Rapid communication: Microsatellites isolated from BAC clones containing v-akt1 murine thymoma viral oncogene homolog 1 and bradykinin receptor B2 assigned to sheep chromosome 18 by linkage analysis

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Genus and Species. Ovis aries.

Locus. Ovine DNA segment OarTMR1 associated with ovine v-akt1 murine thymoma viral oncogene homolog 1 (AKT1) and ovine DNA segment OB2 associated with ovine bradykinin receptor B2 (BDKRB2).

Source and Description of Primers. Primers for AKT1 (GenBank accession no. AF207873) were obtained from Scott Fahrenkrug, USDA-MARC, Clay Center, Nebraska, and were sent to Daniel Vaiman, INRA-Jouy, France, to isolate two positive clones from an ovine BAC library that had an average insert size of 123 kb (Vaiman et al., 1999). The procedure of Munroe et al. (1994) was used to identify microsatellite repeats in these clones and primers were designed for an AC-repeat designated OarTMR1. Primers (OarTMR1 forward: 5′-GCCGCTGGTTCTCCTCCA-3′ and OarTMR1 reverse: 5′-CAGAGCCCTGCGTCCATCTTCT-3′; GenBank accession no. AF224742) were used to amplify the DNA microsatellite OarTMR1. Primers for BDKRB2 (GenBank accession no. AF207860) were designed and used by Scott Fahrenkrug, USDA-MARC, Clay Center, Nebraska, to isolate the DNA microsatellite OB2 (GenBank accession no. AF207876) from a positive clone from an ovine BAC library with an average insert size of 103 kb (Gill et al., 1999). Primers (OB2 forward: 5′-CTGCCCGATCCTTCTGCTT-3′ and OB2 reverse: 5′-AAAGGGGCAGATTCAGTATCCA-3′) were used to amplify the DNA microsatellite OB2.

Method of Detection. Standard PCR conditions were used to amplify both microsatellites, using 32P-labeled reverse primers, Gibco BRL Taq DNA polymerase and buffers (Life Technologies, Grand Island, NY), and 2 mM MgCl2 in a touchdown procedure (Crawford et al., 1995). The PCR products were separated by standard high-voltage electrophoresis in 6% polyacrylamide gels and visualized by autoradiography on Kodak (Rochester, NY) XAR5 x-ray film.

Figure 1. Partial linkage map of the distal region of sheep chromosome 18 showing the positions of OarTMR1 and OB2 relative to other linked markers on OAR18. The centromere is located beyond the dotted lines at the top of the schematic. Distance (Kosambi centimorgans, cM) was calculated by multipoint linkage analysis (CRIMAP; Green et al., 1990). Boxes drawn around loci indicate that these loci have been mapped to the same position on OAR18 and that their relative order cannot, therefore, be determined in this data set.

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**Description of Polymorphism.** Six alleles were detected for OarTMR1 (sizes 124, 126, 130, 134, 136, and 138 bp) and five for OB2 (sizes 206, 210, 121, 214, and 216 bp) in the International Mapping Flock (IMF; Crawford et al., 1995). The two loci had polymorphic information contents calculated from the 15 sampled grandparents of the IMF of 0.68 and 0.70 respectively.

**Inheritance Pattern.** Individuals from the IMF were genotyped for both markers. A total of 193 informative meioses were scored for OarTMR1 and 153 for OB2. Linkage analysis against currently available information in the SheepBase database was performed using CRIMAP (Green et al., 1990). Mapping data for McMA26 and IGHE were from J. F. Maddox (unpublished data).

**Chromosomal Location.** There was strong evidence for linkage with sheep chromosome (OAR) 18 markers for both loci. The maximum two-point LOD scores obtained were 28.2 between OarTMR1 and OY5 (recombination fraction 0.07) and 38.1 between OB2 and McMA26 (recombination fraction 0.01). The multipoint linkage map (Figure 1) showed that OarTMR1 mapped distal to OY15, with the most likely position being 3.5 cM distal to IGHE. OB2 mapped between McM38 and CSSM18, with the most likely position being 2.6 cM distal to McM38.

**Comments.** The mapping of these loci was undertaken as part of a project to fine-map the ribeye muscling locus in sheep, provisionally designated REM (SheepBase, 2000). Previous studies had placed this locus between serine protease inhibitor (PIA) and immunoglobulin heavy chain 7 (heavy chain of IgE) (IGHE) on the distal portion of sheep chromosome 18 (Lord et al., 1998). Comparative genome analysis indicates that this region is equivalent to the region of human chromosome 14 (HSA14) from q32.1 (PI) – q32.33 (IGHE) and mouse chromosome 12 (MMU12), 51 cM (Spi1) – 58 cM (Igh7). BDKRB2 is known to be located on HSA14q32.1–q32.2, and AKT1 on HSA14q32.31. In mice, Bdkrb2 is at 53 cM on MMU12 and Akt is at 58 cM. Although the addition of these loci to the map of sheep chromosome 18 provides additional evidence that confirms that REM is located in a region equivalent to that between HSA14q32.2–q32.31 and MMU12 (53–58 cM), neither locus is in the region to which REM has been mapped.

**Literature Cited**


Green, P., K. Falls, and S. Crook. 1990. Documentation for CRIMAP. version 2.4. Washington Univ. School of Medicine, St. Louis, MO.


**Key Words:** Chromosomes, Gene Mapping, Linkage, Sheep