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Thirty-five new equine microsatellite loci assigned to genetic linkage and radiation hybrid maps

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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of the markers were developed using the PRIMER program (Version 0.5; M.J. Daly, S.E. Lincoln and E.S. Lander, unpublished data).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 genotyping of markers in a reference family (see below) were performed in 15 μl volumes consisting of the following reagents: 25 ng genomic horse DNA, 1× GeneAmp PCR buffer II (Applied Biosystems, Foster City, CA, USA), 1.5 mM $MgCl_2$, 200 μM each dNTP and 1.5 units Amplitaq GOLD™ (Applied Biosystems). Three primers were used for labelling the PCR product: 5 pmol reverse primer (Table 1), 2 pmol forward primer (Table 1) containing the 5′ tail sequence TGACCGG-CAGCAAAATTG, and 10 pmol of fluorescently-labelled TGACCGGCAGCAAAATTG. The PCR conditions, using a MJ Tetrad thermocycler, were: initial 10 min denaturation at 95 °C; 30 cycles of 30 s at 95 °C, 30 s at 60 °C, 1 min at 72 °C; and a final 10 min extension at 72 °C. Alleles were detected using an Applied Biosystems 3100 automated sequencer running GENESCAN 3.7 software (Applied Biosystems).

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.

Mendelian inheritance and chromosomal assignments: Mendelian inheritance for the markers was confirmed by observing segregation of alleles through the Newmarket three generation full-sibling reference family consisting of 71 animals.[3] The chromosomal locations provided in Table 2 were identified by two-point linkage analysis in reference to existing markers on the full-sibling family map using CRIMAP software. The previous chromosomal assignments of two markers by RH mapping (*UMNe362* and *UMNe520*)[4, 5] were confirmed by genetic linkage.

Radiation hybrid mapping and chromosomal assignments: The PCR typing on the 5000 rad equine RH panel and data analysis for 30 of the markers 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 2. Three markers (*UMNe486*, *UMNe504* and *UMNe532*) assigned to the genetic linkage map did not show significant linkage to markers on the current radiation hybrid map. One marker (*UMNe405*) assigned to ECA2 by genetic linkage exhibited significant linkage to markers on the radiation hybrid maps of both ECA2 and ECA9.

Table 1 Equine microsatellite loci UMNe122–UMNe550, including accession number, repeat motif, PCR primers and product sizes, and polymorphism.

