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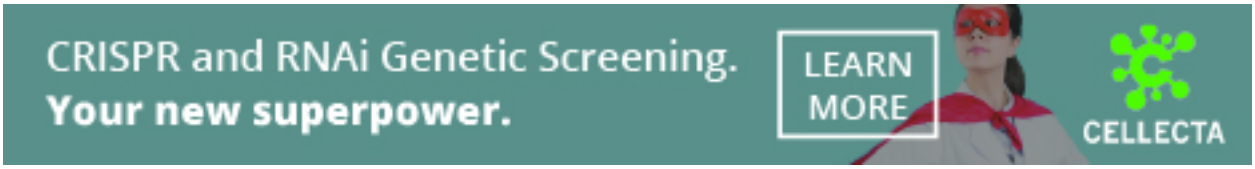

LETTER

A Chromosome-Specific Microdissected Library Increases Marker Density on Bovine Chromosome 1

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Genetic resolution of bovine chromosome 1 (BTA1) linkage group was significantly increased by screening for microsatellite clones in a microdissected library constructed from a bovine cell line carrying a t(1;29) translocation. Eighty-five percent of the microsatellites (ms) (46/54) identified were informative in the USDA/MARC mapping population, and 96% of these ms (44/46) linked to BTA1 (LOD > 3.0). When merged with 40 existing BTA1 markers the genetic map spanned 153.8 cM (sex-averaged interval, 1.9 cM). The fourfold improvement in marker density of BTA1 provides a genetic map that enhances mapping of quantitative trait loci and implementation of marker assisted selection.

[Data regarding type II loci generated from clones obtained from the BTA1 library and their GenBank accession numbers are presented on the journal's WWW site at http://www.cshl.org/gr.]

Twenty-five genes and 45 microsatellites (ms) are currently assigned to BTA1 (Barendse et al. 1993, 1994; Bishop et al. 1994; Harlizius et al. 1995; Ma et al. 1996; H. Hayes, A. Eggen, L.K. Doud, H. Leveziel, and M.D. Bishop, pers. comm.). The most recent BTA1 linkage maps (Barendse et al. 1994; Bishop et al. 1994; Ma et al. 1996) contained 12, 16, and 11 markers, respectively, but have only two loci in common on all three maps. The average intermarker distance for these maps was 11.5 cM. Quantitative trait loci (QTLs) for milk production (Georges et al. 1995) and the polled condition in cattle (Georges et al. 1993; Schmutz et al. 1995) reportedly lie on BTA1. Increased marker density improves the probability of fine-mapping QTLs at a resolution suitable to proceed with positional cloning and ensures informative marker selection for use in QTL analysis and marker-assisted selection (MAS) of various populations (Matise et al. 1994). Continued screening of the random genomic libraries for ms clones (Bishop et al. 1994; Stone et al. 1995) is an inefficient means to increase map resolution in specific regions of the genome. A more efficient strategy is to screen libraries derived from selected chromosomal regions.

Defined regions of the bovine genome can be PCR-amplified and cloned into lambda phage when microdissected from metaphase chromosomes (Ludecke et al. 1989). Microdissection circumvents the problems associated with flow sorting the acrocentric bovine karyotype (Dixon et al. 1991). Such libraries have been used to increase marker density in specific regions of the human, rat, and mouse genomes (Greenfield and Brown 1987; Weber et al. 1990; Fiedler et al. 1991; Bahary et al. 1993; La Pillo et al. 1993; Seki et al. 1993; Kobayashi et al. 1995). Ms isolated from bovine microdissected libraries for BTA11, BTXp, and BTXq (Ponce de León et al. 1996; S. Ambady, P.K. Basrur, R.A. Ma, H.A. Lewin, S.M. Kappes, T.L. Smith, C.W. Beattie, and F.A. Ponce de León, pers. comm.) have improved the resolution of the BTA11 and BTX integrated maps (Ambady et al. 1996; Sonstegard et al. 1997, respectively).

We increased the resolution of the BTA1 genetic map by constructing a BTA1-specific λ library, screening it at high stringency for ms, and merging subsequent genotypes with existing USDA/MARC linkage data (S.M. Kappes, unpubl.). These results

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offer a high-resolution linkage map of BTA1 that will ultimately enhance analysis of QTLs on this chromosome.

