STANDARD OPERATING PROTOCOL · EnGeL-MOLB-DNA-MAG

DNA extraction from bulk soil fauna samples with magnetic beads (Omega Bio-tek Mag-Bind® Blood & Tissue DNA HDQ 96, Invitek InviGenius®)

Protocol ID	EnGeL-MOLB-DNA-MAG
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Area	Molecular biology (MOLB)
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Licence	Text: CC BY 4.0 · Code: MIT
Main hazards	 Guanidine hydrochloride (AL, VHB Buffers): never mix with bleach; Sodium perchlorate (HDQ Binding Buffer): oxidiser; Proteinase K: respiratory sensitiser; Ethanol, isopropanol: flammable

1 Purpose and scope

This protocol produces purified genomic DNA from bulk soil fauna samples for DNA metabarcoding and other PCR-based analyses. DNA is purified in 96-well format on an Invitek InviGenius® automated magnetic-particle extraction robot with the Omega Bio-tek Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (Cat. No. M6399).

Sample types covered. The procedure from lysis onwards is the same for all sample types; only the pre-treatment differs:

- Microarthropods stored in 96% ethanol (non-destructive lysis; specimens can be archived after lysis).
- Nematode suspensions in dH_2O (unfixed aliquot, frozen).
- Macroarthropods: pre-treatment by drying and powdering [TO CONFIRM: procedure to be added].

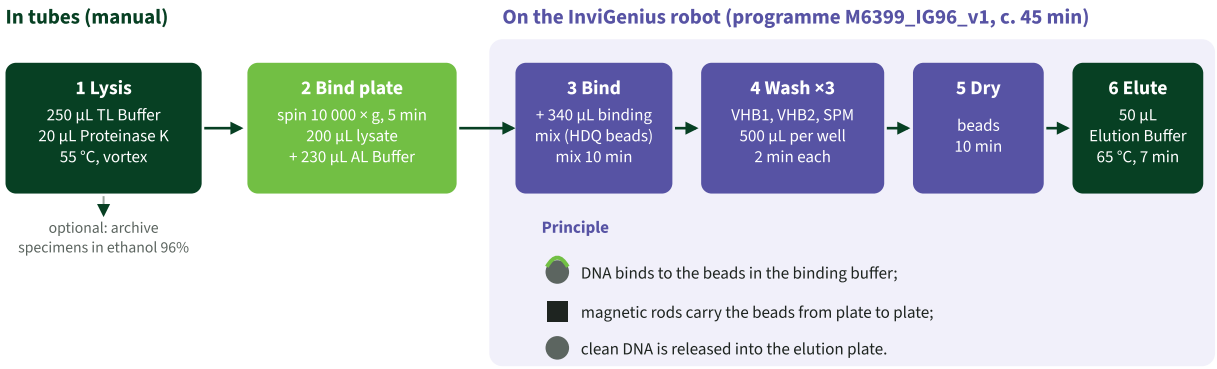
Limits. Lysates are not stored (see Lysis). Throughput is one 96-well plate per run (92 samples and 4 extraction blanks). DNA extraction from soil and from eDNA samples (e.g. airborne eDNA) is not covered and will be de-

scribed in separate protocols. Library preparation is described in EnGeL-MOLB-MB, and detailed DNA quality control in EnGeL-MOLB-NANODROP, EnGeL-MOLB-QUBIT and EnGeL-MOLB-PICOGREEN.

2 Principle

The preservation liquid is removed first: ethanol by centrifugation, pipetting and evaporation of residual ethanol in an IR vacuum concentrator; water from nematode suspensions by centrifugation and pipetting. Samples are digested in TL Buffer with Proteinase K at 55 °C. For microarthropods the approach is non-destructive: the lysate is recovered for DNA extraction and the specimens can be archived in ethanol for the collection.

An aliquot of the lysate is mixed with AL Buffer and with HDQ Binding Buffer containing Mag-Bind® Particles HDQ, to which the DNA binds. The InviGenius® moves the particles with magnetic rods through pre-filled plates: binding, two VHB washes and one SPM wash, bead drying, and elution in Elution Buffer at 65 °C. The robot does not dispense reagents; all plates are pre-filled by the operator (Omega Bio-tek, 2025; Invitek Diagnostics, n.d.).



Schematic. Volumes from EnGeL-MOLB-DNA-MAG, Procedure; lysis time still to be confirmed in the protocol.

