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A Comprehensive Genetic Map of the Cattle Genome Based on 3802 Microsatellites

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A microsatellite-based high-density genetic map facilitates for fine mapping of hereditary traits of interest, characterization of meiosis, and providing a foundation for physical map construction. Here, we developed a comprehensive genetic map on the basis of >880,000 genotypes across the USDA MARC cattle reference families with a potential genetic resolution of 0.8 cM at the 95% confidence level (~800 kb in the bovine genome). We incorporated 2325 microsatellites into the second-generation genetic map by linkage analysis based on sex-averaged two-point LOD scores (>3.0), of which 2293 were fine-mapped by multipoint linkage analysis. The new 3160-cM map comprised of 29 sex-averaged autosomal linkage groups and a sex-specific X-chromosome linkage group includes 3960 markers with 2389 positions, resulting in an average interval size of 1.4 cM. More than half (51%) of the total length of the map is covered with intervals of 2.0 cM or less, and the largest gap is a 10.2-cM interval on the X-linkage group. The new map should accelerate fine mapping and positional cloning of genes for genetic diseases and economically important traits in cattle, as well as related livestock species, such as sheep and goat.

[Supplemental material is available online at www.genome.org. Marker information of new microsatellites is available from DDB] under accession nos. AB164707 to AB166543 including flanking sequences and AB166544 to AB166659 for only primer sequences. Linkage groups for all autosomes and X- and Y-chromosomes are presented at http://www.marc.usda.gov/genome/genome.html.]

Microsatellites are excellent genetic markers because of their high polymorphism and abundant distribution throughout the genome. A microsatellite-based genetic map is an essential tool for linkage mapping of monogenic as well as polygenic traits of interest. Cattle genetic maps have been constructed with 746 markers (Barendse et al. 1997) and 1250 markers (Kappes et al. 1997), respectively. The latter map, spanning 2990 cM, is the current standard genetic map with an average interval of nearly 3.0 cM, similar to the resolution of the most current genetic maps for pig, sheep, and chicken (Rohrer et al. 1996; Groenen et al. 2000; Maddox et al. 2001). This cattle genetic map is probably sufficient to assign hereditary phenotypes to specific chromosomal regions at ~20-cM intervals, but not to fine-map them. An intensive effort to develop more markers from mapped regions to narrow the critical region is commonly required (Takeda et al. 2002; Takeda and Sugimoto 2003). Comparative positional candidate cloning to infer candidate genes from conserved chromosomal segments containing the same complement of genes as in other species is then more feasible and clearly depends on the resolution of physical maps saturated with many well-ordered anchors integrated with genetic linkage maps. However, the time, labor, and cost per marker of isolating DNA markers from a specific chromosomal region is substantially greater than randomly isolating markers. Thus, large-scale, random isolation of microsatellites would be the best choice for enriching markers across the genome, followed by construction of a high-density genetic map to facilitate narrowing regions of interest and identifying genes of agricultural importance.

Furthermore, a high-density genetic map can provide a scaffold for physical map construction, such as radiation hybrid (RH) and BAC contig maps. More framework markers on an RH map make mapping genes much easier and improve reliability of the map. Larkin et al. (2003) recently determined 60,547 cattle BAC end sequences that will contribute to the construction of the BAC contig map, of which 30% could be located on the human genome and assigned to bovine chromosomes in silico using the comparative mapping by an annotation and sequence similarity (COMPASS). Then they placed ~100 BAC ends on the cattle RH map to improve the resolution of synteny borders on HSA11. RH mapping of many BAC ends will be needed for assembling BAC contigs on a whole-genome level, especially for the BAC contigs located around the border of syntenies. A high-density genetic map can provide an alternative method for assembling BAC contigs by screening BAC clones containing microsatellite markers

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and anchoring them on the bovine genome. Construction of a reliable BAC contig map is indispensable for the primary stage of whole-genome sequencing.

We report here a microsatellite-based high-density bovine genetic map consisting of 3960 markers including 3802 polymorphic microsatellites with an average interval of 1.4 cM, covering 3160 cM for 29 sex-averaged linkage groups and a sex-specific X-linkage group. This map can provide powerful resources for fine mapping of QTLs and a genetic backbone for the development of well-annotated gene maps in cattle and other related livestock species.

RESULTS AND DISCUSSION

Isolation and Characterization of Microsatellites

Using microsatellite-enriched libraries (Stone et al. 1995) with minor modification, 5750 previously unreported microsatellites containing (CA) >9 were isolated. Of these, 1750 were heterozygous in the USDA MARC cattle reference population (Bishop et al. 1994). In addition to those, 196 unreported heterozygous microsatellites from other sources and 424 heterozygous microsatellites from public domain sources (BOVMAP database: http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/main.pl?BASE=cattle; Supplemental table) that had already been reported but not genotyped and included in the previous map (Kappes et al. 1997) were also genotyped. A total of 2370 heterozygous microsatellites were subjected to genetic mapping in this work. The average heterozygosity (H) and allele number of 2370 heterozygous microsatellites across 28 parents of the MARC reference population were 0.52 and 5.7, respectively. Of those, 1035 microsatellites had $H \geq 0.60$, and 175 microsatellites were highly polymorphic ($H \geq 0.80$) in the mapping population.

