A simple and fast triplex-PCR for the identification of milk's animal origin in Halloumi cheese and yoghurt

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SUPPLEMENTARY FILE

Table 1: Origin of primers, gene sequence position from start site, nucleotide sequence, melting temperature (T_m), and size of product expected from PCR.

Origin	Gene Sequence Position	Primer Nucleotide Sequence	Amplicon, bp	T_m,°C
Universal, Forward (UN-FW)	400	5′ TGAGGACAAATATCATTYTGAGGRGC 3′	-	61.6
Bos taurus, Reverse (BO-RV)	666	5′ TAAGATGTCCTTAATGGTATAGTAG 3′	287	56.4
Capra hircus Reverse (CA-RV)	693	5′ TTAGAACAAGAATTAGTAGCATGGCG 3′	313	60.1
Ovis aries Reverse (OV-RV)	710	5′ GGCGTGAATAGTACTAGTAGCATGAGGATGA 3′	336	66.8

[a] The UN12S pair of primers were designed to bind and amplify the same complementary DNA sequence of the 12S rRNA gene in all mammalian species (Tobe and Linacre, 2008b)

Table 2. DNA amounts extracted from commercially available Cyprus Halloumi and Yoghurt products

Halloumi ID code*	Milk content Species**	A260/ A280	Total DNA, ng	Yogurt ID code*	Milk content Species**	A260/ A280	Total DNA, ng
H1	100% goat	1.88	540	Y1	50: 50% Sheep: goat	1.94	6.66
H2	100% goat	1.66	675	Y2	100% cow	1.82	1.10
H3	100% goat	1.87	525	Y3	100% cow	1.94	10.05
H4	50: 50, % Sheep: goat	1.86	555	Y4	100% sheep	1.67	1.85
H5	50: 50, % Sheep: goat	1.75	630				
H6	50: 50, % Sheep: goat	1.84	510				

*The Halloumi and yogurt samples obtained from supermarkets were coded to protect the anonymity of the producer;

**The species origin and % milk content are as specified in the marketing labels of each product.