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Sixty-seven new equine microsatellite loci assigned to the equine radiation hybrid map

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Source/description: Horse genomic DNA was digested with 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. DNA was isolated from positive plaques and the inserts were sequenced using an ABI 3100 automated sequencer. Primer pairs for polymerase chain reaction (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of the markers were developed using the PRIMER program (Version 0.5; M.J. Daly, S.E. Lincoln and E.S. Lander, unpublished data). Sequence accession numbers, primer pairs, repeat motif, and PCR product sizes based on the cloned sequences are provided in Table 1.

PCR conditions: The PCR for determination of microsatellite polymorphisms were performed in 15 μl volumes consisting of the following reagents: 25 ng DNA, 1× 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 Hot Start *Taq* polymerase (Qiagen), and 5 pmol 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 to 62 °C for 30 s, and 72 °C for 30 s; and a final 5 min extension at 72 °C. These reaction products were electrophoresed through 7% acrylamide denaturing gels on BioRad SequiGen GT 38 × 50-cm plate sequencing gel units (Hercules, CA, USA), in the presence of 1× TBE, and allele sizes 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 each primer, 2.0 mM $MgCl_2$,0.3 Units HotStar *Taq* Polymerase (Qiagen), and 10× 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: Genotypes were obtained from 12 stallions of the Equine Genome Mapping Workshop International Reference Family,[2] and/or three horses (one stallion, two mares) from the parental generation of the Newmarket full-sibling reference family.[3] The number of alleles of each marker observed in these sample populations is reported in Table 1.

Radiation hybrid mapping and chromosomal assignments: The PCR typing on the 5000 rad equine RH panel and data analysis was performed as described in detail earlier.[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 nearest linked markers for all new equine microsatellites are provided in Table 1.

Acknowledgements: This work was supported by awards from the Morris Animal Foundation and the United States Department of Agriculture National Research Initiate Competitive Grants (JRM, LJA, LCS, BPC), the Texas Higher Education Board (ARP 010366-0162-2001; BPC), Link Endowment (BPC, LCS), and the Dorothy Russell Havemeyer Foundation (BPC).

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Table 1 Equine microsatellite loci UMNe122–UMNe550, including accession number, repeat motif, PCR primers and product sizes, polymorphism, chromosome assignment, nearest marker and distance in cR.

