

Conservation of Gene Order between Horse and Human X Chromosomes as Evidenced through Radiation Hybrid Mapping

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A radiation hybrid (RH) map of the equine X chromosome (ECAX) was obtained using the recently produced 5000_{rad} horse × hamster hybrid panel. The map comprises 34 markers (16 genes and 18 microsatellites) and spans a total of 676 cR_{5000r} covering almost the entire length of ECAX. Cytogenetic alignment of the RH map was improved by fluorescent *in situ* hybridization mapping of six of the markers. The map integrates and refines the currently available genetic linkage, syntenic, and cytogenetic maps, and adds new loci. Comparison of the physical location of the 16 genes mapped in this study with the human genome reveals similarity in the order of the genes along the entire length of the two X chromosomes. This degree of gene order conservation across evolutionarily distantly related species has up to now been reported only between human and cat. The ECAX RH map provides a framework for the generation of a high-density map for this chromosome. The map will serve as an important tool for positional cloning of X-linked diseases/conditions in the horse.

Key Words: horse, X chromosome, gene mapping, RH map, FISH, comparative map

INTRODUCTION

During the past few years coordinated international efforts have significantly contributed to the development of a basic gene map in the horse. Generation of new mapping resources (genomic and cDNA libraries, improved family material, and so on) and isolation of polymorphic markers, genes, and expressed sequence tags (ESTs) for genetic linkage, synteny, and cytogenetic mapping have resulted in over 1000 loci mapped using one or more approaches (<http://locus.jouy.inra.fr/>). To maximize the use of gene maps in analyzing traits of interest, it is essential to integrate maps obtained using different approaches into a consensus map. Such integration also helps to identify regions where more markers must be developed to attain a uniform coverage of the genome. However, in horse, there are currently large subsets of loci that have been mapped using only one of the approaches. This prevents accurate alignment of various maps, which in turn precludes precise positional cloning of important genes.

Radiation hybrid (RH) cell panels have emerged as a highly proficient tool to integrate and expand genome maps of various livestock/domestic species, such as pig [1], cattle [2], dog [3,4], and cat [5–7]. The technique incorporates both polymorphic and non-polymorphic markers into a map, thereby providing improved resolution and accuracy compared with genetic linkage or cytogenetic mapping approaches [8,9]. Recently two RH panels were constructed in the horse (3000_{rad} [10] and 5000_{rad} [11]). Initial analysis provided RH maps for ECA1 and ECA10 [10], and a comprehensive comparative map for ECA11 [11]. The results demonstrate the utility and resolution power of the two panels and underline their potential in rapid expansion of the horse gene map.

The X chromosome, considered as one of the most conserved chromosomes among mammals, has been sparsely studied in the horse especially when compared with other livestock species. The current equine X chromosome (ECAX) genetic linkage map [12] comprises 13 markers (all microsatellites), whereas the syntenic map [13–15] incorporates 23 (10

