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**Development of a novel PCR based analytical protocol for the characterization of the
two variants of prolactin gene that affect milk yield in sheep breeds**

Michael Orford, Ouranios Tzamaloukas, Christakis Papachristoforou and Despoina Miltiadou

Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of
Technology, P.O Box 50329, 3603, Limassol , Cyprus
Corresponding author: michael.orford@cut.ac.cy

Prolactin is a lactogenic hormone which plays a significant role in milk production in mammals, and its depletion in sheep provokes severe reduction of milk secretion. Two different variants within intron 2 of the prolactin gene have been described (A and B) and this polymorphism has been recently proposed as a marker for future breeding schemes in dairy sheep. The present study fully characterized this polymorphism, resulting in a simpler and cost effective PCR-based assay for genetic identification in sheep populations. Up to now, the two variants A and B were identified by their difference in RFLP digestion patterns. This assay, however, is laborious since it requires the generation of a 2.5kb PCR fragment from genomic DNA prior to digestion, which is often difficult to obtain. By sequencing PCR products from AA and BB homozygous animals and performing alignments, we confirmed that the B variant results from a 23bp deletion (sequence: GGTGTTTCTCTTCATAAAGACTC) of the A variant of the prolactin gene. This finding assisted the design of new primers for the identification of prolactin polymorphism based on the size of the PCR product and relinquishes the need of RFLP digestions.

Using these developments, we genotyped an experimental flock of 380 Chios breed sheep and carried out association studies. In contrast to other sheep breeds, such as the East Friesian and the Serra da Estrela, our preliminary data showed no significant effect of this gene on Chios first lactation milk yield. However, the effects of the prolactin gene merit more investigation.