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Cosmid-derived markers anchoring the bovine genetic map to the physical map

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Abstract. The mapping strategy for the bovine genome described in this paper uses large insert clones as a tool for physical mapping and as a source of highly polymorphic microsatellites for genetic typing, and was one objective of the BovMap Project funded by the European Union (UE). Eight-three cosmid and phage clones were characterized and used to physically anchor the linkage groups defining all the bovine autosomes and the X Chromosome (Chr). By combining physical and genetic mapping, clones described in this paper have led to the identification of the linkage groups corresponding to Chr 9, 12, 16, and 25. In addition, anchored loci from this study were used to orient the linkage groups corresponding to Chr 3, 7, 8, 9, 13, 16, 18, 19, and 28 as identified in previously published maps. Comparison of the estimated size of the physical and linkage maps suggests that the genetic length of the bovine genome may be around 4000 cM.

Introduction

Loci anchoring the physical and genetic maps of a species are a very important resource in the development of genome mapping strategies, both in building comprehensive maps and for the eventual use of those maps. The benefits that are derived from integrated physical and linkage maps are numerous, as exemplified by the work on humans. Several aspects of the latest human map (Hudson et al. 1995) should be considered in establishing maps in other species. For example, it was developed with Sequence Tagged Sites (STSs; Olson et al. 1989) and incorporates most of the STSs and Expressed Sequence Tags (ESTs) previously placed on human linkage maps, the latter being crucial for the postitional cloning of disease genes. The amalgamation of physical and genetic maps allows a direct estimation of the distance between the ordered STSs, thereby providing the best coverage of the human genome so far.

Genome maps have been developed over the last few years for several domesticated animal species (Bumstead and Palyga 1992; Fries et al. 1993; Barendse et al. 1994; Bishop et al. 1994; Levin et al. 1994; Archibald et al. 1995; Crawford et al. 1995), the bovine genome map being the most extensive, ranking fourth after the maps available for human, mouse, and rat. An updated bovine linkage map currently comprises almost 800 markers, including 150 type I markers or genes (O'Brien et al. 1993), and is predominantly made up of type II STS markers (W. Barendse, personal communication). The development of a physical map of the bovine genome has lagged behind mainly because of the difficulty in identifying individual bovine chromosomes. Initially, physical mapping data were based on conserved type I markers mapped to syntenic groups with somatic hybrid cell panels (Womack and Moll 1986; Fries et al. 1993). However, direct physical mapping approaches, like fluorescence in situ hybridization (FISH), have recently become available, so that now all the synteny groups have been assigned to chromosomes (Mezzelani et al. 1994; Masabanda et al. 1996). This has been achieved in most cases by combining the localization of large-insert clones to chromosomes by FISH, with the PCR amplification of microsatellites derived from the clones on hybrid cell lines.

Obtaining highly polymorphic markers from cosmid and phage clones is a very efficient way of placing markers on both the genetic and physical maps (Solinas Toldo et al. 1993; Ellegren et al. 1994a; Eggen and Fries 1995; Mezzelani et al. 1995; Thieven et al. 1996). This approach was adopted by the BovMap group as the tool of choice to provide physical anchorage to the developing bovine linkage map, and the results are presented in this paper. To achieve the mapping goals, it was crucial importance to use a common set of animals on which to type the markers, and the work was carried out using the International Bovine Reference Family Panel (IBRP; Barendse et al. 1996).

We report here on the physical mapping of 83 cosmid and phage-derived bovine microsatellite marker loci that were simultaneously placed on the bovine linkage map. Thus, the correspondence between linkage groups, synteny groups, and chromosomes is directly confirmed. In addition, some linkage groups have been

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oriented compared with previously published maps (Barendse et al. 1994; Bishop et al. 1994), so that now all the linkage groups are oriented with respect to the cytogenetic map.

Finally, the set of markers employed allows the estimation of the correspondence between the physical map and the genetic map; this helps to validate the estimates of genome size inferred from chiasmata counts. Estimating genome size has gained a special importance after the finding that the mouse and the porcine genomes are about 2000 centiMorgans (cM) long, which is significantly shorter than that of human despite having the same physical size (Davisson and Roderick 1989; Ellegren et al. 1994b). Our data suggest the converse may be true in cattle, where the length of the genome may be close to that of human, and possibly larger.

Materials and methods

Isolation and physical mapping of cosmids and phages. Cosmids and phage-containing microsatellites were identified with poly d(AC) probes. For the majority of clones (that is, IDVGA and ETH clones), QFQ-banded chromosome preparations were subjected to competitive in situ suppression hybridization (Lichter et al. 1990) and then hybridzed with biotinylated probes. FITC signal detection was performed with a computer-controlled CCD camera device (Photometrics, Tucson, Ariz.), as described by Solinas Toldo et al. (1993) and Mezzelani et al. (1995). Chromosomal band assignments were obtained by determining the fractional length (FLcen) with respect to the proximal border of the chromosome and superimposing the value to the idiogram of the standard chromosomes (Popescu et al. 1996). Details of the physical localization for the other clones can be found in the references in Table 1. Sequencing and development of the microsatellite markers was performed at a later stage, when the chromosomal location of clones was known. From nearly 200 large insert clones that were physically mapped, 83 were eventually selected and the embedded microsatellite regions sequenced either by subcloning or directly from the cosmid (Ferretti et al. 1994) or phage templates (Thieven et al. 1995), for use as genetic markers.

Markers and polymorphisms. For all but five loci, polymorphism in the length of the dinucleotide microsatellite repeat was used for genetic mapping by analysis of the PCR products generated with the primers described in Table I on denaturing acrylamide gel electrophoresis. For five loci that showed low heterozygosity, mapping was by single strand conformational polymorphisms (SSCP) described by Williams and associates (1996b). Table 1 summarizes the details of all the markers that are part of the BovMap database maintained at INRA, which is accessible on the Internet at the address: http://locus.jouy.inra.fr/cgi-bin/bovmap/. A database of D numbers, identifying the anonymous DNA loci, is maintained by R. Fries.

Typing and linkage analysis. A common set of 15 full sib families (330 animals), which are part of the International Bovine Reference Family Panel (IBRP; Barendse et al. 1996), was used for the genetic mapping of the markers. The data are collated in the Cattle Genotypic Database (CGD), managed by W. Barendse. Intervals between markers were estimated by two-point linkage analysis with the CRI-MAP program (Green et al. 1990). The lod scores and recombination fraction data can be obtained from barendse@magic.tap.csiro.au.

Results

Isolation and mapping of microsatellites. The goal of the project was to map large insert clones harboring microsatellite sequences by FISH and to use the microsatellite regions to locate the clones on the genetic linkage map. In the course of the project, 185 cosmid and 10 lambda phage clones were placed on chromosomes by FISH, and microsatellite markers were developed for a subset of 83 of these clones that were suitable distributed across the bovine karyotype. These markers are shown in Fig. 1 and Table 1. Fifteen chromosomes have at least three markers, six chromosomes have two markers, and nine have a single marker. Of the chromosomes with only one marker, only Chr 22 had additional FISH-mapped clones at positions distinct from the one that was genetically mapped, but for which it was not possible to develop a polymorphic marker. On the remaining chromosomes, the additional clones available mapped to the same chromosomal region as the previously genetically mapped marker.

The distribution of the clones described in Fig. 1 is not biased towards the telomeric regions of the chromosomes. However, the overall distribution of the complete set of FISH-mapped clones, that is, the 185 cosmids and 10 lambda phages, suggests a tendency for microsatellite sequences to be more frequent towards the telomeres (54 clones mapped to telomeric or subtelomeric bands out of 195); in addition, more microsatellite-containing clones were obtained from Chrs 15, 16, and 19 than expected compared with other chromosomes (data not shown).

