Available online at www.sciencedirect.com

SCIENCE DIRECT

Genomics 87 (2006) 772–776

GENOMICS

www.elsevier.com/locate/ygeno

A human–horse comparative map based on equine BAC end sequences ☆

Tosso Leeb [a,b,*], Claus Vogl [c], Baoli Zhu [d], Pieter J. de Jong [d], Matthew M. Binns [e], Bhanu P. Chowdhary [f], Maren Scharfe [g], Michael Jarek [g], Gabriele Nordsiek [g], Frank Schrader [g], Helmut Blöcker [g]

[a] *Institute of Animal Breeding and Genetics, University of Veterinary Medicine Hannover, Bünteweg 17p, 30559 Hannover, Germany*
[b] *Institute of Genetics, Vetsuisse Faculty, University of Berne, 3012 Berne, Switzerland*
[c] *Institute of Animal Breeding and Genetics, The Vienna University of Veterinary Medicine, 1210 Vienna, Austria*
[d] *Children's Hospital Oakland, Oakland, CA 94609, USA*
[e] *Veterinary Basic Sciences, The Royal Veterinary College, Royal College Street, London NW1 0TU, UK*
[f] *Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843, USA*
[g] *German Research Centre for Biotechnology, Mascheroder Weg 1, 38124 Braunschweig, Germany*

Received 30 August 2005; accepted 4 March 2006
Available online 17 April 2006

Abstract

In an effort to increase the density of sequence-based markers for the horse genome we generated 9473 BAC end sequences (BESs) from the CHORI-241 BAC library with an average read length of 677 bp. BLASTN searches with the BESs revealed 4036 meaningful hits ($E \leq 10^{-5}$) in the human genome that provide useful markers for the human–horse comparative map. The 4036 BLASTN hits allowed the anchoring of 3079 BAC clones to the human genome, on average one corresponding equine BAC clone per megabase of human DNA. We used the BLASTN anchored BESs for an in silico prediction of the gene content and chromosome assignment of comparatively mapped equine BAC clones. As a first verification of our in silico mapping strategy we placed 19 equine BESs with matches to HSA6 onto the RH map. All markers were assigned to the predicted localizations on ECA10, ECA20, and ECA31, respectively.

Keywords: Horse; Comparative map; Physical map; RH map; BES; BLASTN; Database

Comparative mapping is a powerful tool to transfer the wealth of information from the sequenced genomes of human, mouse, and other sequenced organisms to mammalian genomes for which whole genome sequence data are not yet available. Initial important resources for human–horse comparative mapping included the first gross chromosome-segment-based comparative maps of the horse genome established by Zoo-FISH experiments [1]. This FISH-based comparative map was refined during subsequent years by the assignment of numerous equine genes. A further milestone in the development of more detailed human–horse comparative maps was the construction of the first-generation whole-genome radiation-hybrid (RH) map including 258 comparatively mapped genes [2]. The initial RH map of the horse genome has been rapidly expanded and high-resolution comparative maps of the horse chromosomes (ECA) 7, 10p, 15, 17, 18, 21, 22, and X are now available [3–6]. These high-resolution comparative maps are based on RH-mapped genes that have an average distance of about 1 Mb in the human genome.

For the further enhancement of the equine genome map it would be highly desirable to have clone-based physically ordered maps. Such maps are typically constructed with BAC clones and serve as an important prerequisite for the modified whole-genome sequencing strategy that is currently used for the bovine and porcine genomes (http://bovinegenome.org/; http://www.sanger.ac.uk/Projects/S_scrofa/). BAC end sequences

☆ Sequence data from this article have been deposited with the DDBJ/EMBL/GenBank Data Libraries. A list of accession numbers is available in the supplementary material.

* Corresponding author. Institute of Genetics, Vetsuisse Faculty, University of Berne, Bremgartenstrasse 109a, 3001 Berne, Switzerland. Fax: +41 31 6312640.
E-mail address: Tosso.Leeb@itz.unibe.ch (T. Leeb).

