Technical Note: Direct Genotyping of the Double-Muscling Locus (\(mh\)) in Piedmontese and Belgian Blue Cattle by Fluorescent PCR

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ABSTRACT: A simple PCR-based allele detection system has been developed to assist in the management of the two most prevalent double-muscled (\(mh\)) breeds in the U.S. Application of this assay will permit the implementation of structured mating systems dependent on precise genotypes at the \(mh\) locus. The genetic assay uses standard fluorescent genotyping technology and relies on the unique nucleotide composition of wild-type and mutant alleles of myostatin, the gene underlying the double-muscled phenotype. We present data demonstrating the efficacy of this fluorescent primer-based PCR assay in genotyping animal populations carrying normal and(or) mutant alleles of the myostatin gene.

Key Words: Polymerase Chain Reaction, Alleles

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Introduction

Double muscling is a condition that appears sporadically in many cattle breeds around the world. Double-muscled carcasses have significantly increased muscle mass expressed as retail product yield (RPYD; Shackelford, 1995) and produce a leaner product. However, problems with fertility and calving ease have limited the commercial use of double muscling in the United States. As a result, in some breeds double muscling has been specifically bred for, but in others it has been selected against. The two most prevalent double-muscled breeds in the United States are Piedmontese and Belgian Blue. Recent results indicate that a single copy of the \(mh\) allele can increase RPYD as much as 7 to 8% without the increased dystocia observed when two copies are present (Casas et al., 1999).

Mutations in the myostatin gene (\(MSTN\)) are responsible for double muscling (Grobet et al., 1997, 1998; Kambadur et al., 1997; McPherron and Lee, 1997). In the Belgian Blue breed, there is a 11-base pair (\(bp\)) deletion (\(\Delta 819-829\) relative to the initiation codon), causing a frameshift and premature translational termination. In Piedmontese, the \(MSTN\) mutation is a guanine to adenine transition at position 938 (G938A), causing a substitution of a critical cysteine with a tyrosine in the signaling portion of the protein. The objective of this research was to develop a simple genetic test capable of detecting the most common double muscle allele(s) in the Piedmontese and Belgian Blue breeds to assist in managing \(MSTN\) alleles in a structured mating system. The test makes use of standard fluorescent genotyping technology and relies on the unique nucleotide composition of wild-type and mutant alleles of \(MSTN\). The efficacy of this fluorescent primer-based PCR assay in genotyping animal populations carrying \(MSTN\) mutations is presented.

Materials and Methods

Genomic DNA was prepared from blood or ear notch by salt extraction (Miller et al., 1988). The PCR reactions (11 \(\mu\)L) contained .5 \(\mu\)M each of labeled allele-specific primer and 1 \(\mu\)M of unlabeled reverse primer; other PCR conditions were as described by Kappes et al. (1997) with a 68°C annealing cycle. Portions of the PCR reactions (1 to 3 \(\mu\)L) were diluted with formamide/blue dextran loading buffer (2 to 4 \(\mu\)L) and separated on 5% polyacrylamide gels. Genomic DNA was subjected to PCR that differentially amplified the wild-type and mutant alleles (Figure 1A). Allele-specific forward primers, labeled with the fluorophores TAMRA, ROX, or HEX (Perkin-Elmer, Foster City, CA), were paired with common reverse primers to produce fluorescent PCR products whose length and emission spectra (color) depend on the genotype of the animal at each position. The amplification products were separated by electrophoresis on an ABI377 automated fluorescent sequencer (Perkin-Elmer).
Figure 1. **A**) The sequence of third exon of the bovine myostatin gene and the position of the primers used for this allele-specific PCR assay are given. The 11 nucleotides deleted in some Belgian Blue animals (Δ819–829) is indicated in lower case. The BBNORM primer is specific to the normal allele because its 3'-end extends into the sequence that is deleted in some Belgian Blue animals. The BBDEL primer is specific for the Belgian Blue myostatin allele because it spans (indicated by dashed line) the deletion, with its first 20 nucleotides corresponding to 802 to 818 and the last three nucleotides corresponding to position 830 to 832. The position at which the G938A mutation is found in some Piedmontese animals is indicated in blue. The PNORM primer amplifies this portion of MSTN from animals that are normal at nucleotide 938. The oligonucleotide extends from residue 916 to 938, with the last residue corresponding to a guanosine (green arrow). The PMUT primer extends from nucleotide 913 to 938 and amplifies this portion of MSTN from animals with an adenine at nucleotide 938 (yellow arrow). A destabilizing mismatch has been introduced into both PNORM and PMUT primers to increase specificity at a common annealing temperature. Instead of the normal thymidine residue at position 937, indicated in red, a cytidine has been incorporated into both primers at this position. **B**) The assay was tested with animals of known genotype, either purebred double-muscled Piedmontese (lanes 2, 6, and 7) and Belgian Blue (lane 9) bulls, purebred normal Angus (lane 3) or Angus/Hereford (lane 10), crossbred Piedmontese × Angus (lanes 1, 4, 5, and 8), or Belgian Blue × MARC III (lane 11). The amplicons separated in lanes 1, 4, 5, and 8 derive from animals heterozygous at the position corresponding to the G938A mutation. The amplicons separated in lanes 2, 6, and 7 derive from animals homozygous mutant at this position, and lane 3 represents amplification from a homozygous normal animal. The amplicons separated in lanes 9, 10, and 11 demonstrate the ability of this assay to distinguish between homozygous mutant, homozygous normal, and heterozygous animals at 819 to 829, respectively.
The BBnorm (5'-TGGGCTTGATTGTGATGAAC-3') and BBdel (5'-TTTGCGCTTGATTGTGACAG-3') primers were both HEX labeled and amplified the normal and deleted Belgian Blue allele, respectively, when combined with the unlabeled BBrev primer (5'-ATTCTCCAGAAGCTTAATTGGC-3'). The BBnorm primer corresponded to positions 804 to 824 and was specific to the normal allele because it extended into the sequence that is deleted in Belgian Blue. The BBdel primer was specific for the mutant Belgian Blue allele because it spanned the deletion, with the first 20 nucleotides corresponding to position 802 to 818 and the last three nucleotides corresponding to position 830 to 832. The use of all three primers in the PCR reaction resulted in 134-bp HEX-labeled product in normal animals, 125-bp HEX-labeled product in Belgian Blue animals, and both in Belgian Blue crossbreds.

