



(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/EP2012/061270

(22) International Filing Date:
14 June 2012 (14.06.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/498,317 17 June 2011 (17.06.2011) US
61/553,726 31 October 2011 (31.10.2011) US

(71) Applicant (for all designated States except US): **PROGENIKA BIOPHARMA, S.A.** [ES/ES]; Parque Tecnológico de Vizcaya, 504, E-48160 Derio, Vizcaya (ES).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **OCHOA, Jorge** [ES/ES]; c/o Progenika Biopharma, S.A., Parque Tecnológico de Vizcaya, 504, E-48160 Derio, Vizcaya (ES). **LÓPEZ, Mónica** [ES/ES]; c/o Progenika Biopharma, S.A., Parque Tecnológico de Vizcaya, 504, E-48160 Derio, Vizcaya (ES). **ESCORZA, Sergio** [ES/ES]; c/o Progenika Biopharma, S.A., Parque Tecnológico de Vizcaya, 504, E-48160 Derio, Vizcaya (ES). **TEJEDOR, Diego** [ES/ES]; c/o Progenika Biopharma, S.A., Parque Tecnológico de Vizcaya, 504, E-48160 Derio, Vizcaya (ES). **MARTÍNEZ, Antonio** [ES/ES]; c/o Progenika Biopharma, S.A., Parque Tecnológico de Vizcaya, 504, E-48160 Derio, Vizcaya (ES). **SIMÓN, Laureano** [ES/ES]; c/o Progenika Bio-

pharma, S.A., Parque Tecnológico de Vizcaya, 504, E-48160 Derio, Vizcaya (ES).

(74) Agents: **CASLEY, Christopher S.** et al.; Mewburn Ellis LLP, 33 Gutter Lane, London, Greater London EC2V 8AS (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: DISCRIMINATION OF BLOOD TYPE VARIANTS

(57) Abstract: The present invention provides a method for detecting the presence or absence of, or for discriminating between, blood type variants, including RHD*r's, RHD*DIIfa, RHD*DIVa-2, RHCE*cs^s and RHCE*ce733G. The method comprises genotyping a sample obtained from a human subject at one or more positions in intron 7 of the RHD gene and/or in intron 7 of the RHCE gene. The invention also provides products, in particular, probes, primers and kits for use in the method of the invention.



Discrimination of Blood Type Variants

Field of the Invention

The invention relates to methods for genotyping and blood cell antigen determination, which in particular may discriminate the *RHD***r*'s or *RHD***r*'s-like blood type variants, which encode C^{+W} antigen and no D antigen, from *RHD***DIIIa*, *RHD***DIVa-2* and other blood type variants. The invention also relates to products, in particular, probes, primers and kits for use in such methods.

Background to the Invention

The success of blood transfusion often depends on the degree of compatibility between donor and recipient. The degree of compatibility, in turn, is a function of the similarity in Red Blood Cell (RBC) antigen content between donor and recipient. Expression of many RBC antigens in an individual can be predicted from the analysis of their genomic DNA. Therefore, analysis of donor and/or recipient DNA can be used to facilitate blood matching and thus enable proper blood transfusion practice.

Hemolytic reactions are more common in multi-transfused than in singly transfused individuals, not only because of the increased probability of such an event as the number of transfused units increases, but also because of the accumulative nature of the immune response in the recipient. An example of a condition whose treatment includes repeated blood transfusions is Sickle Cell Disease (SCD). From the above follows that a high degree of compatibility with donor blood is often critical for the long-term success of transfusion in SCD patients.

While SCD is more prevalent among individuals of African ancestry, the blood donor population in the USA and other Western countries is largely Caucasian. As a consequence of this disparity, differences in RBC antigens between both racial groups often become responsible for blood transfusion failures in SCD patients.

The genetic variant *RHD***DIIIa-CE(4-7)-D*, also known as *RHD-CE-D^S*, *RHD-CE(4-7)-D*, (*C*)*ce^S*, or *r^S*, (*RHD***r^S* henceforth) can be found in up to 5-10% of the African-American population, but is extremely rare in Caucasians. This variant poses a special challenge to blood transfusion because it encodes a rather complex antigen profile, which includes absence of D antigen, altered forms of C (C^{+W}) and e antigens, expression of low-frequency VS antigen, no expression

of V antigen, and absence of the high-frequency hr^B antigen. Among them, D and C antigens are the clinically more relevant ones.

The antigenic complexity of RHD^*r^S correlates with its genetic complexity, which includes a substitution of part of RHD exon 3, RHD exons 4-7, and the intervening introns by their $RHCE$ counterparts, a G>T substitution at position 186 (exon 2), a C>T substitution at position 410 (hybrid exon 3), a C>G substitution at position 733 (exon 5), and a G>T substitution at position 1006 (exon 7). In addition to the changes in the RHD gene, RHD^*r^S occurs in *cis* with $RHCE^*ce^S1006T$, an $RHCE$ gene that also encodes substitutions C>G at position 733 (exon 5) and G>T at position 1006 (exon 7).

To add to the antigenic and genetic complexity, knowledge about the molecular basis of RHD^*r^S is incomplete. For instance, the precise points of $RHCE/RHD$ recombination have not been reported to date. Furthermore, two types of RHD^*r^S variant have been described and named Type 1 and Type 2, which differ not only in their genetic composition but also in their antigen profiles.

Several publications (Refs. 1-3) have uncovered the genetic similarity between RHD^*r^S and other RHD variants, in particular RHD^*DIIIa and $RHD^*DIVa/RHD^*DIVa-2$ ($RHD^*DIVa-2$ henceforth). A number of molecular methods for the specific detection of RHD^*r^S rely on the detection of single nucleotide polymorphisms (SNPs) located in hybrid exon 3. These SNPs are now known to be shared with variants RHD^*DIIIa and $RHD^*DIVa-2$. Consequently, to date, identification of RHD^*r^S in a sample by DNA analysis requires detection of hybrid exon 3 SNPs and discrimination from RHD^*DIIIa and $RHD^*DIVa-2$. This discrimination is clinically relevant since the latter variants encode a different antigen profile, which includes expression of partial D and absence of C^{+W} .

Antibody reagents commonly used to detect C antigen do not discriminate between C^{+W} and C^+ . Therefore, the phenotype is often reported as C^+ . In cases where the antibody reagent does discriminate between C^{+W} and C^+ but the sample contains a normal $RHCE^*C$ allele in *trans* to a RHD^*r^S allele, C^{+W} is obscured by C^+ , resulting in a C^+ phenotype for the sample. Therefore, $RHCE^*C$ needs to be tested for and shown absent prior to assignment of a C^{+W} phenotype to a sample. Accordingly, there remains a need for further methods for distinguishing RHD^*r^S from RHD^*DIIIa and $RHD^*DIVa-2$. The present invention addresses these and other objects.

Summary of the Invention

The present inventors have now found that intron 7 of the *RHD* locus and/or intron 7 of the *RHCE* locus find use in discriminating bloodtype variants. Genotyping at one or more polymorphic positions of intron 7 of said loci may, in some cases, be advantageously combined with genotyping positions elsewhere, such as in exon 3 of the *RHD* locus. In particular, the inventors have found that a determination of one or more genetic sequences in one or more regions of intron 7 of the *RHD* locus enables discrimination between blood type variants *RHD**r^S or *RHD**r^S-like (i.e. blood type variants which express the C^W antigen and lack a D antigen) and other *RHD*/*RHCE* hybrid exon 3 variants, including but not limited to *RHD**DIIIa, *RHD**DIII_FN and *RHD**DIVa-2. Knowledge about the sequence of each of these *RHD* and *RHCE* variants at polymorphic positions in the intron 7 regions enables the design and development of typing methods that exploit said polymorphisms, individually or in combination, to discriminate among variants. Discrimination among variants, in turn, enables determination of the genotype and prediction of the phenotype of a sample containing them with a high degree of accuracy, and resulting clinical utility.

As described herein in detail, the sequences of *RHD* variants *RHD**r's, *RHD**DIIIa, *RHD**DIII_FN and *RHD**DIVa-2 were determined at sixty single-nucleotide polymorphic positions located in a region of intron 7 of the *RHD* locus (see, in particular, Table 1). This region is delimited on the 5' end by position IVS7+1139, and on the 3' end by position IVS7+4108. The present invention also describes sequences of *RHCE**ce variants *RHCE**ce^S and *RHCE**ce733G at polymorphic positions of intron 7 of the *RHCE* locus. In particular, the region of *RHCE* intron 7 that is delimited on the 5' end by position IVS7+2970, and on the 3' end by position IVS7+4108 includes twenty seven informative single nucleotide polymorphisms. Intron 7 of *RHD* and intron 7 of *RHCE* are structurally similar and can be amplified together, e.g. in a PCR reaction. The present inventors believe that the informative use of sequences of intron 7 of the *RHD*/*RHCE* loci in the discrimination of blood variants (e.g. *RHD**r's, *RHD**DIIIa, *RHD**DIII_FN, *RHD**DIVa-2, *RHCE**ce, *RHCE**ce^S and *RHCE**ce733G) represents a significant unifying contribution over the prior art; there being a special technical relationship between intron 7 of *RHD* and *RHCE*. Moreover, the link between *RHD**r's and the *RHCE* variants (these frequently being found *in cis*) results in a functional linkage.

Moreover, V and VS antigens and absence of hr^B antigen are also encoded by RHCE variants that often present *in cis* with *RHD**r's. The most prevalent among them are *RHCE**ce^S (and related variants), and *RHD**733G. This responds to the genetic similarity between the *RHD**r's portion that encodes antigens V, VS, hr^B and the corresponding portion in RHCE variants

5 *RHCE**ce^S and *RHD**733G. As a consequence of this, discrimination of *RHD**r's from other variants by genetic testing and for the purpose of predicting RHCE antigens, also benefits from the inclusion of polymorphisms that differ from those found in variants *RHCE**ce^S and *RHD**733G.

10 The present inventors have characterized rearrangements in the sequence of intron 7. One such rearrangement, common to *RHD**r^S and *RHD**DIIIa, has its insertion point between positions IVS7+3101 and IVS7+3256, the sequence corresponding to RHCE 5' of IVS7+3101 and to RHD 3' of IVS7+3256 (with the sequence being indistinguishable between RHCE and RHD between both positions). A second rearrangement, which distinguishes *RHD**r^S from

15 *RHD**DIIIa, has its insertion point between positions IVS7+1888 and IVS7+2152: The sequence corresponds to RHCE 5' of IVS7+1888 in the case of *RHD**r^S and to RHD in the case of *RHD**DIIIa. The sequence 3' of IVS7+2152 corresponds to RHCE for both *RHD**r^S and *RHD**DIIIa (with the sequence being indistinguishable between RHCE and RHD between both positions). This is depicted in Figure 1A.

20

A further rearrangement has been identified that distinguishes *RHD**DIIIa from *RHD**DIII_FN (without wishing to be bound by any particular theory, the present inventors believe that *RHD**DIII_FN likely corresponds to *RHD**DIII-type 4, 6 or 7). This rearrangement can be seen in Figure 1B (see also Table 1). In particular, *RHD**DIIIa comprises *RHCE* sequence between

25 positions IVS7+2153 and IVS7+2335, whereas *RHD**DIII_FN comprises *RHD* sequence between between positions IVS7+2153 and IVS7+2335.

Accordingly, in a first aspect the present invention provides a method for determining the presence or absence of, or for discriminating between, blood type alleles, which method comprises determining the identity of the nucleotide at one or more positions in intron 7 of the

30 *RHD* locus and/or in intron 7 of the *RHCE* locus in a sample obtained from a human subject. The method may comprise genotyping the sample to determine the identity of the nucleotide at said one or more positions for one or more alleles.

Preferably, said blood type alleles are alleles which comprise an *RHD*/*RHCE* hybrid exon 3.

In some cases in accordance with the method of this aspect of the invention, said blood type alleles are selected from the group consisting of: RHD*r's; RHD*r's-like; *RHD*r^S* Type 1; *RHD*r^S* Type 2; RHD*DIIIa; RHD*DIII_FN; RHD*DIVa-2; RHD*DIVa; RHD*DIII-type4;
 5 RHD*DIII-type6; RHD*DIII-type7; RHD*DIII-type8; RHCE*ce^S; *RHCE*ce^S1006T*; *RHCE*ce^S1006C* RHCE*ce733G; RHCE*ce48C,733G,1025T; RHCE*ce48C,697G,733G; RHCE*ce340T,733G; and RHCE*ce48C,733G,748A.

In some cases in accordance with this aspect of the invention the method is for detecting the
 10 presence or absence of, or for discriminating between, blood type variants, said blood type variants comprising an *RHD-RHCE* hybrid exon 3, the method comprising: genotyping a sample obtained from a human subject at one or more polymorphic positions in intron 7 of the *RHD* gene locus, said one or more polymorphic positions being selected from:

(i) a first region of said intron 7 that is 5' of IVS7+1887, said first region including said
 15 position IVS7+1887;

(ii) a second region of said intron 7 that lies between IVS7+2153 and IVS7+3101, said second region including said positions IVS7+2153 and IVS7+3101; and

(iii) a third region of said intron 7 that lies 3' of IVS7+3257, said third region including said position IVS+3257,

20 wherein said positions are numbered according to the position numbering shown in Figure 3.

In some cases, the method is for discriminating RHD*r's or RHD*r's-like from RHD*DIIIa, RHD*DIII_FN and/or RHD*DIVa-2, and wherein the method comprises genotyping the sample at one or more polymorphic positions in said first region of intron 7.

25 In some cases, the method is for discriminating *RHD*r^S* from *RHCE variants*, and wherein the method comprises genotyping the sample at one or more polymorphic positions in said first region of intron 7.

30 In some cases, the method is for discriminating RHD*r's, RHD*r's-like or RHD*DIIIa from RHD*DIVa-2, and wherein the method comprises genotyping the sample at one or more polymorphic positions in said second region and/or said third region of intron 7.

In some cases, the method is for discriminating between: (a) *RHD**r's or *RHD**r's-like; (b) *RHD**DIIIa; and (c) *RHD**DIVa-2, wherein the method comprises genotyping the sample at:

- (i) one or more polymorphic positions in said first region of intron 7; and
- (ii) one or more polymorphic positions in said second or said third regions.

5

In cases where the identity of the nucleotide at said one or more polymorphic positions in said first region is that of *RHCE*, the sample may be classified as containing or having a high probability of containing an *RHD**r's allele. In certain cases, where the sample may have been previously determined to lack *RHCE* and *RHCE* variants, the classification as containing an

10 *RHD**r's allele may be made with even greater certainty.

In cases where the identity of the nucleotide at said one or more polymorphic positions in said first region is that of *RHD*, and sample is classified as being or having a high probability of being *RHD**DIIIa or *RHD**DIVa-2. In certain cases, where the sample may have been previously

15 determined to lack *RHD* and *RHD* variants, the classification as containing an *RHD**DIIIa or *RHD**DIVa-2 allele may be made with even greater certainty.

In some cases, the method in accordance with the present invention comprises genotyping the sample at one or more polymorphic positions in intron 7 of the *RHD* gene locus, said one or

20 more polymorphic positions being selected from:

- (i) a first region of said intron 7 that is 5' of IVS7+1887, said first region including said position IVS7+1887;
- (ii(a)) a second (a) region of said intron 7 that lies between IVS7+2153 and IVS7+2335, said second (a) region including said positions IVS7+2153 and IVS7+2335;
- 25 (ii(b)) a second (b) region of said intron 7 that lies between IVS7+2449 and IVS7+3101, said second (b) region including said positions IVS7+2449 and IVS7+3101; and
- (iii) a third region of said intron 7 that lies 3' of IVS7+3257, said third region including said position IVS+3257,

wherein said positions are numbered according to the position numbering shown in Figure 3. In

30 certain cases, in accordance with the present invention, the method is for discriminating between: (a) *RHD**r's or *RHD**r's-like; (b) *RHD**DIIIa; (c) *RHD**DIII_FN; and (d) *RHD**DIVa-2, wherein the method comprises genotyping the sample at:

- (i) one or more polymorphic positions in said first region of intron 7;
- (ii) one or more polymorphic positions in said second (a) region;

- (iii) one or more polymorphic positions in said second (b) region; and
- (iv) one or more polymorphic positions in said third region.

In cases where the identity of the nucleotide at said one or more polymorphic positions in said second (a) region is the same as that of conventional *RHD*, and the identity of the nucleotide at said one or more polymorphic positions in said second (b) region (other than at positions IVS7+2945 and IVS7+3026) is the same as that of conventional *RHCE*, and the sample may be classified as having (or having a high probability of having) a *RHD*DIII_FN* allele.

- 10 In certain cases in accordance with the method of the invention, the method comprises genotyping the sample at one or more polymorphic positions in intron 7 of the *RHCE* locus, said polymorphic positions in intron 7 of the *RHCE* locus being located in a region that lies between IVS7+2970 and IVS7+4108, said region including said positions IVS7+2970 and IVS7+4108, and wherein said method is for detecting the presence or absence of, or for discriminating
- 15 between, *RHCE*, *RHCE*ce^S* and *RHCE*ce733G*, wherein said positions are numbered according to the numbering shown in Figure 3.

In accordance with the method of this aspect of the invention, said polymorphic positions may be selected from the single nucleotide polymorphism (SNP) positions set forth in Table 1, the position numbering being as shown in Figure 3. In some cases, the method may comprise

20 genotyping the sample at, at least, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more SNP positions selected from those set forth in Table 1. In certain cases, the genotype at the one or more SNP positions detected for the sample is used to classify the *RHD* and/or *RHCE* haplotypes present in the sample, according to the classification set forth in Table 1.

25 In some cases, the sample is a sample which has previously been determined to comprise an *RHD-RHCE* hybrid exon 3.

In some cases, the method further comprises determining whether the sample contains an

30 *RHD-RHCE* hybrid exon 3. The method may comprise genotyping the sample at one or more polymorphic positions selected from:

- IVS2-26, IVS2-13, IVS2-8 in *RHD* intron 2;
- IVS3+64, IVS3+69 in *RHD* intron 3; and

position 410 in RHD exon 3. In particular, the method may comprise genotyping the sample at position c.410 of the RHD locus, located in exon 3, wherein presence of T at said position in the context of upstream *RHD* and downstream *RHCE* sequences near that position indicates presence of an RHD/RHCE hybrid exon 3 variant in the sample.

5

In certain cases in accordance with the method of the present invention, the method comprises genotyping not more than 50, such as not more than 40, 30, 25, 20, 15, or not more than 10, single nucleotide polymorphic positions in the *RHD* gene locus and/or the *RHCE* gene locus. Thus, the method of the invention may provide considerable efficiency savings when compared with, for example, complete sequencing of the entire gene(s) or gene region. The method of the present invention advantageously concentrates on those informative polymorphic positions that may be used to determine presence or absence of, or to discriminate between, blood type variants.

10

Preferably, the method of the present invention further comprises the use of genotyping data to predict the phenotype encoded by *RHD* allele(s), and the use of this prediction to enable the determination of an RhD phenotype and/or the RHCE phenotype for the subject based on the genotype of the sample.

15

In accordance with the present invention, the subject may be undergoing, or may be a candidate for, blood transfusion. In some cases, the subject may have SCD, or any other disease requiring repeated blood transfusions, such as Thalassemia major or certain blood cell malignancies.

20

In accordance with the present invention, the subject may be of non-Caucasian race. In particular, the subject may be of African ancestry (e.g. "Black persons"). In certain cases, the subject may have an ancestral origin in a Mediterranean country.

25

In accordance with the method of the invention, the sample may be any suitable biological sample from which it is possible to determine the genotype of the subject at one or more polymorphic positions in intron 7 of the *RHD* gene and/or the *RHCE* gene. In certain case, the sample may conveniently take the form of a blood sample.

30

In accordance with the method of the invention, the sample may, in some cases, be subjected to one or more treatments to extract and/or amplify a nucleic acid prior to or as part of said genotyping. In particular, the sample may be treated to extract genomic DNA. Furthermore, genomic DNA extracted from the sample may be subjected to a PCR reaction in order to amplify a region of interest, for example, all or part of intron 7 of the *RHD* gene and/or the *RHCE* gene.

In certain cases in accordance with the method of the invention, the method may comprise carrying out an Allele-Specific Polymerase Chain Reaction (ASP) and/or Allele-Specific Hybridization (ASH).

In certain cases in accordance with the method of the invention, the method may comprise labelling a nucleic acid obtained from the sample or labelling an amplicon derived from a nucleic acid obtained from the sample. The label is preferably a detectable label. In some cases, DNA derived from the sample, e.g. PCR product resulting from use of the DNA from the sample as template, may be labelled using a fluorescent label or dye (e.g. by conjugating said fluorescent label or dye to the PCR product before or after fragmentation of the PCR product).