Linkage. The STS markers developed from the large insert clones (Table 1) were placed on the linkage map by typing the animals of the IBRP panel. The results are shown in Fig. 1 (N.B., some markers for Chr 15 are not shown, for clarity). Physical and linkage mapping of the clones has been essential to define the orientation of various linkage groups in relation to chromosomes in the continuous process of updating the bovine genetic map. Thus, the linkage groups for the following chromosomes were anchored and in some cases oriented by use of the cosmids and the embedded microsatellite markers described in this paper: Chr 3, *D3S24*; Chr 4, *D4S32*; Chr 7, *D7S20*; Chr 8, *D8S20*; Chr 9, *D9S14*; Chr 10, *D10S36*; Chr 12, *D12S17*; Chr 13, *D13S16*; Chr 16, *D16S5* and *D16S23*; Chr 18, *D18S10*; Chr 19, *D19S4* and *D19S18*; Chr 28, *D28S10*. The physical assignment of marker *D27S14* to 27q23 confirmed the recent identification of the last unassigned linkage group (Masabanda et al. 1996). Additionally, cosmid MAP1B, 20q14-q15, confirmed the orientation of the linkage group for Chr 20.

Finally, the following markers provided the first physical assignments that anchored the linkage groups corresponding to Chr 9, *D9S14*; Chr 12, *D12S17*; Chr 16, *D16S5*; Chr 29, *D29S2*, formerly Chr 25, and *D25S2* (ISCNDA 1989, 1990; Popescu et al. 1996).

Comparison of the physical and genetic maps. The markers defined intervals on 20 chromosomes as illustrated in Fig. 1. Each chromosome is represented with the idiogram of the corresponding G-banding pattern, in accordance to a recently agreed update of the ISCNDA standard (Popescu et al. 1996). All the idiograms are drawn to scale. The markers described in this study are indicated by the D-numbers flanking the idiograms, with the corresponding chromosomal assignments. The location of the markers on the genetic map is shown at the right of the idiograms, with the linkage group that defines each chromosome. Distances are in cM from the physically anchored microsatellite markers to the closest genes to bridge the gaps between the markers on the genetic map. Additional markers defining the ends of the linkage groups are included, where necessary. Thus, the genetic distances between the markers are obtained by adding the distances that separate all the markers for each chromsome. The complete linkage map will be published elsewhere (Barendse et al., 1996).

The intervals defined by the FISH-mapped anchored loci were used to evaluate the alignment of the physical and the genetic map. With respect to physical mapping, the distances between markers were evaluated by measuring the length spanning hybridization peaks, defined by cytogenetic band assignments (Lichter et al. 1990). In the case of assignments to more than one band, the central position was taken. The length of the interval was estimated as a percentage of the total karyotype. Markers separated by less than 1% of the genome were not considered. No data are presented for Chrs 13 or X, which only have two markers mapped at adjoining positions. In total, intervals could be considered for 20 chro-

Table 1. Markers information

Marker	D No.	FISH	Primers (5′ to 3′)	Repeat	Size (BP)	Acc. No./Reference
INRA212	D1S42	1q12	[a]	[a]	200	[a]
HAUT33	D1S63	1q31	CTGTCAATGGTATCGGACTCA GCAAGCTGTCAGCCTGTTCA	(CA)11G(AC)2	208	X89258 / (Thieven et al. 1996)
JAB6	D1S40	1q41	GAGACATGCATCCCAACCACACC GATGACCTAGAAGGATGGGATGGG	(AC)6AT(AC)12	242/248	X98441 / (Williams et al. 1996a)
RI918	D2S32	2q14-q21	ACTTATGAAAAATTACTGAGAGCG TTACTACTCCTGTCGCCTCTGA	SSCP	140	X93164 / (Williams et al. 1996b)
IDVGA80	D2S46	2q42	TCTTGGATGTCTGACAAGATGC CTAAGGCCTGGGCACAGTC	(GT)21	98	X97565 / (Nijman et al. 1996)
IDVGA37	D2S19	2q45	TAACAGGACAAGTCTTCAGGTG CCTCTCTTTCTCTATGCTCACA	(AC)17(TC)10AT(AC)6	210	X85053 / Mezzelani et al. 1995)
IDVGA2	D2S7	2q45	GTAGACAAGGAAGCCGCTGAGG GAGAAAAGCCAAGAGCCAGACC	(AC)10	132	Z27071 / (Ferretti et al. 1994)
IDVGA53	D3S29	3q21	ACGGGACGCCTCGGTCAGTG GAAGGGGAAGGGGAAGATGAAC	(AC)11	221	X85070 / (Mezzelani et al. 1995)
IDVGA35	D3S25	3q35	AAGTGCCATCACCTCCCCATTC CACCTGCTCTTGACCCCTAAAAA	(TG)20CG(TG)6(AG)6	233	X85052 / (Mezzelani et al. 1995)
HAUT31	D3S41	3q36	CAGGTATCGGTGGTGAGAAT AGCATCAGCCTCAGAAGTGG	(AC)21	202	X89258 / (Thieven et al. 1996)
INRA197	D3S26	3q36	TTGCCCTAGAAACCACATGC AGAAACTGCAGGGTGTTGTGG	(AC)23	180	X73947 / (Eggen et al. 1996)
IDVGA27	D3S24	3q37	TGGTCAGTCACAGAGAAGCACAG GAAAGCCTGGTACTCATGGAATA	(AC)9	138	X85046 / (Mezzelani et al. 1995)
IDVGA51	D4S32	4q31	ATGGCAATATTTTGTTCTTTTTC ATTCCTTGATGGTCTAATGGTTA	(AC)11	174	X85068 / (Mezzelani et al. 1995)
ETH10	D5S3	5q25	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	(CA)19	214	Z22739 / (Solinas Toldo et al. 1993)
HAUT29	D5S49	5q33-q34	AGTGGTTAATTTAAGACTGTGC CTCTGCCCTCAGCCCACTTT	(AC)17	157	X89254 / (Thieven et al. 1996)
JAB2	D5S30	5q33-q34	GCTCTCACATACTTGGGCAC GGAATAGCTATCTGGAGAGGC	(AC)9	224/228	X98435 / (Williams et al. 1996a)
ETH2	D5S2	5q35	CCCACAGGTGCTGGCATGGCC CCATGGGATTTGCCCTGCTAGCT	(CA)16	177	Z22743 / (Solinas Toldo et al. 1993)
IDVGA9	D5S27	5q35	GCTCCCTGGCTTATCTGGTTACA CTTCTTCACAGTCCAACTCTCAC	(AC)11	203	Z27075 / (Ferretti et al. 1994)
FBN3	D6S24	6q12-q14	CCAGGGATGTTGAGATTTTTGTG ACACGACTGAAGCGACTTAGCAG	(TG)10	204	Z50734 / (Kühn et al. 1996)
IDVGA65	D6S29	6q22	ACTAATGAAAAGTTAATAAAGC TGATTCCAAGTTGAGGAGA	SSCP	223	Z73084 / (Harlizius et al. 1996)
IDVGA90	D7S30	7q14-q15	TGACAGTCCCCAGGCACTAA GCCCGTACACCACATAGCA	(AC)20	187	X86815 / (Zhang et al. 1995)
IDVGA62	D7S21	7q15	GGGATCTTGGACCAGAAGGTA GGTGACACTCCTCCCATCT	(TA)8(TG)10	163	X86811 / (Zhang et al. 1995)
IOBT930	D7S20	7q23-q24	AGCACCACTATCCATCTGTCTC TGGAGTCTAGTCAGAGCGTCAGAA	(TG)15	264	X03162 / (Anastassiadis et al. 1996)
IDVGA11	D8S20	8q14	CCTTCTGGCAACCACCTATTTGT CCACCTAAGTGTCTCCTGATGGA	(AC)19	230	Z27077 / (Ferretti et al. 1994)
IDVGA52	D8S21	8q23	GAATCGAACCTTTCCTTCTAC AAGCCTCACTTGACCACTGAT	(AC)21	215	X85069 / Mezzelani et al. 1995)
INRA144	D9S14	9q25	TCGGTGTGGGAGGTGACTACAT TGCTGGTGGGCTCCGTCACC	(AC)20	200	X74174 / (Eggen et al. 1994)
JAB10	D10S36	10q15	GCTGACCGTGTTAACTTCAC CTGAGCACCAAGGGTTGCTG	(AC22)	270 / 290	X98445 / (Williams et al. 1996a)
HAUT30	D11S56	11q12-q13	ACGGTAGGAATATGAGGGTGT CCCAACCATCCCCACTTCTG	(AC)2(CA)2T(CA)22	213	X89255 / (Thieven et al. 1996)
INRA177	D11S17	11q16	CAGCAGTAGTCACCTAAAACC AAGGAAACTCCAAAACACCAGG	(AC)24	195	X74201 / (Vaiman et al. 1994)
IDVGA3	D11S27	11q23	GGATAGGTATAATTAAGTTTCTGGC AGGGACTTAGCACATACTCACAC	(AC)11	158	Z27072 / (Ferretti et al. 1994)
INRA195	D11S34	11q25	CTCCACCCTCTGCCCAGTCC AGCACTCGGACCTATACC	(AC)17	220	X73945 / (Eggen et al. 1996)
JAB7	D11S38	11q26	GCGGAAGTACAGAGAGTTCTGTG GCAGATACGTGGATGACCCTGATC	(AC)17	365/377	X98442 / (Williams et al. 1996a)
IDVGA57	D12S29	12q13	ATGGGTCTCTCGGTGCTGTT GAGTTTGTGCAAGGCTGACTT	(TC)12(AC)20	196	X85072 / (Mezzelani et al. 1995)
HAUT1	D12S22	12q25	CTGCAGGGCTACAGTCA GACAGGGAGTTGTTTAACCAG	complex[b]	188	X81349 / (Thieven et al. 1995)
INRA209	D12S17	12q26	TGCTTTACATATCAAGGCAGC GTAGGCAGATTCTTTACTGAG	(AC)16	145	X95390 / (Bahri-Darwich et al. 1994)
ETH7	D13S1	13q21-q22	GTGAGACCCAGCTCCTGCCTGG CGGAGCAGGGTGTCAGAGCCG	(CA)3N15(CA)17	383	Z22746 / (Solinas Toldo et al. 1993)
JAB3	D13S16	13q23	GCAAGGAGGACCCTGTGCG CCTAGTATCCAGTCTGTGTCC	(AC)9	226/230	X98440 / (Williams et al. 1996a)
IDVGA76	D14S35	14q14	ACACGGGACCACTGGGACCT CACCTTGCGAAAGATGAACAT	SSCP	188	Z73083 / Williams et al. 1996c)
JAB1	D15S18	15q14	CAGCCATTAAGGGCTGGGATTCC GATTTCTGGAGGAGGCTCACAGCAG	(AC)18	224/240	X98434 / (Williams et al. 1996a)
JAB8	D15S20	15q21	CACGTCACCCGCTTTCTCTTG GGTGAGTGTAACACCTGTGTGCG	(AC)15	221/225	X98443 / (Williams et al. 1996a)
INRA224	D15S22	15q21	AGAGGCAGTCCAGTGAAAGG	(CA)17	200	U51937 / (Eggen et al. 1996)