Marker	GenBank accession number	Repeat motif	Primer 1 (5′–>3′)	Primer 2 (5′–>3′)	Product size (bp)	Heterozygotes	Alleles	Chromosome	Nearest marker	Distance from nearest marker (cR)
UMNe83	AY735234	CA15 N5 CA10	AGCTCCTGAAAGAAGAGAGCC	TGGAACCCAGTAACATGAAGC	273			ECA19	*HMS08*	0
UMNe92	AY735235	CA11	CCCTGGCAAAAGAAAGGAAAC	CACCCATGTTCAAAATTCATGC	142	2	2	ECA3	*LEX007*	2.43
UMNe116	AY735236	CA20	TCAAATCCCGAGCTAAAATG	GATCACATGGGAGAAAATACAC	153	8	7	ECA11	*NV040*	9.54
UMNe174	AY735237	CA16	TCTGCATCCTCATGTTCAATG	TCGAAAATGGCAAGATTTCC	184	9	5	ECA4	*HTG22*	0.00
UMNe218	AY735238	CA10 CA7 N6	ATTTTGTTACCCATGCCCAG	GCCATCTGTATCCTGCCATC	139	4	2	ECA29	*COR082*	5.34
UMNe221	AY735239	CA19	AGACCTTGAGAAGCTCTTGCC	CCCTAGAGTCCCATCCTTGC	188	2*	2	ECA19	*MPZ003*	0.00
UMNe277	AY735240	CA7 N2 CA5	TGGAAGGATTCTCCCCAAG	GTTGCTCCAATGCCATACTC	114	5	3	ECA16	*TKY279*	6.94
UMNe344	AY735241	CA17	ATCAGCGCACACCTAGTAGAG	GAGACACTCTGTAGGGGATTGG	130	5	6	ECA22	*AHT030*	0.00
UMNe352	AY735242	CA14	TGAAGTGTCTATTTGACAATGGAA	GGCCTTGTTGACCTGACATT	195	3	3	ECA16	*PDCD6IP*	19.97
UMNe358	AY735243	CA15	TACTGCTAAACAAAGAGTGCGC	TCATGCGTTTCTTTTGTGTTAG	138	2	6	ECA22	*HMS47*	3.15
UMNe385	AY735244	CA12	GGATGCCAATCTCCTTTCAC	CTATGTCTTTATAAGCGCGCG	156	7	3	ECA8	*FLJ11021*	15.43
UMNe404	AY735245	CA11	TTGGAACTTTTAGCAAAGAACC	GATCCATTCCCACATATGGC	166	2*	3	ECA4	*GCK*	14.50
UMNe424	AY735246	CA14	GGCATCACAAGAGAGGTAGAGG	TTCACAGCCTAGACCCATCC	168	2*	4	ECA31	*PLG*	10.76
UMNe427	AY735247	CA11	GGAAGTGGAGACTCAGCAGG	TGCATAGACAACTTGCCTGG	128	2*	3	ECA6	*UCD465*	0
UMNe428	AY735248	CA11	AGGTCCCTCTCAACTCTATCCC	TACACTAGCGTATAAGGGCACG	152	2*	2	ECA20	*SGP28*	16.37
UMNe429	AY735249	CA17	ATGTAGTTCCCCCAGCCTG	TCCCTCCCTCCACTCTCC	271	2*	4	ECA1	*1CA16*	16.84
