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(71) Applicant *(for all designated States except US)*: **Stichting Biomedical Primate Research Centre** [NL/NL]; Lange Kleiweg 151, NL-2288 GJ Rijswijk (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BONTROP, Ronald Edward** [NL/NL]; Leidseweg 280, NL-2253 JL Voorschoten (NL). **DOXIADIS, Gabriele Gerda Maria** [DE/NL]; Hoge Rijndijk 256, NL-2314 AK Leiden (NL).

(74) Agent: **HATZMANN, M.J.**; Vereenigde, Johan de Wittlaan 7, NL-2517 JR Den Haag (NL).

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(54) Title: MEANS AND METHODS FOR HAPLOTYPING MHC-DRB LOCI IN MAMMALS AND USES THEREOF

(57) Abstract: The invention relates to the typing of MHC-DRB loci in mammals. In particular, the invention provides a typing procedure for the mammalian DRB region that allows an easy, economical, high resolution, fast and accurate haplotyping protocol. The invention further provides the use of said typing procedure in genetic applications, and provides a kit for typing of MHC-DRB loci.

Title: Means and methods for haplotyping MHC-DRB loci in mammals and uses thereof.

The invention relates to the field of biology and medicine. In particular, the invention relates to the typing of MHC-DRB loci in mammals.

The Major Histocompatibility Complex (MHC) is a large genomic region that is present in most vertebrates, although individual MHC region-linked genes have been identified in invertebrate genomes such as *Drosophila melanogaster* and *Caenorhabditis elegans*. The MHC gene products play a key role in adaptive immunology. Genes of the MHC are among the most polymorphic loci known in vertebrates, due to a high rate of mutation and recombination. Major differences between species reside not only in the number and identity of MHC genes, but also in the degree of allelic polymorphism for individual genes.

In general, three subgroups of MHC molecules can be defined , termed class I, II and III gene products. Molecules encoded by the so-called classical MHC class I and class II genes, present short peptides derived from proteins of pathogens and self antigens to the immune system. MHC class I genes encode molecules that are present on the surfaces of nearly all nucleated cells. These gene products are mainly responsible for facilitating immune responses to intracellularly processed pathogens such as viruses. MHC class II gene products are mainly present in a subset of antigen-presenting cells such as B-lymphocytes and activated T-lymphocytes. Class II gene products are primarily involved in the immune response against extracellular pathogens such as bacteria. Furthermore, they play an important role in recognition of foreign and self-antigens. MHC class III gene products are not involved in

antigen presentation but rather represent other immune-related components such as components of the complement system.

Of particular importance are the MHC genes that encode the cell surface, antigen-presenting molecules. In humans, these genes are referred to as Human Leukocyte Antigen (HLA) genes. The classical MHC genes HLA-A, HLA-B, and HLA-C belong to MHC class I, encoding the alpha chain of the respective MHC molecule. The HLA class II region is divided into -DP, -DQ, and –DR. The classical HLA-DR, -DQ, and -DP molecules are transmembrane heterodimers, composed of an alpha- and beta-chain subunit encoded by the A and B genes, respectively.

Defects in some MHC class II genes have been associated with autoimmune disorders such as arthritis and diabetes (Otsuka et al.. 2006. Proc Natl Acad Sci U S A. 103: 14465-7; Aoki et al. 2005. Autoimmun Rev. 4: 373-9). Similarly, polymorphisms in MHC class II genes have been associated with susceptibility to a range of infectious diseases including malaria, tuberculosis, leprosy, typhoid fever, hepatitis and HIV/AIDS. For instance, experimental autoimmune encephalomyelitis in rhesus macaques, a model for the human disease multiple sclerosis, is known to be influenced by certain MHC class II alleles (Slierendrecht et al. 1995. International Immunology, Vol. 7: 1671-1679).

In the human population, five major -DRB region configurations are classified. These region configurations share an invariant HLA-DRA gene and a -DRB9 gene segment but differ in physical length and also in the composition and number of other DRB loci. Like humans, other primates such as chimpanzees, gorillas, and rhesus macaques have variable numbers of MHC-DRB loci per haplotype (Doxiadis et al. 2000. J. of Immunol. 164: 3193-3199). For example, the DRB region configuration in rhesus macaques (Macaca mulatta), termed Mamu-DRB, is highly plastic and has been subject to various contractions and

expansions (Slierendregt et al. 1994. J Immunol 152: 2298-307). In humans a high degree of polymorphism is observed for the DRB1 locus present on all haplotypes (Robinson et al. 2003. Nucleic Acids Res 31: 311-4). In rhesus macaques, however, a high degree of region configuration polymorphism has been described characterized by marked differences with regard to number and content of distinct loci present per haplotype (Doxiadis et al. 2000. J Immunol 164: 3193-9; Doxiadis et al. 2001. Immunol Rev 183: 76-85). The Mamu-DRB region configurations themselves, however, display a relatively low degree of polymorphism. Most of the Mamu-DRB alleles belong to loci/lineages that are shared with humans (Bontrop et al. 1999. Immunol Rev 167: 339-50), and their alleles have been named accordingly (Klein et al. 1990. Immunogenetics 31: 217-9). Similar observations regarding sharing of certain MHC-DRB loci/lineages with human orthologues have been made for other primate species, whereas DRB alleles are mostly species specific (Bontrop et al. 1999. Immunol Rev 167: 339-50).

It is generally accepted that allelic polymorphisms of the MHC class II genes warrant that different allotypes select distinct peptides for T cell activation, preventing one particular pathogen from sweeping through an entire population. Most sequence variability is confined to exon 2 of the MHC-DPB, -DQA, -DQB, and -DRB genes. One of the most polymorphic regions in humans is the HLA-DRB region with more than 500 alleles described worldwide until now (Bodmer et al. 1999. Eur J. Immunogenet. 26: 81; see also http://www.ebi.ac.uk/imgt/hla/stats.html). In rhesus macaques, a species far less analysed than humans, already more than 135 Mamu-DRB distinct genes/alleles have been determined, a number which will increase rapidly when more animals are analysed.

Due to the complexity of the DRB-region, the existing typing procedures, involving single-strand conformation polymorphism, denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphism analyses,

or sequence analyses, are cumbersome and time consuming. It is therefore an object of the present invention to provide a typing procedure for the mammalian DRB region that allows an easy, economical, high resolution, fast and accurate haplotyping protocol.

Therefore, in one aspect the present invention provides a method for determining a DRB haplotype in a sample comprising nucleic acid from a mammalian cell, said method comprising amplifying at least an intron 2 microsatellite-containing part of at least one DRB-gene from said nucleic acid; determining the type of intron 2 microsatellite present in said at least one DRB-gene; and determining the DRB haplotype of said cell from said type of intron 2 microsatellite present in said at least one DRB-gene.

The inventors have established that the type of intron 2 microsatellite present in said at least one DRB-gene is indicative of the DRB-allele that is associated with said intron in a cis-configuration, thus allowing the identification of said DRB-allele by typing of the associated intron 2 microsatellite. An advantage of said method is that the typing of said intron 2 microsatellite can be performed by relatively simple methods which do not involve complicated methods such as single-strand conformation polymorphism or sequence analyses. Therefore, said method provides an easy and economical method for determining a DRB haplotype in a sample.

A complex dinucleotide repeat or microsatellite, D6S2878, is located in the beginning of intron 2 of the DRB genes of human and non-human primates (Bergstroem et al. 1999. Am J Hum Genet 64: 1709-1718; Epplen et al. 1997. Hum Genet 99: 399-406; Riess et al. 1990. Immunogenetics 32:110-116). This microsatellite displays a complex polymorphism profile concerning repeat length variability as well as sequence variation. According to the present invention, the variability of said microsatellite can be used for typing of the cis-associated DRB-allele. The number of and identity of distinct DRB-alleles

that are present in a sample, as determined by the number and types of intron 2 microsatellites that are present in said sample, is used to determine a DRB-haplotype.

The number and types of intron 2 microsatellites that are present in a sample can be determined by any method known in the art, including but not limited to sequence analyses and sequence-specific amplification. However, a quick and convenient method for determining the number and types of intron 2 microsatellites comprises the determination of the length of the microsatellite, as it was found that the length of the intron 2 microsatellite is indicative for the cis-associated DRB-allele.

Therefore, in a preferred embodiment the invention provides a method for determining a DRB haplotype, wherein the typing of the intron 2 microsatellite is based on the length of said microsatellite.

Thus, a DRB-haplotype can be determined by analysis of the length of intron 2 microsatellite that is present in the amplified fragments from the at least one DRB gene. Each haplotype is identified by a specific number and size of the amplified fragments. Thus by identifying the specific number and size of the amplified fragments, the haplotype can be identified.

The number of distinct fragments obtained after amplification will depend on the number of distinct DRB-alleles that are present in a particular DRB-haplotype. The total number of DRB-alleles that can be present in a sample, and consequently resulting in multiple distinct amplified fragments, also depends on whether the cell is homozygous or heterozygous for a DRB-haplotype, as in the latter case more distinct fragments might be present.

In a preferred embodiment, the type of DRB-allele is determined by comparing the amplified intron 2 microsatellite-derived fragments with a reference. Said

reference can be a sample from a individual of which the types of DRB-alleles and the corresponding DRB-haplotype have been previously determined. Said reference can, for example, be taken along in the amplification reaction. The reference sample can be taken from the same or a different species, however, it is preferred that said reference sample is taken from the same species.

Therefore, the invention also provides a method wherein a DRB haplotype is determined by comparing the detected types of intron 2 microsatellite with a reference.

In preferred embodiment, said reference is taken from a sample from an individual of which the types of DRB-genes and the corresponding DRB-haplotype have been previously determined and of which relevant data, comprising number and length and/or sequence of the amplified intron 2 microsatellite fragments, have been stored in a database.

Therefore, in this embodiment, the invention provides a method wherein a type of intron 2 microsatellite is determined by comparing with a reference comprising a database of DRB haplotypes correlated with the associated intron 2 microsatellite types.

In a preferred embodiment, a database according to the invention is present in an electronic storage device, such as, but not limited to, a computer or a server. It is further preferred that said database comprising said reference can be addressed to compare the amplified intron 2 microsatellite-derived fragments with said reference.

Amplification of at least intron 2, or a microsatellite-containing portion thereof, of a DRB-gene can be performed by any method known in the art including, but not limited to, polymerase chain reaction, strand displacement amplification, nucleic acid sequence-based amplification, rolling circle

amplification technology, and transcription-mediated amplification. Each of these amplification methods uses different approaches to achieve the amplification of nucleic acid molecules to amounts that can subsequently be detected.

In a preferred embodiment, the invention also provides a primer pair that spans the region containing said intron 2 microsatellite, for amplifying at least said intron 2 microsatellite-containing part of at least one DRB-gene.

According to this embodiment, said primer pair is preferably selected to allow amplification of at least 1 DRB gene, more preferred at least 2 DRB genes, more preferred at least 3 DRB genes, more preferred at least 4 DRB genes, more preferred at least 5 DRB genes, more preferred at least 6 DRB genes, more preferred at least 7 DRB genes, more preferred at least 10 DRB genes, that are present within the DRB region of one or more individuals of a species.

Therefore, each of the primers of said primer pair preferably is selected to hybridize to a nucleic acid region that is conserved in most or essentially all DRB-genes and alleles thereof within a species, thereby allowing the amplification of said intron 2 microsatellite of essentially all DRB genes and alleles thereof that are present within the DRB region of one or more individuals of a species.

In a preferred embodiment, said primer pair comprises a first primer of which the nucleotide sequences corresponds to a conserved nucleotide sequence that is present in a position that is adjacent to the microsatellite in intron 2, and a second primer of which the nucleotide sequences corresponds to a conserved sequence that is present on the opposite side of said microsatellite sequence, relative to the first primer.

In a preferred embodiment, said first primer is selected from a region spanning the 3' end of exon 2 and the 5'start of intron 2 of a DRB gene. This region was found to be conserved within species, but not between species. A second primer can be selected from a region comprising a conserved sequence, said region being present within a range of up to 1000 nucleotides from the microsatellite at the opposite side of the microsatellite in intron 2 of a DRB gene relative to said first primer.

In a more preferred embodiment, said second primer is selected from a region comprising conserved sequences that are present within a range of 5 to 60 nucleotides from the microsatellite at the opposite side of the microsatellite in intron 2 of a DRB gene, relative to said one primer.

Therefore, in an even further preferred embodiment, a primer pair according to the invention comprises a first primer of which the nucleotide sequences corresponds to a conserved region spanning the 3' end of exon 2 and the 5' start of intron 2 of a DRB gene, and a second primer of which the nucleotide sequences corresponds to a region comprising conserved sequences that is present within a range of 5 to 60 nucleotides from the microsatellite at the opposite side of the microsatellite in intron 2 of a DRB gene, relative to said first primer.

In yet an even further preferred embodiment, said first primer is chosen from the primers

GAGAGCTTCACAGTGCAGC (SEQ ID NO 1);
TTCACAGTGCAGCGGCGAGGT (SEQ ID NO 2); and
CGTGTCCCCACAGCACGTTTC (SEQ ID NO 6)

and said second primer is chosen from the primers

GAGAGGATTCTAAATGCTCAC (SEQ ID NO 3);
ACACCTGTGCCCTCAGAACT (SEQ ID NO 4); and

ACATCTGTGTCCTCAGACCT (SEQ ID NO 5).

It is furthermore preferred that the amplified nucleic acid molecules comprising at least intron 2 of a DRB gene, or a microsatellite-containing portion thereof that are derived from different DRB-alleles can be discriminated by physical characteristics such as size, isoelectric point, or nucleotide sequence of the molecules. Therefore, the length of the amplified fragment is preferably less than 1000 nucleotides, more preferred less than 900 nucleotides, more preferred less than 800 nucleotides, more preferred less than 700 nucleotides, more preferred less than 600 nucleotides, more preferred less than 500 nucleotides, more preferred less than 400 nucleotides, more preferred less than 300 nucleotides.

An amplified fragment comprising the microsatellite can be detected by any method known in the art. Detection methods include real-time detection methods such as DNA binding fluorophores, 5' endonuclease, adjacent linear and hairpin oligoprobes, and self-fluorescing amplicons.

In another embodiment, detection of the amplified nucleic acid is performed by post-amplification methods, including but not limited to, colorimetric detection, chemiluminescence, and gel electrophoresis detection.

Gel electrophoresis comprises the analysis of nucleic acid molecules by using a polymer such as agarose or polyacrylamide. Known methods include slab gel electrophoresis and capillary gel electrophoresis. Nucleic acid molecules are separated on the polymer based on physical characteristics such as size, shape, or isoelectric point, of the molecules. Visualization of nucleic acid molecules after electrophoreses comprises classic staining procedures, including but not limited to ethidium bromide, silver, SYBR Green (2-[N-(3-dimethylaminopropyl)-N-propylamino]-4-[2,3-dihydro-3-methyl-(benzo-1,3-

thiazol-2-yl)-methylidene]-1-phenyl-quinolinium; Invitrogen), coomassie blue dyes, or inorganic microparticle labels such as nanocrystals or quantum dots.

The length of an amplified nucleic acid can be determined by methods known to a skilled person, including but not limited to comparison to a known standard provided by fragments of known length, measuring of the time that is required to reach a defined position on the gel under standard conditions, and by any other method that determines the size, shape, or isoelectric point of the nucleic acid, such as mass spectrometry.

Sequence analyses of each of the amplified nucleic acid molecules, or of a suitable part thereof, can be performed using methods that are known in the art, including but not limited to, chemical analyses, enzymatic analyses using nucleotide analogues, and hybridization methods.

In a preferred embodiment, amplified nucleic acid molecules according to the invention are generated using labelled nucleotide analogues in the amplification reaction, allowing detection of said amplified nucleic acid molecules after gel electrophoresis without staining and washing procedures. Labelled nucleotide analogues that can be used include, but are not limited to, fluorescently-labelled nucleotide analogues such as cyanine-dyes (Cy3 or Cy5) –labelled analogues, and radioactively labelled nucleotide analogues.

In an alternative embodiment, amplified nucleic acid molecules are detected through the native fluorescence of nucleic acid molecules.

In a more preferred embodiment, the nucleic acid molecules are fluorescently labelled. They can be detected using any method known in the art, such as, for example, laser-induced fluorescence. Detection is based on the excitation and emission spectra of the fluorescent label that is used.

