

## 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** (25 µL reactions using the most basic Platinum Taq)

| Reagent                              | Final Concentration | Volume for 1 Reaction |
|--------------------------------------|---------------------|-----------------------|
| Molecular grade water                |                     | 17.9                  |
| 10X PCR Buffer                       | 1X                  | 2.5                   |
| 50mM MgCl <sub>2</sub>               | 2 mM                | 1.0                   |
| Forward primer (10µM)                | 0.2 µM              | 0.5                   |
| Reverse primer (10µM)                | 0.2 µM              | 0.5                   |
| 10mM total dNTP (2.5mM each)         | 0.2 mM each         | 0.5                   |
| Platinum Taq DNA Polymerase (5 U/µL) | 2 U/rxn             | 0.1                   |

**Total Master Mix Volume (for 1 Rxn):** 23.0 µL

**DNA Template:** 2 µL (1-500ng, ideally about 25)

**Total Reaction Volume:** 25 µL

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

**Program:** NAME on Eppendorf Mastercycler Nexus GSX1

| Step #    | Temp (°C) | Time    |
|-----------|-----------|---------|
| 1 cycle   | 95        | 5 min   |
| 35 cycles | 95        | 30 sec  |
|           | 55        | 40 sec  |
|           | 72        | 60 sec  |
| 1 cycle   | 72        | 10 min  |
| Hold      | 4         | Forever |

Approximate total time: 90 min.