## IDENTIFICATION OF RAPD GENETIC MARKERS IN SHEEP

William T. Cushwa and Juan F. Medrano
Department of Animal Science
University of California
Davis, CA 95616 USA

### SUMMARY

The random amplified polymorphic DNA (RAPD) assay was evaluated as a means of identifying genetic markers in sheep. DNA samples from an AgResearch International Mapping Pedigree, consisting of maternal and paternal grandparents, sire, dam, and twelve full-sib progeny, were screened with 130 10-mer oligonucleotide primers of arbitrary sequence. A total of 96 polymorphisms were identified between the sire and dam, and 56% (54/96) of these markers segregated in the progeny. Screening of the grandparental samples confirmed the origin of the segregating parental polymorphisms and the consistency and reproducibility of the assay. Three candidate Y-linked markers were identified, and their chromosomal assignment was confirmed by screening four other AgResearch pedigrees. These results demonstrate that the RAPD assay is very effective for identifying genetic markers in sheep. We are continuing to screen the AgResearch International Mapping Pedigrees, with the objective of contributing RAPD markers to the sheep genetic map.

### INTRODUCTION

Identifying genes that influence economically important traits is an important research objective for the domestic animal industry. In order to accomplish this objective, genetic maps are being established for a number of domestic animal species, including cattle, chickens, pigs, and sheep (NAGRP News, 1993). The creation of these maps will provide markers to efficiently screen resource populations and identify linkages to traits of economic importance. The identification of closely linked markers is a first step towards eventually cloning and characterizing the loci determining these traits.

A relatively new approach to identifying genetic markers is the random amplified polymorphic DNA (RAPD) assay (Williams et al., 1990). This method is based on the polymerase chain reaction (PCR) and uses short oligonucleotide primers (usually 10-mers), of arbitrary sequence, to amplify discrete DNA segments in an organism's genome. Inherited polymorphisms are detected on the basis of the presence or absence of PCR products from patterns after gel electrophoresis. The RAPD assay represents one of the most powerful strategies for gene mapping because each primer screens approximately 5 to 15 loci during the same PCR reaction (Michelmore et al., 1991; Reidy et al., 1992), and a very large number of easily-scored markers can be generated in a relatively short period of time without any previous knowledge of the DNA sequences of the organism's genome (Woodward et al., 1992).

The RAPD assay has been successfully used in a number of studies to identify molecular markers for plant (e.g. Reiter et al., 1992; Kiss et al., 1993; Foolad et al., 1993) and microbial (e.g. Akopyanz et al., 1992; Fani et al., 1993) genetic analysis. Applications for identifying genetic markers in mammalian domestic animal species have been relatively limited, even though the efficiency and usefulness of the assay have been demonstrated with mice. In three independent studies, a total of 95 RAPD markers were assigned to specific mouse chromosomes using recombinant inbred lines (Nadeau et al., 1992; Serikawa et al., 1992; Woodward et al., 1992). The RAPD markers were dispersed throughout the mouse genome, with at least one marker localized on each of the autosomes, except for chromosome 15. A group of eight previously unmapped RAPD markers was recently assigned to the Y chromosome (Wardell et al., 1993).

The RAPD assay has been successfully used to generate a genetic map of the chicken Z chromosome (Levin et al., 1993), to identify a cattle sex specific marker (Xiong et al., 1993) and a breed specific marker (Kemp and Teale, 1992), and to estimate genetic variation in cattle populations (Bardin et al., 1992).

The purpose of this study was to evaluate the usefulness of the RAPD assay for identifying genetic markers in a sheep reference family.

## MATERIALS AND METHODS

**Sheep reference families:** The AgResearch International Mapping Pedigrees (Crawford, 1992) contains a set of nine, three-generation families (A to I) that was created, using embryo transfer, by mating $F_1$ sires (Texel sire x Coopworth dams) and four-way cross dams ([Merino x Romney sires] x [Perendale x Coopworth dams]). There are approximately 10 full-sib progeny per family (range: 8 to 17) and a total of 97 progeny from all families. International Mapping Pedigree G, which contains 12 progeny, was used in this study.

**RAPD assay protocol:** Reactions were performed in a 15 $\mu l$ volume containing 1X PCR buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100), 0.10 mM of each dNTP, 2.0 mM $MgCl_2$, 0.60 $\mu M$ of primer (Operon Technologies (OP), Alameda, CA, USA; primers OPA02 to OPG11), 0.60 U of Taq DNA Polymerase (Promega Corp., Madison, WI, USA), 25 ng of genomic DNA, and overlayed with one drop of light mineral oil (Sigma, St. Louis, MO, USA). Amplifications were performed in MJ Research PTC-100-96V thermal cyclers (Watertown, MA, USA) programmed for 40 cycles of 94°C for 1 min., 35°C for 1 min., and 72°C for 2 min. An initial denaturation time of 2 min. at 94°C and a final extension time of 10 min. at 72°C were included on the first and last cycles, respectively.

