

# Report of the International Equine Gene Mapping Workshop: male linkage map

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## Summary

The goal of the First International Equine Gene Mapping Workshop, held in 1995, was the construction of a low density, male linkage map for the horse. For this purpose, the International Horse Reference Family Panel (IHRFP) was established, consisting of 12 paternal half-sib families with 448 half-sib offspring provided by 10 laboratories. Blood samples were collected and DNA extracted in each laboratory and sent to the Lexington laboratory (KY, USA) for dispatch in aliquots to 14 typing laboratories. In total, 161 markers (144 microsatellites, seven blood groups and 10 proteins) were tested for all families for which the sire was heterozygous. Genealogies and typing data were sent for analysis to the INRA laboratory (Jouy-en-Josas, France) according to a specific format and entered into a database with input verification and output processes. Linkage analysis was performed with the CRIMAP program. Significant linkage was detected for 124 loci, of which 95 were unambiguously ordered using a multipoint analysis with an average spacing of 14.2 cM. These loci were distributed among 29 linkage groups. A more comprehensive analysis including synteny group data and FISH data suggested that 26 autosomes out of 31 are covered. The complete map spans 936 cM.

*Keywords:* horse, genome, linkage group, segregation, mapping

## Introduction

The domestic horse is economically important for sport, recreation and entertainment. Genet-

ics has been an important part of horse breeding and young horses may command high purchase prices based on their pedigrees. Horse breeders pay close attention to pedigrees, avoid close inbreeding and apply selection to develop a wide range of athletic types. At the same time breeders are aware that many equine health problems have strong genetic components, including infectious diseases, allergic conditions and musculoskeletal diseases. Genomic approaches may be used to identify the genetic basis underlying more complex hereditary traits associated with performance and health and even suggest methods to improve the quality of horses available to horsemen.

An important first step in genomic studies of any species has been development of a genetic map. Gene maps have been created for other agriculturally important animals, including cattle, sheep, goats, pigs, chickens and some aquaculture species. With this in mind, the co-authors of this manuscript enjoined a workshop in Fall 1995, conducted by the Dorothy Russell Havemeyer Foundation, Inc. in order to create a gene map for the horse and to collaborate in the overall area of genomics for the horse. The approaches used were based on those found successful for other species, namely development of microsatellite DNA markers, selection of a set of reference families and testing those families for linkage of the markers. The purpose of this manuscript is to document the development of the International Horse Reference Family Panel (IHRFP) and to report the initial linkage map for the horse generated using this resource.

## Materials and methods

### *Participants*

Participating laboratories are identified in Table 1 along with an acronym which was used in the remaining tables to attribute the contribution of each laboratory to the workshop.

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**Table 1.** Participating laboratories

Code	Location
CAR	Shelterwood Laboratories, Carthage TX, USA
COR	Cornell University, Ithaca, NY, USA
CZB	University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
DAV	University of California, Davis, CA, USA
FOS	Applied Biosystems Inc, Foster City, CA, USA
JEJ	INRA Centre de Recherches de Jouy, Jouy-en-Josas, France
LEX	University of Kentucky, Gluck Cntr., Lexington, KY, USA
MIN	University of Minnesota, St. Paul, MN, USA
MUC	University of Munich, Munich, Germany
NEW	Animal Health Trust, Newmarket, UK
NZM	Massey University, Massey, New Zealand
OSL	Norwegian College of Veterinary Medicine, Oslo, Norway
POZ	Agricultural University of Poznan, Poznan, Poland
QLD	University of Queensland, Brisbane, Australia
TAM	Texas A & M, College Station, USA
UKY	University of Kentucky, Horse Blood-typing, Lexington, KY, USA
UPP	Swedish University of Agricultural Sciences, Uppsala, Sweden
VHL	Dr van Haeringen Laboratorium, Wageningen, The Netherlands
VIC	Victoria Institute of Animal Science, Australia
WLD	Stormont Laboratories, Inc, Woodland, CA, USA

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Conditions of participation included contribu-  
tion of DNA for the reference families and/or  
typing of at least five markers on the reference  
families.

#### Reference family panel

Paternal half-sib families were chosen as the  
basis for the International Horse Reference  
Family Panel (IHRFP) based on recommenda-  
tions of Da & Lewin (1995). There were 12  
reference families consisting of between 21 and  
52 offspring for each family for a total of 448  
offspring. Informative meioses for dams were  
not included in the analysis.

DNA aliquots of the family members were  
provided by the 10 laboratories identified in  
Table 2. DNA was isolated at the laboratory of  
origin using different but commonly accepted  
methods. The workshop requirement was that  
the DNA be of sufficient quality for polymerase  
chain reaction (PCR) amplification and suffi-  
cient quantity for testing many markers (milli-  
gram quantities).

