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Development of a DNA microarray for detection of expressed equine classical MHC class I sequences in a defined population

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Abstract

Development of an accurate and efficient molecular-based equine MHC class I typing method would facilitate the study of T lymphocyte immune responses in horses. Here, a DNA microarray was designed to detect expressed classical MHC class I genes comprising serologically defined equine leukocyte antigen (ELA)-A haplotypes represented in a closed Arabian horse breeding herd. Initially, cloning and sequencing of RT-PCR products were used to identify sequences associated with the ELA- A1, A4, and W11 haplotypes, and one undefined haplotype, in six horses. Subsequently, sequence- specific, conserved (positive control), and random nucleotide (negative control) 23- to 27-mer oligonucleotide microarray probes were designed and spotted onto an epoxy-coated masked slide using a robotic arrayer. Bulk RT-PCR products from each horse were biotinylated by nick translation, hybridized to the array and detected using tyramide signal amplification. The microarray consistently detected eight of nine classical MHC class I transcripts and allowed ELA haplotypic associations to be made. Cloning and sequencing of RT-PCR products were then performed in a group of ELA disparate horses and ponies, in which six novel sequences were identified. This group was used to determine the specificity of the array. Overall, the microarray was more efficient than cloning and sequencing for detecting expressed classical MHC class I sequences in this defined population of horses, and was significantly more specific than serology. These results confirmed the utility of a microarray-based method for high resolution MHC class I typing in the horse. With additional probes the array could be useful in a broader population.

Keywords

MHC class I; ELA typing; horse; microarray

Classical MHC class I molecules are highly polymorphic membrane-bound glycoproteins that present self and non-self endogenous peptides to cytotoxic T lymphocytes (CTL). The extreme polymorphism observed in this group of proteins is predominantly due to nonsynonymous substitutions within the exons that encode the peptide-binding site (Beck and Trowsdale 2000). The structural changes in the peptide-binding site that result from these amino acid differences have a significant effect on peptide binding affinity and dictate the conformation of the MHC/peptide complex presented to CTL. The observation that a single amino acid difference within the α -2 domain of two MHC class I molecules (7-6 and 141; Eqca-N*00602 and Eqca-N*00601) can alter or abolish recognition of equine

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infectious anemia virus CTL epitopes (Mealey et al. 2006) highlights the immunologic importance of MHC polymorphism in the horse.

Equine MHC class I polymorphisms have been historically assessed by serologic typing using reagents derived from multiparous mares and horses alloimmunized with horse blood cells (Bailey 1980). With this method approximately 15 distinct serological specificities were identified and designated equine leukocyte antigen (ELA)- locus specificities (Lazary et al. 1988). More recently, MHC class I transcripts have been characterized at the molecular level through cDNA library screening, reverse transcriptase polymerase chain reaction (RT-PCR) experiments, and genomic sequencing from BAC libraries, with approximately 60 equine MHC class I molecules and fourteen sequence-defined haplotypes reported to date (Barbis et al. 1994; Carpenter et al. 2001; Chung et al. 2003; Ellis et al. 1995; McGuire et al. 2003; Tallmadge et al. 2005; Tallmadge et al. 2010). Despite these efforts, serotyping remains the standard for equine MHC class I typing primarily due to the lack of a uniform, efficient, and accurate nucleic-acid based ELA typing method.

In humans, serotyping has largely been replaced with nucleic-acid based typing methods because these techniques have significantly greater accuracy and reproducibility (Elsner and Blasczyk 2004), and they have the capacity to detect alleles not distinguished by available antisera (Williams 2001). The three molecular methods predominantly used for human leukocyte antigen (HLA) typing are sequence-specific oligonucleotide probe hybridization (SSOPH), sequence-specific primer PCR (SSP-PCR), and direct DNA sequencing of PCR products (Williams 2001). The method of choice is dictated by the level of resolution required, turn-around time, and the volume of samples to be processed. In addition to these techniques, DNA microarrays provide a rapid and economical molecular-based screening method that is ideally suited for leukocyte antigen typing because of their capacity to screen for multiple polymorphic sequences simultaneously, and to distinguish between genes with as little as a single nucleotide difference (Letowski et al. 2004; Warsen et al. 2004). The goals of this study were: 1) to develop a high resolution microarray capable of identifying expressed classical MHC class I sequences comprising the predominant serologically defined ELA-A haplotypes (ELA-A1, A4, W11 and undefined) in a defined population of Arabian horses; and 2) to test the accuracy of this typing method within this population and in a group of unrelated horses.

