PCR PROTOCOL

Purpose: Chloroplast DNA barcoding of matK

Original Source: Ford et al. 2009; Dunning and Savolainen 2010

Document By: BG (1/06/2020).

Notes: Protocols developed and used by the Smithsonian/Guelph with helpful suggestions from Masha Kuzmina. The marker is coding, but length variable. Target lengths ~500-800bp.

Primers:

matK-xf	TAATTTACGATCAATTCATTC
matK-MALP	ACAAGAAAGTCGAAGTAT

Reaction (25 µL)

REAGENTS (Final concentration)	**1 X (µL)**
DMSO (3%)	0.3
MOLECULAR WATER	5.375
5X HF Buffer (w $MgCl_2$)	2
10 mM dNTPs	0.2
PRIMER1 – 10uM (0.2µM final)	0.5
PRIMER2 – 10uM (0.2µM final)	0.5
Phusion Hot Start II DNA Polymerase	0.125
DNA TEMPLATE	**1**

***Make extra master mix and include a negative and positive control.**

Program: Phusion MatK Bar on Eppendorf Mastercycler Nexus GSX1

Process	Temperature (°C)	Time
Initial denaturing	98	45 s
35 cycles: Denaturing	98	10 s
35 cycles: Annealing	54	30 s
35 cycles: Extension	72	40 s
Final Extension	72	10 min
Hold	4	Hold

Approximate total time: 2 hour.