#### Markers

A total of 161 markers were tested on the  
reference family and included seven blood  
group loci, 10 biochemical loci and 144 micro-  
satellite DNA loci. The markers are listed in  
Table 3 with a citation describing the discovery  
of the locus or the test methods (column 2).  
*VHL145* is a microsatellite locus not previously

reported but which was detected using the  
primers: 5'-GCAAGCACAAATGAATACTCATG-  
3' (forward) and 5'-AGTTTGGTTTCTGGA-  
GAATTGTC-3' (reverse) (W van Haeringen & H  
van Haeringen & JA Lenstra, personal commu-  
nication). Blood group loci, also called erythro-  
cyte antigen loci (EA), included *A (EAA)*, *C  
(EAC)*, *D (EAD)*, *K (EAK)*, *P (EAP)*, *Q (EAQ)*-  
*U (EAU)*. Protein loci included *alpha-1-B  
glycoprotein (A1BG)*, *albumin (ALB)*, *vitamin D  
binding protein (GC)*, *serum esterase (ES)*,  
*hemoglobin alpha (HBA)*, *phosphoglucomutase  
(PGM)*, *glucose phosphate isomerase (GPI)*,  
*transferrin (TF)*, *6-phosphogluconate dehydro-  
genase (PGD)* and *protease inhibitor (PI)*. Eleven  
of the 12 families were typed for these loci. For  
many of the families, blood types were available  
for the dams as well and this was information  
incorporated into the CRIMAP analysis, but  
only to determine the genetic contribution by  
the stallion.

#### CRIMAP analysis

Typing data were sent electronically and  
entered into a genotypic database (MAPGENA)  
specially adapted to this function for parentage  
control, storage and treatment. Maximum like-  
lihood estimates of recombination fraction ( $\theta$ )  
were calculated using the two-point option of  
the CRIMAP program version 2.4 (Green *et al.*  
1990), with a significant lodscore threshold > 3  
for the determination of linkage groups. Multi-  
point analysis was then performed on data from

loci included in these linkage groups to determine their best linear order. The BUILD option was used starting with 2–3 loci about 10 cm apart with a threshold of acceptance of 1.5. The best linear order was checked with the FLIPS option. Map distances were calculated using the Kosambi function as reported in the BUILD option. Assignment was accepted if there was not compelling conflicting evidence against linkage, including published and unpublished information.

#### Assignment of linkage groups to chromosomes

Assignment of linkage groups to chromosomes was based on *in situ* hybridization (ISH) or synteny mapping. Chromosome location and the citation of ISH mapped loci are presented in Table 3, columns 5 and 6. The synteny group numbers were assigned based on the best information available with regard to chromosome assignment (Shiue *et al.* 1999). Likewise, linkage groups were assigned to chromosomes, based on published and unpublished information, when they contain a marker either directly mapped by ISH (Table 3, columns 5 and 6), previously linked to chromosome mapped markers (e.g. *HTG4*, linked with FISH mapped *DNA-PKcs* to ECA 9 per Bailey *et al.* 1997), or included in synteny groups anchored to chromosomes through FISH or Zoo-FISH mapping as reported by Raudsepp *et al.* (1996).

### Results

Heterozygosity of each stallion for the 161 loci is presented in Table 2. It ranged from 38% and 39% for the two Thoroughbred stallions (J and E) to 59% for the Selle Français stallion (G) and the crossbred pony stallion (D).

The stallions were tested for 161 markers, of which 160 were informative for at least one family. The number of possible genotypes was 72 128 (161 loci × 448 offspring). Of the 1932 sire genotypes (12 sires × 161 loci), 1003 (52%) were heterozygous and the vast majority of their offspring were tested. The results of this testing produced 24 272 informative meioses (33.6% of the total possible genotypes). Mares transmitted a total of 1003 alleles for an average of 6.2 alleles per locus. However, the number of alleles may be slightly over reported due to artifacts in reporting and testing. The number of informative meioses for each locus is reported in Fig. 1, column 9; the number of families informative for each locus is reported in column 10. The average number of informative meioses per locus was 151.

#### Linkage groups and map coverage

The CRIMAP program was used to compute lod scores based on the reported segregation data and construct the most probable linkage map presented in Fig. 1 and listed in Table 3. Of the 160 markers which were informative, 124 (78%) showed significant linkage to other markers, 95 were unambiguously ordered and all 124 were distributed among 29 linkage groups (Fig. 1). Comparison of the markers to previously reported mapping data, as represented in Table 3, columns 7–9, indicated that these linkage groups were distributed among 26 of the 31 autosomes. Six chromosomes were not assigned a linkage group, specifically ECA 6, 7, 26, 27, 28 and 31. One linkage group (WS-A) was identified but not assigned to a chromosome. Twenty-eight linkage groups were assigned to a chromosome, 18 by FISH and synteny mapped markers to 17 chromosomes

