

Expected number of mutant copies per PCR reaction	Expected mutation frequency	Average observed mutation frequency (n=3)
10	4×10^{-3}	$4.00 \times 10^{-3} \pm 0$
1	4×10^{-4}	$3.69 \times 10^{-4} \pm 4.8 \times 10^{-5}$
0.1	4×10^{-5}	$4.27 \times 10^{-5} \pm 2.0 \times 10^{-5}$
0.01	4×10^{-6}	$4.33 \times 10^{-6} \pm 5.13 \times 10^{-6}$

Table S4: Validation of the sensitivity and specificity of the RMC assay. Wild-type and mutant clones were mixed in known concentrations and the RMC assay carried out. This was done on 3 separate occasions. The observed mtDNA mutation frequencies were compared to the expected mtDNA mutation frequencies. There was no significant difference between the observed and expected frequencies ($p=0.99$ chi-squared test).