*trn*L-P6 c/h PCR Protocol

Purpose: Amplifying plant *trn*L-p6 for DNA metabarcoding of fecal samples
Original Sources: Taberlet et al. 1991; Taberlet et al. 2007
Document By: IJM/BLC (5/19/2022); ACJ (5/27/2025); TRK (10/29/2025)

Notes: This PCR protocol for *trn*L-p6 DNA metabarcoding has been used for many projects in the lab. It amplifies a portion of the chloroplast *trn*L gene (inclusive of the p6) using the c and h primers; this gives longer reads than the more commonly used g and h primers. The primers were modified to include Nextera-XT overhangs to permit Illumina library preps using Nextera protocols. This protocol is modeled after our *trn*L-p6 g/h protocol with reduced $MgCl_2$ and annealing time.
Target size: 110-243 bp

Primers:
F_C_trnL TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGAAATCGGTAGACGCTACG**
R_H_trnL (or "trnL_H") GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CCATTGAGTCTCTGCACCTATC**

Reaction volume (25 µL)

Reagent (final concentration)	**Reagent brand and input concentration (product info)**	**1 X (µL)**
Molecular water	Corning (#46-000-CV)	18.15
Platinum taq buffer -$MgCl_2$ (1x)	Invitrogen 10x (pack #10966026)	2.5
Platinum taq $MgCl_2$ (1.5 mM)	Invitrogen 50 mM (pack #10966026)	0.75
dNTP mix (0.2 mM each)	NEB 10 mM each (#N0447L)	0.5
F_c_trnL (0.2 µM)	IDT 100uM (10 µM working stock)	0.5
R_H_trnL (0.2 µM)	IDT 100 uM (10 µM working stock)	0.5
Platinum Taq DNA polymerase	Invitrogen (pack #10966026)	0.1
DNA TEMPLATE		**2.0**

***Make extra master mix (1x per ~24 rxns); and include a positive and negative control along with the extraction blanks that correlate with the samples you're working with.**

Program: platinum_p6_55 30 s on Eppendorf Mastercycler Nexus GSX1

Process	Temperature (°C)	Time
Initial denaturing	95	5 min
35 cycles: Denaturing	95	30 s
35 cycles: Annealing	55	30 s
35 cycles: Extension	72	1 min
Final Extension	72	10 min
Hold	4	Hold

Approximate total time: 2.0 hours.