PCR PROTOCOL

Purpose: 'Full length' trnL chloroplast DNA sequencing.

Original Source: Taberlet *et al.* 1991

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Notes: This is the standard PCR protocol to develop a reference library to which dietary P6 sequences may be matched (P6 is a short loop within *trn*L). It is often used for intraspecific population genetic or phylogenetic studies requiring variation in chloroplast DNA haplotypes. Target size varies ~600bp.

Primers:

trnL(UAA)c	CGAAATCGGTAGACGCTACG
trnL(UAA)d	GGGGATAGAGGGACTTGAAC

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 (200µM EACH final)	1.00
PRIMER1 – 10uM (0.2µM final)	0.25
PRIMER2 – 10uM (0.2µM final)	0.25
BSA - mg/ml (0.1 mg/ml final)	0.10
DMSO (4% final)	0.05
MOLECULAR WATER	6.28
NEB TAQ	0.06
DNA TEMPLATE	**2.0**

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

Program: *cp50* on Eppendorf Mastercycler Nexus GSX1

Process	Temperature ('C)	Time
Initial denaturing	95	4 min
35 cycles: Denaturing	94	30 s
35 cycles: Annealing	50	30 s
35 cycles: Extension	72	1:15 min
Final Extension	72	5 min
Hold	10	Hold

Approximate total time: 2.0 hours.