RESULTS

A total of 1×10^6 plaques were generated from chromosomal DNA originating from the scraped and amplified BTA1 arm of t(1;29) (cloning efficiency of 1.2×10^8 PFU/μg). Insert sizes ranged from 100 to 2000 bp (average, 700 bp). The library was calculated to contain 3.64 BTA 1 equivalents. Approximately 6×10^4 plaques, taken from a growth amplified aliquot of the library, were screened at high density with a GT_{11} oligonucleotide probe. More than 300 positive plaques (~0.5%) were identified, and 129 replated at low density and screened to yield 73 plaque isolates. Sequence analysis revealed 61 of these 73 clones (84%) contained ms. Of 61 microsatellite clones, 7 (12%) were identical, and 15% of the microsatellite clones were flanked on one side by artiodactyl-type repetitive elements (similarity >80%; Benson et al. 1993; Alexander et al. 1995). One ms clone was similar to HEL 6 (Kaukinen and Varvio 1993), a locus previously assigned to BTA1 (Vaiman et al. 1994). Primer pairs (designated BMS4000 series) designed from the flanking sequence of the 53 remaining ms were screened across the MARC/bovine map reference parents. Forty-seven of the primer pairs amplified an allele size predicted from sequence analysis, and 46 (~0.2% of all clones screened) were informative (http://www.cshl.org/gr). One primer pair, BMS4049, amplified two polymorphic loci. The second locus linked to BTA6 and was designated BMSB4049 (http://www.cshl.org/gr). The efficiency of ms clone isolation and informative marker development compared favorably with the amplified and random genomic libraries screened by Stone et al. (1995) (Table 1).

Table 1. Comparison of ms Markers Developed from BTA1 Library and Genomic Libraries

Library ID	Percent informative	Percent heterozygosity for informative markers	Percent BTA1 linked
BTA1	85	57.6	93.4
Enriched[a]	86	59.2	3.7
M13 Random[a]	76	53.9	3.4

[a]Statistics derived from observations of libraries generated by Stone et al. (1995).

Analysis of genotypic data for the markers developed from the BTA1 library indicated 96% of the ms (44/46) linked to BTA1 (Fig. 1). The two exceptions, BMS4036 and BMS4027, were linked to BTA23 and BTA29, respectively. The linkage rate of BTA1 library derived ms was 30-fold greater than those developed from random and amplified genomic libraries. Marker number increased from 40 to 84 for BTA1 to span 153.8 cM (average interval, 1.9 cM; see Fig. 1) with the addition of BMS4000 ms. The number of marker intervals >5 cM was reduced from 12 to 7. The map resolution of the BTA1 linkage group (S. Kappes, unpubl.) improved twofold after adding the markers from the microdissected library.

DISCUSSION

Our directed strategy to increase marker density on BTA1 by isolation of ms clones from a BTA1 microdissected library reduced average intermarker distance approximately twofold. These results helped to improve marker density four- to fivefold over BTA1 maps reported previously (Barendse et al. 1993, 1994; Bishop et al. 1994; Ma et al. 1996) and produced the highest resolution linkage map of any bovine chromosome to date (Fig. 1).

Development of ms from the BTA1-specific library proved to be an efficient strategy to increase marker density. Moreover, this library appears to contain a majority of the ms on BTA1. An estimated 2000–3000 unique ms sequences reside on BTA1 (~44,000 ms/genome × 5.87% genome per BTA1; Stone et al. 1995), and results from our initial screen suggest the BTA1 library contains 900–1800 (~45–60%) of these ms [(no. of unique microsatellite clones isolated/no. of clones screened) × (% redundant clones) × total clones in library]. The percentage of total microsatellites cloned following microdissection of BTA1 was reduced by several factors. First, the BTA1 DNA/PCR adaptor ligation mix was digested with *Bgl*II to eliminate adaptor dimers. This digest also cleaved some BTA1 template/adaptor molecules, disabling them from annealing the PCR amplification primers, and, thus, the clonal representation was lowered by ~25%. Second, preferential PCR amplification of specific templates within the ligation mix lowered the effi-