In certain cases in accordance with the method of the invention, the method may further comprise carrying out serological analysis on a blood sample that has been obtained from the subject, combining the serological analysis with said genotype and thereby determining the blood type phenotype of the subject. Combining the genotype-based prediction of blood type with a serological-based prediction may be useful, e.g., to improve accuracy or to resolve ambiguous results.

However, it is specifically contemplated herein that the method in accordance with this aspect of the invention may in some cases avoid the use of any serological analysis. This may result in considerable savings in terms of labour, cost and time.

In certain cases in accordance with the method of the invention, DNA may be obtained from the sample and amplified using primers that are selective for *RHD/RHCE* hybrid intron 7. For example, the primers may be selected from: a primer comprising or consisting of the nucleotide sequence of SEQ ID NO: 9, a primer comprising or consisting of the nucleotide sequence of SEQ ID NO: 10, a primer comprising or consisting of the nucleotide sequence of SEQ ID NO: 12 and a primer comprising or consisting of the nucleotide sequence of SEQ ID NO: 13.

Preferably, said primers may be used in pairs, such as the primers of SEQ ID Nos: 9 and 10 as a pair, or the primers of SEQ ID Nos: 12 and 13 as a pair.

In certain cases, in accordance with the method of the present invention, one or more of the primers may be a variant of one or more of the primers of SEQ ID NOs: 9, 10, 12 and 13, having 1, 2, 3, 4 or 5 nucleotide alterations whether by addition, deletion or substitution ("variant primers"). Preferably, a primer variant of the primers of SEQ ID NO: 9, 10, 12 and 13, respectively, comprises one or more additional nucleotides at the 3' and/or 5' end of the sequence set forth in SEQ ID NOs: 9, 10, 12 and 13, respectively, wherein the one or more additional nucleotides provide an extension to the primer sequence, and wherein the extension sequence is complementary to a portion of the sequence of the RHD or RHCE locus (sense or antisense strand), preferably a portion that is contiguous with the portion of sequence recognised by the primer of SEQ ID NOs: 9, 10, 12 and 13, respectively. Specifically contemplated herein are primer variants that are essentially functionally equivalent to the exemplary primers disclosed herein.

In certain cases in accordance with the method of the invention, DNA may be obtained from the sample and amplified using primers that are selective for *RHD/RHCE* hybrid intron 7. For example, the primers may be selected from: a primer comprising or consisting of the nucleotide sequence set forth in Table 7.

In certain cases, in accordance with the method of the present invention, one or more of the primers may be a variant of one or more of the primers set forth in Table 7, having 1, 2, 3, 4 or 5 nucleotide alterations whether by addition, deletion or substitution ("variant primers").

Preferably, a primer variant of a primer set forth in Table 7, comprises one or more additional nucleotides at the 3' and/or 5' end of the sequence set forth in Table 7, wherein the one or more additional nucleotides provide an extension to the primer sequence, and wherein the extension sequence is complementary to a portion of the sequence of the RHD or RHCE locus (sense or antisense strand), preferably a portion that is contiguous with the portion of sequence recognised by the primer set forth in Table 7. Specifically contemplated herein are primer variants that are essentially functionally equivalent to the exemplary primers disclosed in Table 7 herein.

In certain cases in accordance with the method of the invention, the genotyping step may comprise an allele-specific hybridisation of DNA extracted from the sample, or an amplicon derived from DNA extracted from the sample, wherein the allele-specific hybridisation comprises contacting said extracted DNA, or said amplicon, with a probe that hybridises to a portion of
5 intron 7 of the *RHD* gene and/or of the *RHCE* gene, said hybridisation being selective for one allele at a polymorphic position as set forth in Table 1. In preferred case, the probe may be selected from: a probe comprising or consisting of the sequence of SEQ ID NO: 11, a probe comprising or consisting of the sequence of SEQ ID NO: 14 and a probe comprising or consisting of the sequence of SEQ ID NO: 15.

10 In certain cases, in accordance with the method of the present invention, the probe may be a variant of the probe of SEQ ID NO: 11, 14 or 15 having 1, 2, 3, 4 or 5 nucleotide alterations whether by addition, deletion or substitution ("probe variants"). Preferably, a probe variant of the probe of SEQ ID NO: 11, 14 and 15, respectively, comprises one or more additional
15 nucleotides at the 3' and/or 5' end of the sequence set forth in SEQ ID NO: 11, 14 and 15, respectively, wherein the one or more additional nucleotides provide an extension to the probe sequence, and wherein the extension sequence is complementary to a portion of the sequence of RHD/RHCE hybrid intron 7 (sense or antisense strand), preferably a portion that is contiguous with the portion of sequence recognised by the probe of SEQ ID NO: 11, 14 and 15,
20 respectively. Specifically contemplated herein are probe variants that are essentially functionally equivalent to the exemplary probes disclosed herein.

In certain cases in accordance with the method of the invention, the genotyping step may comprise an allele-specific hybridisation of DNA extracted from the sample, or an amplicon
25 derived from DNA extracted from the sample, wherein the allele-specific hybridisation comprises contacting said extracted DNA, or said amplicon, with a probe that hybridises to a portion of intron 7 of the *RHD* gene and/or of the *RHCE* gene, said hybridisation being selective for one allele at a polymorphic position as set forth in Table 1. In preferred case, the probe may be selected from a probe comprising or consisting of a nucleotide sequence set forth in Table 8.

30 In certain cases, in accordance with the method of the present invention, the probe may be a variant of a probe set forth in Table 8, said variant having 1, 2, 3, 4 or 5 nucleotide alterations whether by addition, deletion or substitution ("probe variants"). Preferably, a probe variant of a probe set forth in Table 8 comprises one or more additional nucleotides at the 3' and/or 5' end

of a sequence set forth in Table 8, wherein the one or more additional nucleotides provide an extension to the probe sequence, and wherein the extension sequence is complementary to a portion of the sequence of *RHD*/*RHCE* hybrid intron 7 (sense or antisense strand), preferably a portion that is contiguous with the portion of sequence recognised by a probe set forth in Table 8. Specifically contemplated herein are probe variants that are essentially functionally equivalent to the exemplary probes disclosed in Table 8 herein.

In certain cases in accordance with the method of the invention, the method may further comprise determining whether the sample contains an *RHD*-*RHCE* hybrid exon 3. Accordingly, in certain cases, DNA obtained from the sample is amplified using primers that are selective for *RHD*/*RHCE* hybrid exon 3. Preferably, the primers that are selective for *RHD*/*RHCE* hybrid exon 3 are selected from: a primer comprising or consisting of the nucleotide sequence of SEQ ID NO: 5 and a primer comprising or consisting of the nucleotide sequence of SEQ ID NO: 6. In some cases, the primers that are selective for *RHD*/*RHCE* hybrid exon 3 are selected from: a primer variant having 1, 2, 3, 4 or 5 nucleotide alternations, whether by addition, deletion or substitution, compared with the primer sequence of SEQ ID NOs: 5 and 6, respectively. The method in accordance with this aspect of the invention may further comprise genotyping the sample at a polymorphic position in exon 3 of the *RHD* locus, wherein the method comprises an allele-specific hybridisation of DNA extracted from the sample, or an amplicon derived from DNA extracted from the sample, and wherein the allele-specific hybridisation comprises contacting said extracted DNA or said amplicon with a probe that is selective for an allele present in *RHD*/*RHCE* hybrid exon 3. In certain cases, the probe may be selected from: a probe comprising or consisting of the sequence of SEQ ID NO: 7 and a probe comprising or consisting of the sequence of SEQ ID NO: 8. In certain cases, the probe that is selective for an allele present in *RHD*/*RHCE* hybrid exon 3 may be a variant probe having 1, 2, 3, 4 or 5 nucleotide alternations, whether by addition, deletion or substitution, compared with the probe sequence of SEQ ID NOs: 7 and 8, respectively.

In a second aspect, the present invention provides one or more oligonucleotide probes and/or primers for use in the method of the invention, wherein the one or more oligonucleotide probes and/or primers span, or are able to be used to span, the polymorphic positions in intron 7 of the *RHD* gene and/or the *RHCE* gene, said probes and/or primers being hybridisable to a portion of the sense or antisense strand of said *RHD* and/or *RHCE* gene. Typically, said oligonucleotide probes and/or primers are not more than 50 bases in length, such as 10-50 nucleotides or 15-35

nucleotides or even 19-27 nucleotides in length. Advantageously, the probes and/or primers are perfectly complementary to a portion of the sense or antisense strand of intron 7 or the *RHD* and/or *RHCE* gene, which portion includes a polymorphic position such that the probe and/or primer hybridises more efficiently to one allele (i.e. sequence including one form of the polymorphism) compared with another allele at said positions (i.e. sequence including another or the other form of the polymorphism). In certain preferred cases, the one or more oligonucleotide probes and/or primers span one or more polymorphic positions set forth in Table 1. For example, the one or more oligonucleotide probes in accordance with this aspect of the invention may, in some cases, be selected from the probes comprising or consisting of the nucleotide sequence set forth in one or more of SEQ ID Nos: 11, 14 and 15, or a variant of one of said probes having 1, 2, 3, 4 or 5 nucleotide alternations, whether by addition, deletion or substitution, compared with the nucleotide sequence set forth in SEQ ID Nos: 11, 14 and 15, respectively. Alternatively or additionally, the one or more primers in accordance with this aspect of the invention may, in some cases, be selected from the primers comprising or consisting of the nucleotide sequence set forth in one or more of SEQ ID Nos: 9, 10, 12 and 13, or a variant of one of said primers having 1, 2, 3, 4 or 5 nucleotide alternations, whether by addition, deletion or substitution, compared with the nucleotide sequence set forth in SEQ ID Nos: 9, 10, 12 and 13, respectively.

Alternatively or additionally, the one or more oligonucleotide probes in accordance with this aspect of the invention may, in some cases, be selected from the probes comprising or consisting of a nucleotide sequence set forth in Table 8, or a variant of one of said probes having 1, 2, 3, 4 or 5 nucleotide alternations, whether by addition, deletion or substitution, compared with a nucleotide sequence set forth in Table 8.

Alternatively or additionally, the one or more primers in accordance with this aspect of the invention may, in some cases, be selected from the primers comprising or consisting of the nucleotide sequence set forth in Table 7, or a variant of one of said primers having 1, 2, 3, 4 or 5 nucleotide alternations, whether by addition, deletion or substitution, compared with the nucleotide sequence set forth in Table 7.

In a third aspect, the present invention provides a plurality of oligonucleotide probes in accordance with the second aspect of the invention, wherein the probes are coupled to a solid support. Preferably, the probes are covalently attached (directly or via a linker) to the solid

support. In some cases the solid support may comprise a planar surface, such as a glass surface (e.g. in the form of a DNA chip or "microarray"). In some cases the solid support may comprise a particle, such as a microbead to which one or more of the probes are conjugated.

5 In a fourth aspect, the present invention provides a kit for assessing a subject's blood type, the kit comprising:

one or more probes and/or primers as defined in accordance with the second aspect of the invention;

optionally, one or more probes and/or primers that span one or more polymorphic
10 positions in intron 2, exon 3 and/or intron 3 of the sense or antisense strand of the *RHD* gene;

optionally, one or more reagents and/or standards for use in carrying out a method as defined in accordance with the first aspect of the invention; and

optionally, instructions for carrying out a method as defined in accordance with the first aspect of the invention.

15

In some cases in accordance with this aspect of the invention, the kit comprises:

one or more primers selected from the primers comprising or consisting of one or more nucleotide sequences set forth in SEQ ID Nos: 5 and 6; and/or

one or more probes selected from the probes comprising or consisting of one or more
20 nucleotide sequences set forth in of SEQ ID Nos: 7 and 8.

In a fifth aspect, the present invention provides a system for use in determining a subject's blood type, the system comprising:

a kit as defined in accordance with the fourth aspect of the invention; and

25 at least one detector arranged to detect a signal from detectably labelled DNA obtained from said subject or a detectably labelled amplicon (fragmented or unfragmented) derived from DNA obtained from said subject;

at least one controller in communication with the at least one detector, the controller being programmed with computer-readable instructions to transform said signal into predicted
30 blood type genotypes, and optionally, to transform said predicted blood type genotypes into a predicted blood type phenotype.

In a sixth aspect, the present invention provides a method of blood matching, the method comprising:

carrying out the method in accordance with the first aspect of the invention on a recipient sample from a recipient subject in need of donor blood and a donor sample from a potential donor subject;

5 comparing the blood type alleles present in the recipient sample with those present in the donor subject and thereby determining the compatibility of the recipient subject to receive blood from the donor subject.

In some cases, the method in accordance with the sixth aspect of the invention may be carried out for a plurality of recipient subjects and a plurality of potential donor subjects.

10

The invention will now be described in more detail, by way of example and not limitation, by reference to the accompanying drawings. Many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the invention set forth are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the scope of the invention. All documents cited herein are expressly incorporated by reference.

15

Description of the Figures

20

Figure 1: A) shows a representation of the RHD locus for RHD* r^S (upper row), RHD*DIIIa (middle row) and RHD*DIVa (and others) (lower row), in which certain polymorphic positions are indicated and certain portions are labelled as being shared RHD and RHCE sequence (shaded box or (S)), RHD sequence (D), RHCE sequence (E), non-RHD, non-RHCE sequence (N) and no nucleotide present ($\emptyset$); **B)** shows a representation of the RHD locus for RHD*DIII_FN, in which certain polymorphic positions are indicated and certain portions are labelled as being shared RHD and RHCE sequence (shaded box or (S)), RHD sequence (D), RHCE sequence (E), non-RHD, non-RHCE sequence (N) and no nucleotide present ($\emptyset$);

25

30

Figure 2: shows a representation of the RHCE locus for RHCE* ce^S and RHCE* $ce733G$, in which certain polymorphic positions are indicated and certain portions are labelled as being shared RHD and RHCE sequence (shaded box), RHD sequence (D), RHCE sequence (E) and non-RHD, non-RHCE sequence (N);

Figure 3: shows a sequence alignment of intron 7 of the RHD locus with intron 7 of the RHCE locus. Identical nucleotides are indicated by a dot, gaps are indicated by a dash. The numbered positions referred to herein, e.g. the SNP position numbers set forth in Table 1, are numbered in accordance with the position numbering shown in this sequence alignment. The upper row sequence shows intron 7 of the RHD gene, numbered positions 1101 to 4120 (SEQ ID NO: 1). The lower row sequence shows intron 7 of the RHCE gene numbered positions 1101 to 4120 (SEQ ID NO: 2), according to the alignment with SEQ ID NO: 1.

Detailed Description of the Invention

The present invention finds use in the determination of the clinically relevant RHD- and RHCE-encoded antigen phenotypes of a blood sample. The invention provides a method for detecting the presence or absence of, or for discriminating between, blood type variants, which method comprises genotyping a sample obtained from a human subject at one or more positions in intron 7 of the *RHD* gene and/or in intron 7 of the *RHCE* gene. Advantageously, the method of the present invention may further comprise determining the presence or absence of an *RHD/RHCE* hybrid exon 3 in said sample.

The Rh blood group D antigen is encoded by the *RHD* gene, which comprises 10 exons. The complete *RHD* gene sequence is available at NCBI Reference Sequence: NG_007494.1 No. NG_007494.1, GI:171184448, (SEQ ID NO: 3), the entire contents of which is incorporated herein by reference.

The Rh blood group C antigen is encoded by the *RHCE* gene, which comprises 10 exons. The complete *RHCE* gene sequence is available at NCBI Reference Sequence: NG_009208.2, GI:301336136, (SEQ ID NO: 4), the entire contents of which is incorporated herein by reference.

In certain cases in accordance with the present invention, the method may comprise: (1) determining the genotype of a sample at one or more positions between positions IVS7+2153 and IVS7+3101, (2) determining if the one or more positions from (1) corresponds to an RHD or RHCE sequence, (3) deducing from the genotype whether or not the sample contains or may contain a *RHD***r*^S haplotype, as indicated in Figure 1.

Preferably, the method further comprises: (1 bis) determining the genotype of a sample at one or more positions on the 5' end of position IVS7+1887, (2 bis) determining if the one or more positions from (1 bis) correspond to an RHD or RHCE sequence.

5

Preferably, the method further comprises: (1 tri) determining the genotype of a sample at one or more positions on the 3' end of position IVS7+3257, (2 tri) determining if the one or more positions from (1 tri) correspond to an RHCE*ceS or RHCE*ce733G sequence, and (3) deducing from the genotype whether or not the sample contains or may contain a *RHD*r^S* haplotype, as indicated in Table 1.

10

Preferably, the method further comprises: (4) determining the genotype of a sample at one or more positions in exon 3, and deducing from the genotype whether or not the sample contains or may contain an *RHD/RHCE* hybrid exon 3.

15

Preferably, the method further comprises: determining the genotype of a sample at one or more polymorphic positions in the region between IVS7+2153 and IVS7+2335, said positions IVS7+2153 and IVS7+2335 being included in said region; determining if the one or more polymorphic positions correspond to an RHD or RHCE sequence; and deducing from the genotype determination whether the sample comprises a *RHD*r^S* haplotype or an *RHD*RIII_FN* haplotype, as indicated in Table 1.

20

The value of the new knowledge described herein is furthered by the fact that the aforementioned *RHD* variants are often found in *cis* or together in the same sample with the *RHCE* variants, which may confound determination of a genotype, and consequently determination of presence or absence of certain *RH*-encoded blood group antigens, in the sample harboring them. The confounding effect affects the more common genotyping methods in use, in particular methods whose source material is genomic DNA. Advantageously, the present invention mitigates this undesirable confounding effect.

25

30

The term "sample" as used herein is intended to encompass any material (solid, liquid or aspirate) obtained directly or indirectly from a human subject and from which the identity of one or more nucleotides in a relevant genomic locus (e.g. intron 7 or the RHD locus and/or intron 7 of the RHCE locus) can be determined. In particular, the term "sample" includes any biological fluid such as blood, plasma, urine, saliva, cerebrospinal fluid and interstitial fluid, any solid matter, such as tissue, bone and hair, any cell or cell extract, any derived cell line, such as an immortalised tumour cell line and stem cell line, an extract of any of the preceding sample types, such as fixed or paraffin-embedded tissue. In certain preferred embodiments, the sample is an extract of human genomic DNA, optionally amplified and/or purified.

As used herein, the term "genotyping" is intended to encompass any method for determining the identity of the nucleotide at a particular position such as a polymorphic position at a specified locus. Thus, genotyping includes identifying one or both alleles of a particular gene. Genotyping may employ any of a variety of techniques, including but not limited to, allele-specific hybridisation, allele-specific PCR, sequencing of all or part of a gene.

As described herein in detail, certain blood type alleles are less common and are typically referred to as "variants" (e.g. *RHD***r*^S). Variant blood type alleles are in some cases referred to herein simply as "blood type variants".

Table 1. On the horizontal axis, the variants whose sequences are described in the present invention (*RHD***r*^S, *RHD***DIIIa*, *RHD***DIII_FN*, *RHD***DIVa-2*, *RHCE***ce*^S, *RHCE***ce733G*), and their respective references (*RHD*, *RHCE***ce*). On the vertical axis, the relevant polymorphic positions in intron 7 of the *RHD* and *RHCE* loci (the region of interest). The SNP position numbering is as shown in the alignment of Figure 3. The *RHD* reference sequence column shows the identity of the nucleotide at each of the indicated positions in intron 7 of the reference *RHD* gene. The *RHCE***ce* reference sequence column shows the identity of the nucleotide at each of the indicated positions in intron 7 of the reference *RHCE* gene. The *RHD***r*^S variant sequence column shows the identity of the nucleotide at each of the indicated positions in intron 7 of the *RHD***r*^S variant of the *RHD* gene (*RHD* locus). The *RHD***DIIIa* variant sequence column shows the identity of the nucleotide at each of the indicated positions in intron 7 of the *RHD***DIIIa* variant of the *RHD* gene (*RHD* locus). The *RHD***DIII_FN* variant sequence column

shows the identity of the nucleotide at each of the indicated positions in intron 7 of the *RHD*DIH_FN* variant of the *RHD* gene (*RHD* locus). The *RHD*DIVa-2* variant sequence column shows the identity of the nucleotide at each of the indicated positions in intron 7 of the *RHD*DIVa-2* variant of the *RHD* gene (*RHD* locus). The *RHCE*ce^S* variant sequence column

5 shows the identity of the nucleotide at each of the indicated positions in intron 7 of the *RHCE*ce^S* variant of the *RHCE* gene (*RHCE* locus). The *RHCE*ce733G* variant sequence column shows the identity of the nucleotide at each of the indicated positions in intron 7 of the *RHCE*ce733G* variant of the *RHCE* gene (*RHCE* locus).

