

Total DNA extraction from beehive bottom debris for NGS analysis and innovative management purpose

Silvia Scibetta, Maria La Giglia, Delia Gambino, Pietro Riolo, Davide Pepe, Eugenia Oliveri, Samira Cacciatore, Carmelo Bongiorno & Stefano Reale

Istituto Zooprofilattico Sperimentale della Sicilia, Via G. Marinuzzi 3, Palermo, Italy

ABSTRACT

The hive can be considered a complex and adaptive biological system in which a variety of living species coexist. Within it, there is a precise spatial organization of bees with different roles, as well as areas where unwanted guests accumulate and where waste products and their associated microbiota are concentrated. In the hive, the microbiome is mainly found in the debris at the bottom of the beehive, which is representative of the entire colony. Collecting these samples is easy and requires only the recovery of the material from the bottom collector. The total DNA extracted from this waste reflects all living species present in the hive, including environmental parasites. Next-generation sequencing (NGS) analysis of total DNA can provide profiles that yield information relevant to hive health, such as the presence of known bee symbionts and pathogens. For this reason, we optimized material collection, homogenization, and DNA extraction procedures by comparing various methods, with the goal of obtaining high quality DNA suitable for shotgun sequencing.

KEY WORDS

Debris; DNA extraction; NGS; Shot-gun sequencing; bee health; microbiota.

Received 31.01.2026; accepted 05.03.2026; published online 30.03.2026

INTRODUCTION

DNA extraction is the process of purifying DNA from a biological sample using physical and/or chemical methods, separating it from cell membranes, proteins, lipids and other cellular components. An effective DNA isolation technique should yield DNA of high quantity and quality, free from contaminants such as RNA and proteins. Both manual methods and commercially available kits are commonly used for DNA extraction.

DNA extraction generally involves cell lysis and DNA solubilization, followed by chemical or enzymatic purification steps. Extraction techniques can be very simple (Boom et al., 1990) or may in-

clude organic extraction methods (phenol–chloroform method), nonorganic methods (salting out and proteinase K treatment), adsorption-based methods (silica–gel membrane), magnetic separation (in which DNA binds reversibly to magnetic beads coated with DNA-binding antibody), anion exchange technology, or salting out.

Since Next-Generation Sequencing (NGS) requires highly purified input DNA, extraction methods that provide superior DNA purity are generally preferred over rapid extraction protocols. Waste material accumulating at the bottom of beehives can reveal the experiences of families and their relationship with the surrounding environment over a given period (Panasci et al., 2011; Tlak Gajger et al., 2024). It functions as a “black box”,

collecting evidence of events occurring within the hive through the analysis of its microbiota.

It permits to:

Monitor bee health. Hive debris contains traces of bees, pathogens, and parasites. Analyzing DNA helps detect diseases or pests affecting the colony, (*Varroa destructor* Anderson et Trueman, 2000, *Nosema* Nägeli, 1857, *Aethina tumida* Murray, 1867, Fungy, Bacteria).

Assess biodiversity. DNA can reveal which bee species or subspecies are present, as well as other insects or microorganisms living in the hive.

Non-invasive sampling. Instead of capturing or harming bees, collecting debris from the hive floor allows researchers to study the colony without disturbing it.

Environmental DNA (eDNA). Plants, pollen, and microorganisms from the surrounding environment leave DNA traces in the hive debris, giving scientists information about local ecosystems.

Analysis of DNA extracted from hive debris enables the investigation of multiple aspects of colony biology and environmental interactions. In particular, it allows the monitoring of bee health through the detection of pathogens and parasites (*Varroa destructor*, *Nosema*, *Aethina tumida*, Fungi, Bacteria) affecting the colony, the assessment of biodiversity within the hive by identifying bee species, subspecies, and associated organisms, and the implementation of non-invasive sampling strategies that do not disturb or harm bees (Olivieri et al., 2025; Roberts et al., 2025; Biová et al., 2021). In addition, hive debris contains environmental DNA derived from plants, pollen, and surrounding microorganisms, providing valuable information on the ecological context in which the colony operates (Evrán et al., 2023). Overall, extracting DNA from beehive bottom debris represents a practical and non-destructive approach to studying bees, their health, and the environment around them (Batchelor et al., 2023; Boardman et al., 2024).