Table 1. *Continued.*

Marker	D No.	FISH	Primers (5′ to 3′)	Repeat	Size (BP)	Acc. No./Reference
JAB4	D15S19	15q21-q22	GCCAGTTTTGTGTACATTATGG GATTACCAGGTACACCTCCAAGC	(AC)13	190/196	X98438 / (Williams et al. 1996a)
INRA046	D15S5	15q25	CAAGTGTCTTCTAAGGTACTGG CAGCTCATGTGTTTACATGGC	(AC)10	115	X71495 / (Vaiman et al. 1993)
IDVGA10	D15S13	15q25	AGTCTCTGGAACTTCTCCTAC GGCTAAGACCAGCACCTTGAATA	(AC)23	190	Z27076 / (Ferretti et al. 1994)
IDVGA32	D15S16	15q25	GGGACCTTGAGGAGGAGACAG CAGGTGTTGGAGGATGAGAAAGG	(AC)15	205	X85051 / (Mezzelani et al. 1995)
HAUT23	D15S29	15q27	CAGTCTATGGCACAGTCTTGGTA CTTTCGTTGAGTTGGGATATCT	complex[b]	451	X89249 / (Thieven et al. 1996)
IOBT395	D15S23	15q27-q28	TCTTACACCGCAGTCCTGAA ACAACAGGAAAGCTCTGCCA	(CA)17	92	X95067 / (Nijman et al. 1996)
IDVGA66	D16S34	16q12	ACATGTAGCTGTTGATACAGAT TATCTTCAGGTGGCAGGAGAC	SSCP	209	Z73082 / (Williams et al. 1996c)
IDVGA68	D16S23	16q16	GGCAGTCCTCATCAGACACTC TCAGAGGGGCAGACAGTGAT	(AC)14	200	X85081 / (Mezzelani et al. 1995)
ETH11	D16S5	16q21	GAGGGACTTGGGAGGGAAAC GGCGGACAAGAGTTCTCAGAGAC	(GT)16GC(GT)4	214	Z22740 / (Solinas Toldo et al. 1993)
JAB9	D16S25	16q17	CTTGACACATCATTGAGCACATGC CAACTTCAAATTCTCTTGG	(AC)10 INTERRUPTED	192/200	X98444 / (Williams et al. 1996a)
IDVGA40	D17S24	17q23	CAGACCTGCAGGCAGCCTGCATC TTCAGTCAAGCAAACAGGACAT	(AC)16	240	X85055 / (Mezzelani et al. 1995)
IOZARA975	D17S29	17q26	TGGGTTCAGTTGCTGGGTGGAGA TGAAGGAGATGTGGGTTTG	(CA)2TG(CA)10(CT)2	233	U47616 / (Martin-Burriel et al. 1996a)
IDVGA31	D18S10	18q12-q13	TTGACGAGACCCAAGAAGTAAACC CCTTGAGATGAATGTTTGAGGATG	(AC32)	214	X85050 / (Mezzelani et al. 1995)
HAUT14	D18S17	18q21	AACGCAGCCAGCAGGGTCAGG TGACCTTCACTCATGTTATTAA	complex[b]	161	X81350 / (Thieven et al. 1995)
IDVGA55	D18S16	18q24	CCAGGGAAGATGAAGTGACC GTGACTGTATTTGTGAACACCTA	(AC)12	199	X85071 / (Mezzelani et al. 1995)
IDVGA46	D19S18	19q16	TCTAAAACGGAGGCAGAGATG AAATCCTTTCAAGTATGTTTTCA	(AC)11	205	X85062 / (Mezzelani et al. 1995)
IOBT34	D19S4	19q22	ACTCACTCCAGTATTCTTGTCTG TCCTTCCATCAATGTGTCAGTCC	(GC)12AT(GT)20	190	U31002 / (Olsaker et al. 1996b)
IDVGA44	D19S17	19q22	TTGGGATTCGGTGGTCAGTCTG GGGAGAATGGATGGAACCAAAT	(AC)19	211	X85059 / (Mezzelani et al. 1995)
ETH3	D19S2	19q23	TTCGAAGACGGGCAGACAGG GAACCTGCCTCTCCTGCATTGG	(CA)26	122	Z22744 / (Solinas Toldo et al. 1993)
COSMAP1B	MAP1B	20q14-q15	ACTTCGCCTGTGGCCAAGTAGG TACTGATTCTGACTGATTATGTCT	(CA)12	280	(Eggen 1992)
IDVGA45	D21S25	21q15	GGCTGTGCCTCGATAGATGGTGCT GTGGTGGCAAAGAGTCAGA	(AC)12	149	X85060 / (Mezzelani et al. 1995)
HAUT28	D21S46	21q15-q16	AACAGCCCTGATTTCCATA GCTTGTTCCTATCACCCATTTA	(AC)5G(CA)2TTCC(CA)7	359	X89253 / (Thieven et al. 1996)
IDVGA39	D21S24	21q23prox	GAAGGCTTGGAAAATGTAATC ACGGTGGGAACATCTTGTCACTA	(AC)17	191	X85054 / (Mezzelani et al. 1995)
IDVGA30	D21S23	21q23	CCAGTATTCTTCCTGCGAAAAATC GCATCTGGGAGCCTCGTATCTC	(AC)40	240	X85049 / (Mezzelani et al. 1995)
INRA194	D22S19	22q13	TTGTAAAACTCGGGGCATAAGCA ATCCTGTTTCAGAATAAATTCCAG	(AC)12	185	X73944 / (Eggen et al. 1996)
IOBT528	D23S27	23q14	ACAGTTCTGGAGTTGCTTGATG TGCTTCTGTAAATGACGTTCCG	(GT)16	182	X95068 / (Nijman et al. 1996)
RI1479	D23S38	23qter	AACTCCAGGAGTTGGTGATGG CCAGTAACGATCTAGCAAAGGGATC	SSCP	111	U59512 / (Olsaker et al. 1996c)
IOBT1401	D24S13	24q21	CATCAACACTGCTGCCAACTCAG CATAGGTTTGTTTAATGCTCAG	(CA)14	110	X95069 / (Nijman et al. 1996)
IDVGA71	D25S12	25q12-q13	TGATAGATAGCATTTGATTGGC GCTAGGCATCTGGCAAATAG	(AC)2AG(AC)16	192	X85053 / (Zhang et al. 1995)
