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Detection
of different *Apis* species
with real-time PCR

SOP

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1. ABSTRACT

1.1. PURPOSE

This method describes a practice for detection of desoxynucleinacids (DNA) from different apis species with real-time PCR.

1.2. AREA OF APPLICATION

With the method describe it is possible to detect DNA from *Apis cerana, Apis mellifera, Apis florae and Apis dorsata with real-time PCR* .

1.3. Origin

This method was designed by Mandy Schöne-Michling.

2 PRINCIPLE

The real-time PCR systems are based on detection of mitochondrial rRNA.

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.

- ABI Prism® Sequence Detection System 7700, Applied Biosystems
- Rotor-Gene 3000, Corbett Research
- Mikrocentrifuge
- Pipettes
- Gloves, powder free
- PCR using material

3.2. Reagents

(Use only DNase and Rnase free reagents.)

- Sterile H_2O bidest
- 2x RT PCR Master Mix

Name of primer and probe	Sequenz 5' – 3'	Target organism
A.cera F	CCTTAGGATAACAGCGTAATAT	***Apis cerana***
A.cera R	CAGACTTAAAATTTTAAACTCCT	
A.cera probe	TTGATAGACCACATTGATAAAGATGT (VIC)	
A.dors F	CTTAGGATAACAGCGTAATATC	***Apis dorsata***
A.dors R	ATTTAAACTTCTGCATTTAACTTTA	
A.dors probe	TTGATAGACCTTATAGATAAAGATGAT (VIC)	
A.florea F	GATTAGAAGATATAGAAATGATTT	***Apis florea***
A.florea R	CAATCATCTTTATCAATATGACC	
A.florea probe	TAATTTATAAATATAAGAATAAATTACCTTA (VIC)	
A.meli F	CTTAGGATAACAGCGTAATATC	***Apis mellifera***
A.meli R	AAACTTAAACTACTGCGCCTA	
A.meli probe	TAGACCATATAGATAAAGATGTTTGC (FAM)	

3.3. Stock solutions

all primers: 8 µM primer solution
all probes: 8 µM probe solution

3.4. Reference material

- Reference DNA from *Apis cerana*
- Reference DNA from *Apis dorsata*
- Reference DNA from *Apis florae*
- Reference DNA from *Apis mellifera*

4 EXECUTION

4.1. Preparation of Mastermix solution

Components	1	10	Endconcentration
2x RT PCR Master Mix	12.5	125	1 x
Forward Primer	1	10	320 nM
Reverse Primer	1	10	320 nM
Probe	0.5	5	160 nM
H_2O	5	50	
Summe	20	200	

4.2. Adding of DNA extract to the Mastermix solution

- Vortex the mastermix solution after preparation.
- Do 20µl of mastermix solution in a sterile tube for ABI Prism 7700 or Rotor-Gene 3000.
- Add 5µl of template DNA (10 fold solution) to the tubes.
- For a negative control add 5µl of sterile H_2O bidest.
- Close the tubes and spin short.

4.3. Real-time-PCR conditions

Step	
UNG activity	2 min. / 50 °C
Activation of HotStarTaq	10 min. / 95 °C
Amplification (50 cycles)	15 sec. / 95 °C 60 sec. / 60 °C

5 ANALYSIS

6 ADVICE