Thirteen microliters of PCR product were electrophoresed in 1.3% agarose (International Biotechnologies, New Haven, CT, USA) and 0.5% Synergel (Diversified Biotech, Boston, MA, USA) gels at 2.5 V/cm for 6 hrs. The products were detected by staining with ethidium bromide and were visualized under UV light. Figure 1 shows the results obtained using primer OPD02 to screen International Mapping Pedigree G.

## RESULTS

Forty-eight percent (63/130) of the primers screened produced at least one polymorphism between the sire and dam. Sixty-three percent (40/63) of these primers produced one polymorphism, 27% (17/63) produced two, and 5% (3/63) produced three. Two of the remaining three primers (OPC08 and OPF05) produced four polymorphisms each, while primer OPF06 produced five.

Using the panel of 130 primers, a total of 96 polymorphisms was identified between the sire and the dam. Fifty-six percent (54/96) of these polymorphisms segregated in the progeny. Two of the polymorphisms (OPD15-490 and OPE02-1000) appeared to segregate as intensity polymorphisms (bright vs. faint), while the others were scored as present vs. absent. The remaining 44% (42/96) of the polymorphisms did not segregate due to parental homozygosity. If the parent is heterozygous for the RAPD marker, the probability of detecting heterozygosity is 99.9% ($1-[0.5]^{12}$) when 12 progeny are screened. The grandparental DNA samples were also screened with the primers that produced segregating polymorphisms. No parental genotype discrepancies were observed.

Three candidate Y chromosome-linked markers (OPA20-1230, OPD02-550, and OPE04-1107) were identified, and their chromosomal assignment was confirmed by screening four other AgResearch International Mapping Pedigrees (A, B, D, and E). These RAPD markers were scored as present in all of the males (n = 41) and absent in all of the females (n = 37).

## DISCUSSION

These results indicate that the RAPD assay is a useful approach for identifying genetic markers in sheep. On average, two segregating polymorphism were detected for every five primers screened in this AgResearch pedigree. The RAPD markers were reproducible and easily scored. However, based on our experience, an initial time investment is required to develop optimal PCR and electrophoresis protocols.

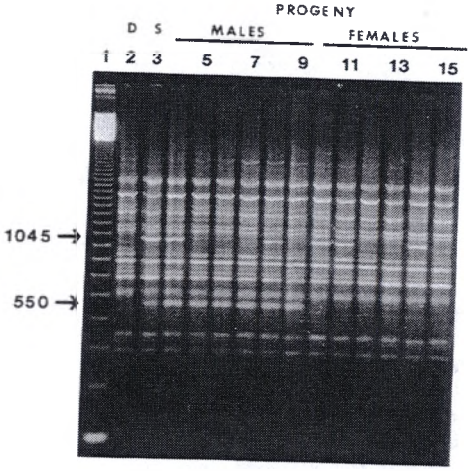


**Figure 1:** Gel electrophoresis of the RAPD products produced using primer OPD02 to screen International Mapping Pedigree G. Lane 1: 123 bp marker; lanes 2 and 3: dam (D) and sire (S), respectively; lanes 4 to 9: male progeny; lanes 10 to 15: female progeny. The approximate sizes (bp) of the two polymorphic RAPD markers, OPD02-550 and OPD02-1045, are indicated. Marker OPD02-550 has been confirmed to be linked to the Y chromosome.

We have established a high throughput screening protocol that utilizes 96-well microtiter plates for PCR, and multichannel pipetters for PCR setup and gel loading. The total "hands-on" time required for a person to screen 12 individuals with 16 primers (including PCR setup, and preparing, loading, and photographing gels) is approximately 3 hrs.

Preliminary data suggest that the three Y chromosome-linked RAPD markers are conserved in at least three sheep breeds (Finn, Javanese, and Suffolk). The primer used to identify one of these markers, OPE04, also produced a Y-linked marker in mice that is approximately 123 bp larger than the corresponding sheep marker (Wardell et al., 1993). The AgResearch pedigree was also screened with two other primers (OPF05 and OPG02) that were reported to produced Y-linked mouse RAPD markers. However, no Y-linked sheep markers were identified using these two primers.

We are presently converting the three Y chromosome-linked RAPD markers to sequence characterized amplified regions (SCARs; Paran and Michelmore, 1993). This conversion is accomplished by cloning the specific RAPD fragment, sequencing the ends, and designing pairs of extended, strand-specific oligonucleotide primers. The pairs of SCAR primers contain the original 10 bases of the RAPD primer plus the next 12 to 14 bases from the end. The longer, sequence complementary primers permit a more stringent annealing temperature than that used with the RAPD assay ($\geq$ 60°C vs. 35°C). The resulting SCAR marker is therefore less susceptible than the original RAPD marker to varying reaction and thermal cycler conditions and represents a universally-reproducible genetic marker.

We are continuing to identify and map RAPD markers in the AgResearch International Mapping Pedigrees, with the objective of contributing these markers to the sheep genetic map.

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