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Radiation hybrid mapping of 75 previously unreported equine microsatellite loci

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Source/description: Horse genomic DNA was digested with the restriction enzyme *Mbo*I, size selected by gel electrophoresis for fragments between 200 and 1200 bp, and ligated into the *Bam*HI site of the M13 phage vector. Clones containing a potential microsatellite were identified by screening the library with [^{32}P] 5′ end-labelled oligo $[dCA]_{16}$ and oligo $[dGT]_{16}$ probes. The DNA was isolated from positive plaques and the inserts were sequenced using an ABI 3100 automated sequencer. Primer pairs for PCR amplification of the markers were developed using the PRIMER program (Version 0.5; M. J. Daly, S. E. Lincoln and E. S. Lander, unpublished). Sequence accession numbers, repeat motif, PCR primer pairs and product sizes based on the cloned sequences are provided in Table 1.

PCR conditions: Polymerase chain reactions for determination of microsatellite polymorphisms were performed in 15 μl volumes consisting of the following reagents: 25 ng DNA; 1X PCR buffer (Qiagen, Valencia, CA, USA); 1.5 mM $MgCl_2$; 25 μM each of dCTP, dGTP and dTTP; 6.25 μM dATP; 0.125 μCi [α-^{32}P] dATP; 0.45 U HotStar *Taq* polymerase (Qiagen) and 5 pmol of each primer. Amplification was done using a MJ Research PTC100 thermocycler (Watertown, MA, USA) under the following conditions: initial 20 min denaturation at 95 °C; 30 cycles of 94 °C for 30 s, annealing temperatures that ranged from 54–62 °C for 30 s and 72 °C for 30 s; and a final 5 min extension at 72 °C. Reaction products were electrophoresed through 7% acrylamide denaturing gels on BioRad SequiGen GT 38 cm ×50-cm plate sequencing gel units (Hercules, CA, USA), in the presence of 1X TBE, and fragments detected using autoradiography.

The PCR for typing markers on the 5000 rad whole genome equine radiation hybrid panel comprising 92 horse × hamster hybrid cell lines[1] contained 50 ng DNA; 5 pmol of each primer; 2.0 mM $MgCl_2$; 0.3 U HotStar *Taq* Polymerase (Qiagen) and 10X PCR buffer which contained Tris-Cl, KCl and $(NH_4)_2SO_4$ (Qiagen). Markers were typed in duplicate, separated by electrophoresis on 2.5% agarose gels, and scored manually.

Allele frequencies: The DNA from 12 stallions of the Equine Genome Mapping Workshop International Reference Family,[2] and/or three horses (one stallion and two mares) from the parental generation of the Newmarket full-sibling reference family,[3] was amplified. The number of alleles observed in this sample population ranged from 1 to 10 (Table 1).

Radiation hybrid mapping and chromosomal assignments: The PCR typing of the 75 microsatellite markers on the 5000 rad equine RH panel and data analysis was performed as described in detail by us.[1] Two point analysis with the RHMAPPER software was carried out to assign markers to map positions on the current equine radiation hybrid map[1] at LOD ≥ 12.0.[1] Chromosomal assignments and the nearest linked marker for all new equine microsatellites are provided in Table 1.

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References

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Table 1 Equine microsatellite loci *UMNe84–UMNe376*.