Figure 1: Magnetic-bead DNA extraction. Lysis and transfer of the lysate to the Bind plate are done by hand; binding, three washes, bead drying and elution run on the InviGenius robot. Volumes from the Procedure. Figure: EnGeL-UC, CC BY 4.0.

3 Before you start

Item	Details
Hands-on time (full plate)	[TO CONFIRM: to be filled in by Luísa Fraga and Andreia Quaresma]
Total time (full plate)	[TO CONFIRM: to be filled in by Luísa Fraga and Andreia Quaresma]. Fixed times in the procedure: ethanol evaporation 15 min per concentrator load; lysis 1 h 30 min; AL mixing 10 min; robot run c. 45 min.
Run size	92 samples + 4 extraction blanks (positions H3, H6, H9, H12) per 96-well plate.
Sample amount	[TO CONFIRM: maximum amount per extraction]. Specimens must be fully covered by the TL Buffer.
Sample condition and storage	Microarthropods: in 96% ethanol at room temperature; ideally in the fridge if there is space. Nematodes: unfixed aliquot in dH_2O in 1.5 mL tubes, frozen at −20 °C or −80 °C. Macroarthropods: [TO CONFIRM].
Prepare in advance	Kit buffers diluted with ethanol / isopropanol (Reagent preparation); plate map (Annex 1); dry bath at 55 °C; bench cleaned and UV on.

Item	Details
Key safety points	Guanidine-containing buffers (AL, VHB): never mix with bleach. HDQ Binding Buffer contains sodium perchlorate. See Safety and waste.

NOTE The IR vacuum concentrator rotor (NB-503-1524) holds 24 × 1.5 mL tubes, so a full plate needs four concentrator loads.

4 Materials

4.1 Equipment

- Invitek InviGenius® extraction robot (Invitek Diagnostics; magnetic-rod system comparable to KingFisher™-type processors) with program “M6399_IG96_v1” (Annex 2)
- IR vacuum concentrator: N-Biotek Micro-CenVac NB-503CIR with rotor NB-503-1524 (24 × 1.5 mL tubes)
- Microcentrifuge (≥ 10 000 × g)
- Dry bath / heat block set to 55 °C
- Vortex mixer and plate vortex/shaker (1 500 rpm)
- Multichannel pipettes, pipettes
- Forceps and spatulas
- UV lamp in the working area
- Spectrophotometer (e.g. NanoDrop) and fluorometer (e.g. Qubit) — see EnGeL-MOLB-NANODROP and EnGeL-MOLB-QUBIT

4.2 Consumables

- InviGenius tip plate
- 96 deep-well plates (5 per run: Bind, VHB1, VHB2, SPM, Elution)
- 96-well microplates with red frame for DNA storage, and seals
- 1.5 mL Eppendorf tubes and 2 mL nuclease-free microcentrifuge tubes
- 15 mL / 50 mL Falcon tubes (buffer and master-mix preparation)
- Filter tips; wide-bore tips (or regular tips with the end cut off) for ethanol removal; fine tips for water removal from nematode pellets
- Reagent reservoirs
- Laser-printed coded labels and 96-position cryoboxes for archiving specimens after non-destructive lysis

4.3 Reagents and kits

- Omega Bio-tek Mag-Bind® Blood & Tissue DNA HDQ 96 Kit, Cat. No. M6399-01 (4 × 96 preps), containing:
  - TL Buffer
  - Proteinase K Solution
  - AL Buffer
  - HDQ Binding Buffer
  - Mag-Bind® Particles HDQ
  - VHB Buffer
  - SPM Buffer
  - Elution Buffer
- RNase A (optional) [TO CONFIRM: supplier and concentration; not listed among the M6399 kit contents]
- Ethanol, 100% (VHB and SPM Buffer preparation)
- Isopropanol, 100% (HDQ Binding Buffer preparation)
- Ethanol, 96% (archiving specimens; cleaning tools)
- Sodium hypochlorite, 3% (cleaning tools), and bleach for bench cleaning

Models and catalogue numbers of general equipment and consumables are kept generic in this version.

5 Reagent preparation

When opening a new kit, dilute the concentrated buffers as below and mark the bottles once done. Volumes are for kit M6399-01, from the Omega Bio-tek manual revision v8.2 [TO CONFIRM: check against manual v9.0, the revision cited in References].

