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A 2.5-Mb contig constructed from Angus, Longhorn and horned Hereford DNA spanning the polled interval on bovine chromosome 1

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Summary

The *polled* locus has been mapped by genetic linkage analysis to the proximal region of bovine chromosome 1. As an intermediate step in our efforts to identify the *polled* locus and the underlying causative mutation for the polled phenotype, we have constructed a BAC-based physical map of the interval containing the *polled* locus. Clones containing genes and markers in the critical interval were isolated from the TAMBT (constructed from Angus and Longhorn genomic DNA) and CHORI-240 (constructed from horned Hereford genomic DNA) BAC libraries and ordered based on fingerprinting and the presence or absence of 80 STS markers. A single contig spanning 2.5 Mb was assembled. Comparison of the physical order of STSs to the corresponding region of human chromosome 21 revealed the same order of genes within the *polled* critical interval. This contig of overlapping BAC clones from horned and polled breeds is a useful resource for SNP discovery and characterization of positional candidate genes.

Keywords BAC contig, bovine chromosome 1, polled, STS.

The presence of horns within commercial cattle populations increases the chances of injuries, particularly during transportation. Quality defects in the form of bruised carcasses, arising from these injuries, cost the industry millions of dollars every year. Producers and packers ranked bruising as one of their top ten concerns for the fed steer and heifer industry in the National Beef Quality Audit-2000 (McKenna *et al.* 2002). Bruising was also the number two 'quality challenge' of the market cow and bull beef industry (Roeber *et al.* 2001). Dehorning cattle provides a recurrent managerial solution to the problem. However, there are negative stress effects to the animal (Graf & Senn 1999) and concerns that dehorning may be an inhumane treatment.

The polled (hornless) condition in cattle has existed since domestication, and it has been selected because of its economic importance and ease of management. A single, dominant mutation is believed to cause the polled phenotype, but the causative gene remains unknown. Georges *et al.* (1993) localized the *polled* locus to the centromeric end of bovine chromosome 1. Brenneman *et al.* (1996) refined the location to a region proximal to the centromere and 4.9 cM from microsatellite *TGLA49*. Using the same population, the interval was further refined to a 1.7-Mb region between *IFNAR* and *SOD1* (unpublished data). Recently, Drögemüller *et al.* (2005b) localized the *polled* locus to a 1-Mb region between markers *BM6438* and *RP42-218J17_MS1*.

As an intermediate step towards identifying the causative mutation, we have built a contig spanning the interval between *SLC5A3* and *SOD1* using bacterial artificial chromosome (BAC) clones from the CHORI-240 (http://bacpac.chori.org/bovine240.htm) and TAMBT (Cai *et al.* 1995) BAC libraries. The TAMBT library now consists of 69 696 Angus BAC clones and 11 328 Longhorn BAC clones, which have been pooled for PCR-based screening. The CHORI-240 library was constructed using DNA from a horned Hereford bull (L1 Domino 99375), which sired the horned Hereford cow used to produce the bovine whole-genome shotgun sequence (http://www.hgsc.bcm.tmc.edu/projects/bovine/).

To begin contig construction, ten markers (*AGLA17*, *TAMU199*, *IFNAR*, *TAMU202*, *BM6438*, *TAMU222*, *TAMU223*, *SOD1*, *SOD1M1* and *SOD1M2*; Table S1) were

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used to isolate 16 BAC clones from the Angus portion of the TAMBT library as described by Cai *et al.* (1995). Overlap between these BAC clones was determined by *Hin*dIII digestion. Additional Angus and Longhorn BAC clones within the critical interval were next isolated by plasmid-end rescue (Cai *et al.* 1995), and BAC-end sequences (BESs) were generated through direct sequencing of BAC DNA with ABI Big Dye v.3 terminators using SP6 and T7 primers. New primers designed from the BESs were used to screen the TAMBT BAC library. Angus BAC clones from the TAMBT library and associated mapping data were subsequently contributed to the International Bovine BAC Map Consortium (IBBMC; http://www.bcgsc.ca/platform/mapping/bovine). TAMBT and CHORI-240 clones were fingerprinted as part of that project. Using the IBBMC fingerprint data, we identified 186 BACs from the CHORI-240 library predicted to map to the critical interval using iCE v3.3 (Fjell *et al.* 2003). A total of 90 of these BACs appear in the final contig and, where available, corresponding BESs were recovered from GenBank. An STS map of the region was assembled using primers for 30 previously mapped microsatellites or SSCPs, 23 gene-specific STSs, 22 STSs derived from BESs and five anonymous STSs (DQ886274–DQ886354; Table S1).

A single contig of ca 2.5 Mb, with an average marker spacing of 31.25 kb, was constructed, and 40% of the STSs were represented by Hereford and Angus clones; 27.5% by clones from all three breeds; 17.5% by Hereford and Longhorn clones; and 15% by only Hereford clones (Fig. 1). Alignment of STSs and BESs to the human genome sequence (Build 36.1) using BLASTN (Altschul *et al.* 1990) allowed us to anchor 22 annotated human genes from the corresponding region on HSA21 to our physical map, including seven genes not anchored by Drögemüller *et al.* (2005a). No rearrangements of genes within this interval were found, which is in agreement with Drögemüller *et al.* (2005a) but in contrast to earlier results based on RH mapping and early assemblies of the human genome sequence (Rexroad *et al.* 1999; Drögemüller *et al.* 2002).