0888-7543/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygeno.2006.03.002

(BESs) can be used to anchor BAC clones to the human genome, which greatly facilitates the building of large clone contigs and detailed physical maps. Large-scale BES projects were reported in human, mouse, chimpanzee, and cattle [7–10]. Complementary to the generation of BES data, bioinformatics tools were developed that allow the efficient comparative in silico mapping of livestock sequence information with respect to the human genome [11,12].

Here, we report the generation and comparative analysis of the first equine BES data that will add to the expanding map of the horse genome. As a validation of the in silico comparative mapping process we experimentally confirmed the equine chromosome predictions of BESs with homology to HSA6 by RH mapping.

Results

BAC end sequencing

BESs were attempted from both ends (SP6 and T7) of 6144 BAC clones of the CHORI-241 BAC library (Table 1). We generated a total of 9473 equine BESs with an average read length of 677 bp. All sequence data totaling 6.4 Mb were submitted to the EMBL nucleotide database. The BESs contained 34.8% repetitive sequences, of which the majority (21.2%) belonged to the LINE family. Other important classes of repeats were LTRs (4.8%), SINEs (5.4%), and interspersed DNA elements (2.3%). The GC content of the equine BESs was 40.27%.

Comparative analysis of equine BESs with respect to the human genome

The repeat-masked equine BESs were subjected to a BLASTN search against build 35.1 of the human genome sequence (Table 2 and Supplementary Tables 1 and 2). For the BLASTN search a significance threshold of $E = 10^{-5}$ was used. BESs with hits of $E \leq 10^{-5}$ on more than one chromosome were filtered out. In the BLASTN analysis 4211 BESs showed significant hits. After filtering 175 BESs with multiple hits, the remaining 4036 unique and significant hits were termed "meaningful hits." The meaningful hits were used for the comparative analysis with respect to the human genome. A total of 3079 BAC clones could be anchored to the human genome (Table 3). On average one BAC clone per megabase was thus mapped to the human genome. The comparatively anchored BAC clones are fairly evenly distributed on all human chromosomes with the exception of chromosomes 19, 22, X, and Y, on which they are underrepresented.

Among the BLASTN hits were 895 pairs of matching BES read pairs, of which 833 matched within 325 kb on a single human chromosome. The average distance of these paired BLASTN hits in the human genome was 214 kb, the median

Table 1
Horse BAC end sequencing statistics

Sequencing results	
BAC clones attempted	6144
BESs [a]	9473
Read pairs	4171
Overall success rate [b]	77%
Average read length	677 bp
Repetitive sequences [c]	34.79%
GC content	40.27%
Total sequenced bases	6,413,735 bp

[a] Number of sequences, free of *Escherichia coli* DNA, with ≥200 bp after vector and low-quality data trimming.
[b] Success rate in relation to the number of total attempted clones. The main reasons for failure were no growth of the bacterial clones, nonrecombinant clones, and insufficient DNA quality.
[c] Repetitive sequences were detected by the RepeatMasker software.

Table 2
Comparative analyses of equine BESs with respect to the human genome

BLASTN results	
Query sequences	9473
BESs with BLASTN hits ($E \leq 10^{-5}$)	4211
BESs with meaningful BLASTN hits [a]	4036
BAC clones with at least one BES	5302 (100%)
BAC clones with one matching BES	2246 (42%)
BAC clones with two matching BESs [b]	895 (17%)
Comparatively mapped BACs total [c]	3079 (58%)

[a] BESs with multiple hits in the human genome were filtered out.
[b] 833 (93%) of these 902 hit pairs matched within 325 kb on one human chromosome in the correct (opposite) orientation.
[c] 62 clones with two matching BESs that did not match within 325 kb on one human chromosome were not comparatively mapped.