UMNe431	AY735250	CA20	CAAGGTAAGGGTCAATCAGAGG	AAGGGTCAGGTGCTAGAAAGC	270	2*	4	ECA22	*AHT030*	14.27
UMNe443	AY735251	CA18	GTGAATAGTAACCAGCCCATCC	GTATATGTGAAAGTGGGGGTGG	300	0*	1	ECA11	*SKIP*	27.31
UMNe446	AY735252	CA12	AGCATCCAGTCCACTGCAG	GGCAGGTAAGTTTGAGAACCC	378	0*	1	ECAX	*MAGE-E1*	7.47
UMNe465	AY735253	CA12	CTGGCTGCATTCTGCATG	ACCCTGAGACAGTGTGTGACC	339	0	1	ECA28	*IGF1*	21.2
UMNe470	AY735254	CA14	TCTGTGTGTGGGTGTGGG	CTCACATGCTTAGCTGCAGC	130	4	3	ECA23	*TLE4*	15.32
UMNe475	AY735255	CA17	TTTTTTCATGCTCAATTCATGG	TGTCAACAGCATTCTCTCAGTG	122	2*	3	ECA17	*UCD014*	2.53
UMNe502	AY735256	CA19	ATTTCAGGAAGTAACCTTCCCC	AGTTTTTACCGAGAGCTTTTGC	176	3*	5	ECA4	*LEX050*	15.55
UMNe506	AY735257	CA20	AGGGACGTGACCTAACATGG	CAGCACAGCCTCCTCTCC	142	2	7	ECA10	*RYR1*	0.00
UMNe521	AY735258	CA13	CTCTGAATACGCCTGGTTTAGC	GAAGATTTGTGAAAGAGGGGG	137	1*	2	ECA17	*LEX076*	13.12
UMNe525	AY735259	CA14	CAACCACTCCCTTTCTCCAG	AATGGCCAAAGGTAGCCAC	152	2*	3	ECA25	*TXN*	3.05
UMNe526	AY735260	CA15	GGCTGTCAGGGGTCACAC	ACATTGATACAATCCATCCACC	147	2*	3	ECA8	*GALR1*	0
UMNe527	AY735261	CA13	GCAAACGTAGTGTACTTGTGCC	TCTCATCCACAGCACCAAAG	209	2*	2	ECA3	*HNRPDL*	3.15
UMNe534	AY735262	CA14	ATGTTGTTGCAAATGGTAGGG	TCCATCAATCCCTCTTCTGG	249	0*	2	ECA5	*SH3GLB1*	10.09
UMNe542	AY735263	CA13	TGAAAGAGACCATACACGATGC	CACGACTTAGAGACGTGTGAGC	252	4	4	ECA26	*NV070*	15.55
UMNe543	AY735264	CA13	GGCAGGATTTCCTTCTTTCC	TCTGCACTCCTGTGTTCACTG	190	6	4	ECA10	*AMD1*	12.67
UMNe545	AY735265	CA19	TCGCTCTCCTCTCTCTGTCTC	TATGGCTGCAGATTCCACTG	197			ECA8	*AMD1*	2.94
UMNe551	AY735266	CA14	GATCTAGTGAGGGTTGGAGGG	AACCCAGAAAGACACGTTCTG	215	4	3	ECA4	*HTG07*	17.55
UMNe554	AY735267	CA14	ACTTGTCAAGCCATGCTGC	ATGCCAAATTAACCACTATGCC	157	8	5	ECA2	*TKY003*	4.81
UMNe555	AY735268	CA11	AGACCTCTCTCATGAACTTCAG	GGAATCAGGATTTCAGTTATGG	120	5	2	ECA5	*CTSK*	2.63