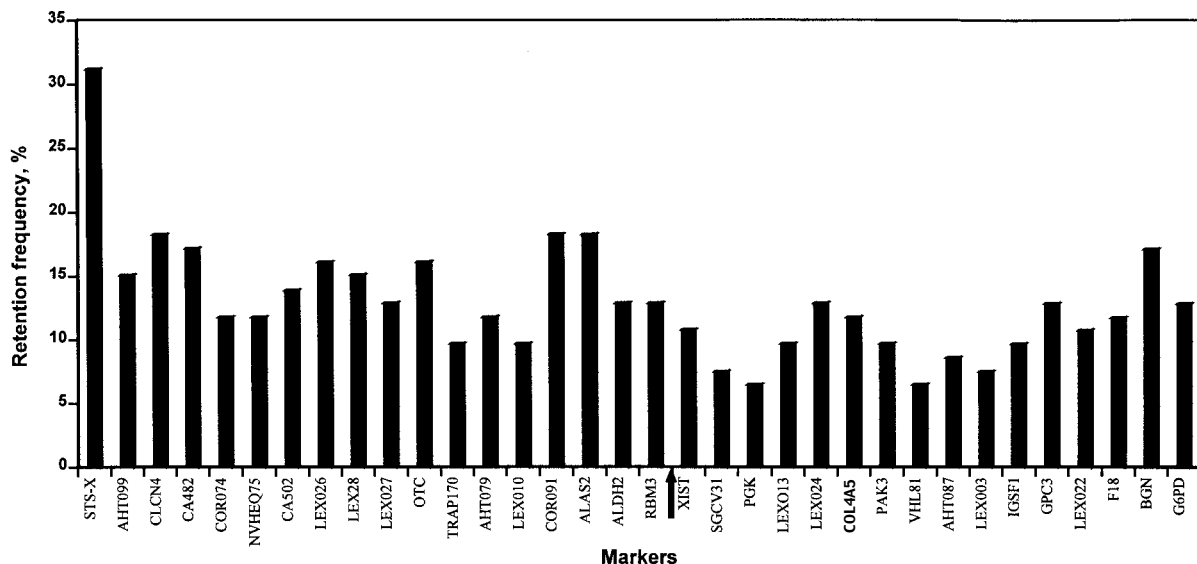


FIG. 1. Retention frequency of ECAX markers in the RH panel. Of the total of 34 markers typed, 18 are located on the short arm (*STS-X*-*RBM3*) and 16 on the long arm (*XIST*-*G6PD*). The arrow represents the centromere. Retention frequency was relatively higher on the terminal parts of long and short arms and close to the centromere. Overall retention was slightly higher on the short arm.

type I and 13 type II from the genetic linkage map). The cytogenetic map includes sublocalization of only 10 loci. Overall, the relative order of all mapped loci is difficult to deduce and the comparative status inferred between horse and human X chromosomes is rudimentary. To overcome these limitations, we used the recently produced 5000_{rad} horse × hamster RH panel [11] to construct a comprehensive map of ECAX.

RESULTS

RH Panel Typing and Analysis

We typed 36 markers (20 microsatellites and 16 genes) on the panel. Retention frequency (RF) for the haploid X chromosome ranged from 6.5% to 31.2% with an average of 12.8% (Fig. 1). This is approximately half of the average RF of the whole RH panel (26%) [11]. A relatively high retention was observed at the telomeric end of the short arm (*STS-X*, RF = 31.2%).

Two-point linkage analysis resulted in a single linkage group at lod score 4 (2PT-RHMAP) [16]. Two pairs of microsatellite markers (*UM038*-*SGCV31* and *LEX024*-*UM001*) showed the same retention pattern and appeared to be totally linked. The markers are available in the NCBI database under different accession numbers and were submitted by different research groups (Table 1). However, sequence comparison revealed that *UM038* and *SGCV31*, and *LEX024* and *UM001* are the same. Hence, only one marker from each pair was included in the final analysis. The resulting map thus comprised 34 markers that spanned a total of 676 cR₅₀₀₀ and covered almost the entire length of ECAX (see legend of Fig. 2 for details on map construction).

BAC Clones and Cytogenetic Mapping

PCR screening of two equine genomic BAC libraries with primer pairs from 20 ECAX markers gave only nine positive BAC clones. Of these, markers *UM038* and *SGCV31* corresponded to the same clone, confirming the panel typing results. Hence FISH analysis was carried out only for eight BACs. Among these, two BACs (corresponding to *LEX024* and *LEX027*) gave a strong hybridization signal only on autosomes, suggesting predominance of autosomal sequences in the insert. The BAC clone containing *STS-X* sequence also appeared chimeric with FISH signal at the expected site on ECAXpter and an additional signal on ECAXqter. Based on synteny data [15], we considered the former to be the true signal for *STS-X*. The remaining five clones showed clear FISH signals along ECAX. Thus the six new cytogenetically mapped loci together with earlier FISH assignments (Fig. 2) provided physical anchor points in constructing the RH map.