Table 3
Distribution of comparatively anchored BAC clones

HSA	Chromosome size (Mb)	No. of hits	Average distance (Mb)
1	245	209	1.2
2	243	288	0.8
3	201	217	0.9
4	191	209	0.9
5	181	230	0.8
6	171	192	0.9
7	159	183	0.9
8	146	172	0.8
9	138	149	0.9
10	135	156	0.9
11	135	149	0.9
12	133	132	1.0
13	114	119	1.0
14	106	112	0.9
15	100	97	1.0
16	89	80	1.1
17	81	78	1.0
18	76	80	1.0
19	65	38	1.7
20	62	54	1.1
21	47	39	1.2
22	50	29	1.7
X	155	66[a]	2.3
Y	58	3[a]	19.3
Total (excl. Y)	3023	3079	1.0

[a] Including two BAC clones that had hits on the pseudoautosomal region of HSA X and HSA Y.

distance was 213 kb, compared to the average insert size of 171 kb of the CHORI-241 BAC library. We manually inspected the 62 BLASTN hit pairs that did not match within a 325-kb interval in the human genome. Five clones belonging to such hit pairs could be unambiguously mapped to a single location in the human genome; however, the automatic map assignment had failed because in each case one BES had multiple BLASTN hits on the same chromosome and therefore was filtered out by our bioinformatics pipeline. Six hit pairs matched in correct orientation within 700 kb on the same human chromosome. Twenty-seven matched on the same chromosome but with parallel orientation and/or farther than 700 kb apart, and 24 BLASTN hit pairs matched on different human chromosomes. These hit pairs might be located in regions of evolutionary breakpoints of conserved synteny or they may reflect rearrangements of the respective BAC clones.

In silico mapping and gene annotation

We used the existing data on the human–horse comparative map to make predictions of the assignment to specific equine chromosomes of BESs that had a match to the human genome (Supplementary Table 1). Although the human–horse comparative map does not yet provide high resolution across all chromosomes, for 3604 or 89% of the 4036 BLASTN hits a prediction of the equine chromosomal assignment could be made. The chromosomal assignment was also in silico predicted for the BAC clones; however, the automatic prediction was suppressed for clones with two matching BESs if the hit pair was not within 325 kb in correct orientation on a single human chromosome (Supplementary Table 2).

To provide a putative gene content prediction of the equine BAC clones, we used the human genome gene annotation (NCBI, build 35.1) to infer the gene content of all BAC clones with BLASTN hits in the human genome (Supplementary Table 2). For BAC clones with two paired hits, the hit end points were taken as the corresponding interval in the human genome. In cases in which only one end of a BAC clone matched to the human genome we conservatively assumed that the BAC clone spanned 170 kb (≈ average BAC insert size) in the human genome. If the interval that an equine BAC clone spanned in the human genome overlapped the coordinates of an annotated gene, we assumed the equine BAC clone to contain the equine ortholog of the human gene. We thus identified BAC clones for 4964 (19%) of the 26,602 genes that are currently annotated in the human genome (NCBI human genome build 35.1).

Validation of the in silico mapping process

As a confirmation of the in silico mapping procedure, 19 random BESs that had BLASTN hits evenly distributed on HSA6 were used to design PCR primer pairs. These primers were then RH mapped to provide an experimental confirmation of the computer-predicted equine chromosome assignments. In the RH mapping, each of the 19 tested primer pairs mapped to the computer predicted localization on the syntenic horse chromosomes 10, 20, and 31, respectively (Table 4).