BESs from the TAMBT and CHORI-240 clones were aligned to the bovine genome sequence (Build 2.1; http://www.hgsc.bcm.tmc.edu/projects/bovine/) to verify the orientation of BAC clones. Of 157 BESs, 105 (66.9%) aligned uniquely to scaffolds on BTA1, while the remainder were repetitive, not yet placed on chromosomes, or shared sequence similarities with scaffolds currently assembled on other chromosomes. Assembly of sequence contigs within scaffolds was mostly consistent with our physical order, but

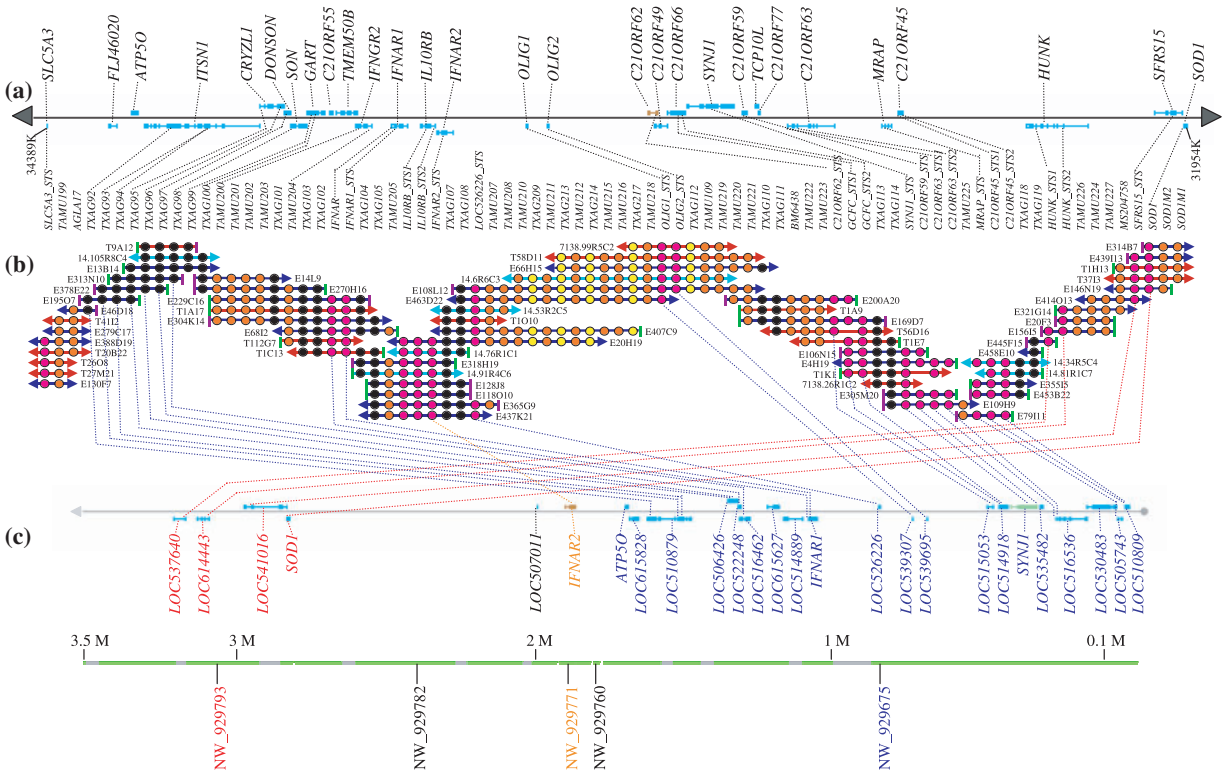

Figure 1 BAC contig and STS map of the polled critical interval relative to human and bovine sequences. (a) Genes from HSA21 (Build 36.1). (b) BAC contig and STS map with Angus BACs shown as red lines, Longhorn BACs as turquoise lines and horned Hereford BACs as blue lines. Forward (T7) BAC ends are represented by green vertical lines, and reverse (SP6) BAC ends are represented by purple vertical lines. Orange dots indicate a microsatellite or SSCP marker, pink dots are gene-specific STSs, black dots are STSs derived from BESs and yellow dots are anonymous STS. Marker names are indicated above the contig. (c) Predicted genes from BTA 1q12 with scaffolds (green and grey lines) from the bovine genome sequence (Build 2.1).

the scaffolds themselves were misassembled (Fig. 1). This is not surprising because this early assembly of the bovine genome is based solely on whole-genome shotgun sequences. Caution should be used when making evolutionary inferences about gene order until genome assemblies have stabilized.

A 4-Mb contig spanning the *polled* critical interval (from *KRTAP8P1* to *CLIC6*) was previously published based on Holstein BAC clones from the RPCI-42 library (Drögemüller *et al.* 2005a). Our contig is unique in that it is tied directly to the bovine genome sequence through the use of the horned Hereford BAC clones from the CHORI-240 library. In addition, the combination of BAC clones from horned and polled breeds makes this new contig a useful resource for SNP discovery and the characterization of positional candidate genes.

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Supplementary Material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2006.01538.x

Table S1 PCR primers used to screen CHORI-240 and TAMU-BT BAC clones.

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