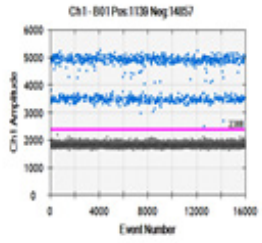
User Manual

Digital Duplex PCR dHoSh

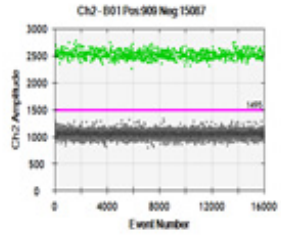
Principle

This method describes a routine procedure for the quantitative detection of horse and sheep DNA. Using the ratio of the concentrations of positive droplets (copies/ul) together with a conversion factor, the weight of the sample can be determined in percent by weight.

Sheep **FAM**



Horse **HEX**



Contents and Storage

4 tubes of primer mix, lyophilized, for 4x24 reactions. Shipped at ambient temperature, store at -20°C

Supplied on demand: EXCEL table for results calculation

Reagents to be Supplied by User

QX200 ddPCR Supermix for Probes 5ml (Cat No. 186-3024) and other consumables for ddPCR with the QX200 system.

Protocol

1. Add 153 µl PCR grade water per tube of primer-mix, vortex vigorously and incubate for 5 min at 60°C (store solution at 4°C, stable for 1 week).
2. Add 282 µl Supermix for probes (2x) and mix well.

Yields 435 µl ready-to-use Mastermix

3. Mix 17 µl ready-to-use Mastermix with 5 µl sample solution in an appropriate PCR reaction vessel. Recommended amount of DNA: 2 ng, measured by absorbance and/or 1:10 diluted isolated DNA.
4. Use the following thermal cycling profile:

1	10 min, 95°C
2	30 s, 95°C
3	60 s, 55°C
4	Repeat steps 2 to 3 **50 times in total**
5	10 min, 98°C
6	Hold, 4°C

6. Set the probes parameter for the droplet reading.
7. Analyse the results with QuantaSoft software. To obtain quantifiable results the droplets/µl value for the total amount of DNA (the lowest threshold) should be over 100cp/µl. The value of the empty droplets should be under 100cp/µl.

Contact

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Further Information

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

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