*rbc*L PCR PROTOCOL

Purpose: rbcLa for standard plant DNA barcoding.
Original Source: Levin et al (2003); Kress et al (2009)
Document By: TRK (10/12/21); updated IJM/BLC (05-19-2022)

Notes: Chloroplast DNA barcode protocol for *rbc*La as adapted from Smithsonian Institution's standard plant DNA barcoding protocol.

Target size: 555 bp
Primers:

rbcLa-F ATGTCACCACAAACAGAGACTAAAGC
rbcLa-R GTAAAATCAAGTCCACCRCG

Reaction volume (12.5 µL)

Reagent (final concentration)	Reagent brand and input concentration (product info)	1 X (µL)
Molecular water	Corning (#46-000-CV)	6.625
10X buffer -$MgCl_2$ (1x)	NEB standard buffer (#M0320L pack or #B9015S)	1.25
$MgCl_2$ (2.50 mM)	NEB standard 25 mM $MgCl_2$ (#M0320L pack or #B9015S)	1.25
dNTP mix (200 µM EACH)	NEB 10 mM EACH (#N0447L)	0.25
rbcLa-F (0.20 µM)	IDT 100uM (10 µM working stock)	0.25
rbcLa-R (0.20 µM)	IDT 100uM (10 µM working stock)	0.25
DMSO (4.00%)	NEB 100% DMSO (#B0515AVIAL)	0.5
BSA - mg/mL (0.10 mg/mL)	NEB 20 mg/mL (#B9000S)	0.0625
Taq polymerase (25.0 u/mL)	NEB Standard Taq 5000 u/mL (#M0320L)	0.0625
DNA TEMPLATE		**2.0**

***Make extra master mix (1x per ~24 rxns); and include a negative and positive control.**

Program: *NEBTaqrbcL55* on Eppendorf Mastercycler Nexus GSX1

Process	Temperature (°C)	Time
Initial denaturing	95	4 min
35 cycles: Denaturing	94	30 s
35 cycles: Annealing	55	1:00 min
35 cycles: Extension	72	1:00 min
Final Extension	72	10 min

Hold	10	Hold

Approximate total time: 2 hours 10 mins.