Buffer	Add	Volume (M6399-01)	Storage
SPM Buffer	100% ethanol	350 mL	Room temperature
VHB Buffer	100% ethanol	290 mL	Room temperature
HDQ Binding Buffer	100% isopropanol	160 mL	Room temperature

Storage and stability of kit components (manual v8.2)

- Mag-Bind® Particles HDQ: 2–8 °C for long-term use.
- Proteinase K Solution: room temperature for up to 12 months; 2–8 °C for long-term storage.
- All other components: room temperature, away from bright light. Precipitates formed in cool conditions are dissolved by warming at 37 °C with gentle shaking.

Binding mix (prepare fresh for each run). Prepare a master mix of HDQ Binding Buffer and Mag-Bind® Particles HDQ only for the samples to be extracted in this run (the mix does not keep). Resuspend the Mag-Bind® Particles thoroughly before pipetting. Volumes include 10% excess (see Calculations).

Component	Per sample	24 samples (+10%)	48 samples (+10%)	96 (92 samples + 4 blanks, +10%)
HDQ Binding Buffer	320 µL	8 448 µL	16 896 µL	33 792 µL
Mag-Bind® Particles HDQ	20 µL	528 µL	1 056 µL	2 112 µL
Total binding mix	340 µL	8 976 µL	17 952 µL	35 904 µL

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.
- Set the dry bath to 55 °C.
- Check that the kit buffers have been prepared (Reagent preparation).
- Samples are processed in 96-well format: each run comprises 92 samples and 4 extraction blanks (negative controls) at positions H3, H6, H9 and H12, processed exactly like the samples.
- Prepare the plate map (sample sheet) before starting and record it in the database and the lab book (Annex 1).
- When establishing a new starting material or community, include an extraction positive control (e.g. a mock community of known composition) and record its well in the plate map [TO CONFIRM: composition].

The steps below apply to 1.5 mL Eppendorf tubes. Specimens must be fully covered by the TL Buffer; for bulk samples with high biomass, scale up TL Buffer and Proteinase K proportionally or split the sample into two or more extractions (the standard 250 µL has been sufficient so far).

6.2 Pre-treatment (choose by sample type)

A. Microarthropods in ethanol: ethanol removal

> **CRITICAL** Microarthropods are stored in 96% ethanol. Complete removal of ethanol is crucial before proceeding with DNA extraction, to avoid inhibition of downstream steps.

1. Centrifuge the microcentrifuge tubes at 6 000 × g for 3 min.
2. Carefully pipette out the supernatant ethanol without disturbing the pellet containing the microarthropods. It might be beneficial to use wide-bore tips or cut off the tip when using regular pipette tips.
3. Place the open tubes in the IR vacuum concentrator (N-Biotek Micro-CenVac NB-503CIR, rotor NB-503-1524; 5 000 rpm, 15 min, temperature [TO CONFIRM]) to evaporate residual ethanol. Monitor to ensure complete ethanol removal, but do not over-dry the sample.

B. Nematode suspensions in water: water removal

4. Thaw the frozen tubes containing the nematode suspension [TO CONFIRM: thawing conditions].
5. Centrifuge the tubes at 6 000 × g for 3 min to pellet the nematodes.
6. Carefully remove the supernatant with a pipette without disturbing the nematode pellet, leaving 50 µL of water with the nematode pellet. It might be beneficial to use fine tips and to work against a dark background to check the pellet.

C. Macroarthropods: drying and powdering

[TO CONFIRM: drying conditions, powdering method, and amount of powder per extraction.]

6.3 Lysis

7. Add 250 µL of TL Buffer to each tube.
8. Add 20 µL of Proteinase K to each tube. Vortex at 1 500 rpm to mix.
9. Incubate the tubes in a dry bath at 55 °C for 1 h 30 min [TO CONFIRM: 1 h 30 min in the UC v2.0 protocols; “usually 2 h” stated on 2026-11-16], vortexing at 1 500 rpm every 30 min.

> **CRITICAL** For non-destructive lysis of microarthropods, do not extend the incubation, so that specimens remain intact for archiving. Shorter times give less but higher-quality DNA; longer times give more but degraded DNA, particularly for soil animals.

