GENETIC VARIATION IN GROWTH AND THE OPTIMISATION OF SNP MOLECULAR MARKERS FOR PARENTAGE ASSIGNMENT IN PASTURE-BASED CROSSBRED SHEEP

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SUMMARY:

Genetic variation in post-weaning growth and body conformation of first cross Merino prime lambs sired by Dorset, White Suffolk and Black Suffolk rams and the optimisation of single and multiplex SNPs for parentage assignment was investigated. Significant variations ($P<0.01$) attributable to sire genetics, gender and their interactions were detected; White Suffolk × Merino lambs had the highest average daily gains, chest girth and body condition scores of 0.17 kg/day, 83cm and 3.1, respectively.

Genomic DNA extracted from wool had the highest yield and purity ranging from 385-425 ng/µl (purity ratio of 1.6-1.9) than blood genomic DNA. The optimal annealing temperature for PCR interrogation primers in a multiplex combination of 4 SNPs was 65ºC, with PCR products run on a 3% agarose gel for 90 minutes. Furthermore, SNP primers 375, 382, 497 and 586 proved reliable in obtaining clear-cut bands. It was concluded that there is scope for utilizing a multiplex of up to 10 SNPs in a Beckman Coulter Platform to genotype and successfully assign crossbred sheep to their parents.

INTRODUCTION:

The combination of genetic selection and good management can deliver improved productivity gains as a result of the choices dual-purpose sheep farmers make when selecting rams and supplementary feed levels (Malau-Aduli and Holman 2010). However, incorrect paternity assignment can have a major effect on these rates of genetic gains (Weller *et al.* 2010). Thus, parentage testing is desirable for preservation of precise pedigree information (Fisher *et al.*, 2009), enhancing the rates of genetic gains, and managing livestock population (Kazuhiro *et al*. 2010).

Single Nucleotide Polymorphic (SNP) markers are known to have lower mutation rates (Kim and Misara 2007), lesser genotyping errors (Weller *et al.* 2010), more genetic stability (Donthu *et al.* 2010), more amenability to high-throughput automated analysis (Lin *et al.* 2010) and more robustness in laboratory handling and data interpretation than microsatellites (Allen *et al.* 2010). Our objectives were to optimise the utilisation of single and multiple SNPs for parentage assignment on the Beckman Coulter platform and to evaluate post-weaning growth variation due to sire genetics, gender and their interactions in first cross prime lambs under pasture-based management.

MATERIALS AND METHODS:

Animals. The experimental flock at the University of Tasmania Farm Cambridge, comprised five hundred first cross Merino weaners sired by 16 White Suffolk, Dorset and Black Suffolk rams. All the animals were maintained on ryegrass pastures. Fortnightly liveweight (LWT), body condition score (BCS) on a scale of 1-5, body length (BL), withers height (WH), chest girth (CG) and average daily gain (ADG) over a ten-week duration were recorded. Wool and blood samples from the 16 sires and 80 weaners were taken for SNP genotyping and parentage assignment.

Genomic DNA extraction. DNA was extracted from wool and blood samples using Ultraclean Tissue and Blood Spin DNA Isolation Kits (MoBio, Solana Beach, CA). DNA purity was quantified using the Nanodrop 8000 (NanoDrop, Wilmington, DE).

Primer design and PCR. PCR amplification primer pairs were selected from a panel of 32 SNP designed by the Australian Genome Research Facility. Flanking interrogation primers were designed using the Schmick Software to minimize crossover between different primer sets. The PCR fragments were amplified from 7.5 ng of genomic DNA in a total volume of 10 µl with 10 µm of each dNTP, 2 mm $MgCl_2$, PCR primers in various concentrations (7–24 fmol/µl) and 0.5 U of *HotStart*Taq *DNA polymerase* (Qiagen, Inc.). The PCR cycling profile was: initial denaturation at 95 °C for 15 min, followed by 55 cycles of: denaturation at 94 °C for 30 s; primer annealing at 65 °C for 30 s; and elongation at 72 °C for 1 min. Final extension was at 72 °C for 3 min. To remove remaining single-stranded primers and dNTPs, 1.5 µl of the PCR products was treated with 4 U of Exonuclease I and 0.8 U of antarctic phosphatase and then incubated at 37 °C for 60 min.

