TNO Triskelion bv ruminant PCR test

This method was validated on the 9th March 2012, after a successful collaborative study gathering 12 European laboratories (eurl.craw.eu/index.php?page=24&id=10).

Before to use the test for routine analysis, the laboratory has:

1. To check that the mastermix used is free from ruminant DNA by performing PCR replicates on a negative control (e.g. PCR grade water)[1].
2. To set the cut-off of the PCR platform (a combination thermocycler-PCR reagents) on which to run the TNO Triskelion bv ruminant test. Each cut-off is specific of a platform and cannot be transferred to another one even with a thermocycler from the same brand and inside a laboratory.

The DNA extraction method to be used with the ruminant PCR test is the Wizard® Magnetic DNA Purification System for Food (Promega, Madison, WI, USA – www.promega.com) on a representative 100 mg test portion of sample. The use of the Promega method cited here is mandatory as the way the cut-off linked to this ruminant PCR test has been determined is "extraction method dependent".

1. PRIMERS AND PROBE SEQUENCES

Primer A : 5'-CCA GCA TCA GAG TCT TTT CCA AAT-3'
Primer B : 5'-GAA GGA ATG ATG CTA AAG CTG AAA C-3'
Probe : 5'-CAA CTC TTC GCA TGA GGT GGC CAA A-3'
Reporter dye : FAM (position 5' of the probe)
Quencher dye : TAMRA (position 3' of the probe)

2. REAL-TIME PCR MIX

In a DNAse free microfuge tube, mix in the following order:

	1 reactions	96 reactions	105 reactions (1 plate)
PCR grade water	5.00 µl	480.00 µl	525.00 µl
Primer A (10 µM)	1.10 µl	105.60 µl	115.50 µl
Primer B (10 µM)	1.10 µl	105.60 µl	115.50 µl
Probe (5 µM)	0.73 µl	70.08 µl	76.65 µl
Master Mix 2x	12.07 µl	1158.72 µl	1267.35 µl
Total PCR mix volume/reaction	20.00 µl	1920.00 µl	2100.00 µl

DNA to be added in each PCR : 5.00 µl
Total reaction volume = 25 µl / well

[1] Universal Mastermix (ref. code: GMO-UN-A600, Diagenode, Liège, Belgium – www.diagenode.com) and qPCR Mastermix Plus No ROX (ref.code: RT-QP2X-03NR, Eurogentec S.A., Seraing, Belgium – www.eurogentec.com) are 2 mastermixes fit for this PCR method.

3. THERMAL PROGRAM

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 (**50 cycles**)			
Step 1	Denaturation	00:15	95
Step 2	Annealing and elongation	01:00	**60**

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	Publication date		
	10.09.2012		