In an even more preferred embodiment, the amplified nucleic acid molecules are generated using at least one primer that is labelled. Said at least one primer can be labelled with, for example, luminescent method system such as lanthanide ions, or fluorescence-based dyes such as 6-FAM, HEX, NED, ROX, 5-FAM, JOE, Cy3, and Cy5. The use of at least one primer that is labelled will also allow detection of the amplified nucleic acid molecules by gel electrophoresis without staining and washing procedures.

Therefore, a preferred method according to the invention comprises amplifying at least an intron 2 microsatellite-containing part from a DRB gene with a primer pair, wherein one or more of the primers is labelled.

Said sample comprising nucleic acid molecules of the cell that is used for amplifying at least intron 2, or a microsatellite-containing portion thereof, may comprise any nucleic acid molecule that comprises intron 2, or at least a microsatellite-containing portion thereof. These nucleic acid molecules include, but are not limited to, unspliced RNA, such as, for example, nuclear RNA, and genomic DNA.

In a preferred embodiment, the invention provides a method for determining a DRB haplotype in a sample comprising genomic DNA.

The sample can be derived from any mammal that comprises a microsatellite in intron 2 of a DRB gene. The present invention is suitable for designing breeding programs such as used in animal husbandry and for endangered species such as whales and elephants. Non-limiting examples of a mammal comprise pets such as dog and cat; ungulates including sheep, goat, and cattle such as cow; horse; and primates.

In a preferred embodiment, the sample is derived from any human or non-human primate that comprises a microsatellite in intron 2 of a DRB gene. Not

limiting examples of human or non-human primates include apes such as chimpanzee, gorilla, gibbon, siamang, orangutan, and human, Old World monkeys such as mandrill, macaque and baboon, New World monkeys , and Prosimians.

In a further preferred embodiment, a method according to the invention provides determining a DRB-haplotype in a sample that is derived from a human.

In another aspect, the invention relates to a kit for determining a DRB haplotype in a cell, said kit comprising means for amplifying at least an intron 2 microsatellite-containing part of at least one DRB-gene.

In one embodiment, said kit comprises a pair of primers that span a region of a DRB gene flanking the intron 2 microsatellite-containing part of at least one DRB-gene.

In a preferred embodiment, a first primer of said primer pair comprises sequences selected from a conserved region spanning the 3' end of exon 2 and the 5'start of intron 2 of a DRB gene, while a second primer comprises sequences selected from a conserved region that is present on the opposite side of said microsatellite sequence, relative to the first primer.

In a further preferred embodiment, said first and second primers are selected such that the length of the amplified fragment from the intron-2 of a DRB-gene is less than 1000 nucleotides, more preferred less than 900 nucleotides, more preferred less than 800 nucleotides, more preferred less than 700 nucleotides, more preferred less than 600 nucleotides, more preferred less than 500 nucleotides, more preferred less than 400 nucleotides, more preferred less than 300 nucleotides.

In another embodiment, the kit comprises primers of which a 5' primer comprises the nucleotide sequence of SEQ ID NO 1; and a 3' primer comprises the nucleotide sequence of SEQ ID NO 3.

In yet another embodiment, the kit comprises primers of which a 5' primer comprises the nucleotide sequence of SEQ ID NO 2; and a 3' primer comprises the nucleotide sequence of SEQ ID NO 4 or SEQ ID NO 5.

In a further embodiment, the kit comprises primers of which a 5' primer comprises the nucleotide sequence of SEQ ID NO 6; and a 3' primer comprises the nucleotide sequence of SEQ ID NO 3, SEQ ID NO 4 or SEQ ID NO 5.

In another aspect, the invention relates to the use of a method for determining a DRB haplotype in a sample for a genetic application.

The genes of the MHC are known the be among the most polymorphic loci known in human or non-human primates, due to a high rate of mutation and recombination. Major differences between species reside not only in the number and identity of MHC genes, but also in the degree of allelic polymorphism for individual genes. Therefore, the method for determining a DRB-haplotype in a sample, as provided by the current invention, can be used in genetic tests. Genetic test comprise tests to look for a possible predisposition to disease before any symptoms appear.
The most widespread type of genetic testing is newborn screening. Genetic test can be helpful in several areas: early detection, diagnosis, prognosis, and treatment.

In a further embodiment, the method for determining a DRB-haplotype in a sample according to the present invention can be used a genetic application comprising paternity testing.

Paternity tests can be performed postnatally by, for example, testing of blood or testing of a buccal swab, or testing of the umbilical cord, or testing of other sample collection including but not limited to semen, tissue and hair. Paternity tests can also be performed pre-natally by testing of a sample obtained by amniocentesis or by chorionic villus sampling.

In yet a further embodiment, the method for determining a DRB-haplotype in a sample according to the present invention can be used in a genetic application comprising forensic testing.

Forensic testing includes the application of method for comparison of biological samples in criminal investigations. Typically, the samples are processed in a dedicated forensic laboratory. Short tandem repeats such as provided by the microsatellite in the intron 2 of a DRB-gene may be included in a standard battery of core loci. This may increase the discriminatory power and decrease the probability of a match between profiles of two unrelated persons.

In another embodiment, the method for determining a DRB-haplotype in a sample according to the present invention can be used in a genetic application comprising tissue typing for transplantation testing.

MHC molecules play a crucial role in T-cell activation by antigen presenting cells. Antigenic recognition depends on the interaction between the antigenic peptide-binding MHC molecule and the T-Cell Receptor (TCR), an immunoglobulin-like heterodimeric protein expressed on T-lymphocytes. The transplanted organ represents a continuous source of antigens that can induce a rejection response at any time post-transplant. Furthermore, donor-derived immuno-competent lymphocytes may react with MHC-incompatible recipient cells and induce inflammatory responses in host tissues such as the skin and gastrointestinal tract. This complication is frequent after bone marrow

transplantation, but may also affect recipients of liver and other organ transplants and even blood transfusions.

Therefore, transplants with well-matched antigens, especially matching of DRB-haplotype, may function significantly better than those with a poor match, due to different rates of initial immune reactions, graft rejection, and graft failure due to infection or other causes.

In yet another embodiment, the method for determining a DRB-haplotype in a sample according to the present invention can be used in a genetic application comprising testing for chronic or infectious diseases.

Determining a DRB-haplotype may help to determine a possible predisposition to a disease before any symptoms appear. These diseases include, but are not limited to, autoimmune disorders such as arthritis, diabetes, multiple sclerosis (Gregersen et al., 2006. Nature 443: 574-7), bowel diseases such as ulcerative colitis and Crohn's disease, psoriasis, and chronic fatigue syndrome. Furthermore, polymorphisms in MHC class II genes such as DRB genes, have been associated with susceptibility to a range of infectious diseases including malaria, tuberculosis, leprosy, typhoid fever, hepatitis and HIV/AIDS.

In yet another aspect, the invention relates to the use of a kit for determining a DRB haplotype in a cell, said kit comprising means for amplifying at least an intron 2 microsatellite-containing part of at least one DRB-gene, in a genetic application comprising paternity testing, forensic testing, tissue typing for transplantation testing, or testing for chronic or infectious diseases.

The *DR* region of humans (*HLA-DR*) as well as other primates as chimpanzees and macaques and other mammals as sheep consist of one *DRA* and one to several *DRB* loci on each chromosome (here called '*DRB* region configurations'). These *DRB* region configurations vary in number and content of *DRB* loci present. Examples are given for humans (Fig. 3), chimpanzees

(Fig. 4), and rhesus macaques in comparison to humans (Fig. 5). Certain *DRB* loci are highly polymorphic. Thus, *DRB* region configurations may display allelic polymorphism and are then called '*DRB* haplotypes'. In humans only 5 *DRB* region configurations are known that are highly polymorphic especially at the *DRB1* locus, whereas in rhesus macaques more than 30 region configurations are described which display a low degree of polymorphism (Fig. 5).

The present invention describes the possibility to amplify the STR present in intron 2 of all *DRB* loci, not only *DRB1*, that contain an exon 2/intron 2 sequence thus all except *DRB2* and *DRB8*. The result of this high-throughput, high-resolution microsatellite typing is a DRB-STR pattern that specifies a given *DRB* haplotype. Examples for such a DRB-STR length analysis are given for humans (Fig. 6), chimpanzees (Fig. 7), rhesus macaques (Fig. 8) and cynomolgus macaques (Fig. 9). The resulting *DRB* haplotypes are color-coded. Extensive DRB-STR length analyses of *Mhc* homozygous individuals or of pedigreed animals of more than 3 generations resulted in the definition of *DRB* haplotypes of humans and rhesus macaques. Additionally, definition of *DRB* haplotypes was feasable for chimpanzees (*Patr-DRB,* Table 4) and cynomalogus macaques (*Mafa-DRB,* Table 5). Comparison of Figure 7 and Table 4 shows that the present application allows a definition of 13 instead of six *DRB* region configurations in the chimpanzee.

The present invention describes the possibility of high resolution typing via *DRB* haplotyping, not DR/DQ haplotype association studies as claimed by US-A-5908749 and US 2003/108940 A1. The present invention allows *DRB* typing in even a higher resolution than exon 2 sequencing, the method-of-choice used for high-resolution typing (van Dijk et al. 2007). An example is given in Fig. 10 for a pedigreed chimpanzee family in which the differentiation between haplotype b of father (Frits) and haplotype d of mother (Sonja) was only possible by DRB-STR typing. Thus, DRB-STR typing as described in the present invention is a powerful method for paternity testing (examples given

in Fig. 10 and 11). Additionally, the present invention allows powerful and quick parentage analysis as needed for example in macaque colony management (Fig. 12).

Furthermore, *DRB* high resolution typing is useful for disease association studies. In humans it is known that genes of the Major Histocompatibility Complex (Mhc) encode proteins important for activating antigen-specific immune responses. Alleles of the human Mhc class I as well as class II genes are known to be associated with susceptibility as well as resistance to certain autoimmune diseases. Alleles of the *HLA-DRB* loci are described to be tightly linked with various immune-related diseases as sarcoidosis (Grosser et al. 2005) and chronic pancreatitis (Cavestro et al. 2003). Especially for autoimmune diseases as Addisons' disease, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), narcolepsia, multiple sclerosis (MS), and type 1 diabetes (T1D) (Carrington 1999; Shiina et al. 2004)(summarized in Shiina, TA, 2006 and reviewed by Carrington 1999) specific *DRB* alleles or *DR/DQ* allele combinations are known to be associated with disease susceptibility and/or protection. In RA and Addisons' disease, specific *DRB1*04* alleles have been reported to be linked to disease susceptibility (Fries et al. 2002; Gombos et al. 2007). Thus, a high resolution typing for *DRB* is necessary for the screening of patients for both disorders. Numerous studies have shown a high linkage disequilibrium with *HLA-DRB1*1501* with susceptibility to SLE (Graham et al. 2007), Graves' disease (Sasaki et al. 2007), narcolepsy (Planelles et al. 1997) as well as to multiple sclerosis (MS). In narcolepsy and MS disease susceptibility has been fine mapped to the HLA class II haplotype including the *HLA-DRB5*0101* allele (Dyment et al. 2004; Dyment et al. 2005; Fogdell et al. 1995; Ramagopalan et al. 2007). The DRB-STR typing method presented here allows high resolution typing and therefore can discriminate between the susceptible alleles *DRB1*1501* and *DRB5*0101* and their variants *DRB1*1502* and *DRB5*0201*, respectively. This discrimination is otherwise only possible by time-consuming and expensive sequencing techniques. Furthermore, both *DRB* alleles are shown to encode

DR beta chains, which form two different HLA-DR dimers that are both, expressed at significant levels on the cell surface (Prat et al. 2005). Additionally, recent publications showed that epistatic interactions of the two DR molecules have a major influence on the disease development in MS patients (Gregersen et al. 2006). Thus, typing only for the *DRB1* allele that is mostly performed by *DRB* typing procedures may not be sufficient for MS research in the future. On contrast, several chromosomal regions have been linked to T1D susceptibility in humans using modern genome screening methods. The largest contribution comes, however, from several genes in the Mhc complex, formerly known as *DR3/DR4*. There is evidence that certain residues important for structure and function of both HLA-DQ and -DR peptide binding pockets determine disease susceptibility and resistance (Pociot and McDermott 2002; Zavattari et al. 2001). A number of susceptible, neutral, and protective DR-DQ haplotypes have been identified recently by the T1D Genetics Consortium (Erlich et al. 2008). The most susceptible haplotypes involve *DRB1*0301* and *DRB1*0405/0401/0402*, whereas the most protective ones are *DRB1*1501* and *DRB1*1401*. However, allelic variation of DRB can be observed for disease association in different ethnic populations (Al-Harbi et al. 2004; Almawi et al. 2004). Thus, for screening of T1D patients as well as for further fine mapping analysis of other, probably disease associated, loci as *DRB5*, a quick typing method as described here will be needed.

Definitions

The term DRB-gene is defined as a functional unit whose inheritance can be followed experimentally. The term DRB gene comprises both an active gene and a pseudogene, the latter being defined as a sequence that closely resembles a known DRB-gene but which is not expressed.

The term DRB-allele is defined as an alternative form of a DRB-gene that can be distinguished from other forms of said DRB-gene by, for example, sequence analyses.

The term DRB-locus is defined as a position on a chromosome that corresponds to a DRB-gene.

The term DRB-haplotype is defined as the unique combination of one or more DRB genes or their evolutionary equivalents that are in most mammals associated in a cis-configuration to the MHC-DRA gene or its evolutionary equivalent on the same chromosome. A DRB-haplotype is determined by the number of DRB-genes, and the specific DRB-alleles that are present of each of the DRB-genes, within the DRB region.

Brief description of drawings

Figure 3

Organization of the human (HLA) DR region.

Supplemental Figure 3

Partial nucleotide alignement of *DRB* exon 2 to intron 2.

Primer sequences of different publications are indicated (green, Doxiadis et al., PNAS 2007, 104, 8907-8912)

Figure 4

Organization of the *DRB* region of the chimpanzee (*Patr-DRB*).

Figure 5

Organization of the *DRB* region of humana and rhesus macaques: a comparison

Figure 6

Results: HLA-STR

Example of genotyping of human *DRB* alleles (*HLA*) by DRB-STR microsatellite analysis

DRB-STRs belonging to one haplotype are color-coded

Figure 7

Example of genotyping of DRB alleles of the chimpanzee (*Patr*) by DRB-STR microsatellite analysis

DRB-STRs belonging to one haplotype are color-coded

Figure 8

Example of genotyping of DRB alleles of the rhesus macaque *(Mamu)* by DRB-STR microsatellite analysis

DRB-STRs belonging to one haplotype are color-coded

Figure 9

Example of genotyping of DRB alleles of the cynomolgus macaque *(Mafa)* by DRB-STR microsatellite analysis

DRB-STRs belonging to one haplotype are color-coded

Figure 10

Example of a paternal and maternal haplotype discrimination by DRB-STR analysis in the chimpanzee (*Patr*)

Figure 11

The use of DRB-STR typing for paternity testing: Example of 2 possible chimpanzee fathers

Figure 12

Pedigree of a cynomolgus macaque family

Example 1

Materials and Methods

Samples

The 167 rhesus monkeys analyzed, housed at BPRC's breeding colony, originated mostly from India, but some are also of Burmese or Chinese origin. Seven of these animals are completely homozygous for their MHC region and derived from consanguineous matings; two additional animals are homozygous for their Mamu-A, -B, and -DR serotypes. Genomic DNA of human individuals or rhesus macaques was extracted from EDTA blood samples or from immortalized B-cell lines using a standard salting out procedure. Of the 160 human samples tested, 64 were HLA-DRB homozygous, 17 of which belong to a thoroughly characterized homozygous typing cell panel of the XIV International Histocompatibility Workshop, 2005.

STR-DRB genotyping

The relevant DNA segment in rhesus macaques was amplified with a forward primer located at the end of exon 2 (5'Mamu-DRB-STR: TTC ACA GTG CAG CGG CGA GGT) and 2 labeled reverse primers in intron 2 (3'Mamu-DRB-STR_VIC: ACA CCT GTG CCC TCA GAA CT and 3'Mamu-DRB-STR_FAM_1007: ACA TCT GTG TCC TCA GAC CT). For human samples a labeled forward primer located at the end of exon 2 (5'HLA-DRB-STR_VIC: GAG AGC TTC ACA GTG CAG C) and one reverse primer in intron 2 (3'HLA-DRB-STR: GAG AGG ATT CTA AAT GCT CAC) was used. The labeled primers were synthesized by Applied Biosystems (Foster City, USA) and the unlabeled primers by Invitrogen (Paisley, Scotland). The PCR reaction for rhesus macaques was performed in a 25 ml reaction volume containing 1 unit of Taq polymerase (Invitrogen, Paisley, Scotland) with 0.6 mM of the unlabeled forward primer, 0.4 mM of the VIC labeled reversed primer, 0.2 mM of the

FAM labeled reversed primer, 2.5 mM MgCl2, 0,2 mM of each dNTP, 1 x PCR buffer II (Invitrogen, Paisley, Scotland) and 100 ng DNA.