HAUT39	D25S19	25q17	CCTCAGAGAAGCCTGGTCAT GCTTGAAAGAAAAATGCCAAAGA	CTN(AC)11GC	145	X89260 / (Thieven et al. 1996)
INRA222	D25S11	25q19	GAGCCTCCCACCCCAACAG GTGGAGGAGTCTTACAGTCCAG	(GT)11	160	U51935 / (Eggen et al. 1996)
IDVGA59	D26S14	26q22	GATCTCTGGATTGAAGGAAC AACCCAAATATCCATCAATAG	(AC)23	256	X85074 / (Mezzelani et al. 1995)
IOBT313	D27S14	27q23	CAGTCCCTCAACCCTCTTTTC GAATCAATAAAGAAGATGCAGCACG	(CA)12	124	U59511 / (Olsaker et al. 1996a)
INRA201	D28S17	28q12	GCCCTCTAGCTCTATCTGTGTTTGC CTCACTAAGCTGACTCAGGC	(AC)11	148	U51932 / (Eggen et al. 1996)
IDVGA29	D28S12	28q13	GGACTCCTTTGGGTGCTTGG CCCACAAGGTTATCTATCTCCAG	(AC)23	149	X85048 / (Mezzelani et al. 1995)
IDVGA43	D28S18	28q17	CCAAGAAGGTCCAAAGCATCCAC GGGGGGTTGGAAGTATTATCTG	(AC)14	171	X85058 / (Mezzelani et al. 1995)
IDVGA8	D28S10	28q18-q19	AGAAGGGTCTTGGTCTCCTCACT CTCTTGGGGGCGTGTTGTCT	(AC)19	224	Z27074 / (Ferretti et al. 1994)
IDVGA7	D29S2	29q19	TAGCAGAAAGCACAGGAGTC GGGTGGGCTTCATTTCTATG	(AC)9	195	Z27073 / (Ferretti et al. 1994)
JAB5	D29S12	29q12	CAGCCACTGTCTCCTCCCAC CCTAGCGATCTCTTGATTGAG	(AC)17	292/302	X98440 / (Williams et al. 1996a)
INRA211	D29S24	29q16	CCTTTGGAACTTCTGATGGTGC TTACAGGTGCAGCTCAGATGCTG	(CA)8C(CA)4	206	U51933 / (Eggen et al. 1996)

Table 1. *Continued.*

Marker	D No.	FISH	Primers (5′ to 3′)	Repeat	Size (BP)	Acc. No./Reference
			CCTACTGTTTAGCACAGGGAACTC			
IDVGA82	DXS30	Xq34	ACAATGATGAGGGGCTCTG GGCAAACCATTCCAGTATTC	(AC)12	191	X86813 / (Zhang et al. 1995)
IOZARA1489	DXYS4	Xq42-q43	ATGCACCAGACACGGAATGG TGCGGCAGCGTCAAAATGAC	complex[b]	292	U47615 / (Martin-Burriel et al. 1996b)

Symbols for anonymous DNA loci: *IDVGA,* Istituto per la Difesa e la Valorizzazione del Germoplasma Animale, CNR, Italy; *JAB* and *RI,* Roslin Institute (Edinburgh), Roslin, Scotland; *INRA,* Institute National pour la Recherche Agronomique, Jouy en Josas, France; *HAUT,* Department of Animal Breeding and Genetics, Hannover School of Veterinary Sciences, Hannover, Germany; *ETH,* Swiss Federal Institute of Technology, Zurich, Switzerland; *IOZARA,* Norwegian College of Veterinary Medicine, Oslo, and Facultad de Veterinaria, Zaragoza, Spain; *IOBT,* Norwegian College of Veterinary Medicine, Oslo, Norway.

[a]The marker is available upon request. Contact A. Eggen.
[b]The complete repeats are as follows: *HAUT1*, (TG)3A(GT)5A(TG)5A(GT)5A(TG) 10(CG)2; *HAUT23,* (AC)6CAGT(AC)6CAGTTCAGGA(AC)9CCCAGTT(CA)17C GNGTTCAGG(AC)13CCCAGTT(CA)17CGNGTTCAGG(AC)18CCCAGTTCT (CA)14CGNGTTCAGG(AC)18GNGTTCAGG(AC)17; *HAUT14,* (AT)2(GT)15G(GT)9G(GT)13C(TG)2; IOZARA1489, (CT)5GA(GT) 2(CT)5(CA)12CT(CA)4.

mosomes, covering 40.6% of the whole genome (Table 2). In some cases, the size of the interval used was increased by including genes for which physical and linkage mapping data were available. Thus, on Chr 6 the interval was between *D6S24* and *CAS@* (Gallagher et al. 1994), on Chr 7 between *D7S30* and *RASA* (Eggen et al. 1992), on Chr 11 between *D11S56* and *LGB* (Hayes and Petit 1993), and on Chr 17 between *D17S29* and *FGG* (Johnson et al. 1993). An interval could be defined on Chr 9 despite the presence of only one cosmid-derived, physically anchored locus: connexin 43 (*GJA1*) was used as a second marker, which was recently mapped by FISH to 9q15-q16 (Castiglioni et al. 1996).