SNP position	Reference Sequence		Variant Sequence					
	RHD	RHCE*ce	RHD*r ^S	RHD*DIlla	RHD*DIll_FN	RHD*DIVa-2	RHCE*ce ^S	RHCE*ce733G
IVS7+1139	C	T	T	C	C	C		
IVS7+1148	G	A	A	G	G	G		
IVS7+1234	C	C	T	T	T	T		
IVS7+1241	A	T	T	A	A	A		
IVS7+1276	C	G	G	C	C	C		
IVS7+1319	A	Ø	A	A	A	A		
IVS7+1356	T	C	C	C	C	T		
IVS7+1758	T	G	G	T	T	T		
IVS7+1765	A	G	G	A	A	A		
IVS7+1782	A	Ø	Ø	A	A	A		
IVS7+1850	G	A	A	G	G	G		
IVS7+1869	G	A	A	G	G	G		
IVS7+1880	A	G	G	A	A	A		
IVS7+1886	A	G	G	A	A	A		
IVS7+1887	T	C	C	T	T	T		
IVS7+2153	G	C	C	C	G	G		
IVS7+2160	T	C	C	C	T	T		
IVS7+2200	A	T	T	T	A	A		
IVS7+2235	C	T	T	T	C	C		
IVS7+2276	C	A	A	A	C	C		
IVS7+2282	A	G	G	G	A	A		
IVS7+2323	C	T	T	T	C	C		
IVS7+2335	A	G	G	G	A	A		
IVS7+2449	T	C	C	C	C	T		
IVS7+2472	A	G	G	G	G	A		
IVS7+2514	A	G	G	G	G	A		
IVS7+2539	C	T	T	T	T	C		
IVS7+2591	G	A	A	A	A	G		
IVS7+2620	C	G	G	G	G	C		
IVS7+2782	T	A	A	A	A	T		
IVS7+2802	A	T	T	T	T	A		
IVS7+2846+1	Ø	T	T	T	T	Ø		
IVS7+2945	G	T	G	G	G	G		
IVS7+2970	C	T	T	T	T	C	T	T
IVS7+2973	G	T	T	T	T	G	T	T
IVS7+2977	A	G	G	G	G	A	G	G
IVS7+3026	C	T	C	C	C	C	C	C
IVS7+3046	C	T	T	T	T	C	T	T
IVS7+3099	C	G	G	G	G	C	G	G
IVS7+3101	C	C	T	T	T	C	T	T
IVS7+3257	C	Ø	C	C	C	C	C	C

SNP position	Reference Sequence		Variant Sequence					
	<i>RHD</i>	<i>RHCE*ce</i>	<i>RHD*r^S</i>	<i>RHD*DIIIa</i>	<i>RHD*DIII_FN</i>	<i>RHD*DIIa-2</i>	<i>RHCE*ce^S</i>	<i>RHCE*ce733G</i>
IVS7+3258	C	Ø	C	C	C	C	C	C
IVS7+3259	C	Ø	C	C	C	C	C	C
IVS7+3260	C	Ø	C	C	C	C	C	C
IVS7+3261	A	Ø	A	A	A	A	A	A
IVS7+3262	A	Ø	A	A	A	A	A	A
IVS7+3349	C	C	T	T	T	C	T	T
IVS7+3489	G	A	G	G	G	G	G	G
IVS7+3521	G	C	C	C	C	G	G	G
IVS7+3523	T	A	A	A	A	T	T	T
IVS7+3525	T	T	T	T	T	T	G	G
IVS7+3648	T	C	T	T	T	T	C	C
IVS7+3961	T	G	T	T	T	T	T	T
IVS7+3975	T	G	T	T	T	T	T	T
IVS7+3981	G	A	G	G	G	G	G	G
IVS7+4052	A	G	A	A	A	A	G	G
IVS7+4105	G	A	G	G	G	G	A	A
IVS7+4106	T	C	T	T	T	T	C	C
IVS7+4107	G	T	G	G	G	G	T	T
IVS7+4108	G	C	G	G	G	G	C	C

Key to Table 1

Ø: No nucleotide (position corresponds to a gap in the alignment set forth in Figure 3).

- 5 Broadly, the present invention provides methods and products for the identification by molecular techniques of genetic variants *RHD*r^S* or *RHD*r^S*-like, which encode no D antigen (D⁻), an altered form of D antigen (partial D), altered C antigen (C^{+W}), altered e antigen (e^{alt}), VS antigen (VS⁺), V antigen (V⁺), and/or no hr^B antigen (hr^{B-}) in blood cells. The present inventors have found that a determination of one or more genetic sequences in a region of intron 7 of the RHD
- 10 locus enables discrimination between variants *RHD*r^S* or *RHD*r^S*-like and other RHD/RHCE hybrid exon 3 variants, including but not limited to *RHD*DIIIa*, *RHD*DIII_FN* and *RHD*DIIa-2*. The present inventors have also found that a determination of one or more genetic sequences in a region of intron 7 of the RHCE locus enables discrimination between variants *RHCE*ce^S* or *RHCE*ce733G* and conventional RHCE. Determination of said sequences in turn, enables
- 15 prediction of the D antigen and C antigen phenotype for a large majority of samples containing RHD/RHCE hybrid exon 3, as well as prediction of e antigen, VS antigen, V antigen and hrB antigen for a large number of samples containing variants *RHCE*ce^S* or *RHCE*ce733G*. In certain embodiments, the method of the invention provides considerable efficiency savings in comparison with, for example, full DNA sequencing, or genotyping of a large number of

polymorphisms, or determining the phenotype by serological methods. Nevertheless, it is specifically contemplated that the method of the invention may, in some cases, involve DNA sequencing in order to genotype the sample obtained from the subject.

5 The *RHD* and *RHCE* variant sequences described herein have been determined by standard DNA sequencing, classified and consensuated from data for a set of samples known from serological and/or molecular typing methods to contain said variants. More specifically, the sample set consisted of seven samples containing the *RHD***r*^S variant, eleven samples containing the *RHD***DIIIa* variant, two samples containing the *RHD***DIVa-2* variant, nine
 10 samples containing the *RHCE***ce*^S variant, and three samples containing the *RHCE***ce733G* variant.

“Consensuated” as used herein specifically includes establishing by compilation and comparison of multiple highly-similar, non-identical sequences and selection of a single
 15 consensus sequence. “Consensuation” advantageously responds to the existence of sequence variations among samples containing the same variant.

In certain cases in accordance with the method of the invention, presence/absence of variant *RHD***DIIIa* or similar variants (including but not limited to *RHD***DIII*_FN, *RHD***DIII*-type4,
 20 *RHD***DIII*-type6, *RHD***DIII*-type7, *RHD***DIII*-type8) is determined by the combined identification of the nucleotide sequence at polymorphic positions IVS7+1887 and IVS7+2153, located in intron 7 of the *RHD* locus. Further determination of, e.g., one or more of the nucleotide sequence at one or more of polymorphic positions IVS7+2449, IVS7+2472, IVS7+2514, IVS7+2539, IVS7+2591, IVS7+2620, IVS7+2782, and IVS7+2802 may be employed in order to
 25 distinguish *RHD***r*^S from *RHD***DIII*_FN (in view of the fact that *RHD***r*^S and *RHD***DIII*_FN appear identical at the aforementioned positions IVS7+1887 and IVS7+2153 (see Table 1). As will be appreciated by reference to Table 1 and Figure 1, the identification of the nucleotide at polymorphic position IVS+1887 may be substituted with a determination of the nucleotide sequence at one or more polymorphic positions located 5' of IVS7+1887, including but not
 30 limited to one or more of positions IVS7+1886, IVS7+1880, IVS7+1869, IVS7+1850 and IVS7+1782. Alternatively or additionally, the identification of the nucleotide at polymorphic position IVS7+2153 may be substituted with a determination of the nucleotide sequence at one

or more polymorphic positions located 3' of IVS7+2153, including but not limited to one or more of positions IVS7+2160, IVS7+2200, IVS7+2235, IVS7+2276 and IVS7+2282. Each and every pair-wise or multiple combination of the polymorphic positions 5' of IVS7+1887 and 3' of IVS7+2153 is specifically contemplated herein. The determination that variant RHD*DIIIa or similar variants (including but not limited to RHD*DIII-type4, RHD*DIII-type6, RHD*DIII-type7, RHD*DIII-type8 and/or RHD*DIII_FN) is present in the sample may be made by positive identification of:

(i) *RHD* sequence at said position IVS7+1887 and/or at one or more polymorphic positions 5' of IVS7+1887 (including but not limited to one or more of positions IVS7+1886, IVS7+1880, IVS7+1869, IVS7+1850 and IVS7+1782); in combination with

(ii) *RHCE* sequence at said position IVS7+2153 and/or at one or more polymorphic positions 3' of IVS7+2153 (including but not limited to one or more of positions IVS7+2160, IVS7+2200, IVS7+2235, IVS7+2276 and IVS7+2282).

Presence of RHD*DIII_FN may be distinguished from RHD*r^S by the presence of *RHCE* sequence at one or more polymorphic positions 3' of IVS7+2449 (including but not limited to one or more of positions IVS7+2449, IVS7+2472, IVS7+2514, IVS7+2539, IVS7+2591, IVS7+2620, IVS7+2782, IVS7+2802).

As will be appreciated by reference to Figure 1 and Table 1, such pair-wise or multiple identification of (i) *RHD* sequence and (ii) *RHCE* sequence at the indicated intron 7 positions is characteristic of RHD*DIIIa and similar variants (including but not limited to RHD*DIII-type4, RHD*DIII-type6, RHD*DIII-type7, RHD*DIII-type8), but is not characteristic of *RHD*r's* or *RHD*DIVa-2*.

In certain cases in accordance with the method of the invention, presence/absence of variants RHD*r^S or RHD*DIIIa or similar variants (including but not limited to RHD*r^S-like, RHD*DIII_FN, RHD*DIII-type4, RHD*DIII-type6, RHD*DIII-type7, RHD*DIII-type8) is determined by the combined identification of the nucleotide sequence at polymorphic positions IVS7+3523 and IVS7+3648, located in intron 7 of the *RHD* locus. As will be appreciated by reference to Table 1 and Figure 1, the identification of the nucleotide at polymorphic positions IVS7+3523 and IVS7+3648 may be substituted by determination of the nucleotide sequence at one or more polymorphic positions located 5' of IVS7+3523 and 3' of IVS7+3648, respectively. The latter

positions include but are not limited to IVS7+3521, IVS7+3099, IVS7+3046, IVS7+2977, IVS7+2973 on the 5' of IVS7+3523 and IVS7+3961, IVS7+3975, IVS7+3981, IVS7+4052, IVS7+4105 on the 3' of IVS7+3648. Each and every pair-wise combination of the polymorphic positions 5' of IVS7+3523 and 3' of IVS7+3648 is specifically contemplated herein.

5

In certain cases in accordance with the method of the invention, presence/absence of variants RHCE*ce^S or RHCE*ce733G or similar variants (including but not limited to RHCE*ce48C,733G,1025T, RHCE*ce48C,697G,733G, RHCE*ce340T,733G, RHCE*ce48C,733G,748A) is determined by the combined identification of the nucleotide
 10 sequence at polymorphic positions IVS7+3981 and IVS7+4052, located in intron 7 of the RHCE locus. As will be appreciated by reference to Table 1 and Figure 1, determination of the nucleotide sequence at polymorphic positions IVS7+3981 and IVS7+4052 may be substituted by determination of the nucleotide sequence at one or more polymorphic positions located 5' of IVS7+3981 and 3' of IVS7+4052, respectively. The latter positions include but are not limited to
 15 IVS7+3975, IVS7+3961, IVS7+3523, IVS7+3521, IVS7+3489 on the 5' of IVS7+3981 and IVS7+4105, IVS7+4106, IVS7+4107, IVS7+4108, IVS7+4127 on the 3' of IVS7+4052. Each and every pair-wise combination of the polymorphic positions 5' of IVS7+3981 and 3' of IVS7+4052 is specifically contemplated herein.

20 In certain cases in accordance with the method of the invention, presence/absence of variants RHD*r^S, RHD*DIIIa, RHD*DIVa-2 or similar variants (including but not limited to RHD*r^S-like, RHD*DIII_FN, RHD*DIII-type4, RHD*DIII-type6, RHD*DIII-type7, RHD*DIII-type8, RHD*DIVa) can be determined by the identification of the nucleotide sequence at a single polymorphic position, such as IVS7+1234, located in intron 7 of the RHD locus. As shown in Table 1 and
 25 Figure 1, the identity of the nucleotide at position IVS7+1234 of the RHD locus (T in the case of the variants RHD*r^S, RHD*DIIIa, RHD*DIII_FN, RHD*DIVa-2) differs from both the RHD reference sequence and the RHCE reference sequence. Therefore, the identification of a nucleotide that is non-RHD and non-RHCE at position IVS7+1234 of the RHD locus is indicative of the sample containing a variant selected from RHD*r^S, RHD*DIIIa, RHD*DIVa-2 and similar
 30 variants (including but not limited to RHD*r^S-like, RHD*DIII_FN, RHD*DIII-type4, RHD*DIII-type6, RHD*DIII-type7, RHD*DIII-type8 and RHD*DIVa).

In certain cases in accordance with the method of the invention, presence/absence of variants RHCE*ce^S or RHCE*ce733G or similar variants (including but not limited to RHCE*ce48C,733G,1025T, RHCE*ce48C,697G,733G, RHCE*ce340T,733G, RHCE*ce48C,733G,748A) is determined by the identification of the nucleotide at a single polymorphic position, such as IVS7+3525, located in intron 7 of the RHCE locus. As shown in Table 1 and Figure 1, the identity of the nucleotide at position IVS7+3525 of the RCE locus (G in the case of the variants RHCE*ce^S and RHCE*ce733G) differs from both the RHD reference sequence and the RHCE reference sequence. Therefore, the identification of a nucleotide that is non-RHD and non-RHCE at position IVS7+3525 of the RHCE locus is indicative of the sample containing a variant selected from RHCE*ce^S, RHCE*ce733G and similar variants (including but not limited to RHCE*ce48C,733G,1025T, RHCE*ce48C,697G,733G, RHCE*ce340T,733G, RHCE*ce48C,733G,748A).

As will be apparent to the skilled person having regard to Table 1 and Figure 1 herein, the RHD and RHCE variants exhibit characteristic and in some cases unique combinations of nucleotide identities at the specified SNP positions in intron 7 of RHD and of RHCE. These may be exploited to discriminate among RHD and/or RHCE variants, as required. The various combinations and variations that are made available by the information disclosed herein are within the scope of the present invention as set out in the appended claims.

A wide variety of techniques are suitable and may be used to detect these genetic sequences, e.g. to determine the identity of the nucleotide at one or more polymorphic positions in intron 7 of RHD and/or RHCE for a sample under consideration. The following are presented as non-limiting examples of such techniques. A suitable technique to detect the herein mentioned genetic sequences is mutation analysis by restriction digestion after a PCR reaction for amplifying the region of interest, if the genetic variant or polymorphism results in the creation or elimination of a restriction site. Sequence analysis, such as direct manual or fluorescent automated sequencing, directly or after selection of the region of interest by PCR, can also be used to detect specific sequences. Allele-specific oligonucleotides, for example, used in a competitive or non-competitive PCR (ASP henceforth), can also be used to detect genetic variants. Another suitable technique to detect specific sequences in a sample is testing that sample for the presence of a nucleic acid molecule comprising all or a portion of the region of interest, consisting in contacting said sample with a second nucleic acid molecule or probe

under conditions for selective hybridization. In any of these techniques, all or a part of the region of interest can be amplified prior to performing the specific technique used for detection of the genetic variants.

- 5 The method makes use of the detection or lack of detection of one or more specific nucleotide sequences within the region of interest or within a "functional segment", such as an intron or an exon (e.g. intron 7 of RHD or RHCE) or part thereof.

10 In certain cases, the method of the invention makes use of Allele-Specific Hybridization (ASH henceforth), and may make use of synthetic oligonucleotide probes usually 10-50 nucleotides long, preferably 19-27 nucleotides long, the sequences of which are designed to be complementary to the interrogated sequence. Complementarity of sequences enables pairing of genomic DNA and oligonucleotide probe molecules. Specific pairing, i.e. pairing of probes to their complementary sequence and to no other sequence, can be made to occur under
15 appropriate conditions, which include but are not limited to time of incubation, temperature of incubation, concentration of probe and complementary sequences, and mixing. Specific pairing to probes allows detection of sequences in a mix of sequences. Detection or lack of detection of specific sequences, in turn, allows determination of presence versus absence of functional segments or regions of interest.

20

Synthetic oligonucleotide probes can be used for the detection of particular conserved, non-variant regions and/or allelic variants in an individual's genomic DNA. Often, allelic variants are single nucleotide polymorphisms (SNPs), i.e. nucleotide positions at which the DNA composition may vary across individuals.

25

In some cases, the synthetic oligonucleotide probes described herein are designed and used to detect the presence or absence of functional nucleic acid segments and also, both to detect allelic variants located within sequences and to determine the presence or absence of functional segments or regions of interest.

30

Given a particular nucleotide at a particular position of a locus of genomic DNA, synthetic oligonucleotide molecules, or probes, can be designed to detect said nucleotide in a test sample. Probes can be designed in pairs such that one member of the probe pair is complementary to one strand of the sequence, whereas the other member of the probe pair is complementary to the other strand of the sequence. Probes can also be designed in sets so that they have different lengths and be complementary to one strand or the two strands of the sequence of interest.

In accordance with any aspect of the present invention, probes may be attached to a chemically-functionalized solid support. An example of a solid support is a flat glass surface, on which probe molecules are placed by contact deposition. Another example of a solid support is a micrometer-size polymer bead, to which probe molecules are attached by conjugation. Another example of a solid support is a nanometer-size particle to which probe molecules are attached by one of various means. An exemplary description herein relates to the procedure performed wherein the probes are immobilised on a flat glass surface. Attachment of probe molecules to the surface is performed at multiple individual locations referred to as replicate features or "replicates". The number of replicate features for each probe species is usually ten, although it may vary. Another exemplary description herein relates to the procedure performed wherein the probes are immobilised on a micron-size spherical surface. Attachment of probe molecules is performed at multiple individual spherical surfaces, referred to as replicate features or "replicates". The number of replicate features for each probe species is usually one hundred, although it may vary.

In accordance with any aspect of the present invention, functional segments or their portions may be amplified, for example by PCR, using as a template genomic DNA. Amplified functional segments or their portions can be labeled (e.g. with a fluorescent label) to allow for their detection, and optionally fragmented to facilitate their pairing with oligonucleotide probes.

In accordance with any aspect of the present invention, labelled and fragmented functional segments or their portions may be incubated under conditions that maximize the sensitivity and specificity of pairing with probes attached to the solid support. The presence of probe-paired functional segments or their portions may be determined indirectly from the measurement of

label, usually a fluorochrome, attached to the solid support. This measurement is referred to herein as signal intensity. By way of example, the fluorescence emitted by the fluorochrome may be collected by means of a fluorescence detection device, such as a confocal scanner.

5 **Examples**

Example 1

Discrimination among genetic variants that share a RHD/RHCE hybrid exon 3 but encode different forms of D Ag (Partial D Ag vs. No D Ag) and RhC Ag (Normal C Ag vs.

10 Altered/Weakened C Ag, sometimes abbreviated as C^{+W})

The following example relates to a method of discriminating among *RHD/RHCE* hybrid exon 3 variants RHD*r's, RHD*DIlla and RHD*DIVa-2. The method is based on the interrogation of the nucleotide composition of the genomic DNA of a sample at three locations in the RHD locus.

15

Identification of the nucleotide at a first location enables discrimination between variants RHD*r's/RHD*DIlla and variant RHD*DIVa-2, and involves an ASP. In particular, this ASP interrogates polymorphic positions IVS7+3349, IVS7+4105, IVS7+4106, IVS7+4107, IVS7+4108, IVS7+4127 in RHD intron 7.

20

Identification of the nucleotide composition at a second location enables discrimination between variants RHD*r's and RHD*DIlla, and involves an ASP. In particular, this ASP interrogates polymorphic positions IVS7+1869, IVS7+1880, IVS7+1886, IVS7+1887, IVS7+2276, IVS7+2282 in RHD intron 7.

