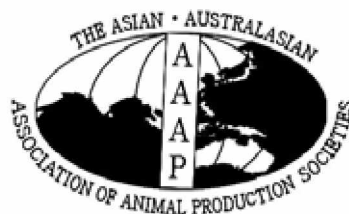

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Chromosomal Localization of Korean Cattle (Hanwoo) BAC Clones via BAC end Sequence Analysis

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ABSTRACT : In this study, a Korean native cattle strain (Hanwoo) evidencing high performance in terms of both meat quality and quantity was employed in the generation of 150,000 BAC clones with an average insert size of 140 kb, and corresponding to about a 6X coverage of bovine chromosomal DNA. The BAC clones were pooled in a mini-scale via three rounds of a pooling protocol, and the efficiency of this pooling protocol was evaluated by testing the accuracy of accessibility to the positive clones, via a PCR-based screening method. Two sets of primers designed from each of two known genes were tested, and each yielded 2 or 3 positive clones for each gene, thereby indicating that the BAC library pooling system was appropriate with regard to the accession of the target BAC clones. Analyses of 3.3×10^6 base pairs obtained from the 7,090 BAC end sequence (BES) showed that 34.88% of the DNA sequence harbored the repetition sequence. Analysis of the 7,090 BES to the 1st and 2nd generation radiation hybrid map of the cattle genome, using the COMPASS program designed for the construction of a cattle-human comparative mapping, resulted in the localization of a total of 1,374 clones proximal to 339 1st generation markers, and 1,721 clones proximal to 664 2nd generation markers. Collectively, the BAC library and pooling system of the BAC clones from the Korean cattle, coupled with the chromosome-localized BAC clones, will provide us with novel tools for the excavation of desired clones for genome mapping and sequencing, and will also furnish us with additional information regarding breed differences in cattle. (**Key Words :** Bovine BAC Library, Pooling System, BAC End Sequence, Comparative Mapping, Chromosomal Localization)

INTRODUCTION

The Hanwoo native Korean cattle breed, which has its origin in a hybrid of *Bos taurus* and *Bos zebu*, is an indigenous breed that has been localized within the Korean peninsula since 4000 BC (Han, 1996). This breed has adapted to the local environment, and is believed to have diverged genetically from other breeds (Mason, 1984). In-depth genetic characterizations of the Hanwoo breed, then, should enable us to determine the DNA sequences unique to Korean cattle, many of which may prove to be economically important with regard to the cattle industry.

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A BAC (bacterial artificial chromosome) library contains a wealth of genetic information, and the sequencing of DNA inserts results in random DNA sequences which can be employed in the generation of comparative physical maps. In the recent past, BAC has become a preferable method to the use of the YAC (yeast artificial chromosome) vector as a means for the cloning of large fragments of genomic DNA (Song et al., 2001). This is because BAC libraries are propagated in *E. coli*, which allows for the ready isolation and manipulation of DNA inserts. Also, BAC libraries are characterized by very low levels of chimerism (Ioannou et al., 1996). In addition, BAC clones have large insert sizes, are known to be stable, grow quickly, and can be readily screened via either hybridization or PCR, which renders them a favorable alternative for both physical mapping and high-quality sequencing (Shizuya et al., 1992; Kim et al., 1996).

The initiative to construct cattle-human or cattle-mouse comparative radiation hybrid maps had its origins in the 1980s with the construction of a cattle, human, and mouse radiation hybrid map (Womack et al., 1997). Information

made available by the Human Genome Project has also contributed significantly to the construction of bovine genome maps, particularly for cattle. These advances have resulted in the construction of several cattle-human whole genome RH comparative maps, including one with a total of 1,913 markers (Everts-van der Wind et al., 2004) and the other with 3,802 bovine microsatellite markers (Ihara et al., 2004). The first BAC clone-based cattle and human comparative map was completed in 2003, and achieved a 32% coverage of the cattle genome (Larkin et al., 2003). The development of high-density genetic maps of cattle carries the added advantage of the possibility of localizing ETL (Economic Trait Loci) and QTL (Quantitative Trait Loci) on these maps, which makes possible marker-assisted selective breeding for the improvement of cattle breeds (Kim et al., 2004; Choi et al., 2006). These maps also augment our understanding of mammalian evolution, via analyses of the conserved genomes prevailing in mammalian chromosomes (Murphy et al., 2001; Everts-van der Wind et al., 2004).

In order to further analyze a constructed BAC library, a variety of methods are available for the accession or screening of the library for the desired clones. One common method involves the pooling of the entire BAC library. In this study, the library was pooled into three tiers (primary, secondary, and tertiary) in an effort to augment the efficiency of finding the desired clones. PCR was employed in the screening of the pooled library, as it has proven to be simple, rapid, cost effective, and accurate (Green and Olson 1990; Kurnit 1995; Munroe et al., 1995). It also facilitates the screening of multiple genes with appropriate primers in the same PCR procedure (Henegariu et al., 1997; Regan et al., 2000).

Thus, we have compiled a bovine synteny map via the construction of a large insert genomic DNA BAC library of Hanwoo Korean cattle, and subjecting it to genomic analysis. A total of 1.5×10^5 BAC clones of Korean cattle were constructed with an average insert size of 140 kb. Further efforts were then focused on the localization of the found DNA sequences, via *in silico* homologous comparative mapping. The results will provide us with a greater understanding of the localization of specific DNA sequences on the Korean cattle chromosomes, and allow us to conduct comparisons of genetic diversity with other cattle breeds, in order to delineate the economically relevant traits in this breed.

