Table S1. Full list of plasmids, bacteria strains used:

| Name                     | Relevant genotype or features  | Reference                    |
|--------------------------|--|------------------------------|
| Plasmids                 |  |                              |
| pMP96                    | $pSC101rep^{TS} oriT_{RP4} [int_{\lambda}-xis_{\lambda}, int_{HK}-xis_{HK}]$   | Val et al.<br>2012           |
| pCP20                    | pSC101 <i>rep</i> <sup>TS</sup> [ <i>flp</i> ]   | Cherepanov<br>et al. 1995    |
| pASB11                   | pCR-BluntII-Topo:: lox66-Zeo-lox71   | Soler-Bistué<br>et al. 2015  |
| pJBA28                   | delivery plasmid for mini-Tn5-Km-PA1/04/03-RBSII-<br>gfpmut3*-T0-T1, Amp <sup>R</sup> and Kan <sup>R</sup>   | Andersen<br>JB et al<br>1998 |
| pASB21                   | pCR-BluntII-Topo:: (gfpmut3* <i>Not</i> I fragment-lox66-Zeo <sup>R</sup> -lox71)  | This study                   |
| Escherichia coli         |  |                              |
| DH5a                     | $F^-$ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG<br>Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(r <sub>K</sub> <sup>-</sup> m <sub>K</sub> <sup>+</sup> ), $\lambda^-$  |                              |
| Vibrio cholerae          |  |                              |
| N16961 <i>ChapR∆lacZ</i> | N16961::mTn7 <i>hapR</i> <sup>+</sup> $\Delta lacZ$ using pMEV69   | Val et al. 2012              |
| Parental -1120           | PGB-A192:: <i>attB'-lox66-dfrB1-lox71</i> inserted in the intergenic region between VC1508-VC1509.   | Soler-Bistué<br>et al. 2015  |
| Parental C2+479          | PGB-A192:: <i>attB'-lox66-dfrB1-lox71</i> inserted in the intergenic region between VCA0543-VCA0544.   | Soler-Bistué<br>et al. 2015  |
| PGB-B393                 | N16961 $\Delta lacZ$ , [lox2272- <i>ere</i> ( <i>A</i> )-lox2272] (at the intergenic space VC2569-VC2570), [ <i>lox66-zeo<sup>R</sup>-lox71</i> ] (at the intergenic space VC2599-VC2600) flanking the region VC2569 and VC2599 encompassing <i>S10-spc-a</i> locus. | Soler-Bistué<br>et al. 2015  |
| S10Tnp+166               | S10 relocated closer to <i>oriC1</i> in the intergenic region between VC2739-VC2740.   | Soler-Bistué<br>et al. 2015  |
| S10Tnp-35                | S10 relocated next to its original location in the intergenic region between VC2536-VC2537.  | Soler-Bistué<br>et al. 2015  |

| S10Tnp-510                         | S10 relocated at the middle of the left replichore of chromosome 1 in the intergenic region between VC2075-VC2076.   | Soler-Bistué<br>et al. 2015 |
|------------------------------------|--|-----------------------------|
| S10Tnp-1120                        | S10 relocated near the <i>dif</i> region of chromosome 1 in the intergenic region VC1508-VC1509.   | Soler-Bistué<br>et al. 2015 |
| S10TnpC2+37                        | S10 relocated near the <i>oriC2</i> in the intergenic region between VCA0030-VCA0031.  | Soler-Bistué<br>et al. 2015 |
| S10TnpC2+479                       | S10 relocated near the <i>dif</i> sequence of chromosome 2 in the intergenic region between VCA0543-VCA0544.   | Soler-Bistué<br>et al. 2015 |
| PGB-B393                           | N16961 $\Delta lacZ$ , [lox2272- <i>ere(A)</i> -lox2272] (at the intergenic space VC2569-VC2570), [ <i>lox66-zeo<sup>R</sup></i> - <i>lox71</i> ] (at the intergenic space VC2599-VC2600) flanking the region VC2569 and VC2599 encompassing <i>S10-spc-a</i> locus. | Soler-Bistué<br>et al. 2015 |
| S10Md(-510;-1120)                  | Merodiploid bearing $S10$ -spc- $\alpha$ copies at the intergenic sequences of VC2075-VC2076 and VC1508-VC1509.  | Soler-Bistué<br>et al. 2015 |
| S10Md(-1120;C2+479)                | Merodiploid bearing $S10$ -spc- $\alpha$ copies at the intergenic sequences of VC1508-VC1509 and VCA0543-VCA0544.  | Soler-Bistué<br>et al. 2015 |
| Parental +166<br>$\Delta(aph,cat)$ | PGB-A192:: $attB$ '- $lox66$ - $dfrB1$ - $lox71 \Delta(aph, cat)$ inserted in<br>the intergenic region between VC2739-VC2740.<br>Kanamycin and chloramphenicol resistance cassettes were<br>deleted using a flipase expressing plasmid.                              | This study                  |
| S10Tnp+166<br>$\Delta(aph,cat)$    | S10 relocated closer to <i>oriC1</i> . Derived from Parental+166 $\Delta(aph, cat)$ .  | This study                  |
| S10Tnp-35 Δ( <i>aph</i> )          | S10Tnp-35 kanamycin resistance cassettes were deleted using a flipase expressing plasmid.  | This study                  |
| S10TnpC2+37 Δ( <i>cat</i> )        | S10TnpC2+37 chloramphenicol resistance cassettes were deleted using a flipase expressing plasmid.  | This study                  |
| S10M2(+166;0)                      | Merodiploid bearing 2 <i>S10-spc-a</i> copies at the intergenic regions between of VC2739-VC2740 and at its original location.   | This study                  |
| S10M2(+166;-35)                    | Merodiploid bearing 2 $S10$ -spc- $\alpha$ copies at the intergenic regions between of VC2739-VC2740 and VC2536-VC2537.  | This study                  |

| S10M2(+166;C2+37)           | Merodiploid bearing 2 <i>S10-spc-</i> $\alpha$ copies at the intergenic regions between of VC2739-VC2740 and VCA0030-VCA0031.  | This study |
|-----------------------------|--|------------|
| S10M3(+166;0;C2+37)         | Merotriploid bearing 3 <i>S10-spc-a</i> copies at wild type location plus two copies at the intergenic sequences between ORFs VC2739-VC2740 and VCA0030-VCA0031.   | This study |
| S10M3(+166;0;-35)           | Merotriploid bearing 3 <i>S10-spc-a</i> copies at wild type location plus two copies at the intergenic sequences between ORFs VC2739-VC2740 and VC2536-VC2537.   | This study |
| S10M4(+166;0;-<br>35;C2+37) | Merotetraploid bearing 4 <i>S10-spc-α</i> copies, one at wild type location and 3 others at the intergenic sequences between VC2739-VC2740, VC2536-VC2537 and VCA0030-VCA0031.   | This study |
| V.cholerae::gfpmut3*        | NotI fragment from pJBA28 (Andersen et al. (1998)) containing promoter $P_{A1/04/3}$ , RBSII and <i>gfpmut3</i> * linked to Zeo <sup>R</sup> from pASB21 gene was inserted in the intergenic region between VC0696-VC0697. | This study |
| Parental-<br>1120::gfpmut3* | <i>gfpmut3*-</i> ZeoR cassette was inserted in the intergenic region between VC0696-VC0697 in Parental-1120 strain.  | This study |