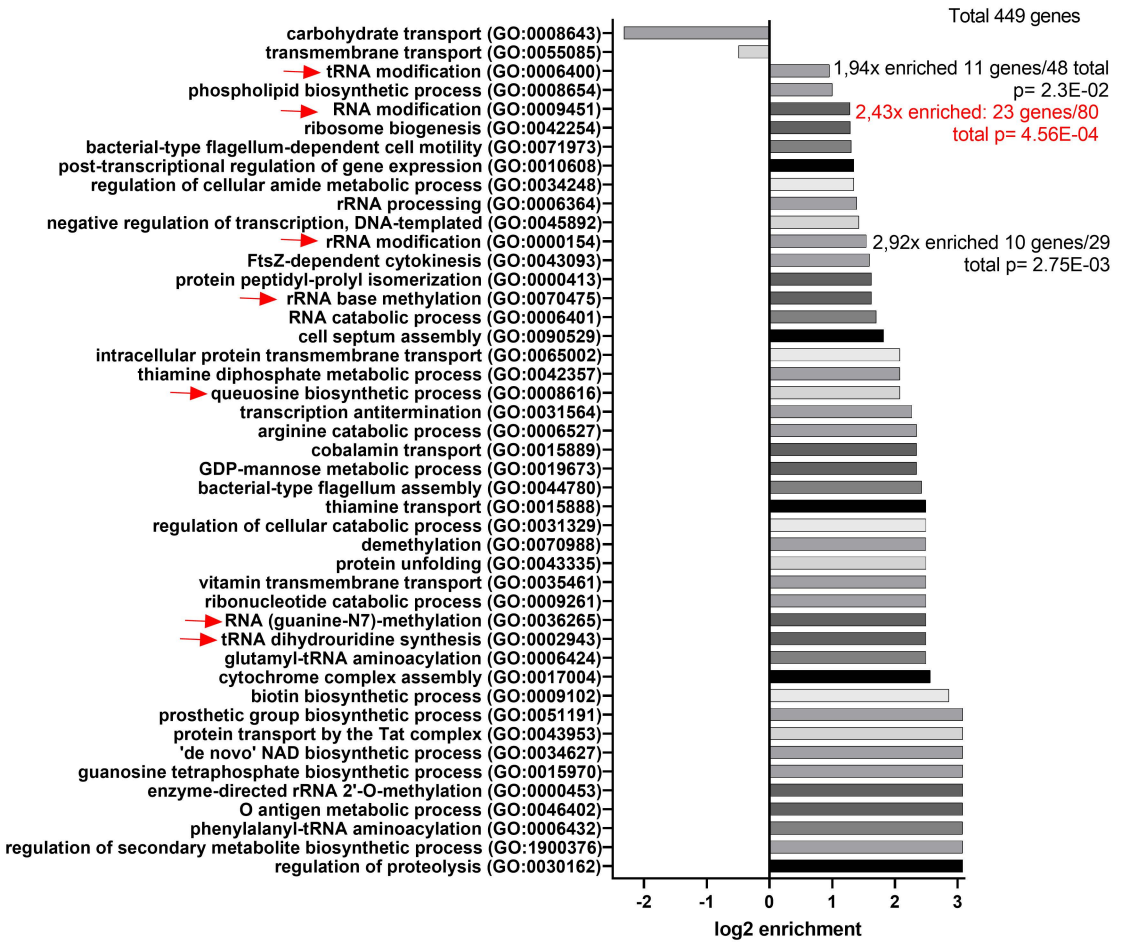
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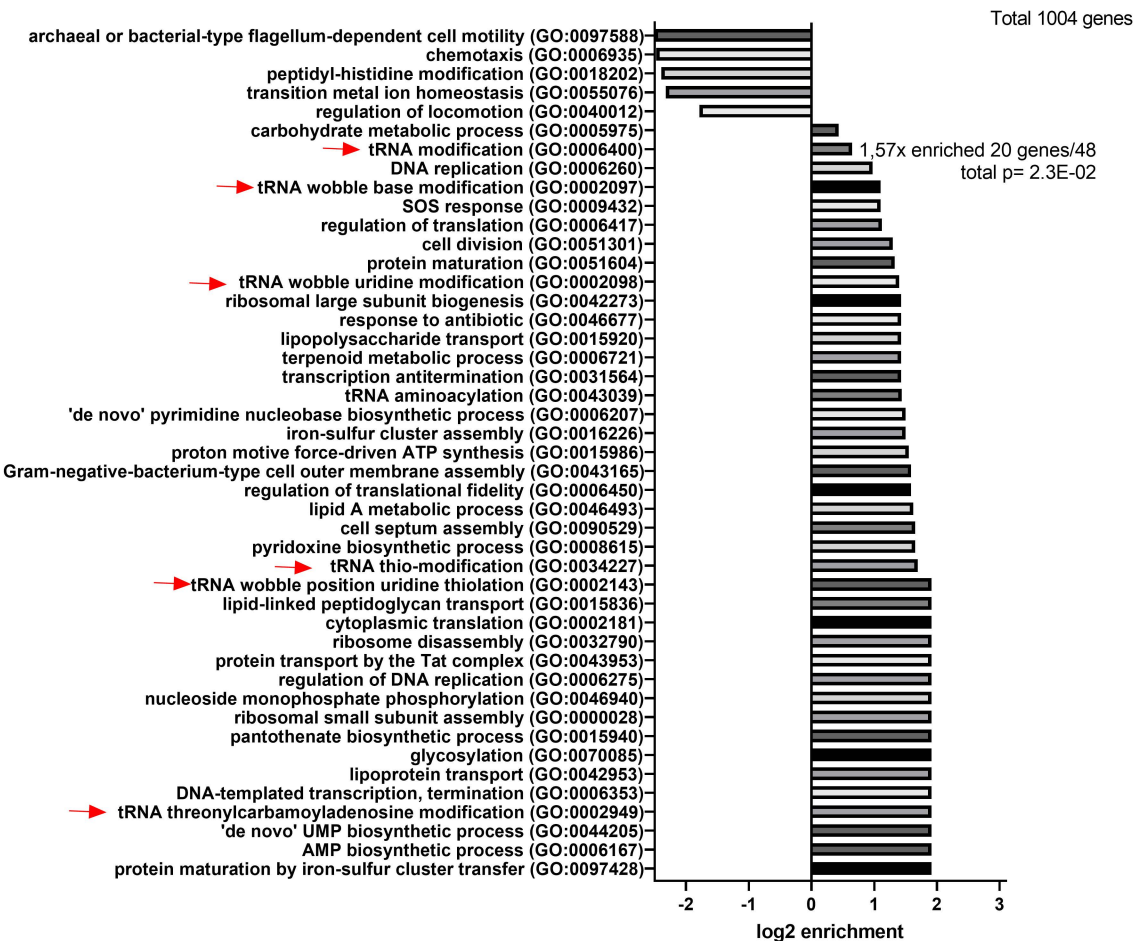
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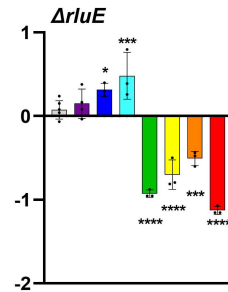
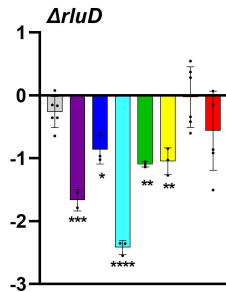
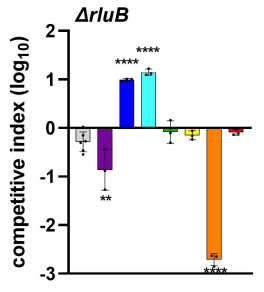
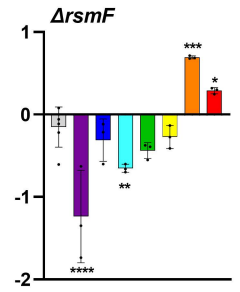
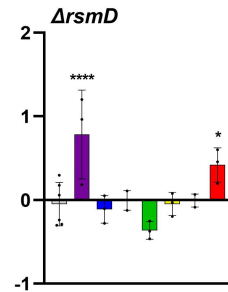
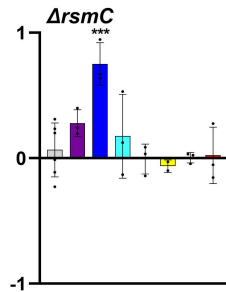
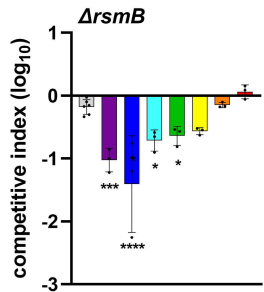
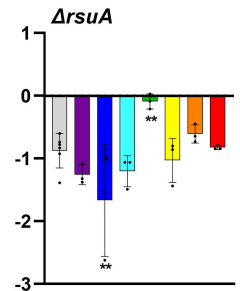
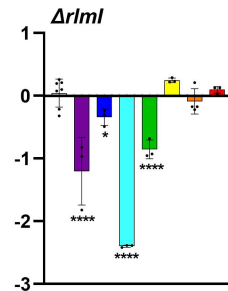
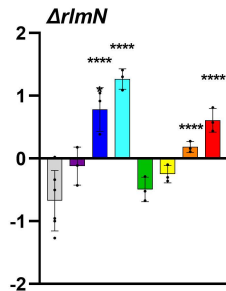
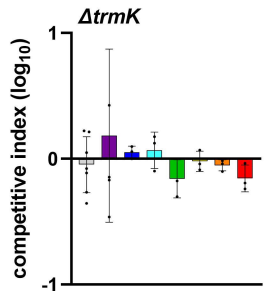
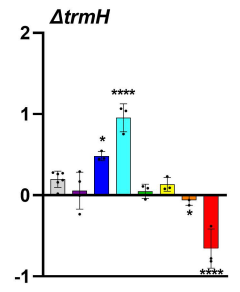
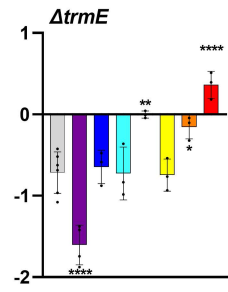
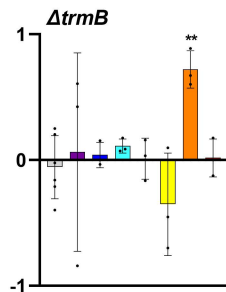
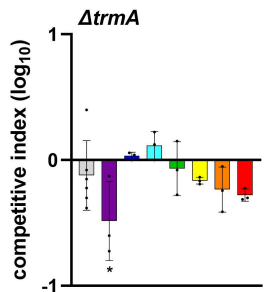
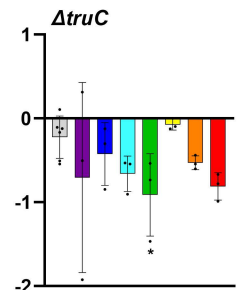
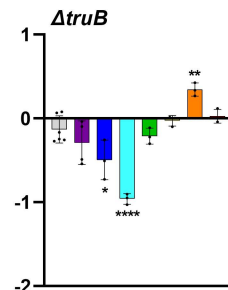
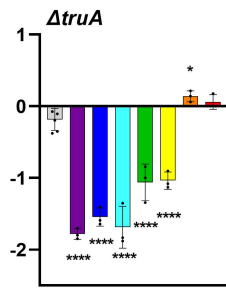
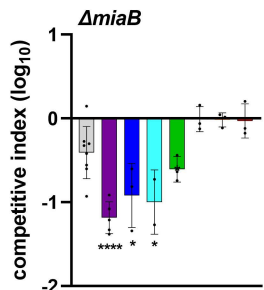
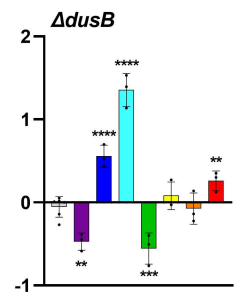
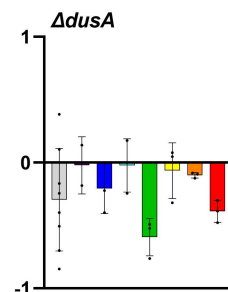
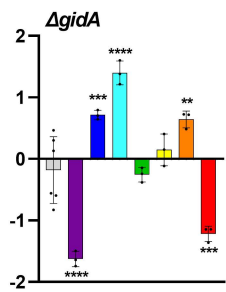
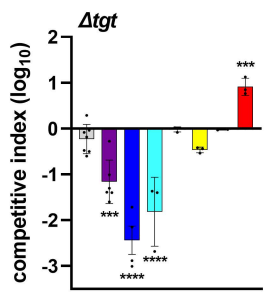
**A****B**

GO biological process: 16gTOB vs 16g MH

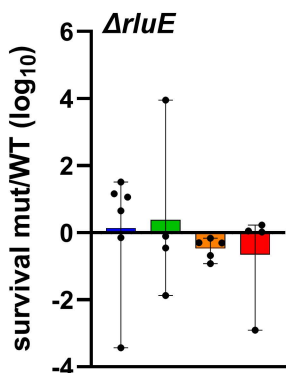
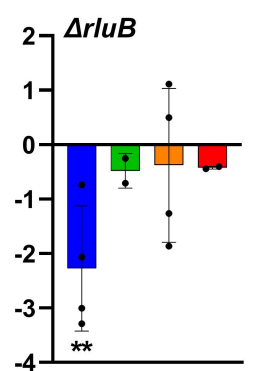
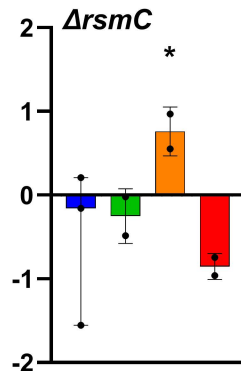
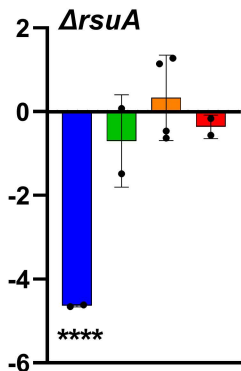
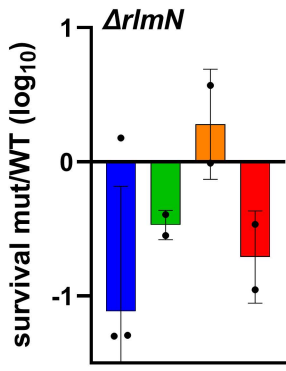
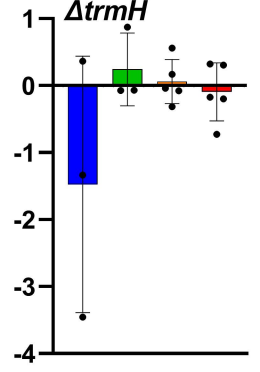
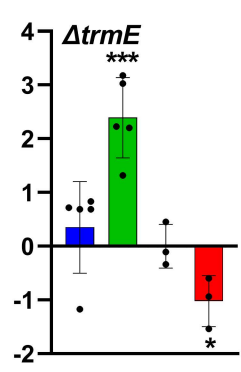
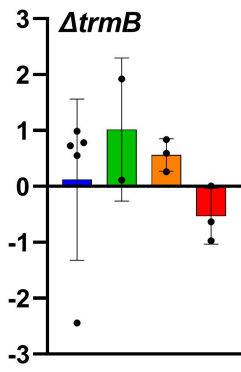
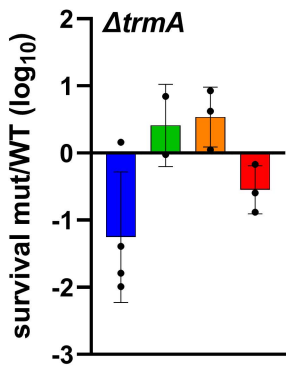
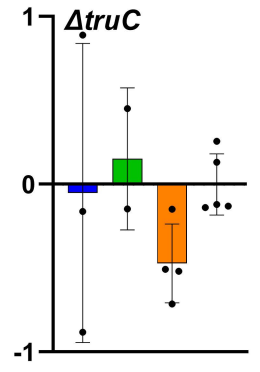
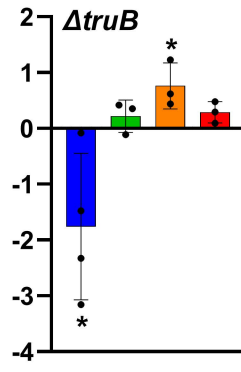
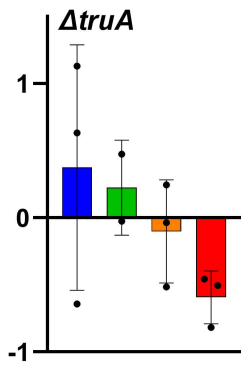
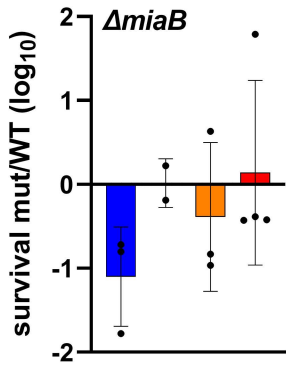
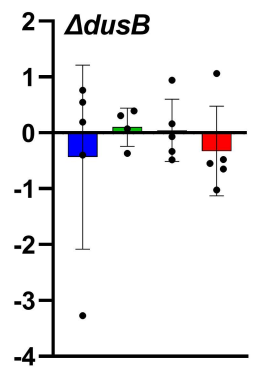
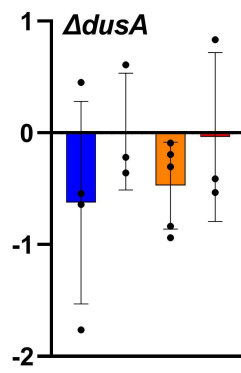
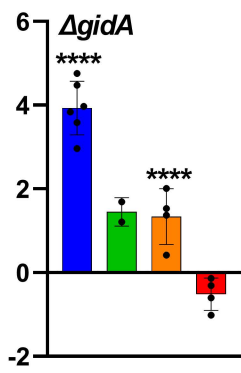
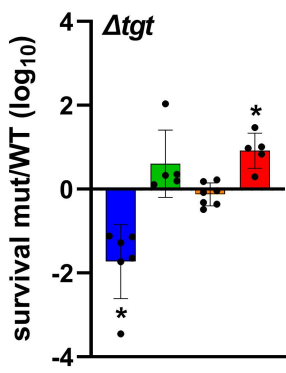


