Supplementary material

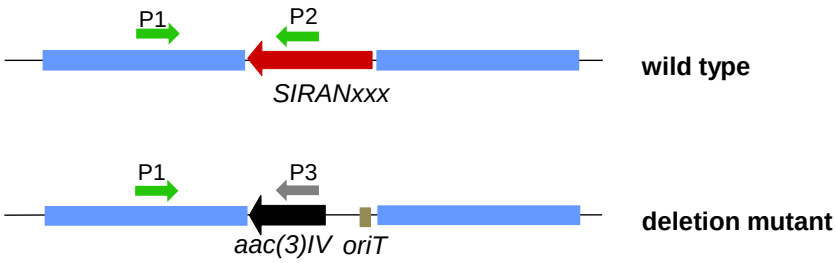

Figure S1. Screening for stable double-cross-over candidates by PCR (schematic overview). Genomic DNA of *S. iranensis* HM 35 wild type and deletion mutants, respectively, was used as template. A 500 – 700 bp PCR fragment obtained with primers P1 and P2 is indicative of the wild-type locus and is only expected for single cross-over mutants as well as for wild type controls. In contrast, primers P1 and P3 form a PCR product of a comparable size only in double-cross-over mutants due to binding of primer P3 to the apramycin resistance gene, which replaced the locus of the gene under investigation.

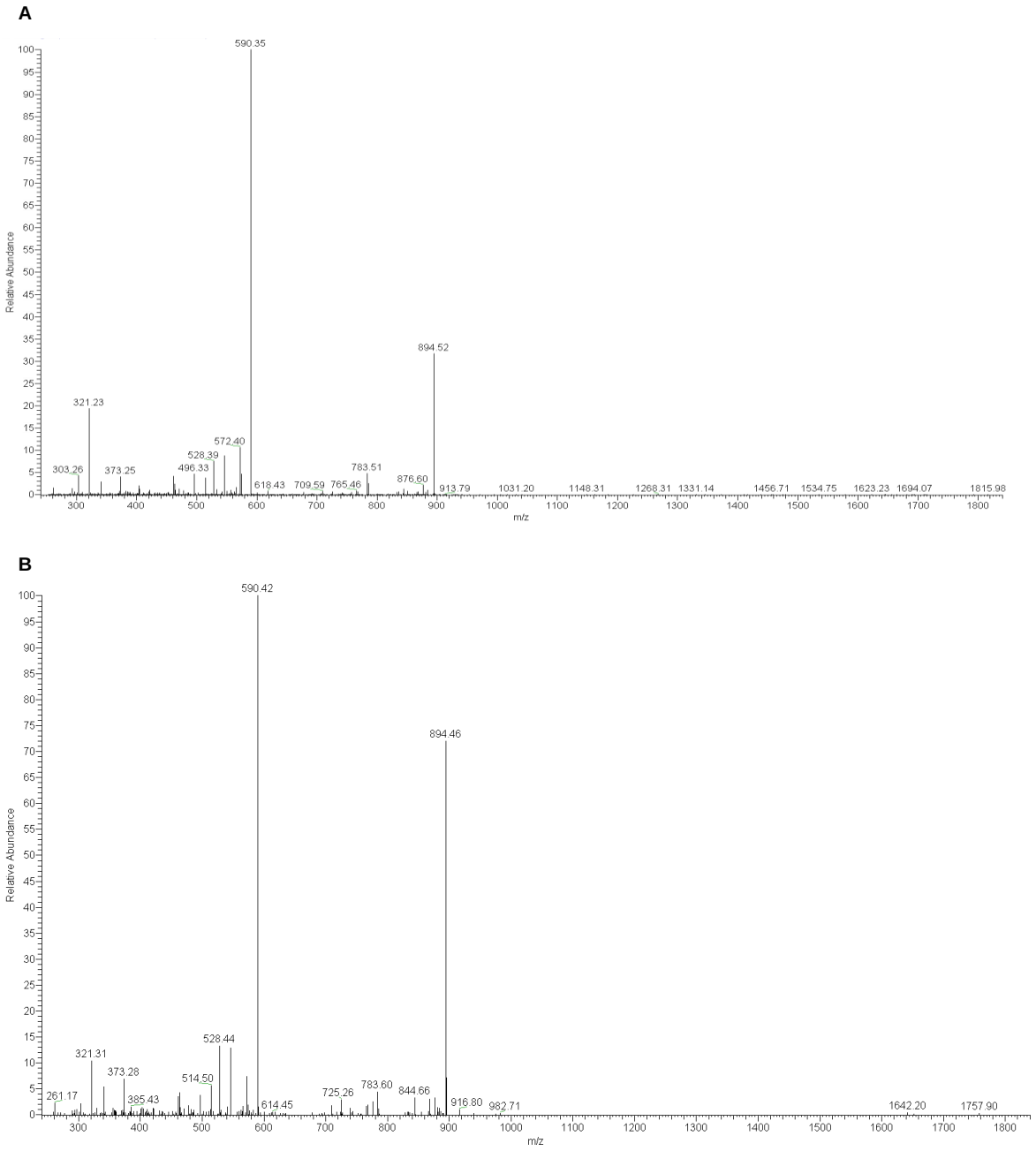

Figure S2. Tandem mass spectrum of **(A)** rapamycin standard (precursor ion *m/z* 912.65 [M-H]-) and **(B)** of rapamycin produced by *S. iranensis* HM35 (precursor ion *m/z* 912.53 [M-H]-).