Genetic Linkage Mapping

Of 2370 microsatellites heterozygous in the mapping population, 2325 microsatellites were assigned to chromosomes by two-point linkage analysis with LOD scores >3.0 using CRIMAP (Green et al. 1990). From this, a total of 2293 (99%) microsatellites were fine-mapped by multipoint linkage analysis. The new genetic map is composed of 3960 markers including 3802 microsatellites and 79 SNPs (Supplemental table), and spans a genetic distance of 3160 cM for 29 sex-averaged linkage groups and a sex-specific X-chromosome linkage group. The markers are distributed among 2389 distinct positions, thus the average marker interval is 1.4 cM. This reference population includes a maximum of 391 informative meioses, resulting in an estimated potential map resolution of 0.8 cM at the 95% confidence level, which suggests that additional markers may be incorporated. The diagram and the parameters of each linkage group are shown in Figure 1 and Table 1, respectively. The average marker interval of each autosome ranged from 0.9 cM for BTA29 to 1.9 cM for BTA26. Approximately 51% of the whole genome was covered with <2.0 cM between markers and 91% of the length by intervals of <5.0 cM. No gaps >10.0 cM have remained in the 29 autosomes. Several large gaps in the previous map, such as centromeric portions of BTA3, BTA4, BTA13, and BTA19, were dramatically reduced by saturating with new markers (Fig. 1).

A difference in size between male and female genetic maps has been reported in human, cattle, sheep, and other species (Beever et al. 1996; Mohrenweiser et al. 1998; Maddox et al. 2001; Kong et al. 2002). Similar to the previous map (Kappes et al. 1997), the overall sizes of the male and female maps are comparable (male, 3158 cM; female, 3132 cM). It has been shown that the male pseudoautosomal region has a nearly 10-fold increase in recombination frequency compared with any other autosomal regions and is due to obligate crossover in this region (Rappold 1993). Consistent with human, considerable size expansion in the map of the male pseudoautosomal region was also observed in this work (Fig. 1; Table 1). The state of the coverage of the genome with markers was considered reasonable from the view of the human genetic map reported by Dib et al. (1996). In most regions, the order of the markers on the new map was consistent with the previous map, although some regions were rearranged through incorporating the new markers and correcting old marker data.

Genotype Validation

Microsatellites yielding ambiguous genotypes or poor amplification were excluded because they can produce unlikely double or more crossover events through genotyping errors and inflate the map length as a result. To detect potential genotyping errors, double or more crossover events within a small chromosomal region (<20 cM) were identified using the CHROMPIC option of CRIMAP (Green et al. 1990). Intensive inspection and a second PCR validation of marker genotypes with potential genotyping errors were done in this work. Following a second PCR validation, genotypes were removed from the data if multiple likely errors for a marker suggested amplification problems or multiple likely errors for an animal suggested DNA problems. As a result, the total size of the new map (3160 cM) was 170.4 cM larger than the 1997 map. Extensions of the map beyond previous centromeric and telomeric ends sum to 171.2 cM, similar to the 170.4 cM overall increase. Therefore, the intensive effort to correct markers with potential genotyping errors, such as unlikely double or more crossover events, generally worked well.

METHODS

Isolation of Bovine Microsatellites

Most of the microsatellites were isolated using microsatellite-enriched libraries (Stone et al. 1995), with some modifications. Briefly, microsatellite-enriched libraries were constructed using $(CA)_{11}$ or $(TG)_{11}$ as a primer, producing unidirectional CA (or TG)-dinucleotide repeat-containing libraries. Clones colony-hybridized with Poly(dA-dC)Poly(dG-dT) (Amersham Biosciences) were submitted to colony PCR, followed by sequencing. Sequencing was performed on an ABI 3700 Capillary Sequencer, using BigDye Ver. 3.0 and dGTP BigDye Ver. 3.0 Cycle Sequencing Ready Reaction Kits (Applied Biosystems). Microsatellites containing (CA) ≥ 9 were submitted to primer design, using Primer 3 (Rozen and Skaletsky 2000) and targeting a product size of 100 to 300 bases. Redundant microsatellites were identified by BLASTN searches against GenBank (Benson et al. 2002) and the sequences of previously developed microsatellites, and were eliminated. These data manipulations were performed using DNA space software (Hitachi Software Engineering Co., Ltd.).

Isolation using the magnetic beads method was essentially as described by Takeda and Sugimoto (2003). Focused isolation from chromosome specific libraries was used for BTA14 and BTA20 essentially as described by Reed et al. (2002a,b). Markers isolated from YAC/BAC DNA are described in the Supplemental table.

PCR Amplification of Microsatellites and Data Collection

Microsatellites were amplified by means of PCR using fluorescent-labeled primers as described (Sugimoto et al. 2003). Markers were genotyped across the MARC reference population (Bishop et al. 1994). Marker heterozygosity was determined only for the reference mapping population as described (Kappes et al. 1997). PCR conditions for each marker are available from the Supplemental table.