25

Optionally, but in many cases very preferably, identification of the nucleotide composition at a third location may be carried out in order to determine the presence or absence of a RHD/RHCE hybrid exon 3 in the test sample, and involves molecular techniques known in the art as ASP and ASH. In particular, the RHD/RHCE hybrid exon 3 ASP interrogates polymorphic positions

IVS2-26, IVS2-13, IVS2-8 in RHD intron 2 and polymorphic positions IVS3+64, IVS3+69 in RHD intron 3, while the ASH interrogates polymorphic position c.410 in RHD exon 3.

5 The method described above is applied to 252 samples selected to contain either no variant in either copy of the RHD gene, one variant in one copy of the RHD gene, or two variants, one on each copy of the RHD gene. Determination of the presence or absence of variants is made from DNA sequencing data as well as from genotyping data at polymorphisms in the RHD locus including certain positions shared with the present method and certain positions other than the ones described in the present method. Said samples are selected to include RHD variants
10 encoding the major RhD phenotypes, namely RhD⁺, Partial D, Weak D, RhD⁻.

The process described below proceeds from the genotyping of said samples and the posterior analysis of said samples grouped by genotype and/or predicted phenotype. The serotype associated to a group corresponds to analysis performed only on a subset of the samples in
15 said group.

Materials & Methods

Genomic DNA is extracted from nucleated cells in a blood sample by cell lysis. Extracted DNA is purified on an affinity column. Both, cell lysis and DNA purification are performed with a
20 QIAamp Blood kit (Qiagen, Germany) by following manufacturer protocols and recommendations. Purity of DNA is determined by spectrophotometry on a Nanodrop instrument (Nanodrop, DE). Only DNA solutions with an OD_{260}/OD_{280} 1.8 ± 0.2 proceed to subsequent analysis.

25 Purified DNA is used as a template for multiplexed Polymerase Chain Reaction (PCR) amplification of the gene segments of interest in a GeneAmp 9700 thermal cycler (Perkin-Elmer, CA). Primer sequences for the different segments are listed in the Technical Description section. Cycling conditions consist of a denaturation/polymerase activation step at 95°C for 15 min, followed by 38 cycles of denaturation at 95°C for 45 sec, annealing at 60°C for 60 sec,
30 extension at 72°C for 90 sec, and a final extension step at 72°C for 10 min.

Amplified DNA is enzymatically fragmented by incubation with DNase I (Promega, WI) and alkaline phosphatase (Roche, Germany) at 37°C for 30 min, followed by enzyme inactivation at 95°C for 10 min.

- 5 Fragmented DNA is labeled by incubation with TdT enzyme (Roche, Germany) and biotin-ddUTP (Perkin-Elmer, CA) at 37°C for 60 min.

10 Labelled DNA is placed on a proprietary microarray (Progenika Biopharma, S.A.). The microarray comprises a modified crystal surface to which allele-specific oligonucleotide probes are covalently attached. Probes are designed to interrogate multiple allelic variant positions in the amplified genomic segments. Each allelic variant is interrogated by 2 probes, for a total of 4 probes per SNP. Each probe is printed 10 times on the microarray, for a total of 40 features (spots) per SNP. Probe sequences are listed in the Technical Description section. The labelled DNA/microarray interface is placed in an incubation chamber of a HS 4800 Pro station (Tecan, Switzerland) and is incubated at 47°C for 30 min and at 45°C for 60 min in buffer containing SSPE, dextran, and deionized formamide to allow for probes to hybridize (bind) to their cognate sequences, when present. Unbound DNA is washed off by incubation at 23°C for various times with buffer containing SSC with or without SDS. A streptavidin-Cy3 conjugate (Invitrogen, CA) diluted in buffer containing PBS and Tween-20 is added to the microarray surface and further incubated at 37°C for 10 min. Unbound conjugate is washed off as before. The microarray is dried by flushing high-pressure liquid nitrogen through the incubation chamber.

Microarray-bound Cy3 fluorescence is detected on InnoScan 710, a confocal scanner (Innopsys, France) and is quantitated by ad hoc software.

25

Proprietary software (Progenika Biopharma, S.A.) is used to transform fluorescence intensity values for the particular allelic variants detected, singly or in combination, into blood group genotypes, and from genotypes into predicted blood group phenotypes.

30 **Technical description**

Amplifications and hybridizations for determination of the three genetic sequences are performed as follows:

- **Amplification of RHD/RHCE hybrid exon 3 by ASP**

5

Oligonucleotide primers that bind to intron 2 and intron 3 sequences in the RHD locus are used. The target sequence of the forward (upstream) primer, located in intron 2, is RHD-specific. The target sequence of the reverse (downstream) primer, located in intron 3, is RHCE-specific. The size of the PCR product is 270 base pairs when the following sequences are used:

- 10 - Forward primer: 5'-CGTCCTGGCTCTCC**CTCTCT**-3' (SEQ ID NO: 5)
 - Reverse primer: 5'-TATTTTTCAAAACCCCGGAAG-3' (SEQ ID NO: 6)

In boldface, RHD-specific nucleotides (forward primer) and RHCE-specific nucleotides (reverse primer).

- 15 It is possible to use different primers provided that the primers enable specific, partial or complete, amplification of the RHD/RHCE hybrid exon 3 that characterizes variants RHD*r's, RHD*DIIIa, RHD*DIVa-2, among others.

- **Hybridization to RHD/RHCE hybrid exon 3 by ASH**

20

Oligonucleotide probes that specifically bind to the non-coding strand of either RHD/RHCE hybrid exon 3 sequences or conventional RHD/RHCE exon 3 sequences are employed. The specificity of these probes hinges upon the allelic form present at a single polymorphic position, usually, but not necessarily, located at the center of the target sequence. One allelic form is
 25 detected by the RHD/RHCE hybrid exon 3-specific probe, the other by the consensus RHD/RHCE exon 3-specific probe. The size of the target sequence is 23 base pairs when the following sequences are used:

- RHD/RHCE hybrid exon 3-specific probe: 5'-ggTcaacttgg**T**gcagttggtgg-3' (SEQ ID NO: 7)

- reference RHD/RHCE-specific probe: 5'-gg^tcaacttgg**C**gcagttggtgg-3' (SEQ ID NO: 8)

5 In boldface, the two allelic forms at the exon 3 polymorphic position.

It is possible to use oligonucleotide probes that differ from the previously described probes in sequence, length, or any other feature, provided that they enable specific, partial or complete, hybridization to the RHD/RHCE hybrid exon 3 that characterizes variants RHD*r's, RHD*DIlla,
10 RHD*DIVa-2.

- **Amplification of a segment ("segment #2") of RHCE/RHD hybrid intron 7 by ASP**

Oligonucleotide primers that bind to intron 7 sequences in the RHD locus are used. The target
15 sequence of the forward (upstream) primer is RHD specific. The target sequence of the reverse (downstream) primer is RHCE specific. The size of the PCR product is 438 base pairs when the following sequences are used:

- Forward primer: 5'-CAAGCT**G**TCAAGGAGAC**AT**CACT**AT**-3' (SEQ ID NO: 9)

- Reverse primer: 5'-CATTACATAGAGATGTCC**CC**CATACT**T**-3' (SEQ ID NO: 10)

20 In boldface, RHD-specific nucleotides (forward primer) and RHCE-specific nucleotides (reverse primer).

It is possible to use oligonucleotide primers that differ from the previously described primers in sequence, length, or any other feature, provided that they enable specific, partial or complete,
25 amplification of the RHCE/RHD hybrid intron 7 that characterizes variants RHD*r's, RHD*DIlla.

- **Hybridization to segment #2 of RHCE/RHD hybrid intron 7 by ASH**

An oligonucleotide probe that specifically binds to the non-coding strand of RHCE intron 7 sequences within the segment amplified by ASP is used. The specificity of this probe hinges upon the allelic forms present at two polymorphic positions, usually but not necessarily located around the center of the target sequence. The probe hybridizes to the target sequence when the appropriate allelic forms are present on it, but does not when replaced by other allelic forms, whether found in the population or not. The size of the target sequence is 27 base pairs when the following sequence is used:

Hybrid RHD/RHCE intron 7-specific probe: 5' -CCTGGTACTCAAACCTCTAAATCTCA-3' (SEQ

ID NO: 11)

It is possible to use an oligonucleotide probe or probes that differ from the previously described probe in sequence, length, or any other feature, as long as such probe or probes enable specific, partial or complete, hybridization to the RHD/RHCE hybrid intron 7 that characterizes variants RHD*r's, RHD*DIIIa, RHD*DIVa-2.

- Amplification of a further segment ("segment #4") of RHD/RHCE hybrid intron 7 by ASP

Oligonucleotide primers that bind to intron 7 sequences in the RHD locus are used. The target sequence of the forward (upstream) primer is RHD specific. The target sequence of the reverse (downstream) primer is RHCE specific. The size of the PCR product is 305 base pairs when the following sequences were used:

- Forward primer: 5' -GCCACTTATTACTTAAAAAAACCCC-3' (SEQ ID NO: 12)

- Reverse primer: 5' -AAGGCCCTTCCTCCAAAATAG-3' (SEQ ID NO: 13)

In boldface, RHD-specific nucleotides (forward primer) and RHCE-specific nucleotides (reverse primer).

It is possible to use oligonucleotide primers that differ from the previously described primers in sequence, length, or any other feature, provided that they enable specific, partial or complete, amplification of the RHD/RHCE hybrid intron 7 that characterizes variants RHD*r's, RHD*DIIIa, RHD*DIVa-2.

- Hybridization to segment #4 of RHD/RHCE hybrid Intron 7 by ASH

Oligonucleotide probes that specifically bind to the non-coding strand of hybrid RHCE/RHD intron 7 sequences within the segment amplified by ASP are used. The specificity of these probes hinges upon the particular allelic form usually but not necessarily located at the central position, which hybridizes to the target sequence when present on the probe, but does not when replaced by another allelic form, whether found in the population or not. The size of the target sequence is either 27 or 25 base pairs, respectively, when the following sequences are used:

- RHD/RHCE hybrid intron 7-specific probe: 5' -AATTT**CATGTGCTG**GAAACTTAATCCT-3' (SEQ ID NO: 14)

- RHD/RHCE hybrid intron 7-specific probe: 5' -AATTT**CATGTGCTG**GAAACTTAATCC-3' (SEQ ID NO: 15)

In boldface, the allelic form at the intron 7 polymorphic position.

It is possible to use oligonucleotide probes that differ from the previously described probes in sequence, length, or any other feature, provided that they enable specific, partial or complete,

hybridization to the RHD/RHCE hybrid intron 7 that characterizes variants RHD*r's, RHD*DIIIa, RHD*DIVa-2.

A total of 252 selected samples known to contain consensus RHD, consensus RHCE, variant RHD or variant RHCE are analyzed as a test of the genotyping method described herein. The results can be shown in Table 2. Expected results correspond to data generated through the analysis of genomic DNA by one or more of the following:

- A genotyping microarray that interrogates 72 polymorphic positions in the *RHD* locus and 8 polymorphic positions in the *RHCE* locus.
- 10- ASP to determine the presence or absence of Hybrid exon 3 (Hyb ex03).
- ASP to determine the presence or absence of Hybrid intron 7 segment #2 ("Hyb in07 sg02").
- ASP to determine the presence or absence of Hybrid intron 7 segment #4 ("Hyb in07 sg04").

Observed results correspond to data generated through the analysis of genomic DNA by a flow-cytometry-based genotyping test that combines ASP and ASH, as described in the Technical

- 15 Description section above, to interrogate the three polymorphisms at Hyb ex03, Hyb in07 sg02, Hyb in07 sg04 that characterize variants *RHD**r's, *RHD**DIIIa, *RHD**DIVa-2.

Table 2 lists samples alphabetically and includes for each one of them the following information:

- *RHD* genotype at both alleles.
- Known Hyb ex03 genotype.
- 20- Reference Method Hyb in07 sg02 genotype.
- Method of the invention Hyb in07 sg02 genotype.
- Reference Method Hyb in07 sg04 genotype.
- Method of the invention Hyb in07 sg04 genotype.
- Reference Method *RHD**r's (r's) call.
- 25- Method of the invention r's call.

Table 2

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03	Hyb in07 sg02		Hyb in07 sg04		RHD ^{r_s}	
					Reference	Method of the invention	Reference	Method of the invention	Reference	Method of the invention
1	A2Y53	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
2	AYU48	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
3	AYU53	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
4	AZJ10	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
5	BAT15	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
6	BBA07	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
7	BC0078	wD13	wD13/RHDdel	Absent	Absent		Absent		No r's	
8	BC0079	DAR	DAR/RHDdel	Absent	Absent		Absent		No r's	
9	BC0080	DAR	DAR/RHDdel	Absent	Absent		Absent		No r's	
10	BC0081	DAR	DAR/RHDdel	Absent	Absent		Absent		No r's	
11	BC0082	DAR	DAR/RHDdel	Absent	Absent		Absent		No r's	
12	BC0083	r's	r's/RHDdel	Present	Absent		Present		r's	
13	BC0084	r's	RHDdel	Present	Absent		Present		r's	
14	BC0085	r's	r's/RHDdel	Present	Absent		Present		r's	
15	BC0086	r's	RHD	Present	Absent		Present		r's	
16	BC0087	r's	r's/RHDdel	Present	Absent		Present		r's	
17	BC0088	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
18	BC0089	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
19	BC0090	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
20	BC0091	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
21	BC0092	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
22	BC0093	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
23	BC0094	DV	DV/RHDdel	Absent	Absent		Absent		No r's	
24	BC0095	DV	DV/RHDdel	Absent	Absent		Absent		No r's	
25	BC0096	DV	DV/RHDdel	Absent	Absent		Absent		No r's	
26	BC0097	DV	DV/RHDdel	Absent	Absent		Absent		No r's	
27	BC0098	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
28	BC0099	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03	Hyb in07 sg02		Hyb in07 sg04		RHD ^{r,s}	
					Reference	Method of the invention	Reference	Method of the invention	Reference	Method of the invention
29	BC0100	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
30	BC0101	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
31	BC0102	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
32	BC0103	DIVa-2	RHD	Present	Absent		Absent		No r's	
33	BC0104	DIIIt4/DIIIt8	RHD	Present	Absent		Absent		No r's	
34	BC0105	DIVa-2	Psi	Present	Absent		Absent		No r's	
35	BC0106	DIVa-2	DIVa-2/RHDdel	Present	Absent		Absent		No r's	
36	BC0107	DIVa-2	RHD	Present	Absent		Absent		No r's	
37	BC0108	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
38	BC0109	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
39	BC0110	RHD Variant	RHDdel	Absent	Absent		Absent		No r's	
40	BC0111	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
41	BC0112	RHD Variant	RHDdel	Absent	Absent		Absent		No r's	
42	BC0113	wD14.0/4.1	RHD	Absent	Absent		Present		No r's	
43	BC0114	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
44	BC0115	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
45	BC0116	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
46	BC0117	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
47	BC0118	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
48	BC0119	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
49	BC0120	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
50	BC0121	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
51	BC0122	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
52	BC0123	DAR	RHD	Absent	Absent		Absent		No r's	
53	BC0124	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
54	BC0125	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
55	BC0126	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
56	BC0127	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
57	BC0128	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03	Hyb in07 sg02		Hyb in07 sg04		RHD r's	
					Reference	Method of the invention	Reference	Method of the invention	Reference	Method of the invention
58	BC0129	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
59	BC0130	DV	DV/RHDdel	Absent	Absent		Absent		No r's	
60	BC0131	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
61	BC0132	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
62	BCGEN446	DIVa-2	DIVa-2/RHDdel	Present	Absent		Absent		No r's	
63	BGG-09-0032	DIIla	DIIla/RHDdel/r's	Present	Present		Present			
64	BGG-09-0084	r's	RHD	Present	Absent		Present		r's	
65	BGG-09-0216	r's	RHD	Present	Absent		Present		r's	
66	BGG-09-0275	DIIla	RHD Variant	Present	Present		Present		No r's	
67	BGG-09-0281	DIIla	RHD	Present	Present		Present		No r's	
68	BGG-09-0287	DIIla	DAR	Present	Present		Present		No r's	
69	BGG-09-0300	DIIla	RHD	Present	Present		Present		No r's	
70	BGG-09-0333	DIIla/r's	wD14.0	Present	Present		Present			
71	BGG-10-0041	DIIla	Psi	Present	Present		Present		No r's	
72	BGG-10-0052	DIVa-2	RHD	Present	Absent		Present		No r's	
73	BGG-10-0056	r's	RHD	Present	Absent		Present		r's	
74	BGG-10-0074	DIIla	RHD	Present	Present		Present		No r's	
75	BGG-10-0075	r's	RHD	Present	Absent		Present		r's	
76	BGG-10-0085	r's	r's/RHDdel	Present	Absent		Present		r's	
77	BGG-10-0097	r's	RHD	Present	Absent		Present		r's	
78	BGG-10-0107	DIIla	RHD	Present	Present		Present		No r's	
79	BGG-10-0118	r's	RHD	Present	Absent		Present		r's	
80	BGG-10-0177	r's	r's/RHDdel	Present	Absent		Present		r's	
81	BGG-10-0187	DIIla	DIIla/RHDdel/r's	Present	Present		Present			
82	BGG-10-0210	r's	RHD	Present	Absent		Present		r's	
83	BGG-10-0280	r's	RHD	Present	Absent		Present		r's	
84	BGG-10-0281	DIIla	DIIla/RHDdel	Present	Present		Present		No r's	
85	BGG-10-0284	r's	RHD Variant	Present	Absent		Present		r's	
86	BGG-10-0367	r's	wD12/2.1	Present	Absent		Present		r's	

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03	Hyb in07 sg02		Hyb in07 sg04		RHD r ^s	
					Reference	Method of the invention	Reference	Method of the invention	Reference	Method of the invention
87	BGG-10-0371	r's	RHD	Present	Absent		Present		r's	
88	BGG-10-0373	r's	RHD	Present	Absent		Present		r's	
89	BGG-10-0376	r's	RHD	Present	Absent		Present		r's	
90	BGG-10-0380	r's	RHD	Present	Absent		Present		r's	
91	BGG-10-0389	r's	RHD	Present	Absent		Present		r's	
92	BGG-10-0396	r's	RHD	Present	Absent		Present		r's	
93	BGG-10-0420	DIIa	RHD	Present	Present		Present		No r's	
94	BGG-10-0425	DIIa	RHD	Present	Present		Present		No r's	
95	BGG-10-0428	r's	RHD	Present	Absent		Present		r's	
96	BGG-10-0443	r's	r's/RHDdel	Present	Absent		Present		r's	
97	BGG-10-0476	r's	RHD	Present	Absent		Present		r's	
98	BGG-10-0481	DIIa	Psi	Present	Present		Present		No r's	
99	BGG-10-0512	DIIa	RHD	Present	Present		Present		No r's	
100	BGG-10-0523	DIIa	RHD	Present	Present		Present		No r's	
101	BGG-10-0543	DIIa	RHD	Present	Present		Present		No r's	
102	BGG-10-0575	r's	RHD	Present	Absent		Present		r's	
103	BGG-10-0579	DIIa	RHD	Present	Present		Present		No r's	
104	BGG-10-0590	DIIa	RHD	Present	Present		Present		No r's	
105	BGG-10-0598	r's	RHD	Present	Absent		Present		r's	
106	BGG-10-0628	DIIa	RHD	Present	Present		Present		No r's	
107	BGG-10-0635	r's	RHD	Present	Absent		Present		r's	
108	BGG-10-0638	DIVa-2	RHD	Present	Present		Absent		No r's	
109	BGG-10-0642	DIIa	RHD	Present	Present		Present		No r's	
110	BGG-10-0654	r's	RHD	Present	Absent		Present		r's	
111	BGG-10-0656	r's	r's/RHDdel	Present	Absent		Present		r's	
112	BGG-10-0669	DIIa	Psi	Present	Present		Present		No r's	
113	BGG-10-0715	r's	r's/RHDdel	Present	Absent		Present		r's	
114	BGG-10-0717	DIIa	RHD	Present	Present		Present		No r's	
115	BGG-10-0723	DIVa-2	RHD	Present	Present		Absent		No r's	

