PCR PROTOCOL

Purpose: Chloroplast DNA sequencing *rbc*L*a*

Original Source: Kress et al 2009

Document By: PTF (1/13/2020)

Notes: This is the PCR protocol that has been used with the NEB Taq for plant DNA barcoding. Target size is 550 bp (coding region).

Primers:

rbcLa-F: ATGTCACCACAAACAGAGACTAAAGC
rbcLa-R: GTAAAATCAAGTCCACCRCG

Reaction (12.5 µL) using standard NEB Taq buffer without $MgCl_2$.

REAGENTS (Final concentration)	**1 X (µL)**
10X Buffer without Mg	1.25
25 mM $MgCl_2$ (2.5 mM final)	1.25
dNTPs mix -mM EACH (200**u**M EACH final)	0.25
PRIMER1 – 10uM (0.2**u**M final)	0.25
PRIMER2 – 10uM (0.2**u**M final)	0.25
BSA - mg/ml (0.1 mg/ml final)	0.10
DMSO (4% final)	0.05
MOLECULAR WATER	7.075
NEB TAQ	0.063
DNA TEMPLATE	**2.0**

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

Program: *Stand50* on Eppendorf Mastercycler Nexus GSX1

Process	Temperature (‘C)	Time
Initial denaturing	95	10 min
35 cycles: Denaturing	94	30 s
35 cycles: Annealing	50	30 s
35 cycles: Extension	72	45 s
Final Extension	72	10 min
Hold	4	Hold

Approximate total time: 2.0 hours.