STANDARD OPERATING PROTOCOL · EnGeL-MOLB-DNA-BEE

Genomic DNA isolation from honey bee guts (NZY Soil gDNA Isolation Kit)

Protocol ID	EnGeL-MOLB-DNA-BEE
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Status	draft
Area	Molecular biology (MOLB)
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Main hazards	Sodium hypochlorite (cuticle disinfection): corrosive; Kit buffers may contain chaotropic salts: never mix with bleach [TO CONFIRM against the NZY SDS] Proteinase K: respiratory sensitiser; Flame-sterilised tools: burn and fire risk

1 Purpose and scope

This protocol isolates genomic DNA from the guts of honey bees (*Apis mellifera*), pooled in groups of three individuals, for analyses of the gut community (e.g. gut symbionts). It uses surface disinfection of the cuticle, aseptic dissection, enzymatic digestion, bead beating and the NZY Soil gDNA Isolation Kit, which removes PCR inhibitors.

2 Principle

Bee cuticles are disinfected with dilute sodium hypochlorite to remove external DNA. Guts are dissected aseptically and digested with Proteinase K in Buffer NSL1, then disrupted by bead beating. Contaminants are precipitated (Buffer NSL3) and removed with an inhibitor-removal column; DNA is bound to a silica column, washed, dried and eluted in preheated Buffer NSE (NZYtech, NZY Soil gDNA Isolation kit).

3 Before you start

Item	Details
Hands-on time	[TO CONFIRM]
Total time	Digestion 2 h at 56 °C plus c. [TO CONFIRM]
Sample	Bees stored at −80 °C; three individuals per extraction
Prepare in advance	0.05% sodium hypochlorite; Buffer NSE (40 µL per sample) preheated at 56 °C; ice; flame-sterilised tools
Key safety points	See Safety and waste

4 Materials

4.1 Equipment

- Bead-beating homogeniser: BeadBlaster 24R (Benchmark)
- Microcentrifuge (≥ 11 000 × g)
- Heat block at 56 °C
- Vortex mixer
- Stereomicroscope [TO CONFIRM], dissection tools and flame for sterilisation
- Ice bucket

4.2 Consumables

- 1.5 mL disposable centrifuge tubes
- NZYSpin Soil Bead Tubes, NZYSpin Soil Inhibitor Removal Columns (red ring), NZYSpin Soil Columns (green ring) and 2 mL collection tubes (in the kit)
- Filter tips

4.3 Reagents and kits

- NZY Soil gDNA Isolation Kit (NZYtech, Cat. No. MB21802), containing:
  - Buffer NSL1
  - NS Enhancer
  - Buffer NSL3
  - Buffer NSB
  - Buffer NSW1
  - Buffer NSW2
  - Buffer NSE
- Proteinase K [TO CONFIRM: from the kit or separate; concentration]
- Sodium hypochlorite, 0.05% aqueous solution (or 1% household bleach)
- Distilled water

5 Reagent preparation

- **Sodium hypochlorite 0.05%:** [TO CONFIRM: dilution from the stock, preparation frequency].
- **Buffer NSE:** add the necessary amount (40 µL per sample) to a separate tube and preheat at 56 °C.

6 Procedure

6.1 Before starting

- 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].

6.2 1. Sample preparation

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.

6.3 2. Sample lysis

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.

6.4 3. Contaminant precipitation

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.

6.5 4. Lysate filtration

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.

6.6 5. DNA binding

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.

6.7 6. Washing and drying

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.

6.8 7. Elution

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.

6.9 DNA storage and quality assessment

22. Store the eluted DNA at 4 °C for short-term use or at −20 °C for long-term storage.

> **PAUSE POINT** Eluted DNA can be stored at 4 °C (short term) or −20 °C (long term) before quality assessment and downstream use.

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.

6.10 After finishing

- 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.

7 Calculations

Not applicable: volumes are per sample. For n samples, prepare $V = v \times n \times 1.1$ of each buffer (v: volume per sample, µL; 1.1: 10% excess).

8 Quality control

Control	Purpose	Acceptance criterion
Extraction blank	Detect contamination (dissection tools, reagents)	Processed through the PCR with the samples: no band on the gel, and < 5 ng/µL on the NanoDrop (regarded as noise)
DNA purity (EnGeL-MOLB-NANODROP)	Quality of the extract	Ideal: A_{260}/A_{280} 1.8–2.0 and A_{260}/A_{230} 2.0–2.2. Values outside are flagged, not failed: the PCR decides
DNA concentration	Enough DNA for PCR	General rule: ≥ 10 ng/µL success; < 5 ng/µL fail; between 5 and 10 ng/µL the PCR result decides

9 Data and metadata

- **Sample labels:** EnGeL labels from the sample master data file (sample nature `DNA` for the extract; source codes of the three bees kept in a separate column).
- **Where:** [TO CONFIRM: project records, Lab Pipeline workbook].

