

Insertion of a spectinomycin resistance cassette into RD2 does not alter the transcript abundance of the upstream or downstream genes. The resistance cassette was inserted in the intergenic region between the RD2 genes M28.1329 and M28.1330. Note that this region was selected, in part, due to bioinformatic analyses not predicting any unannotated open-reading-frame (ORF) or small non-coding regulatory RNAs (sRNAs) in this region (data not shown). The relative abundance of transcripts from M28.1329 and M28.1330 were determined, via Taqman-based quantitative RT-PCR, between the parental and RD2-modified GAS strains. The experiment was performed in triplicate with mean (± standard deviation) shown. Data was tested for significance using the *T*-test.