European Commission - Directorate General Joint Research Centre

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

Standard Operating Procedure
DNA Extraction from honey and pollen – CTAB

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Document history			
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	05/07/2012	I. Matetovici	Creation of the document

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1 Object

This method is used to extract genomic DNA from maize, soy, rapeseed pollen present in honey.

2 Applicability domains

The method for DNA extraction described below is suitable for the isolation of genomic DNA from maize, soy, rapeseed pollen in honey. Application of the method to other substances may require adaptation and possible further specific optimatisation.

3 Reference documents

Folloni S., Kagkli D.M., Rajcevic B. Guimarães N., Valicente F., Van Droogenbroeck B., Van den Eede G. Van den Bulcke M. Detection of airborne genetically modified maize pollen by real-time PCR (*in press*)

4. Abbreviations used

CTAB**:** Cetyltrimethylammonium Bromide
DNA: Deoxyribonucleic acid
EDTA: Ethylenediaminetetraacetic acid
RNase: Ribonuclease
Tris: Tris[hydroxymethyl] aminomethane

5. Equipment

Water bath or heating block
12µM membrane filter (cellulose nitrate)
Vacuum filtration system (Sartorius)
Microcentrifuge (Eppendorf or equivalent)
Micropipettes
Vortexer
Microcentrifuge tubes
Rack for reaction tube
Vinyl or later gloves
Vacuum device
Mini-shaker IKA (or equivalent)
1 mm glass beads

NOTE:
Plastic ware has to be sterile and free of DNAses, RNases and nucleic acids. Filter pipette tips protected against aerosol should be used.

6. Reagents

2% CTAB- buffer
Chloroform (99% at least)
Isopropanol (99, 7 % at least)
Ethanol (96% at least)
Ethanol-solution 70% (v/v)
Proteinase K solution 20 mg/ml
RNase A solution 0.5 mg/ml
Sterile molecular biology grade water

7. Procedure

PRELIMINARY REMARKS:

- The procedure requires sterile conditions. Contamination may be avoided during sample preparation by using single-use equipment, decontamination solutions and by avoiding the formation of dust.

- During all steps of DNA preparation, wear gloves and change gloves regularly.

- Avoid extensive vortexing of genomic DNA.

Step 1: Honey solubilisation and pollen fraction collection

1) Heat the honey jar at 40-50 °C until the matrix becomes fluid.

2) Weight the desired amount of honey to a proper recipient (e.g. a 50 ml Falcon tube when analysing 10g of honey)

3) Add 4 volumes of preheated (40-50 °C) sterile deionised water.

3) Put the samples on an agitator and rotate them until the honey is completely dissolved.

4) After the honey is completely dissolved filter the wet solution on a 12µM filter using a Vacuum filtration system (Sartorius) or equivalent.

5) Transfer the filters into an appropriate recipient (e.g. a 15 ml Falcon tube).

Notes:

- When using large amounts of honey, the 12µM filter may become saturated with material and filtering hampered. Take the filter our of the filter unit, transfer to the Falcon tube and replace by a new 12µM filter and continue the filtration. This handling can be repeated until the complete honey solution has been filtered.

- Honey contains in general low amounts of pollen (in the range of 1000 pollen grains/gram honey). A suitable amount for analysis may be 10-50g, although larger amounts may be required (e.g. 500g).

- Filters with pollen material can be stored for short periods at 4ºC. Storage for longer periods is to be avoided but if the case, air-dried filters can be stored in a Falcon tube at minus 20 ºC

Step II: Isolation and Purification of genomic DNA of collected pollen fraction

1) Take the filter(s) in the Falcon tube

2) Add 1 ml of molecular biology grade water

3) Add 1 volume of glass beads (1 mm glass beads)

4) Add 2 ml of 2% CTAB-buffer

5) Shake at maximum strength using a mini-shaker IKA (or equivalent) to disrupt the pollen grains; first shake for about 1 min, and then allow settling for about 30 sec on ice and finally shake again for 2 min

6) Add 50 µl Proteinase K and incubate with agitation at least 2 hours (or over night) at 60° C

7) Add 1 µl of RNase and incubate 30 min at room temperature

8) Centrifuge for 15 min at 5000 *x* g

9) Transfer the supernatant in 1 ml fractions to a 2 ml Eppendorf tube and add 900 µl of chloroform (or repeat in different tubes until the supernatant is completely collected)

10) Mix for 30 sec and centrifuge at 12000 *x* g for 10 min

11) Transfer the aqueous phase to a new tube and add 0.8 volume of Isopropanol

12) Incubate for 30 min at room temp

13) Centrifuge at 12000 *x* g for 10 min

14) Discard the supernatant

15) Wash the pellet with 1 ml of ethanol 70%

16) Mix by inverting, centrifuge at 12000 *x* g for 5 min

17) Discard the supernatant and repeat the washing step

18) Dry the pellet (room temp or concentrator)

20) Re-dissolve the DNA in 60 µl sterile molecular biology grade water. Add the water in the first tube and resuspend the DNA, then take the obtained solution and added to the next tube until all the tubes are pooled.

Optional
21) Purify the DNA samples using the Genomic DNA Clean& Concentrator™ kit (Zymo Research) according with manufacturers' instructions.

8. Procedure Buffer preparation and required reagents

The following reagents/buffers/solutions are required for the DNA extraction procedure:

8.1 Equipment

Weighting equipment
pH meter
Heating stirrer
Autoclave
Pipettes and micropipettes

8.2 Reagents

CTAB**:** Cetyltrimethylammonium Bromide (Ultrapure grade)
Tris: Tris[hydroxymethyl] aminomethane (Molecular Biology grade) or 1 M Tris-HCl pH 8.0
EDTA: Ethylenediaminetetraacetic acid (titration 99.0%)
Ethanol (98% at least)
NaCl (99% at least)
NaOH (98% at least, anhydrous)
Sterile molecular biology grade water

8.3 Formulations

2% CTAB-buffer

20 g/l CTAB……...4 g
1.4 M NaCl……16.4 g
0.1 M Tris*……3.15 g
20 mM Na_2EDTA……1.5 g

* Alternatively 20 ml of 1M ready-to-use solution of Tris-HCl pH 8.0 can be used.
Preparation:

a. add 100 ml distilled water
b. adjust pH to a value of 8.0 with 1M NaOH
c. fill up to 200 ml and filter sterilise
d. store buffer at 4°C for max. 6 months.

NaOH 1 M

Dissolve 2 g of NaOH in 50 ml sterile water

Ethanol-solution 70% (v/v)

70 ml of pure ethanol are mixed with 30 ml of distilled sterile water.