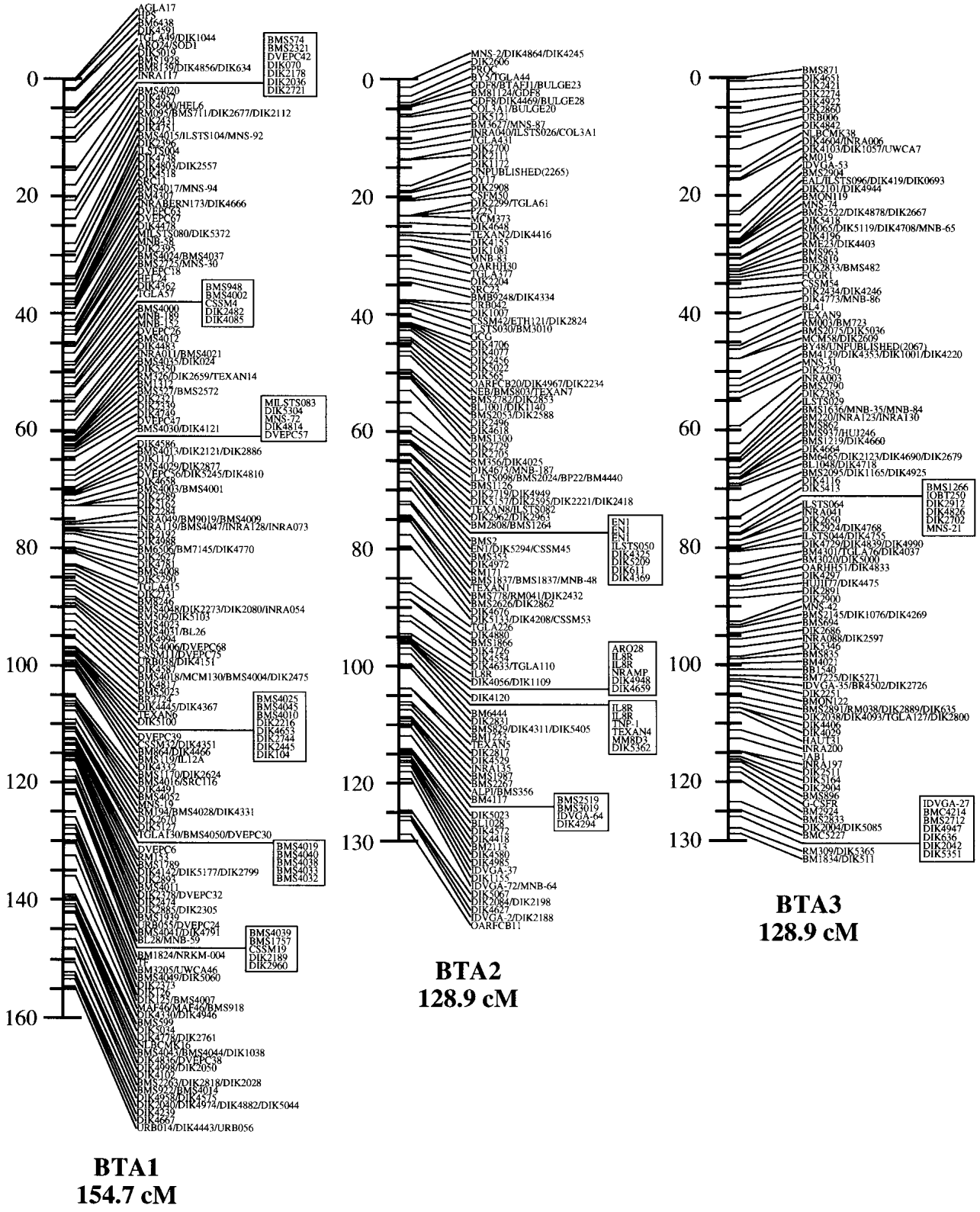
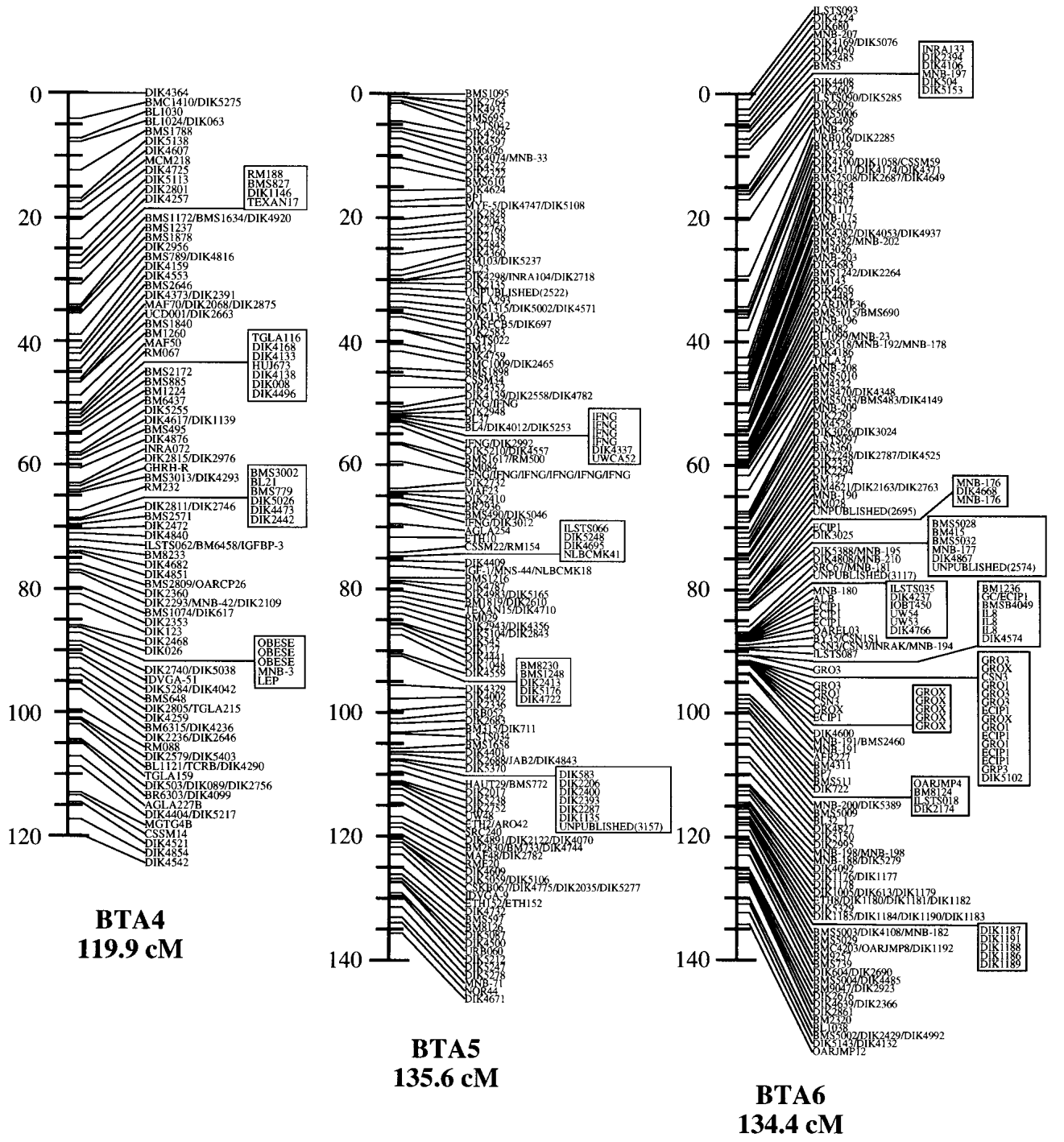