To meet the first goal, six interrelated Arabian horses (172, 174, 176, 184, A2140, and A2152) were selected from the Washington State University research breeding herd to determine the expressed classical MHC class I sequences comprising ELA-A haplotypes of interest (Table 1). ELA-A haplotypes were determined serologically by lymphocyte microcytotoxicity (Bailey 1980; Terasaki et al. 1978) using reagents provided by Dr. E. Bailey (University of Kentucky, Lexington, KY). Three of the six Arabian horses (mares 172, 174, and 184) and the dam of horse A2152 (mare 162) had only a single serologic haplotype (Table 1). Mares 172 and 174 were heterozygous with a haplotype not recognized by available antisera based on ELA-A serotyping of offspring and/or parents. Haplotype heterozygosity or homozygosity for mare 162 could not be determined (only one offspring available for analysis) and mare 184 was likely homozygous by descent. The other horses were heterozygous for the ELA-A1, A4, or W11 haplotypes. Table 1 additionally shows MHC class II haplotypes for DRA and DQA based on data generated by single-strand conformational polymorphism (SSCP) as described previously (Albright-Fraser et al. 1996; Fraser and Bailey 1998). Unrelated MHC disparate horses used for validation of the microarray included three mixed breed ponies (H605, H621, and H623), one outbred Arabian stallion (Stallion D), and two Thoroughbred horses (0834 and 3909) (Carpenter et al. 2001; Tallmadge et al. 2005; Tallmadge et al. 2010) (Table 2). In addition, an Arabian-

pony cross (H600), sired by Sire B, was included because the A1 haplotype in this pony was inherited from an unrelated pony mare (Table 1).

Peripheral blood mononuclear cells were isolated from whole blood and the total RNA was extracted using an RNeasy Kit (Qiagen, Valencia, CA). RT-PCR was performed with the isolated RNA using a One-Step RT-PCR Kit (Invitrogen, Carlsbad, CA). Primers designed to conserved regions in the MHC class I α 1-domain and 3' UTR were used as described (Chung et al. 2003; Tallmadge et al. 2005) with slight modification to amplify classical equine MHC class I genes (forward: 5'-GTG GAC GAC ACG CAG TTC-3'; reverse: 5'-CAG CAA GGA AGC AAA TGA TC-3'). RT-PCR was carried out with SuperScript II RT/Platinum *Taq* mixture (Invitrogen) using the following conditions: 1 cycle at 50°C for 50 min, 1 cycle at 94°C for 2 min, 30 cycles at 94°C for 30 sec, 60°C for 30 sec, and 68°C for 2 min, and a final cycle at 68°C for 7 min. Amplified RT-PCR products of the appropriate size (~1250 bp) were gel-purified, cloned into the pCR2.1 TA TOPO cloning vector (Invitrogen), and sequenced as described (Chung et al. 2003). The number of clones sequenced per horse ranged from 28–90. Most MHC class I sequences were identified in multiple clones, verifying the accuracy of those sequences. Only sequences that were cloned multiple times in an individual horse or in more than one horse were considered for analysis. Sequences were analyzed with Vector NTI software (Invitrogen) while alignments and sequence identity tables were generated with ClustalW2 and Boxshade programs. Expressed classical MHC class I sequences for the two Thoroughbred horses (Table 3) had been previously determined and assigned to equine MHC class I loci (Tallmadge et al. 2010) (Supplementary Figure 1). All MHC class I sequences were named according to MHC nomenclature guidelines (Ellis et al. 2006). Designations conformed to the Eqca-L*XXXXX format, where Eqca represents *Equus caballus*, L represents the MHC class I locus, and X represents the allele.