DISCUSSION

RH cell panels [10,11] are relatively new tools in equine genomics. Their potential effectiveness in generating RH maps for individual horse chromosomes is evident through the preliminary analysis and mapping of ECA1 and ECA10 (3000_{rad}) [10]. More recently, a comprehensive RH map for ECA11, together with comparative information in relation to human and mouse maps [11], confirmed the usefulness of the RH approach and the recently generated 5000_{rad} panel. Continuing our work on this panel, we have presented a RH map for the equine X chromosome. In addition to eight new

TABLE 1: ECAX markers typed in the 5000_{rad} horse × hamster radiation hybrid panel

Symbol	Name	Location in horse	Primers	Product size (bp)	MgCl ₂ (mM)	T _a	Reference	Acc. no.
Type I markers								
ALAS2	δ-aminolevulinate synthase 2	Xp	F: GGCTACGCATGAACCAATCT R: GTTAGGCCAGCTCATTCTGC	195	2.0	58	[15]	AF133200
P ^h ALDH2	Aldehyde dehydrogenase pseudogene (mitochondrial X)	Xp13-p14	F: TTGCCATTATCCAGGGTCTC R: GGGAGGTCATCTGTGGGTA	202	2.0	58	[15]	AF133203
BGN	Biglycan	X	F: CACTGTTCACCAGGGGATTC R: GGGGGCTAGTGGTCTTGAAC	226	2.0	58	[15]	AF135019
CLCN4	Chloride channel 4	Xp23-p24	F: CATCTGCTTGGGGTCTTT R: GAAATGAGCTCGCTTGTGCT	192	2.0	58	[15]	AF133199
COL4A5	Collagen, type IV, α 5	X	F: CTCITACCACATTAGATGCCCC R: GATTACATAGGAAAGAGATTGGG	322	3.0	58	R. Brandon, pers. comm.	M31115
F18=CXorf6	F18 gene, open reading frame 6	Xq29	F: TCAITGTAATCTGCATGTGC R: CAGGTTGGCAAGAAITGGAGT	200	2.0	58	[43]	AB009590
G6PD	Glucose-6-phosphate dehydrogenase	Xq1	F: CCAGAAITCTATGGTGTGTA R: GATGACACAGGCGATGTGT	199	2.0	65	[15]	AF133202
GPC3	Glypican 3	Xq27	F: CTGACTTCTAGTGGCCAGCTC R: CCATGTTCTAGAAGCCAAACATAG	273	2.0	58	[31]	G62152, NM_004484
IGSF1	Immunoglobulin super-family, member 1	Xq27	F: CCTCATCAATCTCCAGGCAAC R: GFACAGTGGAGTTACAGGGCCA	~ 300	2.0	58	R. Brandon, pers. comm.; FISH by T. Lear, pers. comm.	NM_001555
OTC	Omithine carbamoyl-transferase	Xp15-p16	F: TCAGATCTGCTGATAGCCA R: GTGTGGACAACCACTACAAA	~ 200	2.0	58	[29]	ECA000935
PAK3	p21 (CDKN1A)-activated kinase 3	X	F: TACTCCCTCGGATTAITGTAITTC R: GGAGGTTGGAATGCAAGAGGA	238	2.0	58	R. Brandon, pers. comm.; FISH by T. Lear, pers. comm.	U39738
PGK	Phosphoglycerate kinase	X	F: AGTAACTGCTGTGTGTGCTC R: CCTAAGAAAATGCAATCGGATCC	~ 300	2.0	58	R. Brandon, pers. comm.; FISH by T. Lear, pers. comm.	M11968
RBM3	RNA binding motif protein 3	X	F: ACCTTTAGAAAAGCTCCATGTGTTT R: ACCAGGCGAGTATCTTGAGTAA	~ 300	2.0	58	R. Brandon, pers. comm.; FISH by T. Lear, pers. comm.	NM_016809
STS-X (ARSC1)	Steroid sulfatase	Xp25	F: CTCTTG CAGGGTCTTGGTGT R: GCACCAATGGATGTTTTTC	200	2.0	Touch-down 60-50	[15]	AF133204
TRAP170=CRSP2	Thyroid hormone receptor-associated protein, cofactor required for Spi transcriptional activation	X	F: TTTCATCAAAAAGACCATCAGTCTTT R: TTGCTTCCITGGATGAGTTTATAAC	~ 300	2.0	58	R. Brandon, pers. comm.	AF135802
XIST	Xist gene	Xq13	F: ACCCATGAAAAACCCATTGA R: GGGGGTGGAGGAAGTAGAAG	200	2.0	Touch-down 60-50	[15]	U50911

