



S5 Fig. Construction and confirmation of the *sigD::ermB* mutation in *C. difficile* R20291. (A) Schematic diagram of the Group II intron disruption of *sigD* in *C. difficile* R20291 (CDR20291_0270). The Targetron construct was designed previously to insert at nucleotide position 228 of the *sigD* gene in the sense orientation. The *sigD* gene is 702 bp. The forward and reverse primers, R1887 (red arrow) and R1888 (green arrow), partially flank *sigD* and produce a PCR product of 716 bp. Insertion of the Group II intron into *sigD* (*sigD::ermB*) results in a PCR product of ~2500 bp. A second PCR reaction was used to confirm *sigD::ermB* by using R991, a Group II intron specific primer called EBS Universal. A PCR reaction with the R1887 and R991 yields a product of ~450 bp if the Group II intron is in *sigD*. (B) Image of an EtBr stained agarose gel with PCR products for the indicated strains with primers detailed in (A).