A total of nine different classical MHC class I transcripts were detected within the defined population of Arabian horses, with between two and five sequences identified in each horse (Table 3). All nine sequences have been previously reported (Chung et al. 2003; McGuire et al. 2003; Tallmadge et al. 2010) (Table 3) and assigned to MHC class I loci (Tallmadge et al. 2010) (Supplementary Figure 1). In horses with the ELA-A1 haplotype originating from mare 162 or 172, sequence Eqca-N*00602 (along with Eqca-N*01301) was repeatedly identified. Although it was unknown whether mares 162 and 172 shared a recent common ancestor, their ELA-A1 haplotype (designated A1.1 in Table 3) was associated with class II alleles DRA*0201 and DQA*0901 in both cases. In contrast, the ELA-A1 haplotype from mare 169 was associated with sequence Eqca-N*00601. Notably, this ELA-A1 haplotype (designated A1.2 in Table 3) was associated with class II alleles DRA*0101 and DQA*1901. Haplotypes ELA-A4 (associated with class II alleles DRA*0301, DQA*0501), and W11 (associated with class II alleles DRA*0101, DQA*0501) were inherited from a common sire (Sire B) in all horses. Comparison of the sequence data from horses heterozygous for these class II alleles indicated that the W11 haplotype was associated with sequences Eqca-1*00701, Eqca-16*00601, and Eqca-N*00201, and the A4 haplotype was associated with sequences Eqca-N*00801 and Eqca-1*00401. The full complement of sequences associated with the W11 haplotype could not, however, be detected in all horses bearing this haplotype (i.e. horse 176). This finding reflected the fact that certain sequences were cloned with disproportionate frequency in some of the horses, either due to increased expression of that sequence or bias in the RT-PCR or cloning reaction. For the purposes of this study, it was not necessary to overcome this bias with exhaustive sequencing in all horses because identification of any sequence with known haplotypic association (i.e. Eqca-1*00701) in conjunction with the horse's pedigree was sufficient to confirm the serologic typing.

Both sequences Eqca-N*00602 (associated with the ELA-A1.1 haplotype) and Eqca-N*00601 (associated with the ELA-A1.2 haplotype) were identified in the outbred Arabian stallion (Stallion D) (Table 3). This suggested that although Stallion D was serologically ELA-A1 homozygous, he was likely heterozygous for this haplotype (A1.1/A1.2). MHC class II data were not available for Stallion D.

In the ponies, seven transcripts were detected, five of which had not been previously reported. In the Arabian-pony cross (H600), four transcripts were identified including three ELA-W11 associated sequences (Eqca-N*00201, Eqca-1*01001, Eqca-16*00601) inherited from Sire B, and one new sequence associated with the maternal ELA-A1 haplotype (now designated A1.3). The latter sequence was nearly identical to two other ELA-A1 associated sequences (Eqca-N*00601 and Eqca-N*00602), and was assigned to locus 18 based on phylogenetic analysis as described (Tallmadge et al. 2010). The other new sequences were also assigned to loci by phylogenetic analysis (Supplementary Figure 1). Designations for these new sequences conformed with MHC nomenclature guidelines (Ellis et al. 2006).

For horses in which only a single ELA haplotype was detected serologically, serologic typing alone could not distinguish whether they were homozygous for that haplotype or heterozygous with a haplotype not defined by available antisera. This determination could be made using the cloning and sequencing results in conjunction with the breeding information. For horse 184, only sequences associated with the ELA-A4 haplotype were detected, increasing the likelihood that this horse was homozygous by descent. For horses 172 and 174, a common set of sequences (Eqca-1*01001, Eqca- Eqca-N*00201, Eqca-16*00601) were detected which were associated with an inherited serologically undefined haplotype (Table 3). The only detectable difference between this undefined haplotype and the W11 haplotype was the substitution of sequence Eqca-1*01001 for sequence Eqca-1*00701 (both locus 1 alleles based on phylogenetic analysis). This suggested that in these horses, the W11 serotype was defined by the presence of sequence Eqca-1*00701, while the absence of Eqca-1*00701 resulted in the lack of serologic reactivity. The inability to detect all haplotypes by serologic means has also been observed in other species and is presumed to be due to the absence of the serologically undefined alleles in the population from which the antisera was derived, or due to low surface expression of the encoded class I molecules (Hurley et al. 1999). In addition to this limitation, serologic reagents lack the specificity to resolve minor nucleotide polymorphisms (i.e. SNPs) that can have significant functional implications, such as the presentation of CTL epitopes (McGuire et al. 2003; Mealey et al. 2006). Therefore, more robust nucleic acid-based MHC class I typing methods are needed to advance the study of equine T cell immunology, autoimmunity, and tissue transplantation in the horse.