The PCR mixture for the human STR amplification was the same as that used for rhesus macaques with 0.1 mM of the VIC labeled forward primer and 0.1 mM of the unlabeled reverse primer. The cycling parameters for both amplifications were a 5 min 94°C initial denaturation step, followed by 5 cycles of 1 min at 94°C, 45 s at 58°C, and 45 s at 72°C. Then the program was followed by 25 cycles of 45 s at 94°C, 30 s at 58°C and 45 s at 72°C. A final extension step was performed at 72°C for 30 min. The amplified DNA was prepared for genotyping according to the manufacturer's guidelines and analyzed on an ABI 3130 genetic analyzer (Applied Biosystems). STR analysis was performed with the Genemapper program (Applied Biosystems) and all samples were analyzed at least twice.

PCR, cloning and sequencing

Seventy-five different Mamu-DRB alleles and 38 HLA-DRB alleles were sequenced from exon 2 to intron 2 including the microsatellite with a generic 5' DRB-exon 2 primer CGT GTC CCC ACA GCA CGT TTC together with the same 3' primers as used for DRB-STR genotyping but without label. The PCR reactions for rhesus monkey and human DRB were performed in a 100 ml volume containing 4 units of Taq polymerase (Invitrogen, Paisley, Scotland) with 0.2 mM of each primer, 2.5 mM MgCl2, 0,2 mM of each dNTP, 1 x PCR buffer II (Invitrogen, Paisley, Scotland) and 200 ng DNA. The cycling parameters were the same as described for STR-DRB genotyping. The resulting amplicons were cloned and sequenced as described recently (Doxiadis et al. 2006. Immunogenetics 58: 259-268; Penedo et al. 2005. Immunogenetics 57, 198-209). The resulting sequences were analyzed using the Sequence Navigator program (Applied Biosystems).

Phylogenetic analyses

Multiple sequence alignments of exon 2 of human and rhesus macaque -DRB sequences were created using MacVector™ version 8.1.1 (Oxford Molecular Group) and phylogenetic analyses was hen performed using PAUP version 4.0b.10 (Swafford 2002. PAUP*.Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4 (Sinauer Associates, Sunderland, Massachusetts)). Pairwise distances were calculated using Kimura-2 parameter and the neighbor-joining method for creating a phylogram. Confidence estimates of grouping were calculated according to the bootstrap method generated from 1,000 replicates.

Results

Mamu-DRB region configuration definition by microsatellite markers

The rhesus macaques studied are part of a large self-sustaining colony that has been thoroughly pedigreed based on segregation of serologically and molecularly defined markers (Doxiadis et al. 2001. Immunol Rev 183: 76-85; de Groot et al. 2004. J Immunol 172: 6152-7). The current panel covers 22 different region configurations characterized by unique combinations from two to six distinct Mamu-DRB genes (Table 1). Limited allelic polymorphism is detected within region configurations # 1, 11, 15, 18, 19, and 21. To denote allelic variation observed, for instance, in region configuration #1, the relevant haplotypes have been designated 1a and 1b (Table 1). The gene frequencies of the different region configurations as encountered in our population of animals are provided as well, in concert with the number of animals tested. Some haplotypes are frequently observed whereas others appear to be rare (Table 3).

Nearly all Mamu-DRB loci/lineages possess the D6S2878 microsatellite, and the relevant DNA segment could be amplified by means of the unique primer set developed for this protocol (Table 1). Amplification failures have only been observed for a few DRB6/DRBW pseudogenes for which the corresponding

amplicons could be scarcely detected, most probably due to primer inconsistencies. The overall results illustrate, however, that the lengths of the amplified STR products are highly variable and range from 153 to 293 bp (Table 1). Additionally, various STR markers seem to be predictive for the presence of a particular Mamu-DRB allele and, moreover, family studies demonstrated that they segregate in a Mendelian manner. Subsequent sequencing of the DNA segment ranging from exon 2 to intron 2 was conducted to unequivocally link each D6S2878 allele to an individual DRB gene/allele. This approach proved that diverse STR lengths could distinguish even highly similar alleles, differing for only one or two nucleotides. An instance is provided by the Mamu-DRB1*07032 and 07033 alleles, which are part of the two haplotypes belonging to configuration #18 (Table 1).

On average, most of the STRs linked to an individual allele appear to be rather conservative in composition and length. In the case of the frequently observed haplotype 11a (Table 3), for example, no length variability for both DRB1 gene-associated D6S2878 alleles is observed, which also holds true for the STR that is linked to DRB5*0301 as seen in haplotype 1a (Table 1). The DRB1*0406-linked STR of the latter configuration, however, may slightly vary in length. Such differences, as can be seen for example in haplotype 12, are reproducible, and do segregate in families with a particular haplotype as defined by serological methods (Bontrop et al. 1995. Immunol Rev 143: 33-62). As each region configuration is composed of an exclusive combination of different Mamu-DRB genes, the most essential conclusion that can be drawn is that the combination of STR markers appears to be unique for a given region configuration/haplotype.

HLA-DRB haplotype definition by microsatellite analysis

Genotyping for the highly divergent (GT)x(GA)y microsatellite allows speedy and accurate DR haplotyping in rhesus macaques, a species known to possess a high number of region configurations; these display, however, low levels of allelic variation. Next, it was investigated whether the

same approach would work in a species, that possesses a relatively low number of region configurations that parade abundant levels of allelic polymorphism. Therefore, in the first instance, a thoroughly characterized panel of 160 unrelated human samples of Caucasoid origin was chosen to conduct such an analysis. This selection also comprised 64 homozygous typing cells, facilitating the simple definition of haplotypes. Furthermore, the panel included the five known HLA-DRB region configurations, designated DR8, DR1, DR51, DR52, and DR53 (Schreuder et al. 2005. Tissue Antigens 65: 1-55), but also covered the 13 most common DR serotypes present in the Caucasoid population, differing for their DRB1 alleles (Table 2). To denote allelic variation as observed within some serotypes, the different DRB haplotypes have been designated a, b, c and so on (Table 2). The number of samples tested per haplotype, as well as the gene frequencies of the serotypes (Marsh et al. 2000. The HLA FactsBook (Academic Press, London, UK, San Diego)), have also been summarized (Table 3).

Again, a specially designed generic primer pair allowed amplification of the relevant intron 2 segment for virtually all HLA-DRB genes/alleles. Amplification failed only for one particular HLA-DRB5 allele present on DR16 haplotypes. This may be due to a mutated primer site. Subsequent extensive sequencing of exon 2-intron 2 DNA segments verified for each of the HLA-DRB alleles the unique linkage to its adjacent D6S2878 allele. The lengths of the respective repeats are highly polymorphic in the human population as well and range from 135 to 220 bp (Table 2). Moreover, STR lengths are highly predictive for the presence of individual HLA-DRB alleles, as was also shown earlier for rhesus monkeys. Again, differential STR lengths can make the distinction between highly similar -DRB alleles, differing for a few nucleotides: for example in the case of DRB1*0801(03) and DRB1*080302 (Table 2).

The DR53 region configuration contains a pseudogene, named HLA-DRB7, which is absent in macaques. This pseudogene displays no allelic variation, and indeed the associated D6S2878 is invariant in length independent of the adjacent -DRB1 allele. Thus, the DRB7-associated STR typifies all the

haplotypes belonging to the DR53 region configuration (Table 2). The same holds true for the DRB6 allele and its adjacent STR, which are characteristic for the DR1 region configuration, covering the DR1 and DR10 serotypes. As observed in rhesus macaques, some HLA-DRB alleles appear to be associated with multiple D6S2878 variants. A case is provided by the HLA-DRB1*140101 allele observed within the DR14 haplotypes, but some of the repeats grouping in the DR11a and DR13c family of haplotypes also show length differences (Table 2). Family analyses are needed to demonstrate their segregation. Most of the human repeats, however, appear to be conservative in composition and length, and are linked to an individual allele. Within this panel of 30 different HLA-DRB haplotypes, all but two can be readily dissected based on by their D6S2878 profiles. Thus, complex DR region configuration/haplotype information, as present in at least two populations of primate species, is readily obtained and defined based on D6S2878 genotyping after a simple amplification protocol conducted with one primer set.

Genetic stability and evolutionary history of the STR-DRB complex

The D6S2878 microsatellite has a composite character in primates (Riess et al. 1990. Immunogenetics 32: 110-6; Bergstrom et al. 1999. Am J Hum Genet 64: 1709-18; Kriener et al. 2000. Immunogenetics 51: 169-78; Trtkova et al. 1995. Mol Phylogenet Evol 4: 408-19) and phylogenetic comparisons of different DRB1 sequences obtained from humans and chimpanzees indicated that the ancestral structure most likely must have been a (GT)x(GA)y dinucleotide repeat. The HLA-DRB1 associated microsatellite comprises three sections exhibiting different evolutionary stabilities. The 5'(GT)x repeat represents the longest segment and evolves most rapidly, which is a known feature for long, uninterrupted dinucleotide repeats. The middle section or the (GA)z part is shorter and interrupted; its constellation appears to correlate well with different lineages/loci and its length seems to segregate with specific DRB1 alleles. The length of the 3'(GA)y part appears to be specific for a certain DRB lineage/locus (Bergstrom et al. 1999. Am J Hum Genet 64: 1709-18). All HLA-

DRB exon 2 sequences described in this study have been subjected to phylogenetic analyses, on which the D6S2878 sequences have been superimposed (Fig. 1). As can be seen, this report extends the knowledge on this particular microsatellite but also underscores its compound character (Epplen et al. 1997. Hum Genet 99: 399-406; Bergstrom et al. 1999. Am J Hum Genet 64: 1709-18). Additionally, a short dinucleotide (GC)1-3 part could be observed at the 3'end of the microsatellite of all HLA-DRB genes except for those belonging to the DRB6 and DRB7 pseudogenes. The variation seen in this newly recognized section of the microsatellite seems to be prognostic for certain HLA-DRB lineages or loci, respectively. The 5'(GT)x part is indeed the most polymorphic, which may especially in the case of fairly long (GT)x repeats evolve faster than the mutation rate operative on exon 2 itself (Fig. 1). This phenomenon, already described for DQCAR (Lin et al. 1998. Tissue Antigens 52: 9-18) could explain why, for instance, the HLA-DRB1*1302 allele is associated with a STR displaying length variation (Table 2).

The ancient HLA-DRB6 and -7 pseudogenes appear to miss the middle, interrupted (GA)z or the (GA)y part, respectively. The HLA-DRB6*0101 gene harbors a genetically stable D6S2878 showing no length differences at all. This is most probably due to its composition represented by a short and interrupted 5'(GT) and 3'(GA) part (Petes et al. 1997. Genetics 146: 491-8; Wierdl et al. 1997.Genetics 146: 769-79). For HLA-DRB6*0201 the opposite is true, and the long and uninterrupted (GT)x and (GA)y parts are indicative for unstable STR lengths (Table 2 and Fig. 1) (Jin et al. 1996. Proc Natl Acad Sci U S A 93: 15285-8).

The Mamu-DRB exon 2 sequences have also been subjected to phylogenetic analyses and the genetic composition of the D6S2878 sequences has been superimposed (Fig. 2). Like in humans, D6S2878 has a compound character. The newly described fourth 3'(GC) dinucleotide part is also present in rhesus macaques with repeat lengths ranging from 1 to 5. Comparable to the human situation, Mamu-DRB6 alleles form a distinct clade in the phylogenetic tree. Since the DRB6 locus is thought to predate the divergence of Old World

monkeys, great apes, and homonoids, it is not surprising that the same ancestral (GT)x(GA)y structure was detected in rhesus monkeys just as in humans. In contrast to the human microsatellite, some Mamu-DRB lineages/loci are characterized by a multipart (GT)x and/or a (GA)z middle segment. In general, the rhesus macaque STR appears to be even more complex than its human equivalent. This multifaceted composition seems to correlate with the high number of DRB region configuration/haplotype diversity observed in rhesus macaques. Despite the complex microsatellite patterns observed in the rhesus macaque, the composition and length of repeats associated with known Mamu-DRB alleles, are as expected, highly similar. The rhesus macaque DRB region has been subject to several rounds of duplication and contraction processes. For that reason, it is difficult to understand which highly related genes located on different region configurations/haplotypes represent separate loci or whether these sequences have an allelic affiliation. This microsatelite will be helpful in sorting out such genetic relationships.

Discussion

Microsatellite D6S2878 is considered to represent a promising marker for the development of a quick and accurate DRB haplotyping protocol in primate species, on the condition that one single set of informative primers can be developed. Indeed, a specifically designed primer pair allowed amplification of the relevant intron 2 segment for virtually all HLA- as well as Mamu-DRB genes/alleles. For both species, amplification artifacts have rarely been observed and such types of problems are easily to overcome by designation of specific primers that can be added to the same reaction mixture. The D6S2878 STR was proven to be highly variable in length, not only in humans but also in the rhesus macaque, thus verifying this microsatellite as a useful marker for DRB typing of both species. Phylogenetic analyses of human as well as rhesus macaque exon 2 sequences have been performed and

compared with microsatellite composition. For humans, the evolutionary relationships of exon 2 and the adjacent microsatellite seem to segregate closely together (Fig. 1). In rhesus macaques, the microsatellite composition was far more variable than in humans and a comparison of microsatellite and exon 2 phylogeny does not always seem to match the microsatellie composition (Fig. 2). One explanation for these results may be that rhesus macaque DRB loci/lineages are much older than their human equivalents, and this can be the reason for the higher diversity of the adjacent microsatellite as well. Furthermore, preliminary results of intron sequences illustrate that some of the Mamu-DRB sequences that are considered alleles of a given locus probably represent monomorphic loci themselves. However, truly allelic variants manifest the reliability of the comparison of exon 2 and the D6S2878 marker not only in humans but also in rhesus macaques.

Microsatellite typing was, in the first instance performed on large human and rhesus macaque panels for which the typing information was known. The most essential conclusion to be drawn (Table 1 and 2) is that in both species the combination of STR markers appears to be unique for a given haplotype. Within the rhesus macaque panel of 31 haplotypes all could be defined unambiguously, within the human panel of 30 haplotypes all but two could be thus defined. As stated earlier, ambiguities can easily be solved by development of additional primer pairs. As a control, a blind test was performed with 47 human and 26 rhesus monkey samples. More than 90% of the samples were scored correctly for their Mhc-DRB haplotypes. Some of the samples were not scored properly because they contained allotypes that were not present in the original test panel. Thus, this D6S2878 typing protocol provides a highly reliable method for Mamu- and HLA-DRB haplotyping, with its main advantage being simplicity and speed. Since differently labeled primers can be used for microsatellite typing, multiplexing is possible and 96 samples or even more can be analyzed within one test panel. The simplicity of the test is especially useful for Mamu-DRB haplotyping, which is otherwise extremely time consuming due to the unprecedented high number of different -

DRB region configurations. For the human situation, this approach is very helpful in the analysis of large amounts of samples, as they are needed, for example, in population and/or disease association studies. Furthermore, this method may also be of use in forensic medicine as well as in paternity-testing protocols. Additionally, high-resolution -DRB haplotyping will simplify donor-recipient matching in organ as well as bone marrow transplantation. As the D6S2878 STR is an old entity, it may also be used to study other populations of primate species.

The evolutionary stability of this microsatellite has been a matter of debate (Epplen et al. 1997. Hum Genet 99: 399-406; Riess et al. 1990. Immunogenetics 32: 110-6; Bergstrom et al. 1999. Am J Hum Genet 64: 1709-18; Trtkova et al. 1995. Mol Phylogenet Evol 4: 408-19; Maueler et al. 1999. Gene 226: 9-23). The HLA-DRB7 associated D6S2878 allele is especially remarkable, as this repeat has the shortest (GT)x as well as the (GA)y part and is highly stable in length, showing no polymerase slippage at all. This is in accordance with the fact that short and/or interrupted repeats are more stable than long, uninterrupted dinucleotides (Petes et al. 1997. Genetics 146: 491-8; Wierdl et al. 1997. Genetics 146: 769-79; Jin et al. 1996. Proc Natl Acad Sci U S A 93: 15285-8). To what extent the repeat composition may have a direct or indirect influence on the low mutation rate of the adjacent exon 2 segment of the DRB7 pseudogene is not well understood at present. It has been proposed, for example, that microsatellites near genes may increase and probably also decrease local mutation rates (Vowles and Amos 2004. PLoS Biol 2: E199). Interestingly, exon 2 of the DRB7 pseudogene, present on the only shared DR region configuration of humans and chimpanzees, is highly conserved between both species. Moreover, the D6S2878 sequence is completely identical (Fig. 1). It has been suggested that the intron 2 segment containing the (GT)x(GA)y repeat may bind a zinc-dependent protein and forms non B-DNA structures; thus, functionality of these so-called 'junk' DNA sequences cannot be ruled out and should be subjected to further analysis (Maueler et al. 1999. Gene 226: 9-23).