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03	Hyb in07 sg02		Hyb in07 sg04		RHD ^{r,s}	
					Reference	Method of the invention	Reference	Method of the invention	Reference	Method of the invention
116	BGG-10-0735	DIIa	RHD	Present	Present		Present		No r's	
117	BGG-10-0752	r's	RHD	Present	Absent		Present		r's	
118	BGG-10-0770	DIIa	RHD	Present	Present		Present		No r's	
119	BGG-10-0773	r's	RHD	Present	Absent		Present		r's	
120	BGG-10-0790	r's	Psi	Present	Absent		Present		r's	
121	BGG-10-0842	DIIa	RHD	Present	Present		Present		No r's	
122	BGG-10-0847	r's	r's/RHDdel	Present	Absent		Present		r's	
123	BGG-10-0849	r's	DVt1/DAU5	Present	Absent		Present		r's	
124	BGG-10-0853	r's	r's/RHDdel	Present	Absent		Present		r's	
125	BGG-10-0867	r's	RHD	Present	Absent		Present		r's	
126	BGG-10-0876	DIVa-2	RHD	Present	Absent		Absent		No r's	
127	BGG-10-0900	r's	r's/RHDdel	Present	Absent		Present		r's	
128	BGG-10-0933	r's	RHD	Present	Absent		Present		r's	
129	BGG-10-0942	DIIa	RHD	Present	Present		Present		No r's	
130	BGG-10-0972	r's	RHD	Present	Absent		Present		r's	
131	BGG-10-1233	DIIa	RHD	Present	Present		Present		No r's	
132	BGG-10-1374	r's	r's/RHDdel	Present	Absent		Present		r's	
133	BGG-10-1379	DIIa	DIIa/RHDdel	Present	Present		Present		No r's	
134	BGG-10-1391	r's	RHD	Present	Absent		Present		r's	
135	BGG-10-1413	DIIa	RHD	Present	Present		Present		No r's	
136	BGG-10-1423	r's	RHD	Present	Absent		Present		r's	
137	BGG-10-1455	r's	r's/RHDdel	Present	Absent		Present		r's	
138	BGG-10-1458	DIIa	RHD	Present	Present		Present		No r's	
139	BGG-10-1468	DIIa	RHD	Present	Present		Present		No r's	
140	BGG-10-1500	r's	RHD	Present	Absent		Present		r's	
141	BGG-10-1532	r's	r's/RHDdel	Present	Absent		Present		r's	
142	BGG-10-1574	DIIa	RHD	Present	Present		Present		No r's	
143	BGG-10-1577	r's	r's/RHDdel	Present	Absent		Present		r's	
144	BGG-10-1588	r's	r's/RHDdel	Present	Absent		Present		r's	

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03	Hyb in07 sg02		Hyb in07 sg04		RHD r ^s	
					Reference	Method of the invention	Reference	Method of the invention	Reference	Method of the invention
145	BGG-10-1591	r's	RHD	Present	Absent		Present		r's	
146	BGG-10-1599	r's	RHD Variant	Present	Absent		Present		r's	
147	BGG-10-1621	DIIa	DIIa/RHDdel	Present	Present		Present		No r's	
148	BGG-10-1628	DIIa	DIIa/RHDdel	Present	Present		Present		No r's	
149	BGG-10-1634	DIIa	RHD	Present	Present		Present		No r's	
150	BGG-10-1643	r's	RHD	Present	Absent		Present		r's	
151	BGG-10-1649	r's	r's/RHDdel	Present	Absent		Present		r's	
152	BGG-10-1653	r's	RHD	Present	Absent		Present		r's	
153	BGG-10-1658	DIIa	RHD	Present	Present		Present		No r's	
154	BGG-10-1661	r's	r's/RHDdel	Present	Absent		Present		r's	
155	BGG-10-1683	DIIa	RHD	Present	Present		Present		No r's	
156	BGG-10-2038	r's	RHD	Present	Absent		Present		r's	
157	BGG-10-2142	DIIa	DIIa/RHDdel	Present	Present		Present		No r's	
158	BGG-10-2144	DIVa-2	RHD	Present	Absent		Absent		No r's	
159	BGG-10-2153	r's	RHD	Present	Absent		Present		r's	
160	BGG-10-2155	r's	DVt1/DAU5	Present	Absent		Present		r's	
161	BGG-10-2212	r's	RHD	Present	Absent		Present		r's	
162	BGG-10-2215	DIIa/r's	Psi	Present	Present		Present		No r's	
163	BGG-10-2270	DIIa	RHD Variant	Present	Present		Present		No r's	
164	BGG-10-2335	r's	r's/RHDdel	Present	Absent		Present		r's	
165	BGG-10-2347	r's	RHD	Present	Absent		Present		r's	
166	BGG-10-2366	r's	RHD	Present	Absent		Present		r's	
167	BGG-10-2379	DIIa	RHD	Present	Present		Present		No r's	
168	BGG-10-2391	DIIa	RHD	Present	Present		Present		No r's	
169	BGG-10-2433	DIIa	RHD	Present	Present		Present		No r's	
170	BGG-10-2435	DIIa	RHD	Present	Present		Present		No r's	
171	BGG-10-2456	DIIa	RHD	Present	Present		Present		No r's	
172	BGG-10-2470	DIIa	RHD	Present	Present		Present		No r's	
173	BGG-10-3386	r's	r's/RHDdel	Present	Absent		Present		r's	

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03	Hyb in07 sg02		Hyb in07 sg04		RHD r ^s	
					Reference	Method of the invention	Reference	Method of the invention	Reference	Method of the invention
174	BGG-10-3387	r's	RHD	Present	Absent		Present		r's	
175	BGG-10-3400	r's	RHD	Present	Absent		Present		r's	
176	BGG-10-3409	D11a	RHD	Present	Present		Present		No r's	
177	BGG-10-3417	r's	RHD	Present	Absent		Present		r's	
178	BGG-10-3426	r's	RHD	Present	Absent		Present		r's	
179	BGG-10-3427	r's	r's/RHDdel	Present	Absent		Present		r's	
180	BGG-10-3461	r's	r's/RHDdel	Present	Absent		Present		r's	
181	BGG-10-3486	r's	RHD	Present	Absent		Present		r's	
182	BGG-10-3529	RHD	RHD/RHDdel	Present	Absent		Present		No r's	
183	BGG-10-3539	r's	r's/RHDdel	Present	Absent		Present		r's	
184	BGG-10-3545	r's	RHD	Present	Absent		Present		r's	
185	BGG-10-3546	D11a	RHD	Present	Present		Present		No r's	
186	BGG-10-3561	r's	r's/RHDdel	Present	Absent		Present		r's	
187	BGG-10-3714	D1Va-2	D1Va-2/RHDdel/r's	Present	Present		Absent			
188	BGG-10-3809	r's	RHD	Present	Absent		Present		r's	
189	BGG-10-4060	r's	r's/RHDdel	Present	Absent		Present		r's	
190	BGG-10-4080	D11a	RHD	Present	Present		Present		No r's	
191	BGG-10-5191	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
192	BGG-10-5434									
193	D114	DVI	DVI/RHDdel	Absent	Absent		Absent		No r's	
194	D122	DFR	DFR/RHDdel	Absent	Absent		Absent		No r's	
195	D123	D1Va-2	D1Va-2/RHDdel	Present	Absent		Absent		No r's	
196	D126	r's	DVt1/DAU5	Present	Absent		Present		r's	
197	D129	Psi	Psi/RHDdel	Absent	Absent		Absent		No r's	
198	D131	DOL	DOL/RHDdel	Absent	Absent		Absent		No r's	
199	D133	DAU0	DAU0/RHDdel	Absent	Absent		Absent		No r's	
200	D138	RHD Variant	RHDdel	Absent	Absent		Absent		No r's	
201	D140	wDt5	wDt5/RHDdel	Absent	Absent		Absent		No r's	
202	D159	RHD Variant	RHDdel	Absent	Absent		Absent		No r's	

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03	Hyb in07 sg02		Hyb in07 sg04		RHD r ^s	
					Reference	Method of the invention	Reference	Method of the invention	Reference	Method of the invention
203	D161	RHD Variant	RHDdel	Absent	Absent		Absent		No r's	
204	D171	r's	DAR	Present	Absent		Present		r's	
205	D190	r's	DAR	Present	Absent		Present		r's	
206	D193	DIVa-2	DIVa-2/RHDdel	Present	Absent		Absent		No r's	
207	D195	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
208	D196	DIVa-2	DIVa-2/RHDdel	Present	Absent		Absent		No r's	
209	D202	RHD	RHDdel	Absent	Absent		Absent		No r's	
210	D204	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
211	D213	r's	r's/RHDdel	Present	Absent		Present		r's	
212	D214	wD115	wD115/RHDdel	Absent	Absent		Absent		No r's	
213	D221	wD14.0/4.1	wD14.0/4.1/RHDdel	Absent	Absent		Present		No r's	
214	D225	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
215	D226	DAU5	DAU5/RHDdel	Absent	Absent		Absent		No r's	
216	D227	DAU5	DAU5/RHDdel	Absent	Absent		Absent		No r's	
217	D229	DAU5	DAU5/RHDdel	Absent	Absent		Absent		No r's	
218	D230	wD14.0/4.1	wD14.0/4.1/RHDdel	Absent	Absent		Present		No r's	
219	D231	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
220	D237	RHD Variant	RHDdel	Absent	Absent		Absent		No r's	
221	D239	DAU5	DAU5/RHDdel	Absent	Absent		Absent		No r's	
222	D245	wD111	wD111/RHDdel	Absent	Absent		Absent		No r's	
223	D249	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
224	D261	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
225	D262	wD15	wD15/RHDdel	Absent	Absent		Absent		No r's	
226	D288	DMH	DMH/RHDdel	Absent	Absent		Absent		No r's	
227	D289	RHD Variant	RHDdel	Absent	Absent		Absent		No r's	
228	D290	wD11	wD11/RHDdel	Absent	Absent		Absent		No r's	
229	D291	wD12	wD12/RHDdel	Absent	Absent		Absent		No r's	
230	D298	r's	DAR	Present	Absent		Present		r's	
231	D300	RHD Variant	RHDdel	Absent	Absent		Absent		No r's	

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03	Hyb in07 sg02		Hyb in07 sg04		RHD r ^s	
					Reference	Method of the invention	Reference	Method of the invention	Reference	Method of the invention
232	D323	DVI	DVI/RHDdel	Absent	Absent		Absent		No r's	
233	D328	wD14.0/4.1	wD14.0/4.1/RHDdel	Absent	Absent		Present		No r's	
234	D329	wD14.0/4.1	wD14.0/4.1/RHDdel	Absent	Absent		Present		No r's	
235	D330	DAU5	DAU5/RHDdel	Absent	Absent		Absent		No r's	
236	D331	wD14.0/4.1	wD14.0/4.1/RHDdel	Absent	Absent		Present		No r's	
237	GAL 10701360	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
238	GAL 10701364	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
239	GAL 10701365	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
240	GAL 10701366	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
241	GAL 10706636	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
242	GAL 10752569	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
243	GAL 10816074	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
244	GAL 10833160	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
245	GKO 10532283	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
246	GKO 32588	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
247	JCX33	r's	RHD	Present	Absent		Present		r's	
248	L22	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
249	L23	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
250	L28	RHD	RHD/RHDdel	Absent	Absent		Absent		No r's	
251	PGK53	RHDdel	RHDdel	Absent	Absent		Absent		No r's	
252	ZGZ 51979	r's	r's/RHDdel	Present	Absent		Present		r's	
253	ZGZ 52739	DIIa	RHD	Present	Present		Present		No r's	

The above results can be summarized in Table 3.

Table 3

	DIIIa	r's	DIVa-2
Total	49	82	14
Called	33	58	12
OK	23	54	12
FN	10	4	0
FN [sq02+ sq04+]			0
FN [sq02+ sq04-]		0	
FN [sq02- sq04-]	1		
FN [sq02+]		2	0
FN [sq04+]			0
FN [sq02-]	7		
FN [sq04-]	2	2	
Universe	96	63	131
Called	70	45	91
FP	3	8	5

5

Key to Table 3

Shaded cells: does not apply

FN: false negative

FP: false positive

10 Universe: non-DIIIa, non-r's, non-DIVa-2

Called: calls made

Example 2

Discrimination among genetic variants that share a RHD/RHCE hybrid exon 3 but encode
 15 different forms of RhD Ag (Partial D Ag vs. No D Ag) and C Ag (Normal C Ag vs.
 Altered/Weakened C Ag, sometimes abbreviated as C^W)

The following example relates to a method of discriminating among *RHD/RHCE* hybrid exon 3
 variants *RHD*r's*, *RHD*DIIIa* and *RHD*DIVa-2*. The method is based on the interrogation of the
 20 nucleotide composition of the genomic DNA of a sample at three discrete and separate
 locations in the RHD locus by means of a molecular technique known in the art as Allele-
 Specific Polymerase Chain Reaction (ASP). Amplification of a DNA segment at one location
 enables determination of the presence of RHD/RHCE hybrid exon 3 in the test sample.
 Amplification of a DNA segment at another location enables determination of the presence of
 25 variants *RHD*r's* or *RHD*DIIIa* and absence of variant *RHD*DIVa-2*. Amplification of a DNA

segment at yet another location enables determination of the presence of variant *RHD*DIlla* and absence of variant *RHD*r's*.

The method outlined above and described in further detail below has been applied to 252
5 samples selected to contain in the *RHD* gene either no variant allele (i.e. two conventional alleles), one variant allele (and one conventional allele), or two variant alleles (i.e. no conventional allele). The samples were also selected to include *RHD* reference and *RHD* variant alleles encoding the major RhD phenotypes, namely RhD⁺, Partial D, Weak D, RhD⁻. In order to generate the reference dataset, presence vs. absence of variant allele(s) was
10 determined for each sample by standard DNA sequencing and by genotyping, the latter following methods other than the method described herein. Polymorphic positions interrogated by the reference genotyping methods include positions shared with the present method as well as positions not shared with the present method.

15 **Materials & Methods**

According to the present example, genomic DNA was extracted from nucleated cells in a blood sample by cell lysis. Extracted DNA was purified on an affinity column. Both, cell lysis and DNA purification were performed with a QIAamp Blood kit (Qiagen, Germany) by following manufacturer protocols and recommendations. Purity of DNA was determined by
20 spectrophotometry on a Nanodrop instrument (Nanodrop, DE). Only DNA solutions with an $OD_{260}/OD_{280} = 1.8 \pm 0.2$ were used for subsequent analysis.

Purified DNA was used as a template for multiplexed Polymerase Chain Reaction (PCR) amplification of the gene segments of interest in a GeneAmp 9700 thermal cycler (Perkin-Elmer,
25 CA). Primer sequences for the different segments are listed in the Technical Description section. Cycling conditions consisted of a polymerase activation step at 95°C for 15 min, followed by 38 cycles of denaturation at 95°C for 45 sec, annealing at 60°C for 60 sec, extension at 72°C for 90 sec, and a final extension step at 72°C for 10 min.

30 Amplified DNA was separated by electrophoresis on a 2% agarose gel, stained with SYBR Safe dye (Invitrogen, OR), and photographed under UV illumination. Positive and negative control

template DNA for each of the three DNA segments was included in every ASP assay. Amplification vs. No Amplification of a segment was determined visually by a trained laboratory technician.

5 Technical description

According to the present example, amplification of each of the three DNA segments was performed as follows:

10 - Amplification of RHD/RHCE hybrid exon 3 by ASP.

This step can make use of oligonucleotide primers that bind to intron 2 and intron 3 sequences in the *RHD* locus. The target sequence of the forward (upstream) primer, located in intron 2, is RHD specific. The target sequence of the reverse (downstream) primer, located in intron 3, is RHCE specific. The size of the PCR product was 270 base pairs when the following sequences were used:

- Forward primer: 5'-**CGTCCTGGCTCTCCCTCTCT**-3' (SEQ ID NO: 5)

- Reverse primer: 5'-TATTTTTCAAAACCCCGGAAG-3' (SEQ ID NO: 6)

In boldface, RHD-specific nucleotides (forward primer) and RHCE-specific nucleotides (reverse primer). Specifically, the polymorphic positions exploited by the oligonucleotide primers used in this ASP are IVS2-26, IVS2-13, IVS2-8 (forward primer) in RHD intron 2 and polymorphic positions IVS3+64, IVS3+69 (reverse primer) in RHD intron 3.

It is possible to use different primers provided that the primers enable specific, partial or complete, amplification of the *RHD/RHCE* hybrid exon 3 that characterizes variants *RHD*r's*, *RHD*DIIIa*, *RHD*DIVa-2*, among others.

- Amplification of a segment (segment #2) of *RHCE/RHD* hybrid intron 7 by ASP.

Oligonucleotide primers that bind to intron 7 sequences in the RHD locus were used. The target sequence of the forward (upstream) primer is RHD specific. The target sequence of the reverse (downstream) primer is RHCE specific. The size of the PCR product was 438 base pairs when the following sequences were used:

5

:

- Forward primer: 5'-CAAGCT**GT**CAAGGAGAC**AT**CACT**AT**-3' (SEQ ID NO: 9)
- Reverse primer: 5'-CATTACATAGAGATGT**CCCC**CATACT-3' (SEQ ID NO: 10)

In boldface, RHD-specific nucleotides (forward primer) and RHCE-specific nucleotides (reverse primer). Specifically, the polymorphic positions exploited by the oligonucleotide primers used in this ASP are IVS7+1869, IVS7+1880, IVS7+1886, IVS7+1887 (forward primer) and IVS7+2276, IVS7+2282 (reverse primer).

It is possible to use oligonucleotide primers that differ from the previously described primers in sequence, length, or any other feature, provided that they enable specific, partial or complete, amplification of the RHCE/RHD hybrid intron 7 that characterizes variants RHD*r's, RHD*DIIIa.

- Amplification of another segment (segment #4) of *RHD/RHCE* hybrid intron 7 by ASP.

Oligonucleotide primers that bind to intron 7 sequences in the *RHD* locus were used. The target sequence of the forward (upstream) primer is RHD specific. The target sequence of the reverse (downstream) primer is RHCE specific. The size of the PCR product was 305 base pairs when the following sequences were used:

- Forward primer: 5'-GCCACTTATTACTTAAAAA**ACCCC**-3' (SEQ ID NO: 12)
- Reverse primer: 5'-AAGGCCCTTCCTCAA**ATAG**-3' (SEQ ID NO: 13)

In boldface, RHD-specific nucleotides (forward primer) and RHCE-specific nucleotides (reverse primer). Specifically, the polymorphic positions exploited by the oligonucleotide primers used in this ASP are IVS7+3349 (forward primer) and IVS7+4105, IVS7+4106, IVS7+4107, IVS7+4108, IVS7+4127 (reverse primer).

It is possible to use oligonucleotide primers that differ from the previously described primers in sequence, length, or any other feature, provided that they enable specific, partial or complete, amplification of the RHD/RHCE hybrid intron 7 that characterizes variants RHD*r's, RHD*DIIIa, RHD*DIVa-2.

A total of 252 selected samples known to contain conventional *RHD*, conventional *RHCE*, variant *RHD* alleles or variant *RHCE* alleles were analyzed as a test of the genotyping method described herein. The results are shown in Table 4.

Reference results (Reference) correspond to data generated through the analysis of genomic DNA by one or more of the following:

- A genotyping microarray that interrogates 72 polymorphic positions in the *RHD* locus and 8 polymorphic positions in the *RHCE* locus.
- A microsphere- and flow cytometry-based genotyping test that interrogates 1 polymorphic position in the *RHD* locus and 7 polymorphic positions in the *RHCE* locus.
- DNA sequencing by the standard Sanger dideoxy method

Method results (Method) correspond to data generated by the method described herein. Specifically, this dataset was obtained from the analysis of genomic DNA by ASP and agarose gel electrophoresis, as described in the Technical Description section of this document.

Table 4 lists samples alphabetically and includes for each one of them the following:

- *RHD* genotype at both alleles.
- Reference *Hyb ex03* genotype.
- Method *Hyb ex03* genotype.
- Reference *Hyb in07 sg02* genotype.
- Method *Hyb in07 sg02* genotype.
- Reference *Hyb in07 sg04* genotype.
- Method *Hyb in07 sg04* genotype.
- Reference *RHD*r's* Presence/Absence call.
- Method *RHD*r's* Presence/Absence call.

