

PCR-based assay for genotyping of the slick mutation in cattle

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Introduction

Several mutations exist in the prolactin receptor gene (PRLR) of cattle that cause a short, sleek hair coat (Porto-Neto et al., 2018). The mutation is dominant so slick-haired cattle can have either one or two copies of the mutation. The assay described here detects the most-well studied mutation (SLICK1) that causes translation of a truncated PRLR of 461 amino acids. Originally described in Senepol cattle, this mutation has also been introduced into Holsteins in Puerto Rico, Florida and elsewhere. Note that the assay will not detect other mutations in PRLR that also cause the slick phenotype.

Part 1: DNA Extraction

DNA is extracted from whole blood samples (fresh or previously frozen at -20°C) using Thermo Fisher's PureLink Genomic DNA Mini Kit (cat# K182001). For additional product details, alternate procedures (i.e., for cells), and troubleshooting refer to the product manual which can be found on the ThermoFisher website at

https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets/LSG/manuals/purelink_genomic_man.pdf

Note that there are more inexpensive methods of DNA extraction available. The method below is used because of its simplicity and speed.

Materials & Supplies Needed

Pure Link Genomic DNA Mini Kit

96-100% Ethanol (Sigma cat# E7023)

Water bath set to 55°C

Sterile 2 mL snap cap tubes – 2 per sample (Corning Costar microcentrifuge tubes, Fisher cat# 3213)

Microcentrifuge

Pipettors and sterile pipette tips

Set Up

1. Add ethanol to Wash Buffer 1 and Wash Buffer 2 as described on label (if not already completed).
2. Set water bath to 55°C.
3. Vortex all solutions to dissolve precipitate is present.

Procedure

1. Add 200 µL whole blood to sterile microcentrifuge tube (labeled with sample ID).
2. Add 20 µL Proteinase K to the sample.
3. Add 200 µL Lysis/Binding Buffer and mix by vortexing 5 sec to obtain homogenous solution.
4. Incubate in water bath at 55°C for 10 min to promote protein digestion.
5. Add 200 µL ethanol to the lysate. Mix by vortexing 5 sec to obtain homogenous solution.
6. Remove PureLink spin column from kit. Label lid with sample ID.
7. Pipette entire volume of Lysate (~640 µL) into spin column.
8. Centrifuge for 1 minute at 10,000 x g, room temperature.
9. Discard collection tube (bottom portion of the spin column set) and place the spin column into a clean collection tube, obtained from the kit.
10. Add 500 µL Wash Buffer 1 to the spin column.
11. Centrifuge for 1 min at 10,000 x g, room temperature.
12. Discard collection tube (bottom portion of the spin column set) and place the spin column into a clean collection tube, obtained from the kit.
13. Add 500 µL Wash Buffer 2 to the spin column.
14. Centrifuge for 3 min at maximum speed (~21,000 x g), room temperature.
15. Discard collection tube and place spin column into sterile 2 mL microcentrifuge tube.
 - a. Label this tube with any ID information on the sample. This is the final sample used for PCR.
16. Add 50 µL of Genomic Elution Buffer to the spin column.
 - a. See figure regarding elution buffer guidelines on pg 13 in manufacturer's handbook for more details on selecting amount of Elution Buffer to add.
17. Incubate for 1 min at room temperature.
18. Centrifuge (with 2 mL tube top open, point to side or to bottom of centrifuge) for 1 min at maximum speed (~21,000 x g), room temperature.
19. Purified DNA will be in the bottom of the 2 mL tube. Remove and discard the spin column portion.
 - a. If additional DNA is desired perform a second elution in an additional 2 mL microcentrifuge tube.
20. DNA is ready for use in PCR or for storage at -20°C

Part 2: PCR

Materials

KASP Assay primer (Cat #KBS-2100-100 LGC Genomics)

Primer ID: Indel 1

Primer Allele 5'-FAM GGACCAAACAGACCAACATGC-3' (wild-type allele)

Primer Allele 5'-HEX CTGGACCAAACAGACCAACATGT-3' (slick allele)

Primer Common 5'-CCTTCCCTGCCAGTTTCAATGGTTT-3'

KASP V4.0 2x Master Mix (cat #KBS-1016-002-US LGC Genomics)

RNAse free water (Cat# 1017979 Qiagen)

96 well PCR plates (Cat#MLL9601 Bio-Rad)