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Table 1 *Mamu-DRB* haplotypes defined by exon 2 sequencing and D6S2878 genotyping

hapl	1st *DRB* locus	STR	2nd *DRB* locus	STR	3rd *DRB* locus	STR	4th *DRB* locus	STR	5th *DRB* locus	STR	6th *DRB* locus	STR
1a	*DRB1*0406*	205, 207, 211	*DRB5*0301*	169								
1b	*DRB1*0411*	223	*DRB5*0304*	153								
2	*DRB3*0403*	192	*DRB*W305*	227								
3[a]	*DRB1*0412*	199	*DRB*W3701*	235								
4[a]	*DRB1*1010*	205	*DRB6*0121*	216								
5[a]	*DRB3*0408*	203	*DRB*W404*	293								
6[a]	*DRB3*0411*	190	*DRB*W314*	219								
7[b]	*DRB4*0102*	291	*DRB5*0306*	173								
8[b]	*DRB1*0321*	181	*DRB1*0322/3*	209								
9[b]	*DRB1*0321*	181	*DRB1*0322/3*	209	*DRB1*1003*[c]	175?						
10[b]	*DRB1*0309*	229	*DRB*W2507*	181	*DRB6*0109*	?						
11a	*DRB1*0303*	195	*DRB1*1007*	197	*DRB6*0103*	(202-206)						
11b	*DRB1*0312*	195	*DRB1*1007*	201	*DRB6*0103*	?						
11c	*DRB1*0306*	226	*DRB1*1007*	197	*DRB6*0103*	?						
11d	*DRB1*0306*	226-230	*DRB1*1003*	199-213	*DRB6*0107*	182						
12	*DRB1*0309*	233-247	*DRB6*0101*	?	*DRB*W201*	263 (261, 267)						
13	*DRB6*0112*	214	*DRB*W2501*	218?	*DRB*W2002*	218	*DRB6*0106?*	205?				
14	*DRB6*0114*	218	*DRB*W303*	193	*DRB*W401*	187						
15a	*DRB1*0403*	229	*DRB6*0107*	184	*DRB*W501*	?	*DRB6*?*	208				
15b[b]	*DRB1*0403*	229	*DRB6*0107*	183	*DRB*W502*	?	*DRB6*0120*	208				
16	*DRB1*0404*	225	*DRB6*0102*	164	*DRB*W307*	195	*DRB*W702*	188				
17	*DRB1*0701*	197	*DRB3*0405*	237	*DRB5*0303*	169	*DRB6*0123*	184				
18a[a]	*DRB1*0326*	214	*DRB1*07032*	211	*DRB6*0124*	170	*DRB*W2603*	177	*DRB6*0120*	?		
18b[b]	*DRB1*0326*	214	*DRB1*07033*	207	*DRB6*0124*	168	*DRB*W2604*	177	*DRB6*0120*	208		

	19a (220)	*DRB1*0318*	181	*DRB6*0105*	190, 192	*DRB*W604*	201, 205	*DRB*W603*	275, 277	*DRB6*0104*(+ALU)	222
	19b	*DRB1*0318*	181	*DRB6*0105*	194	*DRB*W604*	197	*DRB*W611*	275	*DRB6*01?*	222
	20[a]	*DRB3*0403*	182	*DRB*W402*	185	*DRB*W2701*	227	*DRB6*0116*	206	*DRB6*0107*	182
	21a	*DRB6*0111*	178	*DRB*W606*	211, 213, 197	*DRB*W2104*	211, 213	*DRB*W2603*	177, 187	*DRB6*0122*	236
	21b[b]	*DRB6*0108*	176	*DRB*W606*	213	*DRB*W2104*	213	*DRB*W2604*	177	*DRB6*0122*	232
	22	*DRB1*0310* *DRB6*0106*	241 192	*DRB6*0118*	210	*DRB*W101*	219, 223	*DRB*W602*	279 (285)	*DRB*W609*	219 (223)

[a] Animals with Chinese, [b] animals with Burmese origin. [c] *DRB1*1003* belong to haplotype 8 or 9; () STR length observed in some (1-3) animals; ? STR not or rarely detected but presence of gene ascertained by sequencing; 205?, 175? STR's detected but not confirmed by sequencing

Table 2 *HLA-DRB* haplotypes defined by sequencing and D6S2878 genotyping

Haplotype		*DRB1* locus	STR	2nd *DRB* locus	STR	3rd *DRB* locus	STR
DR8	DR8a	*DRB1*0801(03)*	170 (172)				
	DR8b	*DRB1*080302*	176				
DR1	DR1a/DR103	*DRB1*010101/0103*	154 (156)	*DRB6*010101*	136		
	DR1b	*DRB1*010201*	160	*DRB6*0101*	136		
	DR10	*DRB1*100101*	161	*DRB6*0101*	136		
DR52	DR17a	*DRB1*030101*	172	*DRB3*010101(2)*	180		
	DR17b	*DRB1*0301*	178-184	*DRB3*0202(01*	208 (206, 210)		
	DR11a	*DRB1*110101*	182-192	*DRB3*020201*	206-218		
	DR11b	*DRB1*110201*	186	*DRB3*020201*	214		
	DR11c	*DRB1*110401*	188	*DRB3*020201*	208 (210)		
	DR12a	*DRB1*120101*	200	*DRB3*010102*	180		
	DR12b	*DRB1*1201*	206 (208)	*DRB3*02(0201)*	210, 212		
	DR12c	*DRB1*120201*	216	*DRB3*030101*	186		
	DR13a	*DRB1*1301(01)*	194	*DRB3*0101(02)*	180		
	DR13b	*DRB1*1301(01)*	194, 196	*DRB3*02(0201)*	204-214		
	DR13c	*DRB1*1302(01)*	188, 206-218	*DRB3*0301/0101*	186		
	DR14a	*DRB1*140101*	192	*DRB3*0211*	212		
	DR14b	*DRB1*140101*	186, 188, 196	*DRB3*020201*	210		
	DR14c	*DRB1*140101*	198	*DRB3*0201*	214		
DR53	DR4a	*DRB1*0401(01)*	180, 182, 184	*DRB4*(010101)*	178, 180, (176, 182/4)	*DRB7*010101*	135
	DR4b	*DRB1*0402*	194	*DRB4*010101*	176	*DRB7*010101*	135
	DR4c	*DRB1*0404*	192 (196)	*DRB4*010101*	178	*DRB7*010101*	135
	DR4d	*DRB1*040701*	184	*DRB4*010101*	178	*DRB7*010101*	135
	DR4e	*DRB1*0408*	180	*DRB4*010101*	178	*DRB7*010101*	135
	DR7	*DRB1*070101*	149	*DRB4*010101*	170, 176, 180	*DRB7*010101/2*	135
	DR9	*DRB1*09(0102)*	170 (196)	*DRB4*010101*	180	*DRB7*010101*	135
DR51	DR15a	*DRB1*150101*	186	*DRB5*0101(01)*	180	*DRB6*(0201)*	154
	DR15b	*DRB1*150101*	186, 190 (184, 188)	*DRB5*0101(01)*	184 (190)	*DRB6*(0201)*	152
	DR15c	*DRB1*150201*	202-208	*DRB5*0102*	220	*DRB6*(0201)*	174
	DR16	*DRB1*160101*	178	*DRB5*?*	184	*DRB6*0202*	144

() indicates STR lengths detected only once or twice

Table 3. Gene frequencies and numbers (#) of *Mamu*- and *HLA-DRB* haplotypes tested by D6S2878

Mamu			***HLA***		
hapl[a]	**#**	**gf[b] (2n = 240)**	**hapl[a]**	**#**	**gf[c]**
1a	43 (4)[d]	0,104	DR8a	5 (2)[d]	0.039
1b	4	0,004	DR8b	2 (2)	
2	20 (2)	0,017	DR1a-DR103	42 (20)	0.094
3	1	0,004	DR1b	8 (2)	
4	2	0,004	DR10	4	0.013
5	2	0,004	DR52-DR17a	34 (16)	0.111
6	2	0,004	-DR17b	12 (6)	
7	1	0,004	-DR11a	25 (2)	
8	3	0,004	-DR11b	2 (2)	0.134
9	1	0,004	-DR11c	6 (6)	
10	4	0,008	-DR12a	1	
11a	32	0,063	-DR12b	5 (2)	0.023
11b	7	0,017	-DR12c	1	
11a or b[e]		0,083	-DR13a	13 (6)	
11c	7 (4)	0,004	-DR13b	16 (6)	0.102
11d	21	0,096	-DR13c	12 (4)	
12	58 (2)	0,163	-DR14a	1	
13	10	0,008	-DR14b	4 (2)	0.032
14	7 (4)	0,021	-DR14c	2 (2)	
15a	7 (2)	0,033	DR53-DR4a	23 (6)	
15b	2	0,008	-DR4b	2 (2)	
16	3	0,029	-DR4c	11 (6)	0.128
17	7	0,025	-DR4d	2	
18a	3	0,004	-DR4e	1	
18b	2	0,004	-DR7	28 (12)	0.132
19a	5	0,071	-DR9	10 (2)	0.014
19b	3	0,013	DR51-DR15a1	5	
20	1	0,008	-DR15a2	39 (16)	0.107
21a	20 (2)	0,050	-DR15b	4 (2)	
21b	2	0,004	-DR16	2 (2)	0.036
22	14 (2)	0,042			
other	n.t.	0,092	others	n.t.	0.035
total	294	1,000	total	322	1.000

[a]*Mhc-DRB* haplotypes correspond to those defined in Table 1 and 2, respectively. [b]Gene frequencies (gf) of *Mamu-DRB* haplotypes of the rhesus macaque breeding colony. [c]Gene frequencies (gf) of caucasoid *HLA-DRB* haplotypes according to Marsh and coworkers (39). [d] () refer to # of homozygous typing cells included in the total #. [e]Haplotypes 9a and b could only be descriminated by D6S2878 genotyping and sequencing; n.t. not tested.

Table 4. *Patr-DRB* haplotypes defined by exon 2 sequencing and DRB-STR genotyping

hapl*	1st *DRB* locus	STR	2nd *DRB* locus	STR	3rd *DRB* locus	STR	4th *DRB* locus	STR	5th *DRB* locus	STR
1	*DRB1*0205*	214	*DRB3*0214L*	182						
2	*DRB4*0201*	138	*DRB*W902*	192,194						
3	*DRB1*02new*	228	*DRB3*0102L*	186	*DRB5*0101L*	170				
4a	*DRB1*0302*	190 (184)	*DRB3*0102*	186	*DRB6*0305*	155				
4b	*DRB1*0308*	178	*DRB3*0102*	186	*DRB6*0305*	155				
4c	*DRB1*0309*	176	*DRB3*0102*	186	*DRB6*0305*	155				
5a	*DRB1*0701*	157	*DRB4*0104*	186	*DRB7*0101*	135				
5b	*DRB1*0702*	153	*DRB4*0104*	186	*DRB7*0101*	135				
6	*DRB1*1001*	168	*DRB5*0310*	174	*DRB6*0108*	145				
7	*DRB1*0309*	176	*DRB6*0305*	155	*DRB*W903*	190				
8a	*DRB1*0201*	207, 211, 217 (209)	*DRB3*0201*	186	*DRB5*0301*	174	*DRB6*0108*	144		
8b	*DRB1*0201*	209	*DRB3*0208*	220	*DRB5*0306*	174	*DRB6*0109*	145		
8c	*DRB1*0201*	209	*DRB3*0208*	204	*DRB5*0304*	174	*DRB6*0108*	145		
8d	*DRB1*0202L*	226	*DRB3*0209L*	206	*DRB5*0306L*	195	*DRB6*0109*	145		
8e	*DRB1*02new*	211	*DRB3*0208*	212	*DRB5*0311*	172	*DRB6*0109*	145		
8f	*DRB1*0204*	205	*DRB3*0201*	188	*DRB5*0301*	174	*DRB6*0108*	144		
8g	*DRB1*0204*	205	*DRB3*0208*	208	*DRB5*0101L*	170	*DRB6*0109*	145		
8h	*DRB1*0204*	205	*DRB3*0208*	208 (200)	*DRB5*0102*	170	*DRB6*0109*	145		
8i	*DRB1*0204*	205	*DRB3*0208*	210	*DRB5*0102L*	170	*DRB6*0109*	145		
8j	*DRB1*0204*	221,225	*DRB3*0208*	212	*DRB5*0101L*	170	*DRB6*0109*	145		
9a	*DRB1*0302*	182	*DRB3*0102*	186	*DRB5*0312*	174	*DRB6*0305*	155		
9b	*DRB1*0307*	164, 174	*DRB3*0208*	186	*DRB5*0301*	174	*DRB6*0305*	155		
9c	*DRB1*0307*	166	*DRB3*0102*	186	*DRB5*0306*	174	*DRB6*0305*	155		
9d	*DRB1*0307*	172	*DRB3*0102*	186	*DRB5*0102*	170	*DRB6*0305*	155		
10	*DRB1*1001*	170	*DRB3*0208*	208	*DRB5*0310*	174	*DRB6*0108*	155		
11	*DRB1*0311*	184	*DRB3*0208*	202	*DRB5*0307*	174	*DRB*W901*	190		
12	*DRB1*0305*	166, (168)	*DRB3*0208*	214,218,222,(208)	*DRB5*0310*	174	*DRB6*0108*	145	*DRB6*03?*	155
13	*DRB1*0201*	205	*DRB3*0208*	202	*DRB5*0307*	174	*DRB6*0108*	145	*DRB6*0305*	155

*Thirteen region configurations (1 – 13) could be defined. Letter surfixes (for example 7a – 7k) indicate that a region configuration displays allelic polymorphism and can thus be split in different haplotypes.
Data in parentheses indicate STR lengths detected only once or twice

Table 5. *Mafa-DRB* haplotypes defined by exon 2 sequencing and DRB-STR genotyping

hapl	1st *DRB* locus	STR	2nd *DRB* locus	STR	3rd *DRB* locus	STR	4th *DRB* locus	STR
1	*DRB1*0306*	193	*DRB5*0309*	227	*DRB*W6501*	*225*	*DRB6*0112*	*188*
2	*DRB*W405*	211	*DRB*W2504*	209	*DRB6*0114*	226		
3	*DRB1*0309*	209	*DRB*W2001*	283	*DRB6*0107*	*?*		
4	*DRB*W6601*	193	*DRB*W2001*	274	*DRB6*0108*	204		
5	*DRB1*0411*	229	*DRB*W360202*	229	*DRB6*0115*	214		
6	*DRB1*0312*	247 (239, 251)	*DRB*W2502*	185	*DRB6*0107*	208		
7	*DRB1*0313*	211	*DRB*W3601*	227	*DRB6*0105*	210	*DRB*W102?*	304
8	*DRB1*0314*	181	*DRB1*0315*	211	*DRB6*0112*	176		
9	*DRB1*0316*	195	*DRB*W4001*	203	?	181?		
10	*DRB1*0317*	193 (195)	*DRB*W601*	203	*DRB*W2001*	274	*DRB*W6701*	189 (195)
11	*DRB1*0317*	193	*DRB*W601*	203	*DRB6*0106*	*?*		
12	*DRB1*0401*	189	*DRB5*0303*	169	*DRB4*0102*	231	*DRB6*011302*	182
13	*DRB1*0401*	189 (187)	*DRB5*030101*	169	*DRB*W303*	247		
14	*DRB1*0401*	189	*DRB5*030101*	169	*DRB4*0101?*	255, 259		
15	*DRB1*0403*	207	*DRB*W3701*	241	*DRB6*011301*	?		
16	*DRB1*0403*	201	*DRB*W3701*	225	*?*			

17	*DRB1*0704*	191	*DRB*W605*	281	*DRB1*0308*	234
18	*DRB1*0704*	?	*?*	172?	*DRB*W5301*	219
19	*DRB1*1002*	218, 208, (204)	*DRB*W4901*	259, 263, (265)	*DRB6*0109*	184
20	*DRB3*0401*	239	*DRB5*0306*	169	*DRB6*0110*	186
21	*DRB*W6801*	209	*DRB6*0111*	178		
22	*DRB*W2101*	227 (229)	*DRB6*0101*	204 (206)	*DRB*W501?*	189 (221)

Data in parentheses are STR length observed in some (one or two) animals. Question marks indicate STRs not detected or rarely detected but presence of gene ascertained by sequencing. 172?, 181? Indicate STRs detected but not confirmed by sequencing. *DRB4*0101?* And *DRB*W501?* detected on cDNA, but not on gDNA most likely due to primer inconsistency.

