Bench sheet · EnGeL-MOLB-DNA-BARC

DNA extraction from single arthropod specimens for DNA barcoding (Qiagen DNeasy Blood & Tissue Kit) · version 1.1.2 (2026-11-20). Full protocol: EnGeL-MOLB-DNA-BARC. Tick each step when done; write deviations in Notes.

Date	
Operator	
Plate ID / samples	
DNeasy Blood & Tissue Kit lot	

Before starting

☐	No.	Step
☐	•	Clean the working space and material with bleach and water and leave the UV light on for at least 15 min.
☐	•	Use filter tips at all steps.
☐	•	Clean forceps and spatulas with 3% sodium hypochlorite and 96% ethanol between samples.
☐	•	Perform all centrifugation steps at room temperature (15–25 °C).
☐	•	Include an extraction blank [TO CONFIRM: number per batch].

Lysis

☐	No.	Step
☐	1	Specimens > 1 cm: remove 3 legs from one side (usually the left) [TO CONFIRM: the source says the legs were “macerated one 1h after incubation”: when exactly are they macerated?]. Specimens < 1 cm: use the whole individual (non-destructive approach).
☐	2	Add 180 µL of Buffer ATL and 20 µL of Proteinase K. Mix by vortexing.
☐	3	Incubate at 56 °C until completely lysed (overnight, 10–12 h), vortexing occasionally.
☐	4	Vortex for 15 s.
☐	5	Optional, for non-destructive extraction: recover the specimen for the collection [TO CONFIRM: storage of the specimen].

DNA purification

☐	No.	Step
☐	6	Add 200 µL of Buffer AL (or Buffer NL, NZYtech). Mix thoroughly by vortexing.
☐	7	Add 200 µL of 96–100% ethanol (210 µL if Buffer NL was used). Mix thoroughly by vortexing.
☐	8	Pipette the mixture into a DNeasy Mini spin column in a 2 mL collection tube. Centrifuge at ≥ 6 000 × g for 1 min. Discard the flow-through.
☐	9	Place the column in a new 2 mL collection tube. Add 500 µL of Buffer AW1. Centrifuge at ≥ 6 000 × g for 1 min. Discard the flow-through.
☐	10	Place the column in a new 2 mL collection tube. Add 500 µL of Buffer AW2. Centrifuge at 20 000 × g for 3 min. Discard the flow-through and the collection tube.
☐	11	Dry spin: place the column in a new collection tube and centrifuge at 20 000 × g for 2 min.
☐	12	Transfer the column to a new 1.5 mL or 2 mL tube. Add 200 µL of Buffer AE (previously warmed [TO CONFIRM: temperature]) to the centre of the membrane.
☐	13	Incubate at room temperature (15–25 °C) for 1 min. Centrifuge at ≥ 6 000 × g for 1 min to elute.

DNA storage and quality assessment

☐	No.	Step
☐	14	Store the eluted DNA at 4 °C for short-term use or at −20 °C for long-term storage.
☐	15	Assess DNA purity by spectrophotometry (e.g. NanoDrop; A_{260}/A_{280} and A_{260}/A_{230} ratios) and quantify DNA concentration by fluorometry (e.g. Qubit), following EnGeL-MOLB-NANODROP and EnGeL-MOLB-QUBIT (or EnGeL-MOLB-PICOGREEN for plates). Record values in the database and the lab book.

After finishing

☐	No.	Step
☐	•	Clean the bench with 70% ethanol.
☐	•	Empty the small bench bin into the right waste bin.
☐	•	Wash your hands.
☐	•	Turn off equipment and lights.
☐	•	Lock doors, fridge and freezer.

Notes / deviations
