Evaluation of the sensitivity of a mini-barcode

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Abstract

We developed a mini-barcode based on the 12S rRNA gene to identify predator from scats in Tasmania.

To test the sensitivity of our primers to detect low template DNA samples, we set up serial dilutions of six DNA extracts originating from museum samples, representing each of the six mammal predators that might be detected in Tasmania (Tasmanian devil, eastern quoll, spotted tail quoll, cat, dog and fox).

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Materials

- BSA-Molecular Biology Grade - 12 mg B9000S by New England Biolabs
- Magnesium Chloride (MgCl2) Solution - 6.0 ml B9021S by New England Biolabs
- dNTPs by Contributed by users
  Gold buffer by Applied Biosystems
  SYBR Green by Life Technologies
  AmpliTaq Gold by Applied Biosystems

Protocol

Set up starting dilutions

Step 1.

The DNA concentration of each original DNA extraction was determined using a QuBit Fluorometer and the Qubit dsDNA BR Assay Kit (Thermo Fisher) and diluted with ddH_2O if necessary to obtain a starting concentration of 90 ng / µl

Set up 10 x dilutions

Step 2.

set up a series of six 10 X dilutions from each of these “undiluted” (90 ng / µl) samples

from 90 ng / µl to 0.09 pg/µl

qPCR replicates

Step 3.

For each dilution of each sample, perform three qPCR replicates (on a Viia7 Real-Time PCR system (Thermo Fisher Scientific)) : total volume of 25µl including 1X Gold buffer (Applied Biosystems), 2 mM $MgCl^2$, 0.4 mg / ml BSA, 0.4 µM of each primer, 0.6 µl SYBR green (1:2000 Life Technologies nucleic acid gel stain), 0.25 mM of each dNTP, 1 unit of AmpliTaq Gold™ (Applied Biosystems) and 2 µl of the appropriate DNA dilution.

qPCR protocol: initial step of 95 °C for 5 mins; followed by 40 cycles of 95 °C for 30 s, 57 °C for 30 s and 72 ° for 30 s.

Comparative CT analysis

Step 4.

Using the ViiA7 software v1.2.4 with a threshold of 5,000 ΔRn, conduct a comparative CT analysis by calculating the mean CT value and the standard deviation across qPCR replicates for each dilution of each DNA sample.

Evaluate to which dilution the mini-barcode is effective.