Fast and Reliable Genotyping of Scrapie-Resistent/Sensitive Sheep

LightTyper Sheep PrP Gene Mutation Detection Kit

Michael Knoll, Hella Buck, Ingrid Engel-Stephan, Jürgen Becker, Thomas Metzler, Tobias Schnitzer, Yvonne Knack, Birgit Forsthuber, Steffi König, and Walter Eberle*
Roche Applied Science, Penzberg, Germany
**Corresponding author: michael.knoll@roche.com*

Introduction

Several scientific studies support the claim that natural scrapie is associated with polymorphisms at three codons within the sheep prion protein (PrP) gene. Genotyping of these codons (codon 136 [alanine, valine], 154 [arginine, histidine], and 171 [glutamine, arginine, histidine]) might enable breeding of sheep flocks with high resistance to scrapie. Fast and reliable high-throughput genotyping of sheep is a major issue, since scrapie is a serious problem in several European countries. To address this, an experimental procedure for DNA preparation and PCR-based detection of codons 136, 154, and 171 polymorphisms was established.

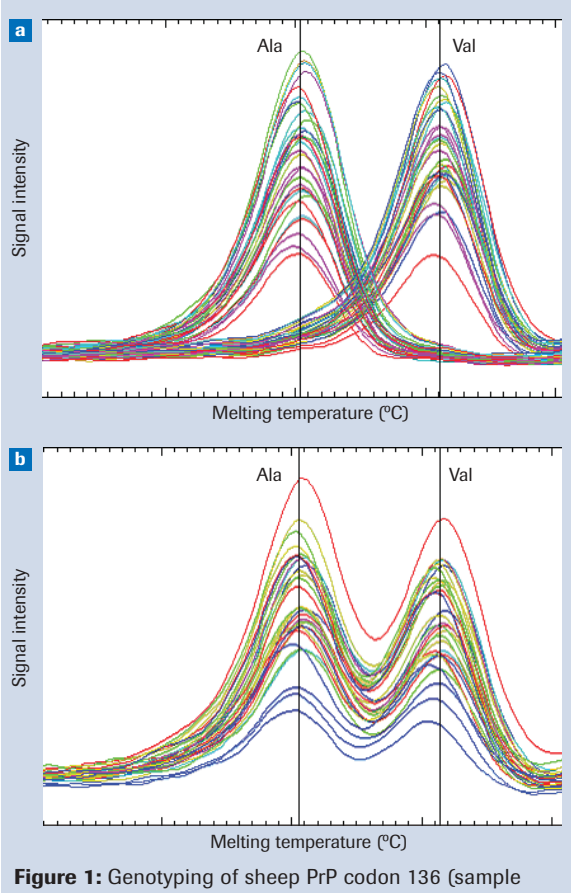

Figure 1: Genotyping of sheep PrP codon 136 (sample material: kit control templates and DNA from sheep whole blood) (a) homozygous genotypes alanine and valine (b) heterozygous genotypes alanine/valine. Each line within the graphs represents an individual sample.

DNA was purified from sheep whole blood and from sheep ear tissue using the MagNA Pure LC Instrument. PCR amplification and PrP genotype detection was performed in two steps:

- Amplification of a PrP-specific PCR product in a 384-well thermocycler.
- Detection of polymorphisms 136, 154, and 171 by hybridization of oligonucleotides (LightCycler Hybridization Probes) using the LightTyper 384 Instrument.

Materials and Methods

Samples

Prior to DNA preparation, whole blood samples were processed in two different ways:

- Sheep blood samples treated with EDTA as anticoagulant were frozen immediately after collection.
- Sheep blood samples treated with EDTA were mixed with two parts of RNA/DNA Stabilization Reagent for Blood/Bone Marrow.

Samples were stored at -20°C.
Ear tissue samples of sheep were collected and stored at -20°C.

DNA preparation from whole blood

DNA from whole blood samples was prepared using the MagNA Pure LC DNA Isolation Kit – Large Volume. Using the MagNA Pure LC Instrument and the respective purification protocol, 200 µl whole blood (or 200 µl mixture of blood and RNA/DNA Stabilization Reagent) were processed. DNA was recovered in 100 µl elution buffer.