RHD: conventional RHD

RHDdel: RHD deletion variant

*RHD** has been omitted from the rest of RHD variants for simplicity

Table 4

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03		Hyb in07 sg02		Hyb in07 sg04		RHD-r's call	
				Reference	Method	Reference	Method	Reference	Method	Reference	Method
1	A2Y53	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
2	AYU48	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
3	AYU53	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
4	AZJ10	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
5	BAT15	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
6	BBA07	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
7	BC0078	wD13	wD13/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
8	BC0079	DAR	DAR/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
9	BC0080	DAR	DAR/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
10	BC0081	DAR	DAR/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
11	BC0082	DAR	DAR/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
12	BC0083	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
13	BC0084	r's	RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
14	BC0085	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
15	BC0086	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
16	BC0087	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
17	BC0088	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
18	BC0089	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
19	BC0090	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
20	BC0091	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
21	BC0092	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
22	BC0093	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
23	BC0094	DV	DV/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
24	BC0095	DV	DV/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
25	BC0096	DV	DV/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
26	BC0097	DV	DV/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
27	BC0098	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
28	BC0099	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
29	BC0100	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03		Hyb in07 sg02		Hyb in07 sg04		RHD'r's call	
				Reference	Method	Reference	Method	Reference	Method	Reference	Method
30	BC0101	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
31	BC0102	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
32	BC0103	DIVa-2	RHD	Present	Present	Absent	Absent	Absent	Absent	No r's	No r's
33	BC0104	DIII4/DIII8	RHD	Present	Present	Absent	Absent	Absent	Absent	No r's	No r's
34	BC0105	DIVa-2	Psi	Present	Present	Absent	Absent	Present	Present	r's	r's
35	BC0106	DIVa-2	DIVa-2/RHDdel	Present	Present	Absent	Absent	Absent	Absent	No r's	No r's
36	BC0107	DIVa-2	RHD	Present	Present	Absent	Absent	Absent	Absent	No r's	No r's
37	BC0108	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
38	BC0109	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
39	BC0110	RHD Variant	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
40	BC0111	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
41	BC0112	RHD Variant	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
42	BC0113	wD4.0/4.1	RHD	Absent	Absent	Absent	Absent	Present	Present	No r's	No r's
43	BC0114	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
44	BC0115	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
45	BC0116	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
46	BC0117	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
47	BC0118	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
48	BC0119	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
49	BC0120	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
50	BC0121	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
51	BC0122	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
52	BC0123	DAR	RHD	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
53	BC0124	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
54	BC0125	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
55	BC0126	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
56	BC0127	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
57	BC0128	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
58	BC0129	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
59	BC0130	DV	DV/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03		Hyb in07 sg02		Hyb in07 sg04		RHD r's call	
				Reference	Method	Reference	Method	Reference	Method	Reference	Method
60	BC0131	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
61	BC0132	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
62	BCGEN446	DIVa-2	DIVa-2/RHDdel	Present	Present	Absent	Absent	Absent	Absent	No r's	No r's
63	BGG-09-0032	DIIla	DIIla/RHDdel/r's	Present	Present	Present	Present	Present	Present	No r's	No r's
64	BGG-09-0084	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
65	BGG-09-0216	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
66	BGG-09-0275	DIIla	RHD Variant	Present	Present	Present	Absent	Present	Present	No r's	r's
67	BGG-09-0281	DIIla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
68	BGG-09-0287	DIIla	DAR	Present	Present	Present	Absent	Present	Present	No r's	r's
69	BGG-09-0300	DIIla	RHD	Present	Present	Present	Absent	Present	Present	No r's	r's
70	BGG-10-0041	DIIla	Psi	Present	Present	Present	Absent	Present	Present	No r's	r's
71	BGG-10-0052	DIVa-2	RHD	Present	Present	Absent	Absent	Present	Present	No r's	No r's
72	BGG-10-0056	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
73	BGG-10-0074	DIIla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
74	BGG-10-0075	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
75	BGG-10-0085	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
76	BGG-10-0097	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
77	BGG-10-0107	DIIla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
78	BGG-10-0118	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
79	BGG-10-0177	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
80	BGG-10-0187	DIIla	DIIla/RHDdel/r's	Present	Present	Present	Present	Present	Present		
81	BGG-10-0210	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
82	BGG-10-0280	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
83	BGG-10-0281	DIIla	DIIla/RHDdel	Present	Present	Present	Present	Present	Present	No r's	No r's
84	BGG-10-0284	r's	RHD Variant	Present	Present	Absent	Absent	Present	Present	r's	r's
85	BGG-10-0367	r's	wDf2/2.1	Present	Present	Absent	Absent	Present	Present	r's	r's
86	BGG-10-0371	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
87	BGG-10-0373	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
88	BGG-10-0376	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
89	BGG-10-0380	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03		Hyb in07 sg02		Hyb in07 sg04		RHD'r's call	
				Reference	Method	Reference	Method	Reference	Method	Reference	Method
90	BGG-10-0389	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
91	BGG-10-0396	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
92	BGG-10-0420	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
93	BGG-10-0425	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
94	BGG-10-0428	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
95	BGG-10-0443	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
96	BGG-10-0476	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
97	BGG-10-0481	Dlla	Psi	Present	Present	Present	Present	Present	Present	No r's	No r's
98	BGG-10-0512	Dlla	RHD	Present	Present	Present	Absent	Present	Present	No r's	r's
99	BGG-10-0523	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
100	BGG-10-0543	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
101	BGG-10-0575	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
102	BGG-10-0579	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
103	BGG-10-0590	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
104	BGG-10-0598	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
105	BGG-10-0628	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
106	BGG-10-0635	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
107	BGG-10-0638	DIVa-2	RHD	Present	Present	Present	Present	Absent	Absent	No r's	No r's
108	BGG-10-0642	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
109	BGG-10-0654	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
110	BGG-10-0656	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
111	BGG-10-0669	Dlla	Psi	Present	Present	Present	Present	Present	Present	No r's	No r's
112	BGG-10-0715	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
113	BGG-10-0717	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
114	BGG-10-0723	DIVa-2	RHD	Present	Present	Present	Present	Absent	Absent	No r's	No r's
115	BGG-10-0735	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
116	BGG-10-0752	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
117	BGG-10-0770	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
118	BGG-10-0773	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
119	BGG-10-0790	r's	Psi	Present	Present	Absent	Absent	Present	Present	r's	r's

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03		Hyb in07 sg02		Hyb in07 sg04		RHD r's call	
				Reference	Method	Reference	Method	Reference	Method	Reference	Method
120	BGG-10-0842	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
121	BGG-10-0847	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
122	BGG-10-0849	r's	DV11/DAU5	Present	Present	Absent	Absent	Present	Present	r's	r's
123	BGG-10-0853	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
124	BGG-10-0867	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
125	BGG-10-0876	DIVa-2	RHD	Present	Present	Absent	Absent	Absent	Absent	No r's	No r's
126	BGG-10-0900	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
127	BGG-10-0933	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
128	BGG-10-0942	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
129	BGG-10-0972	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
130	BGG-10-1233	Dlla	RHD	Present	Present	Present	Absent	Present	Present	No r's	r's
131	BGG-10-1374	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
132	BGG-10-1379	Dlla	Dlla/RHDdel	Present	Present	Present	Present	Present	Present	No r's	No r's
133	BGG-10-1391	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
134	BGG-10-1413	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
135	BGG-10-1423	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
136	BGG-10-1455	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
137	BGG-10-1458	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
138	BGG-10-1468	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
139	BGG-10-1500	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
140	BGG-10-1532	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
141	BGG-10-1574	Dlla	RHD	Present	Present	Present	Absent	Present	Present	No r's	r's
142	BGG-10-1577	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
143	BGG-10-1588	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
144	BGG-10-1591	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
145	BGG-10-1599	r's	RHD Variant	Present	Present	Absent	Absent	Present	Present	r's	r's
146	BGG-10-1621	Dlla	Dlla/RHDdel	Present	Present	Present	Present	Present	Present	No r's	No r's
147	BGG-10-1628	Dlla	Dlla/RHDdel	Present	Present	Present	Present	Present	Present	No r's	No r's
148	BGG-10-1634	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
149	BGG-10-1643	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03		Hyb in07 sg02		Hyb in07 sg04		RHD r's call	
				Reference	Method	Reference	Method	Reference	Method	Reference	Method
150	BGG-10-1649	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
151	BGG-10-1653	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
152	BGG-10-1658	Dlla	RHD	Present	Present	Present	Absent	Present	Present	No r's	r's
153	BGG-10-1661	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
154	BGG-10-1683	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
155	BGG-10-2038	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
156	BGG-10-2142	Dlla	Dlla/RHDdel	Present	Present	Present	Present	Present	Present	No r's	No r's
157	BGG-10-2144	DIVa-2	RHD	Present	Present	Absent	Absent	Absent	Absent	No r's	No r's
158	BGG-10-2153	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
159	BGG-10-2155	r's	DVt1/DAU5	Present	Present	Absent	Absent	Present	Present	r's	r's
160	BGG-10-2212	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
161	BGG-10-2215	Dlla	Psi	Present	Present	Present	Present	Present	Present	No r's	No r's
162	BGG-10-2270	Dlla	RHD Variant	Present	Present	Present	Present	Present	Present	No r's	No r's
163	BGG-10-2335	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
164	BGG-10-2347	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
165	BGG-10-2366	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
166	BGG-10-2379	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
167	BGG-10-2391	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
168	BGG-10-2433	Dlla	RHD	Present	Present	Present	Absent	Present	Present	No r's	r's
169	BGG-10-2435	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
170	BGG-10-2456	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
171	BGG-10-2470	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
172	BGG-10-3386	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
173	BGG-10-3387	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
174	BGG-10-3400	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
175	BGG-10-3409	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
176	BGG-10-3417	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
177	BGG-10-3426	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
178	BGG-10-3427	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
179	BGG-10-3461	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03		Hyb in07 sg02		Hyb in07 sg04		RHD r's call	
				Reference	Method	Reference	Method	Reference	Method	Reference	Method
180	BGG-10-3486	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
181	BGG-10-3529	RHD	RHD/RHDdel	Present	Present	Absent	Absent	Present	Present	No r's	No r's
182	BGG-10-3539	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
183	BGG-10-3545	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
184	BGG-10-3546	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's
185	BGG-10-3561	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
186	BGG-10-3714	DIVa-2	DIVa-2/RHDdel	Present	Present	Present	Present	Absent	Absent	No r's	No r's
187	BGG-10-3809	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
188	BGG-10-4060	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
189	BGG-10-4080	Dlla	RHD	Present	Present	Present	Present	Present	Present	No r's	r's
190	BGG-10-5191	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
191	BGG-10-5434	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
192	D114	DVI	DVI/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
193	D122	DFR	DFR/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
194	D123	DIVa-2	DIVa-2/RHDdel	Present	Present	Absent	Absent	Absent	Absent	No r's	No r's
195	D126	r's	DV11/DAU5	Present	Present	Absent	Absent	Present	Present	r's	r's
196	D129	Psi	Psi/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
197	D131	DOL	DOL/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
198	D133	DAU0	DAU0/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
199	D138	RHD Variant	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
200	D140	wDt5	wDt5/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
201	D159	RHD Variant	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
202	D161	RHD Variant	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
203	D171	r's	DAR	Present	Present	Absent	Absent	Present	Present	r's	r's
204	D190	r's	DAR	Present	Present	Absent	Absent	Present	Present	r's	r's
205	D193	DIVa-2	DIVa-2/RHDdel	Present	Present	Absent	Absent	Absent	Absent	No r's	No r's
206	D195	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
207	D196	DIVa-2	DIVa-2/RHDdel	Present	Present	Absent	Absent	Absent	Absent	No r's	No r's
208	D202	RHD	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
209	D204	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03		Hyb in07 sg02		Hyb in07 sg04		RHD r's call	
				Reference	Method	Reference	Method	Reference	Method	Reference	Method
210	D213	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
211	D214	wD115	wD115/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
212	D221	wD14.0/4.1	wD14.0/4.1/RHDdel	Absent	Absent	Absent	Absent	Present	Present	No r's	No r's
213	D225	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
214	D226	DAU5	DAU5/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
215	D227	DAU5	DAU5/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
216	D229	DAU5	DAU5/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
217	D230	wD14.0/4.1	wD14.0/4.1/RHDdel	Absent	Absent	Absent	Absent	Present	Present	No r's	No r's
218	D231	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
219	D237	RHD Variant	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
220	D239	DAU5	DAU5/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
221	D245	wD111	wD111/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
222	D249	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
223	D261	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
224	D262	wD15	wD15/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
225	D288	DMH	DMH/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
226	D289	RHD Variant	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
227	D290	wD11	wD11/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
228	D291	wD12	wD12/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
229	D298	r's	DAR	Present	Present	Absent	Absent	Present	Present	r's	r's
230	D300	RHD Variant	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
231	D323	DVI	DVI/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
232	D328	wD14.0/4.1	wD14.0/4.1/RHDdel	Absent	Absent	Absent	Absent	Present	Present	No r's	No r's
233	D329	wD14.0/4.1	wD14.0/4.1/RHDdel	Absent	Absent	Absent	Absent	Present	Present	No r's	No r's
234	D330	DAU5	DAU5/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
235	D331	wD14.0/4.1	wD14.0/4.1/RHDdel	Absent	Absent	Absent	Absent	Present	Present	No r's	No r's
236	GAL 10701360	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
237	GAL 10701364	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
238	GAL 10701365	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
239	GAL 10701366	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's

#	Sample ID	RHD allele #1	RHD allele #2	Hyb ex03		Hyb in07 sg02		Hyb in07 sg04		RHD r's call	
				Reference	Method	Reference	Method	Reference	Method	Reference	Method
240	GAL 10706636	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
241	GAL 10752569	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
242	GAL 10816074	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
243	GAL 10833160	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
244	GKO 10532283	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
245	GKO 32588	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
246	JCX33	r's	RHD	Present	Present	Absent	Absent	Present	Present	r's	r's
247	L22	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
248	L23	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
249	L28	RHD	RHD/RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
250	PGK53	RHDdel	RHDdel	Absent	Absent	Absent	Absent	Absent	Absent	No r's	No r's
251	ZGZ 51979	r's	r's/RHDdel	Present	Present	Absent	Absent	Present	Present	r's	r's
252	ZGZ 52739	Dilla	RHD	Present	Present	Present	Present	Present	Present	No r's	No r's

For the subset of *RHD***r*'s samples analyzed by ASP in this example, Table 5 shows:

- Number of samples
- Number of genotype calls made
- Number of correct calls made, as determined by comparison to the reference dataset
- 5 - Number of False Negative (FN) calls, total
- Number of FN calls, by type:
 - Amplification of segment #2 (sg02)
 - No amplification of segment #4 (sg04)
 - Amplification of sg02 and no amplification of sg04
- 10 - FN rate in the detection of the *RHD***r*'s variant.

Table 5. False Negative (FN) calls and rate for the determination of *RHD*r*'s.**

	<i>RHD*</i> <i>r</i> ^S
Samples Analyzed	82
Calls Made	82
Correct Calls	82
FN Calls	0
• FN [sg02+ sg04-]	0
• FN [sg02+]	0
• FN [sg04-]	0
FN Rate	0.000

For the subsets of non-*RHD***r*'s samples, Hybrid exon 3 (Hex03) samples, and non-*RHD***r*'s & Hex03 samples analyzed by ASP in this example, Table 6 shows the following:

- Number of samples
- Number of genotype calls made
- 20 - Number of correct calls made, as determined by comparison to the reference dataset
- Number of false positive (FP) calls
- FP rate in the detection of the *RHD***r*'s variant.

Table 6. False Positive (FP) calls and rates for the determination of *RHD*r*'s.**

	non- <i>RHD</i> * <i>r</i> 's	Hex03	Hex03 & non- <i>RHD</i> * <i>r</i> 's
Samples Analyzed	170	146	64
Calls Made	170	146	64
Correct Calls	159	135	53
FP Calls	11	11	11
FP Rate	0.065	0.075	0.172

Table 7. Primer Sequences

Primer No.	Sequence	SEQ ID NO:
1	CAAGAGCCACCCAGTCCA	16
2	CCAAGAGCCACTCATTCCG	17
3	ACATGGAGAAGTCATCACACA	18
4	CATGGAGAAGTCATCACACG	19
5	TGTTGAGCTGAGGGTCTGGT	20
6	TTGAGCTGAGGGTCTGGC	21
7	TTGAGCTGAGGGTCTCGC	22
8	GAATCTTCTGATATCCCCAT	23
9	GAATCTTCTGATATCCCCAC	24
10	GGTTCTGGGTGTTGGTTATA	25
11	GGTTCTGGGTGTTGGTTATG	26
12	CAAAGGCCCTTCCTCCAC	27
13	CAAAGGCCCTTCCTCCAA	28
14	AAAGGCCCTTCCTCCAAAAAC	29
15	AGATTACAGGTGTGCGCCACCAC	30
16	AAAGGCCCTTCCTCCACAAAC	31
17	CAAGCTGTCAAGGAGACATCACTAT	9
18	TTCTGGTAAAGGATTAACCTTAACAAA	32
19	ATAGAAAAGTGAAGTTTAGGGAGTTTG	33
20	CATTACATAGAGATGTCCCCATACT	10
21	GGATTACAGGTGTGCGCCAGAGT	34
22	TGCTGGTAGAGGATTAACCTTAACAAC	35
23	TGCTGGTAGAGGATTAACCTTAACAAC	36
24	CAACTGGCTTTCCAAGAAAATAA	37
25	GCCACTTATTACTTAAAAAAACCCCA	38
26	AAGGCCCTTCCTCCAAATAG	13
27	TTAATCCTTCAATTCATATGTTGATGCTA	39
28	GCCACTTATTACTTAAAAAAACCCC	12
29	GATTATAAGCCTGAGACACCGTGCCTGGCA	40
30	GTGAAGCAAGGTGCCTCTGTA	41
31	ATCTCTTCCCAAGTCCATGTGC	42
32	GAAGCAAGGCGCCTCTGTG	43
33	TCTCATCTCTTCCCAAGTCCATGTAT	44
34	TTTCCCTAGTATATGTAACCATCA	45
35	GGAAAAGGTTTGAGAGGAATTATAC	46
36	TTTCCCTAGTATATGTAACCATCG	47
37	GGAAAAGGTTTGAGAGGAATTATAT	48
38	CACATTACATAGAGATGTCCCCATACT	78
39	GCCACTTATTACTTAAAAAAACCCC	79
40	CTGAGTCAGATGCTGTGATCAGAG	81

Table 8. Probe Sequences

Probe No.	Sequence	SEQ ID NO:
1	GGTACTCAAACCTCCCTAAATC	49
2	GATTTAGGGAGTTTGAGTACC	50
3	GTACTCAAACCTCCCTAAAT	51
4	ATTTAGGGAGTTTGAGTAC	52
5	TACTCAAACCTCCCTAAA	53
6	TTTAGGGAGTTTGAGTA	54
7	TGGTACTCAAACCTCCCTAAATCT	55
8	CTGGTACTCAAACCTCCCTAAATCTC	56
9	CCTGGTACTCAAACCTCCCTAAATCTCA	11
10	TGCCAGGCACGGTGTCTCAGGCT	57
11	TGCCAGGCACGGTGTCTCAGG	58
12	AGCCTGAGACACCGTGCCTGGCA	59
13	CCTGAGACACCGTGCCTGGCA	60
14	TGCCCTCTCACTGTGTGATGACT	61
15	GCCCTCTCACTGTGTGATGAC	62
16	AGTCATCACACAGTGAGAGGGCA	63
17	GTCATCACACAGTGAGAGGGC	64
18	TTATGCCAGGCACGGTGTCTCAGGCTT	65
19	GAAGTCATCACACAGTGAGAGGGCAGA	66
20	AGGCACGGTGTCTCAGGCTTATAAT	67
21	TCTGATATCCCCATATAACCAACACCC	68
22	GGGTGTTGGTTATATGGGGATATCAGA	69
23	CTGATATCCCCATATAACCAACACC	70
24	GGTGTGTTGGTTATATGGGGATATCAG	71
25	CCCCCAAATTTTCATGTGCTAGAACTT	72
26	AAGTTTCTAGCACATGAAATTTGGGGG	73
27	CCCAAATTTTCATGTGCTAGAACTT	74
28	AAGTTTCTAGCACATGAAATTTGGG	75
29	AATTCATGTGCTGGAACTTAATCCT	14
30	AGGATTAAGTTTCCAGCACATGAAATT	76
31	ATTCATGTGCTGGAACTTAATCC	15
32	GGATTAAGTTTCCAGCACATGAAAT	77
33	ATTCATGTGCTGGAACTTAATCC	80

References

1. DIIIa and DIII Type 5 are encoded by the same allele and are associated with altered
5 RHCE*ce alleles: clinical implications. Connie M. Westhoff, Sunitha Vege, Christine
Halter-Hipsky, Trina Whorley, Kim Hue-Roye, Christine Lomas-Francis, and Marion E.
Reid. Transfusion (2010) 50, pp. 1303-1311.
2. Heterogeneous molecular background of the weak C, VS+, hrB-, HrB- phenotype in
black persons. Bach-Nga Pham, Thierry Peyrard, Genevieve Juszcak, Isabelle
10 Dubeaux, Dominique Gien, Antoine Blancher, Jean-Pierre Cartron, Philippe Rouger, and
Pierre-Yves Le Pennec. Transfusion (2009) 49, pp. 495-504.
3. *RHC* and *RHc* genotyping in different ethnic groups. Martine G.H.M. Tax, C. Ellen van
der Schoot, Rene' van Doorn, Lotte Douglas-Berger, Dick J. van Rhenen, and Petra A.
Maaskant-van Wijk. Transfusion (2002) 42, pp. 6234-644.