Table 1 continued on next page

TABLE 1: Continued

Symbol Type II markers	Name	Location in horse	Primers	Product size (bp)	MgCl ₂ (mM)	T _a	Reference	Acc. no.
AHT079	microsatellite	X	F: CTTTCCCGAACCCTCCTAC R: TTGGATGCTCCGAGAAGAGT	125-137	3.0	58	[12,15]	U67407
AHT087	microsatellite	X	F: CCITGGGCTTIFAGCAACT R: CCATITGGAAACTGAGAGG	155-167	3.0	58	[15,32]	U67420
AHT099	microsatellite	X	F: GAAAAGCGTATCTTCTTAGTC R: GGGCGAITTCATCAGAAITCTA	281-291	2.0	58	[33]	AF142611
CA428	microsatellite	Xp	F: CTTTCCCGAACCCTCCTAC R: TTGGATGCTCCGAGAAGAGT	125-137	3.0	58	[12,15]	U67407
CA502	microsatellite	Xp	F: CCITGGGCTTIFAGCAACT R: CCATITGGAAACTGAGAGG	155-167	3.0	58	[15,32]	U67420
COR074	microsatellite	Xp23-p24	F: GAAAAGCGTATCTTCTTAGTC R: GGGCGAITTCATCAGAAITCTA	281-291	2.0	58	[33]	AF142611
COR091	microsatellite	X	F: GGTGATTCACAGGTTAATGGC R: TGTATCTGTCCACAGCATGG	205	2.0	58	[34]	AF154944
LEX003	microsatellite	Xq	F: ACATCTAACAGTGCTCAGACT R: GAAAGGAAAAAAGGAGGAAGC	143-164	3.0	58	[35]	AF075607
LEX010	microsatellite	Xp	F: TGGGCTAAAAITTAATTTGGG R: ACCAAAACATATGCAAAITTA	198-206	2.0	Touch- down 60-50	[15,35]	AF075613
LEX013	microsatellite	X	F: TGCTAGAGGAAAGGATAAAGG R: CTCTGCTTCCATTTCTTGC	122-128	2.0	58	[35]	AF07615
LEX022	microsatellite	Xq	F: AACATATCCATCGCCTCAC R: TGCAAAITTCACGTAGAGTGG	101-113	2.0	Touch- down 60-50	[15,36]	AF075624
LEX024 = UM001	microsatellite	Xq	LEX024 F: GGGGTAGAGGGAAAAGAG R: TTGTGGCAGATCCCAGG UM001: F: TCAAAATCCTCAGGCTCCTC R: AACCGGAAACAGGTGCTCAC	131-150	2.0	58	[15,36,37]	AF07626 AF195123
LEX026	microsatellite	Xp	F: CAGAGTGAATGGCAAAATCC R: CAGCCCTCAAAGAGTTTAC	231-243	2.0	58	[15,38]	AF075628
LEX027	microsatellite	Xp	F: ACCACTGGGAAACTGTGTAA R: GCCCAGAAATCCGAACC	187-200	2.0	58	[15,38]	AF075629
LEX028	microsatellite	Xp	F: AACTGGGATCACAACACAT R: TTGGTACAGGAGGCTCTT	268-281	2.0	58	[15,38]	AF0756