Marker	Accession number	Repeat motif	Primer 1 (5′ → 3′)	Primer 2 (5′ → 3′)	Product size (bp)	Heterozygotes	Alleles	Chromosome (by RH mapper)	Closest Marker(s) (by RH mapper)	Distance from closest marker(s) (cR)
UMNe84	AY391284	CA8 N2 CA5	ACAGTTGGATATCCGTGGGAAC	TCTGTTATGTGTTGCCCTCTGC	216	0	1	ECA15	*COR075*	0.00
UMNe90	AY391285	CA18	CGACACTTCCCACAGTCATCTC	TCCGTGCTTTTAAGCAAGTTCC	149	0	1	ECA1	*UCD487*	17.67
UMNe91	AY391286	CA19	GCAACAATAAGATACCCAAAGCAG	GATCTTGGCCAACAACTCGTC	153	7	5	ECAX	*ARSC1*	11.32
UMNe93	AY391287	CA13 N2 CA8	CGCAGAGTTGGAGACACCTG	GGTGCCATCCACTGAAACAC	167	2*	3	ECA7	*LEX045*	0.00
UMNe97	AY391288	CA5 N2 CA5	TGTCAGGTGGCATTCAAATC	TAACCAACAATGCCCAAGTC	202	0	2	ECA18	*NV077*	0.00
UMNe100	AY391289	CA5 N8 CA6	CTGATGCAGAGGCATTTCTG	CGTCAGCTGAGGCAACTATG	183	5	5	ECA7	*HBB*	2.84
UMNe102	AY391290	CA9 N4 CA9	ATGCATGCTCATTGCAGAAC	GCACAAAGAAATTTCCAACTCC	191	0	1	ECA15	*LEX046*	0.00
UMNe104	AY391291	CA15	TGATTTTTGTTCAGCCTCTATGC	CCTAGAAAGGTGGGAAATAAATTG	156	0	1	ECA4	*HTG07*	0.00
UMNe106	AY391292	CA7 N CA3	AATCCTGTGAGGCAGGAGTG	TGTCTGGGCATCTCTTAGGG	200	0	1	ECA19	*AHT052*	11.88
UMNe110	AY391293	CA11	TCTTTCCTACCCTCCATGACC	GATCTAGTACAACACTCCGTGTG	141	9	5	ECA4	*PTPN12*	0.00
UMNe112	AY391294	CA9	TGCCCAAATATTGAGCAAGG	TCTCTCCTCCCCTTTTTGTG	155	0	1	ECA4	*LEX033*	5.23
UMNe114	AY391295	CA5 N2 CA7	AACCCAAAAAGCATTTGAGAAG	TCTGCACCAGTTTTGTACGC	129	1	2	ECA18	*SG07*	19.85
UMNe115	AY391296	CA12 N2 CA27	TCCTCCTACACTGGCCATATC	TTTCCTATCGGAGTGCTTGC	168	4	10	ECA1	*MYO5A*	11.09
UMNe125	AY391297	CA14	TGGGTCCTGAGACCATAAGC	TCCTCCCTACCTCCTCACTG	143	9	5	ECA14	*LEX078*	0.00
UMNe127	AY391298	CA10	TTATAAATCACCACTGTTTACACAC	TCTTGAAGCAGGATGGGC	135	0	2	ECA26	*TKY275*	30.11
UMNe137	AY391299	CA10	CTGCTTTTTACTGCTTCAGTGC	GATTTGAGTCGAGGTCTGCC	114	5	5	ECA22	*HMS47*	2.22
UMNe139	AY391300	CA13	AGACACAGGTTTAGGTGGATGC	GATCAAGCACATAAGGGACAC	100	5	2	ECA21	*C9*	2.53
UMNe143	AY391301	CA13	AGAAAGGGTAAACAAGAAGCCC	GACACCTCTGCATCACACATG	105	3	2	ECA11	*NV090*	2.74
UMNe147	AY391302	CA10	CAGACCTACTCCAGTCATCAGC	AAACAAAGAGACTTGAAGTGGC	180	0	1	ECA21	*HTG10*	8.23
UMNe152	AY391303	CA11	ACAGGTGTTCATCCAAATTTTG	AAAGTAGAGGAAGACAGGCACG	144	5	2	ECA24	*COR087*	0.00
UMNe157	AY391304	CA11	TTCAACTGTGTGAGTGTGAATG	AGCTTTTGTCCAGCAGGAAC	94	5	3	ECA31	*PCMT1*	5.23
UMNe158	AY391305	CA22	AATTGAGAGCCAAGATGACACC	GGCACCATTTGAGGAAGATG	146	8	8	ECA3	*KCC1*	4.60
UMNe160	AY391306	CA7 A2 CA15	TGGAAGGATTCTCCCCAAG	GTTGCTCCAATGCCATACTC	114	0	1	ECA25	*NV043*	9.10
UMNe164	AY391307	CA9	AAAGAGGAGGATTGGCAATG	ATCTCCAGTGTGACAATCTCTACC	105	0	1	ECA8	*DSC2*	11.43
UMNe168	AY391308	CA10	CACCAAACCCCACTGAATTC	CACTACCTTCCCCTACGTTCC	149	1	2	ECA12	*IGF2*	20.58
UMNe170	AY391309	CA7 N2 CA5	GGGTGTTAAGAATCCTGCTCC	CTAGGCAAACTACTGACCCCC	138	0	1	ECA20	*UM011*	0.00
UMNe172	AY391310	CA4 N2 CA6	CCAGACACAGACCTAGCACC	CCAGCAAAGGAGGAGGAAG	190	0	1	ECA4	*HMS62*	6.72
UMNe173	AY391311	CA11	TTGGAGAATTCAAGGGTTTCC	GCCAAGACATGGAAACAACC	132	7	5	ECA17	*COR032*	13.35
UMNe178	AY391312	CA11	TGGCAGATAAAGCCCTTACC	GATCACATAGTGAAGGTGTTAAACG	107	1	2	ECA3	*LEX007*	0.00
UMNe180	AY391313	CA8 N2 CA2	TGGAAAATCCTCACAAACTGC	TATATTTTCCTTTTGCGTGTGC	148	0	1	ECA18	*CHRNA*	5.34
UMNe181	AY391314	CA6 N CA5	TTAAGCAACCCAGTGTGTGG	AGAGAAGAGAATCGGGGGAG	149	0	1	ECA31	*PCMT1*	7.80
UMNe182	AY391315	CA10	TCTCTGCATTTCACTTCTCGG	TGAGAGAAGAGCCCAGGAAG	132	3*	3	ECA3	*KCC1*	4.60
UMNe183	AY391316	CA11	AGAGAACAGGAGAAGACATGCC	ACCCTGTCTCATGGAAGCTG	106	1	2	ECA22	*ADA*	11.32
UMNe188	AY391317	CA8 N CA2	GTTAACAAGGATTGTTTTGGGC	TGCGTTTCTGCTTCTCCC	132	2	2	ECA26	*EB2E8*	18.15
UMNe195	AY391318	CA12	CAAAGAAGTGGCCCTCGTAG	GATCTGGCCCCAGAGAAAAA	104			ECA6	*MAP2*	11.32
UMNe196	AY391319	CA16	ATGAAAAAAGGCATACCCCC	TCTTATCAGGTTGATGGTGTGC	131	7	5	ECA1	*RET*	14.73