DNA preparation from ear tissue

DNA from ear tissue samples was prepared using the MagNA Pure LC DNA Isolation Kit II (Tissue). Prior to DNA preparation, 10 mg sample material was incubated by shaking for 4 hours at 55°C with 110 µl Tissue Lysis Buffer from the kit (including 10 µl Proteinase K). Then

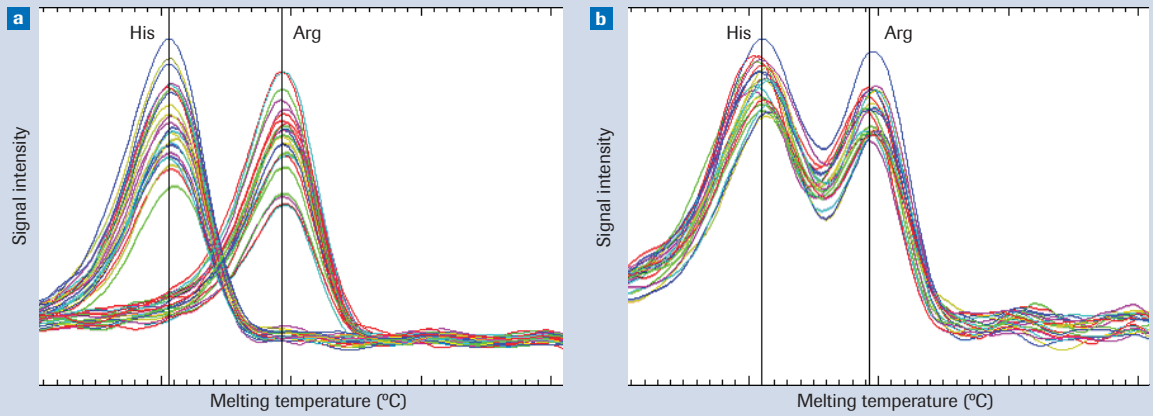

Figure 2: Genotyping of sheep PrP codon 154 (sample material: kit control templates and DNA from sheep whole blood) (a) homozygous genotypes histidine and arginine (b) heterozygous genotypes histidine/arginine. Each line within the graphs represents an individual sample.

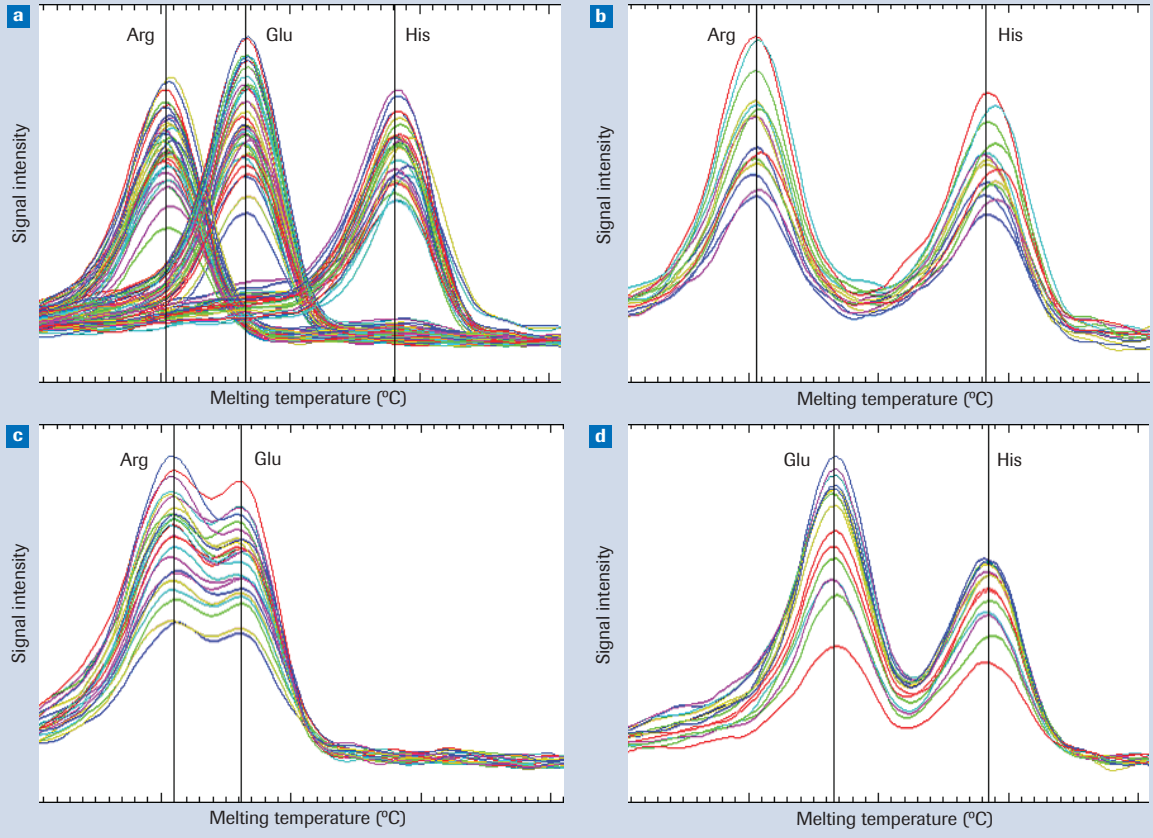

Figure 3: Genotyping of sheep PrP codon 171 (sample material: kit control templates and DNA from sheep whole blood). (a) homozygous genotypes arginine, glutamine and histidine; (b) heterozygous genotypes arginine/histidine; (c) heterozygous genotypes arginine/glutamine; (d) heterozygous genotypes glutamine/histidine. Each line within the graphs represents an individual sample.

