JUN 08, 2018

OPEN ACCESS

DOI:
[dx.doi.org/10.17504/protocols.io.qeidtce](https://doi.org/10.17504/protocols.io.qeidtce)

External link:
<https://doi.org/10.1371/journal.pone.0198819>

Protocol Citation: Loukia Ekateriniadou, Evridiki Boukouvala 2018. Genotyping protocol for detection of polymorphisms at codons 146 (N/S/D), 211 (R/Q) and 222 (Q/K) in the caprine PRNP gene. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.qeidtce>

MANUSCRIPT CITATION: Vouraki S, Gelasakis AI, Alexandri P, Boukouvala E, Ekateriniadou LV, Banos G, Arsenos G (2018) Genetic profile of scrapie codons 146, 211 and 222 in the *PRNP* gene locus in three breeds of dairy goats. PLoS ONE 13(6): e0198819. doi: [10.1371/journal.pone.0198819](https://doi.org/10.1371/journal.pone.0198819)

Genotyping protocol for detection of polymorphisms at codons 146 (N/S/D), 211 (R/Q) and 222 (Q/K) in the caprine PRNP gene

PLOS One

Loukia Ekateriniadou¹, Evridiki Boukouvala¹

¹Veterinary Research Institute of Thessaloniki, Hellenic Agricultural Organization Demeter, Thessaloniki, Greece

Sotiria Vouraki

ABSTRACT

This Real-Time PCR genotyping protocol can be used for the detection of polymorphisms at codons 146 (N/S/D), 211 (R/Q) and 222 (Q/K) in the caprine *PRNP* gene. We developed four separate Real-Time PCR reactions with four different Custom TaqMan[®] SNP Genotyping Assays (Applied Biosystems, Foster City, California, USA). Each SNP Genotyping Assay consisted of a mix of sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest and two TaqMan[®] MGB probes, FAM and VIC dye-labeled to detect the amplified product.

GUIDELINES

- Genotyping Assays (primers and probes) should be stored protected from light and at -20 °C when not in use. Do not perform more than 10 freeze-thaw cycles.
- KAPA PROBE FAST qPCR Master Mix should be stored at -20 °C when not in use. Do not perform more than 30 freeze-thaw cycles.
- Avoid repeated freezing and thawing of all reagents.

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: May 24, 2018

Last Modified: Jun 08, 2018

PROTOCOL integer ID:
12458

Keywords: Real-Time PCR, Custom Taqman SNP Genotyping Assays, PRNP gene, goats

MATERIALS

MATERIALS

KAPA PROBE FAST qPCR Master Mix (2X) Universal Kapa Biosystems, Wilmington, Massachusetts, USA Catalog #07959826001
Custom Taqman SNP Genotyping Assay (40X) (Assay ID: AHMSYKA - codon 146 (N/S)) Applied Biosystems, Foster City, California, USA Catalog #4332077
Custom Taqman SNP Genotyping Assay (40X) (Assay ID: AH89YZI - codon 211 (R/Q)) Applied Biosystems, Foster City, California, USA Catalog #4332077
Custom Taqman SNP Genotyping Assay (40X) (Assay ID: AHABD5B - codon 222 (Q/K)) Applied Biosystems, Foster City, California, USA Catalog #4332077
Custom Taqman SNP Genotyping Assay (40X) (Assay ID: AHN1WQI - codon 146 (N/D)) Applied Biosystems, Foster City, California, USA Catalog #4332077

SAFETY WARNINGS

- Wear appropriate personal protective equipment when handling reagents (gloves, protective clothing)
- Minimize inhalation of reagents
- Use only with adequate ventilation (fume hood)

BEFORE START INSTRUCTIONS

Always ensure that the Genotyping Assays (primers and probes) have been fully thawed and mixed before use.

Real-Time PCR Reactions (12.5 µl mixtures - Separate reac...

- 1 KAPA PROBE FAST qPCR Master Mix (2X): 6.25µl
SNP Genotyping Assay Mix (primers and probes) (40X): 0.3125µl
Genomic DNA: 1µl (50-80ng)
H₂O: 4.9375µl

QPCR cycling conditions

- 2 Initial Denaturation Step: 95°C - 3min

45 cycles
 - Denaturation: 95°C - 3sec
 - Primer annealing/extension: 62°C - 30sec

Genotyping

- 3 Genotypes were detected through amplification plots with QPCR Applied Biosystems Step One Software v2.3