MATERIALS AND METHODS

Library construction

A blood sample from a Korean native Hanwoo steer, evidencing high meat quality and weight, was collected in order to construct the BAC library, in accordance with the previously published protocol (Cai et al., 1995; Osoegawa et al., 1998), with some slight modifications. The blood samples (100 ml) were homogenized in buffer (40 mM sodium citrate and 1% Triton X-100) on ice and centrifuged for 5 minutes at 500×g at 4°C. After the elimination of the supernatant, the pellets were washed twice in washing buffer (0.1 M Tris base, 0.8 M KCl, 0.1 M EDTA, 10 mM spermidine, 10 mM spermine, pH 9.5) and subsequently resuspended in PBS. Agarose plugs were constructed by mixing the cell suspension with an equal volume of 1% InCert agarose (Cambrex, Rockland, ME, USA), followed by melting in PBS and rapid transfer into 10×5×1.5 mm disposable DNA plug molds (Biorad, Hercules, CA, USA), which were then placed on ice for 60 minutes to solidify. The DNA plugs were extruded from the molds and directly placed into 50 ml tubes containing 50 ml proteinase K lysis buffer composed of 0.5 M EDTA, 1% SDS, and 0.1 mg/ml protease K (Roche, Mannheim, Germany), followed by 24 h of incubation at 50°C in a water bath with gentle swirling and periodic replacement with fresh buffer. After the proteinase K treatment, the plugs were rinsed several times with deionized water and stored in 0.5 M EDTA at 4°C until use.

DNA partially digested with either *Eco*RI or *Hind*III was inserted into two different vectors; BACe3.6 and pIndigoBAC-5. Size fractionation was conducted via pulsed field gel electrophoresis (CHEF DR-III, Biorad, Hercules, CA, USA) at 12°C for 16 h at 5.0 V/cm, with an initial switch time of 1 second and a final 12 seconds of pulse time at a 120°C angle in 0.5×TBE buffer. The agarose gel fragments, which harbored 150-250 kb of digested chromosomal DNA fragments, were sliced using a razor, and then washed in electrophoresis buffer. One microliter of 50×GELase (Epicentre, Madison, WI, USA) buffer was then added to every 50mg of the sliced-agarose, and incubated for 3 minutes at 70°C, followed by 10 minutes of additional incubation at 45°C, then digested for 45 minutes with 1 Unit of GELase enzyme per 200 μl of sample. The final DNA concentration was regulated to 10 ng/μl. Approximately 200 ng of size-selected DNA was ligated into the dephosphorylated vector DNA using 5 Units of T4 ligase (Fermentas, Hanover, MD, USA) in a total volume of 50 μl (2 M Tris-Cl, pH 7.6, 50 mM $MgCl_2$, 5 mM ATP, 5 mM dithiothreitol, 25% (w/v) polyethylene glycol 8,000) at 16°C overnight. The enzyme reaction was halted via 30 minutes of incubation at 65°C, followed by storage at 4°C.

The ligated samples were then transformed into bacterial competent cells. 2,000-7,000 colonies per plate were plated on rectangular plates along with 100 μl of host cells. The transformed cells were spread onto 2×LB plates containing 5% sucrose and chloramphenicol, and allowed to grow overnight at 37°C. White colonies were randomly

manually picked and transferred to 96-deep well plates containing 1 ml 2×LB with 12.5 μg/ml chloramphenicol, then incubated for 20 h at 37°C with shaking at 220 rpm. Three copies of the mixed glycerol solution (65% glycerin, 0.1 M $MgSO_4$, 0.025 M Tris-HCl, pH 8.0) (10 μl), combined with 40 μl of bacterial culture products, were prepared and stored at -80°C.

BAC library pooling and screening

The entire BAC library was pooled in accordance with the UWGC BAC pooling protocol (Channakhone 1998; Cai et al., 2001) with some slight modifications. Three levels of BAC DNA pools (primary, secondary, and tertiary) were prepared using these stocks. During primary pooling, a total of 10 plates of 384 wells each were pooled by taking 2 μl of BAC clone glycerol stock from each of the 384 microtiter wells, and mixing them within a reservoir. These mixtures were cultured overnight in 2×LB broth containing chloramphenicol (12.5 μg/ml) in 50 ml tubes in a shaking incubator at 300 rpm. 100 μl of culture was then aliquoted into each well of the 96-well plates and 100 μl of 60% glycerol was added, such that each well of the primary pool harbored 3,840 clones. For secondary pooling, 2 μl samples from each of the wells from a single 384-microtiter plate were pooled into one well of a 96-well plate, such that each plate consisted of 384 different clones per well. In the tertiary pooling, a two-dimensional (vertical and horizontal) pooling system was applied to each plate. The vertical pools were collected from the columns of the same ten 384-microtiter plates that had been used for the primary screening. 5 μl samples from the 16 individual wells (columns) of the 10 plates were mixed in a 12-channel reservoir. Likewise, for horizontal pooling, 5 μl samples from 24 individual wells (rows) of the 10 plates were mixed in an 8-channel reservoir. Therefore, each horizontal and vertical well contained 240 and 160 clones, respectively. Each mixture of the clones obtained in the secondary and tertiary pooling was cultured and aliquoted into a well of a 96-well plate, similarly to the primary pooling procedure.

The BAC clones were selected from the pooled library and inoculated into 1 ml of 2×LB medium containing chloramphenicol (12.5 μg/ml) in a deep 96-well plate, then incubated overnight at 37°C with agitation at 450 rpm (HT-MegaGrow™ Shaking Incubator, Bioneer, Daejeon, Korea). The PCR reaction was conducted in a MyGenie 96 gradient thermal cycler (Bioneer, Daejeon, Korea) in 20 μl volumes (all PCR reagents were purchased from Bioneer Co.) containing 0.6 μl of dNTP mix (each 2.5 mM), 0.8 μl of each primer (10 pmole), 2 μl of 10× reaction buffer (100 mM Tris-HCl, 400 mM KCl, 15 mM $MgCl_2$ at pH 9.0), 1 μl of DNA template of the boiled BAC clone pellet in TE buffer, 14.7 μl of deionized water, and 0.1 μl of Taq DNA polymerase (5 Unit/μl). PCR reactions were conducted for the four different sets of primers under two different conditions. For the X1 and X2 primer sets, the denaturation step was conducted at 94°C for 3 minutes, followed by 35 cycles of 94°C at 30 seconds, 51°C for 30 seconds (primer annealing) and 72°C for 30 seconds (primer extension). Finally, the reaction was terminated after a 5-minute final extension at 72°C. For the Y1 and Y2 primer sets, the denaturation step was conducted at 94°C for 2 minutes, followed by 35 cycles at 94°C for 30 seconds, at 65°C for 30 seconds (primer annealing), and at 72°C for 30 seconds (primer extension). Finally, the reaction was terminated after a 10-minute final extension at 72°C. In order to visualize the amplified products, 12 μl of PCR products were employed for gel electrophoresis in 2% agarose. Primer sets X1 and X2 were designed from different exons of the gene related to mad cow disease in cattle, and primer sets Y1 and Y2 were designed from the bovine AMP-activated protein kinase gene. The primer sequences used were as follows:

Primer X1: 5′-gcaaccgttatccacctcag-3′ (forward) and
5′-tggcttactgggtttgttcc-3′ (reverse),
Primer X2: 5′-gctaaggacaacacggaag-3′and
5′-ttccattccctggaagattg-3′
with product sizes of 203 bp and 239 bp, respectively.
PrimerY1: 5′-acaacagaacgagacagcct-3′ (forward) and
5-′tcgaagatgaccagctgga-3′ (reverse),
PrimerY2: 5′-agatcttggatggtgcggta-3′ (forward) and
5′-ggcttctcaagttcctgcac-3′ (reverse)
with product sizes of 200 bp and 260 bp, respectively.

DNA sequencing

The BAC DNA was isolated with the Montage BAC_{96} Miniprep kit (Millipore, Billerica, MA, USA). Each BAC DNA was amplified in an 11 μl total reaction volume, including 1 μl of BigDye ready reaction mix containing AmpliTaq DNA polymerase (Applied Biosystems, Foster, CA, USA) and 4 picomoles of each forward and reverse primer to 500 ng/μl of BAC DNA. The universal sequences of the primers employed for PCR were as follows: T7 primer (5'-TAATACGACTCACTATAGGG-3') and modified RP2 primer (5'-TACGCCAAGCTATTTAGGTGAGA-3'), with 20 and 23 base pairs, respectively. The reactions were conducted in a thermocycler (MJ research, Waltham, MA, USA) under the following conditions: 95°C for 1 minute and 35 cycles of 95°C for 30 sec, 50°C for 10 sec, and 60°C for 4 minutes. For DNA sequencing, an automated DNA sequencer (ABI 3700) was employed. Each BAC clone of the raw sequence data was analyzed using AB1 data collection software. Sequence chromatograms were processed automatically using sequencing analysis software (Sequencing Analysis, ver. 3.7, Applied Biosystems), and

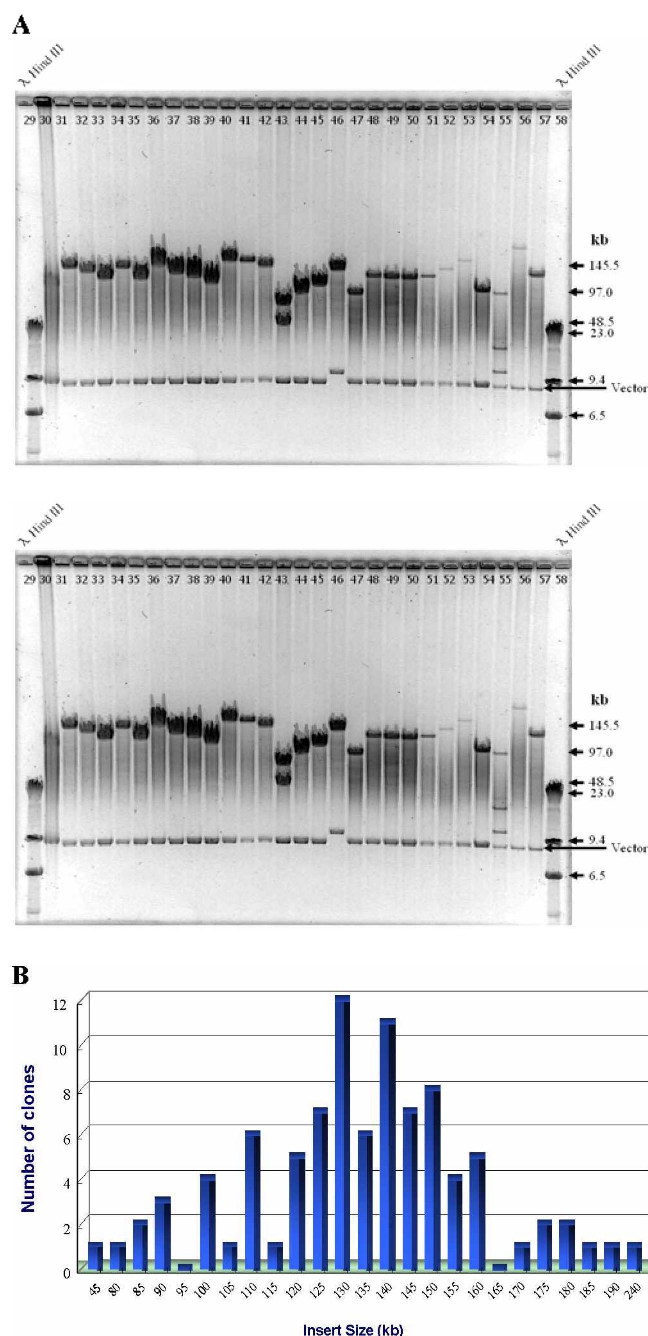

Figure 1. Analysis of the BAC clones. A. The *Not*I-digested DNA was separated via pulsed field gel electrophoresis using DNA markers loaded on both sides. The sizes of the markers are indicated with arrows. Lanes 1 and 28: indicate Lambda ladder (Biorad, CA), Lanes 2, 27, 29 and 58 indicate: Lambda DNA *Hind*III digest (DCC-Bionet, Korea), Lane 3-26 and 30-57 indicate different insert sizes of BAC clones. B. Distribution of insert size in the Hanwoo BAC library. To estimate the insert size range, BAC DNA from 100 randomly chosen clones was analyzed, as is shown in Figure 2A. The results showed that the average insert size was 140 kb, with more than 80% of the clones having insert sizes in the range of 120 kb.

the nonredundant end sequences with a base-call error rate of ≥1% were trimmed (phred quality value ≥20) using the Phred program (Ewing and Green, 1998; version 0.020425.c, Washington University). The DNA sequences of the BAC ends described in this paper were deposited in the GenBank database, and assigned the accession numbers CG839387 through CG844908.