Table 6. *Mafa-DRB* haplotypes of a cynomolgus family (Fig. 10) defined by exon 2 sequencing and DRB-STR typing

hapl	1st *DRB* locus	STR	2nd *DRB* locus	STR	3rd *DRB* locus	STR
a	*DRB1*0403*	201	*DRB*W3701*	225	*?*	
b	*DRB1*0704*	191	*DRB*W605*	281	*DRB1*0308*	234
c	*DRB1*0312*	247 (239, 251)	*DRB*W2502*	185	*DRB6*0107*	208
d	*DRB1*0401*	189	*DRB5*030101*	169	*DRB4*0101?*	255, 259
(e*	*DRB5*0305*)					
f	*DRB1*0411*	229	*DRB*W360202*	229	*DRB6*0115*	214
g	*DRB*W2101*	227 (229)	*DRB6*0101*	204 (206)	*DRB*W501?*	189 (221)
g*	*DRB1*1002*	218, 208, (204)	*DRB*W4901*	259, 263, (265)	*DRB6*0109*	184

Data in parentheses are STR length observed in some (one or two) animals. Question marks indicate STRs not detected or rarely detected but presence of gene ascertained by sequencing. 172?, 181? Indicate STRs detected but not confirmed by sequencing. *DRB*W501?* detected on cDNA, but not on gDNA most likely due to primer inconsistency. * haplotype e only determined by cDNA typing

Claims

1. Method for determining a DRB haplotype in a sample comprising nucleic acid from a mammalian cell, said method comprising
amplifying at least an intron 2 microsatellite-containing part of at least one DRB-gene from said nucleic acid;
determining the type of intron 2 microsatellite present in said at least one DRB-gene; and
determining the DRB haplotype from said type of intron 2 microsatellite present in said at least one DRB-gene.

2. Method according to claim 1, wherein said type of intron 2 microsatellite is determined based on the length of said microsatellite.

3. Method according to claim 1 or claim 2, wherein the DRB-haplotype is determined by comparing the detected types of intron 2 microsatellite with a reference.

4. Method according to claim 3, wherein said reference comprises a database of DRB haplotypes correlated with the associated intron 2 microsatellite types.

5. Method according to claim 4, wherein said database is present in an electronic storage device.

6. Method according to any of the previous claims, wherein said amplification comprises providing said sample with a primer pair that spans the region containing said intron 2 microsatellite.

7. Method according to claim 6, wherein said primer pair comprises a first primer of which the nucleotide sequences correspond to a conserved nucleotide sequence that is present in a position that is adjacent to the microsatellite in intron 2, and a second primer of which the nucleotide sequences corresponds to a conserved sequence that is present on the opposite side of said microsatellite sequence, relative to the first primer.

8. Method according to claim 6 or 7, wherein one or more of the primers used is labelled.

9. Method according to any of the previous claims, wherein the nucleic acid from a mammalian cell comprises genomic DNA.

10. Method according to any of the previous claims, wherein the sample is derived from a primate.

11. A kit for determining a DRB haplotype in a cell, comprising means for amplifying at least an intron 2 microsatellite-containing part of at least one DRB-gene

12. Kit according to claim 11, comprising a pair of primers.

13. Kit according to claim 12, wherein a first primer of said primer pair comprises sequences selected from a conserved region spanning the 3' end of exon 2 and the 5'start of intron 2 of a DRB gene, while a second primer comprises sequences selected from a conserved region that is present on the opposite side of said microsatellite sequence, relative to the first primer.

14. Kit according to any one of claims 11-13, wherein a 5' primer comprises the nucleotide sequence of SEQ ID NO 1; and a 3' primer comprises the nucleotide sequence of SEQ ID NO 3.

15. Kit according to any one of claims 11-13, wherein a 5' primer comprises the nucleotide sequence of SEQ ID NO 2; and a 3' primer comprises the nucleotide sequence of SEQ ID NO 4 or SEQ ID NO 5.

16. Kit according to any one of claims 11-13, wherein a 5' primer comprises the nucleotide sequence of SEQ ID NO 6; and a 3' primer comprises the nucleotide sequence of SEQ ID NO 3, SEQ ID NO 4 or SEQ ID NO 5.

17. Use of a method for determining a DRB haplotype in a sample, said method comprising amplifying at least a microsattellite-comprising region of intron 2 of at least one DRB gene, for a genetic application.

18. Use according to claim 17, wherein the genetic application comprises paternity testing.

19. Use according to claim 17, wherein the genetic application comprises a forensic testing.

20. Use according to claim 17, wherein the genetic application comprises tissue typing for transplantation testing.

21. Use according to claim 17, wherein the genetic application comprises testing for chronic or infectious diseases.

22. Us of the kit of any of claims 11-15 in a genetic application comprising paternity testing, forensic testing, tissue typing for transplantation testing, or testing for chronic or infectious diseases.

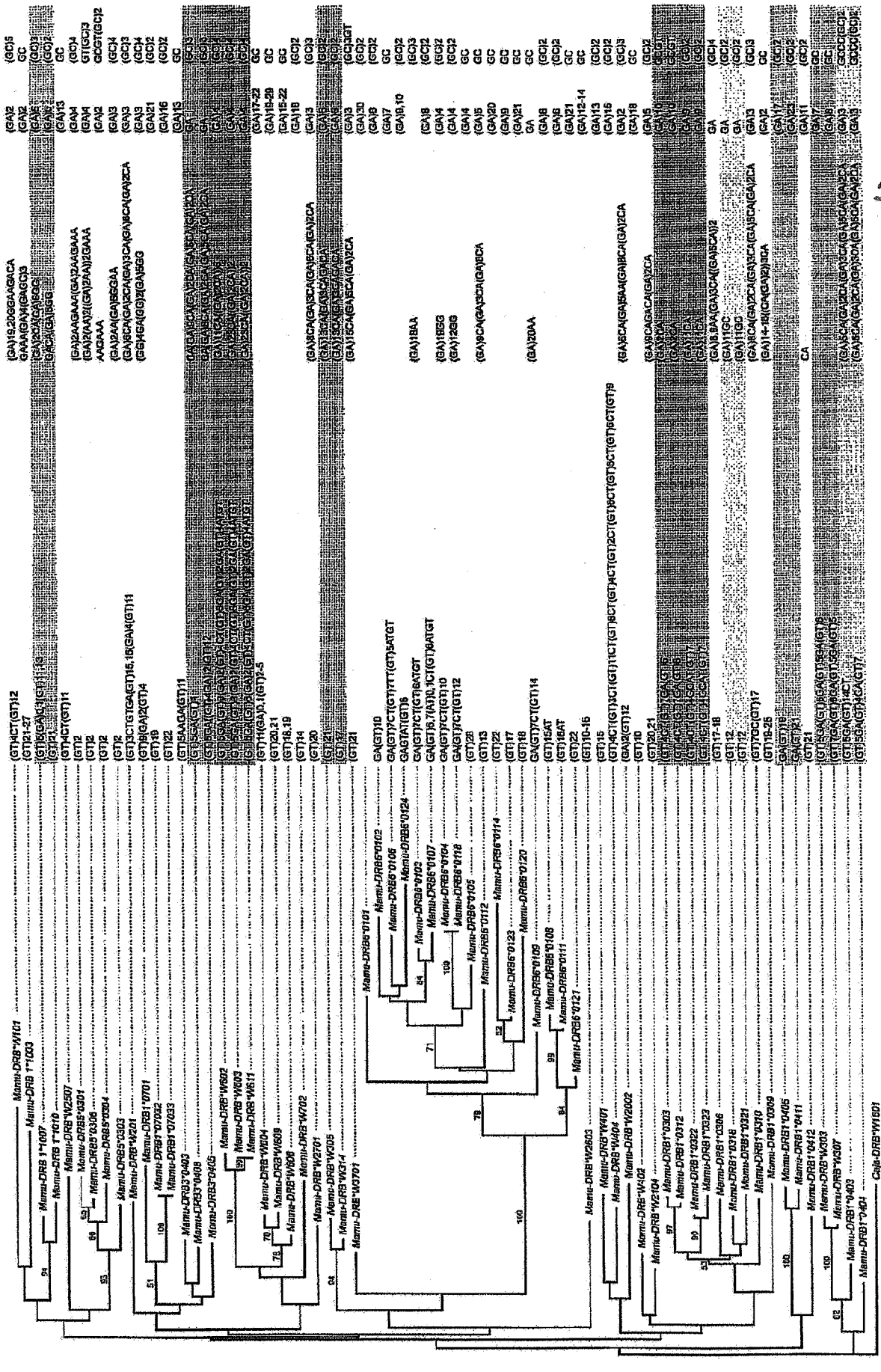
Part 1
(GT)x mixed
Part 2
(GA)z mixed
Part 3
(GA)y
Part 4
(GC)n
Mamu-DRB*W101
Mamu-DRB 1*1007
Mamu-DRB 1*1003
Mamu-DRB 1*1010
Mamu-DRB*W2507
Mamu-DRB5*0301
Mamu-DRB5*0306
Mamu-DRB5*0304
Mamu-DRB5*0303
Mamu-DRB*W201
Mamu-DRB1*0701
Mamu-DRB1*07032
Mamu-DRB1*07033
Mamu-DRB3*0403
Mamu-DRB3*0408
Mamu-DRB3*0405
Mamu-DRB*W602
Mamu-DRB*W603
Mamu-DRB*W611
Mamu-DRB*W604
Mamu-DRB*W609
Mamu-DRB*W606
Mamu-DRB*W702
Mamu-DRB*W2701
Mamu-DRB*W605
Mamu-DRB*W614
Mamu-DRB*W3701
Mamu-DRB*W2603
Mamu-DRB*W401
Mamu-DRB*W404
Mamu-DRB*W2002
Mamu-DRB*W402
Mamu-DRB*W2104
Mamu-DRB1*0303
Mamu-DRB1*0312
Mamu-DRB1*0322
Mamu-DRB1*0329
Mamu-DRB1*0318
Mamu-DRB1*0321
Mamu-DRB1*0319
Mamu-DRB1*0309
Mamu-DRB1*0405
Mamu-DRB1*0411
Mamu-DRB1*0412
Mamu-DRB*W303
Mamu-DRB*W307
Mamu-DRB1*0403
Caja-DRB*W1501
0.01 substitutions/site

Fig. 1

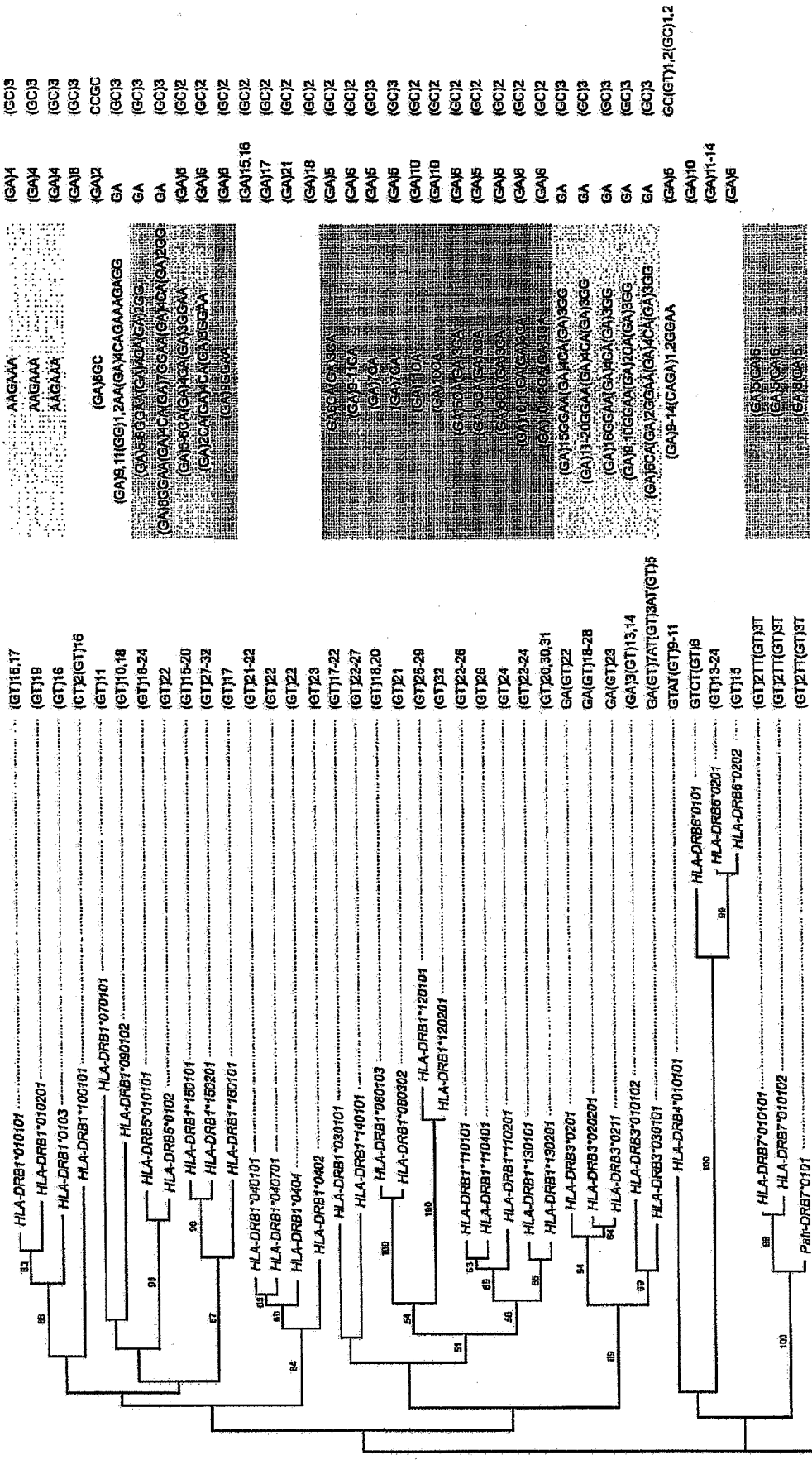
Part 1 (GT)x
Part 2 (GA)z mixed
Part 3 (GA)y
Part 4 (GC)n
HLA-DRB1*010101 (GT)15,17 AAGAAA (GA)4 (GC)3
HLA-DRB1*010201 (GT)19 AAGAAA (GA)4 (GC)3
HLA-DRB1*0103 (GT)16 AAGAAA (GA)4 (GC)3
HLA-DRB1*100101 (CT)2(GT)16 (GA)8 (GC)3
HLA-DRB1*070101 (GT)11 (GA)2 CCGC
HLA-DRB1*090102 (GT)10,18 (GA)9,11(GG)1,2A(GA)4CAGAAAGAGG GA (GC)3
HLA-DRB5*010101 (GT)18-24 (GA)5GCA(GA)4CA(GA)2GCA(GA)4CA(GA)2GC GA (GC)3
HLA-DRB5*0102 (GT)22 (GA)5GCA(GA)4CA(GA)3GGAA GA (GC)2
HLA-DRB1*160101 (GT)15-20 (GA)2CA(GA)4CA(GA)3GGAA (GA)6 (GC)2
HLA-DRB1*160201 (GT)27-32 (GA)3GGAA (GA)6 (GC)2
HLA-DRB1*160101 (GT)17 (GA)8 (GC)2
HLA-DRB1*040101 (GT)21-22 (GA)15,16 (GC)2
HLA-DRB1*040701 (GT)22 (GA)17 (GC)2
HLA-DRB1*0404 (GT)22 (GA)21 (GC)2
HLA-DRB1*0402 (GT)23 (GA)18 (GC)2
HLA-DRB1*030101 (GT)17-22 (GA)8-11CA (GA)5 (GC)2
HLA-DRB1*140101 (GT)22-27 (GA)7CA (GA)5 (GC)2
HLA-DRB1*080103 (GT)18,20 (GA)7TCA (GA)5 (GC)3
HLA-DRB1*080302 (GT)21 (GA)7TCA (GA)5 (GC)3
HLA-DRB1*120101 (GT)25-29 (GA)10CA (GA)10 (GC)2
HLA-DRB1*120201 (GT)32 (GATCA(GA)3CA (GA)10 (GC)2
HLA-DRB1*110101 (GT)23-26 (GATCA(GA)3CA (GA)8 (GC)2
HLA-DRB1*110401 (GT)26 (GA)2CA(GA)3CA (GA)5 (GC)2
HLA-DRB1*110201 (GT)24 (GA)2CA(GA)3CA (GA)8 (GC)2
HLA-DRB1*130101 (GT)23-24 (GA)7CA(GA)3CA (GA)6 (GC)2
HLA-DRB1*130201 (GT)20,30,31 (GA)7CA(GA)3CA (GA)6 (GC)2
HLA-DRB3*0201 GA(GT)22 (GA)15GGAA(GA)4CA(GA)3GG GA (GC)3
HLA-DRB3*020201 GA(GT)18-28 (GA)11-20GGAA(GA)4CA(GA)3GG GA (GC)3
HLA-DRB3*0211 GA(GT)23 (GA)16GGAA(GA)4CA(GA)3GG GA (GC)3
HLA-DRB3*010102 (GA)3(GT)13,14 (GA)8-10GGAA(GA)2CA(GA)3GG GA (GC)3
HLA-DRB3*030101 GA(GT)7AT(GT)3AT(GT)5 (GA)8CA(GA)2GCAA(GA)4CA(GA)3GG GA (GC)3
HLA-DRB4*010101 GTAT(GT)9-11 (GA)8-14(CAGA)1,2GGAA (GA)5 GC(GT)1,2(GC)1,2
HLA-DRB6*0101 GTCT(GT)6 (GA)10
HLA-DRB6*0201 (GT)13-24 (GA)11-14
HLA-DRB6*0202 (GT)15 (GA)8
HLA-DRB7*010101 (GT)12TT(GT)3T (GA)4CA(GA)6
HLA-DRB7*010102 (GT)12TT(GT)3T (GA)7CA(GA)6
Patr-DRB7*0101 (GT)12TT(GT)3T (GA)8(GA)6
Caja-DRB*W1601
0.01 substitutions/site