Micro seal 'B' seals (Cat#MSB1001 Bio-Rad)

Individual 0.2 μ L PCR tubes (Cat#TF110201 Bio-Rad)

Pipettors and sterile pipette tips

Plate centrifuge

Procedure

Plate Setup & Loading

1. Normalize the DNA samples to a final concentration of 10 ng/ μ L using RNAse free water to dilute samples.
2. Prepare master mix stock in sterile tube; (5 μ L KASP Master Mix + 0.14 μ L KASP Primer Indel 1) X # reactions
 - a. Add an extra 2-5 reactions to the calculations to allow for pipetting error.
3. Prepare a plate layout diagram with samples. All samples should be run in duplicate.
4. Add 5 μ L master mix stock solution to each well that will be used in the 96 well plate.
5. Following plate layout diagram, add 5 μ L of each diluted DNA sample to the corresponding well.
 - a. Gently mix or vortex sample to ensure uniformity.
 - b. Change pipette tips between each well.
6. Cover plate with seal tightly, ensuring no edges are lifted.
 - a. Tip: use the blunt end of a marker to run along junctions of the plate to ensure a tight seal.
7. Centrifuge plate for ~30 sec to ensure samples are at the bottom of each well.

PCR Machine Setup, Loading, & Run (SPECIFIC TO CFX MANAGER)

1. Open CFX Manager program and *Create New Run*.
2. Create and save the KASP assay protocol (the saved protocol can be used for future assays)

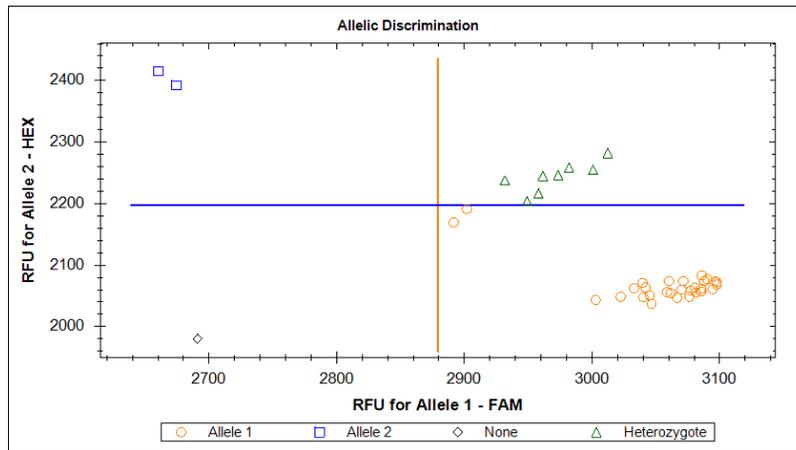
KASP ASSAY PROTOCOL

```
1) 94.0 C for 15:00
2) 94.0 C for 0:20
3) 61.0 C for 1:00
   Decrement temperature by -0.6 C per cycle
4) GOTO 2, 9 more times
5) 94.0 C for 0:20
6) 55.0 C for 1:00
7) GOTO 5, 25 more times
8) 37.0 C for 1:00
   + Plate Read
   END
```

3. Create and save a KASP assay plate layout (Load the plate layout and edit sample names for future assays).
 - a. Each well should have the following selected; Sample Type: NTC, LOAD FAM, HEX, and ROX, LOAD Sample Name (input sample id here).
 - b. Each sample should be run in duplicate.
4. Open PCR machine using the computer controls or button – never force the lid open.
5. Load prepared plates into PCR machine.
6. Close PCR machine using the computer controls or button – never force the lid closed.
7. Press START RUN. The protocol should take roughly 1.5 h to complete.

Part 3: Analysis of Results

When the run has finished, results will display on the main screen of the PCR computer. A result of zero copies of the slick allele indicates a wild type or “non-slick” animal. One (1) copy of the HEX slick allele indicates a heterozygote, and two (2) copies of the HEX slick allele indicates a homozygous animal. Two copies of the FAM allele (zero of HEX) indicate a wild type (non-slick) animal. When samples are all of good DNA quality and integrity the three result possibilities should be clearly grouped on the graph. We recommend running controls of DNA from animals of known genotype (heterozygote and both homozygotes) in each assay if possible. See below for examples of the Allelic Discrimination results.



Note: On occasion, lines on the results graph should be adjusted manually to reflect correct grouping of samples. Use the controls to make these adjustments. When in doubt, run the sample again.

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