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A standardised protocol for extracting environmental DNA (eDNA) from honey samples of diverse origin

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We use this protocol and it's working

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Abstract

We have optimised and validated an in-house protocol for extracting environmental (eDNA) that is applicable to both processed (Commercial honey samples) and unprocessed (backyard hive honey samples) honey samples. The protocol comprises a pre-treatment phase and post-treatment phase for eDNA isolation.

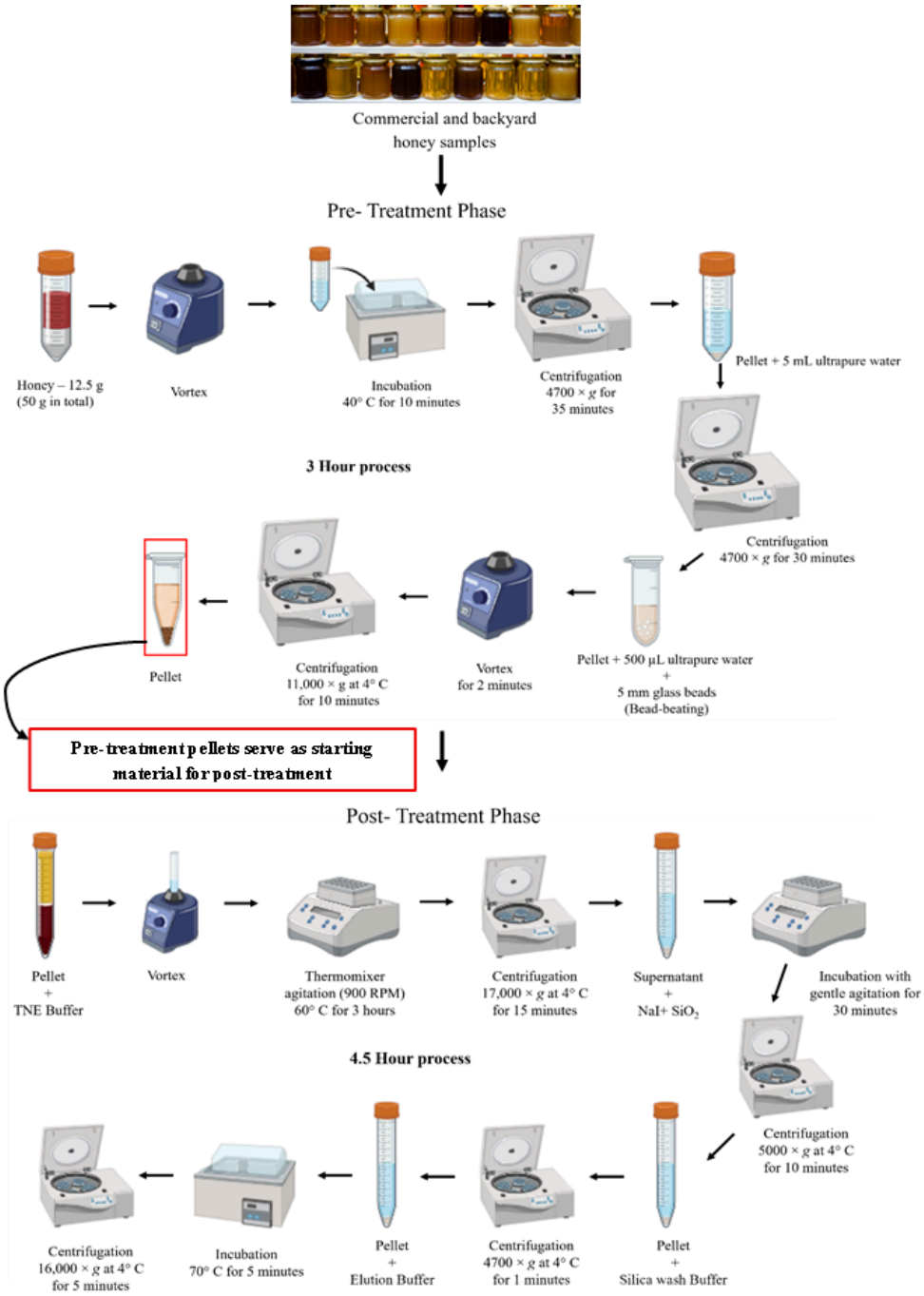
Attachments

DOCX

Protocol- Honey eDNA...

2.7MB

Image Attribution



Flow diagram showing the pre-treatment and post-treatment phase in eDNA extraction from honey samples.

Guidelines

1. Honey needs to be completely homogenized.
2. Vortex well to make sure no clumps of honey are left in the tubes.
3. Complete the whole extraction process in a single day.
4. The samples can be weighed (12g of the same honey sample in 4 different 50 mL tubes), which can be done the day before extraction.
5. The TNE extraction buffer should be pre-heated before adding it to the pellet. Do not heat for more than 5 minutes.