To construct a microarray capable of detecting the nine expressed classical MHC class I sequences in this defined group of Arabian horses, eighteen oligonucleotide probes, 23 to 27 nucleotides in length (Supplementary Table 1), were designed to hybridize conserved (positive control probes) and sequence-specific regions within the $\alpha 1$, $\alpha 2$, and $\alpha 3$ domains (Supplementary Figure 2). All probes had an average melting temperature of 63°C (range: 61–65 °C), 60.5% GC content (range: 51–69%) and were assessed for secondary structure using Oligo Design and Analysis tools (Integrated DNA Technologies). Independent arrays consisting of two replicate spots of each probe and a biotin pseudoprobe (Call et al. 2003) were spotted on each individual well of a 10-well, Teflon/epoxy-silane masked/coated microscope slide by an S3 SciflexArrayer (Scienion AG, Berlin, Germany), as previously described (Call et al. 2003). Following spotting, slides were baked for 1 hr at 130°C (<21 in Hg) and then stored at room temperature until used.

As positive controls for the specificity of the microarray, nine cDNA clones representing each sequence identified within the defined group of Arabian horses were biotinylated by nick translation and hybridized to the microarray at 64 °C. Procedures and chemistry detection steps, which included tyramide signal amplification, were carried out as previously described (Call et al. 2003). Arrays were imaged using an arrayWoRx Autoe scanner (Applied Precision, Issaquah, WA), and the images were processed with softWoRx Tracker software (Applied Percision). The threshold for positive detection was determined using a receiver operator characteristic (ROC; NCSS 2004 Statistical software) curve generated from the binary classification of a core set of probes. Under these conditions the array was sufficiently specific to differentiate the two cloned sequences that differed by only one nucleotide (Eqca-N*00602 and Eqca-N*00601).

To determine the utility of the array for identifying these sequences in individual horses, conserved primers were again used to amplify expressed classical MHC class I genes from all horses and the bulk RT-PCR products were biotinylated by nick translation and hybridized to the array. Within the population of Arabian horses, including outbred Stallion D, it was possible to detect all nine MHC class I sequences identified by cloning and sequencing. However, hybridization to probe 5 (Supplementary Table 1 and Supplementary Figure 2) was not consistent, resulting in a failure to detect sequence Eqca-N*00201 in horses H600 and A2140 (Table 3). Additional complementary probes would likely improve the sensitivity of the array for detecting this sequence.

When RT-PCR products from the non-Arabian horses were hybridized to the array it was evident that probes 4, 5, 12, 15, and 17 (Supplementary Table 1) were not exclusively specific to the MHC class I gene sequence for which they were originally designed. Cloning and sequencing data from these outbred horses indicated that these five probes were hybridizing to regions of segmental sequence identity between the different MHC class I gene sequences (Supplementary Figure 2). In the majority of cases this did not result in false positive detection because it was possible to determine the presence or absence of a specific sequence based on additional redundant probes. For example, target cDNA from ponies H600 and H605 was detected with probe 12, which was complementary to both Eqca-N*00601 and Eqca-N*00602 (neither of which were present in these two ponies by RT-PCR cloning and sequencing). The absence of Eqca-N*00601 and Eqca-N*00602 was confirmed in these ponies by the lack of hybridization to the other probes specific for these sequences (probes 13 and 16). In outbred horse 3903 and pony H623, target cDNA hybridized to probes 5 and 17, respectively. This led to false positive detection of sequences Eqca-N*00201 and Eqca-1*01001, since neither of these sequences were present in these animals by cloning and sequencing. As shown in Supplementary Figure 2, hybridization by these probes was due to shared binding site sequences in Eqca-N*00301 (for horse 3903) and Eqca-1*00901 (for pony H623) (Table 3). Additional probes complementary to non-shared binding regions would be needed to confirm the presence or absence of these class I sequences. Thus, although the array was specific for the MHC class I sequences present in the defined Arabian population for which it was designed to be used, additional probes would be required before the array could be used for typing a larger outbred population.

In summary, the microarray designed in this study consistently identified eight of nine expressed classical MHC class I transcripts in the defined group of Arabian horses. The microarray had a significant advantage in throughput, with the potential to screen 30 or more horses in a 24-hour period. For practical application in this group, the inconsistent results obtained for one of the MHC class I sequences (sequence Eqca-N*00201) is of little consequence since detection of other sequences with the same haplotypic association (i.e. Eqca-1*00701, Eqca-16*00601, and Eqca-1*01001) would support the presence of this sequence and identify those horses that should be retested.