Comparative mapping

DNA sequences of the BAC end sequences were analyzed with the help of a repeat masking program, in order to identify the repeat sequences (RepeatMasker, Smit, 1996). The flanking nucleotide sequence of each clone, after the masking of the repeat sequences, was analyzed using BLASTN and COMPASS (Comparative Mapping by Annotation and Sequence Similarity) software in order to localize the position of each clone on the bovine chromosome (Band et al., 2000).

RESULTS AND DISCUSSION

In this study, a BAC library containing a total of 150,000 clones was constructed from a Korean native steer (Hanwoo) evidencing high performance in terms of both meat quality and quantity. As some regions of the bovine genome are rarely cleaved by one enzyme or are too frequently cut, two different restriction enzymes (*Eco*RI and *Hind*III) were utilized to provide increased coverage of the genome represented in the digested fragment pool. The fragmented genomic DNA was then cloned into BACe3.6 or pIndigoBAC-5 vectors harboring the *Eco*RI restriction digestion site and the *Hind*III restriction digestion site, respectively. Approximately 100,000 clones and 50,000 clones were obtained using the pIndigoBAC-5 vector and BACe3.6 vector, respectively. DNA isolated from 100 randomly selected BAC clones were then digested with the *Not*I restriction enzyme, and separated via pulsed field gel electrophoresis (Figure 1A). This experiment revealed that the average insert size of the BAC clones was 140 kb (Figure 1B). Upon further analysis with other additional clones, less than 2% of the clones were considered to be empty. Assuming that the bovine genome harbors 3×10^9 bp (Eggen et al., 2001), the total constructed library therefore corresponds to six genome-equivalents. The BAC clones are stored in triplicate as glycerol stock in 384-well plates at -80°C.

In order for this library to be useful, it is necessary to be able to access the desired clones easily. Unfortunately, the acquisition of a desired clone among the hundreds of thousands of clones within a BAC library tends to be both difficult and time-consuming. It is, therefore, exciting and useful to develop a technique that can help a researcher to pinpoint the exact location of a desired clone in an efficient and time-saving manner. The first step in this process was the pooling of the constructed Hanwoo BAC library, in an

