

Article S2. Standard curve construction for qPCR assays

To assure reproducibility of the standard curves among our qPCR assays (i.e., 96-well qPCR plates), we constructed plasmids that contain the target amplicon for producing qPCR standard solutions. To do this, we first designed primers to amplify a larger DNA fragment that covers the target region for the Common musk turtle (744 bp fragment on *cytb*):

Forward (5'-3'): ACGGCTGACTCATTCGAAAC

Reverse (5'-3'): TTTGGGTGAATGGTCGGAAC

We then ran conventional PCR assays with the reaction system contained: 25 μ L Taq FroggaMix (Froggabio, USA), 200 nM of both forward and reverse primers, 1 μ L genomic DNA extracted from blood samples, and reverse osmosis H₂O to a final volume of 50 μ L. The thermal cycling profile was as follows: 3 minutes of initial denaturation at 95 °C, 40 cycles of three-step amplification with 30 seconds of denaturation at 95 °C, 1 minute of annealing at 57 °C and 1 minute of extension at 72 °C, and 10 minutes of final extension at 72 °C.

The PCR products were verified on 1.5% (w/v) agarose gel for fragment size. Purified PCR products were then inserted into a pMiniT 2.0 plasmid using the NEB® PCR Cloning Kit (NEB, USA) following the manufacturer's protocol. The insertion was further confirmed via Sanger sequencing using the primers provided by the kit. The plasmids were quantified by Qubit 3.0 fluorometer (ThermoFisher Scientific, USA). Using this plasmid for qPCR, we found minimal degradation of the positive templates for standard curves hence consistence performance across qPCR plates.