Fig. 2

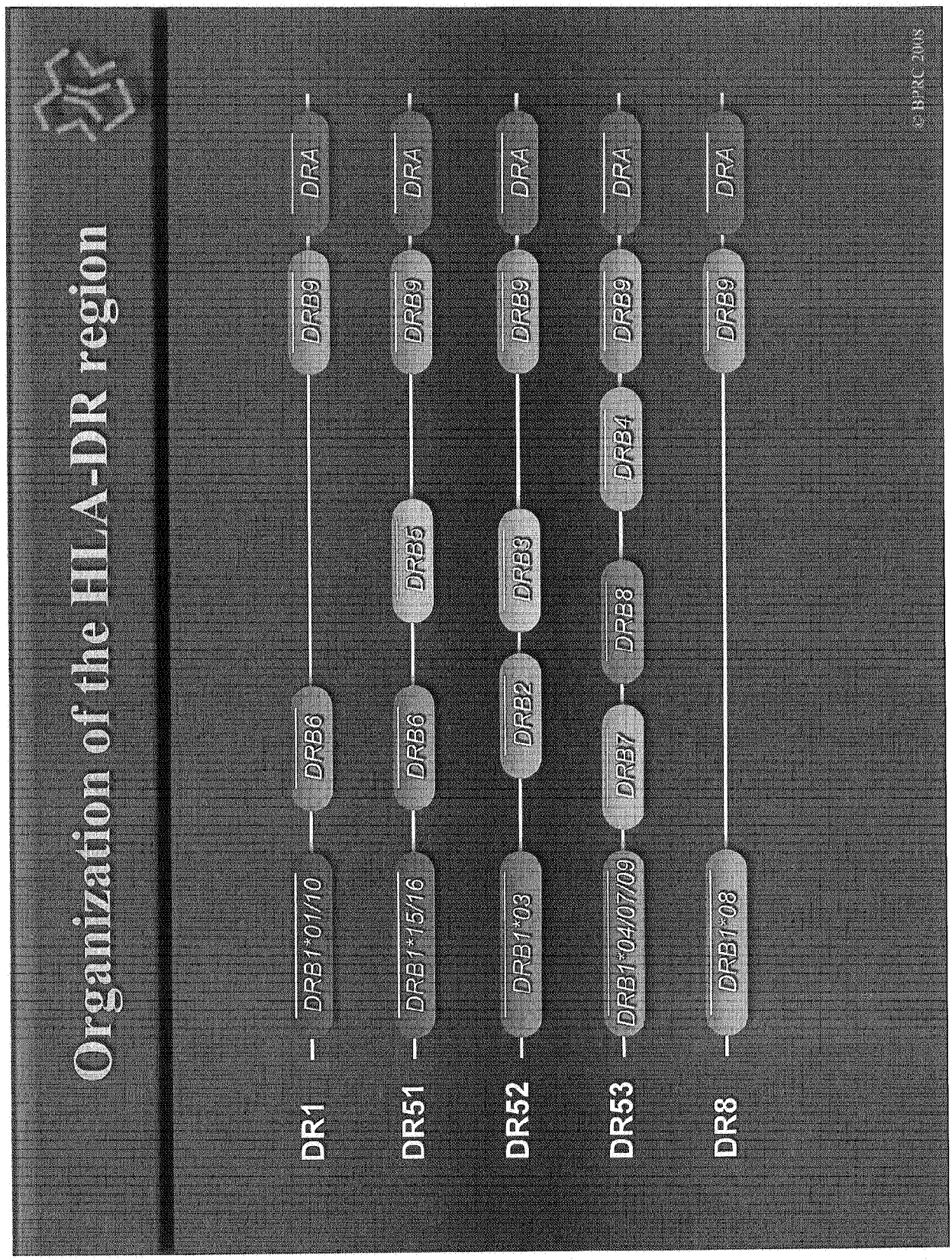
Organization of the HLA-DR region
DR1
DR51
DR52
DR53
DR8
DRA
DRB9
DRB6
DRB5
DRB3
DRB2
DRB4
DRB8
DRB7
DRB1*01/10
DRB1*15/16
DRB1*03
DRB1*04/07/09
DRB1*08
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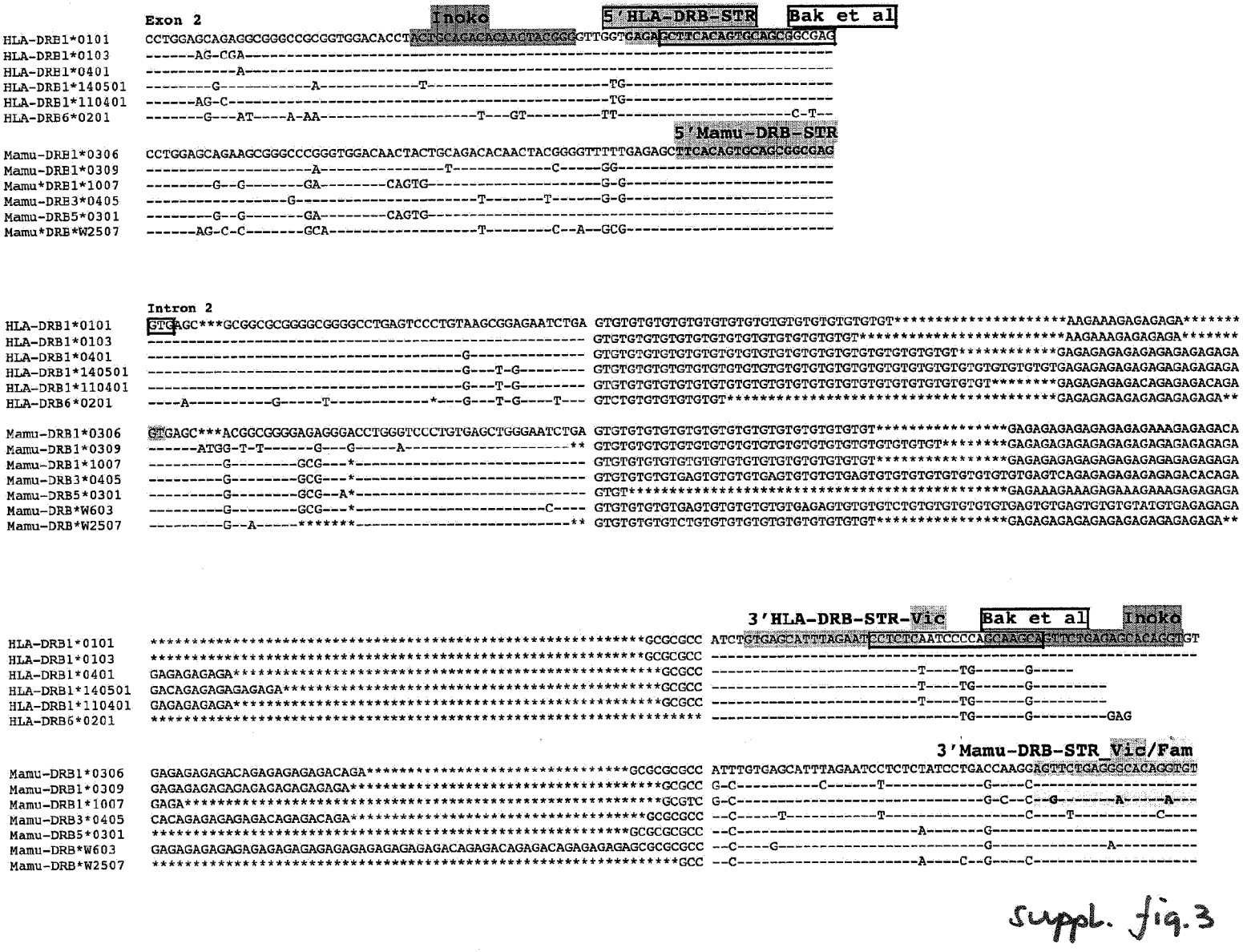
Fig.3


Exon 2
Inoko
5'HLA-DRB-STR
Bak et al
HLA-DRB1*0101
HLA-DRB1*0103
HLA-DRB1*0401
HLA-DRB1*140501
HLA-DRB1*110401
HLA-DRB6*0201
5'Mamu-DRB-STR
Mamu-DRB1*0306
Mamu-DRB1*0309
Mamu*DRB1*1007
Mamu-DRB3*0405
Mamu-DRB5*0301
Mamu*DRB*W2507
Intron 2
Mamu-DRB1*0306
Mamu-DRB1*0309
Mamu-DRB1*1007
Mamu-DRB3*0405
Mamu-DRB5*0301
Mamu-DRB*W603
Mamu-DRB*W2507
3'HLA-DRB-STR-Vic
Bak et al
Inoko
3'Mamu-DRB-STR_Vic/Fam

suppl. fig. 3

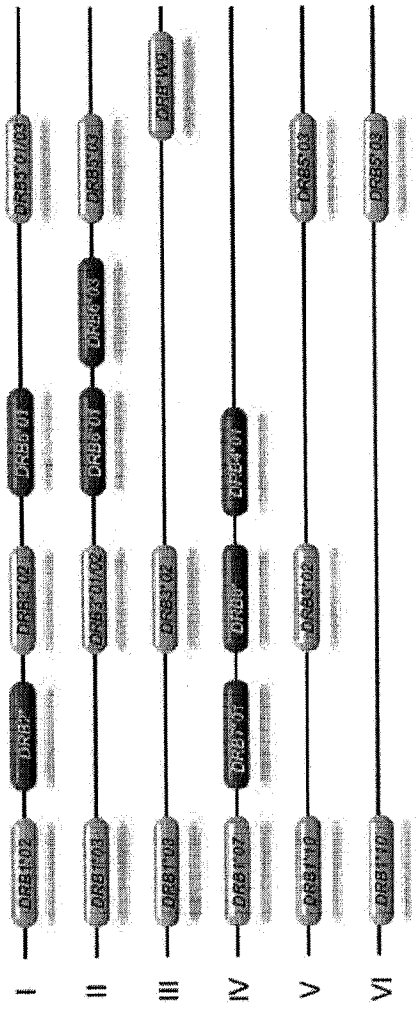


fig.4

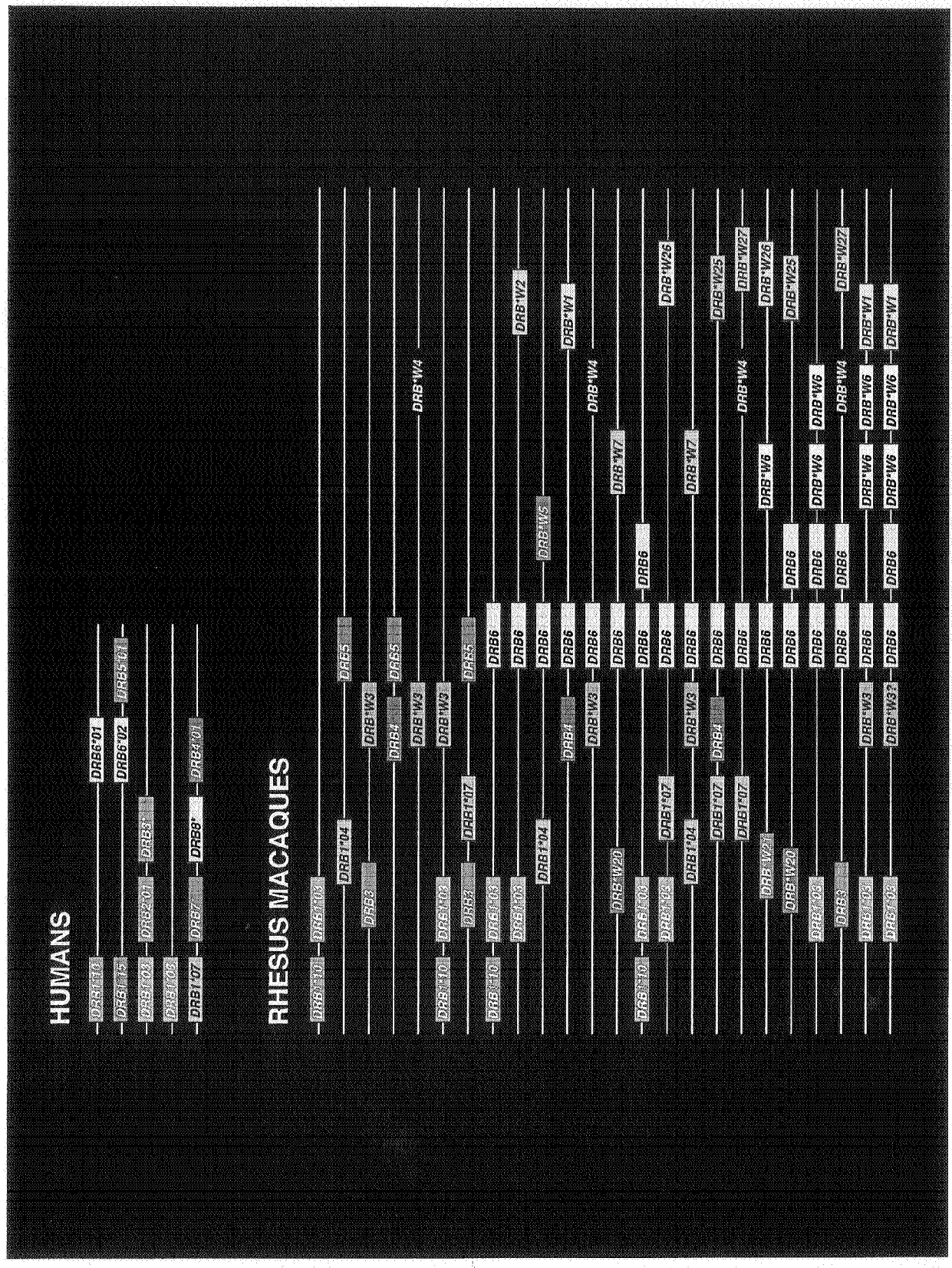

HUMANS
DRB1*16
DRB6*01
DRB1*15
DRB6*02
DRB5*01
DRB1*01
DRB3*
DRB1*02
DRB1*07
DRB7
DRB8*
DRB4*01
RHESUS MACAQUES
DRB1*10
DRB1*03
DRB1*04
DRB5
DRB8
DRB*W3
DRB4
DRB5
DRB*W4
DRB1*10
DRB1*02
DRB*W3
DRB8
DRB1*07
DRB5
DRB1*10
DRB1*03
DRB6
DRB1*03
DRB6
DRB*W2
DRB1*04
DRB*W5
DRB4
DRB6
DRB*W1
DRB*W3
DRB6
DRB*W4
DRB*W7
DRB*W20
DRB6
DRB1*10
DRB1*01
DRB6
DRB1*07
DRB1*03
DRB6
DRB*W25
DRB1*04
DRB*W3
DRB6
DRB*W7
DRB1*07
DRB4
DRB6
DRB*W25
DRB1*07
DRB6
DRB*W4
DRB*W27
DRB1*14
DRB6
DRB*W6
DRB*W26
DRB*W20
DRB6
DRB*W25
DRB1*03
DRB6
DRB*W6
DRB8
DRB6
DRB*W4
DRB*W27
DRB1*13
DRB6
DRB*W6
DRB*W1
DRB1*12
DRB*W3
DRB6
DRB*W6
DRB1*02
DRB*W33
DRB6
DRB*W6
DRB*W1

