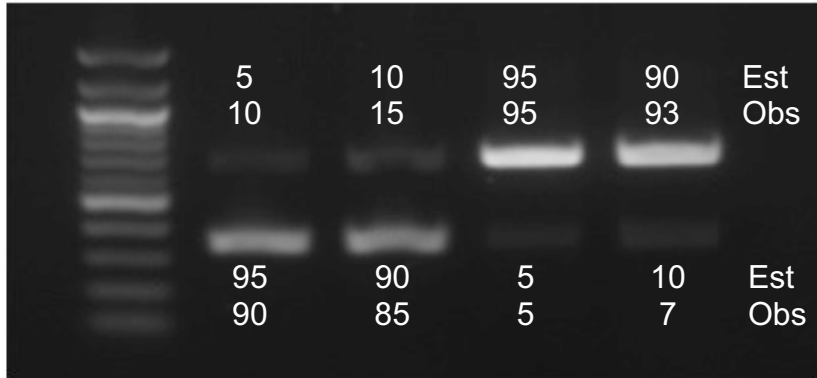


Figure S3: Validation of Restriction Digest-Based Genome Ratio Assessments



CHIKV SL07 RNA, either wild-type containing no restriction enzyme marker or with the E2-R198Q mutation and the Apal marker, was extracted from viral stocks and combined at four different ratios. The RNA mixtures were subjected to RT-PCR and restriction digest analysis as described in the materials and methods. Estimated ratios (on the basis of RNA input as determined by empirically determining the ratio required for a 50:50 observed banding pattern and then scaling the ratios to 5:95, 10:90, 95:5, and 90:10) and observed ratios (on the basis of GelQuant.net software) are noted above and below the relevant bands. An unpaired, two-tailed Student's t-test of the mutant:wild type band ratios found no statistically significant difference between the estimated values and the observed values ($p=0.530101$, ns).