UMNe217	AY391320	CA11	CTTTGAGTTCACCAGTTCTCCC	AACCAAAAGGAACTTTGGTGG	151	3	2	ECA13	*TKY031*	8.34
UMNe223	AY391321	CA10	GTCTGTCCCAGACATATACCCC	AACCTGGGCAAGGGAAAG	112	0*	1	ECA12	*TKY286*	0.00
UMNe225	AY391322	CA17	GATCTCTTGCTGTGTGTTTGTG	TCAATTTCACAAGATGGAAACG	154	2*	4	ECA20	*UM011*	0.00
UMNe227	AY391323	CA13	ATAATTTCCCTTGCCAACACC	CTGTAGACCCAAAGGAAGATGG	188	1*	2	ECA14	*COR104*	41.70
UMNe230	AY391324	CA11	TGGTCTAGAGTACACATACACCAAC	TTTCTCCTTCCACCCCAG	109	0*	1	ECA5	*AHT024*	0.00
UMNe235	AY391325	CA17	TGAAGGCAATGACCAATATCC	CACCAACAAAAGTATCTGCCTG	178	2*	2	ECA17	*LEX055*	13.47
UMNe238	AY391326	CA11	CCAGCACCACTGTTCAAGAC	TCTAGCCATATGTTACCCTGCC	198	0*	1	ECA8	*ASB14*	12.33
UMNe245	AY391327	CA10	TTGCATAGGGACAATCCTACG	ACAAACCCTACCACTCGGC	104			ECA15	*ASB19*	0.00
UMNe253	AY391328	CA23	CAGAAAGATTTCCTTCTTTATGGC	ACTACACTGCCTCTTGGAATATCC	222			ECA29	*LOC91452*	1.41
UMNe256	AY391329	CA14	GGCCACACGACTACTTTCAC	ACTTCTGGAAGACAGCTGGC	142	2*	3	ECA4	*COR047*	10.09
UMNe264	AY391330	CA12	GCAATGCTAGCACAGGGG	CCTCTCTCTCTTTCCCCCTC	101	8	3	ECA16	*PDCD6IP*	7.26
UMNe276	AY391331	CA17	CATGCTCTCCATGACTTTTCC	CACCCAGGACTCCCACAG	154	2*	3	ECA22	*AHT030*	11.65
UMNe301	AY391332	CA5 N2 CA5	TTTTGGGGTCCAGAATAAACC	TAGACAACCAAATGCCAAAGG	140	4	3	ECA20	*NV005*	2.22
UMNe305	AY391333	CA13	ACAGTTCACAGCGGCCTC	GAGAGGGAGCAAGCCCTC	162	2	2	ECA13	*TKY031*	7.04
UMNe313	AY391334	CA15	AGAACCTGTTGGAGATACGAGG	ATCAGAGTGGAGACATGGGG	179	7	5	ECA27	*COR040*	14.62
UMNe314	AY391335	CA10	AGGCCCCTTGTATATCCCAC	GAGCTGGGAGTGGAAGGAG	153	0	1	ECA11	*UCD039*	0.00
UMNe318	AY391336	CA4 N2 CA7	CAGGGCTAGTCAACAGGCTC	TGGGGTGTAATGACTACCAGG	209	0	1	ECA1	*COR046*	4.71
UMNe319	AY391337	CA10	TTCGTGCAATCTGTTGCTTC	GATCACAGTCCCTGGTACTGG	154	1	2	ECA19	*HTG24*	0.00
UMNe323	AY391338	CA8 CA8 CA4 CA6 CA14	GATCCTGCAGGAAAGCATGT	CCGCTCGGAATATTTCATTG	185	4	7	ECA2	*COR037*	0.00
UMNe327	AY391339	CA16	TTTTCCTTCCTCATTGGTGC	GAAATGCAGGGCTAAGGATG	157	4	2	ECA21	*C9*	2.33
UMNe329	AY391340	CA19 N2 CA8	TGTTTGCTTGGAATGGTCAG	TCTCCATCTCCACATAGGCC	104	4	3	ECA12	*TKY286*	8.12
UMNe330	AY391341	CA10	AAACATGGAACCAGAGGGG	TCTCCAGCGTATTTGGTTAGTG	129	0	2	ECA2	*FABP3*	5.55