**Table 2.** International Horse Reference Family Panel (IHRFP)

Family*	Source†	Breed	Number of offspring	Percent informative loci (Stallions)
A	LEX	American Standard bred pacers	31	57
B	POZ	Anglo-Arabian	52	54
C	POZ	Wielkopolska/Hanoverian	35	52
D	UKY	Pony (Shetland crosses)	49	59
E	CZB	Thoroughbred	41	39
F	DAV	Quarter Horse	47	50
G	JEJ	Selle Français	40	59
H	UPP	Swedish Standard bred trotters	36	55
J	NZM	Thoroughbred	40	38
K	MIN	Crossbred (including draft)	23	55
L	MUC	German riding horse	21	45
N	MUC	German riding horse	33	57

\*Family acronym.

†Laboratory providing the family.

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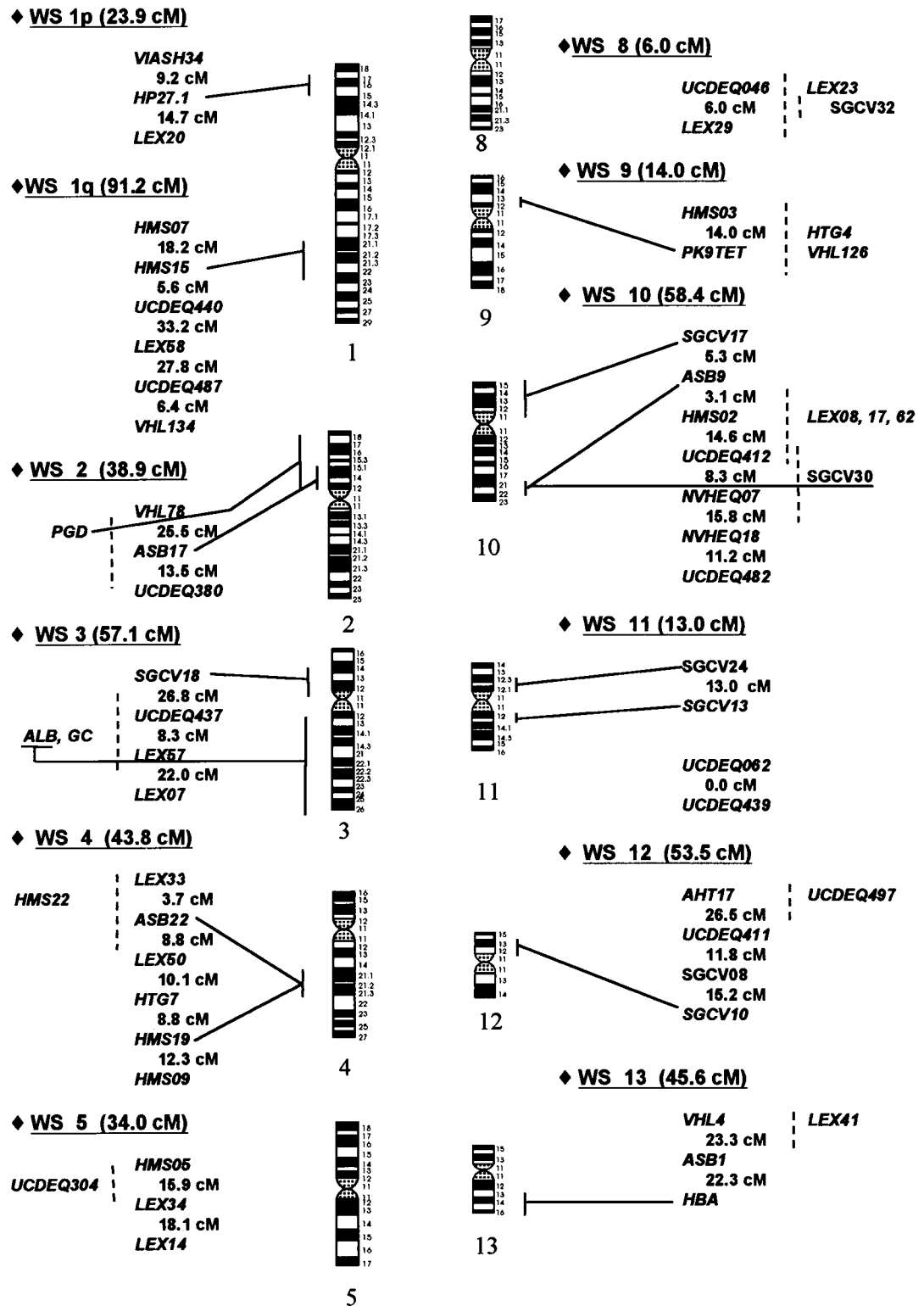


Fig. 1. Markers shown on the map were significantly linked by a two-point analysis at odds greater than 1000 : 1 using the CRI-MAP program (Green *et al.* 1990). Those to the right or left of the main map could not be given a linear position at odds greater than 30 : 1. The dotted line indicates the range of their most likely position. Distances between loci are shown between markers and are expressed in centimorgans (cM) using the Kosambi mapping function. The workshop linkage group assignment is designated with a 'WS' and a number corresponding to the most probable chromosome assignment followed by the total distance spanned by markers on the chromosome. Idio-grams, adapted from ISCNH (1997), are shown for each of the mapped chromosomes and the position of physically mapped markers are indicated.

