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Annex 4

SOP

Preparation and DNA-Extraction from Honey and Pollen

(Applying Invisorb Spin Food Kit II from Invitek)

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Written at Dec. 28th, 2005

1 ABSTRACT

1.1 Purpose

This method describes the preparation of desoxynucleic acid (DNA) from honey and pollen with an optimised sample preparation step for amplification with Polymerase Chain Reaction (PCR).

1.2 Range of application

With the method described it is possible to extract DNA from honey and pollen. Thereby the preparation of both matrices is of special importance. Beside effectiveness, optimization of time- and work-consumption has been played an important role during development of the method.

2 PRINCIPLE

Honey is a concentrated watery solution of invert sugar with other constituents, like different carbohydrates, enzymes, amino acids, organic acids, minerals etc.. In Honey also components from plants, for example pollen are present. It is the goal of the preparation to select pollen components from honey for the following extraction of DNA.

The haploid microspores of seed plants (the pollen) are the male part in sexual reproduction of flowers. Pollen grains got a very hard outer shell, called the exine. It is so durable, that it can be found in fossil deposits millions of years old. The ambition of the preparation of pollen grains means to find an effective method to destroy the exine. From the broken pollen grains the DNA extraction is performed.

3 MATERIAL & METHODS

3.1 Equipment and Materials

Important!

Only use reagents and water approved for molecular biological use. Sterile handling is a must.

Powder free gloves have to be used, if DNA will be used in real time PCR.

- Balance
- Shacking water bath (65°C)
- Incubation oven (68°C)
- Ball mill (Resch)
- Thermomixer
- Centrifuge
- Pipettes
- Vortexer
- Hand gloves, powder free
- 50 ml Greiner Tubes
- sterile Pipettes tips
- Eppendorf Reaction Tubes 1.5 ml und 2 ml

3.2 Reagents

- Invisorb Spin Food Kit II from Invitek
- Proteinase K
- Proteinase K Buffer
- Ethanol (EtOH), 98-100%
- Ultrapure H_2O bidest
- RNAse A

Attention

The reagents from Invisorb Spin Food Kit II are partly noxious.

⇨ **Do work only with gloves and avoid contact with skin.**

3.3 Solutions

3.3.1. Wash Buffer I

- Add 30 ml of Ethanol (EtOH) (98-100%) to Wash Buffer I.

3.3.2. Wash Buffer II

- Add 42 ml of Ethanol (EtOH) (98-100%) to Wash Buffer II.
- Mix thoroughly.

3.3.3. Proteinase K-Solution

- Add 1 ml of dd H_2O to the proteinase K.
- Mix thoroughly.
- Store at –20°C.

4 EXECUTION

- Before starting prewarm Elution Buffer D to 65°C in a drying cupboard (68°C) (e.g. transfer the needed volume into an Eppendorf tube and place the tube in a thermomixer.
- Preheat the thermomixer to 65°C.

4.1 Preparation of Samples

- ***Honey***
  - Transfer 10 g of honey in a 50 ml Greiner Tube.
  - Add H_2O to 50 ml mark.
  - Incubate in a shaking water bath at 65°C for 30 minutes.
  - Centrifuge at 4000 rpm for 30 minutes.
  - Discard supernatant and dry pellet for 5 minutes at room temperature (in a inverted tube).
- ***Pollen***
  - Transfer 200 mg pollen grains in a 2 ml reaction tube.
  - Homogenize with 1 wolfram carbide bead (7 mm) at 30/s for 1 minute in a ball mill.

4.2 Digestion and DNA extraction

- ***For Honey***
  - Dissolve pellet in 400 µl Lysis Buffer P and transfer solution in a 1,5 ml reaction tube.
  - Add 20 µl Proteinase K and vortex briefly.

- ***For Pollen***
  - Add 400 µl Lysis Buffer P and 20 µl Proteinase K.
  - Vortex briefly.

Following procedure is the same for Honey and Pollen

- Incubate at 65°C for 30 minutes in a thermomixer with continuous shaking at 1200 rpm.
- Optional: To remove RNA add 10 µl RNAse (40mg/ml), vortex briefly and incubate for 5 minutes at room temperature
- During incubation place a spin filter in a 2 ml reaction tube.
- Transfer Lysis Solution onto spin filter and centrifuge for 1 minute at 12’000 rpm.
- Discard the Spin Filter.
- Add 200 µl Binding Buffer P to the filtrate and vortex vigorously.
- Place a new Spin Filter in a new 2 ml reaction tube.
- Transfer Solution onto Spin Filter and incubate for 1 minute.
- Centrifuge for 1 minute at 12’000 rpm.
- Discard the filtrate and place the Spin Filter again into the 2 ml reaction tube.
- Add 550 µl Wash Buffer I onto Spin Filter and centrifuge for 1 minute at 12’000 rpm.
- Discard the filtrate and replace the Spin Filter back into the 2 ml reaction tube.
- Add 550 µl Wash Buffer II onto Spin Filter and centrifuge for 1 minute at 12’000 rpm.
- Discard the filtrate and place the Spin Filter again into 2 ml reaction tube.
- Remove residual ethanol by centrifugation for 2 minutes at 12’000 rpm.
- Discard the filtrate and place the Spin Filter into a 1,5 ml reaction tube.
- Add 100 µl prewarmed Elution Buffer D onto Spin Filter and incubate for 3 minutes at room temperature.
- Centrifuge for 1 minute at 10’000 rpm.

5 ANALYSIS

The extracted DNA can be used for PCR reactions.

6 HINT

6.1 Advice for quality control

Each extracted DNA should be tested with a control-PCR. In the case of honey and pollen a conventional or real-time PCR system for plants can be used. Thereby the extracted DNA should show a positive signal.

If the extracted DNA couldn’t be detected with the control PCR, this is a indication for the presence of inhibitors. In this case one has to check the possibility to extract the DNA with an alternative method.

6.2 Control of DNA extraction / Measurement of DNA concentration

For control of DNA extraction PCR, respectively real time PCR, as well as Agarose gel electrophoresis, is a suitable method.

The DNA-concentration is measured photometrically. Typical yield of DNA is in around 10-20 ng/µl for honey. In the case of pollen the yield is about 10 times higher.

6.3 Validation

Validation information forms an own document to make an easier adaptation possible.