The total genetic distance covered by the intervals between markers in 1666 cM, giving an estimate for the whole genome of 4176 cM, which is much larger than the size of 2800 cM predicated on the basis of chiasmata counts (Jagiello et al. 1974; Logue and Harvey 1978) and larger still than 3540 cM suggested in the current version of the CGD linkage map from the distribution of markers (Barendse et al. 1996). Table 2 presents estimates of genome size obtained from the evaluation of individual intervals. There is great variation in the estimated values, which is reflected in a mean of 4176 cM with a standard error of 1360 cM. However, it is interesting to inspect the data for some of the intervals. *D3S29* and *D3S24* span 3.10% of the genome, or more significantly about 66% of Chr 3, which, when extrapolated, gives an estimate of 5063 cM for the total genome. From other chromosomes that are well covered by the anchored markers, generally high values are obtained for the inferred genome size: Chr 18 (61.5%) 6519 cM; Chr 28 and Chr 29 (both 70%), 5868 cM and 7132 cM respectively. On the other hand, the intervals for Chr 1 (68.5% coverage) and for Chr 25 (67.5%) suggest a size for the genome that is much below the average and close to the estimate based on chiasmata counts.

Discussion

In this study cosmid and phage clones have been used as a tool to add to the developing linkage map of the bovine genome by providing cytogenetically mapped anchored loci. Microsatellite markers were derived from 74 cosmid and 9 phage clones and have been placed on the genetic map, while the parent clones have been localized on the bovine chromosomes by FISH. All 30 bovine chromosomes have at least one microsatellite marker mapped by FISH.

The use of microsatellite markers derived from cosmid and phage clones as anchor loci for the genetic map has highlighted the usefulness of combining genetic and physical mapping. Compared with the first published linkage map (Barendse et al. 1994), the set of markers described here allowed the anchoring and orientation of the linkage groups corresponding to Chrs 3, 4, 7, 8, 9, 10, 12, 13, 16, 18, 19, and Chr 28 and led to the identification of four unassigned linkage groups, corresponding to Chrs 9, 12, 16, and 29, formerly Chr 25 (ISCNDA 1989, 1990; Popescu et al. 1996). The linkage group for Chr 27 with marker *D27S14*, 27q23, was assigned and orientated by a single appropriately placed marker, confirming the recent identification of the last unassigned linkage group (Masabanda et al., 1996). Similarly, cosmid MAP1B, 20q14-q15, confirmed the orientation of the linkage group for Chr 20.

Another interesting feature of cytogenetically mapped loci, anchored to the linkage map by means of highly polymorphic STS markers, is that they allow an evaluation of the alignment between the physical map and genetic maps (Bray-Ward et al. 1996). This, in turn, gives an estimate of the rate of genetic recombination and, ultimately, of genome size.

In this study, 83 markers defined intervals on 20 chromosomes, representing 40.6% of the total karyotype, and a genetic distance of 1666 cM. An estimate of 4176 cM was thus obtained for the whole genome, which is greatly in excess of the 2800 cM predicted by chiasmata counts (Jagiello et al. 1974; Logue and Harvey 1978). Our data would also suggest a larger size than the 3540 cM proposed from data in the present bovine linkage map, based on the Cattle Genotype Database (CGD; Barendse et al. 1996). However, care must be taken when considering these estimates as they have large errors associated with them and do overlap at the extremes of the range. Typing errors inflate the estimate of genetic length, particularly considering the relatively low number of markers presented in this study. Nevertheless, our finding is of special interest in light of the results published in a similar study on the porcine genome (Ellegren et al. 1994b). Ellegren and colleagues estimated a genome size of about 2000 cM with a set of markers covering 37.5% of the pig karyotype, a figure much closer to mouse, 1600 cM, than to human, 3800–4000 cM, despite a very similar length of 2.8 billion base pairs (bp). Conversely, our estimate for the size of bovine genome of 4176 cM suggests it might be closer to human and possibly even larger.

The data cannot be taken as conclusive. In particular, it is known from extensive and careful studies in human (Bray-Ward et al. 1996) that the relationship between physical and genetic maps is not uniform along the length of a chromosome. Genetic recombination is generally suppressed in the centromere-proximal regions. Moreover, the striking reduction of genetic recombination in the human acrocentric chromosomes (Bray-Ward et al. 1996) has a special significance here, since all the chromosomes in the bovine karyotype are acrocentric. Conversely, the telomeric regions of several human chromosomes cover very long distances in recombination map units, cM, if compared with the short spacing of the physically mapped markers. Special care should also be taken in evaluating FISH data for telomeric markers, since it is often difficult to interpret the ends of chromosomes by fluorescence microscopy. However, imprecise FISH assignments of telomeric markers cannot alone explain the large estimated genome size presented in this paper. This could be the case for two of the