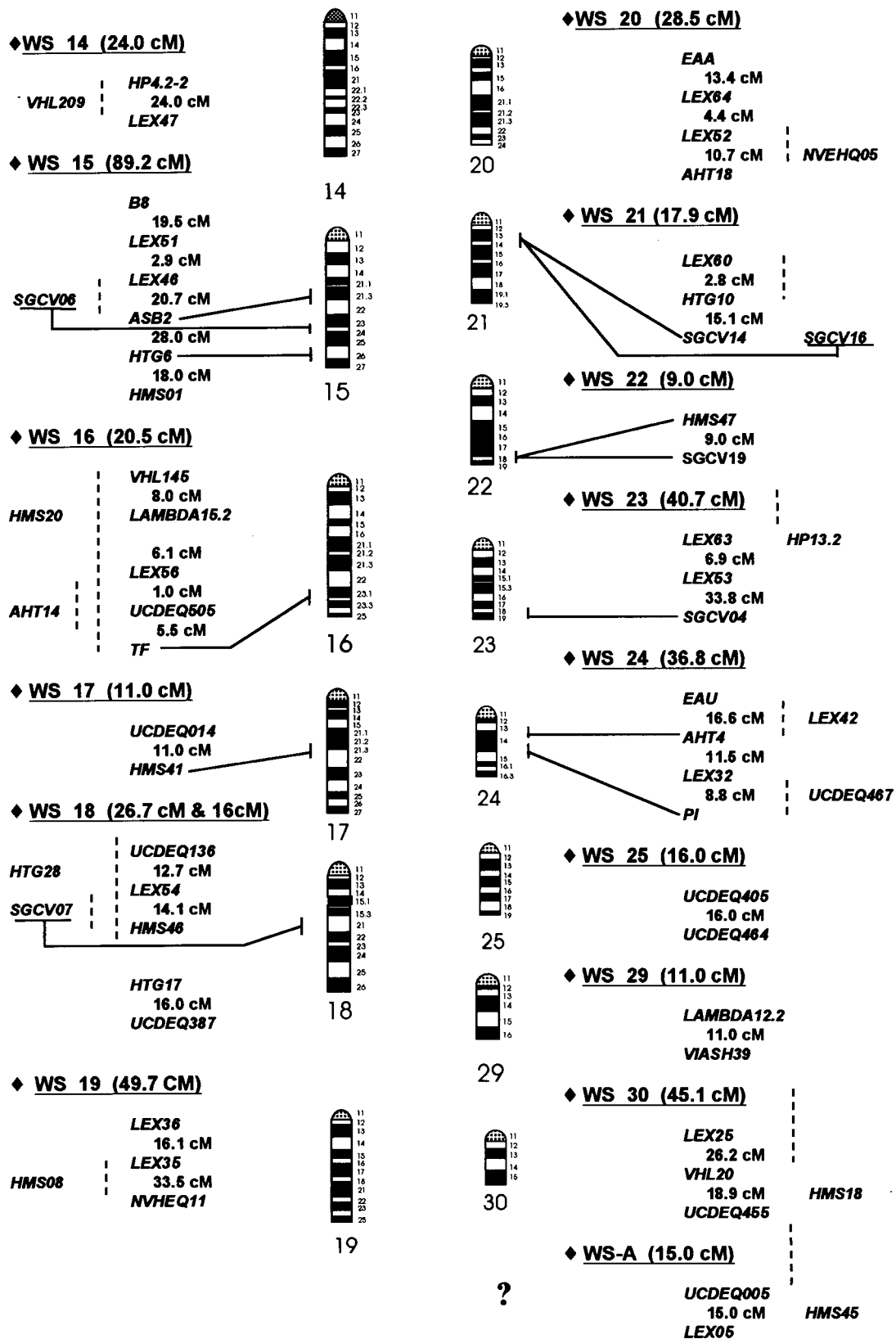


Fig. 1 Continued

and 10 only by synteny mapping. The markers spanned 936.5 cM and represent only the meiotic information from the male. The average

interval between loci is 14.2 cM and ranges from 0 to 33.8 cM. Assuming that an average distance of 15 cM is covered outside each

