Technical note: A novel method for routine genotyping of the G allele of β-casein (CSN2) and T allele of κ-casein (CSN3) in a sheep population using LightCycler

Z. Sztankóová,2 J. Kyselová, J. Rychtářová, and V. Czerneková
Institute of Animal Science, Department of Molecular Genetics, Přátelství 815, 104 00 Prague 10 Uhříněves, Czech Republic

ABSTRACT: The goal of this work was to develop a protocol for rapid genotyping of A and G variants at the CSN2 locus and genotyping of T and C variants at the CSN3 locus in sheep breeds (Sumava and Valachian) by means of PCR and LightCycler analysis. The LightCycler technique combines rapid and efficient in vitro amplification of DNA in glass capillaries with melting curve analysis based on fluorescence resonance energy transfer for the sensitive detection of point mutation. The A variant had a greater frequency (Sumava, 0.778; Valachian, 0.835) than did variant G (Sumava, 0.222; Valachian, 0.165) in both sheep breeds. The CSN3 locus was found to be monomorphic, with no polymorphism identified in either population.

Key words: β-casein, κ-casein, genetic polymorphism, LightCycler

INTRODUCTION
Casein polymorphisms are important and well known because of their effect on the qualitative and quantitative traits and technological properties of milk (Amigo et al., 2000; Moioli et al., 2007; Kuchtík et al., 2008). Ovine β-casein (CSN2) is formed from 209 AA residues. Its primary sequence was described by Richardson and Mercier (1979) and its complete sequence confirmed by Provot et al. (1995). At present, 5 patterns are described: A, B, C, X, and Y (Amigo et al., 2000; Chessa et al., 2010). Ovine κ-casein (CSN3) is formed by 171 AA residues of the mature chain and just 1 phosphate residue (Trujillo et al., 2000; Park et al., 2007). The complete nucleotide sequence of CSN3 was described by Furet et al. (1990). At present, in the ovine CSN3 locus there is described 1 SNP at position 237 (GenBank accession number X51822) of the sheep κ-casein mRNA (Felignini et al., 2005).

The objective of this study was to develop a rapid, efficient, and inexpensive assay to identify the genotypes at the casein loci CSN2 and CSN3 using PCR followed by LightCycler analysis (Roche Applied Science, Indianapolis, IN).

MATERIALS AND METHODS
The experiments were carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010, on the protection of animals used for scientific purposes.

Samples and DNA Sources
A total of 272 individuals from 2 sheep breeds [Sumava (n = 133) and Valachian (n = 139)] were used in this study to evaluate genetic polymorphism at the CSN2 and CSN3 loci. Genomic DNA was extracted from blood using ABI PRISM 6100 analysis (Nucleic Acid PrepStation, Applied Biosystems Co., Foster City, CA) by the standard protocol. Before starting with isolation by standard protocol, we used 200 μL of blood with 0.5 mM EDTA, which was stored in the refrigerator at −24°C.

PCR Amplification

PCR CSN2. A 214-bp fragment containing part of an exon and part of intron 7 of the ovine CSN2 gene was amplified by PCR using primers designed on the basis of ovine sequence GenBank accession number M79703. The PCR assay was performed in a 15-μL reaction mixture consisting of 2 μL genomic DNA (10 to 100 ng), 0.3 units of Taq DNA polymerase, 30 μM of each dNTP, (Top Bio Ltd., Prague, Czech Republic), 0.2 μM of each primer (TIB MOLBIOL, Berlin,
**RESULTS AND DISCUSSION**

The 6 CSN2 and 3 CSN3 samples—yielding, respectively, 3 and 1 different patterns by LightCycler analysis (assumed to be AA, AG, and GG at CSN2, and CC at CSN3; Figure 1)—were sequenced on a genetic analyzer (ABI PRISM 3130, Applied Biosystems) to confirm the results regarding differences in single nucleotide substitution between genetic variants A (EMBL X79703) and G (AY444504) at locus CSN2 and variants C (EMBL X51822) and T (AY4444505) at locus CSN3 (data not shown). At locus CSN2, these variants differ by nucleotide substitution A → G, and AA exchange Met (ATG) → Val (GTG) at position 183. The A variant had a greater (P-value: Sumava = 0.391, and Valachian = 0.111) frequency (Sumava, 0.778; Valachian 0.835) than did variant G (Sumava, 0.222; Valachian, 0.165) in both studied breeds, thus indicating that variant A is more characteristic of sheep populations kept in the Czech Republic compared with variant G. Similar results, with variant A showing a greater frequency (50 to 80%) compared with the G variant, were described by Ceriotti et al. (2004) and Chessa et al. (2010) in Italian sheep breeds. The most common genotype was CCG (Sumava 0.624, Sumava; 0.705 Valachian) followed by CC (0.308 and 0.259) and GG (0.068 and 0.036), respectively, 3 and 1 different patterns by LightCycler analysis (data not shown). At locus CSN3, results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the CSN3 casein, our results showed that variant C, for the AA Ser104 and Leu104 of the mature protein was observed at the
Our study reveals that melting curve analysis via LightCycler could be reliable for detecting mutations and single-nucleotide polymorphisms. LightCycler processing of samples is simple and rapid compared with other techniques currently in use, providing prompt results and allowing high-throughput genotyping. The LightCycler-based assay can be a valuable tool for routine typing of animals independent of single-nucleotide polymorphism, sex, age, stage of lactation, number of animals and origin, as well as for rapid screening of clinical isolates in many acquired and hereditary diseases in microbiology and clinical chemistry. The assay allows the use of different specific oligonucleotides in a single reaction mixture, and amplification/post-amplification analysis is performed in closed glass capillaries, thus minimizing the risk of carryover contamination. Compared with the techniques currently in use, the advantages of LightCycler analysis are its speed, efficiency, and safety (i.e., absence of hazardous chemicals).

**LITERATURE CITED**


**Figure 1.** Melting peak in the sheep casein gene. Melting curve plot of fluorescence signal (F) vs. temperature (T) was transformed into a derivative melting curve plot with −dF/dT vs. temperature T. Genotyping of the sheep: A) Beta-casein (CSN2) loci variant A/G with a specific fluorescent probe by derivative melting curve probes. Melting profiles of representative samples of the 3 detected CSN2 genotypes (AA, AG, and GG). Curves are plotted for homozygous samples variant A (AA), showing a single peak at 62.73°C; for homozygous samples variant G (GG), showing a single peak at 56.87°C; and for heterozygous samples AG, showing double peaks at 56.87°C and 62.73°C, respectively. B) Kappa-casein (CSN3) melting profiles of representative samples of the detected CSN3 genotypes (CC). Curves are plotted for homozygous samples variant C (CC), showing a single peak at 57.83°C. Color version available in the online PDF.