Our results support the utility of a microarray typing method in a defined population of horses, and for broader application, the array could be expanded to include probes for sequences associated with other ELA-haplotypes represented in this herd and in other populations. However, the development and implementation of such a typing method in an outbred population will be hindered because the true level of MHC class I diversity is likely underrepresented by the sequences published to date. The fact that six novel sequences were identified in the small group of horses evaluated in this study is evidence of this. A large scale survey of MHC class I sequences in a diverse population of horses might identify sequence clustering that would allow development of a breed- or region-specific MHC class I microarray. The large scale utility of the array would also be improved if it were optimized for use with genomic DNA because fewer steps would be required and viable cells would not be needed for RNA recovery. For T cell immunology and transplantation applications however, the initial RT-PCR step imparts greater functional relevance by limiting detection to expressed sequences. Regardless, the microarray described herein will be useful in the design of future equine T cell vaccine and immunotherapeutic studies by allowing rapid selection of horses with known sequences of functional significance, or alternatively, to ensure that individual sequences are not over represented in experimental groups. In the case of T cell vaccine development, the microarray will greatly facilitate identification of CTL epitopes that can be presented by a diverse set of MHC molecules, and should be useful in characterizing differential immunologic responses between individuals at the class I sequence level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Albright-Fraser DG, Reid R, Gerber V, Bailey E. Polymorphism of DRA among equids. *Immunogenetics*. 1996; 43:315–7. [PubMed: 9110935]
- Bailey E. Identification and genetics of horse lymphocyte alloantigens. *Immunogenetics*. 1980; 11:499–506. [PubMed: 6242885]
- Barbis DP, Maher JK, Stanek J, Klaunberg BA, Antczak DF. Horse cDNA clones encoding two MHC class I genes. *Immunogenetics*. 1994; 40:163. [PubMed: 8026865]
- Beck S, Trowsdale J. The human major histocompatibility complex: lessons from the DNA sequence. *Annu Rev Genomics Hum Genet*. 2000; 1:117–37. [PubMed: 11701627]
- Call DR, Bakko MK, Krug MJ, Roberts MC. Identifying antimicrobial resistance genes with DNA microarrays. *Antimicrob Agents Chemother*. 2003; 47:3290–5. [PubMed: 14506043]
- Carpenter S, Baker JM, Bacon SJ, Hopman T, Maher J, Ellis SA, Antczak DF. Molecular and functional characterization of genes encoding horse MHC class I antigens. *Immunogenetics*. 2001; 53:802–9. [PubMed: 11862413]
- Chung C, Leib SR, Fraser DG, Ellis SA, McGuire TC. Novel classical MHC class I alleles identified in horses by sequencing clones of reverse transcription-PCR products. *Eur J Immunogenet*. 2003; 30:387–96. [PubMed: 14675391]
- Ellis SA, Bontrop RE, Antczak DF, Ballingall K, Davies CJ, Kaufman J, Kennedy LJ, Robinson J, Smith DM, Stear MJ, Stet RJ, Waller MJ, Walter L, Marsh SG. ISAG/IUIS-VIC Comparative MHC Nomenclature Committee report, 2005. *Immunogenetics*. 2006; 1–6.