Discussion

In this study, we have provided 9473 BESs with an average read length of 677 bp. The long read lengths are partly due to a portion of the BESs being determined on manual slab-gel-based sequencers but the read length of our high-throughput BESs was still 624 bp, which compares favorably to previously reported BAC end sequencing projects. On the other hand the equine BESs had a relatively low repeat content, with only 34.8% compared to 46.4% in a similar bovine study [10]. In the comparison with the human genome using BLASTN, 43% of the equine BESs gave meaningful BLASTN hits on a single human

Table 4
BES-derived primer sequences for RH mapping

Primer pair	Forward sequence (5′–3′)	Reverse sequence (5′–3′)	T_M (°C)	PCR product (bp)	HSA6 position (Mb)	ECA
CH241-102G5_SP6	GATTTCTGTCCGGATTGC	TGGTTAGGCCAGGAAGTC	59	272	30.14	20
CH241-100F12_SP6	AGAACTTCCCAGCCATTG	CAGGTGGGTGTCAGAATG	61	287	31.61	20
CH241-100D3_T7	GCCTTTCAAGTCCTCTGG	TGGCAGCAGTTAGCTCAG	60	208	38.37	20
CH241-100D3_SP6	CTGGTGCCAGTTTGTTTG	ACACAGAAGGAGGGCTTG	58	256	38.59	20
CH241-102E20_T7	AAGCTACCCTACCACCAAC	ACCTGCTCCTTCTTGTGC	60	264	58.65	20
CH241-101N2_SP6	TCCATTTCCTATGCCTGTC	GTCCCTGCCCTAGAATTG	59	201	62.71	20
CH241-102I4_SP6	CCATTTACTAACGGATGCTG	TTTTCTGTGGAAGAACATGC	60	216	65.77	20
CH241-101G24_T7	GCGCTTGATTGTCATTTG	GAATCCTTGGGAATTCTTG	56	249	93.69	10
CH241-102A14_SP6	TTTCCTACGAAGCACAGC	TTCAAGGAAGGGAAATGC	56	216	94.83	10
CH241-101M4_T7	GATTTAGGGCAGGCAGAC	GGAGACGTTGCTGTGATG	61	290	97.62	10
CH241-102D7_SP6	TGGCAGTTCTACCCAAATC	TTGCAATTGTGTATGTTTGC	58	204	101.21	10
CH241-101L6_SP6	TGTCGCTGAACTTTCTTCC	AATGGTGCTGGATTCTCC	59	282	105.26	10
CH241-102D24_SP6	TGGGGGAAATTATTGGAG	ACCACCTTTCCATTTTGG	57	227	110.16	10
CH241-100C6_SP6	GCAGCACAATTACAGATGG	GTCTCCTTTTGCCTGTCC	57	261	114.72	10
CH241-101E16_T7	AGAATGATCCCTCGGATG	TGGGTGATTCTCCACAAC	59	213	121.85	10
CH241-100E2_SP6	TAGACCCTTGGTGGCATC	CAAGATCTTTGTGCCTCATC	60	206	122.80	10
CH241-100J19_SP6	GGAGACACCCTTCACCAG	TGGCAGGAGAAAGAGGAG	61	267	139.95	31
CH241-100B15_SP6	GTGGATCCCATTCTGGAG	GAATGACCTGCTGTTACCC	60	270	150.83	31
CH241-100I20_SP6	CAGCTTCTGCTGTTCAGG	GTGCCTATGGGAAGATGC	59	296	162.56	31

chromosome with $E \leq 10^{-5}$ compared to 23% for a bovine BES collection and 11% for mouse BESs [10,13]. The 1.9-fold higher hit rate in the BLASTN analysis of equine BESs against the human genome compared to bovine BESs is mostly due to the longer read lengths and lower repeat content of the equine BESs. The average number of nonrepetitive bases in the equine BESs was 1.6-fold higher than in the bovine BESs (441 bp vs 276 bp). Thus the long read lengths and the low repeat content of the equine BESs made comparative mapping very efficient.