Materials

1. Honey samples
2. Ultra-pure water
3. 50 mL tubes
4. 5 mL tubes
5. 1.5 mL and 2 mL tubes
6. 5 mm Glass beads
7. TNE Buffer (pH 7.5)
8. 5 M guanidine hydrochloride (w/v)
9. Proteinase K solution (20 mg/ml^{-1})
10. 6M sodium iodide (NaI)
11. 100 mg/mL silica dioxide (SiO_2)
12. Silica wash buffer
13. 10 mM Tris-HCl Elution Buffer (pH 8)

Safety warnings

1. Do not leave the pellet with elution buffer in water bath for more than 5 minutes.

Before start

1. Make sure the TNE buffer is made freshly and stored at 4°C.
2. Proteinase K has to be kept on ice during the process.

Pre-Treatment Phase

1 A total of 50 g of honey was carefully divided into four sterile 50 mL centrifugation tubes, with 12.5 g transferred into each tube. 50 g

2 40 mL of ultrapure water (Milli-Q) is added to each tube and thoroughly vortexed to ensure complete homogenization. 40 mL

3 Samples are incubated in a water bath at 40°C for 10 minutes. 40 °C

4 Samples are then centrifuged at 4700 ×g for 35 minutes. 4700 x g , 35 minutes

5 Following centrifugation, the supernatant is carefully discarded, and each resulting pellet is resuspended in 5 mL of ultrapure water. 5 mL

6 The suspensions were pooled into sterile 50 mL centrifugation tubes corresponding to the original honey samples. 50 mL

7 The pooled samples are then subjected to an additional centrifugation at 4700 ×g for 30 minutes. 4700 x g , 30 minutes

8 After centrifugation, the supernatant is carefully discarded, and the pellet is resuspended in 500 µL of ultrapure water. 500 µL

9 This suspension is then transferred to a 2 mL microcentrifuge tube containing seven 5 mm sterile glass beads.

10 The microcentrifuge tubes are vortexed for 2 minutes.

11 The glass beads are removed using sterile, fine-tipped tweezers.

12 The suspension was then centrifuged at 11,000 × g at 4 °C for 10 minutes.
11000 x g, 4°C , 10 minutes

13 The supernatant is carefully discarded, and the resulting pellet serves as the starting material for the post-treatment phase.

Post-Treatment Phase

14 The pellet obtained from the pre-treatment phase is resuspended in 860 µL of pre-heated (60 °C) TNE extraction buffer (10 mM Tris-HCl, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid, 1% (w/v) sodium dodecyl sulfate, pH 7.5). 860 µL

15 To the lysate, 100 µL of 5 M guanidine hydrochloride (w/v) and 40 µL of proteinase K solution (20 mg/mL^{-1}) were added to facilitate protein denaturation and prevent the enzymatic degradation of DNA. 100 µL
40 µL

16 The lysis mixture is incubated at 60 °C for 3 hours in a Thermomixer Comfort (Eppendorf AG, Hamburg, Germany) with agitation at 900 RPM. 900 rpm, 60°C 3 hour

17 Followed by centrifugation for 15 minutes at 17,000×g at 4° C.
17000 x g, 4°C , 15 minutes

18 The supernatant is collected into a 5 mL tube for DNA purification.

19 The supernatant is mixed with 2 Volumes of 6 M sodium iodide (NaI) and 100 µL of 100 mg/mL silica dioxide (SiO_2). 2 V
100 µL

20 The samples are allowed to mix well by gentle agitation on a rocker for 30 minutes.

21 This is followed by centrifugation for 10 minutes at 5000 ×*g* at 4° C.
5000 x g, 4°C , 10 minutes

22 The supernatant is carefully discarded.

23 The pellet is resuspended in 500 µL of silica wash buffer (50% (v/v) ethanol, 10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8) by vortexing. 500 µL

24 This suspension is then centrifuged for 1 minute at 4700 ×*g* at 4° C.
4700 x g, 4°C , 1 minute

25 The wash step (step 23 and 24) is repeated two more times.

26 The supernatant is carefully discarded.

27 To elute eDNA from the silica matrix, 50 µL of elution buffer (10 mM Tris-HCl, pH 8) is added and incubated at 70°C for 5 minutes. 50 µL

28 Following the incubation, the samples are centrifuged for 5 minutes at 16,000 ×*g*.
16000 x g , 5 minute

29 The supernatant is carefully pipetted into a 1.5 mL microcentrifuge tube and stored in -20 ° C. -20 °C

30 The concentration of isolated eDNA is measured using a Nanodrop Eight spectrophotometer.

31 The isolated eDNA is validated by performing end-point Polymerase Chain Reaction (PCR) targeting *Apis mellifera* mtDNA. Genomic DNA from a healthy adult bee is used as a positive control, and nuclease-free water as a no-template control (ntc).

32 Amplified PCR products are visualized by agarose gel electrophoresis using a 2% (w/v) agarose gel prepared in 1X TBE buffer (0.13M Tris, 45 Mm Boric acid, 2.5 mM EDTA pH 7.6) containing 0.2 µg/mL ethidium bromide.

33 The DNA bands are visualized under a UV illuminator to confirm successful amplification.

Protocol references

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