10. Optional: add 5 µL of RNase A, vortex at 1 500 rpm to mix and leave at room temperature for 2 min.
11. Centrifuge the tubes at 10 000 × g for 5 min to settle the specimens and debris.
12. Transfer 200 µL of the lysate (supernatant) to a 96 deep-well plate (Bind plate), following the plate map, without aspirating specimens.
13. Optional but desired (microarthropods): recover the specimens for archiving. Remove the remaining lysate, refill the tube with 96% ethanol and place a unique, laser-printed coded label inside the tube. Store the tubes in 96-position cryoboxes and archive them in the collection.

> **NOTE** Proceed immediately to the automated purification: lysates are used promptly and are not stored. A hold step for lysates has not been validated for this kit [TO CONFIRM: see Remarks for options from the literature].

6.4 Automated DNA purification (InviGenius®)

14. Add 230 µL of AL Buffer to each well of the Bind plate. Seal the plate and mix on a plate vortex/shaker at 1 500 rpm for 10 min.
15. In the meantime, prepare three deep-well wash plates and one deep-well elution plate:
    - W1 (VHB1 plate): 500 µL VHB Buffer per well
    - W2 (VHB2 plate): 500 µL VHB Buffer per well
    - W3 (SPM plate): 500 µL SPM Buffer per well
    - Elution plate: 50 µL Elution Buffer per well (single elution)
16. Prepare the binding mix (HDQ Binding Buffer + Mag-Bind® Particles HDQ) only for the samples to be extracted in this run (Reagent preparation).

> **CRITICAL** Resuspend the Mag-Bind® Particles thoroughly before pipetting. The binding mix does not keep: prepare it only for the samples of each run.

17. DNA binding: add 340 µL of binding mix to each well of the Bind plate. Mix thoroughly by pipetting up and down 10 times.
18. Load the robot according to the following plate positions:

Plate	Position
Tip plate	1
Bind plate (lysate + AL Buffer + binding mix)	2
VHB1 plate	3
VHB2 plate	4
SPM plate	5
Elution plate	8

CRITICAL Before starting the InviGenius® run, check that all plates are in the correct robot positions.

19. Run the protocol "M6399_IG96_v1" (c. 45 min): binding with 10 min mixing, two VHB washes and one SPM wash (2 min each), 10 min bead drying, and elution for 7 min at 65 °C. Full program parameters are given in Annex 2.
20. Unload the plates. Transfer the eluate (purified DNA) to a labelled 96-well microplate with a red frame.
21. Discard the Bind (lysate) plate and the deep-well elution plate in the biological hazard container. Send the wash plates to be washed and autoclaved.

6.5 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.

6.6 After finishing

- Empty the liquid left in the wash plates into the biological liquid waste before sending the plates to be washed (see Safety and waste).
- 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

Master mix and buffer volumes for a run, with 10% excess:

$$V_{\text{mix}} = v_{\text{sample}} \times n \times 1.1$$

- V_{mix}: volume of a component to prepare for the run (µL)
- v_{sample}: volume of that component per well (µL)
- n: number of wells to process (samples + extraction blanks; dimensionless)
- 1.1: factor for 10% excess (dimensionless)

Worked example (full plate, n = 96: 92 samples + 4 blanks):

- HDQ Binding Buffer: 320 µL × 96 × 1.1 = 33 792 µL
- Mag-Bind® Particles HDQ: 20 µL × 96 × 1.1 = 2 112 µL
- TL Buffer: 250 µL × 96 × 1.1 = 26 400 µL; AL Buffer: 230 µL × 96 × 1.1 = 24 288 µL
- Each wash plate (VHB1, VHB2, SPM): 500 µL × 96 × 1.1 = 52 800 µL

8 Quality control

Control	Where	Purpose	Acceptance criterion
Extraction blanks (4 per plate)	Positions H3, H6, H9, H12; processed exactly like the samples from lysis onwards	Detect contamination during extraction; spread across the plate so that cross-contamination can be traced to a position	Processed through the PCR with the samples: no band on the gel, and < 5 ng/µL on the NanoDrop (regarded as noise)
Extraction positive control (e.g. mock community of known composition)	When establishing a new starting material or community	Check extraction performance for the new material	[TO CONFIRM: composition and criterion]
DNA purity	All extracts	A_{260}/A_{280} and A_{260}/A_{230} (NanoDrop)	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	All extracts	Fluorometry (Qubit)	General rule: ≥ 10 ng/µL success; < 5 ng/µL fail. Depends on the starting quantity and parent material; between 5 and 10 ng/µL the PCR result decides.