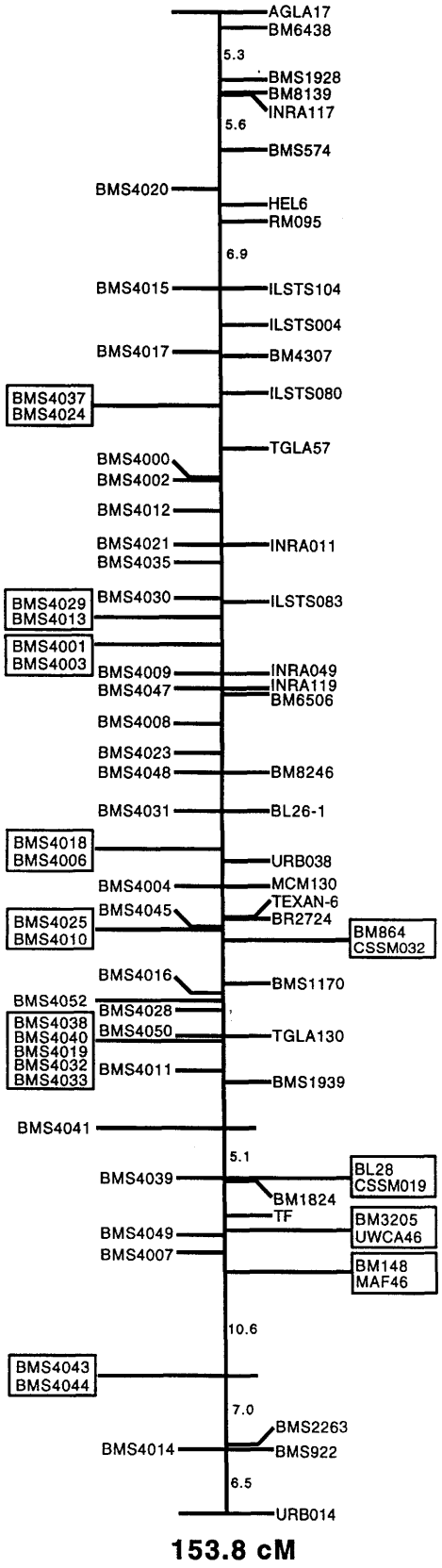

Figure 1 Linkage map distribution of ms clones isolated from a BTA1 microdissected library. Markers developed from the BTA1 library are on the *left* of a sex-averaged linkage map of BTA1 (153.8 cM). Markers on the *right* were obtained from various genomic libraries. Boxed loci indicate markers linked with no detectable recombination. Genetic distances (>5 cM) are to the *right* of the vertical axis of the linkage map (cM).

ciency of amplification and packaging into λ for other clones. The rate of redundancy observed for this library was only 12%. Finally, not all of BTA1 was microdissected during library construction. The relatively low number of ms located near the centromere results from both the t(1;29) translocation and removal of the chromosomal segment containing the translocation during microdissection. When these factors are considered, the clonal representation of ms in this BTA1 library appears maximal. Subsequent screening of this library should prove useful for future development of the BTA1 map, particularly in intervals containing QTL, because only a small fraction (<1/16) of this library was screened.

In summary, the current MARC BTA1 linkage map (Fig. 1) contains 84 markers ensuring the availability of widely distributed, informative, and scorable ms to perform a higher resolution search for QTLs within and between breeds. Because this map contains 28 ms that exist on other BTA1 maps (Barendse et al. 1993, 1994; Ma et al. 1996; J. Taylor, pers. comm.), it serves to integrate these lower resolution maps.

METHODS

Chromosome I-Specific DNA Library Development and Screening

The BTA1 microdissected library was prepared as described (Ponce de León et al. 1996) from a bovine cell line carrying a t(1;29) translocation. Chromosomal specificity of the amplified DNA library inserts was verified by fluorescence in situ hybridization (FISH) of bovine metaphase chromosomes (C. Carpio and F.A. Ponce de León, in prep.). All screening and identification of ms sequences and development of ms primer pairs were as described (Sonstegard et al. 1997).

Linkage Analysis

Genotypic data for each ms marker obtained from the MARC reference population was scored, verified, and entered into an interactive database (Bishop et al. 1994; Keele et al. 1994). Unlikely double recombinants were evaluated, and genotypes were reamplified when necessary. BTA1-linked loci were identified with sex-averaged two-point LOD scores (LOD >3.0) to an existing BTA1 linkage group with 40 markers (sex-averaged interval, 3.8 cM; S.M. Kappes, unpubl.) as described (Bishop et al. 1994; Sonstegard et al. 1997), by use of Cri-Map version 2.4

(Green et al. 1990). The references and number of informative meioses generated for markers obtained from various genomic libraries is presented elsewhere (S. Kappes, J. Keele, R. Stone, R. McGraw, T. Sonstegard, T. Smith, N. Lopez-Corrales, C. Beattie, in prep.).

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