Table 1 continued on next page

TABLE 1: Continued

Symbol	Name	Location in horse	Primers	Product size (bp)	MgCl ₂ (mM)	T _a	Reference	Acc. No
NVHEQ75	microsatellite	X	F: ATAAACCCCTGCTTACCCCTCTGT R: CAGTGACATAATCCCAAGGTGTA	98-112	2.0	58	[39]	AJ245766
UM038=	microsatellite	Xq13-q14	UM038 F: CAAGACAGAAACAGAAGAAGAC R: ATATGGCTCGCTCCTAC	113-137	2.0	58	[15,40,41]	AF195583
SGCV31			SGCV31 F: GACAGAAACAGAAGACCCGG R: CTTTCAATAATGGCTCGCTCCTAC	143	3.0	58		
VHL81	microsatellite	Xq24-q26	F: CAACATATGTACTTTGGGGAGCT R: GTCCATGAAATCTAGTTGTTGC	162-174	2.0	58	[15,42]	Y08443

List of horse chromosome X (ECAX) markers typed in the 5000_{rad} horse X hamster radiation hybrid panel, with the primer sequences and PCR conditions for each of the markers. Where available, FISH locations are presented. Data in bold (FISH location and primer sequences) represent work from this study. Note: CA = UCBEQ (new nomenclature proposed by the laboratory of marker origin).

markers (three microsatellites, five genes), the map incorporates polymorphic markers and genes that have already been assigned to ECAX by genetic linkage, synteny, and/or FISH techniques (Table 1 and Fig. 2). The map thus integrates loci from diverse sources and provides a basis for comparison of the results obtained through different mapping approaches.

The only linkage map hitherto available for ECAX comprises 13 polymorphic microsatellites [12]. Apart from *AHT28*, all other markers are present also on the RH map. Comparatively, the two maps are similar, except the position of *LEX027* is more proximal in the RH than the linkage map. The significant component, however, is that the RH map resolves the physical order for four groups of tightly linked markers that are shown as paired or triple locus clusters in the linkage map (Fig. 2). Similarly, the RH map determines the order of all the loci in the SCH map, where 23 markers are arranged in five hypothetical clusters: three on the short arm and two on the long arm [15] (Fig. 2).

Including the six new loci assigned in this study, the ECAX cytogenetic map now has 17 FISH-mapped markers, of which 11 are also present on the RH map (Fig. 2). The pter to qter arrangement of these markers is the same in the two maps. It is noteworthy that the RH map resolves the order of markers assigned to same chromosomal band by FISH (*CLCN4-COR074*, *IGSF1-GPC3*), implying that the resolution power of the 5000_{rad} RH panel is in the 1- to 3-Mb range that is suggested for overlapping FISH signals in metaphase chromosomes [17]. The above comparisons thus clearly show that the ECAX RH map generated in this study is in broad agreement with the currently available genetic linkage, syntenic, and cytogenetic maps. Despite differences in the marker sets present in each of the three maps, the RH map successfully integrates them and provides a physical order for all available and new markers.

ECAX is the second largest chromosome in the horse karyotype and, as in other mammals, forms about 5% of the total genome [18]. Though the 34 markers placed on the RH map in the present study span almost the entire length of the chromosome, the coverage is not yet even. Some "gaps" in the map are clearly evident, for example, between *LEX027* and *OTC* on the short arm, and *PGK1* and *LEX013* on the long arm, where more markers will be needed to improve the coverage and resolution. Nevertheless, the current RH map will act as a framework and conveniently incorporate new markers to generate dense maps.