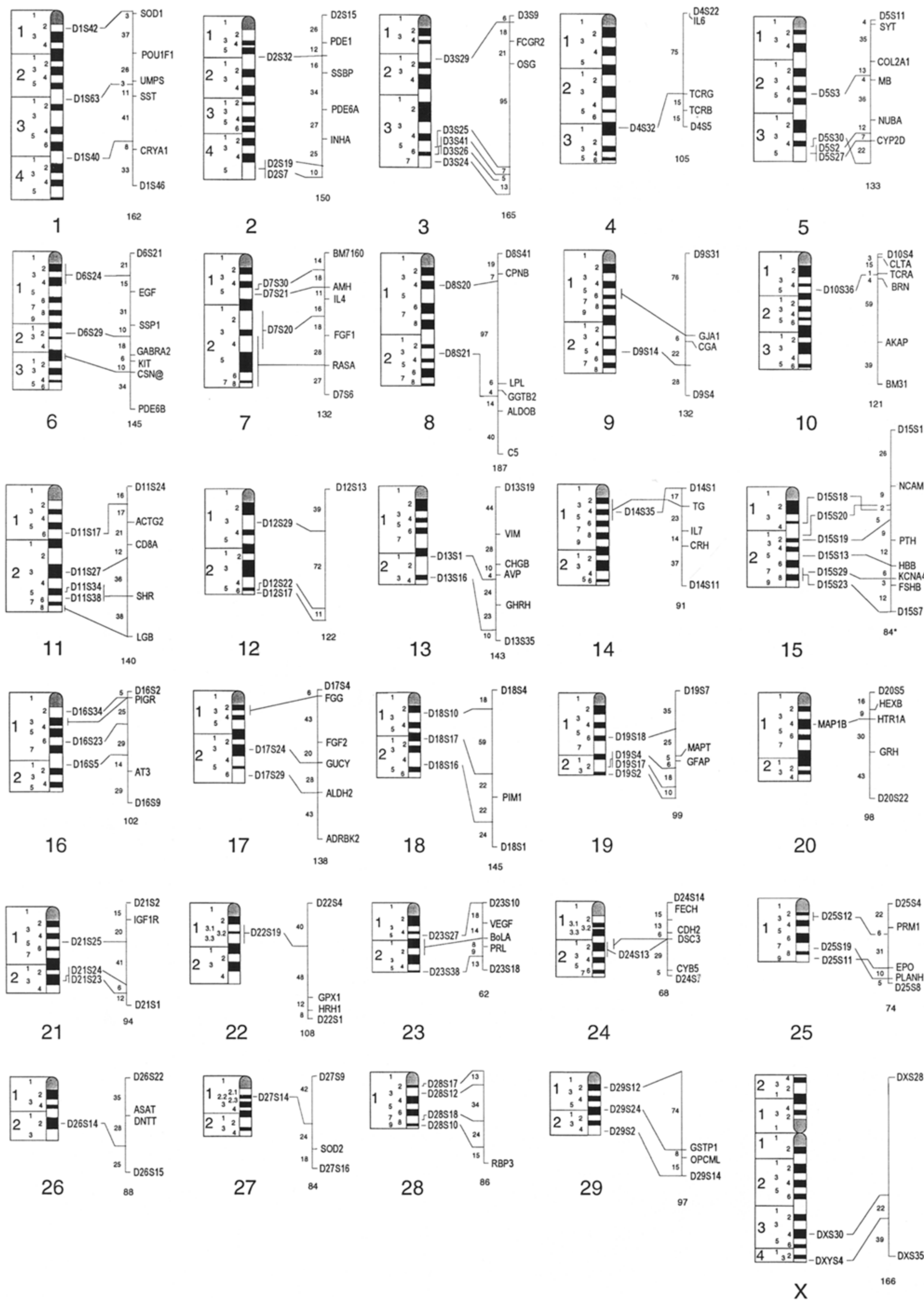

Fig. 1. Integration of the physical and genetic map of the bovine genome Idiograms of G-banded chromosomes (Popescu et al. 1996) are flanked by the corresponding linkage groups. Linkage data are based on the CGD (W. Barendse, CSIRO). Distances are in cM and the linkage groups are drawn to scale, with the total length given at the bottom. D numbers identify anonymous DNA loci. The physically mapped loci anchored on the genetic map are shown in between the idiograms and the linkage groups with the corresponding chromosomal band assignments. For Chr 15 the linkage group is drawn at a 200% scale and only some markers are shown (see Table 1 for a complete list). D numbers without a physical assignment are of markers that define the current ends of the linkage groups. For the following genes the physical mapping information was incorporated in the figure: *CAS@* (Gallagher et al. 1994), *RASA* (Eggen et al. 1992), *LGB* (Hayes and Petit 1993), *FGG* (Johnson et al. 1993), *GJA1* (Castiglioni et al. 1996).

Table 2. Comparison of the bovine physical and genetic maps

Chr	Markers (Longest interval)	Length (% genome)	Genetic distance sex aver. (cM)	Inferred genome size sex aver. (cM)
1	*D1S42–D1S40*	4.02	121	3010
2	*D2S32–D2S7*	3.516	112	3185
3	*D3S29–D3S24*	3.114	159	5063
5	*D5S3–D5S30*	1.635	81	4954
6	*D6S24–CAS@*	2.62	90	3435
7	*D7S30–RASA*	2.27	91	4009
8	*D8S20–D8S21*	2.13	107	5023
9	GJA1–D9S14	1.79	28	1564
11	*D11S17–LGB*	3.09	123	3980
12	*D12S29–D12S17*	2.19	83	3790
15	*D15S18–D15S23*	1.63	47	2883
16	*D16S34–D16S5*	1.63	59	3620
17	*D17S29–FGG*	2.00	91	4550
18	*D18S10–D18S16*	1.58	103	6519
19	*D19S18–D19S2*	1.14	64	5614
21	*D21S25–D21S23*	1.22	47	3852
23	*D23S27–D23S38*	1.07	55	5121
25	*D25S12–D25S11*	1.33	37	2782
28	*D28S17–D28S10*	1.21	71	5868
29	*D29S12–D29S2*	1.36	97	7132
	Total interval	40.66	1666	4176

small chromosomes, namely Chr 28 and Chr 29, and for Chr 5, which has several markers mapped to the telomere, but not for other chromosomes, such as Chrs 17, 18, 7, and 3. As an example, the interval spanning markers *D7S30* and *RASA* on Chr 7 does not include the telomeric region, yet predicts a genome size above 4000 cM. On Chr 3, one could argue that the large estimate of 5063 cM is due to the inclusion of four telomeric markers in the interval; however, a large estimate is still obtained (4980 cM) when considering the interval between markers *D3S29* and *D3S25*, which do not include the telomere region of the chromosome. A chromosome size-dependent control of recombination has been hypothesized in organisms as distant as yeast and human (Kaback 1996). Thus, small human chromosomes recombine at 1.5- to 3-fold higher rates than large chromosomes.

Curiously, there seems to be a better correlation between the genetic size of a genome and the number of chromosomes, rather than size in base pairs. At least, this is what emerges from the analysis of the few mammalian species where substantial linkage data are available: thus, for the mouse (19 autosomes, X and Y) and pig (18, X and Y), the genome size is small (1600 and 2000 cM respectively), compared with human (23, X and Y) with 3800–4000 cM and now for the cattle genome (29, X and Y), which is apparently above 4100 cM (Davisson and Roderick 1989; Weissenbach et al. 1992; Dietrich et al. 1994; Archibald et al. 1995; Hudson et al. 1995). It will be interesting to see the result for sheep, when the linkage map is more complete (Crawford et al. 1995). In chicken the available maps are incomplete (Bumstead and Palyga 1992; Levin et al. 1994); however, the 33 known linkage groups in the chicken seem to cover as much as 3200 cM, and possibly more, despite the genome being 1×10^9 bp, about one-third the size of the mammalian species mentioned above. Indeed, there is evidence that the rate of genetic recombination might be three times higher in chicken than in human.

A better coverage of physically mapped markers is required to assess the alignment of the physical and genetic maps of cattle. However, based on a 40.6% physical coverage of the genome, the data presented in this paper suggest that the current linkage map of the bovine genome may cover less than the hypothesized 95% (Barendse et al. 1996).

The availability of physical resources, such as YAC and BAC libraries (Cai et al. 1995), as well as the development of tools like radiation hybrid somatic cell lines (Gyapay et al. 1996), will be crucial to a better integration of the physical and genetic maps of cattle, and ultimately for the accurate localization of trait loci as a prelude to finding the genes themselves.

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References

Anastassiadis, C., Leyhe, B., Olsaker, I., Friedl, R., Rottmann, O., Hiendleder, S., Erhardt, G. (1996). Three polymorphic microsatellites for bovine chromosomes 7, 12, and 19. Anim. Genet. 27, 125–126.

Archibald, A.L., Haley, C.S., Brown, J.F., Couperwhite, S., McQueen, H.A., Nicholson, D., Coppieters, W., Van de Weghe, A., Stratil, A., Winterø, A.K. Freedholm, M., Larsen, N.J., Nielsen, V.H., Milan, D., Woloszyn, N., Robic, A., Dalens, M., Riquet, J., Gellin, J., Caritez, J.-C., Burgaud, G., Ollivier, L., Bidanel, J.-P., Vaiman, M., Renard, C., Geldermann, H., Davoli, R., Ruyter, D., Verstege, E.J.M., Groenen, M.A.M., Davies, W., Høyheim, B., Keiserud, A., Andersson, L., Ellegren, H., Johansson, M., Marklund, L., Miller, J.R., Anderson Dear, D.V., Signer, E., Jeffreys, A.J., Moran, C., Le Tissier, P., Muladno, Rothschild, M.F., Tuggle, C.K., Vaske, D., Helm, J., Liu, H.-C., Rahman, A., Yu, T.-P., Larson, R.G., Schmitz, C.B. (1995). The PiGMaP consortium linkage map of the pig (*Sus scrofa*). Mamm. Genome 6, 157–175.

Bahri-Darwich, I., Vaiman, D., Olsaker, IL., Oustry, A., Cribiu, E.P. (1994). Assignment of bovine synteny groups U27 and U8 to R-banded Chromosomes 12 and 27, respectively. Hereditas 120, 261–265.