UMNe337	AY391342	CA17	CTTTAATATGCCTGCCACTTCC	TTGGGAAGTCCAGGATCTTG	215	2	3	ECA6	*CUL3*	0.00
UMNe350	AY391343	CA10	GCAAAATAAAAGGGTCACTTGC	AGTGCTCCAGGTGCTTATATCC	153	6	2	ECAX	*COR091*	0.00
UMNe351	AY391344	CA14	TGAGAAGCTCTTGCCAATCC	TAGAGGCCATTTTTGCATCC	150	0	1	ECA19	*HTG23*	2.43
UMNe353	AY391345	CA19	CCACTTTTCCCCTTCCTCTC	AACTTGGAAGCAACCTAAGTGC	322	2*	3	ECA6	*UCD465*	0.00
UMNe355	AY391346	CA13	TCATGCAATTCTGAAAAAGAGC	CAGAGCAAATCTTCCTCACC	182	2*	2	ECA22	*TKY285*	12.90
UMNe357	AY391347	CA10	GATCCAGTAACAAACAAGCTAATTT	TCAAATGATTTCTTACTGAGGATTC	210	1	2	ECA23	*UMNe51*	0.00
UMNe359	AY391348	CA10 N2 CA5 N2 CA20	GTGTGACGGAGGACGAGG	TGGTGCCATCCACTGAAAC	140			ECA7	*LEX045*	0.00
UMNe361	AY391349	CA14	AGGGAATAACTTATGAACAGATGAA	TCTTTATGGAAATTCTCTCTGAACC	169	2*	3	ECA5	*HMS05*	5.13
UMNe362	AY391350	CA10	GAGAGAGAGAGTATGCGCGC	GATTCCAAAGGCTATCCACTC	117	2*	3	ECA25	*TXN*	19.48
UMNe365	AY391351	CA10	CCCTCGTCTCAGACTTCTGG	GAGGCCTTGCATGTGGAC	201	0*	1	ECA11	*PDE6G*	0.00
UMNe366	AY391352	CA11	TGAACTCCAAAGCCAGGC	CATGTTCTGTAGTTTTGCAGGC	174	0*	2	ECA29	*TKY112*	7.15
UMNe368	AY391353	CA15	TCCCAGAAAGCAACACTCAC	TGTCAGTTCAGATTTTTGGCC	292	0*	1	ECA31	*VIP*	0.00
UMNe372	AY391354	CA11	ACGTTACAGGTGGGGAGATG	CTTGGCTTCCAGGAAAACAC	127			ECA10	*CASP8AP2*	3.67
UMNe373	AY391355	CA17	AGGCCACTCAAGCCACAG	CACCTCCTCCTCCAAATGAG	265	9	4	ECA15	*ASB19*	0.00
UMNe374	AY391356	CA16	AATTTCATATGGTTTCCATGCC	TAAGCACTGCGTTAATGTTCTG	194	2*	3	ECA2	*SMARCA5*	31.61
UMNe375	AY391357	CA6 N2 CA12	GGAGCCAGTTCAAAGATTTCC	ATTCAGTGGGTTGTTTTGCC	130	2*	2	ECAX	*FACL4*	3.98
UMNe376	AY391358	CA21	ACGCATATTAAAGTTTGAGAAGTCC	CAGACCTACTGAATTGGAATACACC	155	2*	5	ECAX	*COL4A5*	3.15

Repeat motif, PCR primers and product sizes, polymorphism and radiation hybrid map location (chromosomal assignment along with closest located marker and distance in cR) for these new microsatellite markers were obtained as described in the accompanying text. An asterisk (*) by the number of heterozygous individuals indicates that these markers were only typed on the Animal Health Trust reference family parents.