Figure 1 (Continued on next page)



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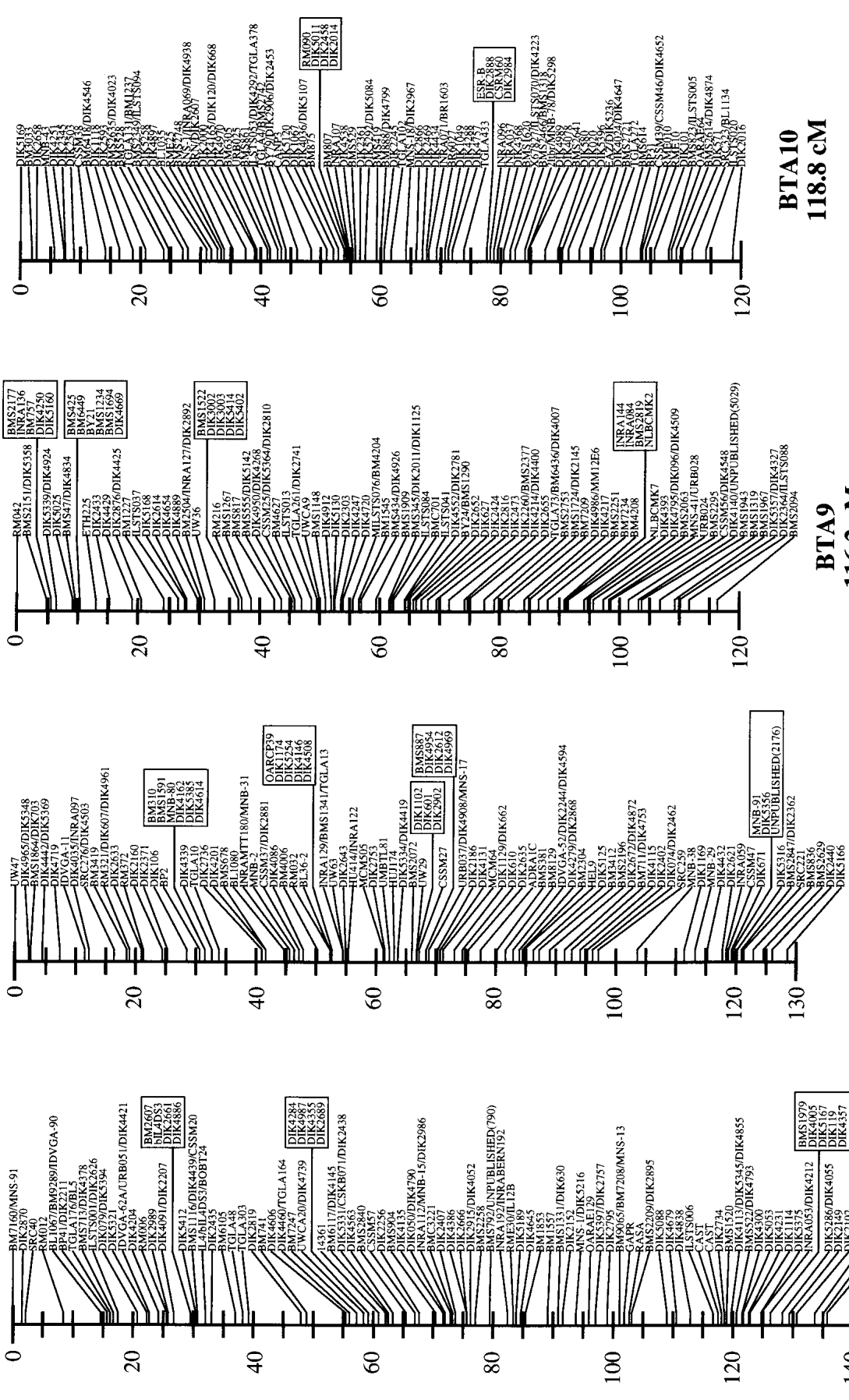
Linkage Analysis

CRIMAP 2.4 software (Green et al. 1990) was used for linkage analysis. Markers were placed into linkage groups based on two-point LOD scores (>3.0) with markers from the previous map. Owing to computational requirements of CRIMAP 2.4, all genotypes for heterozygous progeny resulting from mating like-heterozygous parents were temporarily removed from the data. Ordering the markers within a linkage group began with the previous map. A new marker was inserted into the map by evaluating the LOD at every possible location. The marker was then inserted at the location with the best LOD, and the change in length of the linkage group was evaluated. If the change was <0.75 cM, then the marker was retained. An arbitrary 0.75-cM increase in length was imposed to prevent the inclusion of a large number of probable genotyping errors in the early stages of map building before they had been checked by regenotyping. A single genotyping error typically resulted in a 0.6-cM increase in length. Once the set of markers meeting the 0.75-cM criteria was inserted, then pairs of markers were iteratively interchanged and the LOD was evaluated. If a switched pair improved the LOD, the