SNP assay. The GenomeLab^{TM} SNPStart Primer Extension Kit (PN A23201) was used for SNP assay according to the manufacturer's instructions. 7 µL of Antarctic Phosphatase Buffer and 2 µL Antarctic Phosphatase (5 units/ µL) were added to the PCR reaction and incubated at 37ºC for 15 minutes. The PCR reaction was then incubated at 80ºC for 20 minutes.

Allele separation. Samples were analysed by capillary electrophoresis using an ABI 3100 genetic analyzer (Applied Biosystems) and genemapper software (Applied Biosystems).

Exclusion probability computation. Probabilities of parentage exclusion were based on the probability that the genotypes of the progeny and the 'putative' parent would not conflict with Mendelian rules of inheritance as per Baruch and Weller (2008) and Jamieson and Taylor (1997) as $2(P_i)^2(1 - P_i)^2$, where P_i = minor allele frequency (MAF) for marker i. Thus, the probability of non-exclusion (PN) for a single marker is computed as $1 - 2(P_i)^2(1 - Pi)^2$, and for N markers:

$$\mathrm{PN} = \prod_{i=1}^{N}[1 - 2(P_i)^2(1 - P_i)^2].$$

A generalised linear model (SAS Inst., NC) was utilised in computing the fixed effects of sire breed, sex and their interactions on growth and body conformation parameters.

RESULTS AND DISCUSSION:

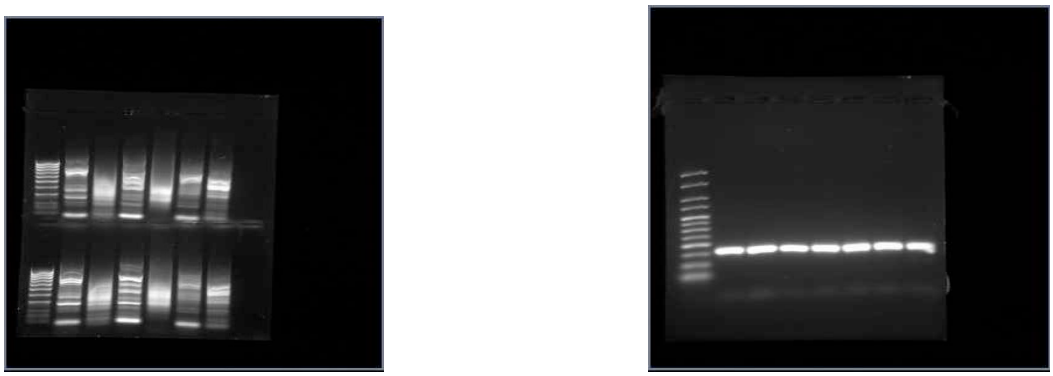
Meat and wool production from the Australian sheep industry are now on an equal footing with the farm gate value of wool production decreasing from over $6 billion to about $2.5 billion and the value of sheep meat increasing from $0.5 to $2.2 billion (Rowe 2010). There is an increasing economic pressure on the Merino industry to grow finer wool and at the same time, produce more sheep meat (Adams and Cronje 2003), hence the extensive utilization of sheep crossbreeding with more than 5 million crossbred ewes mated each year to meat rams and their progeny accounting for more than 30% of the national lamb slaughtered for meat (Afolayan *et al.* 2008). The results depicted on Table 1 followed the expected pattern we had earlier demonstrated (Malau-Aduli and Deng Akuoch 2010; Malau-Aduli and Holman 2010) in which White Suffolk-sired crossbreds and wethers had the highest average daily gains, chest girth and body condition scores. It is an indication that they are likely to grow faster and attain slaughter weight earlier than other crossbreds.