> **Minimum to record**
> - EnGeL labels of the bees pooled and of the extract
> - Dissection and extraction date; operator; kit lot number
> - BeadBlaster programme used; deviations
> - DNA concentration and purity

10 Safety and waste

Follow the manufacturer's instructions and safety data sheets for each reagent. Biological samples are potentially hazardous and are handled following the facility's procedures. EnGeL lab rules:

- Before you enter: wear your lab coat, use appropriate gloves, closed shoes only, tie back long hair.
- Not allowed: eating or drinking; touching phones or laptops with contaminated gloves; bags or coats on benches.
- Label all reagents (name, concentration, date) and treat all chemicals as hazardous.
- Sodium hypochlorite is corrosive; never mix it with the kit buffers or their waste.
- Take care with open flames when sterilising dissection tools.
- Buffers containing guanidine salts: do not add bleach or acidic solutions to guanidine-containing waste, as highly reactive compounds can form. Keep this waste separate from the bleach used for bench and tool cleaning.

Waste

EnGeL waste streams (choose the right bin):

- **Biological solid waste** (black bin, white bag): contaminated gloves, contaminated disposable materials, solid biological laboratory waste, contaminated PPE. No liquids or sharps.
- **Specific hazardous waste** (red bin, red bag): chemicals and pharmaceuticals designated for this waste stream, specific hazardous laboratory waste, anatomical material; sharps only in a rigid sharps container. No loose sharps.
- **Biological liquid waste** (jerrican, UN 3291): liquid biological waste and contaminated biological liquids only. Use a funnel; rinse the original container and the funnel with dH_2O and discard the rinse water in the same jerrican.

For this protocol: bee remains go to biological solid waste (black bin) [TO CONFIRM: or red bin as anatomical material] column flow-through to biological liquid waste (jerrican); used hypochlorite solution [TO CONFIRM: route].

11 Troubleshooting

Problem	Likely cause	Solution
Excess foam after bead beating	Detergent in the lysis buffer	Centrifuge for 2 min at 11 000 × g before adding Buffer NSL3.
Sample volume > 700 µL at filtration	Large lysate	Load the excess through the inhibitor-removal column in a new collection tube and combine the flow-throughs.
Pellet in the filtrate	Carry-over of debris	Transfer the supernatant to another collection tube.

12 Remarks

The source protocol is written for groups of three bees. Standard methods for research on honey bee gut symbionts are in the source folder for reference.

The source protocol was written for the FastPrep-24 5G, which the lab replaced with the BeadBlaster 24R; the BeadBlaster runs the same programme (6.0 m/s, 30 s, one cycle, pause 300 s). The FastPrep-only menu settings of the source (QuickPrep adapter, “lysing matrix A”, “quantity 1 mg”) do not apply.

13 References

NZYtech (n.d.). NZY Soil gDNA Isolation kit (MB21802) – manual [TO CONFIRM: version].

14 Version history and citation

Version	Date	Change	By
1.0.0	2026-11-16	First EnGeL-UC version, from the lab protocol “Standard protocol for isolating genomic DNA from honey bee guts” (2025).	Luís Cunha
1.0.1	2026-11-17	Contributor Yevheniia Shcheka (source protocol, written in the lab).	Luís Cunha
1.0.2	2026-11-17	Authorship: all who contributed are authors, with their roles; lead author first	Luís Cunha

Version	Date	Change	By
		where identified, reviewer (Luís Cunha) last.	
1.1.0	2026-11-17	Bead beating on the Bead-Blaster 24R, which replaced the FastPrep-24 5G in the lab (same programme: 6.0 m/s, 30 s, one cycle, pause 300 s).	Luís Cunha
1.2.0	2026-11-20	Acceptance criteria confirmed by Luís Cunha: extraction blank, purity ranges (ideals; the PCR decides) and concentration rule as for the other DNA extractions.	Luís Cunha
1.2.1	2026-11-20	Author name corrected: Yevheniia Shcheka.	Luís Cunha
1.2.2	2026-11-20	Cross-references to the nucleic acid QC protocols, now split into EnGeL-MOLB-NANODROP, EnGeL-MOLB-QUBIT and EnGeL-MOLB-PICOGREEN.	Luís Cunha

How to cite

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15 Open questions

1. DOI.
2. Proteinase K: from which kit, and at what concentration?
3. Preparation of the 0.05% sodium hypochlorite.
4. Number of extraction blanks per batch.
5. Where results are recorded.
6. Waste route for bee remains and used hypochlorite solution.
7. Hands-on and total time.