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Denaturing Gradient Gel Electrophoresis as a prion protein gene screening method in goats for mutation detection at codons 142, 146, 151, 154, 211, 222 and 240.

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Introduction

Denaturing Gradient Gel Electrophoresis (DGGE) has been applied for prion protein (PRNP) gene allele-specific sequencing in human, sheep and goats. In goats, polymorphisms at PRNP codons 146, 151, 154 and 168 were efficiently detected by DGGE (Papasavva-Stylianou et al., 2009).

Aim of study

The present DGGE protocol aimed to expand the mutation detection in more PRNP codons and particularly at 142, 211 and 222 that are possibly associated with scrapie resistance in goats (Vaccari et al., 2009; Bouzalas et al., 2010).

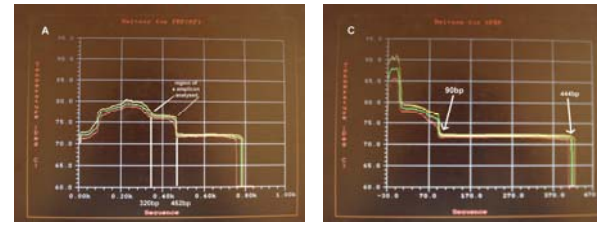
Materials and Methods

Genomic DNA extraction was performed from EDTA-treated blood and brain tissue from reference goat samples carrying the studied polymorphisms. Melting domains of two overlapping PRNP gene regions, referring to the whole ORF were determined by MELT94 software and primer design was optimised (Table 1). DGGE analyzed sequence referred to codons 106-154 (462bp region included in α amplicon) and 178-256 (444bp region included in β amplicon) (Fig.1). DGGE analysis was performed in 6.5% polyacrylamide (37.5:1 acrylamide:bisacrylamide) gels with a linear gradient concentration of 20% to 80% denaturant in 0.5X TAE. Electrophoresis was performed at 84 volts for 19 hours at 60^{0}C. In addition PCR-RFLP with *BspHI* enzyme was used to distinguish polymorphisms at codons 151 and 154 (Fig.3).

Table 1. Characteristics of the PCR assays used for DGGE (α and β amplicons) and RFLP (G1-G2 amplicon) analysis.

Amplicon name	ORF PRNP target (codons)	primers sequence (5'→3')	Reference	Amplicon size (bp)	Cyclic conditions
		M13F: tgtaaaacgacggccagtccctcttttttttgcagagaagtc;	M13F: in-house		94°C/5' -94°C/1'x30
α	*1-154*	P60R: gatagtaacggtcctcatagtc;	P60R: Acutis et al., 2006	462	**55°C/1'x30**-72°C/2,5'x30-72°C / 10′
β	*148-256*	5'P61:gcccgccgtcccggcccgaccccgcgc gtccggcgcccgaaccaacatgaagcatgtgg;	P61: Acutis et al., 2006	484	94°C/5'-94°C/1'x30
		G2: ctatcctactatgagaaaaatgag;	G2: Billinis et al., 2002		**58°C/1'x30**-72°C/2,5'x30-72°C/ 10′
G1-G2	*1-256*	G1: atggtgaaaagccacataggcagt;	G1: Billinis et al., 2002	771	94°C/4' -94°C/1'x39-72°C/10′

Figure 1. Melting map of ORF PRNP region by using MELT94 software.
Fig1A. Melting map of the whole ORFP PRNP. The DGGE analysed sequence of a amplicon (300bp) corresponds to codons 106-154.
Fig1C. Melting map of the b amplicon by using the modified primer lies between 178-256 PRNP codons.



Results

DGGE analysis of the 462bp region revealed four distinct band patterns related to polymorphisms in codons 138, 142, 146, 151 and 154 (Fig.2,4). DGGE analysis of the 444bp region gave five distinct band patterns based on the mutation presence at codons 211, 222 and 240. By combining DGGE results of these two overlapping PRNP gene regions, goat's genotype was determined based on codons 138, 142, 146, 151, 154, 211, 222 and 240.

Figure 2. Interpretation of DGGE bands pattern at PRNP codon and polymorphism level.
α DGGE pattern:1. t138t, I142M, 2. R154R, t138t, 3. R154R, c138c, 4. c138t, R151H or R154H.
β DGGE pattern:1. P240S, 2. Q222K, R211Q, 3. Q222K, P240P, 4. S240S, 5. P240P.

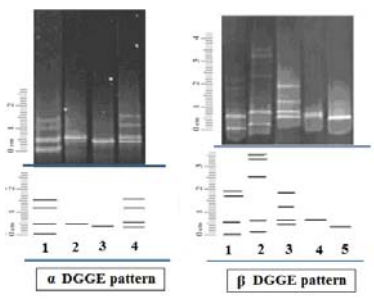


Figure 3. Image of PCR-RFLP gel.
M: molecular weight marker, lanes 1 and 3: G1-G2 amplicons after *BspHI* digestion give two more fragments when R154H is present, lanes 2, 4, 5, 7, 8: G1-G2 amplicons carrying R151R allele give no fragments after enzyme digestion, lane 6: negative PCR control.

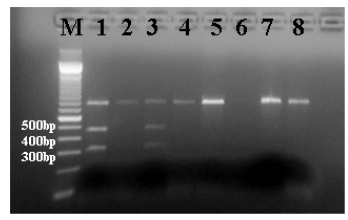
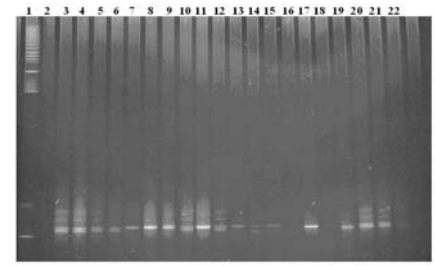


Figure 4. Image of DGGE gel for α amplicon:
1. 100bp ladder; **2, 16, 18.** wild type PRNP samples (NCBI GenBank ID: AJ000739); **3,4,10,12, 20,21.** c138t, R151H or R154H; **7,13,15.** t138t, R154R where 5μl PCR amplicon are loaded; **8, 9, 11**. t138t, R154R where 10μl PCR amplicon are loaded; **17, 19**. c138c, R154R; **14**. t138t, I142M originated from obex sample with bad quality DNA; **4, 5, 6.** same sample where c138t, R151H or R154H are present but are loaded 10μl, 5 μl and 3μl of PCR amplicon respectively; **22**. PCR amplicon using genomic DNA extracted from autolytic obex tissue, no genotyping was possible neither with direct-sequencing nor DGGE.



Discussion

Application of the present DGGE protocol could be used as a PRNP gene screening method in goat herds, using good quality genomic DNA preferably extracted from EDTA-treated blood (Fig.4), for mutation detection at codons 138, 142, 151, 154, 211, 222 and 240. DGGE allows samples grouping based on their pattern and subsequently based on their allele-specific polymorphisms, by using a relative inexpensive and less sophisticated genotyping method. Representative samples from each group could further been direct-sequenced by a reference sequencing method extrapolating data to all group's samples.

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