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

**Luiz Filipe Bartoleti**

## 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).

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## Guidelines

### Mitochondrial gene Citochrome 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 $MgCl_2$ (Thermo-Fisher), 1µL of 10mM dNTP mix (GE Healthcare), 0.5µL of each forward and reverse primer 10µM, and $ddH_2O$ to the volume of 25µL.

PCR cycling: a denaturation step of 3 min at 94ºC, 30 cycles of a denaturation step at 94ºC for 45s, an annealing step at 51ºC for 45s and an extension step at 72ºC for 2 min, and a final extension step at 72ºC 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 $MgCl_2$ (Thermo-Fisher), 0.5µL of 10mM dNTP mix (GE Healthcare), 0.5µL of each forward and reverse primer 10µM, and $ddH_2O$ to the volume of 25µL.

PCR cycling: a denaturation step of 3-4 min at 94ºC, 30 cycles of a denaturation step at 94ºC for 45s, an annealing step at 46ºC for 1 min and an extension step at 72ºC for 1 min, and a final extension step at 72ºC 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 $MgCl_2$ (Thermo-Fisher), 0.5µL of 10mM dNTP mix (GE Healthcare), 0.4µL of each forward and reverse primer 10µM, and $ddH_2O$ to the volume of 25µL.

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

## Protocol