Marker	GenBank accession number	Repeat motif	Primer 1 (5′–>3′)	Primer 2 (5′–>3′′)	Product size (bp)	Heterozygotes	Alleles
UMNe122	AY731378	CA12	CCATGTGTTCACAAACTCGG	TGGAACTTTCCAGTCCAAGG	102	4	3
UMNe189	AY731379	CA16	GATCAAATACCCTCTGCAAAGC	GGCTTCATATCCTCCCTTACC	148	9	8
UMNe201	AY731380	CA15	TATGTGTGAACTTGGTGGTAGC	TGCATAAACATACGCTCATGC	143	7	3
UMNe267	AY731381	CA10	AGCCTCCATATGCTTTGTCG	GGAATTGACTCAGTCCTGAAGG	150	2*	2
UMNe269	AY731382	CA10	GATCAAAGGCGGGATGTG	ATGACACCTGATGAGGACCTG	150	1*	2
UMNe307	AY731383	CA12	ATTGTCCCTGCTCCATGAAG	GTCCCCATGCTCTCCATG	139	5	4
UMNe311	AY731384	CA8 N2 CA6	GATATCCTACACCGAGGACTGC	TCGTTCTTTGCTGTCTGGG	179	4	3
UMNe336	AY731385	CA12	ACCTTCTCTTTGGAAGGAAATG	GAGACCTAAGCATGGAGCATG	119	4	3
UMNe340	AY731386	CA14	TCTGTCTGTCTCGCGCTG	ACTGTGTACTGGTCCAGGCC	133	2*	4
UMNe341	AY731387	CA11	TCCAACTTTTTGCATATGTTGC	ACGTTTGAACTTCAATCCAACC	223	2	2
UMNe342	AY731388	CA11	TCCACTCTCCCAAACTCCTG	TGCAGCTATCATTTTACCATGG	207	3	3
UMNe345	AY731389	CA17	CCAATCTGCTTTTTCACTTGC	ATACTGGTAAGGGCACCGTG	196	5	5
UMNe354	AY731390	CA15	AACTGTCCCAATTTTCCCG	CTTCCGTTCCTCCCTCTACC	270	1*	3
UMNe362	AY391350	CA10	GAGAGAGAGAGTATGCGCGC	GATTCCAAAGGCTATCCACTC	117	2*	2
UMNe384	AY731391	CA19	AGTCAGGAGAGACAGTGTAGGC	TCATGGAAGCATTTCCCTAG	251	2*	4
UMNe405	AY731392	CA19	TTTTCCATCTGGGAATCAGG	ACATAATGCTTGGAAGAGGTGG	234	3*	4
UMNe426	AY731393	CA11	TGGCTTTCATCTGAACTGTTC	TGGCCTGGGAGGTTATTTTG	139	2*	4
UMNe432	AY731394	CA11	CTGACGTCACTGGCAACG	GCGGAGTCCCACATAACG	282	2*	3
UMNe441	AY731395	CA13	GATAAACTGTCCTCCTGCTGAG	GGCAGAAAGTTCTGTGGAGTG	150	2*	3
UMNe448	AY731396	CA13	CCATTCTGCCCTGATTGG	TTCAAGACCCCTCAATCTGC	164	3*	4
UMNe454	AY731397	CA18	GGGAGAAACACCTACAGAGTGG	AGACTGAATTGCCCCATTTG	244	2*	3
UMNe455	AY731398	CA12	TGAGGTACTGTGCCTTGCTG	CTGGGAAGACAGAGCCAGTC	130	1*	3
UMNe474	AY731399	CA15	CCAAAGGGTGGAAATTGATG	TTTTGCCTCTCTCACCATCC	217	2*	3
UMNe476	AY731400	CA11	GAGTCATGTTCACCATCCCC	TTAGACGGTCAGCCAGCAG	227	1*	2
UMNe479	AY731401	CA22	TACGGTAGGACCAATGCCTC	GCCGTCAGAGAAAAGACAGG	175	7	4
UMNe486	AY731402	CA19	CAAATTATTGTTCCCTCTTGCC	GACTACTGGGCTTCACAGGC	217	3*	4
UMNe493	AY731403	CA15	GAACAGAAGGAAAAGCCATCC	ATGGAAGCTTTGCTCCTAAGG	108	2*	3
UMNe501	AY731404	CA16	CCCATGATTTAATTGTCTGCTG	TCCAAGTTTTTCCCGAAATG	296	2*	3
UMNe504	AY731405	CA13	ACCATCGACCAGTTTCCTTG	AAGAGCCCTTTCTTTGGCTC	283	3*	5
UMNe520	AY464515	CA11	AACAACTGCTTTCTTTCTGCC	TCGTAGCATCCCTCACATAC	139	2*	3
UMNe523	AY731406	CA12	GTTCCTGATATTGAAAACACCC	TTTTTTGGCATTACTTCCCTAC	243	1*	2
UMNe530	AY731407	CA13	AATGAAGCCAAGTGAATGGG	GTCTGAACCCTGTAACCTCACC	224	2*	2
UMNe532	AY731408	CA13	TAACATCAATTGGGCAAAACG	GTAGCAAAAGGGGTGTGTGC	233	2*	4
UMNe537	AY731409	CA14	TGCATTACGGGAAAGGAATC	CTGAACTGCACACTGAGAAATG	129	4	5
UMNe539	AY731410	CA19	GGTTTCCAAATGAGTGGTTTG	CAATTTAAGATTCATTGGGGAG	126	7	6
UMNe544	AY731411	CA14	GCCAGGTGTCCCACGTAC	CTGGACCAGCCTCACTCTTC	189	2*	4
UMNe550	AY731412	CA18	GAGTATCACTGCTCCCAGGC	TTTTGGGGACTGTCATTTAACC	122	7	4

Markers were typed on the 12 stallions of the Equine Genome Mapping Workshop International Reference Family unless otherwise noted.
*Markers that were only typed on the Animal Health Trust reference family parents.