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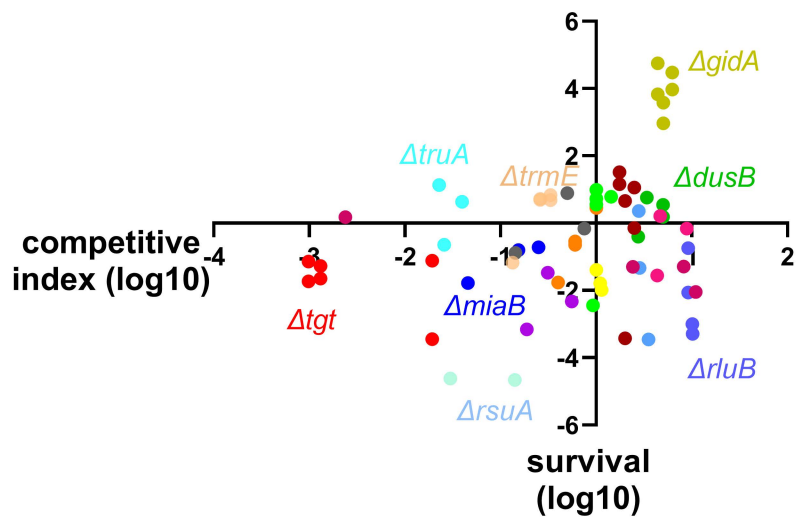




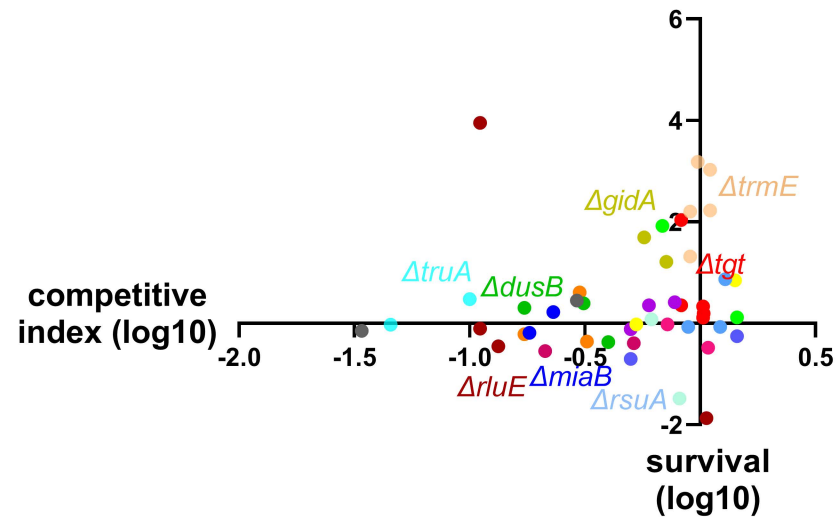




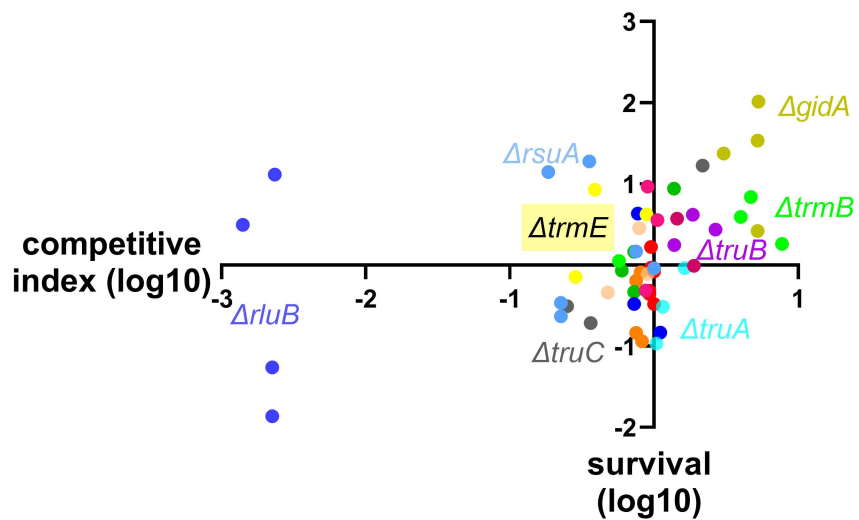
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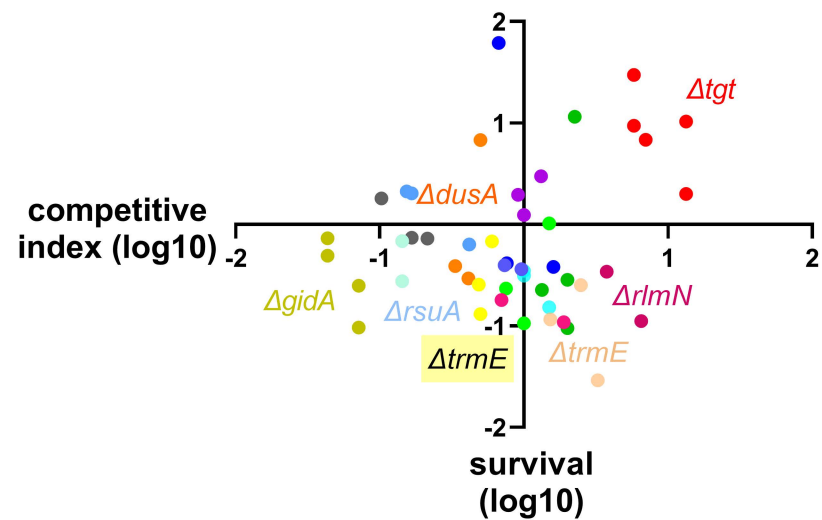
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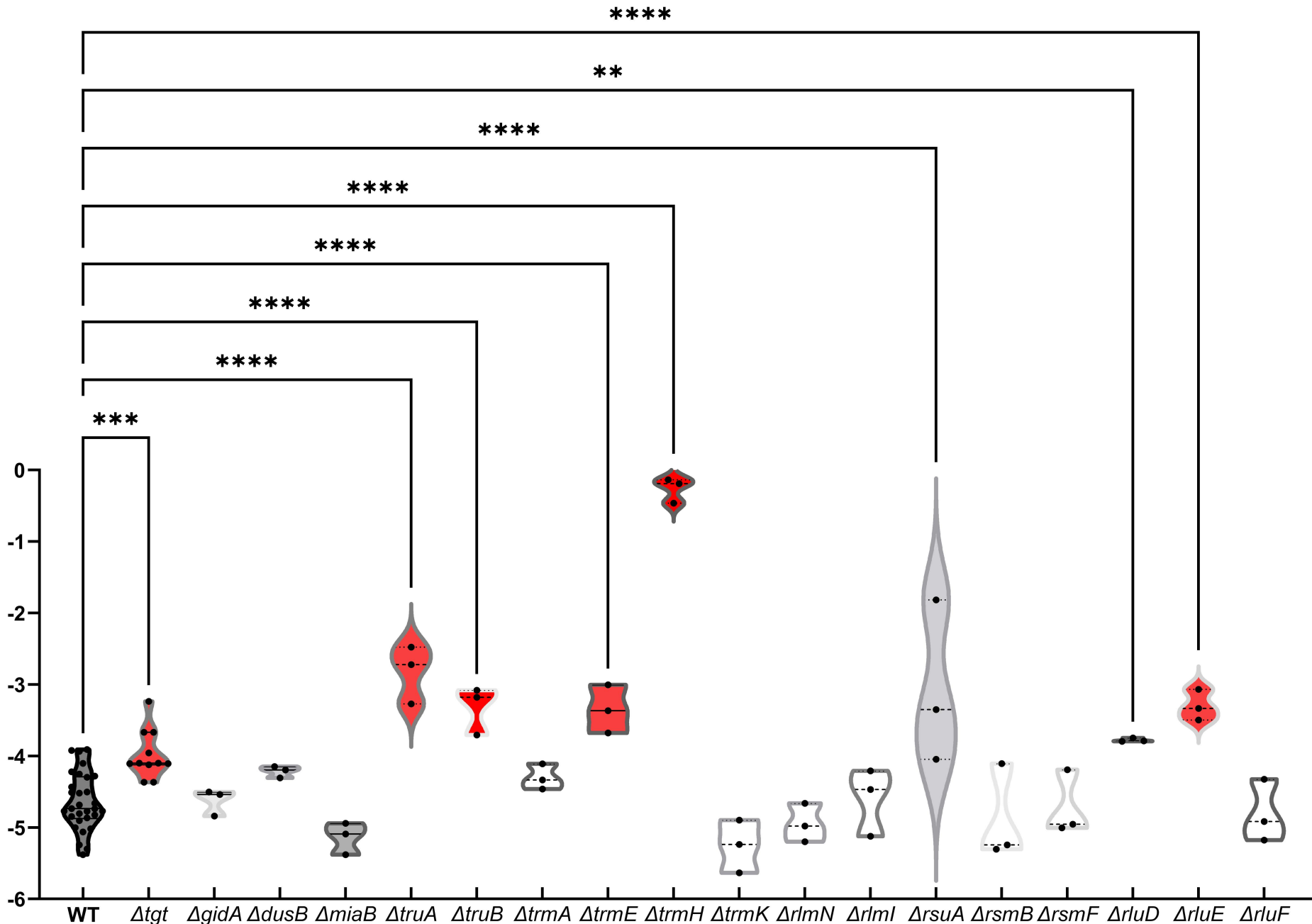
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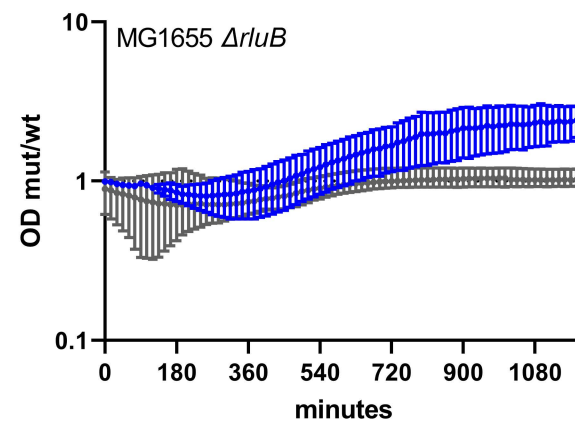
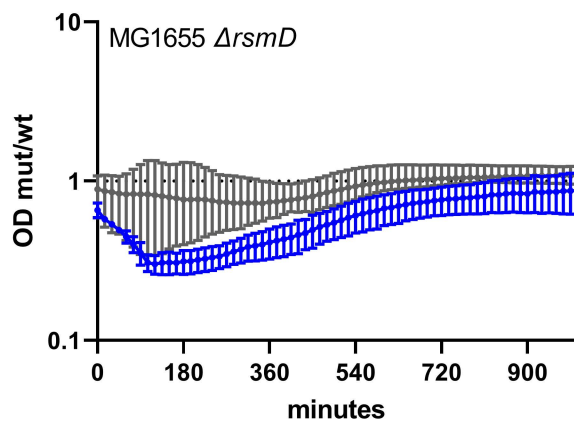
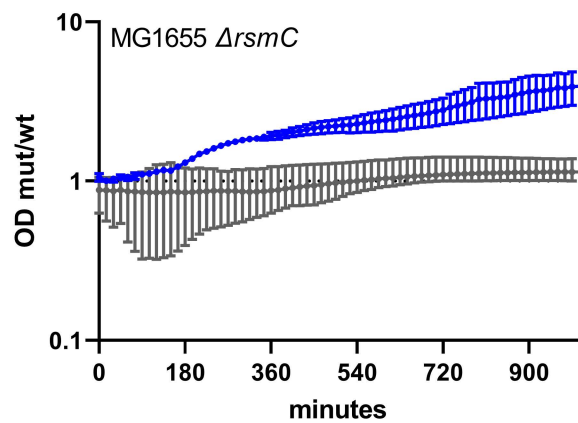
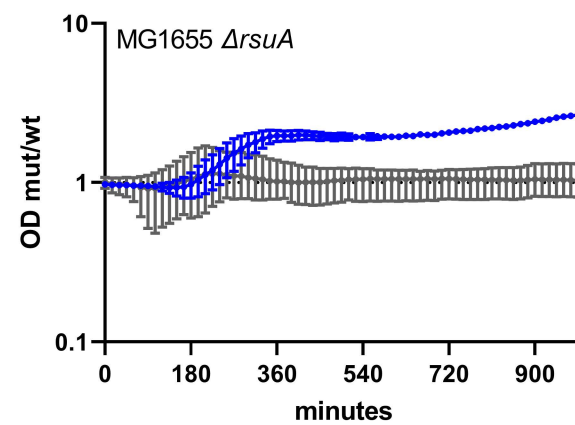
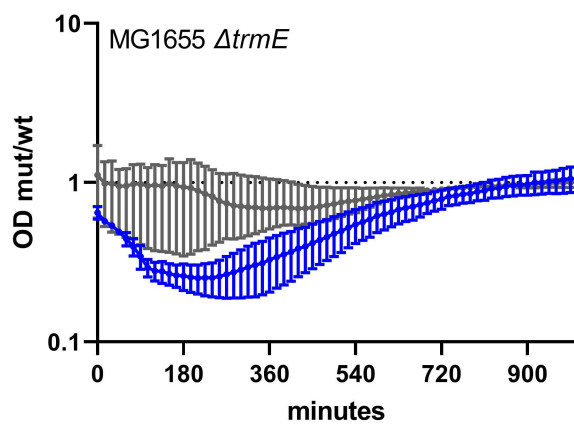
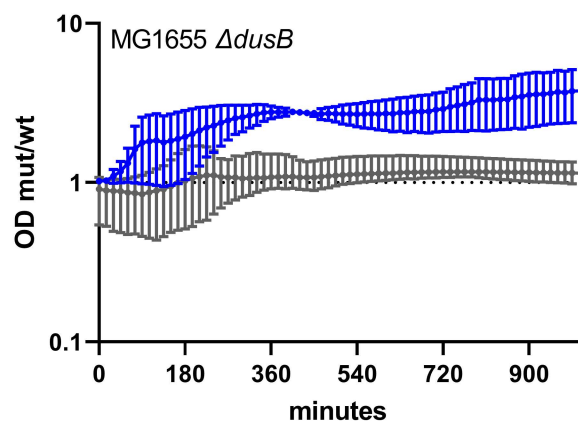
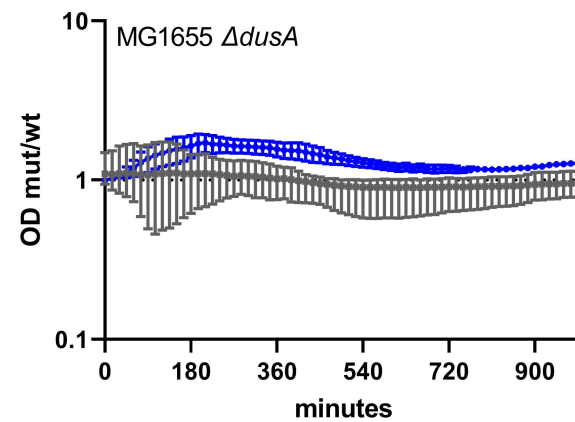
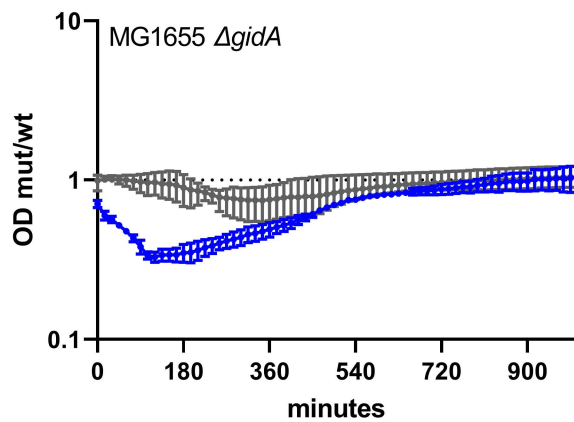
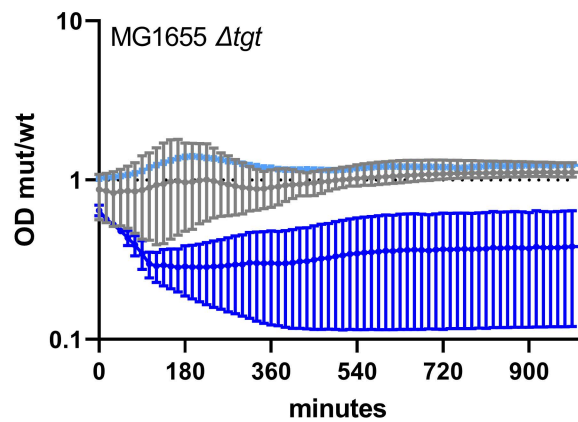


