Rapid communication: Mapping of Oxytocin (OXT) to the central region of bovine chromosome 13 by linkage analysis using SSCP

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Polymorphism. Single strand conformation polymorphism (SSCP) was detected within PCR amplification products of the bovine Oxytocin (OXT) gene.

Source and Description of Primers. Primers were derived from the published genomic sequence (Ruppert et al., 1984) based on primer positions given by Neibergs et al. (1991) and Dietz et al. (1992).

Primer Sequences. The forward primer was 5′-GCCATTAGCCGACATAACCTTGACC-3′ and the reverse primer was 5′-ACGGTGCTAAGAGGGCAGCCGCAT-3′.

Method of Detection. The primers amplified a 125-bp product under the following conditions: 15-μL reactions made up of 50 to 100 ng of genomic DNA, 200 μM dNTP, 10 pmol of each primer, 1.5 mM MgCl₂, and 0.75 U Taq polymerase (Amersham Pharmacia biotech, Freiberg, Germany) in reaction buffer supplied by the manufacturer. The PCR was carried out after an initial denaturation step at 94°C (1 min), then 60°C (1 min) and 72°C (1 min) for 30 cycles. The fragment was verified by restriction analysis.

For SSCP analysis, 4 μL of the PCR reactions was mixed with 6 μL formamide dye (95% [wt/vol] formamide, 0.025% [wt/vol] xylene cyanol, 0.025% [wt/vol] bromophenol blue, 20 mM EDTA), denatured for 2 min at 90°C, and chilled on ice prior to loading 3 to 5 μL of the mixture on nondenaturing 12% polyacrylamide (29:1), 0.5×TBE gels. These were run at 16°C constant temperature for 14 h at 350 V in a Penguin P9DS electrophoresis unit (OWL Scientific, Woburn, MA) connected to an RTE-111 cooling unit (Neslab, Frankfurt, Germany). After electrophoresis, gels were fixed for 30 min in 10% acetic acid/15% ethanol and silver-stained following the protocol of Bassam et al. (1991). The developer solution was modified (45 g/L NaCO₃, 0.0555% formaldehyde, 0.04 g/L Na₂S₂O₃) and staining was stopped by transferring the gels into cold (16°C) 0.04 M EDTA.

Inheritance Pattern. Three codominantly inherited alleles (Figure 1) were observed in the International Bovine Reference Panel (IBRP) (Barendse et al., 1997).

Chromosomal Location. Genetic linkage analysis (CRI-MAP; Green et al., 1990) in the IBRP placed OXT.

Marker   cM (Kosambi)
BMC1222  30.5
HUJ616   12.1
DIK54   4.9
OXT     12.0
ETH7     15.3
CSSM30

Figure 1. Screening gel with IBRP parents showing different oxytocin (OXT) genotypes with SSCP alleles 1, 2, and 3.

Figure 2. Sex-averaged linkage map of the central portion of BTA13 with the most likely position of oxytocin (OXT).
in the central region of bovine chromosome 13. Oxytocin was significantly linked (threshold LOD score 6) to four marker loci with recombination frequencies from 0 to 0.07 and LOD scores from 7.53 to 11.73. Five public BTA13 framework markers (BMC1222, HUJ616, DIK54, ETH7, and CSSM30) were used in a multipoint analysis to produce a sex-averaged BTA13 map (Figure 2), including OXT. The most likely position for OXT is between DIK54 and ETH7, although this position was only slightly better than the location between DIK54 and HUJ616, with a difference in odds of –2:1.

Allele Frequencies. The frequencies of the OXT alleles in 34 IBRP parents were 0.77 (allele 1), 0.15 (allele 2), and 0.08 (allele 3).

Comments. The neuropeptide hormone oxytocin is synthesized in the hypothalamus together with the carrier protein neurophysin (Ruppert et al., 1984). It is responsible for reproductive success in mammalian species (Mori et al., 1990). Prepro-Oxytocin-Neurophysin I is a type-I comparative anchor locus (O’Brien, 1993) and has previously been physically mapped to bovine chromosome 13 (BTA13) using hybrid somatic cells (Neibergs et al., 1991).

Literature Cited


Green, P., K. Fails, and S. Crooks. 1990. Documentation for CRI-Map, version 2.4. Washington Univ. School of Medicine, St. Louis, MO.


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