



Supplemental Fig. 1. The *rctA* promoter is located within the coding region of *yhd0053*. **A.** Scheme of the regions used in β -glucuronidase transcriptional fusions to map the *rctA* promoter and results from transcriptional activity assays. Fragment pVT encompasses the whole region, whilst fragments B, C and D are shortened derivatives from pVT. Specific β -glucuronidase activity is expressed as $\text{nmol min}^{-1}\text{mg of protein}^{-1}$. Data are means from at least three independent experiments \pm standard deviation. **B.** Detailed view of the boundaries of fragments B, C and D. The sharp reduction in β -glucuronidase activity observed with fragments C and D supports the predicted location of the *rctA* promoter. Moreover, the lack of activity observed with fragment D indicates that there are no additional promoters for *rctA* in the intergenic region. Fragments B, C and D were amplified using oligonucleotide pairs 38T1 (Table 1) and Bu (CTCGGCCCGCGCTTT), 38T1/Cu (CGCTTTGCTGCCCAAATAA) and 38T1/Du (GGTGGCGCAACGAAGCAG), respectively, and cloned in pCR2.1-TOPO yielding plasmids pSSH05, pSSH06 and pSSH07. To construct the transcriptional fusions each plasmid was digested using *SpeI/XhoI* to liberate the insert. Then, each fragment was cloned separately in plasmid pBBR1MCS53, linearized with *SpeI/XhoI*, resulting in the *rctA* promoter of each fragment cloned in the direction of the reporter gene.