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Detection of horse DNA using real-time PCR

EURL-AP recommended protocol

Method development:
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1.0	18.02.2013	18.02.2013	

1. Reagents and protocol

1.1. Primers and probe sequences

Forward primer: 5'- CGA TCC CCT ATC AGC C - 3'
Reverse primer: 5'- TCC TTA GAT AGA TGG TGT TGG - 3'
Probe: 5'- TTC TGG TGT TGA CAA CAT GAC TAC TG - 3'

Reporter dye: FAM (position 5' of the probe)
Quencher dye: TAMRA (position 3' of the probe)

The target has a size of 87 bp and is of mitochondrial origin.

1.2. Protocol

1.2.1. Real-Time PCR Mix

After complete thawing of the reagents, in a DNAse free microfuge tube, the reagents are mixed in the following order for a final volume of 35 µl:
PCR grade water, 12.5 picomoles of forward primer and reverse primer, 12.5 picomoles of probe, mastermix with $MgCl_2$ at the final concentration of 4 mM.

The following mixes are given as examples:

	1 reaction	96 reactions	105 reactions (1 plate)*
PCR grade water	8.75 µl	840.00 µl	918.75 µl
Forward primer (10 µM)	1.25 µl	120.00 µl	131.25 µl
Reverse primer (10 µM)	1.25 µl	120.00 µl	131.25 µl
Probe (10 µM)	1.25 µl	120.00 µl	131.25 µl
Master Mix 2x†	17.50 µl	1680.00 µl	1837.50 µl
Total PCR mix volume/reaction	30.00 µl	2880.00 µl	3150.00 µl

DNA to be added in each PCR: 5.00 µl
Total reaction volume = 35 µl / well

* A larger volume than the one required to fill the wells has to be prepared (add ~ 10 % more)
† Master mix used at EURL-AP is Universal mastermix (Diagenode, Liège, Belgium) but any other equivalent master mix of another brand should be fine.

1.2.2. Thermal program

The thermal program to follow is outlined in Table 1.

Table 1 : Thermal program of the horse PCR assay

Process		Time [min:s]	Temperature [°C]
Pre-PCR: decontamination (optional)		02:00	50
Pre-PCR: activation of DNA polymerase and denaturation of template DNA (mandatory)		10:00	95
PCR (**40 cycles**)			
Step 1	Denaturation	00:15	95
Step 2	Annealing and elongation	01:00	**50**