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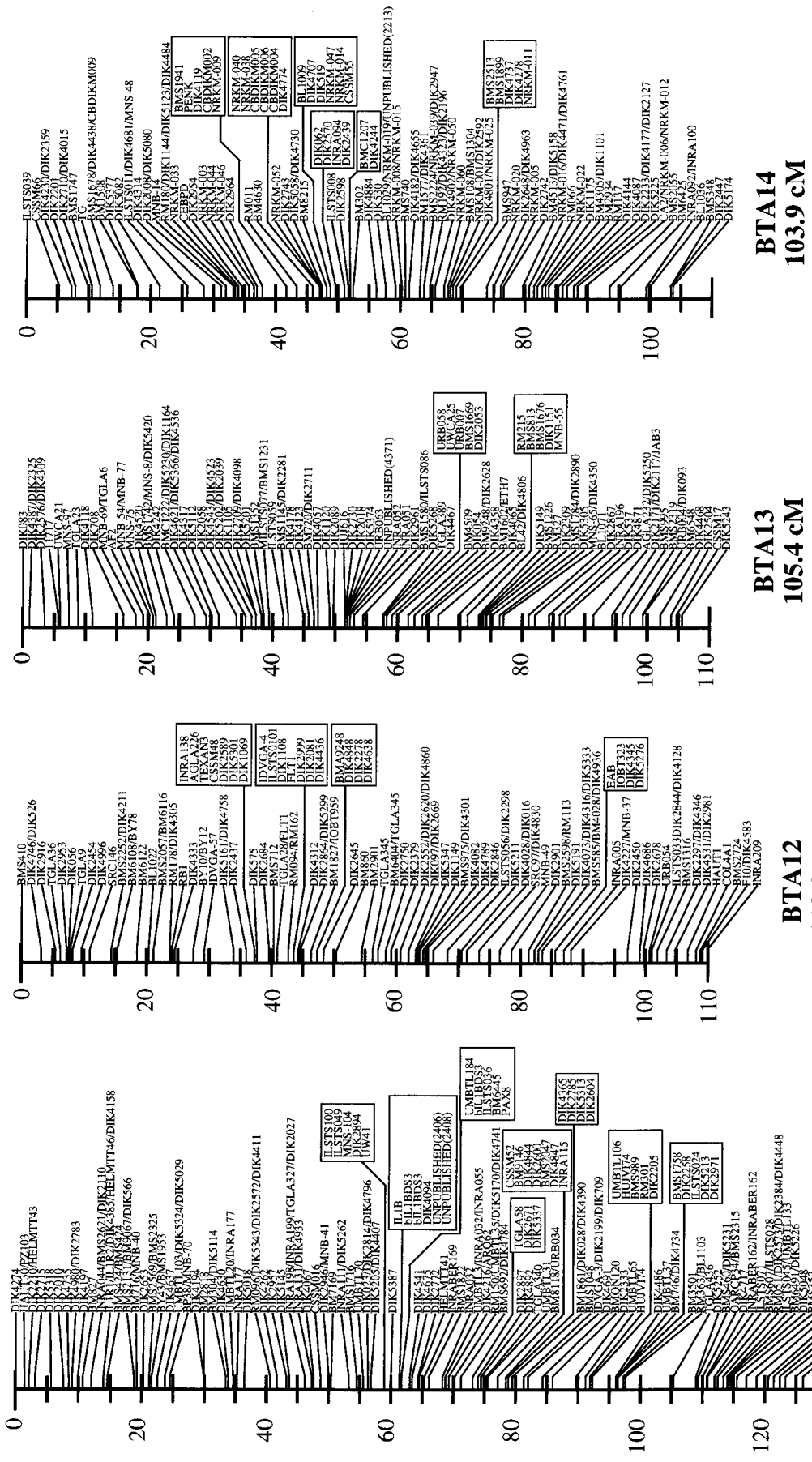