Table 3. Description of loci used in this study

Loci	Reference†	Test lab‡	Inform. meioses§	No. infom. sires¶	WS linkage††	UCD synteny##	UPP linkage\$\$	Physical map location	Reference¶¶
A1BG	6	*	0	0			UPP10		
AHT14	37	NEW	31	1	WS16				
AHT17	37	NEW	275	9	WS12				
AHT18	37	NEW	115	6	WS20				
AHT19	37	NEW	89	7					
AHT4	3	FOS	159	8	WS24	UCD24	U	24q14	20
AHT5	3	FOS	188	8		UCD06	U		
ALB	35	*	80	3	WS03		UPP03	3q14·3	20
ASB1	7	QLD	244	10	WS13	UCD13	UPP13		
ASB2	7	FOS	181	9	WS15	UCD15	UPP15	15q21·3-q23	7
ASB9	7	QLD	297	11	WS10	UCD10	UPP10	10q21-q23	7
ASB17	7	QLD	319	11	WS02	UCD02	UPP02	2p14-p15	7
ASB22	7	QLD	265	10	WS04		UPP04	4q21	7
B8	31	NEW	260	8	WS15		U	15q14-q21	31
EAA	36	*	81	8	WS20		U		
EAC	36	*	26	7					
EAD	36	*	278	9			U		
EAK	36	*	37	3					
EAP	36	*	94	7					
EAQ	36	*	35	3			UPP-C		
EAU	36	*	114	8	WS24				
ES	18	*	191	6			U		
GC	26	*	22	1	WS03				
GPI	2	*	40	2				10pter	25
HP13·2	32	TAM	205	9	WS23			24q15	32
HP27·1	32	TAM	159	6	WS01			1p16-p15	32
HP4·2-2	32	TAM	116	4	WS14				
HBA	4	*	63	3	WS13		UPP13	13qter	33
HMS01	22	JEJ	230	8	WS15	UCD15	UPP15		
HMS02	22	JEJ FOS	290	11	WS10	UCD10	UPP10		
HMS03	22	JEJ FOS	276	11	WS09	UCD09	UPP09		
HMS05	22	JEJ	148	6	WS05	UCD05	UPP05		
HMS06	22	JEJ FOS	186	7		UCD04	UPP04		

Table 3. Continued

Loci	Reference†	Test lab‡	Inform. meioses§	No. infom. sires¶	WS linkage††	UCD synteny‡‡	UPP linkage§§	Physical map location	Reference¶¶
HMS07	22	JEJ FOS	237	8	WS01	UCD01	U		
HMS08	22	JEJ	166	7	WS19	UCD19			
HMS09	19	JEJ	63	3	WS04	UCD04			
HMS15	23	JEJ	196	8	WS01	UCD01		1q21-q23	20
HMS18	19	JEJ	124	7	WS30	UCD30	UPP30		
HMS19	19	JEJ	50	3	WS04	UCD04	UPP04	4q21	20
HMS20	23	JEJ	157	10	WS16	UCD16	UPP16		
HMS22	19	JEJ	133	8	WS04	UCD04			
HMS23	19	JEJ	46	2		UCD10	UPP10		
HMS25	19	JEJ	98	5		UCD17			
HMS41	19	JEJ	164	7	WS17	UCD17		17q21.3-q22	21
HMS45	19	JEJ	37	2	WS-A	UCD-A	UPP-B		
HMS46	19	JEJ	165	7	WS18	UCD18	UPP18		
HMS47	19	JEJ	91	5	WS22	UCD22	UPP22	22q19	20
HTG4	15	FOS	161	8	WS09	UCD09	UPP09		
HTG6	15	FOS	160	7	WS15	UCD15	UPP15	15q26-q27	20
HTG7	30	FOS	138	5	WS04	UCD04	UPP04		
HTG10	30	FOS	253	9	WS21	UCD21	UPP21		
HTG17	28	UPP	152	6	WS18		U		
HTG20	28	UPP	136	5					
HTG21	28	UPP	187	7					
HTG28	28	UPP	40	2	WS18				
HTG31	28	UPP	97	4					
LAMBDA12-2	32	TAM	243	8	WS29				
LAMBDA15-2	32	TAM	138	6	WS16				
LEX05	42	UKY	45	5	WS-A	UCD-A	UPP-B		
LEX07	42	DAV	126	9	WS03	UCD03	UPP03		
LEX08	42	DAV	121	6	WS10	UCD10	UPP10		
LEX14	42	LEX	190	7	WS05	UCD05	UPP05		
LEX17	8	UKY	113	9	WS10	UCD10	UPP10		
LEX20	8	UKY	217	8	WS01	UCD01	UPP01		
LEX23	8	DAV	256	3	WS08	UCD06	UPP06		
LEX25	8	UKY	184	7	WS30	UCD30	U	30q	5