Claims

1. A method for determining the presence or absence of, or for discriminating between, blood type alleles, which method comprises identifying the nucleotide at one or more positions in intron 7 of the *RHD* locus and/or in intron 7 of the *RHCE* locus in a sample obtained from a human subject.
2. A method according to claim 1, wherein said blood type alleles are alleles that comprise an *RHD/RHCE* hybrid exon 3.
3. A method according to claim 1 or claim 2, wherein said blood type alleles are selected from the group consisting of: *RHD**r's; *RHD**r's-like; *RHD**r^S Type 1; *RHD**r^S Type 2; *RHD**DIIIa; *RHD**DIII_FN; *RHD**DIVa-2; *RHD**DIVa; *RHD**DIII-type4; *RHD**DIII-type6; *RHD**DIII-type7; *RHD**DIII-type8; *RHCE**ce^S; *RHCE**ce^S1006T; *RHCE**ce^S1006C; *RHCE**ce733G; *RHCE**ce48C,733G,1025T; *RHCE**ce48C,697G,733G; *RHCE**ce340T,733G; and *RHCE**ce48C,733G,748A.
4. A method according to claim 2 or claim 3, wherein said method comprises genotyping the sample at one or more polymorphic positions in intron 7 of the *RHD* gene locus, said one or more polymorphic positions being selected from:
 - (i) a first region of said intron 7 that is 5' of IVS7+1887, said first region including said position IVS7+1887;
 - (ii) a second region of said intron 7 that lies between IVS7+2153 and IVS7+3101, said second region including said positions IVS7+2153 and IVS7+3101; and
 - (iii) a third region of said intron 7 that lies 3' of IVS7+3257, said third region including said position IVS+3257,
 wherein said positions are numbered according to the position numbering shown in Figure 3.
5. A method according to claim 4, wherein said method is for discriminating *RHD**r's or *RHD**r's-like from *RHD**DIIIa and/or *RHD**DIVa-2, and wherein the method comprises genotyping the sample at one or more polymorphic positions in said first region of intron 7.
6. A method according to claim 4, wherein said method is for discriminating *RHD**r's, *RHD**r's-like or *RHD**DIIIa from *RHD**DIVa-2, and wherein the method comprises

genotyping the sample at one or more polymorphic positions in said second region and/or said third region of intron 7.

7. A method according to claim 4, wherein said method is for discriminating between: (a) *RHD**r's or *RHD**r's-like; (b) *RHD**DIIIa; and (c) *RHD**DIVa-2, wherein the method comprises genotyping the sample at:

- (i) one or more polymorphic positions in said first region of intron 7; and
- (ii) one or more polymorphic positions in said second or said third regions.

8. A method according to any one of claims 4 to 7, wherein the identity of the nucleotide at said one or more polymorphic positions in said first region is the same as that of conventional *RHCE*, and wherein the sample is classified as being or having a high probability of having a *RHD**r's allele.

9. A method according to any one of claims 4 to 7, wherein the identity of the nucleotide at said one or more polymorphic positions in said first region is that of conventional *RHD*, and wherein sample is classified as being or having a high probability of having a *RHD**DIIIa or *RHD**DIVa-2 allele .

10. A method according to claim 2 or claim 3, wherein said method comprises genotyping the sample at one or more polymorphic positions in intron 7 of the *RHD* gene locus, said one or more polymorphic positions being selected from:

- (i) a first region of said intron 7 that is 5' of IVS7+1887, said first region including said position IVS7+1887;
 - (ii(a)) a second (a) region of said intron 7 that lies between IVS7+2153 and IVS7+2335, said second (a) region including said positions IVS7+2153 and IVS7+2335;
 - (ii(b)) a second (b) region of said intron 7 that lies between IVS7+2449 and IVS7+3101, said second (b) region including said positions IVS7+2449 and IVS7+3101; and
 - (iii) a third region of said intron 7 that lies 3' of IVS7+3257, said third region including said position IVS+3257,
- wherein said positions are numbered according to the position numbering shown in Figure 3.

11. A method according to claim 10, wherein said method is for discriminating between: (a) *RHD**r's or *RHD**r's-like; (b) *RHD**DIIIa; (c) *RHD**DIII_FN; and (d) *RHD**DIVa-2, wherein the method comprises genotyping the sample at:

- (i) one or more polymorphic positions in said first region of intron 7;
- (ii) one or more polymorphic positions in said second (a) region;
- (iii) one or more polymorphic positions in said second (b) region; and
- (iv) one or more polymorphic positions in said third region.

12. A method according to claim 10 or claim 11, wherein the identity of the nucleotide at said one or more polymorphic positions in said second (a) region is the same as that of conventional *RHD*, and wherein the identity of the nucleotide at said one or more polymorphic positions in said second (b) region, other than positions IVS7+2945 and IVS7+3026, is the same as that of conventional *RHCE*, and wherein the sample is classified as being or having a high probability of having a *RHD**DIII_FN allele.

13. A method according to any one of the preceding claims, wherein the method comprises genotyping the sample at one or more polymorphic positions in intron 7 of the *RHCE* gene locus, said polymorphic positions in intron 7 of the *RHCE* locus being located in a region that lies between IVS7+2970 and IVS7+4108, said region including said positions IVS7+2970 and IVS7+4108, and wherein said method is for detecting the presence or absence of, or for discriminating between, *RHCE*, *RHCE**ce^S and *RHCE**ce733G, wherein said positions are numbered according to the numbering shown in Figure 3.

14. A method according to any one of the preceding claims, wherein said polymorphic positions in intron 7 of the *RHD* gene and/or in intron 7 of the *RHCE* gene are selected from the single nucleotide polymorphism (SNP) positions set forth in Table 1, the position numbering being as shown in Figure 3.

15. A method according to claim 14, wherein the method comprises genotyping the sample at 2, 3, 4, 5, 6, 7, 8, 9, 10 or more SNP positions selected from those set forth in Table 1.

16. A method according to claim 14 or claim 15, wherein the genotype at the one or more SNP positions detected for the sample is used to classify the *RHD* and/or

RHCE alleles present in the sample, according to the classification set forth in Table 1.

17. A method according to any one of the preceding claims, wherein the sample is a sample which has previously been determined to comprise an *RHD-RHCE* hybrid exon 3.

18. A method according to any one of claims 1 to 16, wherein the method further comprises determining whether the sample contains an *RHD-RHCE* hybrid exon 3.

19. A method according to claim 18, wherein the method comprises genotyping the sample at one or more polymorphic positions selected from:

IVS2-26, IVS2-13, IVS2-8 in *RHD* intron 2;
IVS3+64, IVS3+69 in *RHD* intron 3; and
position 410 in *RHD* exon 3.

20. A method according to claim 19, wherein the method comprises genotyping the sample at position 410 of the *RHD* exon 3, wherein presence of T at said position indicates presence of an *RHD/RHCE* hybrid exon 3 variant in the sample.

21. A method according to any one of the preceding claims, wherein the method comprises genotyping not more than 50, 40, 30, 25, 20, 15, or not more than 10, single nucleotide polymorphic positions in the *RHD* gene locus and/or the *RHCE* gene locus.

22. A method according to any one of the preceding claims, wherein the method further comprises predicting an *RHD* phenotype and/or an *RHCE* phenotype for the subject based on the genotype of the sample.

23. A method according to any one of the preceding claims, wherein the subject is undergoing, or is a candidate for, blood transfusion or bone marrow transplantation.

24. A method according to any one of the preceding claims, wherein the subject has sickle cell disease (SCD) or Thalassemia major.

25. A method according to any one of the preceding claims, wherein the subject has non-Caucasian ancestry.

26. A method according to claim 25, wherein the subject has African ancestry.
27. A method according to any one of the preceding claims, wherein the sample comprises a blood sample.
28. A method according to any one of the preceding claims, wherein the sample is subjected to one or more treatments to extract and/or amplify a nucleic acid prior to or as part of said genotyping.
29. A method according to any one of the preceding claims, wherein the method comprises Allele-Specific Polymerase Chain Reaction (ASP) and/or Allele-Specific Hybridization (ASH).
30. A method according to any one of the preceding claims, wherein the method comprises labeling a nucleic acid obtained from the sample or labeling an amplicon derived from a nucleic acid obtained from the sample.
31. A method according to any one of claims 28 to 30, wherein genomic DNA is extracted from the sample and amplified using PCR to amplify at least a portion of intron 7 of the *RHD* gene and/or intron 7 of the *RHCE* gene.
32. A method according to any one of the preceding claims, wherein the method further comprises carrying out serological analysis on a blood sample that has been obtained from the subject, combining the serological analysis with said genotype and thereby determining the blood type phenotype of the subject.
33. A method according to any one of the preceding claims, wherein DNA obtained from said sample is amplified using primers that are selective for *RHD/RHCE* hybrid intron 7.
34. A method according to claim 33, wherein the primers are selected from: a primer comprising the nucleotide sequence of SEQ ID NO: 9, a primer comprising the nucleotide sequence of SEQ ID NO: 10, a primer comprising the nucleotide sequence of SEQ ID NO: 12, a primer comprising the nucleotide sequence of SEQ ID NO: 13, and one or more primers comprising or consisting of a nucleotide sequence set forth in Table 7.

35. A method according to any one of the preceding claims, wherein said genotyping comprises an allele-specific hybridisation of DNA extracted from the sample, or an amplicon derived from DNA extracted from the sample, and wherein the allele-specific hybridisation comprises contacting said extracted DNA or said amplicon with a probe selected from: a probe comprising the sequence of SEQ ID NO: 11, a probe comprising the sequence of SEQ ID NO: 14, a probe comprising the sequence of SEQ ID NO: 15, and one or more probes comprising or consisting of a nucleotide sequence set forth in Table 8.

36. A method according to any one of the preceding claims, wherein the method further comprises determining whether the sample contains an *RHD-RHCE* hybrid exon 3, and wherein DNA obtained from said sample is amplified using primers that are selective for *RHD/RHCE* hybrid exon 3.

37. A method according to claim 36, wherein said primers that are selective for *RHD/RHCE* hybrid exon 3 are selected from: a primer comprising the nucleotide sequence of SEQ ID NO: 5 and a primer comprising the nucleotide sequence of SEQ ID NO: 6.

38. A method according to claim 36, wherein said method further comprises genotyping the sample at a polymorphic position in exon 3 of the *RHD* locus, and wherein the method comprises an allele-specific hybridisation of DNA extracted from the sample, or an amplicon derived from DNA extracted from the sample, and wherein the allele-specific hybridisation comprises contacting said extracted DNA or said amplicon with a probe selected from: a probe comprising the sequence of SEQ ID NO: 7 and a probe comprising the sequence of SEQ ID NO: 8.

39. One or more oligonucleotide probes and/or primers for use in the method of any one of claims 1 to 38, wherein the one or more oligonucleotide probes and/or primers span, or are able to be used to span, one or more of the polymorphic positions in intron 7 of the *RHD* gene and/or the *RHCE* gene, said probes and/or primers being hybridisable to a portion of the sense or antisense strand of said *RHD* and/or *RHCE* gene.

40. One or more oligonucleotide probes and/or primers according to claim 39, wherein the oligonucleotides and/or primers span one or more polymorphic positions set forth in Table 1.

41. One or more oligonucleotide probes and/or primers according to claim 39 or claim 40, wherein:

the oligonucleotide probes are selected from the probes of SEQ ID Nos: 11, 14 and 15, and the probes comprising or consisting of one or more nucleotide sequences as set forth in Table 8; and/or

the oligonucleotide primers are selected from the primers of SEQ ID Nos: 9, 10, 12 and 13, and the primers comprising or consisting of one or more nucleotide sequences as set forth in Table 7.

42. A plurality of oligonucleotide probes according to any one of claims 39 to 41, wherein the probes are coupled to a solid support.

43. A plurality of oligonucleotide probes according to claim 42, wherein the solid support comprises a planar surface or a particle.

44. A kit for assessing a subject's blood type, said kit comprising:

one or more probes and/or primers as defined in any one claims 39 to 43;
optionally, one or more probes and/or primers that span one or more polymorphic positions in intron 2, exon 3 and/or intron 3 of the *RHD* gene;
optionally, one or more reagents and/or standards for use in carrying out a method as defined in any one of claims 1 to 38; and
optionally, instructions for carrying out a method as defined in any one of claims 1 to 38.

45. A kit according to claim 44, wherein the kit comprises:

one or more primers selected from the primers of SEQ ID Nos: 5 and 6;
one or more probes selected from the probes of SEQ ID Nos: 7 and 8;
one or more primers selected from the primers set forth in Table 7; and/or
one or more probes selected from the probes set forth in Table 8.

46. A system for use in determining a subject's blood type, the system comprising:
a kit as defined in claim 44 or claim 45; and

at least one detector arranged to detect a signal from detectably labelled DNA obtained from said subject or a detectably labelled amplicon derived from DNA obtained from said subject;

at least one controller in communication with the at least one detector, the controller being programmed with computer-readable instructions to transform said signal into predicted blood type genotypes, and optionally, to transform said predicted blood type genotypes into a predicted blood type phenotype.

47. A method of blood matching, the method comprising:

carrying out the method according to any one of claims 1 to 38 on a recipient sample from a recipient subject in need of donor blood and on a donor sample from a potential donor subject;

comparing the blood type alleles present in the recipient sample with those present in the donor subject and thereby determining the compatibility of the recipient subject to receive blood from the potential donor subject.

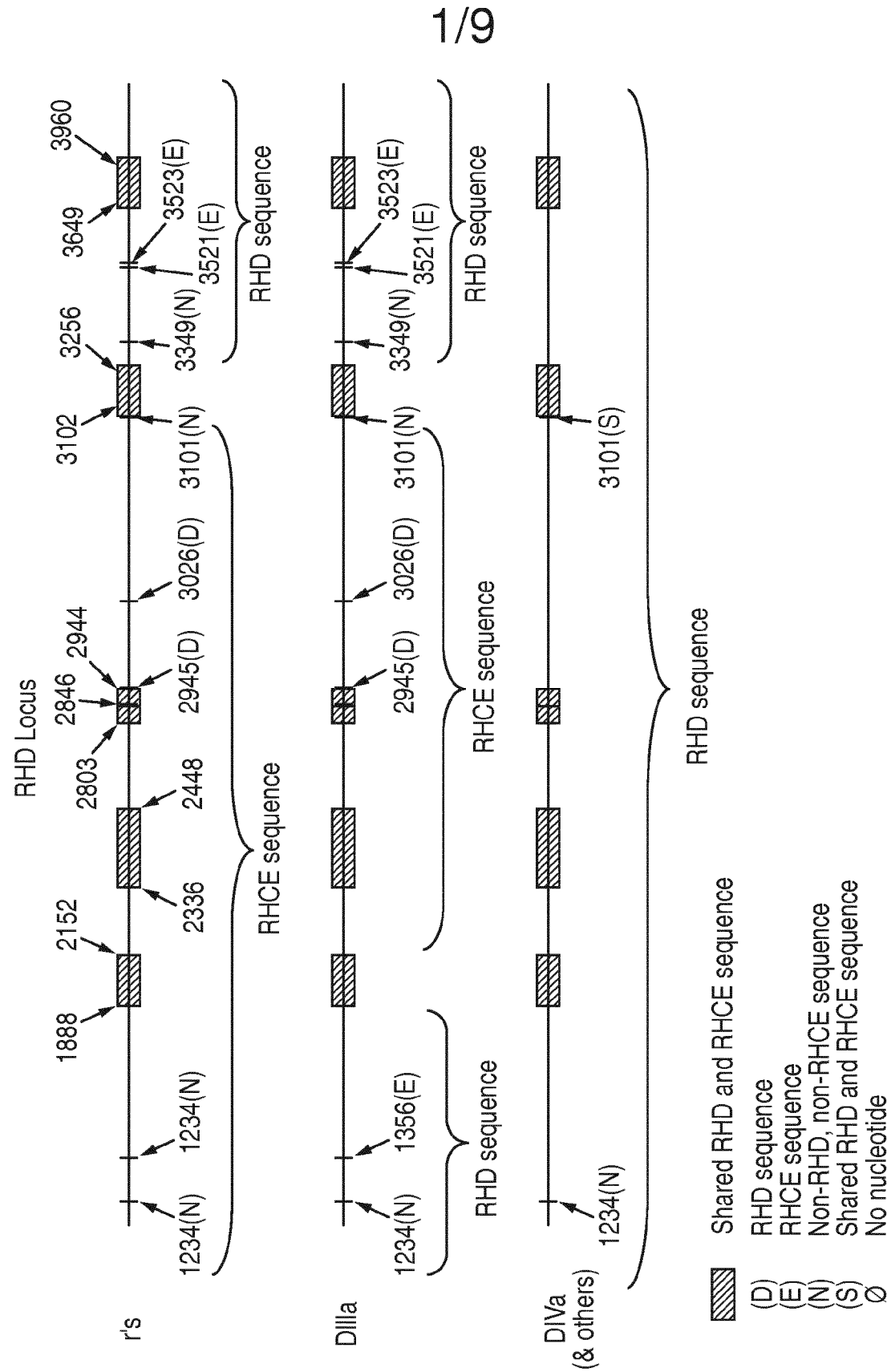


FIG. 1A

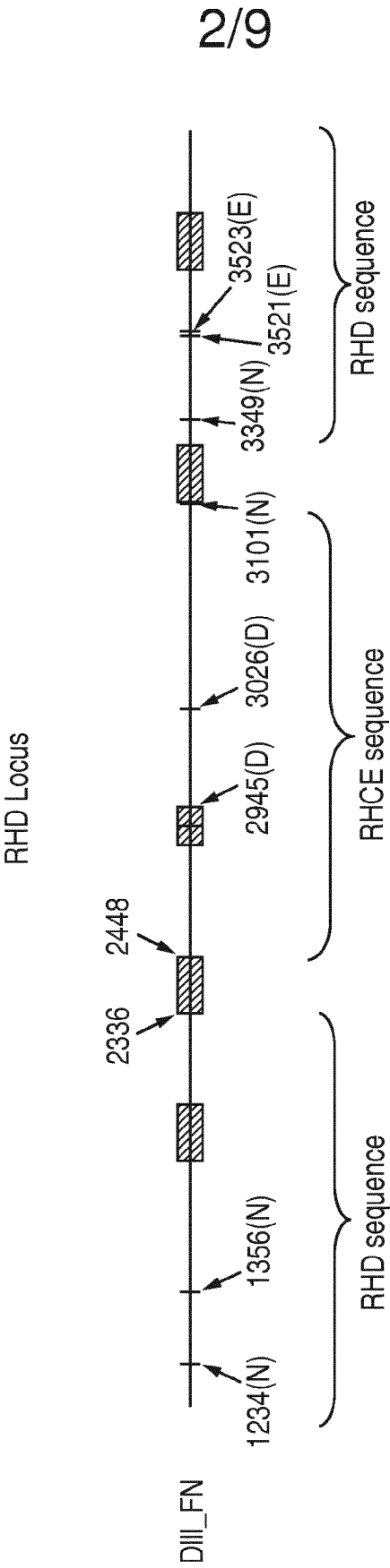


FIG. 1B

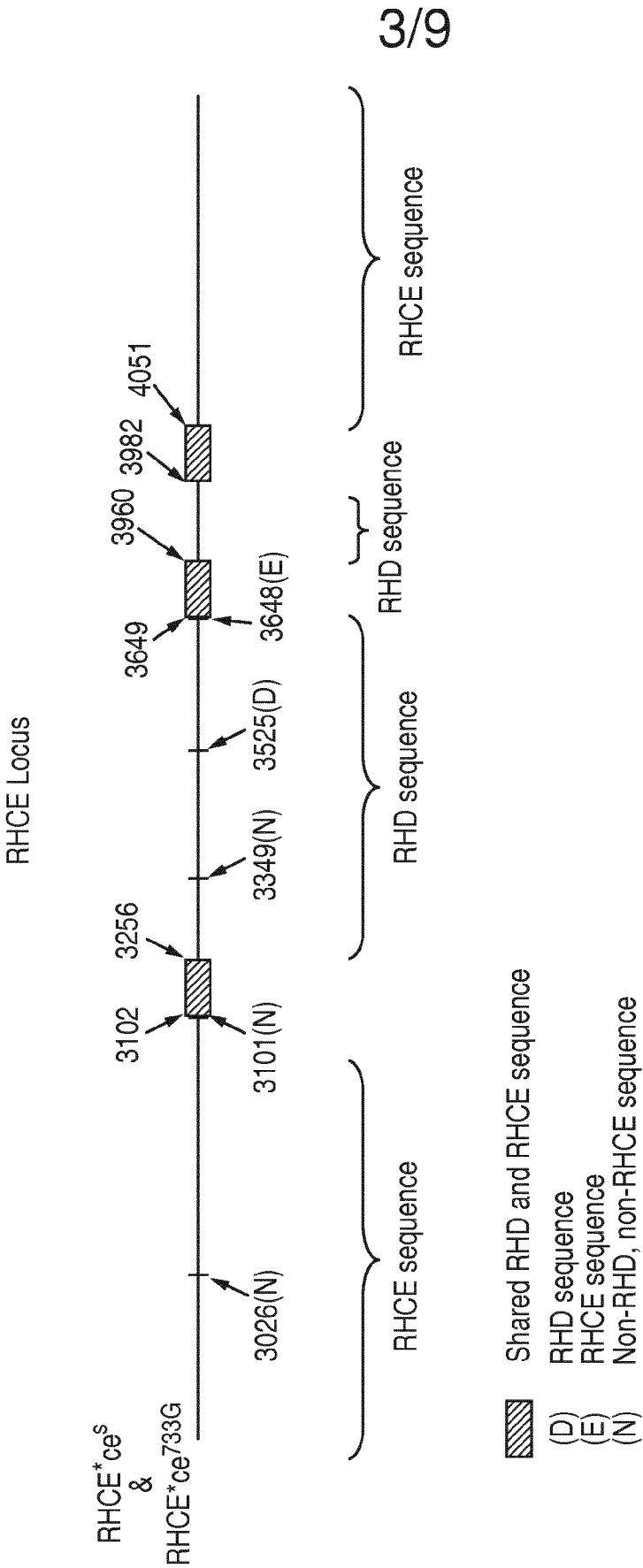


FIG. 2

4/9

	Intron7				Intron7
Position	1110	1120	1130	1140	1150
RHD*00101	GCTTGGGATG	AGGAGGGGAT	GAGCTGTGTG	AAGCAAGGCG	CCTCTGTGAT
RHCE*ce_BN000065				T.	A..