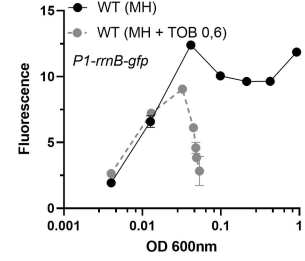
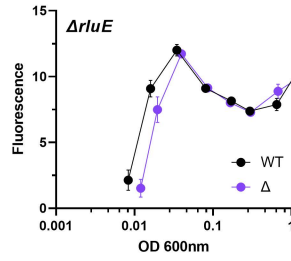
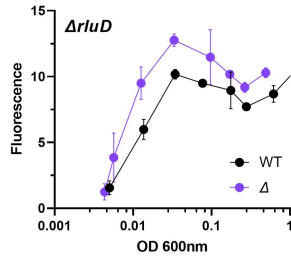
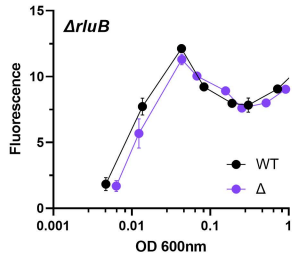
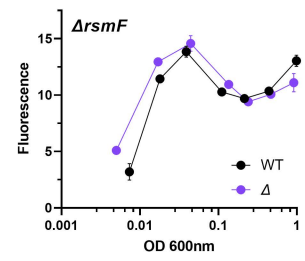
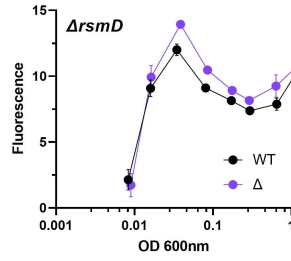
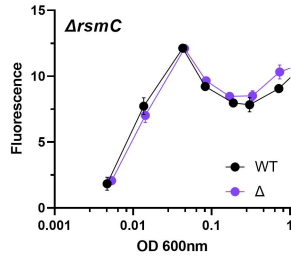
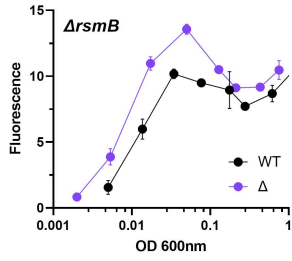
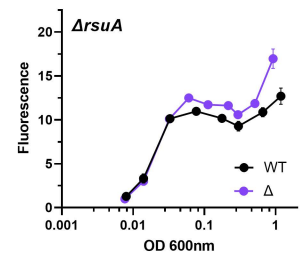
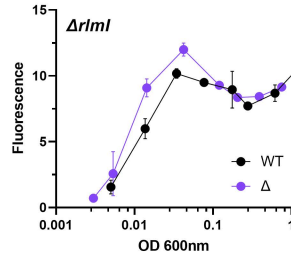
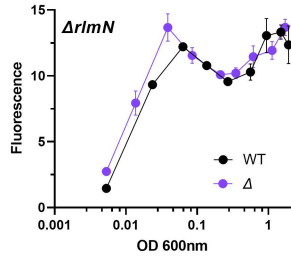
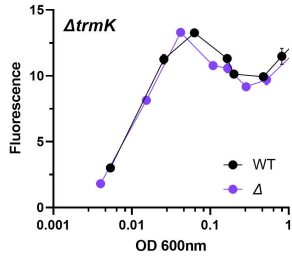
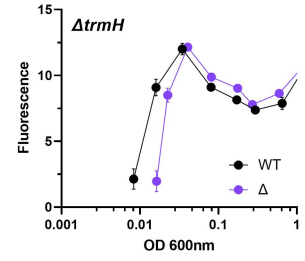
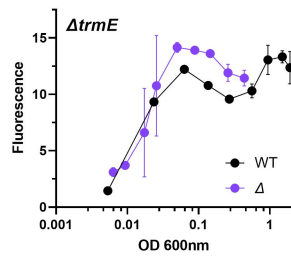
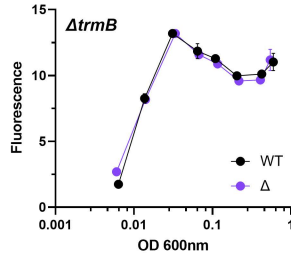
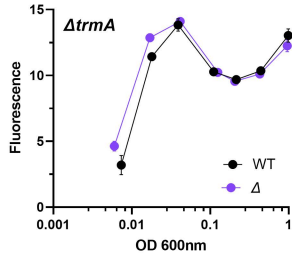
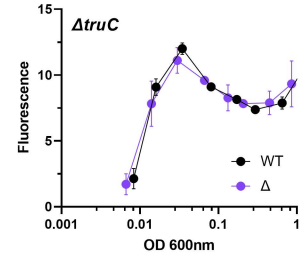
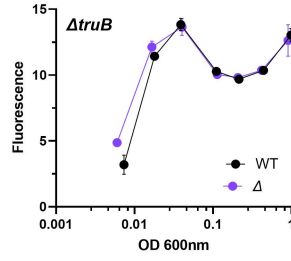
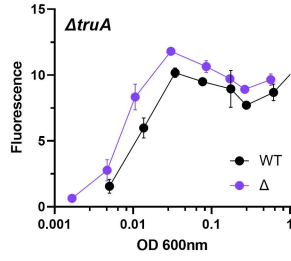
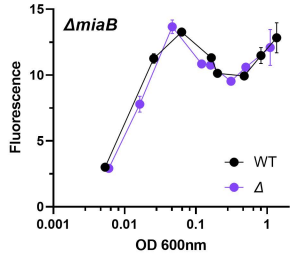
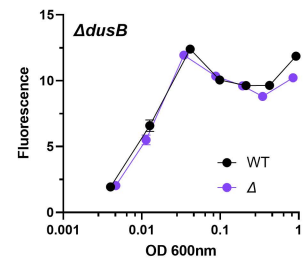
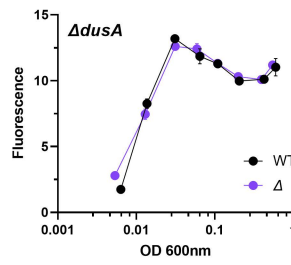
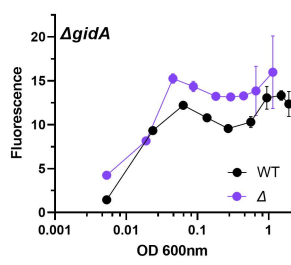
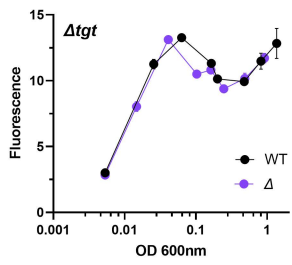
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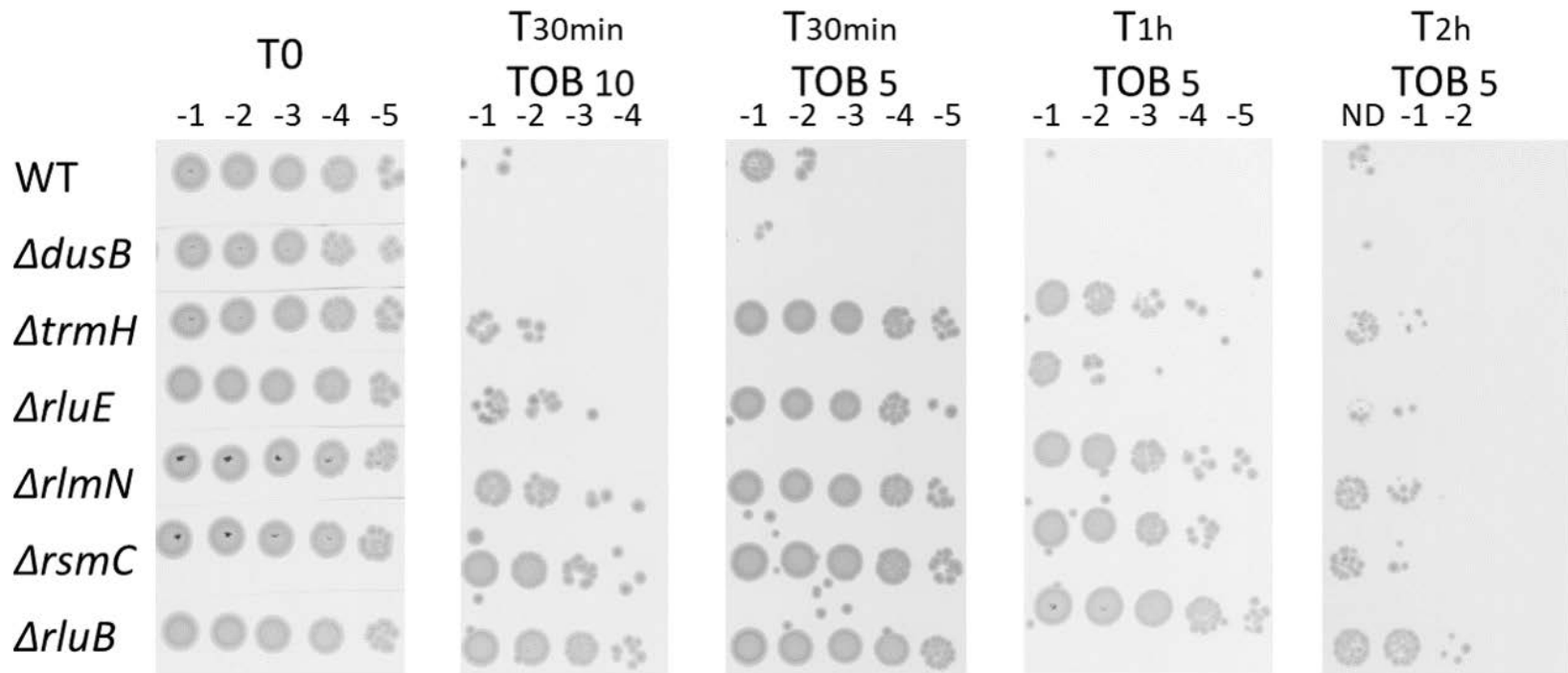


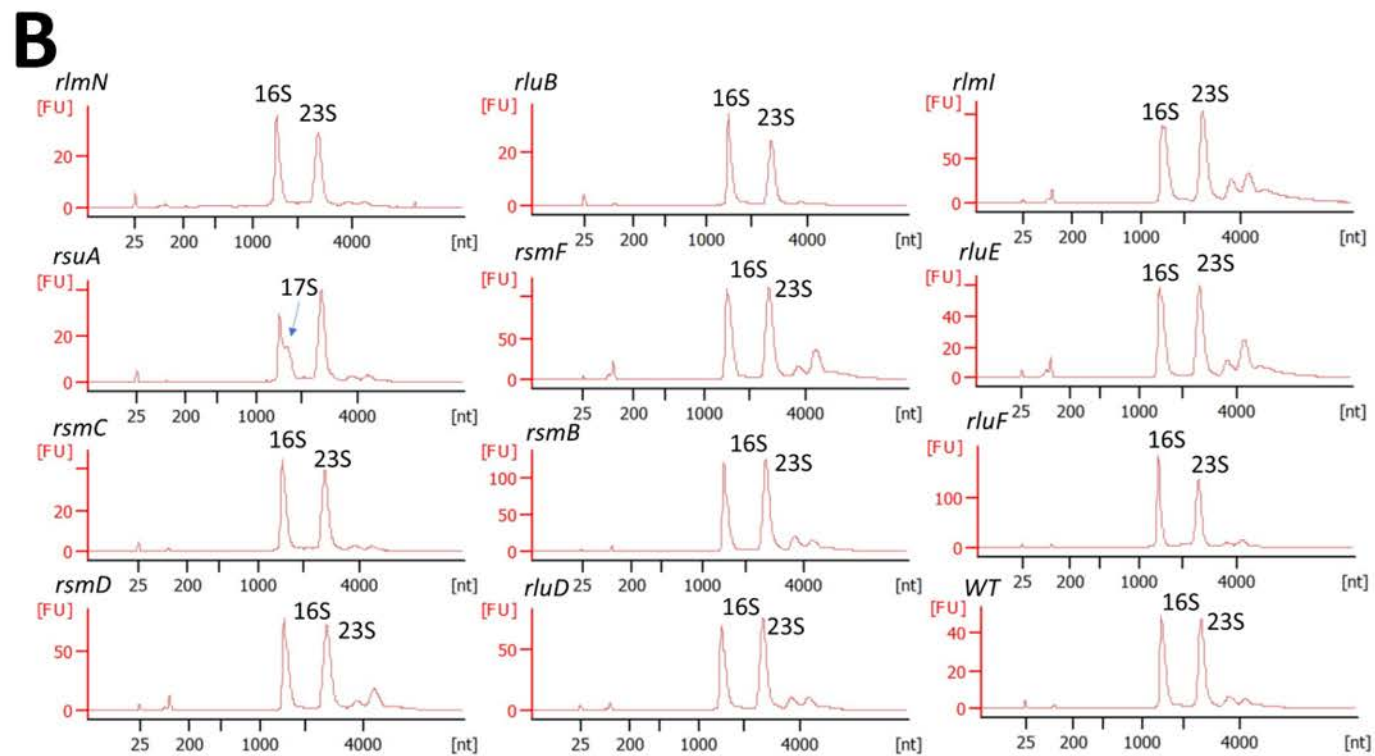
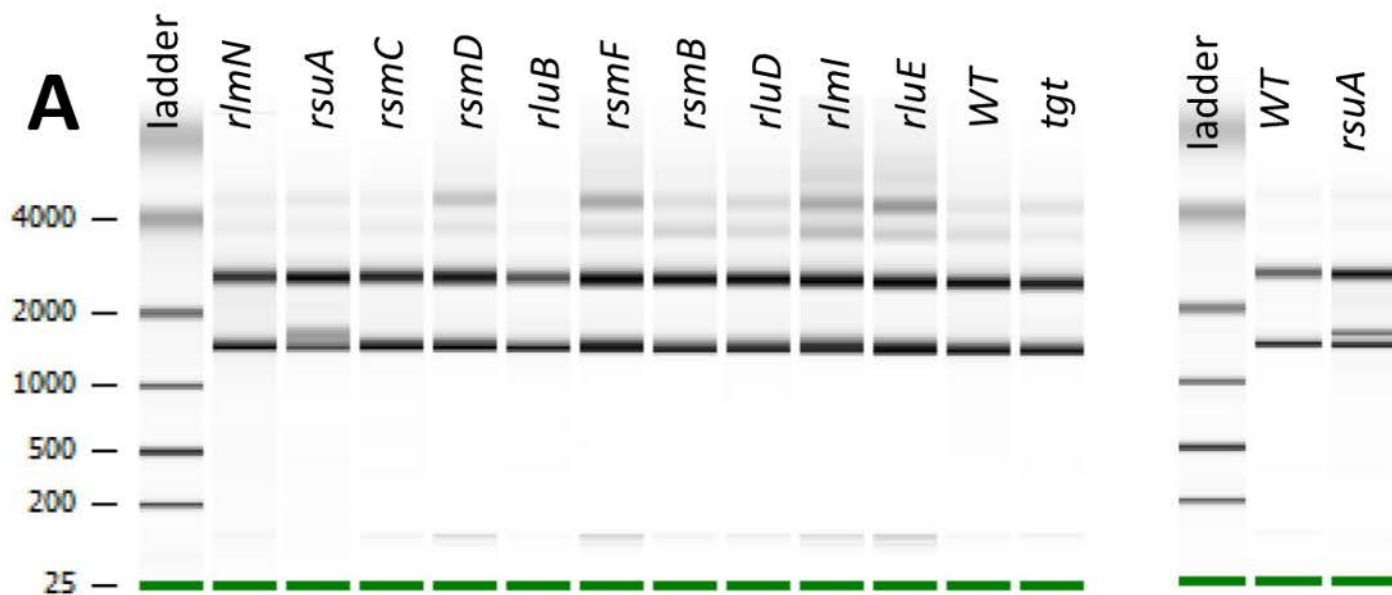
log<sub>10</sub> survival UV irradiation 60J





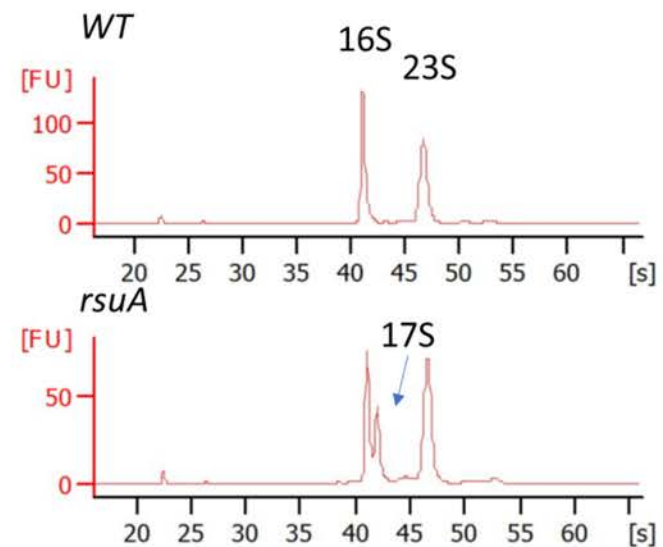






**C**

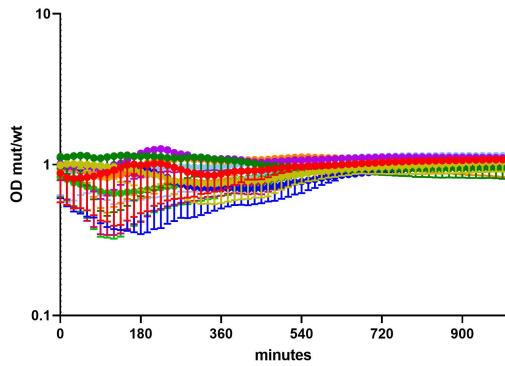
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<i>rlmN</i>	1
<i>rsuA</i>	3.1/1.9
<i>rsmC</i>	1
<i>rsmD</i>	1.1
<i>rluB</i>	0.9
<i>rsmF</i>	1
<i>rsmB</i>	1.1
<i>rluD</i>	1.1
<i>rlmI</i>	1
<i>rluE</i>	0.9
<i>rluF</i>	1





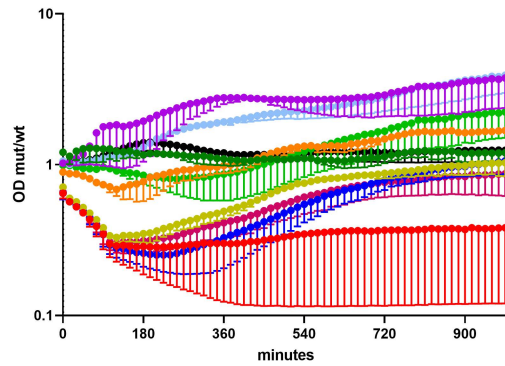


No antibiotic



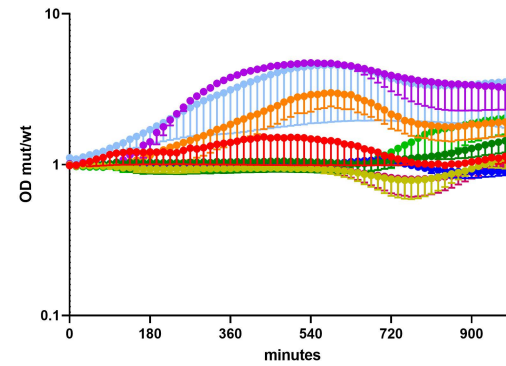
- *Δtgt*
- *ΔgidA*
- *ΔdusA*
- *ΔdusB*
- *ΔtrmE*
- *ΔrsuA*
- *ΔrsmC*
- *ΔrsmD*
- *ΔrluB*

TOB 0.2 μg/ml



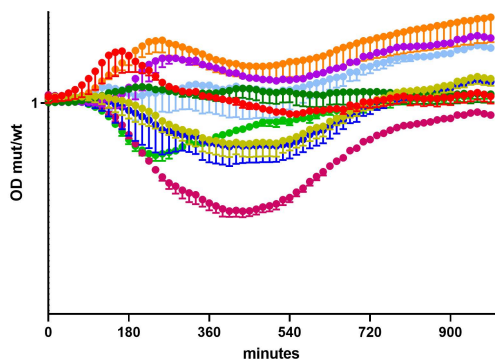
- *Δtgt*
- *ΔgidA*
- *ΔdusA*
- *ΔdusB*
- *ΔtrmE*
- *ΔrsuA*
- *ΔrsmC*
- *ΔrsmD*
- *ΔrluB*
- *tgt bis*