Table 3. Continued

Loci	Referencet	Test lab†	Inform. meioses\$	No. infom. sires¶	WS linkage††	UCD synteny‡‡	UPP linkage§§	Physical map location	Reference¶¶
LEX29	8	LEX	202	8	WS08	UCD06	UPP06		
LEX32	8	DAV	240	10	WS24	UCD24	U		
LEX33	8	DAV	215	10	WS04	UCD04	UPP04		
LEX34	9	LEX	98	8	WS05	UCD05	UPP05		
LEX35	9	UKY	176	9	WS19	UCD19	UPP19		
LEX36	9	CAR	184	7	WS19	UCD19			
LEX37	9	CAR	286	1		UCD21	U		
LEX38	9	CAR	283	6		UCD07	UPP07		
LEX41	9	UKY	174	9	WS13	UCD13			
LEX42	9	WLD	242	2	WS24	UCD24			
LEX43	9	CAR	162	4		UCD14			
LEX44	9	UKY	16	10		UCD26			
LEX46	9	WLD	141	5	WS15	UCD15			
LEX47	9	UKY	226	8	WS14	UCD14			
LEX50	10	UKY	67	7	WS04	UCD04			
LEX51	10	LEX	261	9	WS15	UCD15			
LEX52	10	UKY	188	9	WS20	UCD20			
LEX53	10	UKY	131	10	WS23	UCD23			
LEX54	10	WLD	182	5	WS18	UCD18			
LEX55	10	CAR	239	1		UCD17			
LEX56	10	UKY	237	10	WS16	UCD16			
LEX57	10	LEX	265	4	WS03	UCD03			
LEX58	10	LEX	39	9	WS01	UCD01			
LEX59	10	CAR	265	1		UCD16			
LEX60	10	WLD	72	7	WS21	UCD21			
LEX62	10	WLD	280	7	WS10	UCD10			
LEX63	10	WLD	16	9	WS23	UCD10			
LEX64	11	LEX	238	9	WS20	UCD23			
NVHEQ05	34	OSL	114	6	WS20				
NVHEQ07	34	OSL	140	7	WS10				
NVHEQ11	34	OSL	199	9	WS19				
NVHEQ18	34	OSL	281	12	WS10				
NVHEQ24	34	OSL	49	2					

Table 3. Continued

Loci	Reference†	Test lab‡	Inform. meioses§	No. infom. sires¶	WS linkage††	UCD synteny‡‡	UPP linkage§§	Physical map location	Reference¶¶
<i>PGD</i>	2	*	55	2	WS02		U	2p	24
<i>PGM</i>	2	*	38	1			UPP05		
<i>PI</i>	18	*	197	8	WS24			24q15-q16	20; 27
<i>PK9TET</i>	32	TAM	108	5	WS09			9p14-p12	1
<i>SGCV01</i>	19	JEJ	31	2		UCD22	UPP-A	19q21	19
<i>SGCV04</i>	19	JEJ	94	4	WS23	UCD23	U	23q19	19
<i>SGCV06</i>	19	JEJ	91	5	WS15	UCD15	UPP15	15q24	19
<i>SGCV07</i>	19	JEJ	181	8	WS18	UCD18	UPP18	18q21	19
<i>SGCV08</i>	19	JEJ	177	6	WS12		U	19	19
<i>SGCV10</i>	19	JEJ	127	5	WS12	UCD12	U	12p13	19
<i>SGCV13</i>	19	JEJ	190	8	WS11	UCD11	UPP11	11q12	19
<i>SGCV14</i>	19	JEJ	104	3	WS21	UCD21	UPP21	21q13	19
<i>SGCV16</i>	19	JEJ	221	9	WS21	UCD21	UPP21	21q13	19
<i>SGCV17</i>	19	JEJ	80	5	WS10	UCD10	UPP10	10q15-q21	20
<i>SGCV18</i>	19	JEJ	149	6	WS03	UCD03	UPP03	3p13-p14	19
<i>SGCV19</i>	19	JEJ	140	6	WS22		UPP22	22q19	19
<i>SGCV23</i>	19	JEJ	233	10		UCD04	UPP04	4q27-cen15	19
<i>SGCV24</i>	19	JEJ	152	6	WS11		UPP11	11p12	19
<i>SGCV28</i>	19	JEJ	91	5		UCD07	UPP07		
<i>SGCV30</i>	19	JEJ	195	8	WS10	UCD10	UPP10	10qter	20
<i>SGCV32</i>	19	JEJ	141	7	WS08		UPP06	9q15	19
<i>TF</i>	18	*	194	7	WS16		UPP16	16q23	27
<i>UCDEFQ005</i>	12	DAV	152	6	WS-A	UCD-A			
<i>UCDEFQ014</i>	12	DAV	188	8	WS17	UCD17			
<i>UCDEFQ046</i>	12	DAV	195	8	WS08	UCD06			
<i>UCDEFQ062</i>	12	DAV	95	4	WS11	UCD11			
<i>UCDEFQ136</i>	13	DAV	254	9	WS18	UCD18			
<i>UCDEFQ304</i>	14	DAV	171	6	WS05	UCD05			
<i>UCDEFQ380</i>	14	DAV	171	7	WS02	UCD02			
<i>UCDEFQ387</i>	14	DAV	91	4	WS18	UCD18			
<i>UCDEFQ405</i>	13	DAV	235	7	WS25	UCD25			
<i>UCDEFQ411</i>	14	DAV	198	7	WS12	UCD12			
<i>UCDEFQ412</i>	13	DAV	209	8	WS10	UCD10			

