PCR PROTOCOL

Purpose: trnH-psbA DNA barcoding of plants

Original Source: Sang et al. 1997; Tate and Simpson 2003

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

Notes: Protocols developed/implemented by Smithsonian/Guelph. Targets a non-coding/length-variable locus.

Primers:

psbA3_f	GTTATGCATGAACGTAATGCTC
trnHf_05	CGCGCATGGTGGATTCACAATCC

Reaction (25 µL)

REAGENTS (Final concentration)	**1 X (µL)**
DMSO (3%)	0.3
MOLECULAR WATER	6.320
5X HF Buffer (w $MgCl_2$)	2
10 mM dNTPs	0.056
PRIMER1 – 10**µ**M (0.2µM final)	0.1
PRIMER2 – 10**µ**M (0.2µM final)	0.1
Phusion Hot Start II DNA Polymerase	0.125
DNA TEMPLATE	**1**

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

Program: Phusion Trnh-Psba Bar on Eppendorf Mastercycler Nexus GSX1

Process	Temperature (°C)	Time
Initial denaturing	98	45 s
35 cycles: Denaturing	98	10 s
35 cycles: Annealing	64	30 s
35 cycles: Extension	72	40 s
Final Extension	72	10 min
Hold	4	Hold

Approximate total time: 80 min.