The DNA concentration rule is a general guide: the PCR is the best way to evaluate whether the DNA in a sample is viable. The Metabarcoding Lab Pipeline workbook uses 10 ng/µL as its “adequate DNA” threshold when classifying PCR failures.

Negative controls are carried through library preparation and sequenced alongside the samples, so that contaminant reads can be identified and removed during bioinformatic processing (EnGeL-MOLB-MB). A PCR positive control is always included at the PCR stage (EnGeL-MOLB-MB). Details of the measurements: EnGeL-MOLB-NANODROP and EnGeL-MOLB-QUBIT (EnGeL-MOLB-PICOGREEN for plates).

9 Data and metadata

- **Outputs:** purified DNA in labelled 96-well red-frame microplates; one plate map per plate (Annex 1); DNA purity and concentration values.
- **Sample labels** (DNA extracts have sample nature `DNA`):

Every sample carries its EnGeL label from the sample master data file (`ENGEL_master_data`, lab Google Drive): `EnGeL_<year>_<unique code>_<project>_<sample nature>_<type of sampling>`, e.g. EnGeL_24_AAA_SYBER_Raw_Soil. Codes are assigned by the data curator; lab members fill in their own sheet and the curator validates and generates the labels. A sample derived from another (for example a DNA extract) is entered as a new sample with its own unique code and label; the code of the source sample is kept in a separate column.

- **Plate map:** spreadsheet (.xlsx/.csv) with one row per well plus a visual 8 × 12 grid, with a unique plate ID (e.g. Project_Marker_Plate##_YYYYMMDD). The same file is carried forward to library preparation (EnGeL-MOLB-MB).
- **Where:** extraction results go to the *Sample_Tracking* sheet of the Metabarcoding Lab Pipeline workbook (`EnGeL_LabPipeline_mesofauna_TEMPLATE`, one copy per project) and to the lab book; sample information to the sample master data file. Instrument use is recorded in the equipment user logs (NanoDrop, Qubit).

Minimum to record

- Plate ID and well; EnGeL sample label (master data file), also on any archived tube; sample type (sample, extraction blank, positive control)
- Pre-treatment used (A, B or C) and any deviation (split extraction, scaled-up lysis, optional RNase A)
- Operator
- Lot numbers: Mag-Bind® Blood & Tissue DNA HDQ 96 Kit; RNase A (if used); ethanol and isopropanol used for buffer preparation
- Robot program name (M6399_IG96_v1) [TO CONFIRM: also the robot run ID?]
- As in the Lab Pipeline *Sample_Tracking* sheet: Extraction_pipeline, Extraction_date, Equipment, DNA_ngul, A260/280, A260/230, Elution_vol_ul, DNA_total_ng
- MIxS fields: not produced at this step; recorded at sequence submission (EnGeL-MOLB-MB)

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.

Kit hazards (Omega Bio-tek M6399 manual; to be confirmed by the laboratory against the current kit and reagent SDS):

- AL Buffer and VHB Buffer contain guanidine hydrochloride.
- 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.
- HDQ Binding Buffer contains sodium perchlorate (strong oxidiser; may cause damage to organs through prolonged or repeated exposure). Keep away from heat and combustible materials.
- Proteinase K may cause allergy or asthma symptoms if inhaled; TL Buffer may cause eye irritation and skin sensitisation.
- Ethanol and isopropanol are flammable (to be confirmed against the SDS). Keep away from flames and heat sources.

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:

- Leftover lysates, buffers that have been in contact with samples, and the liquid left in the wash plates: biological liquid waste (jerrican), with a funnel. Never add bleach to this waste.
- Used Bind and elution deep-well plates, tips, tubes and gloves: biological solid waste (black bin).
- Wash plates (VHB1, VHB2, SPM): emptied into the biological liquid waste, then washed and autoclaved for reuse.
- Unused or expired kit reagents: specific hazardous waste (red bin).