There are 16 genes incorporated in the ECAX RH map. The physical order deduced for these genes facilitates comparison of their order on the X chromosomes of human, mouse, and other mammals. Despite minor deviations involving the pseudoautosomal region in mouse [19,20] and prosimians [21], synteny of the X chromosome is evolutionarily conserved across a wide range of eutherian (placental) mammals. The comparative map presented in Fig. 2 shows that this is true also for the horse. It is, however, remarkable that the order of the genes in the horse ECAX RH map is almost the same as that observed in humans. The only dif-

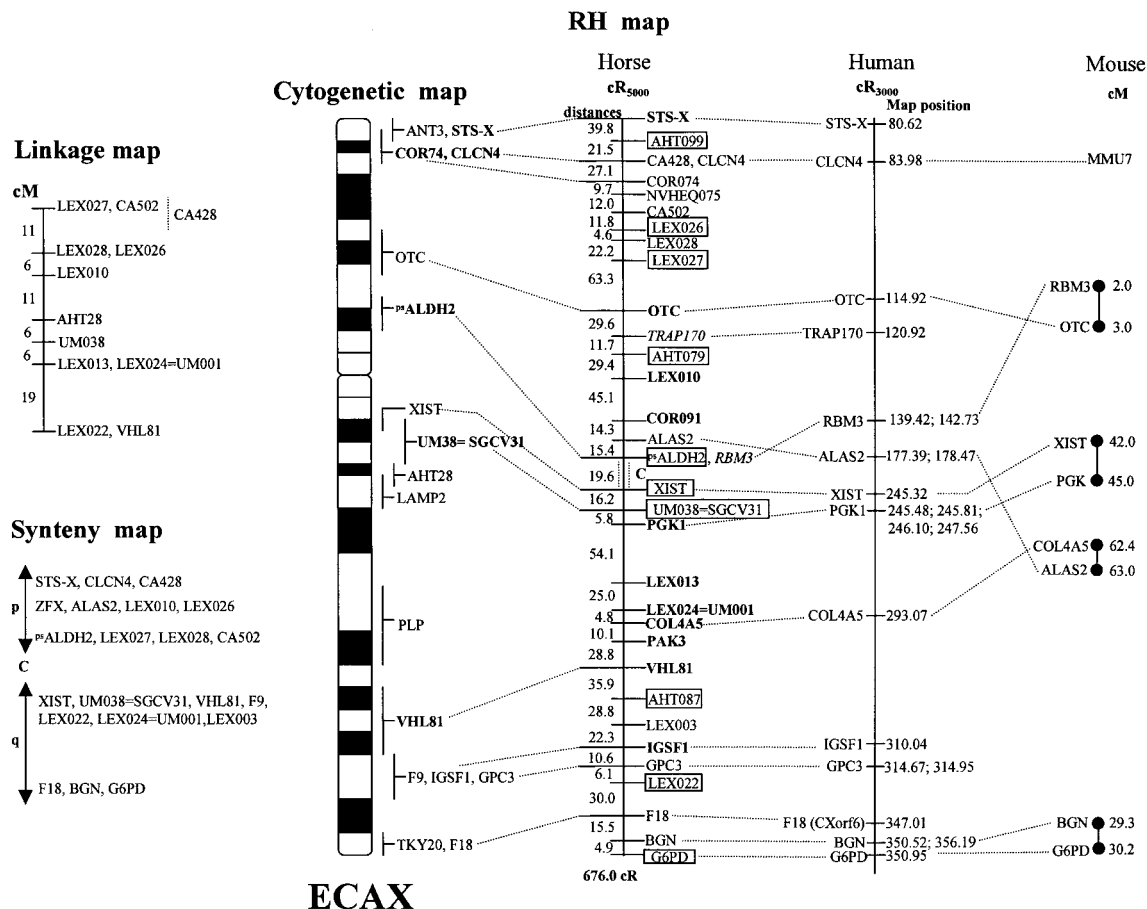


FIG. 2. A comprehensive RH map of the X chromosome in the horse (ECAX) showing the available genetic linkage [12], syntenic [15], and cytogenetic maps (left), and the comparative status of this chromosome in relation to human and mouse X chromosome maps (right). Markers in bold on the cytogenetic maps represent localizations carried out in this study. Using the RHMAP program, the 2-point analysis showed that the 34 markers formed one linkage group at lod 4. The markers were then ordered into a contiguous map using the RHMAXLINK approach. Ten markers formed the framework (in boxes) and could be ordered with odds 1000:1; further, 11 markers were added with odds 100:1 (bold) followed by another 11 with odds 10:1 (normal font). One marker, *TRAP170*, was placed at odds < 10:1. Markers *ALDH2* and *RBM3* were tightly linked and therefore placed together. On the RH map, the distances are shown in centirays (cR). Details on loci are presented in Table 1. Comparative RH mapping information in human and linkage map information for mouse was obtained from <http://www.ncbi.nlm.nih.gov/genemap/query.cgi> and http://www.informatics.jax.org/searches/marker_form.shtml.

ference lies in the relative order of *ALAS2* and *RBM3*. In horse, *ALAS2* is positioned with a maximum likelihood ratio of only 10:1. This placement might change with more markers added to the region. As far as is known, the only other species where a similar degree of conservation of gene order is observed in relation to the human X chromosome is the cat [22]. In all other species, including mouse [22–24], rat [25], goat, and cattle [26,27], several rearrangements relative to the human X chromosome have been observed.

Extensive conservation between horse and human X chromosomes was first proposed on the basis of identical banding patterns observed between the two species [28]. Molecular evidence that these banding pattern similarities reflect similarity

