Short communication

Production of an MHC class II B molecular probe in the turkey, Meleagris gallopavo

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Abstract. Genetic selection for disease resistance may be facilitated by molecular markers of the major histocompatibility complex (MHC) of poultry. We describe the first sequence variation documented at the MHC Class II B region of turkeys, and provide specific probe optimization conditions for studying RFLP polymorphisms in this species.

Key words: MHC, turkey.

The major histocompatibility complex (MHC) consists of clusters of highly polymorphic genes, some associated with disease resistance and production traits in poultry. In the domestic fowl (Gallus domesticus) (LAMONT 1998), it has been investigated intensively. By contrast, the immunogenetics of turkeys (Meleagris gallopavo), despite its commercial importance, has been the subject of only preliminary studies. Those studies examined restriction fragment length polymorphism (RFLP) by probing restriction-digested, genomic turkey DNA with a 2.3 kb fragment from a genomic clone of a chicken MHC Class II B gene (EMARA et al. 1992, ZHU et al 1996). Class II molecules are used in cell-mediated immunity to distinguish between normal tissue and that infected with viral, fungal or protozoan parasites (STEVENS 1996). Below we describe the first nucleotide sequence information for the MHC Class II B of turkeys, demonstrate its similarity to the se-

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quences of related species, and describe generation of a molecular probe that reveals MHC RFLP polymorphisms of this recently domesticated bird. The primer sequences may be of value to domestic turkey breeding programmes, to the study of management of wild turkeys, and also to our understanding of the evolution of the MHC in birds of the order Galliformes (VON SCHANTZ et al. 1996, EDWARDS et al. 1999).

Genomic DNA was extracted from the blood of wild turkeys purchased from a game farm (Quail Co., Dora, New Mexico, USA). We chose game farm turkeys because they are likely to have a greater genetic diversity than domestic turkeys, and we wanted a probe that was broadly applicable. The MHC Class II B region was amplified from one female (MG6801) with degenerate peptide-binding region (PBR) primers known to amplify a 146 bp MHC class II region in the budgerigar (following the conditions of EDWARDS et al. 1999), producing a 124 bp PCR product (AF487249) in the turkey. This product was subjected to six-fold, double-stranded sequencing by MWG Biotech Inc., and demonstrated strong homology with MHC Class II B sequences of domestic fowl (Gallus gallus) and pheasant (Phasianus colchicus) (JONES 2000). The resultant sequence was used to design specific primers for the wild turkey region and were as follows: sense 5' ACTTCCAGAATCTCGGTGTT 3' and antisense 5' GGAATTCGTGGCGATACAC...
These primers were used to amplify the MHC Class II B region of one female (MG6801) and one male (MG6877) wild turkey, generating 75 bp PCR products (Figure 1a).

PCR reaction conditions were optimized with the Epicentre PCR Optimization kit and consisted of 12.5 pmoles of each primer, 30 ng of template DNA, and 2.5 U of Master Amp™ Taq (Epicentre) in a 50 μl final reaction volume. Amplifications were performed on a Primus 96 HPL thermal cycler (MWG Biotech, Inc.), by employing hot-start PCR at 96°C, followed by 30 cycles at 96°C for 30 seconds, 45°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 6 minutes. Using MHC-specific primers for the turkey showed that buffer D from the MasterAmp PCR Optimization kit was best suited for amplification.

PCR products were purified by using Qiagen PCR Purification Spin Kits for subsequent cloning protocols.

One μl of the purified PCR product was used for cloning into a pCR2.1-TOPO vector (Invitrogen). Following insertion of the PCR product into the vector, bacterial transformation was accomplished by using One Shot chemically competent cells. IPTG and Xgal were used for blue/white selective plating on Luria-Bertani (LB) agar to distinguish the transformed cells. Bacterial propagation was accomplished overnight at 37°C. Transformants were verified prior to sequencing by using vector primers M13F and M13R (Invitrogen) that flank the cloning cassette. DNA sequencing reactions were performed from the vector by MWG Biotech, Inc., on a LiCor 4200XL Read IR DNA sequencer. BLAST searches were performed with the use of the sequence from the contig of MG6801 as a template (Figure 1b). Results of the searches showed a 98 % sequence similarity between the two Meleagris gallopavo MHC fragments and an 84 % or 81 % sequence similarity between the MHC fragment of MG6801 and the domestic fowl or pheasant, respectively.

Southern hybridization probe preparation was accomplished via incorporation of DIG-dUTP during PCR with the use of the PCR DIG-probe synthesis kit.

![Figure 1b. Sequence alignments of a PCR-amplified fragment from Meleagris gallopavo with MHC class II B genes from Gallus gallus (Gaga) and Phasianus colchicus (Phco). Mega01 (AF487260) and Mega77 (AF487261) represent contigs for fragments generated via PCR from Meleagris gallopavo. They were aligned with Gaga (AF026562.1) and Phco (AJ224352.1) sequences via BLAST. The underlined regions within the sequence of Mega01 represent sense and antisense primer binding sites.](image-url)
PCR reactions to DIG-label the MHC class II fragment from the wild turkey were as follows: 25 μl MasterAmp Premix D (Epicentre Technologies), 12.5 pmol sense primer, 12.5 pmol antisense primer, 2.6 U Expand™ High Fidelity Enzyme Mix (Roche Molecular Biochemicals), 5 μl PCR DIG Probe Synthesis Mix (Roche Molecular Biochemicals), and 30 ng template DNA with a final reaction volume of 50 μl. Reaction conditions were as described above. Labeled PCR products were confirmed by agarose gel electrophoresis and UV transillumination.

Ten μg of genomic wild turkey DNA were digested with 20 U of the restriction enzyme PvuII for 3 hours at 37°C. The enzyme chosen was consistent with those of previous RFLP analyses using a 2.3 kb molecular probe from the domestic fowl (ZHU et al. 1996). Samples were then precipitated overnight at 0°C by using a mixture of 3M sodium acetate and 95% ethanol (1:20 v/v). Fragments were separated on a 1.0% agarose gel for 22 hours at 30V and then denatured, neutralized, and transferred to nylon membranes according to the manufacturer suggested protocols (Roche Molecular Biochemicals). Hybridization was performed in a Micro-4 hybridization oven (Hybaid Unlimited), and followed by colorimetric detection using NBT (4-Nitroblue tetrazolium chloride) and BCIP (5-Bromo-4-chloro-3-indolyl-phosphate) (Roche Molecular Biochemicals).
Following colorimetric detection, fragments were visible at 7.8 Kb, 5.9 Kb, 3.4 Kb, 3.0 Kb, 2.8 Kb, and 2.2 Kb (Figure 1c). The band patterns from the seven wild turkeys sampled are similar to the MHC class II RFLP patterns for the A, B, C, D, X, Y, and Z haplotypes of commercial and wild turkeys (ZHU et al. 1996) resolved with the use of the large MHC Class II B probe from the domestic fowl. The advantage of using our probe in RFLP generation will be that it is specific to turkeys, and may provide more detailed knowledge of nucleotide variation in this species. The sequence information and RFLP probe may be useful in evaluating the genetic diversity of domestic turkey lines and bottlenecked populations of this widely reintroduced game species, the good genes advantages of parasite-mediated sexual selection by wild turkey hens (BUCHHOLZ 1995) as well as in comparative studies of the evolution of the avian MHC.

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REFERENCES