TOB 0.4 μg/ml



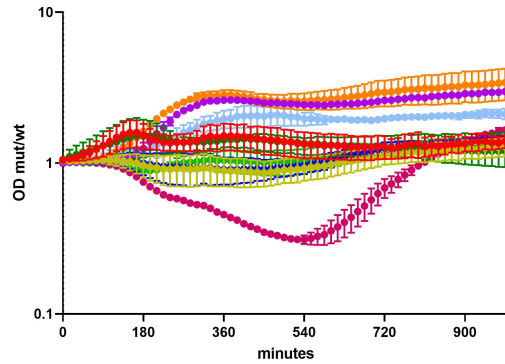
- *Δtgt*
- *ΔgidA*
- *ΔdusA*
- *ΔdusB*
- *ΔtrmE*
- *ΔrsuA*
- *ΔrsmC*
- *ΔrsmD*
- *ΔrluB*

GEN 0.3 μg/ml



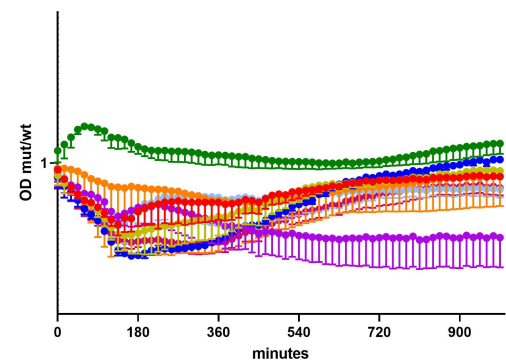
- *Δtgt*
- *ΔgidA*
- *ΔdusA*
- *ΔdusB*
- *ΔtrmE*
- *ΔrsuA*
- *ΔrsmC*
- *ΔrsmD*
- *ΔrluB*

GEN 0.4 μg/ml



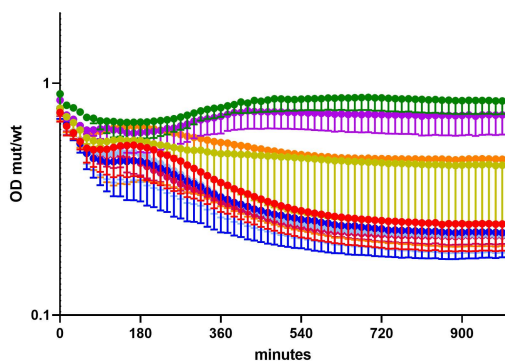
- *Δtgt*
- *ΔgidA*
- *ΔdusA*
- *ΔdusB*
- *ΔtrmE*
- *ΔrsuA*
- *ΔrsmC*
- *ΔrsmD*
- *ΔrluB*

CIP 0.006 μg/ml



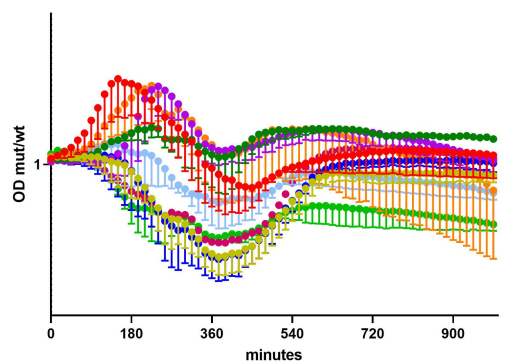
- *Δtgt*
- *ΔgidA*
- *ΔdusA*
- *ΔdusB*
- *ΔtrmE*
- *ΔrsuA*
- *ΔrsmC*
- *ΔrsmD*
- *ΔrluB*

TRM 0.15 μg/ml

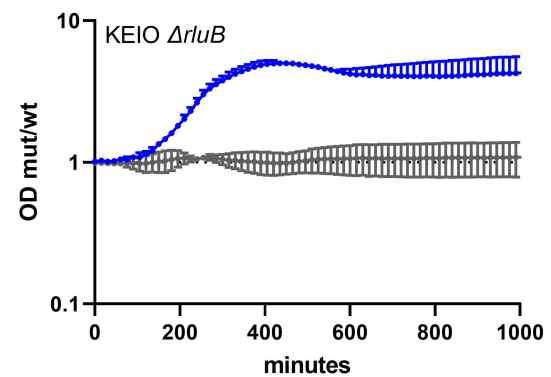
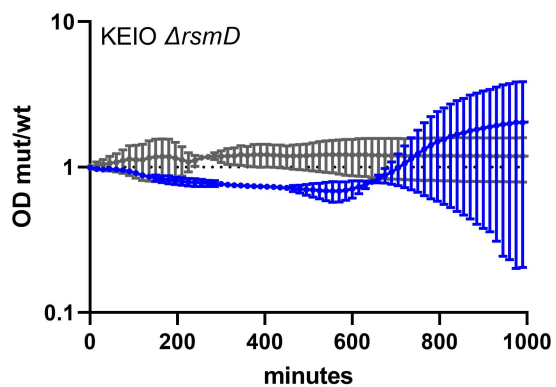
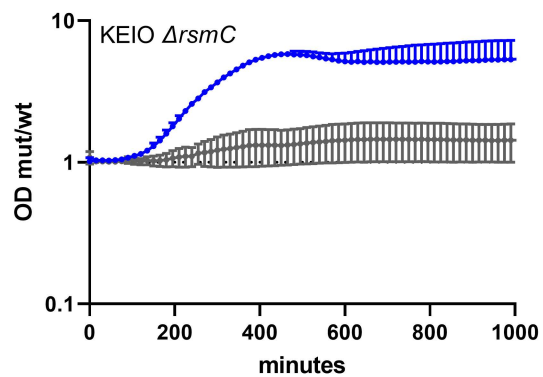
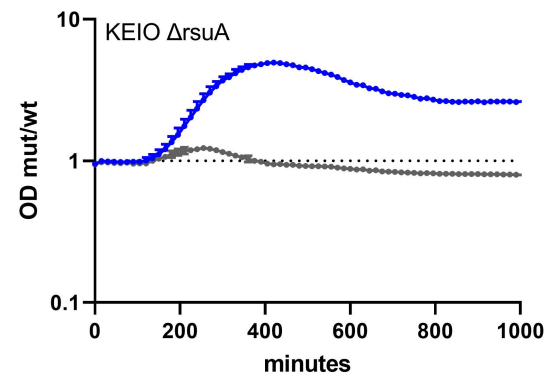
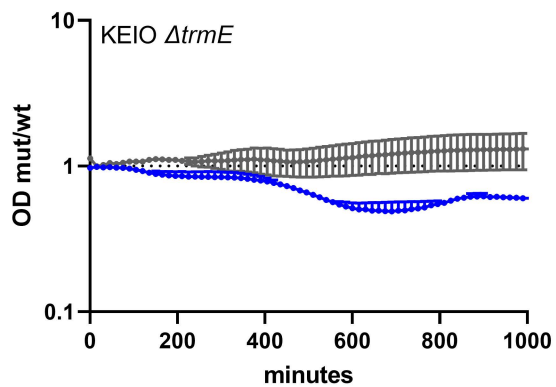
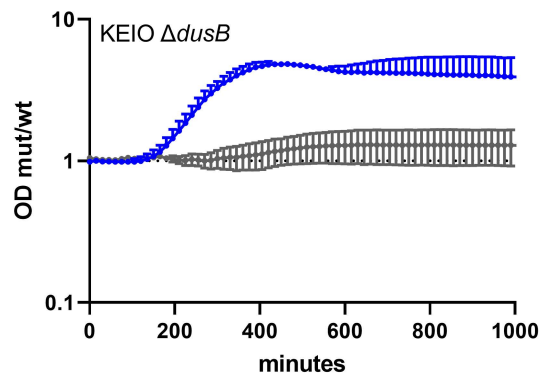
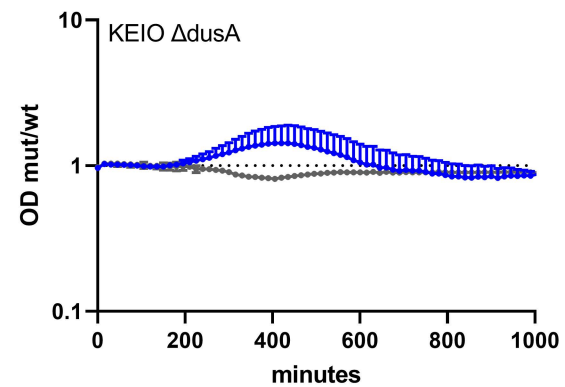
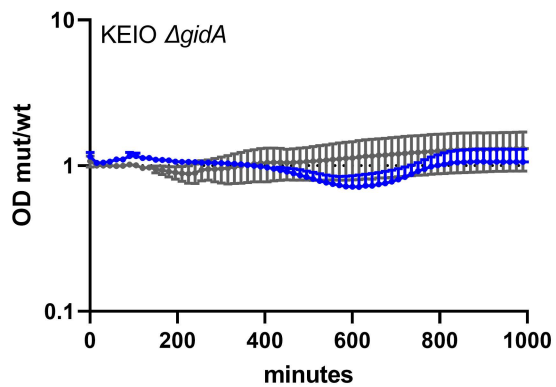
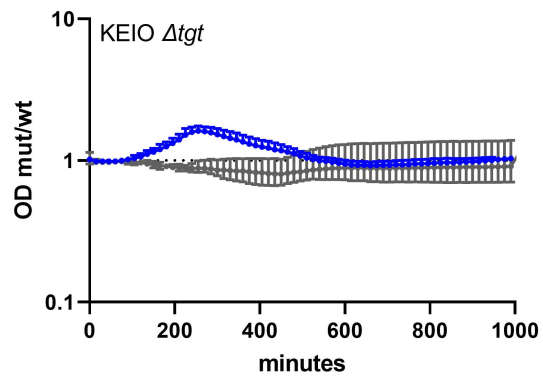


- *Δtgt*
- *ΔgidA*
- *ΔdusA*
- *ΔdusB*
- *ΔtrmE*
- *ΔrsuA*
- *ΔrsmC*
- *ΔrsmD*
- *ΔrluB*

CRB 3 μg/ml



- *Δtgt*
- *ΔgidA*
- *ΔdusA*
- *ΔdusB*
- *ΔtrmE*
- *ΔrsuA*
- *ΔrsmC*
- *ΔrsmD*
- *ΔrluB*



1 **Table 1. Phenotypes associated to RNA modification genes identified by TN-seq.** “N/A” means: not  
 2 selected for further study. NS : non-significant p value.

<i>gene</i>	Function	Known physiological phenotypes In literature	Known effects on translation In literature	TN-seq Fold Change (T16 antibiotic/ T16MH) - This study
tRNA modification				
<b><i>tgt</i></b>	tRNA-guanine transglycosylase GUN anticodon tRNAs	No significant biological difference but defect in stationary phase viability (Noguchi et al., 1982)	UAG readthrough (Frey et al., 1989) reading frame maintenance defect (Urbonavicius et al., 2001)	<b>TOB: -15.8 (p=0.002)</b> <b>CIP: -2 (p=0.04)</b>
<b><i>gidA</i></b> <b><i>mnm</i></b> <b><i>G</i></b>	5- carboxymethyla mino- methyluridine- tRNA synthase. (m <sup>5</sup> U34)	Pleiotropic phenotypes on DNA replication, cell division, tmRNA tagging defect, reviewed in (Nedialkova and Leidel, 2015)	decoding NNG codons (Kurata et al., 2008) reading frame maintenance defect (Urbonavicius et al., 2001)	TOB: +1.9 (NS) <b>CIP: -2.3 (p=0.05)</b>
<b><i>dusA</i></b>	tRNA- dihydrouridine synthase A	N/A (Bou-Nader et al., 2018)		<b>TOB : +3.8 (p=0.0002)</b> CIP : +1.4 (NS)
<b><i>dusB</i></b>	tRNA- dihydrouridine synthase B	N/A (Bou-Nader et al., 2018)		<b>TOB : +12.2 (p=10<sup>-4</sup>)</b> CIP : +1.3 (NS)
<b><i>miaA</i></b> <b>N/A</b>	tRNA dimethylallyltra nsferase (i6A37)	Mutator phenotype (Zhao et al., 2001). Reduced tetracycline resistance (Taylor et al., 1998). Evolvability of resistance to ciprofloxacin (Mehi et al., 2013). Stress specific phenotype: RpoS/IraP translation (Aubee et al., 2016)	reading frame maintenance defect (Urbonavicius et al., 2001)	<b>TOB: -18 (p=10<sup>-4</sup>)</b>
<b><i>miaB</i></b>	isopentenyl- adenosine A37 tRNA methylthiolase (ms2i6A37) U- starting codons	Stress specific phenotype: Fur translation and iron levels (Vecerek et al., 2007)	(Taylor et al., 1998)	<b>TOB : -5.9 (p=0,0007)</b> CIP: +1.1 (NS)
<b><i>truA</i></b> <b><i>hisT</i></b>	tRNA pseudouridine3 8-40 synthase	growth defect in minimal medium (Tsui et al., 1991)	reading frame maintenance defect (Urbonavicius et al., 2001) mistranslation (Bruni et al., 1977; Parker, 1982)	TOB: +2.4 (NS) CIP: -2.1 (NS)
<b><i>truB</i></b>	tRNA pseudouridine5 5 synthase	sensitivity to heatshock (Kingham et al., 2002)		TOB: -1.4 (NS) CIP: -1.9 (NS)
<b><i>truC</i></b>	tRNA pseudouridine6 5 synthase tRNA <sup>Ala1</sup> and tRNA <sup>Asp</sup>	N/A (Del Campo et al., 2001)		TOB: 1 CIP: 1
<b><i>trmA</i></b>	tRNA m <sup>5</sup> U54 methyltransfera se and Y341 of tmRNA	N/A (Bjork and Neidhardt, 1975) Sensitivity to heatshock of <i>truB</i> <i>trmA</i> (Kingham et al., 2002)		<b>TOB: -2.3 (p=0.003)</b> CIP: -1.2 (NS)

<b>trmB</b>	tRNA m <sup>7</sup> G46 methyltransferase	N/A (De Bie et al., 2003)		<b>TOB: +2.7 (p=0.008)</b> CIP: +3.4 (p=0.06 NS)
<b>trmE mnmE</b>	5-carboxymethylaminomethyluridine-tRNA synthase m <sup>5</sup> U modification of U34 in tRNA	Pleiotropic, acts with <i>gidA</i> , see <i>gidA</i> above	reading frame maintenance defect (Urbonavicius et al., 2001) UAG readthrough (Elseviers et al., 1984) Mistranslation (Hagervall et al., 1998)	<b>TOB: +3.9 (p=0.01)</b> CIP: +2.4 (NS)
<b>trmH</b>	tRNA (Gm18) 2'-O-methyltransferase	N/A (Persson et al., 1997)		<b>TOB : +5.4 (p=0,0002)</b> CIP : -1.3 (NS)
<b>trmK</b>	tRNA (m <sup>1</sup> A22)methyltransferase	(Kimura et al., 2020)		TOB : 1 CIP : -1.3 (NS)
rRNA modification (positions described in <i>E. coli</i> )				
<b>rlmB</b>	23S rRNA 2'-O-ribose G2251 methyltransferase	No obvious growth defect (Lovgren and Wikstrom, 2001)		<b>TOB: -2.6 (p=0,001)</b> <b>CIP: +2 (p=0.05)</b>
<b>rlmI</b>	23S rRNA m5C1962 methyltransferase	Decrease in biofilm formation (Herzberg et al., 2006) Slight growth defect (Purta et al., 2008b)		TOB: -1.4 (NS) CIP: +1.3 (NS)
<b>rlmH N/A</b>	23S rRNA m3Ψ1915 methyltransferase	Slight growth defect (Purta et al., 2008a)		<b>TOB: -3 (p=0.003)</b> CIP: -1.3 (NS)
<b>rlmE/ rrmJ N/A</b>	23S rRNA 2'-O-ribose U2552 methyltransferase	Decreased growth rate (Caldas et al., 2000; Pletnev et al., 2020; Toh and Mankin, 2008) Sparsomycin and tiamulin sensitive (Toh and Mankin, 2008) Lincomycin sensitive (Caldas et al., 2000)	Frameshift and stop codon readthrough (Widerak et al., 2005) Accumulation of ribosomal subunit intermediates (Pletnev et al., 2020)	TOB: +1.2 (NS) <b>CIP: +825 (p=0.003)</b>
<b>rsmB</b>	16S rRNA m <sup>5</sup> C967 methyltransferase	No obvious growth defect (Gu et al., 1999; Pletnev et al., 2020)	Accumulation of 17S rRNA (Pletnev et al., 2020) Translation initiation (Arora et al., 2013; Burakovsky et al., 2012)	<b>TOB: -6.7 (p=0.007)</b> CIP: -2.9 NS
<b>rsmC</b>	16S rRNA m <sup>2</sup> G1207 methyltransferase	No obvious growth defect (Pletnev et al., 2020)	Correct folding of 16S rRNA (Gc et al., 2020)	<b>TOB: +2.3 (p=0.05)</b> CIP: -1.2 (NS)
<b>rsmD</b>	16S rRNA m <sup>2</sup> G966 methyltransferase	No obvious growth defect (Lesnyak et al., 2007; Pletnev et al., 2020)	Translation initiation (Arora et al., 2013; Burakovsky et al., 2012)	<b>TOB: -5.7 (p=0.006)</b> CIP: 1.1 (NS)
<b>rsmF /yeb U</b>	16S rRNA m <sup>5</sup> C1407 methyltransferase	No obvious (Pletnev et al., 2020) or slight (Andersen and Douthwaite, 2006) growth defect	Role in translation initiation (Das et al., 2008)	<b>TOB: -2.5 (p=0.002)</b> CIP: -1.8 (NS)