	Intron7				Intron7
Position	1160	1170	1180	1190	1200
RHD*00101	GGGTTCCAGT	GATGTGTCTG	CCACTGTCTT	AATAACTGTG	CAATTCTAAG
RHCE*ce_BN000065					

	Intron7				Intron7
Position	1210	1220	1230	1240	1250
RHD*00101	CAGAACCCTT	CCTGTCTCTG	GGCCTGAGAG	TTCCCCTCTG	AAAGATGAGG
RHCE*ce_BN000065				T.	

	Intron7				Intron7
Position	1260	1270	1280	1290	1300
RHD*00101	ACTTGACCTA	GCAAGGTCCT	ACTCACATGC	CTGTAGAGAA	CAGGCAGGGG
RHCE*ce_BN000065			G....		

	Intron7				Intron7
Position	1310	1320	1330	1340	1350
RHD*00101	AAGTTAGAAA	AAAAAAAAAAG	CCAGTGAAGG	AAGGGAGCTC	TTCAGCTTGC
RHCE*ce_BN000065		-			

	Intron7				Intron7
Position	1360	1370	1380	1390	1400
RHD*00101	ACCCATCATC	ACAGTGCAGG	GACCCAGGCT	CAGTGTGCTC	AGATCCAATG
RHCE*ce_BN000065	C....				

	Intron7				Intron7
Position	1410	1420	1430	1440	1450
RHD*00101	ACTTCTCAAG	AGCTCAAAAT	CTAGAGTTTT	GCATGTGCTC	TCCCAAGTAC
RHCE*ce_BN000065					

	Intron7				Intron7
Position	1460	1470	1480	1490	1500
RHD*00101	TGGCAGAAAA	TTCAAGATTG	TTAGTAACAC	TGTGTGGCTA	AATTCTGCTT
RHCE*ce_BN000065					

	Intron7				Intron7
Position	1510	1520	1530	1540	1550
RHD*00101	GTGGGCTGCC	TAGATTCCCA	ATTCTGTGAT	TCTGTGGTTC	TCTGGAAGCA
RHCE*ce_BN000065					

	Intron7				Intron7
Position	1560	1570	1580	1590	1600
RHD*00101	TTGGTTCTCC	ACAGCACCTG	CATCACTTGG	AAACTTGTTA	GAAATGCAAG
RHCE*ce_BN000065					

	Intron7				Intron7
Position	1610	1620	1630	1640	1650
RHD*00101	CCCTACCTAC	GGCCCCACCC	CAGACCTACC	CAGTTAGAAA	TCTGGGGGTG
RHCE*ce_BN000065					

	Intron7				Intron7
Position	1660	1670	1680	1690	1700

FIG. 3A

5/9

RHD*00101	GGACCTATCA	GTCCATGTTT	GAACAAGCCC	CACAAGTGTT	CTCTTGCAAG
RHCE*ce_BN000065					
	Intron7				Intron7
Position	1710	1720	1730	1740	1750
RHD*00101	CTCAAGTTT	AGAACCACTG	ACCTATAGCC	AAAAAAGAAA	AAGCCAATCA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	1760	1770	1780	1790	1800
RHD*00101	GTGGTTTCT	GGTAAAGGAT	TAACTTAACA	AACTGGCTTT	CCAAGAAAAT
RHCE*ce_BN000065	G..	G.....		..-.....	...T.....
	Intron7				Intron7
Position	1810	1820	1830	1840	1850
RHD*00101	AAAGCCTTGA	TTGGTAGCAC	TTGCAATTTT	TATGGTACAA	ACGCTTCCCG
RHCE*ce_BN000065					A
	Intron7				Intron7
Position	1860	1870	1880	1890	1900
RHD*00101	CATGACTGAG	TTCAAGCTGT	CAAGGAGACA	TCACTATACA	TGGACTTGGG
RHCE*ce_BN000065		A.	G	GC...	
	Intron7				Intron7
Position	1910	F31920	1930	1940	1950
RHD*00101	AAGAGATGAG	AACAATCAGC	CCACTGAGCC	TATGGGAAC	GGCTCCAGCA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	1960	1970	1980	1990	2000
RHD*00101	CATCCCTGCA	AGTCAACTCT	CATCAGGGTG	AGTGAGTTGA	GGACCAAGAA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	2010	2020	2030	2040	2050
RHD*00101	GCAGTTATCC	TCTTGCCTTT	GCAGGACCCA	GGCAAAGGGA	AGGGCATAGT
RHCE*ce_BN000065					
	Intron7				Intron7
Position	2060	2070	2080	2090	2100
RHD*00101	GACAGTGATG	ATCTCTCTTC	CGGAAGTCTT	TGGTTTGCTG	AGAGTAAAG
RHCE*ce_BN000065					
	Intron7				Intron7
Position	2110	2120	2130	2140	2150
RHD*00101	GCGTGGGCTT	CACCAGTGGT	GAAGCCAGTC	ATGCAGCCTT	AGTCCTGGTA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	2160	2170	2180	2190	2200
RHD*00101	CTGAAACTCT	CTAAATCTCA	GTTTTCTATC	TGTAAATGG	GAAAATAAGA
RHCE*ce_BN000065	..C.....C				T
	Intron7				Intron7
Position	2210	2220	2230	2240	2250
RHD*00101	CCTATGTCAC	AGGGTTGCTG	TGCAGATTTA	GCAACAGAAC	ATAGCCCCGT
RHCE*ce_BN000065				T.....	

FIG. 3B

6/9

	Intron7				Intron7
Position	2260	2270	2280	2290	2300
RHD*00101	TCTTTATGAT	GACTGATGCT	GCATCCGTAT	GAGGACATCT	CTATGTAATG
RHCE*ce_BN000065			A.....	.G.....	
	Intron7				Intron7
Position	2310	2320	2330	2340	2350
RHD*00101	GAAAGATGGA	GAGAGGATTA	AGCGCAAAGT	CACAACACTT	AATGGGAACT
RHCE*ce_BN000065			..T.....	G.....	
	Intron7				Intron7
Position	2360	2370	2380	2390	2400
RHD*00101	GTGGATTAGC	TACTTGGTGG	CATTGGGCAA	GTCAGTTGAC	TTTGCATTAA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	2410	2420	2430	2440	2450
RHD*00101	TTCCACAAAC	AATATTTCCC	AATTTCTTAT	TCAGATGAGC	ATATGTGATT
RHCE*ce_BN000065					C.
	Intron7				Intron7
Position	2460	2470	2480	2490	2500
RHD*00101	GAGTCAGATG	CTGTGATCAG	AACCAGGATG	GAGCATTTCC	CACAAACTGT
RHCE*ce_BN000065			.G.....		
	Intron7				Intron7
Position	2510	2520	2530	2540	2550
RHD*00101	GGGATTTTTA	AGTAATGGGA	AGGCACACTG	AAATGGCACT	GAATCATGCA
RHCE*ce_BN000065		...G.....		T.	
	Intron7				Intron7
Position	2560	2570	2580	2590	2600
RHD*00101	GTTGCAGATA	CTCTTTTTTCA	ATTCTCAGTC	CTTTGATTAC	GTCAGGGAGA
RHCE*ce_BN000065					A.....
	Intron7				Intron7
Position	2610	2620	2630	2640	2650
RHD*00101	AAAGAAAGTC	CCCACTTGGC	CTGAGAAATCT	CTGCACCCTT	CTAGCTCTTG
RHCE*ce_BN000065		G			
	Intron7				Intron7
Position	2660	2670	2680	2690	2700
RHD*00101	TTAACCCTC	TTTTGAATAG	CAGAGAAAAC	CTCAGACTGC	CATATCTGGG
RHCE*ce_BN000065					
	Intron7				Intron7
Position	2710	2720	2730	2740	2750
RHD*00101	AGAGATTTTA	GCAACATTTT	GTTTTTCATTG	TATCTCTTTT	TACAGCTACC
RHCE*ce_BN000065					
	Intron7				Intron7
Position	2760	2770	2780	2790	2800
RHD*00101	TCCCATTTCC	CTTCTATTTT	AAGCTAGTAA	CTCAGTTTTT	TTTTAAATTC
RHCE*ce_BN000065				.A.....	
	Intron7				Intron7
Position	2810	2820	2830	2840	2850
RHD*00101	AATTATTTAA	ATGTAAAAAT	AAGTCTATTT	GGAGAAAAAA	AATTTT-AATA

FIG. 3C

7/9

```

RHCE*ce_BN000065 .T.....T....

Intron7
Position      F4 2860      2870      2880      2890      2900
RHD*00101    GCATCTCTGG AATGCCAGTA TGGCTAAATT CATGAATGTT GTCCTCAAAT
RHCE*ce_BN000065 .....

Intron7
Position      2910      2920      2930      2940      2950
RHD*00101    GCTGAAATCT GGAAGCATC TGGCCAAGCT TTGTGGACAG GCCTGCCTAG
RHCE*ce_BN000065 .....T....

Intron7
Position      2960      2970      2980      2990      3000
RHD*00101    TTTGAATCCC AAGAGCCACC CAGTCCAAGC CACAAAACAT TGGAAATCTT
RHCE*ce_BN000065 .....T..T...G...

Intron7
Position      3010      3020      3030      3040      3050
RHD*00101    GGTTCACCTC CCTAACCTGA ACTTGCCCTC TGTGAAATAG GGACACTAAT
RHCE*ce_BN000065 .....T....

Intron7
Position      3060      3070      3080      3090      3100
RHD*00101    AGCTCACTCA CAGGGCTGCT GTGAGGACAT GTGTTGAGCT GAGGGTCTCG
RHCE*ce_BN000065 .....G.

Intron7
Position      3110      3120      3130      3140      3150
RHD*00101    CCAGGGGAGA CCCTGTGCAG GGAGACTGTT ATCATGGTGA TGGATTTCTG
RHCE*ce_BN000065 .....

Intron7
Position      3160      3170      3180      3190      3200
RHD*00101    CTTCAATCAT TTCTTTTCC AGACAGCATC ATATAGAATG AGTTGTGGGG
RHCE*ce_BN000065 .....

Intron7
Position      3210      3220      3230      3240      3250
RHD*00101    TGGCAGTCAG CAGGTTTGGG TTTATCCTCT ATTCTGCCAC TTATTACTTA
RHCE*ce_BN000065 .....

Intron7
Position      3260      3270      3280      3290      3300
RHD*00101    AAAAAACCCC AAAAAACCCA ACTTATATAG TATAAGCTAT ATCCAGAAAA
RHCE*ce_BN000065 .....----

Intron7
Position      3310      3320      3330      3340      3350
RHD*00101    GTGCAAAATAT CATACAAGTA CCATTTGATG AATCTTCTGA TATCCCCACA
RHCE*ce_BN000065 .....

Intron7
Position      3360      3370      3380      3390      3400
RHD*00101    TAACCAACAC CCAGAACCTC TTCTTGCTCT ATTCCAGGAT AACCACTAAC
RHCE*ce_BN000065 .....

Intron7
Intron7

```

FIG. 3D

8/9

Position	3410	3420	3430	3440	3450
RHD*00101	CTGACTTCTA	ACAGCATCAG	TCAGTTTGT	CTGTTTTTGT	ACATTATATA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	3460	3470	3480	3490	3500
RHD*00101	TGTGATGGTT	TGAATGTGTC	CCCCAAATTT	CATGTGCTGG	AAACTTAATC
RHCE*ce_BN000065				A.	
	Intron7				Intron7
Position	3510	3520	3530	3540	3550
RHD*00101	CTTCAATTCA	TATGTTGATG	GTTTTTGGAG	GAAGGGCCTT	TGGGAAGTAA
RHCE*ce_BN000065			C.A.....		
	Intron7				Intron7
Position	3560	3570	3580	3590	3600
RHD*00101	TTAGGATTAG	ATAAGGTCAT	GGGGTGAGGT	ATGATGGCAC	TGGTGACTTA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	3610	3620	3630	3640	3650
RHD*00101	TAAGAAGAGA	AAGAGAAATC	TGAGCTGGCA	TGCTCTTGCC	CTCTCACTGT
RHCE*ce_BN000065					C..
	Intron7				Intron7
Position	3660	3670	3680	3690	3700
RHD*00101	GTGATGACTT	CTCCATGTCA	TGATGCAGCA	AGAAGGCCCT	CACCAGATGG
RHCE*ce_BN000065					
	Intron7				Intron7
Position	3710	3720	3730	3740	3750
RHD*00101	TGGCACCATG	CTTTTGGACT	TCCCAGCCTC	TAGAACTGTG	AGCTAAATCA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	3760	3770	3780	3790	3800
RHD*00101	ATTTATTTTC	TTTATAATCA	CCCAGTTTGA	TATTTTGTCA	TAGCAACAGA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	3810	3820	3830	3840	3850
RHD*00101	ATATGGACAA	AGAAAGAAAA	TTAATGCAAG	AAGTAGAGTT	TTTACTGTAA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	3860	3870	3880	3890	3900
RHD*00101	CAGATTCCTG	AAAATGTGGA	AGTGGCTTTG	GAAC TGGGTG	ATGGGAATAG
RHCE*ce_BN000065					
	Intron7				Intron7
Position	3910	3920	3930	3940	3950
RHD*00101	GTTGGAAGAG	TTTTGAGGAG	CAGGCTAGAA	AAAGCCTGTA	TTGTCAAGAA
RHCE*ce_BN000065					
	Intron7				Intron7
Position	3960	3970	3980	3990	4000
RHD*00101	TGGAGCATT	TGCCAGGCAC	GGTGTCTCAG	GCTTATAATC	CCAGCACTTT
RHCE*ce_BN000065		G.....	G.....	A.....	

FIG. 3E

9/9

	Intron7					Intron7
Position	4010	4020	4030	4040		4050
RHD*00101	GGGAGGCCAA	AGCAGGTGGA	TCACCTGAGG	TCAGGAGTTC	GAGACCAGCC	
RHCE*ce_BN000065						

	Intron7					Intron7
Position	4060	4070	4080	4090		4100
RHD*00101	TAGCTAACAT	GGTGAAACGC	TGTTTCTACC	AAAAATACAA	AAAATTAGCT	
RHCE*ce_BN000065	.G.....					

	Intron7	
Position	4110	4120
RHD*00101	GGGCGTGGTG	GCGCACACCT
RHCE*ce_BN000065	ACTC..	

FIG. 3F

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/061270

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12Q1/68

ADD.

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 practicable, search terms used)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/32702 A2 (DRK BLUTSPENDEDIENST BADEN WUE [DE]; FLEGEL WILLY A [DE]; WAGNER FRANZ) 10 May 2001 (2001-05-10) page 13, paragraph 2 - page 22 page 49 - page 50, paragraph 1 table 4 claims 11-36,38-43,48-53,60,62-64,69-72 examples 2,13-17 ----- -/--	1-3,39, 42-44, 46,47



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15 August 2012

Date of mailing of the international search report

27/08/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Bruma, Anja

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/061270

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHIARONI J ET AL: "Groupes sanguins erythrocytaires", EMC - HEMATOLOGIE, ELSEVIER, vol. 2, no. 2, 1 June 2005 (2005-06-01), pages 53-112, XP027839689, ISSN: 1638-6213 [retrieved on 2005-06-01] page 72, column 1, paragraph 3 - page 81, column 2, paragraph 1 tables 14-20 figures 6-8 -----	1-47
X	LIU H C ET AL: "Aberrant RNA splicing in RHD 7-9 exons of DEL individuals in Taiwan: A mechanism study", BIOCHIMICA ET BIOPHYSICA ACTA - GENERAL SUBJECTS, ELSEVIER SCIENCE PUBLISHERS, NL, vol. 1800, no. 6, 1 June 2010 (2010-06-01), pages 565-573, XP027018781, ISSN: 0304-4165 [retrieved on 2010-02-25] the whole document -----	1-3,39, 42-44, 46,47
X	HILLYER ET AL: "Prevalence, donation practices, and risk assessment of blood donors with hemochromatosis", TRANSFUSION MEDICINE REVIEWS, GRUNE AND STRATTON, ORLANDO, FL, US, vol. 16, no. 3, 1 July 2002 (2002-07-01), pages 270-271, XP005469007, ISSN: 0887-7963, DOI: 10.1016/S0887-7963(02)80080-3 page 270, column 1, paragraphs 2,3 -----	1-3,39, 44,46
E	EP 2 471 949 A1 (PROGENIKA BIOPHARMA SA [ES]) 4 July 2012 (2012-07-04) the whole document -----	1-47
A	WO 2006/075254 A2 (PROGENIKA BIOPHARMA SA [ES]; SIMON BUELA LAUREANO [ES]; MARTINEZ MARTI) 20 July 2006 (2006-07-20) page 11, paragraph 3 - page 12, paragraph 1 page 36, paragraph 3 - page 38 page 93 - page 105 -----	1-47
A	WO 2006/032897 A2 (UNIV BRISTOL [GB]; UNIVERSITETSSJUKHUSET I LUND B [SE]; OLSSON MARTIN) 30 March 2006 (2006-03-30) the whole document -----	1-47

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/061270

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0132702	A2	10-05-2001	AT 356828 T 15-04-2007
			AT 462722 T 15-04-2010
			AU 784319 B2 09-03-2006
			AU 2006202359 A1 29-06-2006
			CN 1413219 A 23-04-2003
			DE 60033954 T2 17-04-2008
			EP 1226169 A2 31-07-2002
			EP 1780217 A1 02-05-2007
			ES 2283324 T3 01-11-2007
			ES 2342837 T3 15-07-2010
			JP 4620920 B2 26-01-2011
			JP 2003513620 A 15-04-2003
			US 2011172406 A1 14-07-2011
			WO 0132702 A2 10-05-2001
EP 2471949	A1	04-07-2012	EP 2471949 A1 04-07-2012
			US 2012172239 A1 05-07-2012
WO 2006075254	A2	20-07-2006	AU 2006205571 A1 20-07-2006
			CA 2594730 A1 20-07-2006
			EP 1836322 A2 26-09-2007
			JP 2008526249 A 24-07-2008
			US 2008070253 A1 20-03-2008
			WO 2006075254 A2 20-07-2006
WO 2006032897	A2	30-03-2006	CA 2581240 A1 30-03-2006
			EP 1802767 A2 04-07-2007
			ES 2302667 T1 01-08-2008
			JP 2008513036 A 01-05-2008
			US 2009186340 A1 23-07-2009
			WO 2006032897 A2 30-03-2006