Specific amplification of the two Piedmontese alleles was more difficult because they differ by only a single nucleotide. The allele-specific primers used in this assay, in addition to possessing allele-specific bases at their 3' termini, also contained intentional destabilizing mismatches at the 5' terminus. This added instability resulted in a primer/template interaction of sufficient specificity to conclusively distinguish alleles upon PCR amplification. The PNORM primer (5'-GCCAATTACTGCTCTGGAGAACG-3') was labeled with TAMRA and corresponded to position 916 to 938, with the last two bases being CG. The C residue was an intentional mismatch at the C residue, ensured that under the assay conditions the mutant allele would amplify. In combination with the PIEDREV primer (5'-AAGGCCAATTACTGCTCTGGAGAAC-3'), labeled with ROX, corresponded to positions 913 to 938, with the last two bases being CA. The terminal A residue, in combination with the destabilizing mismatch at the C residue, ensured that under the proper stringency only the mutant allele would amplify. In combination with the PIEDREV primer (5'-GGGCGCGCTGAACCTCT-3'), corresponding to position 997–975, the PNORM and PMUT primers made 99-bp and 102-bp products from the normal and mutant alleles, respectively.

Results and Discussion

The ability of this assay to distinguish normal and mutant myostatin alleles was assessed using DNA from animal populations known to segregate the mh allele. Development of the assay relied on DNA from eight animals with genotypes previously determined by DNA sequencing. As demonstrated in Figure 1B, the test was capable of distinguishing the selected myostatin alleles (Δ819–829 or G938A), whether homozygous normal, heterozygous, or homozygous mutant genotypes.

The ability of the assay to differentiate normal and Δ819–829 alleles was examined in an additional 20 animals of Belgian Blue descent. All of the genotypes predicted with the assay were in concordance with that predicted by flanking microsatellites for all of these animals.

To confirm the ability of this test to differentiate alleles at G938A, the genotype predicted with our assay was compared with that predicted by flanking microsatellite markers in 204 offspring from a Piedmontese × Angus sire. The genotype predicted with our assay was corroborated by flanking markers in 201 animals (98.5% of the individuals examined). The assay-derived genotype was at odds with that predicted by flanking markers for three animals. This disagreement may result from error in either assay. Alternatively, these three animals may represent recombinants in the interval between flanking microsatellites, with the genotype predicted with the allele-specific assay being correct. These results support the feasibility of identifying animals harboring the G938A allele with this test.

Implications

The ability of our tests to provide accurate assessment of MSTN status of cattle will promote efficient management of the positive aspects of double-muscled breeds and facilitate specific introgression of these alleles into other breeds with genetic backgrounds that might minimize the negative effects of the syndrome.

Literature Cited