However, beehive bottom debris is an extremely heterogeneous material due to its mixed composition. Samples typically contain organic components, such as animal tissues, pollen, and microorganisms, as well as inorganic materials, including dust or sand. This complexity make DNA

extraction particularly challenging. In addition, substances such as wax, propolis, polyphenols, and lipids can inhibit PCR amplification and DNA sequencing by interfering with enzymatic reactions, thereby reducing DNA quality or yield. Furthermore, heterogeneous samples contain particles with very different physical properties. Some cells are fragile and easily lysed, whereas others possess robust protective structures, such as fungal spores or insect exoskeletons (Zizka et al., 2022). This variability necessitates careful selection of extraction methods to ensure efficient DNA recovery from all sample components.

Therefore, sampling and homogenization are crucial steps for successful DNA extraction. Proper sample collection ensures representativeness and minimize contamination, effective homogenization promote uniformity, enhances cell disruption, and improves DNA recovery (Hestetun et al., 2021). Inadequate homogenization can lead to inconsistent or reduced DNA yield and quality (Hart et al., 2015). For these reason, various extraction methods were compared to identify protocols capable of producing high-quality input DNA suitable for PCR and NGS sequencing.

MATERIAL AND METHODS

The general approach to DNA extraction involved a combination of lysis methods including mechanical, thermal, and enzymatic disruption to try and ensure that DNA from plant, microbe, and human sources would be extracted for sequencing. The bee debris can be diluted in a 1:5 ratio of grams of bee debris to ml of ultrapure water. The mixture is then heated in a water bath at 70 °C for 5 minutes in order to soften the debris and have it disperse in the liquid and then spun on the vortex vigorously. The homogenate is placed in the – 20 °C freezer so that a freeze-thaw cycle would help disrupt the cell membranes. The bee debris material is then ground with a Stomacker and resuspended in 1X PBS to bring all of the tubes to a final volume of 20 ml. Subsequently, the material is further crushed in 2 ml tubes with ceramic beads with repeated cycles in a tissue lyser (Qiagen). The supernatant is then used for DNA extraction. Actually, we can choose through Commercial kits for column, silica magnetite,

Prepman (Applied Biosystems), phenol-chloroform purification.

Silica column method

Some methods are specific for stool materials and can be employed on debris to enhance the yield (Kim et al., 2020). In synthesis: add a specific buffer to each, vortex continuously until the sample is thoroughly homogenized to ensure maximum DNA concentration recovery. Heat the suspension for 5 min at 70 °C–95 °C to complete the lyses for Gram positive and other parasites. Vortex and centrifuge at full speed for 1 min to pellet stool particles. Pipet the supernatant into a new 2 ml microcentrifuge tube and discard the pellet. Add specific inhibitor tablet to remove inhibitory substances to each sample and vortex immediately. Incubate suspension at room temperature to allow inhibitors to be adsorbed. Centrifuge at full speed for 3 min and discard the pellet. Pipet 15 µl proteinase K into a new 1.5 ml microcentrifuge tube, add 200 µl lysis buffer and vortex. Incubate at 70 °C for 10 min. Add 200 µl of ethanol to the lysate, and mix by vortexing. Label the lid of the spin column placed in the collection tube. Carefully apply the complete lysate to the spin column close the cap and centrifuge at full speed for 1 min. Place the spin column in a new 2 ml collection tube, and discard the tube containing the filtrate. Wash two times with wash buffer by centrifugation and discard the collection tube containing the filtrate. Finally centrifuge at full speed for 1 min to eliminate the wash buffer carryover. Transfer the spin column into a new, labeled 1.5 ml microcentrifuge tube and pipet 200 µl elution buffer, then centrifuge at full speed for 1 min to elute DNA.

