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Genomic DNA Isolation Methods From Honey Bee (*Apis mellifera* L.) Spermatheca

Carlos A. Yadró¹, Ana R. Lopes¹, Dora Henriques¹, Chiraz Soltani¹, Manoela Marques¹, Jakob Wegener², Eduard Musin², M. Alice Pinto¹

¹Centro de Investigação de Montanha- Instituto Politécnico de Bragança, Bragança, Portugal

² Institute for Bee Research Hohen Neuendorf, Hohen Neuendorf, Germany

Corresponding author: carlosgarcia@ipb.pt

Abstract:

The honey bee queen (*Apis mellifera* L.) has a polyandrous mating system, meaning that the queen mates on average with 17 drones from the surroundings in a congregation area. After the mating event, the spermatozoa of the drones are stored in an organ called spermatheca. Genetic analysis of the spermathecal content can provide an estimate of the genetic diversity and purity of the surrounding honey bee populations. This can be particularly useful for conservation and mating centers that need to monitor their populations' genetic backgrounds. However, isolating enough DNA for genomic applications from such a small and complex matrix can be a challenge. Here, we compared the quantity and quality of DNA isolated using five methods: (i) phenol-chloroform-isopropanol, (ii) QIAamp DNA Minikit, (iii) QIAamp DNA Microkit, (iv) Macherey-Nagel Nucleospin Tissue, and (v) NEB Monarch Genomic DNA Purification Tissue. For each kit, when appropriate, variations including different isolation protocols, lysis incubation times, and the addition of RNA carrier were assayed. The quantity and quality of DNA extracted was assessed by spectrophotometric (SpectroStar®Nano LVis Plate) and fluorometric methods (Quantus™ Fluorometer). Spectrophotometric quantification indicated nucleic acid concentrations ranging from 2.00 to 55.58 ng/μL, and in 91.43% of the cases, the A260/280 ratios were over 2.00, indicating an elevated presence of RNA. The fluorometric quantification, specific for double-stranded DNA, provided values ranging from 0.02 to 2.30 ng/μL. From the five methods, two alternative protocols of the commercial kit QIAamp DNA Microkit produced a sufficient DNA quantity (≥ 1.7 ng/μL measured by Quantus) for applications involving SNP genotyping, namely: the Tissue protocol with 6 hours of lysis incubation and the Tissue protocol with 3 hours of incubation, both with addition of RNA carrier. In contrast, overnight lysis decreased the DNA yield. The other methods generally produced low and/or inconsistent DNA recovery. According to our results, QIAamp DNA Microkit with the use of RNA carrier and lysis incubation times between 3 to 6 hours produce the required DNA quantities for SNP genotyping.

Keywords: DNA isolation, *Apis mellifera*, spermatheca, conservation

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