11 Troubleshooting

Problem	Likely cause	Solution
Specimens aspirated with the ethanol	Narrow tip opening; pellet disturbed	Use wide-bore tips or cut off the tip of regular tips; do not disturb the pellet.
Downstream PCR inhibition	Residual ethanol after evaporation	Ensure complete ethanol removal; monitor drying in the concentrator, without over-drying.
Specimens not covered by TL Buffer	High biomass	Scale up TL Buffer and Proteinase K proportionally, or split the sample into two or more extractions with suffixed IDs (e.g. S045a, S045b).
Low DNA yield	Mag-Bind® Particles not resuspended; binding mix prepared too early	Resuspend the particles thoroughly; prepare the binding mix only for the current run. Other causes and solutions: [TO CONFIRM].
Specimens damaged after lysis	Incubation extended	Keep the standard incubation time for non-destructive lysis.
Run fails or wells empty	Plates in wrong positions	Check plate positions against the loading table before starting the run.
DNA detected in extraction blanks (band on the gel, or ≥ 5 ng/µL)	Contamination during extraction or handling	Identify what amplified (bioinformatics); check reagents and handling; samples of the same run are interpreted with this in mind [TO CONFIRM: when a run is repeated]

12 Remarks

When handling specimens, all materials used such as forceps and spatulas are cleaned with sodium hypochlorite (3%) and 96% ethanol.

The protocol may require optimisation and validation for specific taxonomic groups and sample types.

Lysis time. The standard incubation is used for soil fauna. Overnight lysis is needed for some eDNA samples (e.g. airborne eDNA), which will be described in separate eDNA protocols. Shorter times give less but higher-quality DNA; longer times give more but degraded DNA, particularly for soil animals.

Holding lysates: options from the literature, not validated in the lab [TO CONFIRM]. The lab recommendation is to use lysates promptly. Options reported elsewhere:

- Qiagen states that, after Proteinase K digestion, tissue samples can be stored in its Buffer ATL (DNeasy Blood & Tissue Kit) for up to 6 months at ambient temperature without loss of DNA quality (Qiagen, n.d.). This applies to a different kit buffer, not to Omega TL Buffer.
- In the LIFEPLAN non-destructive Malaise-trap protocol, part of each lysate is archived for future analysis, but no storage temperature or duration is given (LIFEPLAN, 2024).
- The Omega Bio-tek manual (v8.2) gives no lysate storage step; it gives storage of the purified DNA at −20 °C.

Robot volume setting. The Bind step volume setting is 770 µL (200 µL lysate + 230 µL AL Buffer + 340 µL binding mix). The program file exported on 2026-06-30 shows 980 in this field: check the program on the robot [TO CONFIRM].

13 References

Invitek Diagnostics (n.d.). InviGenius® 96 – User Manual. Invitek Diagnostics, Berlin, Germany.

LIFEPLAN (2024). LIFEPLAN Malaise sample metabarcoding. protocols.io. https://www.protocols.io/view/lifeplan-malaise-sample-metabarcoding-dbfq2jmw

Omega Bio-tek (2025). Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (M6399) – Product Manual, revision v9.0 (December 2025; QMF27.0118.M6399). Omega Bio-tek Inc., Norcross, GA, USA. Revision v8.2 used for the reagent preparation volumes and storage conditions.

Qiagen (n.d.). FAQ-3362: Is it possible to stop the DNeasy tissue protocol and store the tissue lysates after digesting in buffer ATL and Proteinase K? https://www.qiagen.com/us/resources/faq/3362

14 Version history and citation

Version	Date	Change	By
1.0.0	2026-11-16	First EnGeL-UC version. Derived from the InviGenius robot protocol (reviewed 2026-07-06 by Luísa Fraga) and the DNA extraction sections of the UC BENCHMARKS protocols BM-BIO-MAR-MB and BM-BIO-NEM-MB v2.0 (2026-09-28); scope widened to bulk soil fauna samples.	Luísa Fraga, Andreia Quaresma, Luís Cunha
1.1.0	2026-11-16	Lab contact added; waste routes from the EnGeL waste streams; DNA concentration rule (≥ 10 ng/µL success, < 5 ng/µL fail); records in the sample master data file and the Metabarcoding Lab Pipeline workbook; EnGeL sample labels; lab rules and end-of-work routine.	Luís Cunha
1.1.1	2026-11-16	DNA extracts get their own EnGeL code in the master data file (source sample code kept in a separate column); unused or expired kit reagents go to the red bin.	Luís Cunha
1.1.2	2026-11-16	Schematic of the extraction steps (figure).	Luís Cunha
1.1.3	2026-11-17	Authorship: all who contributed are authors, with their roles; lead author first where identified, reviewer (Luís Cunha) last.	Luís Cunha
1.1.4	2026-11-17	Sample-label text moved to a shared include (also used by the lab handbook); no change in content.	Luís Cunha
1.2.0	2026-11-17	Storage of microarthropods in ethanol: room temperature, ideally in the fridge if there is space.	Luís Cunha