- Ellis SA, Martin AJ, Holmes EC, Morrison WI. At least four MHC class I genes are transcribed in the horse: phylogenetic analysis suggests an unusual evolutionary history for the MHC in this species. *Eur J Immunogenet.* 1995; 22:249–60. [PubMed: 8547231]
- Elsner HA, Blasczyk R. Immunogenetics of HLA null alleles: implications for blood stem cell transplantation. *Tissue Antigens.* 2004; 64:687–95. [PubMed: 15546342]
- Fraser DG, Bailey E. Polymorphism and multiple loci for the horse DQA gene. *Immunogenetics.* 1998; 47:487–90. [PubMed: 9553156]
- Hurley CK, Wade JA, Oudshoorn M, Middleton D, Kukuruga D, Navarrete C, Christiansen F, Hegland J, Ren EC, Andersen I, Cleaver SA, Brautbar C, Raffoux C. A special report: histocompatibility testing guidelines for hematopoietic stem cell transplantation using volunteer donors. *Tissue Antigens.* 1999; 53:394–406. [PubMed: 10323348]
- Lazary S, Antczak DF, Bailey E, Bell TK, Bernoco D, Byrns G, McClure JJ. Joint report of the fifth international workshop on lymphocyte alloantigens of the horse, Baton Rouge, Louisiana, 31 October–1 November 1987. *Anim Genet.* 1988; 19:447–56. [PubMed: 2466424]
- Letowski J, Brousseau R, Masson L. Designing better probes: effect of probe size, mismatch position and number on hybridization in DNA oligonucleotide microarrays. *J Microbiol Methods.* 2004; 57:269–78. [PubMed: 15063067]
- McGuire TC, Leib SR, Mealey RH, Fraser DG, Prieur DJ. Presentation and binding affinity of equine infectious anemia virus CTL envelope and matrix protein epitopes by an expressed equine classical MHC class I molecule. *J Immunol.* 2003; 171:1984–93. [PubMed: 12902502]
- Mealey RH, Lee JH, Leib SR, Littke MH, McGuire TC. A single amino acid difference within the alpha-2 domain of two naturally occurring equine MHC class I molecules alters the recognition of Gag and Rev epitopes by equine infectious anemia virus-specific CTL. *J Immunol.* 2006; 177:7377–90. [PubMed: 17082657]
- Tallmadge RL, Campbell JA, Miller DC, Antczak DF. Analysis of MHC class I genes across horse MHC haplotypes. *Immunogenetics.* 2010; 62:159–72. [PubMed: 20099063]
- Tallmadge RL, Lear TL, Antczak DF. Genomic characterization of MHC class I genes of the horse. *Immunogenetics.* 2005; 57:763–74. [PubMed: 16220348]
- Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y. Microdroplet testing for HLA-A, -B, -C, and -D antigens. The Phillip Levine Award Lecture. *Am J Clin Pathol.* 1978; 69:103–20. [PubMed: 75690]
- Warsen AE, Krug MJ, LaFrentz S, Stanek DR, Loge FJ, Call DR. Simultaneous discrimination between 15 fish pathogens by using 16S ribosomal DNA PCR and DNA microarrays. *Appl Environ Microbiol.* 2004; 70:4216–21. [PubMed: 15240304]
- Williams TM. Human leukocyte antigen gene polymorphism and the histocompatibility laboratory. *J Mol Diagn.* 2001; 3:98–104. [PubMed: 11486048]

Table 1

Pedigree of Related Horses Used for Classical MHC Class I Microarray Design and Validation

Horse	Breed	ELA-A, DRA and DQA Haplotype *	Dam	Dam ELA-A, DRA and DQA Haplotype	Sire	Sire ELA-A, DRA and DQA Haplotype
I72	Arabian	A1/Undefined; DRA *0201/*0201; DQA *0701/*0901	Not Available	Not typed	Not A available	Not typed
I74	Arabian	A4/Undefined; DRA *0201/*0301; DQA *0501/*0701	172	A1/Undefined; DRA *0201/*0201; DQA *0701/*0901	Sire B	A4*W11; DRA *0101/*0301; DQA *0501/*0501
I76	Arabian	A1/W11; DRA *0101/*0101; DQA *0501/*1901	169	A1/A4; DRA *0101/*0301; DQA *0501/*1901	Sire B	A4*W11; DRA *0101/*0301; DQA *0501/*0501
I84	Arabian	A4/A4; DRA *0301/0301; DQA *0501/0501	174	A4/Undefined; DRA *0201/*0301; DQA *0501/*0701	A2135 [‡]	A4*W11; DRA *0101/0301; DQA *0501/unknown
A2140	Arabian	A1/W11; DRA *0101/*0201; DQA *0501/0901	172	A1/Undefined; DRA *0201/*0201; DQA *0701/*0901	Sire B	A4*W11; DRA *0101/*0301; DQA *0501/*0501
A2152	Arabian	A1/A4; DRA *0201/*0301; DQA *0501/*0901	162	A1; DRA *0101/*0201; DQA *0901/*1201	Sire B	A4*W11; DRA *0101/*0301; DQA *0501/*0501
H600	Arabian-Pony cross	A1/W11 DRA *0101/*0101; DQA *0401/0501	504	A1/A1 DRA *0101/*0101; DQA *0401/1201	Sire B	A4*W11; DRA *0101/*0301; DQA *0501/*0501

* All ELA-A haplotypes were determined serologically. It was not known if mare 162 with only a single detectable haplotype was homozygous or heterozygous with a haplotype not recognized by available antisera. Class II DRA and DQA haplotypes were determined by SSCP analysis.

