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Detection
of *Apis mellifera*
with conventional PCR

SOP

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1. ABSTRACT

1.1. PURPOSE

This method describes a practice for detection of desoxynucleinacids (DNA) from apis with conventional PCR.

1.2. AREA OF APPLICATION

With the method describe it is possible to detect DNA from *Apis mellifera* from other apis species.

1.3. Origin

This method was designed by Mandy Schöne-Michling.

2 PRINCIPLE

This conventional PCR system for *Apis mellifera* was designed on the mitochondrial genome.

3 TEST EQUIPMENT

3.1. Equipment and Material

Important!

Only use analyze clean, for molecular biology grade reagents and water. The handling must be done under sterile conditions.

Powder free gloves have to be used.

- Mastercycler
- Apparat for agar gel electrophoresis
- Mikrocentrifuge
- Pipettes
- Gloves, powder free
- PCR using material

3.2. Reagents

(Use only DNase and Rnase free reagents.)

- Sterile H_2O bidest
- 10 x Buffer
- $MgCl_2$ (50 nM)
- dNTP's (25 nM)
- Taq polymerase

Name of primer	Sequenz 5' – 3'
A.mellifera F	GAAACCAATCTGACTTACGTCGATT
A.mellfera R	TGTTTGCGACCTCGATGTTG

3.3. Stock solutions

A.cera F: 10 µM primer solution
A.cera R: 10 µM primer solution

3.4. Reference material

- Reference DNA from *Apis mellifera*

4 EXECUTION

4.1. Preparation of Mastermix solution

Components	1	10	Endconcentration
10 x buffer	2.5	25	
$MgCl_2$	1.25	12.5	
dNTP's	0.25	2.5	
F Primer A.mellifera	0.5	5	200 nM
R Primer A.mellifera	0.5	5	200 nM
Taq polymerase	0.25	2.5	
H_2O	17.75	177.5	
Summe	23	230	

4.2. Adding of RNA extract to the Mastermix solution

- Vortex the mastermix solution after preparation.
- Do 23 µl of mastermix solution in a sterile tube for Mastercycler.
- Add 2 µl of template DNA (10^0 or 10^{-1}) to the tubes.
- For a negative control add 2 µl of sterile H_2O bidest.
- Close the tubes and spin short.

4.3. PCR conditions

Step		
Denaturation	2 min. / 95 °C	
	30 sec. / 94°C	35 x
Annealing	45 sec. / 57 °C	
Elongation	45 sec. / 72 °C	
	∞ / 4°C	

5 ANALYSIS

Put PCR fragments on an agarose gel (1%).

6 ADVICE