Version	Date	Change	By
1.3.0	2026-11-20	Acceptance criteria confirmed by Luís Cunha: extraction blanks go through the PCR and must show no band and < 5 ng/µL on the NanoDrop; purity ranges A260/A280 1.8–2.0 and A260/A230 2.0–2.2 are ideals (values outside flagged, the PCR decides).	Luís Cunha
1.3.1	2026-11-20	GHS pictograms for ethanol and isopropanol (harmonised EU classification); kit buffers still to be checked against their safety data sheets.	Luís Cunha
1.3.2	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. Lysis time: 1 h 30 min (UC v2.0) or 2 h (stated 2026-11-16)?
2. IR vacuum concentrator: temperature setting (instrument range 35–60 °C).
3. Macroarthropod pre-treatment: drying conditions, powdering method, amount per extraction.
4. Thawing conditions for frozen nematode aliquots.
5. Hands-on and total time for a full plate; maximum sample amount per extraction (Luísa Fraga, Andreia Quaresma).
6. Composition of the extraction positive control.
7. Robot program: the Bind volume field shows 980 in the exported file; the setting should be 770 µL.
8. Reagent preparation volumes: check against Omega manual v9.0.
9. RNase A: supplier and concentration.
10. Holding lysates: adopt one of the literature options, or keep “no storage”?
11. Record the robot run ID?
12. Troubleshooting: when a run with DNA in a blank is repeated; other causes of low yield.
13. Author order; DOI.
14. Materials list 2 mL nuclease-free microcentrifuge tubes (from the UC protocols), but no step uses them: keep or remove?

Annex 1 – Plate map guidance

Each laboratory should keep its own plate map template, but every plate map must contain at least the elements below. The plate map is the key that links each sample to its well, its DNA extract, its index combination and its

sequencing reads. It must therefore be completed before extraction starts and kept up to date through every step of the workflow.

1. Plate layout and identification

- Use one plate map per 96-well plate (rows A–H, columns 1–12). Give each plate a unique plate ID, e.g. Project_-Marker_Plate##_YYYYMMDD.
- Keep the same layout from the Bind plate through the elution plate, the PCR plates, indexing and pooling. The well position is the sample's tracking key throughout the workflow.
- Always orient plates with A1 at the top left, and mark the A1 corner on every plate.

2. Controls

- Extraction blanks: 4 per plate, at fixed positions H3, H6, H9 and H12. They are spread across the plate so that cross-contamination can be traced back to a position.
- Extraction positive control (when establishing a new starting material or community): record its well and composition.
- All controls are recorded in the plate map, carried through library preparation and sequenced. PCR controls and index assignment: see EnGeL-MOLB-MB.

3. Sample placement

- Fill the plate column by column (A1→H1, A2→H2, …), skipping the blank positions. This matches multichannel pipetting and the robot's orientation.
- Where possible, randomise or interleave samples from different sites or treatments rather than grouping them in one area of the plate. This avoids confounding position or batch effects with treatment effects.
- If a sample is split into two or more extractions, give each extraction its own well and its own suffixed ID (e.g. S045a, S045b).

4. Information recorded for each well (one row per well)

- Plate ID and well position
- EnGeL sample label from the sample master data file (e.g. EnGeL_24_AAA_SYBER_Raw_Soil), matching the label on any archived tube; DNA extracts get their own label (sample nature DNA), with the source sample code kept in a separate column
- Sample type: sample, extraction blank, or positive control / mock community
- Taxonomic group and pre-treatment used, and a link to site/treatment metadata
- Extraction date and operator
- DNA concentration (Qubit), A_{260}/A_{280} and A_{260}/A_{230} ratios, and the dilution used for PCR
- Index IDs and sequences, and pool ID (filled in during library preparation, EnGeL-MOLB-MB)
- Notes (e.g. low biomass, split extraction, failed PCR, re-run)

5. Format and storage

- Keep the plate map as a spreadsheet (.xlsx/.csv) with one row per well, plus a visual 8 × 12 grid. Store it in the lab database and keep a printed copy in the lab book.
- Use the same file to generate the sequencing sample sheet, so that sample and index information is never transcribed twice.
- Version the file whenever it changes (e.g. after a re-run), and record the date and the person who made the change.