Silica magnetite

Pipet 20 µl Proteinase K into the bottom of a 2 ml sample tube. Add 200 µl of liquid phase of the samples and 150 µl lysis buffer, mix carefully by vortexing and incubate at room temperature for 30 min and then incubate for 10 min at 65 °C. Briefly centrifuge the 2 ml sample tube and add 15 µl of resuspended silica magnetite to the sample. Add the linking buffer to the sample and place the tube with the sample in the tube holder of the Magnetic Rack. Place the tube holder of the Magnetic Rack

onto the mixer and incubate at room temperature. Place the tube holder on its magnetic base, wait until bead separation has been completed and remove the supernatant. Aspirate the supernatant, while avoiding to disturb the magnetic bead pellet. Remove the supernatant completely and wash the samples two times employing the magnetic rack and removing the supernatant. Rinse the particles with 700 µl distilled water while the tube holder is on the magnetic base and the beads are fixed to the wall of the sample tube. Incubate for 1 min at room temperature, and remove the supernatant completely. All pipetting steps are performed carefully to avoid disturbing the fixed bead pellet. Remove the tube holder of the magnetic rack from its magnetic base and add an appropriate volume of elution buffer or water (100–200 µl). Place the tube holder onto the mixer and incubate at room temperature for 3 min at 1400 rpm. Place the tube holder on magnetic rack, wait until bead separation has been completed and transfer the supernatant with the DNA to a new tube.

Prepman protocol

Weigh 20 mg of each sample or a reference standard into a 2 ml microcentrifuge screw-cap tube. Transfer the appropriate quantity of PrepMan™ Ultra Sample Preparation Reagent (add 400 µL of PrepMan™ Ultra Sample Preparation Reagent to each 20 mg sample). Tightly cap the tubes, then vigorously vortex to the tissue lyser (Qiagen). Place the microcentrifuge screw-cap tubes in a heat block set to 100 °C for 10 minutes. Allow them to cool to room temperature for 2 minutes, spin the tubes in the microcentrifuge at 12,000 rpm for 2 minutes and then transfer 50 µl of the supernatant from the spun tubes into a second set of labeled-microcentrifuge screw-cap tubes and discard the remaining supernatant. Dilute 1/10 and use 5 µl of supernatant per assay reaction.

Phenol-choloroform purification

This method is labor intensive and time consuming (Sambrook et al., 1989). Cell lysis can be done using non-ionic detergent (sodium dodecyl sulfate), Tris-Cl, and Ethylene diamine tetraacetic acid (EDTA), and this step is followed by removal of cell debris by centrifugation. Protease treatment

is then used to denature proteins. Organic solvents such as chloroform, phenol, or a mixture of phenol and chloroform (phenol/chloroform/isoamyl alcohol ratio is 25:24:1) are used for denaturation and precipitation of proteins from nucleic acid solution, and denatured proteins are removed by centrifugation and wash steps. Precipitation with ice-cold ethanol is performed for concentrating DNA. Nucleic acid precipitate is formed, when there is moderate concentration of monovalent cations (NaCl 4 M). This precipitate can be washed in 70% ethanol, recovered by centrifugation and, finally, is redissolved in TE buffer or double-distilled water. The method can be employed to purify an extracted DNA containing inhibitors for PCR. The quality and yield of DNA are assessed by spectrophotometry or by gel electrophoresis. Absorption peak for nucleic acids is at ~260 nm. The A_{260}/A_{280} ratio is ~1.8 for dsDNA. A ratio of less than 1.7 indicates protein contamination.

RESULTS

The four DNA extraction methods differ significantly in terms of efficiency, purity, and ease of use. The silica column method, designed for waste material, provides highly purified DNA suitable for sensitive downstream applications such as PCR or sequencing. However, it requires multiple handling steps and can be relatively expensive.

