PCR PROTOCOL
Purpose: trnL-P6 DNA metabarcoding with Nextera-XT overhangs
Original Source: Taberlet et al. 2007
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Notes: Locus specific primers target trnL-P6 locus of plant chloroplast DNA and have been modified to include Nextera-XT overhangs. These overhangs permit Illumina library preps using Nextera protocols. The locus specific primers from Taberlet et al. 2007 were (g: A49425) and (h: B49466).

Primers:

trnL g	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGGCAATCCTGAGCCAA
trnL h	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCATTGAGTCTCTGCACCTATC

Reaction (15 µL reactions using Amplitaq Gold II); load reagents in the order that follows.

Reagents	**Final Concentration**	**Volume for 1 Rxn (µL)**
Molecular grade water		7.99
10X Amplitaq Gold II Buffer (w/o $MgCl_2$)	1X	1.50
25 mM $MgCl_2$	2.5 mM	1.50
10 mM dNTPs (2.5 mM each)	200 µM each	1.20
10 µM forward primer (trnL(UAA)g)	0.2 µM	0.30
10 µM reverse primer (trnL(UAA)h)	0.2 µM	0.30
100% DMSO	4%	0.060
20 mg/mL BSA	0.1 mg/mL	0.075
Amplitaq Gold Polymerase		0.075

Total Master Mix Volume (for 1 Rxn): 13 µL
DNA Template: 2 µL (1-500ng, ideally about 25)
Total Reaction Volume: 15 µL

***Make extra master mix and include a negative and positive control. Use extraction blanks and 'mock' mixes if possible.**

Program: Standard55 on Eppendorf Mastercycler Nexus GSX1

Step #	**Temp (°C)**	**Time**
1 cycle	95	10 min
35 cycles	95	30 sec
	55	30 sec
	72	30 sec
1 cycle	72	2 min
Hold	4	Forever

Approximate total time: 65 minutes