fig. 5

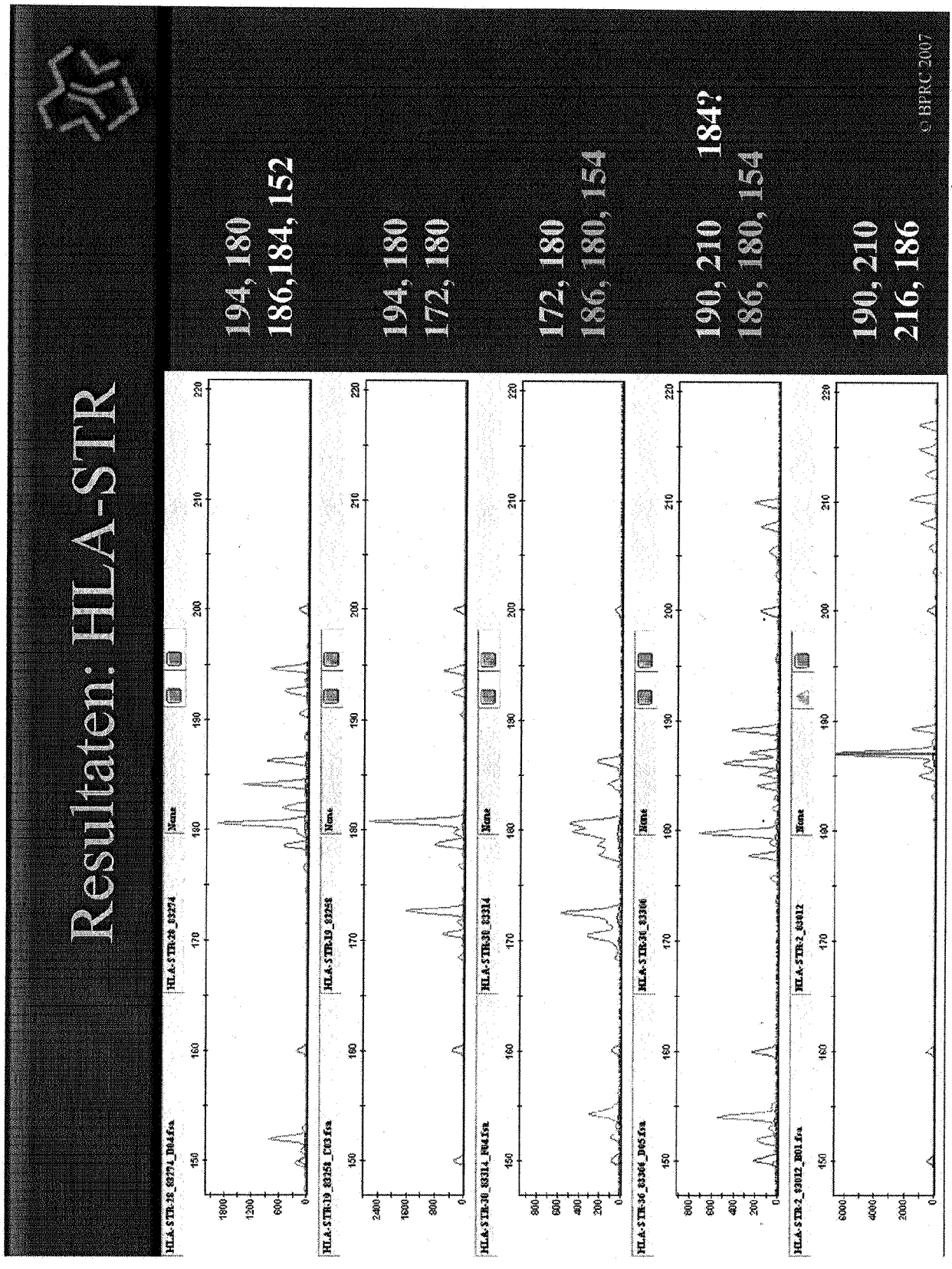

Resultaten: HLA-STR
194, 180
186,184, 152
194, 180
172, 180
172, 180
186, 180, 154
190, 210
186, 180, 154
184?
190, 210
216, 186
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Fig. 6

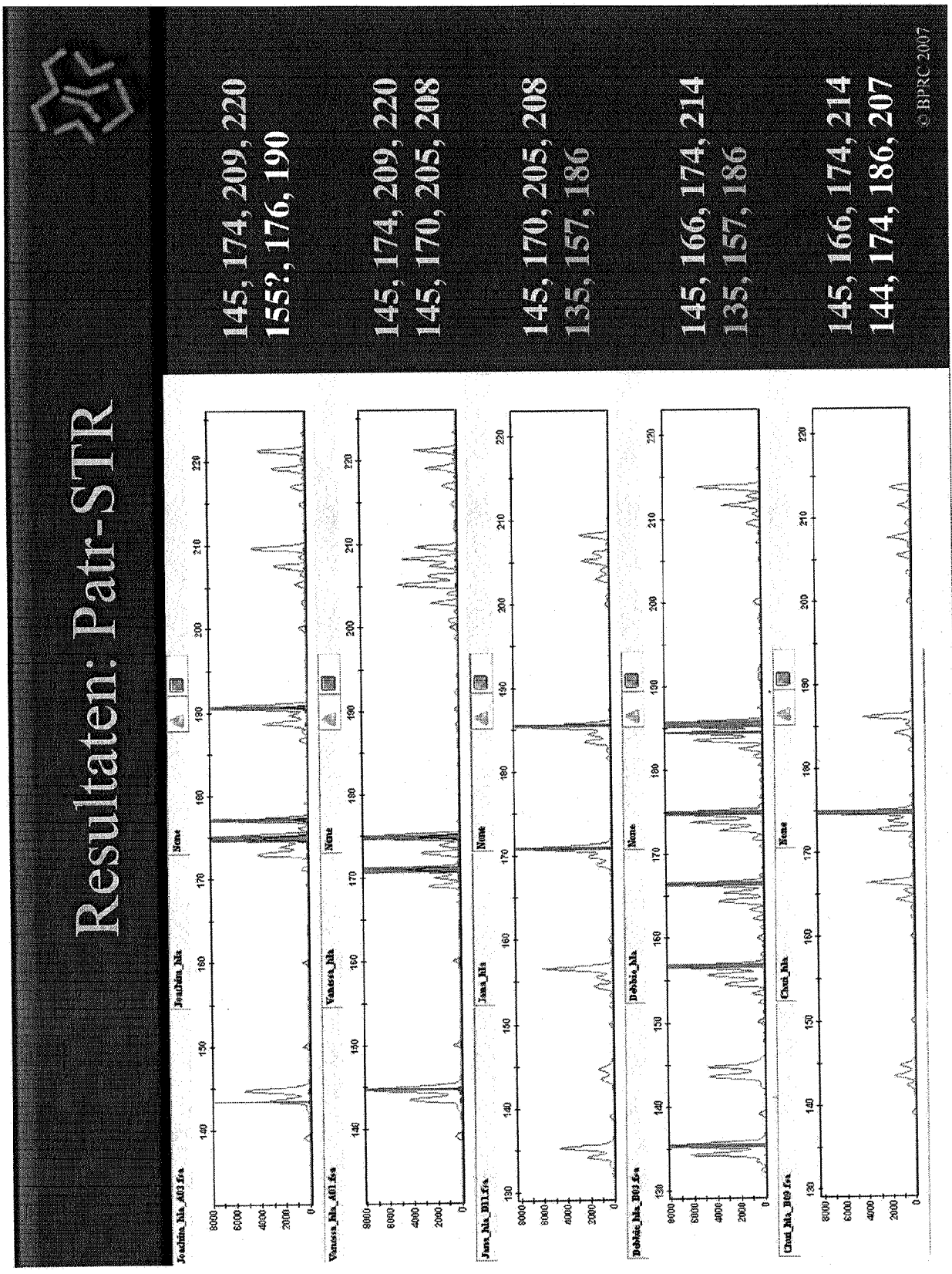
Resultaten: Patr-STR
145, 174, 209, 220
155?, 176, 190
145, 174, 209, 220
145, 170, 205, 208
145, 170, 205, 208
135, 157, 186
145, 166, 174, 214
135, 157, 186
145, 166, 174, 214
144, 174, 186, 207
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Fig. 7

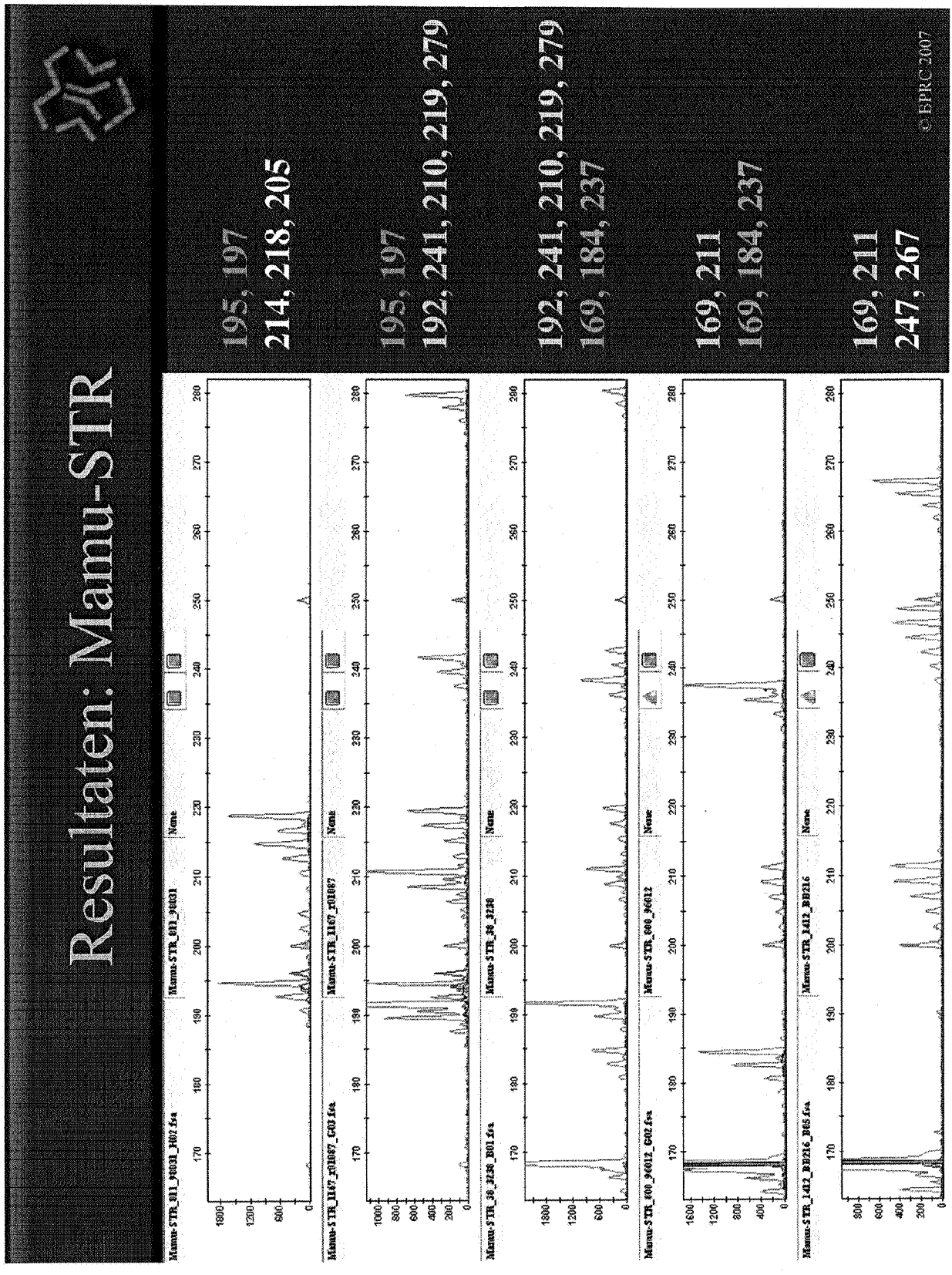
Resultaten: Mamu-STR
195, 197
214, 218, 205
195, 197
192, 241, 210, 219, 279
192, 241, 210, 219, 279
169, 184, 237
169, 211
169, 184, 237
169, 211
247, 267
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fig. 8