another 110 µl Tissue Lysis Buffer (including 10 µl Proteinase K) was added and the samples were further incubated for 1 hour. Using the MagNA Pure LC Instrument and the respective purification protocol, 110 µl of each lysate was processed. DNA was recovered in 200 µl elution buffer.

Amplification of the sheep PrP gene by polymerase chain reaction (PCR)

PCR amplification of a 212-bp fragment of the sheep PrP gene was performed using a 384-well thermocycler. The PCR protocol for amplification was programmed according to the kit package insert.

Analysis of genotypes using the LightTyper Instrument

Following PCR, the 384-well plate was transferred directly into the LightTyper 384 Instrument. Melting-curve analysis was performed according to the kit package insert: by continuous increase of temperature a melting-curve profile was generated. The specified polymorphisms within the sheep PrP gene were detected by melting peaks with a defined temperature. The LightTyper Software automatically matched the melting peaks with the corresponding polymorphisms.

Results and Applications

Melting-curve analysis using the LightTyper Instrument

The LightTyper melting-curve analysis results in peak patterns specific for the specified sheep PrP genotypes. Homozygous (Figures 1a, 2a, 3a) and heterozygous polymorphisms (Figures 1b, 2b, 3b, 3c, 3d) for codon 136, codon 154, and codon 171 are shown.

Number of animals tested

For validation of the kit, 695 whole blood samples of individual sheep were genotyped at codons 136, 154, and 171. The reliability of results was proven by match of melting-curve analysis results and DNA sequencing information available for 32 animals (corresponding to 96 individual genotypes at codons 136, 154, and 171).

Additionally, ear tissue samples of 227 sheep (at codons 136, 154, and 171) resulted in conclusive melting curves comparable to those of the blood-derived preparations (data not shown).

Summary

The LightTyper Sheep PrP Gene Mutation Detection Kit (Codons 136, 154, and 171) from Roche Applied Science is an excellent tool for genotyping sheep prion protein using whole blood or ear tissue samples. Automated DNA preparation using the MagNA Pure LC Instrument and automated genotype identification using the LightTyper 384 Instrument is an easy, fast, and reliable test format for this application. ■

Order INFO

Product	Pack Size	Cat. No.
LightTyper Sheep PrP Gene Mutation Detection Kit	1 kit (768 reactions)	04 365 194 001
LightTyper 384 Instrument	1 instrument	03 357 414 001
MagNA Pure LC DNA Isolation Kit – Large Volume	1 kit	03 310 515 001
MagNA Pure LC DNA Isolation Kit II (Tissue)	1 kit	03 186 229 001
MagNA Pure LC Instrument	1 instrument	12 236 931 001
RNA/DNA Stabilization Reagent for Blood/Bone Marrow	1 bottle	11 934 317 001

Imprint

Roche

Roche Diagnostics GmbH
Roche Applied Science
Sandhofer Straße 116
D - 68305 Mannheim

Managing Editor: Dr. Burkhard Ziebolz
Editorial Team: Dr. Friedhelm Huebner
Dr. Ulrich Jellinghaus
Padma Rao

Biochemica is published quarterly by Roche Applied Science and Springer Verlag and is intended to disseminate information that is of interest and useful to the research community.

We always welcome your comments and suggestions. Contact your local affiliate for a FREE subscription or to change your address.

Biochemica 3, 2004, Cat. No. 04 390 377 001

Springer

Springer Verlag GmbH & Co. KG
Corporate Publishing
PD Dr. Beate Fruhstorfer,
Ulrike Hafner, Ursula Hilpert,
Dr. Sabine Höschele,
Dr. Friederike Holthausen,
Sabine Jost, Dr. Claudia Krekeler,
Dr. Christine Leist, Katrin Stobbe,
Sandra Thake (resp.),Teresa Windelen

We welcome manuscripts on any life science topic in which Roche Applied Science's products are used in novel techniques, improvements of common techniques, or simplified protocols. Manuscripts and correspondence concerning editorial matters should be addressed to:

Springer Verlag GmbH & Co. KG
Editorial Office Biochemica
Sandra Thake
Tiergartenstraße 17
D – 69121 Heidelberg
E-mail: thake@springer.de

 ISSN 0946 1310