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).