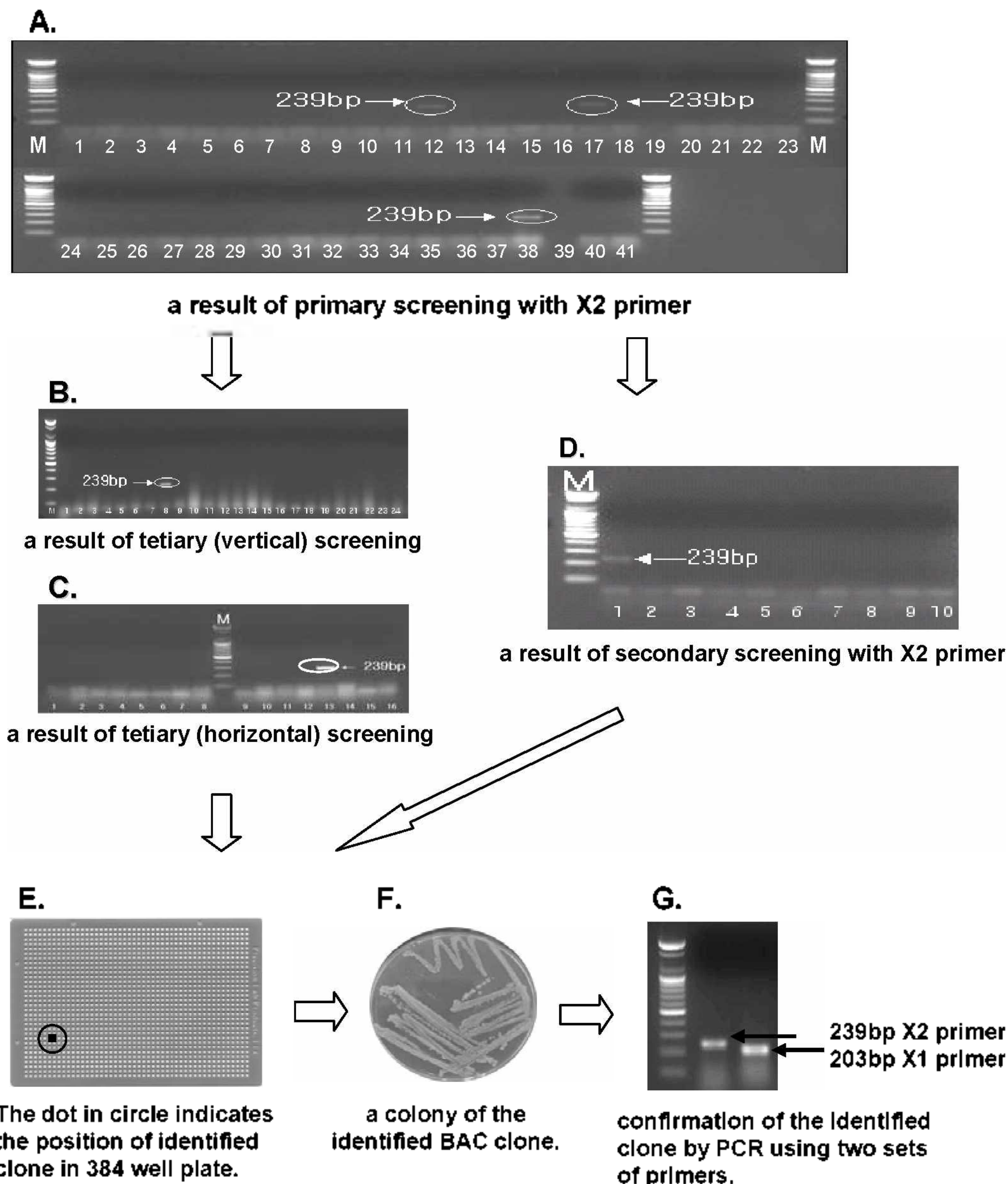

Figure 2. A procedure for PCR-screening of the BAC library. Primers (X2) designed from the bovine gene known to be involved in MCD (mad cow disease) were used here for PCR screening. A. The result of primary screening showing three positive bands for MCD gene (239 bp in size). The numbers 1 to 41 represent the number of primary pooled-wells, each of which harbors 3,840 clones. B and C. Represent tertiary (vertical and horizontal) screening. Numbers 1-24 (B) and 1-16 (C) indicate the pooled-clones from 10 columns, and the rows of the same ten 384-microtiter plates used for the primary pooling of lane #12. D. A result of the secondary screening of the first positive well (#12 in A) identified in the primary screening. Numbers 1-10 refer to the number of secondary pooled-wells, each of which harbored 384 clones. G. Confirmation of the identified clone via PCR using two sets of primers. M in each gel picture represents the 100 bp DNA markers. The circled bands indicate the expected size of the PCR amplification product with X2 primer.

effort to simplify the process by which a specific clone could be identified. Normally, the pooling of a large-insert library results in large volumes of cultured material being handled (Shan et al., 2004). By way of contrast, in this study we devised and employed a modified pooling technique, which reduces the amount of sample to be cultured, the total amount of culture media to be used, and the time required for the preparation of the pooled library, thus ultimately resulting in lower overall experimental costs. In addition, the cultured clones were pooled into three tiers: primary, secondary and tertiary pools, with each clone being represented in each pooling tier. This significantly simplified the screening process (see the materials and methods section for details). The pooled library reduced the total number of PCR reactions required for successful screening, from 410 384-well plates to 25 96-well plates, and the arrangement of the library was systematic, such that it became quite simple to track the source of each clone.

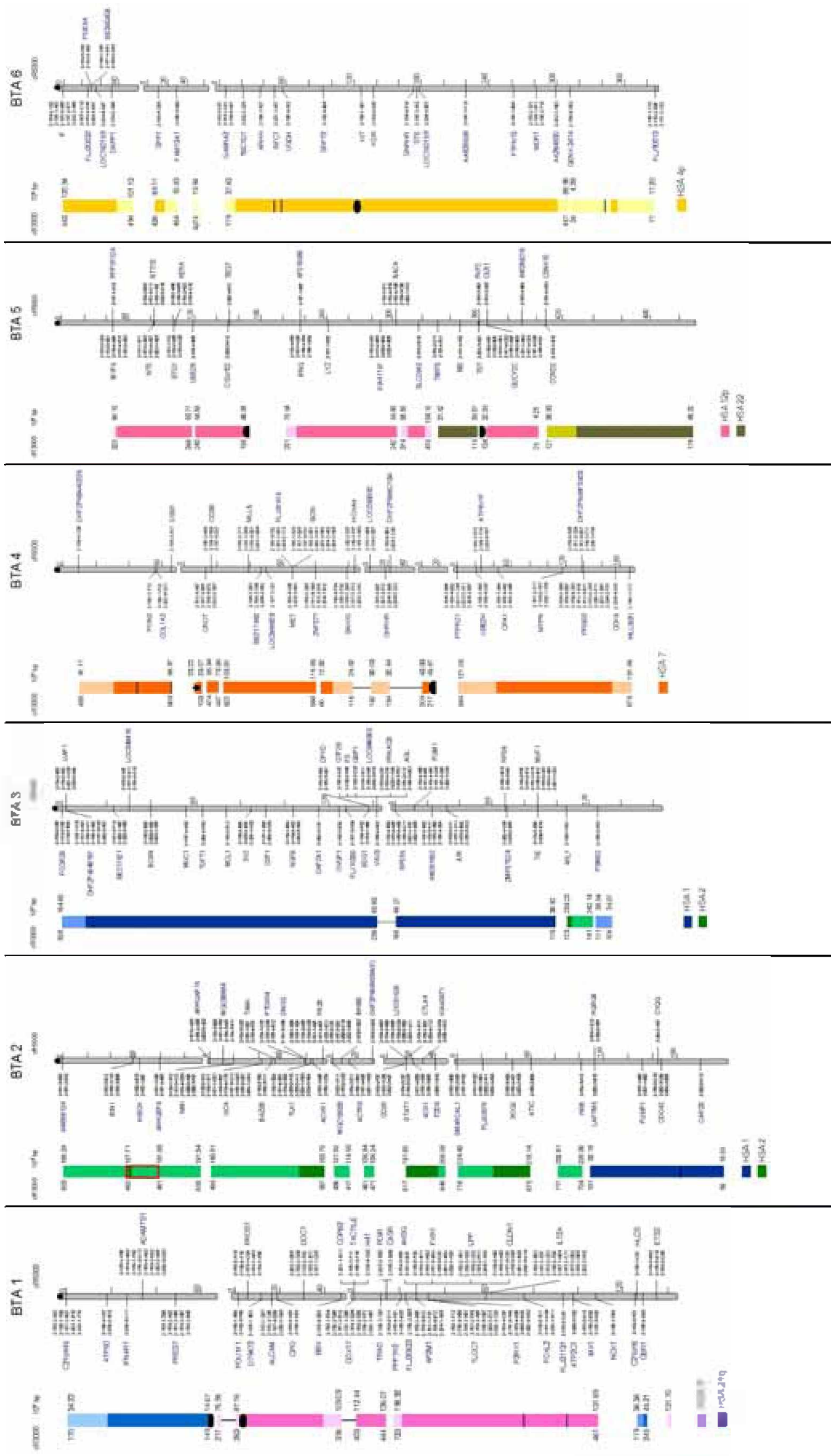

Figure 3. The 2nd generation comparative physical map of the bovine BAC clones. Highlighted markers are marked as previously identified.

