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Development of a cost effective direct DNA sequencing method for rapid SNP detection and genotyping of candidate genes

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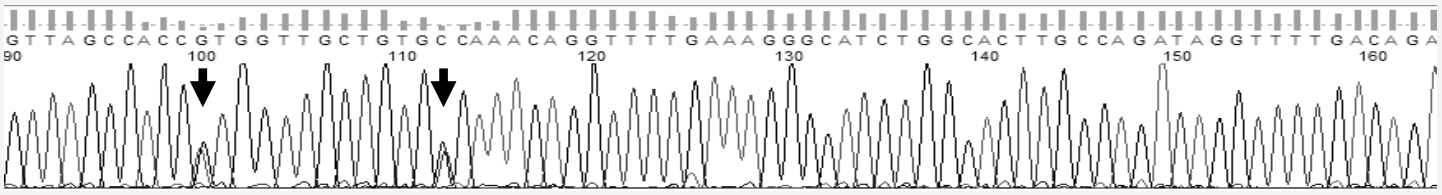
Introduction The search for novel polymorphisms which can either have an impact on animal health, or be utilized for the identification of genotype/phenotype correlations is of paramount importance in the area of animal genetics and genomics. Although high throughput SNP detection and genomic selection tools are now available for production animals such as cattle and sheep (Matukumalli et al., 2009), these methods are very expensive mainly due to high instrumentation and operating costs and hence uneconomical for the evaluation of many regional breeds of sheep around the world. The candidate gene approach alone or in combination with data from genome wide association studies however requires DNA sequencing from at least twenty individual animals for SNP identification. Common direct DNA sequencing methodologies used for SNP identification within a gene first require the PCR amplification of the region of interest followed by electrophoresis and gel extraction prior to performing actual DNA sequencing, thereby increasing the processing costs and limiting the sample throughput rate. This study therefore aimed at the development of a rapid and cost effective direct sequenced based genotyping method that would not require gel extraction or PCR clean up kits prior to sequencing. To illustrate the effectiveness of the technique, we have analysed the entire coding region of the ovine prolactin gene consisting of 5 exons, including both the 5′ and 3′ UTRs

Materials and methods Genomic DNA was isolated from Chios sheep breed using standard methods. After designing appropriate primers and optimizing PCR conditions to yield clean, high intensity bands, individual exons were PCR amplified in 25μl reactions from 20 individual animals in a single 96 well PCR plate. The PCR products were purified using isopropanol precipitation in the presence of sodium acetate, collected by centrifugation and washed with 70% ethanol.. Following a second centrifugation to remove the ethanol, the wells were briefly dried at 50°C in a PCR block prior to resuspension in 50μl of water. DNA sequencing reactions were then set up in a duplicate plate using 1/16th reactions of Big-Dye 3.1 chemistry and cycled according to the manufacturers recommendations. Following cycle sequencing, the termination products were subsequently purified by ethanol precipitation in the presence of EDTA, collected and washed by centrifugation as before, resuspended in 10μl of formamide and read on an ABI 3130 genetic analyzer. The sequence data obtained was confirmed by blast search against both the partial sheep genome database as well as the bovine database.

Results The prolactin exons sequenced ranged in size from 82-341bp. In a similar fashion to samples processed using conventional techniques, all traces showed an excellent signal to noise ratio with quality values ranging from 48-62 (0.0015% to > 0.0001% read error values). In addition, heterozygosity was easily detected as shown for example in figure 1 (black arrows). In addition to the ovine prolactin gene sequence presented here as an illustration, we have also used the method to evaluate three other genes from unrelated species, ranging from plants to microbes with reliable read lengths ranging at present up to 500-600bp
Using the suggested method, 96 samples were easily processed from genomic DNA to final sequence data in approximately 7 hours in a micro-titer plate format. By circumventing the need for electrophoretic and expensive PCR cleanup or gel extraction procedures and by reducing the amount of sequencing reagents necessary for sufficient quality reads, the cost for genotyping a 96 well plate was reduced approximately 20 times.

Figure 1 Representative sequencing chromatogram of ovine prolactin exon 2



Conclusions A rapid and cost effective sequencing protocol supporting large scale sequencing in a 96 well format has been presented which performs with equal efficacy to DNA sequencing performed by conventional methods in terms of reliability and sensitivity. However by circumventing the need to perform gel electrophoresis and subsequent gel purification to prepare the sample for subsequent sequence analysis it provides clear advantages in terms of reduced cost, , minimum sample handling and the ability to process multiple samples simultaneously. In addition, the resolution of the reads generated are equal in quality to those generated using conventional techniques thereby facilitating polymorphism detection and subsequent genotyping with a high degree of accuracy.

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References

Matukumalli, L.K., Lawley, C.T., Schnabel, R.D., Taylor, J.F., Allan, M.F. et al. 2009. PLoS ONE 4, e5350