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Rapid Communication: Mapping of an X-Linked Porcine Microsatellite

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Abstract

Source and Description of Probe. Microsatellite locus S0511, originally identified from a porcine genomic library screened with d(CA)9, was used in these studies. Primers MS6F: 5¢AAAAACACGGAA GCAATAGAAATGTC-3¢ and MS5R: 5¢-GTCCATC CATGTTGCTCCAAATGG-3¢ were used in a PCR protocol to amplify sequence specific alleles. Primer MS6F was labeled using [g-32P]dATP (3,000 Ci/mmol, DuPont NEN) and polynucleotide kinase (New England Biolabs).

Keywords

Porcine, Dinucleotide Repeat, Microsatellite, PCR, X-linked

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PCR Conditions. PCR reaction conditions were 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* Tris-HCl, pH 9.0 (at 25°C), 100 m*M* dNTPs, 50 n*M* each primer, and 12.5 ng genomic DNA for a 10-μL reaction volume. Samples were denatured at 94°C for 3 min and amplified using a thermal profile of 94°C for 1 min, 65°C for 1 min, 72°C for 1 min repeated 34 times, followed by a final extension of 8 min at 72°C.

Description of Polymorphism. Polymorphisms consisting of six alleles ranging in size from 152 to 178 bp were observed in the PiGMaP reference families.

Inheritance Pattern. Codominant inheritance of the alleles was observed in five three-generation pedigrees used for mapping. Segregation of the alleles for one three-generation pedigree is shown in Figure 1.

Chromosomal Location. Locus S0511 mapped to the X chromosome using linkage mapping and the PiGMaP reference family pedigree.

Frequency. S0511 is an X-linked microsatellite, so females contribute twice as many alleles as males. Therefore, only females were examined for a population study. A total of 10 Landrace females, 17 Duroc females, 10 Yorkshire females, 10 Chester White females, and 9 Hampshire females were genotyped using marker S0511. The overall frequencies of each allele in this population are .13 for 178-bp allele, .05 for 174-bp allele, .08 for 170-bp allele, .12 for 169-bp allele, .48 for 154-bp allele, and .12 for 152-bp allele. The 169-bp and 170-bp alleles were observed in PiGMaP reference animals and unrelated population study animals. One would expect allelic differences in multiples of the dinucleotide repeat. The reason for the one base pair difference in alleles 3 and 4 is not known.

Availability. Limited quantities of primers are available from G. Plastow.

Key Words: Porcine, Dinucleotide Repeat, Microsatellite, PCR, X-linked

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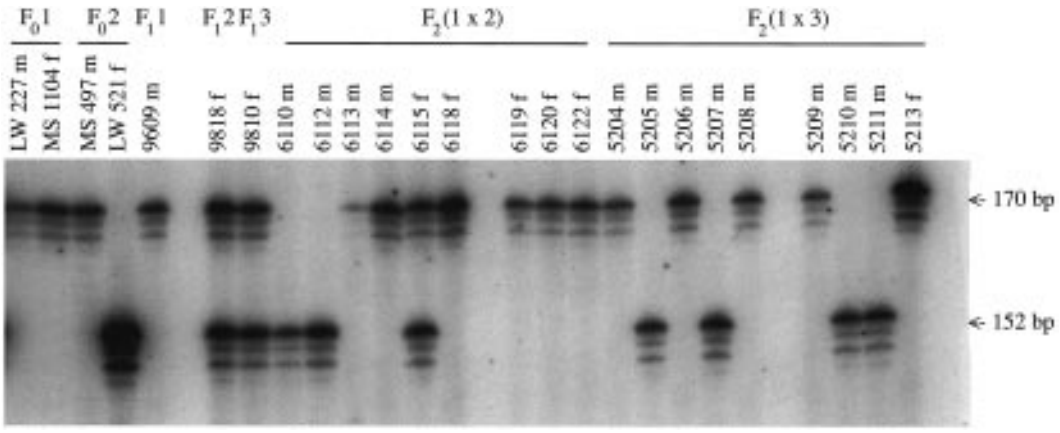

Figure 1. Autoradiograph showing the inheritance pattern observed using one of the PiGMaP pedigrees. Alleles were separated using a 6% polyacrylamide gel. A sequencing ladder was used to determine allele sizes. Animal identification followed by designation of its sex (m = male, f = female) is listed above each lane. F_0, F_1, and F_2 are the grandparents, parents, and offspring of the three-generation pedigree. $F_0$1 produced $F_1$1. $F_0$2 produced $F_1$2 and $F_1$3. $F_1$1 × $F_1$2 = F_2(1 × 2) and $F_1$1 × $F_1$3 = F_2(1 × 3).