POLYMORPHISM AND LOCATION OF MINISATELLITE SEQUENCES IN SHEEP

R.D. Drinkwater, R. Hediger and D.J.S. Hetzel
Molecular Genetics Group, CSIRO, Division of Tropical Animal Production, Rockhampton, Qld. 4702 Australia

SUMMARY

Ten minisatellite sequences were assessed for their ability to detect restriction fragment length polymorphism (RFLP) markers. The sequences detected multiple marker loci with the mean number of variable bands for each sequence ranging from 2.4 to 12.2 per animal. However there was extensive band sharing between some of the minisatellite probes thereby reducing the number of independent loci analysed. *In situ* hybridisation of one minisatellite probe revealed that 33% of the loci were telomeric. However the remaining loci were randomly scattered across autosomes. Despite these limitations, the multi-locus minisatellite approach to linkage analysis was considered the most effective currently available for sheep.

INTRODUCTION

There is a concerted effort underway to develop DNA markers for specific traits in domestic livestock. DNA markers have the inherent attributes that they can be unequivocally measured on all animals at any age post-conception without requiring expression of the associated gene(s). Markers will therefore provide animal breeders with a means of more accurately and rapidly identifying genetically superior animals for certain traits. The majority of variation in production traits in domestic animals has yet to be associated with specific gene products. Hence the development of DNA markers for these traits is dependent on the reverse genetics approach (Orkin, 1986). This method involves the initial determination of genetic linkage of a genetic marker or markers with a specific gene or genes, which is followed by isolation of the gene.

Strategies for screening appropriate families for markers linked to a desired characteristic have developed rapidly. The choice of strategy will depend on the trait and the animal species involved. One strategy is to screen a large number of markers, providing 'significant' genome coverage for linkage analysis. Depending on marker spacing, 100-300 informative markers are required to ensure a high (> 95%) probability of detecting linkage. However many of the conventional markers are lowly polymorphic in livestock populations and therefore frequently uninformative for linkage analysis. In this paper we assess the use of minisatellite sequences to define highly polymorphic markers in sheep, a strategy we are employing to search for a marker linked to the Booroola high fecundity (F) gene in Merino sheep.

SATELLITE SEQUENCES

Polymorphic satellite DNA markers currently offer the most effective means of genome screening in sheep. The types available are the "micro", mini, and "major" satellites. The microsatellites have great potential for linkage analysis (Weber and May, 1989) e.g. high heterozygosities, and are well suited to rapid and automated analysis, but have yet to be isolated and characterised in sheep. Polymorphic major satellite DNA sequences have also been found in humans (Fowler *et al.* 1988) and in cattle (R.D. Drinkwater, unpublished). Minisatellite or variable number tandem repeat (VNTR) sequences are characterised by extreme individual variability in the number of copies of a short repeat sequence (Jeffreys *et al.* 1985). Their high level of polymorphism and ease of use make them potentially ideal for linkage analysis.

Under certain hybridisation conditions, minisatellite and major satellite sequences can simultaneously detect a range of related sequences with varying degrees of homology. Since segregation of these sequences can be followed, this approach permits simultaneous screening of multiple highly polymorphic markers, a potentially very efficient strategy for genome screening. In this study, we have evaluated such markers in sheep.

MINISATELLITE SEQUENCES

A total of 15 minisatellite sequences of human, mouse and bacterial origin were evaluated for their ability to detect homologous sequences in sheep based on the strength of cross hybridisation. Ten (66%) of the sequences gave repeatable and resolvable banding patterns and were selected for further investigation. These are listed in Table (1).

(i) Level of polymorphism

To analyse minisatellite sequences in the sheep genome, the 10 minisatellite probes were sequentially hybridised at low stringency using standard methods to a panel of nine unrelated animals. The patterns were assessed for the number of resolvable polymorphic bands and their polymorphism index (Table 1). Family analysis confirmed Mendelian inheritance of the bands.

Table 1 Mean number, frequency and heterozygosity of polymorphic bands for ten minisatellite probes in sheep. (Calculated as per Jeffreys *et al.* 1985)

Probe Designation	Minisatellite Sequence	Av. Number bands/animal	Mean band frequency	Prob. of heterozygosity
POLY-CA-	synthetic $(CA)_n$	12.2	0.19	0.90
BOV Z.I.	bovine zetaglobin related	12.2	0.19	0.90
INSULIN	human insulin gene	11.4	0.15	0.92
PER	mouse period repeat	10.6	0.20	0.89
M13	M13 phage	10.0	0.19	0.90
APO	human apolipoprotein C-11 gene	9.4	0.14	0.92
3'-αGLO	human alpha-gobin gene 3' repeat	6.9	0.17	0.91
pUCJ	human myoglobin gene	6.7	0.16	0.91
HRAS	human C-Ha-ras-1 gene	5.8	0.16	0.91
pYNZ22	human zeta-globin related	2.4	0.24	0.93

The minisatellite probes varied greatly in the number of polymorphic bands detected as well as in the strength of hybridisation, the latter relating to the degree of homology. Bands below 3 kilobases were not scored. The six most useful probes averaged 11.2 bands per individual with each band occurring in 18% of animals. Thus the sheep minisatellite sequences related to the probes used were in general slightly less abundant and less polymorphic than in humans (e.g. Jeffreys *et al.* 1985), but were similar to a report in cattle (Georges *et al.* 1989).

