

## DLA Typing

### Canine MHC Class I Typing

DLA-88 is the most polymorphic Class I locus in the dog (51 known alleles), and is the primary focus of DLA class I typing. The most reliable method of analysis of DLA-88, to date, was developed in the lab of Rainer Storb, and is described in Venkataraman et al. (2007) Tissue Antigens 70:53-57. In this assay, a 1.1 kb region of canine DLA-88, containing the polymorphic exons, 2 and 3 and flanking introns, is amplified with a high-fidelity DNA polymerase mix (currently Velocity DNA Polymerase from Bionline), TOPO-cloned, and sequenced. The primers used for amplification are as follows: GCGGCGCGGCCAGTGCCCGGAG, and CCCTAGTGGAGGGAGATCGGGGAG. The sequence is then compared to a reference allele (DLA-88\*00101: see link below) for specific polymorphisms, and to the growing list of alleles for classification. For descriptions of most known alleles, to date, see Kennedy et al., (2001) Animal Genetics 32:193-199; Hardt et al. (2006) Tissue Antigens 67:163-165; and Venkataraman et al. (2007) Tissue Antigens 70:53-57.

### Canine MHC Class II Typing

The most polymorphic canine class II genes are DLA-DRB1 (51 known alleles) and DLA-DQBI (36 known alleles), and to a lesser extent, DLA-DQAI (16 known alleles). The former two loci are the focus of most routine DLA class II typing. At Fred Hutchinson, Class II identification is done in two steps. Initial identification is done by microsatellite analysis, as described in Wagner et al (1996) Transplantation 62: 876-877 The alleles are then confirmed by PCR-SSCP and sequencing as described in Wagner, Works and Storb ,(1998), Tissue Antigens 52:397-401. Primers for DRB1 are: CCGTCCCCACAGCACATTTTC and TGTGTCACACCTCAGCACCA. Primers for DQB1 are: TCACTGGCCCGGCTGTCTCC and GGTGCGCTCACCTCGCCGCT. Sequences are compared to the DLA typing data base indicated below.

### Canine MHC Genotyping Database

A listing of known DLA genes and alleles, as well as a suite of tools and references for DLA typing, can be found at <http://www.ebi.ac.uk/ipd/mhc/dla>. Several useful links within the DLA data base page are worth mentioning: Choose "Nomenclature" to observe sequences and references for all the known Class I and Class II alleles. Choose "Alignments" to align your new sequence with known DLA alleles. For a different type of alignment format, choose "Phylogeny" and go to "ClustalW2". Choose "Downloads" to download any of the known DLA allele sequences. Choose "Submissions" to submit a new sequence.