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Table 1 Continued.

Marker	GenBank accession number	Repeat motif	Primer 1 (5′–>3′)	Primer 2 (5′–>3′)	Product size (bp)	Heterozygotes	Alleles	Chromosome	Nearest marker	Distance from nearest marker (cR)
UMNe556	AY735269	CA15	CTAAATGAATGGAGGGAAGGC	TGACTCCAGCCAGAGGATG	325	6	4	ECA6	*UCD465*	6.94
UMNe565	AY735270	CA13	GAGAGCTAACCAGAATTGCCC	CTATACCGTCAACTCTCCTGGG	333	6	6	ECA2	*TKY282*	22.06
UMNe566	AY735271	CA15	TGCTGTGACTATGCTGTGTCC	ATCAGCTGGTCAATGATGAGG	252	8	6	ECA16	*LEX048*	5.02
UMNe567	AY735272	CA14	GGTGCAGCTGCTAGCTCAG	AGACCCAGTCATTGGGAGG	226	7	5	ECA5	*GBA*	16.84
UMNe569	AY735273	CA11	ATGTTATACACTTCATATATATGCTT	TGAGGAAAAGATGGAGTTGT	171	0*	2	ECA24	*UM012*	0
UMNe570	AY735274	CA15	CGTTTCCCTATCATCTCCTCC	ACCTCAAGGTTGGTGGTTTG	192	3*	4	ECA30	*AHT062*	10.54
UMNe572	AY735275	CA11	GGCTGCTGAATAATATTCCAGG	GAGTCCAAACTATGATCCAGCC	221	2	4	ECA9	*UM037*	2.12
UMNe574	AY735276	CA15	AAACTGGTTTCTGCCACCAC	ATTGGATATTGGTCAGAGTGGG	187	2	3	ECA7	*UMNe074*	0
UMNe575	AY735277	CA12	CTTTTGAGGAATCTCCATACCG	GGACCAGGCTGTTTCTGAAC	265	0*	2	ECA15	*TKY104*	10.87
UMNe577	AY735278	CA14	CCGTGCCTTTTTCTCTCATC	ATGAATGGTTACTGGTGACCG	244	3	4	ECA9	*OAZIN*	27.84
UMNe578	AY735279	CA14	TTCACACGCTCGTTCTCATC	TTGTGCAAAAAGAGCCTACTTG	163	2	2	ECA8	*VAPA*	16.72
UMNe580	AY735280	CA11	CCCTGTTGCCACCAACTC	ACAACCCAAGTGTCCTTGATG	242	0*	1	ECA9	*UM037*	8.99
UMNe581	AY735281	CA13	TCATCAAGCCATGTTTTAGTGG	TTGCTGACAGTCAGGGTGAG	199	3*	3	ECA14	*LEX047*	16.72
UMNe582	AY735282	CA18	TCTTGACCTTCTTTACTTAGTACACA	CCTGGGCATAGACCTACACA	173	2*	4	ECA5	*VCAM1*	3.77
UMNe584	AY735283	CA16	AAATTTGTCCCATGTAATTCCC	TCACTGTTGGGAAAGGAACC	119	1*	2	ECA14	*AHT083*	0
UMNe585	AY735284	CA11	ATGAGAACACTGGGTGGGAG	AAGGCCCTCAGGGAAATG	364	0*	1	ECA16	*LEX048*	5.76
UMNe587	AY735285	CA19	TTGGTCAGAATAGGTAGTTATG	AGATGGATATGTACATGGATAC	254	1*	2	ECA4	*HMS06*	0
UMNe590	AY735286	CA11	GGGAGATATTTCTTGGCTTGC	TGAAAATACACCCAGCTACGC	156	2*	4	ECA19	*ZNFU69274*	4.71
UMNe591	AY735287	CA16	CAGCAGAAAGAAATGTTGATGG	ACCTACACACTGCTCATCAAGC	283			ECA1	*1CA22*	5.55
UMNe592	AY735288	CA12	TCCAAACTGTGTGTGTGTTTGT	TGCACATAGAACAGGGCAAG	163	0*	1	ECA19	*COR092*	2.53
UMNe595	AY735289	CA11	TCATTTTCATCTTCTCCGGG	GACCTGCCCAACACAAGG	199	0*	3	ECA14	*LEX047*	17.91
UMNe598	AY735290	CA17	CTGTCCTTAGTCTGGTCC	GATCCTCATTTTATACCC	146	1*	3	ECA25	*NV043*	20.46
UMNe599	AY735291	CA14	AGTCCATCCTTGCATCCAAG	AGACCTTGAGAAGCTCTTGCC	184	2*	2	ECA19	*MPZ003*	0
UMNe600	AY735292	CA14	GAGCAGTGGTGCAGAATTTAC	TAGAAGAATGCATGGCTCATAC	148	3*	3	ECA3	*LEX007*	15.20
UMNe602	AY735293	CA20	AATAGTCCAAAGGAGCAAGACG	TTCAGAGTAAGAGAGTGGCGTG	161	2*	4	ECA29	*COR021*	2.53
UMNe604	AY735294	CA18	AGACTTGCCCAGCAGCAC	ACCTCCAGCCCAGCTTTC	240	3*	5	ECA9	*CTSK*	5.10
UMNe607	AY735295	CA10	AAGACCATCTTTGCAGTGTGG	TTCCTTTGCCTTGGGATATG	220	0*	1	ECA22	*HMS47*	19.00
UMNe608	AY735296	CA13	AATTGTTATTCCCCAACCCC	GAACCTTCCTAAGGACGTTGG	215	2*	3	ECA15	*ASB15*	0.3
UMNe609	AY735297	CA13	CAAAGGTTATAACAGAAGCGGG	CAAGCCTCATCCCCACAC	160	1*	2	ECA1	*SG25*	5.66
UMNe611	AY735298	CA11	GATCTTCTCTAGGTTTGACTG	AAGTAGAAAAGTTGTTCAATTG	241	1*	2	ECA1	*1CA16*	25.75
UMNe612	AY735299	CA13	TTCAAAACCAGAATCACGTCC	AGGCACTGCACGTAACCAG	357	0*	1	ECA7	*AHT019*	6.72
UMNe614	AY735300	CA21	GAGAGCAGAGCAGGCCAG	TGCCCCCTTAGCCTCATAC	278	3*	3	ECA16	*GLB1*	2.22

*Markers that were only typed on the Animal Health Trust reference family parents.