Using the 4036 meaningful BLASTN hits to the human genome we were able to anchor 3079 equine BAC clones to the human genome. Thus we provide roughly one horse BAC clone for every megabase of human DNA sequence and cover about 17% of the human genome with comparatively anchored equine BAC clones. We further used the human gene annotation to predict the putative gene content of the comparatively anchored equine BAC clones. Based on current knowledge, about 90% of the genes are conserved in a 1:1 orthologous fashion between different mammalian species [14], and we expect that our in silico gene prediction should at least correctly identify these genes. The comparatively mapped equine BAC clones and the gene predictions can be used by other researchers without the need for tedious library screenings and will thus facilitate future equine research.

The BLASTN anchored clones were nearly equally distributed among the human chromosomes. HSA19 and HSA22 had a slightly lower coverage than the other autosomes. This is most likely due to their higher than average GC content. As the CHORI-241 library was constructed with *Eco*RI, which has an AT-rich recognition sequence (GAATTC), fewer BLASTN hits may be expected on average to GC-rich target sequences. Additionally, fewer hits are expected on the smaller human chromosomes as these chromosomes have a higher proportion of telomeric and centromeric repeats relative to their single-copy sequences. The coverage of the X chromosome is roughly 50% of the coverage of the autosomes as the CHORI-241 library was prepared from a male horse. The Y chromosome, finally, has only one BLASTN hit in the nonrecombining region, which is probably due to the extremely high repeat content of this particular chromosome.

The analysis of BES read pairs, in which both BESs of a single clone had BLASTN hits to the human genome, provided striking evidence for the general feasibility of the applied comparative mapping approach as 93% of the paired BLASTN hits matched with correct orientation within 325 kb at a single human genome location. The remaining 7% of paired BLASTN hits might indicate comparative mapping artifacts but would also be expected for clones that span breakpoints of conserved synteny or clones harboring regions with microrearrangements between the human and the horse genomes. Alternatively, such results would also be expected with chimeric BAC clones.

The analysis of paired hits also indicates that the horse genome might be significantly smaller than the human genome. The average insert size of the sequenced BAC clones is 171 kb compared to an average distance of 214 kb between paired BLASTN hits in the human genome. When we extrapolate these values to the whole genome the horse genome would be 20% smaller than the human genome. This is consistent with our observation that the repeat content of the 6.4 Mb of the determined horse sequences is only 34.8% compared to ~50% in the human genome. However, it must also be kept in mind that the repeat analysis may be biased because of the *Eco*RI cloning of the BAC inserts and because of the incomplete coverage of equine repetitive sequences in the RepeatMasker database.

In conclusion, we generated an initial set of equine BESs and provide a comparative analysis of these BESs with respect to the human genome sequence. The comparatively anchored BESs can serve as a starting point for a high-resolution clone-based physical map of the horse genome. The availability of closely spaced comparatively mapped clones with gene predictions will facilitate future investigations of specific genome regions and reduce the need for experimental library screenings.

Materials and methods

BAC end sequencing (high-throughput)

All BAC clones were from the CHORI-241 library (http://bacpac.chori.org). For plates 105–115 we prepared DNA in 96-well format either using a modified REAL Prep 96 method (Qiagen, Hilden, Germany) or applying a modified method as provided by another manufacturer (Millipore, Schwalbach, Germany). We used 50–100 ng of purified BAC DNA for 100 cycles of dye-terminator sequencing with the BigDye Terminator v3.1 kit (Applied Biosystems, Darmstadt, Germany). Routinely, the primers were modified SP6 (5′-CGTCGACATTTAGGTGACACTAT-3′) and T7 (5′-CGAGCTTGA-CATTGTAGGACTATA-3′) oligonucleotides. The reaction mixtures were purified by ethanol precipitation and separated on an ABI 3730*xl* capillary sequencer (Applied Biosystems) applying standard conditions.

