

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

Loukia Ekateriniadou, Evridiki Boukouvala

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.

Citation: Loukia Ekateriniadou, Evridiki Boukouvala 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**

dx.doi.org/10.17504/protocols.io.qeidtce

Published: 24 May 2018

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.

Before start

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

Materials

KAPA PROBE FAST qPCR Master Mix (2X) Universal 07959826001 by [Kapa Biosystems, Wilmington, Massachusetts, USA](#)

Custom Taqman SNP Genotyping Assay (40X) (Assay ID: AHMSYKA - codon 146 (N/S)) 4332077 by [Applied Biosystems, Foster City, California, USA](#)

Custom Taqman SNP Genotyping Assay (40X) (Assay ID: AH89YZI - codon 211 (R/Q)) 4332077 by [Applied Biosystems, Foster City, California, USA](#)

Custom Taqman SNP Genotyping Assay (40X) (Assay ID: AHABD5B - codon 222 (Q/K)) 4332077 by [Applied Biosystems, Foster City, California, USA](#)

Custom Taqman SNP Genotyping Assay (40X) (Assay ID: AHN1WQI - codon 146 (N/D)) 4332077 by [Applied Biosystems, Foster City, California, USA](#)

Protocol

Real-Time PCR Reactions (12.5 µl mixtures - Separate reactions for each Genotyping Assay)

Step 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

Step 2.

Initial Denaturation Step: 95°C - 3min

45 cycles

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

Genotyping

Step 3.

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

Warnings

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