Barendse, W., Vaiman, D., Kemp, S.J., Sugimoto, Y., Armitage, S., Williams, J.L., Sun, H.S., Eggen, A., Agaba, M., Aleyasin, S.A., Band, M., Bishop, M.D., Buitkamp, J., Byrne, K., Collins, F., Cooper, L., Coppettiers, W., Denys, B., Drinkwater, R.D., Easterday, K., Elduque, C., Ennis, S., Erhardt, G., Ferretti, L., Flavin, N., Gao, Q., Georges, M., Gurung, R., Harlizius, B., Hawkins, G., Hetzel, D.J.S., Hirano, T., Hulme, D., Joergensen, C.,, Kessler, M., Kirkpatrick, B.W., Konfortov, B., Kostia, S., Kuhn, C., Lenstra, J., Leveziel, H., Lewin, H.A., Leyhe, B., Li, L., Martin Buriel, I., McGraw, R.A., Miller, J.R., Moody, D.E., Moore, S.S., Nakane, S., Nijman, I., Olsaker, I., Pomp, D., Rando, A., Ron, M., Shalom, A., Soller, M., Teale, A.J., Thieven, I., Urquhart, B., Vage, D-I., Van de Weghe, A., Varvio, S., Velmala, R., Vilkki, J., Weikard, R., Woodside, C., Womack, J.E., Zanotti, M., and Zaragoza, P. (1996). A medium density genetic linkage map of the bovine genome. Mamm. Genome, 8, 21–28.

Barendse, W., Armitage, S.M., Kossarek, L.M., Shalom, AL., Kirkpatrick, B.W., Ryan, A.M., Clayton, D., Li, L., Neiberg, H.L., Zhang, N., Grosse, W.M., Weiss, J., Creighton, P., McCarthy, F., Ron, M., Teale, A.J., Fries, R., McGraw, R.A., Moore, S.S., Georges, M., Soller, M., Womack, J.E., Hetzel, D.J.S. (1994a). A genetic linkage map of the bovine genome. Nature Genet. 6, 227–235.

Bishop, M.D., Kappes, S.M., Keele, J.W., Stone, R.T., Sunden, S.L.F., Hawkins, G.A., Solinas-Toldo, S., Fries, R., Grosz, M.D., Yoo, J., Beattie, C.W. (1994). A genetic linkage map for cattle. Genetics 136, 619–639.

Bray-Ward, P., Menninger, J., Lieman, J., Desai, T., Mokady, N., Banks, A., Ward, D.C. (1996). Integration of the cytogenetic, genetic, and physical maps of the human genome by FISH mapping of the CEPH YAC clones. Genomics 32, 1–14.

Bumstead, N., Palyga, Y. (1992). A preliminary linkage map of the chicken genome. Genomics 13, 690–697.

Cai, L., Taylor, J.F., Wing, R.A., Gallagher, D.S., Woo, S.-S., Davis, S.K. (1995). Construction and characterization of a bovine bacterial artificial chromosome library. Genomics 29, 413–425.

Castiglioni, B., Ferretti, L., Tenchini, M.L., Mezzelani, A., Simonic, T., Duga, S. (1996). Physical mapping of connexin 32 (GJB1) and 43 (GJA1) genes to bovine chromosomes Xq22 and 9q15-16 by fluorescence in situ hybridization. Mamm. Genome 7, 634–635.

Crawford, A.M., Dodds, K.G., Ede, A.J., Pearson, C.A., Montgomery, G.W., Garmonsway, H.G., Beattie, A.E., Davies, K., Maddox, J.F., Kappes, S.W., Stone, R.T., Nguyen, T.C., Penty, J.M., Lord, E.A., Broom, J.E., Buitkamp, J., Schwaiger, W., Epplen, J.T., Matthew, P.,

Matthews, M.E., Hulme, D.J., Beh, K.E., McGraw, R.A., Beattie, C.W. (1995). An autosomal genetic linkage map of the sheep genome. Genetics 140, 703–724.

Davisson, M.T., Roderick, T.H. (1989). Linkage Map. In *Genetic Variants and Strains of the Laboratory Mouse*, ed. 2, M.F. Lyon, A.G. Searles, eds. (Stuttgart: Gustay Fischer Verlag), pp. 416–427.

Dietrich, W.F., Miller, J.C., Steen, R.G., Merchant, M., Damron, D., Nahf, R., Gross, A., Joyce, D.C., Wessel, M., Dredge, R.D., Marquis, A., Stein, L.D., Goodman, N., Page, D.C., Lander, E.S. (1994). A genetic map of the mouse with 4006 simple sequence length polymorphisms. Nature Genet. 7, 220–245.

Eggen, A. (1992). Recherche de marqueurs polymorphes dans la région putative de l'Amyotrophie spinale bovine a l'aide de la cartographie comparée. Ph.D. Thesis ETHZ Nr. 9835.

Eggen, A., Fries, R. (1995). An integrated cytogenetic and meiotic map of the bovine genome. Anim. Genet. 26, 215–236.

Eggen, A., Solinas-Toldo, S., Dietz, A.B., Womack, J., Stranzinger, G., Fries, R. (1992). RASA contains a polymorphic microsatellite and maps to bovine syntenic group U22 on chromosome 7q24-qter. Mamm. Genome 3, 559–563.

Eggen, A., Bahri-Darwich, I., Mercier, D., Vaiman, D., Cribiu, E.P. (1994). Assignment of bovine synteny group U2 to chromosome 9. Anim. Genet. 25, 183–185.

Eggen, A., Bahri-Darwich, I., Elduque, C., Petit, E., Oustry, A., Cribiu, E.P., Leveziel, H. (1996). New cosmids containing microsatellites for mapping the bovine genome. Mamm. Genome, in preparation.

Ellegren, H., Chowdhary, B., Johansson, M., Andersson, L. (1994a). Integrating the porcine physical and linkage map using cosmid-derived markers. Anim. Genet. 25, 155–164.

Ellegren, H., Chowdhary, B.P., Johansson, M., Marklund, L., Fredholm, M., Gustavsson, I., Andersson, L. (1994b). A primary linkage map of the porcine genome reveals a low rate of genetic recombination. Genetics 137, 1089–1100.

Ferretti, L., Leone, P., Pilla, F., Zhang, Y., Nocart, M., Guérin, G. (1994). Direct characterization of bovine microsatellites from cosmids: polymorphism and synteny mapping. Anim. Genet. 25, 209–214.

Fries, R., Eggen, A., Womack, J.E. (1993). A bovine genome map. Mamm. Genome 4, 405–428.

Gallagher, D.S., Schelling, C., Groenen, M.A.M., Womack, J. (1994). Confirmation that the casein gene cluster resides on cattle chromosome 6. Mamm. Genome 5, 524.

Green, P., Falls, K., Crooks, S. (1990). Documentation for CRI-MAP, version 2.4 (ST Louis: Washington University School of Medicien).

Gyapay, G., Schmitt, K., Fizames, C., Jones, H., Vega-Czarny, N., Spillet, D., Muselet, D., Prud'Homme, J.-F., Dib, C., Auffray, C., Morissette, J., Weissenbach, J., Goodfellow, P.N. (1996). A radiation hybrid map of the human genome. Hum. Mol. Genet. 5, 339–346.

Harlizius, B., Guérin, G., Ferretti, L. (1996). IDVGA65 (D6S29), an SSCP marker assigned to BTA6 by means of FISH, genetic and synteny mapping. Anim. Genet., in press.