		Increased resistance to some aminoglycosides reported (Gutierrez et al., 2012)		
<i>rsmG</i> <b>N/A</b>	16S rRNA m7G527 methyltransferase	Mutations found in streptomycin resistant MTB clinical isolates (Okamoto et al., 2007)		<b>TOB: -7.7 (p=0.0009)</b>
<i>rsmH</i> <b>N/A</b>	16S rRNA m <sup>4</sup> C1402 methyltransferase	No obvious growth defect (Dassain et al., 1999) <i>ΔrsmH ΔrsmI</i> has a growth defect (Kimura and Suzuki, 2010)	Decoding fidelity (Kimura and Suzuki, 2010)	<b>TOB: -8.4 (p=0.0009)</b>
<i>rsmI</i> <b>N/A</b>	16S rRNA 2'-O-ribose C1402 methyltransferase	No obvious growth defect <i>ΔrsmH ΔrsmI</i> has a growth defect (Kimura and Suzuki, 2010)	Decoding fidelity (Kimura and Suzuki, 2010)	<b>TOB: -8.4 (p=10<sup>-4</sup>)</b>
<i>rsuA</i>	16S rRNA pseudouridine516 synthase	No obvious growth defect (Conrad et al., 1999) Overexpression leads to resistance to HOCl (Chen et al., 2021)	Accumulation of 17S rRNA (the present study)	<b>TOB: -2.3 (p=0.01)</b> CIP: 1
<i>rluB</i>	23S rRNA pseudouridine2605 synthase	No obvious growth defect (Del Campo et al., 2001; Toh and Mankin, 2008) Increased CM and linezolid sensitivity (Toh and Mankin, 2008)	50S subunit maturation (Jiang et al., 2007)	<b>TOB: +9.4 (p=10<sup>-4</sup>)</b> CIP: +1.2 (NS)
<i>rluC</i> <b>N/A</b>	23S rRNA pseudouridine955/2504/2580 synthase	No obvious growth defect (Conrad et al., 1998) Cold sensitivity (Jiang et al., 2007) clindamycin, linezolid, and tiamulin sensitivity (Toh and Mankin, 2008)		<b>TOB: +1.8 (p=10<sup>-4</sup>)</b> CIP: +1.3 (NS)
<i>rluD</i>	23S rRNA pseudouridine1911/1915/1917 synthase	Reported to cause a large growth defect in <i>E. coli</i> but independently of pseudouridines (Gutgsell et al., 2001)	ribosome assembly (Gutgsell et al., 2005)	<b>TOB: +1.4 (p=0.03)</b> <b>CIP: +6.6 (p=0.002)</b>
<i>rluE</i>	23S rRNA pseudouridine2457 synthase	No obvious growth defect (Del Campo et al., 2001)		<b>TOB: -1.5 (p=0.002)</b> CIP: -2.4 (NS)
Modification of both tRNA and rRNA				
<i>rluF</i>	23S rRNA pseudouridine2604 / tRNA <sup>Tyr</sup> pseudouridine35 synthase	No obvious growth defect (Del Campo et al., 2001; Pletnev et al., 2020; Toh and Mankin, 2008) Decreased linezolid resistance (Toh and Mankin, 2008)	Effect on translation of Tyr codons (Addepalli and Limbach, 2016)	TOB: -1.2 (NS) CIP: -1.2 (NS)
<i>rlmN</i>	tRNA m2A37 methyltransferase / 23S rRNA m2A2503 methyltransferase	No obvious growth defect (Benitez-Paez et al., 2012) Slightly increased susceptibility to certain peptidyl transferase-targeting antibiotics (Toh and Mankin, 2008)	UAG readthrough (Benitez-Paez et al., 2012)	<b>TOB: +2.3 (p=0.005)</b> <b>CIP: -2.2 (p=0.0003)</b>

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126



1 **Table 2. Summary of the competitive index of the *V. cholerae* deletion mutants compared to WT**  
 2 **during growth in sub-MIC antibiotics**

<i>gene</i>	TN-seq TOB	TOB fitness	GEN fitness	Ec TOB	TN-seq CIP	CIP fitness	CM fitness	TRM fitness	CRB fitness	TCL fitness
<i>tgt</i>	-	-	-	+/-	-				+	-
<i>gidA/mnmG</i>	(+)	+	+	-	-			+	-	-
<i>dusA</i>	+			+						
<i>dusB</i>	+	+	+	+		-			+	-
<i>miaB</i>	-	-	-	na		-				-
<i>truA</i>	(+)	-	-	na	(-)	-	-			-
<i>truB</i>	(-)	-	-	na	(-)			+		
<i>truC</i>				na		-				
<i>trmA</i>	-			na						-
<i>trmB</i>	+			na	(+)			+		
<i>trmE/mnmE</i>	+			-	(+)	+			+	-
<i>trmH</i>	+	+	+	na					-	
<i>trmK</i>				na						
<i>rlmI</i>	(-)	-	-	na		-				-
<i>rsmB</i>	-	-	-	na	(-)	-				-
<i>rsmC</i>	+	+	+	+						
<i>rsmD</i>	-			-					+	+
<i>rsmF</i>	-	-	-	na	(-)			+	+	-
<i>rsuA</i>	-	-	-	+		+				
<i>rluB</i>	+	+	+	+	(+)			-		-
<i>rluD</i>	+	-	-	na	+	-	-			-
<i>rluE</i>	-	+	+	na	(-)	-	-	-	-	
<i>rlmN</i>	+	+	+	na	-			+	+	

3 “-” means that deletion of the gene decreases fitness (>2-fold change). “+” means deletion of the gene  
 4 increases fitness (>2-fold change). Parentheses indicate that the p value was not significant. An empty  
 5 cell means that no significant effect of deletion was observed. “Ec TOB”: column summarizing results  
 6 from *E. coli* growth curves in sub-MIC TOB (figure 4), “na” means that the corresponding mutant was  
 7 not tested in *E. coli*.

1 Table 3. Minimal inhibitory concentrations (MICs) determined using Etests.

<i>V. cholerae</i>	TOB	CIP	AMP	TRM
<i>WT</i>	0.75 - 1.2	0.0020 - 0.0030	4 +/-1	0.4 +/- 0.1
<i>tgt</i>	0.75 - 1	0.0020	4	0.58
<i>dusA</i>	1 - 1	0.0020 - 0.0030	4	0.5
<i>dusB</i>	1 - 1	0.0020 - 0.0030	4	0.48
<i>gidA</i>	<b>2 – 2*</b>	<b>0.0039*</b>	4	0.37
<i>trmA</i>	0.9 - 1	0.0020	3.9	0.37
<i>trmB</i>	1 - 1.2	0.0020	4.3	0.48
<i>miaB</i>	0.9 - 1	0.0020	4	0.38
<i>rsmF</i>	1	0.0020	4.5	0.4
<i>rlmI</i>	1	0.0020	4.2	0.4
<i>truA</i>	0.8	0.0020	3.5	0.39
<i>rluD</i>	0.75	<b>0.0036*</b>	4	0.5
<i>trmE</i>	0.75 - 1.2	<b>0.0036*</b>	5	<b>0.8*</b>
<i>trmK</i>	1 - 1.2	0.0020	4	0.38
<i>rsmB</i>	1	0.0020	4.1	0.48
<i>truB</i>	1 - 1.2	0.0020	4	0.47
<i>rluF</i>	1 - 1.2	0.0020	4	0.43
<i>truC</i>	0.75	0.0035	5	<b>0.8*</b>
<i>trmH</i>	1	0.0025	4.3	0.5
<i>rlmN</i>	1	0.0025	<b>5.5*</b>	0.38
<i>rlmI</i>	<b>0.7**</b>	0.0028	4.5	0.44
<i>rsuA</i>	0.75	0.0030	3.5	0.4
<i>rsmC</i>	1.5	0.0028	4.8	0.38
<i>rsmD</i>	<b>0.7**</b>	0.0028	3.5	0.38
<i>rsmF</i>	0.8	0.0032	3.8	0.38
<i>rluB</i>	<b>1.5*</b>	0.0030	4	0.5
<i>rluE</i>	0.75	0.0028	5	0.5

2 Green: increase MIC. Blue: decrease. **TOB**: tobramycin. **CIP**: ciprofloxacin. **AMP**: ampicillin (used for  
3 CRB). **TRM**: trimethoprim. \* shows increase. \*\* shows decrease.

4

5

1 **Table S2. Gene ontology enrichment analysis for gene with at least 2-fold change in TN-seq data at**  
 2 **16 generations compared to non-treated condition, and with an adjusted (Bonferroni correction) p-**  
 3 **value <0,05.**

GO biological process complete TOB 16 gen TOB vs 16 gen MH	Vibrio cholerae REFLIST (3782)	upload_1 (447)	expected	fold overrepresentation	P-value
regulation of proteolysis (GO:0030162)	2	2	.24	8,46	2.39E-02
regulation of secondary metabolite biosynthetic process (GO:1900376)	2	2	.24	8,46	2.39E-02
phenylalanyl-tRNA aminoacylation (GO:0006432)	2	2	.24	8,46	2.39E-02
O antigen metabolic process (GO:0046402)	2	2	.24	8,46	2.39E-02
enzyme-directed rRNA 2'-O-methylation (GO:0000453)	2	2	.24	8,46	2.39E-02
rRNA 2'-O-methylation (GO:0000451)	2	2	.24	8,46	2.39E-02
regulation of secondary metabolic process (GO:0043455)	2	2	.24	8,46	2.39E-02
guanosine tetraphosphate biosynthetic process (GO:0015970)	2	2	.24	8,46	2.39E-02
nicotinamide nucleotide biosynthetic process from aspartate (GO:0019355)	2	2	.24	8,46	2.39E-02
purine ribonucleoside bisphosphate biosynthetic process (GO:0034036)	2	2	.24	8,46	2.39E-02
O antigen biosynthetic process (GO:0009243)	2	2	.24	8,46	2.39E-02
'de novo' NAD biosynthetic process from aspartate (GO:0034628)	2	2	.24	8,46	2.39E-02
'de novo' NAD biosynthetic process (GO:0034627)	2	2	.24	8,46	2.39E-02
protein transport by the Tat complex (GO:0043953)	4	4	.47	8,46	1.42E-03
prosthetic group biosynthetic process (GO:0051191)	2	2	.24	8,46	2.39E-02
biotin biosynthetic process (GO:0009102)	7	6	.83	7,25	2.15E-04
biotin metabolic process (GO:0006768)	7	6	.83	7,25	2.15E-04
cytochrome complex assembly (GO:0017004)	10	7	1.18	5,92	2.22E-04
glutamyl-tRNA aminoacylation (GO:0006424)	3	2	.35	5,64	4.97E-02
tRNA dihydrouridine synthesis (GO:0002943)	3	2	.35	5,64	4.97E-02
RNA (guanine-N7)-methylation (GO:0036265)	3	2	.35	5,64	4.97E-02

ribonucleotide catabolic process (GO:0009261)	3	2	.35	5,64	4.97E-02
vitamin transmembrane transport (GO:0035461)	3	2	.35	5,64	4.97E-02
protein unfolding (GO:0043335)	3	2	.35	5,64	4.97E-02
demethylation (GO:0070988)	3	2	.35	5,64	4.97E-02
regulation of cellular catabolic process (GO:0031329)	3	2	.35	5,64	4.97E-02
thiamine transport (GO:0015888)	3	2	.35	5,64	4.97E-02
bacterial-type flagellum assembly (GO:0044780)	11	7	1.30	5,38	3.91E-04
vitamin transport (GO:0051180)	8	5	.95	5,29	2.85E-03
GDP-mannose metabolic process (GO:0019673)	5	3	.59	5,08	2.21E-02
cobalamin transport (GO:0015889)	5	3	.59	5,08	2.21E-02
arginine catabolic process (GO:0006527)	5	3	.59	5,08	2.21E-02
transcription antitermination (GO:0031564)	7	4	.83	4,83	1.01E-02
regulation of protein-containing complex disassembly (GO:0043244)	9	5	1.06	4,7	4.67E-03
regulation of cellular component organization (GO:0051128)	12	6	1.42	4,23	3.34E-03
NAD biosynthetic process (GO:0009435)	6	3	.71	4,23	3.51E-02
thiamine-containing compound biosynthetic process (GO:0042724)	8	4	.95	4,23	1.57E-02
queuosine biosynthetic process (GO:0008616)	8	4	.95	4,23	1.57E-02
regulation of DNA-templated transcription, termination (GO:0031554)	8	4	.95	4,23	1.57E-02
queuosine metabolic process (GO:0046116)	8	4	.95	4,23	1.57E-02
thiamine diphosphate metabolic process (GO:0042357)	6	3	.71	4,23	3.51E-02
thiamine diphosphate biosynthetic process (GO:0009229)	6	3	.71	4,23	3.51E-02
thiamine biosynthetic process (GO:0009228)	8	4	.95	4,23	1.57E-02
primary alcohol biosynthetic process (GO:0034309)	10	5	1.18	4,23	7.19E-03
intracellular protein transmembrane transport (GO:0065002)	8	4	.95	4,23	1.57E-02
bacterial-type flagellum organization (GO:0044781)	17	8	2.01	3,98	1.09E-03
cytokinetic process (GO:0032506)	13	6	1.54	3,91	4.90E-03
thiamine-containing compound metabolic process (GO:0042723)	9	4	1.06	3,76	2.30E-02
thiamine metabolic process (GO:0006772)	9	4	1.06	3,76	2.30E-02

nicotinamide nucleotide metabolic process (GO:0046496)	9	4	1.06	3,76	2.30E-02
pyridine nucleotide metabolic process (GO:0019362)	9	4	1.06	3,76	2.30E-02
alcohol biosynthetic process (GO:0046165)	14	6	1.65	3,63	6.94E-03
sodium ion transport (GO:0006814)	28	12	3.31	3,63	1.62E-04
cell septum assembly (GO:0090529)	12	5	1.42	3,53	1.48E-02
primary alcohol metabolic process (GO:0034308)	12	5	1.42	3,53	1.48E-02
cell projection assembly (GO:0030031)	17	7	2.01	3,48	4.54E-03
cell cycle process (GO:0022402)	20	8	2.36	3,38	2.96E-03
cytokinesis (GO:0000910)	20	8	2.36	3,38	2.96E-03
RNA catabolic process (GO:0006401)	13	5	1.54	3,25	2.01E-02
rRNA methylation (GO:0031167)	21	8	2.48	3,22	3.95E-03
protein-containing complex assembly (GO:0065003)	30	11	3.55	3,1	1.07E-03
rRNA base methylation (GO:0070475)	11	4	1.30	3,08	4.29E-02
protein peptidyl-prolyl isomerization (GO:0000413)	11	4	1.30	3,08	4.29E-02
sodium ion transmembrane transport (GO:0035725)	11	4	1.30	3,08	4.29E-02
RNA methylation (GO:0001510)	36	13	4.25	3,06	4.48E-04
protein-containing complex organization (GO:0043933)	36	13	4.25	3,06	4.48E-04
reproduction of a single-celled organism (GO:0032505)	14	5	1.65	3,02	2.66E-02
reproductive process (GO:0022414)	14	5	1.65	3,02	2.66E-02
FtsZ-dependent cytokinesis (GO:0043093)	14	5	1.65	3,02	2.66E-02
purine nucleoside bisphosphate biosynthetic process (GO:0034033)	14	5	1.65	3,02	2.66E-02
ribonucleoside bisphosphate biosynthetic process (GO:0034030)	14	5	1.65	3,02	2.66E-02
reproduction (GO:0000003)	14	5	1.65	3,02	2.66E-02
asexual reproduction (GO:0019954)	14	5	1.65	3,02	2.66E-02
nucleoside bisphosphate biosynthetic process (GO:0033866)	14	5	1.65	3,02	2.66E-02
cell projection organization (GO:0030030)	23	8	2.72	2,94	6.70E-03
non-membrane-bounded organelle assembly (GO:0140694)	26	9	3.07	2,93	4.28E-03
organelle assembly (GO:0070925)	26	9	3.07	2,93	4.28E-03
cellular component assembly (GO:0022607)	81	28	9.57	2,92	6.81E-07
rRNA modification (GO:0000154)	29	10	3.43	2,92	2.74E-03
macromolecule methylation (GO:0043414)	47	16	5.55	2,88	1.98E-04

pyridine-containing compound metabolic process (GO:0072524)	15	5	1.77	2,82	3.42E-02
cellular macromolecule localization (GO:0070727)	25	8	2.95	2,71	1.07E-02
cellular protein localization (GO:0034613)	25	8	2.95	2,71	1.07E-02
negative regulation of nucleic acid-templated transcription (GO:1903507)	22	7	2.60	2,69	1.69E-02
negative regulation of transcription, DNA-templated (GO:0045892)	22	7	2.60	2,69	1.69E-02
negative regulation of RNA biosynthetic process (GO:1902679)	22	7	2.60	2,69	1.69E-02
negative regulation of macromolecule biosynthetic process (GO:0010558)	29	9	3.43	2,63	8.44E-03
rRNA processing (GO:0006364)	42	13	4.96	2,62	1.77E-03
rRNA metabolic process (GO:0016072)	42	13	4.96	2,62	1.77E-03
cellular macromolecule catabolic process (GO:0044265)	26	8	3.07	2,6	1.32E-02
methylation (GO:0032259)	65	20	7.68	2,6	1.28E-04
monocarboxylic acid biosynthetic process (GO:0072330)	39	12	4.61	2,6	2.75E-03
cell cycle (GO:0007049)	46	14	5.44	2,58	1.40E-03
regulation of cellular amide metabolic process (GO:0034248)	20	6	2.36	2,54	3.31E-02
post-transcriptional regulation of gene expression (GO:0010608)	20	6	2.36	2,54	3.31E-02
negative regulation of biosynthetic process (GO:0009890)	30	9	3.55	2,54	1.04E-02
negative regulation of cellular biosynthetic process (GO:0031327)	30	9	3.55	2,54	1.04E-02
negative regulation of macromolecule metabolic process (GO:0010605)	37	11	4.37	2,52	5.20E-03
bacterial-type flagellum-dependent cell motility (GO:0071973)	24	7	2.84	2,47	2.55E-02
organic hydroxy compound biosynthetic process (GO:1901617)	24	7	2.84	2,47	2.55E-02
negative regulation of RNA metabolic process (GO:0051253)	24	7	2.84	2,47	2.55E-02
ribosome biogenesis (GO:0042254)	59	17	6.97	2,44	8.25E-04
RNA modification (GO:0009451)	80	23	9.46	2,43	1.11E-04
cellular component biogenesis (GO:0044085)	161	46	19.03	2,42	4.99E-08
ribonucleoprotein complex biogenesis (GO:0022613)	60	17	7.09	2,4	9.87E-04
negative regulation of metabolic process (GO:0009892)	39	11	4.61	2,39	7.56E-03