Example layout (92 samples + 4 extraction blanks, filled column by column)

	1	2	3	4	5	6	7	8	9	10	11	12
A	S01	S09	S17	S24	S32	S40	S47	S55	S63	S70	S78	S86
B	S02	S10	S18	S25	S33	S41	S48	S56	S64	S71	S79	S87
C	S03	S11	S19	S26	S34	S42	S49	S57	S65	S72	S80	S88
D	S04	S12	S20	S27	S35	S43	S50	S58	S66	S73	S81	S89
E	S05	S13	S21	S28	S36	S44	S51	S59	S67	S74	S82	S90
F	S06	S14	S22	S29	S37	S45	S52	S60	S68	S75	S83	S91

	1	2	3	4	5	6	7	8	9	10	11	12
G	S07	S15	S23	S30	S38	S46	S53	S61	S69	S76	S84	S92
H	S08	S16	**EB1**	S31	S39	**EB2**	S54	S62	**EB3**	S77	S85	**EB4**

S = sample; EB = extraction blank.

Annex 2 – InviGenius® program M6399_IG96_v1

Program exported from the InviGenius® 96 (program date 2026-06-30; 7 steps). Parameters are decoded below following the program editor fields described in the InviGenius® 96 user manual (section 4.3.3, “Manage Program”). The robot does not dispense reagents: all plates are pre-filled as described in the Procedure.

A. Plate positions and mixing parameters

Step	Pos.	Content	Mix time (min)	Mix amp. (%)	Wait (min)	Volume setting (µL)	Mix speed (1–10)	Temp. (°C)
-Load-	1	Tip plate – tip loading	–	–	–	–	–	–
Bind	2	Lysate + AL Buffer + binding mix (770 µL)	10.0	80	0	770 (see note)	7	off
VHB1	3	VHB Buffer, 500 µL	2.0	80	0	500	7	off
VHB2	4	VHB Buffer, 500 µL	2.0	80	0	500	7	off
SPM	5	SPM Buffer, 500 µL	2.0	80	10.0 (bead drying)	500	7	off
Elute	8	Elution Buffer, 50 µL	7.0	80	0	50	5	65
-Unload-	5	Tip unloading	–	–	–	–	–	–

B. Magnetic collection parameters

Step	Segments (0–5)	Cycle times (0–10)	Mag. speed (1–10)	Lip-lvl (s)	Anti-splash (s)	Segment times (s)
Bind	2	2	1	0	0	8 + 8
VHB1	2	2	1	0	0	4 + 4
VHB2	2	2	1	0	0	4 + 4
SPM	2	2	1	0	0	4 + 4
Elute	2	2	1	0	0	8 + 8

Field definitions (InviGenius® 96 manual): Mix time – mixing time in the selected plate; Mix amp. – mixing amplitude (1–100%); Wait time – interval before the next step (after the SPM wash this dries the beads before elution); Volume – liquid volume used by the software to convert mixing amplitude; Mix speed – 1 (slowest) to 10 (fastest); Temp. – heating, available only at positions 2 and 8; Segments/Cycle times – number of magnetic collection segments and repeats; Mag. speed – speed of the magnetic rods below the liquid level; Lip-lvl / Anti-splash – stand-

ing times at / above the liquid surface; Segment times – magnetic collection time per segment (only the first two apply when Segments = 2).

> **NOTE** Mix and wait times are stored in the program file in tenths of a minute (e.g. 100 = 10.0 min), which is consistent with the c. 45 min run time. The Bind volume field in the exported file reads 980; the setting should be 770 µL [TO CONFIRM].

Program file (M6399_IG96_v1.txt)

```
AS180_S
NAME:M6399_IG96_v1
STEP:7
STATUS:10
TIME:2026-06-30 08:52:55
OPTION:1 1 1 1 1 1 1 1 5 0 1 0 1 2 5 0 2
-Load- 1 0 1 0 5 1 0 1 0 1 0 0 0 0 0 0 0
Bind 2 100 80 0 980 7 0 2 2 1 0 0 8 8 1 1 1
VHB1 3 20 80 0 500 7 0 2 2 1 0 0 4 4 1 1 1
VHB2 4 20 80 0 500 7 0 2 2 1 0 0 4 4 1 1 1
SPM 5 20 80 100 500 7 0 2 2 1 0 0 4 4 1 1 1
Elute 8 70 80 0 50 5 65 2 2 1 0 0 8 8 1 1 1
-Unload- 5 0 1 0 5 1 0 1 0 1 0 0 0 0 0 0 0
AS180_E
```