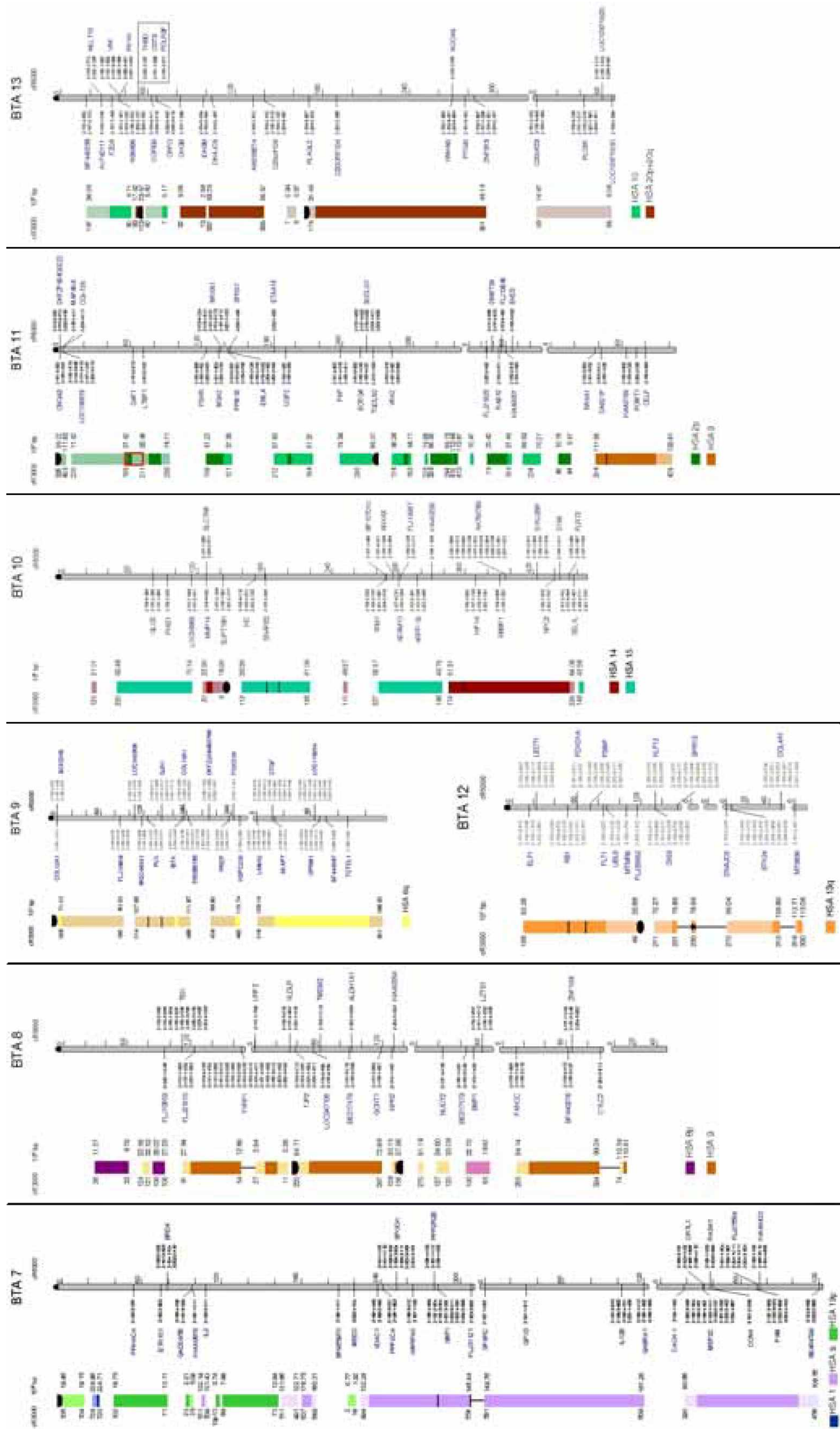

Figure 3. The 2nd generation comparative physical map of the bovine BAC clones. Highlighted markers are marked as previously identified (Continued).