negative regulation of nitrogen compound metabolic process (GO:0051172)	32	9	3.78	2,38	1.51E-02
cilium or flagellum-dependent cell motility (GO:0001539)	25	7	2.95	2,37	3.08E-02
negative regulation of nucleobase-containing compound metabolic process (GO:0045934)	25	7	2.95	2,37	3.08E-02
archaeal or bacterial-type flagellum-dependent cell motility (GO:0097588)	25	7	2.95	2,37	3.08E-02
water-soluble vitamin biosynthetic process (GO:0042364)	44	12	5.20	2,31	6.97E-03
cellular component organization or biogenesis (GO:0071840)	218	59	25.77	2,29	3.81E-09
sulfur compound biosynthetic process (GO:0044272)	52	14	6.15	2,28	4.17E-03
regulation of protein metabolic process (GO:0051246)	26	7	3.07	2,28	3.68E-02
vitamin biosynthetic process (GO:0009110)	45	12	5.32	2,26	8.23E-03
cell division (GO:0051301)	60	16	7.09	2,26	2.50E-03
negative regulation of cellular metabolic process (GO:0031324)	34	9	4.02	2,24	2.14E-02
cellular component organization (GO:0016043)	163	43	19.27	2,23	1.13E-06
establishment of protein localization (GO:0045184)	77	20	9.10	2,2	1.05E-03
alcohol metabolic process (GO:0006066)	27	7	3.19	2,19	4.35E-02
protein localization (GO:0008104)	78	20	9.22	2,17	1.23E-03
RNA processing (GO:0006396)	103	26	12.17	2,14	3.06E-04
organelle organization (GO:0006996)	68	17	8.04	2,12	3.56E-03
ncRNA processing (GO:0034470)	99	24	11.70	2,05	8.95E-04
protein transport (GO:0015031)	71	17	8.39	2,03	5.41E-03
phospholipid biosynthetic process (GO:0008654)	38	9	4.49	2	3.90E-02
phospholipid metabolic process (GO:0006644)	38	9	4.49	2	3.90E-02
water-soluble vitamin metabolic process (GO:0006767)	51	12	6.03	1,99	2.00E-02
ncRNA metabolic process (GO:0034660)	124	29	14.66	1,98	4.79E-04
vitamin metabolic process (GO:0006766)	52	12	6.15	1,95	2.27E-02
nucleobase-containing compound catabolic process (GO:0034655)	39	9	4.61	1,95	4.47E-02
tRNA modification (GO:0006400)	48	11	5.67	1,94	2.97E-02
tRNA processing (GO:0008033)	63	14	7.45	1,88	1.95E-02

metal ion transport (GO:0030001)	78	17	9.22	1,84	1.28E-02
tRNA metabolic process (GO:0006399)	88	19	10.40	1,83	9.68E-03
RNA metabolic process (GO:0016070)	186	39	21.98	1,77	4.66E-04
macromolecule modification (GO:0043412)	202	42	23.87	1,76	3.35E-04
macromolecule localization (GO:0033036)	102	21	12.06	1,74	1.11E-02
lipid biosynthetic process (GO:0008610)	78	16	9.22	1,74	2.53E-02
regulation of gene expression (GO:0010468)	247	46	29.19	1,58	1.72E-03
regulation of macromolecule metabolic process (GO:0060255)	264	49	31.20	1,57	1.30E-03
monocarboxylic acid metabolic process (GO:0032787)	109	20	12.88	1,55	3.73E-02
regulation of metabolic process (GO:0019222)	269	49	31.79	1,54	1.91E-03
gene expression (GO:0010467)	264	48	31.20	1,54	2.20E-03
nitrogen compound transport (GO:0071705)	176	32	20.80	1,54	1.16E-02
organophosphate biosynthetic process (GO:0090407)	142	25	16.78	1,49	3.31E-02
regulation of macromolecule biosynthetic process (GO:0010556)	239	42	28.25	1,49	7.25E-03
regulation of cellular biosynthetic process (GO:0031326)	239	42	28.25	1,49	7.25E-03
regulation of biosynthetic process (GO:0009889)	241	42	28.48	1,47	8.30E-03
regulation of nitrogen compound metabolic process (GO:0051171)	255	44	30.14	1,46	8.22E-03
organic cyclic compound biosynthetic process (GO:1901362)	267	46	31.56	1,46	7.13E-03
regulation of primary metabolic process (GO:0080090)	256	44	30.26	1,45	8.76E-03
regulation of RNA biosynthetic process (GO:2001141)	223	38	26.36	1,44	1.62E-02
heterocycle biosynthetic process (GO:0018130)	253	43	29.90	1,44	1.14E-02
regulation of cellular metabolic process (GO:0031323)	254	43	30.02	1,43	1.21E-02
regulation of RNA metabolic process (GO:0051252)	226	38	26.71	1,42	1.95E-02
regulation of transcription, DNA-templated (GO:0006355)	221	37	26.12	1,42	2.23E-02
regulation of nucleic acid-templated transcription (GO:1903506)	221	37	26.12	1,42	2.23E-02
small molecule biosynthetic process (GO:0044283)	233	39	27.54	1,42	1.93E-02



regulation of nucleobase-containing compound metabolic process (GO:0019219)	231	38	27.30	1,39	2.62E-02
cellular nitrogen compound biosynthetic process (GO:0044271)	355	58	41.96	1,38	7.76E-03
heterocycle metabolic process (GO:0046483)	566	92	66.90	1,38	8.61E-04
organic cyclic compound metabolic process (GO:1901360)	586	95	69.26	1,37	7.57E-04
aromatic compound biosynthetic process (GO:0019438)	235	38	27.77	1,37	3.28E-02
nucleic acid metabolic process (GO:0090304)	316	50	37.35	1,34	2.23E-02
cellular aromatic compound metabolic process (GO:0006725)	557	87	65.83	1,32	3.81E-03
nucleobase-containing compound metabolic process (GO:0006139)	470	73	55.55	1,31	9.30E-03
cellular nitrogen compound metabolic process (GO:0034641)	662	102	78.24	1,3	2.49E-03
organic substance biosynthetic process (GO:1901576)	610	93	72.10	1,29	5.41E-03
cellular macromolecule metabolic process (GO:0044260)	486	74	57.44	1,29	1.37E-02
regulation of biological process (GO:0050789)	469	71	55.43	1,28	1.77E-02
regulation of cellular process (GO:0050794)	418	63	49.40	1,28	2.71E-02
biosynthetic process (GO:0009058)	624	94	73.75	1,27	7.15E-03
cellular biosynthetic process (GO:0044249)	599	89	70.80	1,26	1.26E-02
organonitrogen compound biosynthetic process (GO:1901566)	418	62	49.40	1,25	3.72E-02
biological regulation (GO:0065007)	508	75	60.04	1,25	2.50E-02
cellular metabolic process (GO:0044237)	1232	179	145.61	1,23	5.40E-04
macromolecule metabolic process (GO:0043170)	689	99	81.43	1,22	2.01E-02
metabolic process (GO:0008152)	1391	199	164.40	1,21	4.70E-04
nitrogen compound metabolic process (GO:0006807)	1032	146	121.97	1,2	6.94E-03
organic substance metabolic process (GO:0071704)	1249	174	147.62	1,18	5.03E-03
cellular process (GO:0009987)	1874	261	221.49	1,18	1.09E-04
primary metabolic process (GO:0044238)	1106	149	130.72	1,14	3.34E-02
transmembrane transport (GO:0055085)	371	31	43.85	0,71	2.09E-02
carbohydrate transport (GO:0008643)	43	1	5.08	0,2	3.70E-02

<b>GO biological process complete CIP 16 gen CIP vs 16 gen MH</b>	<b>Vibrio cholerae - REFLIST (3782)</b>	<b>upload_1 (1004)</b>	<b>(expected )</b>	<b>(fold overrepresentation)</b>	<b>P-value</b>
protein maturation by iron-sulfur cluster transfer (GO:0097428)	4	4	1.06	3,77	2.29E-02
tRNA threonylcarbamoyladenosine metabolic process (GO:0070525)	3	3	.80	3,77	4.68E-02
organelle disassembly (GO:1903008)	3	3	.80	3,77	4.68E-02
AMP biosynthetic process (GO:0006167)	4	4	1.06	3,77	2.29E-02
'de novo' UMP biosynthetic process (GO:0044205)	7	7	1.86	3,77	3.02E-03
tRNA threonylcarbamoyladenosine modification (GO:0002949)	3	3	.80	3,77	4.68E-02
DNA-templated transcription, termination (GO:0006353)	5	5	1.33	3,77	1.15E-02
lipoprotein transport (GO:0042953)	4	4	1.06	3,77	2.29E-02
glycosylation (GO:0070085)	3	3	.80	3,77	4.68E-02
AMP metabolic process (GO:0046033)	4	4	1.06	3,77	2.29E-02
pantothenate biosynthetic process (GO:0015940)	3	3	.80	3,77	4.68E-02
pantothenate metabolic process (GO:0015939)	4	4	1.06	3,77	2.29E-02
ribosomal small subunit assembly (GO:0000028)	5	5	1.33	3,77	1.15E-02
nucleoside monophosphate phosphorylation (GO:0046940)	4	4	1.06	3,77	2.29E-02
regulation of DNA replication (GO:0006275)	3	3	.80	3,77	4.68E-02
protein transport by the Tat complex (GO:0043953)	4	4	1.06	3,77	2.29E-02
ribosome disassembly (GO:0032790)	3	3	.80	3,77	4.68E-02
cytoplasmic translation (GO:0002181)	4	4	1.06	3,77	2.29E-02
lipid-linked peptidoglycan transport (GO:0015836)	3	3	.80	3,77	4.68E-02
tRNA wobble position uridine thiolation (GO:0002143)	5	5	1.33	3,77	1.15E-02
aminoacyl-tRNA metabolism involved in translational fidelity (GO:0106074)	7	6	1.86	3,23	1.19E-02
tRNA thio-modification (GO:0034227)	7	6	1.86	3,23	1.19E-02
ribosome assembly (GO:0042255)	14	12	3.72	3,23	4.74E-04
pyridoxine biosynthetic process (GO:0008615)	6	5	1.59	3,14	2.32E-02
pyridoxine metabolic process (GO:0008614)	6	5	1.59	3,14	2.32E-02
regulation of DNA metabolic process (GO:0051052)	6	5	1.59	3,14	2.32E-02
ribonucleoprotein complex subunit organization (GO:0071826)	12	10	3.19	3,14	1.67E-03

cell septum assembly (GO:0090529)	12	10	3.19	3,14	1.67E-03
lipooligosaccharide biosynthetic process (GO:1901271)	12	10	3.19	3,14	1.67E-03
lipooligosaccharide metabolic process (GO:1901269)	12	10	3.19	3,14	1.67E-03
vitamin B6 biosynthetic process (GO:0042819)	6	5	1.59	3,14	2.32E-02
vitamin B6 metabolic process (GO:0042816)	6	5	1.59	3,14	2.32E-02
ribonucleoprotein complex assembly (GO:0022618)	12	10	3.19	3,14	1.67E-03
lipid A metabolic process (GO:0046493)	11	9	2.92	3,08	3.15E-03
lipid A biosynthetic process (GO:0009245)	11	9	2.92	3,08	3.15E-03
cytochrome complex assembly (GO:0017004)	10	8	2.65	3,01	5.94E-03
regulation of translational fidelity (GO:0006450)	10	8	2.65	3,01	5.94E-03
translational initiation (GO:0006413)	5	4	1.33	3,01	4.58E-02
Gram-negative-bacterium-type cell outer membrane assembly (GO:0043165)	10	8	2.65	3,01	5.94E-03
cell envelope organization (GO:0043163)	10	8	2.65	3,01	5.94E-03
membrane assembly (GO:0071709)	10	8	2.65	3,01	5.94E-03
division septum assembly (GO:0000917)	10	8	2.65	3,01	5.94E-03
membrane biogenesis (GO:0044091)	10	8	2.65	3,01	5.94E-03
membrane lipid biosynthetic process (GO:0046467)	14	11	3.72	2,96	1.59E-03
reproduction of a single-celled organism (GO:0032505)	14	11	3.72	2,96	1.59E-03
reproductive process (GO:0022414)	14	11	3.72	2,96	1.59E-03
FtsZ-dependent cytokinesis (GO:0043093)	14	11	3.72	2,96	1.59E-03
reproduction (GO:0000003)	14	11	3.72	2,96	1.59E-03
glycolipid biosynthetic process (GO:0009247)	14	11	3.72	2,96	1.59E-03
asexual reproduction (GO:0019954)	14	11	3.72	2,96	1.59E-03
ATP biosynthetic process (GO:0006754)	9	7	2.39	2,93	1.12E-02
proton motive force-driven ATP synthesis (GO:0015986)	9	7	2.39	2,93	1.12E-02
ribosomal small subunit biogenesis (GO:0042274)	9	7	2.39	2,93	1.12E-02
chromosome segregation (GO:0007059)	9	7	2.39	2,93	1.12E-02
cytokinetic process (GO:0032506)	13	10	3.45	2,9	2.95E-03
regulation of cell shape (GO:0008360)	30	23	7.96	2,89	9.51E-06
regulation of cell morphogenesis (GO:0022604)	30	23	7.96	2,89	9.51E-06

regulation of anatomical structure morphogenesis (GO:0022603)	30	23	7.96	2,89	9.51E-06
translation (GO:0006412)	109	82	28.94	2,83	1.40E-16
nucleoside triphosphate biosynthetic process (GO:0009142)	16	12	4.25	2,83	1.47E-03
iron-sulfur cluster assembly (GO:0016226)	12	9	3.19	2,83	5.47E-03
metallo-sulfur cluster assembly (GO:0031163)	12	9	3.19	2,83	5.47E-03
cell cycle process (GO:0022402)	20	15	5.31	2,83	4.01E-04
'de novo' pyrimidine nucleobase biosynthetic process (GO:0006207)	8	6	2.12	2,83	2.13E-02
cytokinesis (GO:0000910)	20	15	5.31	2,83	4.01E-04
regulation of developmental process (GO:0050793)	31	23	8.23	2,79	1.58E-05
glycolipid metabolic process (GO:0006664)	15	11	3.98	2,76	2.68E-03
purine nucleoside triphosphate biosynthetic process (GO:0009145)	11	8	2.92	2,74	1.02E-02
pyrimidine nucleoside monophosphate biosynthetic process (GO:0009130)	11	8	2.92	2,74	1.02E-02
isoprenoid metabolic process (GO:0006720)	11	8	2.92	2,74	1.02E-02
isoprenoid biosynthetic process (GO:0008299)	11	8	2.92	2,74	1.02E-02
purine ribonucleoside triphosphate biosynthetic process (GO:0009206)	11	8	2.92	2,74	1.02E-02
purine ribonucleoside triphosphate metabolic process (GO:0009205)	11	8	2.92	2,74	1.02E-02
tRNA aminoacylation for protein translation (GO:0006418)	25	18	6.64	2,71	1.82E-04
tRNA aminoacylation (GO:0043039)	25	18	6.64	2,71	1.82E-04
cell cycle (GO:0007049)	46	33	12.21	2,7	5.22E-07
transcription antitermination (GO:0031564)	7	5	1.86	2,69	4.06E-02
isopentenyl diphosphate metabolic process (GO:0046490)	7	5	1.86	2,69	4.06E-02
terpenoid metabolic process (GO:0006721)	7	5	1.86	2,69	4.06E-02
terpenoid biosynthetic process (GO:0016114)	7	5	1.86	2,69	4.06E-02
lipopolysaccharide transport (GO:0015920)	7	5	1.86	2,69	4.06E-02
membrane organization (GO:0061024)	14	10	3.72	2,69	4.91E-03
response to antibiotic (GO:0046677)	7	5	1.86	2,69	4.06E-02
ribosomal large subunit biogenesis (GO:0042273)	7	5	1.86	2,69	4.06E-02
isopentenyl diphosphate biosynthetic process (GO:0009240)	7	5	1.86	2,69	4.06E-02
ribonucleoside triphosphate biosynthetic process (GO:0009201)	14	10	3.72	2,69	4.91E-03

DNA topological change (GO:0006265)	7	5	1.86	2,69	4.06E-02
heme transport (GO:0015886)	7	5	1.86	2,69	4.06E-02
isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway (GO:0019288)	7	5	1.86	2,69	4.06E-02
peptide biosynthetic process (GO:0043043)	116	82	30.79	2,66	4.10E-15
membrane lipid metabolic process (GO:0006643)	17	12	4.51	2,66	2.40E-03
pyrimidine ribonucleoside monophosphate biosynthetic process (GO:0009174)	10	7	2.65	2,64	1.88E-02
pyrimidine nucleobase biosynthetic process (GO:0019856)	10	7	2.65	2,64	1.88E-02
tRNA wobble uridine modification (GO:0002098)	10	7	2.65	2,64	1.88E-02
UMP biosynthetic process (GO:0006222)	10	7	2.65	2,64	1.88E-02
protein-containing complex assembly (GO:0065003)	30	21	7.96	2,64	8.14E-05
amino acid activation (GO:0043038)	26	18	6.90	2,61	2.90E-04
pyrimidine ribonucleotide biosynthetic process (GO:0009220)	13	9	3.45	2,61	8.95E-03
pyrimidine nucleotide biosynthetic process (GO:0006221)	16	11	4.25	2,59	4.32E-03
cellular macromolecule biosynthetic process (GO:0034645)	173	118	45.93	2,57	3.28E-20
oligosaccharide biosynthetic process (GO:0009312)	22	15	5.84	2,57	1.04E-03
pyrimidine nucleoside monophosphate metabolic process (GO:0009129)	12	8	3.19	2,51	1.63E-02
protein maturation (GO:0051604)	18	12	4.78	2,51	3.76E-03
cellular component disassembly (GO:0022411)	9	6	2.39	2,51	3.48E-02
external encapsulating structure organization (GO:0045229)	42	28	11.15	2,51	1.39E-05
protein-containing complex organization (GO:0043933)	36	24	9.56	2,51	5.54E-05
ribonucleoside triphosphate metabolic process (GO:0009199)	15	10	3.98	2,51	7.77E-03
amide biosynthetic process (GO:0043604)	143	94	37.96	2,48	2.76E-15
peptide metabolic process (GO:0006518)	132	86	35.04	2,45	7.34E-14
lipopolysaccharide biosynthetic process (GO:0009103)	20	13	5.31	2,45	3.25E-03
cell division (GO:0051301)	60	39	15.93	2,45	5.72E-07
ribonucleotide biosynthetic process (GO:0009260)	58	37	15.40	2,4	1.69E-06
pyrimidine ribonucleoside monophosphate metabolic process (GO:0009173)	11	7	2.92	2,4	2.94E-02