Table 2 Chromosomal assignments for equine microsatellite loci UMNe122–UMNe550, including meiotic linkage and radiation hybrid map assignments.

Marker	Chromosome	Nearest marker	Recom. fraction	Lod	Chromosome (by RH)	Nearest marker (by RH)	Distance (cR)
UMNe122	ECA16	*AHT063*	0	10.24	ECA16	*AHT081*	4.71
UMNe189	ECA9	*ASB005*	0.08	5.95	ECA9	*NOV*	12.90
UMNe201	ECA11	*SGCV013*	0	6.02	ECA11	*COL1A1*	19.97
UMNe267	ECA10	*LEX017*	0.04	10.26	ECA10	*NV007*	0.00
UMNe269	ECA27	*ASB038*	0.03	9.14	No significant linkage		
UMNe307	ECA18	*HMS047*	0.03	13.91	ECA18	*SG07*	19.12
UMNe311	ECA3	*UM068*	0	10.84	ECA3	*HMS61*	36.96
UMNe336	ECA2	*UM226*	0	3.61	ECA2	*SMARCA5*	31.61
UMNe340	ECA30	*UM088*	0.01	24.7	ECA30	*UCD455*	9.21
UMNe341	ECA20	*HTG005*	0	7.22	ECA20	*LEX064*	9.65
UMNe342	ECA9	*AHT053*	0.02	12.55	ECA9	*OAZIN*	20.21
UMNe345	ECA28	*UCDEQ425*	0.04	13.11	ECA28	*UCD425*	3.15
UMNe354	ECA28	*UM166*	0.07	14.3	ECA28	*IGF1*	4.40
UMNe362	ECA25	*COR018*	0	9.03	ECA25	*TXN*	19.48
UMNe384	ECA17	*UM052*	0	22.28	ECA17	*LEX076*	1.92
UMNe405	ECA2	*AHT012*	0	19.27	ECA2 and ECA9		
UMNe426	ECA10	*LEX017*	0.05	11.46	ECA10	*UM040*	8.77
UMNe432	ECA21	*AHT078*	0	12.04	ECA21	*AHT078*	0.80
UMNe441	ECA22	*SGCV019*	0	11.74	ECA22	*GNAS1*	6.83
UMNe448	ECA1	*1CA030*	0	12.04	ECA1	*VIASH34*	7.26
UMNe454	ECA5	*LEX004*	0	11.74	ECA5	*AHT024*	0.00
UMNe455	ECA5	*LEX014*	0	5.12	ECA5	*LEX014*	8.56
UMNe474	ECA14	*UM032*	0	18.36	ECA14	*UM032*	8.12
UMNe476	ECA27	*UCDEQ005*	0	3.61	ECA27	*UCD005*	0.10
UMNe479	ECA8	*SGCV032*	0	7.22	ECA8	*DSC2*	26.4
UMNe486	ECA1	*HMS015*	0	15.95	No significant linkage		
UMNe493	ECA9	*ASB004*	0.04	5.67	ECA9	*NOV*	0.00
UMNe501	ECA18	*TKY101*	0.02	9.69	ECA18	*COL3A1*	0.00
UMNe504	ECA8	*AHT025*	0.03	18.86	No significant linkage		
UMNe520	ECA1	*UM002*	0	13.25	ECA1	*ASB12*	6.94
UMNe523	ECA27	*UCDEQ005*	0	3.31	ECA27	*ASB38*	0.00
UMNe530	ECA30	*UM078*	0	7.22	ECA30	*UMNe078*	36.82
UMNe532	ECA8	*UM034*	0.05	13.69	No significant linkage		
UMNe537	ECA22	*AHT031*	0.07	7.65	ECA22	*HMS47*	2.22
UMNe539	ECA21	*HTG010*	0	24.99	ECA21	*SG16*	32.57
UMNe544	ECA4	*HTG007*	0.04	5.4	ECA4	*HTG07*	19.85
UMNe550	ECA14	*UM239*	0	6.92	ECA14	*CSPG2*	7.04

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

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