The silica-magnetite-based kit offers a good balance between throughput and automation. Magnetic bead technology simplifies the workflow, reduces the need for centrifugation, and supports high-throughput processing. DNA purity is generally comparable to column-based methods, although performance may vary depending on the sample type (Melzak et al., 1996).

The single-reagent heat treatment is the fastest and most cost-effective approach requiring minimal reagents and handling, which makes it suitable for rapid screening or preliminary analyses. However, DNA obtained with this method is generally of lower purity and stability, limiting its suitability for more complex molecular applications.

In our study, DNA extraction was performed in ten replicates for each method using the same debris material. Replicates within each method produced

highly consistent DNA yields, indicating good methodological reproducibility. Nevertheless, the comparison of DNA extraction methods revealed notable differences in both DNA yield and purity (Table 1).

The PrepMan reagent produced the highest DNA concentration (210 ng/ μ l), indicating a strong extraction efficiency in terms of quantity. However, this method showed the lowest $A_{260}/230$ ratio (1.1), suggesting the presence of contaminants such as salts or organic compounds, which may interfere with downstream applications.

The column kit and silica resin kit produced similar DNA yields (125 ng/ μ l and 130 ng/ μ l, respectively). The silica resin kit showed a higher $A_{260}/230$ ratio (1.9) compared to the column kit (1.6), indicating higher DNA purity and fewer residual contaminants.

The phenol-chloroform extraction resulted in the highest DNA yield (245 ng/ μ l) with highest $A_{260}/230$ ratio (2.2), suggesting excellent DNA purity. Despite the use of these hazardous chemicals may limit its routine application.

Overall, while the PrepMan reagent provided higher DNA yield, the silica resin and phenol-chloroform methods produced DNA with higher purity, which may be more suitable for sensitive downstream applications such as NGS. Both the column-based and silica resin-based kits demonstrated consistent and balanced performance. While their DNA yields were lower than those obtained with the PrepMan reagent, the improved $A_{260}/230$ ratios indicate a more efficient removal of inhibitory substances. Notably, the silica resin kit showed slightly higher purity values compared

DNA extraction method	DNA yield (ng/ μ L)*	DNA purity ($A_{260}/280$)*
Column kit	125	1.6
Silica resin kit	130	1.9
PrepMan reagent	210	1.1
Phenol/chloroform	245	2.2

Table 1. DNA concentration (ng/ μ L) and purity ($A_{260}/280$) of four extraction methods. *Values represent the mean (mean \pm SD) of 10 replicates per method.

to the column kit, suggesting that magnetic silica-based purification may provide a more effective washing and recovery process under the tested conditions. Phenol/chloroform extraction result confirms the well-established effectiveness of organic extraction in removing contaminants. Nevertheless, the method presents several practical limitations, including the use of toxic reagents, increased handling time, and reduced suitability for high-throughput or routine laboratory workflows (Costa et al., 2017). Additionally, the lower yield observed may be related to DNA loss during phase separation and precipitation steps.

DISCUSSION

Currently, the validated methods for DNA extraction most widely used in the laboratories can be classified into three groups on the basis of their purification strategies: organic extraction (phenol–chloroform), solid-phase DNA extraction methods (silica based), and ionic chelating resins (Chelex). Specific protocols based on these principles (or a combination of them) have been developed according to sample type. These include differential lysis for selective sperm DNA extraction, specialized procedures for bone and teeth, and DNA extraction, purification from reference samples spotted on FTA paper. However, applications on bee debris are scarce in the literature (Agetsuma-Yanagihara et al., 2017; Clark et al., 2013).

Automated DNA extraction using robotic platforms has been implemented for high-throughput sample preparation, avoiding manual errors while improving sample tracking and reproducibility, especially when processing large numbers of samples. Quality standards for DNA extraction include preventive measures against contamination, as well as the use of appropriate positive and negative controls.