(ii) Band sharing

Band sharing, i.e. the visualisation of bands of the same fragment size by different probes, was evident between a number of the minisatellite probes (Table 2). The strongest overlaps were between poly-CA, PER, BOV Z.1, INSULIN and HRAS generated patterns, which may relate to the presence of CA motifs in these sequences.. Other significant sharing was observed between pYNZ22 and pUCJ as well as between M13 and APO.

Table 2 Sharing of polymorphic bands between minisatellite probes in sheep. Band sharing was scored as follows: A = 75-100%; B=50-75%; C=25-50%; D = 1-25%; - = no sharing.

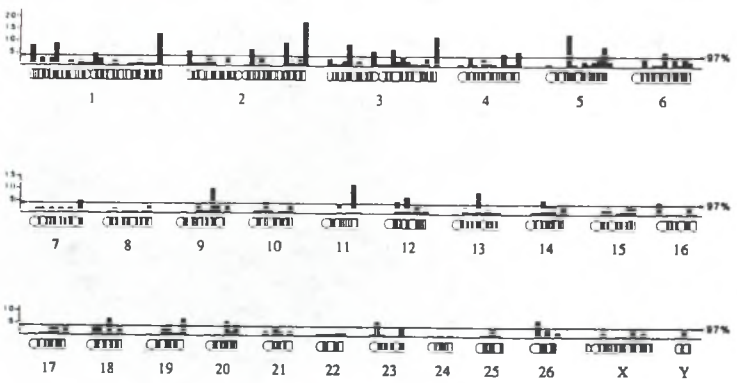
	Poly-CA	Bov.z.1	Per	Insulin	M13	Apo	3'.a.Glo	pUCJ	Hras
Bov.z.1	A								
Per	A	A							
Insulin	C	D	D						
M13	-	-	-	-					
Apo	-	-	-	D	B				
3'.a.Glo	-	-	-	-	-	-			
pUCJ	-	-	-	-	-	D	-		
Hras	A	A	A	D	-	-	-	-	
pYNZ22	-	-	-	-	-	-	-	B	D

Band sharing would be normally be caused by common homology between minisatellite probes and DNA fragments or in some instances by comigration of unrelated sequences. Clearly where significant band sharing exists, different probes will be monitoring segregation at common loci, thereby reducing expected genome coverage.

(iii) Location

The chromosomal location of minisatellite loci is crucial to their use in linkage studies. In man, there is evidence that minisatellite loci are concentrated at chromosome termini or telomeres (Royle *et al.* 1988). The BOV.Z.1 probe was hybridised at low stringency to ovine metaphase spreads as described by Hediger *et al.* (1990). Of all silver grains scored on chromosomes, 29% were located at the proterminal ends (Figure 1). Of the statistically significant ($P < 0.03$) peaks recorded, 33% (11/33) were telomeric. The remaining 22 peaks were distributed evenly over most of the chromosomes. Thus although the minisatellites tended to be concentrated at the telomeres, the majority of loci were distributed along the chromosomes.

Figure 1 Histogram showing the number of silver grains scored on ovine metaphase spreads following *in situ* hybridisation with the BOV.Z.1 (Zetaglobin) minisatellite probe.



CONCLUSIONS

This study has demonstrated that heterologous minisatellite sequences can be used to define multiple polymorphic markers in sheep. However the efficiency of the probes, in terms of the number of markers detected was variable. For any one probe, the effective number of polymorphic marker loci is reduced by band allelism and non-random distribution of markers across chromosomes. Allelism for these probes is currently being analysed. There was evidence provided by *in situ* hybridisation of one probe BOV.Z.1, for some minisatellite loci to be clustered at telomeres. However, the majority of loci were well distributed. Significant band sharing between minisatellite probes reduced the number of markers explored. Finally, it should be remembered that for markers defined by multilocus minisatellite probes linkage analysis can only be carried out within families, since marker bands of a given size cannot be related from one family to another. Thus this approach is only suitable for large family sizes.

In cattle, Georges *et al.* (1990) reported allelism will on average result in a 17% loss in the effective number of markers explored with minisatellite probes, while close linkage of loci both within and between probes will account for a further 18% reduction. If similar parameters apply in sheep, the best six probes in this study would each effectively monitor 7.6 independent marker loci. If 250 loci are required for a minimum spacing of 20 cM between markers, then 33 minisatellite probes would be necessary. This number will increase with band overlap between probes. Therefore it is difficult to envisage complete genome coverage with such probes. In the future, a panel of polymorphic single locus probes, chosen from a genetic map to ensure even distribution, will be needed for efficient linkage screening. Nevertheless, the multi-locus probes probably represent the most efficient means of marker screening in sheep at the present time.

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