

## PCR based detection of bovine myostatin Q204X mutation

E Antoniou, M Grosz

Fort Keogh Livestock and Range Research Laboratory, Route 1, Box 2021, Miles City, MT 59301, USA

Accepted 10 November 1998

*Description:* The bovine myostatin gene (*GDF8*)<sup>1, 2, 3</sup> is responsible for the double-muscléd phenotype observed in the Charolais breed<sup>4</sup>. The mutant allele contains a T instead of a C at nucleotide position 610 from the start codon. The mutation creates a stop codon and the truncated protein is expected to be inactive<sup>4</sup>. A PCR based test was designed to differentiate between the normal and mutant alleles. This test is faster and less expensive to perform than sequencing PCR products, which was used previously to resolve the two alleles. The sequence of the reverse primer used contains a mismatch with the cDNA sequence of the gene (G for T at position 21). When the *Q204X* allele is amplified, the presence of a T (instead of a C) at position 610 of the cDNA sequence, in conjunction with the mismatch introduced in the reverse primer, will eliminate a site (from gcngc to gtngc) for the restriction endonuclease *Fnu4HI*.

### Primer sequences:

Forward: TGAGGCCTGTCAAGACTCCT

Reverse: CACTGTCTTCACATCAATGCGCT

*PCR conditions:* reactions (12 µl) were performed with the following conditions: 80 ng genomic DNA, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 10 mM Tris-HCl pH 9.0, 30 µM each of dNTP, 0.4 µM each of two oligonucleotide primers, and 0.35 units of *Taq* polymerase. PCR profile was 3 min at 92 °C, followed by 35 cycles of 92 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s.

The predicted size of the PCR product is 155 bp. The product was cloned and sequenced to verify the specificity of the primers used. The PCR product sequence is completely identical to the published cDNA sequence (GenBank number AF019620).

*Restriction reaction:* The presence of the *Q204X* mutation will eliminate the *Fnu4HI* restriction site in the amplified fragment. Therefore, the homozygous 'normal' genotype will produce fragments of 23 and 132 bp (the 23 bp band is generally not visible; Fig. 1), homozygous *Q204X* genotype will produce a single fragment of 155 bp, and *Q204X/normal* heterozygotes will produce 23, 132, and 155 bp fragments. The fragments are separated on a 3% Metaphor agarose (FMC bioproducts) gel.

### References

- 1 Grobet *et al.* (1997) *Nat Genet* 17, 71-4
- 2 Kambadur *et al.* (1997) *Genome Res.* 7, 910-15
- 3 Mc Pherron *et al.* (1997) *Proc Natl Acad Sci USA* 94, 12457-61
- 4 Grobet *et al.* (1998) *Mamm. Genome* 9, 210-13

Correspondence: E Antoniou (e-mail: eric@larrr.ars.usda.gov).

---