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A human-horse comparative map based on equine BAC end sequences (BESs)

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Running title: Horse BAC end sequences (BESs)

The sequence data described in this paper have been deposited in the DDBJ/Genbank/EMBL data library. A list of accession numbers is available in the supplementary files.

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Abstract

In an effort to increase the density of sequence based markers for the horse genome we generated 9,473 BAC end sequences (BESs) from the CHORI-241 BAC library with an average read length of 677 bp. BLASTN searches with the BESs revealed 4,036 meaningful hits ($E \leq 10^{-5}$) in the human genome, which provide useful markers for the human-horse comparative map. The 4,036 BLASTN hits allowed the anchoring of 3,079 BAC clones to the human genome, on average one corresponding equine BAC clone per Mb 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.

Key words: horse, comparative map, physical map, RH-map, BES, BLASTN, database

Introduction

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 where 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, respectively, 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 (WGS) 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 (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 6,144 BAC clones of the CHORI-241 BAC library (Table 1). We generated a total of 9,473 equine BESs with an average read length of 677 bp. All sequence data totaling 6.4 Mb were submitted to the EMBL nucleotide data base. 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 4,211 BESs showed significant hits. After filtering 175 BESs with multiple hits, the remaining 4,036 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 3,079 BAC clones could be anchored to the human genome (Table 3). On average one BAC clone per Mb 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, where 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 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 either with parallel orientation and/or further 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 3,604 or 89% of the 4,036 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, where 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 4,964 genes (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 for the *in silico* mapping procedure, 19 random BESs that had BLASTN hits evenly distributed on HSA 6 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 9,473 BESs with an average read length of 677 bp. The long read lengths are partly due to a portion of the BESs 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 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 non-repetitive 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 4,036 meaningful BLASTN hits to the human genome we were able to anchor 3,079 equine BAC clones to the human genome. Thus we provide roughly one horse BAC clone for every Mb 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 the 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. HSA 19 and HSA 22 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 non-recombining region, which is probably due to the extremely high repeat content of this particular chromosome.

The analysis of BES read pairs, where both BES 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 micro-rearrangements between the human and 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 then 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 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 using either a modified R.E.A.L. 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'-CGAGCTTGACATTGTAGGACTATA-3') oligonucleotides. The reaction mixtures were purified by ethanol precipitation and separated on an ABI 3730*xl* capillary sequencer (Applied Biosystems, Darmstadt, Germany) applying standard conditions.

BAC end sequencing (high-quality)

For plates No. 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, Hilden, Germany). 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-labelled SP6 (5'-TTTTTGCGATCTGCCGTTTC-3') and IRD800-labelled 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 (Smit, A.F.A. and Green, P. 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 map viewer, human genome build 35.1). In those cases, where 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].

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Table 1: Horse BAC end sequencing statistics

Sequencing results	
BAC clones attempted	6,144
BESs[a]	9,473
read pairs	4,171
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 *E. 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, non-recombinant 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	9,473	
BESs with BLASTN hits ($E \leq 10^{-5}$)	4,211	
BESs with meaningful BLASTN hits[a]	4,036	
BAC clones with at least one BES	5,302	(100%)
BAC clones with one matching BES	2,246	(42%)
BAC clones with two matching BESs[b]	895	(17%)
comparatively mapped BACs total[c]	3,079	(58%)

[a] BESs with multiple hits in the human genome were filtered out.
[b] 828 (92%) of these 902 hit pairs matched within 325 kb on one human chromosome in 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	Chr. 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)	3,023	3,079	1.0

[a] Including 2 BAC clones that had hits on the pseudoautosomal region of HSA X and HSA Y.

Table 4: BES derived primer sequences for RH mapping

Primer pair	Forward sequence (5´-3´)	Reverse sequence (5´-3´)	T_M	PCR-product	HSA 6 position (Mb)	ECA
CH241-102G5_SP6	GATTTCTGTCCGGATTGC	TGGTTAGGCCAGGAAGTC	59 °C	272 bp	30.14	20
CH241-100F12_SP6	AGAACTTCCCAGCCATTG	CAGGTGGGTGTCAGAATG	61 °C	287 bp	31.61	20
CH241-100D3_T7	GCCTTTCAAGTCCTCTGG	TGGCAGCAGTTAGCTCAG	60 °C	208 bp	38.37	20
CH241-100D3_SP6	CTGGTGCCAGTTTGTTTG	ACACAGAAGGAGGGCTTG	58 °C	256 bp	38.59	20
CH241-102E20_T7	AAGCTACCCTACCACCAAC	ACCTGCTCCTTCTTGTGC	60 °C	264 bp	58.65	20
CH241-101N2_SP6	TCCATTTCCTATGCCTGTC	GTCCCTGCCCTAGAATTG	59 °C	201 bp	62.71	20
CH241-102I4_SP6	CCATTTACTAACGGATGCTG	TTTTCTGTGGAAGAACATGC	60 °C	216 bp	65.77	20
CH241-101G24_T7	GCGCTTGATTGTCATTTG	GAATCCTTGGGAATTCTTG	56 °C	249 bp	93.69	10
CH241-102A14_SP6	TTTCCTACGAAGCACAGC	TTCAAGGAAGGGAAATGC	56 °C	216 bp	94.83	10
CH241-101M4_T7	GATTTAGGGCAGGCAGAC	GGAGACGTTGCTGTGATG	61 °C	290 bp	97.62	10
CH241-102D7_SP6	TGGCAGTTCTACCCAAATC	TTGCAATTGTGTATGTTTGC	58 °C	204 bp	101.21	10
CH241-101L6_SP6	TGTCGCTGAACTTTCTTCC	AATGGTGCTGGATTCTCC	59 °C	282 bp	105.26	10
CH241-102D24_SP6	TGGGGGAAATTATTGGAG	ACCACCTTTCCATTTTGG	57 °C	227 bp	110.16	10
CH241-100C6_SP6	GCAGCACAATTACAGATGG	GTCTCCTTTTGCCTGTCC	57 °C	261 bp	114.72	10
CH241-101E16_T7	AGAATGATCCCTCGGATG	TGGGTGATTCTCCACAAC	59 °C	213 bp	121.85	10
CH241-100E2_SP6	TAGACCCTTGGTGGCATC	CAAGATCTTTGTGCCTCATC	60 °C	206 bp	122.80	10
CH241-100J19_SP6	GGAGACACCCTTCACCAG	TGGCAGGAGAAAGAGGAG	61 °C	267 bp	139.95	31
CH241-100B15_SP6	GTGGATCCCATTCTGGAG	GAATGACCTGCTGTTACCC	60 °C	270 bp	150.83	31
CH241-100I20_SP6	CAGCTTCTGCTGTTCAGG	GTGCCTATGGGAAGATGC	59 °C	296 bp	162.56	31