Release of DNA from cells is a critical step for any DNA-based assay. Cells can be lysed by heating, followed by centrifugation to remove cell debris. This approach generates a crude extract suitable for PCR-based applications but often contains inhibitors that can reduce the amplification efficiency. Higher quality and purer DNA is required for downstream applications, which is typically achieved using solid-phase extraction

methods, such as silica-based columns (Gupta et al., 2019). A variety of commercial column-based kits are available to address challenges posed by different sample matrices, including food, soil, and biological waste. The choice of method ultimately depends on the required DNA quality, available resources, and the intended downstream applications. NGS requires highly purified input DNA to guarantee efficient library preparation, reduce potential inhibitors, and ensure high-quality and reliable sequencing results.

Our comparative analysis of four DNA extraction methods highlights the critical balance between yield and purity, which is particularly important for NGS applications. The PrepMan reagent produced the highest DNA concentrations; however, its markedly low A260/230 ratio indicates co-purification of contaminants, such as salts, proteins, or residual reagents. These impurities are known to inhibit enzymatic reactions during library preparation, potentially compromising sequencing efficiency, read quality, and overall data reliability. Consequently, despite its rapid workflow and high yield, this method is less suitable for NGS. Column-based and silica resin-based kits demonstrated consistent performance, achieving moderate DNA yields with higher purity levels. The silica resin kit, in particular, exhibited slightly higher A260/230 ratios than the column kit, suggesting more effective removal of inhibitory substances and efficient DNA recovery. Such characteristics make both methods well suited for NGS, where high DNA purity is essential for reproducible library preparation and reliable sequencing results. High-purity DNA is advantageous for NGS, the method's labor-intensive protocol, use of hazardous reagents, and potential for DNA loss limit its practicality, particularly for routine or high-throughput applications. Overall, these results indicate that DNA extraction methods for NGS should prioritize purity without excessively compromising yield. Silica-based purification approaches provide an optimal balance, ensuring sufficient DNA quantity while minimizing inhibitors, thereby supporting efficient and reliable downstream sequencing workflows. The selection of an extraction method should be guided by the intended downstream application: rapid extraction protocols are suitable for preliminary screening or applications tolerant to impurities, whereas column-based or silica magnetic purifica-

tion methods offer a reliable compromise between DNA yield, purity, safety, and workflow efficiency. Organic extraction, while producing highly purified DNA, remains less practical for routine laboratory use.

