Genetics and Molecular Biology, 29, 3, 496-497 (2006)

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Short Communication

Assessment of the myostatin *Q204X* allele using an allelic discrimination assay

Ana M. Sifuentes-Rincón, Herlinda E. Puentes-Montiel, Víctor R. Moreno-Medina and Xóchitl F. de la Rosa-Reyna

Laboratório de Biotecnología Animal I, Centro de Biotecnología Genómica, Tamaulipas, México.

Abstract

An allelic discrimination assay was designed and used to determine the genotypic and allelic frequencies of the myostatin (*MSTN*) gene *Q204X* allele from two Mexican Full-French herds. The assay is a simple high throughput genotyping method that could be applied to investigate the effect of the *Q204X* allele on the Charolais breed.

Key words: cattle, Charolais, carcass marker, allelic form, SNP detection.

Received: April 25, 2005; Accepted: December 21, 2005.

The myostatin (*MSTN*) gene is known to affect bovine carcass traits (Wilton 2003), the *Q204X* allele of this gene being present in French Charolais cattle (Grobet *et al*. 1997). Because of the impact of the *MSTN* gene on meat quality it is important to develop a highly sensitive, specific, cost-effective and easy to use DNA diagnostic test to determinate the presence of this allele. Current methods for *Q204X* genotyping in Charolais employ the restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) to identify the allele (Antoniou and Grosz 1999) and an oligonucleotide ligation assay for the simultaneous detection of bovine MSTN alleles is also available (Karim *et al.* 2000).

Compared with traditional PCR-based methods fluorescence-based single nucleotide polymorphism (SNP) detection assays offer several important advantages, including the absence of extensive post amplification manipulation (enzyme digestion, sequencing, etc.) and high throughput capacity (Livak *et al.* 1999). In this paper we describe the use of the allelic discrimination assay to genotype the *MSTN* gene *Q204X* allele in Mexican Charolais Full-French cattle derived from the European Charolais breed which segregates the MSTN *Q204X* allele.

Blood samples were obtained from 151 Mexican Full-French cattle from two herds kept at Nuevo Leon, Mexico. Herd 1, had been selected for improved lean beef and calving ease using founders from England, France and Ireland while herd 2 had been selected for conformation and weight gain traits using founders from France. Both herds provide dams, semen and natural service sires for use in nucleus and commercial herds and are considered as seed-stock for the Mexican Charolais Full-French in Mexico and in some Central America countries. Herds were evaluated according to their origin (Table 1).

Send correspondence to AM Sifuentes. Laboratorio de Biotecnología Animal I, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Boulevard del Maestro esq. Elías Piña, Col. Narciso Mendoza, 88710 Reynosa, Tamaulipas México. E-mail: ana@mail.cbg.ipn.mx.

Primers and probes were designed using the reported MSTN sequence (GenBank: AF01962), the forward-primer being 5'-GGAATCCGATCTCTTGAAACTTGAC A-3' and the reverse-primer 5'-GCTCTGCAACACTG TCTTCAC-3' while the two probes used were Q204Vic (5'-CAATGCTCTGCCAAATA) and Q204Fam (5'-ATC AATGCTCTACCAAATA). The *Q204X* synthetic DNA probe (5'-TATACTGGAATCCGATCTCTGAAACTTG ACATGAACCCAGGCACTGGTATTTGGTAGAGCAT TGATGTGAAGACAGTGTTGCAGAGCTGGCTC) containing the *Q204X* allele was used as a positive control during each allelic discrimination assay. Primers and probes were obtained from Applied Biosystems.

The PCR amplification was performed using a reaction mixture containing 250 ng of sample DNA, 12.5 μL of Taqman PCR master mix (Applied Biosystems) and 0.625 μL of the assay mix (containing primers and probes for allelic discrimination) on an ABI Prism 7000 Sequence Detection System using 96-well optical reaction plates under the following conditions: one cycle of 2 min at 50 °C and 10 min at 95 °C followed by 40 cycles of 15 s at 92 °C and 1 min at 60 °C. Analysis of each genotype was performed by using the ABI Prism 7000 Sequence Detection System software, each sample being visually examined and false positives eliminated.

Table 1 - Allelic and genotypic frequencies of the myostatin *Q204X* allele.

			Genotype frequency (%)			Allele frequencies	
Herd	Origin of founders	n	AA	AB	BB	A	B
1	England, France and Ireland	80	-	8.75	91.25	0.04	0.96
2	France	71	-	2.80	97.20	0.01	0.99

A = *Q204X* allele, B = normal allele.

We found the *Q204X* allele in both herds but only the AB (A = *Q204X* allele, B = normal allele) heterozygous genotype was present in the cattle sampled (Table 1), which showed genotypic and allelic frequencies similar to those reported in Charolais populations from Eastern European countries (Dvorak *et al.* 2002). The Chi-squared test showed that there were significant differences in AB genotype frequencies between herds ($p < 0.05$), which may have been caused by selecting for the phenotypic and productive traits of interest within each herd.

Dunner *et al.* (2003) has proposed that because *MSTN* gene presents a high allelic diversity it should be evaluated in all cattle populations.

We have found that the allelic discrimination approach is not only a simple, rapid and reproducible method for assessing the *Q204X* allele, but that the results for an individual genotype for this mutation can be quickly obtained in laboratories lacking access to real-time PCR equipment because allelic discrimination can be determined using traditional PCR and agarose gel electrophoresis. This method could be applied to asses the effect of the *MSTN Q204X* allele on double muscling as well as to establish mating systems focused on evaluating any association of this allele with growth and carcass traits.

Acknowledgments

The authors thank Dr. E. Casas for revising the manuscript and G.R. Cloud and R.M. González for the technical assistance and support in obtaining the samples. We acknowledge financial support from research grants CGPI-IPN 2004319 and SEP-CONACYT 39841.

References

Antoniou E and Grosz MD (1999) PCR based detection of bovine myostatin *Q204X* mutation. Anim Genet 30:231-232.

Dunner S, Miranda ME, Amigues Y, Cañon J, Georges M, Hanset R, Williams J and Ménissier F (2003) Haplotype diversity of the myostatin gene among beef cattle breeds. Genet Sel Evol 35:03-118.

Dvorak J, Filistowicz A, Hruska D, Horák P, Vrtková I, Kúbek A, Szulc T and Pomichal S (2002) The polymorphism of MSTN, PRNP and CSN3 genes in Charolais cattle. Anim Sci Reps 20:19-23.

Grobet L, Royo Martín LJ, Poncelet D, Pirottin D, Brouwers B, Riquet J, Shoeberlein A, Dunner S, Ménissier F, Massabanda J, Fries R, Hanset R and Georges M (1997) Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. Nat Genet 17:71-4.

Karim L, Coppieters W, Grobet L, Valentini A and Georges M (2000) Convenient genotyping of six myostatin mutations causing double-muscling in cattle using a multiplex oligonucleotide ligation assay. Anim Genet 31:396-399.

Livak KJ (1999) Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal 14:143-149.

Wilton JW (2003) Genomic and computing strategies in the optimization of the genetic component of speciation beef Journal of Animal Science 81(E.Suppl.2):E24-E27.

Associate Editor: Pedro Franklin Barbosa