

## **Genotyping of Horse MHC class I genes**

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Consideration of the polymorphism of horse MHC class I molecules is critical for evaluating antigens that induce protective CTL responses, because each allele presents different peptides. Horses have significant polymorphism in MHC class I, but the number of alleles identified is less than for the human (225 HLA-A, 444 HLA-B, 111 HLA-C alleles) and mouse. To date, 24 horse MHC class I alleles have been defined using a microcytotoxicity test. However, this does not completely define the polymorphism in individual horses.

This study describes an improved typing method for horse polymorphic MHC class I genes. It is based on PCR amplification of allele-specific polymorphic peptide binding region and conserved transmembrane and cytoplasmic regions, followed by cloning and sequencing. This method excluded nonpolymorphic class I genes from polymorphic genes because of the primers and PCR conditions used. In addition, possible functional polymorphic genes were analyzed by translation pattern. MHC class I genes from serologically typed equine A4 haplotypes were defined. Two genes, 7-4 and 7A1A genes were found in two horses [2152(A1/A4) and 2153 (A4/?)] with serologically defined A4 MHC class I haplotypes. Interestingly, A1 horses that recognized the same epitope did not have any similar polymorphic class I genes. Transcribed pseudogenes with frame-shifts, premature stop codons, and large fragment deletions were found. Five horses with different serological haplotypes did not share any polymorphic MHC class I genes, indicating that this method can be used to select horses with different MHC class I genes for immunization experiments.