lipopolysaccharide metabolic process (GO:0008653)	22	14	5.84	2,4	2.78E-03
lipopolysaccharide core region metabolic process (GO:0046401)	11	7	2.92	2,4	2.94E-02
liposaccharide metabolic process (GO:1903509)	33	21	8.76	2,4	2.90E-04
UMP metabolic process (GO:0046049)	11	7	2.92	2,4	2.94E-02
lipopolysaccharide core region biosynthetic process (GO:0009244)	11	7	2.92	2,4	2.94E-02
pyrimidine nucleobase metabolic process (GO:0006206)	11	7	2.92	2,4	2.94E-02
cellular component assembly (GO:0022607)	81	51	21.50	2,37	2.91E-08
nucleoside monophosphate metabolic process (GO:0009123)	40	25	10.62	2,35	1.06E-04
nucleobase biosynthetic process (GO:0046112)	16	10	4.25	2,35	1.18E-02
cell wall organization (GO:0071555)	32	20	8.49	2,35	4.98E-04
macromolecule biosynthetic process (GO:0009059)	238	148	63.18	2,34	1.49E-21
cellular component macromolecule biosynthetic process (GO:0070589)	34	21	9.03	2,33	4.25E-04
glycosaminoglycan biosynthetic process (GO:0006024)	34	21	9.03	2,33	4.25E-04
aminoglycan biosynthetic process (GO:0006023)	34	21	9.03	2,33	4.25E-04
peptidoglycan biosynthetic process (GO:0009252)	34	21	9.03	2,33	4.25E-04
cell wall macromolecule biosynthetic process (GO:0044038)	34	21	9.03	2,33	4.25E-04
ribose phosphate biosynthetic process (GO:0046390)	60	37	15.93	2,32	3.61E-06
ribonucleoside monophosphate biosynthetic process (GO:0009156)	33	20	8.76	2,28	7.23E-04
purine nucleotide biosynthetic process (GO:0006164)	48	29	12.74	2,28	5.60E-05
ribonucleoside monophosphate metabolic process (GO:0009161)	35	21	9.29	2,26	6.12E-04
purine ribonucleotide biosynthetic process (GO:0009152)	45	27	11.95	2,26	1.11E-04
nucleoside triphosphate metabolic process (GO:0009141)	20	12	5.31	2,26	8.34E-03
nucleoside monophosphate biosynthetic process (GO:0009124)	35	21	9.29	2,26	6.12E-04
pyrimidine ribonucleotide metabolic process (GO:0009218)	15	9	3.98	2,26	2.06E-02
pyrimidine nucleotide metabolic process (GO:0006220)	20	12	5.31	2,26	8.34E-03
nucleotide biosynthetic process (GO:0009165)	77	46	20.44	2,25	5.81E-07
oligosaccharide metabolic process (GO:0009311)	27	16	7.17	2,23	2.91E-03

purine ribonucleoside monophosphate metabolic process (GO:0009167)	22	13	5.84	2,23	7.00E-03
purine nucleoside monophosphate metabolic process (GO:0009126)	22	13	5.84	2,23	7.00E-03
carbohydrate derivative biosynthetic process (GO:1901137)	149	88	39.55	2,22	6.68E-12
nucleobase metabolic process (GO:0009112)	17	10	4.51	2,22	1.72E-02
protein localization to membrane (GO:0072657)	17	10	4.51	2,22	1.72E-02
localization within membrane (GO:0051668)	17	10	4.51	2,22	1.72E-02
regulation of cellular component organization (GO:0051128)	12	7	3.19	2,2	4.35E-02
peptidoglycan-based cell wall biogenesis (GO:0009273)	36	21	9.56	2,2	8.67E-04
cell wall biogenesis (GO:0042546)	36	21	9.56	2,2	8.67E-04
cell wall macromolecule metabolic process (GO:0044036)	36	21	9.56	2,2	8.67E-04
nucleoside phosphate biosynthetic process (GO:1901293)	79	46	20.97	2,19	1.15E-06
purine-containing compound biosynthetic process (GO:0072522)	50	29	13.27	2,18	1.12E-04
nucleoside diphosphate metabolic process (GO:0009132)	19	11	5.04	2,18	1.43E-02
phospholipid biosynthetic process (GO:0008654)	38	22	10.09	2,18	7.29E-04
regulation of translation (GO:0006417)	19	11	5.04	2,18	1.43E-02
phospholipid metabolic process (GO:0006644)	38	22	10.09	2,18	7.29E-04
purine ribonucleoside monophosphate biosynthetic process (GO:0009168)	21	12	5.57	2,15	1.19E-02
purine nucleoside monophosphate biosynthetic process (GO:0009127)	21	12	5.57	2,15	1.19E-02
SOS response (GO:0009432)	14	8	3.72	2,15	3.57E-02
tRNA wobble base modification (GO:0002097)	14	8	3.72	2,15	3.57E-02
purine nucleoside bisphosphate biosynthetic process (GO:0034033)	14	8	3.72	2,15	3.57E-02
ribonucleoside bisphosphate biosynthetic process (GO:0034030)	14	8	3.72	2,15	3.57E-02
nucleoside bisphosphate biosynthetic process (GO:0033866)	14	8	3.72	2,15	3.57E-02
cellular component organization (GO:0016043)	163	93	43.27	2,15	1.02E-11
DNA conformation change (GO:0071103)	23	13	6.11	2,13	9.86E-03
cellular amide metabolic process (GO:0043603)	181	102	48.05	2,12	1.91E-12
ribonucleoside diphosphate metabolic process (GO:0009185)	16	9	4.25	2,12	2.94E-02

pyrimidine-containing compound biosynthetic process (GO:0072528)	25	14	6.64	2,11	8.19E-03
organophosphate biosynthetic process (GO:0090407)	142	79	37.70	2,1	1.23E-09
lipid biosynthetic process (GO:0008610)	78	43	20.71	2,08	9.52E-06
regulation of cellular amide metabolic process (GO:0034248)	20	11	5.31	2,07	1.99E-02
post-transcriptional regulation of gene expression (GO:0010608)	20	11	5.31	2,07	1.99E-02
fatty acid biosynthetic process (GO:0006633)	20	11	5.31	2,07	1.99E-02
ATP metabolic process (GO:0046034)	33	18	8.76	2,05	3.91E-03
DNA-templated DNA replication (GO:0006261)	22	12	5.84	2,05	1.64E-02
gene expression (GO:0010467)	264	143	70.08	2,04	8.32E-16
ribonucleotide metabolic process (GO:0009259)	85	46	22.56	2,04	7.47E-06
cellular component biogenesis (GO:0044085)	161	87	42.74	2,04	6.82E-10
cellular nitrogen compound biosynthetic process (GO:0044271)	355	190	94.24	2,02	1.65E-20
purine ribonucleoside diphosphate metabolic process (GO:0009179)	15	8	3.98	2,01	4.97E-02
purine nucleoside triphosphate metabolic process (GO:0009144)	15	8	3.98	2,01	4.97E-02
purine nucleoside diphosphate metabolic process (GO:0009135)	15	8	3.98	2,01	4.97E-02
RNA phosphodiester bond hydrolysis (GO:0090501)	19	10	5.04	1,98	3.31E-02
DNA replication (GO:0006260)	44	23	11.68	1,97	2.07E-03
cellular macromolecule localization (GO:0070727)	25	13	6.64	1,96	1.83E-02
cellular protein localization (GO:0034613)	25	13	6.64	1,96	1.83E-02
chromosome organization (GO:0051276)	31	16	8.23	1,94	1.02E-02
purine ribonucleotide metabolic process (GO:0009150)	70	36	18.58	1,94	1.88E-04
cell wall organization or biogenesis (GO:0071554)	43	22	11.42	1,93	3.28E-03
ribose phosphate metabolic process (GO:0019693)	90	46	23.89	1,93	2.98E-05
cellular component organization or biogenesis (GO:0071840)	218	111	57.87	1,92	8.06E-11
purine nucleotide metabolic process (GO:0006163)	77	39	20.44	1,91	1.40E-04
nucleobase-containing compound biosynthetic process (GO:0034654)	162	82	43.01	1,91	3.62E-08
non-membrane-bounded organelle assembly (GO:0140694)	26	13	6.90	1,88	2.41E-02
organelle assembly (GO:0070925)	26	13	6.90	1,88	2.41E-02
regulation of cellular protein metabolic process (GO:0032268)	24	12	6.37	1,88	2.94E-02



cellular lipid metabolic process (GO:0044255)	101	50	26.81	1,86	3.05E-05
regulation of biological quality (GO:0065008)	84	41	22.30	1,84	2.05E-04
cellular protein metabolic process (GO:0044267)	239	116	63.45	1,83	4.77E-10
ribonucleoprotein complex biogenesis (GO:0022613)	60	29	15.93	1,82	1.88E-03
purine-containing compound metabolic process (GO:0072521)	81	39	21.50	1,81	3.75E-04
cellular polysaccharide biosynthetic process (GO:0033692)	27	13	7.17	1,81	3.12E-02
organonitrogen compound biosynthetic process (GO:1901566)	418	201	110.97	1,81	9.82E-17
pyruvate metabolic process (GO:0006090)	25	12	6.64	1,81	3.81E-02
carbohydrate biosynthetic process (GO:0016051)	48	23	12.74	1,8	5.79E-03
tRNA metabolic process (GO:0006399)	88	42	23.36	1,8	2.72E-04
DNA recombination (GO:0006310)	44	21	11.68	1,8	8.45E-03
ribosome biogenesis (GO:0042254)	59	28	15.66	1,79	2.87E-03
protein transmembrane transport (GO:0071806)	34	16	9.03	1,77	2.20E-02
organelle organization (GO:0006996)	68	32	18.05	1,77	1.72E-03
glycosaminoglycan metabolic process (GO:0030203)	51	24	13.54	1,77	6.05E-03
peptidoglycan metabolic process (GO:0000270)	49	23	13.01	1,77	7.30E-03
organic substance biosynthetic process (GO:1901576)	610	286	161.94	1,77	6.03E-23
carbohydrate derivative metabolic process (GO:1901135)	242	113	64.24	1,76	6.75E-09
polysaccharide biosynthetic process (GO:0000271)	30	14	7.96	1,76	3.25E-02
regulation of cellular macromolecule biosynthetic process (GO:2000112)	30	14	7.96	1,76	3.25E-02
cellular biosynthetic process (GO:0044249)	599	279	159.02	1,75	6.84E-22
cellular carbohydrate biosynthetic process (GO:0034637)	28	13	7.43	1,75	3.97E-02
aromatic compound biosynthetic process (GO:0019438)	235	109	62.38	1,75	1.81E-08
regulation of protein metabolic process (GO:0051246)	26	12	6.90	1,74	4.85E-02
biosynthetic process (GO:0009058)	624	286	165.65	1,73	1.70E-21
cellular polysaccharide metabolic process (GO:0044264)	33	15	8.76	1,71	3.36E-02
aminoglycan metabolic process (GO:0006022)	53	24	14.07	1,71	9.38E-03
organophosphate metabolic process (GO:0019637)	208	94	55.22	1,7	5.72E-07
nucleotide metabolic process (GO:0009117)	125	56	33.18	1,69	1.43E-04

heterocycle biosynthetic process (GO:0018130)	253	113	67.16	1,68	6.76E-08
cellular macromolecule metabolic process (GO:0044260)	486	216	129.02	1,67	2.03E-14
organic cyclic compound biosynthetic process (GO:1901362)	267	118	70.88	1,66	5.83E-08
establishment of protein localization (GO:0045184)	77	34	20.44	1,66	3.39E-03
pyrimidine-containing compound metabolic process (GO:0072527)	34	15	9.03	1,66	4.16E-02
monocarboxylic acid biosynthetic process (GO:0072330)	39	17	10.35	1,64	3.48E-02
protein localization (GO:0008104)	78	34	20.71	1,64	4.11E-03
nucleoside phosphate metabolic process (GO:0006753)	129	56	34.25	1,64	3.09E-04
lipid metabolic process (GO:0006629)	120	52	31.86	1,63	5.20E-04
RNA biosynthetic process (GO:0032774)	44	19	11.68	1,63	2.91E-02
ncRNA metabolic process (GO:0034660)	124	53	32.92	1,61	6.27E-04
nucleic acid-templated transcription (GO:0097659)	40	17	10.62	1,6	4.23E-02
transcription, DNA-templated (GO:0006351)	40	17	10.62	1,6	4.23E-02
cellular nitrogen compound metabolic process (GO:0034641)	662	281	175.74	1,6	1.53E-16
protein metabolic process (GO:0019538)	330	140	87.60	1,6	3.34E-08
nucleic acid phosphodiester bond hydrolysis (GO:0090305)	52	22	13.80	1,59	2.44E-02
protein transport (GO:0015031)	71	30	18.85	1,59	1.01E-02
tRNA modification (GO:0006400)	48	20	12.74	1,57	3.51E-02
RNA metabolic process (GO:0016070)	186	77	49.38	1,56	1.09E-04
electron transport chain (GO:0022900)	58	24	15.40	1,56	2.44E-02
nucleobase-containing small molecule metabolic process (GO:0055086)	160	66	42.47	1,55	3.68E-04
water-soluble vitamin metabolic process (GO:0006767)	51	21	13.54	1,55	3.50E-02
macromolecule localization (GO:0033036)	102	42	27.08	1,55	4.17E-03
nucleobase-containing compound metabolic process (GO:0006139)	470	191	124.77	1,53	1.70E-09
vitamin metabolic process (GO:0006766)	52	21	13.80	1,52	4.14E-02
nucleic acid metabolic process (GO:0090304)	316	127	83.89	1,51	2.50E-06
macromolecule metabolic process (GO:0043170)	689	275	182.91	1,5	6.20E-13
cellular aromatic compound metabolic process (GO:0006725)	557	220	147.87	1,49	7.87E-10

monocarboxylic acid metabolic process (GO:0032787)	109	43	28.94	1,49	7.70E-03
heterocycle metabolic process (GO:0046483)	566	222	150.25	1,48	1.17E-09
DNA metabolic process (GO:0006259)	136	53	36.10	1,47	4.24E-03
organic cyclic compound metabolic process (GO:1901360)	586	228	155.56	1,47	1.30E-09
generation of precursor metabolites and energy (GO:0006091)	107	41	28.41	1,44	1.41E-02
organonitrogen compound metabolic process (GO:1901564)	724	272	192.20	1,42	5.70E-10
cellular metabolic process (GO:0044237)	1232	455	327.06	1,39	3.05E-17
nitrogen compound metabolic process (GO:0006807)	1032	376	273.96	1,37	1.58E-12
RNA processing (GO:0006396)	103	37	27.34	1,35	4.27E-02
carbohydrate metabolic process (GO:0005975)	148	53	39.29	1,35	1.91E-02
primary metabolic process (GO:0044238)	1106	393	293.61	1,34	1.21E-11
organic substance metabolic process (GO:0071704)	1249	441	331.57	1,33	4.31E-13
phosphate-containing compound metabolic process (GO:0006796)	325	114	86.28	1,32	1.58E-03
phosphorus metabolic process (GO:0006793)	337	118	89.46	1,32	1.39E-03
carboxylic acid metabolic process (GO:0019752)	312	109	82.83	1,32	2.27E-03
metabolic process (GO:0008152)	1391	480	369.27	1,3	6.14E-13
oxoacid metabolic process (GO:0043436)	317	109	84.15	1,3	3.69E-03
organic acid metabolic process (GO:0006082)	319	109	84.68	1,29	4.44E-03
small molecule metabolic process (GO:0044281)	514	175	136.45	1,28	3.47E-04
regulation of metabolic process (GO:0019222)	269	88	71.41	1,23	2.68E-02
cellular process (GO:0009987)	1874	608	497.49	1,22	1.61E-12
regulation of cellular metabolic process (GO:0031323)	254	82	67.43	1,22	4.10E-02
regulation of macromolecule metabolic process (GO:0060255)	264	85	70.08	1,21	4.00E-02
cellular response to stimulus (GO:0051716)	258	51	68.49	0,74	1.39E-02
response to stimulus (GO:0050896)	328	64	87.07	0,74	4.31E-03
cell communication (GO:0007154)	175	27	46.46	0,58	1.15E-03
response to chemical (GO:0042221)	117	18	31.06	0,58	7.33E-03
peptidyl-amino acid modification (GO:0018193)	68	10	18.05	0,55	2.85E-02
phosphorelay signal transduction system (GO:0000160)	97	14	25.75	0,54	7.99E-03
intracellular signal transduction (GO:0035556)	97	14	25.75	0,54	7.99E-03

amino acid transport (GO:0006865)	46	6	12.21	0,49	3.98E-02
signal transduction (GO:0007165)	157	19	41.68	0,46	5.30E-05
organic acid transport (GO:0015849)	58	7	15.40	0,45	1.38E-02
signaling (GO:0023052)	158	19	41.94	0,45	4.53E-05
carboxylic acid transport (GO:0046942)	42	5	11.15	0,45	3.36E-02
response to external stimulus (GO:0009605)	94	11	24.95	0,44	1.31E-03
cell adhesion (GO:0007155)	30	3	7.96	0,38	4.28E-02
organic acid transmembrane transport (GO:1903825)	31	3	8.23	0,36	3.57E-02
carboxylic acid transmembrane transport (GO:1905039)	31	3	8.23	0,36	3.57E-02
negative regulation of bacterial-type flagellum-dependent cell motility (GO:1902201)	24	2	6.37	0,31	4.68E-02
protein phosphorylation (GO:0006468)	48	4	12.74	0,31	4.32E-03
cell-substrate adhesion (GO:0031589)	24	2	6.37	0,31	4.68E-02
single-species submerged biofilm formation (GO:0090609)	24	2	6.37	0,31	4.68E-02
submerged biofilm formation (GO:0090605)	24	2	6.37	0,31	4.68E-02
cell adhesion involved in single-species biofilm formation (GO:0043709)	24	2	6.37	0,31	4.68E-02
cell adhesion involved in biofilm formation (GO:0043708)	24	2	6.37	0,31	4.68E-02
negative regulation of cell motility (GO:2000146)	24	2	6.37	0,31	4.68E-02
regulation of cell motility (GO:2000145)	24	2	6.37	0,31	4.68E-02
negative regulation of cellular component movement (GO:0051271)	24	2	6.37	0,31	4.68E-02
regulation of cellular component movement (GO:0051270)	24	2	6.37	0,31	4.68E-02
regulation of bacterial-type flagellum-dependent cell motility (GO:1902021)	24	2	6.37	0,31	4.68E-02
negative regulation of locomotion (GO:0040013)	25	2	6.64	0,3	3.85E-02
regulation of locomotion (GO:0040012)	26	2	6.90	0,29	3.15E-02
localization of cell (GO:0051674)	29	2	7.70	0,26	1.71E-02
cell motility (GO:0048870)	29	2	7.70	0,26	1.71E-02
movement of cell or subcellular component (GO:0006928)	29	2	7.70	0,26	1.71E-02
locomotion (GO:0040011)	86	5	22.83	0,22	6.74E-06
transition metal ion homeostasis (GO:0055076)	19	1	5.04	0,2	3.86E-02
peptidyl-histidine phosphorylation (GO:0018106)	39	2	10.35	0,19	2.00E-03

peptidyl-histidine modification (GO:0018202)	39	2	10.35	0,19	2.00E-03
taxis (GO:0042330)	64	3	16.99	0,18	3.71E-05
chemotaxis (GO:0006935)	64	3	16.99	0,18	3.71E-05
bacterial-type flagellum-dependent cell motility (GO:0071973)	24	1	6.37	0,16	1.24E-02
cilium or flagellum-dependent cell motility (GO:0001539)	25	1	6.64	0,15	9.85E-03
archaeal or bacterial-type flagellum- dependent cell motility (GO:0097588)	25	1	6.64	0,15	9.85E-03

