## LOCALIZATION OF SOMATOSTATIN TO BOVINE CHROMOSOME 1q23 - q25 BY IN SITU HYBRIDIZATION

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### SUMMARY

Somatostatin is physically mapped to bovine chromosome 1q23-q25 by in situ hybridization. A genomic somatostatin probe was isolated from a bovine 1;29 library, prepared previously in our lab, with human somatostatin genomic DNA. Chromosomes were prepared from blood taken from a Charolais bull (59,XY 1;29,tt). The probe was radiolabelled with tritium for in situ hybridization. After autoradiography the slides were developed and chromosomes Giemsa-stained and examined for points of hybridization. A histogram was constructed, recording the number and position of dark grains on the 1;29t chromosomes. A peak was found to occur at chromosome 1q23-q25. The results were statistically analyzed with a $Z_{max}$ test and were found to be significant at the 5% level. This is the second type I locus to be mapped to the center of chromosome 1, providing a valuable marker for this chromosome, and fulfilling the goal for three well-spaced markers on each bovine chromosome. It also provides further evidence for sequence homology between bovine chromosome 1 and the q arm of human chromosome 3.

### INTRODUCTION

Physical mapping of the bovine genome is progressing at a rapid rate, and the goal of locating three well-spaced markers on each chromosome (Fries et al., 1991) will soon be achieved. To date, two type I loci (O'Brien et al., 1993) have been mapped to chromosome 1: Collagen 6A1 at 1q12-q14, near the centromere (Schmutz et al., in press) and uridine monophosphate synthase (UMPS) in the middle at 1q31 (Ryan et al., 1994) and at 1q34-q36 (Friedl and Rottmann, 1994). An anonymous gDNA sequence, BTA D1S11, has been mapped to the telomeric end at 1q43-q46 (Schmutz et al., in press).

Somatostatin is a potent, non-competitive inhibitor of growth hormone secretion; it also has diverse inhibitory effects on the gastrointestinal tract and other hormones. Somatostatin has been mapped to human chromosome (HSA) 3q28 (Zabel et al., 1983; Naylor et al., 1983), and its localization to bovine chromosome 1 will enhance the picture of sequence conservation between these two species.

### MATERIALS AND METHODS

Chromosomes were prepared from a Charolais bull homozygous for the 1;29 translocation (58,XY,tt) to enable rapid and positive identification of chromosomes 1 and 29. Slides with good mitotic indices were stored at 4°C for at least two weeks. Some metaphase spreads were R-banded (Verma and Babu, 1989) to ensure accurate band placement of hybridization signal.

In situ hybridization was performed following a modification of the method of Pardue (1985). The slides were placed in 2XSSC for 30 minutes at 65°C and dehydrated through an alcohol series. The slides were then treated with 0.2mg RNase/ml 2XSSC for 30 minutes at 37°C, followed by four room temperature washes in 2XSSC. Chromosomal DNA was denatured in 0.07N NaOH for 3 minutes at room temperature and dehydrated in an alcohol series.

The 3.7 kb bovine genomic somatostatin probe was isolated from a bovine 1;29 chromosome library (Schmutz et al., in press) using the human somatostatin genomic sequence (ATCC clone pgHS7-2.7). The bovine probe was tritium labelled using nick translation following the BioNick procedure outlined by Gibco with $^{3}H$ CTP (DuPont). A 1000-fold concentration of herring sperm DNA was included in the hybridization mix to compete with the repetitive sequences within the genomic probe. Hybridization was carried out in a moist chamber for 18 hours at 40°C.

Post-hybridization washes included three 7 minute washes in 2XSSC at room temperature, one for 15 minutes in 2XSSC at 65°C, and ten for 10 minutes in 0.1XSSC, followed by alcohol dehydration prior to autoradiography. The slides were dipped in autoradiographic emulsion and exposed at room temperature for 7 - 12 days.

After autoradiography slides were developed, Giemsa stained and analyzed for points of hybridization. When a grain was located on a 1;29 chromosome, it was scored on an idiogram from ISCNDA (1989) according to its position along the chromosome. Grains seen on other chromosomes where tallied for data analysis. A $Z_{max}$ test (Ewens et al., 1992) was used to determine the statistical significance of the data.

## RESULTS

In situ hybridization of the bovine genomic somatostatin probe revealed hybridization at the middle of BTA (bovine chromosome) 1. Thirteen of the 155 grains counted (9%) were located on chromosome 1 and seven of these (54%) were located over the 1q23-q25 region. This peak was found to be significant at the 5% level when analyzed with the $Z_{max}$ test ($Z_{max} = 2.63$).

## DISCUSSION

When using tritium-labelled probes for in situ hybridization, the chromosome on which the gene in question falls usually carries 10 - 20% of the total number of grains scored (Chrisman et al., 1991). The results reported here are comparable to those reported by Zabel et al. (1983), in the mapping of somatostatin to human chromosome 3, where 10% of the total number of grains were found on chromosome 3. The addition of cotDNA, as well as herring sperm DNA to the hybridization mix could aid in decreasing background grains and increase the significance of the peak obtained when using this probe for in situ hybridization.

In situ hybridization allows for the precise localization of genes on chromosomes. This method is being used extensively for the production of physical maps in many species. Comparative gene mapping depends on linkage relationships of homologous genes among different species and allows for analysis of the evolution of genome organization.

The gene for uridine monophosphate synthase (UMPS) has been mapped to human 3q13 (Qumsiyeh et al., 1989), and the bovine UMPS gene has been localized to BTA 1q31 (Ryan et al., 1994) and BTA 1q34-q36 (Friedl and Rottmann, 1994). This provides evidence for homology between bovine chromosome 1 and the q arm of human chromosome 3. The localization of bovine somatostatin to the middle of chromosome 1 provides additional evidence for this homology and suggests an inverted orientation of human 3 to cattle 1. If the localization of UMPS to 1q34-q36 is the more accurate assignment, then the placement of SST above it is valid in comparison to the human locations on chromosome 3. If UMPS is more accurately placed at 1q31, then its close proximity to SST at 1q23-q25 makes it more difficult to suggest an orientation of human chromosome 3. This could be determined with fluorescence in situ hybridization probing with the two genes labelled different colors, enabling simultaneous visualization of both

probes on the chromosome.

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