*trn*L-P6 g/h PCR protocol using KAPA HiFi polymerase

Purpose: Amplify plant *trn*L-p6 using high fidelity polymerase for dietary DNA metabarcoding of fecal samples.
Document By: CPH and ACJ
Original Source: B.L. Petrone et al., Diversity of plant DNA in stool is linked to dietary quality, age, and household income, Proc. Natl. Acad. Sci. U.S.A. 120 (27) e2304441120

Notes: This protocol is based on ones we developed previously and used in the Kartzinel Lab (Taberlet et al. 2007), but it was subsequently improved upon by the "FoodSeq" team at Duke for applications involving human diets. Petrone et al. reported this high fidelity polymerase (KAPA HiFi) together with other tweaks to the reaction and thermocycler protocols enhanced PCR success rates and increased target read counts with difficult samples.

Target size: ~8-160 bp

Primers: Actual primer in **bold**

Forward Primer	trnL_g	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GGGCAATCCTGAGCCAA**
Reverse Primer	trnL_h	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CCATTGAGTCTCTGCACCTATC**

Reaction Volume: (20 µL)

Reagent (final concentration)	**Reagent brand and input concentration (product info)**	**1 X (µL)**
Molecular water	Corning (#46-000-CV)	10
KAPA HiFi Buffer (5x)	KAPA HiFi Fidelity Buffer (5X)	4
KAPA dNTPs (300 µM each)	KAPA dNTP mix (10 mM each)	0.6
NEB DMSO (1%)	DMSO 100% (#00619733)	0.2
F_G_trnL (0.5 µM)	IDT 100 µM (10 µM working stock)	1
R_H_trnL (0.5 µM)	IDT 100 µM (10 µM working stock)	1
KAPA HiFi Polymerase (10 U/mL)	KAPA HiFi HotStart DNA Polymerase (1 U/µL)	0.2
DNA TEMPLATE		**3.0**

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

Program: "foodseq_hifi_trnL" on Eppendorf Mastercycler Nexus GSX1

Process	Temperature (°C)	Time
Initial denaturing	95	3 min
35 cycles: Denaturing	98	20 s
35 cycles: Annealing	63	15 s

35 cycles: Extension	72	15 s
Final Extension	72	5 min
Hold	12	Hold

Approximate total time: 1.0 hours.