Table 3. Continued

Loci	Reference†	Test lab‡	Inform. meioses§	No. inform. sires¶	WS linkage††	UCD synteny‡‡	UPP linkage\$\$\$	Physical map location	Reference¶¶
UCDEQ425	13	DAV	231	8		UCD-C			
UCDEQ437	13	DAV	253	9	WS03	UCD03			
UCDEQ439	14	DAV	21	1	WS11	UCD11			
UCDEQ440	14	DAV	195	7	WS01	UCD01			
UCDEQ455	14	DAV	101	4	WS30	UCD30			
UCDEQ457	14	DAV	80	3		UCD11			
UCDEQ464	14	DAV	159	6	WS25	UCD25			
UCDEQ465	14	DAV	82	4		UCD-D			
UCDEQ467	13	DAV	160	7	WS24	UCD24			
UCDEQ482	14	DAV	130	5	WS10	UCD10			
UCDEQ487	13	DAV	190	7	WS01	UCD01			
UCDEQ497	14	DAV	129	6	WS12	UCD12			
UCDEQ505	13	DAV	288	10	WS16	UCD16			
VHL20	38	FOS	261	10	WS30	UCD30	UPP30	30q	5
VHL47	39	VHL	180	9	WS13		U		
VHL78	39	VHL	97	5	WS02		U		
VHL81	39	VHL	13	1					
VHL123	39	VHL	66	3					
VHL126	39	VHL	29	2	WS09				
VHL134	39	VHL	162	8	WS01				
VHL137	39	VHL	35	1					
VHL145	41	VHL	258	9	WS16				
VHL150	40	VHL	53	3					
VHL161	39	VHL	20	1					

Table 3. Continued

Loci	Reference†	Test lab‡	Inform. meioses§	No. infom. sires¶	WS linkage††	UCD synteny##	UPP linkage\$\$	Physical map location	Reference¶¶
VHL204	39	VHL	37	1					
VHL209	39	VHL	70	4	WS14				
VHL219	39	VHL	34	2					
VIASH34	16	VIC	173	8	WS01	UCD01	U		
VIASH39	17	VIC	131	7	WS29	UCD29	U		

†Citation numbers correspond to the following: 1. Bailey *et al.* (1997); 2. Bengtsson & Sandberg (1973); 3. Binns *et al.* (1995); 4. Bowling *et al.* (1988); 5. Bowling *et al.* (1997); 6. Braend (1970); 7. Breen *et al.* (1997); 8. Coogle *et al.* (1996b); 9. Coogle *et al.* (1996c); 10. Coogle & Bailey (1999); 11. Coogle & Bailey (1999); 12. Eggleston Stott *et al.* (1996); 13. Eggleston Stott *et al.* (1997); 14. Eggleston Stott *et al.* (1999); 15. Ellegren *et al.* (1992); 16. Ewen & Matthews (1994b); 17. Ewen & Matthews (1994a); 18. Gahne *et al.* (1966); 19. Godard *et al.* (1997); 20. Godard *et al.* (1998); 21. Godard *et al.* (1999); 22. Guerin *et al.* (1994); 23. Guerin & Bertaud (1996); 24. Gu *et al.* (1992); 25. Harbitz *et al.* (1990); 26. Juneja *et al.* (1978); 27. Lear *et al.* (1998); 28. Lindgren *et al.* (1998); 29. Lindgren *et al.* (1999); 30. Marklund *et al.* (1994); 31. Marti *et al.* (1998); 32. Mathiason *et al.* (1993); 33. Oakenfull *et al.* (1993); 34. Røed *et al.* (1997); 35. Stormont & Suzuki (1963); 36. Stormont & Suzuki (1964); 37. Swinburne *et al.* (1997); 38. van Haeringen *et al.* (1994); 39. van Haeringen *et al.* (1998a); 40. van Haeringen *et al.* (1998b); 41. W. & H. Van Haeringen and J.A. Lenstra, this report; 42. Coogle *et al.*, (1996a).

‡Test labs identified by acronyms (Table 1). Asterisk denotes testing of blood typing markers by DAV, JEJ, MUC, NZM, UKY and UPP.

