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.

Frangkiadaki Eir.1,2, Ekateriniadou L.1, Kominakis A.2, Rogdakis E.2
1 National Agricultural Research Foundation, Veterinary Research Institute, Thermi, Thessaloniki, Greece
2 Agricultural University of Athens, Faculty of Animal Science and Aquaculture, Iera Odos 75, 11855, Athens, Greece
First author e-mail address: irfraq@yahoo.com

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°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 ORF (G1-G2 amplicon) analysis.

<table>
<thead>
<tr>
<th>Amplified target</th>
<th>PCR primer target (nucleotides)</th>
<th>primers sequence (5’-3’)</th>
<th>Reference</th>
<th>Annealed amplicon size (bp)</th>
<th>Cycle conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>α G1-G2</td>
<td>126-126</td>
<td>G1: atggtgaaaagccacataggcagt; G2: ctatcctactatgagaaaaatgag</td>
<td>Billinis et al., 2002</td>
<td>224</td>
<td>72°C/2,5’x30-</td>
</tr>
<tr>
<td>β G1-G2</td>
<td>178-256</td>
<td>G1: ggtttttgtctggggtcgctggc; G2: ctatcctactatgagaaaaatgag</td>
<td>Billinis et al., 2002</td>
<td>224</td>
<td>72°C/2,5’x30-</td>
</tr>
</tbody>
</table>

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.

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 be direct-sequenced by a reference sequencing method extrapolating data to all group’s samples.

Acknowledgements
The first author would like to acknowledge financial support from Greek Scholarship Foundation (I.K.Y.) and scientific support from Dr G. Vaccari and Dr U. Agrimi’s team for full CDS PRNP gene sequencing of reference goat samples.

References