Hayes, H., Petit, E.J. (1993). Mapping of the β-lactoglobulin gene and of an immunoglobulin M heavy chain-like sequence to homoeologous cattle, sheep and goat chromosomes. Mamm. Genome 4, 207–210.

Hudson, T.J., Stein, L.D., Gerety, S.S., Ma, J., Castle, A.B., Silva, J., Slonim, D.K., Baptista, R., Kruglyak, L., Xu, S.H., et al. (1995). An STS-based map of the human genome. Science 270, 1945–1954.

ISCNDA 1989 (1990). International system for cytogenetic nomenclature of domestic animals (Di Berardino D, Hayes H, Fries R, Long S, eds.). Cytogenet. Cell Genet. 53, 65–79.

Jagiello, G.M., Miller, W.A., Ducayen, M.B., Lin, J.S. (1974). Chiasma frequency and disjunctional behaviour of ewe and cow oocytes matured in vitro. Biol. Reprod. 10, 354–363.

Johnson, S.E., Barendse, W., Hetzel, D.J. (1993). The gamma fibrinogen gene (FGG) maps to chromosome 17 in both cattle and sheep. Cytogenet. Cell Genet. 62, 176–180.

Kaback, D.B. (1996). Chromosome-size dependent control of meiotic recombination in humans. Nature Genet. 13, 20–21.

Kühn, C., Weikard, R., Goldammer, T., Olsaker, I. (1996). FBN3 (D6S24)—a bovine microsatellite derived from cosmid cIOBT475 at chromosome 6q12-14. Anim. Genet. 27, 61.

Levin, I., Santangelo, L., Cheng, H., Crittenden, L.B., Dodgson, J.B. (1994). An autosomal genetic linkage map of the chicken. J. Hered. 85, 79–85.

Lichter, P., Tang, C.-J., Call, K., Hermanson, G., Evans, G.A., Housman, D., Ward, D.C. (1990). High resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. Science 247, 64–69.

Logue, D.N., Harvey, M.J.A. (1978). Meiosis and spermatogenesis in bulls heterozygous for a presumptive 1/29 Robertsonian translocation. J. Reprod. Fertil. 54, 159–165.

Martin-Burriel, I., Chowdhary, B.P., Prakash, B., Zaragoza, P., Olsaker, I. (1996a). A polymorphic bovine dinucleotide repeat D17S29 (IOZARA975) at chromosome 17q26. Anim. Genet. 27, 287.

Martin-Burriel, I., Chowdhary, B.P., Prakash, B., Zaragoza, P., Olsaker, I. (1996b). A polymorphic bovine dinucleotide repeat DXYS4 (IOZARA1489) located at Xq42-q43 and linked within the pseudoautosomal region of the sex chromosomes. Anim. Genet. 27, 287.

Masabanda, J., Kappes, S.M., Smith, T.P.L., Beattie, C.W., Fries, R. (1996). Mapping of a linkage group to the last bovine chromosome (BTA27) without an assignment. Mamm. Genome 7, 229–230.

Mezzelani, A., Solinas Toldo, A., Nocart, M., Guérin, G., Ferretti, L., Fries, R. (1994). Mapping of syntenic groups U7 and U27 to bovine chromosomes 25 and 12, respectively. Mamm. Genome 5, 574–576.

Mezzelani, A., Zhang, Y., Redaelli, L., Castiglioni, B., Leone, P., Williams, J., Solinas-Toldo, S., Wigger, G., Fries, R., Ferretti, L. (1995). Chromosomal localization and molecular characterization of 53 cosmid-derived bovine microsatellites. Mamm. Genome 6, 629–635.

Nijman, I.J., Lenstra, J.A., Schwerin, M., Olsaker, I. (1996). Polymorphisms and physical locations of three bovine microsatellite loci: IOBT395, IOBT528 and IOBT1401. Anim. Genet. 27, 221–222.

O'Brien, S.J., Womack, J.E., Lyons, L.A., Moore, K.J., Jenkins, N.A., Copeland, N.G. (1993). Anchored reference loci for comparative genome mapping in mammals. Nature Genet. 3, 103–112.

Olsaker, I., Prakash, B., Guttersrud, O.A., Chowdhary, B.P. (1996a). D27S14 (IOBT313): a genetic and physical marker for bovine chromosome 27, submitted.

Olsaker, I., Solinas Toldo, S., Fries, R. (1996b). A highly polymorphic bovine dinucleotide repeat D19S4 (IOBT 34) at chromosome 19q21. Anim. Genet. 27, 58–59.

Olsaker, I., Williams, J.L., Chowdhary, B.P., Karlsson, L., Urquhart, B.G.D., Prakash, B. (1996c). Physical and genetic mapping of a bovine dinucleotide repeat marker D23S38 (IOBT1479) to chromosome 23q24-25. Anim. Genet., in press.

Olson, M., Hood, L., Cantor, C., Botstein, D. (1989). A common language for physical mapping of the human genome. Science 245, 1434–1435.

Popescu, C.P., Long, S., Riggs, P., Womack, J.E., Schmutz, S., Fries, R. (1996). Standardization of cattle karyotype nomenclature. Report of the committee for the standardization of the cattle karyotype. Cytogenet. Cell Genet., in press.

Solinas Toldo, S., Fries, R., Steffen, P., Neibergs, N.H., Barendse, W., Womack, J.E., Hetzel, D.J.S. (1993). Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. Mamm. Genome 4, 720–727.

Thieven, U., Harlizius, B., Simon, D. (1995). Dinucleotide repeat polymorphism at the bovine HAUT1 and HAUT14 loci. Anim. Genet. 26, 123.

Thieven, U., Solinas Toldo, S., Fries, R., Barendse, W., Simon, D., Harlizius, B. (1996). Polymorphic CA-microsatellites for the integration of the bovine genetic and physical map. Mamm. Genome, in press.

Vaiman, D., Bahri-Darwich, I., Mercier, D., Yerle, M., Eggen, A., Leveziel, H., Guérin, G., Gellin, J., Cribiu, E.P. (1993). Mapping of new bovine microsatellites on cattle chromosome 15 with somatic cell hybrids, linkage analysis, and fluorescence in situ hybridization. Mamm. Genome 4, 676–679.

Vaiman, D., Mercier, D., A., E., Bahri-Darwich, I., Grosh, C., Cribiu, E.P., Dolf, G., Oustry, A., Guérin, G., Levéziel, H. (1994). A genetic and physical map of bovine chromosome 11. Mamm. Genome 5, 553–556.

Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morissette, J., Millasseau, P., Vaysseix, G., Lathrop, M. (1992). A second-generation linkage map of the human genome. Nature 359, 794–801.

Williams, J.L., Morgan, A.L.G., Guérin, G., Urquhart, B.G.D. (1996a). Ten cosmid-derived bovine microsatellite markers. Anim. Genet. 27, 380.

Williams, J.L., Olsaker, I., Teres, V.M. (1996b). Using SSCP to facilitate mapping microsatellite loci. Mamm. Genome 8, in press.

Williams, J.L., Urquhart, B.G., Castiglioni, B., Ferretti, L. (1996c). Mapping two microsatellite loci, IDVGA66 (D16S34) and IDVGA76 (D14S35), by SSCP. Anim. Genet., in press.

Womack, J.E., Moll, Y.D. (1986). Gene map of the cow: conservation of linkage with mouse and man. J. Hered. 77, 2–7.

Zhang, Y., Redaelli, L., Castiglioni, B., Mezzelani, A., Ferretti, L. (1995). Five polymorphic bovine microsatellite loci, IDVGA-62A, IDVGA-71, IDVGA-82, IDVGA-88, IDVGA-90. Anim. Genet. 26, 365–366.