Wool genomic DNA had the highest yield and purity (385-425 ng/µl, (purity ratio of 1.6-1.9). Optimisation of PCR requires testing a number of variable components, the most important being primer annealing temperature (Li *et al.* 2010). When the annealing temperature is too low, non-specific DNA fragments are amplified which causes the appearance of multiple bands on agarose gel (as indicated in Figure 1a at 45°C). In contrast, when the annealing temperature was raised to 65°C (Figure 1b), clearly distinguishable bands were obtained.

Table 1. Variation in post-weaning growth and body conformation in crossbred sheep

Effect	LWT(kg)	BCS (cm)	CG (cm)	BL (cm)	WH (cm)	ADG (kg)
Sirebreed						
WS	32.9[a]	3.1[a]	83.1[a]	76.8[a]	59.4[a]	0.17[a]
Dorset	31.9[a]	2.8[b]	79.9[b]	78.4[a]	57.6[a]	0.08[b]
BS	33.2[a]	2.6[b]	78.4[b]	79.2[a]	59.6[a]	0.08[b]
p-value	*0.7209*	*0.0048 ** *	*0.0462*	*0.6951*	*0.4102*	*0.0332**
Sex						
Male	34.6[a]	3.2[a]	83.3[a]	78.0[a]	60.1[a]	0.15[a]
Female	30.7[b]	2.6[b]	79.2[b]	77.5[a]	57.5[a]	0.10[b]
p-value	*0.0158**	*0.0002***	*0.0061**	*0.2771*	*0.109*	*0.0065**

LWT= Liveweight, BCS=Body condition score, CG=Chest girth, BL=Body length, WH=Withers height, ADG=Average daily gain, WS=White Suffolk, BS=Black Suffolk. Least square means in columns bearing different superscripts significantly differ (*P<0.05, **P<0.01, ***P<0.001)



1a (45°C) 1b (65°C)

Figure 1. Optimisation of annealing temperatures of interrogation primers at 45-65°C

Table 2. SNPs, flanking primer sequences, minor allele frequencies, and parentage exclusion probabilities in White Suffolk (WS), Dorset and Black Suffolk (BS) sired first crosses

						Allele frequency		
SNP	Genebank no.	MAF	Flanking Sequence	Allele 1	Allele 2	WS	Dorset	BS
375	DU470132	0.49	GAGGG-[G/C]-CCAGT	G	C	0.46	0.37	0.44
382	DU271929	0.48	AGGAC-[A/C]-GGTTG	A	C	0.31	0.48	0.27
497	DU310703	0.45	ATGAC-[A/G]-AGGTC	A	G	0.42	0.40	0.50
586	DU469454	0.33	GGCAG-[T/C]-TGTGT	T	C	0.32	0.29	0.33
Exclusion probability given one putative parent (Jamieson and Taylor 1997)						0.901	0.872	0.893

Van Eenennaam *et al.* (2007) computed an exclusion probability of 0.956 for a set of 28 cattle SNPs, with the lowest MAF being 0.18. Heaton *et al.* (2002) found exclusion probabilities of 0.999 and 0.994 for a multi-breed composite and a purebred Angus population respectively, using a panel of 32 SNPs. The exclusion probability values in this study ranged from 0.87 to 0.90 (Table 2). This slightly lower values could be attributable to the fewer SNPs we used and the fact that we genotyped only one putative parent. However, our values were in close agreement with those of Karniol *et al*. (2009). In computing exclusion probabilities, it is assumed that the distribution of marker loci is independent, all markers are in Hardy–Weinberg equilibrium and there is a uniform distribution of allelic frequencies. Baruch and Weller (2008) reported that if the distribution tends towards a preponderance of markers with higher than expected MAFs, then non-exclusion probabilities will be lower than expected. This observed pattern has been confirmed in our study.

CONCLUSION:

White Suffolk x Merino crosses were the fastest growing and best conditioned weaners under pasture-based management and the 4-SNP multiplex for parentage assignment was a reliable, albeit, preliminary tool that warrants further investigation with more SNPs on a Beckman Coulter platform.

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