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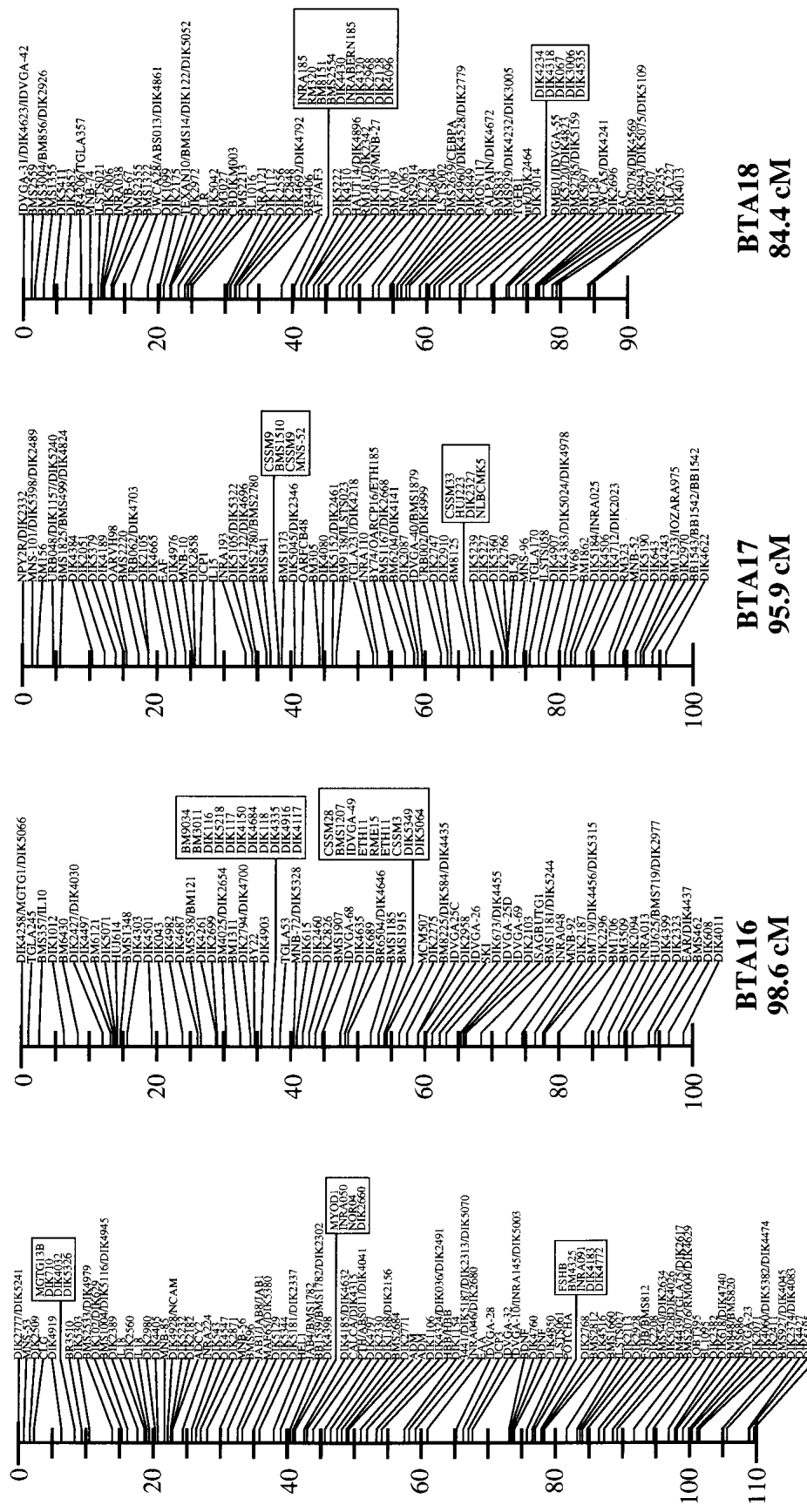

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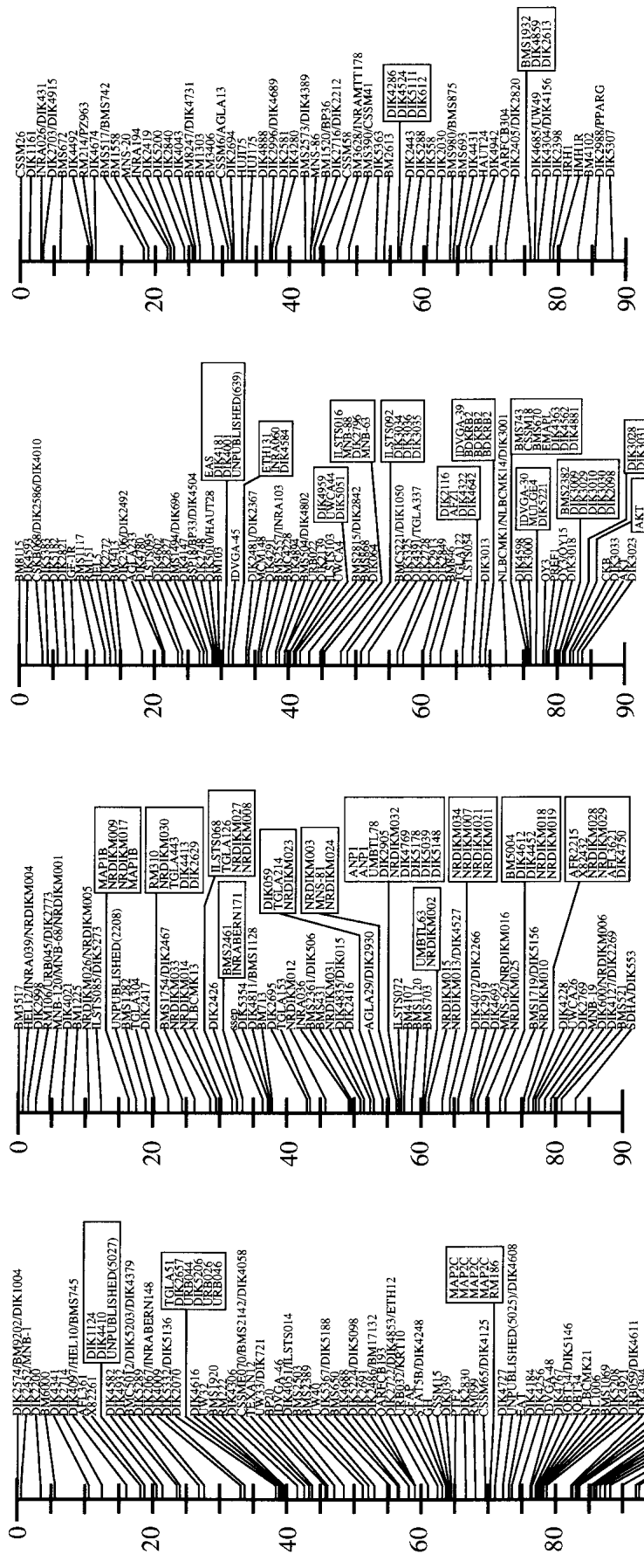