BAC end sequencing (high-quality)

For plates 100–102 and 104 of the CHORI-241 library we prepared BAC DNA from individual clones using the Qiagen Midi or Mini plasmid kit according to the modified protocol for BACs (Qiagen). We used 5–10 μg of purified BAC DNA for 25 cycles of bidirectional dye-primer sequencing with the Thermosequenase kit (Amersham Biosciences, Freiburg, Germany). Sequencing primers were IRD700-labeled SP6 (5′-TTTTTGCGATCTGCCGTTTC-3′) and IRD800-labeled T7 (5′-TAATACGACTCACTATAGGG-3′). The reaction products were separated on 41-cm-long 0.2-mm gels prepared from Sequagel solution (Biozym, Hess. Oldendorf, Germany) on a LICOR 4200L automated sequencer (LICOR Biosciences, Bad Homburg, Germany).

Sequence processing and bioinformatics

Repetitive sequences were masked with RepeatMasker (A.F.A. Smit and P. Green, http://repeatmasker.genome.washington.edu/). Masked sequences were subjected to BLASTN searches against build 35.1 of the human genome (ftp://ftp.ncbi.nlm.nih.gov/BLASTN/db/) using the default word size and a cutoff of $E \leq 10^{-5}$ [15]. The BLASTN results were parsed into a relational SQL database. Syntenic regions between the human genome and a BLASTN-anchored equine BAC clone were inferred as follows: If both BESs of a BAC clone gave BLASTN hits within 325 kb on a single human chromosome, the BAC clone was assumed to be syntenic to the interval defined by the two BLASTN hits. If only one BES of a BAC clone gave a BLASTN hit to the human genome, a syntenic region was inferred by adding 170 kb in the orientation according to the BLASTN hit. Using these syntenic regions, the BAC clone gene content was predicted according to the human gene annotation (NCBI MapViewer, human genome build 35.1). In those cases in which the two BESs of a BAC clone had

significant matches exceeding a 325-kb interval on a single human chromosome, the in silico prediction of gene content and equine chromosome was suppressed.

RH mapping

Primer pairs were designed with primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) from the repeat-masked BESs (Table 4). Amplification of the markers was done in duplicate on the TAMU equine radiation hybrid panel [16] using 38 cycles of PCR. To ascertain the chromosomal location of the markers a two-point linkage analysis (http://equine.cvm.tamu.edu/cgi-bin/ecarhmapper.cgi) was conducted to find associations between the analyzed BES markers and the known RH markers of the first-generation whole-genome RH map [2,17].

Acknowledgments

We thank J. Kanter, H. Klippert-Hasberg, D. Seinige, T. Unger, C. Berg, A. Conrad, T.-H. Löhnert, S. Severitt, C. Schindewolf, and S. Thies for expert technical assistance. Technical advice from B.W. Plumb (Sanger Institute, UK) is gratefully acknowledged. This study was supported by a grant from the German Research Council (DFG LE1032/20-1).

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ygeno.2006.03.002.

References

[1] T. Raudsepp, L. Frönicke, H. Scherthan, I. Gustavsson, B.P. Chowdhary, Zoo-FISH delineates conserved chromosomal segments in horse and man, Chromosome Res. 4 (1996) 218–225.

[2] B.P. Chowdhary, T. Raudsepp, S.R. Kata, G. Goh, L.V. Millon, V. Allan, F. Piumi, G. Guerin, J. Swinburne, M. Binns, et al., The first-generation whole-genome radiation hybrid map in the horse identifies conserved segments in human and mouse genomes, Genome Res. 13 (2003) 742–751 (Published erratum in Genome Res. 13 (2003) 1258).

[3] C. Brinkmeyer-Langford, T. Raudsepp, E.-J. Lee, G. Goh, A.A. Schäffer, R. Agarwala, M.L. Wagner, T. Tozaki, J.R. Mickelson, J.E. Womack, et al., A high-resolution physical map of equine homologues of HSA19 shows divergent evolution compared to other mammals, Mamm. Genome 16 (2005) 631–649.

