Figure S1.



Engineering steps of the initial, intermediate construct harboring a new copy of *rrnB* with a downstream marker gene.

- I.a. Insertion of GH homology box (using pST76-A plasmid) next to the *rrnB* operon/donor site.
- I.b. Insertion of AB homology box (using pSG76-C plasmid) next to the acceptor/new insertion site.
- II. PCR amplification of the modified *rrnB* region (AB-rrnB-pST76-A-GH), and insertion into the modified acceptor site using AB and GH homology boxes.
- III. Replacing the pST76-A plasmid with a pSG76-CS-derived linear fragment containing the Cam<sup>R</sup> gene, two I-Scel sites, and a short homology region to GH (G').