[‡] ELA-A4 / DRA *0301 / DQA *0501 of Sire 2135 inherited through Sire B.

Table 2

Outbred Horses Used for MHC Class I Microarray Validation

Horse	Breed	ELA-A Haplotype
H605	Pony	A5/A6
H621	Pony	A3
H623	Pony	W11
Stallion D	Arabian	A1
0834	TB	A2/A2
3909	TB	A9/A9

Table 3

Classical MHC class I sequences identified by cloning/sequencing and microarray

Horse	MHC class I haplotype (ELA)	Sequences identified by sequencing (Eqca prefix)	No. of clones	Sequence identified by microarray	Locus	GenBank accession
172 ^a	A1.1	N*00602	1	Yes	18	AY225155
		N*01301	>4	Yes	19	AY176101
	Undefined	I*01001	>4	Yes	1	AY176104
		N*00201	2	Yes	17	AY176094
174 ^a	A4	16*00601	>4	Yes	16	EF577070
		N*00801	>4	Yes	N	AY225151
	Undefined	I*00401	>4	Yes	1	AY225153
		I*01001	2	Yes	1	AY176104
176 ^a	A1.2	N*00201	3	Yes	17	AY176094
		16*00601	2	Yes	16	EF577070
	A1.2	N*00601	>4	Yes	18	AY374512
				N*01301 ^c	19	AY176101
184 ^a	W11	N*00201	3	Yes	17	AY176094
				I*00701 ^c	1	EF577068
	A4	N*00801	>4	16*00601 ^c	16	EF577070
		I*00401	>4	Yes	N	AY225151
A2140 ^a	A1	N*00602	>4	Yes	1	AY225153
		N*01301	>4	Yes	18	AY225155
	W11	I*00701	2	Yes	19	AY176101
		N*00201	>4	Yes	1	EF577068
A2152 ^a	A4	16*00601	1	No	17	AY176094
		N*00801	4	Yes	16	EF577070
	A1.1	I*00401	>4	Yes	N	AY225151
		N*00602	>4	Yes	1	AY225153
H600 ^b	A1.3	N*01301	1	Yes	18	AY225155
		N*01401	4	Yes	19	AY176101
	A1.3		>4	No ^d	18	HM450667

Horse	MHC class I haplotype (ELA)	Sequences identified by sequencing (E _{qca} prefix)	No. of clones	Sequence identified by microarray	Locus	GenBank accession
H605 ^b	W11	1*00701	>4	Yes	1	EF577068
		N*00201	3	No	17	AY176094
		16*00601	>4	Yes	16	EF577070
		N*01101	>4	No ^d	18	HM450668
H621 ^b	A3	1*00502	4	No ^d	1	HM450669
		N*01201	>4	No ^d	18	HM450670
		1*00801	>4	No ^d	1	HM450671
		1*00601	>4	No ^d	1	EF577072
H623 ^b	W11	N*01001	>4	No ^d	N	DQ145602
		1*00901	>4	Yes ^e	1	HM450672
		N*00602	>4	Yes	18	AY225155
		N*00601	>4	Yes	18	AY374512
Stallion D ^b	A1.1/A1.2	N*01301	1	Yes	19	AY176101
		2*00201	Tallmadge et al. 2010	No ^d	2	DQ145607
		5*00101		No ^d	5	DQ145611
		7*00101		No ^d	7	DQ145616
0834 ^b	A2	16*00101		No ^d	16	M95410
		N*00101		No ^d	N	M95409
		1*00201	Tallmadge et al. 2010	No ^d	1	DQ145597
		2*00102		No ^d	2	DQ145609
3903 ^b	A9	5*00101		No ^d	5	DQ145612
		6*00201		No ^d	6	DQ145614
		7*00101		No ^d	7	DQ145618
		16*00301		No ^d	16	DQ145598
		N*00301		Yes ^e	N	DQ145595
		N*00701		No ^d	N	DQ145596

^a Arabian horses used for microarray probe design

^b Horses used for microarray validation

^c These sequences were not identified by cloning and sequencing but were known to be present based on haplotype inheritance data

^d Array did not contain probes to specifically detect this sequence

^e Additional sequence-specific probes needed to confirm the presence of this sequence