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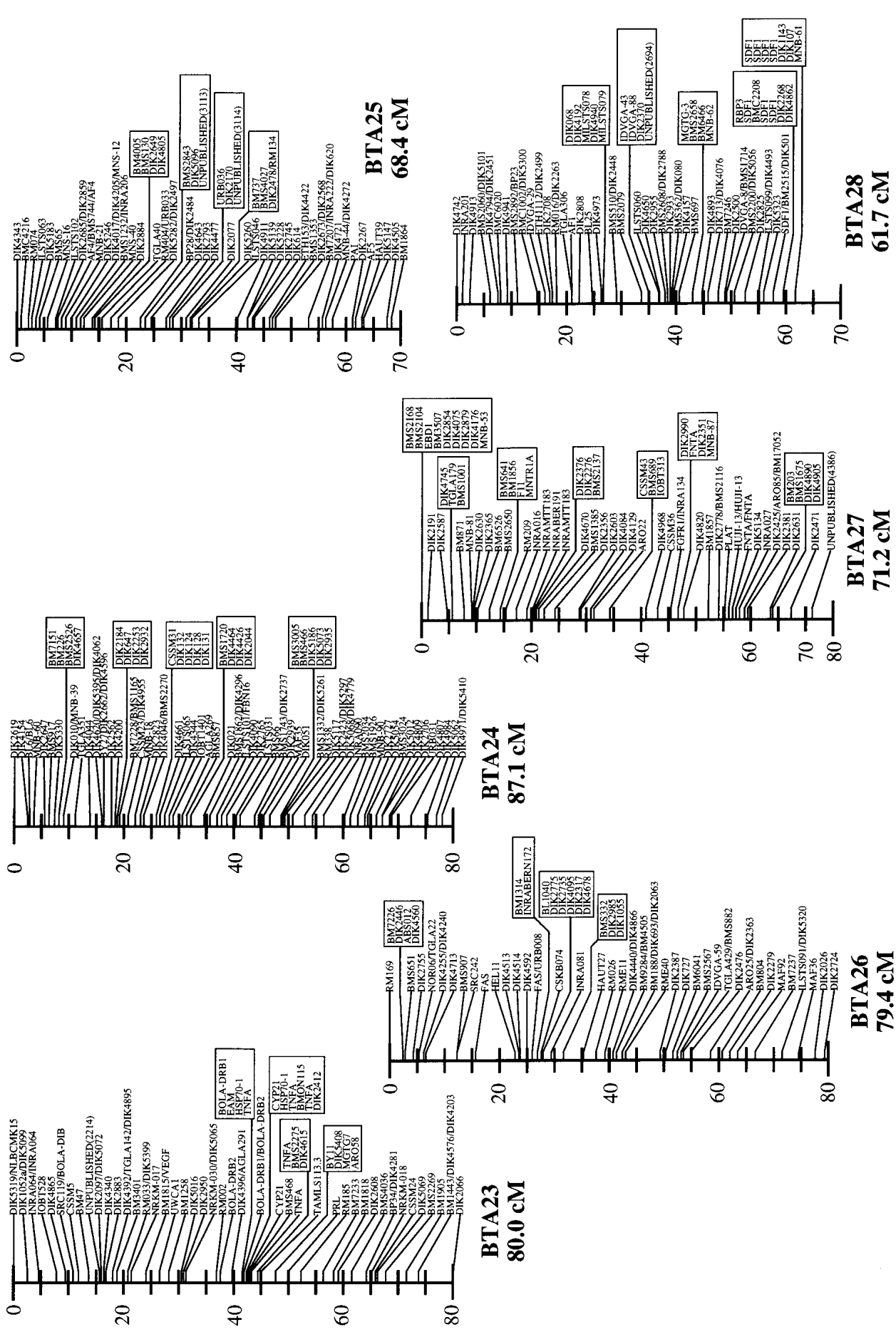

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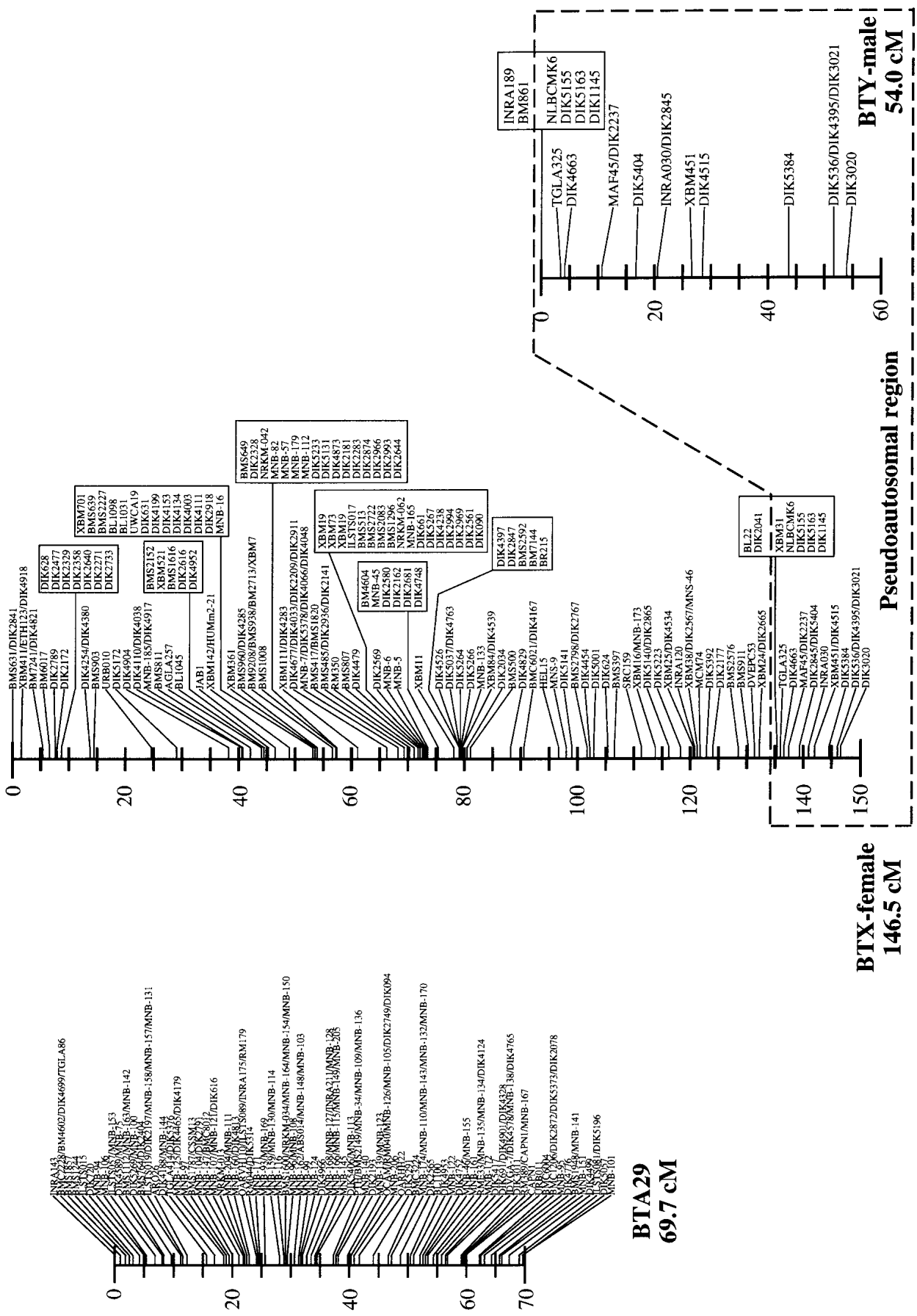