4

1 Table S3. Strains and plasmids

Strain	Strain number	Construction
<i>Vibrio cholerae</i>		
N16961 hapR+ wt strain	F606	Gift from Melanie Blokesch
N16961 hapR+ wt strain $\Delta lacZ$	K329	deletion of lacZ by plasmid integration and excision by sucrose counterselection as described
$\Delta tgt$ (VC0741)	J420	PCR amplification of 500bp up and down regions of VC0741 using primers ZIP431/432 and ZIP433/434. PCR amplicification of aadA7 conferring spectinomycin resistance on pAM34 using ZB47/48. PCR assembly of the VC0741::spec fragment using ZIP431/434 and allelic exchange by natural transformation.
$\Delta tgt$ (VC0741)	M087	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 L910, replacing the gene with frt::kan::frt as described previously (Val et al PLoS Genetics 2012, Negro et al, mBio 2019)
$\Delta gidA$ (VC2775)	H244	PCR amplification of 500bp up and down regions of VC2775 using primers ZIP316/317 and ZIP318/319. PCR amplicification of aadA7 conferring spectinomycin resistance on pAM34 using ZIP320/321. PCR assembly of the VC2775::spec fragment using ZIP316/319 and allelic exchange by natural transformation.
$\Delta dusA$ (VC0379)	L607	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 L024, replacing the gene with frt::kan::frt
$\Delta dusB$ (VC0291)	L606	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 L416, replacing the gene with frt::kan::frt
$\Delta miaB$ (VC0962)	K013	(Negro et al, mBio 2019)
$\Delta truA$ (VC0999)	N095	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 970, replacing the gene with frt::kan::frt
$\Delta truB$ (VC0645)	M562	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 M347, replacing the gene with frt::kan::frt
$\Delta truC$ (VC0888)	P638	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 O651, replacing the gene with frt::kan::frt
$\Delta trmA$ (VC0154)	M564	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 M423, replacing the gene with frt::kan::frt
$\Delta trmB$ (VC0453)	M096	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 L974, replacing the gene with frt::kan::frt
$\Delta trmE$ (VC0003)	H218	PCR amplification of 500bp up and down regions of VC0003 using primers 1640/1641 and 1642/1643. PCR amplicification of aadA7 conferring spectinomycin resistance on pAM34 using 1644/1645. PCR assembly of the VC0003::spec fragment using 1640/1643 and allelic exchange by natural transformation.

<i>ΔtrmH (VC0803)</i>	Q062	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 P493, replacing the gene with <i>frt::kan::frt</i>
<i>ΔtrmK (VCA0634)</i>	K650	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 K440, replacing the gene with <i>frt::kan::frt</i>
<i>ΔrlmN (VC0757)</i>	M094	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 L912, replacing the gene with <i>frt::kan::frt</i>
<i>ΔrlmI (VC1354)</i>	N031	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 M969, replacing the gene with <i>frt::kan::frt</i>
<i>ΔrsuA (VC1635)</i>	H497	Negro et al, mBio 2019
<i>ΔrsmB (VC0044)</i>	N033	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 M771, replacing the gene with <i>frt::kan::frt</i>
<i>ΔrsmC (VC0623)</i>	L601	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 L577, replacing the gene with <i>frt::kan::frt</i>
<i>ΔrsmD (VC0146)</i>	M088	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 L565, replacing the gene with <i>frt::kan::frt</i>
<i>ΔrsmF (VC2223)</i>	N045	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 M769, replacing the gene with <i>frt::kan::frt</i>
<i>ΔrluB (VC1179)</i>	L559	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 L020, replacing the gene with <i>frt::kan::frt</i>
<i>ΔrluD (VC0709)</i>	N097	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 N035, replacing the gene with <i>frt::kan::frt</i>
<i>ΔrluE (VC1140)</i>	Q061	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 P346, replacing the gene with <i>frt::kan::frt</i>
<b><i>Escherichia coli</i></b>		
MG1655 wt strain		laboratory collection
<i>Δtgt</i>	J233	P1 transduction from KEIO strain JW0396-3
<i>ΔgidA</i>	J193	P1 transduction from KEIO strain JW3719-1
<i>ΔdusA</i>	J196	P1 transduction from KEIO strain JW5950-5
<i>ΔdusB</i>	J243	P1 transduction from KEIO strain JW3228-1
<i>ΔtrmE</i>	J194	P1 transduction from KEIO strain JW3684-1
<i>ΔrsuA</i>	H243	P1 transduction from KEIO strain JW2171-1
<i>ΔrsmC</i>	J192	P1 transduction from KEIO strain JW4333-1
<i>ΔrsmD</i>	J241	P1 transduction from KEIO strain JW3430-4
<i>ΔrluB</i>	J235	P1 transduction from KEIO strain JW1261-3
<b>Plasmids pMP7-Δgene::kan</b>		gibson assembly using primers MV450/451 for the amplification of pMP7 vector, primers indicated below for up and down regions of the gene, and primers MV268/269 on pKD4 plasmid for the resistance gene ( <i>frt::kan::frt</i> ).
<i>Δtgt (VC0741)</i>	L910	VC0741tgt5/7 for up region and VC0741tgt6bis/8 bis for down region
<i>ΔdusA (VC0379)</i>	L024	VC0379dusA5/7 for up region and VC0379dusA6/8 for down region
<i>ΔdusB (VC0291)</i>	L416	VC0291dusB5/7 for up region and VC0291dusB6bis/8 bis for down region

<i>ΔtruA (VC0999)</i>	M970	VC0999truA5/7 for up region and VC0999truA6bis/8bis for down region
<i>ΔtruB (VC0645)</i>	M347	VC0645truB5bis/7bis for up region and VC0645truB6/8 for down region
<i>ΔtruC (VC0888)</i>	O651	VC0888truC5/7 for up region and VC0888truC6/8 for down region
<i>ΔtrmA (VC0154)</i>	M423	VC0154trmA5/7 for up region and VC0154trmA6bis/8 bis for down region
<i>ΔtrmB (VC0453)</i>	L974	VC0453trmB5/7 for up region and VC0453trmB6/8 for down region
<i>ΔtrmH (VC0803)</i>	P493	VC0803trmH5/7 for up region and VC0803trmH6bis/8 for down region
<i>ΔtrmK (VCA0634)</i>	K440	VCA06345/7 for up region and VCA06346/8 for down region
<i>ΔrlmN (VC0757)</i>	L912	VC0757rlmN5/7 for up region and VC0757rlmN6/8 for down region
<i>ΔrlmI (VC1354)</i>	M969	VC1354rlmI5bis/7bis for up region and VC1354rlmI6/8 for down region
<i>ΔrsmB (VC0044)</i>	M771	VC0044rsmB5/7 for up region and VC0044rsmB6/8 for down region
<i>ΔrsmC (VC0623)</i>	L577	VC0623rsmC5/7 for up region and VC0623rsmC6/8 for down region
<i>ΔrsmD (VC0146)</i>	L565	VC0146rsmD5/7 for up region and VC0146rsmD6/8 for down region
<i>ΔrsmF (VC2223)</i>	M769	VC1502rsmF5/7 for up region and VC1502rsmF6/8 for down region
<i>ΔrluB (VC1179)</i>	L020	VC1179rluB5/7 for up region and VC1179rluB6/8 for down region
<i>ΔrluD (VC0709)</i>	N035	VC0709rluD5ter/7ter for up region and VC0709rluD6bis/8ter for down region
<i>ΔrluE (VC1140)</i>	P346	VC1140rluE5/7 for up region and VC1140rluE6/8 for down region

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1 **Table S4. Primers**

<b>primers</b>	
ZB47	CCC GTTCCATACAGAAGCTGGGCGAACAAACGATGCTCGC
ZB48	GACATTATTTGCCGACTACCTTGGTGATCTCGCCTTTCACG
ZIP431	CACCTCAGTACGTTCACTCG
ZIP432	GCGAGCATCGTTTGTTCCGCCAGCTTCTGTATGGAACGGGGAAGCCTCCAATGTCA GAGAAACAGTCTGACC
ZIP433	CGTGAAAGGCGAGATCACCAAGGTAGTCGGCAAATAATGTCTGATTTTCGTGCACT GGTTGGATTGTC
ZIP434	CTGCAACGCGCATGGTTAACATCACC
ZIP316	GGCTCAAACGCGTCATCAATCG
ZIP317	GTTCTGGACCAGTTGCGTGAGCGCATGAACGACCTCAGGTATTCATCG
ZIP318	GAAAGGCGAGATCACCAAGGTAGTCGGCAAATAATGAATCCGTTACGAGTCAAAC TCG
ZIP319	GGCTAAAATACCCAGCATTTCGG
ZIP320	CCCATAGTCTCGATGAATACCTGAGGTCGTTTCATGCGCTCACGCAACTGG
ZIP321	AGGCGTCGAGTTTGACTCGTAACGGATTCATTATTTGCCGACTACCTTGGTGATCT CG
1640	TCCACAATCCAAAAAAGAGCGGCATTAAGCCGC
1641	GCGAGCATCGTTTGTTCCGCCAGCTTCTGTATGGAACGGGGTAACGCTATGATTCA TATCATTACTGGCAGC
1642	CGTGAAAGGCGAGATCACCAAGGTAGTCGGCAAATAATGTCTGTAATCAGCAGCC AACAAAAAGGCGACCTTTTGG
1643	ATGCGTGAGCGGATTGGCGATGATCGCCAGCGC
1644	CCGCACCGCCTAGGGTGCTGCCAGTAATGATATGAATCATAGCGTTACTCCCGTTC CATACAGAAGCTGGGCGAACAAACGATGCTCGC
1645	AGAGGCGGCCAAAAGGTGCGCTTTTTGTTGGCTGCTGATTACAGACATTATTTGCC GACTACCTTGGTGATCTCGCCTTTCACG
VC0741tgt5	CTATTATTTAAACTCTTTCggtgcgcgcaagatgctgctg
VC0741tgt7	CTACACAATCGCTCAAGACGTGagcctccaatgtcagaga
VC0741tgt8bis	CTAATTTCCATGTCAGCCGTTGCGGTGGGTGAGCCAAAAG
VC0741tgt6bis	TACGTAGAATGTATCAGACTGTGCACGAAATCAGGCTTTG
VC0379dusA5	CTATTATTTAAACTCTTTCagcagttcatagcacttgat
VC0379dusA7	CTACACAATCGCTCAAGACGTGaggtgaacgtccccatttca
VC0379dusA8	CTAATTTCCATGTCAGCCGTaaaacaccaacattagcgaatg
VC0379dusA6	TACGTAGAATGTATCAGACTcagtgcggtgactagcgccg
VC0291dusB5	CTATTATTTAAACTCTTTCgcatcaaacttggtgccg
VC0291dusB7	CTACACAATCGCTCAAGACGTGaacgtcttcagcttcgtac
VC0291dusB8bis	CTAATTTCCATGTCAGCCGTTAGCCGAAGACTGCGGCATA
VC0291dusB6bis	TACGTAGAATGTATCAGACTcggctagctcttctcttaaTT
VC0999truA5	CTATTATTTAAACTCTTTCGGAGCCGCAAGCGAAGCCTG
VC0999truA7	CTACACAATCGCTCAAGACGTGCTGTTTGTCTCAATAAAAATGG
VC0999truA8bis	CTAATTTCCATGTCAGCCGTCGATTCAGTGCCACTGCACG
VC0999truA6bis	TACGTAGAATGTATCAGACTTAGTTCAAATTATCAGGCAAG
VC0645truB5bis	CTATTATTTAAACTCTTTCGAATCTTGTCTGGCAGCATTG

VC0645truB7bis	CTACACAATCGCTCAAGACGTGCGTTTGTACCACTTGACCATC
VC0645truB8	CTAATTCATGTGTCAGCCGTattcgtggaaggctcgctg
VC0645truB6	TACGTAGAATGTATCAGACTgtacaaactgtgctttgcagc
VC0888truC5	CTATTATTTAAACTCTTTCCGGCGGTATCCATCGCTTGC
VC0888truC7	CTACACAATCGCTCAAGACGTGCTATTGGCACACCTTGTCGA
VC0888truC8	CTAATTCATGTGTCAGCCGTGATTTTCTCAATCAGCCCA
VC0888truC6	TACGTAGAATGTATCAGACTAATCGGCAAAGGCCGCTTAA
VC0154trmA5	CTATTATTTAAACTCTTTCCgcttcgttgccgtgtaaataa
VC0154trmA7	CTACACAATCGCTCAAGACGTGtcgggtaccttttatgggc
VC0154trmA8bis	CTAATTCATGTGTCAGCCGTGGGCGGTGATTGTAATCAA
VC0154trmA6bis	TACGTAGAATGTATCAGACTgctggTTACTTTTTGCGCTCT
VC0453trmB5	CTATTATTTAAACTCTTTCCagattgctgctctggcgt
VC0453trmB7	CTACACAATCGCTCAAGACGTGttctattgctgcacattactaaa
VC0453trmB8	CTAATTCATGTGTCAGCCGTtttgaccctaatcctcaagc
VC0453trmB6	TACGTAGAATGTATCAGACTtagagaggcactttgcagca
VC0803trmH5	CTATTATTTAAACTCTTTCCAAAAGCTTAGAACTGTTGTTGC
VC0803trmH7	CTACACAATCGCTCAAGACGTGGTGTGTCCGAGATAAATG
VC0803trmH8	CTAATTCATGTGTCAGCCGTAAATCCATTTACCTAAAACGAAT
VC0803trmH6bis	TACGTAGAATGTATCAGACTTATCGAACTCAACGCTGAG
VCA06345	CTATTATTTAAACTCTTTCCaagtgcagcaaataccagcc
VCA06347	CTACACAATCGCTCAAGACGTGctgtacctacatgctgtaa
VCA06348	CTAATTCATGTGTCAGCCGTgctagaagatgaaagcactc
VCA06346	TACGTAGAATGTATCAGACTcgtcgttagaatgcacag
VC0757rlmN5	CTATTATTTAAACTCTTTCCattgcccgaatgggtcac
VC0757rlmN7	CTACACAATCGCTCAAGACGTGgtcctctcaagaagacgg
VC0757rlmN8	CTAATTCATGTGTCAGCCGTttttgcctaatatggcaacaaatc
VC0757rlmN6	TACGTAGAATGTATCAGACTggttctctcaatgctttgga
VC1354rlmI5bis	CTATTATTTAAACTCTTTCCGCATAGCCGAAATCCACGTT
VC1354rlmI7bis	CTACACAATCGCTCAAGACGTGCTTGATATTTGTCGATAGTAATG
VC1354rlmI8	CTAATTCATGTGTCAGCCGTGCGATGGCGTGAGCGAACGT
VC1354rlmI6	TACGTAGAATGTATCAGACTCAAACAAGCCTTTTCTCG
VC0044rsmB5	CTATTATTTAAACTCTTTCCGTACGACAACTGGCAGAAC
VC0044rsmB7	CTACACAATCGCTCAAGACGTGCCGACGACGAGCGGCGCG
VC0044rsmB8	CTAATTCATGTGTCAGCCGTCTATTACGCCGTATTAAGC
VC0044rsmB6	TACGTAGAATGTATCAGACTCACCCTTGCAGCGCGCCC
VC0623rsmC5	CTATTATTTAAACTCTTTCCtttttaaccgcatcgcg
VC0623rsmC7	CTACACAATCGCTCAAGACGTGacagcctactcaagaaaa
VC0623rsmC8	CTAATTCATGTGTCAGCCGTctcgttacattttgctgcttt
VC0623rsmC6	TACGTAGAATGTATCAGACTggaagcattcaccagacc
VC0146rsmD5	CTATTATTTAAACTCTTTCCttgttcaacaatcgtgcagg
VC0146rsmD7	CTACACAATCGCTCAAGACGTGagattttttgaccgctaattaagtg
VC0146rsmD8	CTAATTCATGTGTCAGCCGTAagcactactctggttagcga
VC0146rsmD6	TACGTAGAATGTATCAGACTggtgaactgtggctacaagag
VC1502rsmF5	CTATTATTTAAACTCTTTCCAGAGTGAAAAACGCGGCTCG
VC1502rsmF7	CTACACAATCGCTCAAGACGTGAAGCGAGTTCCGTAGATGAAT

VC1502rsmF8	CTAATTCCCATGTCAGCCGTTGGCTGAACTATAGTATTAATTC
VC1502rsmF6	TACGTAGAATGTATCAGACTCATGATTTTCTCCTCAGATGTC
VC1179rluB5	CTATTATTTAAACTCTTTCCgtttcgtttgtaaaaaacaata
VC1179rluB7	CTACACAATCGCTCAAGACGTGttatctacctatgtgtcgtctt
VC1179rluB8	CTAATTCCCATGTCAGCCGTgctgtttattgttgaagctg
VC1179rluB6	TACGTAGAATGTATCAGACTcaatacttgcttgccga
VC0709rluD5ter	CTATTATTTAAACTCTTTCCATAGGTGCGTAAACCGCGCGC
VC0709rluD7ter	CTACACAATCGCTCAAGACGTGCGATAATGTCGTCATCTTCATA
VC0709rluD8ter	CTAATTCCCATGTCAGCCGTCTCGACCAAACGTACTTTAA
VC0709rluD6bis	TACGTAGAATGTATCAGACTTAATACTCTTCAGTGCGGTT
VC1140rluE5	CTATTATTTAAACTCTTTCCGATACGTTGTTCTTCGGTC
VC1140rluE7	CTACACAATCGCTCAAGACGTGTTAAGCACCTTGCAAAAATGT
VC1140rluE8	CTAATTCCCATGTCAGCCGTACTCTGATTAATCAAAAATATATAG
VC1140rluE6	TACGTAGAATGTATCAGACTGATGCACAAGAGGAGTCACA

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