REFERENCES

- Agetsuma-Yanagihara Y., Inoue E. & Agetsuma N., 2017. Effects of time and environmental conditions on the quality of DNA extracted from fecal samples for genotyping of wild deer in a warm temperate broad-leaved forest. *Mammal Research*, 62: 201–207. <https://doi.org/10.1007/s13364-016-0305-x>.
- Batchelor K.L., Bell K.L., Campos M. & Webber B.L., 2023. Can honey bees be used to detect rare plants? Taking an eDNA approach to find the last plants in a weed eradication program. *Environmental DNA*, 5: 1516–1526. <https://doi.org/10.1002/edn3.471>
- Biová J., Charrière J.-D., Dostálková S., Škrabišová M., Petřivalský M., Bzdil J. & Danihlík J., 2021. *Melissococcus plutonius* Can Be Effectively and Economically Detected Using Hive Debris and Conventional PCR. *Insects*, 12: 150. <https://doi.org/10.3390/insects12020150>
- Boardman L., Marcelino J.A.P., Valentin R.E., Boncristiani H., Standley J. M. & Ellis J.D., 2024. Novel eDNA approaches to monitor Western honey bee (*Apis mellifera* L.) microbial and arthropod communities. *Environmental DNA*, 6, e419. <https://doi.org/10.1002/edn3.419>
- Boom R., Sol C.J., Salimans M.M., Jansen C.L., Wertheim-van Dillen P.M. & van der Noordaa J., 1990. Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology*, 28: 495–503. <https://doi.org/10.1128/jcm.28.3.495-503.1990>
- Clark D.P. & Pazdernik N.J., 2013. *Molecular Biology, Polymerase Chain Reaction*. 2nd ed. United States of America (USA): Elsevier, pp. 163–93.
- Costa V., Rosenbom S., Monteiro R., O'Rourke S.M. & Beja-Pereira A., 2017. Improving DNA quality extracted from fecal samples—a method to improve DNA yield. *European Journal of Wildlife Research*, 63: 1–7. <https://doi.org/10.1007/s10344-016-1058-1>
- Evran E., Durakli-Velioglu S., Velioglu H.M. & Boyaci I.H., 2023. Effect of wax separation on macro- and micro-elements, phenolic compounds, pesticide residues, and toxic elements in propolis. *Food Science & Nutrition*, 12: 1736–1748. <https://doi.org/10.1002/fsn3.3866>
- Gupta N., 2019. DNA Extraction and Polymerase Chain Reaction. *Journal of Cytology*, 36: 116–117. https://doi.org/10.4103/JOC.JOC_110_18
- Hart M.L., Meyer A., Johnson P.J. & Ericsson A.C., 2015. Comparative evaluation of DNA extraction methods from feces of multiple host species for downstream next-generation sequencing. *PLoS ONE* 10(11): e0143334. <https://doi.org/10.1371/journal.pone.0143334>
- Hestetun J.T., Lanzén A., Skaar K.S. & Dahlgren T.G., 2021. The impact of DNA extract homogenization and replication on marine sediment metabarcoding diversity and heterogeneity. *Environmental DNA*, 3: 997–1006. <https://doi.org/10.1002/edn3.223>
- Kim S.E., Van Tieu M., Hwang S.Y. & Lee M.H., 2020. Magnetic particles: their applications from sample preparations to biosensing platforms. *Micromachines*, 11: 302. <https://doi.org/10.3390/mi11030302>
- Melzak K.A., Sherwood C.S., Turner R.F.B. & Haynes C.A., 1996. Driving forces for DNA adsorption to silica in perchlorate solutions. *Journal of Colloid and Interface Science*, 181: 635–644.
- Olivieri S., Carisio L., Mogliotti P., Guasco C., Franzin A., Garrone A. & Brusa F., 2025. Use of Hive Debris to Detect Acute Bee Paralysis Virus, Chronic Bee Paralysis Virus and Deformed Wing Virus in Honey Bees: An Innovative and Non-Invasive Approach. *Veterinary Medicine and Science*, 11(3):e70343. <https://doi.org/10.1002/vms3.70343>
- Panasci M., Ballard W.B., Breck S.W., Rodriguez D., Densmore L.D., Wester D.B. & Baker R.J., 2011. Evaluation of fecal DNA preservation techniques and effects of sample age and diet on genotyping success. *The Journal of Wildlife Management*, 75: 1616–1624. <https://doi.org/10.1002/jwmg.221>
- Roberts J.M.K., Hall R.J., Shams F., Encinas-Viso F., Bravo F., Soroka J., Milla L., Snape N., Martoni F., Walford A., Gleeson D. & Trujillo-González A., 2025. Environmental DNA Methods for Detection of *Varroa destructor* in Honey Bee (*Apis mellifera*) Hives. *Environmental DNA*, 7: e70109. <https://doi.org/10.1002/edn3.70109>
- Sambrook J., Fritsch E.R. & Maniatis T., 1989. *Molecular Cloning: A Laboratory Manual*, 2nd Edition Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 600 pp.
- Tlak Gajger I., Bakarić K., Toplak I., Šimenc L., Zajc U., Pislak Očepk M., 2024. Winter Hive Debris Analysis Is Significant for Assessing the Health Status of Honeybee Colonies (*Apis mellifera*). *Insects*, 15: 350.

<https://doi.org/10.3390/insects15050350>
Zizka V.M.A., Geiger M.F., Hörren T., Kirse A., Noll
N.W., Schäffler L., Scherges A.M. & Sorg M., 2022.
Repeated subsamples during DNA extraction reveal

increased diversity estimates in DNA metabarcoding
of Malaise traps. *Ecology and Evolution*, 12(11):
e9502.
<https://doi.org/10.1002/ece3.9502>