Figure 1 (Legend on next page)

Table 1. Parameters of the Bovine Linkage Map

BTA/X[a]	Length (cM)	No. of markers	No. of positions	Average interval (cM)	Maximum interval (cM)	No. of intervals 10–15 cM	No. of intervals 5–10 cM
1	154.7*	263*	150*	1	4.6	0	0
2	128.9	186	112	1.2	6.2	0	2
3	128.9	171	101	1.3	5.2	0	1
4	119.9	130	81	1.5	4.7	0	0
5	135.6	181	115	1.2	4.7	0	0
6	134.4	232	128	1.1	9.2	0	3
7	135.6	138	88	1.6	8.6	0	5*
8	128.6	123	84	1.5	7.9	0	4
9	116.2	127	79	1.5	4.9	0	0
10	118.8	125	88	1.4	4.4	0	0
11	131	195	103	1.3	7.6	0	2
12	110	122	74	1.5	8.9	0	1
13	105.4	114	80	1.3	4.9	0	0
14	103.9	133	77	1.4	5.1	0	1
15	109.8	144	94	1.2	5.6	0	1
16	98.6	103	67	1.5	4	0	0
17	95.9	103	69	1.4	5.5	0	1
18	84.4	111	70	1.2	5.1	0	1
19	109.6	134	89	1.2	6.7	0	3
20	82.9	120	64	1.3	5.2	0	1
21	83.8	125	72	1.2	4	0	0
22	88.1	79	57	1.6	7.1	0	1
23	80	76	48	1.7	6.3	0	2
24	78.1	94	61	1.3	3.8	0	0
25	68.4	72	48	1.5	6.8	0	2
26	79.4	64#	43#	1.9*	7.4	0	3
27	71.2	72	43#	1.7	6.2	0	1
28	61.7#	80	43#	1.5	4.7	0	0
29	69.7	154	78	0.9#	3.3#	0	0
X	146.5	189	83	1.8	10.2*	1	5*
Total	3159.9	3960	2389	1.4 (average)	10.2 (maximum)	1	40

Maximum number and minimum number in each column were shown by * and #, respectively.
[a]Data for 29 sex-averaged autosomal linkage groups and a sex-specific X-chromosome linkage group.

pair was switched and the process repeated until no better LOD could be found. Once this occurred, each new marker was temporarily removed from the linkage group, and the change in length was evaluated. If removing the marker reduced the length by <0.75 cM, then the marker was evaluated at all possible positions and reinserted at the position with the highest LOD. If removing the marker reduced the length by 0.75 cM or more, the marker was removed from the linkage group. The process of switching in pairs and repositioning markers was repeated until the LOD no longer changed. If this process changed marker order, another attempt was made to insert markers that did not meet the 0.75 cM criterion. After no more markers could be inserted and the algorithms did not reveal a more likely marker order, computation proceeded using the entire data set, including heterozygous progeny genotypes from like-heterozygote matings. The process of switching the order of marker pairs was repeated to finalize marker order. The final maps represent the most likely marker order identified with the complete data set, although an exhaustive evaluation of all possible orders was not conducted.

After determining the final marker order, the CHROMPIC option of CRIMAP 2.4 was used to identify potential genotyping errors in the data for all markers, including markers removed because they increased map length by >0.75 cM. When corrections to genotypes were made, the entire process was repeated, starting from data with genotypes for heterozygous progeny from like-heterozygote parents removed. Special attention was given to new markers that appeared to map on the ends of linkage groups. Because new markers on the ends of the linkage groups are often expected to increase the length by >0.75 cM, CHROMPIC outputs for these markers were closely scrutinized. Following a second genotyping and re-examination of crossovers at the ends of linkage groups, these markers were included in linkage groups even though they increased lengths by >0.75 cM. After determining marker order, markers genotyped by other groups that were not published and had not completed checking potential errors were eliminated, and distances were calculated.

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Figure 1 Diagrams of linkage groups for 29 sex-averaged and a female-specific X-chromosome with a male-specific pseudoautosomal region. All markers are indicated as the locus name based on the MARC database, whose detailed information is available from the Supplemental table. The markers in a row or within a box indicate no recombination between them. Genetic distances between markers are given in centimorgans (cM).

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