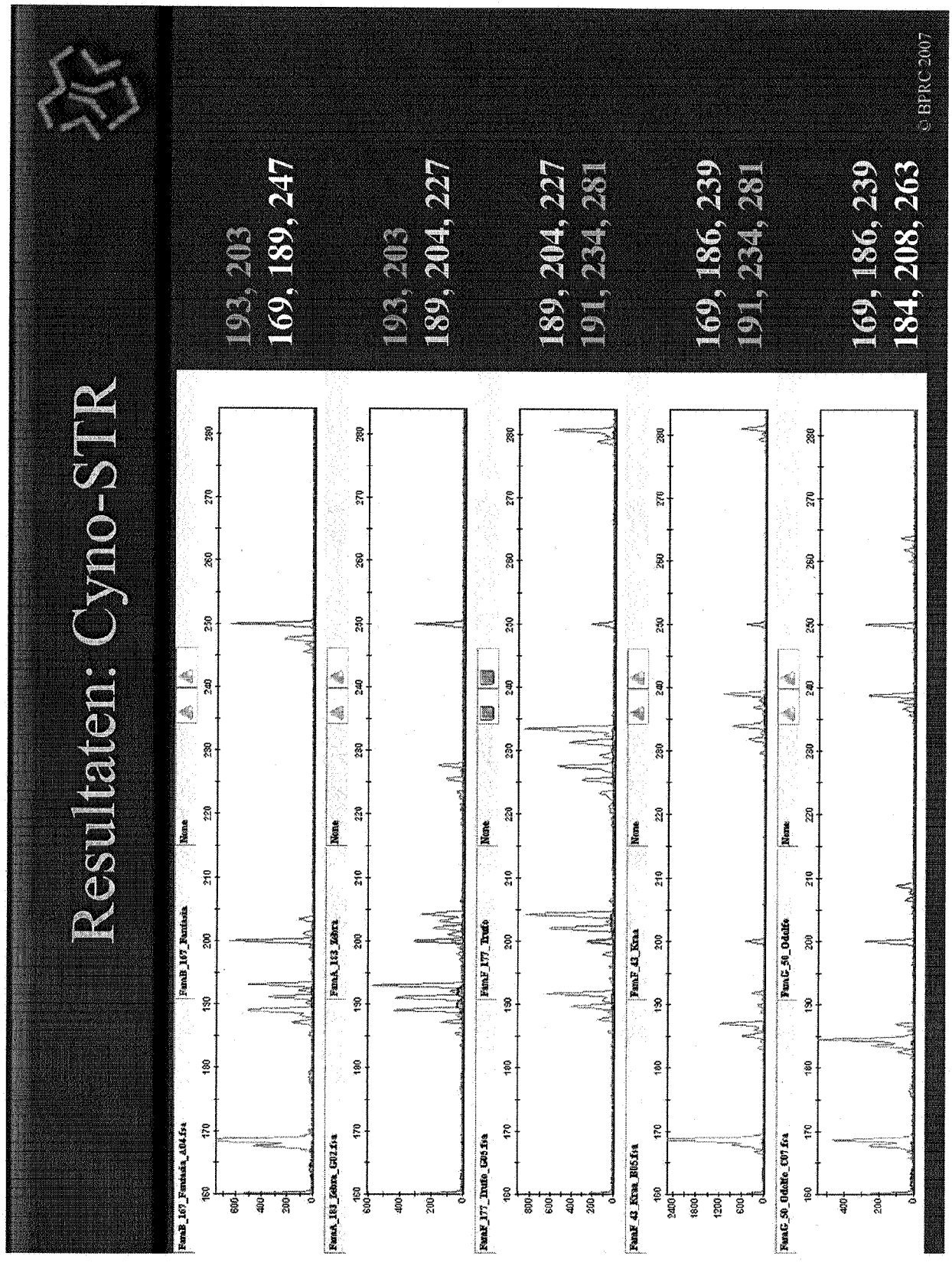
Resultaten: Cyno-STR
193, 203
169, 189, 247
193, 203
189, 204, 227
189, 204, 227
191, 234, 281
169, 186, 239
191, 234, 281
169, 186, 239
184, 208, 263
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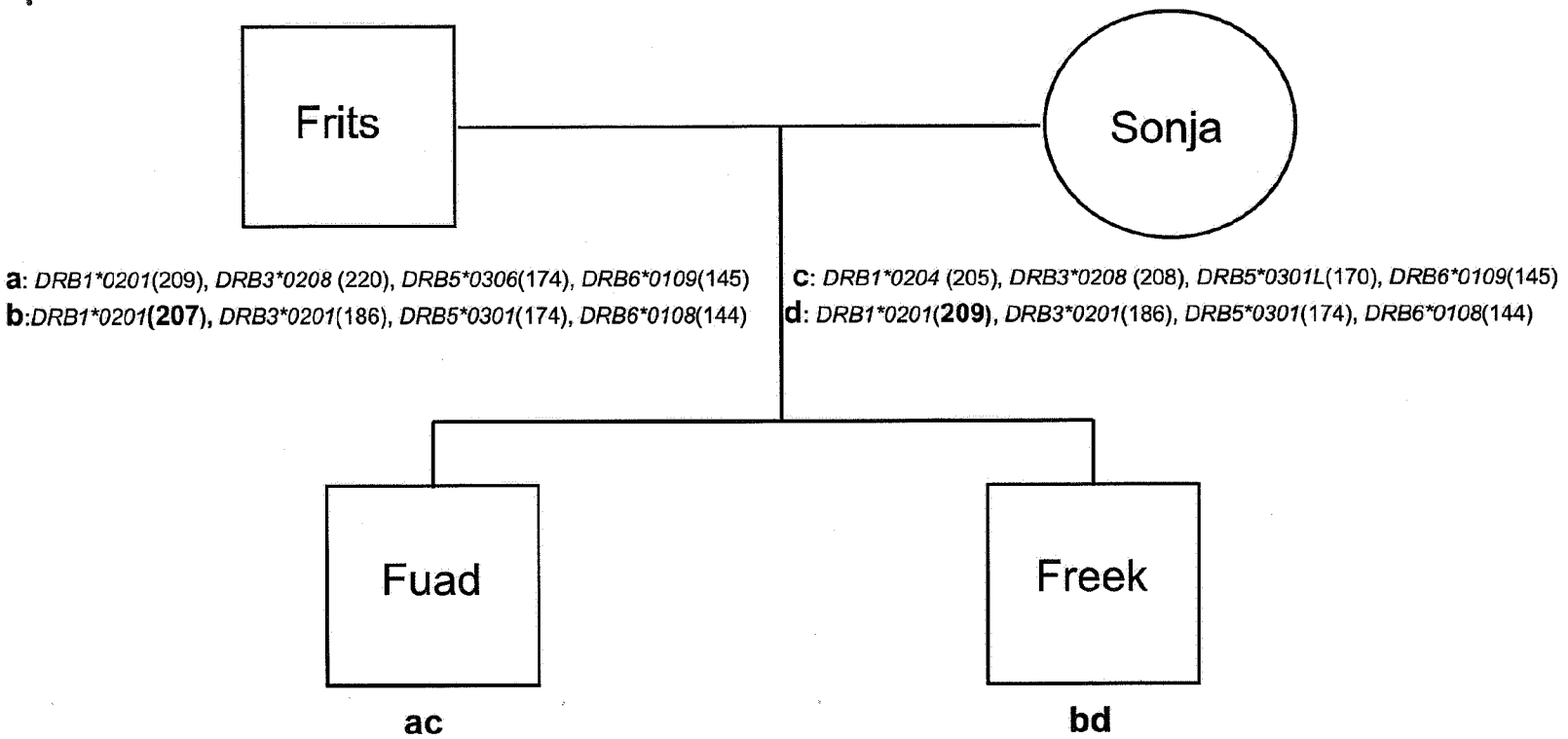
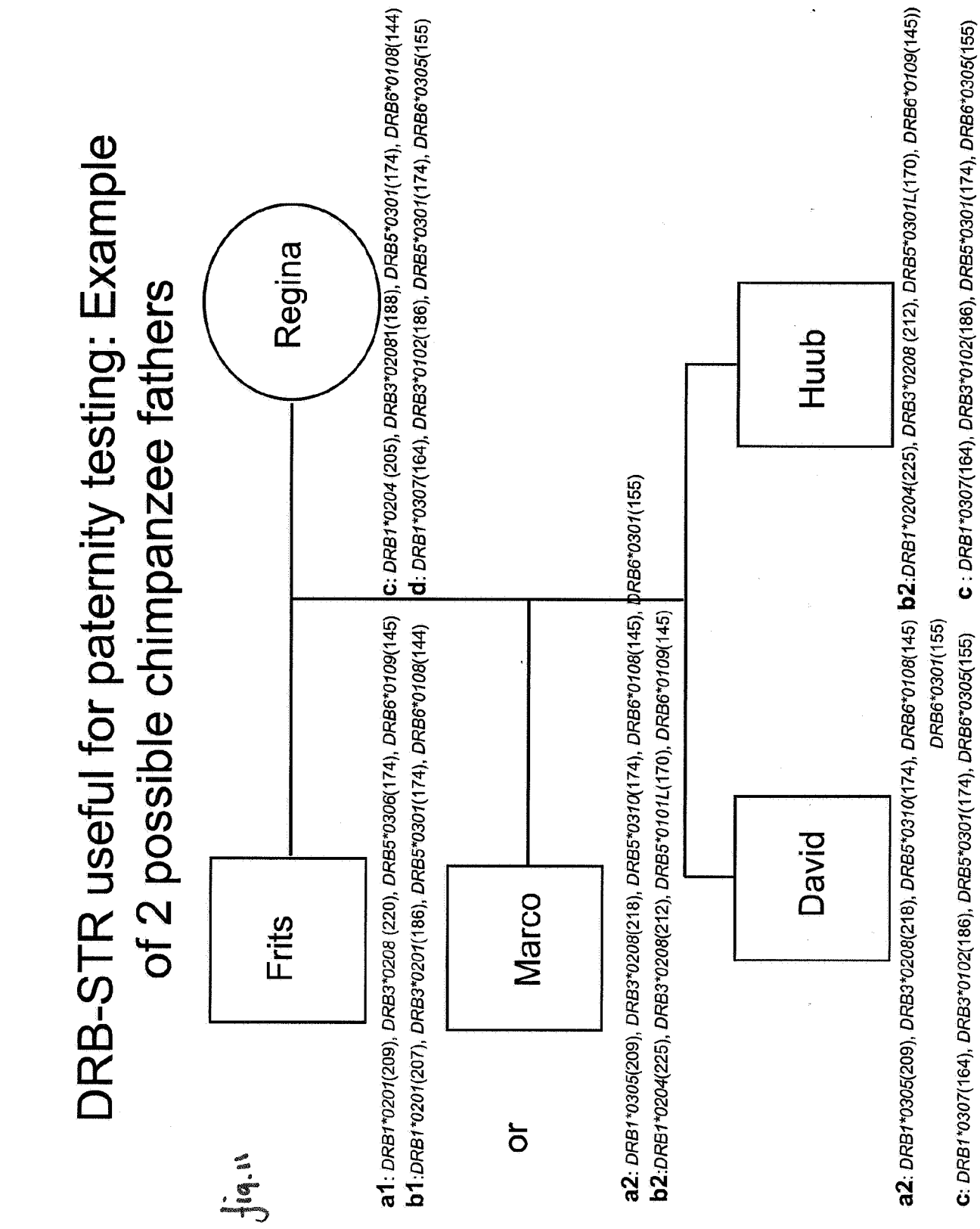
Fig. 9

DRB-STR discriminates between paternal and maternal *Patr-DRB* genotype/haplotype

fig.10



DRB haplotypes b (Frits) and d (Sonja) can only be distinguished by different lengths of the STR of *DRB1*0201*


DRB-STR useful for paternity testing: Example of 2 possible chimpanzee fathers
fig.11
Frits
Regina
a1: DRB1*0201(209), DRB3*0208 (220), DRB5*0306(174), DRB6*0109(145)
b1:DRB1*0201(207), DRB3*0201(186), DRB5*0301(174), DRB6*0108(144)
c: DRB1*0204 (205), DRB3*02081(188), DRB5*0301(174), DRB6*0108(144)
d: DRB1*0307(164), DRB3*0102(186), DRB5*0301(174), DRB6*0305(155)
or
Marco
David
Huub
a2: DRB1*0305(209), DRB3*0208(218), DRB5*0310(174), DRB6*0108(145), DRB6*0301(155)
b2:DRB1*0204(225), DRB3*0208(212), DRB5*0101L(170), DRB6*0109(145)
a2: DRB1*0305(209), DRB3*0208(218), DRB5*0310(174), DRB6*0108(145)
b2:DRB1*0204(225), DRB3*0208 (212), DRB5*0301L(170), DRB6*0109(145))
DRB6*0301(155)
c: DRB1*0307(164), DRB3*0102(186), DRB5*0301(174), DRB6*0305(155)
c : DRB1*0307(164), DRB3*0102(186), DRB5*0301(174), DRB6*0305(155)

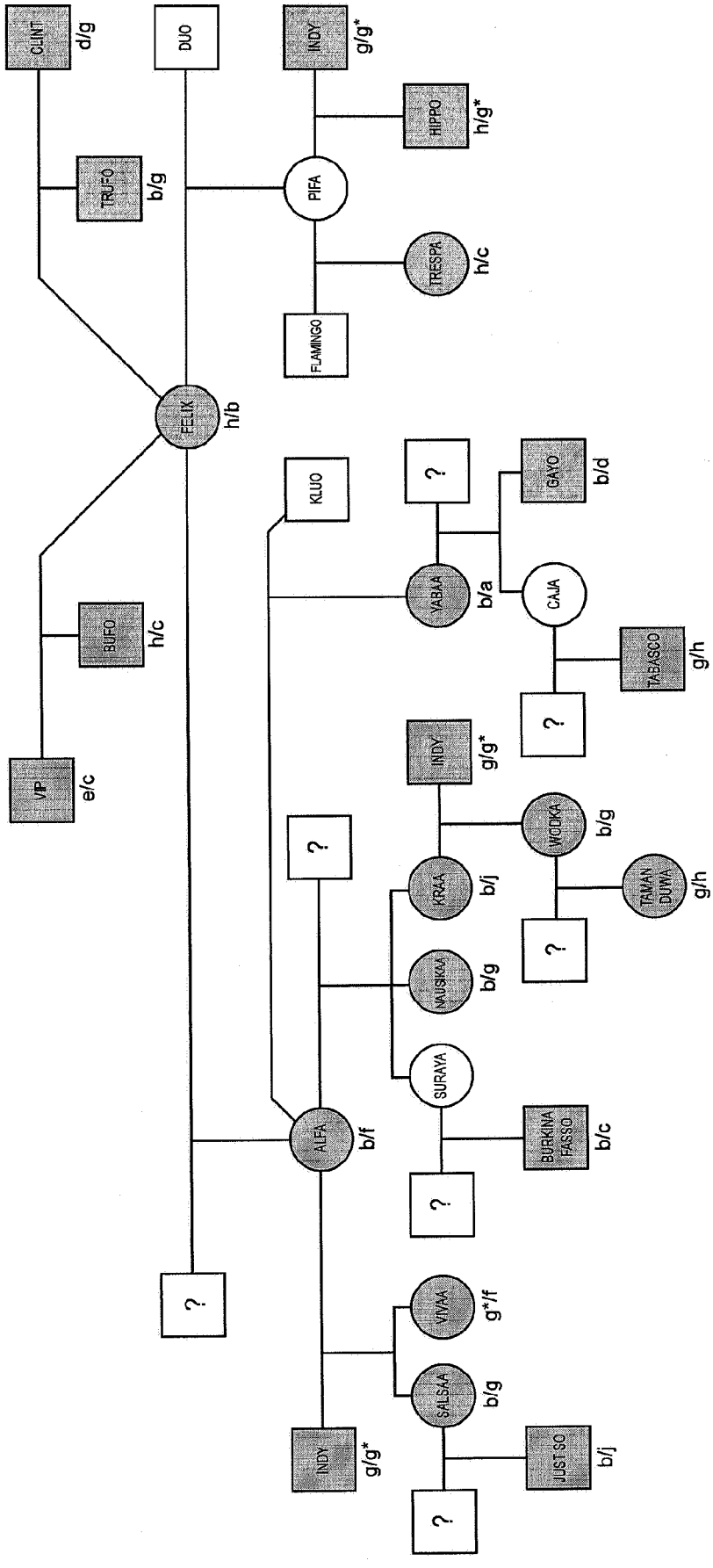

CLINT
d/g
DUO
INDY
g/g*
HIPPO
h/g*
TRUFO
b/g
PIFA
TRESPA
h/c
FLAMINGO
FELIX
h/b
KLUO
?
GAYO
b/d
YABUA
b/a
CAJA
BUFO
h/c
TABASCO
g/h
VIP
e/c
?
INDY
g/g*
WIDIKA
b/g
TAMAN DUWA
g/h
KRAA
b/j
?
MAUSKAA
b/g
SURAYA
ALFA
b/f
BURKINA FASSO
b/c
?
?
VIVAA
g*/f
INDY
g/g*
SALSAA
b/g
JUST SO
b/j
?

fig. 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2008/050199

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BERGSTRÖM T F ET AL: "Tracing the origin of HLA-DRB1 alleles by microsatellite polymorphism." AMERICAN JOURNAL OF HUMAN GENETICS JUN 1999, vol. 64, no. 6, June 1999 (1999-06), pages 1709-1718, XP002447783 ISSN: 0002-9297	11-13
Y	the whole document	1-22
Y	US 5 908 749 A (MIGNOT EMMANUEL J M [US] ET AL) 1 June 1999 (1999-06-01) example 3	1-22
Y	US 2003/108940 A1 (INOKO HIDETOSHI [JP] ET AL) 12 June 2003 (2003-06-12) the whole document	1-22

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 August 2008	20/08/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Dolce, Luca

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2008/050199

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EPPLEN C ET AL: "Coding versus intron variability: extremely polymorphic HLA-DRB1 exons are flanked by specific composite microsatellites, even in distant populations." HUMAN GENETICS MAR 1997, vol. 99, no. 3, March 1997 (1997-03), pages 399-406, XP002447784 ISSN: 0340-6717 abstract	1-22
A	EP 0 414 469 B1 (GENETYPE AG [CH]) 30 October 1996 (1996-10-30) the whole document	1-22
X,P	DOXIADIS GABY G M ET AL: "A highly divergent microsatellite facilitating fast and accurate DRB haplotyping in humans and rhesus macaques" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 104, no. 21, May 2007 (2007-05), pages 8907-8912, XP002447785 ISSN: 0027-8424 the whole document	1-22

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2008/050199

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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US 2003108940 A1	12-06-2003	NONE	
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		AU 672519 B2	03-10-1996
		AU 7285094 A	24-11-1994
		CA 2023888 A1	26-02-1991
		DD 299319 A5	09-04-1992
		DE 69029018 D1	05-12-1996
		DE 69029018 T2	22-05-1997
		DK 414469 T3	14-04-1997
		EP 0414469 A2	27-02-1991
		ES 2095859 T3	01-03-1997
		GR 3022410 T3	30-04-1997
		HK 1008053 A1	30-04-1999
		IL 95467 A	31-07-1995
		JP 3139300 A	13-06-1991
		JP 3206812 B2	10-09-2001
		JP 2001309796 A	06-11-2001
		NZ 235051 A	29-01-1992
		SG 47747 A1	17-04-1998
		US 5192659 A	09-03-1993
		US 5612179 A	18-03-1997