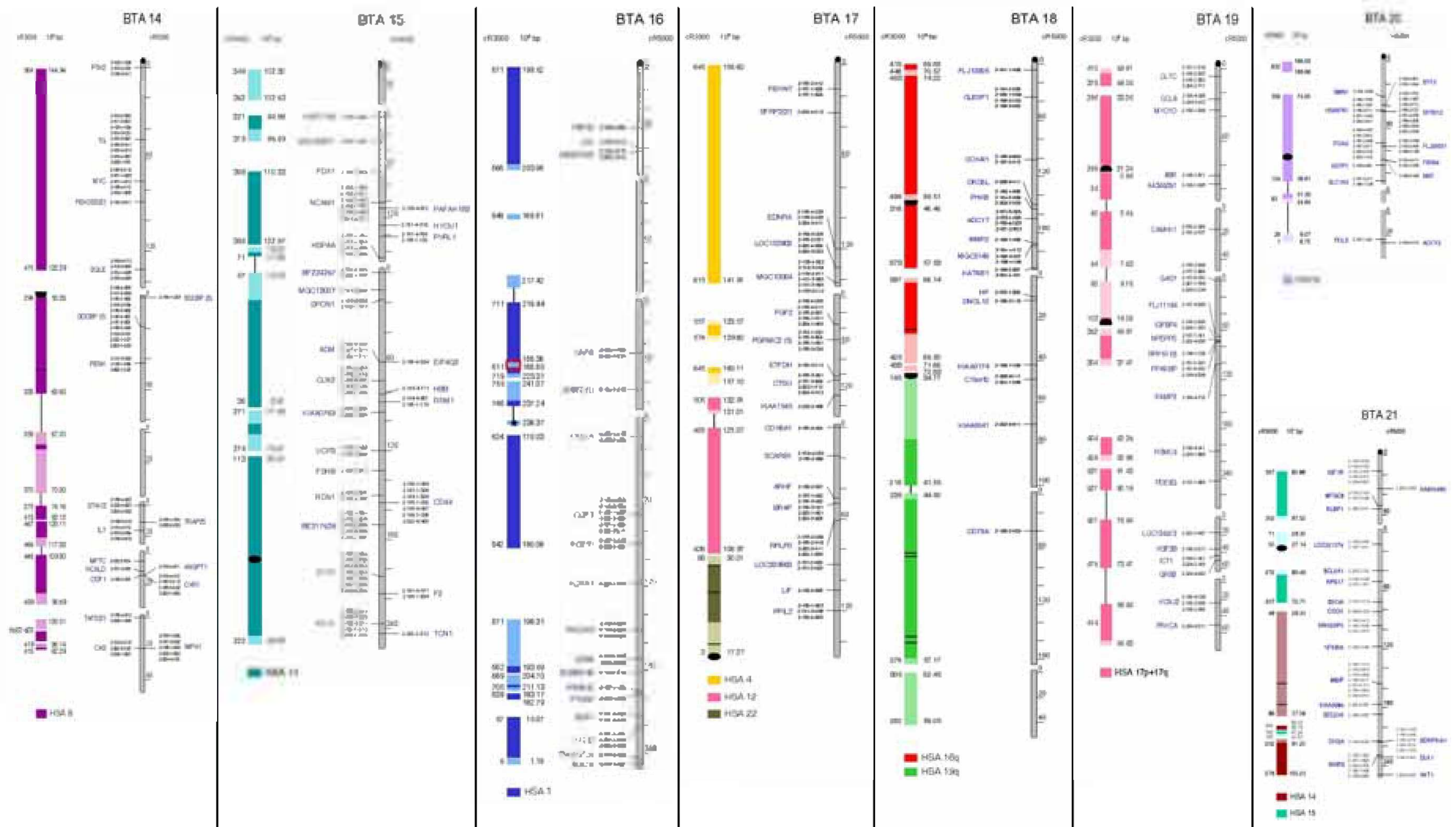

Figure 3. The 2nd generation comparative physical map of the bovine BAC clones. Highlighted markers are marked as previously identified (Continued).

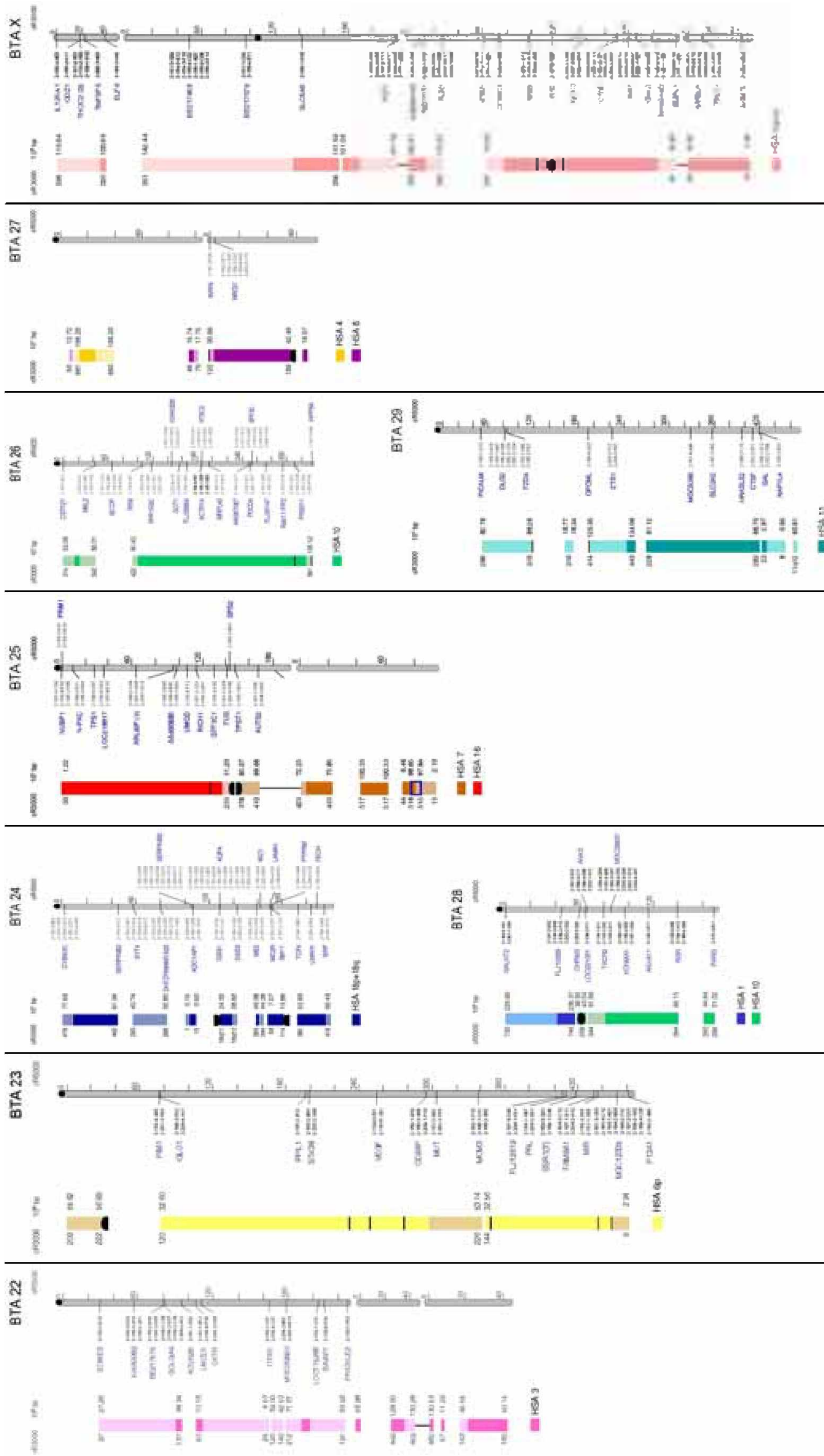

Figure 3. The 2nd generation comparative physical map of the bovine BAC clones. Highlighted markers are marked as previously identified (Continued).

