Bench sheet · EnGeL-MOLB-DNA-BEE

Genomic DNA isolation from honey bee guts (NZY Soil gDNA Isolation Kit) · version 1.2.2 (2026-11-20). Full protocol: EnGeL-MOLB-DNA-BEE. Tick each step when done; write deviations in Notes.

Date	
Operator	
Plate ID / samples	
NZY Soil gDNA Isolation 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.
☐	•	Include an extraction blank [TO CONFIRM: number per batch].

1. Sample preparation

☐	No.	Step
☐	1	Remove the bees from the −80 °C freezer and put them in groups of three individuals in 1.5 mL disposable centrifuge tubes. Keep the tubes at 0–4 °C until disinfection.
☐	2	Disinfect the cuticles: soak each group of three bees for 2–3 min in 0.05% aqueous sodium hypochlorite (or 1% household bleach), then rinse three to five times in distilled water. If necessary, remove excess water from the bodies.
☐	3	Add 180 µL of Buffer NSL1 plus 20 µL of Proteinase K to a 1.5 mL tube and mix gently.
☐	4	Dissect the bees with flame-sterilised tools under aseptic conditions and on ice.
☐	5	Remove the guts and submerge them, by group of three bees, in the Buffer NSL1 + Proteinase K mixture.
☐	6	Vortex for 5 s. Incubate for 2 h at 56 °C.
☐	7	Vortex for 5 s. Centrifuge at 3 000 × g for 20 s.
☐	8	Add 500 µL of Buffer NSL1 to an NZYSpin Soil Bead Tube and transfer the sample into it by pipetting.

2. Sample lysis

☐	No.	Step
☐	9	Add 150 µL of NS Enhancer and close the cap.
☐	10	Place the bead tube in the BeadBlaster 24R and run: speed 6.0 m/s; time 30 s; 1 cycle; pause time 300 s.

3. Contaminant precipitation

☐	No.	Step
☐	11	Centrifuge for 2 min at 11 000 × g to eliminate the foam.
☐	12	Add 150 µL of Buffer NSL3 and vortex for 5 s. Incubate for 5 min at 0–4 °C. Centrifuge for 1 min at 11 000 × g.

4. Lysate filtration

☐	No.	Step
☐	13	Load up to 700 µL of supernatant onto an NZYSpin Soil Inhibitor Removal Column (red ring) in a 2 mL collection tube. Centrifuge at 11 000 × g for 1 min and discard the column. If there is more than 700 µL, load the rest through the column in a new collection tube and combine the flow-throughs. If a pellet is present in the flow-through, transfer the supernatant to another collection tube.

5. DNA binding

☐	No.	Step
☐	14	Add 250 µL of Buffer NSB and vortex for 5 s.
☐	15	Load 550 µL of sample onto an NZYSpin Soil Column (green ring) in a collection tube. Centrifuge at 11 000 × g for 1 min. Discard the flow-through. Load the remaining sample and repeat.

6. Washing and drying

☐	No.	Step
☐	16	Add 500 µL of Buffer NSB, centrifuge for 50 s at 11 000 × g and discard the flow-through.
☐	17	Add 550 µL of Buffer NSW1, centrifuge for 30 s at 11 000 × g and discard the flow-through.
☐	18	Add 700 µL of Buffer NSW2, close the lid, vortex for 2 s and centrifuge at 11 000 × g for 30 s. Discard the flow-through and repeat this step once.
☐	19	Dry the membrane: centrifuge for a further 2 min at 11 000 × g.

7. Elution

☐	No.	Step
☐	20	Place the column in a clean microcentrifuge tube and add 40 µL of Buffer NSE (preheated at 56 °C).
☐	21	Incubate with the lid open for 1 min at room temperature. Close the lid and centrifuge at 11 000 × g for 30 s.

DNA storage and quality assessment

☐	No.	Step
☐	22	Store the eluted DNA at 4 °C for short-term use or at −20 °C for long-term storage.
☐	23	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
