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# Protocol for amplification of three DNA regions from *Nephila clavipes* (Araneae: Araneidae). V.2

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## Abstract

Protocol for amplification of the mitochondrial gene "Citocrome Oxidase I" and the nuclear genes "subunit a from the Histone 3" and "Internal Transcribed Spacer 2" from *Nephila clavipes* (Araneae: Araneidae).

## Attachments



Protocol for ampli...

13KB

## Guidelines

### **Mitochondrial gene Cytochrome Oxidase I (COI)**

Primers: LCO1490 and HCO2198

Reference: Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**(5), 294–299.

PCR Reaction: 1µL of template DNA, 1U of Taq DNA Polymerase (Thermo-Fisher), 2.5µL of 10X KCl Buffer (Thermo-Fisher), 2.8µL of 25mM MgCl<sub>2</sub> (Thermo-Fisher), 1µL of 10mM dNTP mix (GE Healthcare), 0.5µL of each forward and reverse primer 10µM, and ddH<sub>2</sub>O to the volume of 25µL.

PCR cycling: a denaturation step of 3 min at 94°C, 30 cycles of a denaturation step at 94°C for 45s, an annealing step at 51°C for 45s and an extension step at 72°C for 2 min, and a final extension step at 72°C for 3 min.

### **Nuclear gene for the subunit a of the Histone 3 (H3a)**

Primers: H3aF2 and H3aR2

Reference: Colgan, D.J., McLauchlan, A., Wilson, G.D.F. *et al.* (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, **46**, 419–437.

PCR Reaction: 1µL of template DNA, 1U of Taq DNA Polymerase (Thermo-Fisher), 2.5µL of 10X KCl Buffer (Thermo-Fisher), 2.3µL of 25mM MgCl<sub>2</sub> (Thermo-Fisher), 0.5µL of 10mM dNTP mix (GE Healthcare), 0.5µL of each forward and reverse primer 10µM, and ddH<sub>2</sub>O to the volume of 25µL.

PCR cycling: a denaturation step of 3–4 min at 94°C, 30 cycles of a denaturation step at 94°C for 45s, an annealing step at 46°C for 1 min and an extension step at 72°C for 1 min, and a final extension step at 72°C for 10 min.

### **Nuclear intron internal transcribed spacer 2 (ITS2)**

Primers: 5.8S and ITS4

Reference: White, T.J., Bruns, T., Lee, S., Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, *et al.*, eds. *PCR Protocols: A Guide to Methods and Applications*. New York: Academic Press, 315–322.

PCR Reaction: 1µL of template DNA, 1U of Taq DNA Polymerase (Thermo-Fisher), 2.5µL of 10X KCl Buffer (Thermo-Fisher), 3.5µL of 25mM MgCl<sub>2</sub> (Thermo-Fisher), 0.5µL of 10mM dNTP mix (GE Healthcare), 0.4µL of each forward and reverse primer 10µM, and ddH<sub>2</sub>O to the volume of 25µL.

PCR cycling: a denaturation step of 4 min at 95°C, 30 cycles of a denaturation step at 95°C for 45s, an annealing step at 61°C for 45s and an extension step at 72°C for 2 min, and a final extension step at 72°C for 10 min.