In order to estimate the quality of the BAC library and pooling system, the pooled BAC clones were screened via PCR. Prior to the PCR screening of the library, primer sets X1 and X2 and primer sets Y1 and Y2, both specific for the bovine gene believed to predispose cattle to mad cow disease (MCD) and the bovine AMP-activated protein kinase (AMPK) gene, respectively, were designed (Corton et al., 1994; Weissmann et al., 1998). These primers were then employed in the screening of the clones using PCR, and resulted in three positive clones for primer set X2 from the primary pooled plates being identified (Figure 2). Of these three clones, only two were also amplified by primer set X1. Similarly, amplification by primer sets Y1 and Y2 resulted in the identification of three positive clones from the primary pooled plates (results not shown). The results obtained with the primary pools indicated which of the secondary and tertiary pooled plates should be subjected to further PCR screening, and a combination of the results from these two pooling tiers allowed us to identify the actual wells from the original BAC library harboring the desired clones (Figures 2B, C and D). In order to verify that we had the correct well address, a colony from the identified BAC library well was cultured in a petri dish (Figures 2E and F), and a PCR reaction was conducted using a single colony from the petri dish as a template. Two differently-sized primer sets for each of the genes were then employed in order to confirm that the positive clones in the library actually harbored the desired gene sequences (Figure 2G). The sequencing of the DNA inserts from the identified clones further verified that they were the desired clones. This resulted in the six identified clones being confirmed as actually harboring the genes of interest. It is, therefore, confirmed that the pooling strategy employed in this study, when coupled with a PCR-based system, enables a high-throughput screening of large numbers of BAC clones in a time-efficient and cost-efficient manner, thus circumventing the disadvantages inherent to other screening processes, which tend to be laborious and time-consuming.

A total of 4,296 BAC clones were sequenced using two universal primers (T7 and RP2) and 3,299,298 base pairs

Table 1. Comparison of 1st and 2nd generation map

1st generation map				Chr. No.	2nd generation map			
Pair hit	Single hit	Total	Maker		Pair hit	Single hit	Total	Marker
50	52	102	15	1	61	88	149	45
20	29	49	14	2	44	64	108	37
46	38	84	21	3	35	56	91	36
42	36	78	12	4	31	53	84	27
42	48	90	20	5	26	41	67	26
35	35	70	8	6	21	29	50	29
29	32	61	16	7	36	47	83	29
33	26	59	12	8	28	34	62	22
14	12	26	6	9	29	47	76	24
24	25	49	19	10	22	41	63	24
43	41	84	16	11	24	46	70	33
34	33	67	7	12	28	50	78	17
32	30	62	16	13	28	44	72	31
11	16	27	13	14	18	46	64	19
21	34	55	13	15	21	48	69	27
19	14	33	14	16	13	20	33	18
13	22	35	11	17	15	34	49	18
7	11	18	8	18	6	22	28	16
18	20	38	17	19	18	24	42	23
17	14	31	6	20	11	27	38	13
6	17	23	11	21	16	27	43	19
3	2	5	4	22	9	14	23	13
13	21	34	8	23	12	24	36	16
24	28	52	9	24	20	37	57	18
8	12	20	7	25	9	16	25	14
14	20	34	12	26	15	30	45	18
2	4	6	3	27	4	7	11	4
6	12	18	8	28	9	21	30	11
2	1	3	3	29	5	12	17	12
21	40	61	10	X	15	43	58	25
649	725	1,374	339	Total	629	1,092	1,721	664

The table compares initial COMPASS results of bovine BES on the 30 bovine chromosomes - 1st Generation maps with the results obtained after mapping of the same BES on a new version of COMPASS (2nd generation map).

(3.3 Mega bp approximately) of the Hanwoo genome length sequence. Of the 3.3 Mega bp, 1,150,852 bp (34.88%) have been determined to harbor repeated sequences, consisting of 8.52% SINEs, 19.8% LINEs, and 1.41% LTR elements. In excess of 60% of the Hanwoo BAC end sequences are therefore completely free of repetitive DNA, and can be utilized as a source of new STS (sequence tagged site) markers, as the information gleaned from analyses of human BAC end sequences has revealed that BAC end sequences without repetitive DNA are useful in the detection of new STS (Zhang et al., 1999).

Each Hanwoo BAC end sequence was analyzed using the Repeatmasker program. A mini-database from the unmasked versions of all sequences matched with BLAST (Basic Local Alignment Search Tool) software was constructed via query search in BLASTN against the repeat-masked nucleotide sequence database. Initially, the same repeat-masked DNA sequences were analyzed with COMPASS (Comparative Mapping by Annotation and Sequence Similarity, Band et al., 2000) software, which localized the positions of 1,374 BAC clones within the 30 bovine chromosomes, and located them in terms of their proximities to previously known genetic markers. The same repeat masked DNAs were then analyzed using the 2nd generation COMPASS software, which localized 1,721 BAC clones within the 30 bovine chromosomes (Figure 3). In this 2nd analysis and mapping, 25 chromosomes evidenced a higher number of BESs hits on the 2nd generation map than were seen in the 1st generation map. The number of BESs with double hits on different chromosomes was also reduced by 259, from 581 in the 1st map to 359 in the 2nd map. However, 612 clones maintained their position on both the 1st and 2nd generation maps, and are therefore likely to be very accurately positioned on the chromosomes. Apart from these anomalies, it is clear that the 2nd generation map is more detailed and accurate than the 1st generation map. The 2nd generation map shows 325 more markers and 347 more BAC clones than the 1st generation map. This is essentially consonant with previous findings, which revealed an increase of 826 markers in a 2nd generation bovine chromosomal map as compared to a 1st generation map (Band et al., 2000; Everts-van der Wind et al., 2004). A recent study indicated that the bovine chromosomes, as assembled in the 2nd generation RH map, were longer than those in the 1st generation RH map (Everts-van der Wind et al., 2004). This strongly suggests that the 2nd generation map has a larger surface area for the BESs to be able to find a hit match than does the 1st generation map, which results in higher BESs hit numbers. For the same reason, this allows for the localization of more markers on the chromosomes, and the presence of more markers results in a more precise localization of the BAC clones. The application of the COMPASS method for the localization and mapping of the DNA sequences generated in this study resulted in the construction of a more detailed syntenic map of the Korean cattle genome assembled from random DNA sequences, and also permitted us to compare the accuracy of the 1st and 2nd generations of the COMPASS software.

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