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High-resolution RH map of horse chromosome 22 reveals a putative ancestral vertebrate chromosome

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Abstract

High-resolution gene maps of individual equine chromosomes are essential to identify genes governing traits of economic importance in the horse. In pursuit of this goal we herein report the generation of a dense map of horse chromosome 22 (ECA22) comprising 83 markers, of which 52 represent specific genes and 31 are microsatellites. The map spans 831 cR over an estimated 64 Mb of physical length of the chromosome, thus providing markers at ~770 kb or 10 cR intervals. Overall, the resolution of the map is to date the densest in the horse and is the highest for any of the domesticated animal species for which annotated sequence data are not yet available. Comparative analysis showed that ECA22 shares remarkable conservation of gene order along the entire length of dog chromosome 24, something not yet found for an autosome in evolutionarily diverged species. Comparison with human, mouse, and rat homologues shows that ECA22 can be traced as two conserved linkage blocks, each related to individual arms of the human homologue—HSA20. Extending the comparison to the chicken genome showed that one of the ECA22 blocks that corresponds to HSA20q shares synteny conservation with chicken chromosome 20, suggesting the segment to be ancestral in mammals and birds.

Keywords: Horse; ECA22; Gene mapping; RH map; FISH; Comparative map; Human; Mouse

The first-generation horse whole genome radiation hybrid (RH) and comparative map represents the most comprehensive framework of mapped loci in the equine genome [1]. It contains both gene-specific and polymorphic markers and integrates the syntenic, cytogenetic, and genetic linkage maps into a single consensus map. Despite serving as an important foundation for understanding the organization of the equine genome, the resolution of the current map is inadequate for rapid discovery of markers closely linked to traits of interest in the horse. Additionally, it is also insufficient for use in a candidate gene approach to identify genes responsible for traits significant to the equine industry. Among the most apparent drawbacks associated with the map are: (i) presence of regions on a number of equine chromosomes that lack adequate numbers of mapped gene-specific and polymorphic markers and (ii) absence of uniformity in the distribution of markers over individual chromosomes.

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To date very few genes controlling traits of economic significance have been identified in the horse. Among the genes identified include those governing monogenic traits, e.g., coat color and some inherited diseases [2]. The discoveries of most of these genes were facilitated by knowledge of homologous genes for similar phenotypes in humans or other mammalian species. Thus an a priori knowledge of the causative gene and its mutation led to the identification of the equine ortholog and its causative mutation. However, most of the future research aimed at identifying genes governing economically important traits in the horse will require a set of genome-wide polymorphic markers (genome scan panel) appropriate to the population to be analyzed and high-resolution physically ordered comparative maps to align the horse map accurately with its mammalian counterparts. The latter will be critical for the candidate gene approach used for isolating genes potentially implicated in the hereditary condition analyzed. The recent development of the high-resolution RH and comparative map for horse chromosome 17 [3] and the X chromosome [4] represents the first steps toward targeted expansion of gene maps in the horse to obtain dense maps. Though the approach used for these two chromosomes has provided resolutions ranging between 1.4 to 1 Mb per marker, it involved alignment of sequence from multiple species, identification of conserved regions, and tedious development of primer pairs to obtain horse-specific amplification [3,4].

Overgos (overlapping oligonucleotide primer pairs) are probes that hybridize to conserved genomic regions (primarily exons) and can be used for improved multiprobe high-density filter hybridization to enable rapid identification of BAC clones containing specific markers [5,6]. To improve upon the development of expanded gene maps in the horse, we used this approach to generate a high-resolution RH and comparative map of horse chromosome 22 (ECA22). Comparative analysis shows that ECA22 and its homologues in various mammalian species represent one of the most conserved genomic segments across a range of evolutionarily diverged species [7]. The chromosome corresponds to human chromosome 20 (HSA20) [8], which contains genes implicated in, for example, spongiform encephalopathies [9,10], Kindler syndrome [11], and congenital hereditary endothelial dystrophy of the cornea [12,13].

The current map of ECA22 contains a total of 23 markers (7 genes/ESTs and 16 microsatellites), assigned by RH and linkage analysis [1,14,15]. Of the three linkage groups, only two are physically aligned to the chromosome. The proximal one-third of the chromosome has very few mapped markers. To remedy these deficiencies, we undertook development of a high-resolution map of the chromosome with the goal of defining gene-specific markers at every megabase interval. This would finely align ECA22 to the human and mouse chromosomes, facilitate identification of candidate loci for various equine conditions associated with genes located on this chromosome, and help to understand the relative organization and hence evolution of the homologues of this chromosome among different mammals/vertebrates.

Results

Overgo hybridization and isolation of BAC clones containing targeted genes

A total of 45 overgo probes were designed within conserved regions of genes selected at ~1 Mb intervals from the terminal part of the short arm to the terminal part of the long arm of HSA20. The first pooled hybridization with 18 overgo probes for genes located on HSA20p to the high-density filters of the CHORI-241 horse genomic BAC library yielded 63 BACs. The second pooled hybridization with 27 overgo probes representing genes from HSA20q yielded 103 BACs. The rearray and hybridization of the positive BACs with individual overgo probes indicated that 4 of the probes from the short arm, and 8 from the long arm, failed to identify any BACs. Thus, of the 45 overgo probes, BAC clones were obtained for 33 sets, averaging 5.2 BACs/overgo pair (Table 1).

To confirm the identity of the BACs, a single representative BAC clone for each overgo probe was used for direct sequencing with one of the overgo primers as sequencing primer. All representative clones provided on average 450 bp sequence with >80% identity with the respective human ortholog. The BAC for *TCF15* showed no amplification.

BAC end sequencing, sequence-tagged site (STS) development, and optimization of PCR

Analysis of sequence data from one end of the 33 representative clones resulted in the generation of STSs from only 12 of the ends. BACs that did not provide end sequence from one end were sequenced from the other end. This resulted in an additional 20 STS markers. BLAST analysis [16] of the STS sequences showed that none of the end sequences corresponded to coding regions of specific genes. Most of the end sequences that could not be converted to STSs comprised repetitive elements. Following optimization, horse-specific amplification was obtained for 29 of the STSs. Similarly, optimization was also carried out with primer pairs from eight equine ESTs, eight equine orthologs for human genes, and 19 microsatellite markers on ECA22 (Table 1).

Fluorescence in situ hybridization (FISH) mapping

All representative BAC clones isolated for the 33 overgo probes localized to ECA22. One of the clones contained two genes (*BMP2* and *CHGB*). Further, BACs obtained for *AHT030, UMNe276*, and *UMNe344* mapped to ECA13q, in contrast to previous erroneous FISH assignment of *AHT030* [17] and subsequent mapping of loci in this RH group to ECA22 [1]. The remaining five BACs representing *ASIP,*