§Number of offspring for whom the sire contribution could be identified.

¶Number of the 12 sires that were heterozygous.

††Assignment to a workshop linkage group is designated with a 'WS' followed by a letter or a number. Numbers correspond to the expected chromosome assignment. WS-A may correspond to a linkage group on chromosome 27. An empty box denotes the marker was not found to be linked to any other loci.

##Those loci which were also placed on the synteny map reported by Shiue *et al.* (1999) are identified in this column with 'UCD' plus a number or letter designating the synteny group. The number corresponds to the probable chromosome assignment at the time of their report.

\$\$Those loci which were tested by this workshop and by Lindgren *et al.* (1998) are identified in column 4. An empty box indicates the locus was not tested by Lindgren and co-workers. A 'U' denotes that the locus was tested but not found linked. A 'UPP' followed by a number or letter denotes the locus was tested and found to be linked. The numbers correspond to numbers assigned by Lindgren and co-workers in their report and indicate the probable chromosome assignment. Letter designations were added by the authors of this report to differentiate between the unassigned linkage groups.

¶¶References for the physical mapping of loci. Reference numbers correspond to citations in footnote 1.

linkage group by loci located at both ends, the map length is 1661 cM, that is,  $\approx 55\%$  of the genome. The overall density of the map is 7.6 cM when all markers within linkage groups are included in the calculation. Another 37 markers did not show linkage to other markers suggesting that they fell outside the range of statistical detection of linkage. Presumably, these markers will require the addition of new markers to be included in linkage groups.

This map was compared to the male linkage map reported by Lindgren *et al.* (1998). Of the 161 markers tested for this report and 140 markers tested for that report, 73 common markers are identified in Table 3, column 8. Of these 73 markers, 45 were mapped to the same linkage group, 15 were assigned to linkage groups by the workshop (this report) but not by Lindgren and co-workers, nine were assigned to linkage groups by Lindgren and co-workers but not by the workshop and four were not assigned to the linkage map in either study.

## Discussion

One of the major goals of the workshop was to create a male linkage map for the horse based on the use of a common set of reference families. The half-sib, stallion based family model was chosen based on the recommendation of Da & Lewin (1995). Based on the large family sizes and the cooperation among laboratories, the study was effective and detected linkage among 124 of the 160 segregating loci. The linkage map presented in Fig. 1 represents the most probable linkage map based on these data. As the workshop continues to add markers to the linkage map some of the ambiguous linkage orders, identified to the right of the linkage groups, will be resolved.

Lindgren *et al.* (1998) recently reported a linkage map based on investigations of 140 markers in eight half-sibling families with a total of 263 offspring. Based on segregation of markers from the stallions, 100 markers were found in 25 linkage groups, 22 of which could be mapped to 18 autosomes. Comparison of the 73 overlapping markers for the workshop map and the map of Lindgren and co-workers revealed good agreement (Table 3). There were small discrepancies in distances measured between some loci and in the order of genes in the linkage group 4 (specifically, *LEX33-ASB22-HTG7-HMS19* vs. *HMS19-LEX33-ASB22-HTG7*). The differences may reflect variation in the amount of data available for each locus, variation in the recombination frequencies between loci for different stallions or testing

errors. Some of these differences may be resolved with the addition of data for new markers. However, with these few exceptions there was good agreement between the maps.

The 'WS' numbers assigned to linkage groups in Table 3 and Fig. 1 reflect the expected chromosome assignment. In subsequent reports we anticipate describing specific evidence for assigning the linkage groups to chromosomes and replacing the designation 'WS' with 'ECA'. Difficulties have been encountered based on the inadvertent use of chimeric clones for *in situ* hybridization analyses or with difficulties identifying chromosomes. This may explain the discrepancies between the workshop assignments and the cytogenetic assignments observed for *SGCV32* and *SGCV08* (Table 3). To help resolve the problem of chromosome assignment, the workshop published a new standard for karyotypic assignment for the horse (ISCNH 1997). Nevertheless, some uncertainty remained and, for example, the linkage group WS08 is the same as synteny and linkage groups UCD06 and UPP06. The difference in numbering reflects more recently developed information indicating that linkage group formerly thought to reside on ECA06 probably belongs to ECA08 (unpublished data).

In summary, the workshop effort has been the product of generous collaboration and the sharing of information among many laboratories. The International Horse Reference Family Panel DNA will continue to be a valuable resource which will allow laboratories to independently investigate linkage relationships among markers and at the same time contribute to the overall quality of the gene map. Phase II of the workshop is underway with the goal of testing an additional 150 markers against the IHRFP DNA and constructing a linkage map with over 300 markers. Subsequent workshop activities may develop other mapping resources such as a radiation hybrid panel for further development of the equine genome map.

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