GENETIC HOMOGENEITY AND MAJOR HISTOCOMPATIBILITY COMPLEX HAPLOTYPING OF WHITE MICE

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Introduction. Inbred murine strains are generated to insure genetic homogeneity and uniqueness and define immune characteristics, like major histocompatibility complex (MHC) haplotype, of the experimental model. Maintaining of the perfect inbred stock leads to increased level of homozygosity and sometimes encounters a problem of inbreeding depression and consequently deviation from strict inbreeding protocol. Our goals are (i) study genetic homogeneity of mice in the colony, and (ii) haplotyping of H-2 complex (MHC in mice) in this strain.

Materials and methods. White mice colony has been maintained for decades at the Research Institute for Biological Safety Problems, Zhambulskaya oblast, Gvardeisky, Kazakhstan. gDNA from tail clips was isolated using Wizard Genomic DNA Purification Kit (Promega). Oligonucleotide primers were synthesized at the National Center for Biotechnology (NCB, Astana). PCR was carried out using high fidelity Herculase II Fusion DNA Polymerase (Agilent Technologies) for 30-40 cycles. Length polymorphism of PCR products was studied using horizontal agarose gel-electrophoresis. Ultimately, primary nucleotide sequence and amplicon length was analyzed with DNA sequencing (NCB, Astana). Acquired DNA sequences of MHC of target white mice were compared to sequences of known mouse strains available at Mouse Genome Informatics database (Jackson lab, USA).

Results and discussion. In a parallel study in the laboratory of Immunobiology at NURIS, we immunize mice with a cocktail of peptides and test a protective effect of this vaccination against acute infection of mice with human influenza type A virus. Processing of viral proteins, induced immune responses and ultimately a protective effect is reliant upon MHC type, both in mice and in humans. We designed 25 genomic markers (25 forward and 25 reverse primers) that cover the entire H-2 complex including class I, class III and class II regions. Genomic markers were selected for high level of polymorphism in known and widely used white mouse strains. Therefore, PCR product length polymorphism acquired with agarose gel electrophoresis should be sufficient to assign an unknown H-2 haplotype of white mice to certain inbred mouse strain. In case that resolution power of the gel is insufficient, we used direct DNA sequencing of PCR products. Ten mice from different cages to be tested for 25 markers to estimate genetic homogeneity of the strain.

Conclusions. A set of genetic markers was designed to test MHC haplotype and strain inbredness. PCR protocols were optimized to obtain maximum yield and purity of the DNA product.

Acknowledgments. This study was supported by the School of Science and Technology, Department of Biology, Nazarbayev University, and a grant from Ministry of Education and Science, Republic of Kazakhstan.