

User Manual

AssignAnimal

Principle

This SYBR-Green kit allows typification of different species in combination with subsequent sanger-sequencing. The kit amplifies the cytochrom B region of DNA from mammals, birds and fishes for subsequent sequencing.

Animal DNA FAM (ex 494 / em 520)

By performing melt curve analysis it can be judged, if the sample can be directly sequenced without any additional purification.

Contents and Storage

5 tubes of primer-probe mix, lyophilized, for 5x20 reactions. Shipped at ambient temperature, store at -20°C, do not expose to light.

Reagents to be Supplied by User

PCR Mastermix, e.g QuantiFast Multiplex PCR from Qiagen or similar product and PCR water.

Protocol

1. Add 150 µl water (PCR grade) per tube of primer mix, vortex vigorously and incubate for 5 min at 60°C (store solution at 4°C, do not expose to light, stable for 1 week).
2. Add 250 µl QuantiFast Multiplex PCR or respective amount of similar product and mix well. Yields 400 µl ready-to-use mastermix for 20 reactions à 25 µl reaction volume.

3. Mix 20 µl ready-to-use mastermix with 5 µl sample solution (recommended amount of DNA: 10 ng) in a suitable PCR reaction vessel.

4. Set up your Real-Time PCR machine according to the manufacturer in order to be able to measure the used fluorescence dyes.

5. Use the following thermal cycling profile:

- 1 5 min, 95°C
- 2 5 s, 95°C
- 3 15s, 60-50 °C touchdown
- 4 15s, 72°C
- 5 Repeat steps 2 to 4 **10 times in total**
- 6 5 s, 95°C
- 7 15s, 50 °C
- 8 15s, 72 °C
- 9 Repeat steps 6 to 8 **20 times in total**
- 10 Melt curve: 90 sec pre-melt, then ramp from 60 °C to 95 °C by 1°C per 5s

6. Analyze the melt curve. To proceed directly for sequencing a clear melt curve must be visible. If there is more than one melt curve visible, the PCR product can be purified by agarose gel electrophoresis.

7. Dilute the PCR-Product 1:10 in PCR-Water and transfer it into the enclosed 1.5ml Sarstedt tubes for sequencing. Label the tubes by enclosed economy labels.

8. Log into our webshop at www.microsynth.com and order sequencing reactions online. Select for every sequencing reaction the sequencing primer H15149 from the standard primer list within the webshop.

9. Blast the identified sequence to assign the specific animal (www.ncbi.nlm.nih.gov/genbank) or perform the analysis according to the following literature:

Hartmut Rehbein, René Köppel and Thomas Hankeln (2012)

[Leitfaden für die Lebensmittelüberwachung zur Identifizierung der Fischart durch DNA-Sequenzierung von PCR-Produkten](#)

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Contact

Kantonales Labor Zürich
rene.koepfel@klzh.ch

Further Information

<https://www.microsynth.com/food-testing-assays.html>

Microsynth AG
Schützenstrasse 15
CH-9436 Balgach
Telefon +41 71 722 83 33
Fax +41 71 722 87 58
www.microsynth.com