in gene content and order came from recent FISH and syntenic mapping of some HSAX genes to ECAX [15]. However, because the data were limited and the physical order of most of the mapped genes was not resolved, no concrete correlation could be developed. The RH map presented in this study overcomes these limitations and shows that banding pattern similarities between ECAX and HSAX indeed reflect conservation of gene order. Though minor intrachromosomal rearrangements between the X chromosomes of the two species may emerge with further expansion of the equine gene map, data hitherto obtained strongly suggest a high degree of conservation.

This is the first report providing a comprehensive map of ECAX that integrates genetic linkage, cytogenetic, and

syntenic data into a consensus format, adds new loci to the map, and provides a comparative status in relation to human, mouse, and other mammalian species. The map is expected to serve as a basic template for future expansion and generation of a high-density map of this chromosome. It is also anticipated to be an important tool for finding genes and markers related to X-linked diseases/conditions in the horse. The high degree of conservation of gene order between ECAX and HSAX will be useful to address homologous conditions in horse by targeting candidate genes in humans.

MATERIALS AND METHODS

RH panel typing and analysis. A 5000_{ad} whole-genome RH panel comprising 93 hybrid cell lines [11] was used for the study. A set of markers, known to be located on ECAX, was chosen from published data, HorseMap Database, and other sources. Equine sequences submitted to the NCBI database (<http://www.ncbi.nlm.nih.gov/>) were used to obtain new primers for some of the genes that were syntenic mapped using comparative anchor-tagged sequence (CATS) primers [13,15]. PCR conditions for each primer pair were optimized to get horse-specific amplification against hamster background in the hybrid cell lines. Marker names, symbols, primer sequences, PCR conditions, and references are presented in Table 1. All markers were typed in duplicate along with negative and test (horse and hamster genomic DNA) controls. PCR products were resolved on 2.5% agarose gels and scored manually. In cases where two primer pairs had the same PCR conditions and the sizes of expected PCR products differed from each other by more than 50 bp, markers were typed in duplexes. The typing results were subjected to two-point linkage analysis and ordering with RHMAXLIK, using the RHMAP 3.0 software [16].

BAC library screening and FISH. Two equine BAC libraries (INRA and TAMU) were PCR screened for ECAX markers as described [29]. DNA isolated from positive BACs was individually biotin labeled and used as hybridization probe on equine metaphase chromosomes. DNA labeling, *in situ* hybridization, and fluorescent signal detection were carried out as described [30].

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