[4] E.-J. Lee, T. Raudsepp, S.R. Kata, D. Adelson, J.E. Womack, L.C. Skow, B.P. Chowdhary, A 1.4-Mb interval RH map of horse chromosome 17 provides detailed comparison with human and mouse homologues, Genomics 83 (2004) 203–215.

[5] A. Gustafson-Seabury, T. Raudsepp, G. Goh, S.R. Kata, M.L. Wagner, T. Tozaki, J.R. Mickelson, J.E. Womack, L.C. Skow, B.P. Chowdhary, High-resolution RH map of horse chromosome 22 reveals a putative ancestral vertebrate chromosome, Genomics 85 (2005) 188–200.

[6] T. Raudsepp, E.-J. Lee, S.R. Kata, C. Brinkmeyer, J.R. Mickelson, L.C. Skow, J.E. Womack, B.P. Chowdhary, Exceptional conservation of horse–human gene order on X chromosome revealed by high-resolution radiation hybrid mapping, Proc. Natl. Acad. Sci. USA 101 (2004) 2386–2391.

[7] S. Zhao, J. Malek, G. Mahairas, L. Fu, W. Nierman, J.C. Venter, M.D. Adams, Human BAC ends quality assessment and sequence analyses, Genomics 63 (2000) 321–332.

[8] S. Zhao, S. Shatsman, B. Ayodeji, K. Geer, G. Tsegaye, M. Krol, E. Gebregeorgis, A. Shvartsbeyn, D. Russell, L. Overton, et al., Mouse BAC ends quality assessment and sequence analyses, Genome Res. 11 (2001) 1736–1745 (Published erratum in Genome Res. 11 (2001) 1968).

[9] A. Fujiyama, H. Watanabe, A. Toyoda, T.D. Taylor, T. Itoh, S.F. Tsai, H.S. Park, M.L. Yaspo, H. Lehrach, Z. Chen, et al., Construction and analysis of a human–chimpanzee comparative clone map, Science 295 (2002) 131–134.

[10] D.M. Larkin, A. Everts-van der Wind, M. Rebeiz, P.A. Schweitzer, S. Bachman, C. Green, C.L. Wright, E.J. Campos, L.D. Benson, J. Edwards, et al., A cattle–human comparative map built with cattle BAC-ends and human genome sequence, Genome Res. 13 (2003) 1966–1972.

[11] R.Z. Ma, M.J. van Eijk, J.E. Beever, G. Guerin, C.L. Mummery, H.A. Lewin, Comparative analysis of 82 expressed sequence tags from a cattle ovary cDNA library, Mamm. Genome 9 (1998) 545–549.

[12] M. Rebeiz, H.A. Lewin, Compass of 47,787 cattle ESTs, Anim. Biotechnol. 11 (2000) 75–241.

[13] S.G. Gregory, M. Sekhon, J. Schein, S. Zhao, K. Osoegawa, C.E. Scott, R.S. Evans, P.W. Burridge, T.V. Cox, C.A. Fox, et al., A physical map of the mouse genome, Nature 418 (2002) 743–750.

[14] Rat Genome Sequencing Consortium, Genome sequence of the Brown Norway rat yields insights into mammalian evolution, Nature 428 (2004) 493–521.

[15] S.F. Altschul, T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLASTN and PSI-BLASTN: a new generation of protein database search programs, Nucleic Acids Res. 25 (1997) 3389–3402.

[16] B.P. Chowdhary, T. Raudsepp, D. Honeycutt, E.K. Owens, F. Piumi, G. Guérin, T.C. Matise, S.R. Kata, J.E. Womack, L.C. Skow, Construction of a 5000_{rad} whole-genome radiation hybrid panel in the horse and generation of a comprehensive and comparative map for ECA11, Mamm. Genome 13 (2002) 89–94.

[17] D. Slonim, L. Kruglyak, L. Stein, E. Lander, Building human genome maps with radiation hybrids, J. Comput. Biol. 4 (1997) 487–504.